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Stimulated delivery of therapeutic molecules from hydrogels using ultrasound

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THÈSE

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Médicale et de la Complexité - Informatique, Mathématiques et
Applications**
dans l'**École Doctorale Ingénierie pour la Santé, la Cognition et
l'Environnement**

Stimulated delivery of therapeutic molecules from hydrogels using ultrasound

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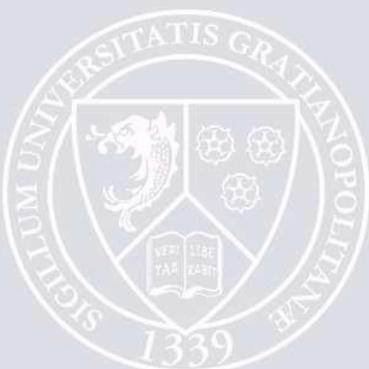
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This document is dedicated to

My Parents

and **My Husband**

for supporting me all the way!

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Abstract

The discovery of new promising therapeutics and development of associated therapies provide the stimulus to discover innovative ways to deliver therapeutic compounds. The invention of novel drug delivery systems (DDS) has an enormous impact on those aspects of medical technology by enabling the delivery of various therapeutic molecules such as small molecules, nucleic acids, peptides, proteins, etc. An ideal DDS fulfills the two functions of targeting and controlling the drug release. Targeting increases the efficiency of the drug and diminishes the side effects. Controlled release reduces or prevents the side effects while its main purpose is releasing the bioactive agents at a predetermined rate in a specified period of time. Efficient delivery of drugs can be achieved with polymeric systems. In such systems a polymer is mixed with an active agent to form a homogeneous system usually referred to as a matrix system. These systems are designed with the dual purpose of providing a drug carrier and a barrier that controls the rate of release. In certain conditions the active agent passes from the polymer matrix into the surroundings.

The aim of this thesis is to provide an insight into an externally stimulated DDS incorporating hydrogels as drug carriers. Various hydrogel formulations with desirable characteristics and structures are developed from which the drug release can be controlled when an external energy is applied. The research described in this thesis utilises low-frequency ultrasound as the external source of energy to provide an efficient non-invasive and controlled release of drug.

The previous literature in this field suggests that hydrogels are good candidates as the drug carrier and release barrier due to the ability to control their porosity and hence the subsequent drug release at a pre-designed rate. Accordingly, hydrogels are selected, modified and characterized with the aim to find a suitable carrier to hold bioactive agents during an ultrasonic stimulated drug delivery process. The hydrogels are 2 natural hydrogels (agarose

and chitosan) and 1 synthetic hydrogel (poly vinyl alcohol). An externally stimulated drug delivery system is fabricated in which ultrasound energy is applied to control the release of the active agent encapsulated in the hydrogel carriers. The fabricated device is characterized and its parameters and performance are analysed.

The effect of various hydrogel formulations on the passive release of the active agent is verified. The model drug to be delivered is theophylline. Next, the stimulated release from these hydrogels is compared with the passive release to understand the effect of different parameters and to select the most desirable condition and formulation for the controlled release. Also, various measurements are carried out to find out and confirm the mechanism by which the ultrasound affects the drug release from the polymer matrix.

The stimulated and passive release of the model drug from hydrogel carriers show how ultrasound as an external energy stimulates and controls the drug release. The results demonstrate that both natural and synthetic hydrogels can be used as drug carriers together with the ultrasonic energy to provide an externally stimulated DDS. However, the ultrasound parameters and the hydrogel drug carrier should be tuned simultaneously based on the therapy required, for example, constant release over a long period or a fast delivery of a volume of some microliters of the drug to be delivered.

Résumé

La découverte de nouvelles thérapies prometteuses et le développement de thérapies associées fournissent le stimulus pour découvrir des moyens innovants de délivrance des composés thérapeutiques. L'invention de nouveaux systèmes de délivrance de médicaments (SDD) a un impact énorme sur les aspects de la technologie médicale en permettant la distribution de diverses molécules thérapeutiques telles que de petites molécules, des acides nucléiques, des peptides, des protéines, etc. Un SDD idéal répond aux deux fonctions de ciblant et contrôlant la libération de médicament. Le ciblage augmente l'efficacité du médicament et diminue les effets secondaires. La libération contrôlée réduit ou empêche les effets secondaires alors que son but principal est de libérer les agents bioactifs à un taux prédéterminé pendant une période spécifiée. La délivrance efficace de médicaments peut être obtenue avec des systèmes polymères. Dans de tels systèmes, un polymère est mélangé avec un agent actif pour former un système homogène habituellement appelé système matriciel. Ces systèmes sont conçus dans le seul but de fournir un vecteur médicamenteux et une barrière qui contrôle le taux de libération. Dans certaines conditions, l'agent actif passe de la matrice polymère dans l'environnement. L'objectif de cette thèse est de donner un aperçu d'un SDD stimulé par l'extérieur incorporant des hydrogels comme supports de médicaments. Diverses formulations d'hydrogel avec des caractéristiques et des structures souhaitables sont développées à partir desquelles la libération du médicament peut être contrôlée lorsqu'une énergie externe est appliquée. La recherche décrite dans cette thèse utilise les ultrasons basse fréquence comme source d'énergie externe pour fournir une libération de médicament non invasive et contrôlée efficace. La littérature précédente dans ce domaine suggère que les hydrogels sont de bons candidats en tant que support de médicament et barrière de libération en raison de la capacité de contrôler leur porosité et donc la libération de médicament subséquente à un taux pré-conçu. En conséquence, les hydrogels sont choisis, modifiés et caractérisés dans le but de trouver un support approprié pour contenir des agents bioactifs lors d'un processus de délivrance

de médicament stimulé par ultrasons. Les hydrogels sont 2 hydrogels naturels (agarose et chitosane) et 1 hydrogel synthétique (alcool polyvinylique). Un système de délivrance de médicament stimulé de manière externe est fabriqué dans lequel de l'énergie ultrasonore est appliquée pour contrôler la libération de l'agent actif encapsulé dans les supports d'hydrogel. L'appareil fabriqué est caractérisé et ses paramètres et performances sont analysés. L'effet de diverses formulations d'hydrogel sur la libération passive de l'agent actif est vérifié. Le médicament modèle livrer est la théophylline. Ensuite, la libération stimulée de ces hydrogels est comparée à la libération passive pour comprendre l'effet de différents paramètres et pour sélectionner l'état et la formulation les plus souhaitables pour la libération contrôlée. En outre, diverses mesures sont effectuées pour détecter et confirmer le mécanisme par lequel les ultrasons affectent la libération du médicament de la matrice polymère. La libération de médicament stimulée et passive du médicament modèle partir des porteurs d'hydrogel montre comment les ultrasons comme énergie externe stimulent et contrôlent la libération du médicament. Les résultats démontrent que les hydrogels naturels et synthétiques peuvent être utilisés comme supports de médicaments conjointement avec l'énergie ultrasonore pour fournir un SDDS stimulé par l'extérieur. Cependant, les paramètres d'ultrasons et le support de médicament hydrogel doivent être considérés conjointement sur la base de l'application souhaitée afin de remplir le contrôle souhaité sur la libération du médicament.

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Chapter 1

Introduction

This chapter provides a general overview and introduction for the research presented in this PhD thesis. The motivation and objectives for the research are described. The organisation of the thesis is outlined.

1.1 Motivation

The discovery of new promising therapeutics and development of associated therapies provide the stimulus to discover innovative ways to deliver therapeutic compounds. The invention of novel drug delivery systems (DDS) has an enormous impact on those aspects of medical technology by enabling the delivery of various therapeutic molecules such as small molecules, nucleic acids, peptides, proteins, etc. (Bridges et al., 2013).

An ideal DDS fulfills the two functions of targeting and controlling the drug release. Targeting increases the efficiency of the drug and diminishes the side effects, particularly when dealing with anti-cancer drugs that may be harmful to healthy cells. Controlled release can also reduce or prevent the side effects while its main purpose is releasing the bioactive agents at a predetermined rate in a specified period of time. The release may be constant or cyclic over a long period, or may be triggered by an external stimulation. In any case, the aim is to achieve

more effective therapies while eliminating the possibility of either under or overdosing (Huang and Brazel, 2001; Peppas et al., 2006). The therapeutics delivered by a drug delivery system, should be delivered at the optimal dosage for an optimal length of time to be effective.

Polymers are important materials for the development of drug delivery systems due to their multitude of possible formulations and physical shapes that can be designed. Indeed, efficient delivery of drugs can be achieved with polymeric systems that are designed with the dual purpose of providing a drug carrier and a barrier that controls the rate of release. In such systems a polymer is mixed with an active agent to form a homogeneous system usually referred to as a matrix system. In certain conditions the active agent passes from the polymer matrix into the surroundings. As the release proceeds, the rate of release decreases due to the progressively longer distance that it has to travel, resulting in a longer diffusion time. The schematic diagram of a generic polymeric DDS is demonstrated in Figure 1.1. Over time, the drug contented initially in the polymer matrix diffuses into the surrounding medium until the concentration of drug remaining in the polymer reaches a steady-state with that in the surrounding medium.

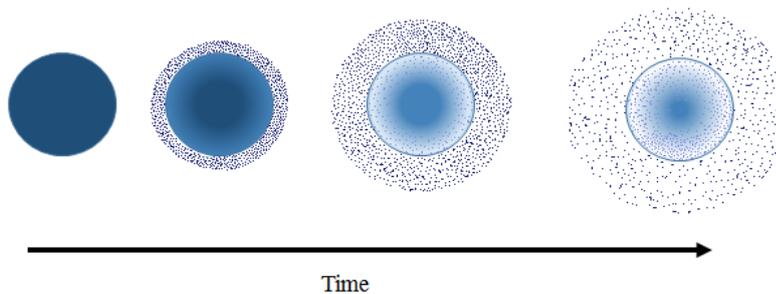


Figure 1.1: Schematic diagram of a polymeric controlled drug delivery system adapted from (Brannon-Peppas, 2008).

Among polymers, hydrogels have gained considerable interest due to their specific properties such as biocompatibility and hydrophilicity (Baroli, 2007; Ganta et al., 2008; He et al., 2008; Hoffman, 2012; Peppas et al., 2000a; Schmaljohann, 2006).

Hydrogels are three-dimensional hydrophilic networks that swell but not dissolve when

brought into contact with water due to the existence of crosslinks in their structure. The porous structure of these hydrophilic polymers facilitates loading of drug into the gel matrix through which the drug is dissolved or dispersed, and then subsequently released at a definite rate. It is notable that the network structure and the nature of hydrogel components affect the diffusional behavior, molecular mesh size changes, and stability of the incorporated bioactive agent (Ghandehari et al., 1997).

Hydrogels can also protect drugs from degradation or inactivation caused by external conditions, e.g. the presence of enzymes and low pH in the stomach. On the other hand, they can control the rate of release by changing the gel structure in response to environmental stimuli, such as pH (Casadei et al., 2007), temperature (Schmaljohann, 2006), ionic strength (Chiu et al., 2001), and electric field (Tavakoli et al., 2006).

The overarching purpose of this PhD research is to provide an insight into an externally stimulated DDS incorporating hydrogels as drug carriers. Based on a literature review, which will be presented in chapter 2, these hydrogels were selected, modified and characterized with the aim to find a suitable carrier to hold bioactive agents during an ultrasonic stimulated drug delivery process. The hydrogels were 2 natural hydrogels (agarose and chitosan) and 1 synthetic hydrogel (poly vinyl alcohol). The model drug to be delivered was theophylline. Theophylline is commonly used in drug delivery experiments according to pharmaceuticals. It is a model drug without any specific characteristics that might affect other parameters under study. Easy detection with the spectrophotometry method was another reason to choose theophylline. Also, theophylline is approximately of the same size as glucose. It should be noted that theophylline was used at a concentration below its solubility limits in both water and DMSO which acted similar to water as a solvent in the PVA hydrogels. Hence, the measurements of theophylline release are of the entire drug content, since no theophylline was likely to have precipitated into the gels. This PhD research reports several novel outcomes and contributions to the body of knowledge in the field of controlled drug delivery.

1.1.1 Non-invasive Controlled Drug Delivery Systems

The oral route is known to be the most preferred way of drug administration by both patients and clinicians. However, this route of delivery has the disadvantages of hepatic first pass metabolism and enzymatic degradation within the gastrointestinal tract, which prohibits oral administration of certain classes of drugs. Nasal drug delivery and injection are the two other frequently employed routes, but they also have some weaknesses, including the poor absorption of polar compounds (Illum, 2002). Injection percutaneously is painful and inconvenient for long-term repeated delivery, so patients prefer to avoid this method.

To overcome those limitations, various alternative routes of drug administration have gained much attention over the past few decades in order to complement the existing drug delivery routes, or to enable the use of those drugs that cannot be delivered orally, nasally or by injection. Those alternative routes include buccal/sublingual, pulmonary, ocular and transdermal routes that can be non-invasive (Mathias and Hussain, 2010). Unlike oral administration, such noninvasive methods avoid the GI tract, and therefore drug degradation and first-pass metabolism are prevented. Another significant factor is the improved patient compliance by using a non-invasive drug delivery route that is painless and easy to administer. Finally, these drug delivery methods facilitate the delivery of the drugs that cannot be easily delivered by traditional techniques (Li et al., 2013).

But, no matter how carefully the non-invasive drug delivery technique is selected and designed, the anticipated therapeutic effects will not be achieved until the drug can reach its target. Moreover, a lot of medications might have undesirable side effects if the drug interacts with parts of the body that are not the target of that drug or when the drug is used less or more than the proper dosage. Hence, the selected non-invasive delivery method needs to be also targeted and controlled to improve the performance of the therapeutic agent. In a controlled drug delivery system, a polymer that can be either natural or synthetic and a drug or an active agent are combined carefully together in such a way that the active agent can be released from the polymer in a predetermined manner. The release kinetics are tuned according to

the therapy required, for example, constant release over a long period, or regulation by the environment or other external stimuli.

In conventional drug administration methods (Figure 1.2 a), the drug level in blood in-

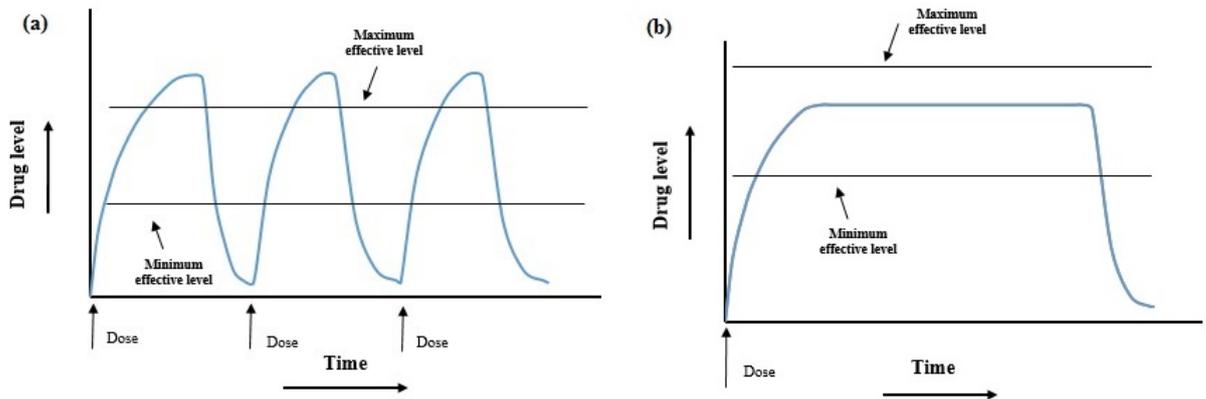


Figure 1.2: Drug levels in the blood with (a) traditional drug dosing and (b) controlled-delivery dosing.

creases sharply soon after each administration and then decreases until the next administration. In contrast, controlled delivery methods (Figure 1.2 b) maintain the drug level between a maximum level at which the drug may cause toxicity and a minimum level below which the drug would be no longer effective. This method can be ideally used for drugs that display high toxicity and/or narrow therapeutic windows. In such cases, the expected therapeutic effect will be attained when the drug concentration reaches the therapeutic levels that do not exceed the maximum tolerable dose and remains at those concentrations for a certain period of time (Gombotz and Pettit, 1995; Langer, 1998; Sinha and Khosla, 1998). In summary, controlled DDS are designed for long-term administration in which the drug level in the blood remains constant between the desired maximum and the effective minimum level for an extended period of time (Peppas and Khare, 1993). The advantages of using controlled delivery systems are then:

- the maintenance of optimum drug levels in the blood with minimum fluctuations;
- predictable and reproducible release rates for extended duration;
- elimination of side effects and no need for frequent drug administration;

- enhancement of therapeutic activity duration for drugs having short biological half-life;
- optimal use of the drug in question;
- and, better patient compliance.

Although these advantages are significant, the potential limitations should not be ignored:

- it is possible that the by-products of degradation be undesirable;
- the materials used may have unpredictable toxicity or non-biocompatibility;
- patient might feel discomfort from the delivery device;
- controlled-release systems may be more costly compared with traditional pharmaceutical formulations.

1.1.2 Hydrogel-based Drug Delivery Devices

A new range of biomolecular therapeutics such as peptides, proteins and nucleic acids have been discovered due to advances in molecular biology and biotechnology. These biotherapeutic drugs require special handling since they are susceptible to degradation or denaturation upon oral intake and may not readily reach their target after parenteral injection (LaVan et al., 2002; Lavan et al., 2003). It is advantageous to encapsulate such biologically active agents within a polymer matrix with well-defined physical and chemical properties which serve as a carrier. These polymers protect the active agents and then target and release them in a controlled and predictable manner after local or systemic administration. Such a usage of polymeric carriers for drug delivery aims at optimizing the therapeutic effects while minimizing undesirable side effects.

As was mentioned previously, hydrogels as water swollen polymer matrices are extremely useful for such applications since they possess unique swelling properties and structures. Hydrogels can be excellent materials for controlled or targeted drug delivery for administration routes such as oral, nasal, ocular, transdermal, epidermal and subcutaneous applications (Peppas et al., 2000b).

1.1.3 Externally Stimulated Drug Delivery from Hydrogels

Combining the basic mechanisms of local drug release by diffusion with a stimulated release system enables finer tuned control of drug delivery for selective treatments. In such delivery systems the active agent remains inside the delivery system until it is released by an external stimulus. For example, the external stimulus can be triggered when the delivery system is placed close to the targeted delivery site or reaches an appropriate environment. The rate of release can be well regulated in these systems such that the drug levels at the local site of action remain effective without exposing the surrounding tissues or the body generally to toxic levels of the drug (Bhattacharai et al., 2010).

The development of controlled-release formulations for bioactive compounds in the last few decades has stimulated a considerable interest to develop stimuli-responsive drug delivery systems utilizing hydrogels (Peppas and Khare, 1993). Since then, a range of hydrogel systems has been investigated for the controlled delivery of a variety of biomolecules in different sizes ranging from small molecular weight drugs to nucleic acids, peptides, and proteins.

The most important property for the hydrogel used for a stimuli-responsive drug delivery system is the sensitivity to the external stimulus, which can include pH, temperature, pressure, ionic strength, electromagnetic radiation, ultrasonic energy, buffer composition, the concentration of glucose, stress and strain, or light (Peppas, 1991; She et al., 2008; Tian et al., 2012). These conditions affect significantly some important parameters in each hydrogel based on its sensitivity, including the swelling behavior, network structure, permeability and mechanical strength of hydrogels. Consequently the hydrogel effectively opens its pores for enhanced diffusion of the entrapped therapeutic (Bhattacharai et al., 2010). Such intelligent materials are promising for novel applications in controlled-release systems.

1.2 Objectives

The thesis aimed to understand the change in polymer physics caused by externally applied energy. To achieve this, various hydrogel formulations are made to control the gel charac-

teristics and structures from which the drug release could be stimulated with the externally applied energy. The research described in this thesis utilised low-frequency ultrasound as the external source of energy to provide an efficient non-invasive and controlled release of drug. The previous literature in this field suggested that hydrogels are good candidates as the drug carrier and release barrier due to the ability to control their porosity and hence the subsequent drug release at a pre-designed rate. The advantage of the externally applied energy (ULS) is to provide an immediate switch to stimulate the drug delivery "on-demand". The research has the following objectives:

1. To chemically reinforce the structure of agarose to improve its mechanical characteristics for controlled drug delivery purposes. Physical crosslinking of this natural polymer with an epoxy crosslinker was investigated by varying the agarose concentration, the pH of the preparation solution and the amount of the crosslinker.
2. To synthesize co-polymerised chitosan hydrogels under environmental conditions. Chitosan and agarose solutions were mixed in different proportions and the resultant hydrogels were characterized.
3. To apply a synthetic hydrogel as drug carrier and investigate its applicability. Several PVA-DMSO hydrogels were prepared and their properties were compared.
4. To fabricate an externally stimulated drug delivery system that applies ultrasound energy to control the release of the active agent encapsulated in the hydrogel carriers. The fabricated device was characterized and its parameters and performance were analysed.
5. To observe the effect of various hydrogel formulations on the passive release of the active agent. Then, to compare the stimulated release from these hydrogels with the passive release to find out the effect of different parameters and select the most desirable condition and formulation for the controlled release.

1.3 Thesis Organisation

The subsequent chapters of this thesis are structured as follows:

- Chapter 2 presents a review of the literature concerning the hydrogels used in this thesis, including their properties and crosslinking principles. A review on the externally stimulated drug delivery systems are also presented in this chapter. Finally, the delivery system developed and used throughout this work is introduced.
- Chapter 3 describes the hydrogel synthesis processes and their characterization methods.
- Chapter 4 presents the characterizations results and a comparison among various samples.
- Chapter 5 outlines the delivery device developed and used in this research. The passive and stimulated drug release experiments are explained, afterwards the obtained results are presented in this chapter.
- Chapter 6 presents the results of drug release experiments and compares different formulation and the release profiles obtained.
- Chapter 7 concludes the thesis, and suggests perspectives for possible future work.

Chapter 2

Research Rationale

Introduction

As was mentioned in Chapter 1, this thesis investigates three different hydrogels as potential drug carriers. These hydrogels are agarose, chitosan and PVA. Each of these hydrogels has different characteristics which affect the rate of release and hence result in different release profiles.

This chapter is divided into three main sections. Section 2.1 is a literature review of the properties and structure of the hydrogels used in this study. Section 2.2 discusses the application of these hydrogels in drug delivery, reviews externally stimulated drug release and describes the drug delivery system used in this study. Section 2.3 provides a summary of this chapter.

2.1 Overview of Hydrogels for Drug Delivery Systems

Drug delivery systems containing a drug carrier have emerged as a powerful technology for the treatment of various pathologies. Hydrogels were chosen as carriers over 30 years ago and have received growing attention since, mainly due to their stability, enhanced loading capabilities and control over physicochemical properties (Sahoo et al., 2009, 2010; Tan et al.,

2010). Drug encapsulation can be achieved with natural polymers such as chitosan, agarose and alginate (Guo and Gao, 2007; Leong et al., 2011) or synthetic polymers such as PVA, pHEMA, PEG, PAA, etc. (Hoffman, 2012; Peppas et al., 2006). Each of these hydrogels has specific properties that enable it to carry a range of drugs or biomolecules.

2.1.1 Important Properties of Hydrogels for Drug Delivery Applications

Hydrogels can be used as a carrier for drugs and other therapeutic biomolecules preferably if they are biocompatible and non-toxic in situ. After synthesis, some important properties like swelling behavior, mechanical properties and toxicity etc. should be evaluated to check if they could be used effectively for the desired application. Some of these properties and their importance are discussed here.

2.1.1.1 Swelling properties

Water in hydrogels not only provides a moist environment but also will have a major influence on a range of properties including mechanical characteristics and drug release. Equilibrium water content in hydrogels is one of the basic properties and it describes the amount of water the material is able to absorb when it is placed into an aqueous environment (Williams et al., 1990). Equilibrium water content is expressed as follows:

$$EWC = \frac{W_w}{W_t} \times 100$$

where W_w is the weight of water in the gel and W_t is the total weight of the hydrated gel. Since water acts as a plasticizer in a hydrogel network, the swelling process of the hydrogel can be considered under a rubbery state. It can be described by the free energy of mixing ΔG_{mix} from the polymer and solvent interaction, and the elastic free energy $\Delta G_{\text{elastic}}$ from the crosslinked network described as (Flory, 1953):

$$\Delta G_{\text{system}} = \Delta G_{\text{mix}} + \Delta G_{\text{elastic}}$$

At the beginning of swelling, the $\Delta G_{\text{mix}} \ll 0$, $\Delta G_{\text{elastic}} > 0$ and $\Delta G_{\text{mix}} + \Delta G_{\text{elastic}} < 0$, so the swelling is favoured and the solvent diffuses into the network. During the swelling process, the ΔG_{mix} and $\Delta G_{\text{elastic}}$ both increase until $|\Delta G_{\text{mix}}| = |\Delta G_{\text{elastic}}|$, thus $\Delta G_{\text{system}} = \Delta G_{\text{mix}} + \Delta G_{\text{elastic}} = 0$ which means there is no driving force for the swelling and equilibrium swelling is reached, so, the swelling process stops.

The kinetics of the swelling process and the equilibrium extent of swelling are affected considerably by several factors, such as the crosslinking ratio, solvent quality, chemical structure of monomers, and synthesis conditions (Hariharan and Peppas, 1996). Hydrogels that contain hydrophilic groups swell to a larger amount compared to those containing hydrophobic groups. Since hydrophobic groups collapse in the presence of water to minimize their exposure to the water molecules, hydrogels containing hydrophobic groups will swell much less. Regarding medical applications, a hydrogel with high water content is generally more advantageous owing to its permeability and biocompatibility (Katime et al., 2001). Nonetheless, a large swelling is usually accompanied by poor mechanical properties which necessitates some modifications to reach a compromise between "large swelling" and "good mechanical behaviour".

2.1.1.2 Mechanical properties

The mechanical properties of hydrogels are extremely important factors in selecting a material that is suitable for a specific pharmaceutical application. These properties must be particularly evaluated for drug delivery purposes, since the mechanical properties of hydrogels should be such that it can maintain its physical texture during the delivery of therapeutic moieties for the predetermined period of time (Anseth et al., 1996).

The mechanical properties depend on various parameters that mainly include monomer composition, the crosslink density, the degree of swelling, and medium conditions. The simplest method to enable control of the mechanical properties of hydrogels is to change the composition of co-monomers used for the preparation of the hydrogels. Another method to obtain desired mechanical properties is copolymerization with co-monomers which may

form hydrogen bonding within the hydrogel. If the hydrogel is a copolymer, increasing the relative amount of physically stronger components results in an increased mechanical strength of the gels. Changing the degree of crosslinking is another option to achieve the required mechanical properties. A mechanically stronger hydrogel will be obtained by increasing the degree of crosslinking, but, since the % elongation of the hydrogel concomitantly decreases, a more brittle structure will be created. Hence, the optimum degree of crosslinking should be targeted to achieve a relatively strong and yet elastic hydrogel. Altering the hydrophilicity of the polymer also affects the mechanical strength of the gels since the swelling properties of the hydrogel changes (Lustig et al., 1991).

2.1.1.3 Biocompatibility

Biocompatibility is one of the essential prerequisites to qualify a material as a biomaterial. Biocompatibility represents the ability of a material to perform with an appropriate host response in a specific application (Williams, 1987). More precisely, this property consists of two basic elements: (a) bio-safety, which means that there is an absence of cytotoxicity, mutagenesis, and/or carcinogenesis both locally at the site of the delivery system and systemically; and (b) bio-functionality, which means the ability of material to perform the specific task for which it is intended (Nair and Laurencin, 2007). Material chemistry, molecular weight, solubility, shape and structure of the implant, hydrophilicity/hydrophobicity, lubricity, surface energy, water absorption, degradation and erosion mechanisms are among the inherent properties of polymeric biomaterials that influence their biocompatibility.

2.1.1.4 Non-toxicity

Non-toxicity means that the biomaterial must be biologically inert and not cause nor trigger acute nor chronic inflammation, bleeding, allergic responses nor disease (including cancers). In addition to a lack of local toxicity, the material must also be non-toxic systemically and should not release toxic degradation products. Thus, the toxic chemicals frequently used in the polymerization of synthetic hydrogels may be problematic if there is not 100% conversion during polymerization. Those chemicals could include initiators, organic solvents, unreacted monomers, stabilizers and cross-linkers used in polymerization and hydrogel

synthesis and may be toxic to host cells if they leach out to tissues or encapsulated cells. Although natural polymers generally have superior biocompatibility over synthetic ones, the presence of synthetic cross-linkers and initiators used in the polymerization of naturally derived monomers causes the same toxicity concerns as for purely synthetic hydrogels. The hazardous and potentially toxic chemicals that may remain in the polymer can be removed using various purification processes such as solvent washing or dialysis.

2.1.1.5 Crosslinking density

In highly hydrophilic hydrogels, the drug releases with a relatively fast rate. To slow down drug release from such hydrogels the macromolecular structure of the hydrogel should be modified, for example by changing the hydrogel structure, by reducing the macromolecular mesh size and hence minimizing the pathways available for drug diffusion (Kim and Lee, 1992).

Increasing the crosslinking density is a means to reduce the macromolecular mesh size and to improve the mechanical properties. This crosslinking density is defined as the ratio of moles of crosslinking agent to the moles of polymer repeating units. The higher the crosslinking ratio, the more crosslinking agent is incorporated in the hydrogel structure. Highly crosslinked hydrogels have tighter structures. For instance, copolymerization of a hydrophilic monomer (which favors swelling) with a less hydrophilic monomer results in a hydrogel with good water absorbance and improved mechanical properties. However, a higher degree of crosslinking makes a more brittle structure and a lower swelling ratio (Korsmeyer and Peppas, 1981). Thus, an optimum degree of crosslinking should be sought to achieve a relatively strong and yet elastic hydrogel (Peppas et al., 2000a).

2.1.2 Hydrogel Crosslinking Chemistry

The molecular weight (MW) of the polymer, charges on the polymers, density of the crosslinking (covalently bonded networks), and physical associations are some parameters that affect the structure of the swollen hydrogels. As polymeric networks, hydrogels contain

crosslinks that avoid dissolution of the hydrophilic polymer chain in aqueous solution. Since hydrogels are most frequently used for controlled release of bioactive agents and for the encapsulation of cells and biomolecules, they should be mechanically strong enough to protect the material that is encapsulated and to control the rate of release. The hydrogel building blocks should be selected to meet this requirement. For instance, high molecular weight polymers typically have multiple cross-linkages per polymer producing more robust hydrogels, while smaller polymers are required at higher concentrations to produce sufficient gel rigidity (Anseth et al., 1996). In the latter case, increasing the hydrogel cross-linking results in an increase in both the elastic moduli and stiffness (Lou et al., 2000; Lou and Chirila, 1999) which are important in the protection of the encapsulated biomolecules. Similarly, the degree of crosslinking controls the most important parameters that regulate diffusion of the encapsulated therapeutics out of a hydrogel, which are the hydrogel's pore or mesh size (Cruise et al., 1998; Lin and Metters, 2006). Depending upon the nature of the polymer, either chemical or physical techniques may be used to crosslink the hydrogel.

It should be noted that even though the chemical crosslinking leads to more mechanical stability, some of the crosslinking agents used may be toxic and give unwanted reactions that could render the hydrogel unsuitable for a drug delivery application. These adverse effects can be avoided with the use of physically crosslinking methods. In physically crosslinked gels, dissolution is prevented by physical interactions between different polymer chains. Both of these methods are used currently for preparation of synthetic hydrogels and are discussed in detail here.

2.1.2.1 Chemical Crosslinking

In this method the chemical structure of the hydrogel is altered through the crosslinking process. Chemically crosslinked gels can be obtained by (i) radical polymerization of low-molecular-weight monomers in the presence of a crosslinking agent, or (ii) covalent linkages between polymer chains by the reaction of their functional groups (mainly OH, COOH, NH_2) with complementary reactivity such as an amine-carboxylic acid or an isocyanate-OH/ NH_2 reaction, or (iii) by Schiff base formation using high energy radiation (in particular gamma

and electron beams) to polymerize unsaturated compounds or enzymatic reaction (Hennink and Van Nostrum, 2012).

As mentioned earlier, chemically crosslinked gels are mechanically quite stable owing to

Table 2.1: Methods of synthesizing physically and chemically crosslinked hydrogels adapted from (Hennink and Van Nostrum, 2012).

Physically crosslinked hydrogels
<ul style="list-style-type: none">• Ionic interactions (alginate etc.)• Hydrophobic interactions (PEO-PPO-PEO etc.)• Hydrogen bonding interactions (PAAc etc.)• Supramolecular chemistry (inclusion complex etc.)
Chemically crosslinked hydrogels
<ul style="list-style-type: none">• Polymerization (acryloyl group etc.)• Radiation (gamma ray etc.)• Small-molecule crosslinking (glutaraldehyde etc.)• Polymer-polymer crosslinking (condensation reaction etc.)

the ionic and covalent bonds which comprise these gels. Although chemically crosslinked hydrogels provide better control over their final physical, structural and mechanical properties, they may contain some residues of the crosslinking agent within the gel matrix that can be harmful in biomedical applications if the compound is toxic (Kuang et al., 2005; Lee and Mooney, 2001; Lutolf and Hubbell, 2005; Peppas et al., 2006; Ruan et al., 2004). In this case, the residues must be completely removed before their use as biomaterials. On the other hand, the utility of chemically crosslinked hydrogels is often limited by the difficulty in manufacturing forms and in post-process modifications. Because of this, shaping is carried out along with their polymerization reaction step (Fazel-Rezai, 2011).

2.1.2.2 Physical crosslinking

The advantage of physically crosslinked hydrogels is that no crosslinking agents are used for their preparation. Moreover, physically crosslinked hydrogels possess higher compressive

strength compared with the corresponding chemically crosslinked hydrogels since the mechanical load can be more uniformly distributed through the crystallites of the three-dimensional structure (Devine and Higginbotham, 2003).

Several methods have been investigated for the preparation of physically cross-linked gels. Cross-linking by ionic interactions is a physical method that can be carried out at ambient temperature and neutral pH. The resultant gel can be used as a matrix for encapsulation of cells and for the release of proteins. In addition to anionic polymers being cross-linked with metallic ions, hydrogels can also be obtained by complexation of polyanions and polycations (Peppas et al., 1987).

Cross-linking by crystallization is another physical method used to prepare PVA hydrogels. In this procedure, an aqueous solution of PVA undergoes freeze-thawing cycles to yield a strong and highly elastic gel. Gel formation is attributed to the formation of PVA crystallites which act as physical cross-linking sites in the network (Yokoyama et al., 1986). The gel properties can be modified by varying polymer concentration, temperature, and the freezing and thawing cycle times.

Cross-linking by forming hydrogen bonds has been used to crosslink poly(acrylic acid) and poly(methacrylic acid) with poly(ethylene glycol) by hydrogen bonding the oxygen of the poly(ethylene glycol) and the carboxylic acid group of poly(methacrylic acid). The hydrogen bonds are only formed when the carboxylic acid groups are protonated (Eagland et al., 1994).

Genetic engineering has also been used for the preparation of hydrogels through cross-linking by protein interaction. The major advantage in this method is that the sequence of peptides and, therefore its physical and chemical properties, can be precisely controlled by the proper design of the genetic code in synthetic DNA sequences (Yoshikawa et al., 1994).

The following section focusses on the hydrogels that were used for the drug delivery applications described in this thesis.

2.1.3 Hydrogels Used in this study

In this study, hydrogels serve as carriers and barriers for the delivery of therapeutic molecules and are coupled with the ultrasound stimulation. It was discussed that hydrogels drug carriers should be of sufficient mechanical stability and have enough water content to make it possible for the drug particles to move within the hydrogel matrix once the external stimulation is applied.

Chemistry of the polymer backbone is another parameter to consider since it might lead to retarded ultrasonic drug delivery. If the polymer contains pendant groups on the backbone to which the drug molecules chemically bond, the ultrasound energy will need to overcome the chemical binding energy. Similarly, in case of a polymer with the surface charge at the backbone, the ultrasonic drug delivery slows down if the drug molecules are inversely charged. Moreover, the characteristics like non-toxicity, biocompatibility, ease of fabrication and the condition of preparation are other factors imposed by future medical applications of the DDS to be considered for hydrogel selection. Nature of the crosslinking molecule required to create the hydrogel with appropriate mechanical and swelling properties could also affect the choice of the drug carrier. Physical gels have simple crosslinking molecules.

Based on the final application of the hydrogels in this study (i.e. to be combined with the ultrasound stimulation for drug delivery) and by considering the intrinsic characteristics of the hydrogels essential for drug delivery purposes, three different hydrogel formulations are selected in this research. Two of these hydrogels are "physical gels" which are known to be biocompatible, non-toxic and their mechanical stability is enhanced by cross-linking. One other hydrogel is a "classic" gel known to be biocompatible.

2.1.3.1 Agarose

As was discussed in sections 2.1.1.3 and 2.1.1.4, the biocompatibility and non-toxicity of the materials are important factors in the development of new polymers. Agarose is known as a non-toxic material in all its forms (Fernández-Cossío et al., 2007). Moreover, the

superior biocompatibility of the agarose hydrogel as well as the ambient conditions for drug encapsulation makes the agarose hydrogel a promising system for drug delivery.

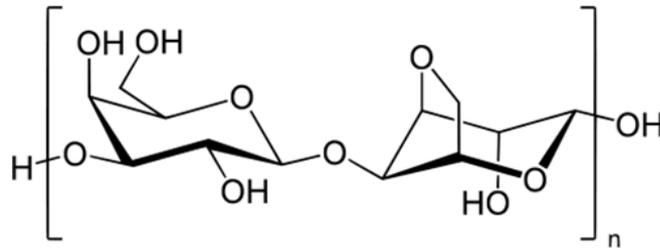


Figure 2.1: Agarose molecular structure

Agarose is a physical gel composed of 1,3-linked-D-galactopyranose and 1,4-linked 3,6-anhydro-L-galactopyranose units (Figure 2.1) (Millán et al., 2002; Normand et al., 2000). The basic agarose units repeat and through hydrogen bonds the two chains transform into double helices which subsequently aggregate into thick bundles (Braudo, 1992). Due to the formation of hydrogen bonding, agarose can form reversible gels even at low concentration merely by cooling the hot aqueous solutions (Phillips and Williams, 2009). Agarose gels contain large pores of water and demonstrate great turbidity and strong elasticity. The macroscopic hydrogel properties such as mechanical properties and water permeability may be altered by changing the agarose concentration (Aymard et al., 2001; Barrangou et al., 2006; Román et al., 2008).

Since there is a large hysteresis between the gelling and melting temperature of the agarose hydrogel, drugs can be loaded readily into the hydrogel matrix by simply mixing the drug solution with the agarose solution at a moderate temperature slightly above the gelling point of the agarose solution. Thus, the agarose hydrogel is a good candidate for delivery of heat sensitive drugs (Wang and Wu, 1997).

Various forms of agarose (micro-spheres, fiber, film etc.) have been developed for using in pharmaceutical industries and medical research (Bao et al., 2010). Iwata et al. applied agarose microbeads to immobilize xenogeneic islets for insulin therapy of type I diabetes. They found that the agarose hydrogel prolonged xenograft survivals (Iwata et al., 1994).

Upadrashta et al. used agarose hydrogel for delivery of ibuprofen and indomethacin (Häglund et al., 1994; Upadrashta et al., 1993).

Nevertheless, agarose has limited application in drug delivery perhaps due to high porosity and low mechanical strength. Blending agarose with either natural or synthetic polymers has been suggested as a useful and efficient means to ameliorate its physical properties to meet the requirements for specific applications (Mahmoud Nasef et al., 2011). In that sense, physical crosslinking is a possible solution to reinforce the structure of agarose gel.

The elasticity is one of the most predominant properties of the gels since it imparts flexibility to the crosslinked chains and facilitates the movement of incorporated molecules. Hence, a reasonable balance must be maintained between mechanical strength and flexibility by selecting the optimum degree of crosslinking when modifying the agarose structure (Hoare and Kohane, 2008).

2.1.3.2 PVA

Poly(vinyl alcohol)(PVA) is a water-soluble synthetic polymer composed mainly of 1,3-diol linkages [-CH₂-CH(OH)-CH₂-CH(OH)-] (Figure 2.2), but a few percent of 1,2-diols [-CH₂-CH(OH)-CH(OH)-CH₂-] occur, depending on the conditions for the polymerization of the vinyl ester precursor.

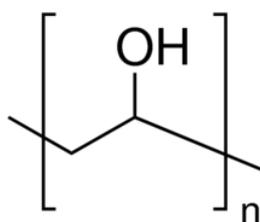


Figure 2.2: Poly(vinyl alcohol) molecular structure

PVA is a polymer of great interest for biomedical and pharmaceutical applications because of its many desirable characteristics such as biocompatibility, drug compatibility, water solubility, non-toxicity, mechanical and swelling properties. These applications include controlled drug delivery systems for oral, transdermal, buccal, intramuscular, or rectal routes of administration

(Muppalaneni and Omidian, 2014; Peppas, 1986).

Apart from the advantages of typical hydrogel materials (ability to absorb water, flexibility and biocompatibility), polyvinyl alcohol (PVA)-based hydrogels are distinguished by their good mechanical properties and the ability to retain water in the structure, which ensures a prolonged moist environment (Gibas and Janik, 2010).

The swelling characteristics of PVA hydrogels depend mainly on the presence of salts and the degree to which the acetate groups are replaced by hydroxyl groups (Morita et al., 2000). However, being quite a hydrophilic system, PVA releases the drug with a relatively high rate. To slow down the drug release from such a system, its macromolecular structure should be modified by cross-linking or using various solvents to reduce the macromolecular mesh size available for drug diffusion (Hassan and Peppas, 2000; Kim and Lee, 1992).

Nonetheless, the problem of using PVA crosslinkers is the same as for any other crosslinking agent, in that residual amounts will be present in the final PVA gel and it becomes extremely time-consuming to do the extraction procedures in order to remove these residues. On the other hand, if the residues are not removed, the gel will not be acceptable for biomedical or pharmaceutical applications due to the presence of the toxic crosslinking agents that could alter the biological activity or degrade the biologically active agent being released. There are also other toxic residual components associated with chemical crosslinking such as initiators, chain transfer agents, and stabilizers (Chang et al., 2003).

Such harmful agents limit the pharmaceutical and biomedical applications of PVA hydrogels, so a possible alternate approach can be preparing these thermoreversible gels in various solvents such as ethylene glycol (Cebe and Grubb, 1985), dimethyl sulfoxide (DMSO) (Tanigami et al., 1994), N-methylpyrrolidone (Hong and Miyasaka, 1991) and mixtures of these solvents with water (Yamaura et al., 1987). A very common method of PVA synthesis is to dissolve PVA in a mixed solvent containing water and dimethyl sulfoxide (DMSO), which results in either transparent or turbid hydrogels based on the concentration of DMSO that is used.

This optical appearance is ascribed to the size and number of crystallites formed upon the crystallization of PVA in DMSO/ H_2O solutions (Cha et al., 1996).

2.1.3.3 Chitosan

Chitosan is an excellent excipient due this hydrogel being non-toxic, stable, biocompatible and biodegradable. These properties have made chitosan a very versatile material with extensive application in the biomedical and biotechnological fields (Kumar et al., 2004; Muzzarelli and Muzzarelli, 2005; Ray and Dinesh, 2000). These attractive properties have also suggested that this natural hydrogel would be an ideal candidate for controlled release formulations (Dodane and Vilivalam, 1998; Singla and Chawla, 2001), non-viral gene delivery (Borchard, 2001) and tissue engineering (Khor and Lim, 2003; Madihally and Matthew, 1999; Suh and Matthew, 2000).

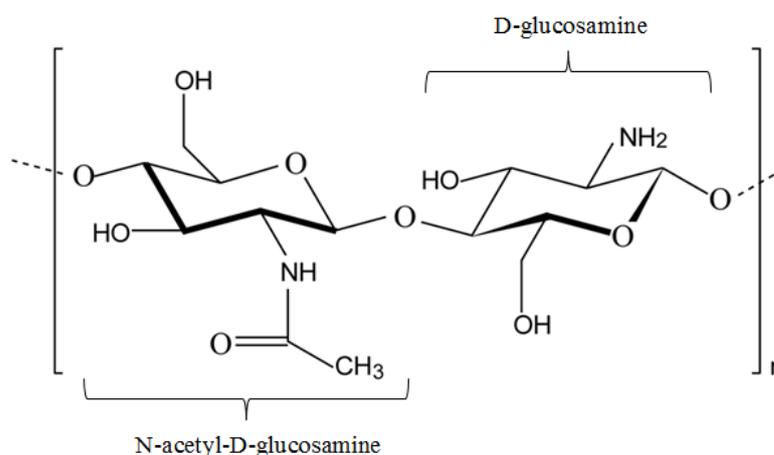


Figure 2.3: Chitosan molecular structure

Chitosan (Cs) is a natural polysaccharide formed during the deacetylation of chitin in alkaline condition. As a linear polycationic polysaccharide, it is composed of randomly distributed (1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units (Figure 2.3). Commercially, it is produced by the exhaustive deacetylation of chitin (>60%). The most easily exploited sources of chitin are the protective shells of crabs and shrimp (Chandy and Sharma, 1992; Edlund and Albertsson, 2002; Jain, 2000; Mathew and Kodama, 1992). The hydrophilicity of the polymer that is due to amine functionality in most repeat units makes the polymer

soluble in dilute acid and yields a rubbery hydrogel in water (Kamath and Park, 1993). Chitosan is insoluble in solutions at neutral or alkaline pH and exhibits a gel-like precipitate when the solution pH is brought close to neutral, which can limit the breadth of its applications.

Polymer blends have been investigated over the years to satisfy the specific needs of the biomedical field. Such blends showed satisfactory performances compared to the individual polymers and the range of application has been extended. These co-polymeric hydrogels are normally composed of two or more types of monomer species where at least one is hydrophilic in nature. In order to increase the possible applications of chitosan hydrogels, composite hydrogels have been formed in which chitosan is blended with other biopolymers such as collagen (Wang and Stegemann, 2010), gelatin (Farshi Azhar et al., 2014) and agarose (Miguel et al., 2014; Wang and Wu, 1997). In particular, the biocompatibility of agarose hydrogels and the mild conditions required for their gelation make agarose a good candidate for the preparation of composite hydrogels suitable for biomedical applications, especially for sustained drug delivery (Wang and Wu, 1997). Moreover, mixing of agarose with chitosan can be used to improve the mechanical properties of agarose (Buckley et al., 2009; Cao et al., 2009).

The gelation of agarose in mixed aqueous solutions of chitosan and agarose leads to the formation of transparent and mechanically stable hydrogels in which chitosan chains remain embedded into the agarose matrix (Zamora-Mora et al., 2014).

In the research described in this thesis a simple straightforward method is developed for the preparation of composite chitosan/agarose gels, without chemical crosslinking, to be applied for the controlled drug delivery system that was developed.

2.2 Drug Delivery Systems Overview

2.2.1 Hydrogels in Drug Delivery Systems

The therapeutic efficacy of drugs depends on their bio-distribution, elimination route and kinetics. The success of a drug delivery system (DDS) is mostly dependent on the carrier that is used to modify the bio-distribution of that drug. The therapeutic efficacy of many drugs can be improved by combining them with hydrogels as drug carriers. This provides control over the release kinetics, the protection of the active agent against degradation or inactivation due to external conditions, prevents side effects and leads to a more effective therapeutic drug action (Kumar and Cotran, 1994).

The performance of a hydrogel in a DDS depends not only on the physical and chemical properties of the gel but also on the nature of therapeutic agent itself. The rate of release from a polymer carrier can be regulated by selecting a suitable hydrogel composition and drug concentration (Chien, 1991; Jain, 1997). In other words, the hydrogel materials, network conformation, drug content and drug loading mechanism must be chosen to complement the properties of the drug (e.g. hydrophobicity, charge) and its mechanism of action (sustained drug release versus rapid, high exposure) (Bhattarai et al., 2010).

Based on the mechanism controlling the release of the drug, drug delivery devices containing hydrogels as drug carriers can be classified as either (i) diffusion-controlled system, (ii) water-penetration-controlled system, (iii) chemically-controlled system, or (iv) modulated release system (Batycky et al., 1997; Brazel and Peppas, 2000; Hoffman, 2012). These systems are described in the following sections.

2.2.1.1 Diffusion-Controlled Devices

The driving force for the movement of drugs in these systems is a concentration gradient. In these devices, the drug movement is either by passing through the pores or between polymer chains, and these are the barriers that control the release rate. The actual pore size of the polymer or the space between the chains may be smaller than the size of the drug molecules,

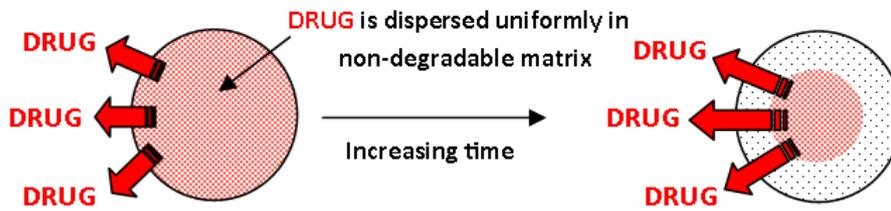


Figure 2.4: A schematic of the monolithic devices (Hoffman, 2012).

but the thermal motion and Brownian motion will facilitate the diffusion of the drug molecules through the polymeric membrane or matrix. The two basic delivery devices that are driven by diffusion are the matrix DDS or the reservoir DDS.

In matrix devices, also called monolithic devices, the drug is uniformly dissolved or dispersed in the polymer and releases by diffusion from the polymer as shown in Figure 2.4. Here, the diffusion depends on the size of the drug and the size of the pores of the polymer (space between the polymer chains). Monolithic devices typically exhibit an initial burst of release from the surface. With passing time, the drug molecules that are in deeper parts of the matrix must diffuse to the surface and since they must travel a much longer path, the amount of drugs available at the surface decrease and the release rate also diminishes. This implies a first-order release behavior which is due to increase in diffusional path length and decrease of the area at the penetrating diffusion front as matrix diffusion proceeds. A zero-order release rate can be achieved by using unconventional geometries, which provides a challenge for the design of the DDS (Peppas and Khare, 1993; Siegel and Rathbone, 2012). Reservoir devices, also called membrane-controlled, are composed of a core and a polymeric

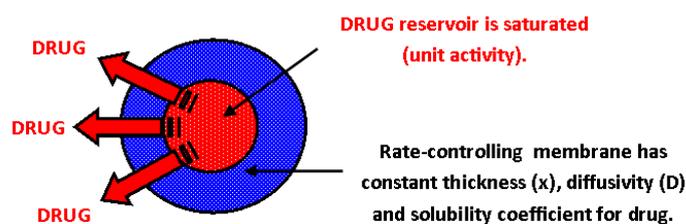


Figure 2.5: Reservoir devices have a coating that controls the release rate(Hoffman, 2012).

membrane. The role of the membrane is to mediate the diffusion of drug. The drug is

encapsulated in the core that is surrounded by the polymer membrane and it is released by diffusion through this rate-controlling membrane (Figure 2.5) (Brannon-Peppas, 2008). The drug transport mechanism through the membrane is usually a solution-diffusion mechanism. The drug first partitions into the membrane from the reservoir, then diffuses to the other side of the membrane where it is released into the receiving medium. In these devices, drug diffusion through the membrane is the rate-limiting step and controls the overall drug release rate.

When the reservoir is saturated, the concentration gradient of drug is constant in the membrane, which results in a constant drug flux or a zero order release in other words. Simplicity of mechanism and the ability to produce zero order release make reservoir systems to be highly advantageous. However, reservoir systems can be difficult to fabricate reliably (Peppas and Khare, 1993; Siegel and Rathbone, 2012).

2.2.1.2 Water Penetration-Controlled Systems

In some controlled drug delivery devices water plays the principal role as the main release controlling agent. In these devices, the drug molecules cannot physically diffuse out of the device until water molecules diffuse inside. There are generally two types of water penetration-controlled systems: swelling-controlled and osmotically-controlled (Heller and Hoffman, 1996; Verma et al., 2002). In swelling-controlled systems drugs are entrapped inside a dried

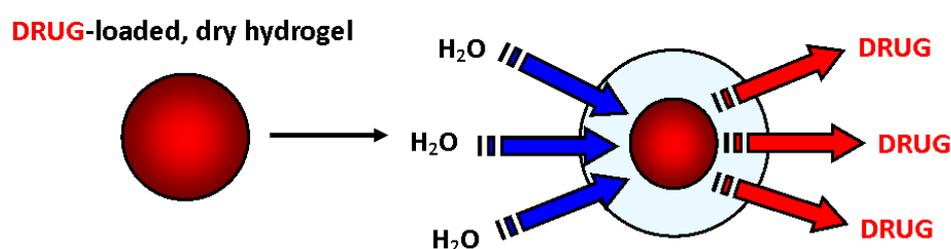


Figure 2.6: A typical oral tablet is a good example of swelling-controlled devices (Hoffman, 2012).

hydrophilic polymer that is stiff or glassy. When placed in an aqueous environment, the glassy polymer at the surface relaxes to a configuration that is more compatible with water and it

swells (Figure 2.6). As the dried polymer is swelling, the drug dispersed inside begins to diffuse out of it while the water molecules diffuse in (Siegel and Rathbone, 2012). Then, the drug trapped inside the polymer will be liberated if it can diffuse through the softened matrix faster than water. In such a system, the drug release depends on the two simultaneous processes of water diffusion into the polymer and chain relaxation (Peppas and Khare, 1993).

A typical oral capsule or pill is usually a swelling-controlled system. Although these devices are easy to manufacture, the release rates are often not steady since a variety of temporal release patterns may occur simultaneously, which further contribute to the complexity of the swelling dynamics (Siegel and Rathbone, 2012).

Osmotically-controlled delivery systems combine the advantage of time-independent release with high delivery rates. Figure 2.7 is schematic of an elementary osmotic drug-delivery system.

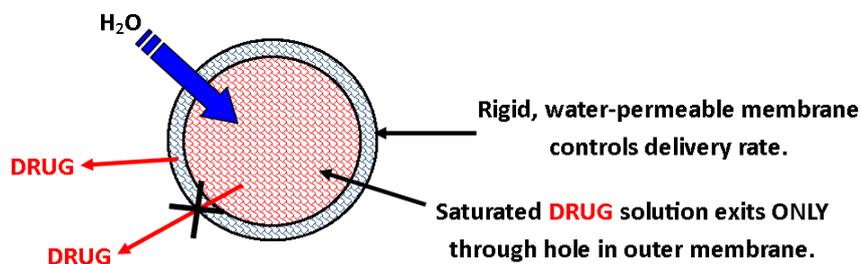


Figure 2.7: Oros is a good example of an elementary osmotic pump (Hoffman, 2012).

In such an osmotically-controlled system, a core of drug is surrounded by a semi-permeable membrane that controls the permeation of water by allowing it to move in, but preventing salt and drug molecules (osmolytes in general) from moving out. A small hole is drilled into the membrane to allow the drug molecules to exit whenever the pressure increases. If the membrane is partially permeable to the osmolytes, the osmotic flow reduces. Water flows osmotically into the core containing drug molecules through the semipermeable membrane, dissolving the drug. A constant osmotic pressure gradient is established between the core and the external medium, causing the water influx, which pushes the drug molecules through the hole at a constant rate. As long as the drug concentration across the membrane is constant the

eroding or degrading (Figure 2.8). Erosion may be either surface or bulk erosion in origin (Peppas and Khare, 1993; Siegel and Rathbone, 2012).

Pendant chain systems, also called prodrugs, are composed of a natural or synthetic polymer carrier to the backbone of which the drug molecule is chemically bonded and released by hydrolytic or enzymatic cleavage. The rate of drug release is regulated by the rate of hydrolysis. These systems are capable of providing an opportunity to target the drug to a particular cell-type or tissue. To do so, the structure of the polymers used can be modified by the incorporation of sugar residues or sulfonyl units to obtain a specific tissue affinity (Peppas and Khare, 1993).

2.2.1.4 Modulated Release Systems

In modulated drug delivery system an external stimuli controls the drug release. These systems are incapable of releasing the active agent or agents until they are placed in an appropriate external environment. Then, the polymer swells as a direct response to the conditions detected and the release occurs (Figure 2.9).

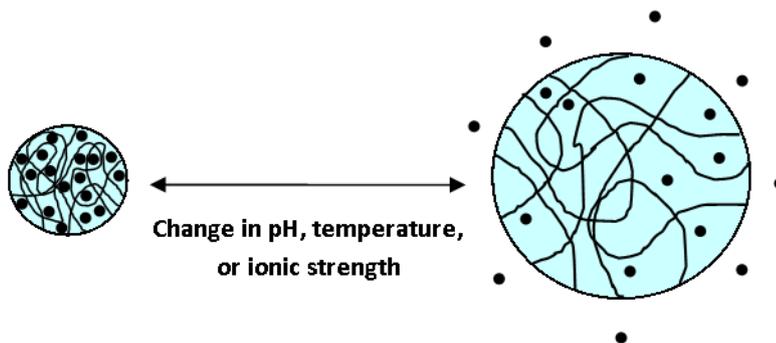


Figure 2.9: Drug delivery from a typical modulated release systems.

The swelling of environmentally sensitive hydrogels can be affected by specific stimuli such as pH, ionic strength, temperature, magnetism, ultrasound and electromagnetic radiation. For example, self-regulating insulin-delivery devices depend on the concentration of glucose in the blood to control the release of insulin (Horbett et al., 1983; Kost et al., 1985).

As mentioned above, the release of drug is affected by its concentration and method of encapsulation. There are at least two main approaches to entrap drugs into hydrogels. One is the chemical crosslinking of the polymer and drug to achieve targeted drug release such as in the case of a prodrug. Drugs with larger size must be added during the gelation process, by mixing the polymer solution and the cross-linking or complexation agent. The chemistry of the drug molecule should be considered to prevent unwanted cross-linking or deactivation of the therapeutic during gelation.

In the second and simplest way, the drug is dissolved or dispersed into the monomer solution or in the formed polymer solution so that the incorporation of drug is directly obtained by polymerization and reticulation. The stability of the drug is ensured by the process in the reaction conditions and the monomers and/or the residual reagents are not removed from the final product. The law of passive diffusion or disintegration of the polymeric matrix controls the release of active ingredient from the surrounding polymeric matrix (Samanta et al., 2003).

2.2.2 Externally Applied Stimulation for Drug Delivery from Hydrogels: Principles and Applications

The main goal of controlled drug delivery and targeting is to improve the efficacy of drug action in the region of the disease and reducing at the same time any undesired side effects in the surrounding healthy tissues (e.g. toxicity). This has led to the idea of utilising an externally applied trigger to stimulate the release of drugs. This approach aims at regulating the drug release and action in the targeted site by an external stimuli such as pH, temperature, pressure, ionic strength or an external energy field, such as light (photodynamic therapy), neutron beam (boron neutron capture therapy), magnetic field (targeted accumulation of magnetic drug carrier in the tissues close to the magnet), or mechanical energy (Hernot and Klibanov, 2008). Some of the commonly used external stimulation techniques that have been proven to effectively control and target the drug release are listed in Table 2.2.

pH-sensitive hydrogels have been known to be suitable candidates as swelling-controlled

Table 2.2: Some of the widely used external stimuli for controlled drug delivery from hydrogels source (Brannon-Peppas, 2008)

Stimulus	Hydrogel	Mechanism
pH	Acidic or basic hydrogel	Change in pH – Swelling – release of drug
Ionic strength	Ionic hydrogel	Change in ionic strength – change in concentration of ions inside gel – change in swelling – release of drug
Chemical species	Hydrogel containing electron accepting groups	electron-donating compounds – formation of charge/transfer complex – change in swelling – release of drug
Enzyme substrate	Hydrogel containing immobilized enzymes	substrate present – enzymatic conversion – product changes swelling of gel – release of drug
Magnetic	Magnetic particles dispersed in alginate microspheres	Applied magnetic field – change in pores in gel – change in swelling – release of drug
Thermal	Thermoresponsive hydrogel poly(N-isopropylacryl)	Change in temperature change in polymer-polymer and water-polymer interactions – change in swelling – release of drug
Electrical	Polyelectrolyte hydrogel	Applied electric field – membrane changing – electrophoresis of charged drug – change in swelling – release of drug
Ultrasound irradiation	Ethylene-vinyl alcohol hydrogel	Ultrasound irradiation – temperature increase – release of drug

release devices. The nature of the pendant groups in hydrogel networks allows the subdivision into either anionic and cationic types. The release mechanism in pH-sensitive hydrogels can be instantaneously or gradual following changes in their dynamic and equilibrium swelling behavior as a result of a change in pH (Bawa et al., 2009; Peppas and Khare, 1993).

Some hydrogels are known to undergo a discontinuous volume change due to the changes in temperature when they are swelling in an aqueous environment. In other words, at a certain temperature range an infinitesimal change in temperature brings about a finite change in hydrogel volume. This phenomenon is known as a phase transition and these type of hydrogels are called temperature-sensitive.

In temperature-sensitive hydrogels, the mechanical strength of the hydrogel and consequently the drug release are altered with the change in the temperature of external environment

(Peppas et al., 2000a). These hydrogels can be classified into three categories based on the transition mechanism, which include (i) negatively temperature-sensitive gels, (ii) positively temperature-sensitive gels, and (iii) thermo-reversible gels (Qiu and Park, 2012).

Negatively thermosensitive hydrogels are known to have a lower critical solution temperature (LCST) behaviour in contrast to positively thermosensitive gels with an upper critical solution temperature (UCST) (Qiu and Park, 2012). The LCST polymers undergo a hydrophilic-to-hydrophobic transition with increasing temperature, while the UCST systems experience the opposite transition. Thermally reversible hydrogels exhibit cyclic phase transitions (sol-gel transition), such as gelatin and other natural polymers (Tian et al., 2012).

A range of hydrogels are sensitive to sugar moieties and exhibit variability in response depending on the presence of glucose. The mechanism of these hydrogels, called glucose sensitive hydrogels, is based on the immobilization of glucose oxidase enzyme. This enzyme catalyzes beta D-glucose to gluconic acid and hydrogen peroxide (Yoshioka and Calvert, 2002). Once gluconic acid is released, the pH of the external environment decreases causing a decrease in hydrogel swelling. The swelling behavior is regulated by the formation of gluconic acid or ionization of amines present in the polymer used for preparation of hydrogels. These smart biomaterials have shown to be able to control the delivery of solute, usually proteins like insulin, lysozyme or BSA (Bovine serum albumin), in response to the external environment (Tang et al., 2003).

In addition to temperature, pH and glucose, other stimuli such as light, electric fields, ultrasound, chemicals and ions have been also used in the formation of responsive hydrogels. Magnetism has been reported to control and enhance the drug release from hydrogels (Kost and Langer, 2012). Magnetically-controlled drug delivery systems are composed of drug-containing hydrogels with small magnetic spheres encapsulated inside. When exposed to an oscillating field a significant amount of drug liberates from the delivery system.

In order to improve the delivery of drugs and therapeutic agents, mechanical energy can

be also applied in the form of ultrasound irradiation. The drug-containing hydrogels can release the entrapped drug rapidly but in a controlled manner when exposed to ultrasound. Ultrasound was also found to enhance erosion and degradation of some biodegradable polymers (Kost et al., 1988) and act as an on-off switch in another applications (Kwok et al., 2000, 2001). Ultrasonic drug delivery devices combine electric fields and ultrasonic waves to deliver biologicals and drugs ranging from small molecules to large proteins systemically or to specific target organs. With such systems, the risks and discomfort associated with injections can be eliminated and the drugs that are inactivated by GI or liver metabolism can be delivered effectively. These systems can also combine the advantageous properties of hydrogels with ultrasonic stimulation to facilitate the release of drugs in regulated doses.

Ultrasonic waves have numerous advantages as a controlling field that make them ideal for the delivery of therapeutic agents and the stimulation of tissues. Ultrasound has been known to improve drug delivery into tissues and cells (Kassan et al., 1996), but perhaps the most important advantage of ultrasound is being a mechanical and yet non-invasive means of delivery that can be applied to a very wide range of therapeutics and target sites (Pitt and Hussein, 2008). Drug release can be triggered easily in an ultrasonic drug delivery device because of a better control on the source of stimulation, which is the pure energy acting on-demand, compared to other stimulus such as pH, temperature, pressure, and ionic strength which depend on the environmental parameters. It does not involve adding specific substances or particles to the drug carrier compared to, for example, enzymatic, chemical or magnetic stimulations, neither is drug specific like electrically stimulated methods.

In the following section, we discuss the characterization of ultrasonic waves, how they are applied for drug delivery purposes and the ultrasonic system used for the research in this thesis.

2.2.3 Delivery System Used in this study

Ultrasound can be applied in a non-invasive system and it has been already used in diagnostic and therapeutic medicine. It is now being investigated for advanced drug delivery, which is based on the observations that ultrasonic waves can be focused onto targeted tissues, they can facilitate the release of pharmacological agents from carriers and they render cell membranes more permeable. Similar to other modulated DDS, ultrasound-based DDS enables the delivery of a large number of drugs, improves the activity and efficiency of drug delivery, and improves the ability to precisely control the amount of drug delivered (Pitt et al., 2004).

Sound is the propagation of pressure waves through some physical elastic medium, such as air or liquid. These pressure waves exist only in a medium, since they are transmitted from one mass to another by direct contact between the masses. Since the elastic property of the medium provides the sustained vibrations required for ultrasonic wave propagation, ultrasonic waves are also called elastic waves (Ensminger, 1988). The pressure waves are generated from mechanical vibrations, thus, they become vibrating pressure waves that can transfer energy to the medium and to the objects that they contact. This transfer of energy causes the intensity of the sound energy to progressively decrease during its propagation.

Ultrasound is the sound wave with the frequency above 20 kHz which is the limit of the human audible range (Suslick, 1988). Unlike microwaves and radio waves which are electromagnetic radiations, ultrasonic waves are completely mechanical in nature. However, it can be reflected, refracted, diffracted and focused like a beam of electromagnetic radiation. Ultrasonic waves are mechanical waves, since they are the actual movement of molecules as the medium is compressed (at high pressure) and expanded (at low pressure), and therefore they can act physically upon biomolecules and active agents. Most importantly, unlike visible light waves, ultrasonic waves are absorbed relatively weakly by water, flesh and other tissues. Significantly, the amount of attenuation is much less when the frequency is low (Husseini and Pitt, 2008). Mitrogotri et al. showed that low-frequency ultrasound can more effectively enhance the drug delivery compared with higher frequencies (Terahara et al., 2002; Tezel et al., 2002).

Ultrasound is generated by a transducer which is the electronic device that converts electrical energy to acoustical energy or vice versa (Asher, 1997). Almost all transducers are piezoelectric materials. They can be either a natural crystalline solid like quartz, or a manufactured ceramic such as lead zirconate titanate. When the frequency of the input voltage reaches the resonance frequency at the piezoelectric material, the transducer responds by undergoing vibrations which are further transmitted to the environment as ultrasonic pressure waves (Ng and Liu, 2002).

Ultrasound, either low- or high-intensity, has several applications in medicine. Low-intensity ultrasound is mainly used to obtain information on the state of matter (e.g. imaging and flow studies), while high-intensity ultrasound is aimed at manipulating the state of matter (e.g. kidney stone shattering or tumor and fibroid ablation) (Schroeder et al., 2009). These two ranges of frequencies, low-frequency in the range of 20–100kHz and high-frequency in the range of 0.7–100MHz, are both commonly used for drug delivery applications (Mitragotri et al., 1995).

Although the intensity of the ultrasound used for both therapeutic and diagnostic applications are non-invasive, the intensity levels required are different for such applications. For diagnostic applications, the intensities are in the range of 0.0001-0.5 (W/cm^2) which is substantially less than the intensity needed for the therapeutic (0.5 - 3 (W/cm^2)) or surgical-based ultrasound (>10 (W/cm^2)) applications (Ng and Liu, 2002).

In this research, ultrasonic waves were used as a non-invasive external stimulus to control and enhance the drug release from various hydrogel formulations. Several hydrogel formulations are chosen to entrap the active agent to protect it from being degraded or damaged, but with a concomitant reduction in the rate of release. Since low-frequency ultrasonic waves have been proven to be more effective for this purpose, 30 kHz ultrasonic waves were used throughout this work. The combination of the mechanical properties of ultrasound with desirable characteristics of hydrogels provided a complete system that has potential to be a

suitable candidate for regulated delivery of a variety of therapeutics and active agents.

2.3 Summary

The importance of controlled drug delivery was discussed in Chapter 1. There is a clear need for a well-designed drug delivery system that control solute release over time. Among various biomaterials that have been investigated to control drug release, hydrogels show distinct advantages that make them ideal for such applications. Some of the reasons why these hydrophilic polymers become very interesting for drug delivery applications are summarised as follows (Chen et al., 2004; Jeong et al., 2002; Peppas et al., 2000a; Risbud et al., 2000; Vaghani et al., 2012):

1. Hydrogels swell significantly in aqueous medium without being dissolved;
2. They can disperse drugs within their matrix readily and release what is entrapped with high degree of control;
3. Drugs can easily diffuse out of the hydrogels;
4. Compared with hydrophobic materials, hydrogels may interact less strongly with active agents;
5. Natural hydrogels have some intrinsic properties including low toxicity and biocompatibility;
6. Synthetic hydrogels can form carriers with required mechanical strengths and biological responses.

To better control the rate of drug release, it is often required to modify the polymer matrix. Since hydrogels are hydrophilic materials sometimes with poor mechanical strength, crosslinking is a common method to enhance their structure. Although chemical cross-linking is a versatile method, the toxicity of the cross-linking agents and unwanted reactions with the bioactive substances present in the hydrogel matrix have made physical cross linking a more advantageous method. In addition to reinforcing the hydrogel structure by crosslinking, the rate of release could potentially be controlled by external stimulation. Ultrasound stimulation is a mechanical and yet non-invasive means of delivery that can be applied to a very wide range

of therapeutics and target sites. The numerous advantageous of this method has encouraged to apply ultrasonic waves combined with hydrogel formulations to present an effectively controlled release device in this research.

Chapter 3

Hydrogel Selection, Synthesis and Characterization

Introduction

In this chapter the synthesis protocols of three different hydrogel formulations and the methods to characterize them are described. The preparation of each hydrogel necessitates a specific process, since they vary significantly in structure and the functional groups are not necessarily the same. Thus, each hydrogel has its specific protocol that will be presented in Section 3.1. It is important to characterise the material prior to utilising the hydrogel in a DDS. The characterisation methods used in this research are described in Section 3.2. Section 4.6 provides the summary of the chapter.

3.1 Hydrogel Synthesis

The advantages and applications of natural and synthetic hydrogels for drug delivery purposes were discussed in previous chapters. In general terms, natural hydrogels offer biocompatibility and non-toxicity while synthetic hydrogels provide mechanical strength. The three hydrogel formulations of agarose, chitosan and PVA were selected as hydrophilic carriers to study the effect of various polymeric structures on drug release. The preparation of each of the gels is described in the following sections.

3.1.1 Agarose Synthesis

Although agarose is widely used in pharmaceutical industries and medical research it has had limited use in drug delivery, due mainly to its highly porous structure and low mechanical strength. Nonetheless, it is a biocompatible material and physical crosslinking is a possible solution to create an agarose gel with desirable mechanical characteristics. In this thesis, agarose in two forms, native and crosslinked, is prepared to investigate the effect of the modifications on hydrogel structure and applications in this work. The preparation of these agarose gels started with low melting temperature agarose (NuSieve GTG Agarose) that was purchased from Lonza Walkersville, Inc. and it is used as received throughout the research. Sodium hydroxide (NaOH), Sodium hydrogen phosphate (Na_2HPO_4) and Potassium dihydrogen phosphate (KH_2PO_4) were obtained from Sigma-Aldrich. The crosslinking agent, Poly (ethylene glycol) diglycidyl ether (average Mn 526), was purchased from Aldrich.

3.1.1.1 Native Agarose Synthesis

Native agarose gels are prepared in distilled water by adding 0.6 g of agarose to 30 mL of distilled water and leaving to soak for some seconds. The bottle was capped and microwaved on a high setting just until the appearance of bubbles. Afterwards, the bottle was removed and swirled slightly for some seconds. It is uncapped if needed to emit the vapor. Heating and swirling is repeated until there is no sign of any solid pieces of agarose remaining. The obtained 2wt% agarose is poured into different shaped moulds for subsequent characterization.

3.1.1.2 Crosslinked Agarose Synthesis

Physically crosslinked gels are suitable for biomedical applications involving entrapment and encapsulation of bioactive substances and molecules, particularly where the gel formation occurs under mild conditions and without any organic solvents. Many compounds such as formaldehyde, epoxy compounds, dialdehyde and starch can be used as crosslinking agents. Several poly-epoxy compounds have been reported as cross-linking agents for biomedical applications such as Ethylene Glycol Diglycidyl Ether (EGDE) which contains two epoxide groups (cyclical ethers constituted by a three-membered ring) at both ends of each molecule

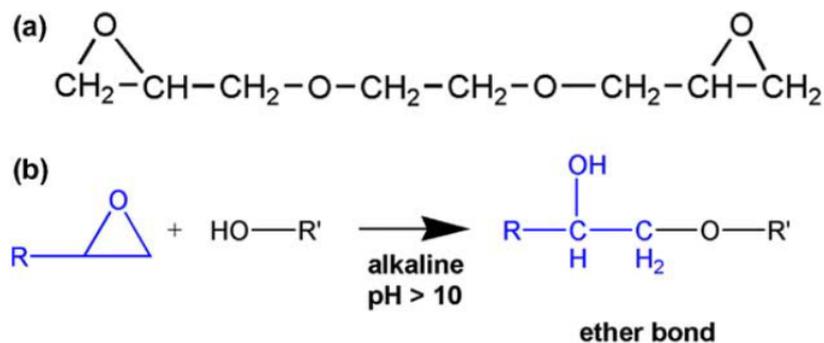


Figure 3.1: Schematic of epoxy reaction dependence on pH . (a) Structure of ethylene glycol diglycidyl ether (EGDE). (b) Under alkaline conditions ($pH > 10$), epoxy groups predominately react with hydroxyl groups to yield stable ether bonds (Sung et al., 1996).

(Figure 3.1 a) (Sung et al., 1996). These ethers are much more reactive than other ethers because of the high energy held by the considerable strains that exist in the three-membered ring (Sung et al., 1996). For this type of cross-linking agents, the opening of the epoxide ring and the chemical interaction with the amino, carboxyl and hydroxyl functional groups can occur in acidic or in basic media and must happen simultaneously. As shown in Figure 3.1 b, epoxy groups react with hydroxyl groups under alkaline pH to yield stable ether bonds (Sung et al., 1996). A physically cross-linked hydrogel was synthesized using agarose and EGDE

Table 3.1: Composition of buffers used for crosslinking agarose with EDGE.

pH	NaOH(0.1M)	Na ₂ HPO ₄ (0.05M)	KH ₂ PO ₄ (0.1M)
8	93.4 mL	-	100 mL
12	53.8 mL	100 mL	-

with the aim to take advantage of the desirable properties of each compound. Since agarose has numerous OH groups at pH 7.5 that are available to react with the crosslinking agent, it was assumed that an alkaline pH must be maintained (Figure 3.1 b) to form ether linkages between agarose hydroxyl groups and EGDE epoxide rings. To investigate the effect of pH on the crosslinking reaction, agarose crosslinked hydrogels were prepared at pH 8 and at pH 12. Depending on the required final pH , either 8 or 12, volumes of $NaOH(0.1M)$ are mixed with $Na_2HPO_4(0.05M)$ or $KH_2PO_4(0.1M)$, (Table 3.1).

A series of cross-linked gels are prepared with different concentrations of EGDE (4, 7 and 10 wt %) and pH according to the proportions shown in Table 3.2. The first step was to prepare an agarose mixture as described in Section 3.1.1.1 by adding 0.6 g of agarose to 30 mL of distilled water and then leaving to soak for some seconds. The bottle was capped and microwaved on a high setting just until the appearance of bubbles. Afterwards, the bottle was removed and swirled slightly for some seconds. It is uncapped if needed to emit the vapor. Heating and swirling is repeated until there is no sign of any solid pieces of agarose remaining. An agarose concentration of 2 wt% in the final mixture was employed throughout the experimental work. Then, the cross-linker was dissolved separately in the buffer solution and thoroughly mixed for 10 min. It was then quickly added to the agarose solution and the whole mixture was stirred for 2 h in a water bath at 55°C. To stop evaporation, the flask cap was closed tightly.

Table 3.2: Composition and designation of agarose crosslinked hydrogels.

Num	Designation	pH	Agarose (wt%)	EDGE (wt%)
1	E4P8	8	2	4
2	E7P8	8	2	7
3	E10P8	8	2	10
4	E4P12	12	2	4
5	E7P12	12	2	7
6	E10P12	12	2	10
7	P8	8	2	-
8	P12	12	2	-
9	P7	7	2	-

As a control for the effect of pH on agarose structure, one non-crosslinked native agarose sample was prepared at low alkaline pH and another one at high alkaline pH. Finally, each solution was poured into a silicon mould appropriate for the desired experiment and was kept at room temperature overnight and transferred to a refrigerator at 4°C for 1 day. The cross-linked gels were washed with acetone (5%) for 30 min and immersed separately in distilled water to be washed (Pitarresi et al., 2000). The washing process took 4 days to elute

unreacted components from each cross-linked agarose gel, with frequent changes to fresh distilled water until the washing medium reached approximately pH 7 for each gel sample. The cross-linked gels were stored at 4°C for further analysis.

3.1.2 PVA Synthesis

PVA is a linear polymer that has to be crosslinked to form hydrogels for the application of drug delivery (Ricciardi et al., 2004a). PVA is insoluble in non-aqueous medium due to strong intermolecular and intramolecular hydrogen bonding. The solubility of PVA in water depends on the degree of hydrolysis and molecular weight (Urbanski, 1977). It is known that PVA solutions show a transition from sol to gel upon cooling when prepared in various aqueous co-solvents (See Section 2.1.3.2). A very common method of PVA hydrogel synthesis is dissolving PVA in a mixed solvent containing water and dimethyl sulfoxide (DMSO). Mixtures of dimethyl sulfoxide (DMSO) and water when used as the co-solvent result in PVA hydrogels with more favourable features (i.e. better transparency, high elasticity, and a high gelation rate) compared with those prepared in aqueous solutions.

Table 3.3: PVA samples and their compositions.

Num	Designation	PVA (wt%)	DMSO (v/v%)	Distilled water (v/v%)
1	PVA1	15	50	50
2	PVA2	15	40	60
3	PVA3	15	30	70
4	PVA4	10	50	50
5	PVA5	10	40	60
6	PVA6	10	30	70

To prepare the PVA hydrogels for the controlled DDS described in this thesis, the concentration of cross-linking agent (DMSO) and PVA content were varied (See Table 3.3).

PVA (Mw=125000) was obtained from SDFine Chem Ltd (Mumbai, India). Dimethyl sulfoxide (DMSO) was purchased from Sigma. A mixture of DMSO/water is prepared as solvent for PVA, where DMSO proportion is varied from 30 wt% to 50 wt% (Table 3.3).

Maximum and minimum concentrations of PVA are determined according to the literature.

DMSO was first warmed and the appropriate proportion of DMSO was then added to distilled water and the whole solution was heated to 90°C in a water bath while being stirred. Then, PVA powder was added to the hot solvent and the mixture was left stirring for 4h in order to obtain a homogenous PVA solution. This solution was then poured into silicon moulds and stored at 4°C for further use.

3.1.3 Chitosan Synthesis

As described in Section 2.1.3.3, polymer blending is one of the useful methods of improving or modifying the physicochemical properties of chitosan polymers to optimise the properties. A polymer blend is defined as a combination of two polymers without any chemical bonding between them (Paul and Newman, 1978). However, in practice, some chemical interaction might occur between components. Polymer blending is a means to enhance the properties of a final material rapidly and economically without requiring the development of a new material (Aminabhavi and Naik, 1998; Mankad et al., 1992).

To prepare the blended hydrogels, chitosan (medium Mw Code:448877) was purchased from Sigma. As mentioned in Section 2.1.3.3 chitosan is insoluble at neutral or alkaline pH so, the pH of synthesis was slightly acidic (pH=6.5). For this, a volume of 40 mL of 1.0 wt% aqueous acetic acid (Sigma) was heated until the temperature in the beaker reached 60°C, then the beaker was removed from the heater and placed on a stirrer at ambient temperature. Then, 0.8 g of chitosan was added slowly to the beaker while stirring to achieve a final concentration of 2 wt% chitosan in solution. The mixture was stirred for a further 2h until chitosan was fully dissolved and a completely transparent solution was obtained. A 2 wt% agarose solution was prepared separately as described in Section 3.2.1.2.

According to the sample under preparation (Table 3.4), the required proportion of agarose

Table 3.4: Chitosan samples and proportions of agarose (2 wt%) and chitosan (2 wt%) that should be mixed.

Num	Designation	Agarose (v/v %)	Chitosan (v/v %)
1	Ch100	0	100
2	Ch75Ag25	25	75
3	Ch50Ag50	50	50
4	Ch25Ag75	75	25
5	Ag100	100	0

solution was then added slowly to the chitosan solution under stirring at room temperature. After a period of time, a homogenous solution was obtained that became progressively more viscous. The polymer blend solution was then poured in silicon moulds and stored at 4°C to allow the mixture to gel.

3.2 Characterization

All hydrogels prepared in this work were further characterized in order to determine and compare their structure, thermal properties and mechanical strength.

3.2.1 Dynamic mechanical analysis (DMA)

The characterization of mechanical properties in polymers typically refers to the measure of the strength. Young's modulus of elasticity, which defines the relationship between stress (force per unit area) and strain (proportional deformation) in a material, is commonly used to describe the mechanical properties of materials. In this thesis, it is used as a measure of the ability of a material to withstand changes in length when under lengthwise compression and describes the elastic properties of a hydrogel undergoing confined compression in only one direction.

Dynamic mechanical analysis is the most common technique used to calculate Young's modulus and characterize the viscoelastic behaviour of a material such as a hydrogel. Vis-

coelasticity is the property of materials that exhibit both viscous and elastic characteristics when undergoing deformation. Elasticity is the tendency of solid materials to return to their original shape after forces are applied on them. When the forces are removed, the object will return to its initial shape and size if the material is elastic. Viscosity is a measure of a fluid's resistance to flow that gives the viscoelastic material a strain rate dependence on time. Hydrogels, due to their large water content, are viscoelastic exhibiting both solid-like and liquid-like traits at different deformation rates and length-scales.

Specifically, viscoelasticity is a molecular rearrangement. When a stress is applied to a viscoelastic material such as a polymer, parts of the long polymer chain change positions. This movement or rearrangement is called creep. Polymers remain solid even when these parts of their chains are rearranging in order to accompany the stress, and as this occurs, it creates a back stress in the material. When the back stress is the same magnitude as the applied stress, the material no longer creeps. When the original stress is taken away, the accumulated back stresses will cause the polymer to return to its original form.

In this thesis, the dynamic mechanical analysis (DMA) was performed with a commercial dynamic mechanical analysis machine that applied cycles of compressive stress to the samples (TA Instruments, RSA 3). Although the complete mechanical behaviours of hydrogels is viscoelastic, we utilised the measure of Young's modulus for small strains to characterise the hydrogels in this thesis.

To prepare samples for compression tests, a hot solution of every hydrogel prepared earlier was poured into cylindrically shaped molds, with dimensions of 15 mm in diameter and 15 mm in height. Using parallel metal plates of 15mm diameter with flat surfaces, the sample was placed on the fixed plate and the oscillating plate applied force in confined compression. Samples were compressed at a rate of 1 mm/min until water started to be exuded to determine the deformation at which the hydrogels were fully elastic and to calculate Young's modulus of the hydrogels.

If we consider one of these samples subjected to a compressive load F along the z -axis

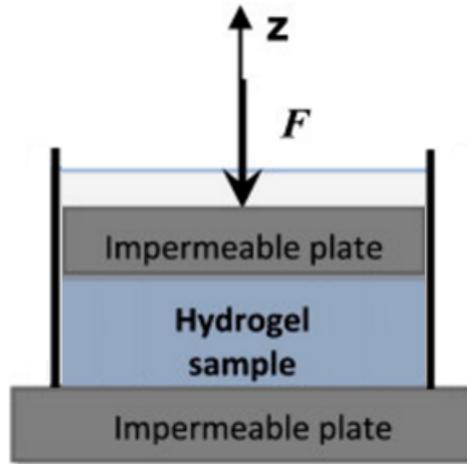


Figure 3.2: Young's modulus calculation for a confined hydrogel under compression.

(Figure 3.2), the load causes time evolution of axial deformations and gradually the movement of liquid towards outside the porous hydrogel (Kazimierska-Drobny et al., 2015). Hence, we maintain on compression until the liquid starts to come out of the sample, otherwise the value obtained will not be the actual elastic modulus.

Simply stated, Young's modulus represents the ease to which a hydrogel can be compressed and can be expressed as equal to the longitudinal stress divided by the strain expressed, as:

$$E(\varepsilon) = \frac{\delta(\varepsilon)}{\varepsilon} = \frac{\frac{F}{A_0}}{\frac{\Delta L}{L_0}} = \frac{FL_0}{A_0\Delta L}$$

where

- E is the Young's modulus [Nm^{-2}];
- F is the force exerted on hydrogel sample [N];
- A is the cross-sectional area through which the force is applied [m^2];
- ΔL is the amount of compression [m];
- L is the original height of the cylinder [m].

A minimum of five samples were examined for each hydrogel formulation. The average of the results from these samples is reported for each hydrogel formulation. The stress and the

strain are calculated by TA Orchestrator 7.0 software. The slope of the first straight line in the elastic region of stress vs. strain curves was used to calculate elastic modulus (in Pa).

3.2.2 Swelling measurement

The amount of water retained in a hydrogel network can provide a suitable pathway for drug diffusion. Hence, the rate and degree of hydrogel swelling are of paramount importance since they tell us about the hydrogel structure and eventual drug release pattern. Swelling is monitored by gravimetric measuring of the water uptake as a function of time (Figure 3.3).

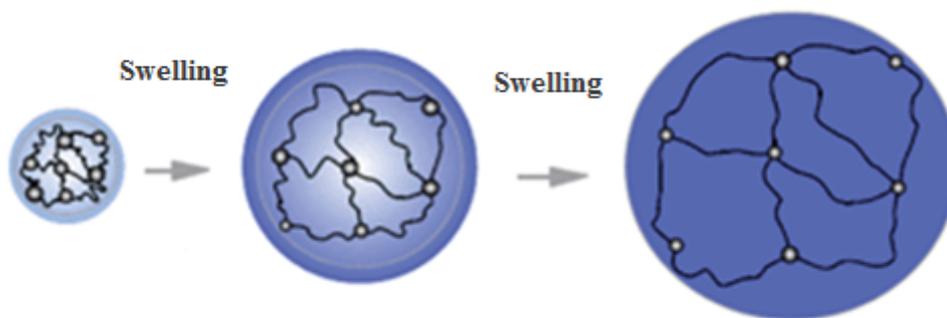


Figure 3.3: Schematic diagram of hydrogel swelling process.

To prepare samples for swelling measurement, hot hydrogel solutions were poured into disc shape silicon molds and then stored at 4°C to form hydrogels. The hydrogel discs were then dried in a vacuum oven until a constant weight was obtained. The swelling ratio of the dried hydrogel disc was measured with gravimetric method in 10ml of PBS (pH 7.4) at room temperature. The dried sample was first weighed with an analytical microbalance and then placed in a petri dish that contains Phosphate-buffered saline (PBS) with 137 mM NaCl, 2.7 mM KCl and 10 mM phosphate buffer solution. Measurements were made every 2min during the first 10min and then every 5min. After the swollen hydrogel has reached its equilibrium swelling, it was removed and gently blotted to remove excess PBS on the hydrogel surface, then immediately weighed with a an analytical balance. The equilibrium swelling ratio (ESR) of hydrogel was calculated as follows:

$$ESR = \frac{W_s - W_d}{W_d}$$

where W_s is the weight of the swollen hydrogel, and W_d is the weight of the dried hydrogel. The experiments were carried out in triplicates for each hydrogel.

3.2.3 Thermal analysis: Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA)

Thermal analysis, particularly differential scanning calorimetry (DSC) is a sensitive method to investigate the composition and structure of polymers. Any change in the compositional and structural parameters of polymers usually affects the melting transitions or glass transitions which can be linked to many performance parameters. Thermogravimetric analysis can also give some information about polymer thermal stability and the effects of additives.

Thermal analysis was performed with a commercial Differential Scanning Calorimetry instrument (DSC, TA instrument 2920, France). The instrument was calibrated using pure indium. A small sample (~10mg) of each hydrogel was precisely weighed and separately heated from 30°C to 90°C at 5 (°C.min⁻¹) in a sealed aluminum pan. Samples were stored under 100% atmospheric RH conditions in order to detect any gel-sol transition. All samples were then cooled to 30°C (Fernández et al., 2010).

Thermogravimetric measurements were performed with TGA (TA instrument 2050, France). About 30mg of agarose samples were precisely weighed and heated up from 30°C to 150°C at 5 (°C.min⁻¹). Samples were stored under 100% atmospheric RH prior to the measurement.

3.3 Summary

Three different hydrogels are used in this research to prepare drug carriers; two natural polymers: agarose and chitosan, and a synthetic polymer: PVA. Agarose is prepared in native form and crosslinked forms at different pH. Various solvents consisting of different proportions of DMSO and water are used to make PVA hydrogels. Chitosan hydrogels were prepared by mixing chitosan and agarose solutions using the method of polymer blending. Several characterizations are performed on samples described above to better know about their structure to be able to assess them for further drug delivery applications. The results of the measurements described in Section 3.2 will be presented and discussed in the following chapter.

Chapter 4

Hydrogel Characterization Results

Introduction

In Chapter 3, the protocols were presented for the synthesis of the three different hydrogel formulations; agarose, chitosan and PVA. The methods used to characterize these hydrogels were described. The results obtained from these characterisations are presented in this chapter. In Section 4.1, the observations after preparing hydrogels and their visual differences are presented. In Section 4.2, the mechanical properties of the hydrogels are extracted from the data obtained. Section 4.3 presents the thermal analysis results which lets us to compare any change or difference among hydrogels structures. The swelling rate and water uptake capacity of each hydrogel is discussed in Section 4.4. In Section 4.5, a comparison of the three series of hydrogels is provided regarding their application for controlled drug delivery. The summary of the chapter is presented in Section 4.6.

4.1 Hydrogel synthesis

4.1.1 Agarose

Crosslinked and non-crosslinked agarose hydrogels were prepared and summarized in Table 3.2. The first indication of difference among these hydrogels is their different turbidities which could be visually recognized. The optical image of all of these hydrogels after preparation

presented in Figure 4.1 demonstrates that they are visually quite different, with some samples being translucent while the others are turbid. Since this difference is due to the light absorption capabilities of the samples, spectrophotometry was used to quantify those differences.

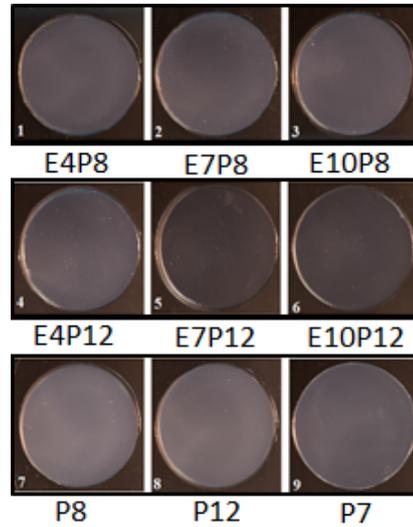


Figure 4.1: Optical images of all agarose hydrogels after preparation.

The UV-vis absorption spectra of the crosslinked and non-crosslinked agarose hydrogels were measured between 400 and 800 nm every 1 nm in a quartz cuvette using a commercial spectrophotometer (mini UV-240 Shimadzu, France). For these tests, samples prepared from hot solutions of agarose hydrogels are poured into rectangular shaped molds of 4 mm of thickness. Turbidity, T , is calculated from the following equation (Aymard et al., 2001):

$$T(\lambda) = 2.3 \frac{AU(\lambda)}{L}$$

where AU is the absorbance unit (optical density) and L the optical path length which is 0.4cm.

The UV-vis results (Figure 4.2) indicated that the samples exhibited different ranges of turbidity. Each data point represent the mean of three measurements. The Standard Error of Mean (SEM) at all points varied between 0.0013 and 0.0067. The changes observed in turbidity suggest an alteration in the structure of the agarose hydrogel prepared under various conditions. The highest turbidity was found in two controls prepared in buffers which lacked crosslinker (P8 and P12), while the turbidity of the control prepared in distilled water (P7) is significantly less. The increase in turbidity of agarose hydrogels in a solvent with pH other

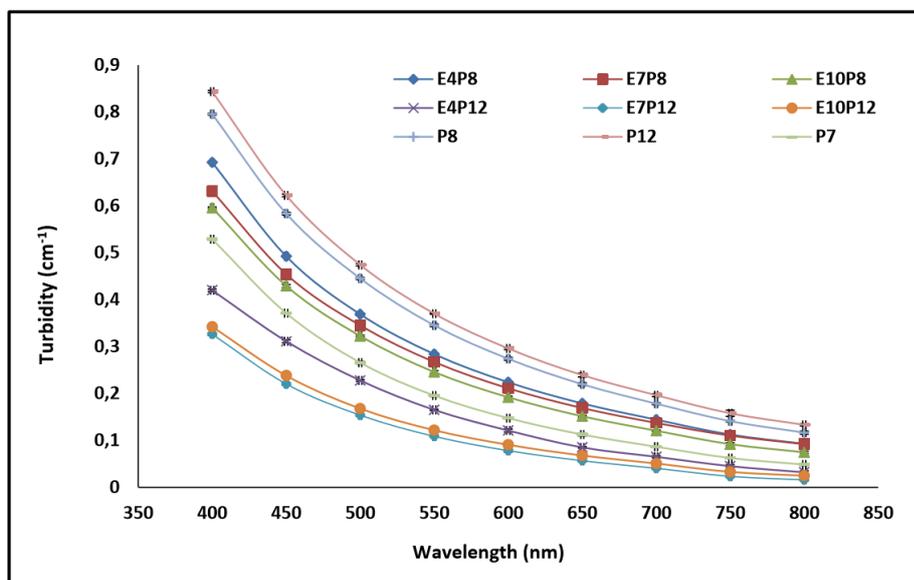


Figure 4.2: Turbidity of crosslinked and non-crosslinked agarose samples (values reported are an average of $n = 3$).

than neutral pH implies the aggregation of the basic units in the gel structure.

Comparing the two controls P8 and P12 with the cross-linked samples, we find that all the cross-linked samples are less turbid than P8 or P12. Although the turbidity is reduced by cross-linking the agarose, the minimum turbidities are for those crosslinked hydrogels prepared at pH12. The samples E7P12 and E10P12 have approximately the same turbidity, which is the least of all values. It implies that increasing the crosslinker concentration to a certain amount decreases the turbidity, which can be also seen by comparing the results of hydrogels prepared at pH8. This implies that the effect of the crosslinking is to reduce the aggregation of the basic agarose units with the result of a more uniformly distributed gel structure. These results are further confirmed by other characterization methods the results of which will be presented in next sections.

4.1.2 PVA

Prior to preparing PVA hydrogels as listed in Table 3.3, we checked if it is possible to prepare a gel by dissolving PVA in water only; without the addition of any other solvent. PVA at 15 wt% and 20 wt% was added to distilled water and the protocol described in section 3.1.2

was followed. It is observed that although PVA dissolves completely in distilled water and a viscous solution is obtained, it does not form any gel.

To verify the minimum proportion of DMSO needed to get 10 wt% and 15 wt% PVA hydrogels, a 15 wt% PVA solution was dissolved in a solvent of 20 v/v% DMSO. The hydrogel obtained had an unstable structure and degraded quickly right after preparation which prevented its use as a drug carrier. The same preparation with 10 wt% PVA in 30 v/v% DMSO solvent resulted a hydrogel with acceptable strength, thus, the minimum concentration of DMSO was set to 30 v/v%. To determine the upper limit of DMSO, a 10 wt% PVA gel prepared in 80 v/v% DMSO resulted in a solid block of gel. Consequently, DMSO proportion was reduced to 50 v/v% which gave a hydrogel with desirable characteristics. The proportion of DMSO was therefore varied between 30 v/v% to 50 v/v%.

4.1.3 Chitosan

The first chitosan sample was pure chitosan, without any agarose added, as indicated in Table 3.4. This results only in a colloidal suspension of chitosan; no gel can be obtained without adding agarose. No more characterization is then performed on this sample, since it cannot be used as a drug carrier.

All samples are characterised and the results will be presented and discussed in the next section.

4.2 Mechanical Analysis Results

The mechanical and structural properties are important parameters in deciding whether a hydrogel is suitable for drug delivery applications or not (Hassan and Peppas, 2000). Hydrogels contain a significant amount of liquid both bound to the backbone and contained within pores, which is an additional load to the structure and significantly affects the hydrogel properties. This liquid however may flow out and this may induce changes in mechanical properties of the materials. Thus, experimental studies performed to determine the mechanical properties of

hydrogels should be divided into two groups; tests without draining and with draining of the liquid from the gel. The mechanical behaviour of hydrogels without draining can be described using theory of rubber elasticity or single-phase models of viscoelastic material (Anseth et al., 1996).

It is useful to have a property of a material that is independent of the size of the material and can be used to compare elastic properties of different materials. So, Young's modulus (E_s) was used as one of such parameters most frequently used to characterize the mechanical properties of materials including hydrogels.

As explained in Section 3.2.1, cylindrical samples of hydrogels are prepared and analysed under compression to measure the Young's modulus. In case of a confined compression, Young's modulus is meaningful only in the range in which the stress is proportional to the strain, and the material returns to its original dimensions when the external force is removed. So, it can be determined by calculating the slope of the initial, linear-elastic part of the stress-strain diagram.

As expected, the deformations of all three hydrogel formulations studied are time-dependent and nonlinear, so resembles some combination of elastic and viscous responses, known as viscoelasticity. However, the degree of viscoelasticity strongly depends upon some structural variables as degree of crystallinity, crosslinking, and molecular mass of hydrogels since the temperature of the test and the rate at which the hydrogels are deformed are the same in all experiments. The results of mechanical analysis are presented in the following sections for three hydrogels used in this research.

4.2.1 Agarose Mechanical Properties

As explained above, the linear (elastic) region of stress-strain curve is selected to calculate the elastic modulus of each agarose hydrogel. These values are similar to what has been reported in literature (Aymard et al., 2001). After plotting the stress-strain curve, it revealed that all samples are fully elastic at maximum 10% deformation (Figure 4.3). This behaviour

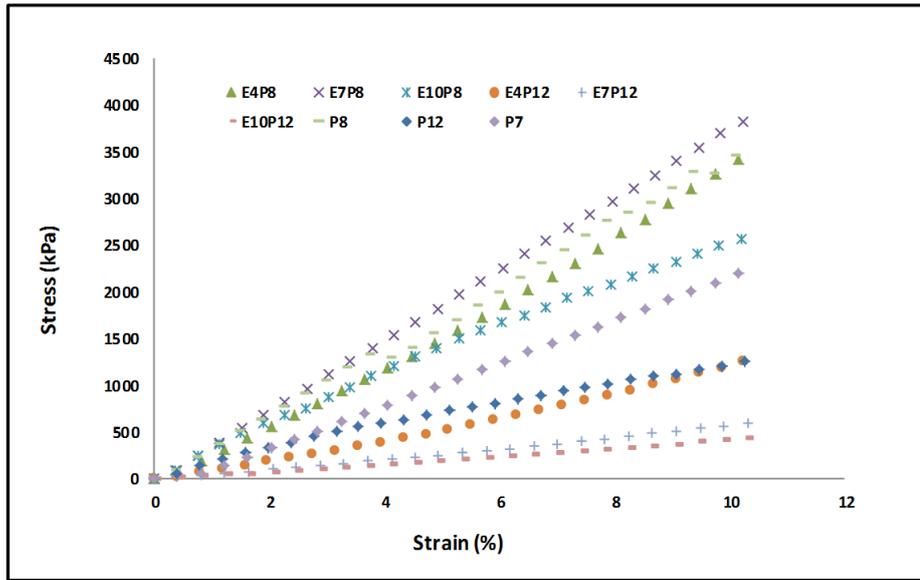


Figure 4.3: Stress vs. strain curve obtained from compression experiments of both crosslinked and non-crosslinked hydrogels.

is consistent with previous measurements of agarose (Mu et al., 2005). The data plotted in Figure 4.3 show that the gels prepared in pH 8 have a higher elastic modulus than the gels prepared at pH 12. These differences are clearer when comparing the summary of the data presented in Table 4.1.

Both controls, prepared in pH 8 and pH 12, revealed different mechanical characteristics than the control prepared in the distilled water; E_Y (P8) > E_Y (P7) > E_Y (P12). These results suggest that the agarose hydrogel is stiffer when prepared in moderate alkaline pH and more flexible when prepared at higher pH values.

The elastic modulus of the non-crosslinked hydrogels prepared in pH 8 and 12 are similar to the crosslinked hydrogels containing 4% EGDE in pH 8 and 12, respectively. This suggests that a concentration of 4% EGDE might not be enough to modify the agarose structure. But we can still say that the presence of EGDE in higher proportions at pH 12 has resulted in a proportionately lower stiffness, as seen by the lower elastic modulus compared to pure agarose gel of the equal concentration. Overall, it can be concluded that the presence of crosslinker molecules has caused the decrease of the elastic modulus of agarose hydrogels in both pH 8

Table 4.1: Summary of mechanical properties of crosslinked and non- crosslinked agarose hydrogels.

Sample	pH	EDGE (wt%)	Youngs Modulus (kPa)
E7P8	8	7	378
P8	8	-	345
E4P8	8	4	339
E10P8	8	10	250
P7	7	-	229
E4P12	12	4	122
P12	12	-	117
E7P12	12	7	57
E10P12	12	10	42

and 12 (Table 4.1). That is, the greater the concentration of the crosslinker, the more flexible is the hydrogel.

This can be rationalized by considering a transformation of the polysaccharide network to a modified structure where substantial parts of the chains are replaced by flexible entities (Norziah et al., 2006). It is possible that the aggregation of helices to bundles has been reduced in the presence of the crosslinker (since the turbidity has also decreased which indicates the formation of less helices in agarose structure) and then the crosslinker flexible entities have been substituted in the polymeric chain or they have occupied the large pores in the agarose network preventing further bundle formation.

The mechanical analysis results are consistent with turbidity measurements and optical images presented in Section 4.1.

4.2.2 PVA Mechanical Properties

It was explained in Section 4.1.2 that PVA hydrogels prepared in DMSO/water solvent are stronger compared to those prepared in only distilled water. Mechanical characterisation has provided further evidence which is discussed here. The results of mechanical compression

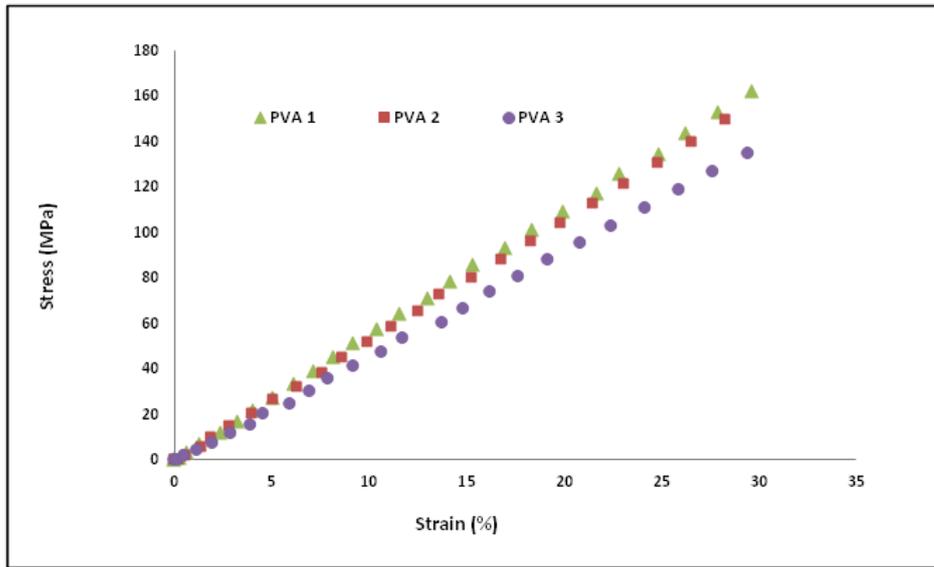


Figure 4.4: Stress vs. strain curve obtained from compression experiments of 15%wt PVA hydrogels with DMSO/water ratio of: PVA1 (50/50), PVA2 (40/60), and PVA3 (30/70).

measurements are plotted in Figure 4.4 and Figure 4.5 for respectively 15 wt% and 10 wt% PVA containing hydrogels.

As can be seen in both Figures 4.4 and 4.5, the gel strength depends on the DMSO/water ratio of the solvent which is in accordance with previous literature, where it was reported that PVA hydrogel properties and especially mechanical properties depend on the ratio of DMSO to water (Ohkura et al., 1992).

The elastic modulus of PVA hydrogels are summarized in Table 4.2. The Young's modulus decreases with a decrease in DMSO content which is in accordance with what has been reported elsewhere (Cha et al., 1996). For example, for a 10% PVA hydrogel, the elastic modulus of the hydrogel with 30 v/v% DMSO content is approximately 25% of that with 50 v/v% DMSO content hydrogel (Stammen et al., 2001). So, stiffer PVA hydrogels are prepared in a higher DMSO/water solvent ratio, most likely as a result of higher gelation rates. This behaviour is reported in the literature, whereby PVA hydrogels prepared from the mixed solvent of water and DMSO exhibit a higher compressive strength and also better transparency than the translucent gel prepared from an aqueous PVA solution (Hyon et al., 1989).

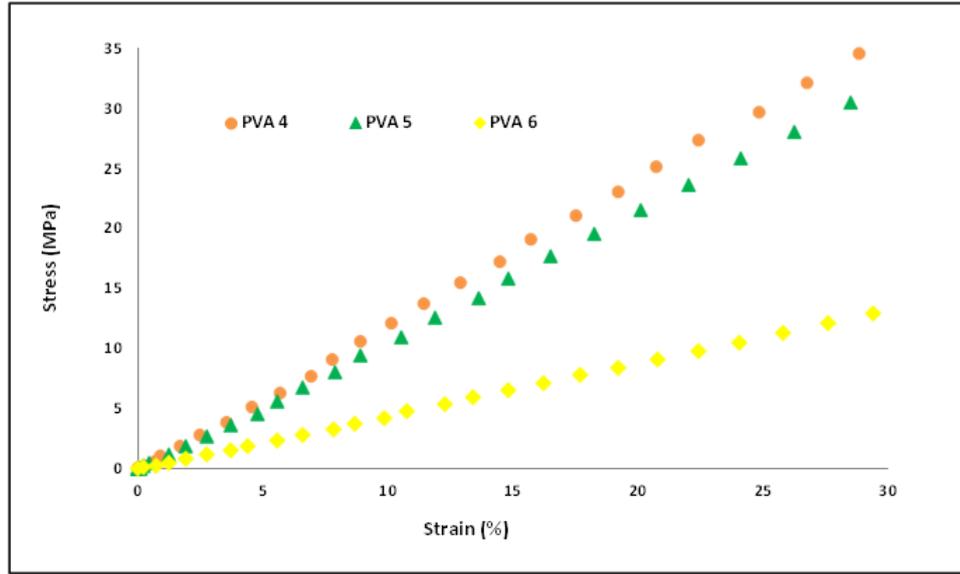


Figure 4.5: Stress vs. strain curve obtained from compression experiments of 10%wt PVA hydrogels with DMSO/water ratio of: PVA4 (50/50), PVA5 (40/60), and PVA3 (30/70).

This can be explained by considering the structure of PVA hydrogels prepared in DMSO/water solvent. This synthetic hydrogel is known as a typical crystalline polymer composed of a network in which the crosslinking or junction points are physically linked by crystallites (Kanaya et al., 1994; Tanigami et al., 1995). Indeed, the physical properties of PVA hydrogel

Table 4.2: Summary of mechanical properties of PVA hydrogels.

Sample	PVA (wt%)	DMSO (v/v%)	DW (v/v%)	Youngs Modulus (MPa)
PVA1	15	50	50	5.48
PVA2	15	40	60	5.28
PVA3	15	30	70	4.61
PVA4	10	50	50	1.20
PVA5	10	40	60	1.07
PVA6	10	30	70	0.43

is derived from its complex structure, where PVA chains and solvent molecules are organized at different hierarchical scales. PVA has a porous structure composed of some pores filled by a polymer-poor phase and highly interconnected regions of a polymer-rich phase that ensure the network scaffolding. The polymer-rich phase is itself organized and consists of small micellar crystalline aggregates of PVA chains and amorphous domains. The PVA chains in the amorphous domains swell by the solvent and connect the fringed micelle-like crystals

(Ricciardi et al., 2004b). The size and amount of crystalline aggregates in PVA hydrogels determine the dimensional stability, the toughness, and strength to external stresses; thus, they play an important role in the performance of these hydrogels. Adding DMSO to the water as the solvent increases the solubility of PVA and induces the formation of the more and smaller crystallites in the PVA gel. On the other hand, the crystallites as physical crosslinking points in the gel can provide higher mechanical property in some extent. Based on a systematic quantitative analysis of the structure of PVA, the degree of crystallinity increases with increasing the PVA concentration (Ricciardi et al., 2004b).

Moreover, it is observed that the PVA hydrogels prepared in a solvent of pure water are translucent, compared to the hydrogels prepared in a solvent composed of dimethyl sulfoxide and water which are transparent. This difference in optical appearance can be due to the difference in size and number of crystallites formed upon crystallization of PVA in aqueous and DMSO/water solutions (Cha et al., 1996; Hyon et al., 1989). Hyon et. al has confirmed this difference demonstrating that the surface of the translucent gel has many irregular large pores while the surface of the transparent gel prepared from the mixed solvent contains very small regular pores distributed densely and homogeneously which will account for the high light transparency (Hyon et al., 1989).

As can be seen in Table 4.2, at a certain DMSO/water ratio of the solvent, the elastic modulus increases by increasing the PVA concentration from 10% to 15%. The same behaviour is observed when the DMSO/water ratio increases at a fixed PVA concentration. Both of these observations can be ascribed to the formation of crystallites and the increase in degree of crystallinity by increasing the PVA and DMSO concentrations.

4.2.3 Chitosan Mechanical Properties

To improve the characteristics of chitosan hydrogels without a complex chemical process, a facile approach of polymer blending is applied in this work to prepare simple yet effective hydrogels as drug carriers. The material of choice for the blend is another natural polymer,

agarose. The elastic modulus based on the linear fit approach was measured for hydrogels at different ratios of chitosan to agarose and listed in Table 4.3. The compressive modulus of these blended hydrogels generally has increased with increasing the agarose concentration, as is consistent with a previous report for agarose gels (Zamora-Mora et al., 2014).

For example, for blended chitosan-agarose hydrogels at weight ratios of 75:25, 50:50 and

Table 4.3: Summary of mechanical properties of chitosan hydrogels

Sample	Agarose (v/v %)	Chitosan (v/v %)	Youngs Modulus (kPa)
Ch100	0	100	-
Ch75Ag25	25	75	65.07
Ch50Ag50	50	50	198.55
Ch25Ag75	75	25	614.29
Ag100	100	0	230.05

25:75 the compressive modulus are 65.07 ± 1.9 , 198.55 ± 11.1 and 614.29 ± 23.7 kPa respectively. Since no gel is formed from a 2 wt% chitosan without addition of agarose, the increasing trend can be attributed to the increased crosslinking densities of the hydrogel blend network due to the increase in agarose content. This increase in the elastic modulus of the composite chitosan/agarose gels is likely to be due to the formation of hydrogen bonding between the chitosan chains and the agarose network (Lake et al., 2011; Ulrich et al., 2010). Considering the mechanical modification of agarose hydrogels, it is observed that the stiffness of the agarose gel is still maintained at lower concentrations of chitosan, although the increase in chitosan content has slightly decreased the elastic modulus of agarose (plain agarose hydrogel: 230.05 kPa; Ch75-Ag25: 65,07 kPa).

To better understand the gelation process of chitosan after addition of agarose, we consider the structural characteristics of chitosan. Two parameters should be provided to obtain a chitosan hydrogel. Since no ordered conformation has ever been observed in chitosan solutions at low concentration, the concentration should be sufficiently high to favour the chain entanglement required for the formation of a continuous gel instead of aggregates (Domard and Rinaudo, 1983).

The second parameter for chitosan hydrogel formation corresponds to the balance between attractive and repulsive interactions along the polymer chain and the ability to favour the

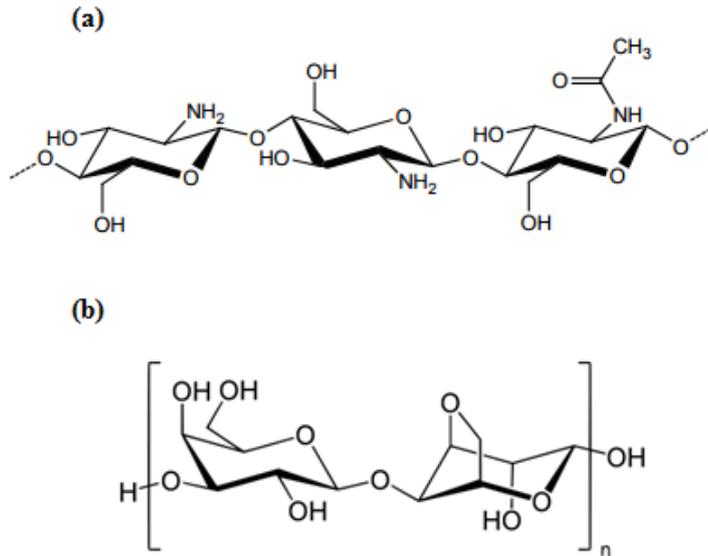


Figure 4.6: Schematic structure of (a) Chitosan (b) Agarose.

formation of junction points by means of ordered domains. This parameter can be decreased by changing the dielectric constant of the media, which controls the ionization state of the amine groups such that it favours the formation of hydrophobic interactions and hydrogen bonding. The latter constitutes the junction points on chains where numerous hydrophilic interactions still exist and contribute to the existence of the chain segments between the junction points (Anthonsen et al., 1994).

Several functional groups present in the structure of chitosan (e.g. alcohol, amine, amide and ether functions) can be involved in the formation of hydrogen bonds (Vachoud et al., 1997) with various substrates such as water or polymers (Roberts, 1992); such as collagen (Taravel and Domard, 1995). In case of collagen, for example, the interaction between the two polymer chains are essentially due to hydrogen bondings (Taravel and Domard, 1995). Hydrogen bonding in the gelation process of chitosan is responsible for the stability of the ordered structure in the crystalline domains and of the interchain interactions which reinforce the stability of these domains (Domard and Domard, 2001).

Chitosan has one primary amino and two free hydroxyl groups for each C6 building unit (Figure 4.6 (a)). This natural polysaccharide is a weak base and is insoluble in water and organic solvents, however, it is soluble in dilute aqueous acidic solution ($pH < 6.5$) (Demarger-Andre

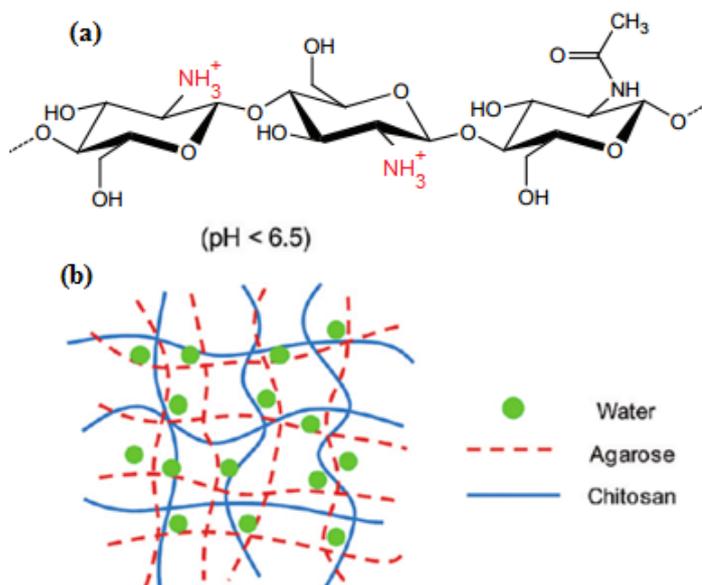


Figure 4.7: Schematic illustration of (a) chitosan structure in acidic pH ($pH < 6.5$), and (b) the blend of chitosan and agarose (Cao et al., 2009).

et al., 1994) and gets precipitated with polyanions and forms a gel at lower pH.

In acidic solutions ($pH < 6.5$) chitosan carries a positive charge because of the presence of free protonated amino groups along the polymer chains (Figure 4.7 (a)). The electrostatic effect among them at this pH enables chitosan to react with many negatively charged surfaces/polymers and allows the formation of ionic cross-linking (Demarger-Andre et al., 1994; Sahoo et al., 2010). In the form of a hydrogel, agarose has a porous structure (Figure 4.6 (b)) while chitosan is linear (Figure 4.6 (a)). In this acidic pH, the blends of chitosan and agarose form a homogeneous polymer blend with extended chitosan chains within the agarose matrix (Figure 4.7 (b)) which displays a clear appearance (Elsabee et al., 2009; Kumirska et al., 2011).

4.3 Thermal analysis: DSC and TGA

Thermal analysis of all hydrogels prepared previously are carried out using DSC and TGA methods. The results are presented and discussed in the following sections.

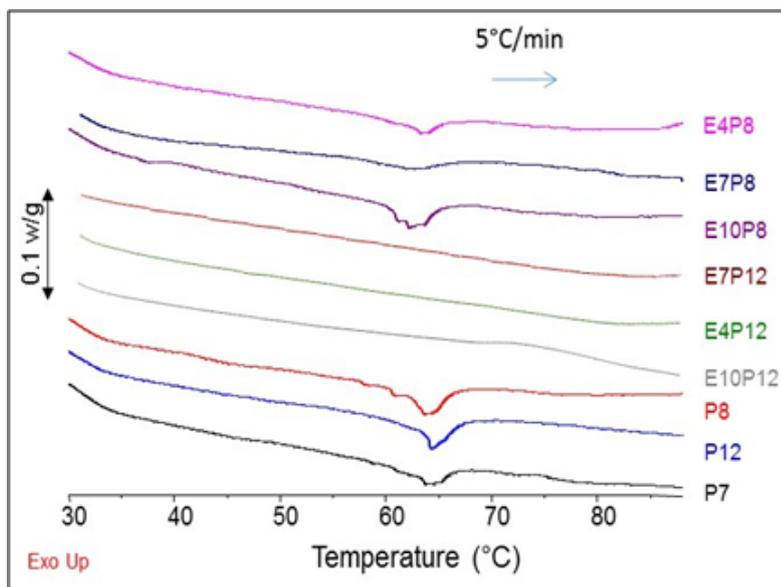


Figure 4.8: Examples of agarose hydrogels DSC thermograms of the crosslinked agarose gels prepared at pH8 (E4P8, E7P8 and E10P8), pH12 (E4P12, E7P12, E10P12) and non-crosslinked agarose gels prepared at pH 7, 8 and 12 (P 7, P 8, P 12).

4.3.1 Agarose Thermal Properties

Figure 4.8 shows the DSC thermograms obtained upon heating (from 30°C to 100°C) of non-crosslinked and crosslinked agarose gels prepared at various pHs.

The non-crosslinked agarose gels (Figure 4.8) exhibit an endothermic event between 56°C and 70°C corresponding to the gel-sol transition of agarose and mentioned herein as melting. This is in agreement with the results found in the literature (Nishinari, 1997). The melting onset temperatures (T_{onset}) shown in Table 4.4 were similar and independent of the pH of preparation for all non-crosslinked samples.

The melting onset temperature of crosslinked hydrogels prepared at pH 8 has shifted to lower values (around 50°C) compared to the T_{onset} of non-crosslinked agarose (56°C) (Figure 4.8, Table 4.4). The T_{onset} shift is similar for all samples crosslinked at pH 8 and independent of the crosslinker concentration. This temperature shift suggests a structural order change of the agarose gel. Since agarose forms a gel by double helix formation and subsequent aggregation of these helices into bundles called suprahelices that determine the stability of the hydrogels, a lower melting onset temperature can be an indication of a lower structure order (i.e a lower

Table 4.4: Summary of thermal, mechanical and swelling results of crosslinked and non-crosslinked agarose hydrogels.

Sample	pH	EDGE (wt%)	$T_{\text{onset}}(^{\circ}\text{C})$	Final weight (%)
E4P8	8	4	50.3	1.88
E7P8	8	7	50.4	1.82
E10P8	8	10	50.7	1.55
E4P12	12	4	-	1.95
E7P12	12	7	-	1.85
E10P12	12	10	-	1.76
P8	8	-	56.9	2.19
P12	12	-	56.5	1.97
P7	7	-	56.9	2.37

content of suprahelices) and consequently lower thermal stability (Ross et al., 2006).

All hydrogels crosslinked at pH 12 showed similar thermograms with no melting. As no melting was observed during these measurements, additional experiments were carried out and these hydrogels were heated to 200°C to check whether the T_{onset} has really shifted to higher values. Even with heating to 200°C the crosslinked hydrogels at pH 12 revealed no melting, suggesting the formation of more thermally stable gels at this pH compared with those hydrogels obtained at pH8 or the non-crosslinked ones. The shift of the endothermic peak to higher temperatures can be attributed to one or both of the following possibilities: (i) the crosslinker added to agarose immobilizes water molecules and the effective free water content in the gel decreases in the presence of the crosslinker, (ii) crosslinker molecules interact directly with agarose molecules and promote the formation and aggregation of helices which play the main role in the network formation.

Indeed, the crosslinked agarose gels prepared at pH 12 showed the most thermal stable gels, followed by the non-crosslinked gels and finally the crosslinked gels prepared at pH8. These results confirm the simultaneous effect of pH and the crosslinker on the gel structure/mechanical behavior.

4.3.2 PVA Thermal Properties

All PVA hydrogels were heated from 30 °C to 250 °C to obtain thermograms. Three endotherms are observed on all PVA hydrogel thermograms, as indicated in Figure 4.9 for a typical PVA sample:

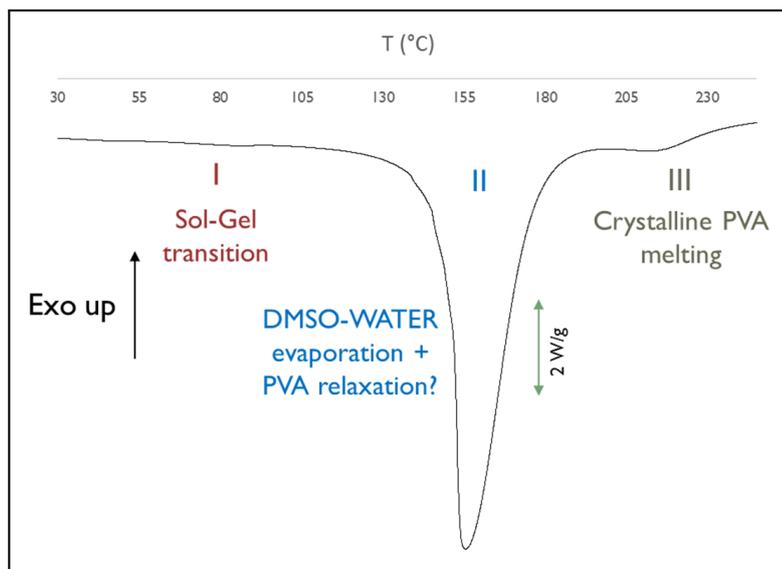


Figure 4.9: Typical PVA DMSO Hydrogels thermogram

- first small endotherm between 57 °C and 90 °C ;
- second large endotherm between 110 °C and 200 °C;
- and, third endotherm between 200 °C and 240 °C.

All 10% PVA hydrogels show a wide endotherm (Figure 4.10(a)) corresponding to the PVA sol- gel transition ranging between 57 °C and 93 °C (Chang et al., 2003).

Among 15% PVA hydrogels, samples with higher contents of DMSO (50 and 40%) show a higher sol-gel transition temperature ranging from 82 to 98 °C (Figure 4.10 (b)). Except 15% PVA hydrogel with 30% DMSO which has a similar behaviour to that of hydrogels with 10% PVA.

The second large endotherm is observed for all samples between 110°C and 200°C corresponding to both solvent evaporation and PVA crystalline domain relaxation (Hassan and

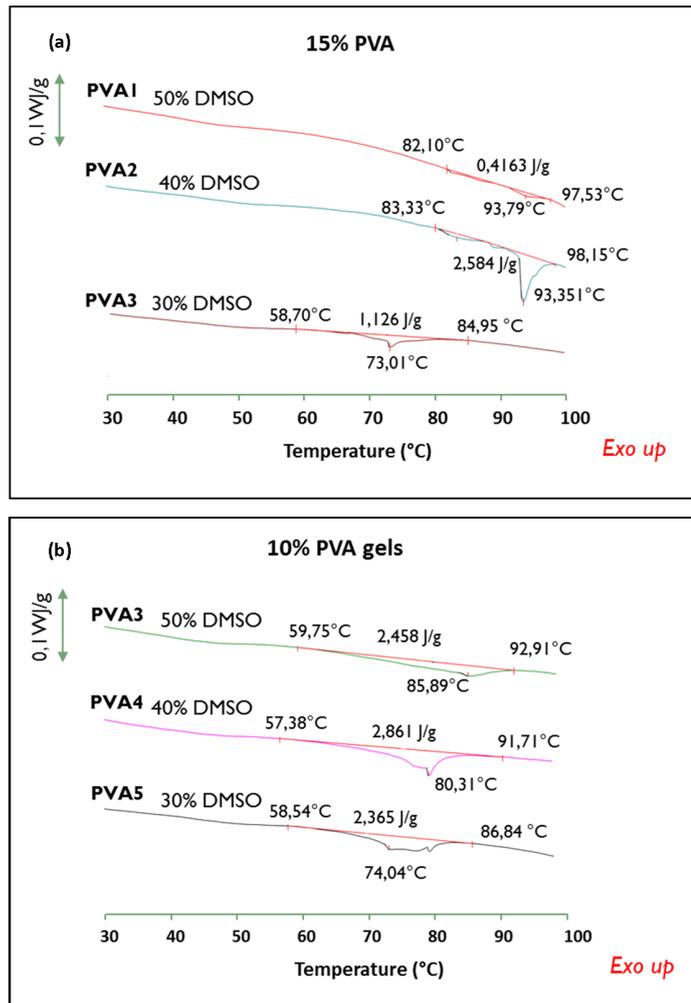


Figure 4.10: Sol-gel transition part of PVA hydrogels DSC thermograms: (a) 15% PVA containing hydrogels; (b) 10% PVA containing hydrogels.

Peppas, 2000).

All PVA hydrogels show a wide endotherm ranging between 187°C and 242°C ((Figure 4.11 (a) and (b)) corresponding to the melting of the PVA crystalline part (Chang et al., 2003). Both 15 and 10% PVA hydrogels exhibit a higher melting enthalpy at higher DMSO contents. Also, the melting enthalpy of 15% PVA hydrogels with 40 and 50 % DMSO contents are higher compared to 10% PVA hydrogels prepared at those DMSO concentrations.

Briefly, hydrogels prepared with high DMSO content (40 and 50%) and high PVA content (15%) show a higher sol-gel temperature and a higher melting enthalpy that according

to the literature can be attributed to a higher crystallinity in the PVA structure (Hassan and Peppas, 2000). These observations are consistent with the results of the mechanical analysis, discussed above, which demonstrate the role of high DMSO and PVA content on the formation of more crystallites in hydrogel network and consequently a stiffer gel.

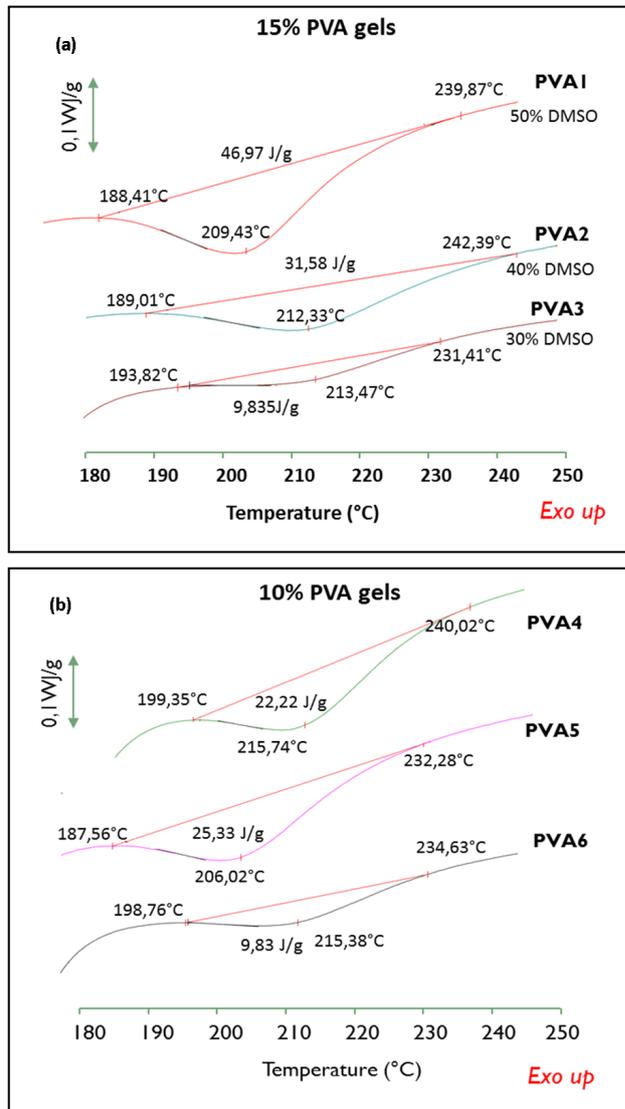


Figure 4.11: Crystalline PVA melting part of PVA hydrogels DSC thermograms: (a) 15% PVA containing hydrogels; (b) 10% PVA containing hydrogels.

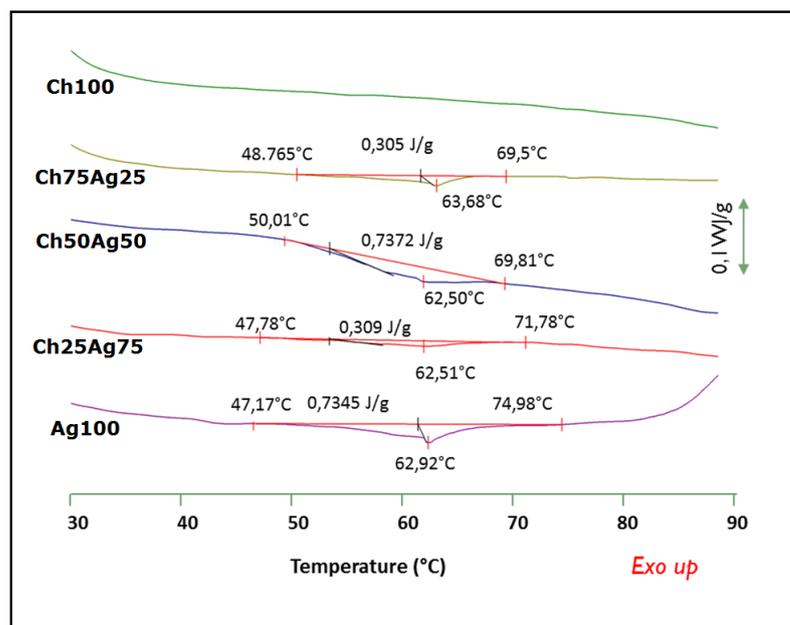


Figure 4.12: DSC thermograms of chitosan-agarose hydrogel blends

4.3.3 Chitosan Thermal Properties

All chitosan-agarose blend hydrogels, except Ch100, show an endotherm between 47 °C and 75 °C (Figure 4.12). Ch100 does not reveal any sol-gel transition since chitosan alone does not form a gel. Ag100 sample prepared with only agarose has a sol-gel transition from 47.17 °C to 74.98 °C. The endotherm observed for gels containing both agarose and chitosan (Ch75-Ag25, Ch50-Ag50, Ch25-Ag75) which corresponds to the agarose sol-gel transition (Fernández et al., 2010; Ross et al., 2006). Ch75-Ag25, Ch50-Ag50 show a similar behaviour with a higher onset temperature (48.7 and 50 °C, respectively) compared to pure agarose gel. The onset temperature of Ch25-Ag75 is 47.8 °C very close to that of pure agarose gel, Ag100.

4.4 Swelling experiments

The water uptake and swelling capacity of hydrogels is another characteristic that represents the differences in the structures of the hydrogels. The swelling results of all hydrogels are presented and discussed in the following sections.

4.4.1 Agarose swelling

It was explained that hydrogels are frequently crosslinked to avoid their solubility in aqueous environments. Additional evidence of agarose crosslinking can be obtained from the swelling behaviour of the hydrogels. Figure 4.13 shows the swelling profile of all agarose samples in PBS (pH 7.4), which provides a measure of the amount of water a material can absorb. All swelling behaviours are plotted as the average of three trials. The hydrogels swelled rapidly and reached equilibrium maximum in 4h (Figure 4.13 presents the first 20 mins of the experiment). The gels can be classified in three distinct groups according to their swelling profiles: non-crosslinked, crosslinked at pH 8 and crosslinked at pH 12.

Due to the hydrophilic nature of the agarose chains, the network is able to absorb wa-

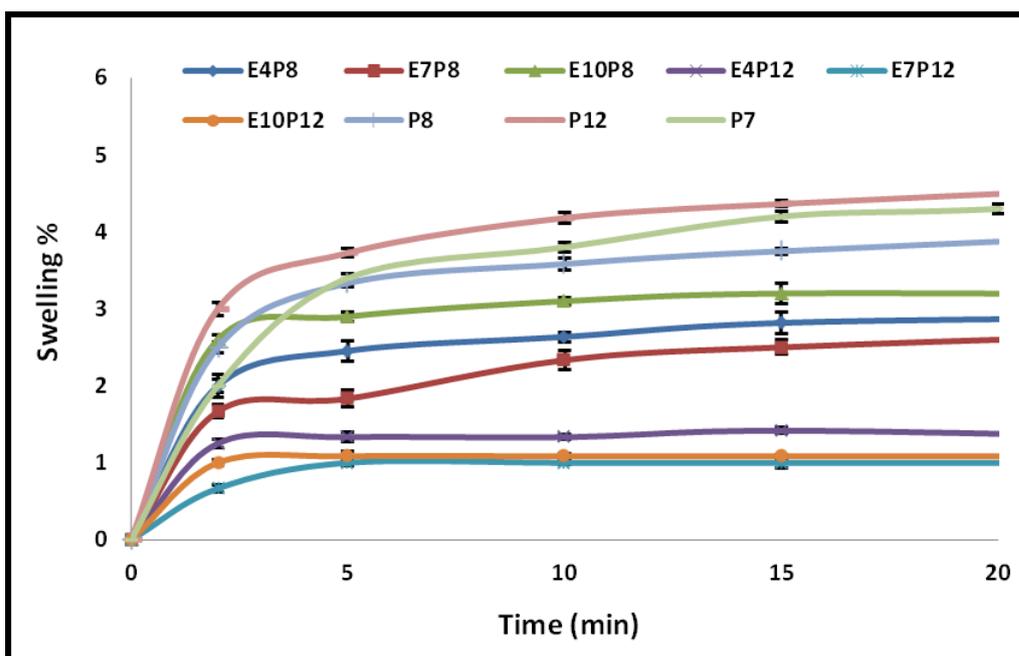


Figure 4.13: Equilibrium swelling ratio of agarose and composite hydrogels as a function of weight ratio incubated in PBS at ambient temperature. (values reported are an average of $n = 3$).

ter from PBS within its structure (Truong et al., 2011). Furthermore, in swelling experiments, water molecules are not only adsorbed into polar sites but also fill in the voids between the chains in the polymer network (Builders et al., 2008). For a better understanding of the swelling behaviour, it is important to review the structure of the agarose hydrogel.

As can be seen in Figure 4.14 (a), agarose takes the form of random coils in solution.

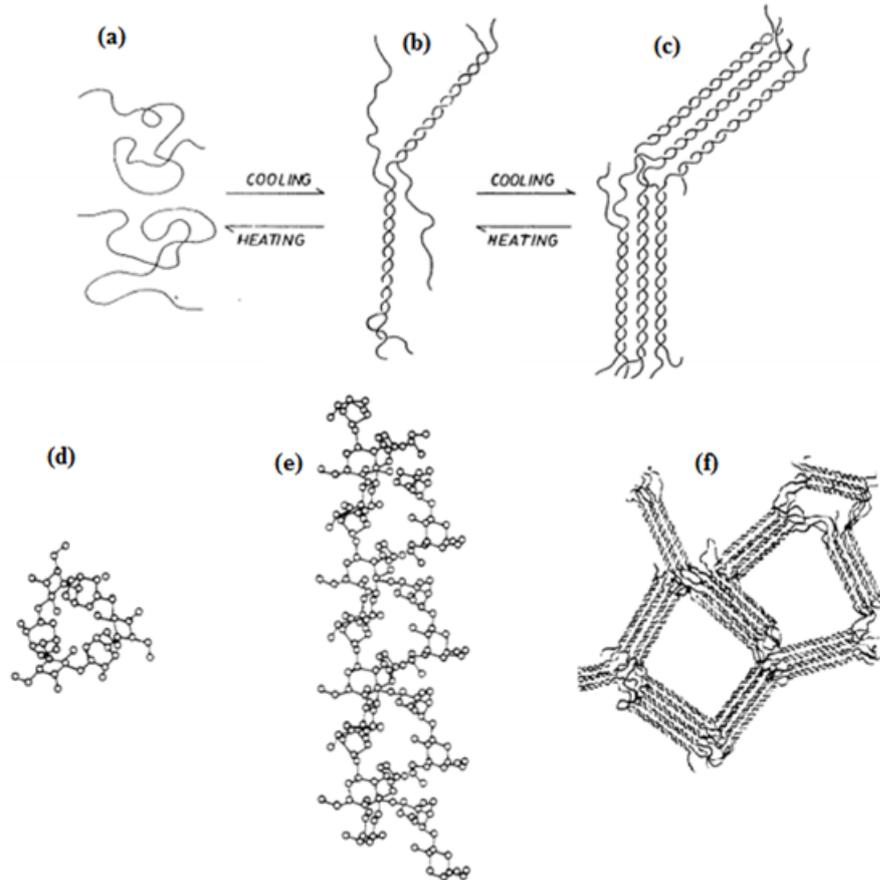


Figure 4.14: Process of gelification in thermoreversible agarose hydrogels: (a) agarose is composed of random coils in solution, (b) random coils join to form double helices, (c) double helices link together to form bundles of doubles (d,e) structure of agarose double helix (f) a schematic of the agarose gel network structure based on Arnot et al. model (Armisen, 1991).

In cooling this solution, random coils are linked to form double helices and an elastic clear agarose gel is obtained (Figure 4.14 (b)). By further cooling, these double helices link together to form bundles of double helices and a turbid hydrogel forms (Armisen, 1991). At junction zones the bundles interact, further forming a three dimensional network (Figure 4.14 (f)). This network has great ability to immobilize water molecules in its interstices (Norziah et al., 2006). Several studies and especially work of (Arnot et al., 1974) has suggested that agarose coil-to-double helix transition is the origin of the gelation process (Norziah et al., 2006), but the agarose gel network is described as arising both by double helix formation and by

subsequent aggregation of these helices into bundles (see also Figure 4.14).

The weak swelling characteristics exhibited by the crosslinked agarose hydrogels could be due to the blocking of the large pores of the agarose polymer network by the monomers of the crosslinker. Thus, the void volume is reduced in the hydrogel and decreasing the movement of water across the porous double helical structure of the agarose gel network (Pang et al., 2007). Another possibility is that the crosslinker molecules interact directly with agarose molecules and increase the formation and aggregation of helices which consequently leads to a denser agarose network formation.

As demonstrated in Figure 4.13, the non-crosslinked hydrogels (P12, P8 and P7) exhibit a

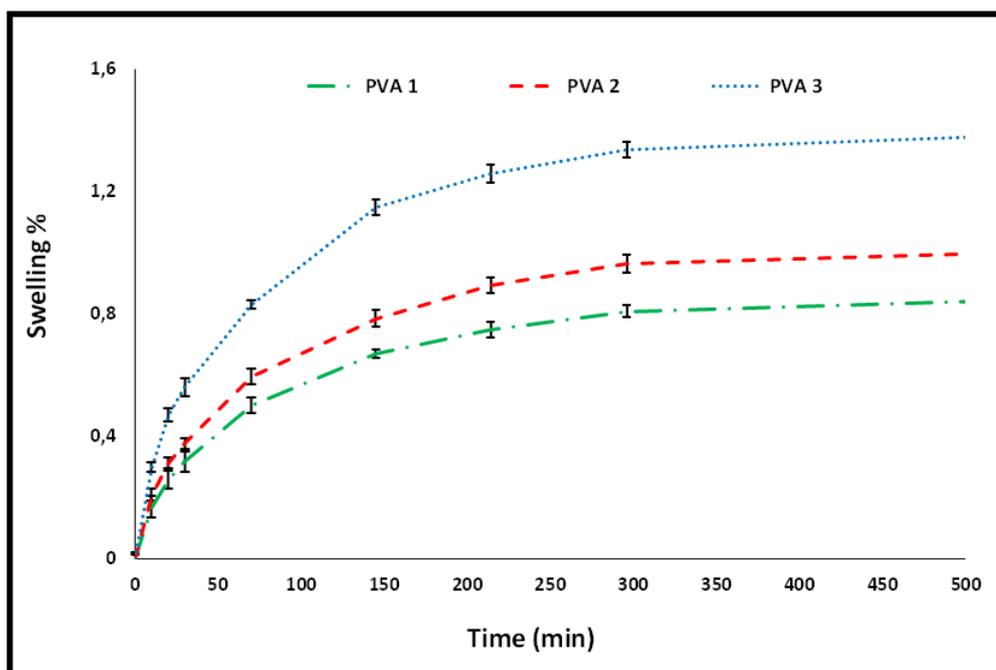


Figure 4.15: Equilibrium swelling ratio of 15 wt% PVA hydrogels (PVA1: DMSO/water 50/50, PVA2: DMSO/water 40/60, PVA3: DMSO/water 30/70) as a function of weight ratio incubated in PBS at ambient temperature. (values reported are an average of $n = 3$)

higher water uptake than the crosslinked ones. Moreover, the hydrogels prepared at pH 12 swell less than the ones prepared at pH 8. For P7, the equilibrium swelling ratio is the highest and the time required to reach equilibrium swelling is the longest. The gels E7P12 and E10P12 had the least amount of swelling. Based on the discussion above, this is due to the formation

of a more compact structured agarose hydrogel in presence of the crosslinker. Moreover, the swelling ratio of crosslinked agarose hydrogels decreases at higher pH, which confirms that the alkaline pH strongly promotes the effect of crosslinker in the agarose structure. At alkaline conditions, the hydroxyl group on the agarose backbone are deprotonated. Consequently, agarose carries negative charges due to its negatively charged oxygens which are highly nucleophilic. This is more significant at higher alkaline pH. Hence, higher alkaline pH favours more the reaction between the epoxide ring and the nucleophilic groups of agarose which then results in the formation of more crosslinks in the agarose network.

4.4.2 PVA swelling

The preparation of PVA hydrogels in mixed aqueous solvents is a way of reducing the molecular mesh size of the gel for drug diffusion, which enables prolonged and controlled drug release (Morita et al., 2000). This consequently decreases the swelling capacity of hydrogels. So, the effect of different DMSO/water ratios was studied on the swelling characteristics of PVA hydrogels. The swelling kinetics of PVA hydrogels were studied over a period of 3 days in PBS and the results are shown in Figures 4.15 and 4.16.

As can be seen in Figure 4.15, increasing the DMSO proportion in the solvent decreases the swelling capacity of 15 wt% PVA hydrogels. For example, the data show that the maximum water uptake of the hydrogel prepared in 50 wt% DMSO is 0.66 of the maximum water uptake of the hydrogel prepared in 30 wt% DMSO. This difference in swelling capacity is greater among the 10 wt% PVA hydrogels (Figure 4.16). These data show that the maximum water uptake of the hydrogel prepared in 50 wt% DMSO is 0.37 of the maximum water uptake of the hydrogel prepared in 30 wt% DMSO (Tanigami et al., 1994). Thus, it can be concluded that by increasing the DMSO/water ratio, the number of crystallites (e. g. junction points or crosslinking) increases and this in turn reduces molecular mesh size and water uptake of the gels.

As demonstrated in Figures 4.15 and 4.16, all PVA hydrogels regardless of the DMSO/water ratio generally show high swelling rates during the initial 4h, a plateau thereafter and finally

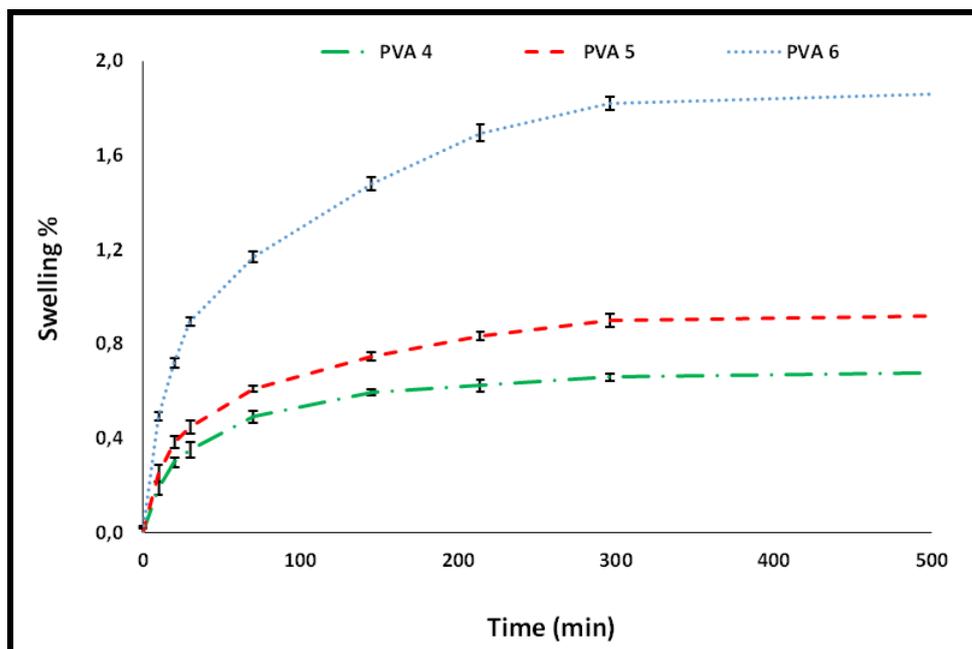


Figure 4.16: Equilibrium swelling ratio of 10 wt% PVA hydrogels (PVA4: DMSO/water 50/50, PVA5: DMSO/water 40/60, PVA6: DMSO/water 30/70) as a function of weight ratio incubated in PBS at ambient temperature. (values reported are an average of $n = 3$)

reaching equilibrium within 3 days (data shown for the initial 10h). The fast swelling kinetics at the beginning and the slowdown within 4h for all these hydrogels can be attributed to the different porosity of the surface and bulk. It has been found that the surface layer is significantly different from the bulk irrespective of the solvent composition. For hydrogels prepared with DMSO as a cosolvent, the three-dimensional fibrillar structure is much porous at the surface than in the bulk. However, a large mesh size is only confined to a thin surface layer (Trieu and Qutubuddin, 1994).

It is observed that at higher concentrations of DMSO (40 wt% and 50 wt%), hydrogels with higher PVA concentration swell more than the ones with lower PVA content. This is due to the fact that as the amount of PVA present in the hydrogels increases, the capacity of hydrogels to imbibe more water also increases, because of the highly hydrophilic nature of the PVA molecules (Lyons et al., 2009).

4.4.3 Chitosan swelling

The swelling kinetics of the chitosan composite hydrogels were studied over a period of 3 days in PBS and the results are shown in Figure 4.17. It was explained in Section 4.2.3 that the chitosan concentration should be sufficiently high so as to favour the chain entanglement required for the formation of a continuous gel. This was found in this study. It was observed that chitosan itself at a 2 wt% cannot form a hydrogel network and thus sample Ch100 could not be used for drug delivery application in this work. Concerning the rest of chitosan composite hydrogels, blending chitosan with agarose has improved the gelation process but the resultant hydrogels continue to dissociate after swelling for a certain time when the concentration of agarose is reduced (i.e. Ch75Ag25 and Ch50Ag50). This dissociation could be partially due to the formation of a relatively loose hydrogel network.

As shown in Figure 4.17, all chitosan blends regardless of chitosan/agarose ratio gen-

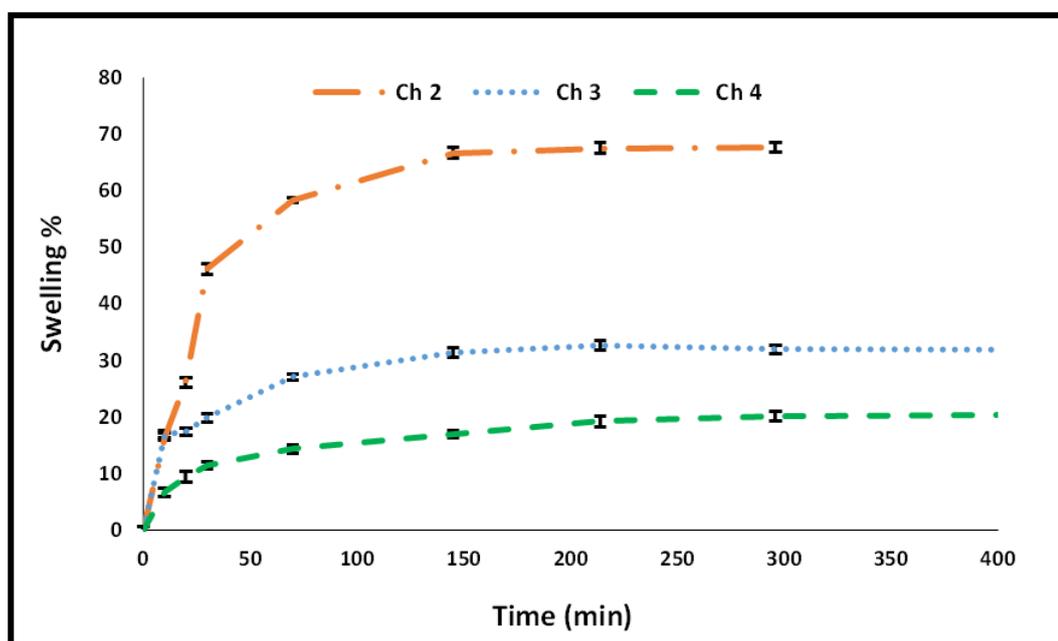


Figure 4.17: Equilibrium swelling ratio of chitosan/agarose composite hydrogels as a function of weight ratio incubated in PBS at ambient temperature. (values reported are an average of $n = 3$)

erally show high swelling rates during the initial 2h, a plateau thereafter and finally reaching equilibrium within 2 days (data shown for the initial 10h). The fast swelling kinetics and high

water retention capability of these hydrogel blends are due to the hydrophilic nature of agarose and especially chitosan. Moreover, the swelling rates of these hydrogel blends are all higher than that of the pure agarose hydrogel and decrease with an increase in agarose content of the hydrogel. This behaviour can be related to the crosslinking density of these chitosan composite hydrogels which affects their swelling capacity, suggesting that the swelling becomes more limited as the crosslinking density increases.

4.5 Comparison of Hydrogel Formulations

Three different series of hydrogels were synthesized from two natural polysaccharides (agarose and chitosan) and a synthetic polymer (PVA), and then characterized as described above. Here, the hydrogels of each series are first compared among themselves and then a comparison among three series of hydrogels is presented.

4.5.1 Agarose

The results reported in previous sections indicate that agarose has some good physical properties that make it an ideal candidate as a carrier for delivery applications. It is soluble in boiling water and forms thermally reversible hydrogels while being cooled below its gelation temperature (31 - 36 °C). The reversible gel-to-sol transition does not occur below the melting point (65 - 85 °C). The large hysteresis between the gelling and melting temperature of the agarose hydrogel makes it a good material for the delivery of protein, peptide and heat sensitive molecules. It is due to the fact that these molecules can be loaded easily into the hydrogel matrix by mixing the drug solution with the agarose solution at a mild temperature slightly above the gelling point of the agarose solution. Being hydrophilic, inert and biocompatible, agarose offers some more advantages for specific delivery purposes. However, in a highly hydrophilic hydrogel such as agarose, the drug releases with a relatively fast rate. Thus, the macromolecular structure of the hydrogel should be modified to slow down drug release. To this end, agarose was crosslinked with EGDE, a bifunctional water-soluble crosslinker for hydroxyl functional polymers.

The crosslinking condition and formulation affects the characteristics of the resultant hydrogel. The characterisations demonstrated that the hydrogels prepared at pH 12 and crosslinked in the presence of EGDE are better candidates for controlled drug delivery applications. The elastic modulus of these crosslinked hydrogels is lower compared to other agarose hydrogels prepared in this study, which indicates better incorporation of crosslinker molecules in agarose polymeric network. This result was confirmed by the limited swelling capacity of these crosslinked gels compared to pure agarose hydrogels and the ones crosslinked at pH 8. Also, the significant difference in melting temperature of crosslinked gels at pH 12 confirms the structural changes and the mesh size reduction in agarose matrix. Although crosslinking agarose allows for better control of drug release, a high degree of crosslinking makes a highly brittle structure. Hence, an optimum degree of crosslinking should be maintained to achieve a relatively strong and yet elastic hydrogel. However, the applicability of the crosslinked hydrogels remains to be verified when coupled with the ultrasound as the external source of energy.

4.5.2 PVA

PVA as a synthetic, biocompatible, and toxicologically safe polymer can be used as a matrix former for sustained release hydrogel drug delivery systems and is well suited for a variety of pharmaceutical applications, including solid, liquid, and semi-solid formulations. In this regard, the polymeric structure of PVA can profoundly affect the drug release. Then, there is a need to adopt a proper preparation protocol and hydrogel formulation that produces a hydrogel with required characteristics.

It is found that high PVA concentration in the formulation could effectively sustain drug/biomolecules release. Incorporation of PVA in microparticles, for example, has significantly decreased the release of bovine serum albumin (Lyons et al., 2009). The same result has been obtained for the release of antibiotics (Lyons et al., 2009). The benefit of such sustained release is that the drugs can be delivered locally with a controlled rate, thus improving therapeutic efficacy due to the modification of drug release by limitation of diffusion (Morita et al., 2000).

As reported by (Hyon et al., 1989), the gelation process using an aqueous solution of DMSO and PVA produces PVA hydrogels with improved mechanical properties as well as optical transparency. The same results were obtained in this study. An increase in PVA concentration and/or the ratio of DMSO in the aqueous cosolvent, improves greatly the mechanical properties of hydrogels. In other words, the number of junction points increases in higher concentrations of PVA and higher DMSO content in the cosolvent. This consequently results in the formation of more crystallites and the increase in degree of crystallinity, which make mechanically stable hydrogel networks.

At low DMSO and PVA concentrations, the PVA solution forms extremely weak and tacky hydrogels which are highly swollen. At the other extreme, at high DMSO and PVA concentrations, the lowest value of equilibrium swelling is observed. Indeed, the water content of PVA hydrogels decreases with increasing DMSO concentration due to the formation of more junction points or crosslinks. This effect is more significant at higher PVA concentrations. The water content also decreases with increasing initial PVA concentration.

Although the mechanical characteristics and water content of PVA has been improved with this method of preparation to be useful for a controlled release, the best formulation remains to be determined when applied in combination with the external energy.

4.5.3 Chitosan

It was explained that chitosan has been the subject of interest for its use as a polymeric drug carrier due to its desirable properties such as low toxicity, biocompatibility, biodegradability and relatively low production cost from abundant natural sources. However, one drawback of using this natural polysaccharide as a drug carrier is its unstable structure that degrades quickly after the gel formation. This becomes even more noticeable when the hydrogel is loaded with drug molecules. Hence, it is important to reinforce the structure of the chitosan hydrogel and to achieve stable polymeric carriers.

As it was stated, to maximize the application of chitosan without a complex chemical process the facile approach of a polymer blend can be adopted. This approach has been taken in this research to prepare simple yet effective drug carriers and stable enough to be used in combination with an external energy source for drug delivery purposes. The material of choice for the blend was another natural polymer, agarose. By changing the concentration of both chitosan and agarose hydrogel, the ability of agarose to enhance the polymeric structure of the chitosan hydrogels was investigated.

It was stated that the chitosan used in this study is only soluble in acidic conditions ($pH < 6.5$) due to the electrostatic effect among the protonated amine groups along the polymer chains. According to (Cao et al., 2009), under this condition, the hydrogels blend forms a homogenous phase with extended chitosan chains within the agarose matrix and retains a transparency (as observed in this study) which suggest the uniform distribution of chitosan in the agarose matrix without noticeable phase separation (Zamora-Mora et al., 2014).

Zamora-Mora et al (2014) examined the nature of the interactions between the chitosan and agarose components in the hydrogel blends of chitosan and agarose using ATR-FTIR. The obtained spectrum suggested intermolecular hydrogen bonding between the chitosan amino groups and agarose hydroxyl groups. In addition, band broadening indicated interaction between polymers (Zamora-Mora et al., 2014).

The structural modification of chitosan caused by adding agarose was reflected in the mechanical analysis of samples. The results of the mechanical analysis revealed that blending chitosan with agarose has greatly improved the stability and mechanical characteristics of chitosan hydrogels. While chitosan itself does not form a hydrogel (as it was observed in this work) and degrades quickly after preparation, the addition of agarose has resulted in the formation of polymeric carriers. The increase in agarose content generally increased the compressive modulus of the blended hydrogels. This is attributed to the hydrogen bonds formed between protonated amino groups of chitosan and hydroxyl groups of agarose.

However, although blending chitosan with agarose has improved the gelation of chitosan but the composite hydrogels obtained at lower concentrations of agarose were not stable enough since they dissociated after swelling for a certain time. Since hydrogen bonding in the gelation process is responsible for the stability of these hydrogel blends, an optimum ratio of chitosan to agarose content must be maintained to ensure the intermolecular interaction and to obtain the desired mechanical characteristics. This ratio corresponds to those of Ch50Ag50 and Ch25Ag75 here.

To conclude this chapter, the three series of hydrogels synthesised and applied during this work are compared. As demonstrated in Figure 4.18, the fastest swelling rates and the higher water uptakes were found for chitosan hydrogels regardless of their agarose content. These hydrogels swelled 20 to 70 times more than other hydrogels prepared in this study. This means although polymer blending has modified the structure of chitosan hydrogels leading to more junction points and crosslinks in polymer blends compared to pure chitosan (has been discussed above), the chitosan hydrogels still have looser polymeric networks compared to other hydrogels from PVA and agarose.

The least swelling corresponds to PVA hydrogels and crosslinked agarose hydrogel (agarose-min). This demonstrates that the crosslinking of agarose has been successfully carried out, since the pure agarose with a higher swelling capacity (agarose-max) is modified such that its water uptake is on the order of highly crystallite containing PVA hydrogels.

Regarding the mechanical stability of three series of hydrogels, PVA hydrogels demonstrated higher elastic modulus, followed by agarose hydrogels generally and then chitosan blend hydrogels with overall least elastic modulus. Hence, drug release is expected to be slowest from PVA hydrogels and fastest from chitosan blends. This remains to be confirmed in the next chapters.

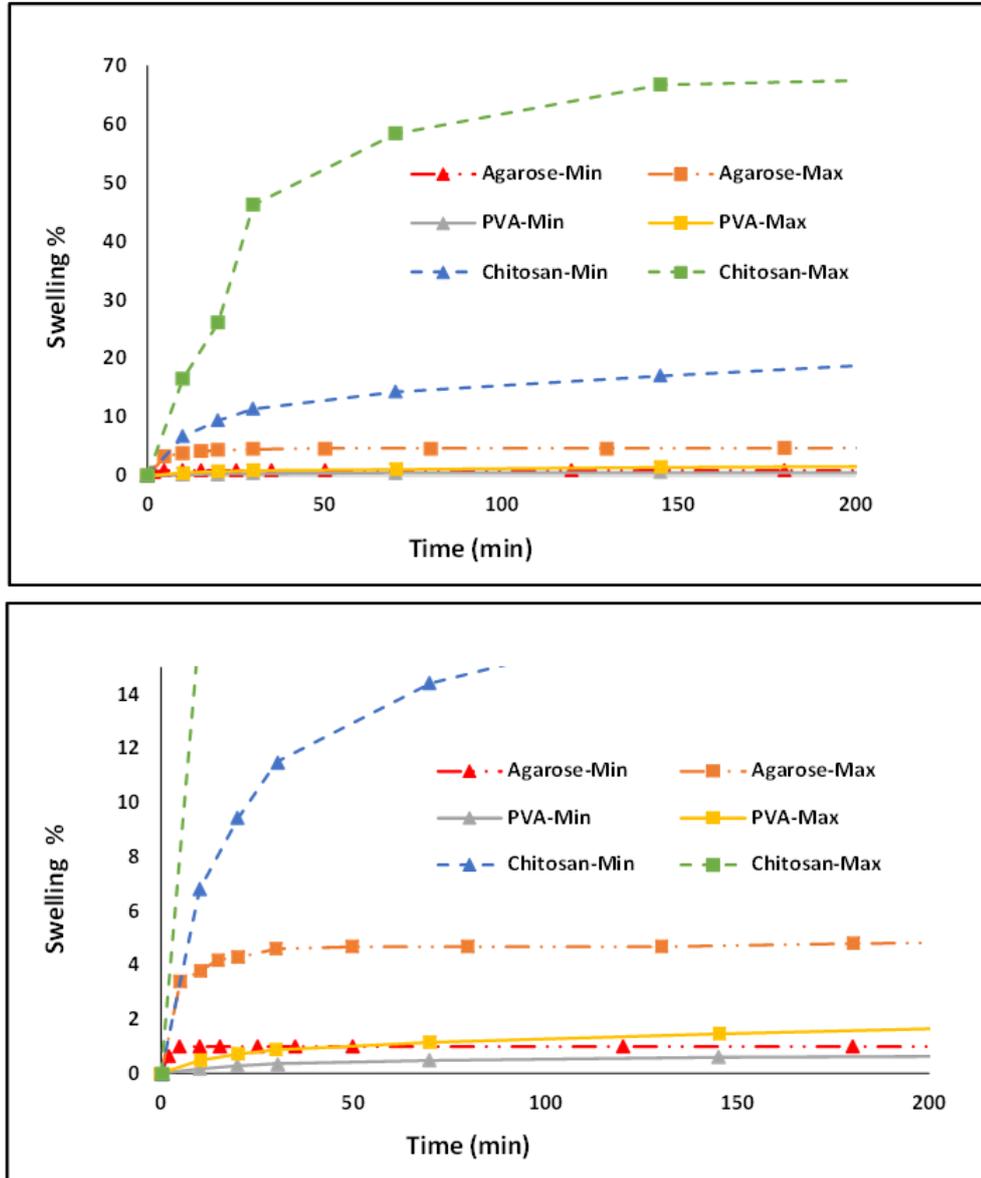


Figure 4.18: Maximum and minimum equilibrium swelling ratio of three sets of hydrogels (agarose, PVA, chitosan) as a function of weight ratio incubated in PBS at ambient temperature (values reported are an average of $n = 3$) (Agarose-min: 2wt% agarose, 10wt% EGDE; Agarose-Max: 2wt% agarose, 0wt% EGDE; PVA-min: 10wt% PVA, 50wt% DMSO/ 50wt% water; PVA-max: 10wt% PVA, 30wt% DMSO/ 70wt% water; Chitosan-min: 0.5wt% chitosan, 1.5wt% agarose; Chitosan-min: 1.5wt% chitosan, 0.5wt% agarose).

4.6 Summary

Three series of hydrogels were synthesized from agarose, PVA and chitosan. It was found that the agarose hydrogels are visually much different. These observations were confirmed by quantification with UV-Vis spectrophotometry and then other characterizations methods. PVA hydrogels also revealed different transparencies which were discussed. No gel was obtained by dissolving 2 wt% chitosan in 1% acetic acid, but adding agarose helped the formation of chitosan polymer blends. It was concluded that the presence of a second polymer is essential for the gel formation of 2 wt% chitosan. The chitosan polymer blends were also visually different due to the modifications in their structures.

Considering these significant differences in appearance of these hydrogels, they needed to be characterized to know about their structures so that the decision can be made for their further applications. Dynamic mechanical analysis of hydrogels was carried out to study the mechanical properties of all samples. PVA was recognized as the most mechanically stable hydrogel, with the elastic modulus in MPa range, followed by agarose and chitosan, with elastic modulus in the order of kPa.

Thermal analysis showed structural differences in each series of hydrogels. The shifts in the melting points and the reasons for these changes were thoroughly discussed.

The swelling behaviour of the hydrogels was another characteristic studied here. As it was expected, chitosan revealed the fastest swelling rates and higher water uptakes.

The next step is using these hydrogels in the drug delivery system applied in this work to assess how different polymeric structures affect their performance as drug carrier.

Chapter 5

Design of Delivery System and Drug Release Experiments

Introduction

In this chapter, the drug delivery system used throughout the experiments and its characteristics are given in Section 5.1. The experiments performed to characterize the energies that will act on hydrogels and drug carriers are explained here. The measurement set-up used for the detection of the amount of the drug release is described in Section 5.2. Section 5.3 discusses the experiments that are designed to evaluate the efficiency of different hydrogel formulations for drug delivery applications. That set of experiments is performed with the ultrasound generator turned off. Section 5.4 explains the experiments carried out to assess the effect of ultrasound on release. Finally, Section 5.5 provides the summary of this chapter.

5.1 Drug Delivery System

The DDS developed for the thesis utilises externally applied ultrasound to provide the stimulus for drug release. Low-frequency ultrasound has been shown to enhance the transport of drugs. The source of this low-frequency ultrasound for our DDS is an ultrasonic device used by dentists to clean teeth, which typically operate at frequencies between 25 and 40 kHz. The



Figure 5.1: Dental scaler used as the sonicator in this research.

dental ultrasonic scaler (Guilin Woodpecker, UDS-K) pictured in Figure 5.1, operating at a planned frequency of $28\text{kHz} \pm 3\text{kHz}$, is used in the following experiments to generate ultrasound. The ultrasonic transducer is enclosed in the handpiece. The end of the handpiece has a screwthread to enable different scaling tips to be attached. For the delivery system described in this thesis, the screwthread was used to attach custom-made delivery tip-cones. A torque wrench was used to tightly screw the sonicator tip-cones and to control the strength of the tip-cone installation.

An optimal selection of ultrasound parameters, such as frequency, intensity, as well as of non-ultrasonic parameters, such as transducer configuration, tip-cone shape and drug concentration, were made to ensure a reproducible, safe and efficacious drug release. This small benchtop sonicator is already designed for easy application and manipulation, which enabled an easy adaptation to our DDS. In addition, these devices could be potentially combined with sensors or another instrument that can monitor drug concentrations to formulate a self-controlled drug (insulin, for example) delivery device that can potentially eliminate the attention required by the patient.

A variety of drugs and bioactive agents, such as proteins, peptides, nucleic acid molecules, genes encoding therapeutic proteins, synthetic organic and inorganic molecules, can be potentially administered by this system. The drug should be typically administered in an appropriate pharmaceutically acceptable carrier having an absorption coefficient similar to water, such as

hydrogels. The site of drug administration should be selected based on convenience to the patient as well as maximum drug penetration.

5.1.1 Ultrasound Intensity Measurement

The ultrasound intensity is one of the parameters that potentially affect the drug release, since an increase in intensity would increase the external force delivered to the drug carrier. To understand the importance of intensity, it should be considered that the ultrasound was used as the external stimulus for the drug release from the hydrogel. The intensity of the oscillating source of ultrasound provides the mechanical energy that is transferred to the hydrogel drug carrier. The energy produced by the ultrasound transducer is imparted to the hydrogel carrier because the tip-cone is in intimate contact with the hydrogel carrier. The intensity of the ultrasound is a measure of the energy imparted to the hydrogel carrier to provide the external stimulation for the drug release.

The ultrasonic scaler device (Figure 5.1) provides a control over the power delivered to the ultrasound transducer in the handpiece of the device. However, this may not necessarily be the amount of energy that is actually delivered to the drug carrier or the recipient tissue. Hence, we measured the energy delivered to the drug carrier with a commonly used calorimetric method (Mitragotri et al., 1995). The measure of applied energy are based on the change of temperature in water exposed to the sonicator (Tezel et al., 2002).

A plastic beaker, thermally insulated with expanded polystyrene, was filled with 40 ml water. The ultrasound tip, corresponding to the position of the drug carrier, was immersed into water and activated for 5 min at various intensities indicated on the sonicator dial. The rate of increase in water temperature was measured using a type N thermocouple (TC S.A., France) with a sensitivity of $39 \mu\text{V}/^\circ\text{C}$ at 900°C .

To make sure that the sonicator casing did not transfer heat to water, the casing temperature was measured during the intensity measurement. No detectable change in its temperature was

observed. Water temperature in the close vicinity of the tip (within 1 mm) was also measured to check whether local heat was generated by the tip. The temperature difference between water close to the tip and the bulk water was negligible. The equation below is then used to calculate the intensity:

$$I = \frac{M_{\text{water}} C_{\text{p,water}} \Delta T}{A \Delta t}$$

where I is the ultrasound intensity (W/cm^2), M_{water} is the mass of water exposed (g), $C_{\text{p,water}}$ is the specific heat of water ($4.18 J/(g \cdot ^\circ C)$), and A is the effective area of the transducer. $\Delta T/\Delta t$ is the rate of temperature change of the water. To use this equation, we assume that the temperature changes of water exposed to the sonicator is due to incident acoustic power, and the acoustic power is completely converted into heat.

5.1.2 Measurement of the Actual Frequency of the Ultrasonic Transducer

Although the frequency generated by the ultrasonic device is designed to be in the range of $28\text{kHz} \pm 3\text{kHz}$, that operating frequency required verification to ensure that every parameter is under control for the DDS. To measure the actual frequency, Polyvinylidene fluoride (PVDF) was used as a sensor for the ultrasound since it is a piezoelectric polymer with high sensitivity. PVDF was in direct contact with the transducer tip, and was also connected to an oscilloscope (Tektronix) to detect and record the frequency at the top corresponding to the position of the drug carrier. Ultrasonic waves are generated by the sonicator which are then transferred to the tip. PVDF in contact with the tip oscillates in resonance with the tip. Since the PVDF has high sensitivity, the frequency of these oscillations recorded by the oscillator provide the measurement of the operating frequency of the sonicator.

5.1.3 Vibration Measurement

We assume that in our drug delivery device, the energy imparted by the longitudinal ultrasonic waves is solely responsible for the changes in the polymeric carrier network and consequently for the release of the active agent particles from polymer matrix. An alternative hypothesis

is that the ultrasound simply causes the lateral displacement of the tip, due to the vibration caused by the ultrasonic waves, and this vibration is simply the stimulus for the drug release from the hydrogel.

To verify this hypothesis, the vibration of the transducer with and without tip was measured using a vibrometer (Keyence, LK-G3000) when ultrasound waves were generated by the sonicator. The vibrometer could measure the displacement at an accuracy of $0.05 \mu\text{m}$ and a linearity of 0.03% F.S. which means that the vibrometer is very sensitive to the vibration.

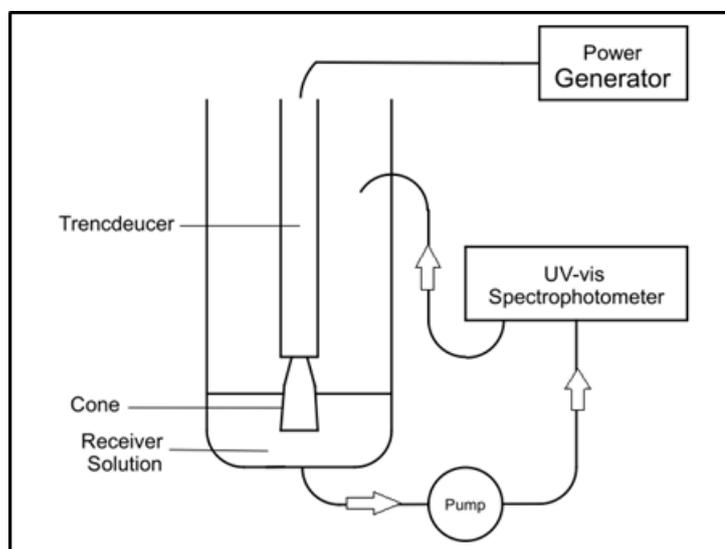


Figure 5.2: Experimental arrangement for detection of the concentration of the drug released under ultrasonic exposure.

5.2 Drug Release Measurement Set-up

The sonicator used to generate the ultrasonic waves was described above. In order to measure the drug release in real-time and investigate the effect of ultrasound on drug release, an experimental set-up (Figure 5.2) was designed that comprised of a custom-made ultrasonic exposure chamber, a spectrophotometer and a peristaltic pump. The thin plastic tubing is used to connect different parts of the set-up and to form a circuit.

The power generator supplies the required electrical energy to the sonicator. The electri-

cal energy is converted to ultrasound waves by the transducer. Since ultrasonic waves are longitudinal mechanical waves, we can say that the transducer converts the electrical energy to mechanical energy. The transducer has a cone shape tip screwed on it, which holds a solid hydrogel containing active agent particles. The generated ultrasonic waves are transferred to this tip, which is then passed to the hydrogel and the encapsulated particles.

As there is no other energy acting on the hydrogel (see following sections for data) and the tip does not vibrate, we can imagine that only the longitudinal ultrasonic waves act on the polymeric network. This is analogous to what happens in a ratchet device, which has a round gear (see Figure 5.3b) or linear rack with teeth that allows continuous linear or rotary motion in only one direction while preventing motion in the opposite direction by a spring-loaded finger, called a pawl (Figure 5.3a).

The polymer chains play the role of the teeth while the ultrasonic waves act like a pawl. This only allows the drug particles to move towards the receiver solution in the direction of the longitudinal waves. Hence, once the power is on, the particles move through the hydrogel (like travelling step by step) until they finally move out of the tip into the receiving solution. The sonicator tip is submerged 1 cm in the receiver solution as shown in Figure 5.2. So, the particles enter the receiver solution which flows through a peristaltic pump to the detector. Indeed, a peristaltic pump maintains a flow of receiver solution coming out of the exposure chamber, passing the detector and returning to the ultrasonic exposure chamber. Since the

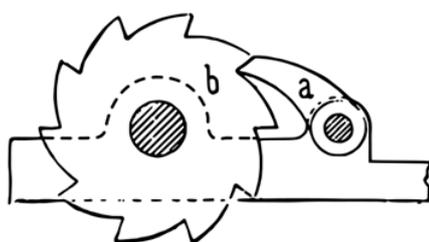


Figure 5.3: Schematic of a ratchet device: a) pawl b)gear.

turbidity of the receiver solution changes as the particles are released into it, measuring the

turbidity of the solution can quantify the concentration of the particles in the solution, which is then related to the amount of the active agent released. A UV-Vis spectrophotometer (Jenway 6305 UV/Visible Spectrophotometer, Genova) was used to measure the turbidity of the receiver solution. The active agent used throughout this research is Theophylline, a methylxanthine drug used in therapy for respiratory diseases, which absorbs UV at 272 nm. Thus, the measurements are made at this wavelength. The spectrophotometer is calibrated with distilled water before each run at the same wavelength. Also, the tubing is washed several times before each run with distilled water until the spectrophotometer shows a zero absorbance.

It was mentioned that the tip screwed into the transducer has a cone shape (Figure 5.4). Three different cones in size are used to cast and retain the hydrogels inside. These cones are made of stainless steel.

5.2.1 Heat generation and its Effects on hydrogel

Molecules and atoms vibrate continuously except at absolute zero. An applied external force causes them to vibrate more rapidly, which increases the friction between the molecules and atoms. This frictional energy is then dissipated in the form of heat. In our delivery system, ultrasonic energy provides that external energy on hydrogel chains and active agent particles which augments the total friction inside the polymeric network and consequently would cause a temperature rise. The delivery system continues to function properly as long as the structure is maintained. The problem is when the hydrogel melts and the physical structure is lost. So, the hydrogel does not work any more like a ratchet (Figure 5.3) when it melts.

The amount of heat produced depends on the intensity of the ultrasound, the time of exposure, and the mode of the application of the ultrasound while the consequences depend mostly on the thermal characteristics of the hydrogel. Increasing the intensity increases the energy of the waves and the energy transferred to the medium which means more vibration and more friction. This can augment the amount of the heat produced by the absorption of ultrasound waves when applying a higher intensity. The same effect is expected for the

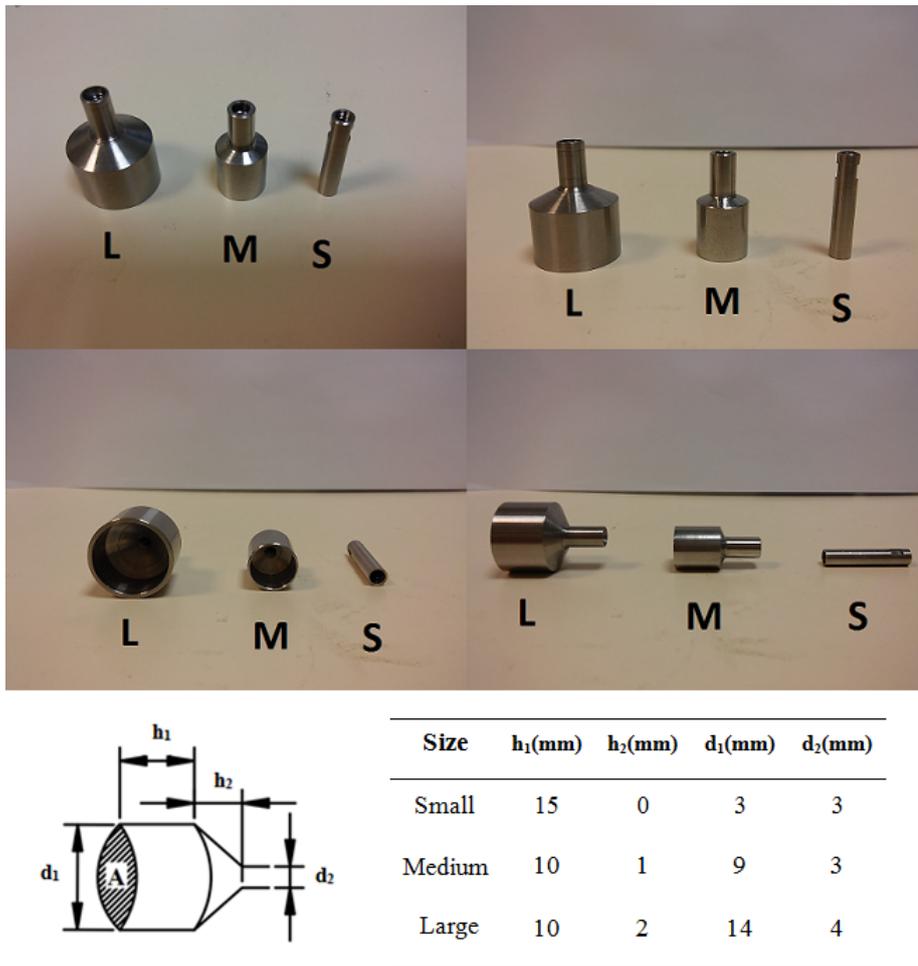


Figure 5.4: Cone shape tips in different sizes that screw into the transducer. L=Large, M=Medium, S=Small

exposure time. If the particles in the medium absorb an amount of energy for a longer duration, the total amount of the energy absorbed and consequently the total friction increase. Such an increase in temperature would eventually lead to melting of the gel in the cone-tip.

To investigate this effect of the heat generated by the ultrasonic waves on the hydrogels, the following parameters were varied: time of exposure, ultrasound intensity and hydrogel formulation. The effect of these parameters were measured as the time required to observe the melting of the gel. For that purpose, a thin metal wire was inserted into the cone and removed after a few seconds. If the gel was melted, a thin viscous layer of hydrogel sol could be observed coating the surface of the wire.

5.2.2 Effect of tip size on drug release

A possible hypothesis is that apart from the mode of ultrasound application, its intensity and the time of exposure, the shape and size of the tip also affects the drug release. So, prior to any experiment, a set of release experiments were performed to select the optimum tip. Since agarose gel prepared in distilled water is a physical gel with a non-complex structure, a 2wt% agarose gel is used in these experiments to entrap the active agent particles inside. For each of the tips, the experiment is repeated with a high and a low ultrasound intensity.

5.2.3 Effect of gravity on drug release

As it was stated earlier, we assume that the only energy in this delivery system acting on hydrogel and active agent particles is ultrasound. Since the ultrasound transducer (hand piece) is mounted vertically and the tip faces downward in the setup (Figure 5.2), it is possible that the gravitational energy also amplifies the release. To measure any effect of gravity on the rate and final amount of drug released from hydrogels, a set of release experiments were carried out with the ultrasound hand piece turned upward. In other words, the handpiece was still mounted vertically but the tip faced upwards. In these experiments, the best tip selected previously is used. The release experiments were carried out at three different intensities (high, medium, low) during 5 minutes. The results were then compared with those measured with the hand piece mounted with the tip facing downwards.

5.3 Non-stimulated Drug Release

To investigate the effect of ultrasound on drug release, as a control we first need to know the release profile from polymeric carriers when the ultrasound generator is off.

5.3.1 Release from gels that do not contain drug and without ULS application

As a further control for the ULS-stimulated experiments, we must ensure that any change in the turbidity detected by the spectrophotometer originates only from the drug released into

the receiver solution. Thus, a set of release experiments are carried out with various hydrogel formulations without active agent particles inside, to check if any traces of polymer diffuse into the receiver solution in contact with polymer. Hydrogel solution was poured into the tip and the gel was directly casted inside. Once the gel was set, the tip was screwed into the transducer which became in contact with the receiver solution as indicated in Figure 5.4. The absorption is measured at 272 nm.

5.3.2 Release from gels containing drug without ULS application

Hydrogels are structurally different which means that they control the rate of drug release differently. Evaluating the amount of drug released from each polymeric network in absence of the ultrasound allows a direct comparison for controlled released application. The drug (theophylline) is loaded into the hydrogel by mixing the hydrogel sol and the drug in solution. This mixture is well stirred to obtain a homogenous solution which is then poured into the tip. Once the gel was set, we performed the experiment as described in Section 5.3.1. A standard curve for Theophylline concentration vs. turbidity is prepared separately using the UV-Vis spectrophotometer.

5.4 Stimulated Drug Release

These experiments were designed to assess the efficiency of ultrasound as the external energy for this delivery system and to see how effectively it controls the drug release.

5.4.1 Release from gels with ULS application

The ultrasound energy affects both the hydrogel network and drug molecules. If the hydrogel traces also diffuse into the receiver solution, for example because of polymeric chain degradation due to ultrasonic exposure, the absorbance measured by the spectrophotometer may be artifactually higher. This hypothesis was tested with the set of control experiments described in Sections 5.3.1 and 5.3.2.

The effect of different ultrasound intensities at continuous mode on the release of theophylline was investigated here. In these release experiments, ultrasound was applied from the initiation of the experiments until no change in turbidity was observed. Each sonication protocol was run 5 times.

5.5 Summary

In this chapter, the ultrasonic delivery system used in this research, the set-up and all its components were described. The delivery system is composed of a power generator, a transducer with cone shaped tips in three sizes, a peristaltic pump for the maintenance of the flow, and a detector which is a UV-Vis spectrophotometer. The mechanism of the release and the method of the quantification of the drug released were discussed. Various parameters that might affect the rate and profile of release were introduced and relevant experiments to assess them were explained. Different sets of experiments that were performed to evaluate the effects of ultrasound waves and different drug carriers used in this study on drug release, are summarized below (Table 5.1):

Table 5.1: Different sets of experiments performed to evaluate the effects of ultrasound and drug carriers on release.

Experiment	Ultrasound	Hydrogel	Active agent	To evaluate
1	×	✓	×	Hydrogel "leakage" to the release media
2	×	✓	✓	Amount of release in absence of ultrasound
3	✓	✓	×	Hydrogel "leakage" caused by ultrasound energy
4	✓	✓	✓	Amount of release stimulated by ultrasound

Chapter 6

Drug Delivery Results and Discussion

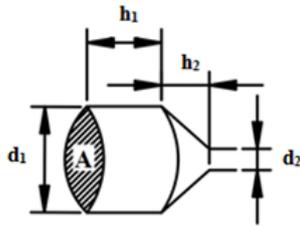
Introduction

In this chapter, the results of various measurements carried out to assess the performance of the ultrasound generator are presented in Section 6.1. The next three sections present the data obtained from drug release experiments respectively for agarose, PVA and chitosan hydrogels. Section 6.5 compares the release behaviour of the three sets of hydrogels with regard to their intrinsic characteristics. Finally, Section 6.6 provides the summary of this chapter.

6.1 Drug Delivery System

As it was explained in Section 5.1.1, a calorimetric method was used to measure the ultrasound intensity. For the ultrasound conditions used in this study, intensities were determined to be in the range of 0.4 - 1.5 (W/cm^2). The measured intensity was in the range that is known to be safe and also required for therapeutic applications (see Section 2.2.3). This range is controlled by the intensity adjustment on the sonicator (see Figure 5.1). It was mentioned that ultrasound cone shape tips in three sizes were used for the drug release experiments. A schematic of these cones with their dimensions are provided in Figure 6.1.

The actual frequency generated by the sonicator was measured as described in Section 5.1.2. Measurements were carried out on three cones with and without hydrogels inside and with



Size	h_1 (mm)	h_2 (mm)	d_1 (mm)	d_2 (mm)
Small	15	0	3	3
Medium	10	1	9	3
Large	10	2	14	4

Figure 6.1: Schematic of the ultrasound tip; 3 cone shape tips and their dimensions. Photographs of the actual cone tips are shown in Figure 5.4

the highest and the lowest powers. It revealed that neither the presence of the hydrogel nor the size of the cone affect the centre frequency, which is 27.93 ± 1.47 kHz. However, there is a difference in the harmonics of the centre frequency. The frequency profiles recorded for large/medium/small cones with/without hydrogel and on minimum/maximum intensity are presented in Figures (6.2-6.4).

The harmonics of the center frequency can be seen in Figures (6.2-6.4). It is important to have harmonics because it helps to have directed motion of particles from the hydrogel. The harmonics increase for a higher intensity with the smaller cone. This may in fact be due to the constructive interference of the ultrasound waves within the cone tip. To clarify, interference is a phenomenon in which two traveling waves that exist in the same medium superpose to form a resultant wave of greater, lower, or the same amplitude, which is equal to the vector sum of the amplitudes of the individual waves. If a crest of a wave meets a crest of another wave of the same frequency at the same point, then the amplitude is the sum of the individual amplitudes; known as constructive interference. If a crest of one wave meets a trough of another wave, then the amplitude is equal to the difference in the individual amplitudes; known as destructive interference.

Reducing the cone size from large to medium, there is a slight improvement in the harmonics. In the small cone, there are very strong harmonics. We can conclude that with the small cone there is an optimization of the ultrasound harmonics to better assist the motion of drug particles. It is notable that at a higher intensity, no matter which cone tip size is utilised, there is a doubling of the harmonics. This can be noticed by comparing Figures 6.2(a)-(b),

6.3(a)-(b), and, 6.4(a)-(b). In comparison, with both the medium and large cones it seems that there is destructive interference within the cone tip, since there are fewer harmonics and no change with intensity. It can be concluded that the prevalence of destructive interference is induced by the design of the cone tip. The practical effect on the drug release is that the destructive interference induced by the medium and the large cones is likely to minimise the amount of ultrasound energy imparted to the hydrogel drug carrier. That is, the small cone the small cone size optimizes the production of harmonics and is expected to be most effective for drug release.

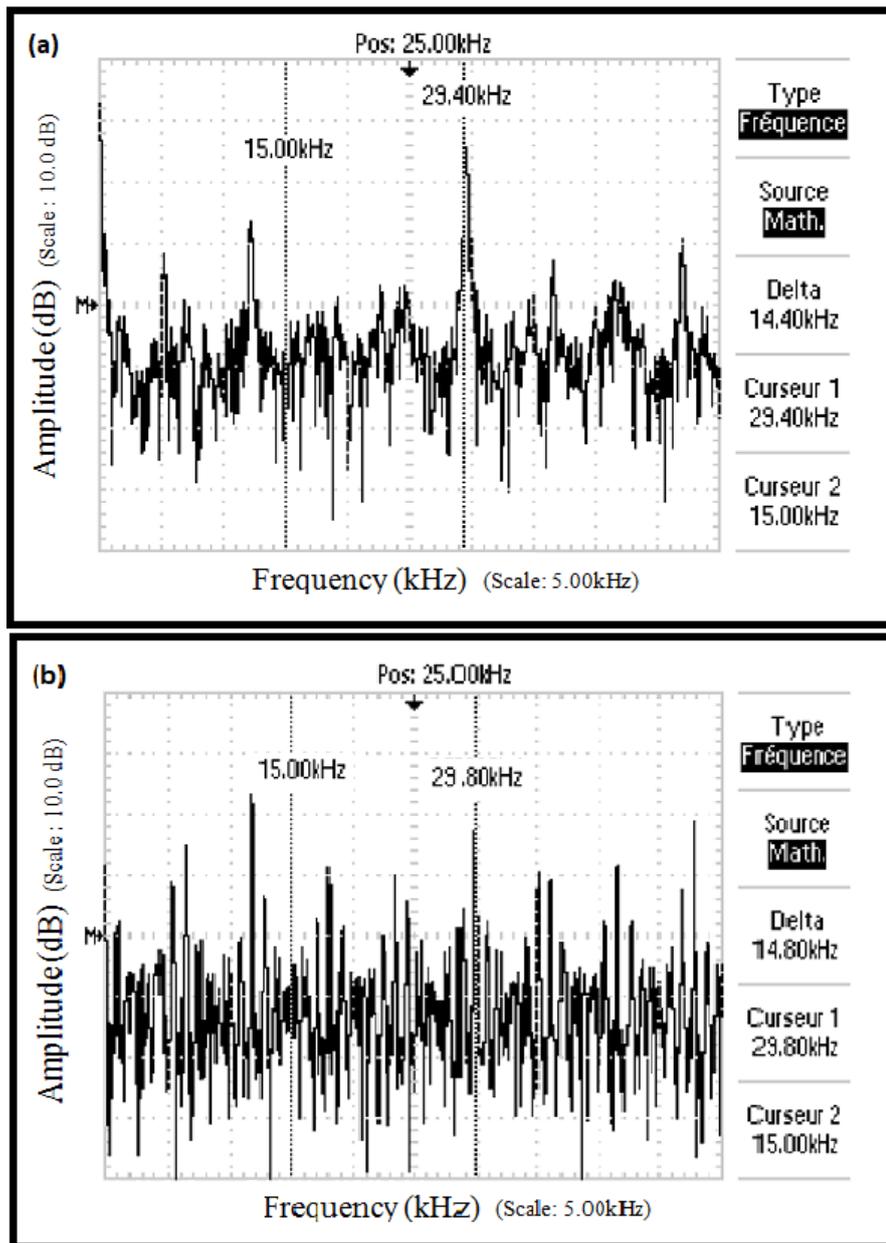


Figure 6.2: Frequency measurement using: large sized cone, without gel, (a) on minimum power, (b) on maximum power.

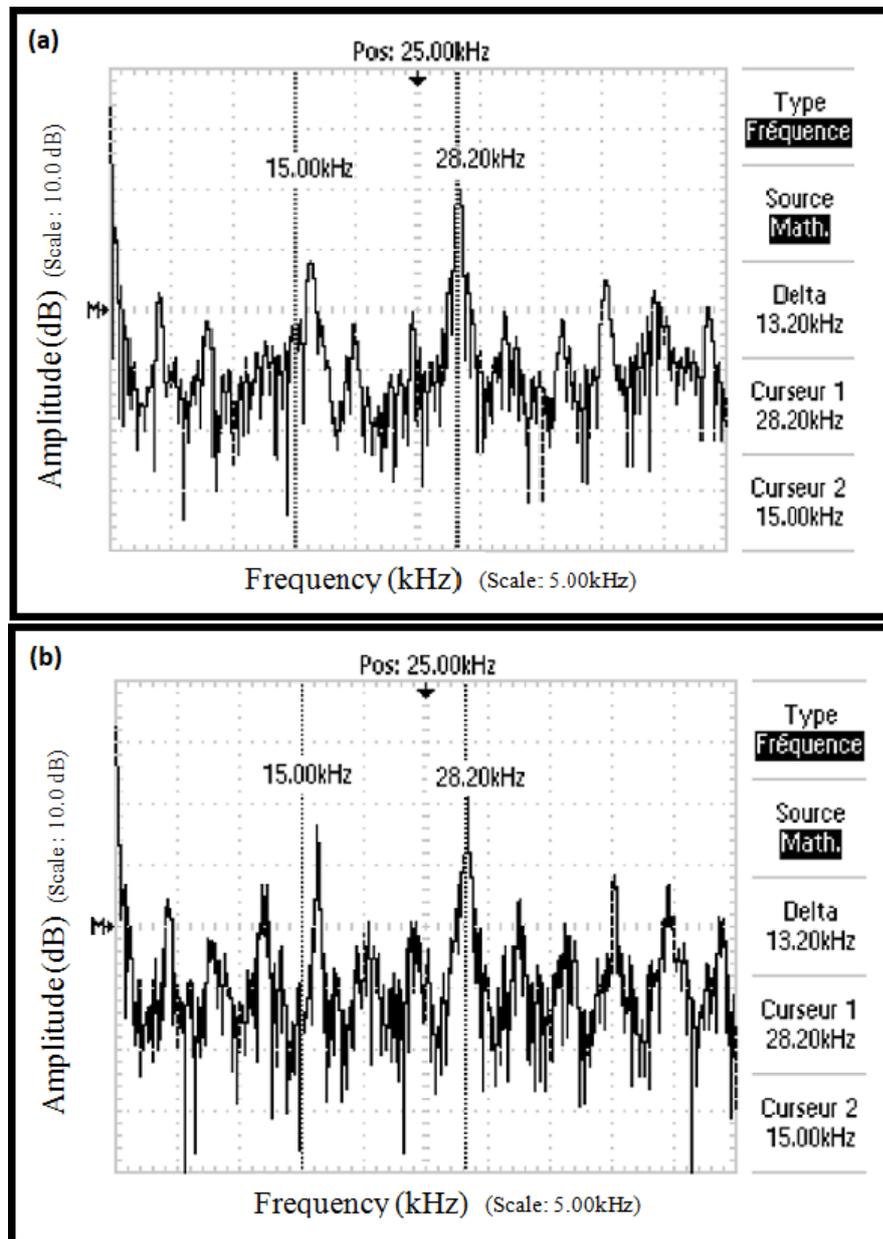


Figure 6.3: Frequency measurement using: medium sized cone, without gel, (a) on minimum power, (b) on maximum power.

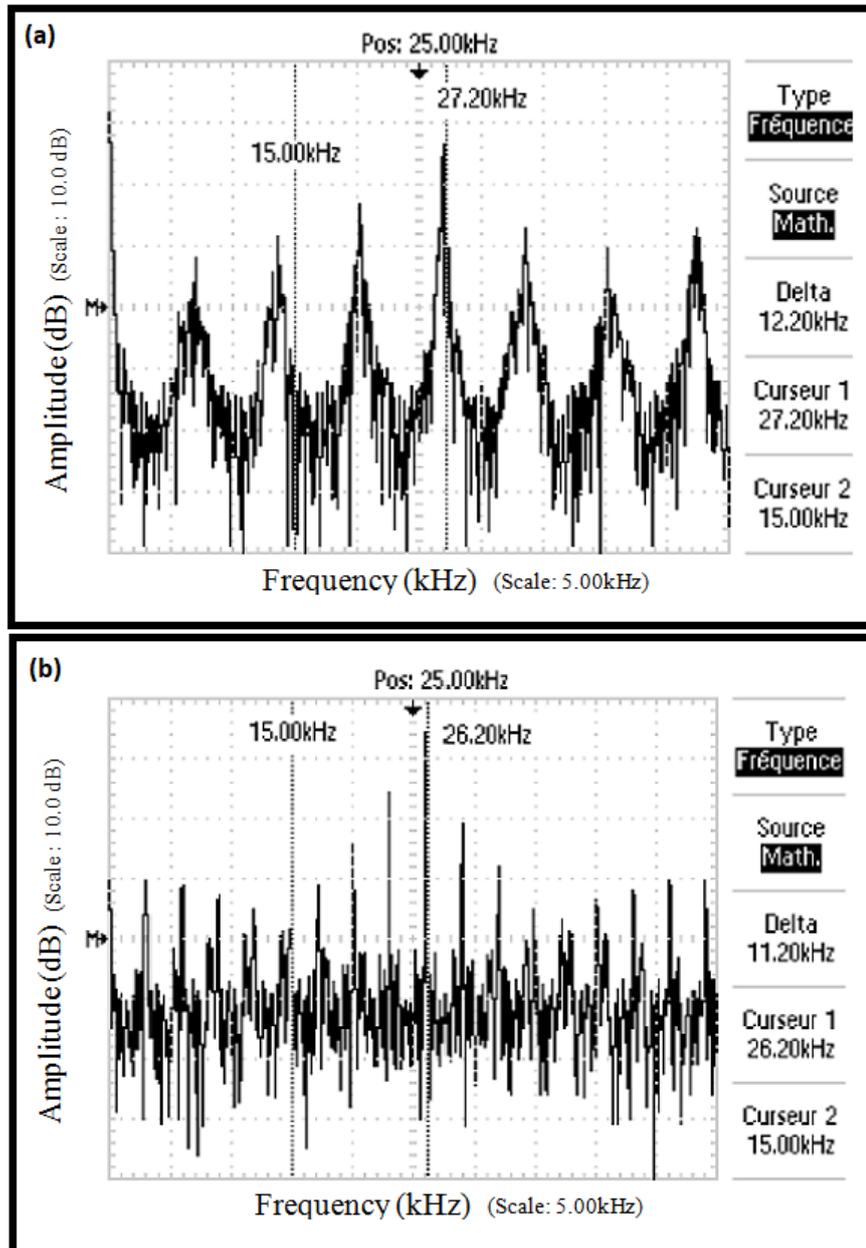


Figure 6.4: Frequency measurement using: small sized cone, without gel, (a) on minimum power, (b) on maximum power.

The vibrometer (Section 5.1.3) detected no displacement of the cone with variations in the intensity. In other words, the vibration of the cone was smaller than the level of the sensitivity of the instrument which is a quite small value. Thus, we can conclude that the influence of the externally applied signal is only due to the frequency of the ultrasound and the harmonics induced in the cone tip. There is no artefactual shaking of the hydrogel due to vibration of the tip. Hence, the sonicator provides a longitudinal energy profile to the hydrogel drug carrier (Section 5.2) as the external stimulation to trigger the drug release.

To select the optimum tip size, theophylline release experiments were conducted using the 2wt% agarose hydrogel prepared in distilled water. This hydrogel was chosen for this set of preliminary experiments because it is a physical hydrogel that drugs can be loaded readily into by simply mixing the drug solution with the agarose solution at a moderate temperature slightly above the gelling point of the agarose solution. Each experiment was carried out at the highest and the lowest ultrasound intensities. The results are shown in Figure 6.5.

In the case of the small cone, using the highest intensity ($1.5 \text{ (W/cm}^2\text{)})$ all the drug was

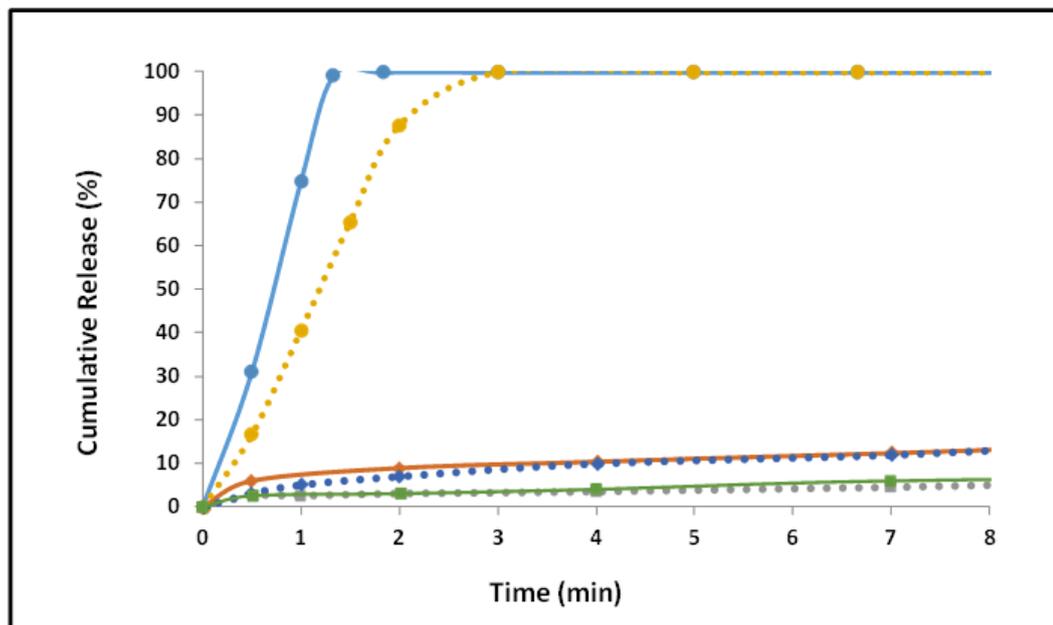


Figure 6.5: Theophylline release from 2wt% agarose gel prepared in distilled water, using three different ultrasound tips: small Cone (●), Medium Cone (◆), Large Cone (■); and, two different ultrasound intensities: $0.4 \text{ (W/cm}^2\text{)}$ (dotted line) and $1.5 \text{ (W/cm}^2\text{)}$ (solid line).

released in less than 90 seconds. For the lowest intensity ($0.4 \text{ (W/cm}^2\text{)})$ all the drug was

released from the small cone after around 3 minutes. For the two other cone sizes, the time needed for complete drug release was more than 24h. It means that the rate of drug release was significantly faster when using the small cone compared to the two other cones.

The reason can be due to the dissipation of ultrasound energy when using bigger Medium and Large cones. As discussed above, there is destructive interference of the ultrasound in both the Medium and Large cones. On the contrary, there is constructive interference of the ultrasound waves when using the small cone and consequently higher energy is delivered to the hydrogel. Since the amount of energy carried by a wave is proportional to the amplitude of the wave, it is clear that the energy of the ultrasound wave is higher with the small cone tip. On the contrary, when using large and medium sized cone tips, the destructive interference of the waves diminishes the energy of the waves (see Figure 6.2 - 6.4). For both the Medium and Large cones there is insufficient energy to trigger the drug release to the media. Thus, the small ultrasound tip was selected to hold drug containing hydrogel samples in the rest of experiments.

It was hypothesised that the gravity could also affect the drug release (see Section 5.2.3). This was tested by maintaining the ultrasound hand-piece upward during the experiments. A 2wt% agarose gel prepared in distilled water was used for all the experiments. Ultrasound power was changed between 0.4, 1, and 1.5 (W/cm^2). The initial concentration of drug was the same in all experiments. The difference in the amount of theophylline released from 2wt% agarose gel when the ultrasound hand piece is kept downward or upward is summarized in Table 6.1.

It can be seen that whatever the ultrasound intensity is, the difference in the amount of drug released from a 2wt% agarose gel is negligible whether the position of the hand piece is downward or upward. In other words, the effect of gravity on drug release can be neglected.

The effect of the heat generated by the ultrasonic waves on the hydrogels were assessed as described in Section 5.2.1 in the form of the time needed for a hydrogel to start melting under ultrasound exposure. Experiments were carried out on all agarose, PVA and chitosan hydrogels. The amount of heat produced depends on the intensity of the ultrasound and the time of exposure while the consequences depend mostly on the thermal characteristics of the

Table 6.1: Effect of gravity on drug release: the difference in the amount of Theophylline released from 2% agarose gel when the ultrasound hand piece is kept downward or upward, at 3 ultrasound intensities.

Time (s)	Difference in the amount of drug released with/without gravity effects (%)		
	Ultrasound Intensity		
	0.4 (W/cm^2)	1 (W/cm^2)	1,5 (W/cm^2)
0	0.00	0.00	0.00
30	-0,965	0,898	-1,03
60	-0,706	-1,187	0,87
90	1,413	-0,148	-0,49
120	0,155	0,554	0,00
180	-0,712	0,000	0,00
300	-0,293	0,000	0,00

hydrogel. The time to start melting varied based on the hydrogel formulation and its melting point, but the effects of the ultrasound intensity and the duration of exposure were the same for all of the hydrogels.

For all samples (Figure 6.6), the time to start melting decreased at higher ultrasound intensities. For example for agarose, note that the drug release is complete in less than 120 seconds at the ultrasound intensity of 1.5 (W/cm^2) and hence unlikely to be influenced by the degradation of the agarose gel (see Figure 6.5). The experiments indicated that increasing the intensity increases the energy of the waves and thus the energy transferred to the medium. This augments the amount of the heat produced by the absorption of ultrasound waves, which causes the hydrogels to start melting more quickly. A similar effect was observed when the particles in the medium absorb an amount of energy for a longer duration. It means that the melting does not begin immediately after the application of the ultrasound, but some time after the ultrasound exposure and is based on the hydrogel formulation. In both cases, the total amount of the energy absorbed is greater and consequently the total friction increases, which results more heat generation. The only exceptions were the agarose hydrogels crosslinked at pH 12 that did not melt after being exposed to ultrasound for 1h. This can be attributed to the high melting temperature that was confirmed during DSC studies.

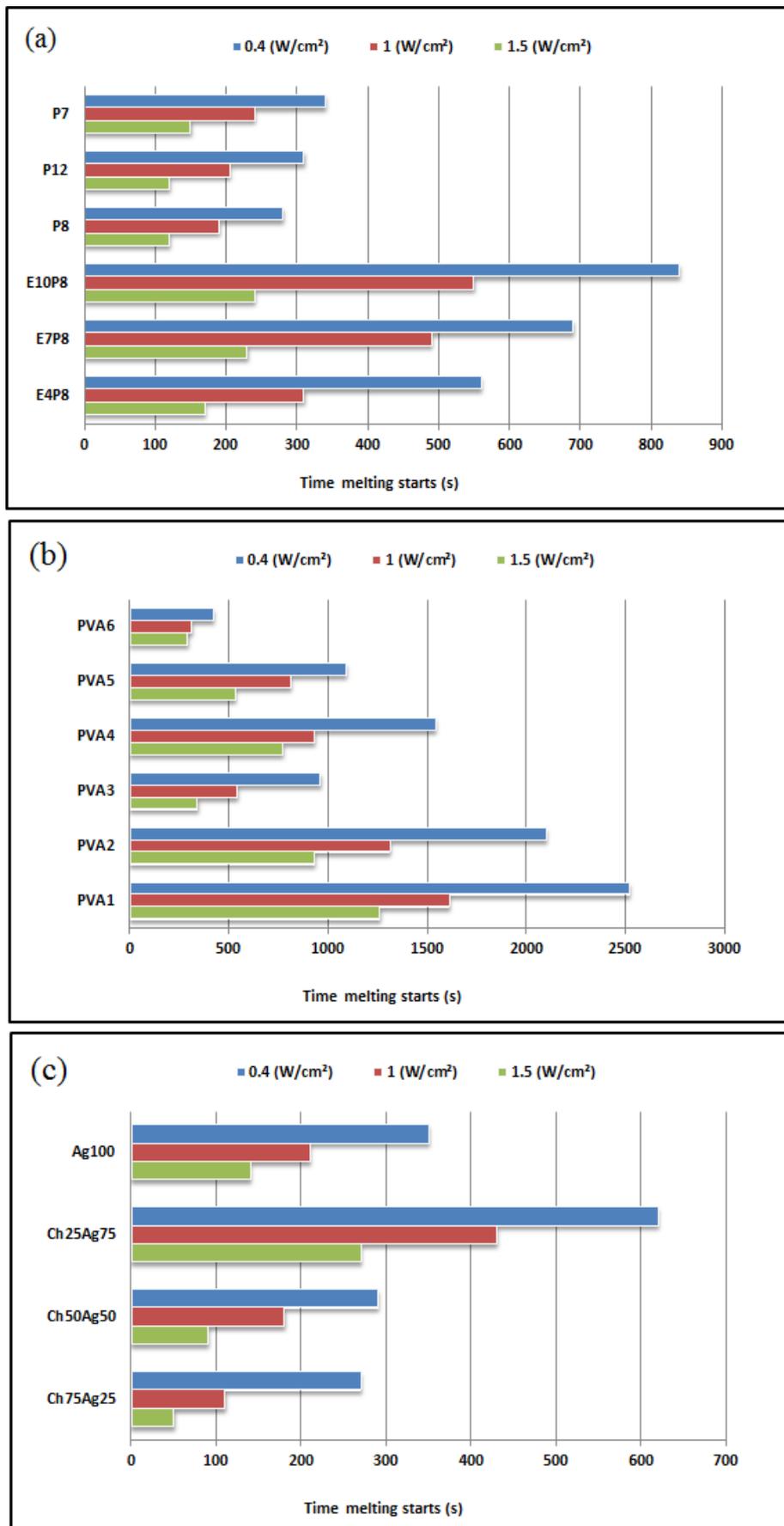


Figure 6.6: The effect of heat generated by the ultrasound application expressed as the time that a hydrogel starts melting after ultrasound exposure at different intensities (0.4 (W/cm²), 1 (W/cm²), 1.5 (W/cm²)): (a) Agarose hydrogels; (b) PVA hydrogels; (c) Chitosan hydrogels.

6.2 Agarose Hydrogels

6.2.1 Non-stimulated drug release

These experiments measured the influence of the level of cross-linking to control the passive diffusion of theophylline from the hydrogels. With no ultrasound applied to the hydrogels that were loaded with theophylline, the amount of theophylline diffusing from the hydrogel was measured in the release media. The controls for these experiments were hydrogels that were not loaded with theophylline.

For the control condition where theophylline was not loaded into the hydrogels, there was no

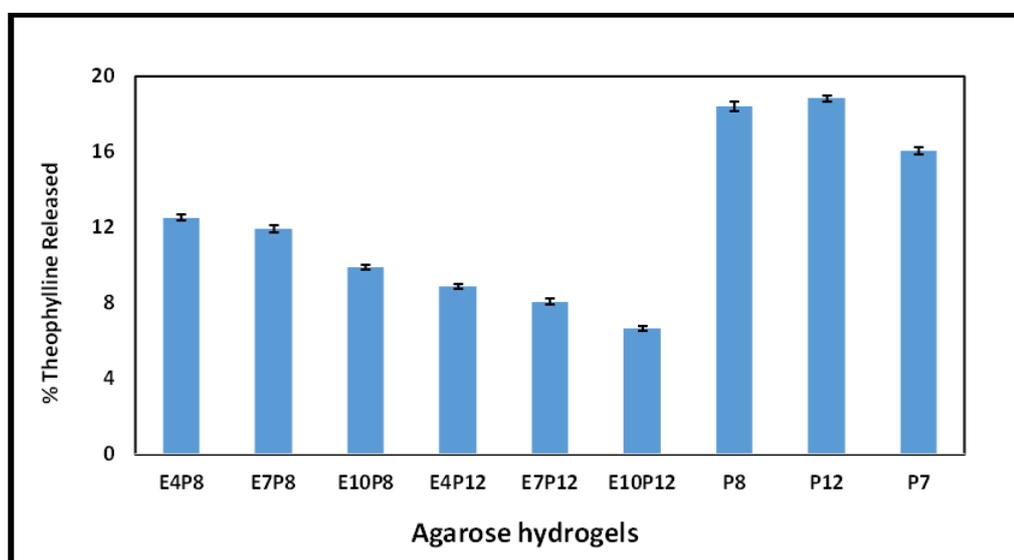


Figure 6.7: Cumulative release (%) of theophylline measured at $t=5\text{min}$ from 2wt% agarose hydrogel samples in absence of the ultrasound (values reported are an average of $n = 5$).

absorbance at 272 nm detected in the release media (N.B.: the absorbance peak of theophylline is 272 nm). These control experiments demonstrate that the hydrogels are stable and do not release any breakdown products at the absorbance peak of theophylline.

Figure 6.7 shows the amount of theophylline released from each agarose hydrogel during a period of 5 minutes when there is no external stimulation from ultrasound. Error bars on this figure represent the Standard Error of Mean. This period of 5 minutes is longer than the time required for stimulated release in the initial experiments, shown in Figure 6.5. The control experiments indicate that this absorbance is only due to the presence of theophylline in the

release media (and not any breakdown products from the hydrogels). There is a larger amount of theophylline released from the non-crosslinked hydrogels compared to the hydrogels crosslinked at alkaline pH. This is in accordance with the characterisation results presented in chapter 4, which demonstrated that crosslinking agarose with EGDE at alkaline pH increases the number of crosslinks in the polymeric network. The results in Figure 6.7 indicate that crosslinking the agarose provides a means to control drug release from the hydrogel matrix.

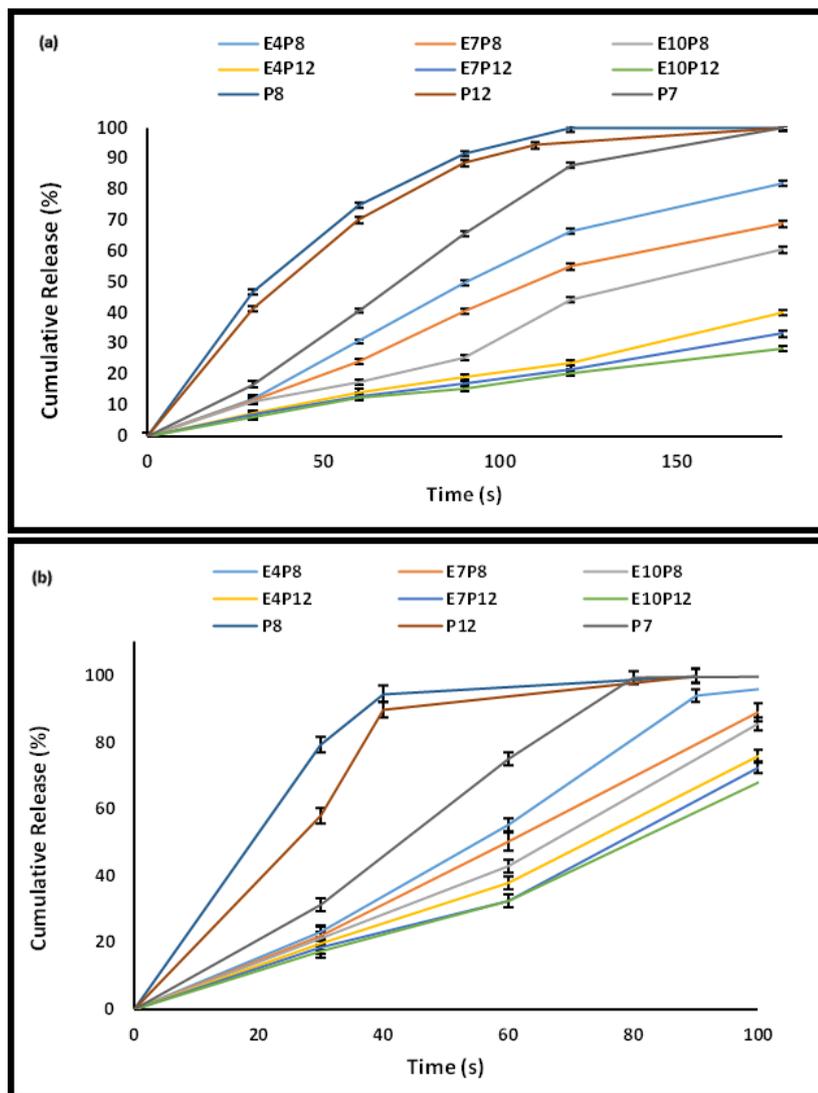


Figure 6.8: The cumulative release profiles of Theophylline from crosslinked and non-crosslinked agarose samples with ultrasound intensity set to (a) 0.4 (W/cm^2) and (b) 1.5 (W/cm^2) (values reported are an average of $n = 5$).

6.2.2 Stimulated drug release with continuous ULS application

As was described in Section 5.4, ultrasound at 27.93 ± 1.47 kHz (center frequency, Section 6.1) is continuously applied until the whole drug content is released. The release of theophylline from the hydrogel matrix is monitored in real-time with UV-vis spectroscopy at 272 nm. Figure 6.8 (the first 180 sec of the release is presented) shows the cumulative release of theophylline with the ultrasound intensity set to 0.4 (W/cm^2). Similar release profiles are obtained with the ultrasound intensity 1.5 (W/cm^2) (Figure 6.8 b)(the first 100sec of the release is presented). Error bars on this figure represent the Standard Error of Mean.

The release rate of theophylline from different agarose hydrogels at different ultrasound intensities (0.4 (W/cm^2), 1.5 (W/cm^2)) is calculated from the first linear part of the release curve (Figure 6.8) and presented in Table 6.2. It can be seen that at both ultrasound intensities, three groups of theophylline release rates can be distinguished based on the pH of the initial solutions in which the agarose hydrogels were prepared and the existence of the crosslinker.

Theophylline is released slower from crosslinked hydrogels than from non-crosslinked ones.

Table 6.2: Rate of release from different agarose hydrogels at different ultrasound intensities (0.4 (W/cm^2), 1.5 (W/cm^2)) calculated from the first linear part of the release curve.

Sample	Release Rate (s^{-1})	
	at 0.4 (W/cm^2)	at 1.5 (W/cm^2)
E4P8	0.542	0.987
E7P8	0.446	0.865
E10P8	0.332	0.810
E4P12	0.223	0.719
E7P12	0.202	0.672
E10P12	0.163	0.648
P8	1.123	2.642
P12	1.064	2.128
P7	0.717	1.205

For example, at an intensity of 0.4 (W/cm^2), the rate for the release of theophylline content from E7P12 (the hydrogel crosslinked at pH12 with 7% of EGDE) into the release media is

nearly five times more than the rate of release from P12 (non-crosslinked gel prepared in pH 12).

To reiterate the composition of the hydrogels, E stands for the percentage of the crosslinker and P designates the pH of the solution in which the hydrogel solution is prepared. Among crosslinked gels themselves, the slowest releases are from the crosslinked gels prepared in high alkaline pH. This suggests that high alkaline pH favors greater crosslinking reactions between the epoxy groups of the crosslinker and the hydroxyl groups of agarose. Among crosslinked gels with different concentration of crosslinker, there is not a significant difference in the amount of drug released.

Overall, a high alkaline pH must be maintained to allow the crosslinking reaction to occur between agarose and EGDE. Also, a crosslinker concentration of 7% seems to be enough to crosslink a 2wt% agarose solution.

Concerning the effect of ultrasound intensity, comparing Figure 6.8 (a) and Figure 6.8 (b), we see that the time of release decreases significantly with increasing the ultrasound intensity from 0.4 (W/cm^2) to 1.5 (W/cm^2). The decrease in time of release is larger for crosslinked hydrogels. For example, increasing the intensity from 0.4 (W/cm^2) to 1.5 (W/cm^2), reduces the time of release four times for E7P12 compared to 1.8 times for P7. This means that although the crosslinked gels are more stable in structure, 1.5 (W/cm^2) ultrasound intensity is sufficiently high that it could overcome the effect of crosslinker addition. In other words, a high ultrasound intensity induces too much diffusion of the drug particles from the agarose hydrogel matrices. Since we are interested to have control over the drug release, it will be more advantageous if lower intensities are applied when using agarose drug carriers.

The hydrogels were examined after each experiment to check if there is any macroscopic change in the gel structure. Since there is no crack on the hydrogel carrier after the experiment, we can say that there is no cavitation inside the polymeric network and it could not be the reason to explain the release.

6.2.3 Agarose hydrogels discussion

It was explained that in highly hydrophilic hydrogels like agarose, the drug releases with a relatively high rate, so, the macromolecular structure of these hydrogels should be modified to enable the control over drug release. It was also mentioned that this can be done by reducing the macromolecular mesh size available for drug diffusion through crosslinking hydrogels. This was confirmed in the release experiments performed on both non-crosslinked and crosslinked hydrogels.

In non-stimulated release experiments, the amount of release dramatically decreases in the presence of the crosslinker (Table 6.3). After 5min, 7.72% of the theophylline is released on average from agarose hydrogels crosslinked at pH 12 while this value increases to 17.76% in non-crosslinked samples. It is also observed that introduction of EGDE in agarose hydrogel structure decreases the release rate of theophylline. For example, the time needed the whole drug content be released into the release media is raised from 10h on average in non-crosslinked samples to 22h in samples crosslinked at pH12.

These results are in accordance with what was observed from swelling experiments whereby

Table 6.3: Average percentage of theophylline release from crosslinked and non-crosslinked agarose hydrogels in 5min in absence of ultrasound and the time needed to achieve 100% release from hydrogels.

Sample	Average Release (%) in 5min	Time for 100% Release (h)
Crosslinked at pH12	7.72	22
Crosslinked at pH8	11.45	15
Non-crosslinked	17.76	10

the addition of the crosslinker molecules to hydrogel structure decreases both the swelling capacity and drug release rate. It was discussed that this behaviour exhibited by the crosslinked agarose hydrogels could be due to the blockage of the large pores in agarose polymeric network by the monomers of the crosslinker, or because of the interaction between the crosslinker molecules with agarose molecules which increases the formation of crosslinks which consequently leads to a denser agarose network formation (Pang et al., 2007). In both cases, this reduces the void volume in the hydrogel structure and consequently decreases the amount of

water in hydrogel structure and its movement across the pores (Holowka and Bhatia, 2014). Since water acts as a plasticizer in the non-covalently crosslinked hydrogels and effectively reduces the cohesive forces between polymer chains, reduction in water amount and movement limit the diffusion of theophylline particles from pore to pore inside the hydrogel structure. Thus, the rate of release decreases in crosslinked hydrogels and allows more control over drug release.

The effect of ultrasound, as an external source of energy to stimulate release, was also

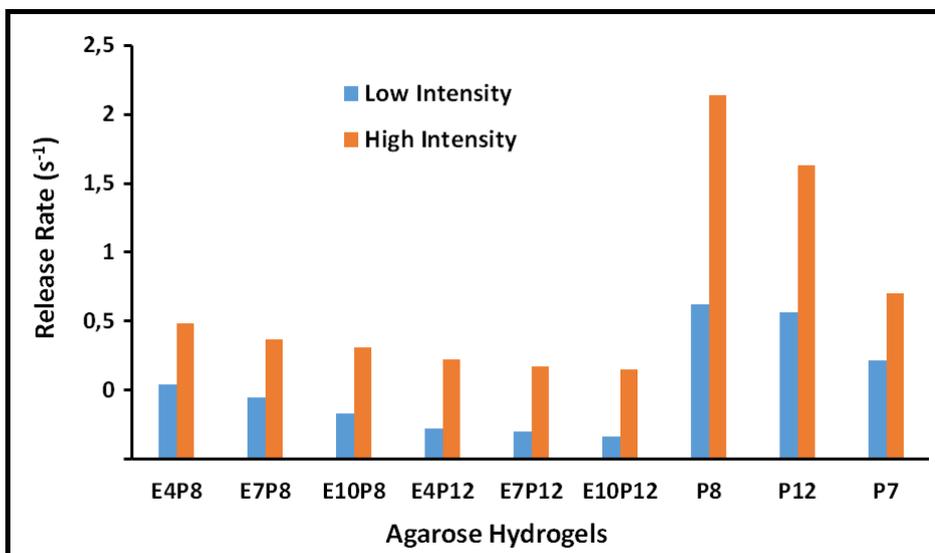


Figure 6.9: Histogram of the release rates from agarose hydrogels at low intensity ($0.4 \text{ (W/cm}^2\text{)}$) and high intensity ($1.5 \text{ (W/cm}^2\text{)}$).

verified on the release from crosslinked and non-crosslinked agarose hydrogels. According to ANOVA results, at an intensity of $0.4 \text{ (W/cm}^2\text{)}$ p-value is less than 0.05, while at the higher intensity of $1.5 \text{ (W/cm}^2\text{)}$ p-value is greater than 0.05. This indicates that there are significant differences among the drug release behaviour of agarose hydrogels when the ultrasound is applied at a lower intensity ($0.4 \text{ (W/cm}^2\text{)}$), but there are not significant differences among agarose hydrogels when the ultrasound intensity increases to $1.5 \text{ (W/cm}^2\text{)}$. This can be observed from the histograms (Figure 6.9) depicted based on the release rates reported in Table 6.2. This observation can indicate that the ultrasound intensity of $1.5 \text{ (W/cm}^2\text{)}$ is high, such that there is no significant difference in the release rate of crosslinked and non-crosslinked agarose hydrogels at this intensity. This also demonstrates the importance of considering the optimization of ultrasound intensity together with the hydrogel parameters.

As can be seen in Table 6.4, regardless of which hydrogel sample is used as the drug carrier, the time needed for the whole drug content to be released from hydrogel into the release media decreases when ultrasound is applied, even at its lowest intensity.

For example, when ultrasound is continuously applied at $0.4 \text{ (W/cm}^2\text{)}$ to a non-crosslinked

Table 6.4: Average time for 100% Theophylline release from crosslinked and non-crosslinked agarose hydrogels in absence of ultrasound, and when the ultrasound is applied at two different intensities: $0.4 \text{ (W/cm}^2\text{)}$, $1.5 \text{ (W/cm}^2\text{)}$.

Sample	Average Time for 100% Release (s)		
	Non-stimulated release	Stimulated release at $0.4 \text{ (W/cm}^2\text{)}$	Stimulated release at $1.5 \text{ (W/cm}^2\text{)}$
Crosslinked at pH12	79,200	570	180
Crosslinked at pH8	54,000	390	120
Non-crosslinked	36,000	180	90

drug loaded hydrogel, the release is over three times faster compared to the case without any ultrasound application. This increase in the rate of release is even greater at a higher ultrasound intensity. At an ultrasound intensity of $1.5 \text{ (W/cm}^2\text{)}$, the release is over six times faster than a non-stimulated release. For crosslinked hydrogels, a similar behaviour is observed (Table 6.4). The higher the intensity of ultrasound, the more drug content is released. Moreover, a similar difference in the rate of release from non-crosslinked (over two times) and crosslinked (over seven times) hydrogels was observed in the absence of ultrasound compared to when ultrasound is applied.

As it was confirmed by previous experiments (Section 6.1), the only external energy acting on the polymeric carrier is due to the ultrasound. Hence, when the ultrasound is turned on, the longitudinal ultrasonic waves act on the polymeric network and consequently on drug particles to push the drug particles to move from chain to chain. The intensity of a beam of ultrasound at a point is the amount of energy passing through unit cross-sectional area perpendicularly to the beam per unit time at that point. Thus, regardless of the existence of the crosslinks or not, increasing the ultrasound intensity from $0.4 \text{ (W/cm}^2\text{)}$ to $1.5 \text{ (W/cm}^2\text{)}$ means that the energy transferred to the drug particles has nearly increased four times, so, they diffuse much faster from one pore to another in the polymeric network and finally are

released at a faster rate.

6.3 PVA Hydrogels

It was explained that preparation of PVA in a co-solvent produces a prolonged-release hydrophilic micromatrix which is suitable for drug delivery applications. As a part of developing a controlled DDS in this research, the effect of structural changes in PVA polymeric network on drug release (theophylline) was investigated.

6.3.1 Non-stimulated drug release

Two sets of experiments in absence of the ultrasound stimulation were carried out, one with the hydrogel without theophylline inside, and the other with the hydrogel containing theophylline. As described in section 6.2.1, in the first set of experiments the aim is to check if there is any release from hydrogel alone. The second set of experiments allows to find out how drugs are loaded in various hydrogels and to compare the hydrogels structures.

No absorbance is recorded by the spectrophotometer when measuring the release from hydro-

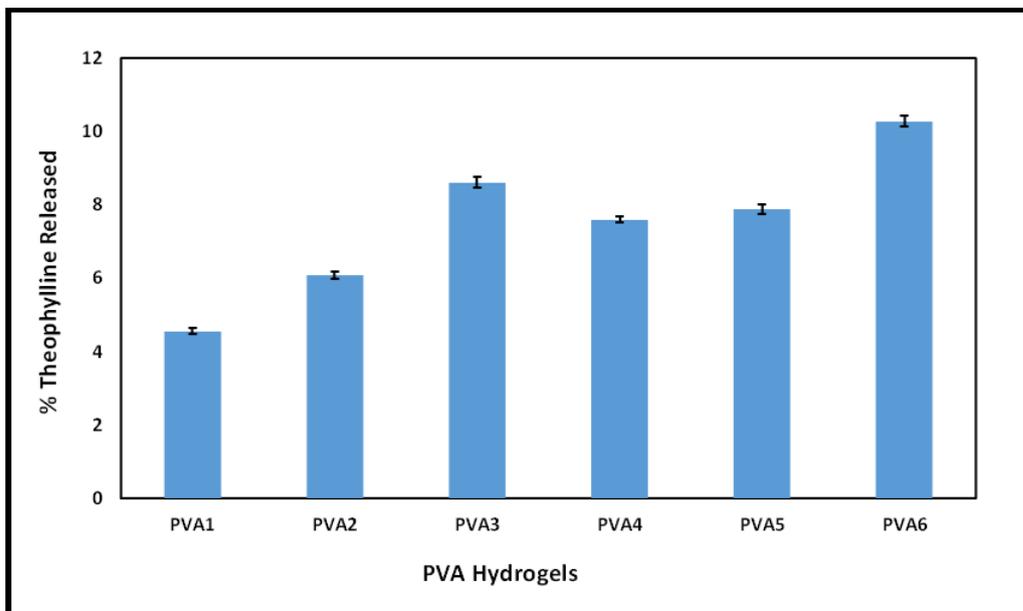


Figure 6.10: Amount of Theophylline released (%) from PVA hydrogels during 24hrs in absence of ultrasound (values reported are an average of $n = 5$).

gels alone. This means that the absorbance detected from theophylline loaded hydrogels into

the release media can be totally attributed to the drug particles released from these hydrogels.

The data obtained from the second set of experiments is depicted in Figure 6.10. This figure shows the amount of theophylline released from each PVA hydrogel during 5min when the ultrasound is off. Error bars on this figure represent the Standard Error of Mean. Note that this absorbance recorded here is only that of theophylline. The maximum releases are respectively from PVA6 with 10wt% PVA/30%DMSO and PVA3 with 15wt% PVA/30%DMSO. The minimum amount of release is from PVA1 with 15wt% PVA/50%DMSO and then from PVA2 with 15wt% PVA/40%DMSO. This is in accordance with the characterisation results presented in chapter 4.

It was already explained that PVA hydrogel is known as a typical crystalline polymer composed of a network in which the crosslinking or junction points are physically linked by crystallites (Kanaya et al., 1994; Tanigami et al., 1995). The size and amount of these crystallites formed by the organization of PVA chains and solvent molecules at different hierarchical scales determine the physical properties of PVA hydrogel including the strength to external stresses. Hence, they play an important role in performance of these hydrogels. It was mentioned that according to a systematic quantitative analysis of the structure of PVA, the degree of crystallinity increases by increasing the PVA and DMSO concentration (Ricciardi et al., 2004b).

So, the minimum release from PVA1 and PVA2 can be attributed to the formation of more crystallites in their structure since the PVA and DMSO content are more in these two hydrogels compared to other PVA hydrogels.

Although PVA3 contains a higher concentration of PVA compared to PVA4 and PVA5, the amount of release from PVA3 is more (Figure 6.10). Since these two hydrogels (PVA4 and PVA5) are composed of a higher concentration of DMSO compared to PVA3, we may conclude that the effect of DMSO in the formation of a more stable and strong polymeric structure is more than PVA. Thus, the amount of release is firstly dominated by DMSO

concentration in the co-solvent and then with PVA.

6.3.2 Stimulated drug release with continuous ULS application

As described in section 5.5, Ultrasound at 27.93 ± 1.47 kHz is continuously applied to the theophylline loaded PVA hydrogels until the whole drug content is released. The release of theophylline from the hydrogel matrix is recorded in real-time with UV-vis spectroscopy at 272nm. The data obtained from cumulative release of theophylline at ultrasound intensities of $0.4(W/cm^2)$ and $1.5(W/cm^2)$ are shown in Figure 6.11 (a) and Figure 6.11 (b), respectively. Error bars on this figure represent the Standard Error of Mean. To better show the results, the data of the first 480min of the experiments are presented.

From Figure 6.11 a, we distinguish quite different rates of drug release from PVA hydrogels at an intensity of $0.4(W/cm^2)$. The fastest release is from the hydrogel containing the least content of both PVA and DMSO solvent, with a 100% release achieved after 3min in PVA6. On the contrary, the rate of release in the hydrogel with the highest PVA and DMSO content is the slowest. Theophylline is totally released after 30min from PVA1. Among hydrogels with the same PVA concentrations, the greater the DMSO content the slower is the release. Also, between each two hydrogel with the same DMSO content, the greater the PVA concentration, the slower is the release. These observations are in accordance with the data obtained from non-stimulated theophylline release from PVA hydrogels. Since the amount of PVA and DMSO in these hydrogels determine their structural stability and strength, the higher the amount of these two substances in the PVA formulation, the polymeric network is denser and consequently results in a slower release.

Considering PVA3, although the PVA content is higher (15%) in this hydrogel compared to PVA4 and PVA5 (10% PVA content), the rate of release from PVA3 is higher. This behavior can be attributed to the role and amount of DMSO in these hydrogels. Since DMSO content in PVA3 is less compared to these two hydrogels, the rate of release from PVA3 is higher despite the higher PVA content. This is consistent with the thermal analysis results that demonstrated

the difference among the hydrogel structures.

Comparing the data of Figure 6.11 (a) and Figure 6.11 (b), we observe that the ranking

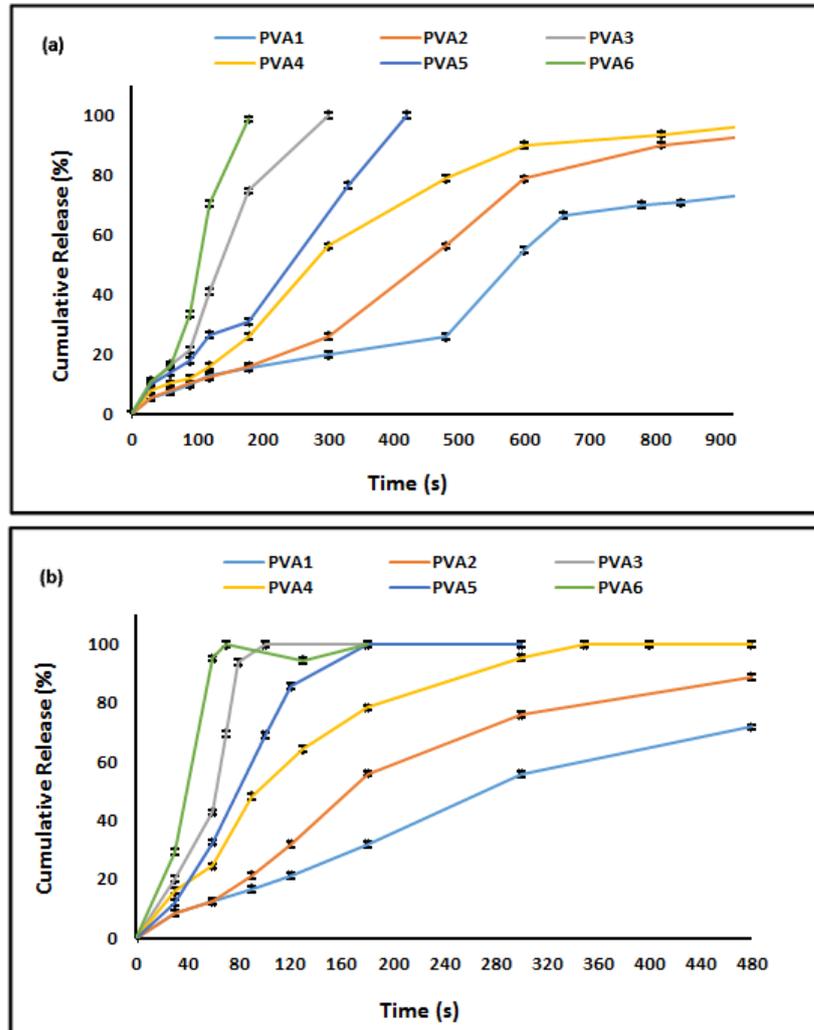


Figure 6.11: The cumulative release profiles of theophylline from PVA hydrogels with ultrasound intensity set to (a) $0.4 \text{ (W/cm}^2\text{)}$ and $1.5 \text{ (W/cm}^2\text{)}$ (values reported are an average of $n = 5$).

of release rates from PVA hydrogels are the same at both intensities, except that the rate of theophylline release is much faster at the higher ultrasound intensity. As discussed in section 6.2.3, this can be ascribed to the higher energy transferred to drug particles at the higher ultrasound intensity. Here, for instance, the time to release the whole drug content from PVA1 has decreased from 30min at an ultrasound intensity of $0.4 \text{ (W/cm}^2\text{)}$ to 15min at $1.5 \text{ (W/cm}^2\text{)}$. Although the time of release decreases with increasing the ultrasound intensity, the time needed for the complete release from some of these PVA hydrogels is still high for an

ultrasound controlled release system. The consequence of this is greater heat generated at higher intensities and longer application times.

Table 6.5: Rate of release from different PVA hydrogels at different ultrasound intensities (0.4 (W/cm^2), 1.5 (W/cm^2)) calculated from the first linear part of the release curve.

Sample	Release Rate (s^{-1})	
	at 0.4 (W/cm^2)	at 1.5 (W/cm^2)
PVA1	0.112	0.182
PVA2	0.114	0.283
PVA3	0.301	0.794
PVA4	0.235	0.462
PVA5	0.268	0.513
PVA6	0.323	1.265

6.3.3 PVA hydrogels discussion

PVA has extensively been used in controlled-release applications (Peppas, 1986). This hydrogel releases the drug with a relatively high rate because of being quite a hydrophilic system. It was explained earlier that as a means of prolonging drug release from such system, its macromolecular structure can be modified by using an aqueous co-solvent of DMSO/water. This results in a reduced macromolecular mesh size available for drug diffusion (Kim and Lee, 1992; Korsmeyer and Peppas, 1981) and consequently slows down the release from this polymeric carrier. This was confirmed during the non-stimulated release experiments using the theophylline loaded hydrogels (Section 6.3.1). Table 6.6 represents a part of the data obtained from non-stimulated release of theophylline from PVA hydrogels. The percentage of drug released from each hydrogel after 24h of non-stimulated release is presented in this Table.

The highest release is from PVA6 (47.8%) which contains the smallest concentrations of PVA and DMSO. The amount of release is the lowest from PVA1; which has the largest PVA and DMSO contents. Moreover, we observe that by increasing the amount of DMSO at a fixed concentration of PVA, there is a decrease in the amount of drug released. Increasing

Table 6.6: Average percentage of Theophylline release from PVA hydrogels in 24hrs in absence of ultrasound.

Sample	PVA (wt%)	DMSO (v/v%)	Maximum non-stimulated release (%) in 24h
PVA1	15	50	23.4
PVA2	15	40	29.6
PVA3	15	30	39.8
PVA4	10	50	32.5
PVA5	10	40	34.7
PVA6	10	30	47.8

the amount of PVA at a certain concentration of DMSO has the same effect. This is probably due to the reduced free space available for water movement to carry the drug particles, which essentially depends on the network structure (Varshosaz and Koopaie, 2002). In other words, the chain entanglement and the degree of crystallinity have increased by increasing the PVA and DMSO concentration. Thus, the macromolecular mesh size available for drug diffusion reduces and the drug release slows down.

The effect of ultrasound stimulation on drug release from PVA hydrogels was also verified.

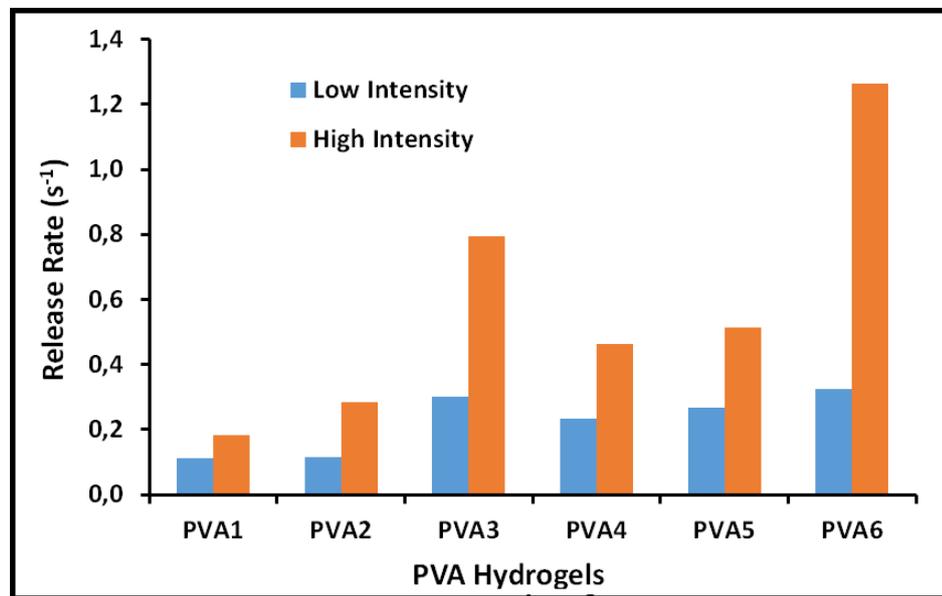


Figure 6.12: Histogram of the release rates from PVA hydrogels at low intensity ($0.4 \text{ (W/cm}^2\text{)}$) and high intensity ($1.5 \text{ (W/cm}^2\text{)}$).

According to ANOVA results, at both high ($1.5 \text{ (W/cm}^2\text{)}$) and low ($0.4 \text{ (W/cm}^2\text{)}$) ultrasound intensities: $p\text{-value} < 0.05$. This indicates that there are significant differences among the drug

release behaviour of PVA hydrogels regardless of the ultrasound intensity. This can be also seen from the histogram (Figure 6.12) depicted based on the release rates from PVA hydrogels reported in Table 6.5.

Table 6.7 represents the data from non-stimulated and stimulated release at two ultrasound intensities, allowing to evaluate the effect of ultrasound on release. The results of the non-stimulated release shows that the theophylline content loaded in PVA hydrogels cannot be totally released during 24h (Table 6.6). But, when applying the ultrasound, the time needed for the whole drug content to be released from hydrogel into the release media decreases dramatically, even at the lowest intensity, regardless of the hydrogel sample used as the drug carrier. This external energy exerted to the drug particles enables them to diffuse faster through pores by the ratchet mechanism described in Section 5.2.

Table 6.7: Time for 100% Theophylline release from PVA hydrogels at two different ultrasound intensities: 0.4 (W/cm^2), 1.5 (W/cm^2).

Sample	PVA (wt%)	DMSO (v/v%)	Time for 100% Release (s)	
			Stimulated release at 0.4 (W/cm^2)	Stimulated release at 1.5 (W/cm^2)
PVA1	15	50	1,800	840
PVA2	15	40	1,380	660
PVA3	15	30	300	100
PVA4	10	50	960	300
PVA5	10	40	420	170
PVA6	10	30	180	70

6.4 Chitosan Hydrogels

It was stated that a polymer blend composed of chitosan and agarose can improve or modify the physicochemical properties. The characteristics of this polymer blend was examined in chapter 3 and the results presented in chapter 4 confirmed the formation of a polymer better suited to the requirements of a drug carrier.

6.4.1 Non-stimulated drug release

Firstly, release experiments were carried out in absence of ultrasound using hydrogels without loading theophylline. It revealed that there is no release from any of the chitosan hydrogels alone that can be detected by the spectrophotometer at 272 nm.

Next, the non-stimulated release experiments were repeated with chitosan hydrogels loaded with theophylline. The data obtained from this set of experiments are shown in Figure 6.13. This figure demonstrates the amount of theophylline released from each chitosan hydrogel during 5min. Error bars on this figure represent the Standard Error of Mean. Since there was no release from chitosan hydrogels alone, the absorbance recorded here can be attributed to the theophylline released from the hydrogel. The maximum releases are respectively from Ch75Ag25 (75% chitosan/25% agarose) and Ch50Ag50 (50% chitosan/50% agarose). The minimum amount of release is from Ch25Ag75 (25% chitosan/75% agarose).

As it was explained, chitosan carries a positive charge at acidic solutions ($pH < 6.5$) due

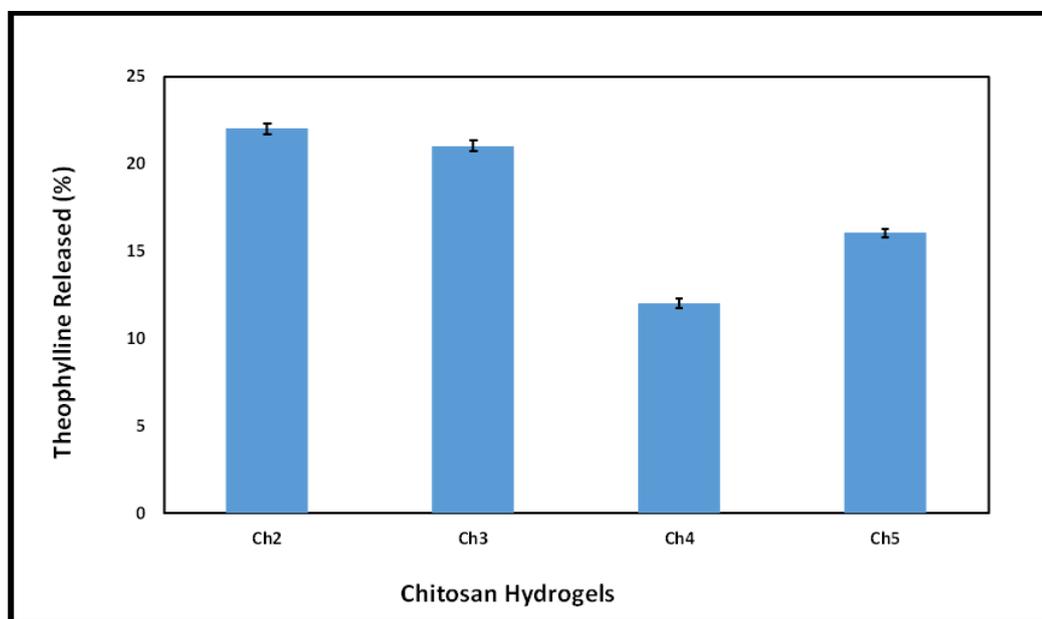


Figure 6.13: Amount of Theophylline released (%) from chitosan hydrogels during 5min in absence of ultrasound (values reported are an average of $n = 5$).

to the presence of free protonated amino groups along the polymer chains. The electrostatic effect among them at this pH enables chitosan to react with many negatively charged

surfaces/polymers and allows the formation of ionic cross-linking (Demarger-Andre et al., 1994; Sahoo et al., 2010). Since agarose contains hydroxyl groups, these functional groups interact with the protonated amino groups of chitosan and form the crosslinks between the two polymers in this pH (Elsabee et al., 2009; Kumirska et al., 2011).

This was also found during the preparation of chitosan hydrogels that a 2wt% chitosan never formed a gel at an acidic pH. However, a gel did form when agarose was added to the chitosan, even at its lowest concentrations. Moreover, from mechanical analysis it appeared that among the polymer blends of chitosan/agarose prepared in this study, Ch25Ag75 has the highest Young's modulus. The swelling experiments on the other hand showed that Ch25Ag75 swells less than other polymer blends. These observations together with the results of release from theophylline-loaded experiments, confirm the advantage of the polymer blend with reduced macromolecular mesh size as providing the better structure for drug diffusion.

6.4.2 Stimulated drug release with continuous ULS application

In stimulated release experiments, ultrasound at 27.93 ± 1.47 kHz is continuously applied to the theophylline loaded chitosan polymer blends until the whole drug content is released. The amount of the theophylline released from hydrogel matrix is measured in real-time using UV-vis spectroscopy at 272 nm. The data obtained from cumulative release of theophylline at ultrasound intensities of 0.4 (W/cm^2) and 1.5 (W/cm^2) are shown in Figure 6.14 a and Figure 6.14 b, respectively. Error bars on this figure represent the Standard Error of Mean. To better show the results, only the data of the first 500sec and 135sec of release are presented.

As demonstrated in Figure 6.14 a, theophylline releases from chitosan hydrogel blends at different rates. The fastest release is from the hydrogel containing the lowest content of agarose (25v/v%), whereby a 100% release is achieved from Ch75Ag25 after 2min. On the contrary, the rate of release in the hydrogel with the highest agarose content (75v/v%) is the slowest, with the total release from Ch25Ag75 taking 8min. These release profiles are in accordance with the profiles obtained from non-stimulated theophylline release from chitosan

hydrogels. The swelling experiments also demonstrated that increasing the proportion of agarose in hydrogel blends decreases the rate and capacity of swelling. Hence, the decrease in drug release with increasing the agarose content of polymer blends could be due to the blocking of the large pores of the chitosan polymer network by the more compact agarose network crosslinked to chitosan. This reduces the movement of water across the porous structure of the chitosan which consequently decreases the drug diffusion from pore to pore. Besides, the rate of drug release from the chitosan blend with 75v/v% agarose content is

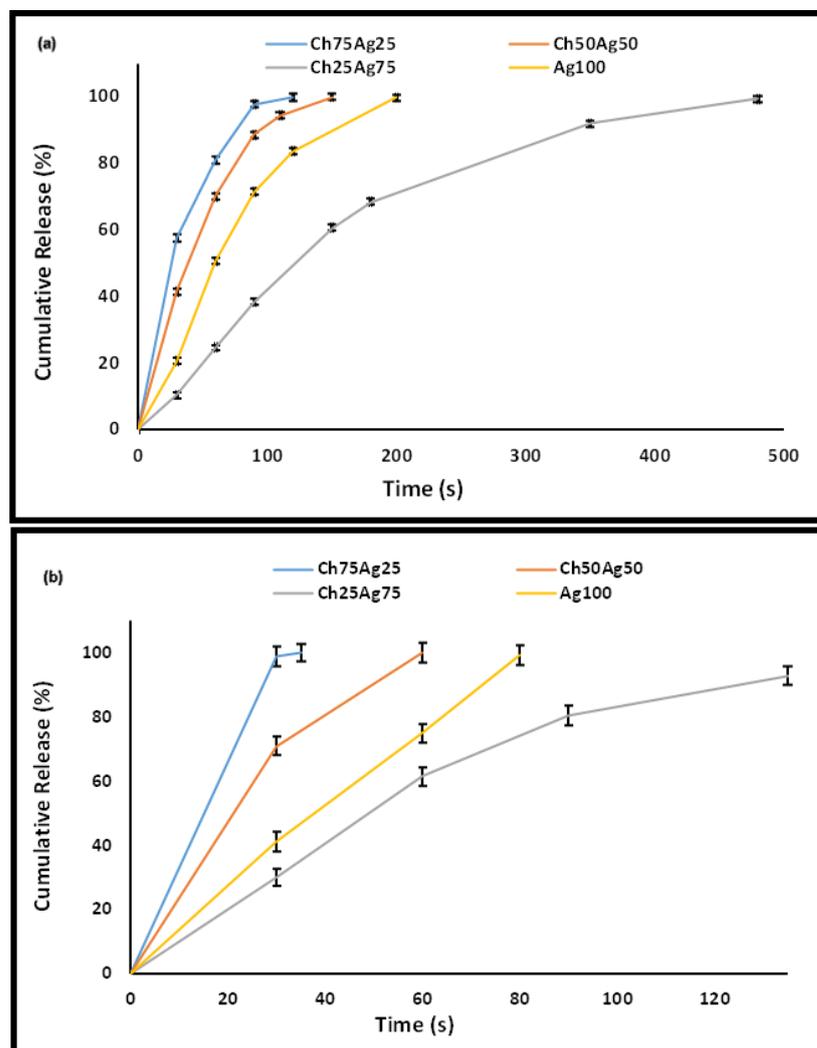


Figure 6.14: The cumulative release profiles of theophylline from chitosan hydrogel blends with ultrasound intensity set to (a) 0.4 (W/cm^2) and (b) 1.5 (W/cm^2) (values reported are an average of $n = 5$).

significantly less compared to the rate of release from pure agarose hydrogel. This may show that blending agarose with chitosan can also improve the control of drug release from agarose

hydrogel.

The effect of ultrasound intensity on release rate can be seen by comparing Figure 6.14 a and Figure 6.14 b. At a higher ultrasound intensity ($1.5 \text{ (W/cm}^2\text{)}$), the rate of release still follows the composition of the drug carriers. Again, the fastest release is from Ch75Ag25, containing the minimum agarose content, and the slowest release is from Ch75Ag25, with the highest agarose concentration. But, the time and consequently the rate of release decreases significantly at the higher ultrasound intensity.

Ch25Ag75 reaches nearly 90% of release 2.5 times faster when the intensity is increased from

Table 6.8: Rate of release from different chitosan hydrogels at different ultrasound intensities ($0.4 \text{ (W/cm}^2\text{)}$, $1.5 \text{ (W/cm}^2\text{)}$) calculated from the first linear part of the release curve.

Sample	Release Rate (s^{-1})	
	at $0.4 \text{ (W/cm}^2\text{)}$	at $1.5 \text{ (W/cm}^2\text{)}$
Ch75Ag25	1.462	3.291
Ch50Ag50	1.064	2.359
Ch25Ag75	0.395	0.987
Ag100	0.725	1.223

$0.4 \text{ (W/cm}^2\text{)}$ to $1.5 \text{ (W/cm}^2\text{)}$. The same increase in the rate of release is observed when using other chitosan hydrogel blends. This behaviour was also observed in the release experiments from agarose and PVA hydrogels and, as described previously, can be attributed to the higher energy transferred to drug particles at the higher ultrasound intensity.

6.4.3 Chitosan hydrogels discussion

During preparation of the chitosan hydrogels we realized that a chitosan solution even at its highest concentrations and highest viscosity cannot form a hydrogel. Thus, it cannot serve as a drug carrier in our experiments. To modify the gelation and some other properties of chitosan hydrogel, polymer blends of chitosan and agarose were developed. It revealed that the presence of agarose in chitosan structure creates polymer blends with interesting properties for controlled drug delivery applications. This was further confirmed in the release

Table 6.9: Average percentage of Theophylline release in absence of ultrasound from polymer blends of chitosan and agarose in 5 min and the time needed to achieve 100% release from hydrogels.

Sample	Agarose (v/v%)	Chitosan (v/v%)	Release (%) in 5 min	Time for 100% Release (h)
Ch100	0	100	-	-
Ch75Ag25	25	75	22.02	6
Ch50Ag50	50	50	21.02	8
Ch25Ag75	75	25	12.00	23
Ag100	100	0	16.03	11

experiments performed with the chitosan hydrogels as the drug carrier. The data obtained are summarized in Table 6.9.

It can be seen that in non-stimulated release experiments, the rate of release dramatically decreases by increasing the proportion of agarose in hydrogel blends (Table 6.9). After 5min, 12.00% of the theophylline is released from Ch25Ag75 (with the highest agarose content) while this value increases to 22.02% for Ch75Ag25 (with the lowest agarose content). Similar results were obtained for release of the entire theophylline content. Indeed, blending chitosan with agarose has decreased generally the release rate. The time needed the entire drug content be released into the release media increased from 6h for the sample with minimum agarose content (Ch75Ag25) to 23h for the sample with maximum agarose content (Ch25Ag75).

The results obtained from release experiments are consistent with the characterization data.

Table 6.10: Time for 100% Theophylline release from crosslinked and non-crosslinked agarose hydrogels in absence of ultrasound, and when the ultrasound is applied at two different intensities: 0.4 (W/cm^2), 1.5 (W/cm^2).

Sample	Agarose (v/v%)	Chitosan (v/v%)	Time for 100% Release (s)		
			Non-stimulated release	Stimulated release at 0.4 (W/cm^2)	Stimulated release at 1.5 (W/cm^2)
Ch100	0	100	-	-	-
Ch75Ag25	25	75	21,600	120	21
Ch50Ag50	50	50	28,800	150	60
Ch25Ag75	75	25	82,800	480	180
Ag100	100	0	39,600	210	90

The structural study of chitosan hydrogels has also demonstrated that a denser structure is

obtained by adding agarose. As noted before, in acidic pH conditions, the free amino groups along the chitosan polymer chains become protonated and the electrostatic effect among them at this pH enables chitosan to react with many negatively charged functional groups and allows the formation of ionic cross-linking among polymers (Demarger-Andre et al., 1994; Sahoo et al., 2010). Hence, the blends of chitosan and agarose form a homogeneous polymer in acidic pH (Elsabee et al., 2009; Kumirska et al., 2011) with reduced pore size which allows a control over drug release.

The effect of ultrasound stimulation on drug release from chitosan blends was also verified. According to ANOVA results, at an intensity of $0.4 \text{ (W/cm}^2\text{)}$ p-value is less than 0.05, while at the higher intensity of $1.5 \text{ (W/cm}^2\text{)}$ p-value is greater than 0.05. This indicates that there are significant differences among the drug release behaviour of chitosan blends when the ultrasound is applied at a lower intensity ($0.4 \text{ (W/cm}^2\text{)}$), but there are not significant differences when the ultrasound intensity increases to $1.5 \text{ (W/cm}^2\text{)}$. This can be easily found out from the histogram (Figure 6.15) depicted based on the release rates reported in Table 6.8.

The data obtained from the stimulated release of theophylline from chitosan polymer blends

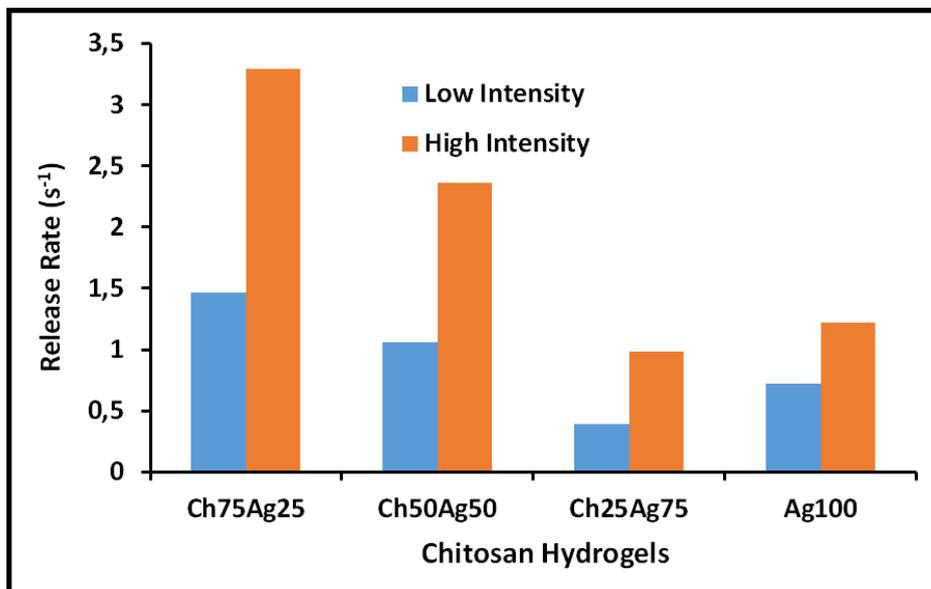


Figure 6.15: Histogram of the release rates from PVA hydrogels at low intensity ($0.4 \text{ (W/cm}^2\text{)}$) and high intensity ($1.5 \text{ (W/cm}^2\text{)}$).

are presented in Table 6.10. For example, for Ch25Ag75 with the slowest release rate, when

the ultrasound is continuously applied at $0.4 \text{ (W/cm}^2\text{)}$, the release rate is significantly much faster compared to the case without any ultrasound application. This increase in the rate of release is greater at the higher ultrasound intensity $1.5 \text{ (W/cm}^2\text{)}$ (Table 6.9). Similar results were obtained in experiments that applied ultrasound stimulation to agarose and PVA hydrogels. The time needed for the entire theophylline content to be released from drug carriers into the release media decreases when ultrasound is applied, even at its lowest intensity. As it was explained earlier, this increase is due only to the ultrasonic stimulation since it is the only external energy acting on the polymeric carrier.

6.5 Comparison of Hydrogels Release Rates

According to Figures 6.7, 6.10 and 6.13, a maximum 18%, 10%, and 22% of theophylline is released respectively from agarose hydrogels, PVA hydrogels and chitosan blends in the absence of the ultrasound. From these data, it can be concluded that agarose and PVA pore sizes are slightly smaller than the molecular size of theophylline (2.2°A); but still not a limiting small size since the drug totally releases from these hydrogels in the presence of the ultrasound. The chitosan pore size is larger than the pore sizes of the two other polymers which make the release from this hydrogel much faster with and without the ultrasound stimulation compared to the two other hydrogels. This was also found out from the swelling capacities of the three groups of hydrogels and their mechanical characterises, which had suggested a higher rate of drug release from chitosan samples compared to agarose and PVA hydrogels.

For all hydrogel samples studied, the release rate of theophylline increased by utilising the ultrasound external stimulus compared to the case without ultrasound stimulation, and the time needed for the whole drug content to be released was much less in stimulated release experiments. These data emphasize the role of ultrasound in drug release as the only source of external energy acting on drug particles without which there will be only a very slow osmotic release (Figures 6.7, 6.10 and 6.13).

The ANOVA test of the cumulative release profile of theophylline from different sets of

hydrogels (Figures 6.8, 6.11, 6.14) revealed interesting results. At an ultrasound intensity of 0.4 (W/cm^2), the ANOVA test suggested significant differences among different hydrogels of the same backbone, while at a higher intensity (1.5 (W/cm^2)) only the PVA hydrogels were different. These results were also confirmed with the magnitude of the Standard Error of Mean calculated at each data point of the cumulative release profiles. SEM was quite small for all sets of hydrogels at the lower intensity and for PVA hydrogels at the higher intensity, but it was larger for agarose and chitosan hydrogels at the higher intensity. These results demonstrate the differences among hydrogel release capacities, and how important are the type of hydrogel carrier used, the optimization of the hydrogel and ultrasound parameters according to the required application.

To conclude this chapter, the release rate of theophylline from three different groups of hy-

Table 6.11: Rate of release from three hydrogel groups at the lower ultrasound intensity (0.4 (W/cm^2)) calculated from the first linear part of the release curve.

Agarose	Release rate (s^{-1})	PVA	Release rate(s^{-1})	Chitosan	Release rate (s^{-1})
E4P8	0.542	PVA1	0.112	Ch100	-
E7P8	0.446	PVA2	0.114	Ch75Ag25	1.462
E10P8	0.332	PVA3	0.301	Ch50Ag50	1.064
E4P12	0.223	PVA4	0.235	Ch25Ag75	0.395
E7P12	0.202	PVA5	0.268	Ag100	0.725
E10P12	0.163	PVA6	0.323	-	-
P8	1.123	-	-	-	-
P12	1.064	-	-	-	-
P7	0.717	-	-	-	-

drogels at different ultrasound intensities (0.4 (W/cm^2), 1.5 (W/cm^2)) are presented altogether in respectively Tables 6.11 and 6.12. The effect of ultrasound, as the external source of energy and the only source of energy to stimulate release, is clear when comparing the data provided in Tables 6.11 and 6.12. Regardless of which hydrogel formulation is used as the drug carrier, the release rate of the drug content from hydrogel into the release media increases when ultrasound is applied at its higher intensity.

Table 6.12: Rate of release from three hydrogel groups at the higher ultrasound intensity (1.5 (W/cm^2)) calculated from the first linear part of the release curve.

Agarose	Release rate (s^{-1})	PVA	Release rate(s^{-1})	Chitosan	Release rate (s^{-1})
E4P8	0.987	PVA1	0.182	Ch100	-
E7P8	0.865	PVA2	0.283	Ch75Ag25	3.291
E10P8	0.810	PVA3	0.794	Ch50Ag50	2.359
E4P12	0.719	PVA4	0.462	Ch25Ag75	0.987
E7P12	0.672	PVA5	0.513	Ag100	1.223
E10P12	0.648	PVA6	1.265	-	-
P8	2.642	-	-	-	-
P12	2.128	-	-	-	-
P7	1.205	-	-	-	-

The slowest release rates correspond to PVA hydrogels and crosslinked agarose hydrogels, whilst the fastest release rates are found for chitosan hydrogels regardless of their agarose content. These hydrogels release the drug content almost 2 to 18 times faster than other hydrogels prepared in this study. As discussed in Chapter 4, this means although polymer blending has improved the structure of chitosan hydrogels and has increased the junction points and crosslinks in the chitosan structure, the chitosan hydrogels still have less stable networks compared to other hydrogels from PVA and agarose.

This suggest that highly crystallite containing PVA hydrogels with higher elastic modulus, least swelling capacities and smaller pore sizes allow a better control over the rate of ultrasonic release of therapeutic molecules. On the contrary, chitosan blends with overall least elastic modulus, higher swelling rates and larger pore sizes can be used when faster rates of drug release is required.

6.6 Summary

In this chapter, the parameters of the ultrasound generator and possible external energies affecting the release of drugs were evaluated. The actual ultrasound intensities generated by the ultrasonic device was measured. The actual frequency of the ultrasound delivered to different sized cones were measured with/without hydrogels inside and at both minimum and maximum powers. In all conditions the frequency was 27.93 ± 1.47 kHz. It implies that there is no frequency fluctuation to affect the drug release during the experiments.

Drug release from all cone sizes, when using the highest and the lowest ultrasound intensities, was measured to select the most appropriate cone(s). While no release was observed from the Medium and the Large cones even at the maximum ultrasound intensity, drug was released from the Small cone at both intensities. Neither gravity nor vibration were measured to contribute to the energy delivered to the hydrogels.

The effect of the heat generated by the ultrasonic waves on the hydrogels was also studied. It appeared that an increase in the ultrasound intensity and time of exposure to the ultrasonic waves, as well as continuous application of ultrasound, increases the amount of the heat generated inside the hydrogel which can consequently accelerate the deformation and melting of the hydrogel carrier.

To assess the applicability of different hydrogel formulations prepared earlier as drug carrier, non-stimulated and stimulated release of theophylline from each hydrogel were measured. Among agarose hydrogels, the samples crosslinked at pH 12 demonstrated better control over the release both in stimulated and non-stimulated experiments. This confirms structural modifications of these samples that has resulted in a denser network. For PVA hydrogels, it revealed that higher PVA and higher DMSO contents both reduce the macromolecular mesh size in hydrogel structure due to the formations of more crystallites in hydrogel matrix. For the chitosan blend polymers, the addition of agarose has reduced the pore size in chitosan structure which allows an improved controlled release.

It was also found out that the amount of release from polymeric drug carriers can be triggered by the ultrasound at different intensities and time of exposure.

Chapter 7

Conclusions and Prespectives

7.1 Summary and Conclusion

This research aimed to develop several hydrogel formulations, of different mechanical and structural characteristics, to be used together with an ultrasound stimulated drug delivery system. A fundamental study of the release system presented in this study was another goal followed in the second stage. To accomplish this, two sets of natural hydrogels from agarose and chitosan and one set of synthetic hydrogels from PVA were fabricated. Parameters that affect the structure of each type of hydrogel were varied in order to study the effect of structural changes on drug loading and release capacity of hydrogels. Next, the hydrogels were applied as the drug carriers at the tip of the ultrasound transducer to study the drug release profile.

Drug encapsulation and retention capacity of the polymeric carriers used in this study were achieved by three methods that included: crosslinking (agarose), preparation of the hydrogel in a co-solvent (PVA) and co-polymerisation (chitosan blend). The characterisation of the obtained hydrogels was performed using DSC, swelling experiments and mechanical compression. These techniques provided information about the mechanical properties, water content and structural specifications of the hydrogels. The next stage of this work was dedicated to study the ultrasound generator specification, release of drug particles from various carriers

and at different ultrasound application conditions. The whole work can be summarized into the following items:

- Preparation and crosslinking of agarose hydrogels with EGDE with the aim of improving its mechanical characteristics for a better controlled release;
- Synthesis of PVA hydrogels with high mechanical strength and verification of the effect of DMSO as a co-solvent of water on hydrogel structure;
- Preparation of a polymer blend of chitosan using agarose to improve its physicochemical properties and to obtain a drug carrier;
- Study of the structure of hydrogel samples using thermal and mechanical analysis as well as swelling properties to assess their drug carrying capacity and drug movement within the polymer matrix;
- Measurement of the parameters of the ultrasound generator and designing several hydrogel holders that screw to the transducer;
- Design of the experimental arrangement to detect the amount of the drug released under ultrasonic exposure;
- Performance of the release experiments in absence/presence of the ultrasound and with/without drug loaded to evaluate the release from each sample and to study the effect of ultrasound stimulation on release.

For agarose hydrogels, the samples prepared at pH 12 and crosslinked in the presence of EGDE are better candidates for controlled drug delivery applications. The low elastic modulus of these crosslinked hydrogels compared to other agarose samples studied here showed the better incorporation of the crosslinker molecules in agarose polymeric network. The limited swelling capacity of these crosslinked gels compared to pure agarose hydrogels and the ones crosslinked at pH 8 also confirms this.

Regarding PVA hydrogels, any increase in PVA concentration and/or the ratio of DMSO in the co-solvent increases the number of junction points and the degree of crystallinity which

consequently increases greatly the elastic modulus. The swelling experiments also revealed that an increase in DMSO content decreases the swelling capacity of hydrogels, which indicates the formation of more junction points or crosslinks in the hydrogel structure.

Although chitosan by itself did not form any gel when dissolved in the proper solvent, blending chitosan with agarose greatly improved the mechanical characteristics of chitosan hydrogels. This stability in the structure of chitosan copolymers is attributed to the formation of hydrogen bonds in the gelation process of chitosan.

Among these three sets of hydrogels, the chitosan/agarose blends were found to have the fastest swelling rates and the higher water uptakes. The lowest swelling corresponded to PVA hydrogels and then agarose hydrogels crosslinked at pH 12. PVA hydrogels had the highest elastic modulus, followed by the agarose hydrogels and then the chitosan co-polymers. Considering these observations, a more mechanically stable structure is obtained from PVA hydrogels while the chitosan samples are the mechanically weakest samples. Hence, the movement of water and consequently the drug release is reduced across the porous structure of PVA hydrogels and is fastest from chitosan copolymers.

The stimulated and passive drug release of a model drug from hydrogel carriers showed how ultrasound as an external energy stimulates and controls the drug release. The measurements carried out confirmed that, in the DDS developed, no energy acts on hydrogels except the longitudinal ultrasonic waves that act on the polymeric network. The mechanism by which the ultrasound affects the release is similar to what happens in a ratchet motor. The polymer chains play the role of the steps that the particles should take to go down while the ultrasonic waves accelerate their movement by pushing them downward to the release media. Hence, once the ultrasound is applied, the particles release from the polymer chain to chain (like going down step by step) until they reach out to the release media. That process results in directed Brownian motion of the particles that is stimulated by the energy imparted by the ultrasound.

That explanation of directed Brownian motion ("ratchet motor") is consistent with the observation that increasing the ultrasound intensity accelerates dramatically the drug release. Indeed a higher intensity equals a higher energy transferred from the ultrasonic waves to the drug particles. This also depends on the type of drug carrier structure. If the hydrogel carrier is mechanically stable like PVA samples studied here or the agarose hydrogels crosslinked at pH 12, the effect of high ultrasound intensity is much less compared to a less mechanically stable carrier like the chitosan blends. Applying ultrasound for a longer period of time increases the amount of drug released with the disadvantage that the amount of the heat generated also increases. When the drug particles in the medium are exposed to certain amount of energy for a longer duration, the total amount of the energy absorbed and consequently the total friction increase, which result in more heat generation. This is an important consideration for heat-sensitive drugs that are to be delivered and the thermal characteristics of the polymeric carrier.

This thesis demonstrated that both natural and synthetic hydrogels can be used as drug carriers together with the ultrasonic energy to provide an externally stimulated DDS. The ultrasound parameters and the carriers selected should be chosen to fulfill the desired control over the drug release.

In summary the strengths and limitations of this ultrasonic DDS are as follows: **Strengths:**

- Ultrasound is an external energy being easily switched on or off that can be applied to the hydrogels;
- This DDS improves the ability to precisely control the amount of drug delivered;
- The drug-containing hydrogels can release the entrapped drug rapidly but in a controlled manner when exposed to ultrasound;
- Various hydrogel formulations can be combined with this ultrasound system according to their intrinsic characteristics, the therapeutics to be delivered and the rate of release needed;

- Biologicals and drugs ranging from small molecules to large proteins can be delivered in regulated doses to specific target organs while reducing undesired side effects in the surrounding healthy tissues;
- Compared to other ultrasonic drug delivery systems which mainly perform at therapeutic frequencies and for transdermal delivery purposes, this system applies low-frequency ultrasonic waves that have been proven to be more effective for this purpose.
- This DDS enables doctors, nurses and potentially patients themselves to easily administer treatments as appropriate. This is extremely beneficial to patients with chronic diseases who may require frequent treatments for many years.

Limitations

- In this DDS, external energy is required to deliver therapeutic molecules; without that, there will be only osmotic release as the data have shown;
- Unfavourable interaction of a particular drug and hydrogel carrier is possible (e.g. chemical interaction between the polymer backbone and drug particles);
- Ultrasound at too high energy can destroy the polymer matrix. Consequently, the ratchet motor system described in section 5.2 will not exist anymore.

7.2 Perspectives for Future Work

Throughout the course of this research several areas of interest for the further optimisation of this DDS have been identified as follows:

1. Agarose drug carriers:

- Optimizing the agarose and EGDE concentration to obtain the best crosslinking density according to the desirable application. Also, the alkaline pH can be determined more precisely to improve the crosslinking reaction.
- Investigating the effect of various agarose powders provided by different suppliers to verify the dependence of the degree of crosslinking reaction on agarose source.

2. PVA drug carriers:

- Synthesising PVA by freeze/thawing method and compare the potential application of this new set of hydrogels as drug carrier with that of PVA prepared in DMASO/water co-solvent.
- Optimizing the formulation of PVA hydrogels prepared in this study to achieve a desired hydrogel with faster release rates and still good mechanical strength.

3. Chitosan drug carriers:

- Synthesising the copolymers of chitosan/agarose with different concentrations of chitosan and chitosan powders with different molecular weights.

4. Ultrasound stimulated release:

- Applying a hydrophobic drug model instead of theophylline, which is a hydrophilic drug model, and verifying how the delivery system present in this work can improve and control the release in this case. This helps to develop the ultrasound controlled delivery for controlled release of any type of drug.
- Designing various tip shapes for the transducer according to the amount of the drug needed to be loaded, and then verifying the efficiency and applicability of the new tips by determining the ultrasound parameters required and measuring the amount of release.
- Changing the mode of the ultrasound application from continuous to pulsed. Since at pulsed mode the ultrasound is applied for short time periods, then turns off and comes back on later for short burst of ultrasonic energy, the pulsed mode produces less heat. Hence, the unwanted effect of ultrasound on drug and carrier might be avoided.
- Developing a mathematical model, applying heat equation, that represents the results obtained to become able to describe the release phenomenon and the effect of the ultrasound on release.

5. In-vivo applications follow from the work in the thesis that has defined the parameters of the hydrogel and the ultrasound. The complete DDS comprises the ultrasound (frequency, energy) and the hydrogel carrier (crosslinking, volume, geometry, type of polymer backbone). An in-vivo application would define parameters such as the size and geometry of the hydrogel carrier (e.g. how much drug is required to be delivered?) and the required speed of delivery. For example, this ultrasonic DDS could be used to replace a hypodermic needle injection, which would require a fast speed of injection but only a stored volume of some microliters of the drug to be delivered. To summarise, the parameters that are available for optimisation to suit the specific in-vivo application include the following:

- Frequency low to high;
 - For example, a low frequency (kHz range) is well-suited for transcutaneous delivery. Whereas, although a higher frequency (MHz range) is better suited to imaging, it is also possible to include a limited drug delivery capability in such an in vivo imaging application
- Intensity of the ultrasound;
 - Short or fast delivery. High ultrasound intensity leads to faster drug release while the ultrasound at its lower intensities slows down the release. Based on the required rate of release, the appropriate intensity should be applied. The parameters of the gel material should be also considered here
- Cone material (Stainless-steel, inert and stable);
 - A solid material that does not react when it becomes in contact with the gel material and therapeutic molecules should be selected
- Cone geometry (Ultrasound delivery horn);
 - Tuneable for different frequencies. The prevalence of whether instructive or destructive interference is induced by the design of the cone tip. For example in this study, the medium and the large cones induced destructive interference while the small cone size resulted in instructive interference.

- Can be modelled to best define the cone (horn) geometry according to the desired frequency
- Gel material.
 - Cross-linking to retard drug diffusion, to strengthen the material
 - The intrinsic parameters of the gel material can accelerate or retard the release rate

Chapter 8

Author's Publications

8.1 Conferences

- F. Gerayeli, Nawel Khale Aziz Bakri, Donald Martin; " Evaluation of the effectiveness of non-invasive drug delivery of bioactive molecules to treat tumor cells"; European Nanomedicine Meeting 2015; 7-9 December 2015, Grenoble, France.
- F. Gerayeli, Nawel Khale Aziz Bakri, Donald Martin; "Study of a non-invasive drug delivery system: Optimization and modelling "; The 3rd International Conference on Bioinspired and Biobased Chemistry and Materials; 16-19 October 2016, Nice, France.

8.2 Publications

- F. Gerayeli, F. Ghosvandi, S.M. Mousavi, S. Yaghmaei, F. Amiri, Screening and optimization of effective parameters in biological extraction of heavy metals from refinery spent catalysts using a thermophilic bacterium, *Separation and Purification Technology*, 18 (2013), 151-161.
- F. Gerayeli, N. Khalef, A. Bakri, D. K. Martin, Controlled drug-release from native and cross-linked agarose gels can be stimulated by ultrasound (to be submitted to *Journal of*

Controlled Release)

- F. Gerayeli, N. Khalef, A. Bakri, D. K. Martin, Strength of binding of theophylline in native and cross-linked agarose gels (in preparation)
- F. Gerayeli, N. Khalef, A. Bakri, D. K. Martin, Ultrasound provides a control for the stimulated release of drugs from PVA and chitosan gels (in preparation)
- F. Gerayeli, P. Benech, D. K. Martin, Empirical model for the effect of ultrasound to stimulate release of drugs and particles from gels (in preparation)

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