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Biodegradable sponge like microparticles for drug delivery and cosmetotextile

Nadiah Zafar

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Microparticules biodégradables à morphologie éponge pour
applications thérapeutiques et cosmetotextile

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Résumé

L'objectif de cette thèse est de préparer et de caractériser les particules cationiques biodégradables à base de cyclodextrine. Ces microparticules doivent également présenter une structure et morphologie éponge et multifonctionnelle. L'encapsulation simultanée de plusieurs actifs cosmétiques (bien être) et thérapeutiques (anti-inflammatoire) dans ces microparticules peut ouvrir de nouvelles applications une fois appliquées sur un textile spécifiquement sélectionnés pour leur fonctionnalisation. Pour atteindre cet objectif, des microparticules à base de polyméthacrylate cationique ont été préparés en utilisant le procédé émulsion double et diffusion de solvant suivi de l'évaporation du solvant. Des études systématiques ont été effectuées pour l'optimisation des paramètres du procédé ce qui a permis de contrôler la taille des particules et leur stabilité colloïdale. Une fois la taille désirée des particules de type éponge a été obtenu, la surface d'un textile model de polyamide a été fonctionnalisé via l'adsorption de ces particules tout en consacrant une attention particulière aux propriétés électrocinétiques du textile avant et après fonctionnalisation en fonction d'un grand nombre de paramètres physico-chimiques tels que; le pH, la salinité, la quantité initiale de particules présente lors de l'adsorption. Enfin, l'encapsulation de la Vitamine E, LIB (Lauryl Isoquinolinium Bromide), IMC (Indomethacin) ainsi que l'encapsulation de complexes de vitamine E-HP β CD, LIB-HP β CD et IMC-HP β CD dans microparticules a été effectuée séparément. Ces particules ont été caractérisées et la pénétration cutanée en utilisant une peau humaine a été examinée.

Summary

The objective of this thesis was to prepare and characterize cyclodextrin based biodegradable, cationic and sponge like multifunctional microparticles that can be used not only for the delivery of cosmetic and therapeutic agent (anti-inflammatory) but can also be potentially applied onto specifically selected textile for its functionalization. For achieving these goals, polymethylmethacrylate based microparticles were prepared using double emulsion-diffusion solvent evaporation technique. Systematic studies were performed for optimization of process control parameters. Once desired size of sponge like particles was obtained from the systematic study, the polyamide textile surface was functionalized with these particles while dedicating special attention to electrokinetic properties of textile as a function of various parameters such as; particles amount present during adsorption process. Finally, encapsulation of pure vitamin E, Lauryl Isoquinolinium Bromide (LIB), Indomethacin (IMC) as well as encapsulation of complexes of Vit E-Hydroxypropyl-beta-cyclodextrin (HPBCD), LIB-HPBCD and IMC-HPBCD into microparticles was done separately. These particles were characterized for skin penetration and encapsulation efficiency.

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Part I
GENERAL INTRODUCTION

Introduction

A textile or cloth is a flexible material which is made of a network of natural or synthetic fibers (yarn or thread). Fibers are usually long thin materials having a length at least one hundred times their diameter. Textiles have used since the dawn of mankind for the protection of human body from surrounding environment. With passing years they have become an integral part of our lives and revolutionary changes have occurred in their properties like improved quality and comfort of wear. A functional textile is any textile material that in addition to retaining its basic function also contains a precise functional load for improved efficiency (water repellency, anti-UV, antibacterial, etc). Textile encompass versatile characteristics due to which they are increasingly employed in diverse fields such as agriculture, marine materials, civil engineering textiles, forestry textiles, filtration materials, textiles for vehicles, sportswear, protective clothing, medical, hygiene and packaging materials. Because of these diverse applications of textiles in almost every field of life as well as increasing demands from market or consumers for introduction of innovative and specifically designed textile products, the surfaces of fabrics are specially and increasingly engineered to impart desired characteristics.

Textile technology includes variety of disciplines like structures of fibers, assembly of fibers into fabrics, surface modification, making, analysis and sale of fabrics. Among these disciplines, surface modification plays a key role in achieving desired textile functionalization. Unmodified textiles cannot be used as such since they might contain properties that could negate their use for humans. There are two major categories of surface modification techniques; physical and chemical. In physical methods, mostly non-chemical ways are used to deposit materials onto textile surface. On the other hand, in chemical techniques, usually a specific chemical agent is added. Some of the commonly used functionalization techniques include chemical grafting, chemical vapor deposition, sol-gel process, electrostatic interactions and electrospinning. Characterization of the surface of textiles is also very important for better understanding and optimization of textile surface modification. There are various techniques available for this characterization optical, microscopic, spectroscopic, thermodynamic and mechanical analysis.

Broadly speaking, two of the most important applications of textiles include their delivery of cosmetic and therapeutic agents. A cosmetotextile is a material containing a cosmetic preparation primarily for dermatology application such as moisturizing, slimming or anti-stretch mark.[1]. Textiles have been used in medical field since long time and they find applications in medical sutures, gauzes, bandages and repair fabrics and artificial aortas. However, the concept of their usage for drug delivery was not matured until the end of last millennium. Transdermal drug delivery is sought for a number of reasons. Certain actives, upon oral delivery, get absorbed in stomach or intestinal tract and on metabolism lose their activity before reaching their target organs. In such cases, dermal drug delivery is preferred to avoid the requirement of higher doses and toxicity risk associated with such higher doses. Moreover in specific patients e.g. children, elderly patients or patients in coma, where drug compliance is relatively low because of forgetfulness or difficulty in swallowing of medications, the drug compliance can be enhanced by dermal drug delivery which requires less frequent dosing.

The simplest method to functionalize textile is to impregnate the fabric directly with the active agents [2]. However, such processes offer no product durability and the effect achieved is not continuous in time. Loss of potent active agents upon washing of fabrics is one of the major drawbacks of such direct attachment of actives onto textile surfaces. Encapsulation of the active agents into particles or colloidal carriers provides a solution to these problems. Recently, variety of encapsulation techniques has attracted considerable attention of the researchers because of useful characteristics they impart to resultant formulations. Some of the useful properties that encapsulation provides include protection of the drugs against degradative effects of enzymes and pH, targeted and controlled release formulations and increased drug efficacy and specificity. A number of colloidal carriers such as liposomes[3], polymeric micelles and polymeric biodegradable nanoparticles, nanorods [4], nanotubes can be constituted from a number of encapsulation techniques available like nanoprecipitation [5], solvent evaporation [6], [7] solvent diffusion, ionic gelation, radical polymerization [8], interfacial polycondensation [9], spray coating [10], spray drying [11] and coacervation [12]. The selection of a particular technique usually primarily depends on the nature of the actives and the targeted application. Multi active particles are quite an interesting platform for drug delivery which can enable the simultaneous loading of active agents of opposing nature within the same formulation. The use of different polymers e.g. PLGA, PLA, Eudragit, to constitute drug carriers further helps to achieve multiple functionalities.

This work was targeted to achieve two main goals, i) elaboration of multifunctional biodegradable microparticles that could encapsulate both therapeutic and cosmetic agents ii) functionalization of specifically selected textile with the prepared active containing microparticles. This thesis is divided into two major parts. The first part deals with the state of art or literature review. This literature survey is necessary to highlight in a comprehensive way, important studies done in the past relating to our subject. An important novelty of this work is the introduction of cyclodextrin (CDs) into our formulation. The first two reviews provide the readers with very useful information about the nature and structure of CDs. The literature also provides an insight into how such structures are used to constitute biodegradable particles and the role CDs play in theranostic agents [13], [14]. Since the technique selected to form particles is double emulsion solvent like process, so the need to do literature survey on this process is also felt. Review about double emulsion informs about the history and mechanism of formation of particles via this process together with recent examples relating to encapsulation of actives [15]. The final application of our particles is adsorption onto textile surface and therefore a literature study about textiles is quite mandatory. The last part of state of art includes a review about textiles, their surface characterization and modification techniques.

The second part of thesis deals with the experimental studies. Firstly, preliminary and a systematic study is performed to optimize the various process control parameters as well as to optimize the size of particles according to our final application [16]. This is followed by an investigation of the electrokinetic properties of the selected textile. Next study is related to encapsulation of two cosmetic agents and one therapeutic agent into the particles and the adsorption of these active encapsulating particles onto the textile surface [17]. Finally, the last part of experimental study was dedicated to encapsulation of active-CD complexes into the

microparticles and their adsorption onto textile. Skin penetration studies of active containing particles were also performed.

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Part II
BIBLIOGRAPHY

Summary

In this part, we do deep analysis of the state of art so that we have profound knowledge and better understanding of research that already has been done by different researchers before our experimental study. It also helps us to decide which pathway would be best to follow to reach our goal and how to bring novelty into our work. An important aspect of this work is the introduction of cyclodextrin (CD) molecules into the particulate formulation. So the state of art was started with the collection of maximum amount of data available relating to CDs. Basic knowledge about nature, structure and properties of CDs are provided. Special focus is made on description of different encapsulation techniques that has been commonly used to prepare CD based particles. These techniques may include solvent evaporation, nanoprecipitation, ionic gelation, solvent diffusion, layer by layer process and emulsion polymerization. The role of CDs in particles formed via these techniques is also highlighted. Here, another aspect of using CDs in particles form is also brought into light. That is, how CDs are used to form theranostic agents. We discuss different types of CD based theranostic systems together with the role of CD in each of the system.

There are several encapsulation techniques available for preparing polymeric colloidal drug carriers. The next part is related to the description of basic background knowledge about double emulsion process. It will describe the mechanism of formation of particles via this technique while mainly focusing on different types of actives that has been encapsulated. Comparison of this technique to other techniques and the commonly employed polymers in this process is also highlighted.

The most important part of literature review is the requirement of basic knowledge about textiles. In the final part, we describe the different types of textiles and how their surfaces are characterized, which is very important for the proper textile functionalization. Moreover, this part will help us to understand different methods commonly adopted to modify the surface of fabrics.

In general, this state of art will also point out that sufficient data is not available on the usage of CD based polymeric particles for the functionalization of textiles.

II.1. Cyclodextrin containing biodegradable particles: From preparation to drug delivery applications

Summary

Encapsulation of medicinal agents in particles enhances drug efficacy, tolerability, specificity and therapeutic index of corresponding drugs. In this respect, variety of colloidal particles has provided enormous applications for both *in vivo* and *in vitro* functions in biomedical and biotechnology field. These vastly classified colloidal carriers furnish diverse alternatives for drug loading, administration and release. Different kinds of colloids employed in medicinal field include liposomes, polymeric micelles and polymeric biodegradable nanoparticles. The use of biodegradable polymers in drug delivery systems have become common due to superb characteristics imparted by these on the final particles obtained. Polymers are now also used as scaffolds in regenerative medicine and tissue engineering. The particles formed using biodegradable polymers can be used as active reservoirs providing local drug therapy or carrying drugs, genes or vaccines into the body for targeted and site specific drug delivery. Biodegradable particles have smaller sizes, are biocompatible with the human body and can also display sustained release properties. The polymeric material of such particles allows some modifications of the particles like changes in; zeta potential of the particles, hydrophobicity or hydrophilicity, bio-adhesion, cellular uptake and drug release. The polymeric particles can be conjugated with antibodies to make them suitable for therapy or diagnosis or the surface of particles can be coated with certain materials to make particles resistant to macrophage uptake. Moreover, biodegradable nanoparticles provide protection to the pharmaceutical actives from early degradation and also enhance the aqueous solubility of more hydrophobic agents.

The useful advantages provided by the polymeric particles can be further improved by the inclusion of different molecular cages in the formulation. Cyclodextrins (CDs) are one example of such cage like molecules. CDs are multifunctional structures that are known to form inclusion complexes with the active molecules leading to alteration in their physical, chemical and biological properties. CDs are composed of a number of glucose units (α -D-glucopyranose) joined together in a ring form. CDs are named depending on the number of glucose units they contain e.g. CD with 6 glucose units is named as α -CD. The glucopyranose units are linked together by α -1-4 linkages. CDs are bucket shaped molecules whose outer part is more hydrophilic due to hydroxyl groups as compared to inner part which is lined by ether like anomeric oxygen atoms. Hence, they possess hydrophobic inner cavity suitable to cage/encapsulate hydrophobic agents leading to inclusion complex formation.

CDs were first discovered by Villiers in 1891. These are obtained by the action of enzymes like amylases on the starch digest. Different types of CDs can be obtained depending on the type of enzymes utilized. Three common types of CDs i.e. α -CD, β -CD and γ -CD have similar structures and are homogeneous, crystalline and non-hygroscopic. However, they vary in their cavity volumes and solubility in water. Many derivatives of CDs have also been introduced. Derivatives can be formed by processes like etherification, esterification and amination of the primary and secondary hydroxyl groups. The derivatives were introduced in order to improve the aqueous solubility and stability of these agents against heat and light. The inclusion complex formation is a reversible process which does not involve any covalent bond formation or breakage. Removal of high energy water from the cavity and van der Waals forces are primarily responsible for the complex formation.

When a certain agent/drug molecule is fully or partially enclosed in the cavity of CDs, they are protected from direct heat, light, water, oxygen and radiation. So, any early degradation is prevented in this way. Some polymers that are water soluble also form ternary complex with the active-CD complexes leading to an increase in the stability of the active-CD complex. Controlled release properties can also be achieved through complex formation. Main advantage of using cyclodextrin is to enhance the aqueous solubility of hydrophobic active molecules by their encapsulation inside the hydrophobic cavity. Interactions between different agents can also be avoided through complexation. Bitter taste or irritancy of certain drugs can also be avoided through CD complexation. Any compound in liquid form can be converted to crystalline form when complexed with CD and hence such compounds can easily be formulated into solid dosage forms. It has also been observed that toxicity of certain drugs was lowered when they were delivered after complexation with CDs.

This review describes in detail the drug delivery systems based on cyclodextrins. Effort has been made to compile majority of the recent work done on cyclodextrin containing biodegradable polymeric delivery systems to provide the readers insight into further possibilities for enhancing research on such systems. Several techniques to constitute cyclodextrin based particles are discussed in detail together with advantages and disadvantages associated with each of the described technique.

Various drug molecules have been complexed with CDs and then formulated into particulate form for drug delivery. Some examples include oxaprozin-CD based nanoparticles (NPs) (emulsification solvent evaporation), bifonazole- β CD NPs (nanoprecipitation), clotrimazole- β CD NPs (nanoprecipitation), camptothecin- β CD NPs (nanoprecipitation), β CD based NPs delivering coumarin 102 (dialysis), chitosan/ β CD NPs containing plasmid DNA encoding for interleukin-12 (ionic gelation), chitosan/sulfobutylether- β CD NPs delivering econazole (ionic gelation), chitosan/carboxymethyl- β CD NPs delivering insulin and heparin (ionic gelation), glutathione-CD encapsulated in Eudragit RS100 based NPs (emulsion solvent diffusion), CD modified magnetic nanoparticles (layer by layer), amphiphilic CDs based vesicles encapsulating siRNA (phospholipid like structures), CD-insulin complex encapsulated in polymethacrylate acid based NPs (emulsion polymerization) and amikacin encapsulated in β CD microparticles (interfacial polycondensation).



Review

Cyclodextrin containing biodegradable particles: From preparation to drug delivery applications



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ABSTRACT

Cyclodextrins (CDs) offer a very broad spectrum of applications in diverse fields of drug delivery. They are a family of cyclic α -(1–4)-linked oligosaccharides of α -D-glucopyranose subunits forming a more hydrophobic central cavity and a hydrophilic outer shell. CDs bear cage like supramolecular structures, similar to calixarenes, cyclophanes and crown ethers. No covalent bonds are required to host a guest molecule in the central cavity. The aim of this review is to throw light on some of the applications and formulation techniques for the novel multifunctional CD based nanocarriers used in diverse areas of drug delivery. Furthermore, this article highlights the molecular structure, chemical, complexation properties and the use of CDs in nanosystems like liposomes, magnetic nanoparticles, biodegradable polymers, micro and nanospheres and capsules.

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1. Introduction

Colloidal particles have provided tremendous applications for both in vivo and in vitro purposes in biomedical and biotechnology field. Different types of colloids used in medicine are liposomes (Laouini et al., 2012), block ionomer complexes, dendrimers, polymeric micelles, polymeric biodegradable nanoparticles (NPs), quantum rods, nanorods (Wang et al., 2012) and nanotubes. Colloids especially liposomal NPs have been used in protective coatings; stain resistant clothing and cosmetic creams. Polymers have a range of medical applications, which include medical supplies, supports or replacement therapy for dysfunctioning parts of body. Recently, polymers are used as scaffolds in regenerative medicines and tissue engineering. The biodegradable polymers interact with biological environment in such a way to initiate/start cell ingrowth and tissue regeneration. This result can be achieved by modifying the surface of scaffold or by loading it with bioactive molecules. Biodegradable particles have gained more importance because they act as drug reservoirs providing local therapeutic effect or carry drugs, genes, and vaccines into the body for site specific delivery (Jahanshahi and Babaei, 2008). NPs can be conjugated with antibodies to combine the individual properties of both (Arruebo et al., 2009). For example, the small size, special thermal, drug carrier or magnetic characteristics of NPs can be combined with specific and selective recognition abilities of antibodies to antigens. Antibody-conjugated NPs are principally used for therapy and diagnosis. In therapy, development of targeted drug delivery is the main use of such particles. Whereas in diagnosis, these particles can be used as contrast agents for magnetic resonance imaging (MRI), for sensing, cell sorting, bioseparation, enzyme immobilization, immunoassays, transfection (gene delivery) and purification (Arruebo et al., 2009). Different natural and synthetic polymers have shown superb encapsulation of drugs like paclitaxel, 9-nitrocamptothecin, cisplatin, insulin, dexamethasone, estradiol, progesterone, tamoxifen, tyrphostins and haloperidol (Kumari et al., 2010).

Biodegradable NPs exhibit controlled/sustained release characteristics, have subcellular size and are biocompatible with human cells and tissues (Kumari et al., 2010; Panyam and Labhasetwar, 2003). Apart from this, biodegradable polymeric encapsulated drugs are non-toxic, stable in blood, noninflammatory and polymeric materials allow the modification of physicochemical characteristics (e.g. hydrophobicity, zeta potential), drug release properties (e.g. delayed, prolonged, triggered) and biological behaviour (e.g. bioadhesion, improved cellular uptake) of the NPs (Kumari et al., 2010; Des Rieux et al., 2006; Galindo-Rodriguez et al., 2005). Furthermore, particles surface can be modulated by coating with certain molecules like PEG which helps in preventing uptake of NPs by macrophages. Biodegradable NPs enhance therapeutic value of many water soluble/insoluble drugs by improving the solubility, bioavailability and retention time (Kumari et al., 2010; Shenoy and Amiji, 2005). Apart from these advantages, the added benefits include protection from early degradation in biological system, enhanced absorption at specific areas and greater intracellular penetration (Kumari et al., 2010; Alexis et al., 2008). The selection of polymer is done considering its final application as well as its toxicity, e.g. biodegradable polymer can be a preferred candidate for temporary application.

The immense characteristics delivered by biodegradable particles can be further enhanced by the use of various molecular cages. Cyclodextrins (CDs) are an example of such cages; which impart useful features to particles drug delivery system. These are potential high performance multifunctional drug carriers having the capability to alter the physical, chemical and biological properties of drug moieties by inclusion complex formation (Vyas et al., 2008). CDs isolate the substance from the external environment

by enclosing it fully or partially inside its bucket shaped cavity, thereby giving it protection against oxygen, water, heat, radiation and hence degradation. Water soluble polymers also form ternary complex with drug/CD complexes which increases the stability constant of drug/CD complex (Citernes and Sciacchitano, 1995; Loftsson and Duchêne, 2007). CD inclusion complexes prevent the natural decomposition of enclosed drug and boost its effect over time. These are not sensitive to pressure stress as inclusion complex reaction is reversible and resultant complexes can exist in crystalline form. Temporary encapsulation of certain active substances allow them to have controlled-release properties (Citernes and Sciacchitano, 1995). Aqueous solubility of poor water-soluble compounds can be increased by CD inclusion complexes with the functional groups. Similarly when low bioavailability is due to poor solubility then CD inclusion complexes are of great value. CDs can also encapsulate drugs, which are irritants to skin, eye or stomach. Inclusion complexes help in preventing interaction between various components of a formulation. Complexation inclusion can convert substances which are liquids at room temperature to crystalline form for easy formulation into solid dosage form (Tiwari et al., 2010; Asai et al., 2002). Bitter taste, bad smell and colour of substance can be easily masked by inclusion complexes (Del Valle, 2004). Toxicity of certain drugs is also lowered when such drugs are placed in NPs containing CD. Local intestinal toxicity of docetaxel was found to be low when it was encapsulated in mucoadhesive NPs comprised of methyl- β -CD (Me- β -CD)/poly(isobutylcyanoacrylate) coated with thiolated chitosan (Mazzafarro et al., 2012). Due to complexation properties, CDs have been employed in the design of drug delivery systems like liposomes, microspheres, microcapsules, magnetic NPs, nanogels, nanosponges, hydrogels, beads and CD containing polymers. Targeted drug delivery can also be achieved by using CD (Vyas et al., 2008).

2. CD chemical structure and properties

CDs are substances composed of sugar (α -D-glucopyranose) molecules joined together in a ring form i.e. cyclic oligosaccharides. These are also called cyclo amyloses, cyclomaltooses or Schardinger dextrins and are non-reducing in nature. CDs nomenclature is based on the number of glucose units in their structure, so that CD with 6 glucose units is called α -CD, heptamer as β -CD and one with 8 units is named as γ -CD. Glucopyranose units in CDs are linked by α -1-4 linkages. The chair formation of glucopyranose units gives CDs a cone like shape (Arun et al., 2008). There is hydrogen bonding between the 2-OH and 3-OH groups around the outer edge. These bonds are weakest in α -CD and strongest in γ -CD. Around the bottom edge, 6-OH groups can also form hydrogen bonds but the bonds are destabilized by dipolar effects and are not normally present in CD crystals. In α -CD, the hydrogen bonding is purely 3-OH (donor), 2-OH (acceptor). But in β and γ -CD the bonding switches between this and 3-OH (acceptor), 2-OH (donor). CDs are amphipathic structures in which 3-OH and 2-OH groups are displayed on the wider rim and 6-OH groups on the narrower rim. The outside of the molecular cavity is lined by these hydrophilic groups and the inner surface is lined by ether like anomeric oxygen atoms. Thus CDs have an inner hydrophobic cavity and an outer hydrophilic surface (Fig. 1; Zhou and Ritter, 2010).

CDs were first discovered by Villiers in 1891. He found a small amount of new crystalline substance from the starch digest of *Bacillus amylobacter*. According to other scientists, Villiers perhaps used impure cultures and CDs were a product of *Bacillus macerans* contamination. Cellulose was the name Villiers chose for this substance. Later on in 1903, Schardinger isolated two crystalline materials named dextrins A and B, which lacked reducing power.

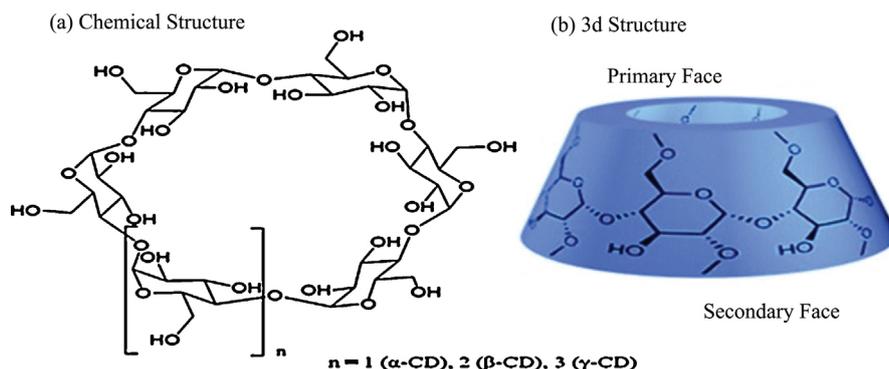


Fig. 1. Chemical structure of cyclodextrin (Zhou and Ritter, 2010).

In 1904, Schardinger separated a new organism that could produce acetone and ethyl alcohol from starch/sugar containing plant materials. In 1911, he found out that this organism called *Bacillus macerans* could also produce large quantities of crystalline dextrans. These dextrans were named as α and β dextrans. γ -CD was separated in 1935. X-ray structures of α and β dextrans were elucidated in 1942 and that of γ -dextrans in 1948. It was also found out that CDs formed inclusion complexes (Del Valle, 2004).

CDs are obtained by the action of enzymes like CD glycosyltransferase (CGTase) and α -amylases on starch. Starch is first liquefied by subjecting to heat or by adding α -amylase. This step is followed by the addition of CGTase, which synthesizes all forms types of CDs in ratios that are dependent on the enzyme type used. The three types of CDs can be easily purified by utilizing their different aqueous solubilities. β -CD can be removed by crystallization as it has very low water solubility. The other two forms are purified by chromatography or by addition of complexing agents like toluene and ethanol. The three common CDs have similar structures and are homogeneous, crystalline and non-hygroscopic in nature. The diameter of the cavities is different depending on the number of glucose units. β -CD has a lower solubility than α -CD even though it contains greater number of hydroxyl groups. This is due to the fact that internal hydrogen bond network is formed between the secondary hydroxyl groups. Many derivatives of CDs have also been synthesized by variety of processes like amination, etherification and esterification of primary and secondary hydroxyl groups. The derivatives have different cavity volume and also possess improved solubility, stability against oxygen and light (Del Valle, 2004). There is rapid equilibrium between the free molecules in solution and the drug in complex (Fig. 2). No covalent bonds are either broken or formed during complexation (Schneiderman and Stalcup, 2000). The forces responsible for complexation are (i) removal of high energy water from the cavity, (ii) release of ring strain especially in α -CD, (iii) van der Waals forces, (iv) hydrogen and hydrophobic bindings. We can achieve greater enhancement of solubility

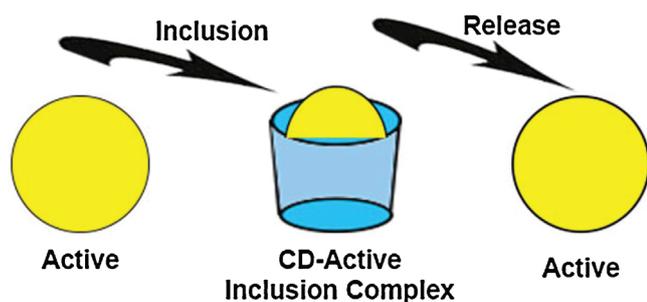


Fig. 2. Inclusion and release of guest molecules into and from the cyclodextrin cavity.

with CD complexation with the drugs that possess lower aqueous solubility (Del Valle, 2004).

3. Techniques

Several techniques are employed for the preparation of NPs. In broad terms there are two techniques namely: dispersion of preformed polymers and polymerization of monomers. The former can be accomplished by methods like solvent evaporation, nanoprecipitation, solvent diffusion, salting out, dialysis and supercritical fluid technology. Whereas polymerization of monomers is carried out through emulsion formation, miniemulsion, microemulsion, interfacial polymerization and controlled/living radical polymerization processes. Sometimes two methods can be combined to get better results. The selection of a method depends upon several factors like size of a particle, particle size distribution and the required application.

3.1. Nanoparticles obtained by dispersion of preformed polymers

3.1.1. Emulsification solvent evaporation method

In 1979, Vanderhoff introduced the idea of emulsion solvent evaporation (El-Aasser et al., 1979). This technique comprises of two prime steps: formation of single or double emulsion and organic solvent evaporation. Evaporation leads to polymer precipitation and particulate formation (Miladi et al., 2013; Fig. 3).

In single emulsification, the selected polymer with desired characteristics is dissolved in an organic, volatile and water immiscible solvent like dichloromethane, ethyl acetate or chloroform. This constitutes an organic phase, which is emulsified into an aqueous phase, which already contains a surfactant. High speed homogenization or sonication is essential for dispersion of organic phase in an aqueous phase (Miladi et al., 2013) and to prevent coalescence of droplets. Single emulsion evaporation is generally used for hydrophobic drug encapsulation (Miladi et al., 2013; O'Donnell and McGinity, 1997). Double emulsion evaporation is a more apt technique for encapsulation of any hydrophilic moiety. In this case, the primary emulsion formed via single emulsification (generally w/o) is again dispersed by homogenization in a second aqueous phase (Giri et al., 2013). In both types of emulsions, evaporation of the organic solvent is achieved by stirring at room temperature or under high temperature and low pressure conditions. The formulated particles can be obtained by ultracentrifugation or filtration and finally are washed and lyophilized (Miladi et al., 2013). More knowledge about the mechanism involved in particulate formation can be acquired from the study of Rosca et al. (2004). Stabilizers are found to play double role in emulsions. They act as film formers and barrier to drug release at the internal interface and as a steric stabilizer on the external aqueous phase (Mora-Huertas et al., 2010; Khoee and Yaghoobian, 2009).

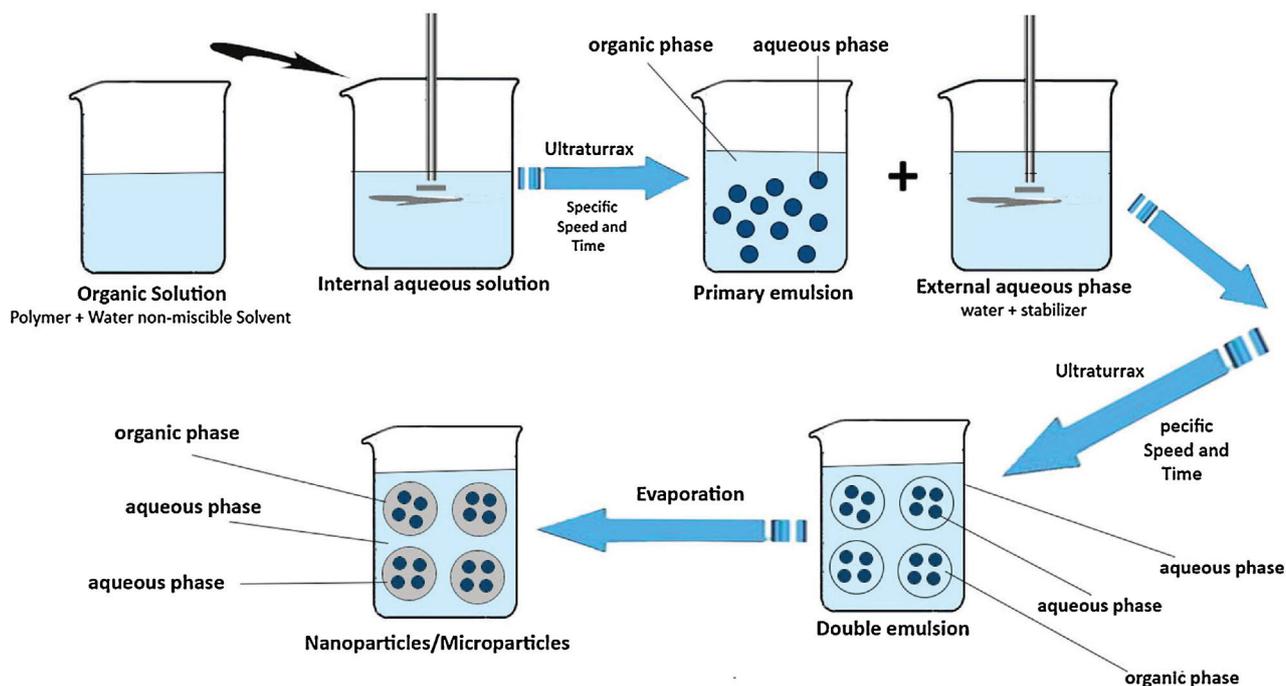


Fig. 3. Basic principle of emulsification solvent evaporation.

An anti-inflammatory drug, oxaprozin's (OXA) therapeutic efficacy was enhanced by complexation with CD and loading into poly(lactic-co-glycolic acid) (PLGA) NPs by double emulsion method (Mura et al., 2010). Ball-milling in a high-energy vibrational micro-mill was utilized to obtain drug-CD equimolar co-ground systems (GR). Water-in-oil-in-water (w/o/w) double emulsion solvent method was employed to construct PLGA NPs. PLGA solution in dichloromethane containing OXA-CD systems was added to an aqueous solution of polyvinyl alcohol (PVA) and emulsified by high speed homogenization. This resultant primary emulsion was again added to an aqueous solution of PVA to form w/o/w double emulsion. It was kept under magnetic stirring for a specific time. The NPs obtained were then isolated by ultracentrifugation. It was found out that presence as well as type of CD markedly influenced the drug release rate from the NPs. The percent of drug released over 24 h varied from 16% (simple drug loaded NPs) to 50% (drug- β CD loaded NPs) up to 100% (drug-methyl β CD loaded NPs). Thus, enhanced bioavailability of the drug and prolonged release characteristics were achieved by using preparation of CD NPs by this technique (Mura et al., 2010).

3.1.2. Nanoprecipitation technique

Nanoprecipitation and interfacial deposition processes are similar as they involve impromptu emulsification of organic internal phase containing the dissolved polymer into the external aqueous phase (Fig. 4). However, the resultant products are different for these processes. Nanospheres/nanocapsules are obtained in solvent displacement and only nanocapsules in interfacial deposition. Solvent displacement (also called as nanoprecipitation) includes precipitation of the selected preformed polymer from an organic solution and the diffusion of organic solvent in the water medium under the presence/absence of surfactants. The polymer e.g. polylactide (PLA) is dissolved in water miscible solvent of intermediate polarity, leading to precipitation of nanospheres. This phase is then introduced into an already stirred aqueous solution containing a stabilizer as a surfactant. Polymer deposition on the interface between organic solvent and water, caused by speedy diffusion of the solvent, induces spontaneous formation of colloidal suspension

(Quintanar-Guerrero et al., 1998). Only water-miscible solvents are employed in this technique (Quintanar-Guerrero et al., 1998) in which the diffusion rate is good enough to give spontaneous emulsification. Furthermore this method is not considered best for encapsulation of hydrophilic drugs and is basically used for lipophilic moieties because of the miscibility of the solvent with the aqueous phase (Barichello et al., 1999). Eudragit[®] RL 100 based nanosuspensions were formulated by nanoprecipitation technique with an aim to improve ocular drug delivery. Average particle size of about 123.5–140.2 nm and zeta potential in the range of +13.1 to 20.8 mV was obtained. Results of the studies showed sustained drug (Brimonidine Tartrate) delivery for about more than 6 h (Khan et al., 2012). In 2012, modified nanoprecipitation method was employed to constitute argan oil based nanocapsules containing Naproxen. The aim was to achieve dual functionalities of cosme-to-therapy in a single platform (Rosset et al., 2012).

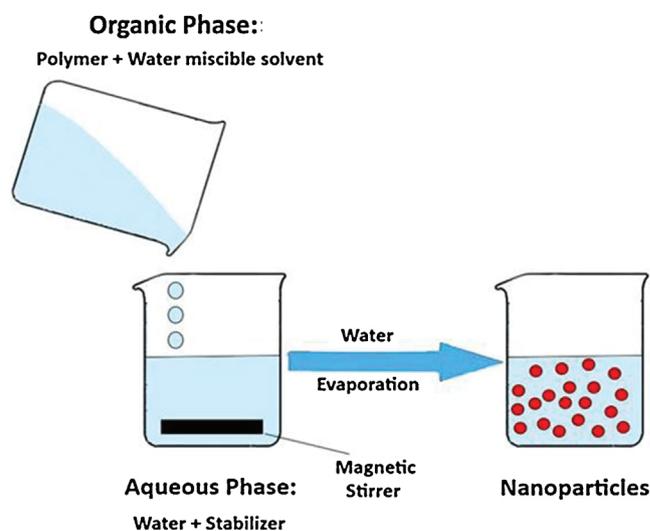


Fig. 4. Basic principle of nanoprecipitation technique.

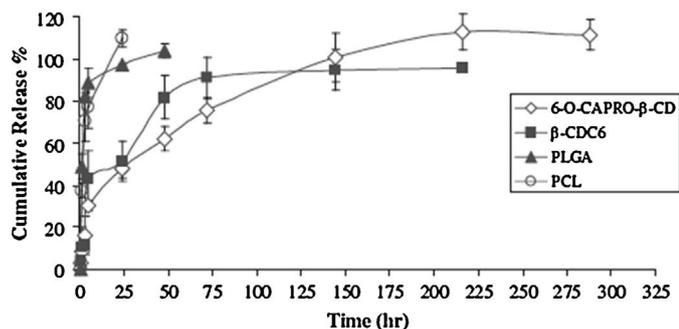


Fig. 5. In vitro release profile of CPT from nanoparticles into isotonic phosphate buffer, pH 7.4 ($n = 3 \pm \text{S.D.}$) (Çirpanli et al., 2009).

Highly loaded nanoparticle systems containing amphiphilic β -CD to promote the parenteral administration of poorly soluble antifungal drugs bifonazole and clotrimazole were prepared by using solvent displacement method (Pinto Reis et al., 2006; Memişoğlu et al., 2003). Camptothecin (CPT) is an anticancer drug and amphiphilic β -CD NPs containing CPT were prepared by nanoprecipitation by Çirpanli et al. (2009). CPT: β -CD₆ complexes were prepared by co-lyophilization method. An organic phase consisting of CPT: β -CD inclusion complexes dissolved in absolute alcohol was prepared. This phase was then added to aqueous phase containing ultrapure water at room temperature. Evaporation was carried out for removal of organic solvent. CPT loaded polymeric NPs were also prepared by this technique. Different polymers like PLGA or poly- ϵ -caprolactone (PCL) were utilized and dissolved in organic solvent like acetone followed by addition of this phase into deionized water. The deionized water already had Pluronic F68 and CPT: hydroxypropyl(HP)- β -CD inclusion complexes. There was immediate formation of NPs by nanoprecipitation. Removal of organic solvent was done by roto-evaporation under reduced pressure (Çirpanli et al., 2009). Amphiphilic CD based NPs displayed markedly higher drug loading values as compared to PLGA and PCL NPs prepared via the same nanoprecipitation technique (Table 1). Furthermore CD based NPs showed a controlled release profile extending up to 12 days and polymeric NPs (PLGA, PCL) showed release profile for 48 h (Fig. 5). In vitro drug release times from the different forms of NPs (Table 2) were also investigated. Comparison between the polymeric NPs and CD NPs for anticancer efficacy revealed that CD loaded NPs had higher efficacy. The cell viability of breast cancer cells was found to be 65% for CPT solution in DMSO, 56% for CPT-loaded 6-O-Capro- β CD, 61% for CPT-loaded β CDC6,

Table 1

Drug-loading values of the amphiphilic β -cyclodextrin nanoparticles loaded with CPT (Çirpanli et al., 2009).

Formulation	Entrapped quantity (μg) \pm S.D.	Associated drug (%) \pm S.D.
CPT: β -CDC6 NPs	28 \pm 2	9 \pm 1
CPT:6-O-Capro- β -CD NPs	48 \pm 4	13 \pm 3
PLGA NPs	13 \pm 0.1	2.6 \pm 0.03
PCL NPs	7.2 \pm 0.5	1.4 \pm 0.2

Table 2

In vitro drug release time of nanoparticles (Çirpanli et al., 2009).

	β -CDC6	6-O-Capro- β -CD	PLGA	PCL
30% release (burst effect)	2 h	5 h	0.5 h	1 h
50% release	5 h	24 h	1 h	2 h
Complete release	144 h	288 h	48 h	48 h

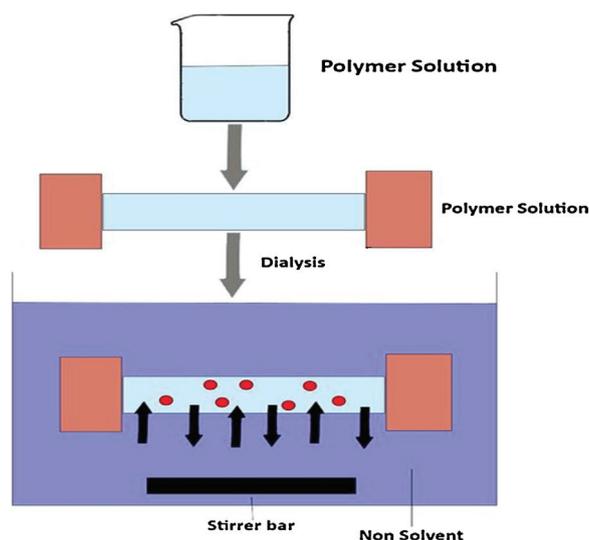


Fig. 6. Principle of dialysis method.

Adapted from Rao and Geckeler (2011).

81% for CPT-loaded PLGA and 68% for CPT-loaded PCL NPs suggesting the better efficacy of CPT when encapsulated into amphiphilic CD NPs especially the 6-O-Capro- β CD NPs. Similarly another anticancer drug Paclitaxel was encapsulated in amphiphilic CD NPs by Bilensoy et al. (2008) through this strategy.

3.1.3. Dialysis method

This technique is based on principle of osmosis and employs a physical barrier, precisely a dialysis membrane or semipermeable membrane, which allows the passive transport of solvents. This slows down the mixing of polymer solution with a non-solvent. The selected polymer dissolved in an organic solvent is placed inside the dialysis membrane (Chronopoulou et al., 2009). Dialysis is done against a non-solvent, which is miscible with the former miscible. The mixing of the solvent and non-solvent inside the membrane gradually decreases the ability of the mixture to dissolve the polymer. There is an increase in interfacial tension, which impels the aggregation of the polymer into spheroidal particles and hence precipitation occurs (Fig. 6). There are many advantageous associated with this method in addition to its low cost and general applicability. Mild conditions are employed for this technique averting the use of emulsifiers and co-stabilizers. Pure products are achieved and recovery of solvents is done easily by distillation (Chronopoulou et al., 2009). Furthermore, this technique allows the formation of NPs along with bioactive molecule immobilization (Masotti et al., 2008).

β -CD based core-shell structured nanoassemblies were prepared via a dialysis procedure using PEG-*b*-PCDs (copolymers) in the presence of a hydrophobic homopolymer poly (β -benzyl L-aspartate) (PBLA) (Zhang and Ma, 2011). The assemblies may serve as novel nanocarriers for the delivery of hydrophobic drugs. In brief, PEG-*b*-PCD and PBLA were dissolved separately in DMSO. A mixture solution with the appropriate weight ratio of PBLA/PEG-*b*-PCD was then dialyzed against deionized water for the whole day (Zhang and

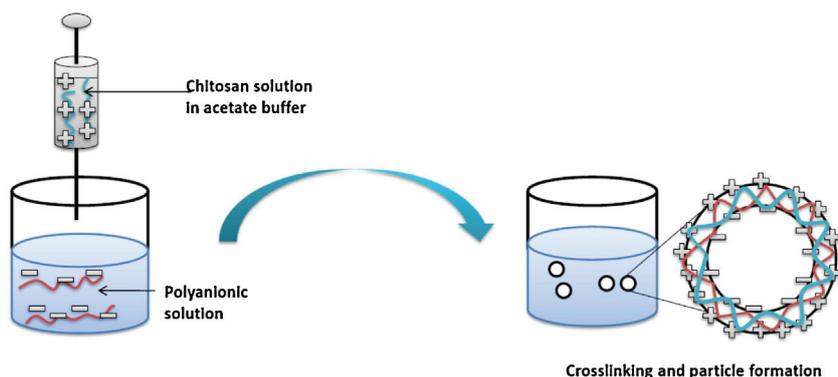


Fig. 7. Ionic gelation method (Miladi et al., 2013).

Ma, 2011). For the preparation of coumarin 102 containing assemblies, C102 was dissolved in DMSO followed by addition of PBLA and PEG-*b*-PCD-3. Then the same dialysis procedure was carried out (Zhang and Ma, 2009).

3.1.4. Ionic gelation method

The basic principle of this technique is the electrostatic interaction between oppositely charged polymer and a polyelectrolyte. The process involves drop wise addition of charged polymeric solution to an oppositely charged polyelectrolyte under constant stirring. Cross linkages between the two materials leads to formation of particles (Doustgani et al., 2012; Fan et al., 2012).

This technique was widely used for preparing chitosan based particles (Miladi et al., 2013; Fig. 7). Electrostatic interaction may occur between positively charged chitosan (in an acidic medium) and negatively charged tripolyphosphate (Jafarinejad et al., 2012; Li et al., 2011) (TPP) or arabic gum (Avadi et al., 2010). Hydrophilic chitosan based NPs were prepared by Calvo et al. (1997) by ionic gelation technique. Similarly, dexamethasone sodium phosphate release from chitosan based NPs prepared via this technique was also investigated (Doustgani et al., 2012). Particles of size 256–350 nm with a loading capacity of 72.2% were achieved.

Chitosan/ β -CD NPs containing plasmid DNA encoding for interleukin-12 were prepared by ionic gelation technique. Two aqueous solutions were prepared (Nahaei et al., 2013). The first one contained chitosan solution and the second one consisted of CD having TPP as a crosslinking agent. Specific amount of pDNA (pUMVC3-hIL12) was incorporated directly into the CD:TPP phase. The two aqueous phases were mixed under vortexing for 10 min. Resultant NPs were spherical in shape with a size in the range of 171.3 ± 2.165 nm. The particles exhibited a positive zeta potential of 34.3 ± 1.55 and an encapsulation efficiency of 83.315 ± 2.067 (Nahaei et al., 2013).

Chitosan CD (CS-CD) NPs can also be utilized for gene delivery into the airway epithelium (Teijeiro-Osorio et al., 2009). In this case, the two aqueous solutions consisting of CS solution and sulfobutylether- β -CD solution (SBE- β -CD) were mixed under magnetic stirring. Loading of CD/TPP phase was done with pSEAP (plasmid DNA model that encodes the expression of secreted alkaline phosphatase). The formation of NPs was due to combined effect of electrostatic interaction between CS and CDs (oppositely charged) and ability of CS to undergo a liquid–gel transition due to its ionic interaction with TPP. The particles showed a size range of 100–200 nm, a positive surface charge (+22 to +35 mV) and very high DNA association efficiency values of more than 90% (Teijeiro-Osorio et al., 2009). In another work, Chitosan/sulfobutylether- β -CD NPs were constructed for ocular drug delivery system (Mahmoud et al., 2011). Econazole was the chosen model drug. NPs were obtained by using ionic gelation

method. The ratio of CS and SBE- β -CD was very important as it controlled the particle size. It was best to establish an optimum ratio (which enabled the formation of NPs) between these two components before the encapsulation of the drug into chitosan NPs. The resultant spherical particles had an average diameter of 60–673 nm and a positive zeta potential of 22–33 mV. Encapsulation efficiency was 13–45% (Mahmoud et al., 2011).

Chitosan-CD NPs can also act as nanocarriers for slow/fast delivery of macromolecules. CS/carboxymethyl(CM)- β -CD/TPP NPs were prepared according to method described by Calvo et al. (1997). Insulin and heparin were used as macromolecular drugs. NPs spontaneously evolved due to interaction of positively charged amino groups of CS and negatively charged CM- β -CD and/or the negatively charged TPP. Size range of resultant particles was 231–383 nm and a positive zeta potential of 20.6–39.7 mV. Association efficiency for insulin was 85.5–93.3% and for heparin was 69.3–70.6% (Krauland and Alonso, 2007).

Chitosan CD NPs developed by ionotropic gelation method also improve the solubility of poor water-soluble drugs e.g. ketoprofen.

3.1.5. Emulsion solvent diffusion technique

This method was developed by Leroux et al. (1995). Basic requirements for this technique are presence of three liquid phases; an aqueous phase (stabilizer dispersed in water), an organic phase (polymer in organic solvent and a hydrophobic drug) and a dilution phase (Miladi et al., 2013). The organic phase employed must be partially soluble in water. The organic and aqueous phases are mutually saturated and emulsified using high speed homogenization. Finally addition of large volume of water allows diffusion of organic solvent from dispersed phase to external water phase. This results in precipitation of polymer and particulate formation (Miladi et al., 2013; Kwon et al., 2001; Fig. 8). Solvent removal is done according to its boiling point either by evaporation or filtration. Encapsulation efficiency is more than 70%. This technique is good for encapsulating hydrophobic drugs (Pinto Reis et al., 2006).

Special NPs consisting of CD and Eudragit RS 100 were formulated by this technique for the encapsulation of glutathione (GSH) (a model peptide drug) which was intended to be absorbed through transmucosal route (Lopedota et al., 2009). The basic aim was to combine mucoadhesive properties of Eudragit RS100 with the penetration enhancement and peptide degradation protective qualities of CD in a single NP formulation for transmucosal drug delivery. α , β , γ -CD, HP- β -CD and Me- β -CD were used. Initial step consisted of mixing equimolar amounts of GSH and CD in deionized water under magnetic stirring to achieve inclusion complex formation. The formed inclusion complexes were then dissolved in appropriate volume of deionized water containing aqueous solution of PVA. Organic phase was prepared by dissolving Eudragit RS100 in acetone. Organic phase was added to aqueous phase and subjected to

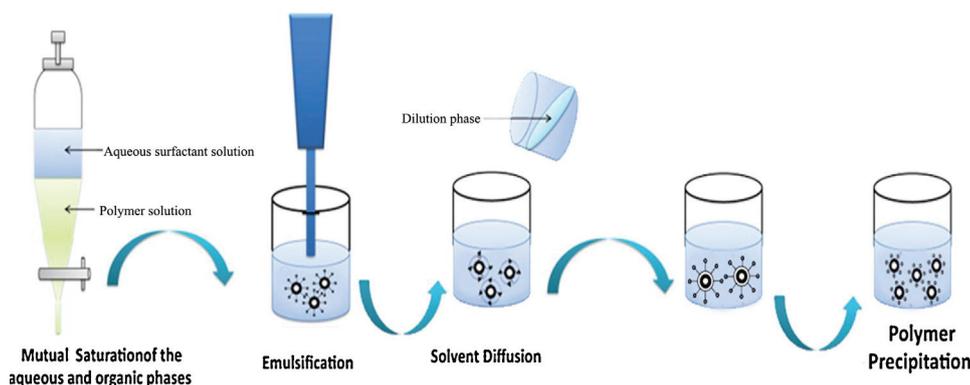


Fig. 8. Emulsion solvent diffusion.

Adapted from Miladi et al. (2013) and Kwon et al. (2001).

homogenization under Ultraturrax. Acetone removal with rotavapor and finally centrifugation resulted in NPs construction. NPs displayed mean diameter between 99 and 156 nm and encapsulation efficiency of peptide between 14.8 and 24%. The in vitro GSH release profile of these NPs was also studied at pH values of 1.2 and 6.8 corresponding to pH values of stomach and intestinal fluids respectively. It was observed that nature of CD employed markedly influenced the GSH released. The greatest GSH release was from γ -CD based NPs at pH 6.8 over 6 h and there was less than 50% release by all the types NPs in acidic environment. The NPs without any CD showed a release of 23% and 15% after 6 h at pH 1.2 and 6.8, respectively (Lopedota et al., 2009). It was concluded that oligosaccharides affects the drug release and to some extent peptide stability as well but had no influence on the drug loading (Lopedota et al., 2009). Tapas et al. (2011) worked on CD based spherical agglomerates. Carvedilol (CAR) was chosen as the model drug. CAR-CD agglomerates were constructed by solvent diffusion technique using acetone, water and dichloromethane as the good solvent, poor solvent and bridging liquid respectively. β -CD/HP- β -CD acted as hydrophilic polymers (Tapas et al., 2011). Indomethacin with CD has been used to make nanocrystals by solvent diffusion (Makhlof et al., 2008). Indomethacin is poorly water-soluble drug. The drug was dissolved in a water miscible organic solvent ethanol to constitute organic phase. This phase was slowly poured into aqueous CD phase with moderate mechanical stirring. Centrifugation followed this step. There was immediate formation of indomethacin particles when ethanol diffused from disperse phase to bulk aqueous phase during initial emulsion formation. CD prevented the growth and aggregation of particles to micro-order. The mechanism suggested was the formation of CD network (by intermolecular interactions) that could conserve and stabilize the resultant nanocrystals. This formed product was compared to commercial drug powder and it showed speedy and complete dissolution of IMC. The particles displayed an average diameter of 300–500 nm (Makhlof et al., 2008).

4. β -CD based nanoparticles induced through layer by layer process

In the last decades, much research has been done to develop and improve the various synthetic procedures for the iron oxide NPs. Many advances have been made to control their shape and improve their stability and biocompatibility. There are various methods described in literature for constitution of magnetic NPs (Ahmd et al., 2012; Rahman and Elaissari, 2012). Whereas the most common methods employed to synthesize magnetic NPs include co-precipitation, thermal decomposition, hydrothermal synthesis, microemulsion and sonochemical synthesis (Wu et al., 2008). Other methods like electrochemical synthesis (Cabrera et al., 2008; Pascal

et al., 1999), laser pyrolysis (Bomatí-Miguel et al., 2008), microorganism or bacterial synthesis can also be utilized to manufacture magnetic NPs (Bharde et al., 2008; Roh et al., 2006). Magnetic NPs are constructed for their immense uses in biotechnology and catalysis. Their wide range of applications includes magnetic bio-separation, iron oxide based novel biothin films (Ahsan et al., 2013), biological molecules detection, clinical diagnosis and therapy (such as MRI), MFH, targeted drug delivery and biological labels. The surface of iron oxide NPs is often modified with different organic and inorganic molecules either to enhance the biological application scope of these particles or to improve their stability and prevent agglomeration by passivating the surface of iron oxide NPs during the manufacturing process (Wu et al., 2008). Xia et al. (2007) prepared water-soluble magnetic nanoparticles with a surrounded layer by using polyethylene glycol nonylphenyl ether (N5) and CD in aqueous medium.

4.1. Preparation of magnetic nanoparticles

The customary procedure to construct Fe_3O_4 particles is by utilizing the co-precipitation technique. In this method ferric and ferrous ions in a mole ratio of 1:2 are mixed in an alkaline solution at room or elevated temperature (Wu et al., 2008). The formed particles can be modified with CDs in some of the following ways to get combined benefits of CDs and magnetic particles.

4.2. β -CD modified Fe_3O_4 magnetic nanoparticles by LBL technique

This technique uses the spontaneous electrostatic attraction between the oppositely charged polymer chains to synthesize magnetic latex. This method can be employed to give coated latex of different shapes and sizes possessing uniform, controlled thickness of the layer without the usage of costly equipments (Rahman and Elaissari, 2010). Introduction of various functional groups onto the surface of such particles gives multifunctional magnetic latexes (Sauledde et al., 1999; Kim et al., 2002). Polyelectrolyte adsorption onto colloidal particles by LBL technique is also a well-established method for magnetic NPs encapsulation and functionalization (Caruso et al., 1999a,b).

Zhu et al. (2011) prepared a novel magnetic nanoparticle whose surface was modified with mono-6-deoxy-6-(p-toluenesulfonyl)-CD (6-TsO- β -CD). In this case monotosyl-CD particles were first prepared by modification of β -CD. NaOH in water was added to β -CD suspended in water. To this mixture, p-toluenesulfonyl chloride (TsCl) (in acetonitrile) was added drop-wise and this resulted in immediate formation of white precipitates of 6-TsO-CD. The surface of magnetic particles was modified with

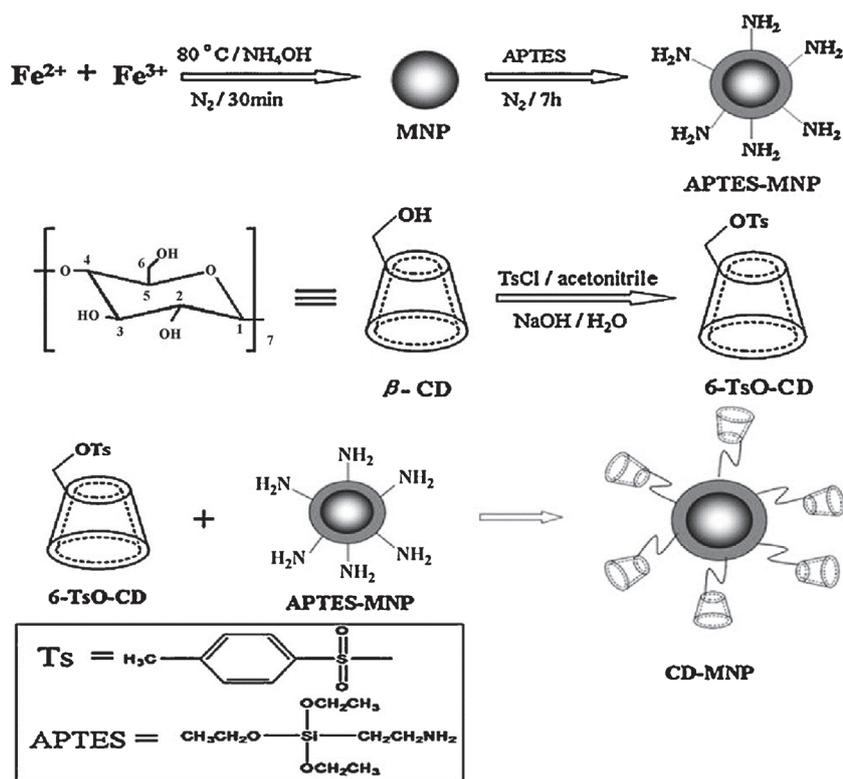


Fig. 9. Schematic illustration of method to construct CD-APTES-MNPs (Zhu et al., 2011).

γ -aminopropyltriethoxysilane (APTES) (Fig. 9). The next step was the covalent bonding of monotosyl-CD onto the surface of modified iron NPs by means of layer-by-layer process. The size of obtained NPs was around 16 nm. β -CD graft on magnetic NPs served as a modified electrode for detection of uric acid with cyclic voltammograms. CD modified MNPs showed better and higher electrochemical performance than the unmodified MNPs. These particles can act as drug carriers, biosensors and bio-separators. These can also be used for recognition and separation of nucleosides by slight modification of grafted CD (Zhu et al., 2011).

4.3. Biodegradable polyelectrolyte nanocapsules for intracellular protein delivery by LBL

In another experiment amino functionalized silica particles were coated with monotosyl-CD (Shu et al., 2010). Gradient cross-linked hollow polyelectrolyte nanocapsules having cysteamine conjugated chitosan, dextran sulfate (DS) were constructed by LBL adsorption on β -CD conjugated silica NPs. Bovine serum albumin (BSA) as a model drug was loaded into the nanocapsules (Shu et al., 2010).

Firstly, amino functionalized silica ($\text{SiO}_2\text{-NH}_2$) particles were prepared according to modified Stöber method (Stöber et al., 1968). Then, β -CD conjugated silica NPs were manufactured by addition of tosyl- β -CD to amino functionalized silica particles (Fig. 10). The next step was the formation of cystine conjugated chitosan (CS-SH) by method described by Krauland et al. (2005) (Fig. 11). The final step involved of cross-linked nanocapsule formation by LBL process (Fig. 12). CD conjugated silica particles were added to the drug solution. This step was followed by adsorption of CS-SH and polymer DS solution. After completion of multiple layers build-up, particles were exposed to chloramine T for formation of cross-linked particles. Dissolving of silica core with $\text{HF}/\text{NH}_4\text{F}$ at specific conditions of temperature and time resulted in formation of hollow cores. The mean size of $\text{Si-NH}_2\text{-CD}@\text{(CS-SH)}_{20}/\text{DS}$ was

about 126.7 ± 8.2 nm. Particles were spherical in shape. Encapsulation efficiency and loading capacity were affected by the initial concentration of BSA (drug) used. The encapsulation efficiency was decreased and loading capacity was increased with an increase in the initial concentration of BSA. The release of the protein from the hollow capsules in the presence and absence of GSH was also investigated and it was found out that there was more BSA release with higher concentration of GSH. These NPs can protect the drugs from destruction in acidic medium. Drug can be easily released by reduction of disulfide bond by the glutathione present in the cells. These nanocapsules hold promising applications in drug delivery and microreactors (Shu et al., 2010).

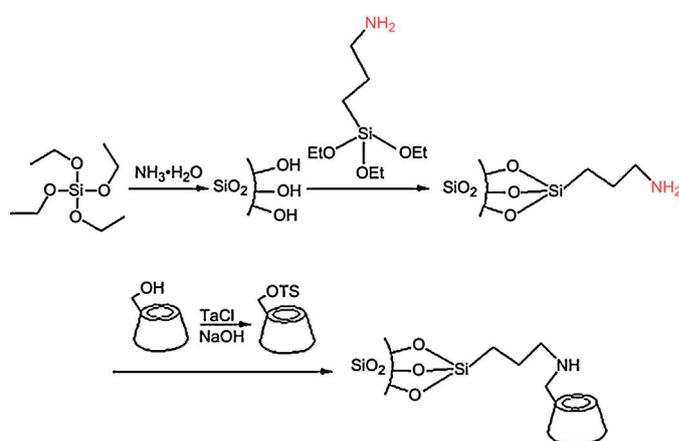


Fig. 10. Synthesis of β -CD conjugated silica nanoparticles (Shu et al., 2010).

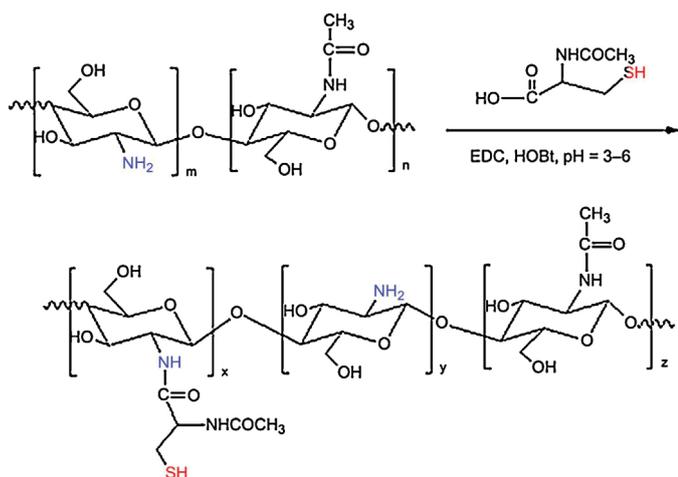


Fig. 11. Synthesis of cysteine conjugated chitosan (Shu et al., 2010).

5. CD containing phospholipids and phospholipids like particles

Nucleic acid based therapy is a special, definitive and powerful therapy for different human diseases. Major problem in the formulation of such therapeutics is the hurdle of systemic delivery. Multifunctional NPs are being worked upon for systemic, non-viral drug delivery systems. This also includes research on CD containing polycations (CDP) and their NPs constructed with nucleic acids including plasmid DNA (pDNA) and small interfering RNA (siRNA) (Bartlett and Davis, 2007). Kostarelos and Miller (2005) introduced a new classification system that helped in selection and comparison of different technologies available with respect to synthetic non-viral vector systems. This ABCD concept was a structural model for modified polymer based carrier systems. Few of the most developmental areas of ABCD vectors are those using CD (Bartlett and Davis, 2008; Davis and Brewster, 2004; Hu-Lieskovan et al., 2005; Davis, 2009). Davis and his colleagues designed a novel ABCD system which was based on the following steps; (i) siRNA was condensed with CD containing polycations (CDPs); (ii) formation of inclusion complexes between CDP-siRNA and adamantane (hydrophobic moiety) which in turn is used for the attachment of functional groups PEG-transferrin (Hu-Lieskovan et al., 2005; Guo et al., 2010). Some light was also thrown on the structural concept of CD-siRNA vesicles (Fig. 13).

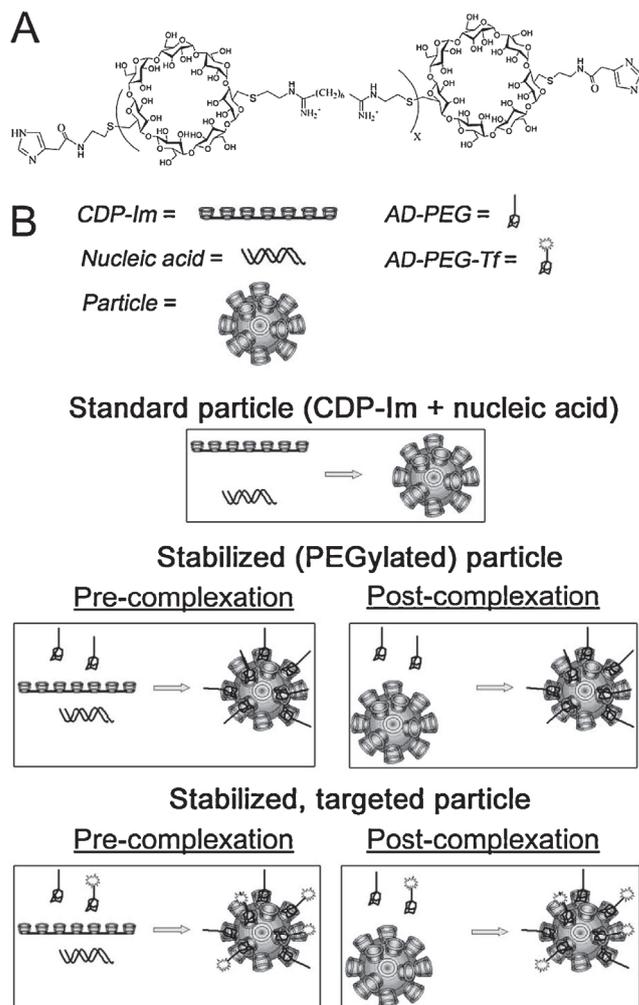


Fig. 13. Formation of nucleic acid-containing particles using CDP-Im. (A) Schematic of the chemical structure of CDP-Im. (B) Schematic of particle assembly (Bartlett and Davis, 2007).

A different approach uses CDs as promising core molecules which can be modified with miscellaneous functional groups like polycations, lipophilic chains, PEG chains and targeting ligands (Sallas and Darcy, 2008; Cryan et al., 2004a,b; McMahon et al.,

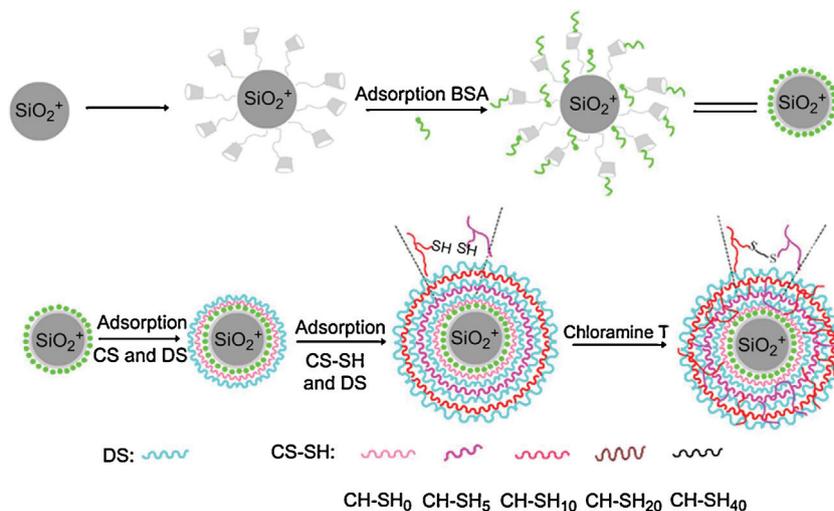


Fig. 12. Schematic representation of loading BSA of gradient cross-linked nanoparticles (Shu et al., 2010).

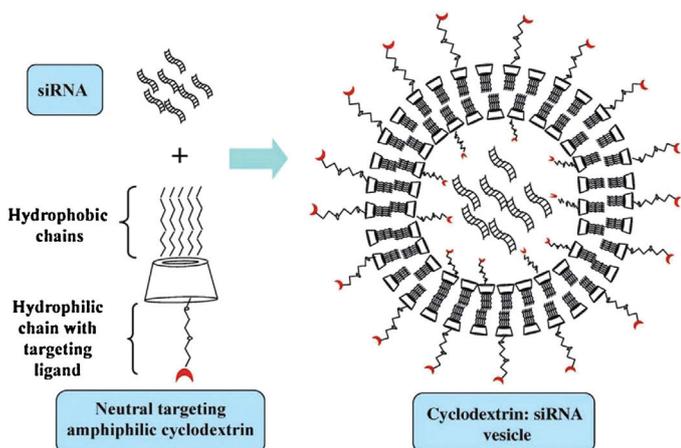


Fig. 14. Cyclodextrin: siRNA vesicles. Uncharged targeting (i.e. galactosylated) amphiphilic cyclodextrins can form a hydrophilic interior. This water interior acts as hosts to possibly encapsulate siRNA inside (Guo et al., 2010; Sallas and Darcy, 2008).

2008). Amphiphilic CDs have been shown to form vesicles that can encapsulate siRNA inside the aqueous core (Guo et al., 2010; Sallas and Darcy, 2008; Donohue et al., 2002; Fig. 14).

The polycation/nucleic acid complexes can be integrated to give particles in the range of 60–150 nm, zeta potential from 10 to 30 mV and molecular weights from approximately 7×10^7 – 10^9 g mol⁻¹. These formulations give protection to nucleic acids from nuclease degradation, avoid aggregation at physiological salt concentrations, give minimal erythrocyte aggregation and complement fixation at the concentrations normally used for in vivo administrations (Bartlett and Davis, 2007). CDs being oligosaccharides possess low immunogenicity and offer multiple sites for the attachment of functional groups. This feature imparts to final formulation an excellent flexibility to accommodate various targeting requirements for a range of therapeutic applications (Guo et al., 2010).

Furthermore, the effect of various delivery systems e.g. CD complexes and liposomes on the topical delivery of an anti-inflammatory agent like Ketorolac has been investigated by Nagarsenker et al. (2008). Ketorolac is usually available as

Table 3

Mean values of cumulative KTRM/KTRA permeated at the end of 10 h (Nagarsenker et al., 2008).

Formulation	KTRM or KTRA permeated at the end of 10 h (% of applied dose)
KTRM solution	17.81 ± 10.81
KTRM liposomes	6.84 ± 2.94
KTRA-HPBCD liposomes	10.53 ± 4.876
KTRA-HPBCD solution	68.51 ± 6.99

Ketorolac tromethamine (KTRM). Ketorolac acid was obtained by acidification of KTRM. Next step was the preparation of KTRA-CD solid dispersion by using kneading method. Liposomal dispersions of KTRM and KTRA-HPβCD were formulated by using the common lipid film hydration method (Fig. 15; Miladi et al., 2013). KTRA-HPβCD liposomes had particle size in the range of 0.9–11.2 μm and possessed encapsulation efficiency of 10.64 ± 1.43%. KTRA-CD showed higher transdermal delivery of the drug as compared to all other systems whereas liposomes considerably reduced the delivery of drug (Nagarsenker et al., 2008). CDs demonstrated such effect due to solubilizing action on drug thus enhancing its availability at the absorption site. Furthermore CD interacted with the free lipids present in stratum corneum and ameliorated transdermal penetration of the active moiety (Nagarsenker et al., 2008; Måsson et al., 1999; Loftsson and Masson, 2001). The liposomal form of the drug promoted dermal localization of the drug (Table 3; Nagarsenker et al., 2008).

6. Nanoparticles obtained by polymerization in disperse media

Polymerization based technology is divided into two types namely; emulsion and interfacial polymerization. Emulsion polymerization is further divided into organic and aqueous (surfactant free), depending on the continuous phase.

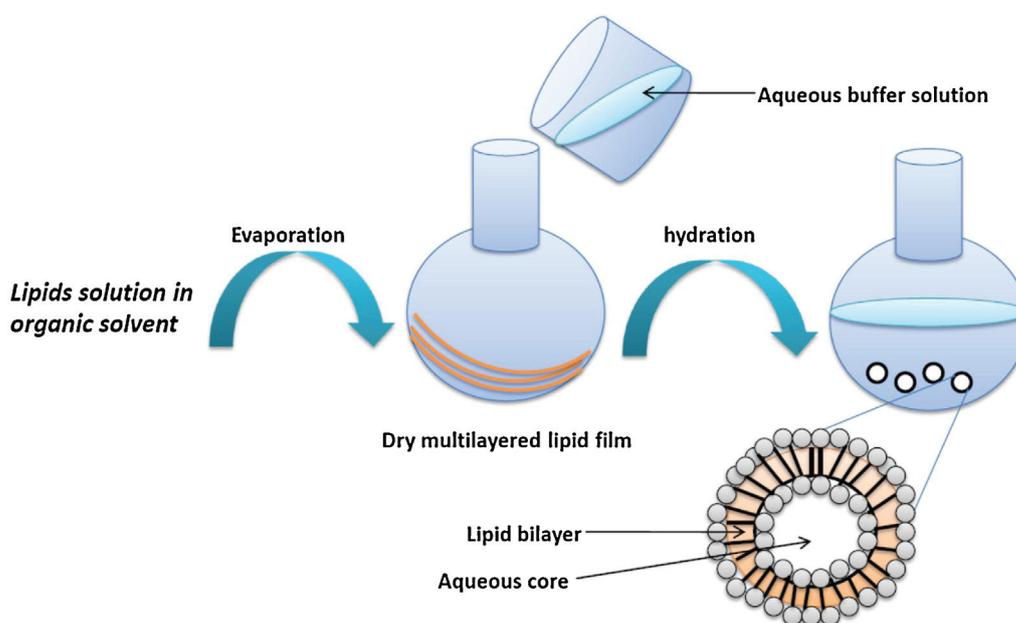


Fig. 15. Lipid film hydration technique (Miladi et al., 2013).

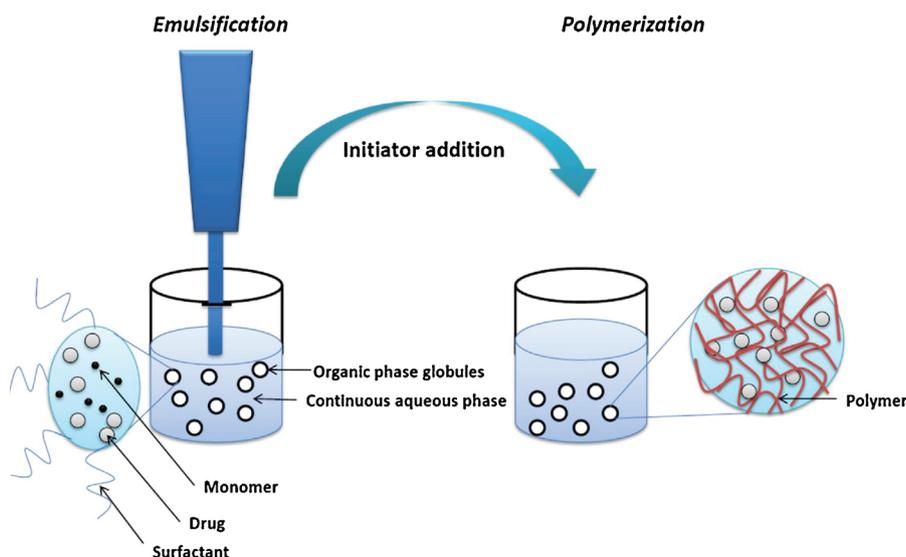


Fig. 16. Basic principle of emulsion polymerization (Miladi et al., 2013).

6.1. Emulsion polymerization process

It is a fast and easy way to prepare NPs. If no surfactant is used then the method is called as surfactant free, emulsifier free or soapless emulsion polymerization technique. The reagents involved in this process include deionized water, water soluble initiator (e.g. potassium persulfate, KPS) and monomers (mostly vinyl or acrylate monomers) (Rao and Geckeler, 2011). Monomer is dissolved in a continuous phase, which is usually water and no surfactants/emulsifiers are added. Initiation of polymerization occurs when the monomer in aqueous solution collides with an initiator molecule (an ion or free radical) (Fig. 16). Alternatively, by using high energy radiations (γ -radiations, ultraviolet or strong visible light), the monomer can be altered into an initiating radical. The chain growth commencement is marked by the collision of initiated monomer ions or radicals with other monomer molecules according to an anionic polymerization mechanism. Separation of phase and solid particle formation can occur before or after the termination of polymerization reaction (Pinto Reis et al., 2006). Very useful information about the latest progress in emulsion polymerization and the important parameters in modelling kinetics of emulsion copolymerization were discussed by Van Herk (2012).

CD-insulin complex encapsulated polymethacrylic acid based NPs have been formulated for the oral delivery of insulin by Sajeesh and Sharma (2006). Insulin complexation with CD improved therapy by stabilization of the protein against aggregation, thermal denaturation and degradation. Hydrophilic β -CD bared the adsorption of insulin to hydrophobic surfaces and averted self-aggregation of insulin at neutral pH. Polymethacrylic acid–chitosan–polyether (polyethylene glycol–polypropylene glycol copolymer) (PMCP) NPs were synthesized by modified inter-ionic gelation process. MAA, EDMA were mixed at room temperature and then chitosan and polyether were added separately into the mixture. Double distilled water was utilized for solution making and magnetic stirring was carried out. Potassium persulfate was added to initiate free radical polymerization. Resultant particles were allowed to settle overnight and then were dried afterwards. HP-CD-I were prepared by mixing insulin solution with CDS. Loading of insulin onto dried NPs was done by diffusion filling method. NPs were exposed to protein solution and insulin diffused mainly by concentration gradient. Approximately 80% encapsulation efficiency was achieved by insulin complexed with CD and uncomplexed insulin

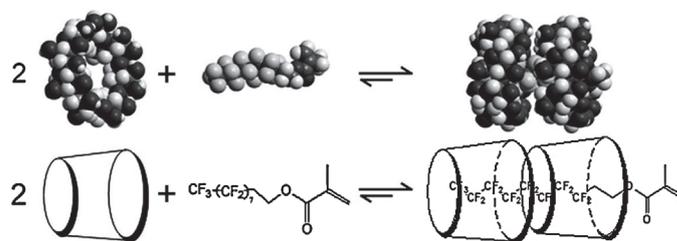


Fig. 17. Formation of 1:2 inclusion complex between FMA and β -CD (Yang et al., 2011).

preparations by this technique (Table 4; Sajeesh and Sharma, 2006). Complexation with CD had no significant effect on the encapsulation efficiency. The in vitro release of the insulin from the complexed and uncomplexed was studied at different pH values and pH dependent release kinetics were observed perhaps due to ionic nature of the materials employed. Furthermore complexation with CD showed no change in release properties of insulin from the NPs (Sajeesh and Sharma, 2006).

The effect of β -CD on polymerization of different vinyl monomers like butyl-methacrylate, styrene, dodecyl-methacrylate, octadecyl-methacrylate have been investigated (Rimmer, 2000). Poly(butyl-methacrylate) (PBMA) particles using CD in place of surfactant were constructed by emulsion polymerization. β -CD provided colloidal stability in polymerization of monomers of BMA and styrene. This was probably due to inclusion of PBMA chains within β -CD. Furthermore, β -CD increased the solubility of highly insoluble dodecyl and octadecyl methacrylate monomers and augmented emulsion homopolymerization of these monomers (Rimmer, 2000). Different forms of CDs such as HP β -CD, β -CD, Me β -CD has been used as phase transfer catalyst to form semi-fluorinated acrylic NPs possessing non fluorinated core and outer layer comprising of copolymers of hydrophobic and

Table 4
Insulin encapsulation efficiency of PMCP nanoparticles (Sajeesh and Sharma, 2006). F1, insulin-CD complexed formulations; F2, uncomplexed insulin formulations.

Formulations	Loading time (h)	Encapsulation efficiency
F1	6	86 \pm 2
F2	6	85 \pm 2

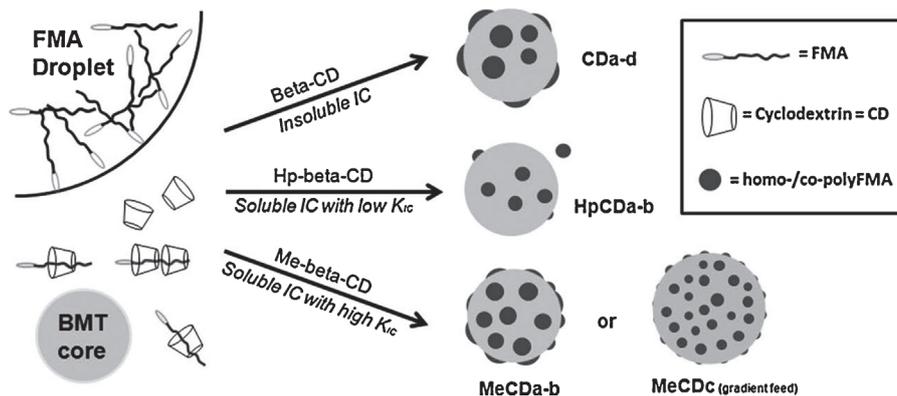


Fig. 18. The formation of various nanoparticles as a function of type of CD employed (Yang et al., 2011).

lipophobic FMA (heptadecafluorodecyl methacrylate). Synthesis was carried out by emulsion polymerization (Yang et al., 2011). All the CDs formed inclusion complexes with FMA (Fig. 17). CD assisted in emulsion polymerization (Fig. 18). CDs have been used to increase the solubility of Saquinavir (a potent HIV-1 and HIV-2 protease inhibitor) (Boudad et al., 2001). Hydroxypropyl β CD combined PIBCA and PIHCA nanospheres were prepared by emulsion polymerization of isobutyl- and isohexyl cyanoacrylate monomers (Monza da Silveira et al., 1998). Freeze-dried HP β CD-saquinavir complexes were prepared before carrying out polymerization. It was observed that there was 400-fold increase in solubility of saquinavir at pH 7.0 in the presence of HP β CDs. In addition to this, a 20-fold increase in saquinavir loading into the NPs was achieved as compared to NPs prepared in the absence of CDs. The diameter of the particles ranged from 250 to 350 nm depending on the concentration of HP β CD.

6.2. Interfacial polycondensation method

Interfacial polycondensation can also be used to construct polymeric NPs from lipophilic monomers like phthaloyldichloride and hydrophilic monomers like diethylenetriamine. The process can be carried under the presence or absence of surfactants (Pinto Reis et al., 2006; Montasser et al., 2002). Resultant nanoparticle size was less than 500 nm. Modified version of this technique was also developed and applied to polyurethane polymer and poly (ether urethane) which successfully acted as drug carriers for α -tocopherol (Pinto Reis et al., 2006; Bouchemal et al., 2004). Polycondensation has been used to prepare polyrotaxanes (Chelli et al., 2009). β -CD were threaded onto a polyether chain by polycondensation of β -CD/bisphenol A (PBA) inclusion complex with aromatic amines. The NMR spectrum displayed the inclusion complex of polyether chains in the β -CD cavity (Chelli et al., 2009).

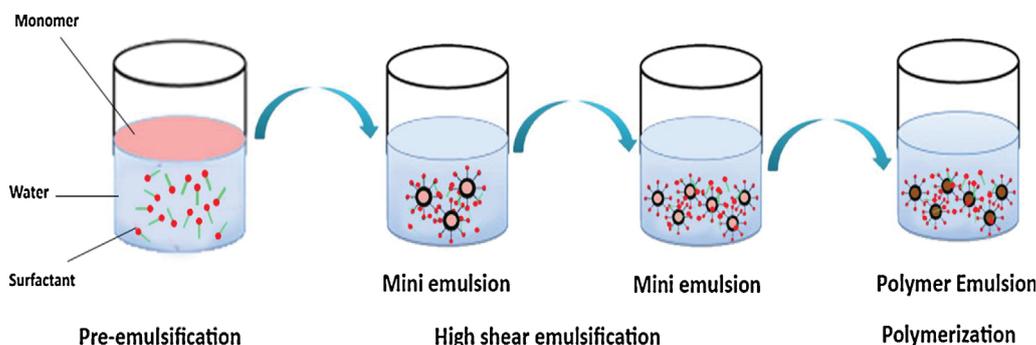


Fig. 19. Diagrammatic representation of miniemulsion technique.

Adapted from (Rao and Geckeler, 2011).

CD based microspheres has also been prepared for delivery of drug into lungs (Skiba et al., 2005). Amikacin encapsulation into approximately 5 μ m β -CD microparticles was achieved and encapsulation yield higher than 50% was observed. Polycondensation of β -CD by terephthaloyl chloride was carried out in the following way: Terephthaloyl chloride was dissolved in a mixture of chloroform/cyclohexane to constitute organic phase. β -CD dissolved in NaOH was emulsified in cyclohexane solution containing sorbitan 85 trioleate. Emulsification was performed under constant stirring. Microparticles were constituted by the addition of organic phase to emulsion under stirring. Cyclohexane was added to stop the cross-linking reaction. Finally centrifugation and freeze-drying resulted in formulation of the desired microparticles. Model drug Amikacin could be loaded at different steps of fabrication like solubilization, emulsification, cross-linking and freeze drying (Skiba et al., 2005).

6.3. Mini-emulsion polymerization

Miniemulsion polymerization provides versatile polymeric NPs by employing variant combinations of co-stabilizers and initiators (Rao and Geckeler, 2011; Wang et al., 2007; Bardajee et al., 2007; Rotureau et al., 2008; Yildiz and Landfester, 2008; Ethirajan et al., 2009; Crespy and Landfester, 2009; Wu et al., 2009; Baruch-Sharon and Margel, 2010; Jiang et al., 2010). The requirements for this technique are; water, monomer mixture, co-stabilizer, surfactant and initiator (Fig. 19). In this process, low molecular mass compound as a co-stabilizer is used and high shear devices (ultrasound etc.) are employed. This feature constitutes the major difference between emulsion polymerization and miniemulsion process. Miniemulsions are critically stabilized and have interfacial tension much greater than zero. Kriwet et al. (1998) constituted polyacrylic acid using a co-emulsifier system, which included a mixture of span 80 and tween 80. Free

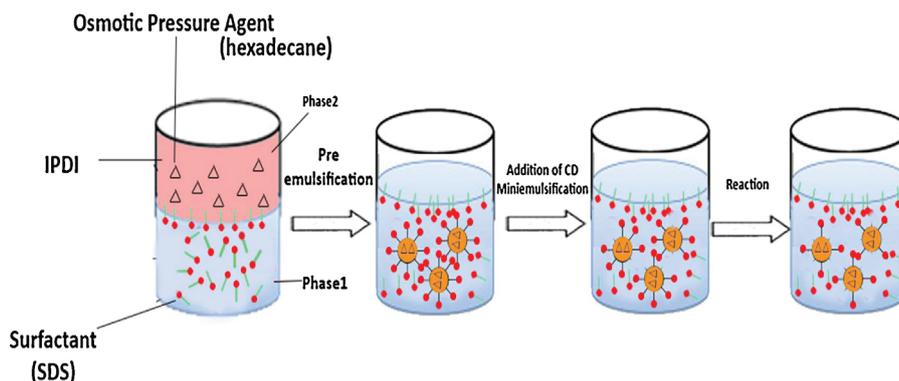


Fig. 20. Diagrammatic representation of miniemulsion polymerization of cyclodextrin nanospheres.

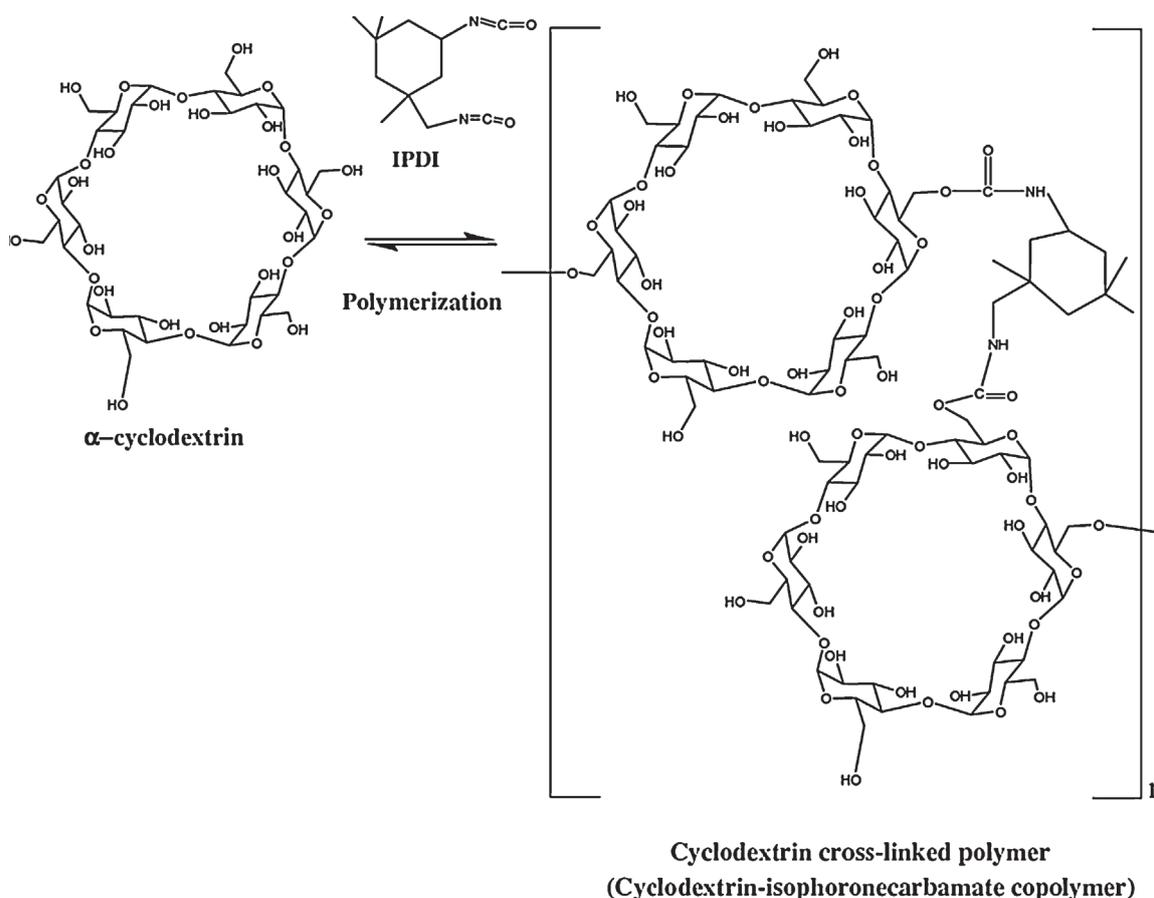


Fig. 21. Copolymerization of CD-isophoronecarbamate copolymer (Baruch-Teblum et al., 2010).

radicals initiated polymerization and size of particles was largely dependent on type of radical initiator utilized. It was possible to construct NPs of diameter in the range of 80–150 nm by utilization of lipophilic radical initiators like azobisisobutyronitrile (AIBN). Though a limited amount of surfactant is used in this process, some amount of it still lingers in the polymer latex which changes the properties of final polymeric NPs. Miniemulsion polymerization (Arias et al., 2001) was successfully utilized to constitute core-shell polymer NPs which had a biodegradable polymeric shell and a magnetic core (magnetite). Polystyrene single walled carbon nanotube composites were prepared by this method (Ham et al., 2006).

This process was used to synthesize cross-linked CD polymeric nanospheres for the purpose of using them as water purification technology (Fig. 20; Baruch-Teblum et al., 2010). These

nanospheres display high ability to absorb aromatic organic compounds such as phenol and toluene. α -CD was modified by a cross-linking agent called isophorone diisocyanate (IPDI) (Fig. 21; Baruch-Teblum et al., 2010). Sodium dodecyl was used as an emulsifier and a co-stabilizer hexadecane acting as an osmotic pressure agent within the droplets was included in the formulation. Droplets of size 30–500 nm could be obtained.

7. Concluding remarks

CDs are multifunctional tools that serve as solubilizing and stabilizing agents for the drugs and play significant role in improving bioavailability, solubility of poorly water-soluble drugs and in masking the smell. CDs enhance the efficiency of encapsulating the guest molecules by inclusion complex formation and effectively

regulate the drug release rate and targeting. Combination of CDs with biodegradable substances gives superior qualities to the final product which includes non-toxicity, low cost, greater specificity, tolerability, protection from earlier degradation in biological systems, prevention of interaction between formulation components and reduction in drug irritancy by encapsulation. CD based biodegradable particles can be constituted by several techniques. Some of which include dispersion of preformed polymers, polymerization of monomers, layer by layer, polycondensation, miniemulsion and liposomal based formulation technique. The selection of these techniques depends upon many factors such as the desired encapsulation efficiency, size of particles and polymer type to be employed keeping in view the few drawbacks associated with different techniques. Only water miscible solvents can be employed for nanoprecipitation technique. Furthermore, this method is not good for encapsulation of hydrophilic drugs and basically used for lipophilic moieties because of the miscibility of the solvent with the aqueous phase. The recovery of CDs from the final suspension is sometimes too low in emulsification evaporation method as compared to nanoprecipitation technique. Moreover, drug release and entrapment efficiency of almost 100% has been achieved in some experiments via double emulsion solvent evaporation. Solvent diffusion technique has been found to be very good for hydrophobic drug encapsulation. Magnetic NPs modified with CD provide particles possessing dual functionalities of being both therapeutic and diagnostic in nature. CD in theranostic is an important area and more research in field would open doors for better treatment and diagnosis via single platform. CDs in liposomal form of drug delivery have been observed to promote dermal localization of the drug molecules and in general the observed encapsulation efficiency is also quite low by this technique. Some of the preparations containing CDs made by emulsion polymerization method showed that CDs had no significant effect on encapsulation efficiency and release kinetics of the active molecules. CDs, because of their continual ability to explore novel applications, are counted on to fix plenty of problems linked with the delivery of several novel drugs through different routes of administration and delivery through various approaches of nanotechnology.

Acknowledgement

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II.2. Colloidal particles containing labeling agents and cyclodextrins for theranostic applications

Summary

This work is an extension of first review published on cyclodextrin containing drug delivery systems. Here, the role of cyclodextrins in theranostic agents has been discussed in detail. The work describes basic theranostic approaches and the integral components of theranostic agents. Most commonly used materials such as iron and gold in constituting theranostic agents are briefly discussed and also how cyclodextrins interaction with such particles gives altered theranostic agents possessing improved characteristics. The review focuses on quoting examples of different kinds of cyclodextrin containing theranostic agents, techniques to constitute such particles as well as advantages and applications of such particles are also highlighted. Furthermore, a small part of review is also dedicated to role of cyclodextrins as pores control motifs in particles accompanied by few examples of such particles.

Theranostics is a relatively new domain of interest for researchers in life sciences as it is an excellent gateway to target therapy and diagnosis simultaneously. In addition, theranostics provide novel personalized drug delivery systems that show enhanced drug efficacy and safety with minimal associated adverse/side effects. Well defined colloidal carriers have special importance in theranostic field especially nanoparticles are superb carriers of drug molecules as well as aid in controlled and targeted delivery at the diseased site. Polymeric particles are commonly employed in medical field as these can be easily manipulated with different functional groups. Such manipulations confer multifunctionality on the particles which is a great assistance in the evolution of theranostics. Carriers like micelles, nanocomposites and liposomes have been used for theranostic applications. There are four basic components of a theranostic agent namely: signal emitter, active molecule, payload carrier like polymeric material and targeting ligand.

Usually, a theranostic agent is formed by using variety of substances like silica, iron oxide, gold and gadolinium. Two of these materials are also used in combination with each other to formulate theranostic particles with better or specific characteristics.

Cyclodextrins (CDs) are sugar molecules that are composed of a number of glucose units joined together in a ring form. These are biocompatible molecules that have no reducing properties. They have non-polar cavity where they encapsulate hydrophobic agents and hence increase the aqueous solubility of hydrophobic agents. CDs have also been used in theranostic agents to achieve certain specific properties.

Magnetic nanoparticles (MNPs) are extensively used in medicine for drugs encapsulation, as contrast agents in magnetic resonance imaging, in local hyperthermia and also for magnetic targeting. The iron oxide used is biodegradable and the small size of superparamagnetic iron oxide NPs helps in their interaction with the human tissues and cancer cells. CDs are principally used in MNPs for theranostic applications. CDs coatings are generally applied to the surface of iron oxide core where they bind through hydrophilic (-OH) units. It has been observed that it was possible to increase the loading of active molecules into the particles by increasing the coatings of CDs. This was possible due to availability of greater number of cavities of CDs for drug encapsulation. Smaller size of particles with sustained and controlled drug release properties were also possible to be achieved by the use of CDs. Moreover, studies show that when CDs are used with iron oxide NPs, commonly associated aggregation phenomenon is reduced and good colloidal stability is provided to the particles.

Mesoporous silica NPs are another versatile drug delivery platforms that can be functionalized in different ways to constitute excellent theranostic particles. Silica NPs are prepared via base-catalyzed sol-gel process. Usually iron oxide cores are coated with silica shell and further functionalized with CDs. Such particles can be loaded with therapeutic agent and diagnostic agent simultaneously. In some cases, CDs just act as gatekeepers for the particles preventing the release of drug molecules loaded into the silica shell over the iron core. Here, the drug release is stimulus based and this type of therapy is very useful in cancer treatment. Whereas, in other cases, fluorescent agents can be loaded in mesoporous silica shell placed over the iron oxide core. In such cases, CDs are coated over silica shell and act as drug carriers e.g. for anticancer drugs. Such particles can be easily monitored and tracked inside the biological fluids and also possess greater drug loading capacity. Particles can also be rapidly accumulated at the tumor site by application of external magnetic field.

Gold NPs are also extensively used for drug delivery and diagnosis. Gold NPs have good biocompatibility, stability and can easily bind with many biomolecules. Moreover, gold NPs display surface Plasmon effect, good light scattering and absorption properties. CDs can be placed on the surface of gold NPs to enhance the drug loading of the prepared particles.

In conclusion, CDs are used in different fields and play an important role in enhancing the properties of theranostic agents. Primarily, CDs helps to increase drug loading and to achieve sustained release formulations.



Review

Colloidal particles containing labeling agents and cyclodextrins for theranostic applications



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ABSTRACT

This review aims to give to the reader some new light on cyclodextrin (CD)-based theranostic agents in order to complete our recently published review dedicated to CD-particles conjugates in drug delivery systems (Zafar et al., 2014). CDs are biocompatible sugar-based macrocycles used in a wide range of biomedical applications. Here, we mainly focus on fundamental theranostic approaches combining the use of cyclodextrin molecules and colloidal particles as theranostic agents. The system's key features are discussed and a few recent pertinent applications are presented. CDs are used in order to enhance theranostic properties by providing apolar cavities for the encapsulation of hydrophobic moieties. Thus, CD molecules are used to enhance the loading capacity of particles by hosting active molecules. The relevance of CDs in enhancing the labeling properties of particles and the preparation of controlled drug release particles is also highlighted.

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1. Introduction

Nowadays, the combination of diagnostic and therapy in life science is of great interest in order to perform in one step both the disease identification and drug delivery. To target such objective and principally to develop novel personalized treatments with

improved safety and efficacy, well-defined colloidal carrier will be of great interest. In this domain, various processes including chemistry, self-assembly and formulation lead to more and more sophisticated nanoparticles (nanospheres and nanocapsules). Nanoparticles act as excellent carriers of therapeutic drugs and genes delivery by virtue of their large surface area to volume ratio and also assist in targeted delivery and controlled release of therapeutics at diseased sites *via* well-defined target. Nanoparticles for medical uses are mainly polymer based and in some case inorganic core coated with polymer layer with different functional groups to provide flexibility of encompassing multiple

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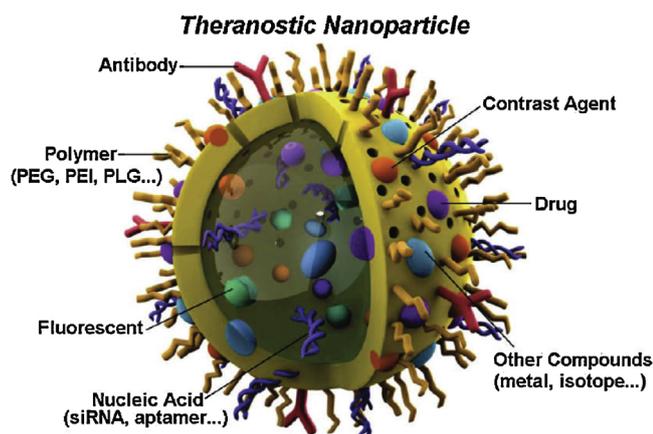


Fig. 1. Theranostic nanoparticle: surface functionalized with antibody and polymers. The interior core of nanoparticle can be encapsulated with active agents, such as nucleic acids, imaging contrast agents, drugs, and fluorescent materials to accomplish different theranostic requirements (Ma et al., 2011).

functionalities. This flexibility is a major boost for emergence of theranostics in nanomedicine. Examples of various drug delivery forms that have been used for theranostic applications include liposomes (Laouini et al., 2012; Tapia et al., 2013), micelles and nanocomposites (Roveimiab et al., 2012; Ahmad, 2013). The integral components of theranostic agents are: signal emitter, therapeutic payload, payload carrier and targeting ligands (Fig. 1 and Table 1).

Iron oxide nanoparticles bearing chlorotoxin (a peptide possessing strong affinity for majority of brain tumors) displayed accumulation in brain tumors six times higher than non-targeted nanoparticles in genetically engineered mice with brain tumors (Veisoh et al., 2009). Similarly, a magnetopolymeric nanohybrid conjugated with an antibody specific to human epidermal growth factor receptor (HER2) showed tumor targeting and growth inhibition three times higher than control nanoparticles having inappropriate antibody (Yang et al., 2007). Multiple ligands can also be attached on single particle surface to achieve multi-valency and multi-functionality (Fang and Zhang, 2010).

The release of the drug at specific site can be controlled by keeping a check/control over certain specific parameters such as pH, ionic strength, temperature, hydrolytic, enzymatic degradation and application of external stimulus like radiofrequency electromagnetic waves and light excitation. In future, theranostic agents may also offer a cost effective course to battle baleful and debilitating illnesses like cardiovascular diseases, neurodegenerative diseases and cancer.

Table 1

Properties of different components of theranostics agents (Fang and Zhang, 2010).

No.	Component	Properties
1.	Signal emitter	a. These agents have optical, magnetic or radioactive properties to assist in imaging b. Embedded/encapsulated/conjugated on carrier surface
2.	Therapeutic payload	a. Genes, proteins, chemotherapy drugs or combination of these agents b. Embedded/conjugated on carrier surface
3.	Payload carrier	a. Mostly polymeric materials are employed which have multiple functional groups for conjugation of signal emitters and therapeutic payloads b. Polymers can be immobilized on nanoparticles surface during synthesis of nanoparticles cores (Veisoh et al., 2009) or by surface modification after synthesis of core c. Biodegradable in nature d. Usually comprise of multiple amide-ester, glycosidic bonds in their backbone which can be easily cleaved hydrolytically/enzymatically when carrier arrive at targeted site e. Examples include: poly(ethylene glycol) (PEG), dextran, carboxydextran, β -cyclodextrin(β -CD), poly(DL-lactide-co-glycolide) (PLGA) and poly(L-lysine) (PLL)
4.	Targeting ligand	a. Bind to certain disease markers on target cells allowing transport of theranostics agent to target site b. Always covalently attached to surface of carrier c. Examples include oligosaccharides, aptamers, peptides, antibodies and specific proteins

Substances like iron oxide, silica, gold and gadolinium are commonly used in the formulation of theranostic agents (Fig. 2) and in some cases two of these are combined together to confer better and specific qualities on the resultant particles.

Cyclodextrins (CDs) first discovered by Villiers in 1891, possess extensive applications in diverse fields of drug delivery and pharmaceutical industry. They contain sugar (α -D-glucopyranose) molecules bound together in a ring form i.e., cyclic oligosaccharides. These are also referred to as cycloamyloses, cyclomaltooses or Schardinger dextrins and have no reducing properties. These are highly biocompatible macrocycles that are capable of encapsulating hydrophobic agents in their non-polar cavity. During their reversible complexation with drugs no covalent bonds are formed or broken (Zafar et al., 2014). In this short review, we focus on the role of cyclodextrins (CDs) in altering the properties of theranostic particles.

2. Iron and cyclodextrin based theranostic agents

Magnetic nanoparticles (MNPs) are widely used for miscellaneous medical purposes such as nanocarriers for drugs encapsulation, drugs delivery, contrast agents in magnetic resonance imaging (MRI) and also in local hyperthermia and magnetic targeting (Yallapu et al., 2012; Laurent et al., 2010; Shubayev et al., 2009). Magnetic particles largely used in numerous *in vivo* and in biomedical application are reported by various authors which include Ahsan et al. (2013); Ahmd et al. (2012); Medeiros et al. (2013) and Rahman and Elaissari (2012). The iron oxide generally used in magnetic particles preparation is biodegradable and upon degradation in body it becomes part of natural iron stores like hemoglobin in red blood cells. The small size of superparamagnetic iron oxide nanoparticles (SPIONS), the most common iron oxide based material used in various *in vivo* applications (Fig. 3), assists their movement in tissues, endocytosis and intracellular interaction with cancer cells. SPIONS also offer various advantages such as easy to prepare *via* classical chemistry and also noninvasive material for MRI application (Kievit and Zhang, 2011). However, high aggregation of generally non-coated magnetic nanoparticles (MNPs) poses a major obstacle not only for *in vitro* application but also for their *in vivo* biomedical usage. This problem can be overcome by surface engineering of MNPs through application of stabilizer coatings such as surfactants, synthetic and natural polymers (Shubayev et al., 2009) and also chemical grafting of some biomolecules in order to induce sterical stabilization. Some of the polymer coatings confer advantage of changing the contrast imaging properties suitable for T_1 and T_2 imaging (Yallapu et al., 2012).

Cyclodextrins have been used principally in conjugation with magnetic nanoparticles to achieve better theranostic applications.

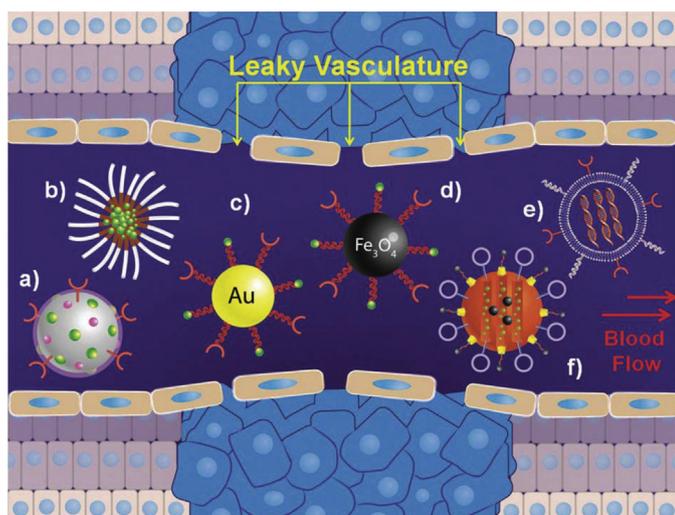


Fig. 2. Various theranostic agents in 'attack mode' over a site of tumor. Conjugated targeting ligands depicted as circles or semicircles. Cargo is shown as green spheres. Purple spheres show embedded contrast agents. A multifunctional (a) polymeric nanogel, (b) polymeric micelle, (c) gold NP, (d) iron oxide NP, (e) siRNA inside liposomal delivery system, (f) stimuli responsive capped mesoporous silica NP are shown (Li et al., 2012). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Yallapu et al. (2012) prepared curcumin loaded MNPs (Fig. 4A) for enhanced cancer therapy. Iron oxide nanoparticles were prepared by chemical coprecipitation process and cyclodextrin was introduced during this process. This process was followed by addition of pluronic polymer F68 to nanoparticle suspension in order to induce and to maintain good colloidal stability. Anticancer moiety curcumin was loaded into CDs molecules by diffusion method. The resultant particles possessed hydrodynamic average particle size of approximately 123 nm. Furthermore, these particles displayed potent anticancer activities and enhanced imaging and magnetic targeting abilities. CD molecules are chemically bonded to iron oxide nanoparticles surface through hydrophilic units ($-\text{OH}$) and provide hydrophobic cavity for loading of drug molecules. Furthermore, CDs with poloxamer F68 polymer conferred to the particles good steric stabilization, hence low aggregation phenomena and also good hydrophilic character, which induced low proteins binding. Approximately 94.2 μg of curcumin per mg of dried nanoparticle was loaded. This formulation allowed controlled and sustained release of the drug molecules (Fig. 4B)

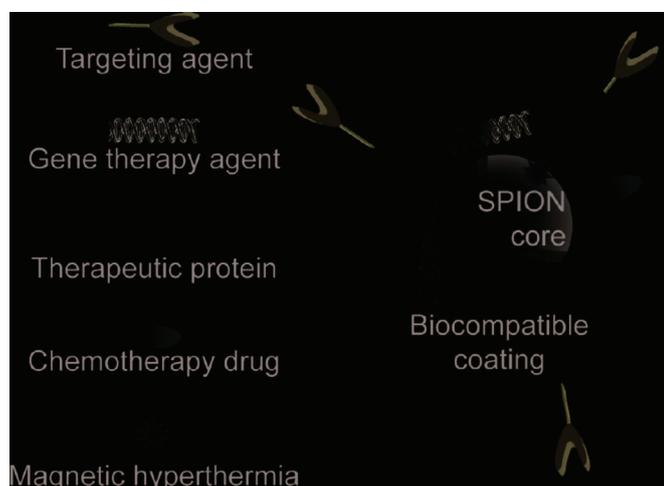


Fig. 3. Structure of SPION for theranostic usage (Kievit and Zhang, 2011).

and was believed to prevent development of drug resistance in cancer (Yallapu et al., 2012).

Earlier Yallapu et al. (2011) had elaborated similar particles (Fig. 5) using the same materials and methodology except pluronic polymer F127 coatings were employed instead of the pluronic F68 coatings. Formulation was water dispersible and intended for hyperthermia, magnetic resonance imaging (MRI) and drug delivery. It was also confirmed that drug loading continuously increased with an increase in cyclodextrin coating amount. These colloidal particles exhibited good colloidal and physical chemistry stability, enhanced cellular uptake, sustained release of anticancer drug and good imaging characteristics (Yallapu et al., 2011).

3. Silica, iron oxide and cyclodextrin based theranostic agents

Mesoporous silica nanoparticles (MSNPs) are robust drug delivery platforms that can be functionalized to give versatile theranostic agents. Such MSNPs have large surface area, pore volume and are stable to broad range of chemical conditions (Li et al., 2012; Ambrogio et al., 2011). Silica nanoparticles have been prepared *via* base-catalyzed sol-gel process. In this approach, organosilane precursors *e.g.*, tetramethyl orthosilicate (TMOS), tetraethyl orthosilicate (TEOS) are used to produce a new phase (sol) *via* hydrolysis and condensation reaction in the presence of highly basic pH.

Hydrolysis: $\text{OH}^- + \text{Si}(\text{OR})_4 - \text{Si}(\text{OR})_3\text{OH} + \text{RO}^-$

Condensation: $\text{SiO}^- + \text{Si}(\text{OH})_4 - \text{Si}-\text{O}-\text{Si} + \text{OH}^-$

Condensation of small colloidal particles within the sol leads to gel phase. The Stober process can be quoted as an example, which has been extensively used for the production of narrowly size distributed particles and also monodispersed silica nanoparticles submicron in size and mainly ranging from 50 to 200 nm. Concentrated ammonia catalyzed hydrolysis of tetraethyl orthosilicate (TEOS) in a water-alcohol solution is carried out as illustrated above (Li et al., 2012).

3.1. Surface functionalization

Mesoporous silica nanoparticles (MSNPs) can be functionalized with molecular, biomacromolecules, supramolecular complexes, mechanically interlocked molecules (MIMs) or polymer moieties to provide versatile theranostic agents. These organic functional groups can be attached through covalent bonding or electrostatic interactions (Li et al., 2012). The development of robust drug delivery system that can control the release of the drug upon stimulation by various external stimuli is important for delivering concentrated payloads of drugs to cancerous sites in the body. Mesoporous silica nanoparticles can store sufficient amount of cargo, but in the absence of suitable machinery or capping agents the early diffusional loss of drugs limits the targeted delivery of drugs. Functionalization helps in attachment of cap like structures to achieve controlled release of cargo from the formulation. Various external stimuli/triggers *e.g.*, light, chemicals, heat, electrical (redox) can be utilized for stimulating the controlled release of cargo at appropriate site (Li et al., 2012). Control over the pores can be achieved by (a) chemical bond formation directly over the pores that are cleavable by stimuli (Fig. 6a), (b) polymer adsorption/covalent bonding on the surface (Fig. 6b), (c) attachment of bulky groups over the pores (Fig. 6c).

Iron oxide nanoparticles are usually coated with silica shell to enhance biocompatibility and enable various chemical functionalities (Li et al., 2012; Cole et al., 2011). The synthesis of magnetic core-mesoporous silica shell nanoparticles can be carried out by

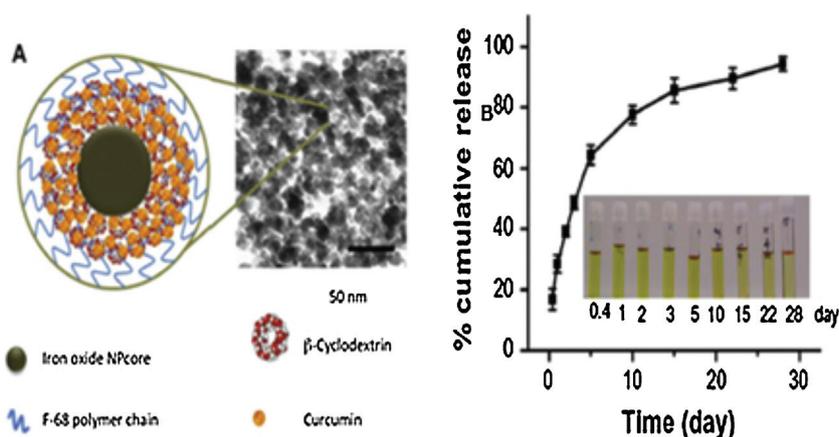


Fig. 4. (A) Hypothetical representation of curcumin loaded single magnetic nanoparticle formulation. (B) *In vitro* release of curcumin from these NPs. Inset tubes show curcumin release at different times (0.4–28 days) (Yallapu et al., 2012).

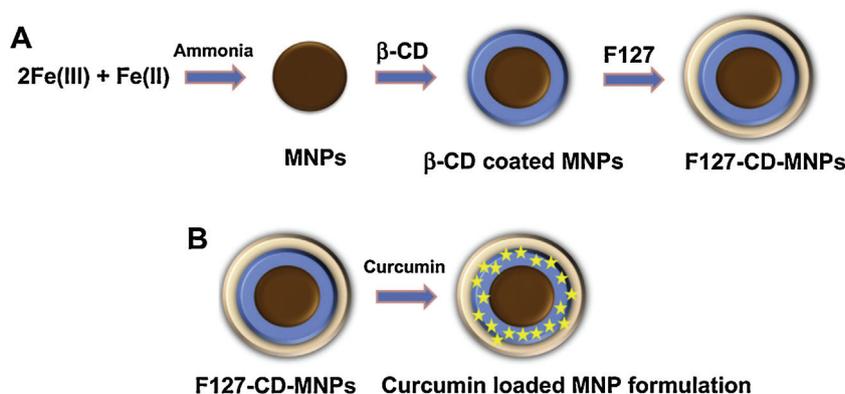


Fig. 5. Synthetic route for MNP formulation and curcumin drug loading. (A) Precipitation of iron salt into iron oxide NPs and coatings of β -CD, F127 polymer leads to F127-CD-MNPs. (B) Loading of curcumin into MNPs via diffusion process (Yallapu et al., 2011).

addition of surfactant coated metal nanocrystal suspension to solution of templating surfactant *e.g.*, cetyltrimethylammonium bromide (CTAB). Then the addition of tetraethyl orthosilicate (TEOS) or TEOS derivatives leads to silica shell (Li et al., 2012). The introduction of cyclodextrins molecules into iron–silica particles constitutes excellent theranostic particles. Lee et al. (2012) achieved a hybrid system of silica nanoparticles comprising of iron oxide in the core, doxorubicin (DOX) inside pores and

cyclodextrin present on the surface of silica nanoparticles acting as gatekeepers that are responsive to glutathione (present in much higher concentrations in cancer cells) (Fig. 7). The various steps involved in the synthesis were as follows:

Cetyltrimethylammonium bromide (CTAB) stabilized iron oxide nanoparticles were synthesized first (Liong et al., 2008). Silica source TEOS and sol–gel process were then used to apply mesoporous silica shell onto the surface of magnetic nanoparticles

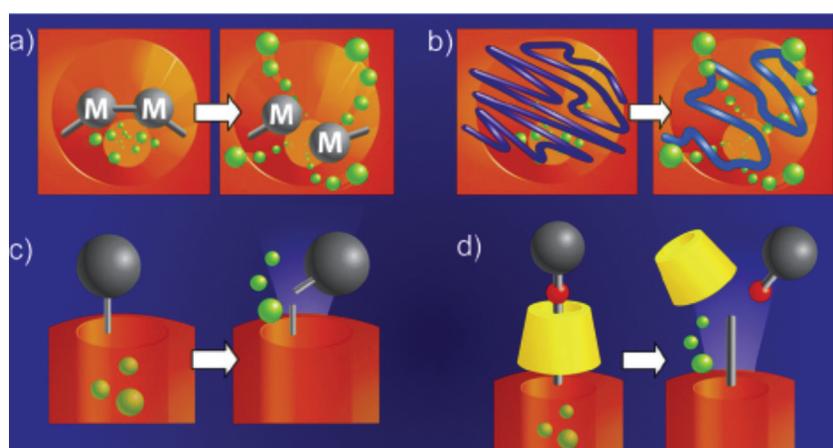


Fig. 6. Pore control system for MSNPs responsive to stimuli. (a) Chemical bond formation based system, (b) shrinking and coiling of polymer chains coatings on the surface, (c) attachment and removal of bulky groups *e.g.*, Au, (d) threading and dethreading of a cyclic molecule like CD (Li et al., 2012).

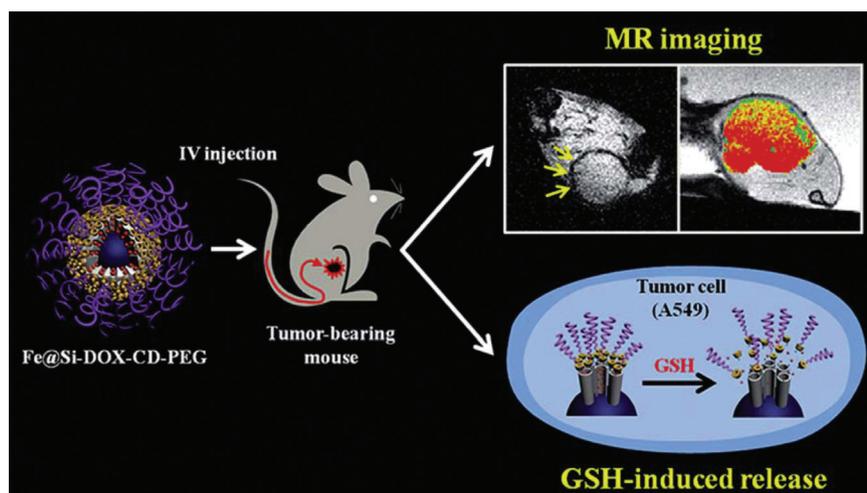


Fig. 7. Illustration of dual functionality of Fe@Si-DOX-CD-PEG (Lee et al., 2012).

(Liong et al., 2008). The reaction between these resultant particles and 3-mercaptopropyltrimethoxysilane resulted in the formation of Fe@Si-SH. Then treatment with S-(2-aminoethylthio)-2-thiopyridine HCl allowed the introduction of disulfide linker (GSH cleavable group) into the nanoparticles. Alkyne groups were applied for click coupling of cyclodextrins, and the anticancer drug doxorubicin was next placed into the pores of Fe@Si-alkyne. Finally, cyclodextrins were introduced by click coupling reaction to give Fe@Si-DOX-CD. The enhancement of solubility and biocompatibility of silica coated nanoparticles was done by surface pegylation of the particles with NCO-PEG (Lee et al., 2012) (Fig. 8A). The added advantage of these particles was the prevention of premature release of drug into the blood vessels in the absence of glutathione (GSH). So these would be good carriers of anticancer drug against tumors possessing high GSH levels e.g., A549 (Kim et al., 2010; Verma et al., 2004). As observed from the results (Fig. 8B), the drug was not released from the particles over a prolonged period in the absence of glutathione. But as soon as glutathione was made available there was cleavage of disulfide

stalk units of cyclodextrin gatekeepers causing release of doxorubicin from the particles (Lee et al., 2012). These particles displayed apoptosis and clonogenic death in cancer cells. The detection of nanoparticles in tumor was made with MRI *in vivo*. On intravenous injection, the surviving fraction of the A547 cells when treated with 1 and 2 μM of Fe@Si-DOX-CD-PEG decreased to 2.6×10^{-1} and 3.0×10^{-2} , respectively.

In another case, water soluble and biocompatible mesoporous $\text{Fe}_3\text{O}_4@m\text{-SiO}_2@Y\text{PO}_4:\text{Tb}^{3+}$ particles were prepared by Sahu and Mohapatra (2013) (Fig. 9). Surface modification of the particles was done with β -cyclodextrin (β -CD) and folic acid utilizing glutathione as a linker. The successful formation of 5-fluorouracil (5-FU) cyclodextrin complexes allowed storage and delivery of this hydrophobic drug more efficiently. Recently, more attention has been focused on employing new photostable and less toxic labeling materials derived from rare earth doped nanoparticles because they impart superb luminescence properties, narrow line-width emission bands, high quantum yields, long lifetimes and better photostability. Superparamagnetism enabled facile and

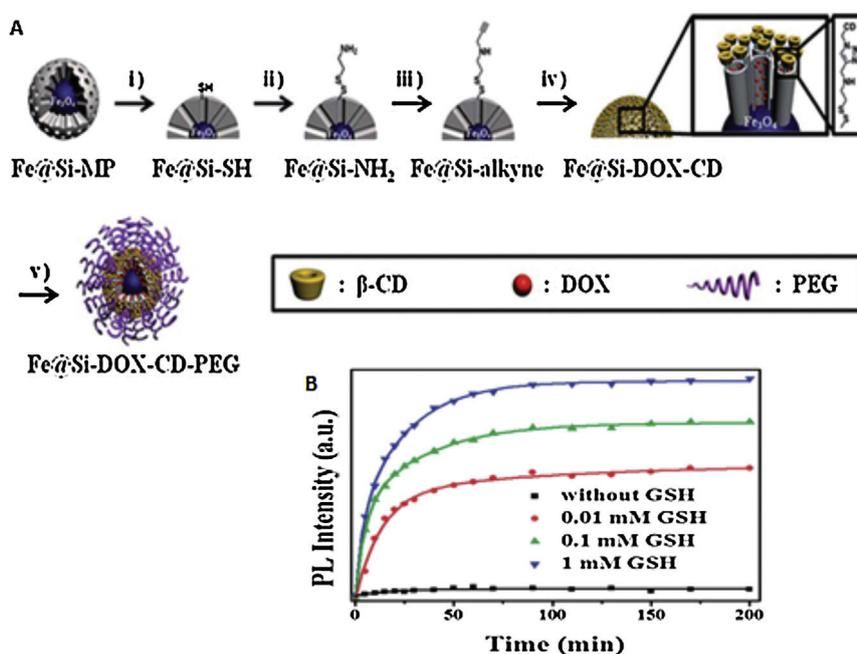


Fig. 8. (A) Synthetic pathway for Fe@Si-DOX-CD-PEG. (B) Release profile of doxorubicin from these nanoparticles (Lee et al., 2012).

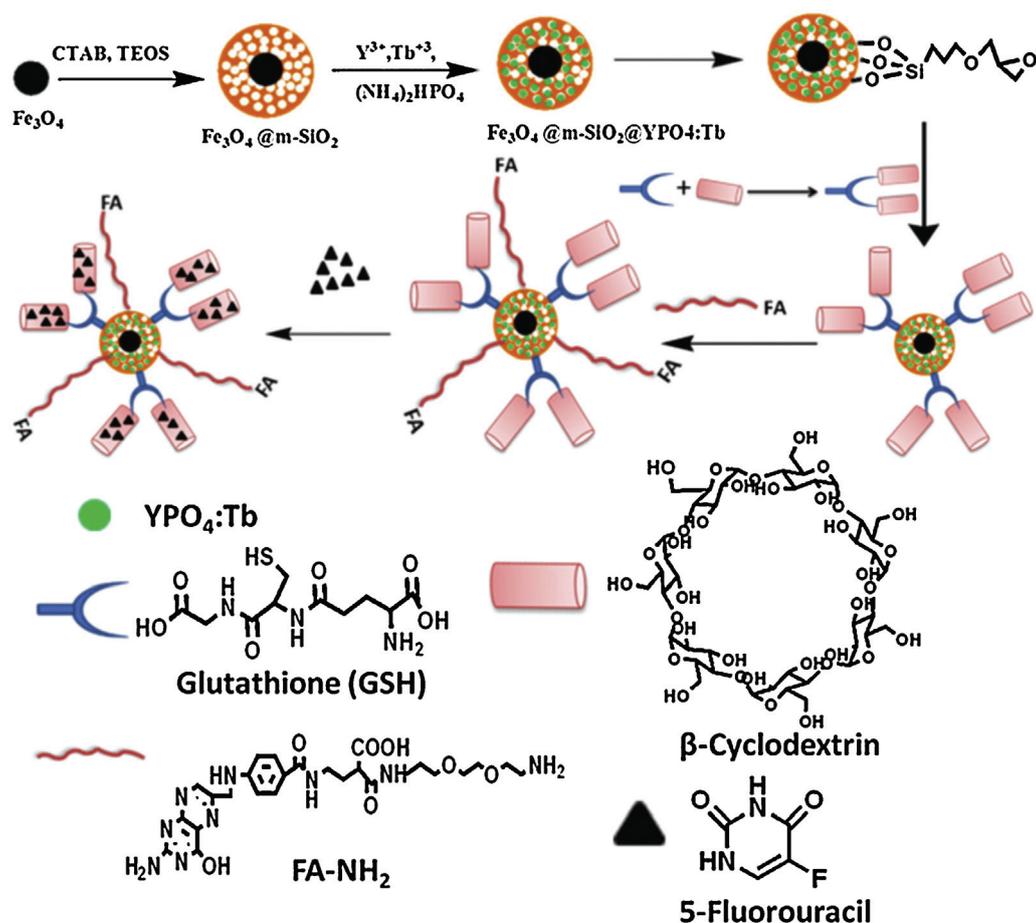


Fig. 9. Synthetic pathway of $\text{Fe}_3\text{O}_4@m\text{-SiO}_2@Y\text{PO}_4:\text{Tb}$ (Sahu and Mohapatra, 2013).

quantitative separation by the application of external magnetic field. Fe_3O_4 nanoparticles were fabricated by using a method described by Sun and Zeng (2002). Then, these particles were coated with mesoporous silica by adopting modified Stober's method. Precipitation of fluorescent material $Y\text{PO}_4:\text{Tb}$ on silica matrix lead to functionalization of these particles. Epoxy groups were introduced on nanoparticles by modification with (3-glycidypropyltrimethoxysilane) (GTPs). This step was followed by the addition of cyclodextrin using GSH as a coupling agent. Then drug was loaded by inclusion complexation with cyclodextrin. Finally, $\text{NH}_2\text{-PEG-FA}$ allowed folates to be added onto particles through epoxide openings. This was done to enable receptor mediated endocytosis (Sahu and Mohapatra, 2013).

The formed particles were highly stable in aqueous medium and displayed overall drug conjugate size of 71 nm. This system acted as probe that could track and monitor the particles in biological fluids and hence allowed monitoring of treatment of disease. This was due to fluorescence of the particles. Coatings of mesoporous silica gave added benefit of lowering the probability of fluorescence quenching and increasing water solubility of hydrophobic iron oxide particles. High drug loading was achieved due to mesoporous nature of silica that promoted conjugation of large amounts of cyclodextrins to carry the drug molecules. These particles displayed pH dependent release profile due to stability of 5-FU CD complexes (Hayashi et al., 2010). At low pH values the complexes were less stable and gave fast release of drug. This property is of particular importance at tumor sites where the pH is low as compared to normal tissues. Hence particles can be accumulated by application of external magnetic field to induce fast and more drug delivery at such tumor sites (Sahu and

Mohapatra, 2013). The formulated particles also displayed sustained release over a long period.

Recently, Badruddoza et al. (2013) prepared magnetically controlled nanoparticles possessing multifunctional characteristics which included magnetism, luminescence, cell targeting and sustained drug delivery (Fig. 10A). The particles had Fe_3O_4 core within a SiO_2 shell, which served as a host for (i) fluorescent dye fluorescein isothiocyanate (FITC), (ii) cancer targeting ligand folic acid (FA) and (iii) $\beta\text{-CD}$ (drug storage and delivery agent). Following the method of Lopez-Lopez, oleic acid stabilized Fe_3O_4 MNPs were prepared. These particles were then coated with layer of silica by micro-emulsion sol-gel process to form $\text{Fe}_3\text{O}_4@m\text{SiO}_2$ core-shell structure. Fluorescent dye conjugated (FITC) silica layer was applied in the next step. The targeting ligand (FA) was attached by silanization with folic acid-(3-aminopropyl)triethoxysilane (FA-APTS) conjugate. Lastly, carboxymethyl $\beta\text{-cyclodextrin}$ ($\text{CM}\beta\text{-CD}$) was added to outermost surface of the particles by the formation of amide bonds between the carboxylic groups of $\text{CM}\beta\text{-CD}$ and free amino groups. Nanoparticles displayed good monodispersity and average particle size in the range of 70 nm. The constructed nanoparticles displayed preference for cancer cells (Hela) as compared to healthy cells when fluorescence imaging of cellular uptake of these nanoparticles was performed. Retinoic acid (RA) as a model hydrophobic drug was loaded into the nanoparticles and inclusion complexation between drug and cyclodextrin was investigated by UV-vis spectroscopy. Increase in the concentration of $\text{CM}\beta\text{-CD}$ shifted the UV absorption peak to shorter wavelengths and resulted in an increase in absorbance as well indicated the RA/ $\text{CM}\beta\text{-CD}$ inclusion complex formation (Fig. 10B). The release

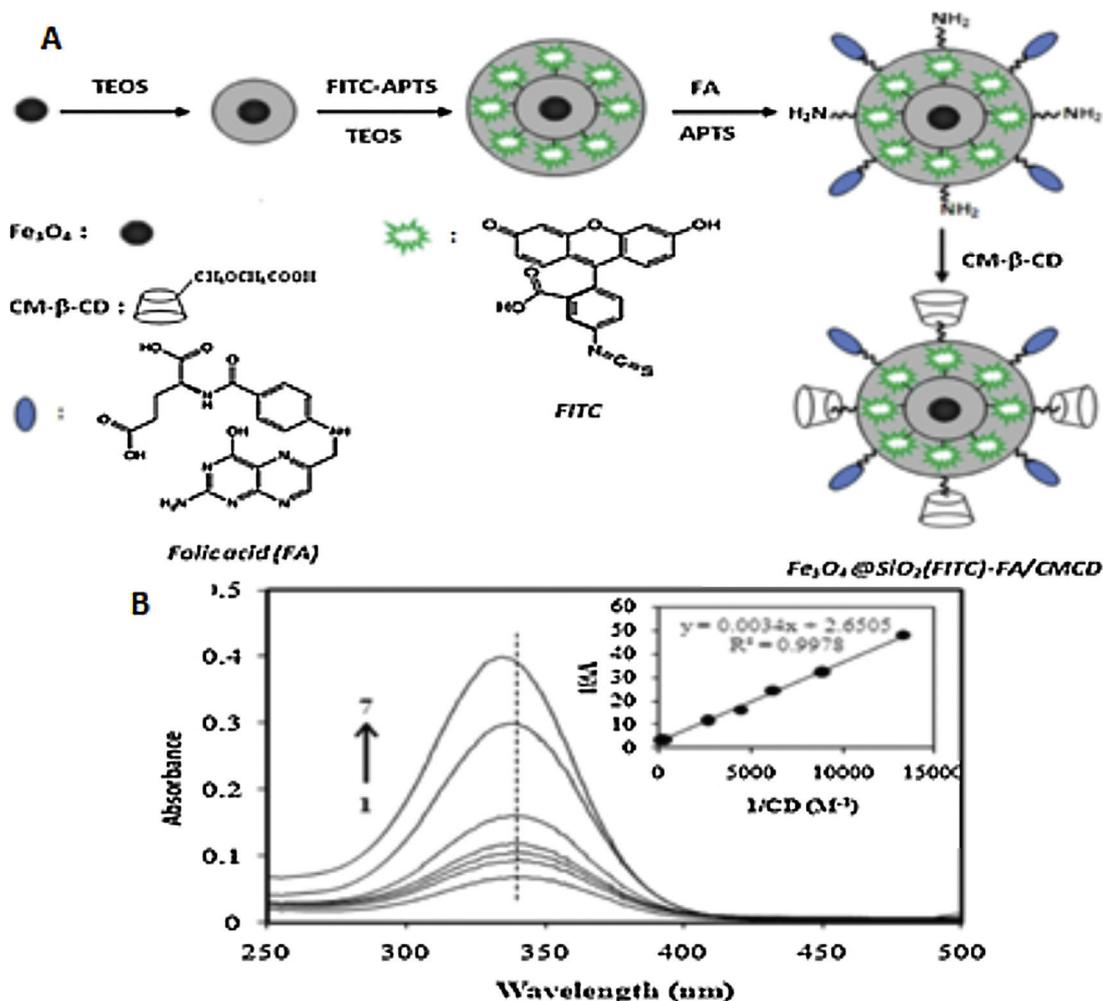


Fig. 10. (A) Synthetic route for synthesis of $\text{Fe}_3\text{O}_4@SiO_2(\text{FITC})\text{-FA/CMCD}$ nanoparticles. (B) Absorption spectra of retinoic acid (RA) ($7.3 \times 10^{-6} \text{ mol L}^{-1}$) in pH 7.4 buffer containing various concentrations of CM β CD. Concentrations of CM β CD: (1) 0, (2) 7.5×10^{-5} , (3) 1.1×10^{-4} , (4) 1.6×10^{-4} , (5) 2.3×10^{-4} , (6) 5.6×10^{-4} and (7) $3.8 \times 10^{-3} \text{ mol L}^{-1}$. Inset: double reciprocal plot for RA complexes with CM β CD (Badruddoza et al., 2013).

profile of the particles showed a rapid drug release in first 60 min with 23% of the drug being released, then the drug release was slowed down. Apart from this, passive drug release by magnetism could be employed as well to get controlled drug release (Badruddoza et al., 2013).

4. Miscellaneous

Gold nanoparticles (AuNPs) (Nalage et al., 2013) have been employed extensively in medicine for variety of purposes such as carrier for drug delivery, vector for cell targeting and for molecular imaging of cancerous cells (Heo et al., 2012). Gold nanoparticles are used because of their high chemical stability, biocompatibility and ease of binding with numerous biomolecules (Lee et al., 2008; Mayavan et al., 2011). Distinct properties which render gold nanoparticles alluring for therapy and diagnosis include unique surface plasmon resonance effects, good light scattering and absorption capability (Ma et al., 2011). Heo et al. (2012) prepared gold nanoparticles (AuNPs) containing PEG on the surface, biotin, model drug Paclitaxel (PTX) and rhodamine linked β -cyclodextrin (Fig. 11). β -cyclodextrin formed inclusion complex with PTX and enhanced its water solubility (Agüeros et al., 2010). Biotin acted as cancer specific targeting ligand and polyethylene glycol (PEG) served as solvated anti-fouling shell (Heo et al., 2012). Polyethylene glycol, biotin and rhodamine linked β -CD surface

functionalized AuNPs displayed marked anti-cancer activity against Hela cancer cells with little cytotoxic effects on NH3T3 cells. Furthermore, fluorescence of rhodamine B helped in cells imaging (Heo et al., 2012).

A novel type of multifunctional pH-disintegrable micellar nanoparticles were formulated from asymmetrically functionalized β -CD based star copolymers which were covalently conjugated with doxorubicin (DOX), folic acid (FA) and chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid DOTA-Gd molecules for combined cancer cell-targeted drug delivery and magnetic resonance imaging contrast enhancement (Liu et al., 2012) (Fig. 12). Asymmetrically functionalized β -CD i.e. $(N_3)_7$ - $(\beta\text{-CD})\text{-}(\text{Br})_{14}$ was synthesized first. It consisted of 7 azide functionalities and 14 α -bromopropionate molecules in the upper and lower rim of the rigid toroidal β -CD core, respectively. The subsequent steps for the formulation of $(\text{DOTA-Gd})_7\text{-CD-(PHPMA-FA-DOX)}_{14}$ were: (i) the atom transfer radical polymerization (ATRP) of *N*-(2-hydroxypropyl) methacrylamide (HPMA), (ii) conjugation with DOX and FA and (iii) finally click reaction with alkynyl-(DOTA-Gd) complex. The formulated star copolymer comprised of 7 DOTA-Gd complex molecules and 14 poly(HPMA) arms covalently anchored with DOX and FA via acid labile carbamate linkages and ester bonds. Approximately 13 DOX moieties were covalently conjugated with poly(HPMA) arms per star copolymer which constituted $\sim 14 \text{ wt.}\%$ loading content. As a

result, amphiphilicity was imparted to initial hydrophilic star copolymers which lead to their self-assembly into micellar nanoparticles in aqueous solution at pH 7.4. Over a period of 42 h, it was observed that DOX release was ~10%, 53% and 89% at pH values of 7.4, 5.0 and 4, respectively. When HeLa cells were treated with these micellar nanoparticles, a drastic decrease in cell viability was observed with merely ~15% of the HeLa cells remaining alive at DOX concentration above 80.0 mg/L. *In-vivo* magnetic resonance imaging assays in rats were also performed. Considerable accumulation of micellar nanoparticles within the rat

liver and kidney was observed along with outstanding positive contrast enhancement. The unified design of these micellar nanoparticles confirms their practical application in image guided chemotherapy (Liu et al., 2012).

Later on, Li et al. (2013) prepared Janus type star copolymers (DOTA-Gd)₇-CD-(PDMA)₁₄ via atom transfer radical polymerization of *N,N*-dimethylaminoethyl methacrylate (DMA) followed by click reaction of alkynyl-functionalized gadolinium (Gd³⁺) complex, DOTA-Gd. These asymmetrically functionalized β-CD based copolymers have been used in both diagnostic and therapeutic

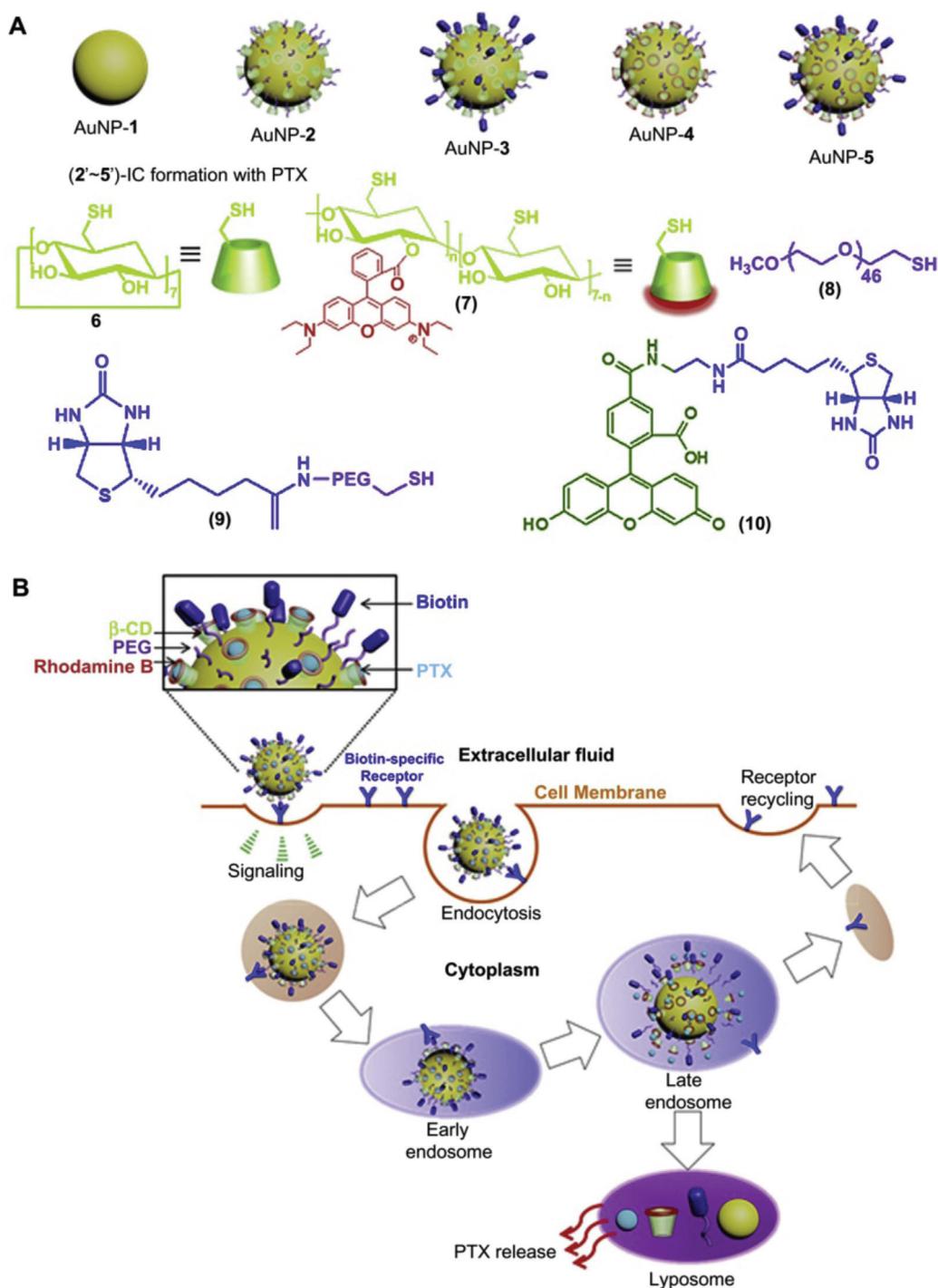


Fig. 11. (A) AuNP (1–5) and compounds (6–10). (B) Schematic illustration of cancer-targeting PTX delivery mechanism of AuNP 5' as a theranostic agent (Heo et al., 2012).

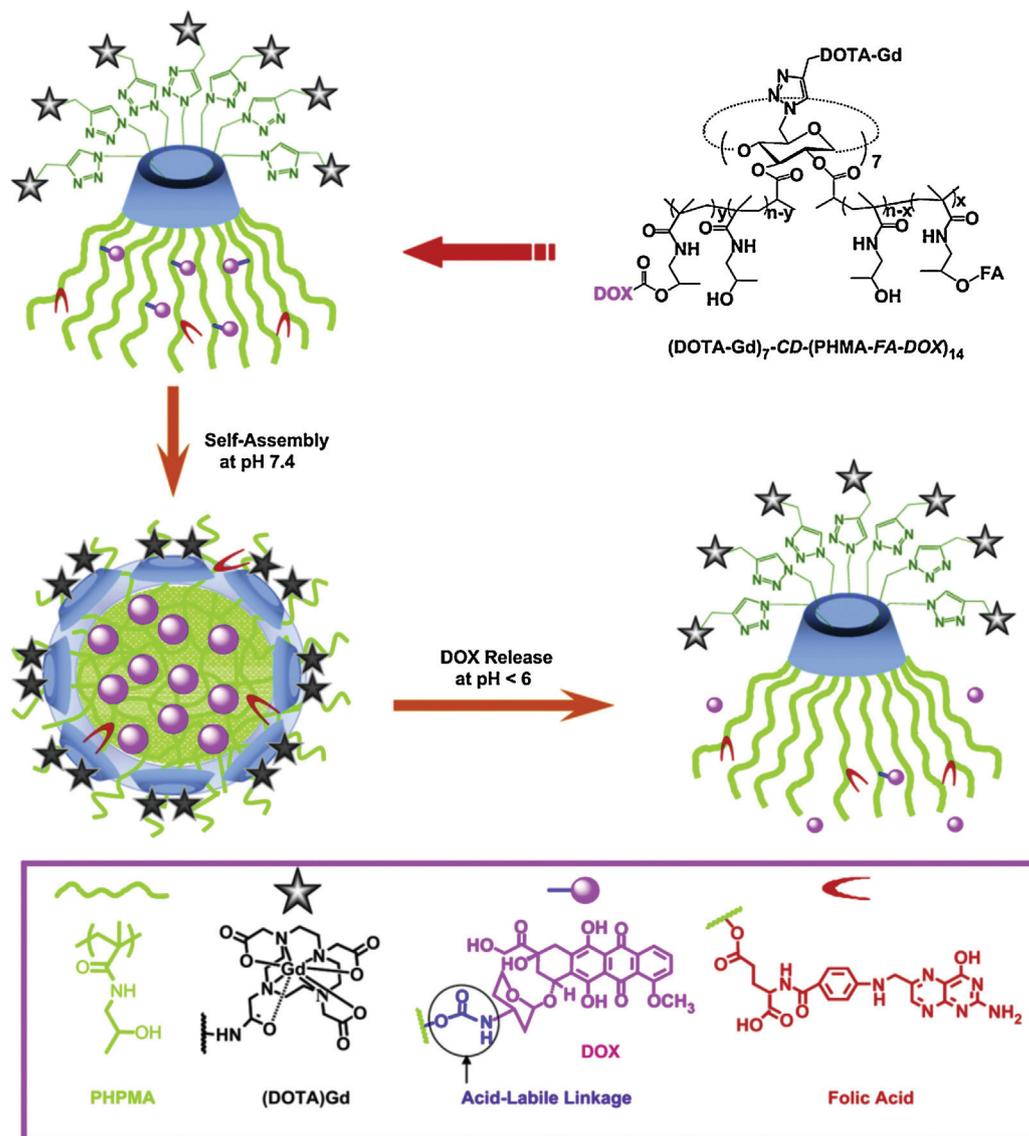


Fig. 12. Illustration of multifunctional micellar NPs possessing targeted drug delivery and MR imaging contrast enhanced functions from β -CD based star copolymers, $(\text{DOTA-Gd})_7\text{-CD-(PHMA-FA-DOX)}_{14}$, covalently conjugated with DOX, FA and DOTA-Gd molecules. It also shows the pH dependent DOX release via the cleavage of acid-labile carbamate linkages between DOX and HPMA arms (Liu et al., 2012).

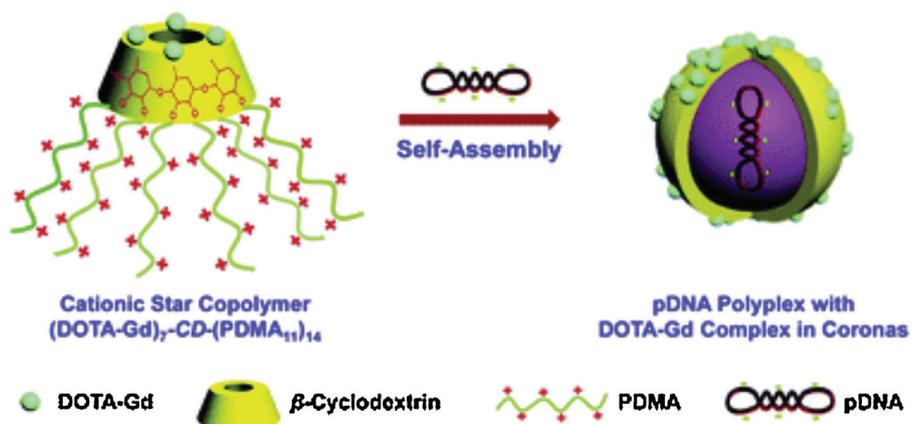


Fig. 13. Schematic illustration of $(\text{DOTA-Gd})_7\text{-CD-(PDMA)}_{14}$ copolymer preparation (Li et al., 2013).

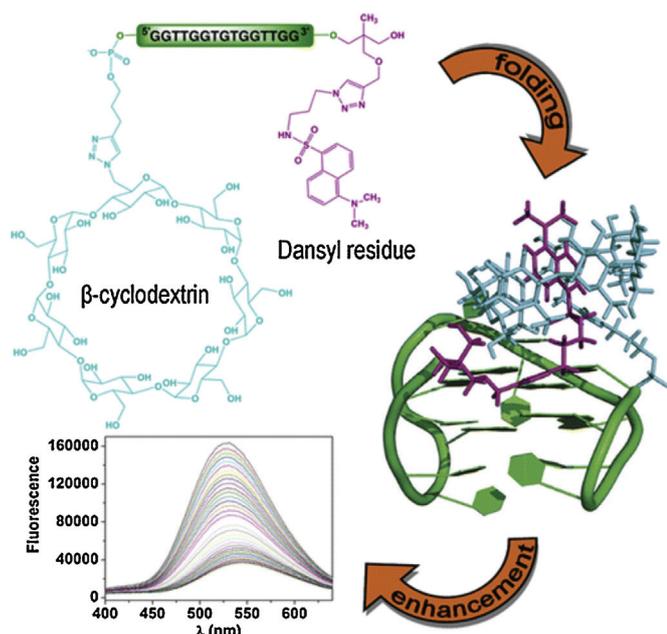


Fig. 14. Bis-conjugated TBA₁₅ (De Tito et al., 2013).

applications such as in image guided gene therapy since these copolymers can easily form complex with plasmid DNA (pDNA) via electrostatic interactions (Fig. 13).

Recently, a novel fluorescent thrombin binding aptamers (TBA) were synthesized by utilizing Cu(I)-catalyzed azide–alkyne cycloaddition processes (De Tito et al., 2013) (Fig. 14). The aptamers

were conjugated with dansyl probe at the 3'-end and β -CD residue at the 5'-end. Among the fluorescent dyes, the dansyl groups are considered exemplary probes for the study of self-assembly or recognition processes, as they are sensitive to external stimuli. Inclusion of dansyl into β -CD gave relevant enhancement of fluorescence. The host-guest system of dansyl-CD was selected for modification of TBA to render it suitable for diagnosis/therapy of coagulation related disorders. Structural characterization of the modified TBA revealed that it not only exhibits a potential application in diagnostic for thrombin recognition but could also provide a suitable basis for the design of aptamers-based devices for theranostic purposes which could allow both detection and inhibition/modulation of thrombin activity simultaneously (De Tito et al., 2013).

5. Cyclodextrin based pore control motifs

5.1. Supramolecular nanovalves

In this system, covalent attachment joins an immobilized stalk molecule to silica matrix and stalk is capped by a cyclic molecule like cyclodextrin, cyclobis and crown ether. Cyclic molecules encircle stalk and bind to recognition site on stalk by non-covalent interactions. Binding between stalk and cap is reversible and whole system act as gatekeepers for the pores allowing cargo release upon any suitable stimuli like chemical, electrical, light, heat *etc.* (Li et al., 2012). Du et al. (2009) introduced a cyclodextrin based pH responsive nanovalve (Fig. 15A and B). It had α -cyclodextrin (α -CD) rings on stalks containing aniline residues. Cargo was loaded into hollow interior of silica nanoparticles (SNPs). At neutral pH, α -CD rings were complexed to the stalk and were

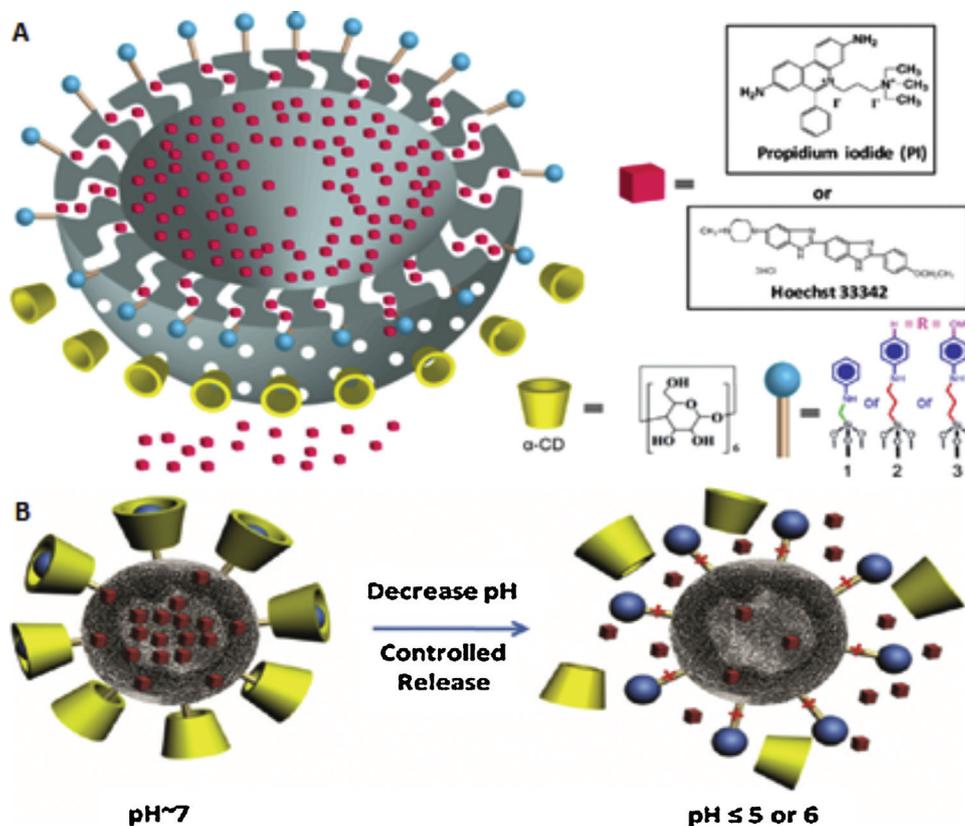


Fig. 15. (A) Illustration of cross-section of hollow cyclodextrin based nanovalve. (B) Stalks and α -CD rings controlling the release of cargo (upon change in pH) are also shown (Du et al., 2009).

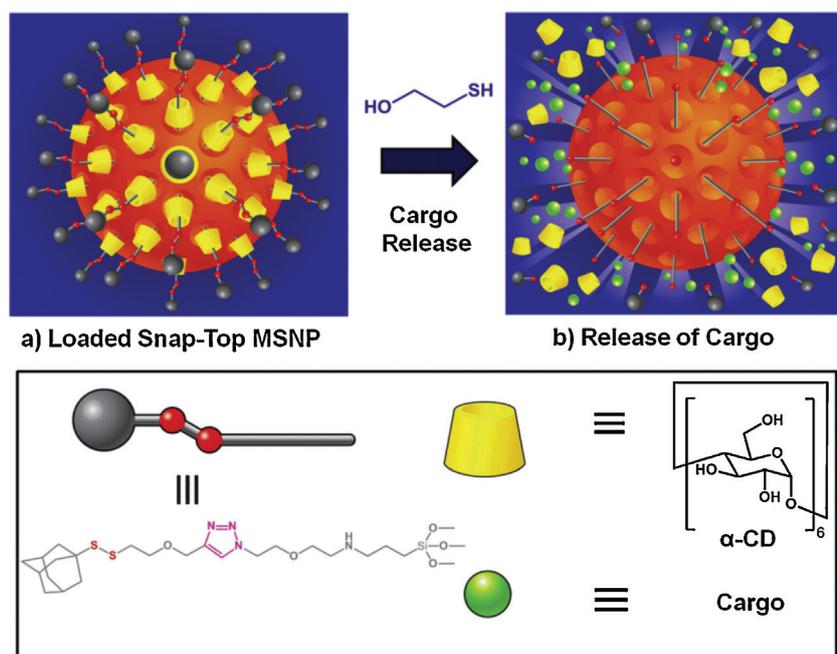


Fig. 16. Snap-top carrier having rotaxane on surface of MSNP. Stalks were made of PEG. α -CD was threaded on stalk and disulfide-adamantyl derivative acted as stopper. 2-Mercaptoethanol stimulation cleaved the disulfide bonds causing α -CD caps and cargo release. Luminescent cargo was loaded into this carrier (Li et al., 2012).

found near the pore openings. On encountering an acidic media, nitrogen atoms of aniline were protonated, binding of α -CD was decreased and this lead to escape of α -CD and hence controlled release of drug molecules (Ambrogio et al., 2011). Only fluorescent molecules have been loaded into such particles. More work can be done on it to check whether both fluorescent molecules and active moieties can be loaded simultaneously for converting these particles into a theranostic agent.

Meng et al. (2010) also prepared another CD-SNPs based agent working on nanovalve principle. Benzimidazole stalks were attached to surface of SNPs and β -CD rings were bound to benzimidazoles at neutral pH conditions. When these particles were placed in squamous carcinoma cell line KB-31, lower pH of the compartment caused protonation of benzimidazoles, escape of β -CD rings and release of cargo into internal compartment of cells. These MSNPs possessed the ability to release either Hoechst 33342 (used for imaging of cells) or doxorubicin (for apoptosis) drug (Ambrogio et al., 2011).

In this case, either the drug molecules *i.e.*, doxorubicin or the contrast agent for imaging *i.e.*, Hoechst 33342 was loaded into the particles. But it would be interesting if both imaging agent and therapeutic active molecule were loaded together in the particles to explore the possibility of their working as a theranostic agent.

5.2. Snap-top like machine

It is similar to nanovalve in consisting of stalks immobilized on silica matrix and cyclic capping molecules. The difference is that, there is minimum interaction between the caps and the stalks. Specific bulky groups referred to as 'stoppers' are utilized for holding the caps in place. These stoppers can snap off the stalks allowing dethreading of the cyclic caps and the release of cargo. A redox responsive snap-top (Li et al., 2012; Ambrogio et al., 2010) consisting of cyclodextrin caps was built (Fig. 16). In this case, only luminescent molecule was loaded into the MSNPs and there exists possibility of exploring these particles for theranostic application

by loading them with any therapeutic moiety in addition to luminescent cargo.

5.3. Nanopiston like system

Nanopiston like system was advanced in 2010 (Zhao et al., 2010) and consists of β - cyclodextrin rings tethered covalently to the surfaces of nanoparticles. This system is pH responsive and stalks (pistons) are freely movable as soon as pH is lowered. This system allows release of molecules smaller in size than β -CD rings and release of larger molecules as well by cleavage of β -CDs from nanoparticles surface upon acidification. Hence, dual drug delivery can be easily achieved by using different stimuli (Li et al., 2012). More research can be done on these types of particles to find out if these can be used as theranostic agent by loading imaging agent and drug molecules simultaneously inside it, instead of loading two drug molecules for dual drug delivery.

6. Conclusion

Nowadays, the target of majority of research in life science is to develop novel personalized therapeutics with improved safety and efficacy. The field of theranostics has opened new roads in achieving such objectives. The combination of diagnosis and therapy allows simultaneous disease identification and drug delivery. Certain specific materials like iron oxide, silica, gold and gadolinium are commonly used in the formulation of theranostic agents and in some cases two of these are combined together to confer better and specific qualities on the resultant particles.

Cyclodextrins possess extensive applications in diverse fields of drug delivery and pharmaceutical industry and play an important role in improving the properties of theranostic agents. The major role of cyclodextrins in theranostics is to enhance the drug loading and to give sustain release formulations. Furthermore, it has been shown to increase fluorescence as well. Cyclodextrins based micellar

nanoparticles usage for theranostics is quite interesting and show promising potential in image guided chemotherapy. It is also proposed to explore the possibility of converting certain systems such as supramolecular nanovalves based systems, where cyclodextrins have been mostly used as pore control agents, into theranostic systems by loading both active and contrast agents simultaneously in one system rather than loading just one of these molecules.

Acknowledgments

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II.3. Double emulsion evaporation in drug delivery systems: from preparation to encapsulation

Summary

Nowadays, pharmaceutical research is being directed towards evolution of innovative pharmaceutical products. The conventional therapies are progressively replaced by more adaptable and well refined drug deliveries by the exploration of advanced diversified technologies. Special focus is paid on tackling the restrictions associated with traditional drug delivery. Some of the most common obstacles encountered are the low bioavailability, poor stability, bitter taste and unpleasant odor of certain active agents. Here, encapsulation plays a vital role in overcoming the aforementioned obstacles. Premature degradation of active agents especially of proteins and peptides can be easily prevented by their encapsulation. Furthermore, encapsulation technologies also aid in achieving controlled and targeted release formulations. Colloidal carriers find immense applications in biomedical and biotechnology field. Various kinds of colloids employed in medicine are dendrimers, block ionomer complexes, liposomes, nanorods, nanotubes, polymeric micelles and polymeric biodegradable particles. There are several encapsulation processes available for the preparation of these colloidal carriers e.g. emulsion polymerization, miniemulsion polymerization, multiple emulsion techniques, nanoprecipitation, spray drying etc.

Microencapsulation by solvent evaporation is commonly employed to obtain controlled release formulations. There are different ways/methods to use microencapsulation by solvent evaporation technique. Mostly hydrophobicity/hydrophilicity of the drug molecules is the determinant for the selection of these methods e.g. oil-in-water is used for the encapsulation of water insoluble active agents and this method fails to encapsulate highly hydrophilic drugs. Multiple emulsions come into view when such cases arise. Multiple emulsions or double emulsions are complex systems, in which droplets of dispersed phase contain one or more smaller droplets inside themselves. In multiple emulsions e.g. in w/o/w type two step emulsification is performed. Firstly, inner aqueous phase is emulsified in organic phase using an external energy source like Ultraturrax[®] or Ultrasound. The primary emulsion formed is then dispersed in the outer aqueous phase using the selected homogenizer. The evaporation of the volatile organic solvent from the emulsion leads to insolubility and hence precipitation of the used polymer. In this way, the active agent is encapsulated in the polymeric particles. Organic solvent evaporation can be done either by stirring at room temperature or by using rotary evaporator.

The organic solvents used in double emulsion should be highly volatile in order to expedite the removal of this solvent from the final double emulsion formulation. The commonly used organic solvents include methylene chloride, chloroform, ethyl acetate and propylene carbonate. Methylene chloride is usually preferred because of its low boiling point (39.8 °C), low solubility in water (2.0% w/v) and its ability to dissolve large number of biodegradable polymers.

Nowadays, polymers are increasingly used to constitute particles. The selection of polymer depends on toxicity and final application of the polymer. Drugs encapsulated in biodegradable polymers show non-toxicity and stability in blood. Moreover, polymeric materials also allow modification in physicochemical properties, drug release properties and biological behaviour. Commonly used polymers for encapsulation include poly (lactic acid), poly (glycolic acid) (PLA) and poly (lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL). An important

advantage of using biodegradable polymers is that the release of encapsulated drugs mainly relies on the degradation kinetics of the polymers such as in case of PLGA; the release rate is controlled by changing the ratio of lactic acid and glycolic acid and also by varying the molecular weight. PCL is widely used for the encapsulation of vast number of drugs with which it shows excellent compatibility. This polymer degrades slowly to release the active agents and hence is used to formulate prolonged release pharmaceuticals. PLGA is another biocompatible and biodegradable polymer which is approved to be used in humans and is commonly employed for drugs encapsulation via double emulsion. However, this polymer also poses certain drawbacks such as risk of toxicity due to its acidic by-products when used for longer periods.

Double emulsion technique is used for the encapsulation of diverse pharmaceuticals and biopharmaceuticals and this process has its own advantages and drawbacks as well. This process is suitable for the encapsulation of both hydrophobic and hydrophilic active agents but is more preferable for the encapsulation of hydrophilic drug molecules. Biomolecules like peptides, proteins and nucleic acids pose a challenge of their efficient delivery to the target site without degradation. Since they are hydrophilic, they tend to diffuse into continuous phase during emulsification and hence lead to their low encapsulation efficiency. To overcome this difficulty, double emulsion like w/o/w type is employed and water soluble agents are dissolved in inner aqueous phase of the double emulsion from where they are released slowly. Moreover, double emulsions are biocompatible, biodegradable and versatile with respect to different oils and emulsifiers. This method is also reproducible and can be scaled up as well. Some drawbacks associated with this technique include high shearing forces from homogenization which can cause damage to structures of biomolecules. The organic solvents used also pose toxicity problems. Stability of multiple emulsions is another problem which is encountered commonly.

Diverse classes of drugs have been encapsulated by double emulsion method e.g. anticancer drugs, anti-inflammatory actives and different antibiotics. For anticancer drugs, targeted drug delivery and prolonged release is achieved by encapsulation via this method. Commonly anti-inflammatory drugs are encapsulated by this method to reduce their systemic irritation and to reduce possible drug interaction with other medications. The ratio of aqueous phase to oil phase is quite important for the stability of the emulsion during encapsulation of the drug molecules. Generally a ratio of 1:10 (water:oil) in an emulsion is considered to produce stable emulsion.

Theranostic is another emerging technology where double emulsions are also finding some applications. A theranostic agent is capable of delivering both a therapeutic and a diagnostic agent simultaneously. Though double emulsion is widely used for encapsulation of peptides, proteins and hydrophobic agents as well, research work on its application in co-delivery of therapeutic and diagnostic agents is limited. Mostly anticancer drug together with some diagnostic agent has been encapsulated by double emulsion technique.



Review

Double emulsion solvent evaporation techniques used for drug encapsulation



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ABSTRACT

Double emulsions are complex systems, also called “emulsions of emulsions”, in which the droplets of the dispersed phase contain one or more types of smaller dispersed droplets themselves. Double emulsions have the potential for encapsulation of both hydrophobic as well as hydrophilic drugs, cosmetics, foods and other high value products. Techniques based on double emulsions are commonly used for the encapsulation of hydrophilic molecules, which suffer from low encapsulation efficiency because of rapid drug partitioning into the external aqueous phase when using single emulsions. The main issue when using double emulsions is their production in a well-controlled manner, with homogeneous droplet size by optimizing different process variables. In this review special attention has been paid to the application of double emulsion techniques for the encapsulation of various hydrophilic and hydrophobic anticancer drugs, anti-inflammatory drugs, antibiotic drugs, proteins and amino acids and their applications in theranostics. Moreover, the optimized ratio of the different phases and other process parameters of double emulsions are discussed. Finally, the results published regarding various types of solvents, stabilizers and polymers used for the encapsulation of several active substances via double emulsion processes are reported.

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1. Introduction

Nowadays, scientific advanced technologies are being directed towards evolution of innovative pharmaceutical products. The conventional therapies are progressively supplemented by more adaptable and well refined drug deliveries by the exploration of these advanced diversified technologies. Special focus is paid on tackling the restrictions associated with traditional drug delivery. Some of the most common obstacles encountered are the low bioavailability, poor stability, bitter taste and unpleasant odor of certain active agents. Here, encapsulation plays a vital role in overcoming the aforementioned obstacles. Premature degradation of active agents especially of proteins and peptides can be prevented by their encapsulation. Furthermore, encapsulation technologies also aid in achieving controlled and targeted release formulations. Colloidal carriers find immense applications in biomedical and biotechnology field. Various kinds of colloids employed in medicine are dendrimers, block ionomer complexes, polymeric biodegradable nanoparticles (NPs), polymeric micelles, liposomes (Laouini et al., 2012; Naseer et al., 2014), nanotubes, nanorods (Wang et al., 2012) and quantum rods.

Recently, polymers are increasingly used to constitute biodegradable particles. Such particles serve as drug reservoirs which bring not only local therapeutic effect but also deliver drugs, genes and vaccines to target organs for site specific delivery (Mohsen Jahanshahi, 2008; Zafar et al., 2014). Polymers are also employed in regenerative medicines and tissue engineering. Polymer selection is primarily based on toxicity and final application of the polymer. Biodegradable polymer encapsulated drugs show superb attributes e.g. non-toxicity and stability in blood. Moreover, polymeric materials permit modification in (a) physicochemical characteristics (e.g. hydrophobicity, zeta potential), (b) drug release properties (e.g. delayed, prolonged, triggered) and (c) biological behaviour (e.g. bioadhesion, improved cellular uptake) of the NPs (Galindo-Rodriguez et al., 2005; Kumari et al., 2010; des Rieux et al., 2006). Examples of commonly used biodegradable polymers for encapsulation include poly (lactic acid), poly (glycolic acid), and poly (lactic-co-glycolic acid) (PLGA). Biodegradable polymers have the advantage that the release of loaded drug depends mainly on the degradation kinetics of the polymers used e.g. the release rate from PLGA carriers can be controlled by altering the lactic acid and glycolic acid ratio and molecular weight (Ye et al., 2010). Successful encapsulation of drugs like paclitaxel, 9-nitrocamptothecin, cisplatin, insulin, dexamethasone, estradiol, progesterone, tamoxifen, tyrphostins and haloperidol (Kumari et al., 2010) has been done by using various natural and synthetic polymers.

Several encapsulation processes have been used in order to protect and to transport active molecules. These techniques can be divided in two major classes: (i) chemistry based processes e.g. emulsion polymerization, miniemulsion polymerization and interfacial polymerization, (ii) physicochemical processes e.g. multiple emulsion techniques, spray drying, emulsion solvent

diffusion and layer by layer process. Different pharmaceutical carriers such as microparticles, nanoparticles and liposomes can be obtained by these techniques (Miladi et al., 2013). Microencapsulation by solvent evaporation is mostly used in pharmaceutical industries to get controlled release formulations. Different methods are available to use microencapsulation by solvent evaporation technique. The selection of a method that will give adequate drug encapsulation usually depends on the hydrophilicity or hydrophobicity of the active molecules (Li et al., 2008). Oil-in-water (o/w) method is generally adopted for the encapsulation of insoluble or poorly water soluble active agents. Few examples of hydrophobic active agents that have been encapsulated via this technique include Cisplatin (Verrijck et al., 1992), 5-Fluorouracil (Boisdron-Celle et al., 1995), Lidocaine (Chung et al., 2001) and Progesterone (Aso et al., 1994; Bums et al., 1993). However, this technique fails when it comes to encapsulation of highly hydrophilic agents. This is because the active agent may diffuse into the continuous phase during the formulation or it may not get dissolved in the organic solvent. Multiple emulsions play a pivotal role in such cases. The most common type of multiple emulsions is water-in-oil (w/o/w) emulsion. Most hydrophilic drugs have been encapsulated via (w/o/w) method (Crotts and Park, 1998; Okochi and Nakano, 2000; Sinha and Trehan, 2003). The water soluble agent is solubilized within the inner W1 phase of the emulsion which then shows prolonged drug release, lesser toxic effects (Nakhare and Vyas, 1996) and high encapsulation efficiency of the active agent. For this reason, proteins have been widely encapsulated by w/o/w emulsion system. The stability and release properties of double emulsions can be greatly improved by a change in the type and concentration of stabilizer employed in the system. Combining targeted delivery and prolonged drug release by using double emulsion system presents tremendous benefits in cancer therapy. Many advantages are associated with the use of double emulsion systems. Such systems are biocompatible, biodegradable and versatile with respect to different oils and emulsifiers being employed. Moreover, both hydrophobic and hydrophilic kinds of drugs can be encapsulated (separately and simultaneously) and protected. However, some difficulties are also linked with multiple emulsions such as difficulty in formulation, bulky and susceptibility to different routes of physical and chemical degradation. Various attempts have been made by researchers to improve the stability of multiple emulsions. Some of these efforts include surfactant concentration modulation, interfacial complexation, polymerization gelling, additives in different phases, steric stabilization and pro-emulsion approach (Garti, 1997; Garti and Aserin, 1996; Hino et al., 2000). The objective of this work is to present a comprehensive literature review on double emulsion technique. The review begins with a brief description of double emulsion and a comprehensive history of this technique. It progresses on to discuss the mechanism involved in formulation of a double emulsion. This review also reports the different polymers, stabilizers, surfactants, drugs

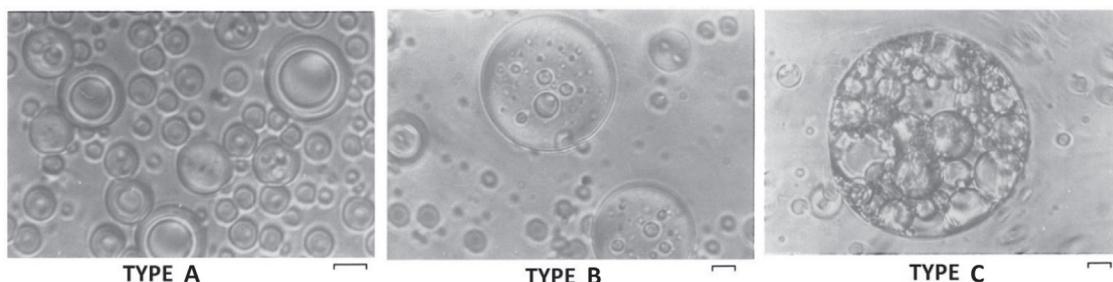


Fig. 1. Types of double emulsion droplets. (Florence and Whitehill, 1981).

encapsulated and average particle size obtained in different studies via this technique.

2. What is a double emulsion?

The first paper on double emulsion dates back 89 years (Seifriz, 1924), but detailed investigation on double emulsions was started at the end of 1970s. We can find several reviews on multiple emulsions, which come primarily from three research groups namely: Florence and Whitehill (1981, 1982), Matsumoto et al. (1980) and Frenkel et al. (1983). Double emulsion also termed as emulsion of emulsion, are complex system, in which the droplets of dispersed phase themselves comprises even small of dispersed phase (Garti and Bisperink, 1998). Double emulsion (DE) droplets are mostly polydispersed in size. In some cases its droplets are big enough containing many small compartments

with 50–100 droplets in each drop of double emulsion, while on the other hand small droplets of DE may consist of one or few droplets. There are two common types of DE; water-oil-water (w/o/w) and oil-water-oil (o/w/o). Two step processes are most commonly used for preparation of double emulsions, in which for w/o/w DE preparation, the inner aqueous phase (W1) is dispersed in oil phase containing lipophilic emulsifier in the first step, which is followed by dispersion of the primary emulsion into outer aqueous phase (W2) containing hydrophilic emulsifier (Garti and Bisperink, 1998; Schuch et al., 2013).

Florence and Whitehill (1981) identified that double emulsion droplets could be of three types (A, B, C), which did not exist exclusively in any one system but usually one of them is predominant. The type A, was found to be the simplest system consists of relatively small droplets with almost single droplet of the internal aqueous phase (Fig. 1A). The droplet size in the type B

Table 1
Advantages and limitation of various techniques used for encapsulation of drug.

Techniques	Advantages	Disadvantages	Examples
Single emulsion solvent evaporation	<ol style="list-style-type: none"> 1. Provides high entrapment of lipophilic actives. 2. Size of particles is adjustable by changing homogenization speed, amount of stabilizer, viscosity of organic and aqueous phases. 	<ol style="list-style-type: none"> 1. Entrapment of hydrophilic drugs is poor. 2. It is difficult to scale up. 	(Khalil et al., 2013) (Seremeta et al., 2013) (Guerreiro et al., 2012)
Double emulsion solvent evaporation	<ol style="list-style-type: none"> 1. It provides an advantage of encapsulation of both hydrophilic and hydrophobic actives. 	<ol style="list-style-type: none"> 1. Large and non-uniform particles (polydisperse). 2. Two step process. 3. Leakage of the hydrophilic active into external aqueous phase. 4. Difficult to scale up. 	(Zakeri-Milani et al., 2013) (Bitar et al., 2015) (Ibraheem et al., 2013)
Emulsion diffusion method	<ol style="list-style-type: none"> 1. It allows incorporation of thermosensitive drugs. 2. Reduces mean particle size and narrow size distribution. 3. Good batch–batch reproducibility. 4. Higher entrapment of lipophilic drugs. 5. Use of nontoxic solvents. 6. Easy scale up process 	<ol style="list-style-type: none"> 1. Concentration of final formulation is required. 2. Possible organic solvent residues in the final formulation. 3. Poor encapsulation of hydrophilic drugs. 4. Longer time of emulsion agitation required. 5. Require larger volume of water for nanoparticles formation. 	(Campos et al., 2013) (Hao et al., 2013) (Souguir et al., 2013)
Emulsion polymerization	<ol style="list-style-type: none"> 1. It is fast and scalable. 	<ol style="list-style-type: none"> 1. Toxic organic solvents and monomers are used. 2. Difficult to remove residual monomers, initiators and surfactants from final product. 	(Tolue et al., 2009) (Wu et al., 2011) (Sajeesh and Sharma, 2006)
Microemulsion Technique	<ol style="list-style-type: none"> 1. Reduces mean particle size and narrow size distribution. 2. Organic solvent free method. 3. No energy consuming process. 4. Easy to scale up. 	<ol style="list-style-type: none"> 1. High concentration of surfactants and co-surfactants. 2. Concentration of final formulation is required 	(Zhang et al., 2006) (Destrée et al., 2007)
Nanoprecipitation	<ol style="list-style-type: none"> 1. Simple and fast. 2. Uses non-highly toxic solvents. 3. High batch–batch reproducibility. 4. Does not require high shear stress. 5. Monodispersed particles. 	<ol style="list-style-type: none"> 1. Mostly limited to hydrophobic active encapsulation. 2. Nanoparticle size mainly depends on polymer concentration. 	(Han et al., 2013) (Katara and Majumdar, 2013), (Siqueira-Moura et al., 2013) (Seremeta et al., 2013)
Salting out	<ol style="list-style-type: none"> 1. No high shear stress is required. 2. Applicable to heat sensitive drugs. 3. High loading efficiency for lipophilic drugs. 4. Easy scale up. 5. High reproducibility. 	<ol style="list-style-type: none"> 1. Extensive nanoparticles washing step. 2. Exclusively applicable for only lipophilic drugs. 	(Miller et al., 1988)
High Pressure Homogenization (Hot and Cold)	<ol style="list-style-type: none"> 1. Good reproducibility. 2. Well established homogenization technology on large scale. 3. Organic solvent free method. 	<ol style="list-style-type: none"> 1. High temperature process. 2. High energy input 3. Complex equipment required. 4. Possible degradation of the components caused by high pressure homogenization. 	(Shi et al., 2011) (Silva et al., 2011) (Kamiya et al., 2009) (Hoyer et al., 2010)
Coacervation method	<ol style="list-style-type: none"> 1. Allows incorporation of thermosensitive drugs 2. Inexpensive for laboratory and industrial application. 3. Possibility to control shape and size of SLNs by reaction conditions. 	<ol style="list-style-type: none"> 1. Possible degradation of the components under acidic conditions. 	(Wieland-Berghausen et al., 2002)
Phase Inversion Temperature Method	<ol style="list-style-type: none"> 1. Organic solvent free method. 2. Non-energy consuming method. 3. Easy to scale up. 	<ol style="list-style-type: none"> 1. Not suitable for thermosensitive molecules like peptides or proteins. 	(Alvarado et al., 2013) (Saberi and McClements, 2015)
Solvent Injection Method	<ol style="list-style-type: none"> 1. Easy to handle. 2. Fast production. 	<ol style="list-style-type: none"> 1. Possible organic solvent residues in the final formulation. 	(Schubert and Müller-Goymann, 2003) (Arica Yegin et al., 2006)

Table 2
Examples of polymers, stabilizers and solvents used in encapsulation of various actives/nonactives via double emulsion method.

Polymer used	Active molecules/solid ingredient	Organic solvent	Stabilizers/surfactants	Use/Objective/Purpose	Average size	References
PLGA	Clonidine (hydrophilic molecule)	Methylene chloride	PVA	Sustained release for intra-articular administration	10–20 µm	(Gaignaux et al., 2012)
PLGA	DNA	DCM	PVA	Parameters optimization (PLA nanoparticles exhibit high release of DNA)	100–300 nm	(Rizkalla et al., 2006)
PLGA	Hydroxyapatite	DCM	PVA	System optimization	–	(Takai et al., 2011)
PLGA	SiO ₂	Chloroform	PVA	System optimization	–	(Takai et al., 2011)
PLGA	Tartrazine (synthetic dye)	Corn oil	Tween 80	Tartrazine encapsulation and release	15–25 µm	(Hattrem et al., 2014)
PLGA	BSA (bovine serum albumin)	Chloroform	PVA	Residual PVA effect on cellular uptake of nanoparticle	380–520 nm	(Sahoo et al., 2002)
PLGA	Vancomycin	Methylene chloride	PVA	Improving intestinal permeability of vancomycin administered via orally	450–466 nm	(Zakert-Milani et al., 2013)
PLGA	Alendinate	Ethyl acetate	PVA	Loading efficiency evaluation	145–223 nm	(Cohen-Sela et al., 2009)
PLGA	MEP421 (peptide) & BSA	DCM-ethyl acetate (3/2, v/v)	PVA	MEP421 (for Alzheimer's disease therapy) encapsulation for sustained drug release	21–25 µm	(Ji et al., 2008)
PLGA	SPf66 (vaccine for preventing malaria)	Methylene chloride	PVA	Evaluation of the effect of γ -irradiation on PLGA microspheres containing SPf66.	1.6–2 µm	(Igartua et al., 2008a)
PLGA	Pingyangmycin (anti-tumor antibiotic)	DCM	PVA	Pingyangmycin encapsulation for sustainably release	2 µm	(Han et al., 2010)
PLGA	BSA	DCM	PVA	Protein loaded polymeric microspheres development for controlled release of drug	300 nm–10 µm	(Ravi et al., 2008)
PLGA	Interferon-alpha (IFN- α) 2b	DCM	PVA	Sustain drug delivery	3–7 µm	(Zhang et al., 2008)
PLGA	Egg-white lysozyme	Methylene chloride	PVA	Preservation of lysozyme via encapsulation	45–110 µm	(Pérez et al., 2002)
PLGA	Ciprofloxacin HCl	DCM	PVA	Ciprofloxacin antibacterial potential evaluation	–	(Jeong et al., 2008)
PLGA	Proteins (tetanus toxoid, lysozyme, and insulin)	Ethyl acetate and methylene chloride	PVA	Process-related stability issues evaluation in encapsulation of proteins	130–353 nm	(Bilati et al., 2005)
PLA	BSA (bovine serum albumin)	Methylene chloride	PVA	Controlled release devices	353–468 nm	(Yang et al., 2001)
PLA	Oligonucleotide (DNA)	Methylene chloride	PVA	Preparation of porous via leaching of pore former (glycerol monooleate), and loading with oligonucleotide for antisense therapy	98–121 µm	(Ahmed and Bodmeier, 2009)
PLA	rhi (recombinant human insulin)	DCM+ Toluene	PVA	Parameters optimization	80–220% m	(Liu et al., 2005a)
PLA	Recombinant human epidermal growth factor (rhEGF)	Methylene chloride	PVA	Encapsulation of rhEGF (peptide) for chronic gastric ulcer healing.	16 µm	(Han et al., 2001)
PLA	HSA	Methylene chloride	PVA	Parameters optimization	75–130 µm	(Zambaux et al., 1998)
PLA	–	Methylene chloride	PVA	Particles formation mechanism	200 nm	(Quintanar-Guerrero et al., 1997)
PLA	Ovalbumin	DCM	PVP/ NaCl	Proteins encapsulation mechanism	169–188 nm	(Chen et al., 2002)
PLA	Acridine orange (fluorescent dye)	Ethyl acetate	Pluro-nic f68	Particles Transition behavior study	14 µm	(Ji et al., 2012b)
PLA	BSA (bovine serum albumin)	Methylene chloride	Tween 80	Nanoparticle preparation without stabilizer	500–1000 nm	(Lu et al., 1999)
PLA	–	Propylene carbonate	PVA: polysorbate 80	Process optimization	200–500 nm	(Quintanar-Guerrero et al., 1996)
PLA	Insulin	DCM	Tween 80	Influence of surfactant on Insulin encapsulation (an antidiabetic agent)	150–450 nm	(Zhu et al., 2005)
PCL	Vancomycin (antibiotic)	Methylene chloride	–	Ophthalmic controlled drug delivery	253 nm	(Petitti et al., 2009)
PCL	Sodium norcantharidate	DCM	PVA	Encapsulation of anticancer drug	39–181 µm	(Wang et al., 2008)
PCL	Streptococcus equi antigens	DCM	PVA	Vaccine adjuvant, to protect animals against strangles	1.43–2.35 µm	(Florindo et al., 2008)
PCL	Miglyol 812 (oil)	Ethyl acetate	PVA	Particles formation mechanism	450 nm	

PCL	Insulin	DCM	PVA	Insulin encapsulation	0.75–30 µm	(Moinard-Chécot et al., 2008)
PCL	–	DCM	PVA	Process parameters optimization	2–8 µm	(Mukejje et al., 2007)
Eudragit	Propranolol HCl (hydrophilic), Nife-dipine (lipophilic)	Methylene chloride	PVA	2 drug encapsulation in non-degradable microparticles for controlled release	85–130 µm	(Ibraheem et al., 2014a)
Eudragit S100	Theophylline	DCM	Spane 80	Drug encapsulation.	122–339 µm	(Hombreiro-Pérez et al., 2003)
Eudragit S100	Enalapril Maleate	DCM: ethanol: Isopropyl alcohol, (5: 6: 4)	Tween 80	developed for hypertension and congestive heart failure, was used as a model peptide	9–18 µm	(Rawat Sing et al., 2011)
PLA-PEG	Plasmid DNA	Ethyl acetate: Methylene chloride (1:1)	PVA	Plasmid DNA encapsulation to avoid its degradation in the body, it has application in therapy and vaccine.	272–296 nm	(Perez et al., 2001)
PLA-PEG-PLA	Insulin	Acetone	Tween 80, dextran T-70	Insulin encapsulation (an antidiabetic agent)	250 nm	(Ma et al., 2001)
PLGA-PCL	BSA	Methylene chloride	PVA	Process parameter optimization	200–900 nm	(Lamprecht et al., 2000)
PLA or PLGA	Melittin (peptide)	DCM	PVA	Development of controlled release injection to deliver the melittin over a month.	5 µm	(Cui et al., 2005)
PLGA/PCL blend (ratio:80:20; 60:40; 40:60)	Doxycycline	DCM	PVA	controlled delivery of doxycycline in the treatment of human periodontal pocket	90–200 µm	(Mundargi et al., 2007)
poly methacrylic Acid polybutyl adipate	Doxonubicin Penicillin-G	Chloroform DCM	PVA Tween	Co-delivery of Anti-cancer drugs Investigation of Surfactants role on particle size of nanocapsules containing penicillin-G	174 nm 131–247 nm	(Chiang et al., 2014) (Khoee and Yaghoobian, 2009)

PLGA=poly(lactic-co-glycolic acid), PLA= poly(lactic acid), DCM=dichloromethane, PVA= polyvinyl alcohol, HSA= human serum albumin, PLA-PEG= poly(lactic acid)-poly(ethylene glycol), BSA=bovine serum albumin, HSA =Human serum albumin.

emulsion system is larger composed of several small droplets (less than 50) of internal aqueous phase (Fig. 1B). The system became more complex (type C) when majority of droplets achieve relatively largest size, encapsulating numerous droplets of internal aqueous phase (W1) (Fig. 1C). The system C, showed slow release of entrapped moiety than A or B, so system C would expected to be more promising in drug delivery system.

Double emulsion has the ability to prepare polymeric particles, which allow the controlled release of active ingredients soluble in the internal aqueous phase or dispersed in the polymeric matrix. Its foremost function is regarded as internal reservoir to entrapped active ingredients whatever you chose into the inner confined space, which can protect the entrapped sensitive ingredient against light, enzymatic degradation, and oxidation. It also allows the slow and sustained release of active ingredients from the internal reservoir to the external dispersion media and their key importance is due to their capability to encapsulate some water-soluble flavors and active ingredients (Benichou et al., 2004). In cosmetic field, it offers the possibility of combining incompatible ingredients in the same formulation to enhance the efficacy. Regarding disadvantages of double emulsion, it a complex process and thermodynamically unstable. The particles produced by this technique are comparatively heterogeneous and particle size is sensitive to various process parameter of double emulsion technique. Few prominent advantages and limitation of various techniques for encapsulation of drug are given in the table (Table 1). Single emulsion solvent evaporation, double emulsification, nanoprecipitation, emulsion diffusion and salting out techniques can be used to encapsulate lipophilic active agents. Double emulsion is a unique process encompassing the advantage of encapsulating both lipophilic and hydrophilic drug molecules. Both emulsion diffusion and coacervation techniques are used for incorporation of thermosensitive drugs whereas phase inversion temperature method cannot be utilized to encapsulate thermolabile actives like peptides and proteins. Techniques that generally require high shearing stress and high pressure homogenization include single and double emulsification. Moreover, double emulsion generally gives polydisperse particles as compared to other techniques. Lastly, the techniques that do not require the use of toxic solvents or organic solvents are; emulsion diffusion, microemulsion, nanoprecipitation, high pressure homogenization and phase inversion temperature technique.

3. Formulated double emulsion based dispersion

The most often used double emulsion technique for preparation of micro- and nanoparticle, and encapsulation of active molecules is double emulsion solvent evaporation technique. Initially, this technique was used for microencapsulation (Alex and Bodmeier, 1990; Pisani et al., 2008). In this method, homogenization is performed in two steps; in the first step, water soluble drugs are incorporated in the inner aqueous phase (W1) and polymer/lipophilic drugs are added into oil phase (O), then both phases are homogenize by proper agitation to form the primary emulsion (W1/O). Then, the primary emulsion is emulsified with the outer aqueous phase containing appropriate stabilizer to form double emulsion (W1/O/W2). Formation of double emulsion (particulate dispersion) is followed by evaporation of the organic solvent (O) from the dispersed phase leading to a point of insolubility and consequently, hardening of the polymer encapsulating the active material. The solvent may be evaporated under reduced pressure via rotary evaporator or by simple stirring at ambient temperature depending upon the boiling point of organic solvent. The outer aqueous phase act as dispersion medium and the agitation can be provided either by mechanical stirring or sonication depending

upon the nature of drug to be encapsulated and the intended particle size.

3.1. Commonly used polymers in double emulsion solvent elimination preparations

Biodegradable polymers have been used tremendously in drug delivery system for encapsulation of pharmaceuticals and pharmaceuticals as they can be degraded into nontoxic components. These polymers are consisting of ester, amide and ether functional groups. Different polymers such as PLA, PLGA and PCL are being intensively researched for encapsulation various active drugs, genes and macromolecules using double emulsion process (Badri et al., 2014; Pillai and Panchagnula, 2001; Rosset et al., 2012). PCL is a nontoxic, biodegradable and biocompatible polymer; with low glass transition temperature and melting point and its metabolites are eliminated from the body by innate metabolic process. It has been extensively study for encapsulation via double emulsion process for control drug delivery system in several formulations due to its compatibility with wide range of drugs and its slow degradation to release drug for extended period of time (Florindo et al., 2008; Wang et al., 2008; Iqbal et al., 2014; Lowery et al., 2010). Poly(lactic-co-glycolic acid) is another biocompatible and biodegradable polymers commonly used in double emulsion techniques (Hattrem et al., 2014; Rizkalla et al., 2006; Takai et al., 2011). After its approval by FDA for use in humans, it has been a popular choice for drug delivery. It degrades gradually into the biocompatible products of lactic and glycolic acid via hydrolysis and thus releases the encapsulated agents slowly over a long period of time (weeks–months). This polymer is available in different PLGA/PLA ratio. As lactic acid is more hydrophobic than glycolic acid and, thus, lactic acid-rich PLGA copolymers are less hydrophilic, and degrade very slowly (Dinarvand et al., 2011). PLGA has been investigated for encapsulation of protein, peptides and nucleic acids, and for attachments of legends to targeting particle to specific tissues and imaging (McCall and Sirianni, 2013).

Poly (L-lactic acid) (PLA) polymers are approved by FDA in humans (Zille et al., 2010) and have been frequently used for encapsulation in drug delivery system via double emulsion process (Hong et al., 2005; Montiel-Eulefi et al., 2014; Nihant et al., 1994). It belongs to the most promising category of biodegradable polymers having excellent mechanical properties, good biocompatibility, and low toxicity. It is semi-crystalline polymer with a melting point of about 180 °C, glass transition temperature of about 55 °C

and tensile strength of 50–70 MPa and commonly prepared by ring-opening polymerization (ROP) (Auras et al., 2010; Södergård and Stolt, 2002). The properties such as crystallinity, hydrophobicity and melting point of PLA can be tailor made by copolymerization (random, block, and graft) with other comonomers, modification in molecular architecture (hyperbranched, star shaped, or dendrimers), functionalization or blending with other polymers. For example, glycolide, ϵ -caprolactone, δ -valerolactone, trimethylene and carbonate have been often used to modify its thermal properties (Gruevård et al., 1998). These modifications also affect drug loaded PLA particles properties such as drug release rate, permeability and degradation rate of the matrix. Beside many advantages of biodegradable PLA polymers in controlled drug delivery systems, they have certain shortcomings such as a risk of toxicity and immunogenicity due to their acidic by-products when used for long period of time (Naraharisetti et al., 2005).

The selection of the appropriate polymer during encapsulation of drug is a critical step, which depends upon the chemical nature of the drug and polymer and their intended application. Table 2 is listing some of the most commonly used biodegradable polymers in encapsulation via double emulsion process.

3.2. Stabilizers

Stabilizers make it possible to maintain the physicochemical state of a dispersion of two or more immiscible phases and prevent the separation of phases, thus making emulsion system more stable. The commonly used stabilizers in double emulsion process include PVA, Tween 80 and Span 80. However, poly vinyl alcohol (PVA) is one of the most frequently used stabilizers in double emulsion process for encapsulation of different active moieties (Liu et al., 2005a; Rizkalla et al., 2006; Yang et al., 2001). It is a well-known hydrophilic, biocompatible polymer and possesses good mechanical strength, low fouling potential, and lasting temperature stability and pH stability. These properties of PVA make it suitable candidate to be used in encapsulation of various pharmaceuticals and biopharmaceuticals (Xia and Xiao, 2012).

4. Encapsulation of active ingredients

The selection of a specific technique for an efficient drug encapsulation is generally determined by the hydrophilicity or

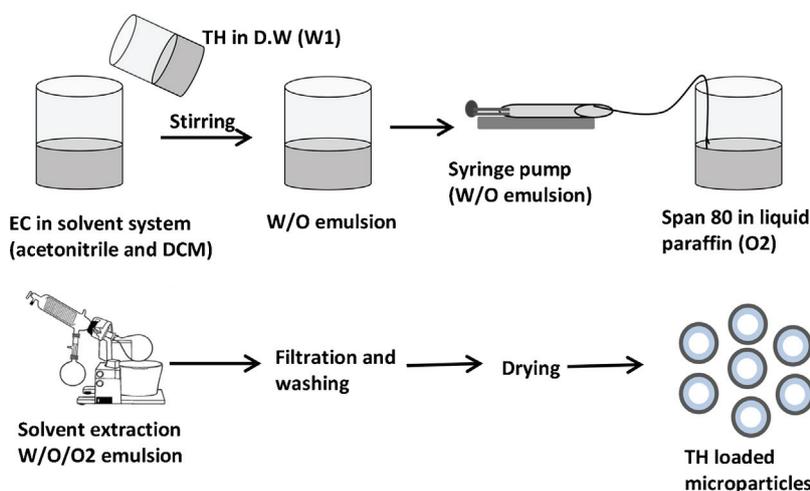


Fig. 2. Ethyl cellulose loaded microparticles prepared via double emulsification (W/O1/O2) technique (Jelvehgari and Montazam, 2012).

hydrophobicity of the drug molecules (Jelvehgari and Montazam, 2012). Despite the fact that various techniques have been described in the literature as well as successfully employed by researchers to encapsulate hydrophobic agents into biodegradable nanoparticles (Ibrahim et al., 2013; Palamoor and Jablonski, 2013, 2014), the encapsulation of hydrophilic drugs into such carriers is not facile. This is because the hydrophilic compound is pushed out from hydrophobic matrix into the dispersing water phase during formulation of the particles (Ibrahim et al., 2013). W/O or O/O emulsion prepared via solvent evaporation methods are quite suitable for biologically active substances encapsulation into microspheres but certain problems are involved in these techniques e.g. it is difficult to remove the large volumes of solvents from the dispersions (Jelvehgari and Montazam, 2012). The mineral or vegetable oil used as external phase in both w/o or o/o emulsion makes washing/collecting of resultant particles difficult. However, more innovative method to encapsulate hydrophilic drugs via emulsion solvent evaporation technique includes double emulsions (multiple emulsions) e.g. in w/o/o emulsion type, the problems associated with w/o or o/o methods are eliminated. Jelvehgari and Montazam (2012) prepared theophylline loaded microparticles via emulsion-solvent extraction or evaporation techniques to compare the results (Fig. 2). In any case, the formulation should be adapted to the chemical nature of used polymer and the chemical stability of the active molecule in the used solvent and encapsulation conditions.

4.1. Encapsulation of common pharmaceutical molecules by double emulsion solvent evaporation process

Some common pharmaceutical drugs encapsulated into particles by double emulsion process such as anticancer drugs, anti-inflammatory and antibiotics are reviewed here. The most encapsulated active molecules are anticancer drugs.

4.1.1. Anticancer drugs

Among anticancer drugs, doxorubicin, cisplatin, fluorouracil and epirubicin have been encapsulated by this technique (Ji et al., 2012a; Matsumoto et al., 1997; Wang et al., 2010; Zhou et al., 2006). Doxorubicin is an antitumor drug and has been frequently incorporated into different polymeric particles prepared by double emulsion process (Amjadi et al., 2013; Jiang et al., 2011). It is an antibiotic commercially available as a water soluble hydrochloride salt, which used in cancer chemotherapy. Tewes et al. (2007) prepared PLGA particles loaded with doxorubicin. The hydrodynamic size of DOX loaded particles was found to be 316 nm and its encapsulation efficiency was 67%. The release rate was slow and over a period of 24 h only 1.5% of drug was released, which indicates the controlled release of formulation. Release rate depends upon several factors such as water-solubility, dissolution, particles size and thickness of polymeric coating (Pearnchob et al., 2003; Siepmann et al., 2004). In another study, Wang et al. (2011) demonstrated that the anti-tumor efficacy of doxorubicin (DOX) and paclitaxel (TAX) can be enhanced by its co-delivery strategy. They prepared drug-loaded Methoxy poly(ethylene glycol)-poly(lactide-co-glycolide) (mPEG-PLGA) nanoparticles by double emulsion process. The hydrophilic doxorubicin was incorporated in inner aqueous phase and the hydrophobic paclitaxel in methylene chloride (oil phase) and emulsified by sanitation. Average size of drug loaded particles (NPs-DOX-TAX) was measured by DLS and found to be 243 nm. The in vitro drug release profile was determined in phosphate-buffered saline (PBS) at pH 7.4 and pH 4.4 and the release rate of both DOX and TAX were pH dependent (Fig. 3). At neutral pH, the release rates of both drugs were slower than acidic pH, and about 90% of drugs were released within 300 h at pH 7.4.

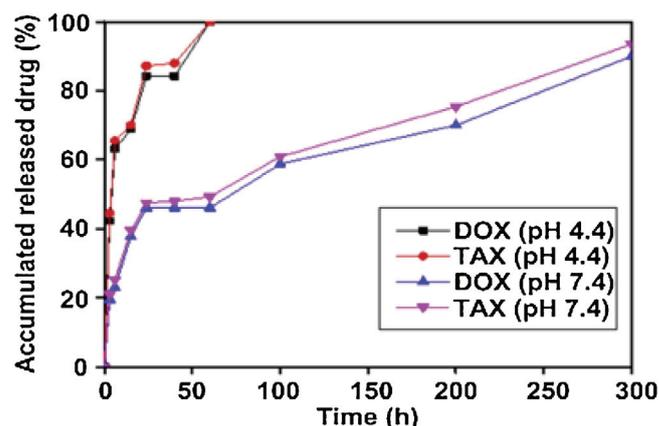


Fig. 3. Release profiles of DOX and TAX from mPEG-PLGA nanoparticles in PBS at 37 °C at pH 4.4 (black, red) and pH 7.4 (blue, purple) (Wang et al., 2011). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.1.2. Anti-inflammatory drugs

Anti-inflammatory drugs encapsulated by double emulsion process included acetaminophen, aceclofenac, diclofenac sodium, aspirin and ketoprofen etc. (Bhatnagar et al., 1995; Lai and Tsiang, 2005; Nagda et al., 2009; Pavanetto et al., 1996). Aspirin is widely utilized nonsteroidal anti-inflammatory drug (NSAID). It may be used to reduce pain and swelling in conditions such as arthritis, to reduce fever and relieve mild to moderate pain from conditions such as muscle aches, toothaches, common cold. Fargnoli et al. (2014) encapsulated aspirin and prednisolone drugs in poly-lactic glycolic acid polymer (PLGA) nanoparticles by double emulsion process for cardiac gene therapy. And it was found that aspirin particles have large size (323 nm) than prednisolone nanoparticles (234 nm), this difference in size was attributable to higher aspirin mass content. Loading Efficiency results were uniform for all nanoparticle types, i.e. PLGA 50:50 Prednisolone (88.9%), PLGA 65:35 Prednisolone (88.2%), PLGA 50:50 Aspirin (89%) and PLGA 65:35 Aspirin (88.8%). The controlled release of drug for over a time period of 5 days are shown in Fig. 4. The release rate of aspirin was faster as compared to prednisolone as shown in the Fig. 4.

4.1.3. Antibiotic drugs

Antibiotics such as, erythromycin, gentamicin, norfloxacin, cephalixin, capreomycin (Chaisri et al., 2009; Fan et al., 2009; Huang and Chung, 2001) Cefazolin, ciprofloxacin, clindamycin, colistin, doxycycline, and vancomycin (Shah et al., 2014) have been encapsulated by double emulsion technique. Encapsulation of highly hydrophilic drugs such as gentamicin has been performed

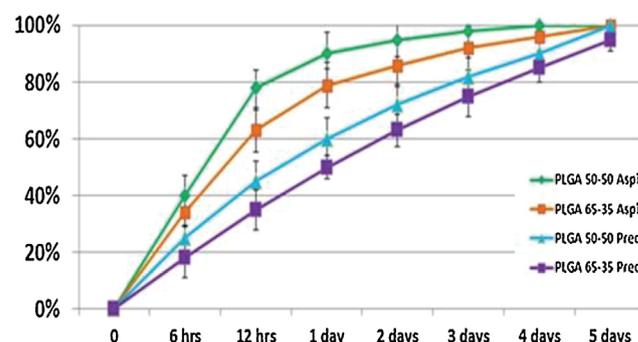


Fig. 4. PLGA Nanoparticle formulations release analysis. Controlled release study results demonstrate that aspirin particles overall release faster than prednisolone types (Fargnoli et al., 2014).

by many researches via double emulsion method (Virto et al., 2007; Yang et al., 2001). Gentamicin is the most important aminoglycoside that has been used widely for the treatment of osteomyelitis and against a wide range of Gram-positive and Gram-negative bacteria (Lecároz et al., 2006). However, a prolonged dosage regimen of antibiotics (4–6 weeks) for the treatment of osteomyelitis is needed, which may cause systemic toxicity. Thus, localized drug delivery of gentamicin has been proposed, in order to minimize the side effects by reducing administered dose and to reduce probability of drug resistance (Huang and Chung, 2001). The in vitro drug release is dependent on the distribution of drug within microsphere, polymer blend ratio, polymer degradation pattern, stabilizer and size of microsphere (Faisant et al., 2002; Lecomte et al., 2005; Luan et al., 2006, 2006). The high initial burst release of drug is often attributed to the associated drug on the particles surfaces (Kassab et al., 1997). On the other hand, larger size particle release drug more slowly and for extended period of time compared to smaller particles (Berkland et al., 2003).

In a study conducted by Shah et al. (2014) cefazolin, ciprofloxacin, clindamycin, colistin, doxycycline and vancomycin were loaded at 10 wt% and 20 wt% into PLGA microparticles using double emulsion solvent evaporation technique. All the antibiotics were first solubilized in the inner aqueous phase at 37 °C and then added to the oil phase (PLGA dissolved in methylene chloride) at a ratio of 1:5.6 v/v. The internal phase/oil phase emulsion was then homogenized with outer aqueous phase (0.3 wt% PVA solution) at 700 rpm. Finally, the solvent was evaporated and the particles were washed. Table 3 shows their loading efficiencies.

The release kinetics of all of the antibiotics loaded PLGA particles were determined in phosphate buffered saline at pH 7.4. The in vitro release curves are shown in Fig. 5. From the release analysis, it was found that during phase-1 (0–1 day), 20 wt% loaded ciprofloxacin formulation has greater burst release as compared to 20 wt% clindamycin, cefazolin and doxycycline loaded formulations, while no significant difference was found between 20 wt% cefazolin, clindamycin and doxycycline formulations. Most of the loaded drugs were released during phase-2 (1–21 days). In this phase, drug release rates were almost same for cefazolin, ciprofloxacin, clindamycin, and doxycycline for both 10 wt% and 20 wt% loaded particles. In case of colistin and vancomycin loaded particles, the drugs were mainly released during phase 3 (24–38 days) and there was no significant difference in release rates between colistin and vancomycin loaded particles (Shah et al., 2014).

4.2. Encapsulation of biopharmaceuticals via double emulsion method

4.2.1. Encapsulation of proteins

Advancement in the field of biotechnology/genetic engineering (Hutchinson and Furr, 1990) and better understanding of the role of peptides and proteins in the physiology and pathology has

enhanced the importance of peptides and proteins as therapeutic agents. However, certain hurdles are associated with the therapeutic usage of the available peptides and proteins such as their short half-life. Moreover, these can be easily degraded and most peptides possess poor passage through biological barriers due to their poor diffusivity and low partition coefficient (Lee, 1988). Due to these reasons, during the past two decades, the researchers have focused their interest in encapsulation of these agents within colloidal particles by employing different biodegradable polymers (Arshady, 1991; Heller, 1993; Jalil and Nixon, 1990; Langer, 1990; Zhou and Wan Po, 1991). Encapsulation protects these agents from degradation, controls their release from site of administration and in some cases, it can also improve the passage through biological barriers. Although the double emulsion encapsulation technique is taken as a complex process, it is still widely employed to encapsulate hydrophilic agents, particularly protein and peptide drugs, into polymeric microspheres resulting in higher encapsulation efficiencies (Alonso et al., 1994; Bley et al., 2009; Kreitz et al., 1997; Leach and Mathiowitz, 1998). Engel et al. (1968) successfully encapsulated insulin via this technique to enhance the efficiency of insulin upon oral administration as well as to facilitate its gastrointestinal absorption.

Protein encapsulation via double emulsion is performed in two steps (Nihant et al., 1994; Tan and Danquah, 2012): In the primary emulsion formation stage, the aqueous solution of protein is added to the polymeric organic solution in the presence of high shearing forces (ultrasonication/homogenization). In the second stage, double emulsion (w/o/w) is formed by dispersing the primary emulsion in an external aqueous phase containing suitably selected stabilizer. Finally, organic solvent removal either by evaporation or extraction results in the formation of protein loaded particles (Fig. 6).

Many proteins have been encapsulated via this technique such as bovine serum albumin (Benoit et al., 1999; Lu et al., 1999; Sah et al., 1995; Jameela et al., 1996; Yang et al., 2001; Youan et al., 1999). Lamprecht et al. (2000) also prepared BSA based nanoparticles via double emulsion pressure homogenization technique with the goal to investigate the influence of miscellaneous process control parameters on the obtained nanoparticles. Some novel methods for the encapsulation of proteins and peptides were also introduced such as Viswanathan et al. (1999) introduced a (water in oil) in oil emulsion. They used oil as processing medium to prevent the hydrophilic proteins from diffusing out of the microspheres before they harden. Whereas, in another case, BSA loaded PCL microparticles were prepared by Lin and Huang (2001a) via w/o/o emulsion solvent evaporation method. They utilized two types of homogenizers and investigated the influence of solvent evaporation rate on the crystallinity and performance of particles. Another study was also performed on similarly prepared particles i.e. via w/o/o emulsion technique (Lin and Huang, 2001b) to study the effect of pluronics on the BSA loaded microparticles.

A luteinizing hormone-releasing hormone (LHRH) agonist i.e. Leuprolide acetate was encapsulated via w/o/w emulsification by Ogawa et al. (1988a) and Ogawa et al. (1988b). Currently this product is available in the market. There are many other examples of proteins encapsulated by double emulsion technique and few of these examples are listed in the following table (Table 4).

Although, this technique is widely used for protein encapsulation, still there are certain limitations associated with this method. The drug encapsulation efficiency is not very good and the production cost of protein drugs is high. Moreover, the use of toxic organic solvents such as dichloromethane and ethyl acetate is also not very favorable. Furthermore, protein drugs tend to denature and form aggregates due to various factors like high shearing forces

Table 3
Loading Efficiencies of Microparticle Formulations (Shah et al., 2014).

	Loading efficiency of 10 wt% antibiotic-loaded PLGA MPs (%)	Loading efficiency of 20 wt% antibiotic-loaded PLGA MPs (%)
Cefazolin	36.4±3.3	51.3±6.1
Ciprofloxacin	86.8±10.9*	38.9±17.8*
Clindamycin	84.9±6.9	89.5±4.0
Colistin	102.0±5.0	105.3±4.4
Doxycycline	71.4±2.3	89.4±0.9
Vancomycin	83.3±4.8	76.9±6.2

Data is presented as mean ± standard deviation, n = 3 per group

*Indicates significant difference between 10 and 20 wt% loaded for a given antibiotic

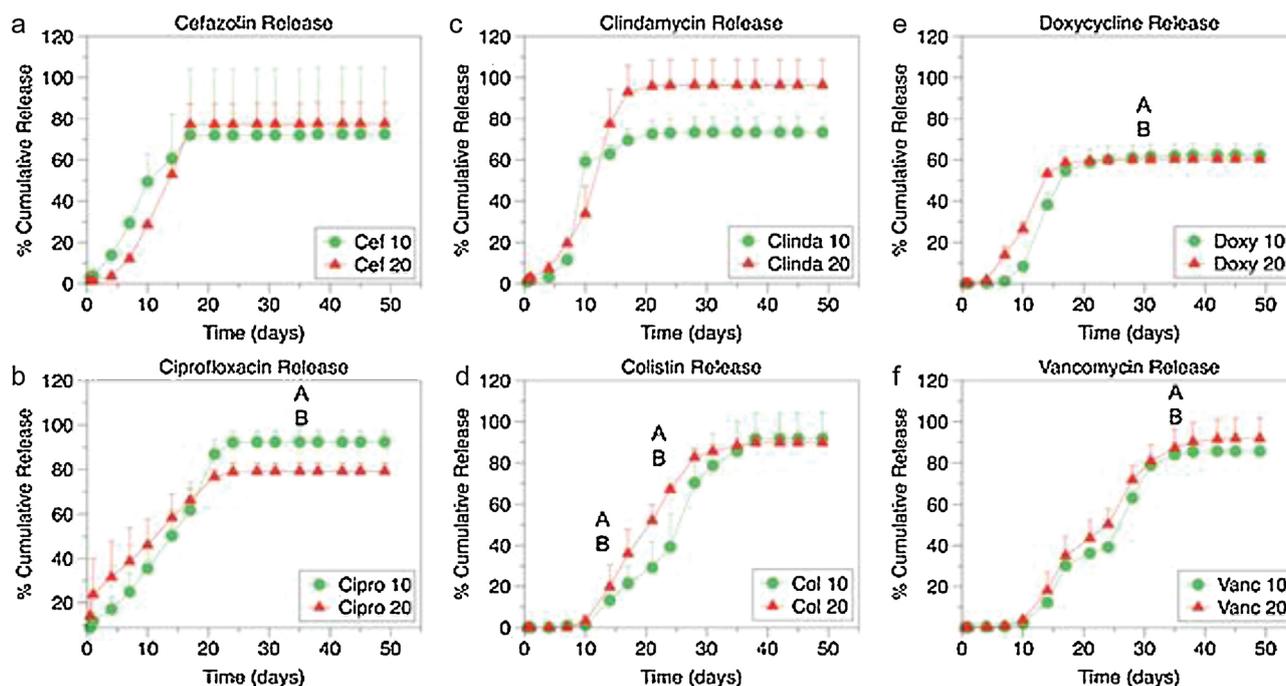


Fig. 5. Release curves for (a) cefazolin, (b) ciprofloxacin, (c) clindamycin, (d) colistin, (e) doxycycline, and (f) vancomycin loaded PLGA microparticles at 10 and 20 wt%. Significant differences between release rates during each phase for the same antibiotic are indicated by letters A and B. For colistin loaded PLGA MPs, Phase 2 and Phase 3 are significantly different between 10 and 20 wt% loaded MPs ($p < 0.05$). Ciprofloxacin and doxycycline 10 wt% loaded MPs demonstrate increased release of antibiotic during Phase 3 compared to 20 wt% loaded MPs, and vancomycin loaded MPs demonstrate increased release from 20 wt% MPs compared to 10 wt% MPs in phase 4 ($p < 0.05$). Cumulative percent release is the same between 10 and 20 wt% for any antibiotic ($p > 0.05$). The three release phases of cefazolin, ciprofloxacin, clindamycin and doxycycline are: phase 1 (0–1 d), phase 2 (1–21 d), phase 3 (21–49 d), and four phases of colistin and vancomycin are: phase 1 (0–10 d), phase 2 (10–24 d), phase 3 (24–38 d) and phase 4 (38–49 d) (Shah et al., 2014).

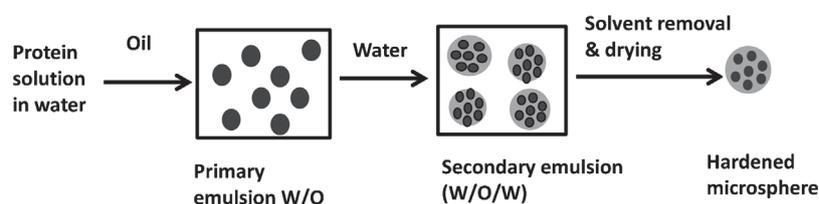


Fig. 6. Double emulsion solvent evaporation method for microencapsulation of proteins (Yeo et al., 2001).

Table 4
Various proteins encapsulated via double emulsion technique using different polymers.

Protein	Polymer	Encapsulation efficiency	Initial release in 1 day	Reference
Carbonic anhydrase	PLGA	45–48%	< 10%	(Lu and Park, 1995)
BSA	PLGA	56–85%	30–50%	(Igartua et al., 1997)
Recombinant human growth hormone (rhGH)	PLGA	40–66%	20%	(Cleland et al., 1997)
Urease	PLGA	17–55%	17–45%	(Sturesson and Carlfors, 2000)
Leuprolide	PLGA	13.4%	10%	(Woo et al., 2001)
Brain derived neurotrophic factor (BDNF)	PLGA, PLL, PEG	-	BDNF delivered for longer than 60 days	(Bertram et al., 2010)
Vascular endothelial growth factor (VEGF)	PLGA	46–60%	-4ng/mL -27ng/mL	(Karal-Yilmaz et al., 2011)
Alpha-1 antitrypsin (α 1AT)	PLGA	Varies with different copolymer ratio of PLGA	-	(Pirooznia et al., 2012)
Lysozyme	PEG/PBT block	89%	<5%	(Bezemer et al., 2000)

and exposure to large interface between aqueous and organic phases.

4.2.2. Encapsulation of nucleic acids

Nucleic acid is an important biomolecule in our body having many applications in gene therapy and diagnostics (Chen et al., 2009). However the major challenge is its effective and efficient delivery to the site of interest without its degradation. Different strategies have been adopted for this purpose such as, complexation of DNA with polycations (Möbus et al., 2012; Putnam, 2006) encapsulation within liposomes (Edwards and Baeumner, 2007; Tsumoto et al., 2001), encapsulation via double emulsion (Ibraheem et al., 2013) and polyelectrolyte capsules (Kreft et al., 2006; Shchukin et al., 2004). In modern drug delivery system, it is a challenge to administered hydrophilic biomolecules such as nucleic acids. Because of their water solubility, they tend to diffuse into continuous aqueous phase during emulsification process (Iqbal and Akhtar, 2013; Luo et al., 1999; Perez et al., 2001; Woodrow et al., 2009) which leads to low encapsulation efficiency of active moiety (Cun et al., 2011). Additionally, the negative charged biomolecules are unable to penetrate the cell membrane appropriately and also labile to degradation by different enzymes such as proteases and nucleases (Amidon et al., 1995; Li et al., 2006; Verdine and Walensky, 2007). In order to overcome these problems, researchers have focused on encapsulation of nucleic acid drugs inside biodegradable polymer, because it protecting the nucleic acid against degradation, facilitating its intracellular penetration, minimizing fluctuation in plasma concentration, enhancing the drug delivery to intended site and controlling the rate of drug release by diffusion (Lecomte et al., 2004; Singh et al., 2010; Streubel et al., 2006)

Various approaches have been used for encapsulation of nucleic acids including emulsion solvent evaporation, coacervation, spray drying and double emulsion techniques (Oster and Kissel, 2005; Tan and Danquah, 2012; Zhao et al., 2014). However double emulsion is technique is considered one of the most appropriate method, especially for encapsulation of hydrophilic nucleic acid drug inside biodegradable polymer (Jeffery et al., 1991, 1993; Mehta et al., 1996), this technique is reproducible and can be scale up for large batches (Jorgensen and Nielson, 2009). Typically in this method, the aqueous solution of nucleic acid is mixed with a solution of polymer dissolved in organic solvent by using sonication or homogenization techniques (Ducheyne et al., 2011). Beside several advantages, double emulsion process has a drawback of shear force used for homogenization of nucleic acid's solution in the organic phase. Which can damage the integrity of biomolecules, thus leads to loss of its biological activity. Moreover, the organic solvent can adversely affect the structure of nucleic acid during homogenization. This damage can be minimized by condensation of nucleic acid with cationic polymers, in order to reduce its size and maintain its supercoiling structure, and thus

preserve its biological activity (Ducheyne et al., 2011). It has been established that during second step of double emulsion emulsification, the applied shearing force disrupts the primary emulsion droplets, thus the inner aqueous phase containing nucleic acid mixed with outer aqueous phase, allowing diffusion of nucleic acid into outer aqueous phase. Which may leads to poor loading efficiency, in systems with low stability of primary emulsions (Jorgensen and Nielson, 2009).

The ratio of aqueous phase to oil phase has a significant effect on double emulsion stability. It has been reported that, an emulsion with ratio of 1:10 (water: oil) is more stable than ratio of 1:5 (water:oil) (Mohamed and van der Walle, 2006). Similarly, the emulsion of smaller particle size can be prepared with water: oil ratio of 1:30 as compared to ratio of 1:10 (water:oil), however beyond water:oil ratio of 1:20 a decrease in encapsulation efficiency has also been observed, thus water: oil ratio in between 1:10 and 1: 20 are frequently used (Hsu et al., 1999).

For encapsulation of nucleic acid, both homogenization and sonication process can be used, which results in almost same percentage encapsulation efficiency. Though, homogenization is the most commonly used technique. In case of sonication technique, there are concerns regarding scale up of process to produce large batches and stability of larger biomolecules (Hsu et al., 1999). The particle size decreases with an increase in homogenization speed (Díez and Tros de Ilarduya, 2006). However, this is not ideal choice to decrease the particle size, because vigorous homogenization in second step of emulsification can disrupt the primary emulsion droplet and consequently, leakage of nucleic acid into the outer aqueous phase may occur (Jorgensen and Nielson, 2009). Encapsulation efficiency of nucleic acid can be further enhanced by addition calcium phosphate to inner aqueous phase of double emulsion. Dördelmann et al. (2014) reported that, by addition of calcium phosphate to inner aqueous phase of double emulsion, the encapsulation efficiency of siRNA and DNA was increase by 37% and 52% respectively, compared to nanoparticle without calcium phosphate (Fig. 7). They obtained nucleic acid loaded PLGA nanoparticles with a diameter of 200 nm and zeta potential of -26 mV, prepared by a double emulsion solvent evaporation technique and stabilized with PVA. The addition of polyethyleneimine has changed the zeta potential to $+30$ mV, thus facilitating the cellular uptake of these particles.

4.2.3. Encapsulation of miscellaneous biopharmaceuticals

Nanoparticles encapsulating enzymes prepared via w/o or w/o/w emulsifications are quite susceptible to denaturation, aggregation, oxidation, cleavage, especially at the aqueous phase solvent interface. This obstacle has been overcome by the addition of stabilizers like carriers proteins (e.g. albumin), surfactants during primary emulsification or by adding molecules like mannitol, trehalose to the protein phase. Three model enzymes i.e. L-asparaginase, catalase and glucose oxidase were encapsulated in

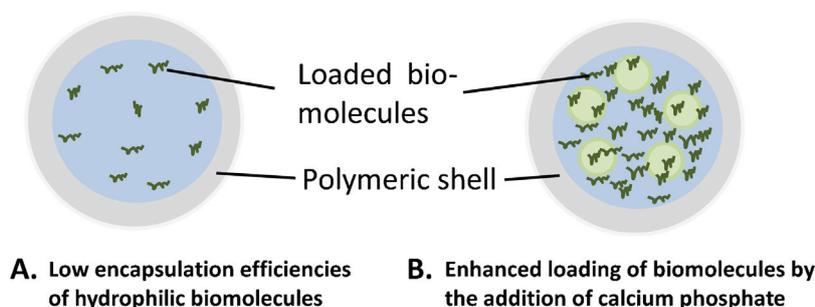


Fig. 7. Schematic illustration of the improved loading efficiency of nucleic acids by the addition of calcium phosphate nanoparticles. (A) PLGA nanoparticles, (B) Calcium phosphate-PLGA nanoparticles (Dördelmann et al., 2014).

poly(3-hydroxybutyrate-co-3-hydroxyvalerate) via w/o/w emulsification (Baran et al., 2002). The enzyme activity was increased upon usage of low molecular weight PHBV and adjusting second aqueous phase to isoelectric point of proteins enhanced the encapsulation yields of catalase and L-asparaginase. L-asparaginase was also encapsulated in PLG nanospheres via w/o/w emulsification by Gasper et al. (1998). They investigated the influence of molecular weight of copolymer and presence of carboxyl-end groups in copolymer chains on the physicochemical and in vitro release characteristics of the nanoparticles. Particles having higher molecular weight PLG displayed larger sizes, higher loading and slower release rates than particles made from low molecular weight PLG. Moreover, nanoparticles made of PLG with free carboxyl-end groups showed high protein loading (4.86%) and continuous delivery of active for about 20 days. α -Chymotrypsin (proteolytic enzyme) was encapsulated into PLGA microparticles by w/o/w double emulsion technique (Pérez-Rodríguez et al., 2003). It was observed that interface induced protein aggregation and inactivation could be reduced by co-dissolving it with maltose and poly ethylene glycol in the primary aqueous phase. (Liu et al., 2005b) loaded lysozyme into PLA microparticles by combining Shirasu porous glass membrane (SPG) emulsification and w/o/w double emulsification techniques. They observed that SPG gave higher encapsulation efficiency than stirring method. In another work, lysozyme were loaded into PLGA microparticles via the same w/o/w double emulsion technique to find out the effect of emulsification of lysozyme solutions with methylene chloride on the structural integrity and recovery of active (van de Weert et al., 2000). Another enzyme Staphylokinase variant K35R (DGR) was loaded into PLGA microparticles via double emulsion technique (He et al., 2006). Results showed that encapsulation efficiency was enhanced from 7% to 78% upon coencapsulation of 2% PVA and introduction of 2.5% NaCl into the external aqueous phase of the w/o/w emulsion.

Double emulsion technique has also been applied to encapsulation of antibodies. Poor stability and low efficiency are major hurdles in the formulation of engineered monoclonal antibodies (mAbs) for different therapeutic uses, which may be severer for application to encapsulation into nanoparticles. Son et al. (2009)

investigated the formulation and stabilizing conditions for encapsulation of mAb (3D8 scFv) into PLGA nanoparticles via double emulsion. It was concluded that mannitol was the most suitable stabilizer to retain stability and activity of 3D8 scFv. Immunoglobulin G is an antibody contributing to 75% of serum immunoglobulin in humans and it provides protection to fetus in uterus. This antibody was encapsulated into PLGA microparticles via s/o/w double emulsion technique (Wang et al., 2004). They investigated the stabilizing effects of different excipients during the period of protein atomization by spray freeze drying and subsequent encapsulation into the particles. They observed that double emulsion solvent evaporation process inactivated approximately 80% of the total IgG. More recently another monoclonal antibody Anti-Annexin A2 (AnxA2) was encapsulated via w/o/w emulsion into microparticles (Gdowski et al., 2015). Nanoparticles were monodispersed, 250 nm in size and had encapsulation efficiency of 18.7%. Particles exhibited sustained release and maintained their functionality upon release. In one study, malarial antigen SPf66 was encapsulated into PLGA microparticles via w/o/w emulsification (Igartua et al., 2008b). They observed the effect of gamma-irradiation on the biopharmaceutical properties of the particles and found out that the irradiation exposure did not affect the integrity of SPf66. Moreover, in vivo activity of malarial antigen was retained for week 27. Wei et al. (2008) used w/o/w emulsification and premix membrane emulsification to encapsulate hepatitis B surface antigen. They observed that usage of diblock copolymer PLA-mPEG provided higher encapsulation efficiency as compared to using triblock copolymer PLA-PEG-PLA. Double emulsion solvent evaporation technique has been applied to encapsulation of other miscellaneous biopharmaceuticals as summarized in Table 5.

5. Theranostic applications

The development of agents for simultaneous diagnosis and treatment of various diseases has gone through extensive investigations in recent years for biomedical applications. These multifunctional theranostic agents allow for feedback mechanism to establish the localization of drug, release of drug, disease phase

Table 5
Various biopharmaceuticals encapsulated via double emulsion technique.

Biopharmaceutical	Polymer	Type of emulsion	Observation	Reference
Recombinant human erythro-poietin (rhEPO)	PLGA	W/O/W	Reduction of rhEPO aggregates by using cyclodextrins, arginine, BSA	(Morlock et al., 1997)
Tetanus toxoid	PLA	W/O/W	Max. entrapment efficiency when serum albumin, sucrose, sodium bicarbonate used in internal aqueous phase and sucrose in external aq. phase	(Katara and Panda, 2006)
Luteinizing hormone releasing hormone (LHRH) antagonist	PLGA	double emulsion single solvent evaporation (S/O/O)	E.E influenced by LA/GA ratio of PLGA, salt conc., solvent mixtures and preparation method	(Du et al., 2006)
LHRH antagonist (ornitide acetate)	PLA and PLGA	W/O/W	Peptide binding to microparticles dependent on pH; higher in phosphate buffer pH 7.4 than in acetate buffer pH 4.0	(Kostanski et al., 2000)
Glial cell-line derived neurotrophic factor (GDNF)	PLGA	W/O/W	Diameter between 8 and 30 μ m & E.E 50-100%	(Garbayo et al., 2008)
Exenatide	Polysaccharide-microparticle (PAMs)	W/O/W	E.E 60-90% Sustained release for 21 days	(Yang et al., 2009)
Exenatide	PLGA	W/O/W	Significant hypoglycemic activity within 1 month	(Liu et al., 2010)
Glucagon like peptide-1 (GLP-1)	PLGA	W/O/W	Optimized formulation achieved, controlled release <i>in vivo</i> for 28 days	(Yin et al., 2008)
Bovine Hb	PELA	W/O/W	E.E 90%, Size 3-5 μ m	(Qiu et al., 2004)
Basic fibroblast growth factor (bFGF)	PLGA	W/O/W	In vitro release from particles 72-47% in 11 days, bioactivity of bFGF preserved	(Shen et al., 2008)
Bovine lactoferrin	Poloxamer	W/O/W	Developed an optimized nanoemulsion formulation for mouth wash elixir	(Balcão et al., 2013)

and efficacy of the treatment (Ahsan et al., 2013; Eissa, 2014; McCarthy, 2010). At present most of the researches in theranostic have been focused primarily on oncology, since, cancer is one of the most fatal diseases and currently the main cause of morbidity and mortality. It is assumed that a by this approach cancer can be managed timely with reduced cost (Ahmed et al., 2012a). Though, double emulsion technique has been frequently used for encapsulation of proteins, peptides hydrophilic and hydrophobic drugs, however limited work has been done in encapsulation of diagnostic agent and therapeutic agent simultaneously via double emulsification process, to the best of our knowledge. Some of the published studies are summarized here.

Theranostic particles fabricated from different polymers have been used for better diagnosis and improved drug delivery, by several groups of researchers such as Yang et al. (2010) fabricated polymer wormlike vesicles loaded with loaded with superparamagnetic iron oxide (SPIO) nanoparticles (as MRI contrast agent) and anticancer drug doxorubicin (DOX) for targeted cancer therapy and MR imaging. The calculated SPIO nanoparticles (NPs) loading content in the vesicles was about 48.0 wt%, while The DOX loading level for these vesicles was about 9.0 wt%. This type of nanocarriers has the advantage that the SPIO and DOX loading amount can be easily changed by adjusting various process parameter during preparation and by changing the chemical structure of the triblock copolymers. This theranostic vesicle nanocarrier system was established to be very efficient, which can provide controlled and targeted drug delivery to the tumor as well as it can be used as an efficient MRI contrast agent, thus providing targeted cancer therapy and diagnosis simultaneously. Similarly, Park et al. (2012) incorporated dexamethasone in PLGA nanoparticles and drug-loaded particles were then complexed with

(PEI)/siRNA. Co-delivery approach of siRNA and dexamethasone was used in the treatment of rheumatoid arthritis. Almost over 50% of dexamethasone was loaded onto PLGA NPs. And, Ngaboni Okassa et al. (2005) prepared biodegradable sub-micron PLGA particles by double emulsion technique, loaded with magnetite/maghemite nanoparticles (Mag NPs) for intravenous drug targeting. Mag NPs were incorporated in the inner aqueous phase and the final particle size was found to be 268–327 nm with the magnetite entrapment efficiency up to 60%. Several bio-molecules have been also encapsulated via double emulsification for theranostic applications such as Ibraheem et al. (2014b) entrapped human albumin protein into biodegradable polymer (PCL) along with fluorescence active molecule i.e. FITC-BSA (albumin-fluorescein isothiocyanate labeled bovine serum albumin). They incorporated human albumin into inner aqueous phase and emulsified in DCM solution containing polycaprolactone polymer using ultra-turrax hominization. The encapsulation efficiency of albumin was sufficiently high (up to 95%) and albumin-loaded particle was about 340 nm. And the confocal microscopy revealed that all the loaded molecules were evenly distributed in the polycaprolactone matrix.

Another study performed by Ahmed et al. (2012b) demonstrates the effective loading of iron oxide and a hydrophilic model drug (stilbene) into polymeric submicron particles for in vivo theranostics. They incorporated the hydrophilic model drug i.e stilbene in the inner aqueous phase (W1) of double emulsion and homogenized with polycaprolactone dissolved in DCM (oil phase). Subsequently the primary emulsion was dispersed in 0.5% PVA solution (outer aqueous phase). Additionally, organic iron oxide nanoparticles were incorporated in oil phase to be used as MRI contrast agent for imaging. The results showed that iron

Table 6
Theranostics particles applications and composition of various components used in their preparation.

Actives/probes (in the inner aqueous phase)	Solvent	Polymer	Stabilizer	Particle Size	Objectives	References
MagNP	Methylene chloride	PLGA	PVA 0.3% (w/v)	268-327 nm	Encapsulation of magnetic particles for intravenous drug targeting.	(Ngaboni Okassa et al., 2005)
Iron oxide, stilbene	DCM	PCL	PVA 0.5%	300-400 nm	Preparation of hybrid particles for in vivo theranostics	(Ahmed et al., 2012b)
HSA, FITC-BSA	DCM	PCL	PVA	340 nm	Albumin encapsulation for in vivo imaging applications	(Ibraheem et al., 2014b)
Dexamethasone	Methylene chloride	PLGA	PVA 1% (w/v)	90 nm	Treatment of rheumatoid arthritis	(Park et al., 2012)
SPIO	Chloroform	FA-PEG ₁₁₄ -PLA _x -PEG ₄₆ -acrylate	PVA 0.1%	100-200 nm	Cancer diagnosis and therapy	(Yang et al., 2010)
DOX	PTX and SPIO in chloroform	PMAA	PVA 2%	174 nm	Dual targeting and co-delivery of hydrophilic and hydrophobic drugs	(Chiang et al., 2014)
Carboplatin and GMO-GMPs	Paclitaxel and PLGA in DCM	PLGA	PVA 5%	100 nm	co-delivery of hydrophilic and hydrophobic anticancer drugs and MRI imaging	(Singh et al., 2011)
DOX	Magnetite in chloroform	chitosan	Tween 80, 4%	100-150 nm	Doxorubicin-loaded MNPs for drug delivery	(Balan et al., 2015)
QDs and cetux-imab antibody (targeting agent)	Camptothecin and QDs in acetone	PLGA	PEG 0.1%	178 nm	Multifunctional nanoparticles with in vitro targeted imaging ability and delivery of camptothecin.	(Deepagan et al., 2012)
DOX, VER and MNPs-CA	DCM	PLGA	PVA 5%	130-140 nm	Polymeric MNPs for targeted dual-drug delivery for cancer therapy	(Shen et al., 2013)
Adriamycin or siRNA	Chloroform	mPEG-PLGA-b-PLL	pluronic F-68	169-151 nm	copolymer carrier for adriamycin and siRNA delivery	(Liu et al., 2012)
siRNA	Chloroform	mPEG-PLA	PVA 1%	170-200 nm,	siRNA encapsulation and delivery for cancer therapy	(Yang et al., 2011)
FNPs	Dichloromethane (DCM)	PCL	PVA 0.5%	342-375 nm	FNPs encapsulation as a model contrast agent	(Iqbal et al., 2015)

MagNP = Magnetite/maghemite nanoparticles, DOX= Doxorubicin, SPIO = Superparamagnetic iron oxide, FA-PEG₁₁₄-PLA_x-PEG₄₆-acrylate= Folate-poly(ethylene glycol)-poly(D,L-lactide)-poly(ethylene glycol)-acrylate, HSA= Human serum albumin FITC-BSA= Albumin-Fluorescein isothiocyanate conjugate bovine serum albumin, PTX= Paclitaxel, PMAA= Poly(methacrylic acid), GMO-GMPs= Glyceryl monooleate magnetic nanoparticles, QDs=Quantum dots (tracking agent), PEG= Polyethylene glycol, MNPs-CA= citric acid modified MNPs, VER= Verapamil, mPEG-PLGA-b-PLL= monomethoxy (polyethylene glycol)-poly (D,L-lactide-co-glycolide)-poly (L-lysine), mPEG-PLA= Poly(ethylene glycol)-b -poly(D,L -lactide), FNPs= Fluorescent nanoparticles.

oxide particles were properly encapsulated by biodegradable polymeric shell of polycaprolactone and the average size of loaded particles were about 300–400 nm. Such hybrid particles owning the dual properties of diagnosis and therapy can be used for co-delivery of multimodal diagnostic agents and a variety of drugs in treatment of fatal disease such as cancer. Some examples of theranostic particle encapsulated via double emulsion process are listed in Table 6.

The double emulsion technique has also been used for particle preparation with dual targeting ability (magnetic and molecular targeting) and co-delivery of hydrophilic and hydrophobic drugs simultaneously. Chiang et al. (2014) encapsulated hydrophilic doxorubicin (DOX) and hydrophobic paclitaxel (PTX) in nanoparticles along with superparamagnetic iron oxide (SPIO), and these particles were further conjugated with trastuzumab (monoclonal antibody) in order to specifically target the HER-2 positive cancer cells. DOX was added into the inner hydrophilic phase I (2 wt% PVA solution) while PTX was added to the hydrophobic phase II (chloroform containing 5 mg SPIO) and sonicated to form the primary emulsion (W/O). The primary emulsion homogenized using sonication with hydrophobic phase III to form a double emulsion (W/O/W). Afterward, organic solvent was removed and particles were collected by centrifugation at 9000 rpm and redispersed in DI-water. The average particles were found to be 174 nm, while the encapsulation efficiency of PTX and DOX in PTX–DOX–nanoparticles were 91% and 72% respectively. They reported that HER-2 positive antigens showed excellent binding ability toward the trastuzumab-conjugated nanoparticles, thus, demonstrating the targeting ability of these nanoparticles. When the magnetic field was applied on the tumor site, there was an increase in amount of particles accumulation. By using magnetic targeting, about 25.8% of PTX and 20% of DOX from the initial dose were accumulated in the tumor for trastuzumab–PTX–DOX–nanoparticles, which was 2.47-fold higher compared to the particle without trastuzumab conjugation. Drug delivery using dual targeting may provide a more specific accumulation of drug-loaded particles at the desired tumor site, which can contribute to lower drug doses and thus reduce the side effects of cancer therapy. Shen et al. (2013) prepared PLGA nanoparticles by using double emulsion technique. Two hydrophilic drugs, doxorubicin and verapamil (VER) were initially combined with chitosan shell coated on magnetic nanoparticles (MNPs), which were then incorporated into PLGA nanoparticles to be used for cancer therapy through dual-drug delivery system (DDDS). Additionally, a tumor-targeting ligand was also conjugated onto the end carboxyl groups on the PLGA-NPs. From morphological observations, drug loaded NPs were found to be spherical and with a narrow range of distribution of the particle sizes (about 130–140 nm). While, the entrapment efficiencies of DOX and VER were about 74.8 and 53.2 wt% respectively. In vitro drug release behaviors were evaluated under dialysis condition at 37 °C in a simulative normal body fluid (50 mM PBS, pH 7.4) and an acidic environment (50 mM PBS, pH 5.3). The cumulative release of DOX and VER at 37 °C was 29.0 and 41.3% respectively at pH 5.3; and 25.0 and 34.5% respectively at pH 7.4. It was demonstrated that the intelligent DDDS could significantly inhibit both, the growth of tumor as well as DOX-induced cardiotoxicity in mice, and potentially offer an approach for safe cancer therapy.

6. Conclusion

Double emulsions have many potential applications. In biomedical field, it can be used in drug delivery systems for encapsulation of both hydrophobic as well as hydrophilic active medicaments, cosmetics, foods, imaging agents and

other high value products. In modern drug delivery system, it is a challenge to administered hydrophilic biomolecules such as nucleic acids and proteins. Because of their water solubility, they tend to diffuse into continuous aqueous phase during emulsification process. However, the double emulsion technique has been extensively used for the encapsulation highly water soluble compounds including protein and peptides. Encapsulation of these compounds prevents its degradation, control the rate and extent of release and enhances the loading efficiency. Nanoparticles made by double emulsion solvent evaporation method are excellent carriers for delivery of hydrophilic molecules such as proteins, peptides and variety of pharmaceutical and biopharmaceutical compounds. It offers stability and controlled release of encapsulated molecules. Beside several advantages, double emulsion process has a drawback of shear force used for homogenization of inner aqueous phase containing biomolecules in the organic phase. Which can damage the integrity of biomolecules, thus leads to loss of its biological activity. Moreover, the organic solvent can adversely affect the structure of biomolecules during homogenization. This damage can be minimized by condensation of biomolecules with cationic polymers, in order to reduce its size and preserve its biological activity. Consequently, the research works reported in this review give information about different polymers, stabilizers and solvents that can be used in this technique, the effects of process parameters and the application of this technique in encapsulation of various pharmaceutical and biopharmaceuticals.

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II.4. Textile in therapy and cosmetic: from characterization to surface functionalization

Summary

Textiles are used by humans in different ways since the beginning of mankind. Apart from being used to cover human skin and act as means of fashion and design, textiles also find applications in many diverse and important fields relating to humans. Fabrics are used as civil engineering textiles, marine textiles, agricultural textiles, forestry textiles, filtration textiles etc. Textiles also hold an important place in medical field. They are being used in medical gauzes, bandages, sutures, patches and repair fabrics.

Since textiles are used extensively by mankind for different functions, more and more research has been devoted to find innovative ways to develop advanced functionalized fabrics suitable for drug and cosmetic agent delivery. The concept of using fabrics for drug delivery has been developed since the end of last century. The need to use textiles for drug delivery arose because of some problems encountered with conventional drug delivery systems. Certain drugs cause irritation in gastric lining or are metabolized before being delivered to the target organ or tissue. On the other hand, in case of children, elderly patients or unconscious patients, the patient compliance is quite low and delivery of medicine in more frequent doses is rather difficult. To solve all these problems, delivery of drugs via fabrics or textiles is quite a logical option. Though textiles are extremely versatile, still not all drug molecules are suitable for application on textiles.

Cosmetotextiles is an interesting field which is extremely explored nowadays. In 1999, a cosmetotextile offering moisturizing and sliming effect was introduced in the French market by a company named DIM. Cosmetotextile functionalization is usually brought about by encapsulation of cosmetic agent in nano-objects and then impregnation or grafting of the textiles with these particles. Variety of cleaning, perfuming and protecting agents have been loaded onto textiles for cosmetic functions.

To bring about the desired functionalization of the textile, a good understanding of the properties of fabric is the foremost essential requirement. Fiber is a basic unit of textile and assembly of fibers forms yarn whose assembly in turn leads to formation of textile. There are different types of fibers depending upon the source from which they are obtained. There are four basic fiber types namely; natural, artificial, synthetic and technical. Each type of fiber has its own particular application. Characterization of textile is dependent on the properties of yarn and fiber. It usually includes characterization of diameter, relative density and moisture uptake.

Desired functionalization or surface modification of the fabric/textile is not possible without prior sound knowledge of the surface characterization techniques for textiles. Using different characterization techniques, the surface of textile is characterized before and after the functionalization in order to verify the presence of correct modification. There are several techniques available for the characterization of textile surface e.g. optical, microscopic and spectroscopic techniques. It is very common to use scanning electron microscopy and transmission electron microscopy to view the surface morphology of the fabrics as well as the cross-sections and interfacial phenomenon in macroscopic detail. Small variations in the surface, surface roughness or film thickness are analyzed by using profilometry and atomic

force microscopy. FTIR and x-ray photoelectron microscopy are other techniques used for analyzing the surface of textiles.

Natural fabrics are normally not suitable for any particular application and require surface modifications to achieve this objective. Primarily, there are two main types of surface functionalization; chemical and physical. In physical method, desired material is deposited onto textile surface without using any chemical agents. Studies show that techniques like physical vapor deposition, chemical vapor deposition, sol-gel process, electrostatic interactions and electrospinning have been used diversely to modify the surfaces of textiles. Literature shows that metal/metal nanoparticles based functionalization is the most commonly performed textile functionalization. Apart from this, polymeric nanoparticles and cyclodextrins have been used to functionalize textiles. Generally, active agents are encapsulated inside polymer based particles which are then directly introduced onto textile surfaces. This mini review also quotes examples of works relating to textile functionalization via such active carrying polymeric nanoparticles.

Textile in therapy and cosmetic: from characterization to surface functionalization

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Abstract

This mini-review aims to provide an overview of textile surface functionalization and characterization. Various methods to characterize the surfaces of textile are discussed briefly. The prime focus of the review was to throw light on commonly used methods for the modification of surface of the textiles so that they are well suited for applications in fields of therapy and cosmetics. The role of polymeric particles, capsules, nanoparticles and cyclodextrins in surface functionalization is explained in detail.

Keywords

Textiles, surface modification, characterization, cyclodextrins, drug release, self-cleaning, cosmeto-textile, polymeric particles, nanoparticles

Introduction

Textiles have been produced since the dawn of mankind and used to protect the human body from the surrounding environment. Over the years, textiles have become a very important commodity of human lives and with time a revolutionary improvement has occurred in the different properties of textiles like quality and wearing comfort. Textile materials with their particular characteristics are often used as agriculture and marine materials, filtration materials, civil engineering textiles, agriculture and forestry textiles, textiles for vehicles and for many other applications. With the passage of time, textiles have also made their way into the medical field e.g. artificial aortas and bandages. A fast growth in the medical textile industry was made possible by the hard research and development of new textile materials and ingenious innovations in biomedical products. Textiles applications in medical sutures, gauzes, bandages, repair and functional fabrics, artificial prostheses represents a bulk fraction of textile materials market and are becoming to a greater extent intricate for the creation of integrated products.

The concept of advanced functional textile drug delivery systems was not matured until the termination of last millennium. Here it is important to highlight that why it was necessary to explore and develop textiles in drug delivery systems. In certain cases as in oral drug delivery, drugs are absorbed in the stomach or intestinal tract and on metabolism they might lose their activity before delivering the desired effect to the target organ. Hence, the need to deliver the drug in higher doses would arise which could ultimately lead to greater risk to side effects or toxic effects associated with the drugs. Drug delivery via skin bypasses the metabolism in the liver and hence makes it possible to deliver particular drug in lower doses. Moreover, there are situations where oral delivery is not very efficient e.g. in children and elderly people, patients with swallowing complications or patients with dementia. Here again, transdermal and *in-vivo* drug delivery systems are an efficient alternative. Such systems are preferable for prolonged drug delivery where daily intake of pills or injections would adversely affect the

patient compliance. Though such delivery systems are good at reducing the dosage and dosing frequency but not all drugs are applicable in such drug delivery systems. Textiles are immensely versatile combining different materials and structures. Their properties and functionalities are affected by physical and chemical characteristics on micro-, nano-, meso- and macroscopic length scales (Fig.1).

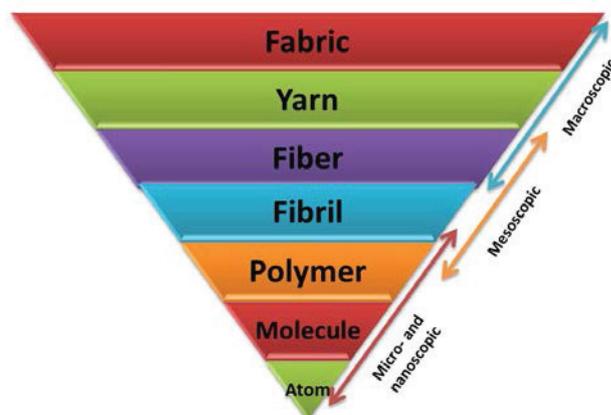


Fig. (1). Different scales of length in textile.

On macroscopic level, though dependent on a actual fabrication process, textiles are nearly ‘open’ and ‘loose’, permeable structures, with absorptive capacities. The open permeable structure and large surface area makes textiles suitable basis for *in-* and *ex-vivo* drug delivery. It is also logical to employ textiles for *ex-vivo* drug delivery in professional and private situations due to the fact that we all are used to wearing textiles in our daily lives. Due to immense progress in nanotechnology, supramolecular chemistry, nanobiotechnology and polymer science and technology, high performance textile drug delivery technologies have been introduced recently [1].

A cosmetotextile is a textile containing a cosmetic preparation primarily for dermatology applications such as moisturizing, slimming or anti stretch mark [2]. According to Textile industry and Clothing Standards Agency (BNITH), cosmetotextile is “a textile item containing a substance or mixture that releases active molecules when in contact with the human body (i.e. on the epidermis, hair and external sexual organs, with the exclusive or primary intention of cleaning, perfuming, changing aspect, protecting, and helping to maintain or correct body odors)”. This particular functionalization of textile is brought about by the physical immobilization or impregnation of the fibers with nano-objects containing active agents. Such nano-objects are mostly prepared by variety of encapsulation processes, which encapsulate active molecules within polymeric matrix (particles or capsules) fixed on the fiber. Physical degradation of the polymeric matrix or shell or molecular diffusion results in release of the active agents. Impregnated and/or chemically grafted textiles have been developed since 1990 e.g. impregnated wipes for hygiene, bactericidal curtains, bed linen releasing essential oils and perfumes [3-4]. In 1999, a company named DIM produced a cosmetotextile for the first time in French market, which offered moisturizing, and slimming tights. Similarly, in 2002, F IRTEX L.L.C. introduced a patent textile functionalization technique by utilizing nanoparticles prepared from starch whose sizes allowed them to enter natural fibers.

This review begins with describing textile properties and proceeds to explain different methods to characterize textile surfaces. The prime focus of the review is to highlight textile surface functionalization techniques. The review also focuses on advanced functional drug delivery systems like textile bearing polymeric particles and cyclodextrins [5-11] and (biodegradable) nano-fibers containing drugs produced by electrospinning [12-13].

Textile properties

A sound knowledge about the textile is very crucial for the development of therapeutic textile or cosmetotextile. Textile is a complex assembly of yarn and yarn can be defined as assemblage of fibers tightly bound together. There are different types of fibers depending upon their source of origin namely: natural, artificial, synthetic and technical fibers [14] (Fig. 2). Natural fibers are obtained from either plants or animals. These fibers find applications in the field of clothes, insulation, automobiles and dressings. The dissolution of natural fibers gives artificial fibers. The resultant fiber solution is spun by extrusion to get uninterrupted filament, which is finally then cut into fibers. Viscose is a typically interesting example of this category as it can be functionalized with particles or active molecules directly in the solution before spinning. Extrusion/spinning of synthetic polymers is used to obtain synthetic fibers like polyesters and polyamides [15-16]. Technical fibers are high performance fibers which are developed for specific requirements like high resistance to traction, temperature and conductivity. All the fibers can be employed for traditional usage except these technical fibers which are only specifically used for technical purposes. Textiles are characterized by the properties of fibers and yarns which include diameter, relative density, strength and moisture uptake.

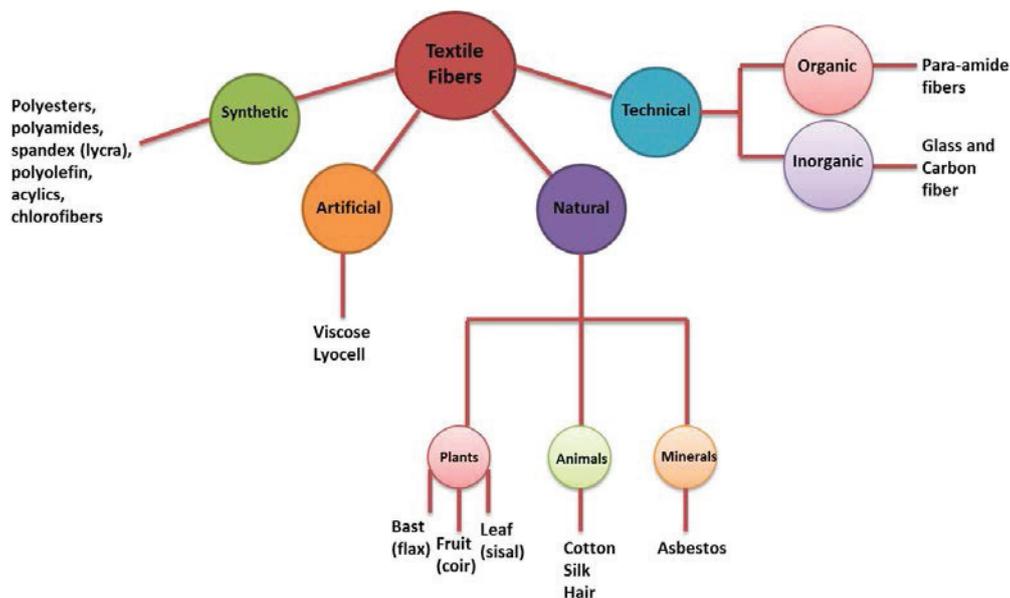


Fig. (2). Different types of textile fibers.

Textile surface characterization techniques

Various disciplines are observed within the field of textile technology e.g. the structure and properties of textiles fibers, techniques to assemble fibers into fabrics, surface modification of fibers and the end use of fabrics. Characterization of textiles by surface analysis is perceived to be an imperative process for better understanding and optimization of surface modification of textiles. Surface modification is of profound importance because it helps to enhance many properties like softness, wettability and adhesion. Textiles can also be functionalized via surface modification. Various techniques are employed to characterize the textile surface. These techniques include optical, microscopic, spectroscopic, thermodynamic and mechanical analyses.

Scanning electron microscopy (SEM)

It is frequently used to examine the surface morphology and structures of textile surfaces [17]. Fig. 3 shows how SEM is used to perform the structural characterization of textiles [18]. Different structures of electrospun nanofibers were observed in this SEM analysis.

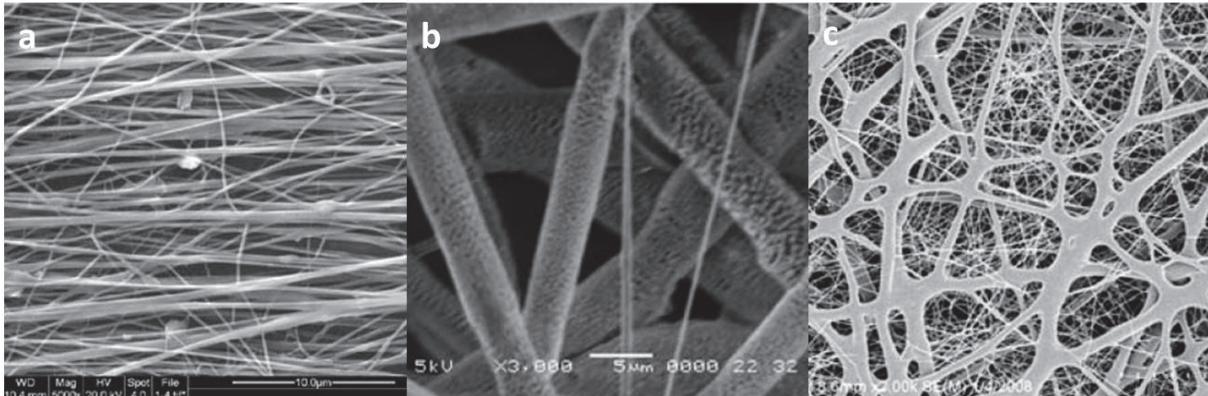


Fig. (3). SEM analysis of structured nanofibers: a) aligned; b) porous; c) gradient [18].

In another study, SEM was used to observe the surface evolution of electrospun polyacrylonitrile (PAN) nanofibers modified by sol-gel coating of zinc oxide [19]. The SEM photos (Fig. 4) revealed the evolution of surface nanostructures during the treatment process.

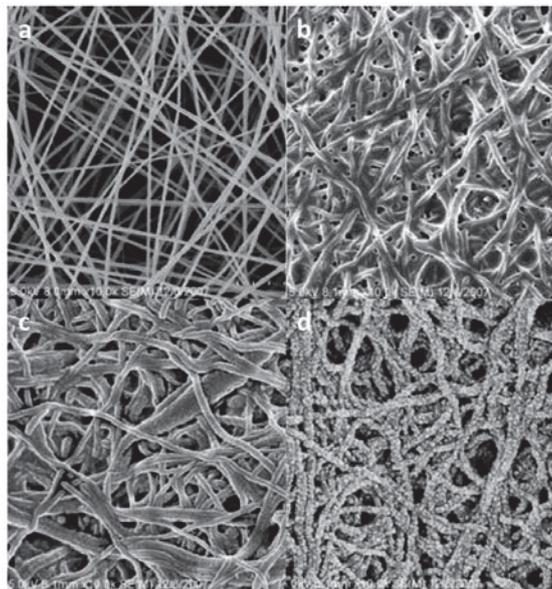


Fig. (4). PAN nanofibers modified by sol-gel coating: a) PAN nanofibers; b) ZnO-coated PAN nanofibers; c) pre-oxidized ZnO-coated PAN nanofibers; d) carbonized ZnO-coated PAN nanofibers [19].

Transmission electron microscopy (TEM)

TEM is primarily used to examine fiber surface morphology, cross-sections and interfacial phenomenon in microscopic detail. Li et al. (2008) used TEM to study the formation of electrospun polyvinyl acetate (PVAc) (Fig. 5a). A PVAc nanofibers and a barrel shaped bead were observed which indicated the formation mechanism of jet stretching in electrospinning. TEM analysis also confirmed the formation of composite nanofibers and the distribution of organically modified montmorillonite (O-MMT) nanoparticles in a polyamide 6 (PA6) nanofibers matrix [20] (Fig. 5b). In another study, TEM analysis was used to study the texture, structure and chemistry of boron nitride (BN) [21].

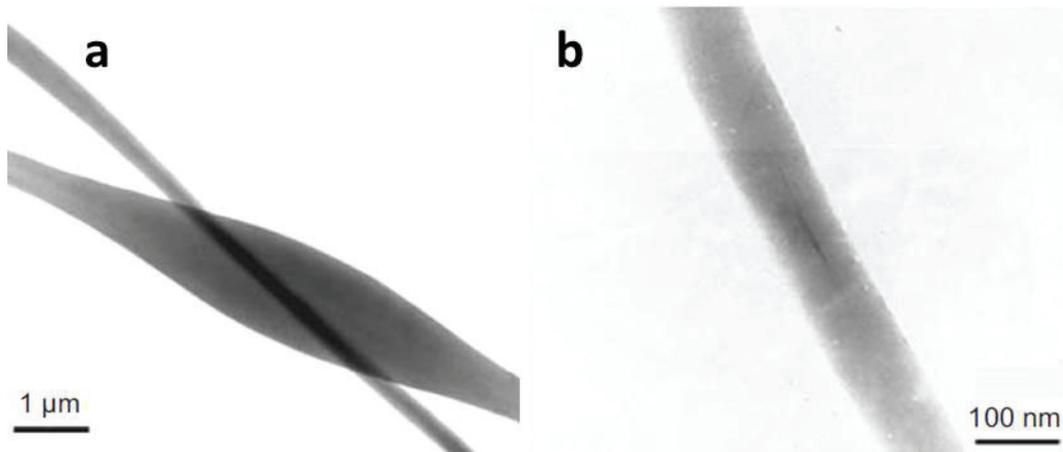


Fig. (5). TEM images: a) PVAc nanofibers; b) PA6-O-MMT nanofibers [20].

Profilometry and atomic force microscopy

Profilometry and atomic force microscopy (AFM) are used to measure film thickness and surface roughness. Profilometry measures small surface variations. In this technique, a diamond tip is brought in contact with the sample and moved laterally for a specified distance with a controlled contact force. AFM when used in different modes presents a novel and dynamic approach to the examination of nanostructures of textile materials. AFM's working depends on a sharp tip (part of a cantilever) which is scanned over a surface to be examined. On scanning, the forces between the surface and the tip cause bending of the cantilever in vertical direction and by measurement of power spectral density of deflection, an image of the surface with atomic resolution can be produced. The forces produced depend on the type of interaction between the tip and the surface under examination. Wei et al. (2007) examined a change in surface morphology of polyethylene terephthalate (PET) fibers caused by plasma treatment. The surface of PET fiber was observed to be roughened after plasma treatment [22] (Fig. 6).

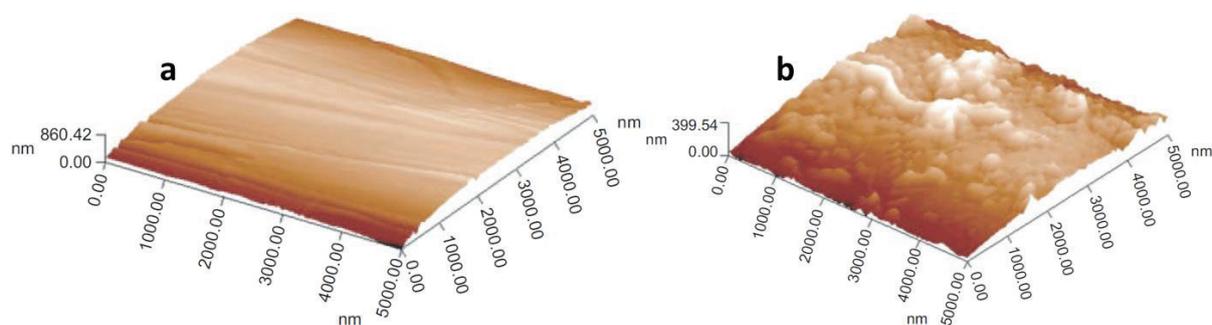


Fig. (6): The surface of PET fiber a) before and b) after plasma treatment [22].

Fourier Transform Infra-Red spectroscopy (FTIR)

FTIR is a qualitative and quantitative technique used to identify different compounds and to reveal molecular structure of a given sample. It relies on the fact that molecules have specific frequencies at which they rotate or vibrate. These frequencies of vibration are associated with particular bond type. Generally, a beam of infrared light is passed through the sample and a transmittance or absorbance spectrum is generated. FTIR find applications in textiles in areas like contamination analysis, identification of unknown materials, comparative analysis and identification of surface chemical structures. FTIR was used to determine the presence of microcapsules in textiles by Monllar et al. (2007) by specific vibration properties of microcapsules [23]. In another study, a PET fabric was modified to impart conductive

properties to the fabric through surface polymerization [24]. FTIR was used to confirm the successful attachment of polyaniline to the fabric. A significant increase in the resolution of FTIR spectroscopy has made it possible to identify contaminated fibers in a textile. Universal attenuated total reflectance-Fourier transform infrared method was used to analyze cotton contaminations as well as to separate sticky and non-sticky cotton [25].

X-ray photoelectron spectroscopy (XPS)

Photoelectron spectroscopy uses photo-ionization of the emitted photoelectrons to investigate the composition and electronic state of the surface part of a sample. XPS is widely used to identify the elemental composition of solid surfaces. Basically, a soft x-ray beam is used to measure the binding energies of electrons emitted by the ionization of atoms. The binding energies of the ejected electrons are characteristics of the elements present at the surface. In a study atmospheric plasma was used to modify fabrics and XPS was employed to identify the surface modifications occurred due to plasma treatment [26]. A hydrophilic polyester fiber was fabricated using photocatalytic reactions. XPS analysis showed that C-O and C=O groups were generated on the surface of polyester [27]. XPS have been used in combination with other technologies e.g. SEM to show the surface changes of wool fibers after plasma treatment [28].

Dynamic Contact angle

Measurement of contact angle helps to assess the inclination of a liquid to spread out on a surface. In this method, the angle between the drop of liquid and surface of substrate is measured. Surface energy of liquid or solid is calculated by using contact angle which helps to assess hydrophobic or hydrophilic behaviour. The method by which substrate /textile is functionalized directly effects the value of contact angle. Dynamic contact angle is mostly measured using the Wilhelmy technique [29]. This method measures the pull force or push force i.e. wetting force, in order to explain the contact angles.

Textile surface functionalization

The composition and structure of the surface of a textile is an integral component in determining a textile's performance for a particular application. Textile surface is certainly an interface or reaction region between the textile and the environment where textile is intended to be used (30). However, the surfaces of textiles without any modification are not suitable for any particular application e.g. for providing anti-static properties. In order to enhance this suitability and compatibility of a textile for the desired application, various textile surface modification techniques are employed. There are two major categories of surface modification techniques: physical and chemical. In chemical methods, usually either specific chemical agent is added or a control on the surface chemical environment is maintained. On the other hand, in physical techniques, mostly non-chemical ways are employed to deposit materials onto textile surface. Table 1 provides a brief summary of some the techniques used for the functionalization of textiles.

Techniques	Objective achieved	Textile	Coating material	References
Vacuum evaporation	Electromagnetic interference Shielding	Poly(ethylene terephthalate (PET) woven fabric	Silver (Ag) layer	Hong et al. (2001) [31]

Ion implantation	Shrinking of nanofibers	PVA nonwoven	Two new functional groups (N-C=O, C-N) introduced	Wong et al.(2006) [32]
	Wear loss and friction of fabric decreased	PET membrane fabric	Ti, W, Ti+N, Cr+N, C+N implanted	Öktem et al. (2008) [33]
Chemical grafting	High water absorbency of textile	Gray cotton	Acrylonitrile monomer grafted	Deo et Gotmare (1999) [34]
	Higher thermal stability Tensile strength increased	Wool fiber	Poly(benzyl methacrylate) grafted	Tsukada et al. (1997) [35]
	Water repellency, chemical/thermal stability, enhanced fiber adhesion	Polyester fiber	Perfluorooctyl-2 ethanol acrylic monomer grafted	Louati et al. (1999) [36]
	Increased chemical resistance and decreased water retention	Silk fibers	Graft copolymerization of methyl methacrylate and acrylamide	Ojah et Dolui (2007) [37]
Sputtering	Antibacterial properties achieved	PET fiber	Ag coating	Wei et al. (2004) [38]
	Light shielding effect	Polypropylene (PP) nonwovens	Cu and Ag coatings	Wei et al. (2007) [39]
	Antibacterial	PP fibers	Ag coatings	Wang et al. (2007) [40]
	Antibacterial and antifungal	SiO ₂ fabrics	Ag, Cu, Au, Pt, Pt/Rh coatings	Scholz et al. (2005) [41]
	Improved hydrophobic properties	Silk fabric	Polytetrafluoroethylene (PTFE) coating	Huang et al. (2007) [42]
	Electrical resistance of fabric decreased	PET nonwovens	Al films deposited	Deng et al. (2007) [43]
Radiation induced grafting	Antibacterial	Polyester fibers	Chitosan and collagen grafted	Jou et al. (2007) [44]
Light induced grafting	Water repellency imparted	Cotton fabric	UV curing of silicone and urethane acrylates	Ferrero et al. (2008) [45]
	Improved antiseptic properties	PET textile	Acrylamide, poly(ethylene glycol) methacrylate	Uchida et al. (1991) [46]

Chemical vapor deposition	Antimicrobial	Nylon fiber	Antimicrobial polymer	Martin et al. (2007) [47]
	Bactericidal and water repellent fabric	Cotton fabric	Hydrophobic polymer + N-alkyl-polyethylenimine layer	Lin et al. (2003) [48]
Plasma treatment	Hydrophobicity of surface enhanced	Cellulose	Fluorocarbon film	Balu et al. (2008) [49]
	Antibacterial	Polyethylene terephthalate (PET)	Carbon films	Wang et al. (2004) [50]
	Antibacterial	PET	Chitosan and quaternized chitosan	Huh et al. (2001) [51]
Sol-gel	Enhanced textile strength, barrier to aggressive environment	-	-	Textor et al. (2003) [52]
	Water repellent textile	Polyamide and polyester fabrics mixed with cotton	Silica sol deposited	Mahltig et al. (2003) [53]
	Antimicrobial	Cotton textile	Silica sol + silver nitrate solution	Xing et al. (2007) [54]
	Antimicrobial	Polyester/cotton fabrics	Silica coating with embedded biocides (Ag + cetyltrimethyl ammoniumbromide CTAB + octenidine)	Mahlting et al. (2004) [55]
	Self-cleaning	Cotton textile	Au/TiO ₂ films	Uddin et al. (2008) [56]
Pad-dry-cure	Photocatalytic & antimicrobial	Cotton & cotton/polyester blended fabrics	ZnO & Ag/ZnO nanocomposites	Ibanescu et al. (2014) [57]
Electrostatic interactions	Antibacterial, self-cleaning, improved hydrophilicity	Wool fabric	SiO ₂ , TiO ₂ , Ag nanoparticles	Mura et al. (2015) [58]
Electrospinning	Tetracycline hydrochloride delivered	Poly(lactic acid) (PLA), poly(ethylene-co-vinyl acetate) (PEVA) fibers	-	Kenawy et al. (2002) [12]
	Hydrophilic protein (cytochrome C) loaded	Poly(L-lactide) nanofibers nonwovens	-	Maretschek et al. (2008) [59]
	Rolipram adsorbed via hydrophobic adsorption	Poly(L-lactide) based nanofibrous patches	-	Downing et al. (2012) [60]

	Antibacterial activity	PCL fibers	Linezolid delivered	Tammaro et al. (2015) [61]
	Antibacterial activity	Zein nanofibrous mats	Ag nanoparticles	Dashdorj et al. (2015) [62]
	Wound healing	Polycaprolactone, hyaluronic acid nanofibrous scaffolds	Epidermal growth factor	Wang et al. (2015) [63]
	Skin care	Silk fibroin nanofibrous mats	Vitamin E	Sheng et al. (2013) [64]
	Keloid treatment	Poly(lactide-co-glycolide) ultrafine fiber meshes	Dexamethasone and green tea polyphenols co-delivery	Li et al. (2014) [65]
	Antibacterial	ultrafine cellulose acetate (CA) and gelatin (GL) blend fibers	Amoxicillin	Kiatyongchai et al. (2014) [66]
	Antibacterial & anticarcinogenic	Microfibers of poly(ethylene glycol) (PEG) and poly(butylene succinate) (PBS)	Triclosan & curcumin delivery	Llorens et al. (2015) [67]
	Anti-inflammatory	PCL/PEG core/sheath fibers	Salicylic acid	Yu et al. (2014) [68]
	-	Drug/zein composite fibers	Ferulic acid (FA) delivery	Yang et al. (2013) [69]
	Antibacterial	Poly(methyl methacrylate)-nylon6 core shell nanofibers	Ampicillin delivery	Sohrabi et al. (2013) [70]
	Inhibition of bacteria	PCL/gelatin nanofiber membrane	Metronidazole	He et al. (2015) [71]

Table 1. Commonly used techniques for the functionalization of textiles.

Role of polymeric particles, nanoparticles and cyclodextrins in textile functionalization

A particle is a tiny mass of material or a conglomeration of discrete matter, having properties distinctively different from its bulk. Mostly particles are composed in a solid state and their simplest shape is a sphere. Particles display some special characteristics due to their unique forms and high surface area/mass ratio. A polymeric particle is a spheriform matter and can be prepared via different techniques. Adaptive/functional particles aimed to serve special applications can be formed by controlling certain factors e.g. synthesis conditions, selection of composition and optimization of the technique. Important properties of particles include their size, morphology, zeta potential and surface group attachment. Polymeric particles have

different sizes, concentrations and properties depending on their source and chemical composition. Physical and chemical composition strongly affects the behaviour and application of particles. Adaptive/functional particles find applications in diverse fields such as colloidal chemistry, biomedical [72], painting materials [73], dyeing materials [74-75] and textiles [76-77]. The particular functionalization of textile can be done by impregnation or physical immobilization of nano-objects containing active agents. These nano-objects are mostly obtained by encapsulating active agents in a polymer matrix (particles or capsules), and are then fixed on a textile material [4,23,78]. Various encapsulation techniques include solvent evaporation [79-80], nanoprecipitation, interfacial polycondensation [81], prilling [82], spray coating [83], spray drying [84], coacervation [85] and inclusion processes [11]. The encapsulated actives are released by processes like mechanical stress, diffusion process, humidity, temperature or pH change, polymer matrix degradation or by contact with the skin. The Textile Nanotechnology Laboratory at the Cornell University modified textile materials by using electrostatic self-assembly and atomic layer deposition techniques and obtained multifunctional and customizable textile surfaces by depositing polyelectrolyte onto textile substrates with a myriad of nanoparticles and non-spherical colloids. Magnetic and electric fields were utilized to precisely control the position of functional nanoparticles inside and outside the polymeric fibers. The functionalized textile could be used for antibacterial, anti-odour and self-decontaminating applications [86]. In another study, Wang et al. (2005) modified cotton surface with chitosan particles to impart protective and self-cleaning properties to the textile material [87]. They also used chitosan based polymeric core-shell particles to functionalize cotton fabric and obtained antibacterial properties [88]. The particles with antibacterial chitosan shells covalently grafted onto polymer cores were prepared by surfactant-free emulsion copolymerization in aqueous chitosan. Two types of core-shell particles were prepared: one with poly(*n*-butyl acrylate) soft core and another with a cross-linked poly(*N*-isopropylamide) hard core (Fig. 7). The particles were applied onto cotton fabric by pad-dry-cure process. The functionalized fabric displayed bacterial reduction higher than 99% and antibacterial activity was maintained upto 90% after 50 times of home washing.

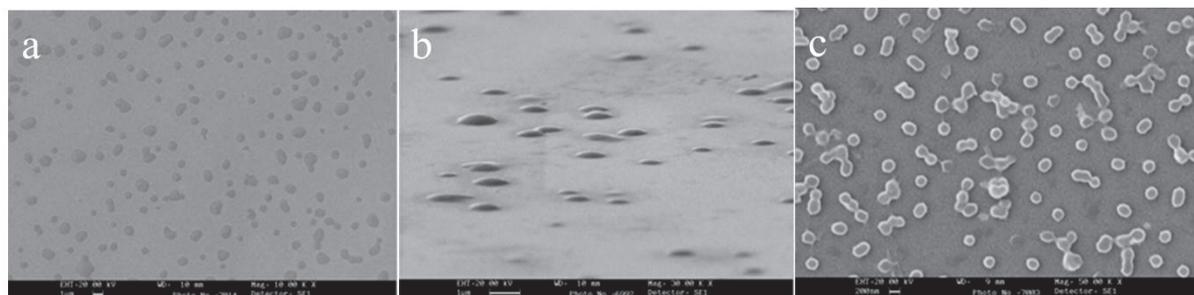


Fig. (7). SEM photos of (a, b) chitosan-poly(butyl acrylate) (CTS-polyBA) particles and (c) chitosan-poly(*N*-isopropylamide) (CTS-polyNIPAM). (a) top view; (b) side view; (c) top view [88].

Yequi et al. (2005) prepared an emulsion of shape memory polyurethane particles and used it to finish cotton fabric. In this experiment, successive additions of polypropylene glycol (PPG) and methyl ethyl ketoxime (MEKO) (blocking agent) to diphenyl-methane-diisocyanate (MDI) was done. Cotton fabric was then treated with the emulsion. Under appropriate temperature blocked polyurethane generated free isocyanates and further reacted with the hydroxyl group of the cotton fabric (Fig. 8). Polyurethane takes a crosslinking structure during the finishing process. The modified fabric was observed to possess improved handling and resistance to creasing and shrinkage [89].

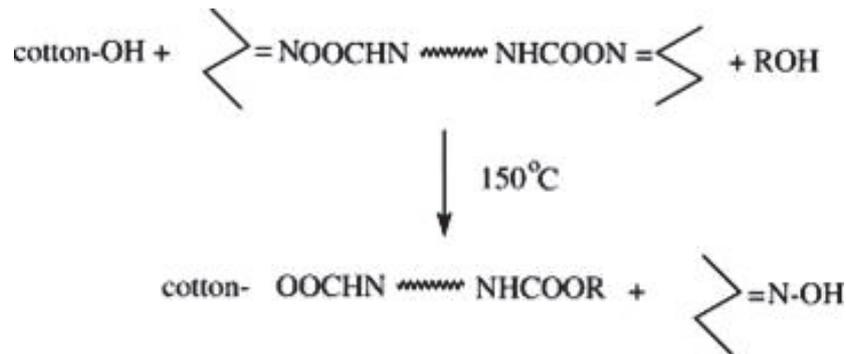


Fig. (8). Reaction of blocked polyurethane with cotton [89].

Rodrigues et al. (2009) introduced Scentfashion[®] (a technology that uses microencapsulated perfumes in textile applications). They used interfacial polymerization to obtain polyurethane/urea (PUU) microcapsules. It was observed that 38% of limonene was lost upon first cycle of dry cleaning and 87% after five dry cleaning cycles [78]. Monllor et al. (2007) analyzed the behaviour of micro-encapsulated peppermint onto cotton fabric. Bath exhaustion and impregnation were used to apply the microcapsules on the surface of cotton fabrics (Fig. 9). It was observed that rubbing test and few washing cycles do not destroy all of the microcapsules [23].

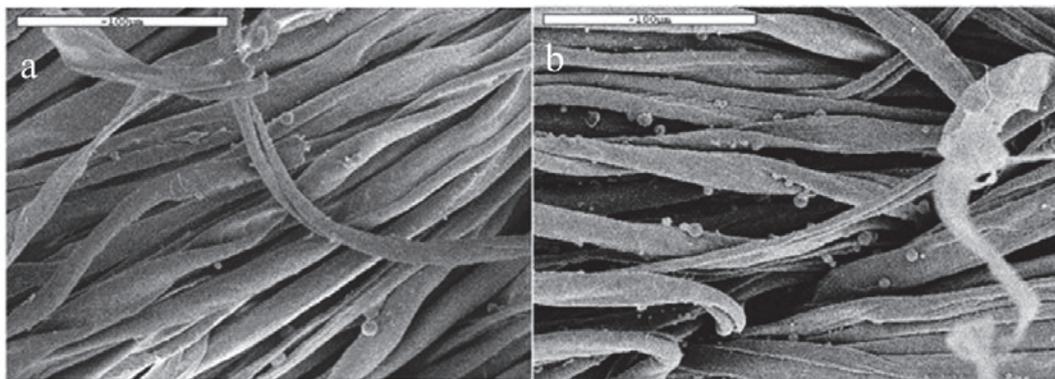


Fig. (9). SEM micrographs of cotton fabric with (a) microcapsules applied by bath exhaustion and (b) microcapsules applied by impregnation [23].

In a research programme under NASA in early 1980s, Nelson and his group developed textiles functionalized with phase change material (PCM) microcapsules to improve the thermal performance of the textiles [90]. The microcapsules were produced by deposition of thin polymer coating on small solid particles or on dispersions of solids in liquids (Fig. 10).

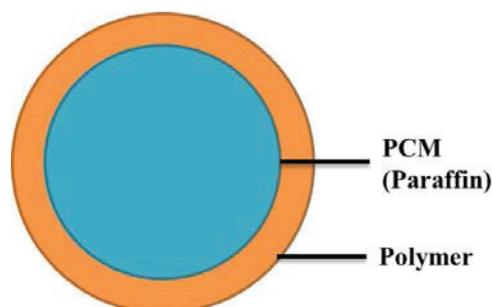


Fig. (10). Microcapsule encapsulating PCM.

Ripoll et al. (2012) investigated the adsorption behaviour of cationic latex particles on synthetic fibers (polyamide based fibers) (Fig. 11). Particles adsorption was investigated as a

function of time, concentration, pH and ionic strength. It was observed that cationic latex particles remained stable between pH 3 and 8. Desorption experiments were also performed and results showed that only 20% of the initially fixed particles were desorbed after ten washing cycles [91].

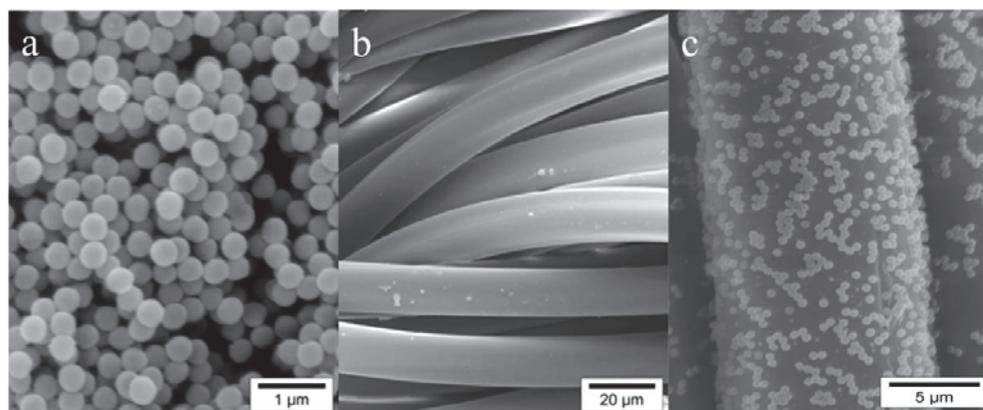


Fig. (11). SEM micrographs of (a) cationic latex particles, (b) textile surface before treatment and (c) textile surface after adsorption of particles [91].

Nanomaterials are substances having at least one dimension in the nanometric scale e.g. nanowires, nanorods and thin films [92]. Recently nanotechnology application into textile field has paved way for development of better and new drug delivery systems. Deposition of silver nanoparticles provides antibacterial properties and gold nanoparticles deposition allows use of molecular ligands, which helps in the detection of biological compounds presence in the surrounding medium. Platinum and palladium nanoparticles impart catalytic properties. Usually, these nanoparticles are impregnated onto textile materials without changing their texture or comfort. Deposition of metal nanoparticles has an added advantage of displaying surface plasmons which display strong optical extinctions that can be tuned to various colors by changing their size and shape e.g. silver nanoparticles create a shiny metallic yellow-dark pink colour. Vigneshwaran et al. (2007) presented a novel one-pot method to prepare silver nanoparticles and also stabilized them by starch on the surface of cotton fabrics. The resultant functionalized cotton fabric displayed strong antibacterial and bacteriostatic activity as well as showed colour due to surface plasmon resonance [93]. In another study, Jiang et al. (2006) coated cotton and polyester fabrics by chemical silver plating with nanolayer of silver. This improved antibacterial, UV light absorption, antistatic and light fastness properties of the fabrics [94]. Metal oxides like ZnO are found to be more stable as UV blockers than organic UV-blocking agents. The nano-ZnO greatly increases UV blocking properties because of increased surface area and intense absorption in the UV region. Vigneshwaran et al. (2006) performed nano ZnO coating of cotton textiles (Fig. 12) and achieved promising antibacterial and UV protection [95]. Similar results were obtained when Gorenssek and Recelj (2007) impregnated cotton fabric with nano silver [96].

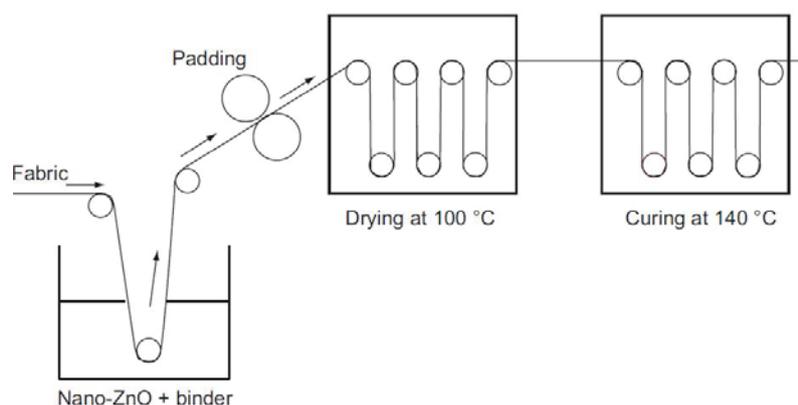


Fig. (12). Coating of cotton textile with nano-ZnO [95].

Nanocrystalline coatings of titanium dioxide have been applied for textile functionalization. Nanocrystalline anatase impregnation on cotton fabrics made the fabric self-cleaning in nature [97]. Similarly photocatalytic self-cleaning characteristics were imparted to cotton fabric when titania layer was deposited through dip-pad-dry-cure process by Qi et al (2006) [98]. In addition to metal and metal oxide nanocoatings, coatings with nanoscale biological materials are also used for adding specific functionality to medical textiles e.g. usage of enzymes and drugs [99]. Specialized nanomaterials functionalized with ligands can be introduced on the textile surface with the objective of absorbing odors, providing strong antibacterial properties, relieving pain and irritation. Moreover, such valued functionalized textiles find applications in tissue engineering, drug delivery and protective clothing.

Cyclodextrins (CDs) is relatively a new class of molecules that is widely investigated since last three decades. A lot of scientific papers on CD based textile slow release systems are being published. The first patent on CD-inclusion complexes dates back to 1953 including the application of CDs in drug formulations [100]. First patent about CDs in textile appeared in 1982 [101]. CDs have the ability to form complexes with dyes [102] and different aliphatic or aromatic agents e.g. pesticides, detergents, hormones, fragrances and vitamins. CDs are cyclic (α -1, 4) linked oligosaccharides, built from a number of D-glucose units. B-CD is usually more often used in CD-textile drug delivery systems as compared to other forms of CDs. The relative hydrophobic cavity of CDs is responsible for the complex formation. Complexation depends on the fact that selected drug molecule should fit into the cavity of the CD and it is independent of the chemical properties of the active agent. The number of drug molecules compatible for complexation is found to be quite large. Van der Waals forces are important for drug-CD complexation and complex formation is found to be enthalpy driven [103]. The fixation of CDs on textile does not affect the complexation and release of active agents from CDs. Dilution and degradation of drugs are controlling factors where drugs have weaker interactions with the host but on the other hand for drugs having stronger interactions with host, competitive displacement affects the release rate too by decreasing the available amounts of uncomplexed CDs. Triggered or delayed release drug delivery can also be achieved by using CD-drug complexes.

At present, textile drug delivery systems involving CDs are mostly designed on basis of experience and trial and error and not by following any specific designed rules. The release pattern is also largely determined by the CD-drug combination. There are different techniques for the production of textiles bearing CDs on their surfaces. The fixation of CDs onto textile surface can be done via permanent or non-permanent techniques. Permanent fixation is brought about either by non-covalent bonding (cross linking) or by covalent bonding (reaction of textile fiber functional groups with the functional groups in the CDs). An example is the reaction of CD hydroxyl groups with the hydroxyl, carboxyl, amide or other acidic or basic

groups [104]. Another comparable fixation technique uses an intermediate molecules e.g. a polymer between the textile fiber and CD. After polymerization, such compounds react with the hydroxyl groups of the CDs and textile functional groups like hydroxyl groups of cellulose. Moreover, some network formation also occurs which leads to both fixation and entrapment of the CDs [104]. Electronic interactions are also helpful in fixation when CD derivatives are ionic in nature e.g. the interaction of hydroxypropyl trimethyl ammonium chloride- β -CD with polyacrylonitril fibers [105]. Fixation can also be brought about by the “anchor groups” present on CD derivatives. The anchor groups penetrate the textile fibers when the latter is present in amorphous state. Anchor groups are mostly hydrophobic tails like alkyl/aryl groups [104], which penetrate well into the fiber’s hydrophobic interior. However, hydrophilic outer surface of CDs avoids complete penetration into the fibers and hence functional cavity still remains available on the textile surface [7]. The mobility of the fiber polymers is restricted by lowering the temperature (below T_g). This captivates the anchor groups and causes fixation of CDs [104-105]. Another promising fixation technique is the grafting of CDs. α -, β - and γ -CDs have been grafted on textiles fibers e.g. cotton, tencell, polyester and wool [106-109]. The scientific literature describes three common approaches for grafting of CDs. These are: acryl-amido-methylated- β -CD [5] based grafting, polycarboxylic acids based grafting [110-111] and monochlorotriazynyl- β -CD based grafting [109,112]. Apart from the above mentioned techniques, CDs can also be spun into the fibers. This is only workable for textiles whose fibers are made by melt or solution spinning e.g. polyamide 6. Cooling of fibers with the spindle left in a “shock-wise” manner causes migration of CDs to the surface. However, CDs cavities remain accessible for drug complexation [104]. The selection of the technique depends on the application and on the fiber material [106]. Biocompatibility and biodegradability of textiles containing CDs is determined by the type of textile material and hence is not considered a factor affecting application of CDs as textile drug delivery systems. Previous studies [105-106] show that fixation does not destroy the complexing power of the CDs. However, CD fixation onto textile, do bring about certain changes. The derivatives containing large hydrophobic side groups increase the diffusion time for water droplets and opposite phenomenon is observed with hydrophilic groups. The surface resistance is also altered by CD fixation and it decreases on increasing the amount of alkyl groups on the surface [105]. The reactivity of the treated fabric is also changed as CDs introduce reactive hydroxyl groups to the textile surface. Apart from the above mentioned functionalization techniques, several new technologies have been introduced as well. These include atom transfer radical polymerization (ATRP), molecular imprinting, ultrasonic waves, ionic liquids and biomimetic approaches.

Conclusion

Textiles are ubiquitous and are widely used every day in the world for diverse purposes. Textiles with their unique performance characteristics are widely used in various industrial areas like civil engineering textiles, medical treatment materials, agriculture and forestry textiles, filtration materials and cosmetic textiles. Natural textiles cannot be used for the above mentioned diverse applications without any particular functionalization or surface modification. There are many different techniques available for the functionalization of textiles like physical vapor deposition, chemical vapor deposition, surface grafting, sol-gel etc. The selection of a particular technique depends on the target application and the type of fiber material. Literature shows that generally the target applications for surface modifications have been properties like antibacterial and antiseptic, water repellency, skin care and wound healing. The proper characterization of textile surface is essential for better understanding of surface functionalization. Various techniques like SEM, TEM and AFM are available to characterize the surfaces. Among these techniques, SEM is usually the foremost and most

commonly used method for initial characterization of the surface. CDs are also now paving their way into the textile industry for better functionalization. They can form complexes with a wide range of drugs and then can be introduced into the textiles by different methods like CDs grafting. Researchers are constantly working to further improve the role of CDs in textiles for enhanced drug delivery and cosmetic applications as well.

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Part III
EXPERIMENTAL STUDIES

Summary

This experimental work is a struggle towards achieving better functionalized fabric by encapsulating active agents inside polymeric microparticles before their adsorption onto textile surface.

Among the many available encapsulation methods like nanoprecipitation, solvent diffusion, ionic gelation, we selected double emulsion-diffusion solvent evaporation technique for the preparation of particles. This method is slight modification of emulsion evaporation and more specifically consists of water/oil/water emulsion. This method was chosen because of a number of reasons. In pharmaceutical field, this is one of the most used industrial processes due to low toxicity of the used solvents. Both hydrophilic and hydrophobic actives can be encapsulated via this process. It also allows flexibility of adjusting the particle size by changing the process control parameters. The process also does not require any special laboratory equipment and is also cost effective. After formation of double emulsion, an excess or large volume of water is used to facilitate the rapid diffusion of organic solvent into the external aqueous media. Evaporation of organic solvent is done to avoid the risk of toxicity. The biodegradable polymer selected for particulate formation is Eudragit RS100 (ammonio methacrylate copolymer). This polymer allows constitution of cationic particles, the charge being contributed by the ammonium groups. Charged particles are required for stability (since no stabilizer is used in the formulation) and for adsorption onto oppositely charged textile. It degrades slowly making it a suitable candidate for prolonged release drug delivery systems. Dichloromethane is the organic solvent selected mainly due to its low boiling point (allows easy removal by rotavapor evaporation), immiscibility with water and solubility of polymer in it. Preliminary and systematic studies are performed in order to evaluate the influence of process control parameters like polymer amount, on the morphology and size of particles. These studies help us to know which homogenizers and process parameters would be suitable to obtain desired particles morphology and size near to 1 micrometer.

A study related to electrokinetic properties of the provided textile is then performed. A polyamide based textile is used and it is kindly provided by Gervaris Company. This study is performed to assess the influence of salinity and pH on the zeta potential of textile and particles so that we might know under which conditions maximum interaction would occur between particles and textile. Moreover, this experiment will also show how the increasing amounts of adsorbed polymeric particles will change the zeta potential of the textile.

The next step is the encapsulation of active agents into the microparticles. Since the intended application of particles is adsorption onto textile surface, so two cosmetic agents i.e. Lauryl Isoquinolinium Bromide (LIB, shaving agent) and vitamin E (antioxidant) are selected. Both these actives are photosensitive and hydrophobic and therefore suitable candidate for encapsulation into particles via double emulsion method. The third active is an anti-inflammatory agent Indomethacin. The active molecules are introduced into the organic phase of w/o/w emulsion. The particles are characterized for size, morphology, encapsulation efficiency and skin penetration. In addition, particles are also adsorbed onto polyamide based textile via electrostatic interactions and are viewed under scanning electron microscopy.

Hydrophobic active agents are specifically chosen in our study since another target of our work is to enhance the aqueous solubility of hydrophobic active molecules. This can be

achieved by the introduction of cyclodextrin molecules into our formulation. The final work is dedicated to this goal. Firstly, solid complexes of each of the three actives are formed with hydroxypropyl-beta-cyclodextrin by physical mixing and coevaporation process. These complexes are characterized by SEM, DSC and FTIR. The complexes are introduced into the internal aqueous phase of w/o/w emulsion rather than into the organic phase. Finally such particles are adsorbed onto the textile. Characterization of these particles includes morphology analysis, size determination and study of their skin penetration. Depending on the results, this could open new pathways to functionalize textiles with therapeutic and cosmetic agents.

III.1. Elaboration of sponge like biodegradable cationic particles via double emulsion solvent evaporation: Preliminary systematic study

Summary

The main goal of pharmaceutical research nowadays is to focus on developing encapsulation techniques rather than to develop new chemicals or actives. Encapsulation provides many important characteristics to the encapsulated drug molecules. The major advantages of encapsulation include concentration, shielding and protection of molecules from degradation, hydrolysis, oxidation and sustained and targeted drug delivery with improved solubility. Different colloidal carriers can be prepared like liposomes, polymeric micelles and microemulsions by using several encapsulation methods. Recently, researchers are more often using biodegradable polymers to make colloidal carriers. These biodegradable polymers are extensively used because these polymers are biocompatible and drug release from polymeric colloidal particles can be controlled by factors such as biodegradation kinetics of the polymers. Commonly used biodegradable polymers for carrier formation include polysaccharides, polyanhydrides and polyesters.

Among the several techniques available for the formation of biodegradable polymeric particles some of the commonly used techniques include double emulsion, nanoprecipitation, ionic gelation, emulsion polymerization, interfacial polycondensation, miniemulsion polymerization etc. The choice of a specific method mainly depends on the nature of the drug molecules. However, few other parameters, like the technique providing maximum encapsulation efficiency with minimum aggregation of the particles, also help in the selection of the encapsulation method. Emulsion solvent evaporation method is popularly used to prepare biodegradable particles and this method is considered significantly suitable for the encapsulation and delivery of hydrophilic actives e.g. vaccines, enzymes, hormones, vitamins and proteins. Controlled release formulations by the active entrapped in the internal droplets are also possible by this method. Both hydrophilic and hydrophobic agents can be encapsulated via this technique. Double emulsions are usually comprised of three phases, and inner and outer layers are of the same phase. W/O/W and O/W/O are the main types of double emulsions. Double emulsions can be prepared in two steps based emulsification brought about by the use of two surfactants.

The experimental work of this PhD project was started with the preparation of spongy cationic biodegradable particles via double emulsion-diffusion solvent evaporation technique. The objective was to optimize or to fix the several process control parameters involved in the technique up to a point where they provide us the desired size of the particles for our future application. The first two parts of thesis are related to achieving this goal. Preliminary studies were performed in the first part where cationic particles were obtained by the use of Eudragit[®] RS100 polymer, which is a co polymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester with quaternary ammonium groups. The ammonium groups are responsible for the positive charges. Particles were formed in three main steps: (i) internal aqueous phase (w_1) was emulsified in organic phase (o) using ultraturrax[®], (ii) primary emulsion formed (w_1/o) was emulsified in external aqueous phase (w_2) using ultraturrax[®], (iii) an excess volume of water was added to the double emulsion formed to facilitate the diffusion of the organic solvent. Fixed amount of polymer was used throughout the study. Various parameters investigated during the study were: the ultraturrax[®] stirring time and

speed and amount of different stabilizers used in the internal and external aqueous phase of the double emulsion.

In general, it was observed that morphology and zeta potential of the particles remained constant irrespective of the different parameters involved. SEM images showed particles to be spherical, polydispersed and porous in nature. Zeta potential of all the particles was found to be $\sim +50\text{mV}$. An increase in ultraturrax[®] stirring time and speed caused a decrease in particles size. The different types as well as different amount of stabilizers showed almost non-existent influence on the size or morphology of the particles. The size of the particles was observed to be quite large and quite far away from the desired targeted size.

In conclusion, study was successful in providing spongy cationic particles with the lowest size near to $\sim 20\mu\text{m}$. There is a need to perform another systematic study to decrease the size and polydispersity of the particles. It should also be noted to exclude the use of any stabilizers from the future formulations.



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Elaboration of sponge like biodegradable cationic particles via double emulsion solvent evaporation

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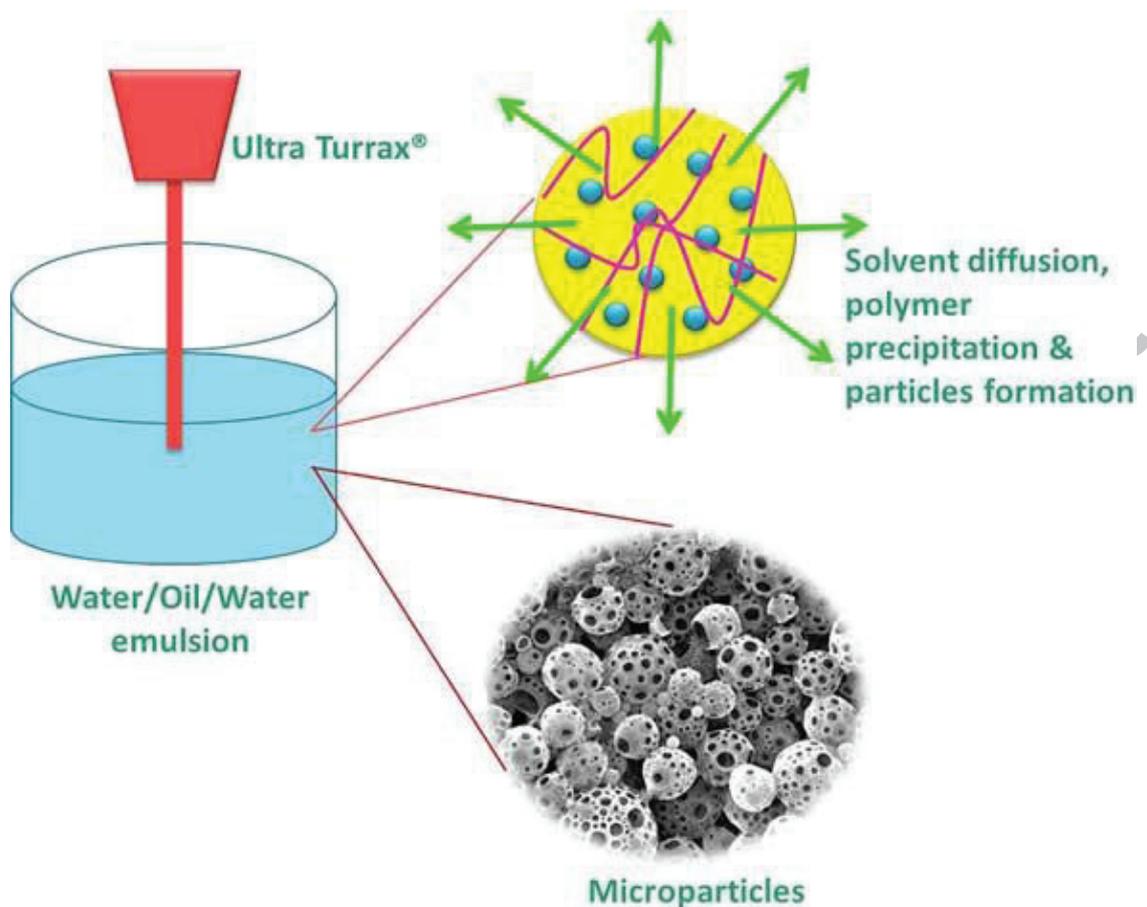
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Abstract

Researchers work on different optimization methods to come up with preparations, which show the desired or targeted outcomes. Moreover, nowadays research is more focused on developing formulations which contain biodegradable polymers. The objective of present systematic study was to achieve the above mentioned goals by the use of cationic Ammonio methacrylate copolymer (Eudragit[®] RS100). Double emulsion solvent evaporation technique was used. This methodology was chosen because the particles formed by this method allow encapsulation of both hydrophilic and hydrophobic active molecules. It also has applications in diverse fields such as drug delivery, food, cosmetics and pharmaceutical industries.

The aim of this research work was to investigate the influence of various process control parameters, such as stabilizer chemical nature and amount, ultra turrax[®] stirring speed and time, on the morphology, size and size distribution of the final dispersions. Then, the prepared particles were characterized using Scanning Electron Microscopy and Laser Diffraction Particle Size Analyzer. This study helped us to know which parameters have drastic effect on the colloidal properties of the particles.



KEYWORDS: Double emulsion solvent evaporation, biodegradable polymer, spongy microparticles, SEM.

1. INTRODUCTION

Nowadays, research is extensively focused on different encapsulation technologies due to increasing interest in the fields of biotechnology, medicine, pharmaceuticals, catalysis, ecology and nutrition. Active molecules are frequently encapsulated to attain variety of specific characteristics. These benefits include shielding and protection of actives by enclosing them in a compartment which separates the active molecules from the

surrounding environment.^[1] One of the aim of advanced drug delivery is to provide drug doses for sustained periods to particular targets in the body.^[2-4] Encapsulating drugs into microparticles and causing their controlled release upon arrival of particles at specific target helps in achieving this goal.^[3,5] Multiple structures like liposomes, microgels, microemulsions, polymer micelles and colloids are widely used as drug carriers.^[6] The colloidal carriers provide advantages of sustained delivery of protein agents like DNA vaccines,^[7] growth factors and other peptides^[8]. Many potent active molecules possess low aqueous solubility and even some of them, when used in their therapeutic concentrations e.g. Paclitaxel, show poor solubility in lipid media.^[9] Encapsulation of such drugs resolves this issue. Added advantages of encapsulation include masking of organoleptic properties like colour, taste, odour of molecules and avoidance of adverse effects like gastric irritation caused by certain drugs.

The trend of drug formulation techniques is increasingly moving towards constitution of microparticles from biodegradable polymers for drug delivery since they can be easily introduced into the body without the requirement of any surgical procedures.^[10] The drug release rates from biodegradable polymers are controlled by biodegradation kinetics of the polymers,^[11-13] physicochemical properties of the polymers and drugs,^[14,15] thermodynamic compatibility between the polymers and drugs^[16] and the shape of the devices.^[17-19]

Biodegradable microparticles can be employed for delivery of variety of drug molecules irrespective of their molecular weights and water solubility.^[20-24] Examples of commonly

used biodegradable polymers in microparticles formation are poly (ortho esters),^[25-27] polyanhydrides,^[28,29] polysaccharides,^[22,30,31] polyphosphazenes^[32,33] and polyesters.^[34,35] Diverse methods are explored for the encapsulation of various active molecules and the nature of the drug^[36] is generally the deciding factor for the selection of the encapsulation technique^[37]. The most commonly used encapsulation methods include; single emulsion process,^[38] double emulsion process, solvent diffusion,^[39] organic phase separation, supercritical fluid technology, spray drying^[34,40] and miniemulsion polymerization^[41] techniques. Few important parameters must be taken into consideration before designing any new technique like stability and biological activity of the drugs should remain unaltered during encapsulation process, encapsulation efficiency should be high, microparticles quality and drug release profile should be reproducible and aggregation of microparticles should be prevented.

Emulsification solvent evaporation method has been broadly used for the preparation of biodegradable microspheres.^[42-44] This technique has been shown in literature to be particularly suitable for encapsulating hydrophilic drugs (vaccines, vitamins, enzymes, hormones) and proteins within microparticles.^[45,46] This method provides many good prospects for the controlled release of molecules initially encapsulated in the internal droplets.^[47] There are two main kinds of double emulsions; water in oil in water (W/O/W) and oil in water in oil (O/W/O) types. Double emulsion comprises of three phases and the inner and outer layers are of the same phase.^[37] Active molecules can migrate from outer to inner phase of multiple emulsions; thus providing a kind of reservoir suitable for detoxification (overdose treatment). Another benefit of this method

is that it requires only mild conditions of ambient temperatures and constant stirring.

Therefore, stable emulsions are easily formulated without compromising the activity of the drugs.^[48]

Double emulsions are prepared in two step emulsification process using two stabilizers. For example, in W/O/W emulsion, a hydrophobic stabilizer is used to stabilize the interface of the W/O internal emulsion and a hydrophilic one for the stabilization of external interface of oil globules.^[37,49] Various drugs and proteins have been encapsulated by this technique e.g. Bovine Serum Albumin^[50] (BSA), Plasmid DNA,^[51] Ciprofloxacin^[52] etc.

The goal of this study is to investigate the influence of various process control parameters on the properties of resultant microparticles (e.g. morphology, size, size distribution and zeta potential). This systematic study is necessary in order to point out the effect of each parameter separately.

2. EXPERIMENTAL SECTION

2.1. Materials

Ammonio methacrylate copolymer type BNF (Eudragit[®] RS100), (Mw ~32,000 g/mol) was purchased from EVONIK (EVONIK, Germany). Dichloromethane (DCM), Poly(vinylalcohol) (PVA) (Mowiol 4-88 Mw = 31,000g/mol) and NaCl were purchased from Sigma-Aldrich. Poloxamer 407 (Lutrol[®] F 68) and poloxamer 188 (Lutrol[®] F 127)

were purchased from Ludwigshafen Germany. All these reactants are used as such.

Deionized water was used throughout the work.

2.2. Method

2.2.1. Preparation Of Microparticles

1 g of Ammonio methacrylate copolymer (Eudragit[®] RS100) was dissolved in 5ml of organic solvent Dichloromethane (DCM) by placing it on magnetic stirrer until polymer was well dissolved. 1 ml of aqueous phase (0.5% PVA solution) was emulsified in the organic phase using Ultra-turrax (T25 basic IKA-WERK) for certain specific speed and time. The resultant primary emulsion (W/O) was then transferred to 50ml of the aqueous phase (0.5% PVA solution) along with stirring using Ultra-turrax at a specific speed and time until the formation of double emulsion. The obtained double emulsion (W/O/W) was diluted with 100 ml of aqueous phase and finally subjected to evaporation by using rotavapor (Buchi rotavapor R-144). To ensure that solvent was completely removed, the volume of diluted double emulsion before and after evaporation was measured. Figure 1 shows illustration of double emulsion evaporation process.

2.3. Characterization Of The Prepared Particles

2.3.1. Size Measurement

The hydrodynamic size of the particles and their size distribution were studied by using LS 13 320 Laser Diffraction Particle Size Analyzer (Beckman Coulter, USA).

2.3.2. Zeta Potential

Zeta potential of each the dispersion was measured at 25° C by using Zetasizer (Nano-ZS, Malvern, UK). The particles were dispersed in a 1mM NaCl before every measurement. Each measurement was performed in triplicate.

2.3.3. Scanning Electron Microscopy

The shape and appearance of the microparticles was examined by scanning electron microscope (SEM) by using Hitachi S800 FEG microscope at the “Centre Technologique des Microstructures” (CTμ) at the University of Lyon (Villeurbanne, France).

3. RESULTS AND DISCUSSION

The objective of this systematic study was to elaborate spongy, hollow and cationic microparticles by using double emulsion-diffusion solvent evaporation process (W/O/W type). Moreover, investigation on the various process control parameters (e.g. stirring time and speed) which can influence the properties (e.g. size, zeta potential) of the resultant microparticles was also performed. The amount of polymer used was kept constant throughout this study so that other parameters could be evaluated more thoroughly.

3.1. SEM, Size Distribution And Zeta Potential

The prepared particles were analyzed for their morphology and size distribution as shown in Figure 2. It was observed that particles have spherical morphology with sponge like structure and very well defined porosity. The porosity can be attributed to the nature of the Ammonio methacrylate copolymer used. Eudragit RS100 has ammonium groups,

which remain hydrated during the precipitation of the polymer. The size distribution shows particles were polydisperse and minimum size observed was around 20 μm . Broad size distribution was due to limitation of the used homogenizer (i.e. Ultra turrax[®]) and also due to the process (i.e. double emulsion solvent evaporation). The zeta potential of the particles was found to be $\sim 50\text{mV}$. The positive zeta values were contributed by the positively charged ammonium groups present in the structure of the used polymer. Since a fixed amount of polymer was used throughout the study, the zeta potential values were observed to be stable and independent of the formulation parameters.

3.2. Effect Of Stirring Speed Of Ultra Turrax[®]

The stirring speed of the homogenizing instrument is a dominant factor affecting the size and the size distribution of the resultant dispersion as too much energy supplied to the system can result in disrapture of the particles.^[53] In this study, several experiments were performed by changing the speed of the used homogenizer (Ultra turrax[®]), while maintaining other parameters constant as shown in Table 1. The results shown in Figure 3 indicated a general decreasing trend in the size of particles with an increase in the stirring speed of the instrument with the lowest size of 22.68 μm obtained at a speed of 13500 rpm.

3.3. Effect Of Stirring Time Of Homogenizer Ultra Turrax[®]

An external energy input is essentially required for the dispersion of one immiscible liquid in another liquid phase. This energy input can be supplied in the form of high stirring forces, ultrasonication, high shearing and membrane-based technology. The

duration for which this external energy is provided, is considered to be very important as low times of stirring can result in inefficient mixing of two immiscible liquids and very long stirring times can cause disruption of the resultant emulsion. So, finding out the optimum duration of stirring is very crucial in order to obtain a stable emulsion with good morphology. This study was carried out to investigate the influence of stirring time on the properties of the final microparticles by using high performance stirring apparatus Ultra turrax[®] T-25. The experiment was divided into two parts; i) stirring time for primary emulsion was varied keeping the stirring time for 2nd emulsion constant and, ii) stirring time for the 2nd emulsion was changed while the stirring time for the primary emulsion was kept constant.

3.3.1. Effect Of Variable Stirring Time For The First (W/O) Emulsion

In this case, five samples of emulsion were prepared using the same amount of polymer (1 g). All the other parameters were kept constant as reported in Table 2. The hydrodynamic size of the particles was measured at time intervals of 5 minutes starting from 5 upto 25 minutes and the obtained results were plotted as shown in Figure 4. As can be seen from Figure 4, particle size did not display any linear trend (an increase or decrease) with increasing stirring time for the first emulsion. These results indicated that stirring time for the formation of primary emulsion do not considerably affect the final particles size. This could be due to smaller volume of primary emulsion, which limits the efficiency of the instrument. However, it was also observed that longer stirring time of 25min caused aggregation or destruction of the final particles and also resulted in high foam formation, which may lead to increase in particle size.

3.3.2. Effect Of Variable Stirring Time For Second Emulsion

The stirring time for the 2nd emulsion is significantly important because if the primary emulsion is not properly dispersed via stirring for appropriate time, then the resultant double emulsion may display large polydispersity and high aggregation of particles. This experiment was performed to verify this fact. Stirring time for 2nd emulsion was varied between 5 minutes to 25 minutes. All the other parameters were unchanged throughout the study as shown in Table 3. The size of the particles measured by Laser Diffraction Particle size analyzer was plotted against stirring time for 2nd emulsion and the obtained results are reported in Figure 5. The results showed that the size of the particles was initially decreased with an increase in stirring time upto a certain time (approximately 15 min.). Lowest particle size of approximately 21 μ m was observed for stirring time of about 15 minutes. This indicates that best stirring time for 2nd emulsification was between 10-15 minutes. However, higher stirring times lead to high foam formation and may provide faulty size measurements.

3.4. Effect Of Stabilizers Nature And Concentration

The inclusion of a suitable stabilizer in a formulation plays an important role in the dispersion of one liquid into another liquid.^[54] The nature and concentration of stabilizer has an important impact on the colloidal stability of the dispersions. The stability of emulsion is very important because when organic solvent is removed from the formulation, a decrease in the volume occurs, which could lead to increased viscosity.

This can affect final particle size and can lead to coalescence or aggregation of particles.^[55]

3.4.1. Effect Of Different Stabilizers In The Internal Aqueous Phase With Fixed Stabilizer In External Aqueous Phase.

In this part, various stabilizing agents were used in order to prepare water in oil emulsion and PVA was used with a fixed concentration (0.5%) in the external aqueous phase (W/O/W). The main objective of this study by introducing stabilizer (PVA, F86, F127) in the internal phase was principally to avoid coalescence during the second step. In addition, the effect of stabilizer amount used in the internal phase was also investigated. Surprisingly, the amount and type of used stabilizer in W/O was found to have no drastic influence on the final particles size and even on size distribution as shown in Figure 6. This can be attributed to immediate use in the second step, the freshly prepared water in oil emulsion. Then in the following study, water in oil emulsion prepared in the first step was stabilizer free in order to examine the effect of stabilizer in the external phase. It was also interesting to notice, that the used stabilizers are not soluble in DCM, and then during the solvents diffusion, the stabilizer may precipitate inside the water phase or inside the polymer matrix.

3.4.2. Effect Of Different Stabilizers In The External Aqueous Phase With No Stabilizer In Internal Aqueous Phase

In order to reduce the particle size, various stabilizers were used in the external phase. Then, in this part, the effect of different amounts of three stabilizers, namely, PVA, F68,

F127 in external aqueous phase was investigated and special attention was dedicated to the final particles size. The obtained result tendencies are reported in Figure 7 in which the hydrodynamic particles size is reported as a function of the stabilizer concentration. As a general tendency, the size of particles was not significantly influenced by the presence or increase in the amount of stabilizer in the external aqueous phase. This can be attributed to non-effect on surface tension and also the used non charged stabilizers were not acting as surfactants, but as depleting agents maintaining only the good colloidal stability of the formed particles. Moreover, the particles were positively charged in nature due to the used polymer containing ammonium groups. Then the particles were electrostatically stabilized and the used non-charged stabilizer had no influence on final particles size. Therefore, it was decided to exclude the use of any stabilizers from our future experiments since they would just unnecessarily complicate the formulation without bringing any significant effects on the colloidal properties. It was interesting also to notice, that the surface morphology was not influenced by the chemical nature of the used stabilizer as examined by scanning electron microscopy Figure 8.

4. CONCLUSION

Multiple emulsions are complex systems composed of two immiscible phases, which are dispersed by using homogenizers and appropriate stabilizers. In this preliminary systematic study, Ammonio methacrylate copolymer type (Eudragit RS100) based spongy particles were formed by using modified double-emulsion solvent evaporation method. Different conditions that affect the properties (particle size, size distribution and morphology) of the formed dispersion were studied.

The stirring speed of the homogenizer (Ultra turrax[®]) decreases the size of particles but speeds higher than 13500 rpm leads to destruction of particles, heating and also to foam formation. The stirring time at primary emulsification does not significantly affect the final particles size but an increase of stirring time at second step of emulsification produces significant decrease in size of the final particles. Minimum particle size of 21 μ m was observed at stirring time of 15min. The size results for different amounts of stabilizers used in internal aqueous phase and external aqueous phase were not different and in both cases did not show any significant changes in the size of the particles. The morphology of the particles, in general, was spherical with multiporous or sponge like structure and particles were also polydisperse as clearly evident by laser diffraction and viewed by scanning electron microscopy. Then such conditions, process and approach can't lead to micrometric or submicron particles. However, this study pointed out that a combination of two different homogenizers like Ultra sonicator and Ultra Turrax[®] or Ultra sonication alone could be more helpful to obtain micrometric, submicron and narrowly size distributed particles.

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TABLE 1 Investigation of effect of homogenizer Ultra turrax[®] stirring speed on the size of the final particles

Obs.	Polymer amount (g)	DCM (ml)	Ultra turrax [®] Stirring time for 1 st & 2 nd emulsion (min.)	Ultra turrax [®] stirring speed (rpm)	Internal aqueous phase (ml)	External aqueous phase (ml)	Dilution phase (ml)	Size (µm)
1	1.0	5.0	2.0	6500	1.0	50	100	70.31
2	1.0	5.0	2.0	9500	1.0	50	100	50.56
3	1.0	5.0	2.0	13500	1.0	50	100	22.68
4	1.0	5.0	2.0	21500	1.0	50	100	24.91
5	1.0	5.0	2.0	24000	1.0	50	100	25.59

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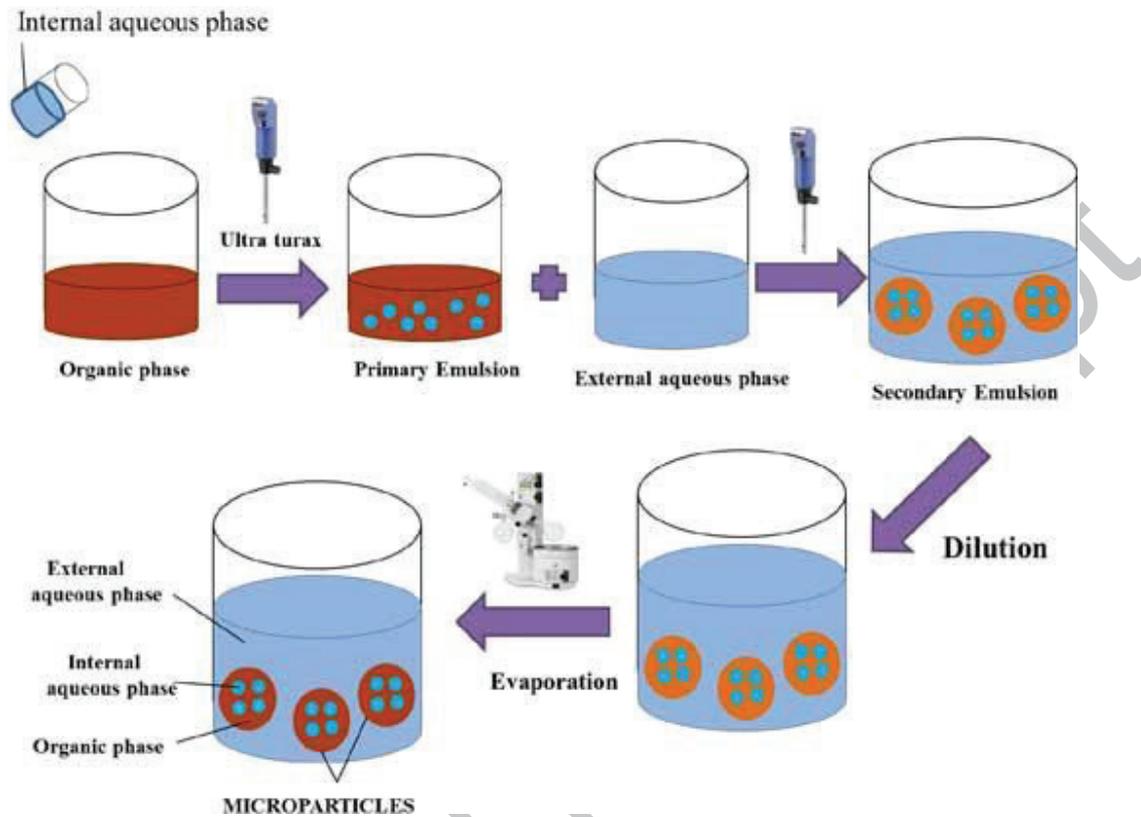
TABLE 2 Investigation of effect of Ultra turrax[®] stirring time for the first emulsion on the size of microparticles

Obs.	Polym er amoun t (g)	DC M (ml)	Ultra turrax [®] Stirrin g time for 1 st emulsio n (min.)	Ultra turrax [®] Stirrin g time for 2 nd emulsio n (min.)	Ultra turrax [®] stirrin g speed (rpm)	Intern al aqueo us phase (ml)	Extern al aqueou s phase (ml)	Dilutio n phase (ml)	Size (μ m)
1	1.0	5.0	5.0	2.0	13500	1.0	50	100	27.9 9
2	1.0	5.0	10.0	2.0	13500	1.0	50	100	28.0 2
3	1.0	5.0	15.0	2.0	13500	1.0	50	100	28.2 1
4	1.0	5.0	20.0	2.0	13500	1.0	50	100	29.0 1
5	1.0	5.0	25.0	2.0	13500	1.0	50	100	48.5 9

TABLE 3 Investigation of effect of Ultra turrax[®] stirring time for the second emulsion on the size of microparticles

Obs.	Polymer amount (g)	DCM (ml)	Ultra turrax [®] Stirring time for 1 st emulsion (min.)	Ultra turrax [®] Stirring time for 2 nd emulsion (min.)	Ultra turrax [®] stirring speed (rpm)	Internal aqueous phase (ml)	External aqueous phase (ml)	Dilution phase (ml)	Size (μm)
1	1.0	5.0	2.0	5.0	13500	1.0	50	100	33.88
2	1.0	5.0	2.0	10.0	13500	1.0	50	100	22.52
3	1.0	5.0	2.0	15.0	13500	1.0	50	100	21.80
4	1.0	5.0	2.0	20.0	13500	1.0	50	100	21.01
5	1.0	5.0	2.0	25.0	13500	1.0	50	100	48.02

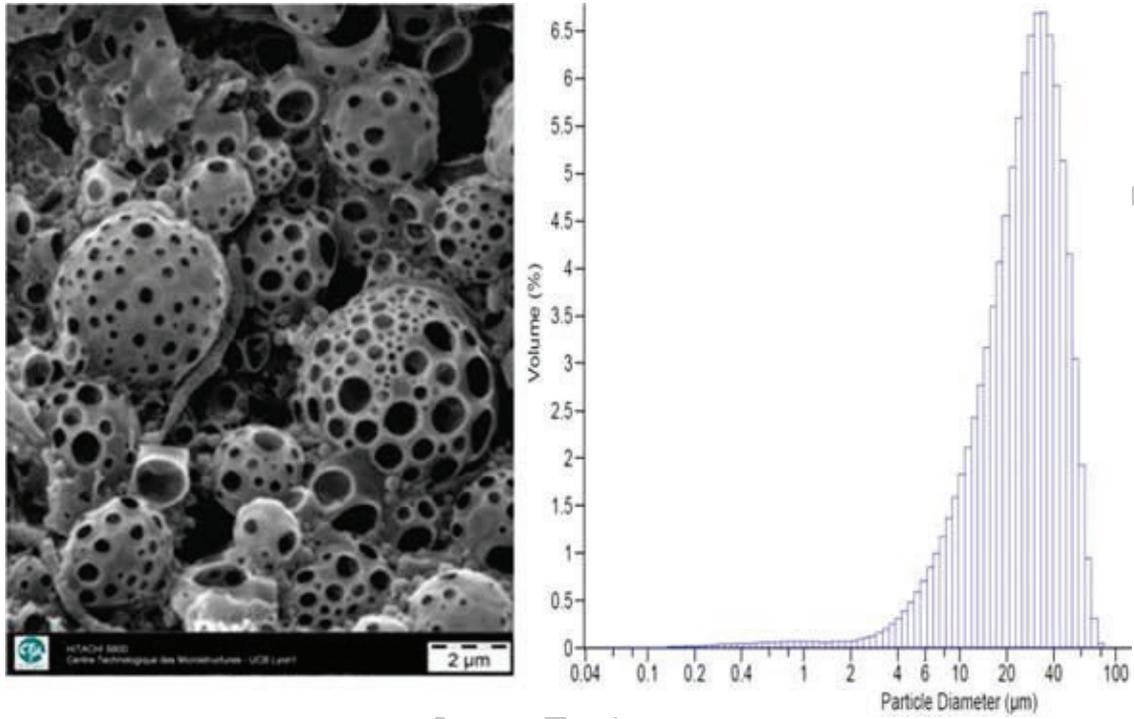
Figure 1. Double emulsion solvent evaporation technique.



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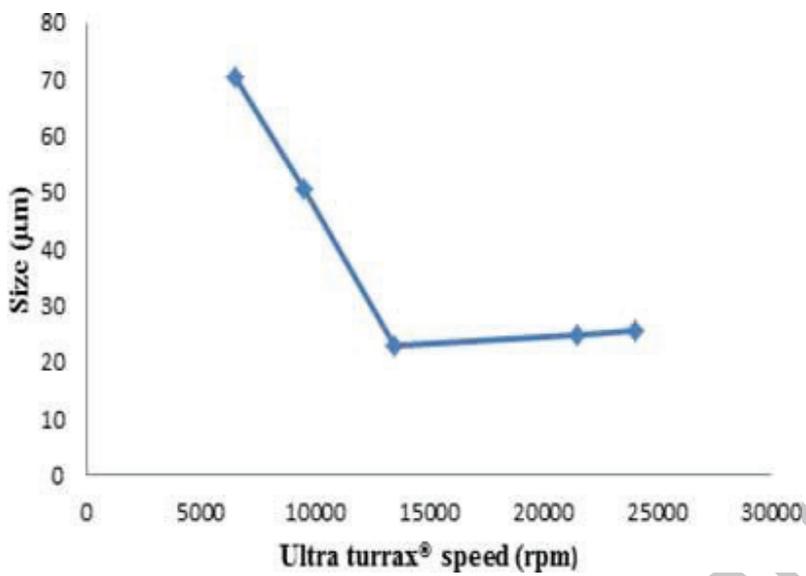
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Figure 2. Morphology and size distribution of particles prepared by emulsion-diffusion solvent evaporation method.



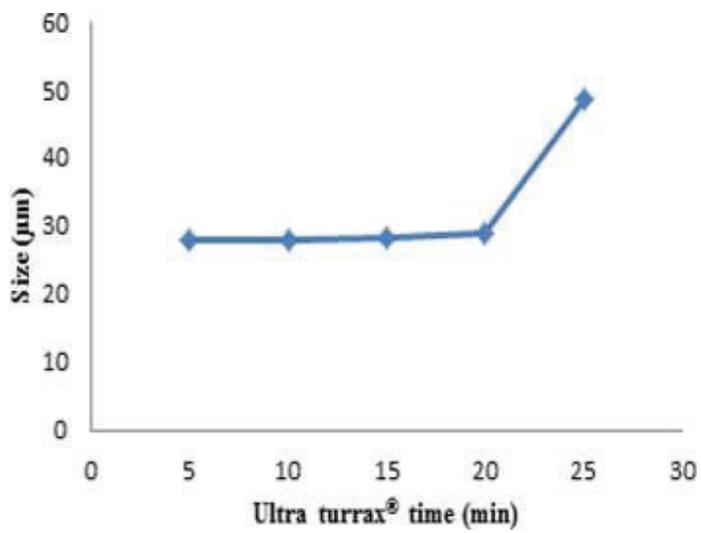
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Figure 3. Effect of stirring speed of homogenizer Ultra turrax® on the final particles size.



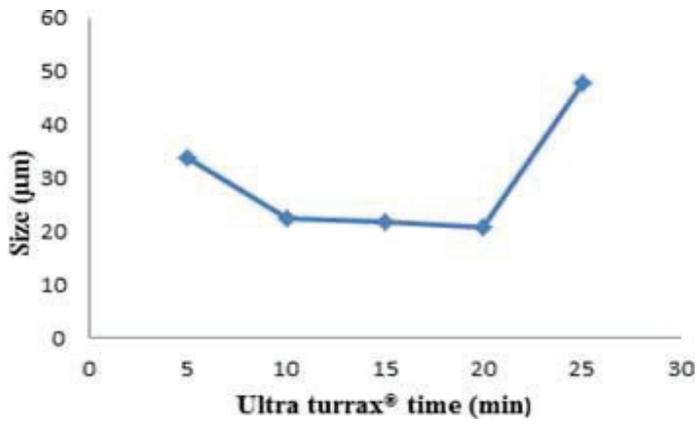
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Figure 4. Effect of Ultra turrax[®] stirring time for the first emulsion on the particles size.



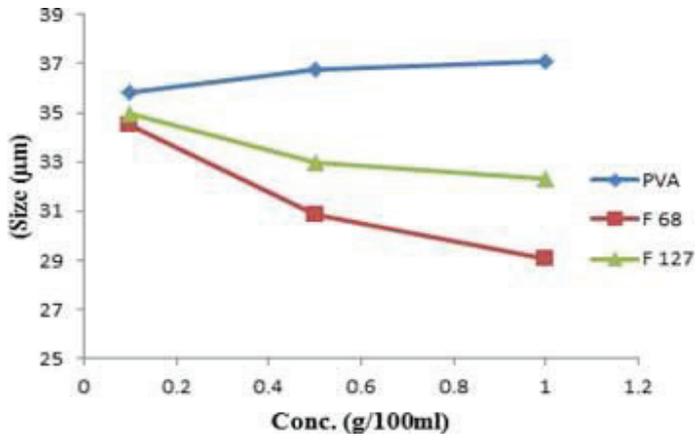
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Figure 5. Effect of Ultra turrax[®] stirring time for the second emulsion on the particles size.



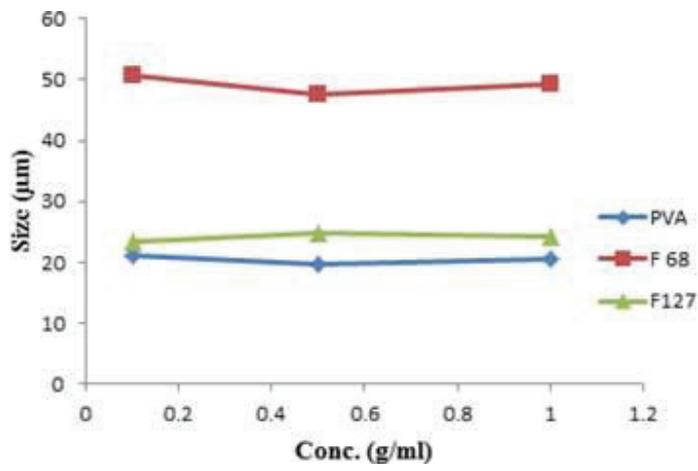
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Figure 6. Effect of different stabilizers used in the internal aqueous phase on the final particle size.



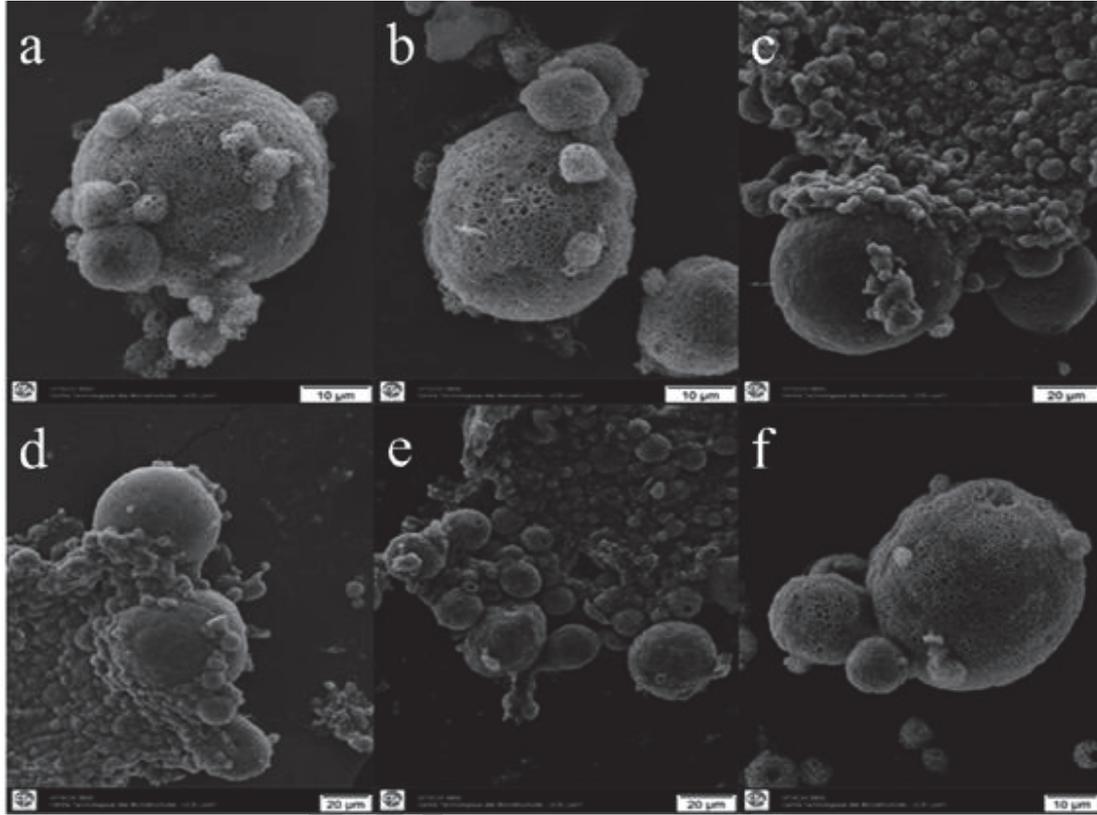
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Figure 7. Effect of different stabilizers in the external aqueous phase on the particle size.



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Figure 8. Morphology of particles when different stabilizers were used in external aqueous phase a) PVA 0.1%, b) F68 0.1%, c) F 127 0.1%, d) PVA 1%, e) F68 1% and f) F127 1%.



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III.2. Systematic study of encapsulation process via double emulsion-diffusion solvent evaporation technique using Ultrasound and Ultraturrax[®]

Summary

Nowadays, pharmaceutical research is highly focused on developing various encapsulation technologies. Encapsulation methods are being developed because they impart many important characteristics to the encapsulated actives. The prime benefits of encapsulation are concentration, shielding and protection of active molecules from early degradation, hydrolysis, oxidation and sustained and targeted drug delivery with enhanced solubility. Different carriers are formed via encapsulation methods like liposomes, polymeric micelles and microemulsions. Biodegradable polymers are extensively used to constitute colloidal carriers because these polymers are biocompatible and drug release from polymeric colloidal particles can be controlled by factors such as biodegradation kinetics of the polymers. Commonly used biodegradable polymers for carrier formation include polysaccharides, polyanhydrides and polyesters.

There are several techniques available for the formation of biodegradable polymeric particles e.g. double emulsion, nanoprecipitation, ionic gelation, miniemulsion polymerization etc. The selection of a particular technique primarily depends on the nature of the drug molecules. However, few other factors, like the technique providing maximum encapsulation efficiency with minimum aggregation of the particles, also help in the selection of the encapsulation method. Emulsion solvent evaporation method is widely used to prepare biodegradable particles and this method is of significant importance for the encapsulation and delivery of hydrophilic actives e.g. vaccines, enzymes, hormones, vitamins and proteins. It also provides controlled release for the active entrapped in the internal droplets. Both hydrophilic and hydrophobic agents can be encapsulated via this technique. Double emulsions are usually composed of three phases and inner and outer layers are of the same phase. W/O/W and O/W/O are the major categories of double emulsions. Double emulsions can be prepared in two steps based emulsification brought about by the use of two surfactants.

The experimental work of this PhD project was started with a preliminary systematic study. The results of preliminary study were used to modify parameters of this systematic study. Firstly, no stabilizer was employed in this study. Secondly, here a combination of Ultra turrax[®] and Ultra sonication was used as homogenization systems to prepare cationic particles. The technique used was the same i.e. double emulsion-diffusion solvent evaporation. Here, various parameters investigated and optimized during the study were: the polymer amount, ultrasound time, Ultra turrax[®] stirring time and speed, volume of external aqueous phase and volume of diffusion phase.

In general, it was observed that morphology and zeta potential of the particles remained constant irrespective of the different parameters involved. SEM images showed particles to be spherical, polydispersed and porous in nature. Zeta potential of all the particles was found to be $\sim +50$ mV. Increase in polymer amount lead to an increase in the size of particles. Any increase in ultrasound stirring time at primary emulsification stage produced insignificant effect on size of particles. However, increase in Ultra turrax[®] stirring time and speed caused a decrease in particles size. External aqueous phase and dilution phase volumes also showed almost non-existent influence on the size of particles.

In conclusion, study was successful in providing spongy cationic particles with a size up to 1 μ m. Such particles are suitable for loading both hydrophilic and hydrophobic active molecules.



Elaboration of ammonio methacrylate copolymer based spongy cationic particles via double emulsion solvent evaporation process



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ABSTRACT

The aim of present work is to investigate systematic study of the preparation of biodegradable particles via double emulsion solvent evaporation technique. The used formation is based on cationic ammonium methacrylate copolymer Eudragit® RS 100, without the use of any stabilizer. The effect of process parameters like ultra turrax® stirring speed and stirring time, ultrasonication time, polymer amount, and volume of outer aqueous phases on the colloidal properties of particles was investigated. All prepared dispersions were characterized in terms of size, size distribution, and electrokinetic properties, and surface morphology was investigated.

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1. Introduction

Increasing interest in the fields of biotechnology, medicine, pharmaceuticals, catalysis, ecology, nutrition, etc. leads research to being more focused on encapsulation technologies. Encapsulation of active molecules is frequently hunted for to attain assorted favorable characteristics. These benefits include concentration, shielding and protection of bioactives by creation of a single compartment which separates the active molecules from the surrounding environment [1]. The contemporary microencapsulation still continues to be an imperative formulation strategy since its initiation about 70 years ago. It was commenced with an objective to shield vitamins from oxidation [2]. One of the objectives of advanced drug delivery is to provide drug doses for sustained periods to specific targets in the body [3]. Loading of drugs into microparticles and causing their controlled release upon arrival of microparticles at specific target helps in achieving this objective. Diverse microstructures like liposomes [4], microgels, microemulsions, polymer micelles and colloids are extensively employed as drug carriers. The colloidal carriers present advantages in sustained delivery of DNA vaccines, growth factors and other peptides [5]. Few of the advantages that these carriers provide include enhanced bioavailability, drug protection against degradation and control over the rate of release of entrapped actives [5]. Many newly synthesized molecules have low aqueous solubility and few of these when used in their therapeutic concentrations e.g. Paclitaxel, also show poor solubility in lipid media [6]. Encapsulation of such agents assists in resolving this problem. Additional benefits of encapsulation are safe handling of toxic agents, masking of

organoleptic properties like color, taste, odor of molecules and evasion of adverse effects like gastric irritation caused by certain active agents.

Microparticles constituted from biodegradable polymers are increasingly utilized for drug delivery because they can be easily implanted into the body without the need of any surgical removal. The drug release rates from biodegradable polymers can be controlled by factors like biodegradation kinetics of the polymers [7], physicochemical properties of the polymers and drugs [8], thermodynamic compatibility between the polymers and drugs and the shape of the devices [9]. Biodegradable microparticles prepared through various formulatory techniques can be employed for delivery of variety of drug molecules regardless of their molecular weights and water solubility [10,11]. Polymeric microparticles also offer potential for evolution of mucosally administered vaccines. Examples of different biodegradable polymers used in microparticle formation are polyesters, polysaccharides [10], polyanhydrides [12], polyphosphazenes [13] and poly (ortho esters) [14]. Injectable controlled release depots such as drug-loaded biodegradable polymer microparticles can serve as an alternative delivery platform for some of the drugs that (i) possess broad therapeutic window, (ii) need a low daily dose, and (iii) are going to be used for long-term disease treatment [2].

Various methodologies are delved into for encapsulation of different active molecules and the nature of the drug [15] mainly determines the selection of the encapsulation technique [16]. The frequently employed encapsulation techniques include: single emulsion process [17], double emulsion process [18], ionic gelation [19], solvent displacement [20], nanoprecipitation [21,22], supercritical fluid technology, spray drying [23], miniemulsion polymerization [24] and solvent diffusion [25] techniques. In designing any new technique, one must take into consideration some important factors like stability and biological activity of the

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drugs must not be altered during encapsulation process, encapsulation efficiency should be high, microparticle quality and drug release profile should be reproducible and aggregation or adherence of microparticles should not be exhibited.

Emulsification solvent evaporation method has been widely employed for the preparation of biodegradable microspheres [26,27,28]. This technique has been shown as the most pertinent method to encapsulate hydrophilic drugs (vaccines, vitamins, enzymes, hormones) and proteins within microparticles by many authors [29]. This method offers many attractive prospects for the controlled release of active molecules initially entrapped in the internal droplets. There are two major kinds of double emulsions: water in oil in water (W/O/W) and oil in water in oil (O/W/O) types. Double emulsion consists of three phases and mostly the inner and outer layers are of the same phase [16]. Active molecules may migrate from inner to outer phase of multiple emulsions; thus providing a kind of reservoir suitable for detoxification (overdose treatment) or be used for the removal of toxic agents from waste water. This technique needs only mild conditions like ambient temperatures and constant stirring. Hence, a stable emulsion can be formulated without a compromise on the activity of the drugs.

Double emulsions are generally prepared via two step emulsification process employing two surfactants e.g. in W/O/W, a low hydrophilic–lipophilic balance (HLB) surfactant is used to stabilize the interface of the W/O internal emulsion and a high HLB one for the stabilization of external interface of oil globules [16,30]. Various drugs and proteins have been encapsulated by this technique e.g. Bovine Serum Albumin [31] (BSA), Plasmid DNA [32], Ciprofloxacin etc.

The objective of this study was to investigate the influence of various process control parameters on the properties of resultant microparticles (e.g. size and zeta potential). The aim was to optimize these parameters for future encapsulation of active molecules via emulsification–diffusion solvent evaporation process. Such studies play an integral role in not only developing better drug delivery systems but also in enhancing human perception of the bioavailability and permeability of the active agents encapsulated in the particles.

2. Materials and methods

2.1. Materials

Ammonio methacrylate copolymer type BNF Eudragit® RS100 ($M_w \sim 32,000$ g/mol) was purchased from EVONIK (EVONIK, Germany). Dichloromethane (DCM) and NaCl were purchased from Sigma-Aldrich, Germany as used as such. Deionized water was used throughout the work.

2.2. Method

Double emulsion–diffusion solvent evaporation method was selected for the preparation of microparticles. This process involves the formation of double emulsion at a first stage followed by an addition of excess volume of water to facilitate the diffusion of organic solvent into external aqueous phase.

2.2.1. Preparation of double emulsion

1 ml of aq. phase was emulsified in the organic phase (1 g polymer in 5 ml DCM) using ultra sound (Optic ivymen system CY-500) for certain specific amplitude and time. The resultant primary emulsion (w/o) was then transferred to 50 ml of the aq. phase along with stirring using ultra turrax® (T-25 basic IKA®-Werke) at a specific speed and time until the formation of double emulsion. No stabilizer was employed in the formulation. The obtained double emulsion (w/o/w) was diluted with 100 ml of aq. phase and finally subjected to evaporation by using rotavapor (Buchi rotavapor R-124). To ensure that solvent was completely removed, the volume of diluted double emulsion was measured before

and after evaporation. Fig. 1 gives a diagrammatic illustration of the process.

2.3. Characterization of the prepared microparticles

The obtained particles were analyzed in three different ways. The hydrodynamic size of the particles and their size distribution were measured after slight dilution. Zeta potential of highly diluted dispersions was investigated as a function of pH. Finally, the morphology of the particles was also carefully examined.

2.3.1. Particles size measurement

The diameter size of the particles and their distribution was studied by using Beckman Coulter LS 13 320 Laser Diffraction Particle Size Analyzer. Prepared dispersions were added dropwisely to the sample cell contained continuous phase (deionized water). The pump speed was adjusted to 31% for proper mixing of the samples. An average diameter of three readings was calculated.

2.3.2. Zeta potential

Zeta potential of each sample was measured at 25 °C by using Zetasizer (Nano-ZS, Malvern®, UK). The microparticles were dispersed in 1 mM NaCl before every measurement. Each measurement was made in triplicate. Zeta potential of the microparticles as a function of pH was measured using auto autotitrator (MPT-2, Malvern®, UK). It was measured at pH range of 3–11 keeping an interval of 1.

2.3.3. Scanning electron microscopy

The shape and appearance of the microparticles was examined by scanning electron microscope (SEM) by using Hitachi S800 FEG microscope at the “Centre Technologique des Microstructures” (CTM) at the University of Lyon (Villeurbanne, France). A drop of each sample was placed on a flat steel holder (cell) and dried at room temperature. The sample was then coated with platinum under vacuum by cathodic sputtering. An accelerating voltage of 15 kv was used for SEM analysis of the samples.

3. Results and discussion

The objective of this research was to obtain stable cationic particles, which could be adsorbed onto different negatively charged textiles for our future work. Moreover, the first aim was, not to use any stabilizer in the formulation to avoid any complexity in the double emulsion–diffusion solvent evaporation process. The influential factors were investigated in order to fix these parameters to those values, which would give spherical, stable porous particles within a specific size range so that such particles can be easily adsorbed onto different textiles after loading of specific active agents. Without investigating individual parameter it is difficult to know e.g. which polymer amount would give micrometer particles size free from any aggregated particles. To target such objective, double emulsification solvent evaporation process using ammonium methacrylate copolymer (Eudragit® RS100) as polymer. Moreover, the discussion under each parameter is done to provide scientific reasons for the results that are obtained.

3.1. Parameters investigated: water in oil emulsion (W₁/O) stage

Two parameters were first investigated: the polymer amount used in the formulation and the ultrasonication time. All other parameters related to the formulation were kept constant (Table 1).

3.1.1. Polymer amount effect

The amount of polymer Eudragit® RS100 can play an integral role in controlling the particle size and encapsulation efficiency. Various amounts of polymer used were 0.25 g, 0.5 g, 0.75 g, 1.0 g, 1.25 g and 1.5 g. Above 1.5 g of the polymer was not feasible due to high viscosity

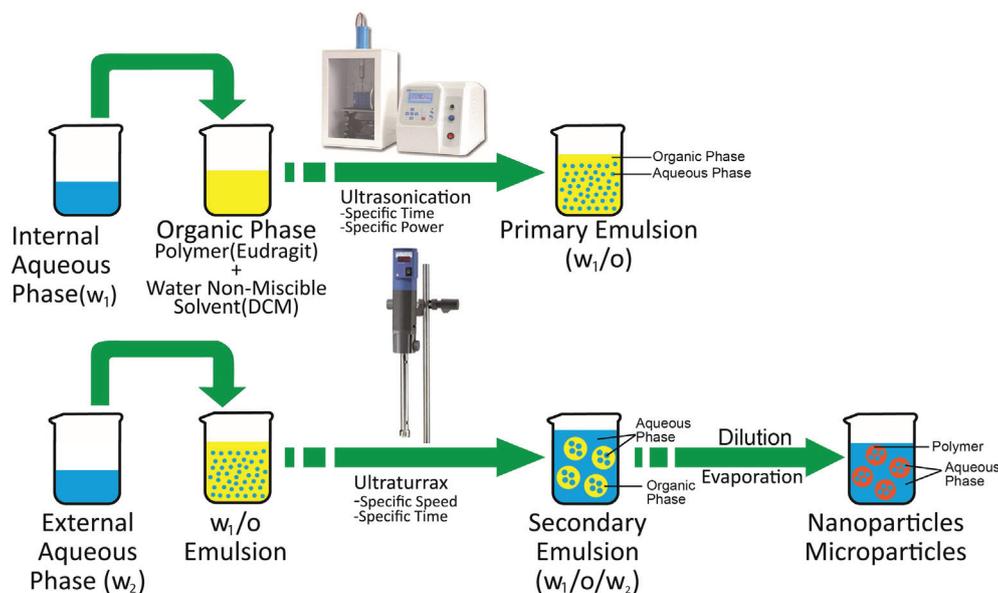


Fig. 1. Basic principle of emulsification–diffusion solvent evaporation technique (W/O/W type).

of the organic solution. The results of this study reported in (Fig. 2 (a)) showed that system behaves in two different regimes: for polymer amount below 1 g the particle size is not affected by this parameter. Above 1 g the size of particles is increased gradually. When small amount of polymer was utilized, the observed size was 0.89 μm but this size increased to 1.10 μm, 1.00 μm, 1.23 μm, 7.20 μm and 13.37 μm as the polymer amount was increased to 0.5 g, 0.75 g, 1.00 g, 1.25 g and 1.50 g respectively. Similar results were also reported by Lamprecht et al. [31] The increasing amount of polymer contributes to an increase in size and number of particles. In order to prove this, a mathematical relationship reported by Ibraheem et al. [33] can be used. The equation: $R = (3/4\pi d N_p)^{1/3} \times (M)^{1/3}$ represents a relationship between the average particle size (R) and the polymer amount (M). In the equation, d is the density of polymer and N_p is the number of particles. According to this equation, if the number of particles is considered constant and independent of the polymer amount, then the slope of the relationship between (R) and $(M)^{1/3}$ should be straight. However, in this study it was observed that the slope increased with the increasing polymer amount above 1 g (Fig. 2 (b)). Therefore, increasing polymer amount leads to both an increase in size and number of particles

above a certain limit. There is also an associated aggregation phenomenon due to higher solid content.

The morphology of the particles was examined by Scanning Electron Microscope. This SEM analysis confirmed that double emulsion–solvent diffusion technique was successful in producing microparticles. Throughout the study, irrespective of the variation in different parameters, the morphology of the particles was spherical without the presence of any aggregation or particles deformation phenomenon. In addition, all the particles also exhibited porosity. The particles are porous due to the nature of the polymer. The positively charged ammonium groups present in the polymer interact with the water molecules. During organic solvent evaporation when polymer precipitates, these ammonium groups are still interacting with the water molecules and hence lead to the formation of hydrophilic cavities and porous particles. Ibraheem et al. [33] prepared polycaprolactone based particles via similar technique. However, the particles obtained had smooth surfaces instead of multipores like morphology. Regarding polymer amount effect, three samples containing 0.25 g, 1.00 g and 1.50 g of Eudragit® RS100 were selected for SEM analysis. The SEM images (Fig. 3 (a, b and c)) for these three samples revealed that microparticles were spherical, non-

Table 1

Parameters that were constant and changed during the investigation of (1) effect of polymer amount and (2) time of ultrasonication at the primary emulsion stage.

Observed parameters	W ₁ /O emulsion					W ₁ /O/W ₂ emulsion			Dilution	Size (μm)
	Vol. of DCM (ml)	Eudragit® RS100 (g)	Vol. of inner aqueous phase (ml)	Sonication power (%)	Sonication time (min)	Ultraturrax time (min)	Ultraturrax speed (rpm)	Vol. of external aqueous phase (ml)		
1 Polymer effect	5.0	Varied	1.0	70	3.0	10	13,500	50	100	0.89
		0.25								1.10
		0.50								1.00
		0.75								1.23
		1.00								7.20
		1.25								13.37
2 Sonication time effect	5.0	1.0	1.0	70	Varied	10	13,500	50	100	1.15
					1.50					1.23
					3.00					1.21
					4.50					1.21
					6.00					1.18

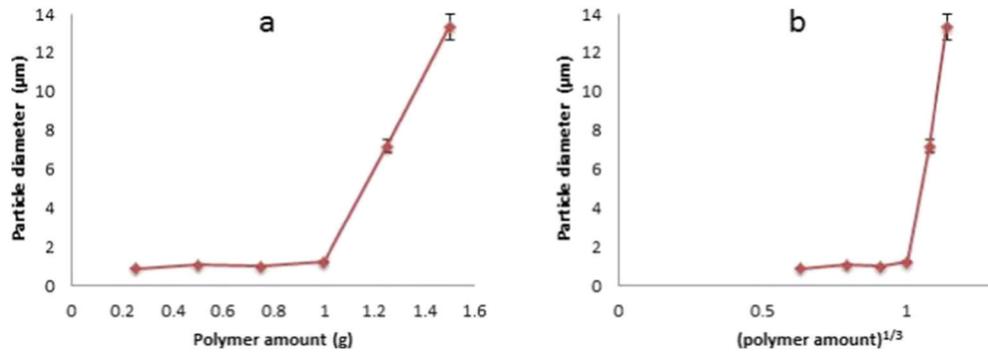


Fig. 2. (a) Effect of polymer amount on the hydrodynamic particle diameter. (b) Average hydrodynamic diameter versus (polymer amount)^{1/3}.

aggregated and polydisperse size. Particles were well formed displaying larger pore size in 1.50 g polymer sample as compared to particles prepared using 0.25 g and 1.00 g polymer. Whereas, a progressive increase in dimensions was observed according to the results of particle size distribution (Fig. 3 (d, e and f)). The size distribution for 0.25 g polymer sample was in the range of 0.25–1.7 μm and the size range for 1.50 g polymer sample was in between 4 and 26 μm. Porosity is more visible in larger particles due to larger size as compared to smaller particles where other characterization techniques are required to get an exact estimation of porosity. It was concluded from this study, that 1.00 g of polymer would be best amount to be used for next experiments as our aim was to achieve positively charged and non-aggregated dispersion near to 1.00 μm in size, which was reached by using 1.00 g of the polymer (Fig. 3 (b and e)).

3.1.2. Ultrasonication time effect

Input of energy is an essential key factor to achieve a uniform dispersion of non-miscible phases and principally emulsions [34]. This required energy can be supplied through various means which include high stirring, ultrasonication, high shearing or membrane based technology [16]. High intensity ultrasound brings out a wide range of physical and chemical consequences. The chemical effects basically result from acoustic cavitations (the formation, growth and collapse of bubbles). Bubble collapse in liquids gives a huge concentration of energy. This energy is produced from conversion of kinetic energy of the liquid motion into heating of the contents of the bubble. The intense local heating and high pressures together with exceptional rapid cooling contribute towards means for driving chemical reactions. Thus, cavitation serves to concentrate the diffuse energy of the sound into a unique set

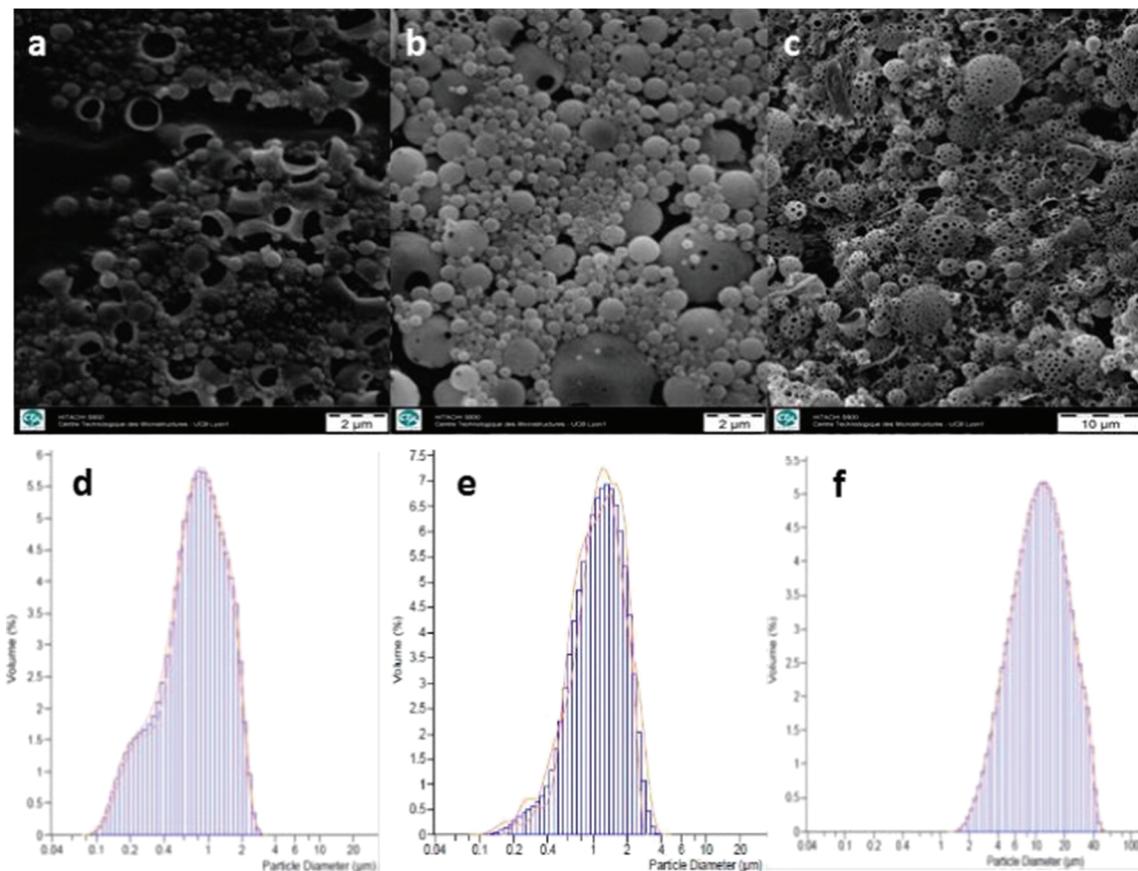


Fig. 3. SEM images and size distribution of samples prepared using (a, d) 0.25 g of Eudragit® RS100 (b, e) 1.00 g of Eudragit® RS100 (c, f) 1.50 g of Eudragit® RS100 respectively.

of conditions. Physical effects of sonication (often having chemical consequences) include improved mass transport, emulsification and bulk thermal heating.

In this study, ultrasonication was used for the formation of primary emulsion (w/o). We investigated the influence of sonication time on the final size and morphology of the particles. 1.00 g of the polymer amount was selected based on results in the first study above described. The sonication time was varied from 1.5 min to 6.0 min. All other parameters for the process were kept constant as already shown in Table 1. The amplitude and pulse of sonication was kept constant to 70% and 10 respectively, throughout the experiment. It was observed that the particles size was not significantly influenced by an increase in sonication time (Fig. 4). Generally, in previous studies relating to double emulsions, it has been observed that any parameters changed at secondary emulsion stage show remarkable influence on the final particle's properties as compared to parameters varied at primary emulsion stage [16]. These results are in accordance with the previous studies as well. The size of droplet at this stage influences the final particle size. The objective of using sonication in this study was to obtain uniform and small droplets at primary emulsion stage as compared to relatively larger droplets that are obtained by using high stirring apparatus like ultra turrax® at this stage. In short, ultra sonication gave smaller droplets as compared to ultra turrax® but any increase in time of ultra sonication showed no effect on size and morphology of the resultant final particles because the droplet size produced by ultra sonication (primary emulsification) is small enough to not influence the final particles size produced by ultra turrax® (secondary emulsification). In other words, final particles size is quite large due to limitation of ultra turrax® as compared to droplet size produced by ultra sonication. So, whatever the effect of increase in ultra sonication time has, will not be observed in the final particles.

Two samples prepared using 1.00 g of the polymer at ultrasonication times of 1.5 min and 6.0 min were selected for the SEM analysis to observe the effect of ultrasonication time on the morphology of the particles. The SEM images (Fig. 5 (a and b)) showed that there was no considerable difference in the morphology of particles. Particles were spherical in shape, non-aggregated and porous. The size analysis (Fig. 5 (c and d)) also displayed no significant difference in the particles sizes for both samples. Consequently, ultrasonication time of 1.5 min was selected for the next parameters investigation.

3.2. Parameters investigated: water in oil in water emulsion ($W_1/O/W_2$) stage

Three parameters were investigated at secondary emulsion formation stage: 1) ultra turrax® stirring time, 2) ultra turrax® stirring speed, and 3) volume of external aqueous phase. All the other parameters were kept constant as shown in Table 2.

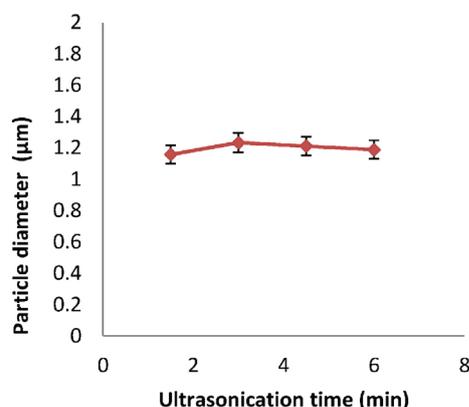


Fig. 4. Effect of ultra sonication on the final size of particles for sample prepared using 1.00 g of the polymer.

3.2.1. Ultra turrax® stirring time effect

The energy required for the formation of second emulsion was provided to the system by the use of high performance stirring apparatus ultra turrax® T-25. The time duration of energy supply is quite important in the formation of double emulsion because second emulsification must be dispersed via a proper stirring process to avoid aggregation of particles as well as to prevent obtaining highly and large polydisperse particles dispersions. So, it is needed to optimize the stirring time to provide sufficient energy to the system for good emulsification. This experiment was carried out with the objective to achieve the above-mentioned goal.

All parameters such as sonication time, ultra turrax® speed, polymer amount etc. were kept constant as shown in Table 2, whereas, only ultra turrax® stirring time was varied from 3 min to a maximum of 15 min. The results of this study reported in Fig. 6 indicated that, in general, the particle size decreased with an increase in stirring time of ultra turrax® and 10 to 15 min were quite suitable enough for second emulsification. The decrease in size occurs due to longer energy supply from longer stirring times. This energy helps in better fragmentation of the polymeric solution into smaller droplets. Here, 15 min were selected for performing the future experiments since the particle size was the lowest at this point. Longer duration for stirring such as more than 20 min was not good due to overheating of the sample and aggregation of particles.

Scanning electron microscopy analysis of two samples stirred for 3.0 min and 15 min was performed. Spongy and large spherical particles were seen when 3.0 min stirring time was used compared to 15 min. Large particle size and large size distribution were observed in 3 min sample stirring time (Fig. 7 (a and c)). But, for 15 min sample, smaller size and narrower size distribution was observed (Fig. 7 (b and d)). This means that polydispersity of particles was also decreased as seen from the SEM images and size distribution data of sample prepared when 15 min stirring period was used. More supply of energy from longer stirring time result in better fragmentation of the polymer solution into smaller droplets and consequently more narrow size distribution with low polydispersity.

3.2.2. Ultra turrax® stirring speed effect

In this experiment, the effect of ultra turrax® stirring speed on the final particles size at second emulsion stage was investigated. As observed in previous studies, the stirring speed is a dominant factor in controlling the particle size in double emulsification technique [34]. The energy supplied by this agitation should not be very severe especially during the second step of emulsification because it may rupture the droplets obtained from the first emulsion [35]. Here, several experiments were performed by changing the stirring speed of ultra turrax® and keeping all other parameters constant as shown in Table 2.

The results of this study reported in (Fig. 8) showed that the average particle size of 17 µm at the lowest speed of the instrument i.e. 6500 rpm was obtained. A marked decrease in size from 17 µm to 1.87 µm was observed with increasing in speed to 9500 rpm. The size further decreased to 0.92 µm when speed was 13,500 rpm. The decrease in size occurs due to better fragmentation of polymer solution into smaller droplets at higher speeds. However, this decrease was observed only up to a certain specific speed. Certain difficulties are faced with respect to working at higher speeds above 13,500 rpm such as 21,000 rpm and 24,000 rpm. In fact, using high stirring speed leads to disruption of formed particles, high foam formation and also heating effect inducing solvent evaporation.

Scanning electron microscopy analysis showed that all the particles obtained at the three used speeds were spherical, porous, non-aggregated and polydisperse in size (Fig. 9 (a, b and c)). Larger particle size and wider size distribution was seen in samples prepared using 6500 rpm and 9500 rpm ultra turrax® speeds compared to 13,500 rpm speed (Fig. 9 (d, e and f)). As evaluated from the size

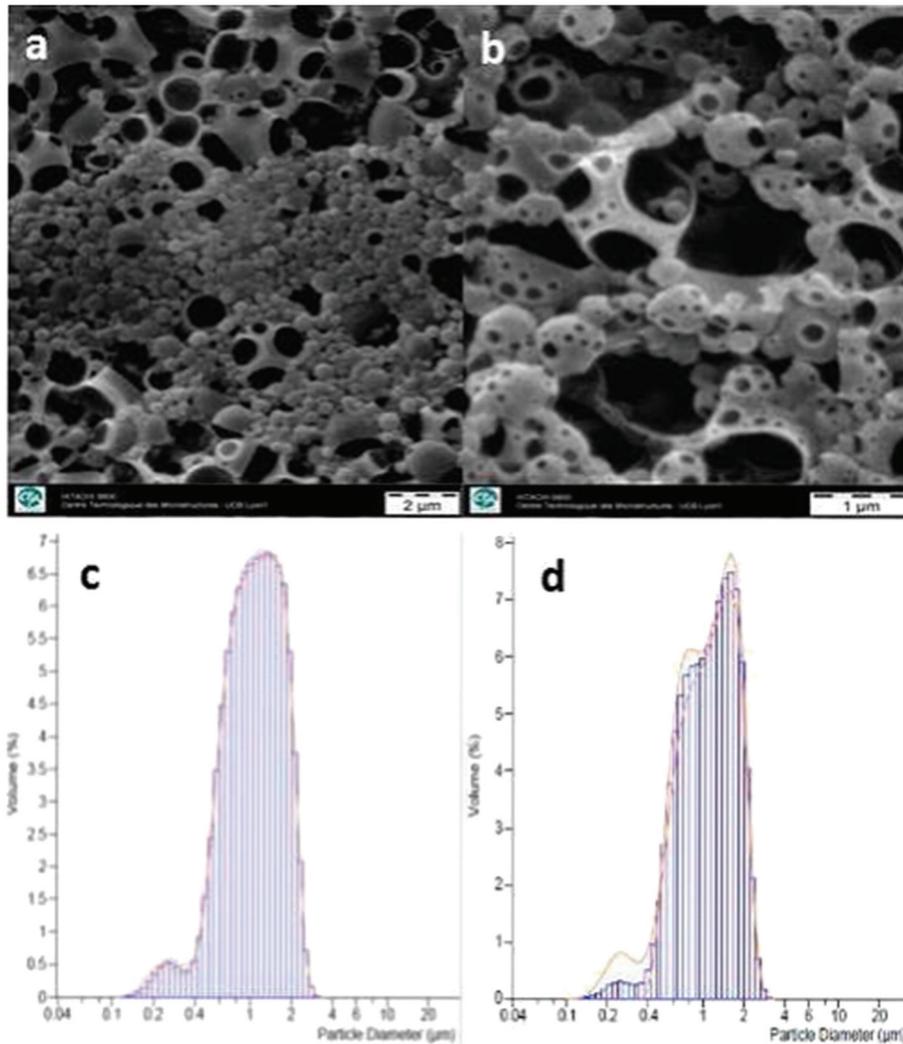


Fig. 5. SEM images and particle size distribution of samples prepared at ultrasonication time of (a, c) 1.5 min and (b, d) 6.0 min.

distribution data, the different ranges within which 90% of the particles fall for the three ultra turrax® speeds were approximately: 0.4–30 μm for 6500 rpm, 0.4–3.53 μm for 9500 rpm and 0.2–1.57 μm for

13,500 rpm. This confirmed that an ultra turrax® stirring speed of 13,500 rpm was appropriate to be use in order to obtain narrowly size distribution in micron diameter range.

Table 2

Parameters (investigated and changed) at second emulsion stage.

Parameters observed	W ₁ /O emulsion					W ₁ /O/W ₂ emulsion			Dilution Vol. of water for dilution (ml)	Size (μm)
	Vol. of DCM (ml)	Eudragit® RS100(g)	Vol. of inner aqueous phase (ml)	Sonication power(%)	Sonication time(min)	Ultra-turrax time(min)	Ultra-turrax speed(rpm)	Vol. of external aqueous phase (ml)		
1 Ultra-turrax stirring time	5	1.0	1.0	70	1.5	Varied	13,500	50	100	9.78
						3				1.56
						6				1.21
						9				1.11
						12				1.04
2 Ultra-turrax stirring speed	5	1.0	1.0	70	1.5	15	Varied	50	100	17.11
							6500			1.87
							9500			0.92
3 Vol. of external aqueous phase	5	1.0	1.0	70	1.5	15	13,500	Varied	100	0.92
								30		1.04
								45		1.25
								60		1.21
								75		1.34

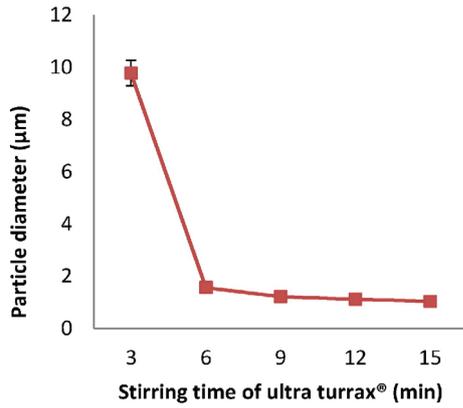


Fig. 6. Effect of ultra turrax® stirring time on the mean particle diameter. (All the parameters for first emulsion were constant).

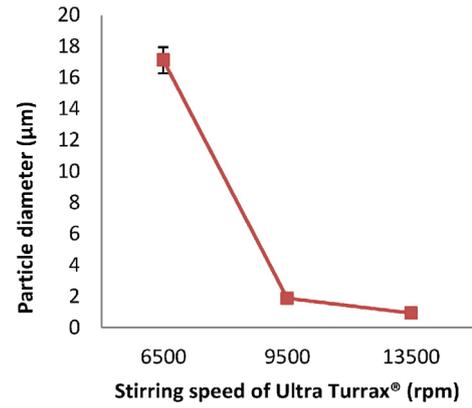


Fig. 8. Effect of ultra turrax® stirring speed on the mean particle diameter. (All the parameters for first emulsion were constant).

3.2.3. Effect of outer aqueous phase at double emulsion stage

The effect of outer aqueous phase volume on the final Eudragit® RS100 based particles prepared by double emulsion was also investigated. Five different samples containing different volumes of external aqueous phases were prepared while keeping all the other parameters

for the formulation constant as displayed in Table 2. It was observed that there was a very small (almost non-existent) increase in particle size with an increase in outer aqueous phase volume (Fig. 10). The particle size was 0.92 µm for 30 ml of outer aqueous phase and the size increased to 1.34 µm for 90 ml of the external aqueous phase. This

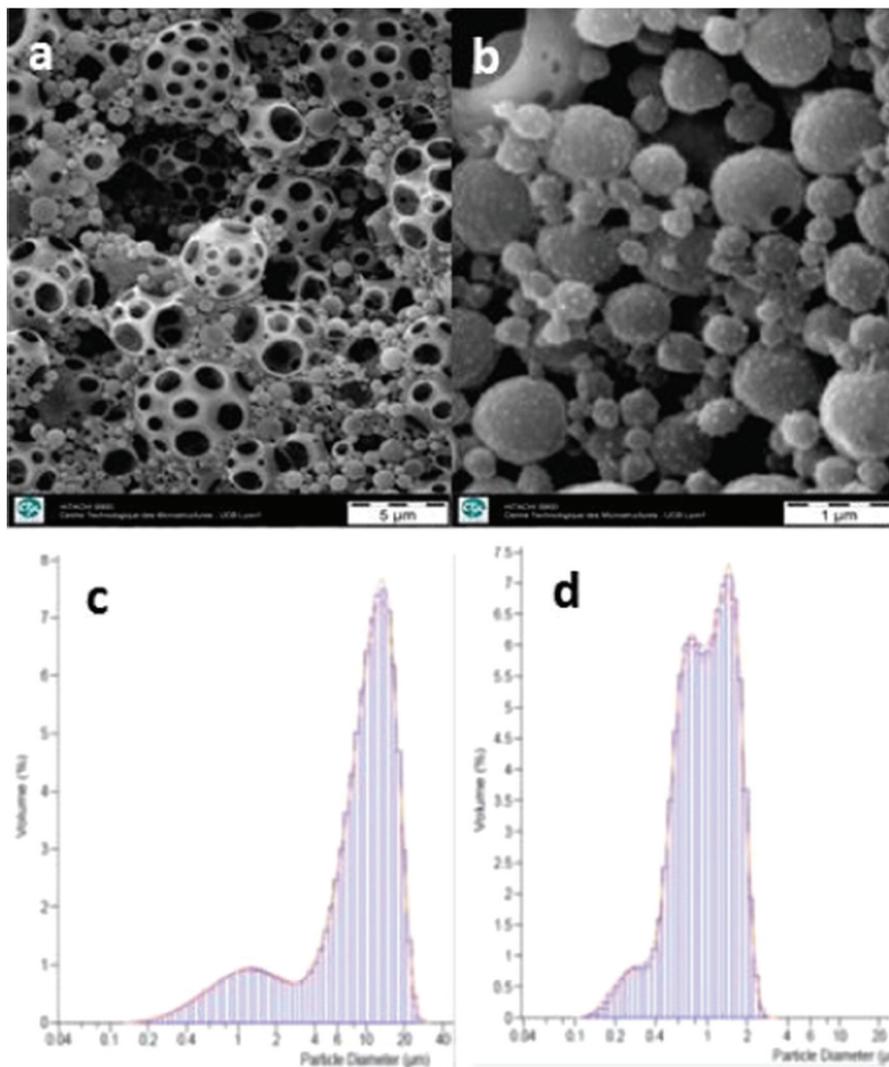


Fig. 7. SEM images and particle size distribution of samples prepared at ultra turrax® stirring time of (a, c) 3.0 min and (b, d) 15.0 min.

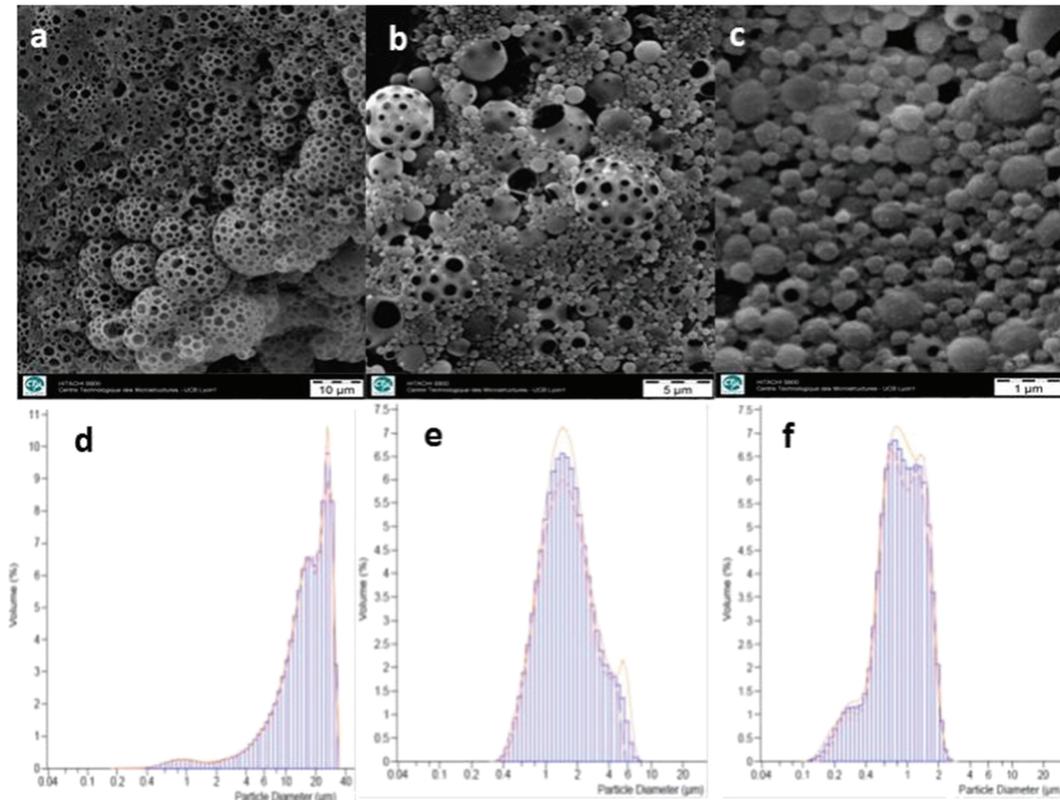


Fig. 9. SEM images and particle size distribution of samples prepared at ultra turrax® stirring speed of (a, d) 6500 rpm (b, e) 9500 rpm and (c, f) 13,500 rpm.

insignificant difference in size can be attributed to greater distance between the shearing forces from the ultra turrax® probe and the droplets because of the larger volume of external aqueous phase or in other words the efficacy of ultra turrax® was decreased. So, it was concluded that external aqueous phase had insignificant effect on the final particles size and 30 ml volume of water was selected for the future experiments. Volume less than 30 ml was not used because of the limitation of the experimental conditions.

Scanning electron microscopy analysis of two samples (containing 30 ml and 90 ml of external aqueous phase) also confirmed that external aqueous phase has almost negligible influence on the particles morphology and size distribution as reported in (Fig. 11).

3.3. Scale up studies

The previous investigations helped us to optimize the different process control parameters in order to get the desired Eudragit® RS100 based microparticles prepared via double emulsion solvent diffusion-

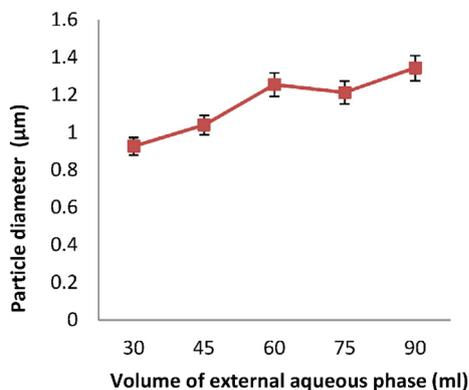


Fig. 10. Effect of outer aqueous phase volume on mean particle diameter.

evaporation technique. These optimized parameters were then further investigated for their applicability on a scale up recipe as shown in Table 3. The recipe was scaled up to four and ten times of its original recipe. Each of four and ten times scale up studies were performed in duplicate to confirm the consistency of results.

It was observed that the recipe which was scaling up four times, displayed mean particles size in between 1.87–2.42 μm. Whereas, the ten times scale-up experiment showed mean particle size between 3.99–4.35 μm. For the four times scale up, the results were totally reproducible since the particle size was found to be 1 μm range. For the ten times scale up, the volume was too large to give the same size ranges. This was due to loss of efficacy of ultra turrax® to prepare microparticles in larger volumes under the same experimental conditions and in the same size range. Hence, the result of this study explains the flexibility of the original recipe to only four times scale up. The SEM analysis and particle size distribution data also confirm the above-mentioned results (Fig. 12). Particles were spherical and stable since no aggregations were observed during the preparation and even after storage for some days.

3.4. Effect of dilution phase

All the previous experiments in this study were performed using dilution phase as the last step to finish off the process. This step was used to facilitate the diffusion of organic solvent from inside the particles to external aqueous phase. We decided to study the influence of this dilution phase on the final particle size. Three experiments were performed to study the influence of dilution phase as detailed in (Table 4). First experiment was performed without the dilution step. This was done to compare its results with that of an experiment done with dilution step. All of these experiments were performed using the four times scaled up recipe.

Table 4 showed that the obtained particles have almost the same diameters, with or without dilution. Due to the poor solubility of DCM in water, the dilution step has no effect on the particles size. Even with

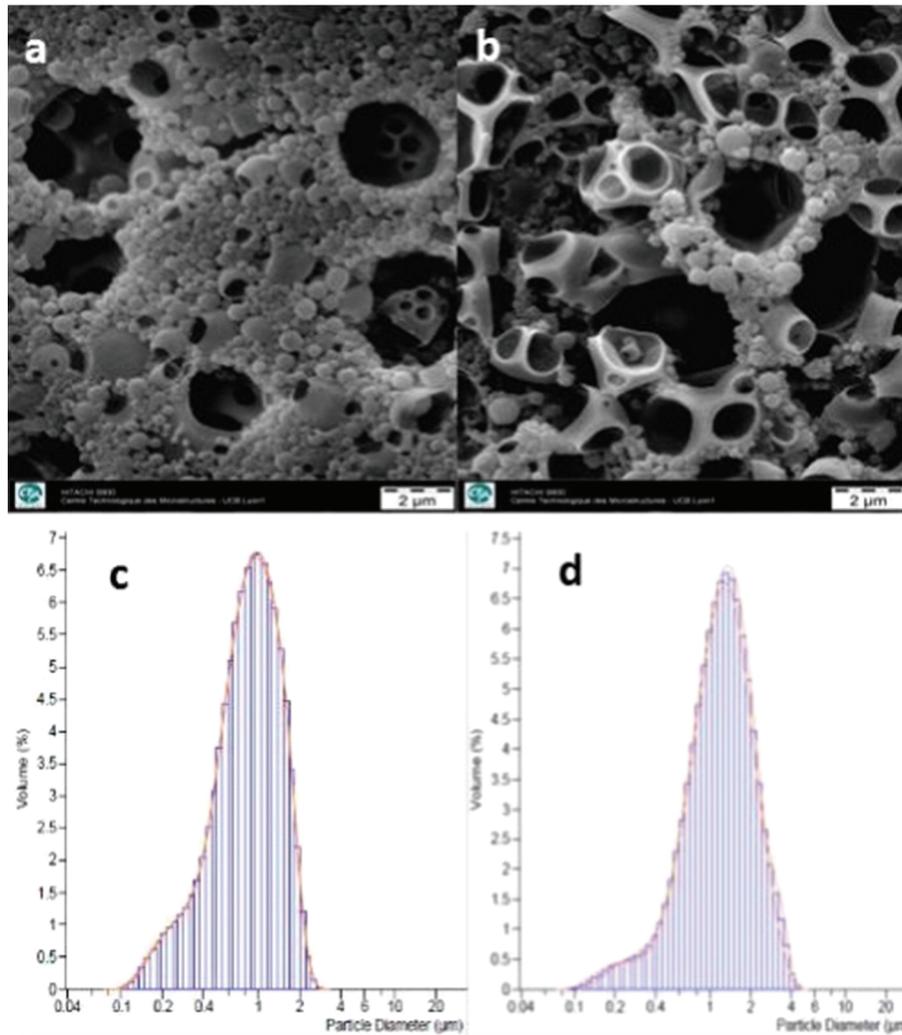


Fig. 11. SEM image and particles size distribution of samples prepared using (a, c) 30 ml and (b, d) 90 ml of external aqueous phase at secondary emulsification stage.

high dilution volume, 800 ml, particles diameter was in the same range like without dilution, which confirms that the diffusion process has no effect on the particles size in these conditions. Furthermore, a set of four experiments were performed to investigate the effect of increasing volume of water phase employed for the dilution phase. The different volumes of water used were 240 ml, 320 ml, 600 ml and 800 ml. The results (Fig. 13) confirmed that the dilution step has no considerable effect on the final particle size. This was probably due to poor solubility of DCM in water [36] i.e. 1.3 g/100 ml at 20 °C. The mean sizes of particles observed for all the different volumes of water were almost the same. Thus, it was concluded that dilution phase just helps in facilitating the diffusion of organic solvent into aqueous phase while having insignificant effect on the final particle size.

A sample containing 240 ml of the dilution phase and another sample containing 800 ml of the dilution phase were selected for SEM and particle size analysis. The SEM images and size distribution data displayed no considerable difference between the particles in both samples (Fig. 14). Morphology and size distribution were observed and found to be similar.

3.5. Zeta potential

Zeta potential of all prepared particles was studied to evaluate the stability of the prepared particles and to investigate how the pH of the surrounding media influences the surface charges of the particles (Fig. 15). Zeta potential (1 mM NaCl, 20 °C) at pH values ranging from

Table 3
Parameters for the optimized and scale up studies.

Studies	W ₁ /O emulsion					W ₁ /O/W ₂ emulsion			Dilution Vol. of water for dilution (ml)	Size (μm)
	Vol. of DCM (ml)	Eudragit® RS100 (g)	Vol. of inner aqueous phase (ml)	Sonication power (%)	Sonication time (min)	Ultra-turrax time (min)	Ultra-turrax speed (rpm)	Vol. of external aqueous phase (ml)		
Optimized recipe	5.00	1.00	1.00	70	1.50	15	13,500	30	100	1.00
4 times scaled up recipe	20.00	4.00	4.00	70	1.50	15	13,500	120	400	1.87
10 times scaled up recipe	50.00	10.00	10.00	70	1.50	15	13,500	300	1000	3.99
										4.35

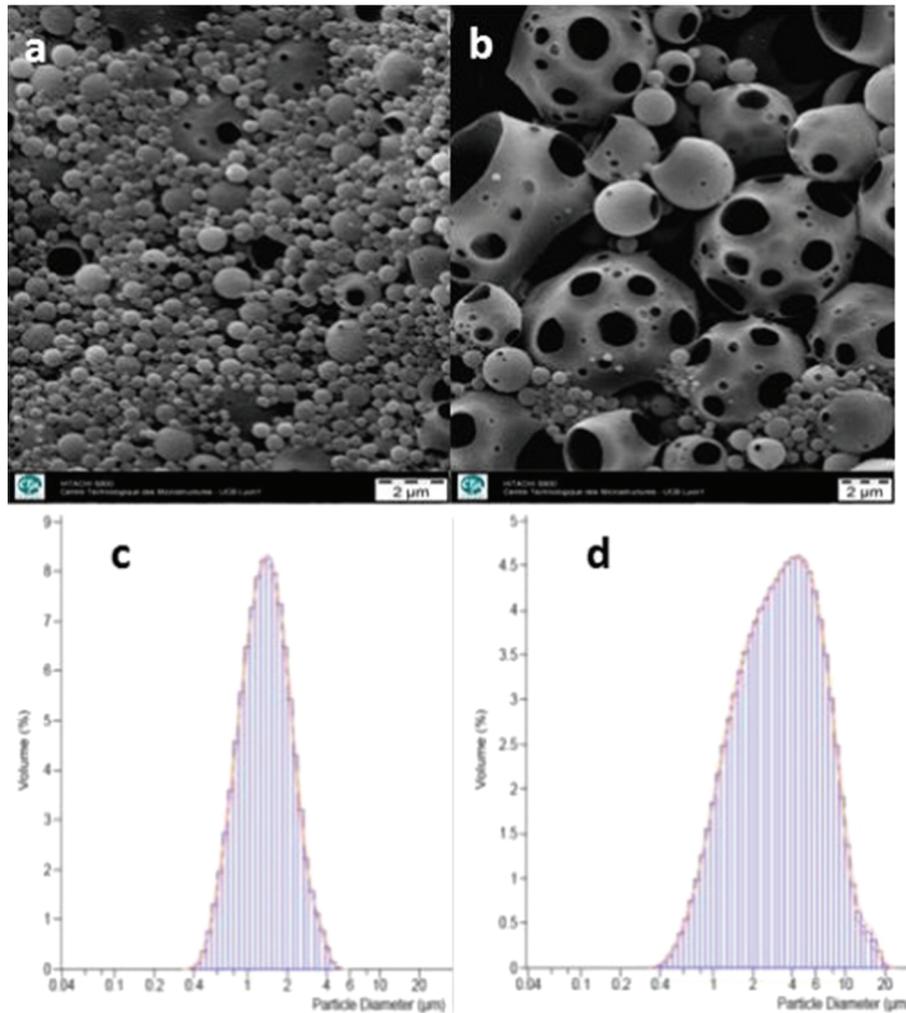


Fig. 12. SEM image and particle size distribution of samples prepared using (a, c) 4 times and (b, d) 10 times scaled up recipe.

3 to 11 was studied. The measured zeta potential of all the particles prepared in this study was found to be + 50 mV, positive and was constant throughout the study irrespective of the various process control parameters variation. This positive zeta value is due to the quaternary ammonium groups present in the structure of Eudragit® RS100.

However, it was observed that zeta potential was influenced by the pH of the surrounding media. The zeta potential values were almost constant up to pH 8 (between 50 and 40 mV) but a decreasing trend in zeta potential with pH values higher than 8 was observed. This behavior can be best explained by the chemical structure of the polymer Eudragit® RS100. It is seen that the polymer has quaternary ammonium

groups, which give positive charge to the particles. However, in basic media, this positive charge is shielded by the condensation of negative charges around the surface of microparticles, contributed by the increasing OH^- ions from the increase in pH of the surrounding media. Hence a decrease in zeta potential is observed with an increase in pH values.

4. Conclusion

Double emulsion–diffusion solvent evaporation technique was used to prepare cationic ammonio methacrylate copolymer Eudragit® RS100

Table 4
Parameters for the experiments investigating the effect of dilution phase.

Studies	W_1/O emulsion				$W_1/O/W_2$ emulsion			Dilution	Size (μm)
	Vol. of DCM (ml)	Eudragit® RS100 (g)	Vol. of inner aqueous phase (ml)	Sonication power & time	Ultra-turrax time (min)	Ultra-turrax speed (rpm)	Vol. of external aqueous phase (ml)		
With dilution	20.00	4.00	4.00	70% 1.5 min	15	13,500	120	400	1.67
Without dilution	20.00	4.00	4.00	70% 1.5 min	15	13,500	120	–	1.64
Serial dilution	20.00	4.00	4.00	70% 1.5 min	15	13,500	120	240 320 600 800	1.69 1.66 1.66 1.59

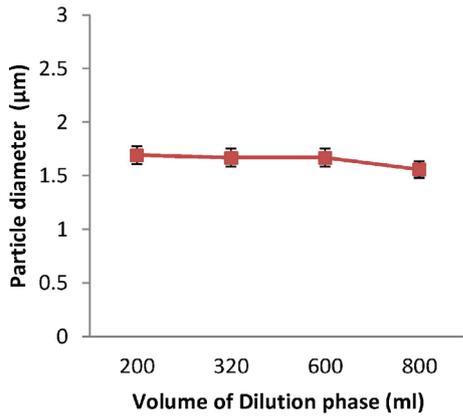


Fig. 13. Effect of dilution phase on mean particle diameter.

based microparticles. A stable emulsion was formed without using of any stabilizer. The influence of various process control parameters such as polymer amount, ultra sonication time, ultra turrax® stirring speed and time, external aqueous phase volume and dilution phase volume were investigated as systematic study. The morphology and size distribution were studied by using Laser Diffraction Size Analyzer and SEM. It was found out that, the amount of polymer Eudragit® RS100,

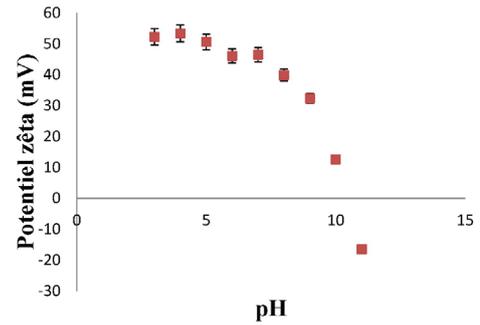


Fig. 15. Zeta potential of the particles against different pH values.

ultra turrax® stirring time and speed were important factors in controlling the size of the particles. This study helped us to fix these controlling parameters and obtain an optimized recipe to prepare cationic microparticles via this Double emulsion–diffusion solvent evaporation technique.

Apart from optimization of this technique, some other important findings also came into light from this systematic study. Most importantly, this process is able to be used for preparing micrometer particles, cationic in nature, multi-hollow and porous surface. In future study, these cationic microparticles will be evaluated for simultaneous loading

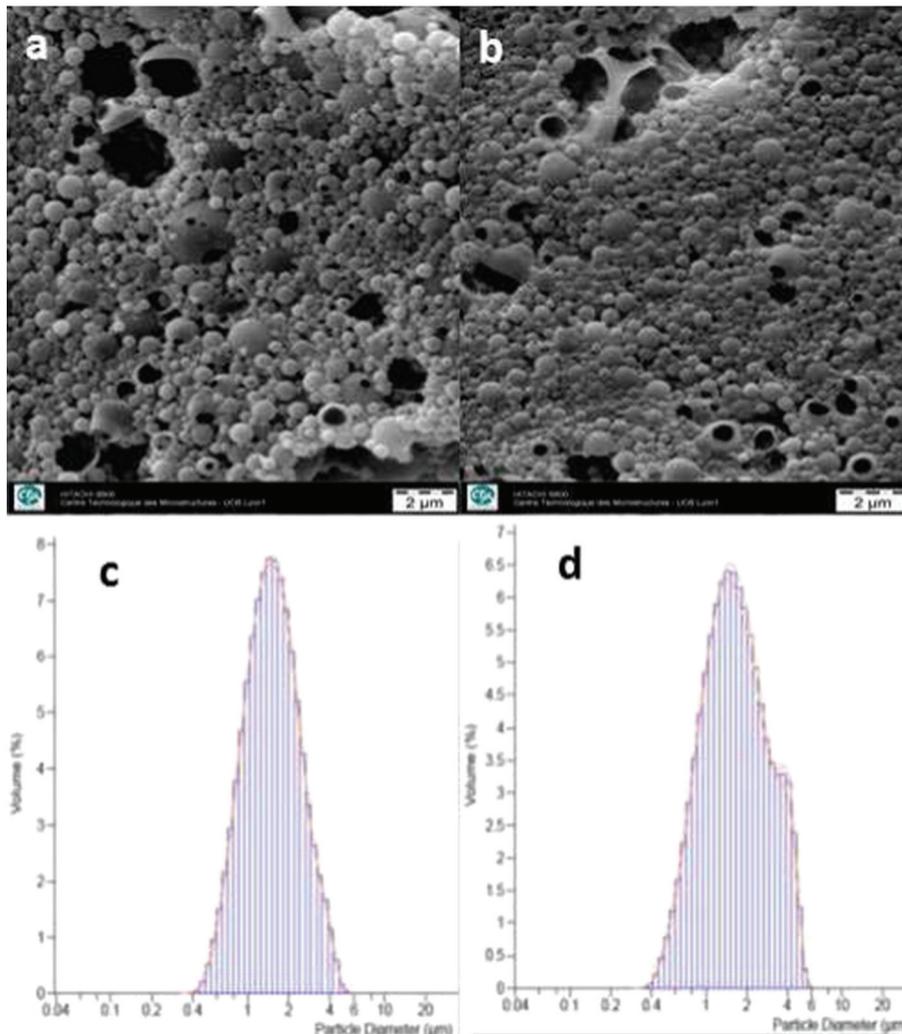


Fig. 14. SEM images and particle size distribution of samples prepared using (a, c) 240 ml and (b, d) 800 ml of deionized water in the dilution phase respectively.

of active agents of opposing nature e.g. hydrophilic and hydrophobic agents.

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III.3. Investigation of electrokinetic properties of selected textile

Summary

Nowadays, technological innovations and commercial requirement for high-quality products is pushing the textile industry towards refined and diverse markets with versatile products that are to be used for many different applications ranging from agriculture, filtration, medical and hygiene, civil engineering, protective clothing, packaging, transport, sportswear, defense and safety. For these progressively increasing applications of textiles, their surfaces are specially engineered to provide a product with desired characteristics. Textile technology includes many disciplines like the structure of fibers, assembly of fibers into fibrous structures and fabrics, surface modification of fibers, making, analysis and sale of fabrics. Surface modification is of profound importance since it imparts variety of useful properties such as dyeability, softness, absorbency and wettability. Functional properties are also imparted to textiles via surface modification. In fact, unmodified textile surface might contain unsuitable properties that might negate their human use. The factors affecting the charges of textile and the technique employed to functionalize them are key parameters to achieve specially functionalized textile.

The electrokinetic properties hold a very important place in textile industries and among them zeta potential or electrokinetic surface charges are playing significant role in textile functionalization. The electrokinetic (zeta) potential is a part of total drop in potential occurring in the intermediate surface layer at the boundary of solid/liquid phases due to distribution of ions from solid surface to the liquid mass. Zeta potential of textiles provides significant information about the dissociation of ions, hydrophobicity/hydrophilicity of the fibers surface. Zeta potential is usually measured by using principles like electrophoresis, streaming current/potential and sedimentation potential. The streaming current/potential is most commonly employed for zeta potential measurement of fibers.

Specific functionalization of the textiles can be achieved by physical immobilization of nano or microparticles encapsulating drug molecules. These colloidal carriers can be prepared from variety of polymers using different encapsulation techniques available like nanoprecipitation, double emulsification etc.

The aim of our study was to investigate the influence of pH and salinity on the zeta potential of the particles and bare textile. Moreover, after adsorption of particles onto textile, the effects of pH, salinity and amounts of particles adsorbed on the zeta potential of particles were also observed. Results showed particles to be spherical and sponge like in morphology with positive zeta potential contributed by the cationic character of the polymer used. The zeta potential of the bare textile deduced from streaming potential was found to be negative irrespective of pH. Increasing salinity seemed to decrease the zeta potential of bare textile. The increasing amount of particles in the adsorption medium resulted in shifting of the negative zeta potential of textile to positive due to progressive adsorption of positively charged particles onto negatively charged textile. Such functionalized textile may find expanded applications in the dermal delivery of cosmetic and therapeutic agents.

Electrokinetic properties of bare and particles containing textile

Nadiah Zafar, Jean Pierre Valour, Geraldine Agusti, Hatem Fessi and Abdelhamid Elaissari*

Functional, reactive and smart textiles are nowadays of great interest in the fields of both cosmetic and pharmaceutical industries. Textile functionalization according to demands of the consumers can only be achieved with better understanding of the properties of the textile surface as well as sound knowledge about the technique to be used to functionalize the textiles. The aim of this work was to investigate the zeta potential of polyamide textile and prepared sponge like particles as a function of pH and salinity of the electrolyte. Special attention has been dedicated to the electrokinetic properties of textile as a function of sponge like particles amount present during adsorption process. As a general tendency, streaming potential measurement showed the changes in surface charge density of textile because of the changes in pH, salinity and adsorbed particles amount. All presented results were discussed in terms of zeta potential. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: streaming potential; zeta potential; adsorption; cationic particles; polyamide textile

INTRODUCTION

Over the past few decades, the needs and market for smart and technical textiles have been progressively growing, leading to further advancements and novelty in textiles for new functionalities. The textile performance according to demands of the consumers is enhanced by encompassing a large range of properties with higher added value.^[1] To accomplish this, formulation and understanding of polymeric surfaces have advanced exceedingly, thus allowing achieving systems well-defined functionality. The properties of textile, factors affecting charges of textile and the technique utilized to functionalize textiles are very important parameters to obtain specially required functionalized textile.

The electrokinetic properties of textile fabrics are very significant, e.g., from dyeing and finishing point of view. Under the electrokinetic properties, the zeta potential, i.e., the electrokinetic surface charge of the fabric plays an integral role. The electrokinetic potential is part of total potential drop which occurs in the intermediate surface layer at the boundary of the solid/liquid phases as a result of the ions distributing from the solid surface to the liquid mass. At the interface of an electrically charged textile and an aqueous solution of electrolyte, dyes or surfactants form an electric double layer. Electrokinetic (zeta) potential results from the movement of one of these two charged surfaces. Zeta (ζ) potential is not a constant value for the textiles, and it provides very useful information about the nature and dissociation of functional groups, hydrophobicity/hydrophilicity of the fiber surface and ions or water sorption. Moreover, this potential is also important for the characterization of textiles for the wet processing.^[2,3] Generally, surface charge is provided by the specific adsorption of ions or dissociation of surface groups in aqueous solution. The surface charge of the fiber depends on the molecular and supramolecular structure, swelling capacity and ionogenicity, structure and concentration of the adsorbate. Therefore, wet treatments result in change in the surface charge of the fibers as well as change their zeta potential

values and adsorption abilities.^[4] Zeta potential measurements are generally made using different principles like electrophoresis, sedimentation potential, electro-osmosis, streaming current/potential, potential of colloidal vibration and electrokinetic sound amplitude. Mostly, streaming current/potential or electro-osmosis is used for the fibers and electrophoresis is commonly used for the dyes. The streaming potential/current is most commonly used for measurement of zeta potential of the fibers as it linearly depends on pressure (both amount applied and direction) and the results obtained are reproducible.

The specific textile functionalization is obtained by impregnation or physical immobilization of nano-objects encapsulating active drug molecules. These nano-objects are obtained by encapsulating active agents in a polymer matrix (particles or capsules), and then are fixed onto textile fibers.^[5–7] There are different encapsulation techniques like radical polymerization,^[8] interfacial polycondensation,^[9] spray coating,^[10] prilling,^[11] spray drying,^[12] coacervation,^[13] nanoprecipitation^[14] and solvent evaporation^[15,16] to prepare microparticles. The selection of particular technique primarily depends on the nature of the active molecules and the final application of the formulation.

The objective of this study was to investigate the influence of the amount of microparticles adsorbed on the zeta potential of a specifically selected textile. To achieve this objective, prior studies of the influence of pH and salinity on the zeta potential of the textile as well as zeta potential of the particles were performed separately. The microparticles were cationic in nature and were prepared by using positively charged polymer Eudragit® RS100 and double emulsion–diffusion solvent

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evaporation technique. The fabric selected for adsorption was polyamide based textile. Polyamide fibers are type of synthetic fibers which have recurring amide groups as an integral part of the main polymer chain commonly named as Nylon. Nylon is probably the most useful synthetic polymer material and one of the most commonly used materials in textile industry.

EXPERIMENT

Materials

Ammonio methacrylate copolymer type BNF Eudragit® RS100 was purchased from EVONIK (EVONIK, Germany) and was used as such. Dichloroethane (DCM), NaCl, NaOH and HCl are from Sigma-Aldrich, Germany. Deionized water was used throughout the work. Polyamide based textile used was kindly provided by GERVARIS company.

Particle preparation

Double emulsion solvent evaporation process was used to prepare polymethacrylate particles based on the following recipe: 1 ml of aqueous solution was first dispersed in organic polymer solution (1-g polymer in 5-ml DCM) using ultrasound system (Optic ivymen system CY-500) for 1.5 min at 70% amplitude. The obtained primary emulsion, i.e., (w/o) was then dispersed in 30-ml water solution using ultra turrax® (T-25 basic IKA®-Werke) for 15 min at a speed of 13,500 rpm. For the preparation of this dispersion, no stabilizer was needed. The obtained double emulsion (w/o/w) was then diluted with 100-ml aqueous solution before DCM evaporation using rotavapor (Buchi-rotavapor R-124).^[17]

Particles size measurement

Particle size and size distribution were measured with a Laser Diffraction Particle Size Analyzer (Mastersizer 3000, Malvern). Samples were prepared by adding drops of aqueous particle suspension to the instrument container, already filled with distilled water. An average diameter of three readings was calculated.

Electrokinetic study of particles

The electrophoretic mobility of highly diluted dispersion was measured as a function pH at a constant ionic strength or as a function of salinity but at constant pH. Each measurement was performed at 25°C using Zetasizer (Nano-ZS, Malvern®, UK). The zeta potential was deduced from the electrophoretic mobility using Smoluchowski zeta potential given by the instrument. Each measurement was made at least in triplicate.

Scanning electron microscopy

The surface morphology and the shape of the used materials (particles and textile) were examined using scanning electron microscope (SEM) a Hitachi S800 FEG microscope at the "Centre Technique des Microstructures" (CTμ) at the University of Lyon (Villeurbanne, France). For particle analysis, a drop of dispersion was placed on a flat steel holder (cell) and dried at room temperature before viewing. Regarding textile, it was analyzed as such after sticking on the SEM grid support. All samples were coated with platinum under vacuum by cathodic sputtering

before imaging. An accelerating voltage of 15 kV was used for SEM analysis of the samples.

Zeta potential of textile

The zeta potential of textile was examined as a function of pH and salinity using ZetaCAD® (CAD instruments). Briefly, this instrument (Zeta CAD) leads to streaming potential measurement which is converted to zeta potential. The pH of the circulating aqueous solutions was prepared at a constant ionic strength using NaCl, and the pH was adjusted using NaOH and HCl.

Adsorption of particles on textile

The adsorption of polymer particles on textile was performed by simple dipping of weighted piece of textile in a given amount of particles at fixed pH and ionic strength. The adsorption was conducted during time of 6 hr. The adsorbed amount was not quantified in this study because this is not the exact aim of this research work. After adsorption time, the textile was removed from the adsorption medium and washed one time in 1 mM NaCl solution by dipping process only.

RESULTS AND DISCUSSION

Particle characterization

The prepared polymethylmethacrylate based particles were characterized using various techniques such as light diffraction for size and size distribution, scanning electron for morphology and electrokinetic properties as a function of ionic strength and pH.

Particle morphology and size distribution

The surface morphology and shape of prepared polymethylmethacrylate based particles were examined using scanning electron microscopy (SEM). As can be seen from SEM images (Fig. 1a), the prepared particles were polydisperse in nature which explains the limitation of the used process based on Ultraturax® homogenization technique and fragmentation shearing energy. This is in good agreement with all prepared particles using double emulsion solvent evaporation and solvent diffusion processes. The morphology of the prepared particles was spherical explaining that the used high shearing process did not break the particles. The surface morphology of the formed particles was found to be sponge like. The presence of holes, cavities and caters like aspect was because of the polarity of the used positively charged polymethylmethacrylate based polymer and this polar part should keep interaction with water, which lead to such unexpected morphology compared to smooth one observed in the non-charged polymer such as polycaprolactone.^[18]

The particle size and size distribution were examined using Laser diffraction method and the obtained size distribution is illustrated below in Fig. 1b. The size distribution was found to be polydisperse as expected and generally reported in literature when such double emulsion based process was used even when the combination of ultrasound and Ultraturax® was used. The average particle size was found to be approximately 0.3 μm. This size distribution revealed the polydisperse character of the prepared particles as also evidenced from SEM analysis.

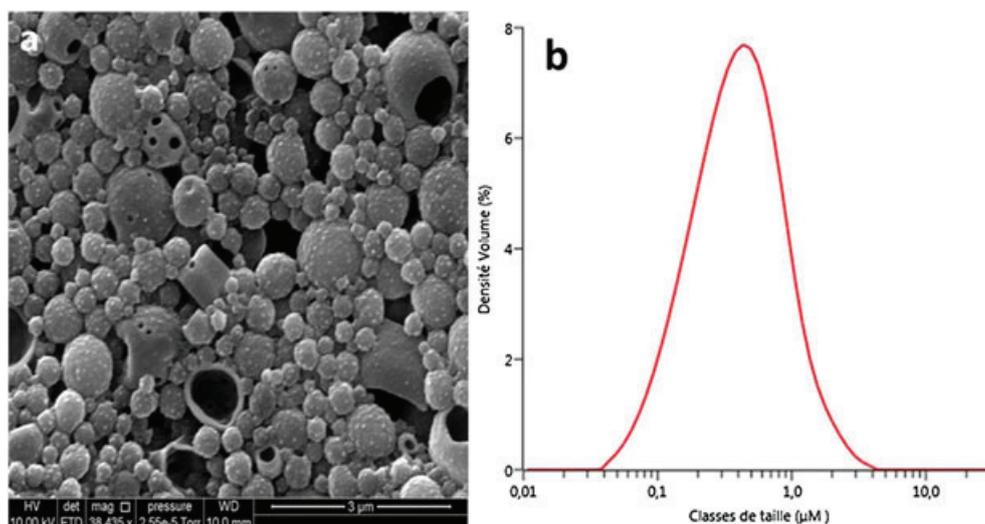


Figure 1. SEM micrographs of microparticles (a) and size distribution (b).

Effect of pH on zeta potential of particles

The zeta potential of the prepared polymethylmethacrylate particles was examined as a function of pH at constant ionic strength and temperature. The Smoluchowski's zeta potential deduced from the measured electrophoretic mobility was found to be positive until pH 10 (Fig. 2). This positive zeta potential is because of the cationic character of the used polymer. In fact, this polymer named EUDRAGIT® RS 100 is a copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester with quaternary ammonium groups. Then the positive zeta potential is attributed to quaternary ammonium group. Above pH 10, the zeta potential was found to be negative. This negative zeta potential can be attributed to hydroxyl group condensation on the particle surface leading to negative character of the particles.

The surface charge density deduced from the corresponding electrophoretic mobility (at pH in between 3 and 5 in 1 mM NaCl) was found to be around 8 mC/cm^2 . This value is close to those reported in the case of polystyrene latexes bearing amine or amidine groups, exhibiting approximately similar electrophoretic mobility at acidic pH.^[19]

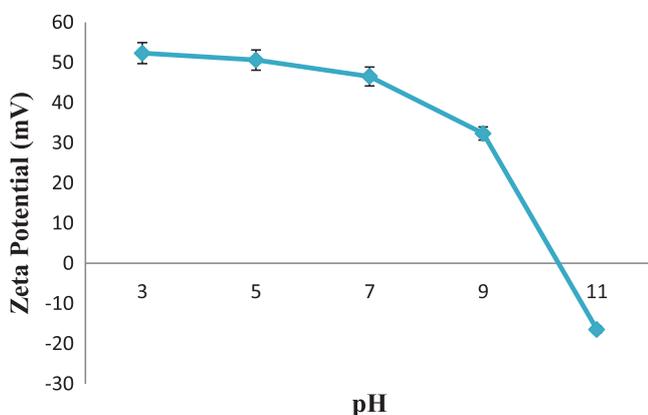


Figure 2. Zeta potential of the prepared particles as a function of pH in 1mM NaCl and at 20°C.

Effect of salinity on zeta potential of particles

First, the zeta potential measured at pH 9 and as function of ionic strength was found to be positive. Second, the determined zeta-potential was also investigated as a function of ionic strength at acidic (NaCl) at pH 9 and the obtained results are reported in Fig. 3. It appears that, no maximum was observed in the investigated salinity range. The decrease in the zeta potential versus ionic strength was observed as basically predicted by the classical electrokinetic theory^[20–22] In the case of smooth like particle bearing low surface charge density, the increase in salinity leads to a decrease in the potential energy (in absolute value); therefore, a decrease in the zeta-potential is observed. Such tendency is generally observed in the case of non-hairy like particles (i.e., polymer latexes prepared without surfactant or water soluble functional monomer).

Electrokinetic properties of textile

Prior to functionalization of the textile surface with the prepared cationic particles, brief characterization of the textile was done. The surface was observed under SEM before after adsorption of particles. The effect of pH and salinity of the electrolyte (NaCl)

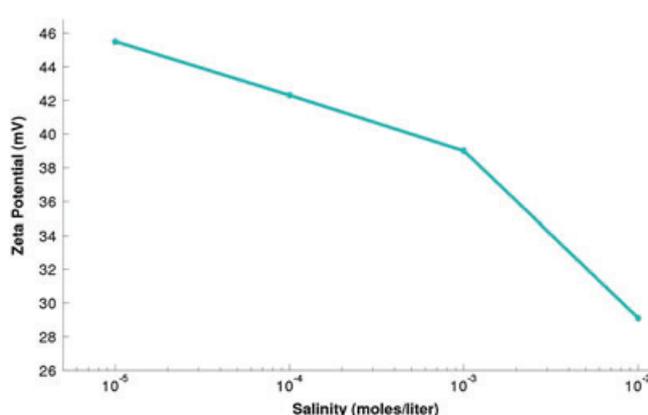


Figure 3. Zeta potential of the prepared particles as a function of ionic strength (NaCl) at pH 9 and at 20°C.

solution) on the zeta potential of the textile was studied by using Zeta CAD instrument. The adsorption of the particles was proved from the effect of different amounts of particles on the zeta potential of the textile.

Morphology analysis of bare and coated textile

The surface morphology of bare textile and textile containing polymethylmethacrylate particles were analyzed using SEM (Fig. 4). For bare textile, the surface was smooth and seemed to be homogeneous. Whereas for textile containing adsorbed particles showed heterogeneous surface exhibiting adsorbed particles. As can be seen also, the surface of textile was far to be saturated and this can be because of the non-homogeneous negative charge distribution of the surface of the fibers. In addition, the dimension of particles is lower than the dimension of textile; then the surface of textile can be considered flat seen by the particles.

Effect of pH on zeta potential of bare textile

The zeta potential of deduced from streaming potential was found to be negative irrespective of pH (Fig. 5). The absolute value of the deduced zeta potential was found to be low at acidic pH and high at basic pH which explains the possible ionization (i.e., dissociation) of surface groups on the textile. Then, from theoretical point of view, the pH affects the surface potential, and therefore, the relationships between the surface potential Ψ_s and charge density σ_d in the diffuse layer can be expressed as follow:

$$\sigma_d = -\sqrt{8} n_o \epsilon k_B T \sinh \left(\frac{e \Psi_s}{2k_B T} \right)$$

where, σ_d is the charge density in the diffuse part of the double layer, e is the Coulombic charge of an electron, k_B is the Boltzmann constant, T is the absolute temperature, ϵ is the permittivity of the medium and n_o is the solution ion concentration. The calculated charge density in the diffuse layer was found to be in between $-7 \mu\text{C}/\text{cm}^2$ at acidic pH and $-14 \mu\text{C}/\text{cm}^2$.

Effect of salinity on zeta potential of textile

The zeta potential of bare textile was also examined as a function of ionic strength at constant pH and temperature and the obtained results are reported in Fig. 6. The zeta potential (in

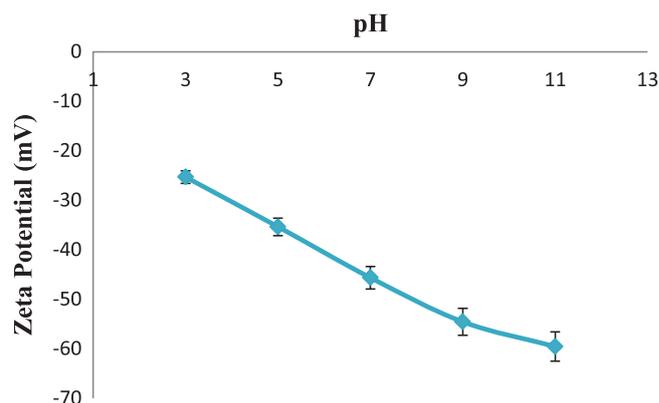


Figure 5. Zeta potential of textile as a function of pH in 1 mM NaCl and at 20°C.

absolute value) was found to decrease with increasing the salinity of the medium as theoretically expected. In fact, if we consider the potential at the shear plane position is the zeta potential, then by increasing the salinity of the medium a decreasing in the potential at the shear plane position is observed as explained by the following theoretical equation:

$$\Psi = \Psi_s \exp(-kx)$$

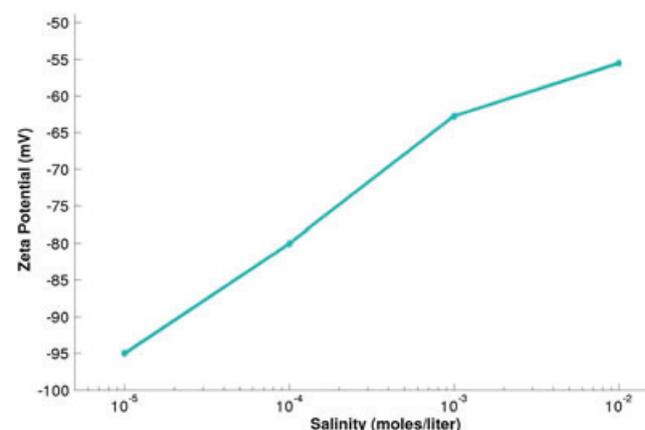


Figure 6. Zeta potential of textile as a function of ionic strength (NaCl) at pH 9 and at 20°C.

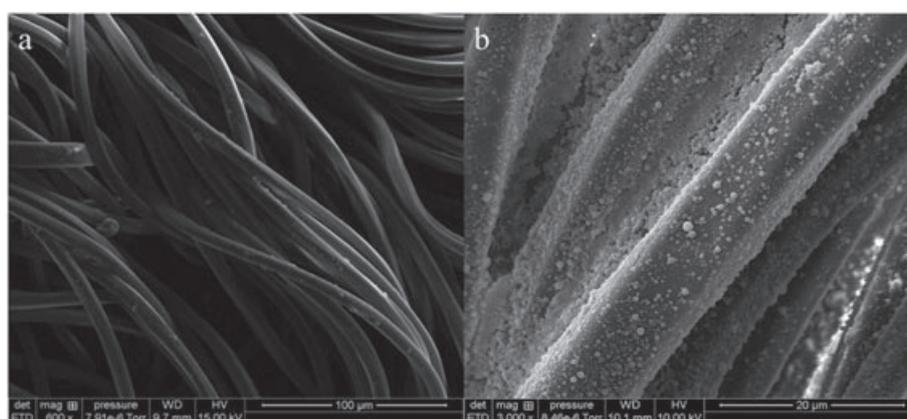


Figure 4. Scanning electron microscopy images of bare (a) and textile bearing adsorbed particles (b).

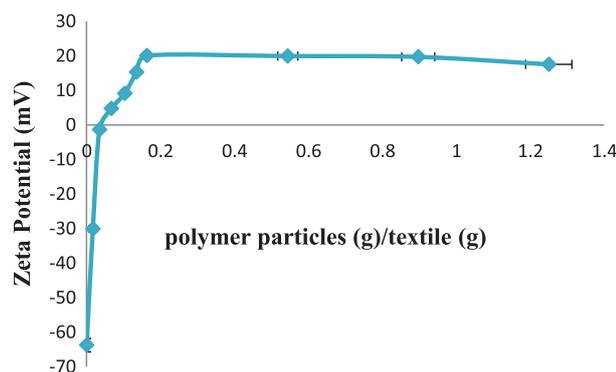


Figure 7. Zeta potential of textile as a function of particles amount/textile weight ratio in 1 mM NaCl, pH 9 and at 20°C.

where, Ψ_s is the surface potential, k is the reciprocal Debye length (equal to $32.5 [\text{NaCl}]^{1/2}$) and x is the distance from the surface.

Zeta potential of textile as a function of particles amount

From the above studies, it was observed that the textile and the particles showed maximum differences in zeta potential at pH 9 (1 mM NaCl concentration) of the circulating electrolyte. Hence, it was deduced that at these conditions of pH and salinity optimum adsorption of particles would occur. Keeping these two parameters of the electrolyte fixed, the effect of initial particle amount in the adsorption medium on the zeta potential of the textile was investigated. An approximately fixed weight of textile was used for each of the different amount of particles used. It was observed as reported in Fig. 7 that with an increase in particle amount (i.e., number of particles) the zeta potential of the textile gradually increases from being negative (−65 mV) to positive (+20 mV). This zeta potential increased until plateau value was reached.

CONCLUSION

This study was carried out with the objective to investigate the influence of electrolyte parameters (pH and salinity) on the zeta potential of the specifically selected polyamide textile as well on the spongy cationic particles.

First, the prepared polymethylmethacrylate particles showed sponge like morphology and polydispersity. The zeta potential of these particles was found to be positive in large pH domain because of the cationic character of the used polymer.

Second, the zeta potential of bare textile was deduced from streaming potential and found to be negative irrespective of pH. In addition, the effect of ionic strength on the zeta potential was also found to decrease in absolute value as theoretically expected.

Finally, the zeta potential of textile as a function of polymeric particles amount was examined. As expected, the zeta potential of bare textile shifts from negative to positive as the amount of particles was increased in the adsorption medium. This has been attributed to the progressive adsorption of positively charged particles on negatively charged textile. In addition, the observed

positive zeta potential measured under circulating electrolyte solution proved that the adsorption is irreversible. In fact, if the desorption occurred during electrolyte circulation, possible negative zeta potential may be obtained.

Hence, this study showed that the polyamide textile was successfully functionalized with positively charged spongy hollow particles. These particles prepared via double emulsion solvent evaporation technique possess the capability to encapsulate both hydrophilic and hydrophobic active agents. They can be used for dual drug delivery, cosmetic actives delivery or for the formation of theranostic agents. Moreover, this method is not very expensive or complicated to use. The selected textiles functionalized with such porous microparticles find significant and extensive applications in the transdermal delivery of therapeutic and cosmetic agents.

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III.4. Encapsulation of cosmetic agents, skin penetration studies and application onto textile

Summary

Recently, the advancement in encapsulation techniques and nanotechnology has opened many new ways to encapsulate the drug molecules, transfer them to the target organ/tissues and to achieve their release at desired site and at the desired rate. To achieve these objectives, colloidal drug carriers play an integral role. Nowadays, biodegradable polymers are extensively used to constitute the colloidal drug carriers. The active agents are loaded inside the polymeric particles from where they are released either by diffusion through matrix or by degradation of the matrix over a prolonged period. Cosmetic industries are also now exploring polymeric biodegradable particles as new ways to deliver cosmetic and drug agents. These industries are using these carriers in order to protect the sensitive cosmetic agents from external environment by hiding them inside the capsules.

These are several techniques for example nanoprecipitation, double emulsion, spray drying etc available to prepare these carriers. In this study double emulsion solvent evaporation method was employed to prepare spongy particles. Eudragit[®] RS100 was used as a polymer to achieve prolonged release of active agents. Eudragit[®] polymers are water insoluble, inert, non-toxic and possess low permeability and pH independent swelling. Two cosmetic agents i.e. vitamin E and lauryl isoquinolium (LIB) were loaded into the particles. Since the active agents are hydrophobic in nature, so they were placed in the organic phase during primary emulsion formation. The active loaded particles were further adsorbed onto negatively charged polyamide based textiles via electrostatic forces. The encapsulation efficiency as well as the cutaneous penetration of the active loaded particles was also analyzed. Different parameters affecting adsorption such as pH of the medium and incubation time were also investigated.

Scanning Electron Microscopy (SEM) analysis showed particles to be spherical, polydispersed, porous and had rough surface. The zeta potential of particles was found to be constant and independent of any formulation parameters. It was generally between 45-55 mV proving the fact that particles were suitable for adsorption onto negatively charged textiles. The encapsulation efficiency was found to be 98% for vitamin E and 100% for LIB. The cutaneous penetration of the active loaded particles was analyzed by using microdialysis, tape stripping and punch-biopsy techniques. Dermal microdialysis for vitamin E showed that penetration kinetics of vitamin E loaded particles was different with slower initial penetration and higher concentration was found at 9 vs 6 h for the control. Results of tape stripping and biopsies showed that after 24 h of application, the content of vitamin E in skin layers was not significantly different between two formulations. On the other hand, for LIB microdialysis displayed slower initial penetration with a higher concentration found at 18 vs 13 h 30 for the control. Tape stripping and biopsy results showed lower content of LIB in the epidermis after 24 h of application. LIB was not detected in stratum corneum.

These results proved that encapsulation process does not modify the cutaneous penetration of the actives. The adsorption study showed complete coverage of the threads with the particles. Hence, we successfully prepared cationic spongy particles which could be loaded with cosmetic agents and could also be adsorbed onto oppositely charged textile.

Elaboration of sponge-like particles for textile functionalization and skin penetration

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Abstract The preparation and characterization of active-loaded microparticles for cosmetic textile applications were studied in this work. The double emulsion diffusion/evaporation technique was served and the Eudragit® RS100 (ethyl acrylate and methyl methacrylate copolymers) was used as biodegradable polymer. Two actives molecules, vitamin E and Lauryl isoquinolinium bromide (LIB) were successfully loaded during the formulation. Obtained particles were spherical in shape with a little variable diameter about 1 μm and zeta potential of 55 mV. The cutaneous penetration of the particles was evaluated by dermal microdialysis, tape stripping, and punch-biopsy techniques from ex vivo human skin. LIB or vitamin E concentrations in the different layers of the skin were analyzed by high-performance liquid chromatography method. Finally, these particles were adsorbed onto a textile in optimized conditions. The scanning electron microscopy is used to confirm the total threads coverage by particles.

Keywords Double emulsion · Eudragit · Microdialysis · Vitamin E and textile

Introduction

Recently, the preparation of particles, as new active carriers, which control the amount of drug released and their localization, has become an important field of research. The facilities provided by the nanotechnologies have opened many ways to present the active molecules, transfer them into the target and release them at the desired site. Nowadays, particle synthesis is widely based on biodegradable polymers, so the active molecules are loaded inside the particles, which allow their controlled release over prolonged time [1, 2]. Encapsulation techniques are used to improve the administration efficacy, reduce the toxicity, protect the drug, and control the release. Drug release from particles occurs either by diffusion through the matrix or by matrix degradation [3].

Cosmetic industries [4], in addition to medicine and pharmacy, are highly interested by nanotechnology and polymer capsules applications as new drug administration methods. In these cases, encapsulation is used to hide the active molecules inside the polymer cavity, protect it from the external environment, and control the amount released during the capsule life.

Eudragit® polymers are used to prepare particles containing active molecules for prolonged release [5]. Whereas, these polymers are insoluble in water, non-toxic, have low permeability, and pH-independent swelling, they are suitable inert carriers of drugs and active molecules [6, 7]. Various techniques have been used to encapsulate active molecules into Eudragit® particles. These techniques include double emulsion [8], nanoprecipitation [9], supercritical fluid [10], and spray drying [11]. Among them, double emulsion method [12] is widely used to synthesize drug-loaded polymeric particles. Here, W/O/W-type double emulsion was selected and several steps for its preparation are required: (1) first emulsion: an aqueous solution of the active molecule (the internal aqueous phase W_1) is emulsified into an organic solution

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consisting of biodegradable polymer and organic solvent (this is the oil phase O). (2) Then, the obtained emulsion (W_1/O) can be emulsified in second aqueous phase (external phase W_2) to get the double emulsion ($W_1/O/W_2$). (3) The next step is the evaporation of organic solvent to solidify the polymer and result in the formation of solid polymeric particles, which entrap the active molecules. (4) The last step is the particles' separation, usually by centrifugation or freeze-drying. In addition, double emulsion technique is suitable for both hydrophilic and hydrophobic [13] molecules.

The objectives of this study were to synthesize active-loaded polymeric particles of Eudragit® RS100 using double emulsion solvent diffusion and evaporation technique. Then, these particles were deposited onto the textile using the electrostatic forces. The adsorbed particles should resist against washing and release the active molecules over a prolonged period.

Firstly, we synthesized blank particles by a systematic study and investigated all the parameters that can affect the size and morphology of particles, such as the polymer amount, the stirring speed and time, the external phase volume and the water volume of final dilution. Obtained particles were then adsorbed onto negatively charged textiles. The textile was incubated with the particles' suspension for a predetermined (or specific) time, and the adsorbed particles' amount was evaluated using SEM and zeta potential. Different parameters of adsorption were investigated such as the pH of medium and the time of incubation. Secondly, we synthesized active-loaded particles; two actives, vitamin E and lauryl isoquinolinium bromide (LIB), were chosen respectively for their hydrating and anti-body hair growth effects. The encapsulation efficiency was analyzed by high-performance liquid chromatography (HPLC) method. The cutaneous penetration of the particles was evaluated by dermal microdialysis [14, 15], tape stripping, and punch-biopsy techniques from ex vivo human skin. LIB or vitamin E concentrations were determined in the different layers of the skin.

Materials and methods

Materials

Eudragit® RS100 (ethyl acrylate and methyl methacrylate copolymers), weight average molar mass: approx. 32,000 g/mol, was purchased from EVONIK (EVONIK, Germany). Vitamin E, LIB, dichloromethane (DCM), and poly(vinyl alcohol) (PVA) were obtained from Sigma-Aldrich. Deionized water was used throughout the study.

Preparation of particles

Eudragit® RS100 particles were prepared using double emulsion W/O/W method. To prepare the first emulsion, two types

of homogenizer were used (Ultra Turrax® T-10, IKA® Werke, Staufen, Germany, and/or ultrasound). The steps of the general experiment were briefly as follows; 1 g of Eudragit® RS100 was dissolved in 5 ml DCM and emulsified with 1 ml water using Ultra Turrax® or ultrasound. The obtained emulsion W/O was injected in 30 ml water (with or without stabilizer PVA) and emulsified using Ultra Turrax® (T-25, IKA® Werke, Staufen, Germany). Then, the second emulsion W/O/W was poured into 100 ml water and magnetically stirred for 15 min (using simple magnetic bar). Finally, the double emulsion was evaporated to remove the organic solvent, DCM, and solidify the particles (rotary evaporator). The obtained particles were filtered on glass wool to remove the potential aggregations.

Optimization of particles' size

We studied the influence of all synthesis parameters on the particles' morphology and size.

The effect of polymer amount

The amount of Eudragit® dissolved in 5 ml of DCM varied from 0.25 to 1.5 g: Emulsions were prepared using 1 ml water for internal aqueous phase (W_1) and 50 ml water for external aqueous phase (W_2). The ultrasound was used to emulsify the first emulsion (70 % power and 1.5 min) and Ultra Turrax® to prepare the second emulsion (13,500 rpm for 15 min). Finally, the double emulsion was diluted in 100 ml water, stirred for 15 min, and evaporated to remove DCM.

The effect of Ultra Turrax speed and time (first emulsion)

The first emulsion was prepared initially using a small-size Ultra Turrax (T-10) and the effects of its speed and emulsification time were investigated. Speed varied from 13,500 to 24,500 rpm and used times were 2, 3, and 4 min.

The effect of ultrasound power and time (first emulsion)

Particles' size of the first emulsion influences the finale particles' size, so we studied the effect of ultrasound power and time on the finale particles' size. One gram of Eudragit® was dissolved in 5 ml DCM and emulsified with 1 ml water using variation of ultrasound time. The second emulsion was prepared as previously described (Ultra Turrax®, 13,500 rpm for 15 min).

The effect of external aqueous phase volume

The volume of external aqueous phase, W_2 was changed from 30 to 90 ml water and the particles were prepared using 1 g Eudragit® in 5 ml DCM. The first emulsion was prepared by

ultrasound (70 % for 1.5 min) and the second emulsion was prepared by Ultra Turrax® (13,500 for 15 min).

The effect of final dilution volume

Particles prepared by double emulsion were diluted in water to help the organic solvent to diffuse outside the particles. The amount of water used for this dilution varied and the effect of this dilution on the particles' size and morphology was investigated. The double emulsions were prepared as previously described.

Characterization of particles

Particles' morphology

Scanning electron microscopy SEM, was performed with a Hitachi S800 FEG microscope, at the "centre technologiques des microstructures" CTμ at the University of Lyon. For the sample preparation, a drop of the sample was deposited on a flat steel holder and dried at room temperature overnight. The samples were coated under vacuum cathodic sputtering with platinum. The particles were examined using SEM under accelerating voltage of 15 kV.

Particles' size analysis

Particles' size and size distribution were measured with a laser diffraction particle size analyzer (Beckman Coulter LS 13 320). Samples were prepared by adding drops of aqueous particle suspension to the instrument container, already filled with distilled water.

Zeta potential analysis

The zeta potential of particles was measured using Zetasizer instrument (Zetasizer Nano S, Malvern®, UK). Particles were highly diluted in NaCl 1 mM and potential zeta was measured using special zeta cell DTS1070 (Malvern®, UK). Potential zeta, of particles, as a function of pH was measured using autotitrator (MPT-2, Malvern®, UK). Sample was highly diluted in NaCl 1 mM and potential zeta was measured between pH 3 and 11.

Cutaneous penetration

Encapsulation efficiency

Particles' suspensions were centrifuged and the supernatants were removed and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Ex vivo microdialysis

Microdialysis system consisted of a CMA/100® syringe pump and a CMA/140 microfraction collector (Phymep, France). Microdialysis probes (CMA/20®) had a 20-kDa cut off with a polycarbonate membrane (length 10 mm). The probes (3 per condition) were inserted into the dermis of ex vivo abdominoplastic skin fragments. Probes were perfused with Ringer's lactate/3 % BSA solution at an optimal flow rate of 3 $\mu\text{l}/\text{min}$. The penetration areas were demarcated on the skin with metallic ring. Five hundred microliters of standard and encapsulated molecules were applied to the demarcated area of the skin. Samples were collected every 90 min over a period of 24 h and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

The depth of microdialysis probes in the skin was measured by ultrasound scanning.

Tape stripping and biopsies

D-squame® tapes (Monaderm, France) were applied and removed. Discs 1–10 were stored at $-20\text{ }^{\circ}\text{C}$ until analysis (disc 1 for skin surface and 2 to 10 for *stratum corneum*). Skin biopsies were realized and epidermis was separated from dermis by dispase incubation. Tissues were stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Sample analysis

Extraction The content of vitamin E and LIB in the tape strips and biopsies was analyzed following extraction procedure [16]. Methanol was added to tape strips. Tubes were shaken during 10 min, centrifuged at $14,000\times g$ for 10 min before collecting the supernatant. Human dermises or epidermises were weighted in glass tube. After homogenization using a dispersing Ultra Turrax®, methanol was added, shaken, and sonicated for 10 min. After centrifugation at $14,000\times g$ for 10 min, the supernatant was collected. The concentration of vitamin E and LIB in dialysates was measured without pretreatment.

Vitamin E quantification Vitamin E was assayed by HPLC using a Shimadzu Prominence HPLC system equipped with a pump model LC-20AB, an autosampler model SIL-20A. Double detection was used: photodiodearray detector model SPD-M20A (Shimadzu, France) coupled with a triple quadrupole TSQ Vantage (ThermoFisher, France). Separation was performed on a Prevail C18 column ($150 \times 4.6\text{ mm}$, $3.1\text{ }\mu\text{m}$, Alltech, Deerfield, IL, USA) using mobile phases composed of acetonitrile:0.05 % phosphoric acid solution (35:65, v/v) at a flow rate of 1 ml/min and of acetonitrile (100 %). UV detection wavelength of 285 nm was used for vitamin E detection and quantification. Standard solutions of the drugs were prepared in methanol or acetonitrile, and

linearity was achieved over the range of 1–100 $\mu\text{g/ml}$ ($r^2 > 0.99$) [17].

LIB quantification LIB was analyzed by HPLC-DAD. The column used was a C18 Xterra, 5 μm (4.6×150 mm, SN^o: T32753G) and the system was a P4000 pump coupled with a UV6000LP diode array detector (Thermo Separation Products, Les Ulis, France). The acquisition software was ChromQuest (Thermo Separation Products, Les Ulis, France). The mobile phase was composed of 60 % acetonitrile and 40 % ammonium formate (0.1 M, pH = 3.5) with a flow rate of 1 ml/min. The quantification was done at 335 nm. The retention time was 4.8 min and the volume of injection was 20 μl . Linearity was obtained in the range 0.1 to 10 $\mu\text{g/ml}$ ($r^2 > 0.99$) [18].

Statistical data

Statistical analyses were performed using a two-way ANOVA (for microdialysis) and one-way ANOVA (for tape stripping and biopsies), followed by a Fischer test.

Active-loaded particles and adsorption onto textile

Two active molecule models used were the vitamin E and lauryl isoquinolinium bromide (LIB). Particles were prepared as described above and the active molecules were added to the first emulsion (W_1/O). In our case, the actives are hydrophobic and soluble in the organic phase. Therefore, the actives were added to the organic phase. In the case of hydrophilic active, they can be added in the internal aqueous phase.

The obtained particles were incubated with small pieces (1 cm^2) of fabrics, polyamide. Next, the pieces were immersed in distilled water to remove the excess of particles. The optimum conditions of adsorption were investigated by testing the pH of medium and incubation time. Particle-treated textiles characterized by SEM and surface zeta potentials were determined before and after particles' adsorption.

Results and discussion

Particles' preparation

Double emulsion diffusion evaporation ($W_1/O/W_2$) technique was used to prepare the particles. The first emulsion W_1/O was formed by emulsification of organic solution of a polymer containing the active molecules with an aqueous solution. Then, the second emulsion formed by emulsifying the first one (W_1/O) with an aqueous solution of PVA. Size and morphology of the obtained particles depend on several factors such as the following:

- The droplet size of the first emulsion.
- The organic solvent and its diffusion into the external aqueous phase.
- The volume of the external aqueous phase.
- Stabilizer and its concentration.
- Homogenizer used, its speed and treatment time.

Usually, particles obtained by double emulsion, using a mechanical homogenizer, have a minimum size of 10 μm [19]. This is due to the limited efficiency of the mechanical homogenizer to produce very small droplets.

In this study, particles were prepared by two different ways, the preparation of the first emulsion only, using the Ultra Turrax[®] and the ultrasound.

The particles prepared using Ultra Turrax[®] were big in size, between 10 and 40 μm , and those that were prepared using ultrasound had sizes in the range of 1 μm , as shown in Fig. 1.

These results are due to the high fragmentation power of ultrasound, which formats small drops (W_1/O). In addition, as it is shown in the Fig. 1, the surface properties are also different. The particles obtained by Ultra Turrax[®] were porous, with big pores, while the particles obtained by ultrasound had smooth surfaces with few and small holes. As the same quantity of polymer was used in both cases, walls of the small particles were thicker than walls of the big particles, so the solvent evaporation is more effective in the case of big particles, which produced bigger holes in the particles walls. The specific surface area of small particles was higher than that in the big particles, so the contact area between organic and aqueous phases in small particles was larger than that in the big particles. Therefore, the organic solvent diffusion to the aqueous phase was faster in the case of small particles, which lead to rapid solidification of the polymer matrix. This is a second reason why big particles were more porous than the small ones. Size distribution in both cases was wide.

As known that the particles prepared by emulsification process have non-uniform (polydisperse) sizes and to improve the uniformity, an expenditure of high energy for longer duration is required. Here, for our desired applications, polydispersity was not a big problem so it was not so necessary to spend high energy for a long time to get better results.

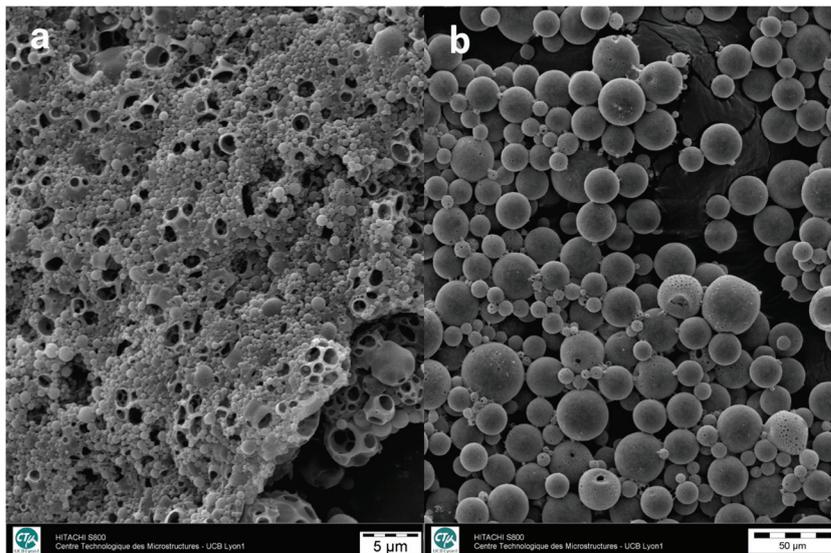
After these preliminary studies, we decided to use ultrasound in the first emulsification step since our target size was 1 μm , which was achieved by the ultrasonication (without any stabilizer).

Particles' size optimization

The effect of polymer amount

Different quantities of polymer were used to prepare particles, 0.25, 0.5, 1, 1.5, and 2 g Eudragit[®] RS100. Table 1 resumes

Fig. 1 SEM photographs of particles obtained using ultrasound (**a**) or Ultra Turrax® (**b**) to emulsify the first emulsion



the results of preparations using different quantities of polymer.

Slight increasing trend in particle size was observed as polymer amount was increased from 0.25 to 1.0 g, but this slight increase was followed by a sudden large increase in particle size (~13 µm) which was observed at 1.5 g of polymer. The viscosity became more important, so the energy needed for the fragmentation is greater than the supplied.

At higher concentration, 2 g and more, particles' formation was not observed due to the high viscosity of organic solution. It is obvious that the particles' size was highly sensitive to the polymer concentration, due to the absence of surfactant or stabilizer agent so the particles' coagulation was effective at high polymer concentration. Yang et al. [19] found similar results where it was observed that the polymer concentrations of 16.7 and 33.3 mg/ml gave particles' diameters of 97.9 and 125.8 µm, respectively.

The effect of other parameters, ultrasound, external aqueous phase, and final dilution phase

Ultrasound was used to emulsify the first emulsion W_1/O ; the drops were prepared without surfactants or stabilizers and poured directly into the external phase to prepare the second

emulsion. Generally, droplets size of the first emulsion does not affect the final particles' size (in condition that the droplets' size is relatively smaller than the final particles [20, 21]). To study the effect of ultrasound onto the particles' size, two polymer concentrations were chosen, 0.25 and 1 g. Particles were prepared using double emulsion diffusion/evaporation; with the first emulsion being prepared using ultrasound at 70 %. Ultrasonication times were varied from 1.5 to 6.0 min and the results are displayed in Table 2.

It was clear that the ultrasound time at these conditions showed no effect on the final particles' size even with different polymer concentrations. By comparing these particles with the particles prepared using Ultra Turrax® over all the experiments, we found that the average particles' size is 40 µm. This was due to the size of first droplets formulated by Ultra Turrax®. Even with high speed (24,000 rpm), these droplets were too big and the final particles were so far from 1 µm. These results suggested that in these conditions, we could not mechanically reach to very small droplets to have submicron particles in the final step.

In addition, the effect of the external phase on the particles' size was investigated. The volume of the external phase varied from 30 to 90 ml. Table 3 shows the average particles' sizes of particles prepared with different external phase volume.

Table 1 Preparations of Eudragit® RS100 particles obtained using different polymer amounts

Preparation	Eudragit RS100 (g)	DCM (ml)	Internal aq. phase (ml)	External aq. phase (ml)	Diameter (µm)	Zeta potential (mV)
1	0.25	5	1	30	0.87	39.5
2	0.50	5	1	30	1.1	44.9
3	0.75	5	1	30	1.1	48.6
4	1.00	5	1	30	1.2	51.8
5	1.50	5	1	30	13.3	51.3
6	2.00	5	1	30	X	X

Table 2 The effect of ultrasound time on the final particle size

Time (min)	1.5	3	4	6
Diameter (μm), (0.25 g)	0.78	0.88	0.41	0.63
Diameter (μm), (1 g)	1.16	1.23	1.21	1.18

Changing the volume of the external phase increased slightly the particles' size. In this case, increasing the external phase volume decreased the efficacy of homogenizer, so the average particles' size was bigger with extra volume of external aqueous phase. Moreover, for completing the investigation of the external water effect on the particles' size, the effect of dilution with excess volume of water was also studied.

The volume of external phase is important in this process of particles' formulation, specially its effect on the size and morphology. The process of polymer solidification depends on removing the organic solvent, which can be done by extraction and evaporation. Extraction step depends on the miscibility between the organic solvent and water [16]. In this study, the double emulsion was diluted with water after the second emulsion preparation to help the organic solvent diffusion into the external aqueous phase and start the polymer solidification. This pre-solidification step has an effect on the final particles' morphology [16]. Table 4 shows the results obtained by variation in the volume of water used to dilute the $W_1/O/W_2$ emulsion.

Results in Table 4 showed that the dilution step had no effect on the average particles' size and adding more water only slightly decreased the average particles' size. This was due to the poor solubility of DCM in water, 1.3 g/100 ml at 20 °C (ethyl acetate 8.2 g/100 ml at 20 °C). Moreover, the used external aqueous phase did not contain any surfactant or a stabilizer agent, which could help to increase the DCM solubility in water.

Characterization of particles

The morphology and the surface characteristics of obtained particles were observed using scanning electron microscope. Figure 2 shows the SEM photographs of particles prepared by double emulsion diffusion/evaporation. It was seen that the particles were spherical in shape, polydisperse, and with rough surface, which can be related to the diffusion and evaporation processes applied after the particles' synthesis.

Table 3 The effect of external aqueous phase volume on the particle sizes

Water (ml)	30	45	60	75	90
Diameter (μm)	0.93	1.0	1.2	1.2	1.3

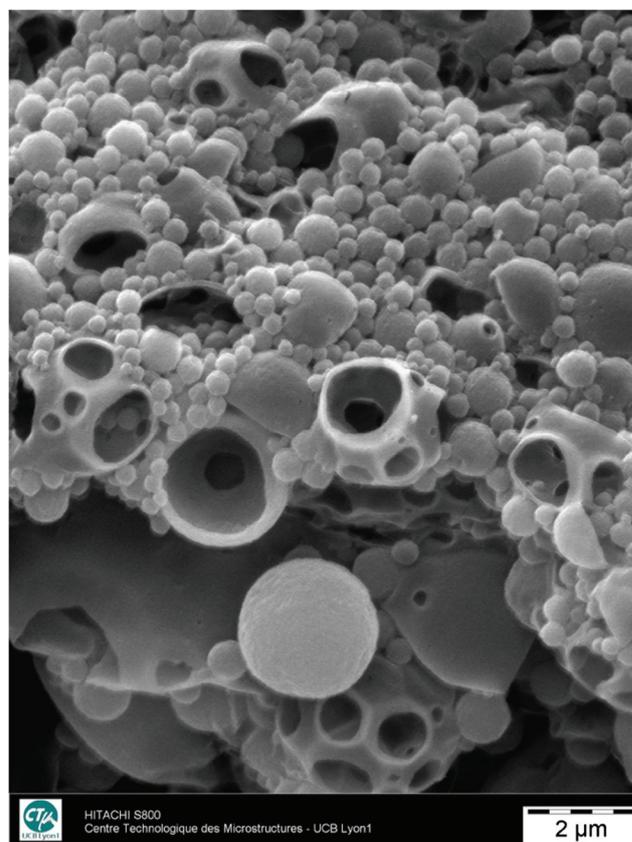
Table 4 The effect of dilution phase volume onto the particle size

Water (ml)	60	80	100	120
Diameter (μm)	1.7	1.7	1.7	1.6

In addition, zeta potential values of prepared particles were measured using ZetaSizer, and it was found that these values were almost stables and independent of formulation recipe. The zeta potential values were between 45 and 55 mV. This independence of formulation method was due to the usage of the same polymer in all preparations and absence of any stabilizer agent or surfactant, which could modify the electrostatic characteristics of particles' surface.

Study of the encapsulation efficiency and skin penetration

Here, we studied the encapsulation efficiency and the penetration of active-loaded microparticles through the skin using microdialysis, tape stripping, and punch-biopsy techniques. Two actives, vitamin E-loaded particles and LIB-loaded particles, and two standard solutions (as control) were used in this work. Particles' suspensions were centrifuged and the supernatants were removed to study the encapsulation efficiency.

**Fig. 2** SEM photographs of obtained particles

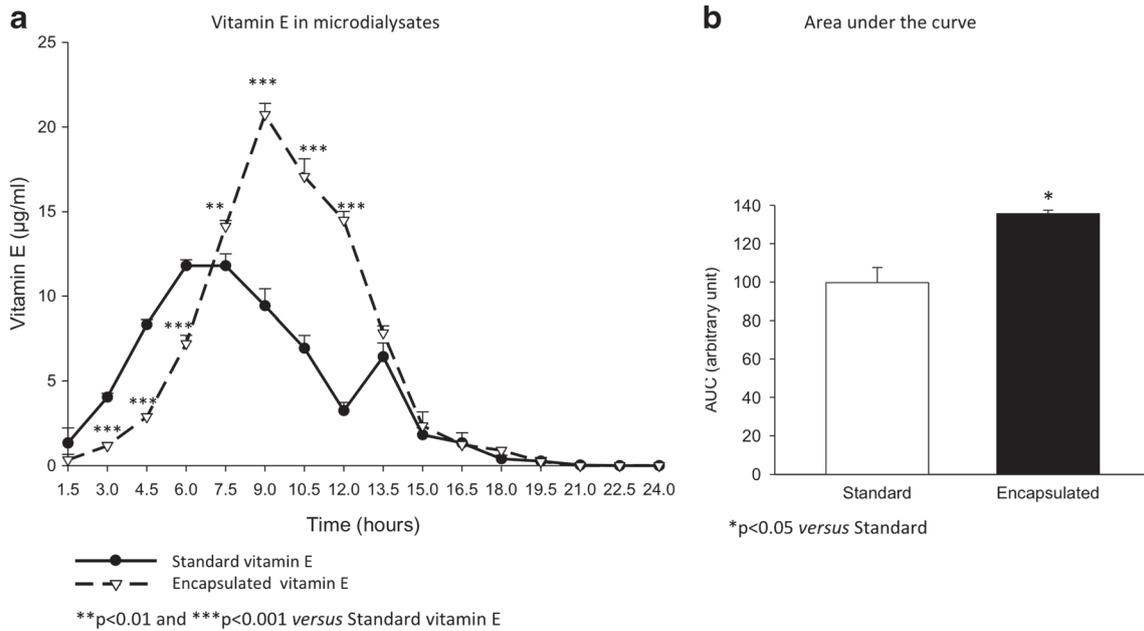


Fig. 3 Microdialysis. **a** Concentration vs time curve for the cutaneous penetration of vitamin E-loaded particles vs standard. Vitamin E concentrations (mean ± S D) are presented for $n = 3$. ** $p < 0.01$ and *** $p < 0.001$. **b** Area under the curve. * $p < 0.05$

The actives' concentration in the supernatants showed that the encapsulation efficiency was 98 % for vitamin E and 100 %

for LIB (data not illustrated). For vitamin E, results obtained by dermal microdialysis are shown in Fig. 3.

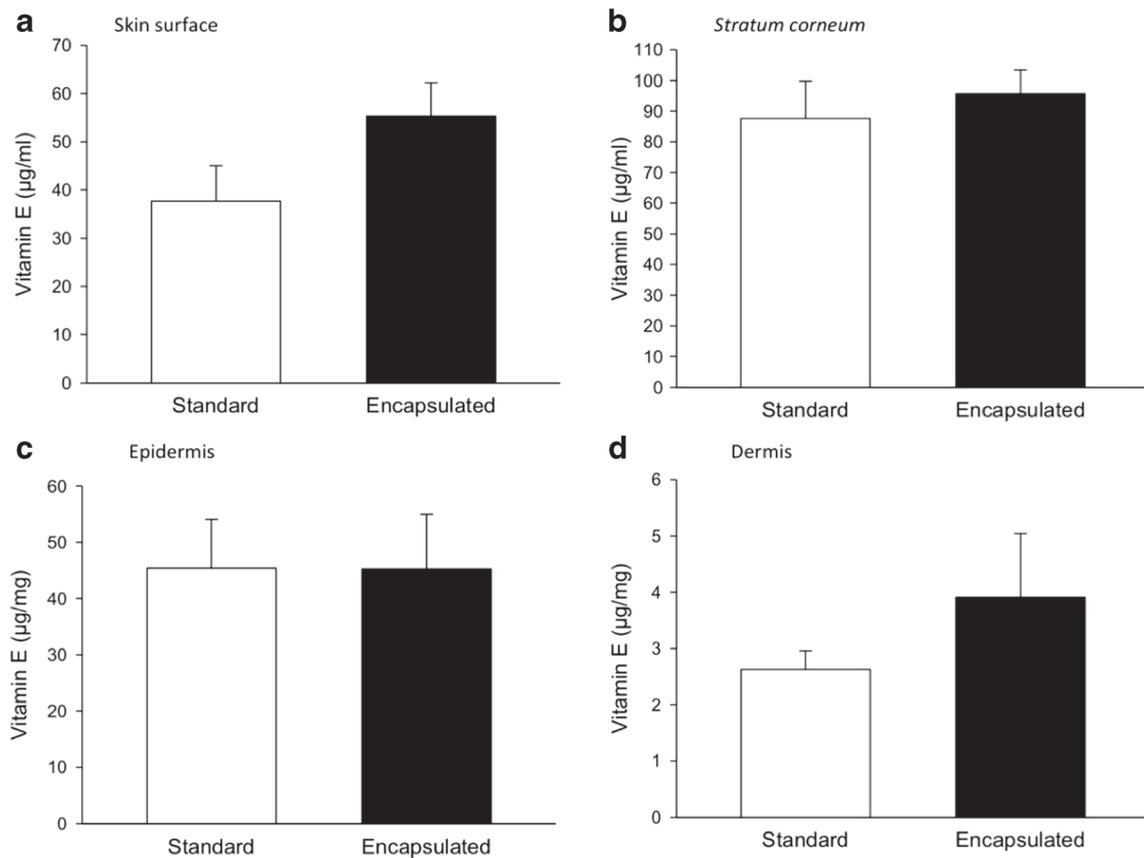


Fig. 4 Tape stripping and biopsies after 24 h of vitamin E-loaded particles and standard application. Vitamin E concentrations (mean ± SD, $n = 3$) in tape strip samples of skin surface (**a**) and stratum corneum (**b**) in biopsies of the epidermis (**c**) and dermis (**d**)

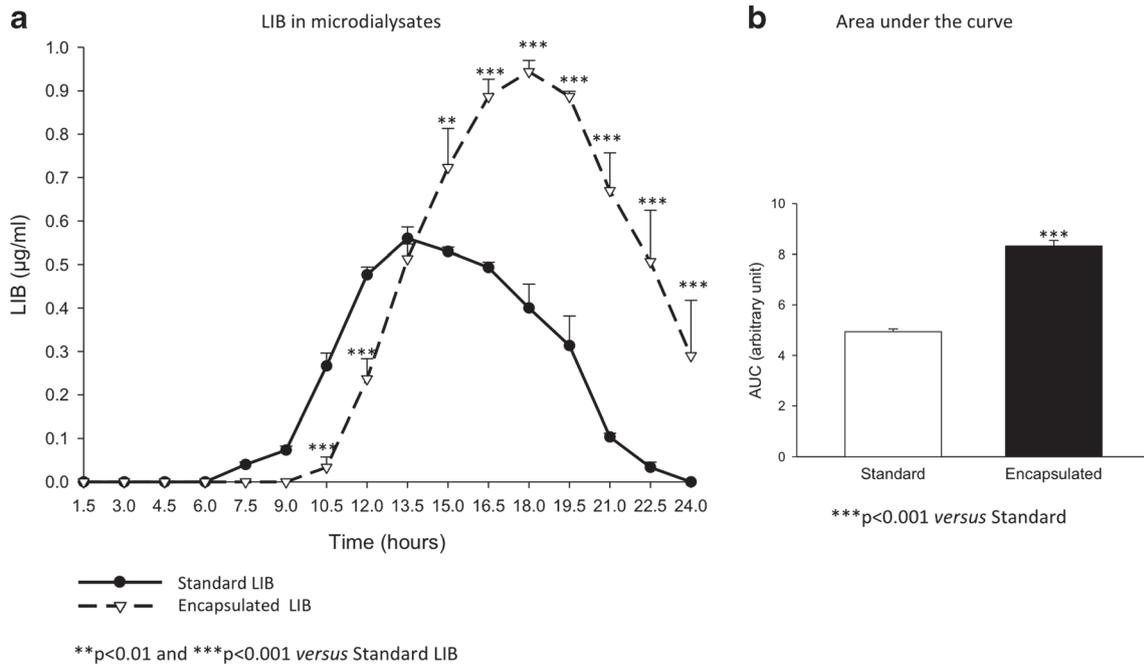


Fig. 5 Microdialysis. **a** Concentration vs time curve for the cutaneous penetration of LIB-loaded particles vs standard. LIB concentrations (mean ± SD) are presented for $n = 3$. ** $p < 0.01$ and *** $p < 0.001$. **b** Area under the curve. *** $p < 0.001$

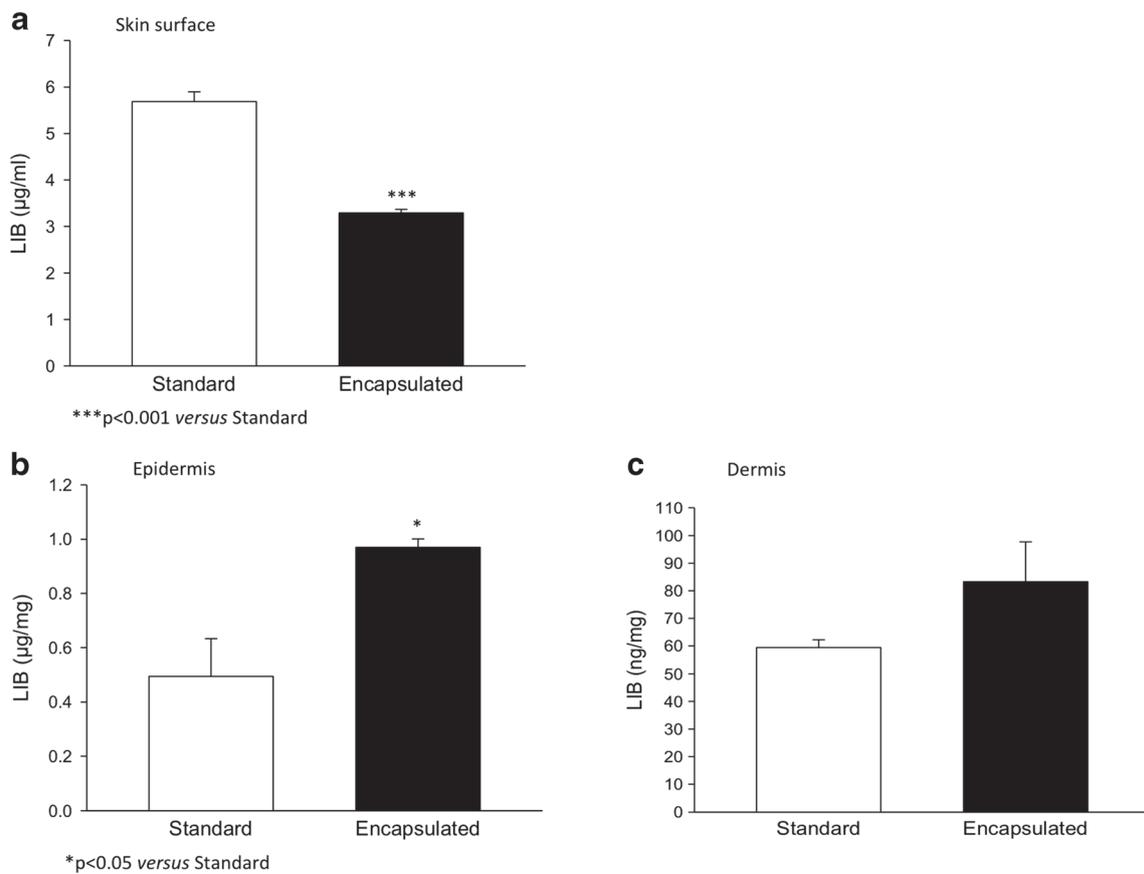
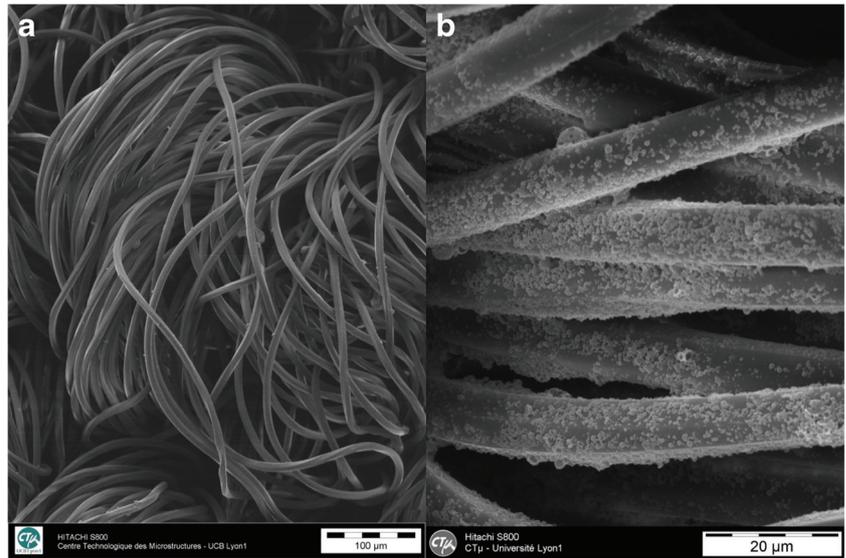


Fig. 6 Tape stripping and biopsies after 24 h of LIB-loaded particles and standard application. LIB concentrations (mean ± SD, $n = 3$) in tape strip samples of skin surface (**a**) and in biopsies of the epidermis (**b**) and dermis (**c**)

Fig. 7 SEM photographs of textile before (a) and after (b) particle adsorption



The dermal penetration kinetic of vitamin E-loaded particles was different from the control, with a significant slower initial penetration and a significant higher concentration found

at 9 vs 6 h for the control (Fig. 3a). The area under the curve was significantly larger with vitamin E-loaded particles compared with that in the control, indicating an increased dermal

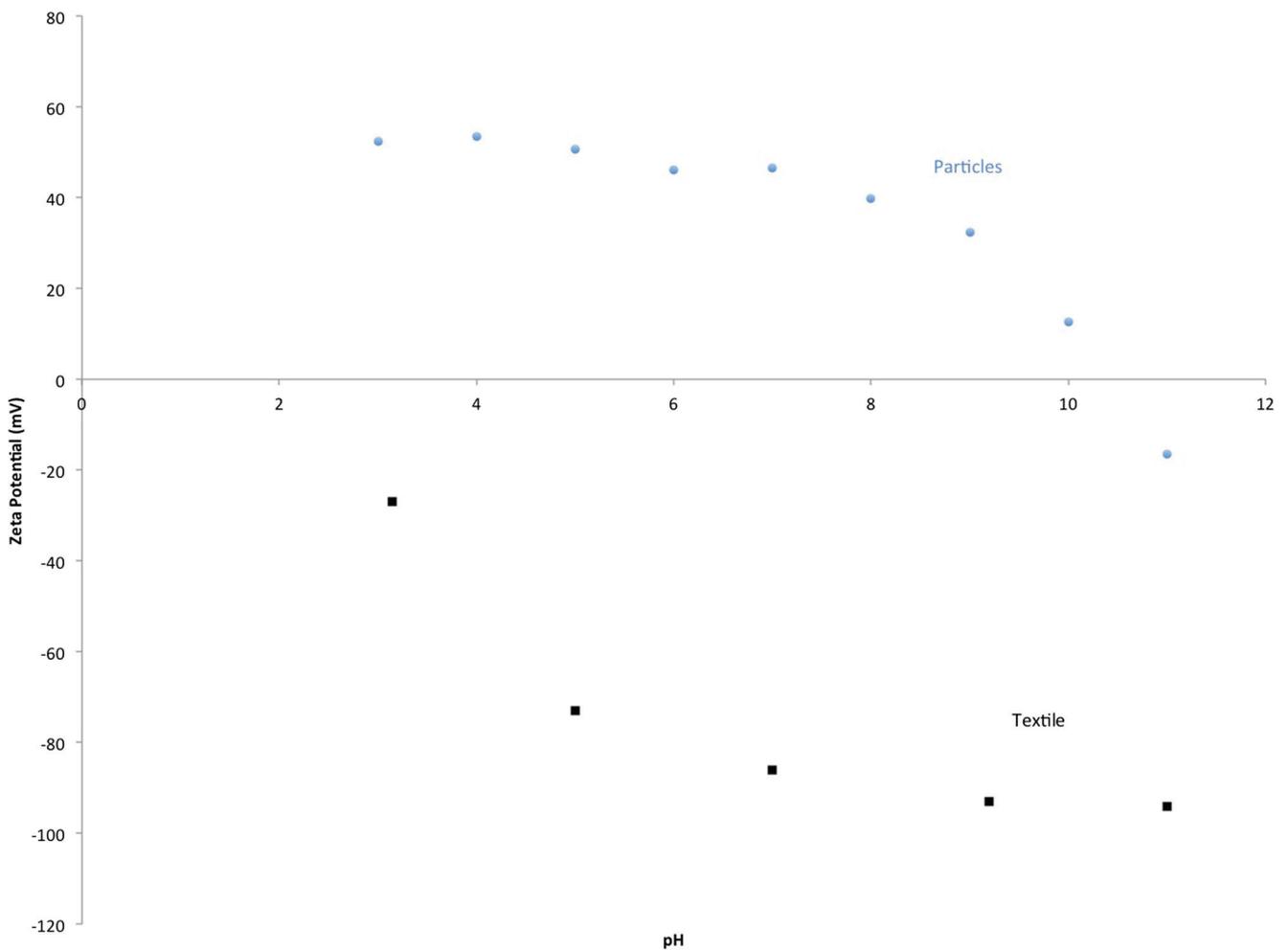


Fig. 8 Zeta potential as a function of pH of obtained particles (blue) and studied textile (black)

penetration (Fig. 3b). Results obtained by tape stripping and biopsies are shown in Fig. 4. The content of vitamin E in the skin layers, after 24 h of application, were not significantly different between the two formulations.

For LIB, results obtained by dermal microdialysis are shown in Fig. 5. The dermal penetration kinetic of LIB-loaded particles was different from the control, with a significant slower initial penetration and a significant higher concentration found at 18 vs 13 h 30 for the control (Fig. 5a). The area under the curve was significantly larger with LIB-loaded particles compared with that in the control, indicating an increased dermal penetration (Fig. 5b). Results obtained by tape stripping and biopsies are shown in Fig. 6. The content of LIB was significantly decreased in the skin surface and increased in the epidermis layer after 24 h of application of LIB-loaded particles compared with that in the control.

The content of LIB in the dermis, after 24 h of application, was not significantly different between the two formulations and LIB was not detected in the *stratum corneum*.

All these results proved that the encapsulation process is good and does not modify the cutaneous penetration of actives.

Adsorption onto the textile

According to the zeta potential results, the obtained particles are positively charged, $\approx +50$ mV and the textiles target of this treatment are negatively charged, so we supposed that the particles could be easily adsorbed by electrostatic interaction onto the textile threads. To study this interaction and get a maximum adsorption of particles onto the threads, the particles were adsorbed by a sample incubation of textile pieces (1 g of pieces of 1 cm²) in 30 ml of aqueous particle suspension for 1 h. Then, the textile pieces were removed and immersed for a short time in distilled water to remove the excess particles. Textile pieces were dried and analyzed by SEM. Figure 7 shows the SEM photographs of textiles before and after particles' adsorption.

In this study, we investigated the effect of time and pH on the adsorption efficiency. Figure 8 shows the zeta potential values of particles and textile as a function of pH. As seen in Fig. 8, the range of pH between 5 and 9 was the best condition to perform the interaction between particles and textile because in this area, the difference of zeta potential values was at maximum. The time effect was studied also and we found that interaction was fast and did not require a long time (30–60 min are enough). Figure 7 shows that the threads were very well coated by particles in these conditions. Hence, these results suggested a very good distribution of loaded actives onto the textile threads.

Conclusion

The successful preparation of active-loaded microparticles was achieved using the double emulsion diffusion/evaporation technique and the polymer Eudragit® RS100. A systematic study of particles' formulation and size optimization was performed to identify the optimum formulation recipe. Obtained particles were characterized and studied by SEM, which confirmed the spherical morphology of particles with rough and porous surfaces. Controlling the particles' diameter could control the particles' surface and the pores' size. Active molecules (vitamin E and LIB) were successfully loaded during particles' synthesis and their skin penetration aptitude was confirmed in addition to the total active conservation. Finally, particles' adsorption onto a negatively charged textile was studied and a complete coverage of threads with particles was accomplished.

In summary, we prepared positively charged microparticles, which can be loaded with active molecules and can be stably adsorbed onto a negatively charged textile. Particle-activated textiles have promoted applications in cosmetic and pharmaceutical industries.

Acknowledgments This work has been achieved in the frame of Texactiv research program and has been supported by French public agency, OSEO.

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III.5. Encapsulation of active-cyclodextrin complexes, skin penetration studies and application onto textile

Summary

As have already been stated in our previous works, nowadays, pharmaceutical research is extensively focused on developing new encapsulation techniques. Encapsulation of active agents provides many beneficial properties which include protection of drug molecules, control over the drug release, reduced toxicity and improved drug efficacy. Diverse drug carriers are prepared using different encapsulation methods such as polymeric particles, micelles and liposomes. Nowadays, biodegradable polymers are popularly used to prepare colloidal drug carriers. Eudragit[®] polymers are mostly used to achieve controlled release formulations. Moreover, these polymers are inert, non-toxic, water insoluble and have pH independent swelling. Here, we used Eudragit[®] RS100 polymer to obtain positively charged particles. Though many different encapsulation techniques are available, we chose double emulsion-diffusion solvent evaporation method to prepare spongy cationic particles.

Encapsulation of active agents has already been done via this technique in our last experiment. The uniqueness of this study is the introduction of cyclodextrin (CD) into the formulation. CDs are cyclic sugar molecules having a bucket shaped structure. They are known to form complexes with hydrophobic active molecules and thereby enhance their aqueous solubility. Our objective was to form complexes of active agents with CDs and then introduce them into the polymeric particles. The active-CD complexes were introduced during the preparation of particles via double emulsification. CD derivative hydroxyl-propyl-beta-cyclodextrin (HP β CD) having higher aqueous solubility was employed in the study.

The active agents selected for encapsulation were Indomethacin (IMC), Vitamin E and lauryl Isoquinolinium Bromide (LIB). Firstly, all of these agents are hydrophobic and are good candidates for complexation. The two cosmetic agents are photosensitive and hence quite suitable for complexation with CD. The complexes of IMC, Vit E and LIB with HP β CD were prepared by physical mixing and coevaporation process. Dried powdered complexes were obtained. SEM, FTIR and DSC analysis were performed for the pure compounds as well as for the physically mixed and coevaporated complexes to show and confirm changes in structures and hence prove successful complexation. The coevaporated complexes of the three actives were used to prepare polymeric particles. The complexes were placed in the internal aqueous phase. On the other hand, pure actives were also encapsulated into polymeric particles for comparison. All the prepared particles were analyzed for size, zeta potential and morphology. All the particles were adsorbed onto selected textiles and analyzed via SEM. Particles encapsulating pure actives and complexes of actives were analyzed for encapsulation efficiency and skin penetration.

SEM analysis of pure compounds and of complexes showed complete complex formation for the coevaporation process. Particles encapsulating IMC, Vit E and LIB were found to be spherical, polydispersed and porous under SEM analysis. These particles also showed good adsorption onto textile. Particles encapsulating IMC-HP β CD, Vit E-HP β CD and LIB-HP β CD were also seen as porous, spherical and polydispersed. CD did not seem to change the morphology of the particles. Adsorption of these particles onto textile was good and was not affected by the presence of CD. Results of FTIR and DSC also confirmed the presence of

complexation between the actives and HP β CD. The zeta potential of all the particles was found to be between 40-55mV and CDs being uncharged molecules did not affect the zeta potential of the particles. The size of all the particles was measured to be $\sim 1\mu\text{m}$ and unaffected by the presence of CDs. Complexes of actives with HP β CD when encapsulated in polymeric particles displayed prolonged release characteristic.

Sponge like microparticles for drug delivery and cosmeto-textile use: Formulation and human skin penetration

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Abstract

This unique work is targeted to achieve three main goals: i) to enhance the aqueous solubility of three specifically selected hydrophobic active agents, ii) to prepare such polymeric biodegradable microparticles which can encapsulate active-cyclodextrin complexes and iii) to functionalize a polyamide base textile with active loaded microparticles and active-cyclodextrin loaded microparticles. To achieve this objective, biodegradable cationic microparticles were prepared via double emulsion solvent evaporation process and were loaded with hydroxypropyl-beta-cyclodextrin based complexes of Indomethacin, α -tocopherol and Lauryl Isoquinolinium Bromide during the formulation process. Inclusion complex based particles were evaluated for their morphology, size distribution, zeta potential, skin penetration aptitude and adsorption onto a selected textile. It was observed that active-cyclodextrin complex encapsulation do not affect the morphology, size and zeta potential of the microparticles as well as adsorption of the microparticles onto textile remains unaltered. However such active-cyclodextrin complex encapsulated particles provided the enhancement in the aqueous solubility of hydrophobic agents and also provided prolonged release formulations.

Keywords

Hydroxypropyl-beta-cyclodextrin (HP β CD), indomethacin (IMC), α -tocopherol (Vit. E), Lauryl Isoquinolinium Bromide (LIB), cosmetology, zeta CAD, double emulsion-solvent evaporation, Eudragit[®] RS100, anti-inflammation, antioxidant, anti-hair growth.

1. Introduction

Nowadays pharmaceutical research is primarily focused on developing drug delivery systems possessing enhanced therapy. This quest of researchers leads them to delve into diverse encapsulation technologies. These technologies assist in (a) improving drug efficacy, (b) reducing toxicity, (c) protecting the active molecule and (d) controlling the drug release (Uhrich et al., 1999). Recently encapsulation technologies are used to prepare colloidal drug carriers which can control the drug release and its localization. Diverse microstructures are available for encapsulation of active agents which include liposomes (Laouini et al., 2012), microgels, microemulsions and polymeric micelles. Biodegradable polymers are widely employed to synthesize particles for drug delivery. The active agents are encapsulated inside

the polymeric particles which allow their controlled release over prolonged time (Freiberg and Zhu, 2004; Tran et al., 2011). Specific factors like, biodegradation kinetics of the polymers (Çiçek et al., 1995), physicochemical chemical properties of the polymers and drug (Calandrelli et al., 2002) and thermodynamic compatibility between the polymers and drugs, generally control the drug release rates. Various biodegradable polymers employed for microparticulate formation include polyesters, polysaccharides (Sinha and Trehan, 2003), polyanhydrides (Kipper et al., 2002), polyphosphazenes (Lakshmi et al., 2003) and poly (ortho esters) (Wang et al., 2004).

Eudragit[®] polymers are generally employed to achieve controlled release formulations (Khan et al., 2012). These polymers act as suitable inert carriers of drugs since they are insoluble in water, non-toxic, have low permeability and pH independent swelling (Pignatello et al., 2002; Ibrahim et al., 2010). Eudragit[®] RS100 is widely used in controlled release formulations. It has quarternary ammonium groups present as salts which make the polymer permeable. Different techniques are used to encapsulate actives into Eudragit[®] based particles e.g. double emulsion (Sipos et al., 2005), nanoprecipitation (Das et al., 2010), supercritical fluid technology (Chattopadhyay et al., 2006) and spray drying (Esposito et al., 2000). In this study double emulsion technique was focused on.

Cosmetic industries (Rosen, 2005) are also working on polymeric particles as new drug administration methods. Several cosmetic agents are susceptible to heat, oxidation and evaporation. These reasons make microencapsulation an attractive technique to develop cosmetotextiles. Cosmetotextile concept appeared in late 1980s with Japan leading the way being followed by European Union in 1995. Microencapsulation provides protection to the cosmetic agents against oxidation or evaporation thereby prolonging their shelf life. A major drawback faced by the cosmetotextiles is the loss of agents loaded on them after few washes. Currently, the researchers are focusing on developing new particles and new fixing techniques to improve this poor resistance to washing. This study is also targeted to achieve the above mentioned objective. Here, one active agent and two cosmetic agents are complexed with cyclodextrin and then encapsulated inside cationic microparticles. Active loaded microparticles are tested for their applicability onto specifically selected textile.

Indomethacin (IMC) a gold standard non-steroidal anti-inflammatory drug (NSAID) is generally used to lessen pain, swelling, stiffness and fever. Despite being potent and extremely effective, the drug shows many drawbacks (Dillon et al., 2003). IMC has poor water solubility (~ 0.01mg/ml) and is soluble only at pH 12 (a pH at which significant hydrolysis of the molecule occurs). This insolubility of the drug in water for the skin pH leads to poor bioavailability. Moreover, IMC is also unstable under UV light (Lin et al., 1994). For these reasons IMC was a good candidate for inclusion complexation with hydroxyl-propyl- β cyclodextrin (HP β CD). α -Tocopherol (Vit. E) is naturally present in human skin providing protection against oxygen species and so is believed to act as anti-aging agent. The other cosmetic agent selected for encapsulation is Lauryl Isoquinolinium Bromide (LIB) which is known to affect hair growth. Both of these cosmetic agents are photosensitive and suitable for complexation and microencapsulation. HP β CD was chosen for inclusion complexation of the actives for its higher water solubility and solubilizing power, low cost and low toxicity. HP β CD causes neither physical nor chemical changes within the skin barrier (Loftsson and Masson, 2001) and enhances drug bioavailability in dermal dosage forms. Penetration of active-encapsulating particles and active-cyclodextrin encapsulating particles into the different layers of the human skin (ex vivo) was investigated by tape stripping, skin biopsy and microdialysis methods.

2. Materials and methods

2.1. Material

Ammonio methacrylate copolymer type BNF Eudragit[®] RS100 was purchased from EVONIK (EVONIK, Germany). Indomethacin (IMC), α -tocopherol (Vit. E), Lauryl Isoquinolinum Bromide (LIB), dichloromethane (DCM) and NaCl were purchased from Sigma-Aldrich, Germany. Hydroxypropyl-beta-cyclodextrin (HP β CD) was purchased from Acros organics. Deionized water was used throughout the work. Polyamide based textile was kindly supplied by Gravis company, France.

2.2. Method

2.2.1. Preparation of IMC-HP β CD, Vit E-HP β CD, LIB-HP β CD complexes

Complexes of IMC-HP β CD were formed at the beginning of the study and powder form of complex was stored for further experiments. Co-evaporation process was used to prepare these complexes. Co-evaporated (CO) complexes were formed by dissolving equimolar amounts of IMC and HP β CD in 40ml of 50% aqueous ethanol. This solution was stirred until the complete dissolution of the added powders. The solvents were then removed by using a rotary evaporator. The obtained solid was ground and sieved. Physical mixture (PM) of IMC-HP β CD was prepared by taking equimolar amounts of HP β CD and IMC in a pestle and mortar and mixing them for about 10 minutes. CO complex of Vit E-HP β CD was prepared by weighing accurately equimolar amounts of Vit E and HP β CD. Solution of each was made separately using absolute ethanol and water as a solvents. The two solutions were mixed under constant stirring and the organic solvent was evaporated to get a dried complex. The same procedure was followed to obtain a dried powdered complex of LIB-HP β CD. On the other hand, the PMs for both Vit E-HP β CD and LIB-HP β CD complexes were prepared in similar manner as described for PM of IMC-HP β CD complex.

2.2.2. Encapsulation of Vit. E, LIB, IMC, Vit E-HP β CD, LIB-HP β CD and IMC-HP β CD

The basic recipe for the experiment was as follows: 1ml of aqueous phase was emulsified in the organic phase (1g polymer in 5ml DCM) using ultrasound (Optic ivymen system CY-500) for 1.5min at 70% amplitude. The resultant primary emulsion (w/o) was then transferred to 30ml of the aq. phase along with stirring using ultra turrax[®] (T-25 basic IKA[®]-Werke) for 15min. at a speed of 13500rpm. No stabilizer was employed in the formulation. The obtained double emulsion (w/o/w) was diluted with 100ml of aq. phase and finally subjected to evaporation by using rotavapor (Buchiotavapor R-124) (Zafar et al., 2016). As all of the actives were hydrophobic in nature so they were incorporated in the organic phase during primary emulsification. Solutions of Vit E and LIB were made by dissolving 0.1g Vit E and 0.05g LIB in 400 μ L of absolute ethanol separately followed by their addition into the organic phase. Whereas, 0.1g IMC was dissolved in 2ml of DCM and then this solution was added to organic phase. On the other hand, the encapsulation of complexes was done by taking specific amount of complex of each active and dissolving it in 1ml of distilled water. This was used as internal aqueous phase of double emulsion. The remaining procedure was same as described above.

2.2.3. Encapsulation efficiency

Active-encapsulating particles were centrifuged and Vit. E, LIB and IMC were quantified by liquid chromatography-mass spectrometry (LC-MS) in the supernatants.

2.2.4. Skin penetration

Skin penetration was performed according to the protocol described previously (Bitar et al., 2015). Cutaneous penetration of active encapsulating particles and active-cyclodextrin complexes was evaluated into fresh excised human skin. Skin surface was cleaned with water and, ultra-thin and semi-permeable probes of microdialysis were implanted in the dermis. Penetration areas were demarcated and 500 μ l of active-encapsulating particles, active-HP β CD complexes and free molecules were deposited. Probes were perfused with Ringer's lactate solution at an optimal flow rate of 3 μ l/min. Dialysate samples were collected every 90 min over a period of 24 h. At the end of the sampling period, penetration areas were tape-stripped 10 times (disc 1 for skin surface; discs 2 to 10 for *stratum corneum*). Biopsies were realized and epidermis was separated from dermis by dispase incubation. Skin extraction was performed with methanol. Levels of Vit E, LIB and IMC were quantified by LC-MS. Statistical analyses were conducted with a two-way ANOVA (for microdialysis) and one-way ANOVA (for tape stripping and biopsies), followed by a Fischer test.

2.2.5. Adsorption onto textile

10ml of emulsion from each of the six prepared samples i.e. Vit E, LIB, IMC, vit.E-HP β CD, LIB-HP β CD and IMC-HP β CD loaded microparticles emulsion was taken in six different test tubes. Approximately 0.5g of the textile (cut into 1cm² pieces) was placed in each of the tubes. The tubes were placed on rotation for about 3hrs to allow sufficient adsorption onto the textile. Afterwards, pieces of textiles were washed with little water to remove the excess emulsion. Adsorption was confirmed by SEM analysis.

3. Characterization

3.1. Scanning Electron Microscopy (SEM)

The shape and surface characteristics of pure actives i.e. Vit E, LIB, IMC, HP β CD powder and PM and CO complexes of Vit E-HP β CD, LIB-HP β CD, IMC-HP β CD were visualized using a FEI Quanta 250 FEG microscope at the "Centre Technologique des Microstructures" (CT μ) at the University of Lyon (Villeurbanne, France). SEM was also used to characterize morphology of particles encapsulating Vit E, LIB, IMC, complexes of Vit E-HP β CD, LIB-HP β CD and IMC-HP β CD. Moreover, the adsorption onto selected textile of particles loaded with Vit E, LIB, IMC, Vit E-HP β CD, LIB-HP β CD and IMC-HP β CD was also confirmed by SEM analysis. Briefly, prior to examination, the samples were fixed on a brass stub using double-sided tape and sputter coated with copper under argon atmosphere. The SEM was set at an excitation voltage of 10kV. The magnification set was sufficient to appreciate in detail the general morphology of the samples before and after complexation, emulsification and adsorption.

3.2. Differential Scanning Calorimetry (DSC)

DSC of pure actives i.e. Vit E, LIB, IMC, HP β CD powders and PM and CO complexes of Vit E-HP β CD, LIB-HP β CD and IMC-HP β CD was performed using differential scanning calorimeter Q200 (TA instruments, USA). Each sample weighing 5.0 \pm 0.2mg was heated in a hermetic aluminium sample pan under nitrogen gas flow over the temperature range of 20-300°C at a heating rate of 10°C min⁻¹. Individual thermograms were recorded and studied.

3.3. Fourier-Transform Infra-Red (FTIR) spectroscopy

FTIR analysis was performed on pure Vit E, LIB, IMC, HP β CD powders and PM and CO complexes of Vit E-HP β CD, LIB-HP β CD, IMC-HP β CD powders. Analysis was carried out by FTIR analyzer IRPrestige 21, Shimadzu, Japan. The scanning was 400-4000 cm⁻¹ and resolution was 2 cm⁻¹.

3.4. Size and Zeta potential

Particles size and size distribution were measured with a Laser Diffraction Particle Size Analyzer (Mastersizer 3000, Malvern). Samples were prepared by adding drops of aqueous particles suspension to the instrument container, already filled with distilled water. The zeta potential of particles was measured using Zetasizer instrument (Zetasizer nanoS, Malvern[®], UK). Particles were highly diluted in NaCl 1mM and potential zeta was measured using special zeta cell DTS1070 (Malvern[®], UK). Zeta potential of the selected textile as a function of pH between 3 and 11 was measured using ZetaCAD[®] (CAD instruments). Briefly, the pH of the circulating fluid in the containers of the Zeta CAD i.e. 1mM NaCl solution was adjusted to different pH from 3 to 11 and zeta potential of the textile against each pH was measured.

4. Results and Discussion

4.1. SEM

4.1.1. Complexation

The morphology of HP β CD, IMC, PM and CO complexes of Vit E-HP β CD, LIB-HP β CD and IMC-HP β CD is presented in Fig. 1. This SEM analysis was done to confirm the presence of complexation by observing changes in the morphology of pure compounds after complexation. HP β CD exist as shrunken, cylindrical particles (Fig. 1a). The micrograph of IMC (Fig. 1b) indicated that it had large plate like crystals with smooth borders but irregularly shaped. The SEM analysis of pure Vit E and LIB could not be performed due to their high viscosity. The complexes showed clear and significant change in the morphology of these agents. The physical mixtures displayed morphology which was comparable with the pure actives. In PM of IMC-HP β CD, cylindrical particles of HP β CD embedded with crystals of IMC could be clearly seen (Fig. 1c). Similarly, PM of both LIB and Vit E showed that original morphology of HP β CD was still somewhat preserved (Fig. 1d and e). In contrast, a drastic change in the morphology and in crystalline nature of IMC was observed in CO IMC-HP β CD complex. The original morphology of IMC and HP β CD disappeared due to an interaction between the two agents during coevaporation process, making it difficult to separate IMC from HP β CD. This suggested successful complex formation. The coevaporated complex displayed medium sized randomly arranged amorphous aggregates (Fig. 1f). Photos for CO LIB-HP β CD and CO Vit E-HP β CD displayed similar results suggesting good complexation (Fig. 1g and h).

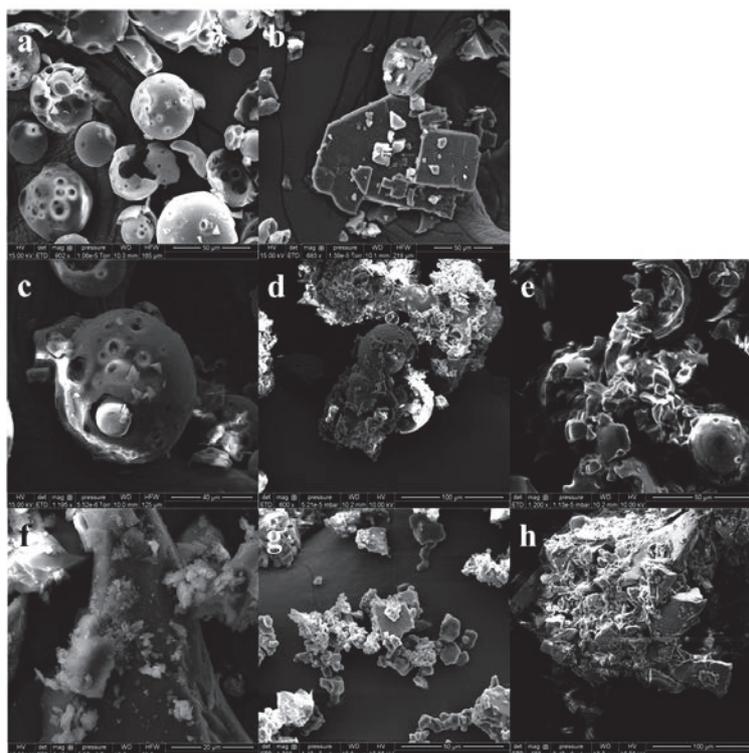


Fig. 1. SEM micrographs of (a) HPβCD powder, (b) IMC powder, (c) PM of IMC-HPβCD, (d) PM of LIB-HPβCD, (e) PM of Vit E-HPβCD, (f) CO complex of IMC-HPβCD, (g) CO complex of LIB-HPβCD and (h) CO complex of Vit E-HPβCD.

4.1.2. Pure active encapsulation and adsorption

The SEM photos of particles encapsulating pure actives i.e. Vit E, LIB and IMC are displayed in Fig. 2. It was observed that all the particles were polydisperse, porous and spherical in shape. Particles encapsulating LIB were observed to be larger with more visible pores (Fig. 2b) as compared to particles encapsulating Vit E and IMC. Both LIB and Vit E are liquid in nature and therefore might cause more swelling of the polymer leading to large particles as seen in SEM photos. Particles seemed to be lesser polydisperse in case of IMC (Fig. 2c) with porosity not very prominent. SEM photos showed that spherical morphology and porosity of particles due to the polymer Eudragit® RS100 (as shown in our previous works: (Zafar et al., 2016) (Bitar et al., 2015)) was unaffected by the encapsulation of the three hydrophobic active agents.

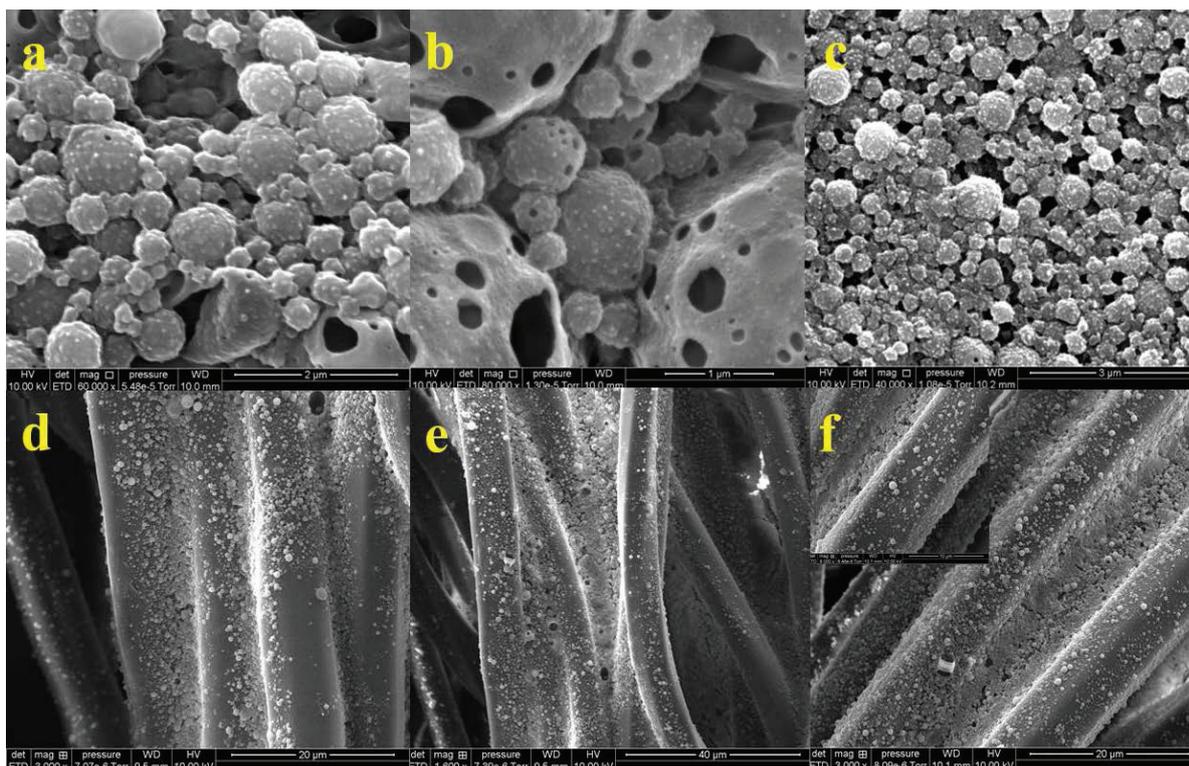


Fig. 2. SEM micrographs of microparticles encapsulating (a) Vit E, (b) LIB, (c) IMC and subsequent adsorption onto a polyamide based textile of (d) Vit E encapsulated particles, (e) LIB encapsulated particles, (f) IMC encapsulating particles.

SEM analysis for the adsorption of particles loaded with Vit. E (Fig. 2d), LIB (Fig. 2e) and IMC (Fig. 2f) onto the selected textile was also done. The SEM photos showed complete coverage of the fibers of textiles with the active loaded particles. This adsorption occurred due to electrostatic interaction between the positively charged particles and negatively charged textile.

4.1.3. Complexes encapsulation and adsorption

Photos for SEM analysis of particles encapsulating Vit E-HP β CD complex, LIB-HP β CD complex and IMC-HP β CD complex are shown in Fig. 3(a, b and c). It was observed that in general, the spherical morphology and multiporosity of the particles was unaffected by the presence of cyclodextrin. Regarding adsorption, the particles displayed complete coverage of fibers of textile Fig. 3(d, e and f). SEM photos also showed that polymer destruction or poor fragmentation was observed in case of cosmetic agent complex encapsulation. IMC-HP β CD complex loaded particles showed the best morphology, porosity and adsorption among the three actives encapsulated particles.

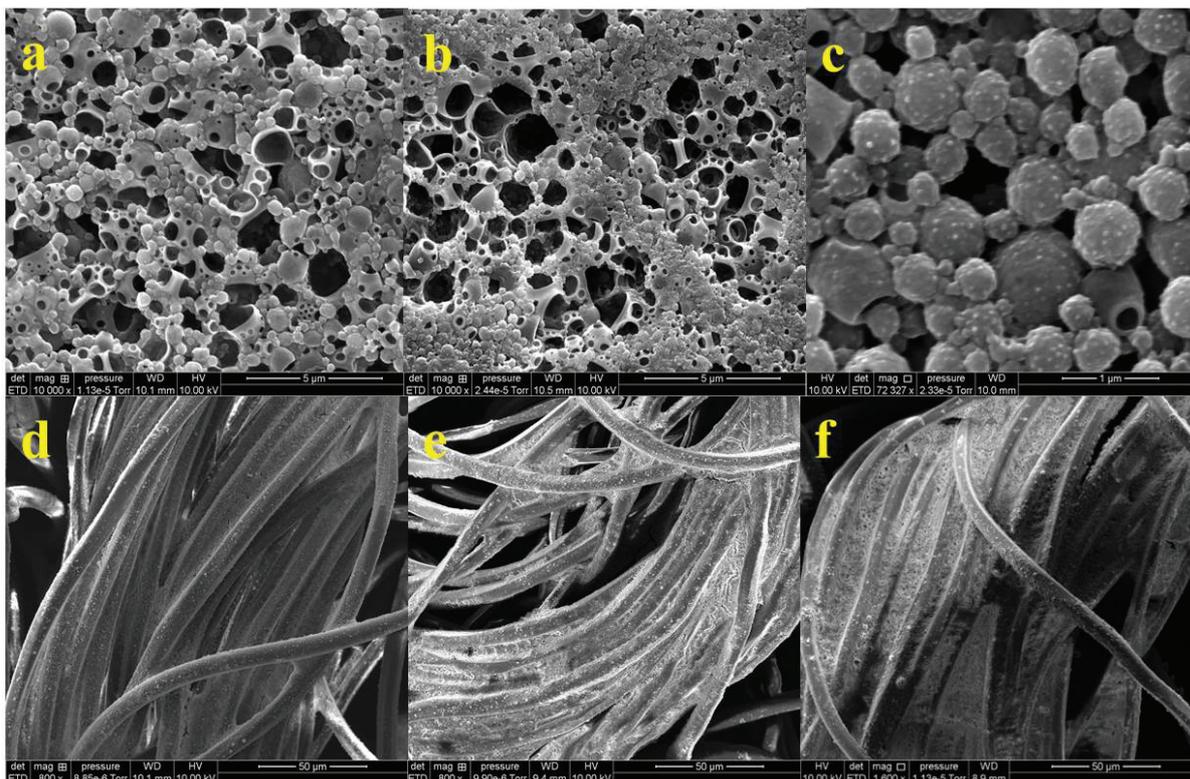
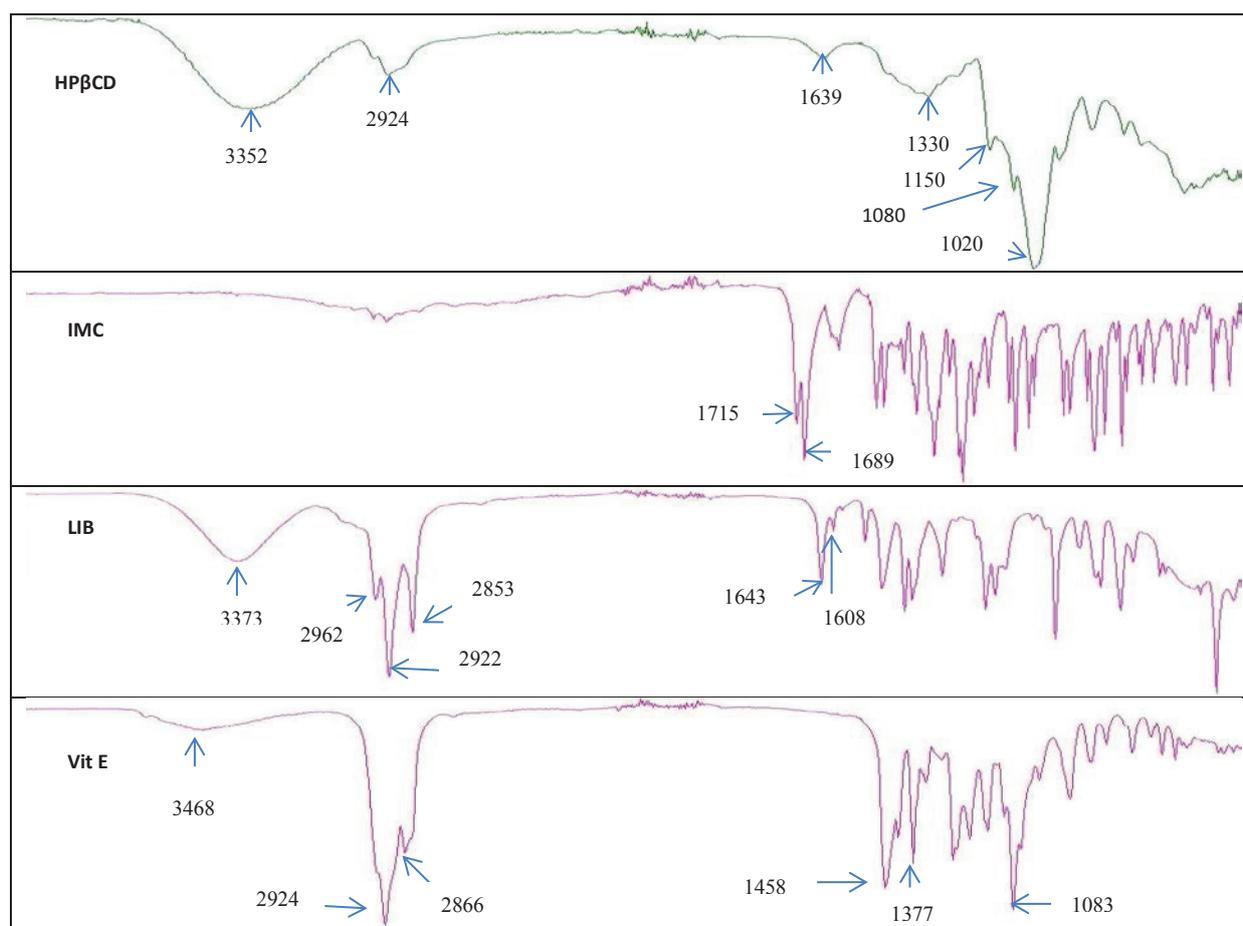


Fig. 3. SEM micrographs of microparticles encapsulating (a) Vit E-HP β CD complex, (b) LIB- HP β CD complex, (c) IMC-HP β CD complex and subsequent adsorption onto a polyamide based textile of (d) Vit E-HP β CD complex encapsulated particles, (e) LIB- HP β CD complex encapsulated particles and (f) IMC-HP β CD complex encapsulated particles.

4.2. Fourier-Transform Infra-Red (FTIR) spectroscopy

The diminutive effect on characteristics peaks or presence/absence of the characteristics peaks associated with the specific structural groups of each active molecule was used to observe the effect of complexation with cyclodextrin. The chemical interaction was reflected by changes in the characteristics peaks of the actives depending upon the degree of interaction. In general, minor peaks (lesser intensity) or peak shifts were observed in the complexes spectra when compared with the pure drugs and HP β CD spectra. The FTIR spectrum of HP β CD (Fig. 4) was characterized by the prominent absorbance peak at 3352 cm^{-1} due to O-H stretching vibrations. The vibration of -CH and -CH₂ groups appeared in the 2800-3000 cm^{-1} region. The peaks at 1080 cm^{-1} and 1020 cm^{-1} corresponded to C-O bond stretching. The peak at 1639 cm^{-1} was due to O-H deformation. Pure IMC displayed two characteristics peaks at 1715 and 1689 cm^{-1} corresponding to carbonyl & benzoyl stretching (C=O) respectively. The PM and CO complexes of IMC-HP β CD displayed characteristics peaks of the HP β CD at 3361 cm^{-1} and 3358 cm^{-1} (O-H), 2926 cm^{-1} and 2927 cm^{-1} (C-H), 1028 cm^{-1} (C-O), 1083 cm^{-1} (C-O) respectively and peaks for IMC at 1712 cm^{-1} and 1710 cm^{-1} , 1690 cm^{-1} showed minor shifting as well as lower intensity. This confirmed successful complexation between IMC and HP β CD. The spectrum of LIB showed peaks at 1643 cm^{-1} (C=N stretching), 1608 cm^{-1} (C=C aromatic stretching), 2853 cm^{-1} (-CH₂ symmetric stretching), 2922 cm^{-1} and 2962 cm^{-1} (-CH₃ stretching) and 3373 cm^{-1} (O-H). It appeared that the compound LIB was not pure and had some impurity present in it since the peak corresponding to O-H group should not be normally present in its spectrum according to its structure. The PM and CO complexes of LIB-HP β CD displayed spectrum similar to each

other. The peaks for LIB at 2962 cm^{-1} , 2922 cm^{-1} , 2853 cm^{-1} , 1643 cm^{-1} and 1608 cm^{-1} disappeared from the complexes spectrum indicating the complete encapsulation of LIB molecule inside the HP β CD cavity. The peaks for HP β CD at 3342 cm^{-1} and 3344 cm^{-1} , 2922 cm^{-1} and 2918 cm^{-1} , 1641 cm^{-1} , 1080 cm^{-1} and 1024 cm^{-1} and 1026 cm^{-1} were all displayed in both PM and CO complex spectrums respectively. This further proved the complete complexation between the two molecules. It was observed that the spectrum of pure Vit E was very similar to the spectrum of pure HP β CD. Hence, due to this similarity it was difficult to make any conclusion about the complexation between Vit E and HP β CD. Scanning the spectrum of Vit E, absorption bands at wavenumbers 3468 cm^{-1} (O-H) (Man and Mirghani, 2000), 2924 cm^{-1} and 2866 cm^{-1} for asymmetric and symmetric stretching vibrations of the $-\text{CH}_2$ and $-\text{CH}_3$ (Guillén and Cabo, 1997), 1458 cm^{-1} for methyl asymmetric bending, 1377 cm^{-1} for methyl symmetric bending and 1083 cm^{-1} for plane bending of phenyl were observed. In the Vit E-HP β CD complex spectrum (PM and CO), it was observed that the peaks corresponding to Vit E at 1452 cm^{-1} , 1379 cm^{-1} and 1377 cm^{-1} , 1261 cm^{-1} respectively were diminished (lower intensity), which proved the encapsulation of Vit E inside the HP β CD. The peaks at 1082 cm^{-1} , 1024 cm^{-1} and 1028 cm^{-1} clearly indicated the presence of HP β CD. However, as stated earlier FTIR results with respect to pure Vit E and HP β CD are similar making it difficult to assess the complex spectrum and to conclude anything about the degree of complexation between the two agents. In general, FTIR results showed that the processes i.e. physical mixing and coevaporation gave similar results with respect to complexation.



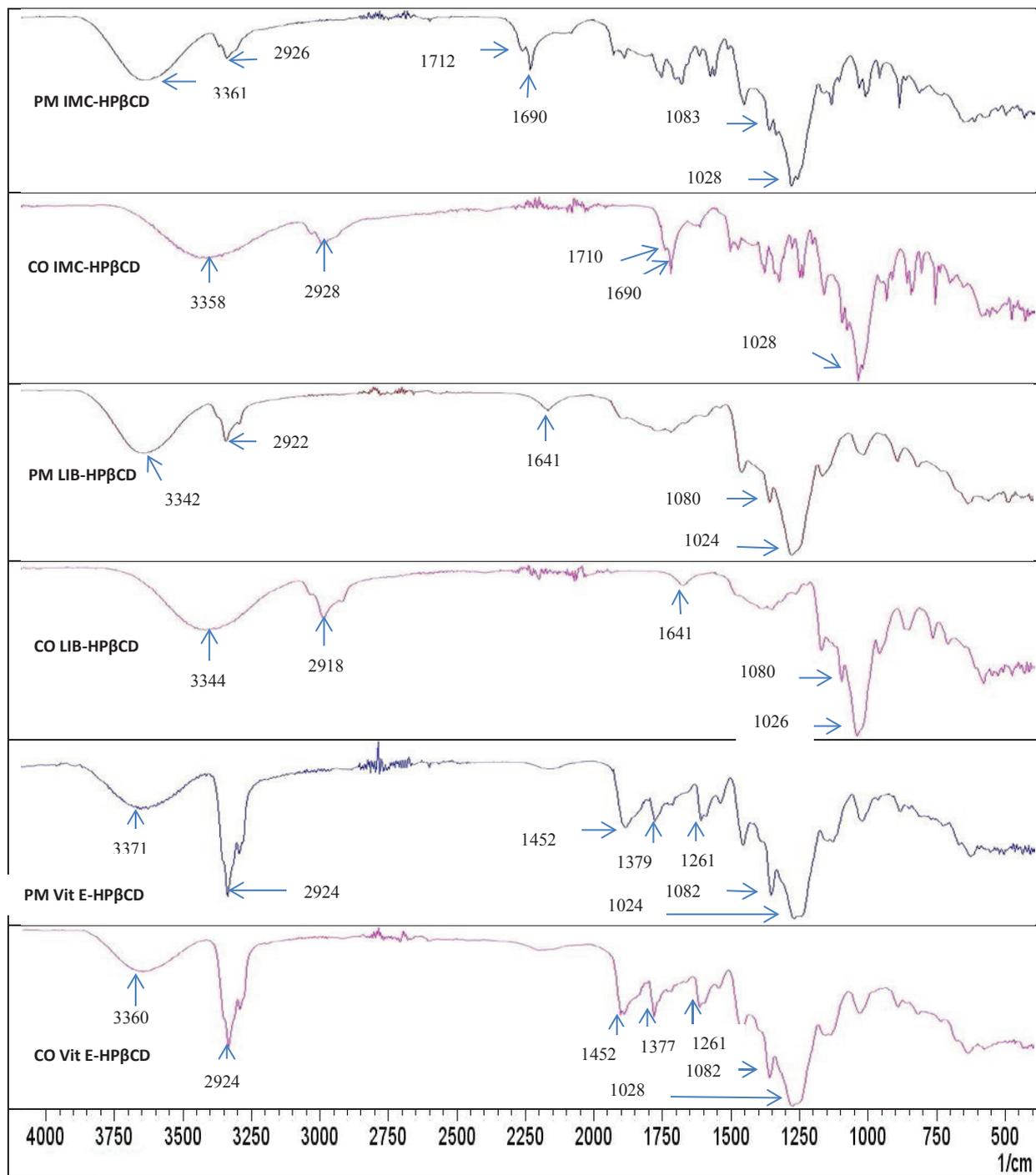


Fig. 4. FTIR spectra for the pure active molecules and their physically mixed and coevaporated complexes.

4.3. DSC

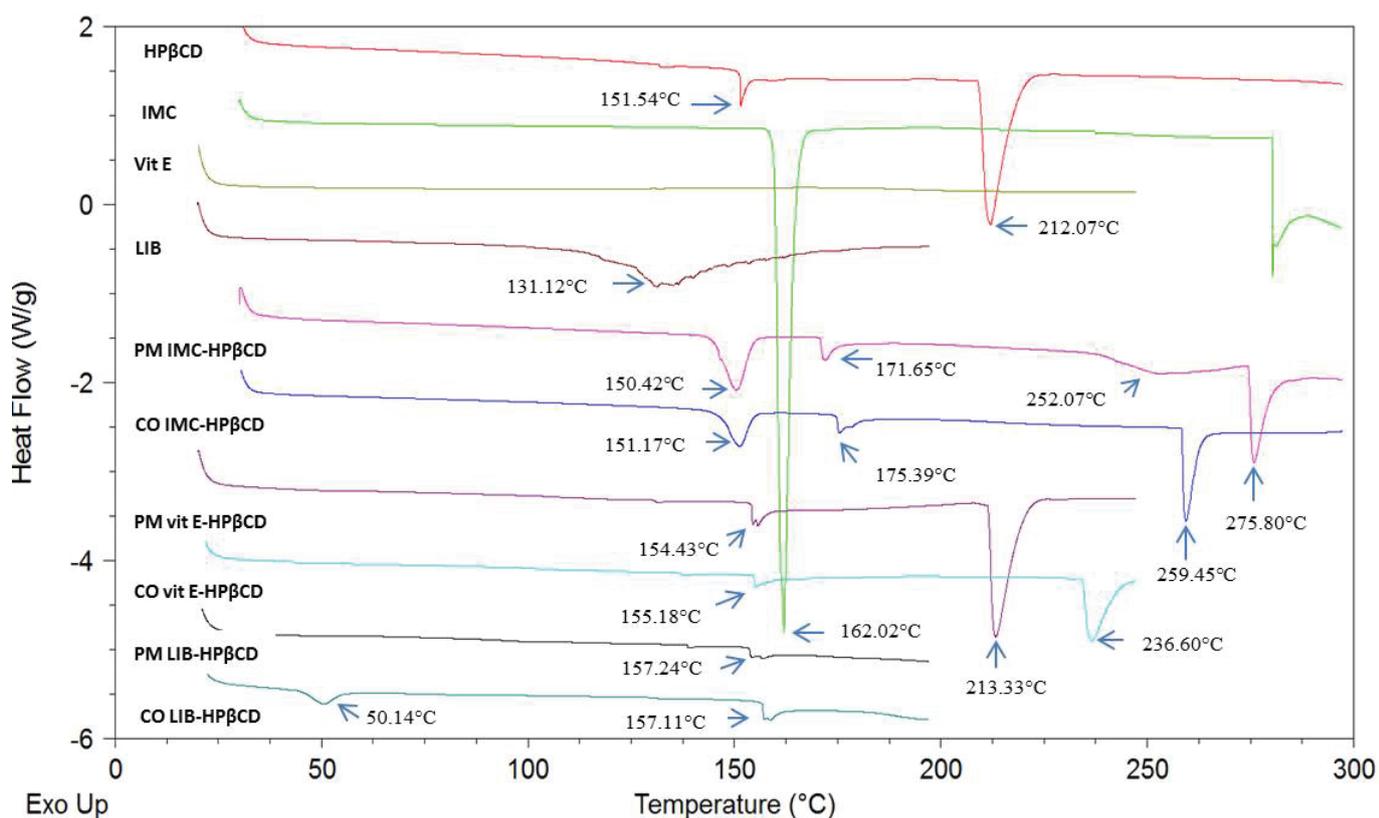


Fig. 5. DSC curves for the pure active molecules and their physically mixed and coevaporated complexes.

DSC can be used to recognize the inclusion complex formation. When molecules are entrapped in the HP β CD cavity, their m.p, b.p or sublimation points are generally shifted to different temperatures. The thermograms for all the samples are presented in the Fig. 5. The decomposition temperature of the pure IMC, Vit E and LIB were determined by TGA (results not displayed) and were found to be 293°C, 262°C and 349°C for IMC, LIB and α -tocopherol respectively. IMC showed a sharp endothermic peak at 162°C which disappeared from both PM and CO IMC-HP β CD complexes suggesting a successful complexation. The spectrum of both PM and CO IMC-HP β CD showed endothermic peaks at ~150°C indicating the presence of HP β CD. Pure Vit E did not show any specific peak at the temperature range used but the curves for both PM and CO Vit E-HP β CD complexes showed peaks at 154°C, 155°C, 213°C and 236°C indicating the presence of HP β CD. We concluded from this that the data was not sufficient enough to assert successful complexation between the two compounds. The peaks at 275°C and 259°C probably indicated the shifting of peak at 212°C (HP β CD) which occurred due to intermolecular interaction between HP β CD and α -tocopherol. LIB displayed a peak at 131°C which completely disappeared from its PM and CO complexes. The complexes spectrum however showed peaks at 157°C which corresponded to HP β CD and hence proved the successful entrapment of LIB molecule in the cavity of HP β CD.

4.4. Size and Zeta potential

Table 1

Size and zeta potential of the particles encapsulating pure actives and their complexes with HP β CD.

Samples	Size (μ m)	Av. zeta potential (mV)
Vit. E	0.41	51.9
LIB	0.72	52.1

IMC	0.37	42.3
Vit. E-HP β CD	0.32	53.6
LIB-HP β CD	0.53	44.6
IMC-HP β CD	0.27	46.5

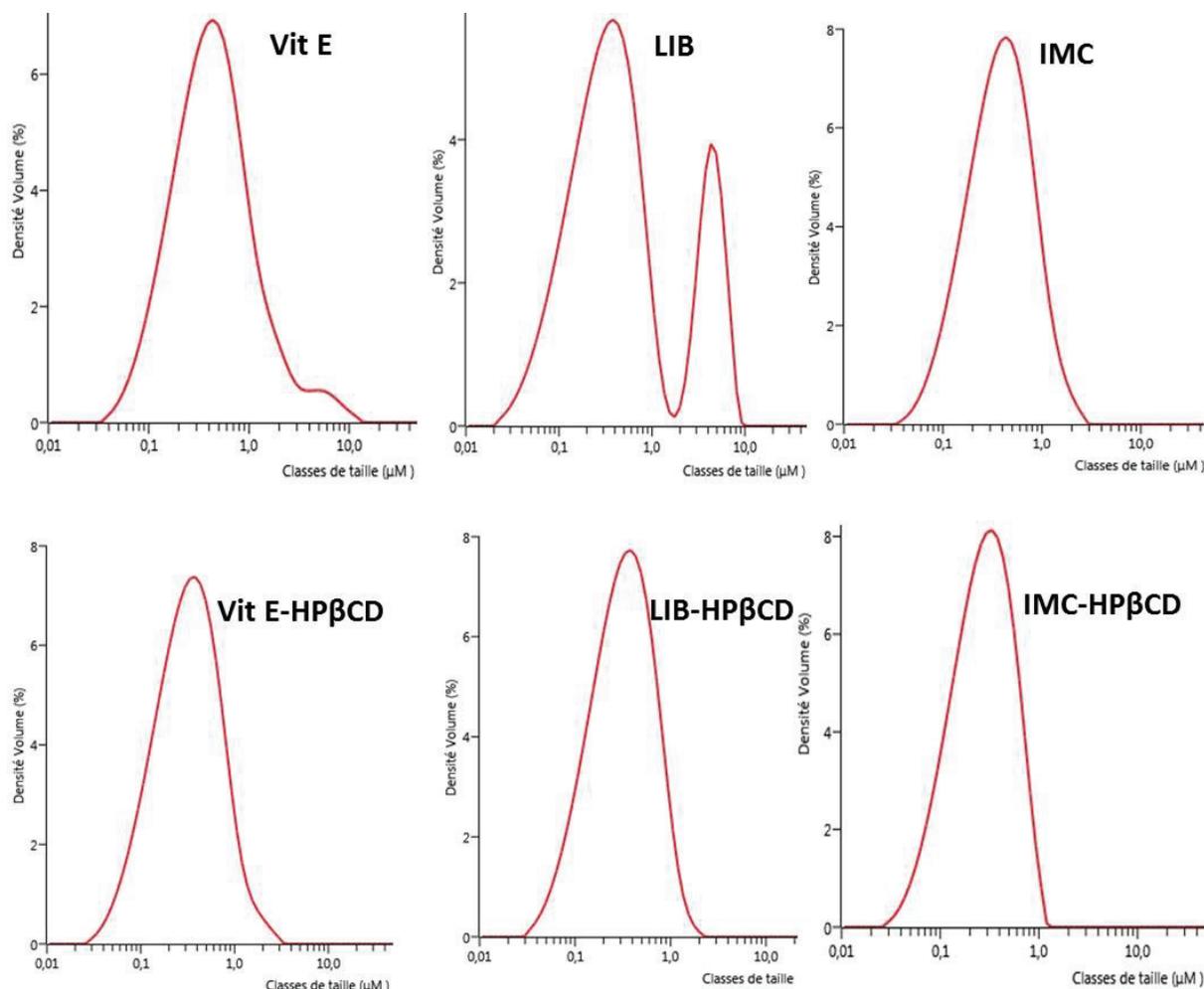


Fig. 6. Size distribution of particles encapsulating pure actives and their complexes with HP β CD

The results for all the samples size and zeta potential are displayed in Table 1 and size distribution in Fig. 6. From Table 1, it was observed that presence of HP β CD did not affect the size of particles after encapsulation. In general, the size range for pure active encapsulating particles and active-complex encapsulating particles was 0.27 μ m-0.7 μ m. The zeta potential of all the particles was observed to be \sim 50mV. The positive charge was due to the presence of positively charged polymer Eudragit RS100 which had quaternary ammonium groups contributing to this charge. Cyclodextrin are neutral compounds and thus did not affect the overall zeta potential of the particles.

4.5. Encapsulation efficiency

The concentrations of actives in supernatants showed that the encapsulation efficiency was 41% for Vit E, 26% for LIB and 20% for IMC (data not illustrated).

4.6 Skin penetration

The cutaneous penetration of active-encapsulating particles and active-cyclodextrin complexes through the skin was studied using microdialysis, tape stripping and punch-biopsy techniques. Standard solutions of Vit E, LIB and IMC were used as controls.

For Vit E, results obtained by dermal microdialysis are shown in Fig. 7. The dermal penetration kinetics of Vit E-encapsulated particles and their complexes with HP β CD were different from the control, with a significant slower initial penetration. A higher Vit E concentration of about 120 μ g/ml at 9 h for Vit E encapsulated particles was observed vs a lower concentration of 80 μ g/ml at 7 h 30 for the control (Fig. 7A). Similarly, Vit E-HP β CD loaded particles produced a higher content of Vit E (163 μ g/ml) at 10 h 30 vs a lower concentration of 100 μ g/ml at 7 h 30 for the control (Fig. 7B). Results obtained by tape stripping and biopsies are shown in Fig. 8 and 9. The content of Vit E in dermis for both Vit E and Vit E-HP β CD loaded particles was observed to be similar to the control. These results indicated that cutaneous penetration of active was unaffected by the encapsulation of active-HP β CD complex.

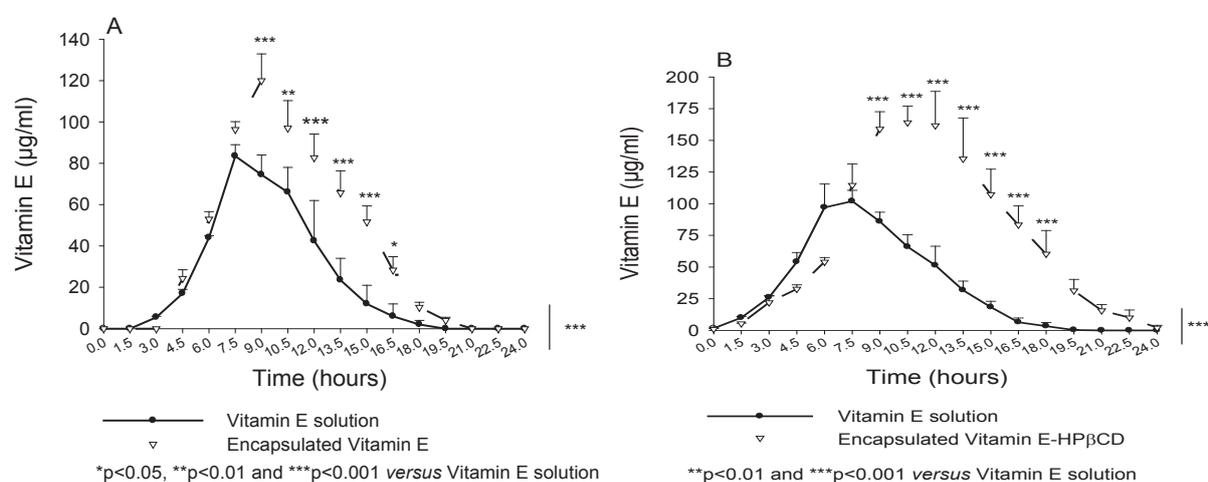


Fig. 7. Vit E concentration in microdialysate in the presence of encapsulated Vit E (A) and encapsulated Vit E-HP β CD (B)

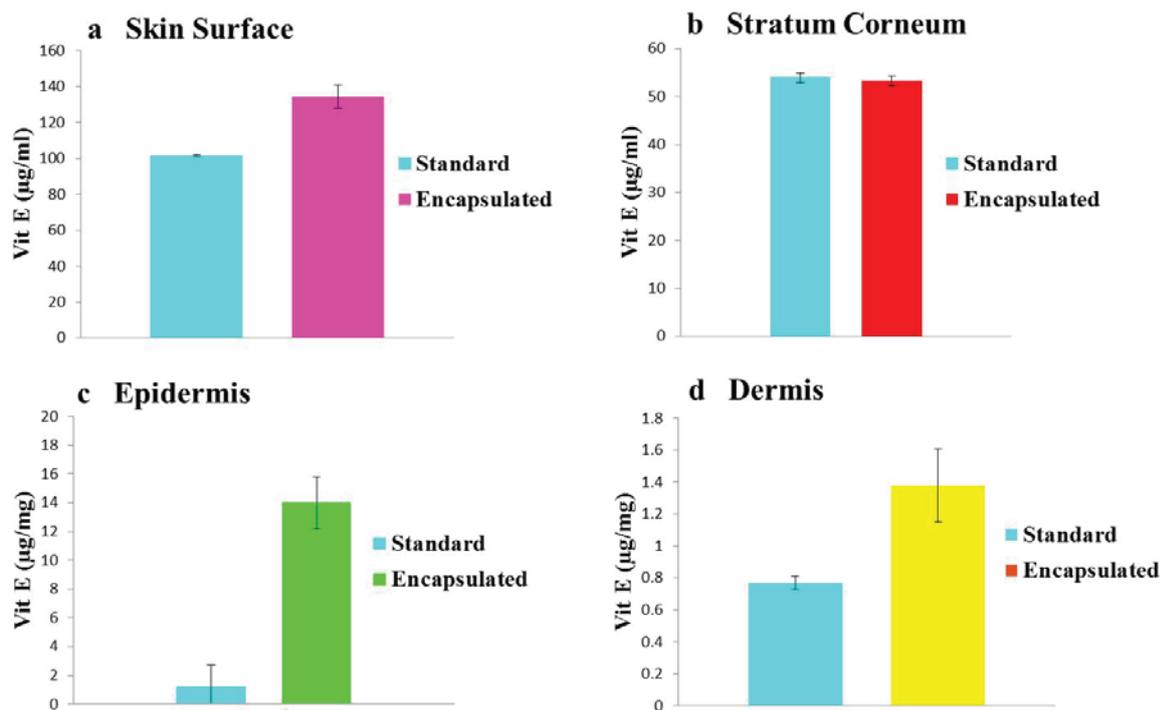


Fig. 8. Tape stripping and biopsies after 24 h of Vit.E loaded particles and standard application. Vit.E concentrations (mean ± SD, n= 3) in tape strip samples of skin surface (a) and stratum corneum (b) in biopsies of the epidermis (c) and dermis (d).

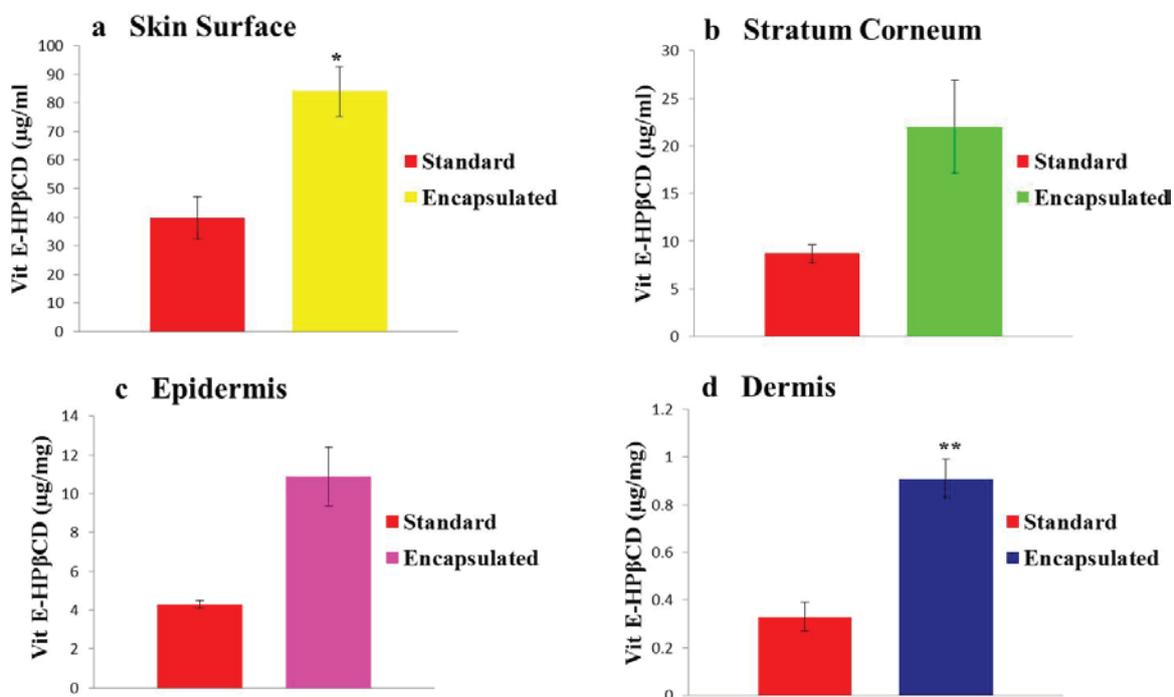


Fig. 9. Tape stripping and biopsies after 24 h of Vit.E-HPβCD loaded particles and standard application. Vit.E-HPβCD concentrations (mean ± SD, n= 3) in tape strip samples of skin surface (a) and stratum corneum (b) in biopsies of the epidermis (c) and dermis (d).

For LIB, results obtained by dermal microdialysis are shown in Fig. 10. The dermal penetration kinetic of LIB-encapsulated particles and their complexes with HP β CD were different from the control, with a significant slower initial penetration. A higher LIB concentration of about 1 μ g/ml at 15 h for LIB encapsulated particles was observed vs a lower concentration of 0.6 μ g/ml at 11h for the control (Fig. 10A). Similarly, LIB-HP β CD loaded particles produced a higher content of LIB (3.7 μ g/ml) at 15 h vs a lower concentration of 0.8 μ g/ml at 11 h for the control (Fig. 10B). Results obtained by tape stripping and biopsies are shown in Fig. 11 and 12. The content of LIB in dermis for both LIB and LIB-HP β CD loaded particles was observed to be similar to the control. These results indicated that cutaneous penetration of active was unaffected by the encapsulation of active-HP β CD complex.

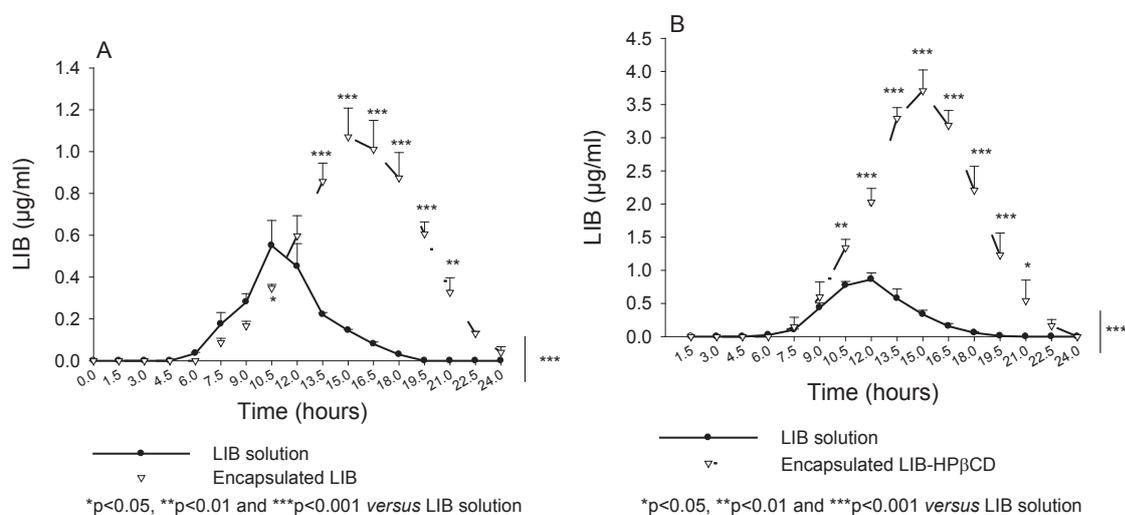


Fig. 10. LIB concentration in microdialysate in the presence of encapsulated LIB (A) and encapsulated LIB-HP β CD (B)

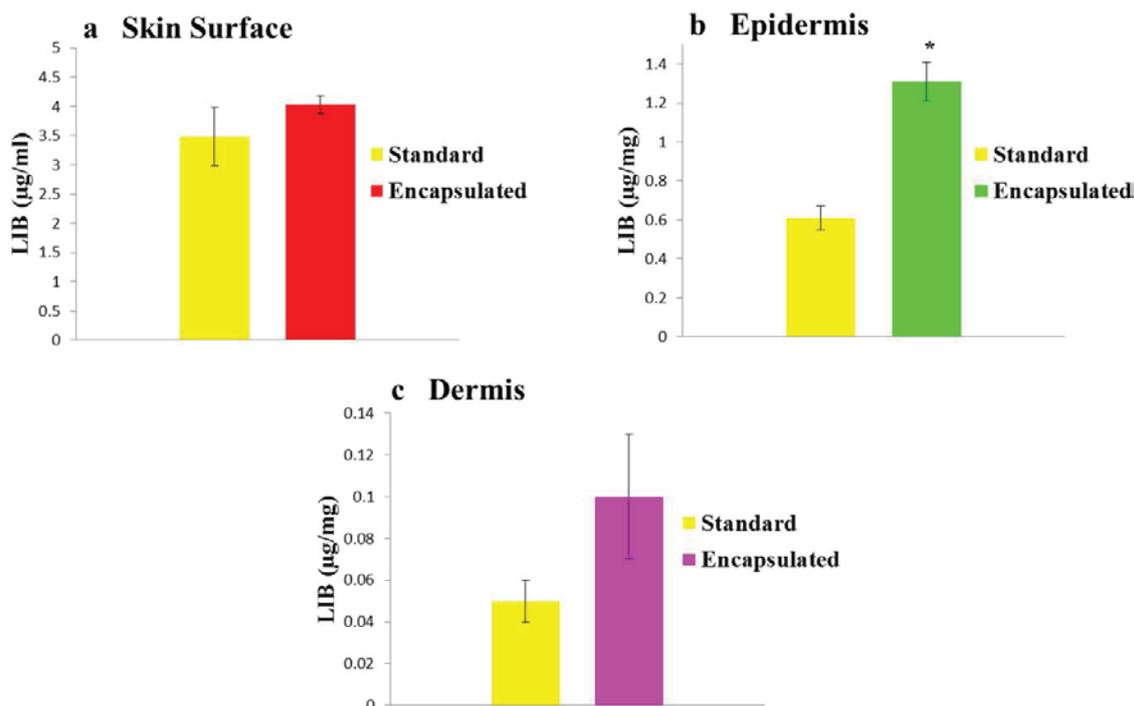


Fig. 11. Tape stripping and biopsies after 24 h of LIB loaded particles and standard application. LIB concentrations (mean \pm SD, n= 3) in tape strip samples of skin surface (a) in biopsies of the epidermis (b) and dermis (c).

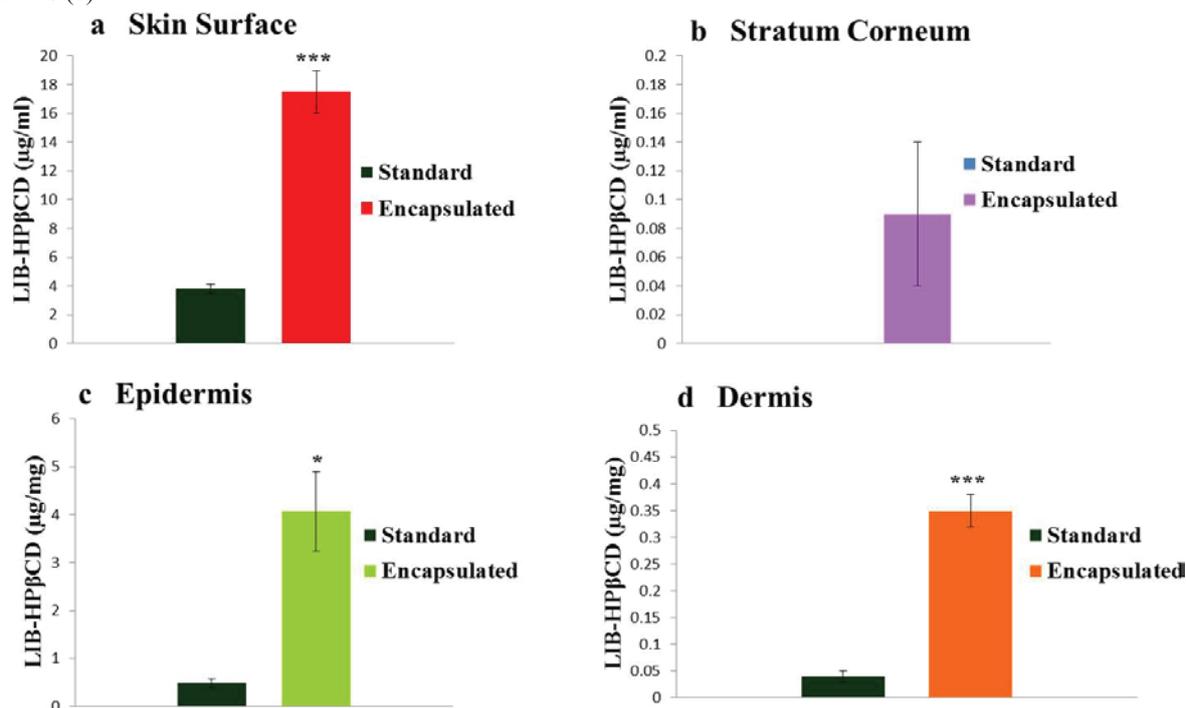


Fig. 12. Tape stripping and biopsies after 24 h of LIB-HPβCD loaded particles and standard application. LIB-HPβCD concentrations (mean \pm SD, n= 3) in tape strip samples of skin surface (a) and stratum corneum (b) in biopsies of the epidermis (c) and dermis (d).

For IMC, results obtained by dermal microdialysis are shown in Fig. 13. The dermal penetration kinetic of IMC-encapsulated particles and their complexes with HPβCD were different from the control, with a significant slower initial penetration and a significant higher concentration found at 7 h30 for IMC-encapsulated particles and HPβCD complexes, vs 6 h for the control (Fig. 13A and B). Results obtained by tape stripping and biopsies are shown in Fig. 14 and 15. The content of IMC in dermis was not significantly higher with HPβCD complexes compared with control, indicating that dermal penetration was not affected by the encapsulation of active-HPβCD complex.

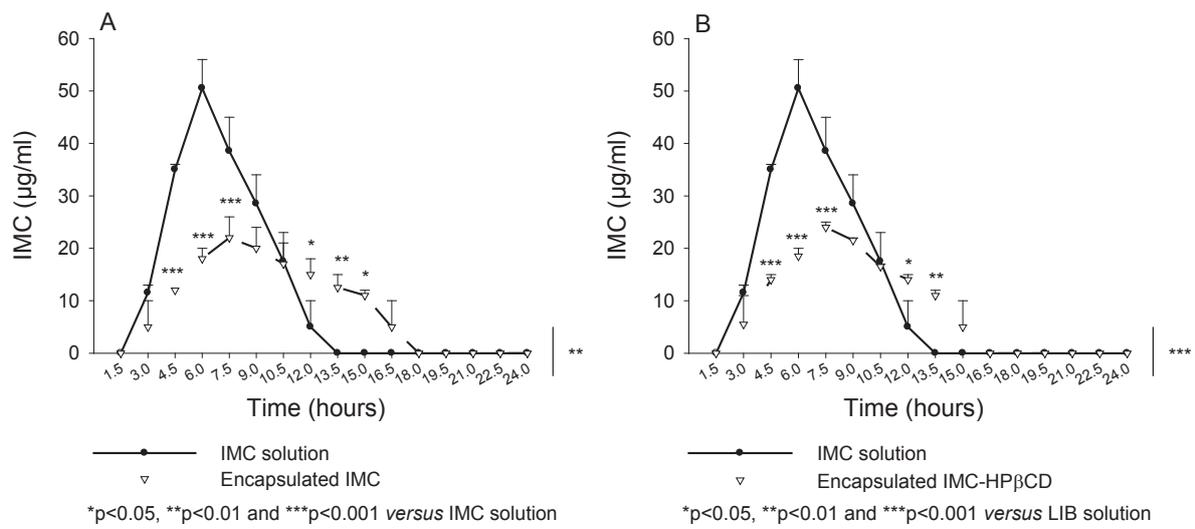


Fig. 13. IMC concentration in microdialysate in the presence of encapsulated IMC (A) and encapsulated IMC-HPβCD (B)

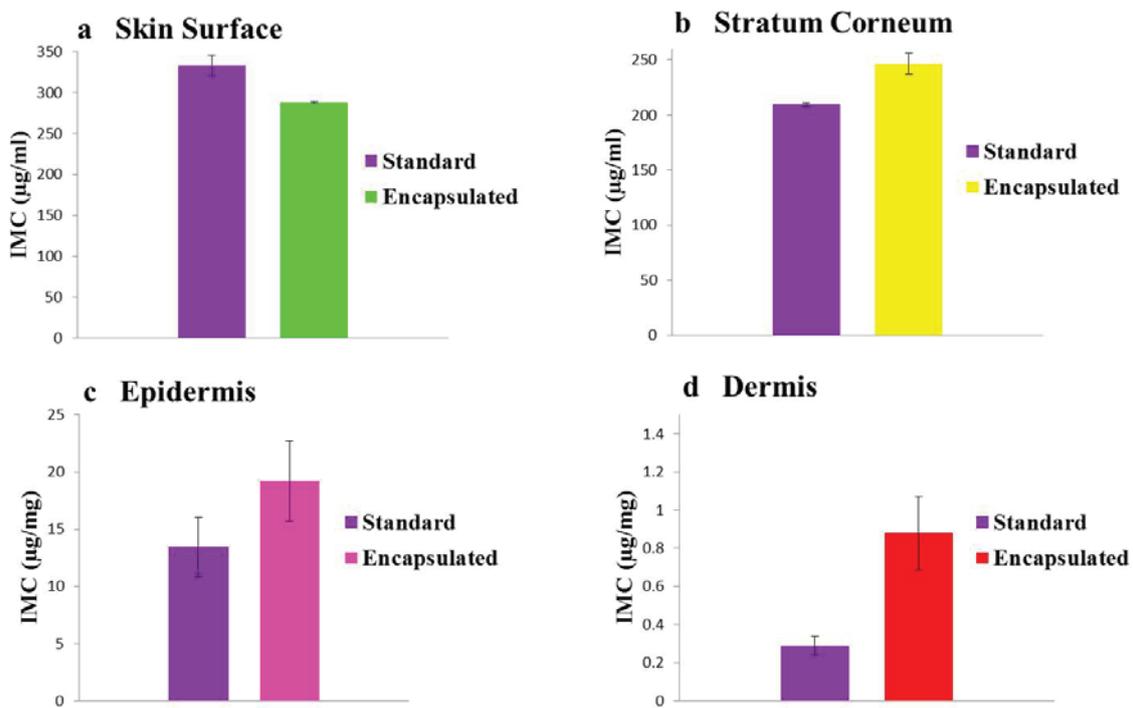


Fig. 14. Tape stripping and biopsies after 24 h of IMC loaded particles and standard application. IMC concentrations (mean ± SD, n= 3) in tape strip samples of skin surface (a) and stratum corneum (b) in biopsies of the epidermis (c) and dermis (d).

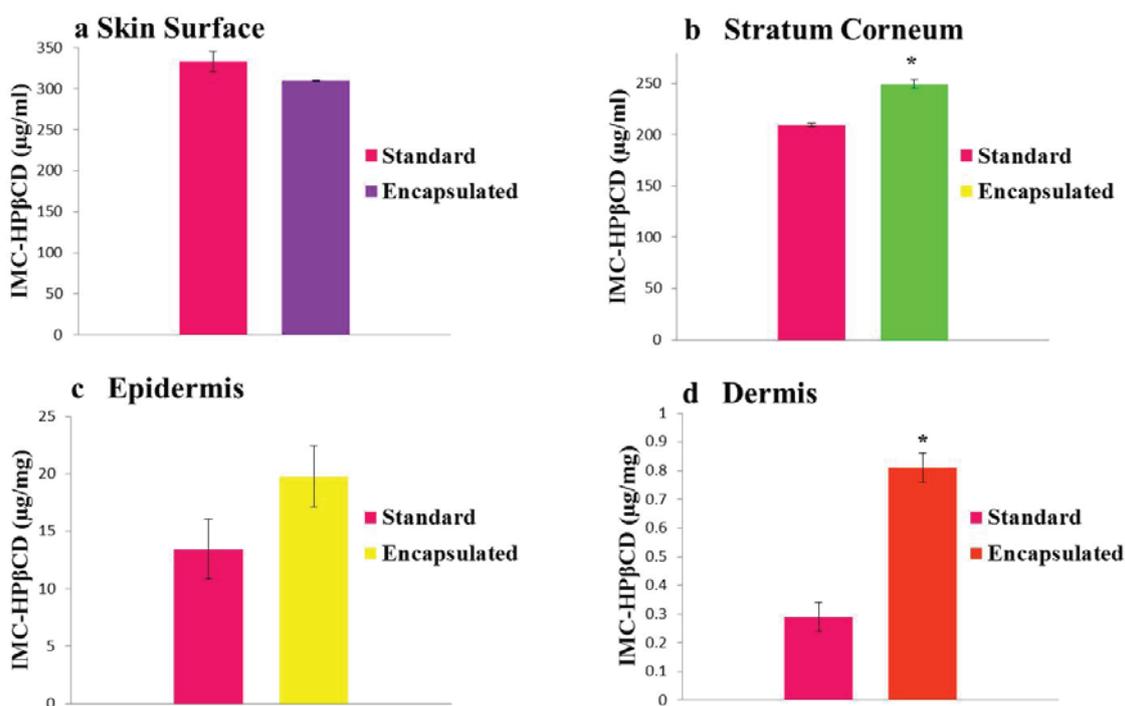


Fig. 15. Tape stripping and biopsies after 24 h of IMC-HPβCD loaded particles and standard application. IMC-HPβCD concentrations (mean ± SD, n= 3) in tape strip samples of skin surface (a) and stratum corneum (b) in biopsies of the epidermis (c) and dermis (d).

Active-encapsulated particles and their HPβCD complexes displayed similar skin penetration profiles. Both do not modify the cutaneous penetration of actives. However, cyclodextrin did help to achieve prolonged release formulation.

5. Conclusion

The successful preparation of active-loaded microparticles was achieved by using double emulsion solvent evaporation technique and the polymer Eudragit® RS100. Active-cyclodextrin complexes were also successfully loaded into the prepared microparticles. Active-cyclodextrin complexes were characterized by SEM, FTIR and DSC which confirmed successful complexation. The obtained particles were characterized by SEM, size and zeta potential measurements. SEM showed particles having spherical, sponge like morphology with rough and porous surfaces. The size of all prepared microparticles was near to 1μm and zeta potential in the range of 45-50mV. Active (Vit E, LIB, IMC) and active-cyclodextrin complexes (Vit. E-HPβCD, LIB-HPβCD, IMC-HPβCD) were loaded during particles' synthesis and their skin penetration aptitude was confirmed. In general, it was observed that cyclodextrin based actives in polymeric particulate form displayed prolonged release properties with unchanged dermal penetration. Moreover, the active loaded microparticles and active-cyclodextrin complex loaded microparticles provided better skin penetration profiles as compared to pure actives. Finally, all prepared particles adsorption onto a negatively charged textile was studied and a complete coverage of fibers with particles was accomplished. In summary, positively charged microparticles were prepared which could be loaded with both cosmetic and therapeutic active molecules and could be adsorbed onto a negatively charged textile. Such functionalized textiles have promoted applications in both cosmetic and pharmaceutical industries especially for achieving prolonged release formulations.

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Part IV
DISCUSSION AND CONCLUSION

Discussion and Conclusion

Functionalized textiles are nowadays in great demand as they are multifunctional materials and are extensively used by pharmaceutical and cosmetic industries. Their pharmaceutical and cosmetic applications include e.g. wound dressings and slimming tights among many other different uses. However, there are still some limitations associated with their preparation techniques that hinder their lasting stay in the markets. The functionalized textile products available in the market produced by using binders for the attachment of microcapsules containing active agents face the problem of poor resistance to washing and hence earlier loss of efficacy. Such problems are commonly overcome by using recharge solutions as proposed by industries. However, this approach is not ideal as it does not allow targeted functionalization of active agents on the textiles and also requires extra manipulations from the consumers.

This thesis was targeted at achieving a new type of functionalized textile which did not involve the use of binders, was functionalized with biodegradable polymeric particles containing therapeutic and cosmetic agents and offered improved resistance to washing.

Various process control parameters were thoroughly investigated since they affect the physicochemical properties of the polymeric particles prepared by the chosen method. The preliminary study performed by using Ultra-turrax[®] as homogenizer (for both primary and secondary emulsification) produced particles with minimum size near to 20 μm . An increase in Ultra-turrax[®] stirring time and speed, in general, led to a decrease in particles size due to better fragmentation of the polymeric particles by the high amount of energy being supplied to the system. Moreover, the investigation about using different stabilizers (PVA, F68, F127) showed that their usage in the formulation just further complicates the formulation while bringing no significant change to the size of the particles. Since our goal was to obtain particles with a size near to 1 μm , so in our next systematic study we did not use any stabilizer and decided to use Ultrasonication for the primary emulsification and Ultra-Turrax[®] for the secondary emulsification.

Increase in Ultrasonication time did not show any significant change in the size of particles whereas polymer amount, Ultraturrax[®] speed and time showed significant influence on the particles size. Effect of external aqueous phase was also investigated and any increase in volume did not significantly change the size of particles. From this systematic study, it was concluded that 1g of Eudragit RS100, 1.5min of sonication time, 15min and 13500 rpm stirring time and speed of Ultraturrax[®], 30ml of external aqueous phase were the optimum parameters that provided spherical and sponge like particles with size of 1 μm . Following this recipe, scale up studies, up to 4 and 10 times of original values were also performed and it was observed that the optimized recipe was only applicable to 4 times scale up. Such studies are quite important from industrial point of view.

After the preparation and optimization, the next was the electrokinetic study of provided textile and adsorption of microparticles. The zeta potential of the microparticles was found to around +50mV in large pH domain due to cationic character of the used polymer. The zeta potential of bare textile was deduced from streaming potential and was observed to be negative irrespective of pH. In addition, salinity of the adsorption medium seemed to decrease the zeta value of the textile due to shielding of the negative charges. It was observed that maximum adsorption of particles occurred at pH 9 and 1mM NaCl concentration conditions of the adsorption medium. Finally, under these conditions of pH and salinity, particles were adsorbed onto the textile and results showed that upon increasing the amounts of particles in the adsorption medium, the zeta potential of the textile was gradually changed from negative to positive values until a plateau value was reached which indicated the adsorption saturation

point of the textile. Hence, polyamide textile was successfully functionalized with spongy, cationic and hollow microparticles.

Two cosmetic agents, vitamin E and LIB, selected for their hydrating and anti-hair growth effect, and an anti-inflammatory active: Indomethacin, were now encapsulated into the spongy microparticles. Since these are hydrophobic in nature, so they were introduced into the organic phase during the formation of primary emulsification. The encapsulated particles were spherical in shape, polydisperse and with rough surface due to diffusion and evaporation processes applied after particles synthesis. The zeta potential value of these particles was ~50mV and this value was independent of the formulation recipe. This independence was due to usage of same amount of polymer as well as absence of any stabilizer which could modify the electrostatic characteristics of particles surface. The encapsulation efficiency was observed by using liquid chromatography-mass spectrometry. Penetration of active loaded particles through the skin was studied using microdialysis, tape stripping and punch biopsy techniques. 41% encapsulation efficiency for Vit E, 26% for LIB and 20% for IMC was observed. Dermal microdialysis results for Vit E showed that slower initial penetration and a significantly higher concentration of 120 μ g/ml at 9 h for encapsulated particles vs a lower concentration of 80 μ g/ml at 6 h for the control was observed. The drug release was upto 21 h for the Vit E encapsulated particles as compared to 19 h 30 release time for the control. On the other hand, in tape stripping and punch biopsy results, Vit E content in the different skin layers after 24 h of application was found to be similar for both encapsulated particles and control. Similarly, for LIB, slower initial penetration and a higher concentration of 1 μ g/ml at 15 h for encapsulated particles vs a lower concentration of 0.6 μ g/ml at 11 h for the control was observed from the dermal microdialysis results. The drug release was upto 24 h for the LIB encapsulated particles as compared to 19 h 30 release time for the control. Results of tape stripping and punch biopsies for LIB showed that content of LIB was not significantly different in different layers of skin after 24 h application of both encapsulated particles and control. LIB was not detected in the stratum corneum. IMC loaded particles showed a significantly lower concentration of 22 μ g/ml at 7 h 30 vs a higher concentration of 50 μ g/ml at 6 h for the control. The drug release was upto 18 h for the IMC encapsulated particles as compared to 13 h 30 release time for the control. However, results of tape stripping and punch biopsy again showed that content of IMC in different layers of skin was not significantly different for both active encapsulated particles and control.

The active loaded particles were also adsorbed onto the provided textile and SEM analysis showed good coverage of textile fibers with the particles. Moreover, it was also observed that adsorption was fast and did not require a long time (30-60 minutes were enough). Hence, it was concluded that encapsulation of the three actives did not alter the morphology, adsorption and the cutaneous penetration of the selected active agents.

The most important and final step of the thesis was the inclusion of cyclodextrin complexes into the polymeric particles. Physically mixed and co-evaporated complexes of the three actives with HPBCD were formed and analyzed by SEM, DSC and FTIR. SEM images showed different morphology of the complexes as compared to the pure actives and HPBCD, confirming the presence of successful complexation. For FTIR, the diminutive effect on characteristics peaks or absence of characteristics peaks associated with specific structural groups of each active molecule was used to observe the effect of complexation with cyclodextrin. In general, minor peaks (lesser intensity) or peaks shift were observed in the complexes spectra when compared with the pure drugs and HPBCD spectra. In DSC, generally, when molecules are entrapped in the cyclodextrin cavity, their m.p, b.p. or sublimation temperature are shifted to different temperatures. The thermograms for complexes of LIB and IMC showed the absence of characteristic endothermic peaks for them but displayed the sharp endothermic peak for HPBCD thus confirming good complexation.

For vitamin E analysis, data was not sufficient to assert successful complexation between the two compounds. Moving onto next step, specific amounts of these complexes were taken, solution made in water and 1 ml of these solutions was used as internal aqueous phase of the w/o/w emulsion. On characterization, all the three active-CD complex loaded particles showed spherical morphology, polydispersity, size near to 1 μm and zeta potential values near to 50mV. The presence of cyclodextrin did not affect the morphology or size of particles which was an added advantage in addition to achieving enhanced aqueous solubility and protection of the actives from surrounding environment. The cutaneous penetration studies revealed that penetration profiles of all the three active-CD loaded microparticles were similar to profiles of pure active loaded microparticles. The active-CD loaded particles also showed good adsorption by displaying complete coverage of the textile fibers in SEM analysis. Complex encapsulation did not alter the morphology, size, zeta potential, adsorption and skin penetration tendencies of the actives.

Hence, polyamide textile was successfully functionalized not only with particles loaded with pure therapeutic and cosmetic agents but was also specifically functionalized with active-cyclodextrin complex loaded polymeric sponge like particles.

Part V
PERSPECTIVES

Perspectives

The promising results of this thesis open new innovative ways to functionalize textiles for efficient drug delivery and cosmetic agent delivery. CDs are known to enhance the drug loading of the particles so in future studies it could be interesting to quantify this improvement in active loading. Another interesting avenue that is opened by this thesis is to explore the possibility of loading two or more active agents of opposing nature within the same formulation and then functionalizing the textile with such particles. Such multifunctional CD based particles could be used for dual drug delivery, drug-cosmetic delivery or formation of theranostic agents as well.

Relating to textiles, there are many other studies that need to be performed for better functionalization. Few of these include investigating the adsorption of particles on several other textiles and amount of adsorbed capsules left after each washing cycle. Study of the adsorption kinetic of the particles on the textiles is also necessary. Moreover, investigation of the drug release from textiles and skin penetration of capsules after particles adsorption onto textiles would be another step towards completion of all the studies performed earlier.

Part VI

LIST OF PUBLICATIONS IN MANUSCRIPT

1. Cyclodextrin containing biodegradable particles: from preparation to drug delivery. Nadiah Zafar, Hatem Fessi, Abdelhamid Elaissari. Published in International Journal of Pharmaceutics (2014).
2. Colloidal particles containing labeling agents and cyclodextrins for theranostic applications Nadiah Zafar, Hatem Fessi, Abdelhamid Elaissari. Published in International Journal of Pharmaceutics (2014).
3. Double emulsion solvent evaporation techniques used for drug encapsulation. Muhammad Iqbal, Nadiah Zafar, Hatem Fessi, Abdelhamid Elaissari. Published in International Journal of Pharmaceutics (2015).
4. Textile in therapy and cosmetic: from characterization to surface functionalization. Nadiah Zafar, Hatem Fessi, Abdelhamid Elaissari. Submitted to Current Organic Chemistry (2016).
5. Elaboration of sponge like biodegradable cationic particles via double emulsion solvent evaporation. Nadiah Zafar, Geraldine Agusti, Hatem Fessi, Abdelhamid Elaissari. Published in Journal of Dispersion Science and Technology (2016).
6. Elaboration of ammonio methacrylate copolymer based spongy cationic particles via double emulsion solvent evaporation process. Nadiah Zafar, Ahmad Bitar, Jean Pierre Valour, Hatem Fessi, Abdelhamid Elaissari. Published in Material Science and Engineering C (2016).
7. Electrokinetic properties of bare and particles containing textile. Nadiah Zafar, Jean Pierre Valour, Geraldine Agusti, Hatem Fessi, Abdelhamid Elaissari. Published in Polymers for advanced technologies (2016).
8. Elaboration of sponge-like particles for textile functionalization and skin penetration Ahmad Bitar¹, Nadiah Zafar, Jean Pierre Valour, Géraldine Agusti, Hatem Fessi, Philippe Humbert, Sophie Robin, Céline Viennet, Nathalie Lévêque, Abdelhamid Elaissari¹. Published in Colloid Polymer Science (2015).
9. Sponge like microparticles for drug delivery and cosmeto-textile use: Formulation and skin penetration. Nadiah Zafar, Sophie Robin, Céline Viennet, Philippe Humbert, Jean Pierre Valour, Geraldine Agusti, Hatem Fessi, Abdelhamid Elaissari. Submitted to International Journal of Pharmaceutics (2016).