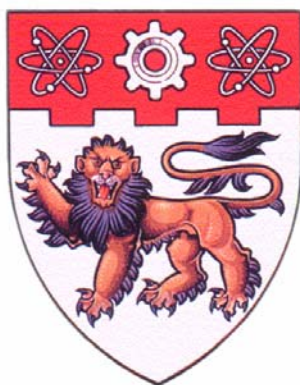


Study of Molecular Diffusion and Release of Drugs from Hydrogels



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Abstract

Hydrogels are useful formulations for the controlled release of drugs. Investigation on the molecular diffusion and release of drugs through hydrogels are of fundamental and practical importance. The diffusion and release of two small molecular drugs through hydrogels made from natural polymers have been studied in this thesis.

The first drug studied is an effective antitumor drug, camptothecin (CPT), which has shown a broad spectrum of activities against various cancers. To better understand the drug's property and prepare for the subsequent release studies, the spectral characteristics of CPT, including the UV-vis absorption, the steady-state fluorescence emission and excitation, and the time-resolved fluorescence emission, were examined and the effects of pH on the spectra of CPT were studied. The calibration curves for the quantitative assay of this drug were also obtained. The UV-vis absorption and steady-state fluorescence emission of CPT were found to obey the Beer's Law in certain ranges of CPT concentration.

The release of CPT from agar hydrogel was studied at different temperatures. The steady-state fluorescence spectrometry was used to monitor the release process and the amount of drug released at time t was evaluated using the calibration curve obtained previously. The release data were then fitted to two well-known Fickian diffusion models and the diffusion coefficients of CPT at each temperature were obtained. Temperature has been shown to be an important factor affecting the diffusion and release behavior of CPT. The relationship between the diffusion coefficient and the releasing temperature was established.

A new approach has been made to overcome the poor water solubility of CPT and the resultant limitations in the controlled release of this drug. CPT was first solubilized in the micelles formed from an ionic surfactant, sodium dodecyl sulfate (SDS) and the micellar drug solution was then used in the preparation of hydrogel drug carrier. It has been found that the presence of SDS in solution greatly increased the aqueous solubility of CPT. For example, in 1 ml of 1.0 wt.% SDS water solution, 0.11 mg CPT could be solubilized (0.318 mM), which was 83 times the solubility of CPT in pure water. It was the hydrophobic cores of the SDS micelles that were able to accept the drug molecule to form stable drug-immobilized micelles. The formulation of a hydrogel using the drug-immobilized micelles has allowed us to obtain a unique and novel system, where the drug molecules are encapsulated in the micelles and the drug-containing micelles are dispersed in the hydrogel networks. The release of CPT from the deliberately fabricated agarose-SDS systems was studied as a function of SDS concentration. The results showed that the presence of SDS in the hydrogel system decreased the diffusion coefficient of the drug and prolonged the release.

The next question worthy to be investigated is whether other surfactants have the similar effects as SDS does on the solubilization, immobilization and the release behavior of CPT. Many typical surfactants, including anionic, cationic, nonionic and several polymeric surfactants, were chosen to solubilize CPT and further immobilize the drug into the hydrogels. The solubility of the drug in each surfactant solution was determined by UV-vis absorption measurements. The location of the solubilized drug molecules in the surfactant micellar solutions was determined using fluorescence spectroscopy. The effects of the hydrophobic drug on the critical micellization concentration (CMC) of the surfactants were also examined.

It has been found that different surfactants have different effects on the solubilization of CPT. For example, the solubility of CPT can be improved 115 times by using a 1.0 wt.% sodium dodecylbenzenesulfonate (SDBS) solution, while a 1.0 wt.% sodium decyl sulfate (SDeS) solution can only increase the solubility of CPT by 15 times. The order of the solubilizing efficiency of several sodium sulfate surfactants is SDBS > SDS > SDeS. Besides, small molecular surfactants are generally more effective than polymeric ones. The different solubilizing abilities of surfactants could be ascribed to a combined effect from the hydrophobicity of the micellar core, the size of the micelles, and the miscibility between CPT and the micellar core for a given surfactant.

The release of CPT from various hydrogel-surfactant systems was investigated. All the release results were fitted to the theoretical models of Fickian diffusion and the diffusion coefficients were obtained. The relationship between the diffusion coefficient and the concentration of surfactant was developed. For the hydrogel - cationic surfactant systems, an additional interaction was developed between the drug molecules and the hydrogel network by introducing the negatively charged κ -carrageenan into the gel system. The drug was immobilized in the surfactant micelle, so the diffusion of the drug in the hydrogels was governed by the diffusion of micelles. Therefore, the coulombic interaction between the cationic surfactant micelles and negative κ -carrageenan could be used to control the release of drug. It was demonstrated that the presence of κ -carrageenan in the gel system have resulted in a remarkable decrease in the release of the drug. A series amount of κ -carrageenan was tested and consecutive decrease in the release was observed.

In addition to the hydrophobic CPT, a water soluble drug, theophylline (TPH) was also

used. TPH is an effective drug for the treatment of symptoms of chronic asthma. The release of TPH from blend hydrogels consisting of two polymers from gelatin, agar and κ -carrageenan has been studied. The experimental results showed that the release of TPH from these hydrogels was dependent upon the composition of the hydrogel, the type of component, the possible interactions between two component polymers, as well as the external temperature. Among the three kinds of blend hydrogels prepared in this study, the slowest release was obtained from the blend hydrogel of gelatin and agar with a weight ratio of 1:1. The drug release patterns and release mechanisms have been discussed by considering the possible molecular interactions and gel network structures.

Thus, a study involving both water insoluble (CPT) and water soluble (TPH) drugs has allowed us to get a profound insight into the mechanism and kinetics of the molecular diffusion and release through hydrogels. This fundamental study is believed to be able to provide a useful knowledge in design and fabrication of effective drug release systems.

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Chapter 1 Introduction

1.1 Overview of the Study

From the discovery of the first controlled drug release device in 1960's [Folkman and Long, 1964], this technology enjoyed a rapid development in the past few decades and has now become more important than ever in modern medication and pharmaceuticals [McCulloch and Shalaby, 1998]. Release-tailored drugs are advantageous over conventional forms of dosage in a number of ways, such as minimizing deleterious side effects, prolonging time of activity, protecting sensitive drugs from enzymatic or acidic degradation in the gastrointestinal tract, taste masking, etc. [Uhrich et al., 1999; Yang and Alexandridis, 2000].

Among various formulations and materials for controlled drug release applications, hydrogels have attracted intensive attentions due to their excellent biocompatibility, flexibility in tailoring physiochemical properties, such as permeability and swelling, and the ability to load drugs without the loss of their bioactivity [Peppas, 1986]. The release of solutes (drug, biological molecules, fragrances, deodorants, etc) from hydrogels is a subject of considerable interest and has received increased attention during the last couple of years [Peppas, 1997]. Hydrogel is a three-dimensional hydrophilic polymeric network capable of imbibing a large amount of water or biological fluids. The networks are composed of homopolymers or copolymers, and are insoluble due to the presence of chemical or physical crosslinks. To the respect of drug release applications, hydrogels obtained from polymers of natural origins, such as polysaccharides and proteins are

particularly desired due to their low cost, wide acceptance and biocompatibility [Brown and Johnsen, 1981; Gehrke, 1993]. Furthermore, their resemblance to human tissues is of value in order to study or mimic solute transport through biological media [Graham et al., 1988].

As a new point of interest, the application of surfactants to controlled drug release has been paid much attention in recent years. Like polymers, surfactants are useful excipients in drug release systems, where they may be used to control drug release rate, enhance effective drug solubility, minimize drug degradation, reduce drug toxicity, or facilitate control of drug uptake [Malmsten, 2002]. Moreover, the amphiphilic properties of surfactants allow the solubilization of lipophilic substances, as well as a potential in increasing the permeability of the drug through biological membranes, resulting in an augmented intracellular drug concentration [Kakemi et al. 1993]. In fact, micellar solutions and microemulsions have been proposed as efficient strategies for drug delivery even earlier [De Oliveira and Chaimovich, 1993; Kreuter, 1994]. They offer particularly several potential advantages as controlled drug release systems, and are able to modulate both the pharmacokinetics and the bioavailability of the drug to result in an overall increase in the drug therapeutic index.

The diffusion and release of two small molecular drugs through hydrogels made from natural polymers of agar/agarose, κ -carrageenan and gelatin were studied in this work. The study mainly focused on the hydrophobic drug, camptothecin (CPT), which shows a broad spectrum of activities against various cancers but suffers a limited therapeutic application because of its poor water solubility. To improve the water solubility of CPT and enhance the drug loading efficiency into hydrogel systems, surfactants were used.

The fabrication of hydrogels using solutions of drug-solubilized surfactant micelles has allowed us to obtain a unique and novel system, where the drugs are immobilized in the micelles and the micelles are dispersed in the hydrogel network. The release of CPT from these deliberately fabricated hydrogel systems was investigated. The effect of surfactant on the solubility of CPT, the effect of releasing temperature, surfactant concentration and interactions between surfactant and hydrogel polymer on the release of CPT were examined. In addition to CPT, the release of an anti-asthma drug, theophylline (TPH) through blend hydrogels has also been studied.

1.2 Objectives and Scopes of the Project

The main objective of this project is to study the molecular diffusion and release of drugs through physical hydrogels. This was achieved using natural polymer hydrogels of various formulations (with / without surfactant, single or blend hydrogels, etc.) to load and release small molecular drugs (including both water-soluble and water-insoluble drugs) under different conditions. To solve the solubility limitation of the hydrophobic drug and to get a better control on the release, surfactants were used. Through these studies, it is hoped that this project will, (1) provide a profound insight into the mechanisms of molecular diffusion and release of drugs through hydrogel systems; (2) develop a new strategy for the administration and controlled release of hydrophobic drugs; and (3) offer a useful knowledge in design and fabrication of novel, simple and effective controlled drug release systems.

The scope of the project includes:

- 1) Selection of materials and preparation of hydrogels.
- 2) Examination of the spectral properties of CPT using UV-visible spectrometer, steady-state and time-resolved fluorescence spectrometer; and the calibration for the UV-vis absorption and fluorescence emission of the studied drugs.
- 3) Release of CPT from hydrogel made from the selected material under different temperatures.
- 4) Solubilization of CPT in various surfactant solutions and determination of the solubility at different surfactant concentrations.
- 5) Release of CPT from various hydrogel-surfactant systems. Investigation of the effect of releasing temperature, surfactant concentration and interaction between surfactant and hydrogels, on the release of the drug.
- 6) Preparation of polymer blends hydrogels and the release of TPH from the blend-hydrogels. Study of the effect of blending, hydrogel composition and external temperature, on the release of the drug.

1.3 Survey of the Thesis

A review on the controlled drug release technology and the applications of hydrogels in this area followed by a brief introduction of surfactants and their applications in modern drug release systems are presented in Chapter 2. The experimental details for the selection of hydrogel materials, the preparation of hydrogels, the spectroscopic measurements of the studied drug, the release of CPT from agar hydrogel under different temperatures, the solubilization of CPT in various surfactants, the release of CPT from surfactant-hydrogel systems, and the release of TPH from polymer blend hydrogels are all given in Chapter 3. In Chapter 4, the spectral properties of CPT are

presented, the calibration curves for the UV-vis absorption and fluorescence emission of CPT are produced, and the release results of CPT from agar hydrogel are given. The release mechanism and the effect of temperature are discussed. From Chapter 5 to Chapter 7, surfactants are used to solubilize the hydrophobic CPT and subsequently load the drug into hydrogel systems. The effects of two anionic surfactants, SDS and SDBS, on the solubilization, immobilization and controlled release of the drug are studied in Chapter 5. Paralleled to Chapter 5, Chapter 6 investigates the effect of cationic surfactants on the solubilization and release of CPT. A negatively charged guest polymer, κ -carrageenan is included in the system, and the effect of κ -carrageenan on the release of CPT is discussed. In Chapter 7, more surfactant candidates, including some small molecular and polymeric surfactants as well as another type of solubilizing agents, cyclodextrins are examined for their potentials to solubilize the hydrophobic CPT. As an additional work, a water-soluble drug, TPH, is used in Chapter 8. The preparation of blend hydrogels from gelatin, agar and κ -carrageenan and the release of TPH from those blend hydrogels are investigated in Chapter 8. Before ending, the conclusions about this project and the recommendation for future work are given in Chapter 9.

Chapter 2 Literature Review

2.1 Introduction to Controlled Drug Release – The Strategies, Formulations, Materials, and Important Release Mechanisms

Controlled drug release technology represents one of the most rapidly advancing areas of science, in which chemists and chemical engineers are contributing to human health care. Research in this field focuses on developing carrier systems for the controlled release of drugs into the target tissue or cell compartment. The objectives of controlled drug release are to improve the effectiveness of drug therapy by increasing therapeutic activity compared to the intensity of side effects, reduce the number of dosages required during treatment, and eliminate the need for specialized drug administration [Uhrich et al., 1999]. Delivery systems are formed by the combination of a drug and an excipient, usually a polymeric material, in such a way as to allow the biologically active agent to go to the target (organ or organism) at controlled rates over a specific period of time [Cowsar, 1974]. Such delivery systems offer numerous advantages compared to conventional dosage forms including improved efficacy, reduced toxicity, and improved patient compliance and convenience.

The first of these controlled drug delivery devices was introduced in 1964 by Folkman and Long who developed a diffusion device with a silicon rubber, rate-controlling membrane [Folkman and Long, 1964]. This led to the development of controlled release steroidal systems for contraception in the late 1960's. Novel controlled drug delivery systems developed by Alza Corporation, and insecticide impregnated polymeric devices

developed by Shell promoted the interest in consumer and agrochemical controlled release devices [Baker, 1987].

Because controlled drug delivery systems offer numerous advantages over conventional dosage forms, controlled release devices are occupying an ever-increasing share of the health-care technology market. Although the introduction of the first clinical controlled release systems occurred less than 25 years ago, 1997 sales of advance drug delivery systems in the United States alone were approximately 14 billion dollars and in 2005, they were estimated to exceed \$35 billion [Mathiowitz, 1999; Uhrich et al., 1999]. Pharmaceutical companies are interested in controlled release systems not only because of their inherent advantages over conventional delivery, but also because these companies can receive patent extensions by simply reformulating their products into a controlled release device. Health care companies favor controlled release devices because this type of delivery greatly improves patient compliance. When the willingness of patient to take every prescribed dose at the right time and finish all medications is improved, health care companies can save an estimated \$100 billion dollars per year [Mathiowitz, 1999].

2.1.1 The Need, the Advantages and the Strategies

The development of a new drug involves more than the synthesis of a substance that has a particular effect on the body. The means by which a drug is introduced into the body is almost as important as the drug itself. The developer must also consider how to transport the drug to the appropriate part of the body and, once there, make it available for use. This is not a trivial issue; in some cases, the development of an appropriate drug

delivery system can be as complex as the development of the drug itself.

In recent years, the dramatic progress in the area of biomedical engineering has resulted in numerous commercially available macromolecular drugs. Most of them exhibit little specificity following systemic administration and they often have limited or no access to their intended sites of action or are prematurely metabolized or excreted. The selective delivery of drugs to their site of action could increase therapeutic effectiveness and reduce harmful systemic effects.

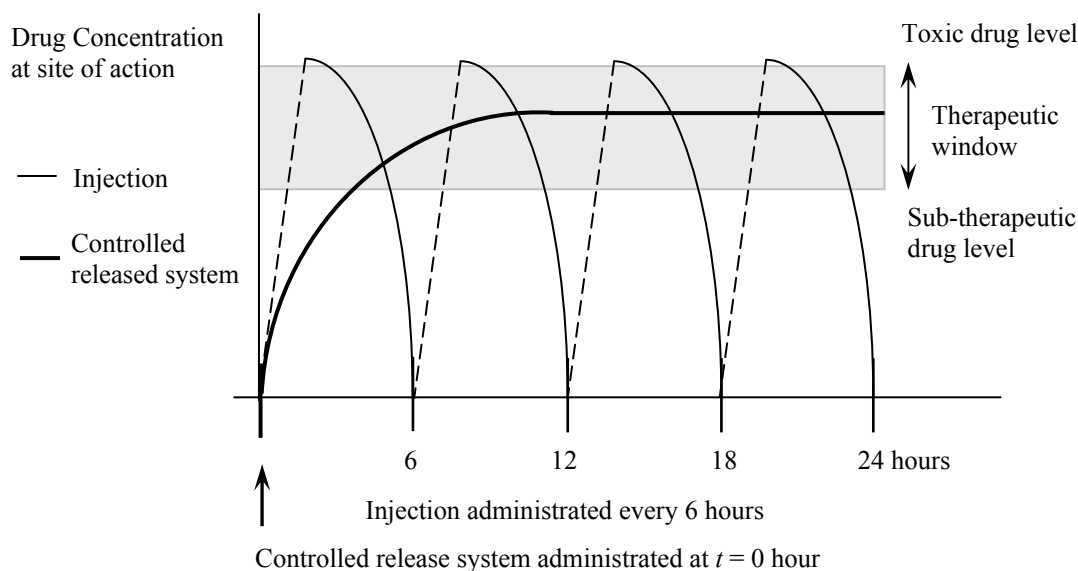


Figure 2-1: Drug concentrations at the site of therapeutic action after delivery as a conventional injection (thin line) and as a temporal controlled release system (bold line)

As the name implies, controlled-release drug delivery systems serve two functions. The first, drug delivery, involves the transport of the drug to a particular part of the body. This may be accomplished in a number of ways: intravenously, transdermally, or orally. The second function is control of release. This describes the rate at which the drug is made available to the body once it has been delivered. Traditionally, delivery systems

have not incorporated means of controlled release. The problem, however, is that with each dose of a non-control released drug, the concentration of drug available to the body immediately peaks and then declines rapidly. Taking four doses of a drug in a day, for instance, would yield an absorption pattern similar to the one shown by the thin line in Fig. 2-1. At some times, the drug concentration is very high, which may contribute to adverse side effects; while at other times, the concentration is too low to provide therapeutic benefit. It is desirable to release drugs at a constant rate, thereby maintaining drug concentration within the therapeutic range and eliminating the need for frequent dosages [Swarbrick and Boylan, 1990].

All controlled release systems aim to improve the effectiveness of drug therapy [Brouwers, 1996; Langer, 1998]. Successful drug delivery requires consideration of numerous aspects. Depending on the route of administration, the requirements of medication, the properties of the drug, and many other aspects, various strategies have to be developed. Without doubt the most generically important aspects of any therapy are its efficacy and safety. First and foremost, the drug concentration should be sufficiently high at the site of action in order to have a therapeutic effect, but at the same time it should not be too high, since this may result in detrimental side effects. For a safe and efficient therapy, the drug concentration should preferably lie essentially constant within this “therapeutic window” over the time of action. Two types of control over drug release can be achieved, temporal and distribution control [Uhrich et al., 1999].

1) Temporal Control

In temporal control, drug delivery systems aim to deliver the drug over an extended

duration or at a specific time during treatment. Controlled release over an extended duration is highly beneficial for drugs that are rapidly metabolized and eliminated from the body after administration. An example of this benefit is shown schematically in Fig. 2-1 in which the concentration of drug at the site of activity within the body is compared after the immediate release from 4 injections administered at 6 hourly intervals and after the extended release from a controlled release system. Drug concentrations may fluctuate widely during the 24 hours period when the drug is administered via bolus injection, and for only a portion of the treatment period the drug concentration in the therapeutic window (i.e., the drug concentration that produces beneficial effects without harmful side effects). With the controlled release system, the rate of drug release matches the rate of drug elimination and, therefore, the drug concentration is within the therapeutic window for the vast majority of the 24 hours period. Clinically, temporal control can produce a significant improvement in drug therapy. For example, when an opioid painkiller is administered to a patient with terminal cancer, any time that the drug concentration is below therapeutic concentrations the patient will experience pain. A temporally controlled release system would ensure that the maximum possible benefit is derived from the drug.

2) Distribution Control

In distribution control, drug delivery systems aim to target the release of the drug to the precise site of activity within the body. The benefit of this type of control is shown schematically in Fig. 2-2 in which drug concentrations at the site of activity and side effect production are compared. There are two principle situations in which distribution control can be beneficial. The first is when the natural distribution causes drug molecules to encounter tissues and cause major side effects that prohibit further

treatment. This situation is often the cause of chemotherapy failure when bone marrow cell death prevents the patient from undergoing a complete drug treatment. The second situation is when the natural distribution of the drug does not allow drug molecules to reach their molecular site of action. For example, a drug molecule that acts on a receptor in the brain will not be active if it is distributed by the patient's blood system but cannot cross the blood-brain barrier [Deboer and Breimer, 1994].

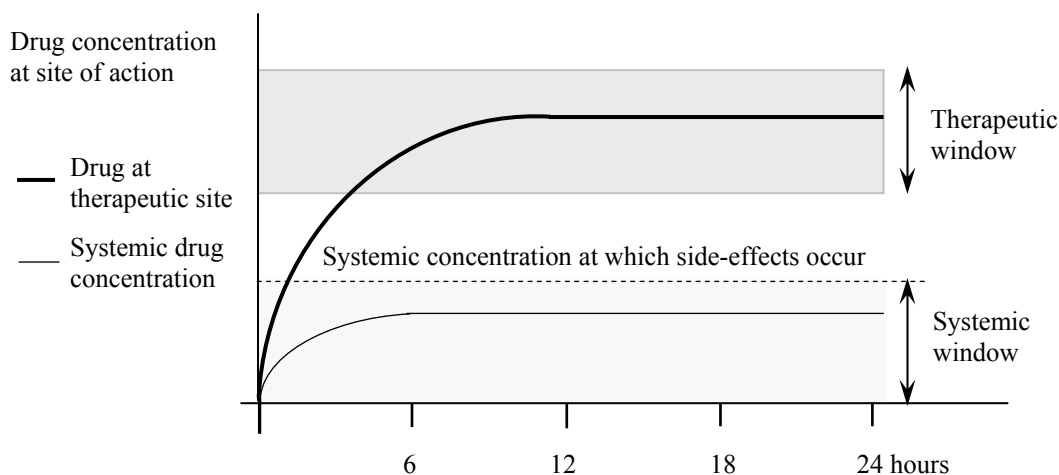


Figure 2-2: Drug delivery from an ideal distribution controlled drug release system. Bold line: drug concentrations at site of therapeutic action; thin line: systemic level at which side effects occur.

2.1.2 Various Formations and Materials

Drug delivery systems have taken a great impetus to deliver drugs to the diseased lesions. Although this concept is not new, great progress has recently been made in the treatment of a variety of diseases. A suitable carrier is needed to deliver a suitable and sufficient amount of the drug to a targeted point, hence, various kinds of formulations are being constantly developed depending upon the end use specification [Kumar et al., 2002].

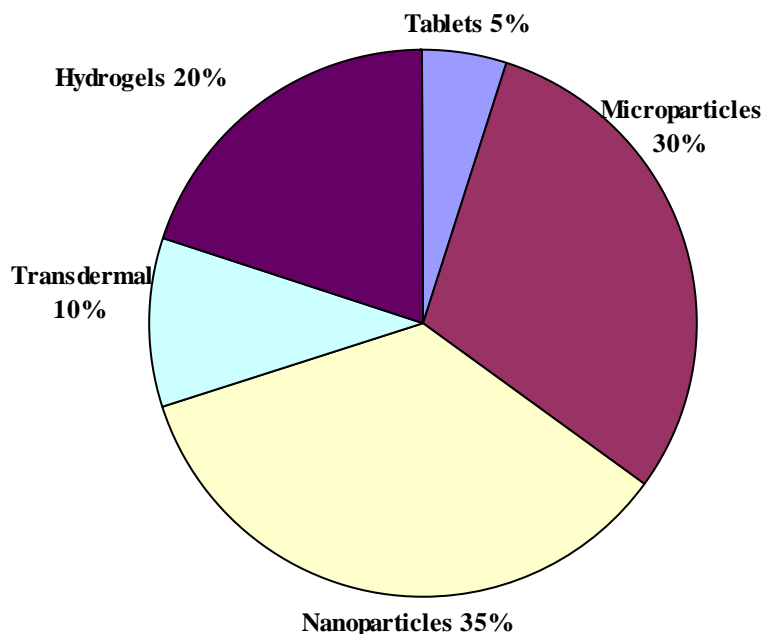


Figure 2-3: Various delivery routes for drugs [Kumar et al., 2002]

As illustrated in Fig. 2-3, commonly used controlled drug release formulations include nanoparticles [Cascone et al., 2002; Diepold et al., 1989; Illum et al., 1989; Liu and Zhou, 2002], micelles [Bromberg and Magner, 1999; Discher et al., 1999; Gref et al., 1994; Soo et al., 2002], microparticles [Qiu et al., 2001; Wong et al., 2001] and hydrogels, etc. Among them, nanoparticles take a predominant proportion of up to 35% of all the controlled drug delivery systems, followed by microparticles and hydrogels. Kumar et al. [2002] reviewed the present state of the art of the synthetic methods and characterization of nanoparticles, the suitability of polymeric systems for various drugs, drug loading and drug release properties of various systems such as nanoparticles, hydrogels, microspheres, film and membranes, tablets, etc. The information available in this area was well summarized in this review. New methods of drug administration can substantially reduce the development cost of new drug entities and can result in an

improved therapeutic index by modifying drug distribution and bioavailability. Reduced dosing frequency can improve patient compliance and minimize side effects.

In addition to the above traditional delivery formulations, new controlled drug delivery systems that respond to changes in environmental conditions, e.g., temperature [Makino et al., 2000], pH [Sideratou et al., 2000; Sumida et al., 2001], ultraviolet [Ishihara et al., 1984] or visible light [Suzuki and Tanaka, 1990], electric field [Kwon et al., 1991], and certain chemicals [Kokufata et al., 1991] have emerged since 1990s. Such systems, which are potentially useful for pulsed drug delivery, experience changes in either their structures or their intra/inter-molecular interactions due to external stimuli as mentioned above [Kost, 1990].

Controlled drug release systems often use polymeric materials as carriers for the drugs. The advent of polymeric systems as release matrices for controlled delivery of drugs occurred in 1964, when Folkman and Long found that triiodtyrosine, isoproterenol and digitoxin diffused slowly through silicon rubber tubing [Folkman and Long, 1964]. Subsequently, the release of atropine, histamine, antimalarial drugs and general anesthetics, was demonstrated using silicon rubber. Since that time, a variety of polymer materials has been exploited for controlled drug release. These polymers can be basically divided into three types, which are water-soluble polymers, biodegradable polymers, and nonbiodegradable polymers. Each type of polymer has certain advantages and limitations and the decision to use a polymer from one of these three types of polymeric materials depends upon the route of administration, the type of drug used, the amount of drug to be delivered, and the duration of release required [Saltman, 2001].

1) Water-soluble Polymers

Water-soluble polymers are typically used for short term (several hours to several days) drug delivery because of their quick dissolution in biological fluids. Their solubility in body fluids is a result of functional groups such as hydroxyl, amine, and carboxylic acid that are present on the polymer chains. These polymers do not have to undergo any chemical degradation to become water soluble; they simply have to become hydrated, ionized or protonated to lose their original forms and dissolve within the body. They can be used in their molecular, water-soluble form as agents to modify other materials or as solid, dissolvable matrices. Alternatively, water-soluble polymers may be cross-linked, by chemical or physical means, into solid materials (gels) that swell in water but do not dissolve. Typical examples are polyethylene glycol [Sawhney et al., 1993], polyethyleneimine, and polyacrylic acid [Katime et al., 2001].

2) Biodegradable Polymers

Biodegradable polymers are those requiring some chemical reactions or alteration in the body before they become water-soluble. Several methods to achieve this change in the body are known. First, the polymer can have a side chain substituent which undergoes hydrolysis in the body to produce hydroxyl, carboxyl, or other hydrating groups to cause the entire polymer to become water-soluble. Second, the polymer may come from a water-soluble polymer crosslinked with a hydrolysable crosslinking agent. When placed in the body, the crosslinking group is hydrolyzed or degraded to give a water soluble polymer. The most frequently used technique is to use water-insoluble polymers that contain hydrolysable functional groups directly in the polymer chain. Early

examples are poly (amino acids) developed by Sidman and coworkers [Langer and Peppas, 1983], the pseudopoly (amino acids) investigated by Kohn, and polyphosphazenes synthesized by Allcock and coworkers [Chasin and Langer, 1990]. But the most commonly used biomaterials for drug delivery and tissue engineering are polylactide [Wong et al., 2001], polyglycolide [Hurrell and Cameron, 2001], and copolymers of lactide/glycolide, (PLGA) [DeLuca et al., 1994; Lewis, 1990]. Because the degradation involves some chemical reactions such as hydrolysis which usually occurs over an extended period of time, these polymers can be used for longer-term drug delivery. Extensive research has produced biodegradable polymers with degradation times ranging from several days to more than one year; thus these polymers afford a wide range of applications for drug delivery.

3) Non-biodegradable Polymers

The third general class of polymers used as matrices for drug delivery is the non-biodegradable polymers. Among the many classes of polymeric materials now available for use as biomaterials, non-biodegradable and hydrophobic polymers are the most widely used materials for drug delivery [Saltman, 2001]. Silicone [Segal, 1987], polyethylene [Lu and Anseth, 2000], polyurethanes [Szycher et al., 1994], PMMA [Padilla et al., 2002], and EVAc [Hsu and Langer, 1985] account for the majority of polymeric materials currently used in clinical applications. These polymers are essentially inert and do not undergo any chemical change in vivo. Because they are essentially inert in the body, they can be used for applications in which extended drug delivery is required. Also, because of their inertness, they will remain in the body indefinitely after the drug is depleted unless they are removed. This is actually a big

disadvantage of this type of polymer, because the after-medication removal will increase the treatment cost and the patient suffering.

2.1.3 General Mechanisms and Mathematic Models

To date, there have been several proposed mechanisms to model the drug release from controlled drug delivery systems [Korsmeyer and Peppas, 1984; Singh and Fan, 1986; Kou and Amidon, 1987]. However, it is obvious that there is no universal drug release mechanism that is valid for all kinds of systems [Rao and Devi, 1988]. In contrast, there are many devices that exhibit various mechanisms that control drug release, such as polymer swelling, drug dissolution, drug diffusion or the combination of them.

2.1.3.1 Higuchi Equation and Its Modification

In 1961, Higuchi [Higuchi, 1961] proposed a mathematical equation to describe the release rate of drugs from matrix systems. Initially, the equation was valid only for planar systems, but it was later modified and extended to cover different geometries and matrix characteristics, including porous structures [Higuchi, 1963]. The equation of the Higuchi model for a homogeneous matrix system is:

$$\frac{M_t}{A} = \sqrt{D(2C_0 - C_s)C_s t} \quad \text{for } C_0 > C_s \quad (2-1)$$

where M_t is the cumulative absolute amount of drug released at time t , A is the surface area of the controlled release device exposed to the release medium, D is the diffusivity of the drug in the polymer matrix, and C_0 and C_s are the initial drug concentration and the solubility of the drug in the polymer, respectively.

Lapidus and Lordi [1968] developed two equations to account for the release of water insoluble and soluble drugs from swellable hydrophilic matrices by modifying the Higuchi equation in terms of the basis that the rate of the drug diffusion controls the rate of drug release:

$$M_t = S[D' \varepsilon C_s (\frac{2M_0}{V} - \varepsilon C_s)t]^{1/2} \quad (2-2a)$$

$$M_t = M_0 \frac{S}{V} (\frac{D't}{\pi})^{1/2} \quad \text{where } D' = \frac{D}{\tau} \quad (2-2b)$$

where M_t denotes the cumulative amount of drug released at time t , M_0 is the initial amount of drug, S represents the surface area of the system, V is the volume of the hydrated matrix, C_s is the drug solubility in the hydrated matrix, and ε is the porosity of the matrix. D' is the effective diffusion coefficient, D is the true self-diffusion coefficient of the drug in the pure release medium, and τ is the tortuosity factor of the capillary system. These two equations can be simplified and expressed as:

$$\frac{M_t}{M_\infty} = k\sqrt{t} \quad (2-3)$$

where M_∞ is the absolute cumulative amount of drug release at infinite time (which should be equal to the absolute amount of drug incorporated in the system at $t = 0$), and k is a constant reflecting the design variables of the system. Thus, the linearity of a plot of the fraction of drug released versus the square root of time gives an indication of the appropriateness of the Higuchi model to describe the mechanism of drug release as well as an evaluation of the effect of dose and other formulation variables on the release rate of the drug [Skoug et al., 1993]. There are numerous studies [Sung et al., 1996; Talukdar and Kinget, 1997] using the Higuchi equation to interpret the experimental drug release data.

2.1.3.2 Power Law

A more comprehensive semi-empirical equation developed by Ritger and Peppas [1987a, b] to describe drug release from a polymeric system has been extensively used due to its simplicity and broader applications [Skoug et al., 1993; Siepmann and Peppas, 2001].

This equation is so-called power law:

$$\frac{M_t}{M_\infty} = kt^n \quad (2-4)$$

where M_t/M_∞ is the fractional drug release, t is the release time, k denotes a kinetic constant incorporating structural and geometric characteristics of the release device, and n is the release exponent, indicative of the mechanism of drug release. The inferences about the mechanism of drug release were made by fitting the drug release data less than 60% to the equation and comparing values of n to the semi-empirical values for various geometries reported by Ritger and Peppas [1987a, b]. The values of the exponent n corresponding with drug release mechanism from polymeric controlled delivery systems of different geometries are shown in Table 2-1.

Table 2-1: The release exponent, n of the power law and drug release mechanism from polymeric matrix systems of different geometries

Release exponent, n			Drug release mechanism	Time – dependence of solute release rate (dM_t/dt)
Thin film	Cylinder	Sphere		
0.5	0.45	0.43	Fickian diffusion	t^{n-1}
$0.5 < n < 1.0$	$0.45 < n < 0.89$	$0.4 < n < 0.85$	Anomalous transport	t^{n-1}
1.0	0.89	0.85	Case-II transport	time - independence

As can be seen from Table 2-1, the classical Higuchi Eq. (2-3) represents the special case of the power law where $n = 0.5$. It is clear from Eq. (2-4) that when the exponent n takes a value of 1.0, the drug release rate is independent of time. This case corresponds to zero-order release kinetics. For slabs, the mechanism that creates the zero-order release is known among polymer scientists as case-II transport [Wang et al., 1968].

Colombo et al. [1999] applied the power law to the experimental drug release data for a highly water-soluble drug, buflomedil pyridoxalphosphate released from hydroxypropyl methylcellulose (HPMC)-based matrices. They found a value of 0.73 for the exponent, n , indicating the anomalous drug transport from the swellable matrix. Dimitrov and Lambov [1999] used the power law to describe the release of verapamil hydrochloride from the tablets based on high molecular weight polyethylene oxide (PEO). The authors investigated the effect of molecular weight of PEO and concentration of the drug loaded into the matrix on the drug release. The results of the study showed that the drug release proceeded as a controlled diffusion ($n = 0.44-0.47$) and the drug release rate was dependent on the molecular weight of PEO. Reduction of the amount of verapamil hydrochloride loaded into the system slowed the release rate sharply, resulting in a drastic change in the nature of the drug release process from a typical abnormal diffusion ($n = 0.65$) to a relaxation controlled diffusion ($n = 1$).

2.1.3.3 Other Empirical and Semi-Empirical Models

Peppas and Sahlin [1989] developed another interesting model that can be utilized to calculate the approximate contribution of the diffusional and relaxational mechanisms to an anomalous process. The equation is expressed as follows:

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \quad (2-5)$$

where k_1 , k_2 and m are constants. The first term of the right-hand side of the equation represents the Fickian diffusional contribution (F), and the second term is the case - II relaxational contribution (R). The ratio of both contributions can be calculated as follows:

$$\frac{R}{F} = \frac{k_2 t^m}{k_1} \quad (2-6)$$

Colombo and co-workers [Colombo et al., 1992] conducted the study to quantify the change of the matrix relaxation and drug diffusion rates from the matrices prepared with hydroxypropyl methylcellulose (HPMC), by measuring the surface exposed during polymer swelling and drug release as a function of coating coverage and location. Drug release data were fitted to Equation (2.5) and the R/F ratio was then calculated using equation (2.6). The results of the study showed that the presence of a coating on the swellable matrix shifted the ratio of the two contributions towards an increase in the relaxation release mechanism.

2.2 Hydrogels

2.2.1 Applications of Hydrogels in Controlled Drug Release

A number of strategies have been proposed to achieve drug delivery systems for efficient therapy [Kumar et al., 2002]. Among them, hydrogels have attracted considerable attention as excellent candidates for controlled release devices, bioadhesive devices, or targetable devices of therapeutic agents.

Hydrogels are three-dimensional, hydrophilic, polymeric networks which are capable of imbibing large amounts of water from 10%-20% up to thousands of times their dry weight without undergoing dissolution [Peppas, 1986; Hoffman, 2002]. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve. They are called “reversible” or “physical” gels when the networks are held together by molecular entanglements, and/or secondary forces including ionic, H-bonding or hydrophobic forces [Campoccia et al., 1998; Prestwich et al., 1998]; while they are called “permanent” or “chemical” gels when they are covalently-crosslinked networks.

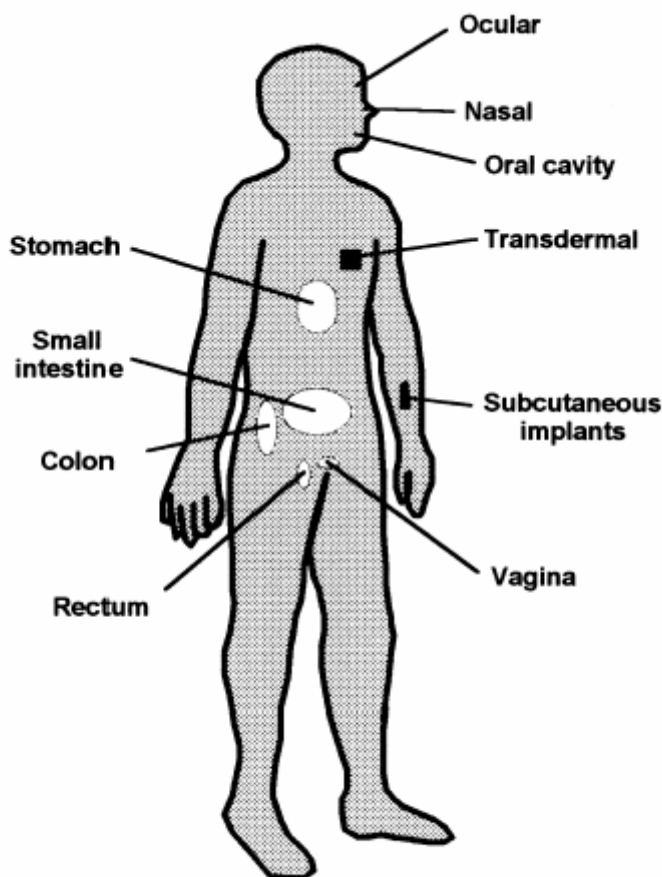


Figure 2-4: Tissue locations applicable for hydrogel-based drug delivery systems.

Hydrogels are being exploited for a variety of biomedical applications in biotechnology

and medicine owing to their physical similarity to living tissues [Chen et al., 1999; Shantha et al., 1995]. Hydrogel-based delivery devices can be used for oral, rectal, ocular, epidermal and subcutaneous application. Fig. 2-4 illustrates various sites that are available for the application of hydrogels for drug delivery. In addition to being used as carriers of bioactive agents, they can also provide protection for proteins or drugs [Peppas, 1997]. Excellent reviews in relation to this topic are readily available [Junginger, 1991; Knuth et al., 1993; Nagai and Machida, 1993; Yang and Robinson, 1998]. Historical research trends on hydrogel formulations for pharmaceutical applications, as well as the anatomy and physiology of each administration site, can be found in these reviews.

Table 2-2: Hydrophilic polymers used to prepare hydrogel matrices

Natural polymer and their derivatives	Anionic	HA, alginic acid, pectin, carrageenan, chondroitin sulfate, dextran sulfate
	Cationic	Chitosan, polylysine
	Amphipathic	Collagen (and gelatin), carboxymethyl chitin, fibrin
	Neutral	Dextran, agarose, pullulan
Synthetic polymers	Polyesters	PEG-PLA-PEG, PEG-PLGA-PEG, PEG-PCL-PEG, PLA-PEG-PLA, PHB, P(PF-co-EG) \pm acrylate end groups, P(PEG/PBO terephthalate)
	Other polymers	PEG-bis-(PLA-acrylate), PEG \pm CDs, PEG-g-P(AAm-co-Vamine), PAAm, P(NIPAAm-co-AAc), P(NIPAAm-co-EMA), PVAc/PVA, PNVP, P(MMA-co-HEMA), P(AN-co-allyl sulfonate), P(biscarboxy-phenoxy-phosphazene), P(GEMA-sulfate)
Combinations	P(PEG-co-peptides), alginate-g-(PEO-PPO-PEO), P(PLGA-co-serine), collagen-acrylate, alginate-acrylate, P(HPMA-g-peptide), P(HEMA/Matrigel), HA-g-NIPAAm	

2.2.2 Materials for Hydrogels

A wide and diverse range of polymers has been used to fabricate hydrogels as summarized in Table 2-2. These materials can be basically divided into natural polymers, synthetic polymers and combinations of the two classes.

Different routes have been used to synthesize hydrogels, and they are summarized in Table 2-3.

Table 2-3: Methods for making physical and chemical hydrogels

Physical gels
<ol style="list-style-type: none"> 1) Warm a polymer solution to form a gel (e.g., PEO-PPO-PEO block copolymers in H₂O) 2) Cool a polymer solution to form a gel (e.g., agarose or gelatin in H₂O) 3) ‘Crosslink’ a polymer in aqueous solution, using freeze–thaw cycles to form polymer microcrystals (e.g., freeze–thaw PVA in aqueous solution) 4) Lower pH to form H-bonded gel between two different polymers in the same aqueous solution (e.g., PEO and PAAc) 5) Mix solutions of a polyanion and a polycation to form a complex coacervate gel (e.g., sodium alginate plus polylysine) 6) Gel a polyelectrolyte solution with a multivalent ion of opposite charge
Chemical gels
<ol style="list-style-type: none"> 1) Crosslink polymers in the solid state or in solution with: 2) Radiation (e.g., irradiate PEO in H₂O) 3) Chemical crosslinkers (e.g., treat collagen with glutaraldehyde or a bis-epoxide) 4) Multi-functional reactive compounds (e.g., PEG₁diisocyanate=PU hydrogel) 5) Copolymerize a monomer crosslinker in solution (e.g., HEMA+EGDMA) 6) Copolymerize a monomer a multifunctional macromer (e.g., bis-methacrylate terminated PLA-PEO-PLA + photosensitizer + visible light radiation) 7) Polymerize a monomer within a different solid polymer to form an IPN gel (e.g., AN + starch) 8) Chemically convert a hydrophobic polymer to a hydrogel (e.g., partially hydrolyse PVAc to PVA or PAN to PAN/PAAm/PAAc)

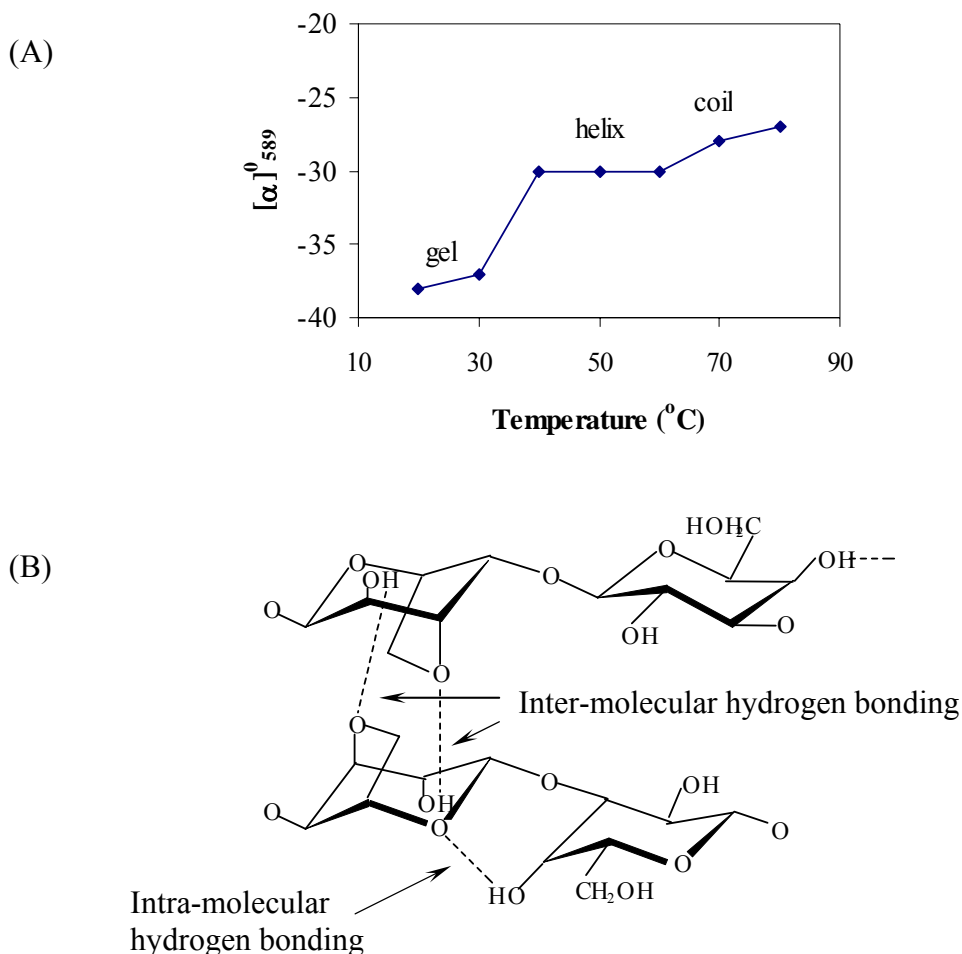


Figure 2-6: Gelation mechanism of agarose: **A.** specific rotation of 0.1 % aqueous solution as a function of temperature; **B.** intra- and inter-molecular hydrogen bonding of agarose in aqueous solution. (Redrawn from Tako and Nakamura, 1988)

Gelation of agarose occurs at temperatures below 40 °C, whereas the melting temperature of agarose gel appears to be around 90 °C. The gel formation in aqueous polysaccharides systems is generally believed to be induced by helix formation, sometimes followed by aggregation of helices [Clark et al., 1987]. Since the helix formation involves a transition from a coil-like isotropic conformation to an optically active conformation, the coil-helix transition and the gelation can be followed, e.g. by

optical rotation or circular dichroism [Dea et al., 1972]. From optical rotation measurements of 0.1 % agarose aqueous solution [Tako and Nakamura, 1988], it appears that transitions are present at 40 and 60 °C (Fig. 2-6 A).

At these temperatures, the mechanical properties of the solutions/gels change. On account of these observations Tako and Nakamura [1988] propose a gelation mechanism. At temperatures below 60 °C intramolecular hydrogen bonding takes place (Fig. 2-6 B), which contributes to the rigidity of the molecular agarose chain. The authors believe that the agarose chains are coiled above 60 °C. In addition, below 40 °C intermolecular hydrogen bonding takes place, in which the anhydro O-3, 6 ring is involved (Fig. 2-6 B). Both the intra- and inter-molecular hydrogen bondings are thought to be responsible for the gelation up to 60 °C. In this hydrogen bonded system water molecules are also bonded. This also contributes to the stiffness of the chains. At temperatures above 90 °C, the agarose molecules are hydrated and dissociation occurs.

2) Gelatin

Gelatin is a denatured, biodegradable protein obtained by acid or alkaline treatment of collagen derived from the skin, white connective tissue or bones of animals. Gelatin is extensively used for industrial, pharmaceutical and medical purposes. The biosafety of gelatin has been proved through its long clinical usage as a plasma expander, in surgical biomaterials and as an ingredient in drugs [Cremers et al., 1990]. Another unique advantage of gelatin as a drug carrier is the electrical nature of gelatin, which can be changed by the collagen processing method [Veis, 1964]. For example, the alkaline process, through hydrolysis of the amide groups of collagen, yields gelatin with a high

density of carboxyl groups, which makes the gelatin negatively charged.

Because of its natural origin, the chemical formula of gelatin is complex and composed of several amino acids. Although the formula of gelatin depends upon its origin, it always contains large amounts of proline (Pro), hydroxyproline (Hyp) and glycine (Gly) (Fig. 2-7).

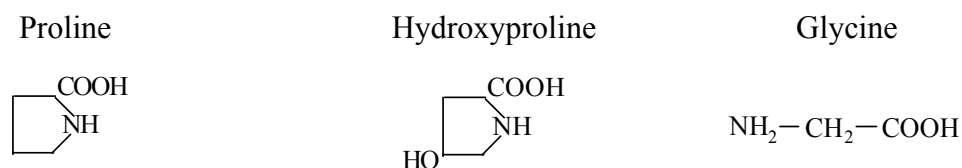


Figure 2-7: Chemical structure of proline, hydroxyproline and glycine

Sequences of (Gly-X-Pro) and (Gly-X-Hyp), in which X is another amino acid, are the commonest. Common industrial gelatins are mixtures of various components, including α -gelatin (one chain), β -gelatin (two chains linked by covalent bonds) and γ -gelatin (as β but with three chains).

Gelation of gelatin solutions has been investigated by several techniques [Chatellier et al., 1985; Pines and Prins, 1973; Finer et al., 1975; Godard et al., 1978]. Since there is little doubt that the partial renaturation of the collagen helical structure occurs during gelation, optical rotation determination has been used as a main tool [Djabourov et al., 1988]. Gel formation and melting behavior are strongly dependent upon temperature, concentration and molecular composition of the gelatin sample [Flory and Weaver, 1960]. In particular, the initial gelation of gelatin is believed to proceed by formation of ordered quasi-crystalline triple-helical junction zones separated along a single polymer chain contour by flexible regions, and these zones (analogous to the collagen triple helix) form on cooling solutions to $\sim 28^\circ\text{C}$ [Clark et al., 1983]

3) Carrageenan

Carrageenans are naturally occurring polysaccharides that are extracted from seaweeds. They have long been widely used as thickening agents, suspending agents and gelling agents in food as well as in the pharmaceutical industry. Carrageenans are high molecular weight polysaccharides made up of repeating units of galactose and 3, 6-anhydrogalactose (3, 6-AG). There are three different classes of carrageenans namely, lambda (λ), iota (ι), and kappa (κ). The structure of κ -carrageenan is shown below:

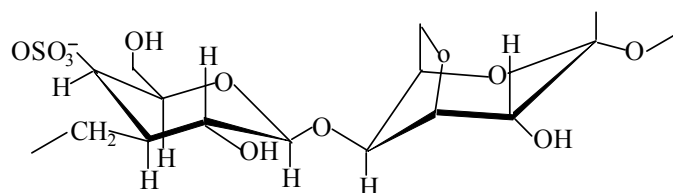


Figure 2-8: Chemical structure of κ -carrageenan

κ -Carrageenan forms gels that are thermally reversible. The κ -carrageenan gels are stiff and strong, which are useful in applications that require shape retention such as otic, vaginal suppository formulation as well as controlled drug delivery. There are several studies that show the potential use of carrageenans in controlled drug delivery due to their swelling property [Picker, 1999a; Hariharan et al., 1997] and possible ionic interaction with certain drugs [Carm-Lelham and Sundelof, 1996; Bonferoni et al., 2000]. The results of their studies showed that carrageenans could be used as either a single polymer or as a combination of various classes of carrageenans, or combination of carrageenans with other types of polymers. Examples of the latter include combination of λ carrageenans with hydroxypropyl methylcellulose (HPMC) or with

crosscarmellose sodium to prepare oral controlled release dosage forms [Bonferoni et al., 1998].

In addition, the release study of the model drug, theophylline monohydrate from the matrices containing κ -carrageenan was evaluated by Picker [1999b]. The results of the study showed that the drug release from these matrices changed with concentration of carrageenan. Increasing the carrageenan concentration resulted in a faster swelling of the tablets, and all formulations containing higher percentages of carrageenan provided more or less zero-order release kinetics or swelling-controlled mechanism (n values were all above 0.89).

2.3 Detective Techniques Used in Drug Release

Several techniques have been ever employed in controlled drug delivery applications to determine the release profiles of drugs, including UV-visible spectrometry [Favre and Girard, 2001; Miyazaki, 2000], fluorescence spectrometry [Yilmza and Pekcan, 1998; Pekcan and Erdoğan, 2002], scanning force microscopy (SFM) [Qiu et al., 2001], confocal laser scanning microscopy (CLSM) [Qiu et al., 2001; Wong et al., 2001], high-pressure liquid chromatography (HPLC) [Katime et al., 2001], gas chromatography [Peppas and Ende, 1997], chemical analysis [Sakiyama et al., 2001] and NMR [Liu and Zhou, 2002] etc. Of all the techniques used in controlled drug delivery, UV-vis and fluorescence spectrometry have been most extensively used and also employed as primary detective techniques in this study, due to their high performance, easy operation, non-destructive properties as well as other merits. Details about these two techniques will be given below.

2.3.1 UV-visible Spectrometry

UV-visible spectrometry refers to the absorption of a specific substance in the ultraviolet and visible light regions. It is now more than 50 years since the first spectrometers working in the ultraviolet and visible light regions of the spectrum came into general use, and over this period they have become the most important analytical instrument in many chemical, biological and clinical laboratories [Perkampus, 1991].

2.3.1.1 Characterization of Compounds

Most organic compounds and many ions and complexes absorb radiation in the UV-vis light region. A plot of this absorption by a compound against wavelength is called its absorption spectrum, which has a shape that is characteristic of a particular compound or class of compounds. The UV-vis spectrum does not usually give enough information to identify an unknown compound, but when combined with other analytical techniques or with chromatographic separation, an unequivocal identification can be achieved.

Absorption spectrometry is a non-destructive technique and is extremely sensitive, and is therefore ideal for the characterization of small amounts of precious compounds. As an extreme example, it is used for the measurement of pigments in the single retinal receptor cells [Knowles et al., 1984]. A custom-built microspectrometer is used which is capable of recording satisfactory spectra from only 4×10^{-16} mol pigment, which is only about 10^8 molecules.

2.3.1.2 Quantitative Assay

The most important application of the technique is as a means of measuring concentration of a known substance and modern instruments are designed to facilitate rapid and accurate measurements. The precision that can be achieved depends upon a number of factors, but single measurements of precision better than $\pm 0.5\%$ should be possible. One particularly important merit of the technique is that trace components can be measured in the presence of high concentrations of other components if there is sufficient difference in their absorption spectra. Similarly, mixtures of compounds with differing spectra can be analyzed.

The technique is best suited to dilute solutions, though gases and solids can be measured by special methods. Thus solubility in a suitable solvent is a prerequisite for the accurate measurement of a particular sample. The optimum concentration range for the measurement of a compound is limited and does not generally exceed a 100-fold range, and so it may be necessary to adjust the concentration of the test solution to bring it into the optimum range. Beyond manipulations of the concentration of solutions, measuring the UV-vis absorption will not change the sample in any way and even UV-sensitive compounds will suffer negligible damage, for the amount of radiation adsorbed by the sample during the measurement is very small.

2.3.1.3 Rapid Assay

The fast response of photodetectors and modern electronic components means that the

measurement of solutes in flowing or rapidly changing systems can be carried out with millisecond time resolution [Knowles et al., 1984]. Absorption measurements are also the most popular means of following the kinetics of reaction systems since they do not interfere with the progress of the reaction in any way [Burgess and Knowles, 1981].

2.3.2 Fluorescence Spectrometry

The application of fluorescence techniques in controlled drug delivery systems has gained more and more attention and increasing interest. These techniques, such as steady-state spectroscopy, fluorescence anisotropy and fluorescence decay measurements are powerful tools for studies of molecular diffusion or molecular interactions. They have been extensively applied in many areas such as analytical chemistry, biochemistry, cell biology, environmental science, etc. due to the high sensitivity, versatility of information and commercially available instrumentation [Tazuke and Winnik, 1986; Lakowicz, 1999]. For example, the in situ steady-state fluorescence (SSF) method has been used to study the slow release processes in gels formed by free radical cross-linking copolymerization of methyl methacrylate and ethylene glycol dimethacrylate [Pekcan and Erdoğan, 2002]. The same technique was also applied for studying the swelling and drying processes of various gel systems [Pekcan and Yilmaz, 1997; Yilmaz and Pekcan, 1998].

2.3.2.1 A Brief Description of a Fluorescence Process

Fluorescence is one type of luminescence, which comes from emission of photons from electronically excited states. In a singlet excited state, the electrons in the higher energy

orbital and the lower energy orbital have opposite spin orientations. They are said to be paired. Return to the ground state from an excited state does not require an electron to change its spin orientation. Fluorescence is the emission that results from the return to the lower orbital of the paired electrons. Such transitions are quantum mechanically allowed and the emissive rates are typically near 10^8 sec^{-1} , which gives fluorescence a lifetime near 10^{-8} sec or 10 ns. The lifetime is the average period of time a fluorophore remains in the excited state. This favorable time scale of phenomenon of fluorescence gives it many advantages, because during this period of time a wide range of molecular process can occur, and these can affect the spectral characteristic of the fluorescent compound.

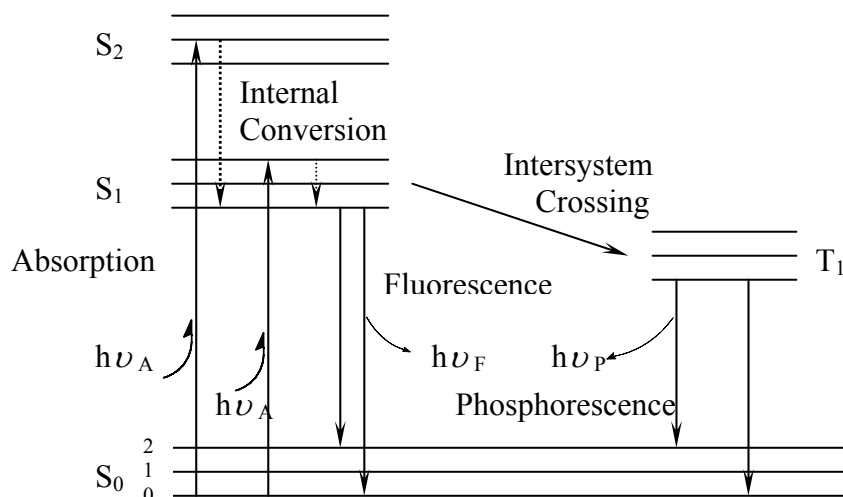


Figure 2-9: Jablonski diagram [Jablonski, 1935]

The absorption and emission of light are nicely illustrated by the energy-level diagram suggested by Jablonski A. [Jablonski, 1935]. The ground, first and second electronic states are depicted by S₀, S₁ and S₂, respectively (Fig. 2-9). At each of these electronic energy levels the fluorophores can exist in a number of vibrational energy levels, depicted by 0, 1, 2, etc. Note that the transitions between the various electronic levels

are vertical. This presentation is used to illustrate the instantaneous nature of light absorption. This process occurs in about 10^{-15} sec, a time too short for significant displacement of nuclei to take place.

There are seven different experiments that one can in principle carry out on any given fluorescent sample. These are listed in Table 2-4. Every dye can emit fluorescence from its lowest singlet state and phosphorescence from its lowest triplet state. Both emissions may not, however, always be observable. For each of these emissions one can run an excitation spectrum by setting the emission monochromator to the appropriate wavelength and scanning the excitation monochromator. Using pulsed sources one can measure the decay profile of fluorescence or phosphorescence. By exciting the sample with plane polarized light, one can measure the steady state polarization, or with a pulsed source, the polarization decay of the sample.

Table 2-4: Types of fluorescence measurements

Steady-state	Transient
Fluorescence spectra	Fluorescence decay time-resolved fluorescence spectra
Phosphorescence spectra	Phosphorescence decay time-resolved fluorescence spectra
Excitation spectra	
Polarization	Polarization decay

2.3.2.2 Merits of Fluorescence Methods

It is well known that fluorescence techniques have many advantages over the traditional methods such as UV spectroscopy [Tazuke and Winnik, 1986].

1) High Sensitivity

Fluorescence can be measured with a great sensitivity. Micromolar quantities of dye can be studied with ease; nanomolar concentrations are within the scope of the method. One can also work with tiny amounts (micrograms) of a sample if necessary. This is why fluorescence methods have been such powerful tools in biological systems where the available amount of a sample is often extremely limited [Wehry, 1976; Chen and Edelhoch, 1985; Lakowisz, 1999]. High sensitivity is essential to reduce the amount of externally added probe or label since these are, of course, contaminants in the system. Fluorescence methods allow one to do meaningful experiments with dyes added at the 1-100 ppm level in the sample.

2) Versatility of Information

Fluorescence method can provide a broad spectrum of information about a particular system. The versatility of the method derives from three separate factors. First, there are the seven different luminescence detection experiments, which were described early in Table 2-4. Second, different dyes are capable of sensing different aspects of the system into which they are introduced. Third and perhaps most important, bimolecular quenching processes provide a battery of high resolution tools that are able to measure molecular distances and study dynamics in macromolecular systems.

2.3.2.3 Applications of Fluorescence Techniques

Fluorescence methods, such as steady-state spectroscopy, fluorescence anisotropy and

fluorescence decay measurements, have been shown quite effective in the investigation of the microscopic environment around a chromophore [Shea et al., 1990]. A few reports have been found on the application of fluorescence techniques to study polymeric gels. Various fluorescence techniques were used to study the gelation, gel swelling, or slow release of drugs from chemical or physical gels.

A fluorescence probe study was carried out by Hu et al. for a gel system [Hu et al., 1992; 1993]. They have successfully applied the fluorescence depolarization technique to investigate the volume phase transition of the acrylamide gel induced by the change in solvent composition and pH. The time-resolved and steady-state fluorescence techniques were employed to study isotactic polystyrene in its gel state where the excimer spectra were determined to monitor the existence of two different conformations in the gel state of polystyrene [Wandelt et al., 1991]. The sol-gel transition in a free radical cross-linking copolymerization process was observed in situ by Pekcan et al. by using the steady-state fluorescence technique [Pekcan et al., 1994; 1996a, b]. The same technique was also applied for studying the swelling of disc-shaped poly(methyl methacrylate) gels made with different crosslinking densities [Yilmaz and Pekcan, 1998], or with different toluene contents [Pekcan and Yilmaz, 1997]. The slow release processes were studied in these gels with various solvents [Pekcan and Erdoğan, 2002]. Pyrene was used as a fluorescence probe in all these experiments and its steady-state fluorescence emission intensity was recorded to monitor the above processes. A single photon counting method in conjunction with direct energy transfer was used to study the diffusion of a dye labeled poly(methyl methacrylate) sterically stabilized by polyisobutylene, where the mean lifetimes of fluorescing donor molecules were measured to monitor molecular diffusion [Pekcan, 1993; 1996].

2.4 Application of Surfactants in Controlled Drug Release

Surfactants and polymers are extensively used as excipients in drug delivery. Surfactant and polymer systems play an important role in modern drug delivery, where they may allow the control of the rate of drug release, enhance effective drug solubility, minimize drug degradation, contribute to reduced drug toxicity and facilitate control of drug uptake [Malmsten, 2002].

2.4.1 Introduction to Surfactants

Surfactants are low to moderate molecular weight compounds which contain one hydrophobic part, which is generally readily soluble in oil but sparingly soluble or insoluble in water, and one hydrophilic (or polar) part, which is sparingly soluble or insoluble in oil but readily soluble in water (Fig. 2-10). Due to this “schizophrenic” nature of surfactant molecules, they experience suboptimal conditions when dissolved molecularly in aqueous solution. If the hydrophobic segment is very large the surfactant will not be water-soluble, whereas for smaller hydrophobic moieties, the surfactant is soluble, but the contact between the hydrophobic block and the aqueous medium nevertheless are energetically less favorable than the water-water contacts.

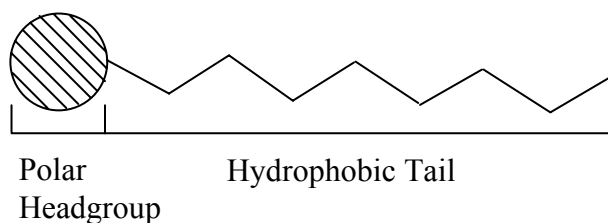


Figure 2-10: Schematic illustration of a surfactant molecule

Alternative to a molecular solution, surfactants could accumulate at the air-water interface (Fig. 2-11), where the contact between the hydrophobic group and the aqueous surroundings is reduced, therefore offers ways for the system to reduce its free energy. Consequently, surfactants are surface active, and tend to accumulate at various interfaces, where the water contact is reduced.

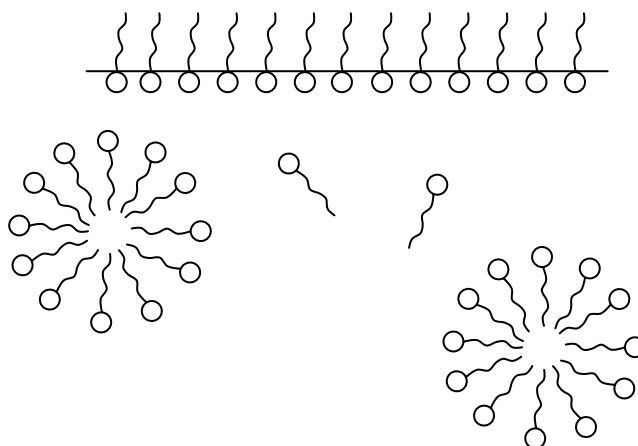


Figure 2-11: Schematic illustration of the adsorption of surfactants at the air-water interface and the formation of micelles

Another way to reduce the oil-water contact is self-assembly, through which the hydrophobic domains of the surfactant molecules can associate to form various structures, which allow a reduced oil-water contact. Various such structures can be formed, including micelles, microemulsions, and a range of liquid crystalline phases. The type of structures formed depends on a range of parameters, such as the size of the hydrophobic domain, the nature and size of the polar head group, temperature, salt concentration, pH, etc. Through varying these parameters, one structure may also turn into another, which offers interesting opportunities in triggered drug delivery.

2.4.2 Classification of Surfactants

Surfactants are classified according to their polar headgroup, i.e., surfactants with a negatively charged headgroup are referred to as anionic surfactants, whereas cationic surfactants contain polar headgroups with a positive charge. Uncharged surfactants are generally referred to as nonionic, whereas zwitterionic surfactants contain both a negatively charged and a positively charged group (Fig. 2-12).

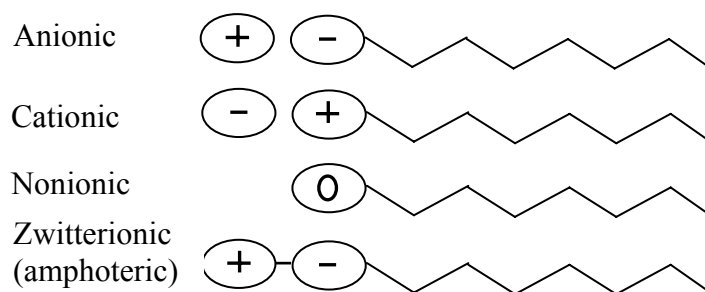


Figure 2-12: Schematic illustration of various types of surfactants

1) Anionic Surfactants

Anionic surfactants constitute the largest group of available surfactants. Examples of such surfactants include fatty acid salts (“soaps”), sulfates, ether sulfates and phosphate esters.

A common feature of all anionic surfactants is that their properties, e.g., surface activity and self-assembly, are quite sensitive to salt, and particularly divalent or multivalent cations. A commonly experienced illustration of this character is the poor solubility, foaming and cleaning efficiency of alkyl sulfate surfactants in salt or hard water.

Naturally, this salt dependence also offers opportunities in drug delivery. Sulfates are also somewhat sensitive toward hydrolysis, particularly at low pH, but are still one of the most frequently used anionic surfactant types in drug delivery.

2) Cationic Surfactants

Cationic surfactants are frequently based on amine-containing polar headgroups. Due to their charged nature, the properties of cationic surfactants, e.g., surface activity or structure formation, are generally strongly dependent on salt concentration, and on the valency of anions present. Cationic surfactants are frequently used as antibacterial agents, which may be advantageous also in certain drug delivery applications, such as delivery systems to the oral cavity. However, cationic surfactants are frequently irritant and sometimes even toxic, and therefore their use in drug delivery is significantly more limited than that of nonionic, zwitterionic, and anionic surfactants.

3) Nonionic Surfactants

Nonionic surfactants, i.e., surfactants with an uncharged polar headgroup, are probably the ones used most frequently in drug delivery applications, with the possible exception of phospholipids. In particular, nonionic surfactants used in this context are often based on oligo(ethylene oxide)-containing polar headgroups. Due to the uncharged nature of the latter, these surfactants are less sensitive to salt, but instead quite sensitive to temperature, which may be used as a triggering parameter in drug delivery with these surfactants. The critical micellization concentration for such surfactants is generally much lower than that of the corresponding charged surfactants, and partly due to this,

such surfactants are generally less irritant and better tolerated than the anionic and cationic surfactants.

4) Zwitterionic Surfactants

Zwitterionic surfactants are less common than anionic, cationic and nonionic ones. Frequently, the polar headgroup consists of a quarternary amine group and a sulfonic or carboxyl group. Due to the zwitterionic nature of the polar headgroup, the surfactant charge changes with pH, so that it is cationic at low pH and anionic at high pH. Due to the often low irritating properties of such surfactants, they are commonly used in personal care products.

2.4.3 Applications in Drug Release

Surfactants are useful as excipients in drug release formulations for a number of reasons [Malmsten, 2002]. For example, through solubilization in the core of the micelles or other types of surfactant self-assembled structures, the effective solubility of a hydrophobic drug may be increased, its hydrolytic degradation decreased, its toxicity decreased, and its bioavailability improved. Also, the properties of the surfactant systems can allow advantageous effects relating to the drug release rate, extent and selectivity of drug uptake, etc.

The most important applications of surfactants in controlled drug release concentrate on two aspects: as solubilizer [De Oliveira and Chaimovich, 1993; Kreuter, 1994; Kakemi et al., 1993] to solubilize hydrophobic and sparingly soluble drugs or as the release

controller [Paulsson and Edsman, 2001a, b] by using the interaction between the surfactant and the polymeric drug carrier. The sustaining effect of surfactants on the drug release was proved by Paulsson and Edsman [2001a, b] by studying the release of several charged or uncharged drugs from polymers in the presence of nonionic Brij 58, the anionic sodium dodecyl sulfate (SDS) and the cationic benzyldimethyldodecyl ammonium bromide (BAB). A novel stimuli-responsive drug delivery system consisting of anionic surfactants and poly(N-isopropylacrylamide) (PNIPAAm) was prepared by Eeckman et al. [2003]. The surfactants was used to modify the dissolution properties of PNIPAAm and of a copolymer with N-vinyl-acetamide (NVA), and so to induce the release of a drug contained in the compression coated tablets. During the last decade in particular, there has been a strong push to develop new surfactants of low toxicity and high biodegradability, particularly from renewable resources. In particular, carbohydrate polar headgroups have been found to be interesting in this respect [Malmsten, 2002].

Based on the above reviews, it has been known that both hydrogels and surfactants find numerous applications in controlled drug release. However, there is lack of reports on studies that incorporate hydrogel and surfactant into one release system. The present study aims to fill in this gap. Incorporating surfactants into the hydrogel drug release system can help to: (i) improve the administration and release capabilities of the hydrogel system for hydrophobic drugs; (ii) enhance the solubility of poorly water-soluble drugs and increase the miscibility between the drug and the hydrogel network; (iii) achieve a better control on the release of drugs based on the interaction between surfactant and hydrogel network.

Chapter 3

Materials and Experimental Details

3.1 Materials

The polymers used in this work were purchased from Sigma-Aldrich (Singapore). The name, commercial product number, molecular weight and chemical structure of these polymers are given in Table 3-1.

Table 3-1: Commercial information of polymers used in this study

Polymer	Product No.	Molecular Weight	Chemical Structure
Agar	05039	3000 ~ 9000	
Agarose	A5093	~12000	
K-Carrageenan	22048	~30000	
Gelatin	48724	~10000	

Other materials purchased from Sigma-Aldrich include xanthan gum (Product No. 95465), theophylline (TPH, $C_7H_8N_4O_2$, Mw. 180.2, anhydrous, 99%, powder), (s)-(+)-camptothecin (CPT, $C_{20}H_{16}N_2O_4$, Mw. 348.35, 95% HPLC, powder), dimethylsulfoxide (DMSO, >99.8%, GC), sodium dodecyl sulfate (SDS, $\geq 99\%$ GC), sodium dodecylbenzenesulfonate (SDBS, approx. 80%), sodium decyl sulfate (SDeS, $\geq 99\%$), triton x-100, dodecyltrimethylammonium bromide (DTAB, $\geq 98.5\%$), cetyltrimethylammonium bromide (CTAB, 99%), α -cyclodextrin (α -CD, $\geq 98.0\%$ HPLC, $C_{36}H_{60}O_{30}$, 972.84), hydrochloric acid (HCl, 1 N), sodium hydroxide solution (NaOH, 1 N) and poly (sodium 4-styrenesulfonate) (PSS; average Mw. ca. 70,000). Pluronic F127 and 25R4 were kindly supplied by BASF. All solvents and chemicals were used as received without further purification unless otherwise mentioned. Deionized (DI) water was obtained by the Millipore purification system (Alpha-Q: CPMQ004R1).

3.2 Selection of Materials

The selection of materials in this study has been focused on polymeric materials that are able to form hydrogels in aqueous solutions, especially those with natural origins. Hydrogels are always preferred in controlled drug release systems due to their established advantages over other materials, which have been reviewed in details in Chapter 2. Hydrogels from natural origins will further enrich these advantages.

Four natural polymers, agar/agarose, gelatin, κ -carrageenan and xanthan gum have been examined. Hydrogels from each of them were prepared and properties of these hydrogels were compared.

3.2.1 Hydrogel Preparation

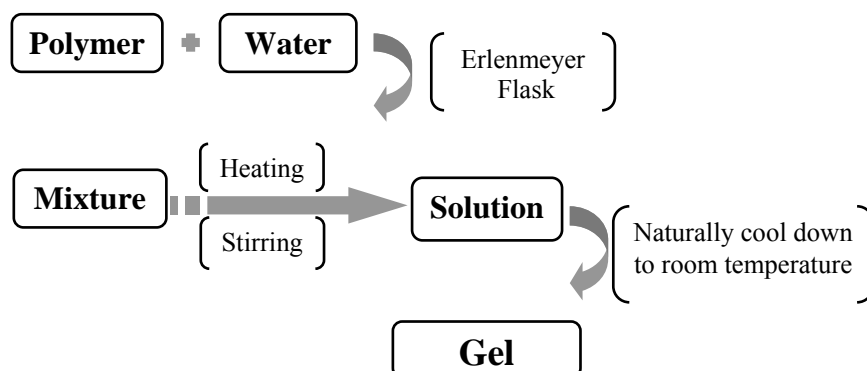


Figure 3-1: Schematic procedure for hydrogel preparation

The procedure for the preparation of hydrogels from agar/agarose, gelatin, κ -carrageenan and xanthan gum was illustrated in Fig. 3-1. A certain amount of one of these polymers was dispersed in DI water and then slowly heated with stirring till the solid powder dissolved. The resulting solution was allowed to cool down naturally at room temperature (25 °C) to form a gel.

3.2.2 Property Examination

Table 3-2 summarizes some basic properties of the four polymers and their resultant hydrogels. Agar, κ -carrageenan and gelatin all have shown certain abilities to be used as the hydrogel carrier for release of drugs. Among them, agar may be the best candidate. Agar is readily soluble in water, easy to form a gel and the resultant hydrogel is stable both at room temperature and in hot water (40°C).

The solubility of xanthan gum in water is too low to form a hydrogel.

Table 3-2: Basic properties of various materials and their hydrogels

Materials	Agar/Agarose	Gelatin	κ -carrageenan	Xanthan gum
Solubility in H ₂ O at 25 °C	15 mg/ml	67 mg/ml	3 mg/ml	Sparely soluble
Gel Point	~ 36 °C	~27 °C	~30 °C	—
Stability at room temperature for 30 days	Stable	Dehydrate	Stable	—
Stability in hot water (40 °C)	Stable	Dissolved quickly	Dissolved after ~7 h	—

3.3 The Spectral Properties of CPT

The drug that has been studied in this work, camptothecin (CPT), is a potent cytotoxic alkaloid possessing a broad spectrum of activity against various cancers.

3.3.1 Preparation of CPT Solutions

CPT (3.48 mg) was dissolved in 50 ml NaOH (0.01N solution), which was obtained previously by 100 times dilution of a 1 N NaOH solution. CPT has a good solubility in basic solution, so the resultant solution was clear and homogenous, with a concentration of CPT of 2×10^{-4} M. This solution was subsequently used as the stock solution to prepare CPT solutions at various pH values. The pH of the solution was adjusted by adding HCl (0.01N) or NaOH (0.01N) and deionized water, the final concentration of CPT was fixed at 1×10^{-5} M. The details for the preparation were given in Table 3-3.

Table 3-3: The preparation of CPT aqueous solutions at various pH values

Sample	Stock Solution (ml)	HCl		NaOH		DI water (ml)	pH Value	CPT Conc. (mM)
		1 N (ml)	0.01N (ml)	1N (ml)	0.01N (ml)			
1	0.50	1.00	0.50			8.00	1.4	0.01
2	0.50		1.50			8.00	3.5	0.01
3	0.50		0.70		0.16	8.64	5.3	0.01
4	0.50		0.50			9.00	6.7	0.01
5	0.50		0.76		0.30	8.44	7.4	0.01
6	0.50		0.50		0.10	8.90	7.8	0.01
7	0.50				0.50	9.00	10.1	0.01
8	0.50		0.50	1.00		8.90	12.9	0.01

3.3.2 Spectroscopic Measurements

The UV-vis absorption spectra were measured at 25 °C using an Agilent 8453 UV-vis spectrometer equipped with thermostated quartz cells (1.0 cm path length). Fluorescence spectra were recorded at 25 °C on a Shimadzu-RF5301PC fluorometer equipped with a jacketed cuvette holder. Both the fluorescence emission and excitation spectra of the samples were measured with the front-face geometry with an excitation wavelength of 369 nm and an emission wavelength of 440 nm, respectively. In both cases, the slit widths for excitation and emission were set to 1.5 nm. The scanning rate was set to be fast. Fluorescence decay measurements were made with an Edinburgh FLA900 single-photon-counting apparatus equipped with a hydrogen pulse lamp. Each decay profile consists of 200 channels of data, with 5000 counts in the maximum channel.

3.3.3 Calibration Curves for CPT

Before one can use UV-vis or fluorescence spectrometry as the detective method in release studies, the calibration curves for the model drug must be known. The calibrations for the UV-vis absorption and fluorescence emission of CPT were made by measuring these two parameters of a series of CPT solutions in dimethylsulfoxide (DMSO), in which the drug has a very good solubility so that the preparation of high concentration stock solution is possible. The stock solution of 4.02 mg CPT in 50 ml DMSO was prepared and the calibration solutions were obtained by dilution. The UV-visible and fluorescent measurements of these solutions were made in the same way as described in the previous section.

3.4 Release of CPT from Agar Hydrogel

3.4.1 Hydrogel Preparation

Cylinder-shaped hydrogel of agar (1 g agar/ 19 g DI water), which contained 3.48 mg CPT (1×10^{-5} M), was prepared. The inner diameter of the cylindrical mold was 22.80 ± 0.02 mm, which represents the diameter of the resultant gel sample. CPT was loaded by physically mixing the CPT solution with the agar gelling solution. This method of drug loading is commonly employed in drug delivery studies [Andreopoulos et al., 2001; Guerra et al., 2001]. The mixture was then heated with stirring to around 90 °C until an optically clear solution was obtained. The temperature was subsequently reduced to 60 °C and held at 60 °C for 1 hour [Lead et al., 2003]. The resultant solution was slowly

poured into a pre-heated (60 °C) cylindrical mold to allow a natural cooling of the solution to room temperature (25 °C). The solution gelled at around 36 °C. Identical disk-shaped release samples (thickness = 2.5 mm) were cut from the cylindrical gel for the subsequent release experiments at different temperatures. The weights of these disks were measured to estimate the accurate amount of drug CPT in each of them.

3.4.2 Release Experiments

The release of CPT from the agar hydrogel was performed in DI water at different temperatures (23, 30, 37 or 44 °C). In all experiments, identical disc-shaped hydrogel samples (volume $\sim 1 \text{ cm}^3$) were used. At each temperature, the sample was totally immersed in a bottle containing 50 ml of DI water so that a perfect sink condition can be assured [Ritger and Peppas, 1987a]. At certain time intervals (typically, every half an hour in the first two hours' release and every one hour later on), 3 ml solution was taken out from each release system (i.e. the surrounding releasing medium) for fluorescence measurements and the same volume of fresh DI water, which was previously kept at the same temperature as the release system, was added in order to maintain a constant volume of the surrounding solution.

3.4.3 Fluorescence Measurement of the Release Solution

The amount of drug released at time t was determined by measuring the fluorescence intensity of the solutions using the Shimadzu-RF5301PC fluorometer. All measurements were made with the front-face geometry with an excitation wavelength of 369 nm and the slit widths for both excitation and emission were set to 1.5 nm. The

emission intensities at 440 nm were recorded. Because the calibration curve for the fluorescence emission of CPT was known previously, the above measurements enabled the drug amount released at time t to be known.

3.5 Anionic Surfactants - Aided Solubilization, Immobilization and Controlled Release of CPT

One of the biggest problems that limit the therapeutic utility of CPT is its poor water solubility, which is mainly due to the hydrophobic property of the drug molecule. To overcome this limitation, amphiphilic surfactants were introduced. CPT was first solubilized in the micellar solutions of surfactant and then loaded into hydrogels together with the surfactant micelles. The release of CPT from these hydrogel-surfactant systems was studied.

Two anionic surfactants, SDS and SDBS were first investigated. Similar work has been done to them. SDS will be taken as an example to explain the experimental details.

3.5.1 Solubilization of CPT with SDS

The SDS aqueous solutions of different concentrations (0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 wt%) were prepared. Since the critical micellization concentration (CMC) of SDS in water is 8.3 mM (about 0.24 wt%), the SDS concentrations used were expected to cover the range from unimers to micelles of SDS. 3.50 mg of CPT was added to 10 ml of these SDS solutions. 3.50 mg is an amount that far exceeds the solubility of CPT in these solutions so that saturation solubilization can be assured. The mixtures were

stirred at room temperature at 700 rpm (RCT basic, IKA[®] Works, Inc.) for 48 hours. After equilibrium, the mixtures were centrifuged at 10000 rpm, 25 °C (IEC Multi-RF, Thermo IEC) for 30 minutes and then the upper solutions were filtered through a nylon syringe filter (pore size 0.20 µm, Whatman Inc. USA). In the centrifugation and filtration processes, the concentration of SDS was assumed to be unchanged because of two reasons: (1) SDS has a good solubility in water, and (2) the syringe filter has much bigger pore sizes (0.20 µm) than the micellar sizes of SDS. The clear solutions obtained were subject to UV-vis measurements using the Agilent 8453 UV-vis spectrometer. The UV-vis absorption at 369 nm was measured at 25 °C. Then the amount of CPT solubilized in each solution could be obtained using the UV-vis calibration curve for CPT developed previously.

Dark-brown glassware was used throughout the solubilization processes to avoid any negative effect due to the possible photodegradation of the drug [Lown and Chen, 1980].

3.5.2 Surface Tension Measurement

A DataPhysics[®] Tensiometer (Model: DCAT21) was used to measure the surface tension of SDS in the presence of CPT at room temperature (25 °C). SDS solution (2.0 wt%) was prepared and used as the stock solution. The stock solution was titrated slowly to 50 ml saturated CPT aqueous solution and the surface tension was measured as a function of the concentration of SDS. The critical micellization concentration (CMC) was determined from the semi-logarithmic plot of the surface tension versus the SDS concentration.

3.5.3 Fluorescence Measurement

The fluorescence emission spectra of the CPT-SDS solution were recorded using the Shimadzu-RF5301PC fluorometer. The measurement was made with the front-face geometry with an excitation wavelength of 369 nm and the slit widths for both excitation and emission were set to 1.5 nm.

3.5.4 Hydrogel Preparation

The agarose hydrogels (3.0 wt%) used in this study as the drug carrier, which contains appropriate amounts of CPT and SDS, were prepared as follows. The homogeneous solution of CPT-SDS was first prepared and mixed with a certain amount of agarose by stirring. The mixture of agarose, CPT and SDS was then slowly heated to around 90 °C until an optically clear solution was obtained. Gentle stirring was needed in the heating process in order to avoid bubbles in the final gelling solution. The resultant solution was poured into a home-made flat mold (square shape, 0.98 mm in thickness), which was kept in an oven at 90 °C before use. The warm polymer solution in the mold was allowed to cool down naturally at room temperature (25 °C) to form a sheet hydrogel. The agarose hydrogels containing different concentrations of SDS were prepared. The sheet hydrogels were then cut into small samples, whose dimensions allowed them to fit in the UV-vis measurement cell (10 mm × 10 mm).

3.5.5 Drug Release Experiments

The drug-loaded gel sample was placed into the UV-vis cell, and 3.0 ml of DI water was

dropped in serving as the release medium. The gel sample was kept in the bottom region of the cell, which was supported by a thin steel frame. It was ensured that the positions of the sample and the frame were always below the position where the UV-vis beams transit, so that the UV-vis absorbance measurements would not be affected. The release system was kept at 37 ± 0.1 °C maintained by the temperature-control unit of the spectrometer. The release of CPT was determined in situ by measuring the UV-vis absorbance at a time interval of 10 minutes.

In addition to 37 °C, the release at the temperatures of 23 °C, 30 °C and 44 °C was also carried out.

3.6 Cationic Surfactants - Aided Solubilization, Immobilization and Controlled Release of CPT

3.6.1 Effect of DTAB

3.6.1.1 Solubilization of CPT with DTAB

The solubilization of CPT in DTAB aqueous solutions followed the procedure described in the Section 3.5.1. A saturated solution of CPT was prepared by adding extra amount of CPT. After stirring for 48 hours, subsequent centrifugation and filtration with a 0.45 µm nylon filter, clear solutions of CPT solubilized in DTAB micelles were obtained. The concentrations of DTAB were assumed to be not changed in the above procedure because of two reasons: (1) DTAB has a good solubility in water, and (2) the syringe filter had much bigger pore sizes (0.45 µm) than the micellar sizes of DTAB.

Dark-brown glassware was used throughout the solubilization processes.

3.6.1.2 UV-vis and Fluorescence Measurement

The clear solutions obtained in the above step proceeded to the UV-vis and fluorescence measurements. The UV-vis absorption at 369 nm was recorded. The amount of CPT solubilized in each DTAB solution was determined by referring to the calibration curve of CPT. The fluorescent measurement was made at the front surface of the solutions at 45° with an excitation wavelength of 369 nm and the slit widths for both excitation and emission were set at 1.5 nm.

3.6.1.3 Hydrogel Preparation

Two types of hydrogels were prepared. In the first type of hydrogels, agarose was mixed with the previously prepared CPT-DTAB solution to make hydrogel in a similar procedure as that described in the preparation of an agarose-SDS hydrogel. The agarose concentration was 3.0 wt% and concentrations of DTAB were 10 mM to 50 mM. In the second type of hydrogel samples, κ -carrageenan was added. The resultant hydrogels were therefore named as agarose-DTAB- κ -carrageenan (A-d- κ for abbreviation), in which the concentrations of agarose and DTAB were fixed at 3.0 wt% and 40 mM, respectively, and the concentration of κ -carrageenan varied from 0 wt% to 0.50 wt%. The designation and composition of all the hydrogel samples were summarized in Table 3-4. Small square samples (approx. 0.1 gram in weight, 0.98 mm in thickness) were cut from the sheet hydrogels, whose dimensions were appropriate to be put in the UV measurement cell ($10 \times 10 \text{ mm}^2$).

Table 3-4: The feed composition and sample designation for the preparation of agarose hydrogels with DTAB and κ -carrageenan (“A” stands for agarose, “d” for DTAB and “ κ ” for κ -carrageenan).

Hydrogels	Gel Composition		
	Agarose	κ -Carrageenan	DTAB solution (with CPT solubilized in)
<i>for DTAB effect</i>			
A-d10	3 wt%	0	10 mM
A-d30	3 wt%	0	30 mM
A-d50	3 wt%	0	50 mM
<i>for κ-carrageenan effect</i>			
A-d- κ 0	3 wt%	0	40 mM
A-d- κ 0.25	3 wt%	0.25 wt%	40 mM
A-d- κ 0.50	3 wt%	0.50 wt%	40 mM

3.6.1.4 Drug Release Experiments

The small square sample of drug-loaded hydrogel was placed in the bottom of the UV-vis measurement cell. 3.0 ml of DI water was dropped in serving as the release medium. It was ensured that the position of the gel sample was always below the transiting UV-vis beam so that the UV-vis absorbance measurements would not be affected. The release temperature was set at 37 ± 0.1 °C and maintained by the temperature-control unit of the UV-vis spectrometer. The release profiles of CPT from the hydrogel in the presence of DTAB were determined in situ by measuring the UV-vis absorbance at a time interval of 10 minutes.

3.6.2 Effect of CTAB

3.6.2.1 Solubilization of CPT with CTAB

CPT (10.0 mg) was added to 50 ml of CTAB water solution (10 mM). 10.0 mg was chosen after performing a saturation test and it turned out to be high enough to ensure a saturation solubilization of CPT. The concentration of CTAB in the solution exceeded the CMC of CTAB, reported to be 0.92 mM in H₂O, and therefore micelles of CTAB were confirmed to exist in the above solution. The mixture was first sonicated (TRU-SWEEP™ ultrasonic cleaner. Model: 175TAG) for 1 hour and subsequently stirred at room temperature at 700 rpm (RCT basic, IKA® Works, Inc.) for 3 days. Since the drug tends to aggregate in aqueous solutions, the sonication process was believed to be able to destroy the aggregates of the drug and promote a better dissolution. After 3 days' stirring, the mixtures were centrifuged and filtered (0.45 µm nylon filter). The clear solution obtained was subsequently investigated by UV-vis measurements. The amount of CPT solubilized was then obtained. The resultant solution of CPT-CTAB was diluted 4 times using the 10 mM CTAB solution for further use.

3.6.2.2 Hydrogel Preparation

The CPT-CTAB solution (CTAB 10 mM, CPT 0.122 mM) obtained in the above step was used to prepare the hydrogels. Certain amounts of agarose (final concentration in the hydrogel samples was 3.0 wt%) and κ-carrageenan (0 wt% ~ 0.3 wt%) were added to the solution of CTAB-CPT. The subsequent procedure was similar to those described previously. Hydrogels with different compositions were prepared as shown in Table 3-5.

Table 3-5: The feed composition and sample designation for the preparation of agarose hydrogels with CTAB and κ -carrageenan (“A” stands for agarose, “c” for CTAB and “ κ ” for κ -carrageenan).

Gels Designation	Gel Composition			
	Agarose	κ -Carreangen	CTAB-CPT solution Used	
			CTAB (mM)	CPT (mM)
A-c- κ 0	3 wt%	0	10	0.122
A-c- κ 05	3 wt%	0.05 wt%	10	0.122
A-c- κ 10	3 wt%	0.10 wt%	10	0.122
A-c- κ 20	3 wt%	0.20 wt%	10	0.122
A-c- κ 30	3 wt%	0.30 wt%	10	0.122

3.6.2.3 Drug Release Experiments

Uniform disk-shaped sample (0.98 mm in thickness; approximately 13 mm in diameter) were cut from hydrogels prepared in the previous step. Each disk was accurately weighed in order to determine the exact amount of drug incorporated in it. The measurement cell from the UV-vis spectrometer was used as the release container so that the release behavior of the drug can be monitored in situ by UV-vis measurement. DI water (3.0 ml) was added to the cell serving as the release solution and the hydrogel sample was immersed in the solution yet kept at the upside of the cell, which was supported by a thin steel frame. It was ensured that both the gel sample and the frame do not affect the UV-vis measurements. The release system was kept at 37 ± 0.1 °C maintained by the temperature-control unit of the UV-vis equipment. The release of CPT was determined by measuring the UV-vis absorption at 369 nm of the release

solution in the cell at a time interval of every 20 minutes.

3.7 Solubilization of CPT in other Surfactants and Additives

More surfactants, including both small molecular and polymeric surfactants, and cyclodextrins (CD), including α -CD, β -CD and γ -CD were examined for their effect on the solubility of CPT. The solubilization of CPT in these agents followed the typical procedure of solubilization, which has been described in details in previous sections.

3.8 Release of TPH from Blend Hydrogels

The release properties of both single-component and two-component blend hydrogels from agar, gelatin, and κ -carrageenan were investigated. Theophylline (TPH) was used as the model drug. TPH was chosen because of several reasons: first, it is an effective drug for the treatment of asthma and pulmonary diseases [Yu et al., 1996] and it has been one of the most frequently used model drugs in controlled drug release studies [Picker, 1999a; Katime, 2001; Coviello, 2003]; second, TPH has a simple UV-vis absorbance spectrum, which is easy to determine; last, although it has a complicated chemical structure, this drug has a relatively good solubility in water, for example, a 10 mM TPH water solution can be easily prepared.

3.8.1 Extinction Coefficient of TPH

The calibration for the UV-vis absorption of TPH was done. 4.51 mg (0.25×10^{-4} mol) of this drug was dissolved in 25 ml DI water in a standard volumetric flask to obtain the

stock solution (1.0 mM). A series of calibration solutions of TPH for the UV-vis analysis were then prepared using dilutions shown in the Table 3-6. The UV-vis absorptions of these solutions were measured and plotted as a function of the concentration of TPH to obtain the calibration curve. The extinction coefficient of TPH was obtained from the slope of the curve.

Table 3-6: Solutions of TPH for calibration

Solution	A	B	C	D	E	F	G	H
Volume of Stock / ml	0.5	0.5	1	1	2	3	4	10
Final diluted volume/ ml	25	10	10	5	5	5	5	10
Concentration ($\times 10^{-4}$ M)	0.20	0.50	1.00	2.00	4.00	6.00	8.00	10

3.8.2 Preparation of Single and Blend Hydrogels

The hydrogels used were prepared from agar, gelatin, and κ -carrageenan or their two-component blends. These hydrogels served as the drug release matrices after incorporating appropriate amounts of the drug. The detailed procedure for the preparation of the hydrogel is described below:

1 gram of a gel material or a mixture of two of them was mixed with 20 ml of TPH aqueous solution (10 mM) prepared previously, so that in all the obtained gel samples, the total polymer concentration was the same, i.e. 4.8 wt%. For the single-polymer systems, the mixture (polymer and TPH solution) was slowly heated to around 60, 80 or

90 °C for the gelatin, κ -carrageenan and agar hydrogels, respectively; for a blend hydrogel, the higher melting temperature of the two component polymers was adopted as the heating temperature. Gentle stirring was needed here in order to avoid bubbles in the final gel solutions. After stirring for about one hour, optically clear solutions were obtained. These solutions were poured into an upright placed glass syringe with top cut off (machined perpendicularly to the cylinder axis), which was kept in an oven at 90 °C before use. The warm polymer solutions in the syringe were allowed to equilibrate at ambient temperature (~ 25 °C) to form the gel.

Table 3-7: The feed composition and sample designation for the preparation of blend hydrogels from gelatin, κ -carrageenan and agar. (“G” stands for gelatin, “ κ ” for κ -carrageenan and “A” for agar)

Gels Designation	Gel Composition			TPH Solution (10 mM)
	Gelatin	κ -carrageenan	Agar	
<i>Single Hydrogels</i>				
G-type	1.0 g			20 ml
A-type			1.0 g	20 ml
κ -type		1.0 g		20 ml
<i>Blend Hydrogels</i>				
GA91	0.9 g		0.1 g	20 ml
GA73	0.7 g		0.3 g	20 ml
GA55	0.5 g		0.5 g	20 ml
G κ 73	0.7 g	0.3 g		20 ml
G κ 55	0.5 g	0.5 g		20 ml
κ A91		0.9 g	0.1 g	20 ml
κ A73		0.7 g	0.3 g	20 ml
κ A55		0.5 g	0.5 g	20 ml

The hydrogel samples based on the single polymers (i.e. gelatin, agar and κ -carrageenan) are designated as G-type, A-type and κ -type, respectively. Those prepared based on

two-component blends are named GA, Gκ and κA hydrogels, in which “G” stands for gelatin, “A” for agar and “κ” for κ-carrageenan, respectively. Table 3-7 shows the sample designation and feeding compositions for all the hydrogels prepared. For example, GA55 represents that the blend hydrogel consists of 50 wt% gelatin and 50 wt% agar. These solid hydrogels were cut into the disk-shaped samples with a sharp blade for the use of following drug release experiments.

3.8.3 Drug Release Experiments

For the drug release studies, disk-shaped hydrogel samples (2.5 mm in thickness, 23 mm in diameter and approx. 1.0 gram in weight) were immersed in 20 ml water and maintained in a thermal water bath (Polyscience G20516). The releases at different temperatures (30, 37, 45 °C) were investigated. The amount of TPH within the disk gel was far below its solubility in 20 ml water so that a sink condition can be assured. At certain time intervals, 3.0 ml solution was taken out from each release system and the amount of TPH released at that time was determined by the UV-vis spectroscopic measurement at 272 nm with the Agilent 8453 UV-visible spectrometer. After each measurement, the sample used was returned to the original medium solution in order to maintain a constant volume of the surrounding release medium.

Chapter 4

Properties of Camptothecin and Its Release from Hydrogel

Camptothecin (CPT) is a cytotoxic plant alkaloid discovered by Wall et al. [1966]. The high antitumor activity seen with this drug is directly related to the lactone ring in its molecular structure and it is already known that the structure of CPT is subject to the environmental pH. To characterize the structural change of CPT under various pHs and get a better understanding of the drug's property, the spectral characteristics of CPT, such as the UV-vis absorption, the steady-state fluorescence emission and excitation, and the time-resolved fluorescence emission, were studied in this chapter. In addition, the calibration curves for the UV-vis absorption and steady-state fluorescence emission of CPT were developed.

The feasibility of using agar hydrogel to load and release CPT was investigated. The release kinetics was studied under different temperatures. Steady-state fluorescence spectrometry was used as a detective technique to monitor the release process. The amount of drug released at time t was evaluated by using the previous calibration curve. Two mathematic models based on the well-known Fickian diffusion equation were employed to fit the release results and the diffusion coefficients of the drug molecule were therefore obtained. The relationship between diffusion coefficient and the external temperature was produced and the mechanism for the release of CPT from hydrogel formulation was discussed.

At this stage of the release study of CPT, no other additives, for example surfactants,

were included in the release system.

4.1 Spectral Properties of CPT

Discovery of the antitumor plant alkaloid, camptothecin (CPT, Fig. 4-1A) goes back as far as 1966 [Wall et al., 1966]. It aroused a great deal of interest immediately because of its strong cytotoxic activity against a variety of experimental tumors in vitro and in vivo [Wall et al., 1966; O'Leary and Muggia, 1998]. CPT and its various derivatives (known as camptothecins) show a broad spectrum of activity against various cancers, including human lung, prostate, breast, colon, stomach, and ovarian carcinomas, as well as melanoma, lymphomas, and sarcomas [Dancey and Eisenhauer 1996; Potmesil and Pinedo 1995; Takimoto et al. 1998]. Unlike many other antineoplastic agents, which inhibit cancer cell proliferation by binding to DNA, the CPTs act by binding to the topoisomerase I-DNA complex, leading to an accumulation of DNA strand breaks upon replication, ultimately causing cell death [Hsiang et al., 1985].

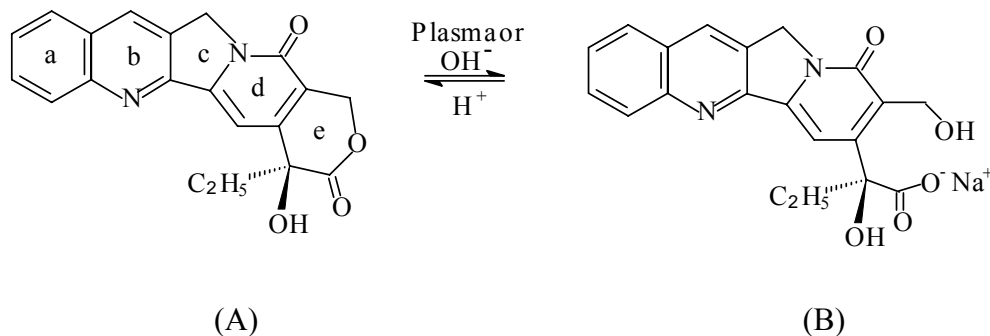


Figure 4-1: Chemical structure of (A) lactone form and (B) carboxylate form of camptothecin (CPT). (“a”, “b”, “c”, “d” and “e” indicate the five rings of CPT)

Despite the high antitumor activity noted *in vitro* and *in vivo*, the full therapeutic potential of CPT has not yet been achieved [Kang et al., 2002]. This is mainly because it is practically insoluble in water, and *in vivo* and in aqueous buffer solutions ($\text{pH} > 7$) it may rapidly convert to the respective water-soluble, pharmacologically less active carboxylate form (Fig. 4-1B) [Chourpa et al., 1998; Scott et al., 1993]. The latter has been suspected as the cause of the severe toxicity observed with this agent [Moertel et al., 1972; Muggia et al., 1972]. Further studies have shown that the closed lactone ring of CPT is essential for passive diffusion into cancer cells and for interaction with the topo-I target based on the established mechanism of action [Hsiang et al., 1985; Jaxel et al., 1989]. The lactone-carboxylate conversion of CPT is reversible, 90% active CPT-lactone being present at pH 4.5 and 90% inactive CPT-carboxylate being present at pH 7.4 respectively [Slichenmyer et al., 1993].

4.1.1 UV-visible Absorption

The CPT molecule (Fig. 4-1) consists of two main aromatic nuclei, quinoline (formed by rings “a” and “b”) and pyridone (ring “d”), linked by ring “c”. To simplify the following discussion, the lactone ring “e” of CPT together with rings “d” and “c” will hereafter be referred to as a pyridone moiety.

Fig. 4-2 shows the UV-vis absorption spectra of CPT in deionized water with different pH. The absorption spectrum of CPT is characterized by two strong bands with the maxima at 370 and 355 nm, which are especially notable for acidic and neutral pH. These two bands of π - π^* nature are considered to be due to the appearance of the quinoline moiety of the drug [Chourpa et al., 1998]. A low intensity absorption band of

$n-\pi^*$ nature near 330 nm overlaps with the stronger bands and is difficult to describe.

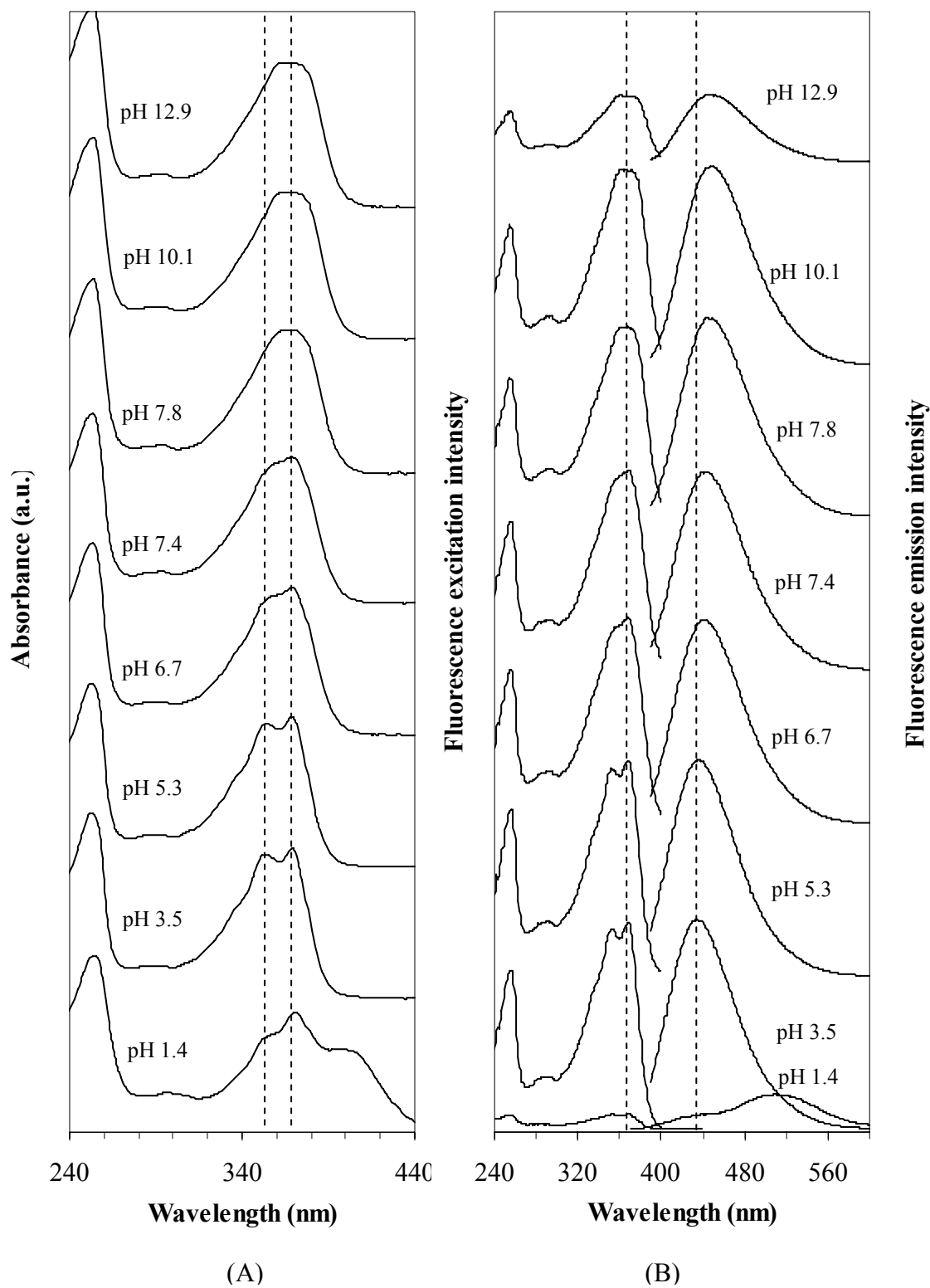


Figure 4-2: (A) UV-vis absorption and (B) steady-state fluorescence emission (right) and excitation (left) spectra of CPT at different pH.

Upon increasing the pH gradually from acidic to neutral and further to alkaline, the shifts of the peak wavelength of the two strong bands in the absorption spectra of CPT could be illustrated in Fig. 4-3.

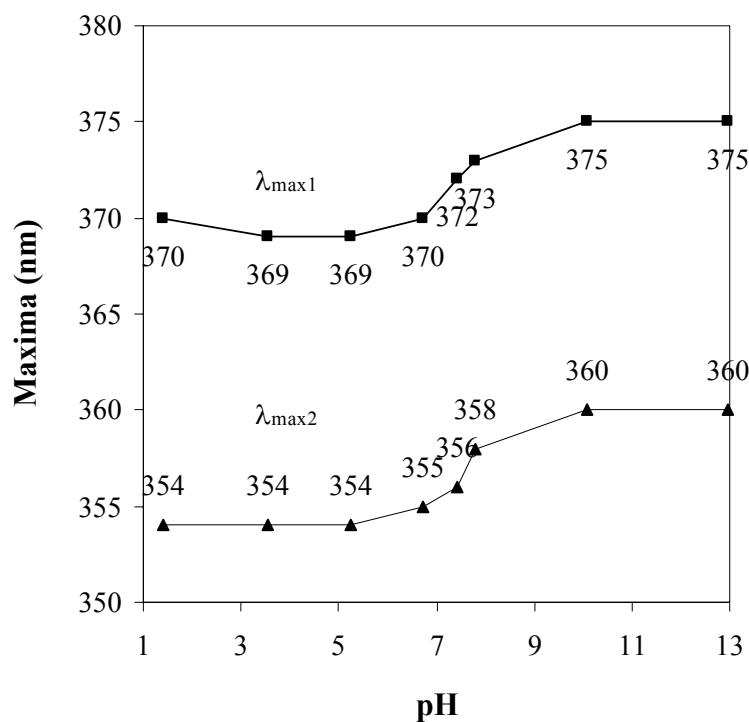


Figure 4-3: Shifts in the UV-vis absorption spectrum of CPT upon increasing the environmental pH. ($\lambda_{\max 1}$ and $\lambda_{\max 2}$ correspond to the two peaks around 370 and 355 nm, respectively)

The two strong absorption bands of CPT underwent red-shifts, $\lambda_{\max 1}$ from 369 nm at pH 3.54 to 375 nm at pH 12.95 and $\lambda_{\max 2}$ from 354 nm to 360 nm, respectively, accompanied by a slight decrease in the absorption intensity when the environmental pH increased. An exception was observed in the case of pH 1.4, in which the absorption of CPT suffered a sudden decrease and a new band around 404 nm was developed. The decrease in the absorption intensity at pH 1.4 could be ascribed, at least partly, to the aggregation of CPT molecules. At this low pH, the CPT molecules exist totally in the

lactone form [Akimoto et al., 1994] and the solubility is quite low, so the hydrophobic CPT molecules tend to aggregate in the aqueous solution. But the reason for the new absorption band at 404 nm is still not clear. What could be known about this band is that it is nonfluorescent, as is manifested in its absence in the excitation spectra (Fig. 4-2, B).

As shown above, the strongest absorption maxima of CPT in the range of 355-370 nm should be related to the quinoline nucleus. Nonetheless, these maxima are also red-shifted as the molecules of CPT going from lactone to carboxylate form upon increasing the environmental pH. This indicated that the extended conjugation of quinoline and pyridone moieties of the CPT molecules. It should be underlined that the hydrolysis-induced changes in the absorption of CPT are different from those caused by the chemical substitutions at the quinoline moiety [Chourpa et al., 1998]. Upon lactone hydrolysis the long-wavelength absorption maximum of CPT at 370-385 nm is less shifted, but more decreased in intensity than the shoulder at 355-365 nm. Generally, the hydrolysis-induced modifications of the absorption bands, related preferentially to the quinoline part of CPT (Fig. 4-1), are rather slight. The conjugation of the quinoline and pyridine moieties of the CPT molecule is slightly displayed in the absorption spectra.

4.1.2 Steady-state Fluorescence

The intrinsic fluorescence emission of CPT, remarkably sensitive to the status of the “e” ring (Fig. 4-1), can be used for a non-invasive ‘real-time’ quantification of the lactone hydrolysis in CPT [Chourpa et al., 1998].

Fig. 4-2 shows the steady-state fluorescence emission spectra of CPT at various pH as

well as its fluorescence excitation ones, which reflect the absorption properties of the fluorescent probe and usually in the same shape as its UV-vis spectra. As can be seen from Fig. 4-2, the fluorescence emission spectrum of CPT consists of only one wide fluorescence band in all the solutions used. The location of the fluorescence band is independent of the excitation wavelength.

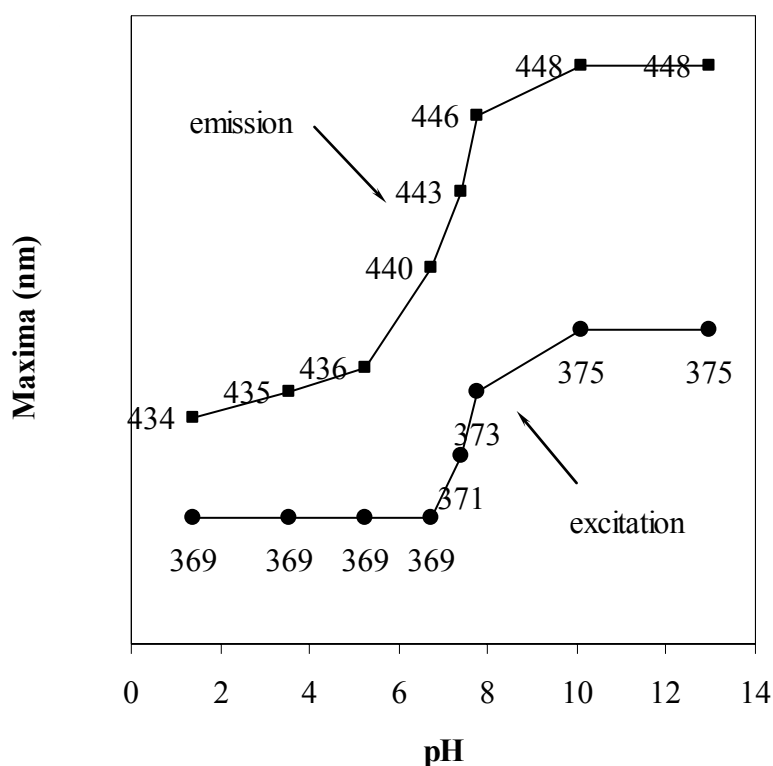


Figure 4-4: Shifts in the fluorescence emission and excitation spectra of CPT upon increasing the environmental pH.

As revealed by Posokhov et al. [2003], the fluorescence emission is more sensitive to the extended conjugation of the two aromatic nuclei of CPT. Consequently, the fluorescence emission spectra of the majority of CPT are dramatically affected by the opening of the lactone ring induced by the increase in the environmental pH. As shown in Fig. 4-4, the emission spectra of CPT are significantly red-shifted on going from their

lactone to carboxylate form with a decrease in the intensity.

It is very important that the differences in the fluorescence emission spectra of the closed and open forms of the majority of the hydrolysable CPT are well pronounced and clearly observed (Fig. 4-2). As proved by Chorupa et al. [1998], these changes in the fluorescence emission are enough to provide a good precision in the determination of the lactone versus carboxylate rate.

Exceptional spectra were observed in the cases of pH 1.4 and pH 12.9. In both of them, especially in the former one, the fluorescence intensities of both emission and excitation are very much lower than those at other pH values. Again, this was explained by the aggregation of CPT molecules. It is a common knowledge that aggregation usually leads to severe quenching of fluorescence [Mandal et al., 1999; Verkman, 1987]. The decrease in fluorescence intensity of CPT could be ascribed partly to the decrease in the population of its monomers. However, this does not seem to be the sole cause. The fluorescence spectra underwent a progressive blue shift with decrease in pH in this range. This might indicate the formation of some new fluorescing species, as explained by Datta et al. in their paper about the pH effect on the aggregation behavior of chlorine p_6 [Datta et al., 2002].

4.1.3 Time-resolved Fluorescence

Time-resolved fluorescence spectroscopy provides a measure of the time dependence of fluorescence intensity of one substance after a short excitation pulse, which can also be made as a function of emission wavelength. The time dependence of the fluorescence

emission is far more sensitive to changes in the environment of the fluorescent residues than steady-state measurements of peak emission wavelength or intensity [Lakowicz, 1999]. There are several ways to perform time-resolved fluorescence measurements. Since the time dependence of the fluorescence emission is typically on a picosecond to nanosecond time scale it is very difficult to achieve using a single laser pulse experiment. To overcome this difficulty either a frequency domain method or the single photon counting approach is used. The later is employed in this work.

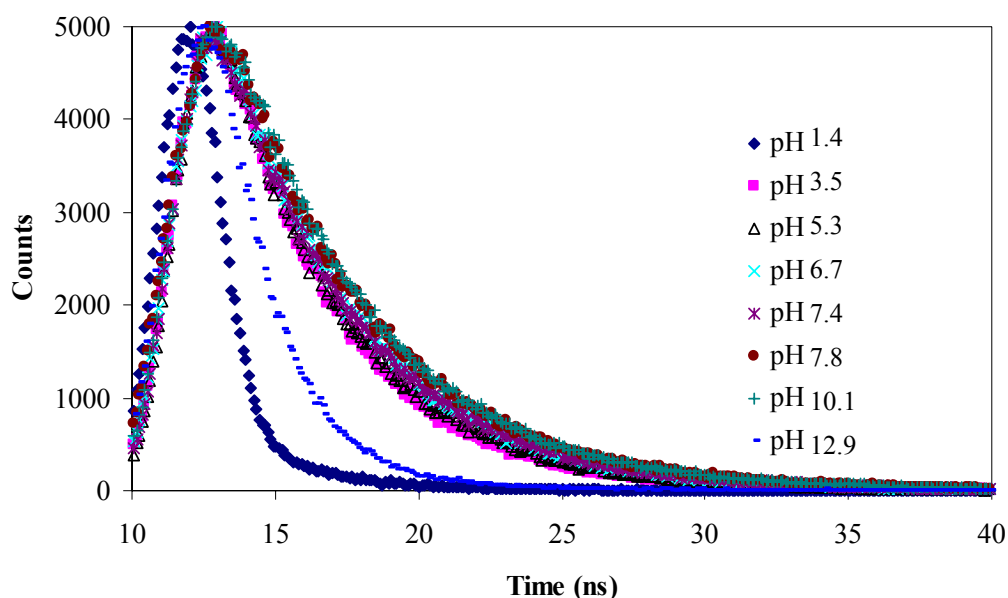


Figure 4-5: Fluorescence decays of CPT solutions (1×10^{-5} M) at pH 1.4, 3.5, 5.3, 6.7, 7.4, 7.8, 10.1, and 12.9. The excitation wavelength was 369 nm, and the emission wavelength was 440 nm.

The pH dependence of the time-resolved fluorescence traces of CPT is shown in Fig. 4-5. The decays at pH 1.4 and 12.9 are singled out. Those at pH 3.5 to 10.1 form another set of decays, with slower decay constants than the previous two. The decay at pH 1.4 is the fastest of all. These decays are fitted to a single-exponential function,

$$I = A + B \exp(-t/\tau) \quad (4-1)$$

where I is the emission intensity, A , B are constants, τ is the lifetime of the fluorescent substance and t is the time. The lifetimes of CPT at various pHs, with parameters A , B and χ^2 , are summarized in Table 4-1. Except for the extreme acidic and alkaline environments, the lifetime of the fluorescence probe showed slight and consecutive increase upon increasing the pH of the solution.

Table 4-1: Lifetimes' (τ) change of CPT upon increasing the solution pH from acidic value (1.4) to alkaline value (12.9).

pH	τ	A	B	χ^2
1.4	1.35	0.620	6175	4.398
3.5	4.09	0.742	5399	1.067
5.3	4.31	0.746	5203	0.892
6.7	4.60	0.820	5315	0.991
7.4	4.70	0.998	5327	1.075
7.8	4.92	0.729	5722	1.163
10.1	4.90	0.828	5530	1.644
12.9	2.19	0.866	6042	1.384

4.2 Calibration Curves of CPT

As non-destructive and effective methods for quantitative assay, UV-vis absorption spectrometry and steady-state fluorescence emission spectrometry are both widely used in controlled drug delivery studies [Favre and Girard, 2001; Katime et al., 2002; Pekcan and Erdoğan, 2002]. But before they can be used, the calibration curves for the UV-vis absorbance or the fluorescence emission of the studied drug must be known, which are usually based on the Lambert-Beer's Law (or Beer's Law) [Knowles and Burgess, 1984;

Lakowicz, 1999].

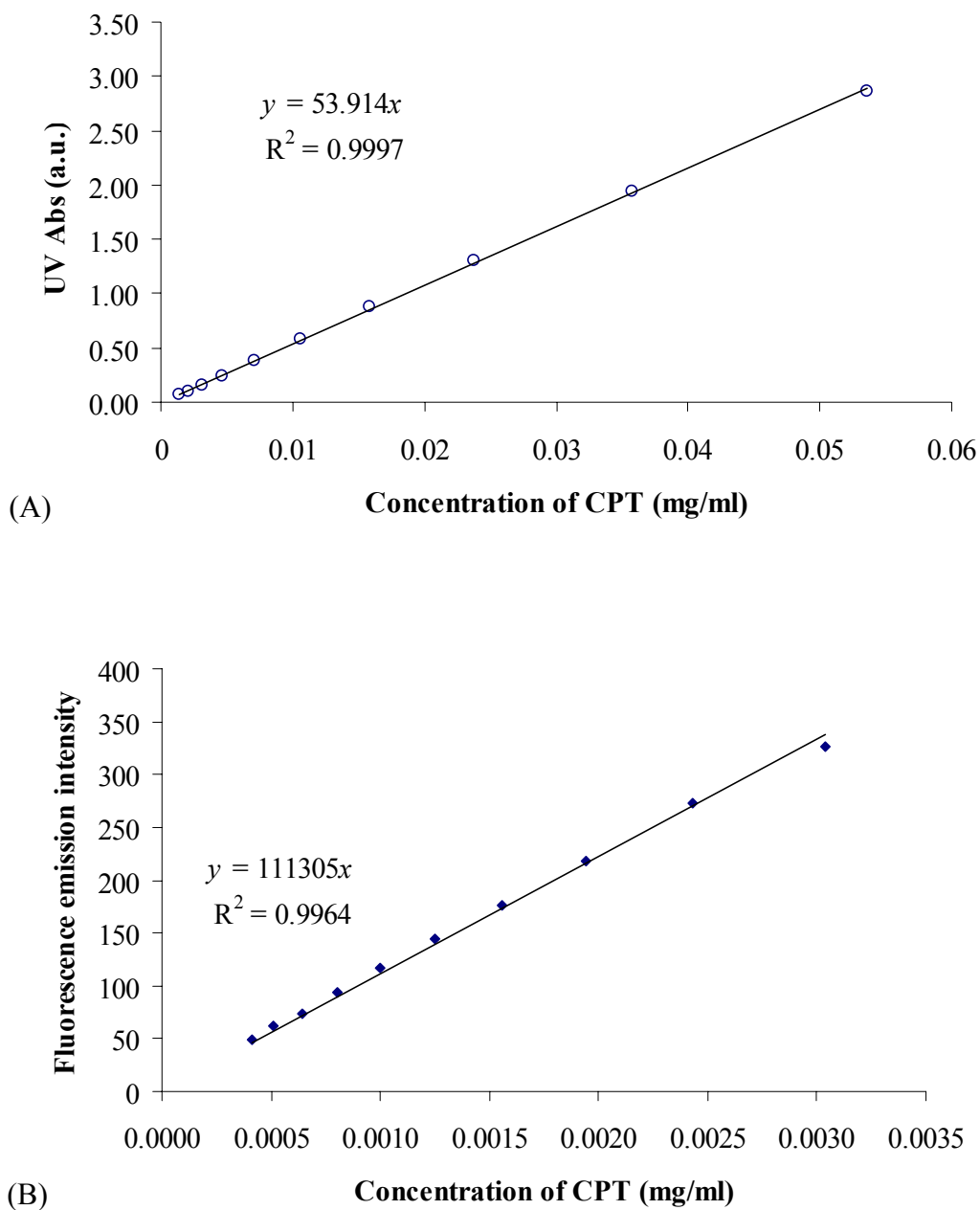


Figure 4-6: The UV-vis absorption (A) and fluorescence emission (B) calibration curves of CPT in dimethylsulfoxide (DMSO) as a function of concentration.

Fig. 4-6, A and B give respectively the UV-vis absorption and fluorescence emission

calibration curves for CPT in dimethylsulfoxide (DMSO), in which the axis Y stands for the UV-vis absorbance (A) or fluorescence emission intensity (B) and the axis X stands for the CPT concentration. In both cases, very good linear relationship could be observed, indicating the validity of the calibrations in the studied concentration ranges, where the Beer's Law is well maintained.

4.3 Release of CPT from Agar Hydrogel

By understanding the basic properties of CPT and knowing its calibration curves, the release of this drug from an agar hydrogel under a series of temperatures was performed. Steady-state fluorescence spectrometry was employed as the detective and monitoring method.

4.3.1 Release Profiles Determined by Fluorescence Spectra

In the four release experiments, which were carried out simultaneously at different temperatures of 23, 30, 37 and 44 °C, the drug molecules incorporated in the hydrogel samples would slowly diffuse out from the hydrogel matrix to the aqueous surrounding medium (deionized water in this study). As time went on, more and more drugs would diffuse out resulting in a concentration increase of the drug in the target solution. The change in the drug concentration could be reflected by the fluorescence spectra, knowing that fluorescence emission is directly related to the concentration of the fluorophore in the solution. Fig. 4-7 shows an example of the change in the fluorescence emission spectra of the surrounding solution as the release process goes on. Fig. 4-7 was obtained from the release experiment at 37 °C and represents the typical fluorescence

spectra result in any of the four release experiments.

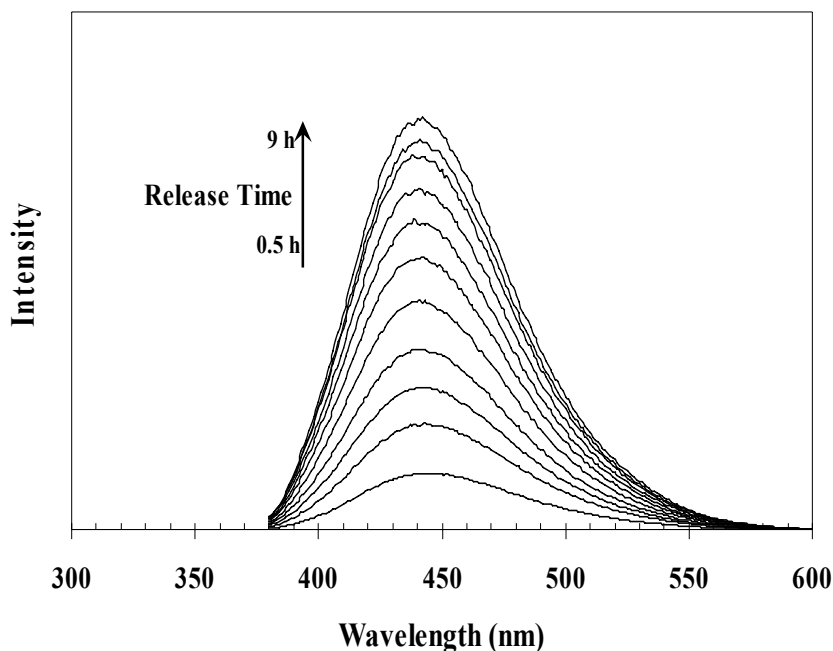


Figure 4-7: Fluorescence emission spectra of the surrounding solution in the release of CPT from the agar hydrogel at 37 °C. The measurements were made at a time interval of 0.5 h initially and 1 h later on. The lowest and highest curves were obtained with the release time of 0.5h and 9 h, respectively.

As shown in Fig. 4-7, the fluorescence emission intensity (I_f) of the surrounding solution increases with the release time. Since I_f is immediately related to the amount of the drug in the solution, the feature of the intensity curves in Fig. 4-7 suggests that the longer the release time, the more CPT molecules have been released to the surrounding solution.

As one of the most important parameters in drug release studies, temperature is considered to be able to cause a significant effect on the diffusion of the substance that is being released. The temperature-induced difference in the release profiles of the four

experiments performed under different temperatures are illustrated in Fig. 4-8 by their fluorescence emission spectra measured at a fixed release time. Samples in Fig. 4-8 were taken from the four parallel experiments when the release time was 8 hours. Similar comparative result of fluorescent curves could be observed throughout the release process except at the very early and very long release time regions, which correspond respectively to the very beginning and equilibrium situations of the release. From Fig. 4-8, it is apparent that a higher temperature led to a faster release of CPT.

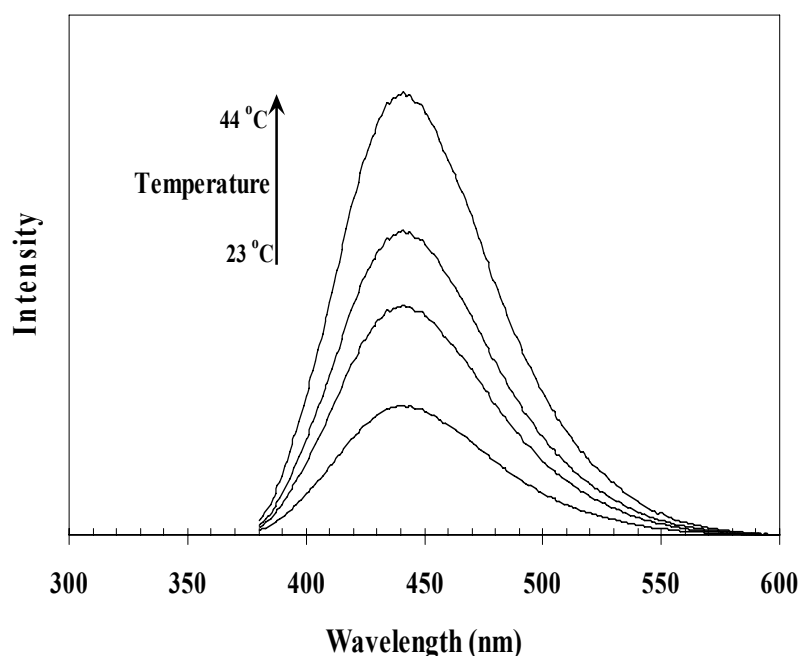


Figure 4-8: Fluorescence emission spectra of the surrounding solutions in the release experiments of CPT from agar hydrogel at 23, 30, 37 and 44 °C. The release time was 8 h. The excitation for the fluorescence spectra was done at 369 nm.

4.3.2 Relationship between M_t/M_∞ and I_t/I_∞

As found from literature [Favre and Girard, 2001; Ritger and Peppas, 1987a; Xiong et

al., 2005], the fraction (M_t/M_∞) of the amount of drug released at time t has been widely employed to interpret the drug release behaviors. Here, M_t is the amount of drug released at time t , and M_∞ is the amount of drug released at infinite long time or alternatively, the total mass of the drug incorporated in the carrier. In this work, only I_t , the emission intensity of the surrounding solution at time t and I_∞ , the emission intensity of the surrounding solution when the release behavior reached an equilibrium (i.e. the intensity approached to a certain value) could be obtained by fluorescent measurements. As suggested by Pekcan et al. [Pekcan and Erdoğan, 2002; Yilmaz and Pekcan, 1998], in a slow release process, fluorescence emission intensity I_t can be considered to be proportional to the number of the probe molecules (CPT in this work) in the solution, i.e. M_t and M_∞ can be proportionally related to I_t , and I_∞ , respectively. In this study, since the concentration of all the solutions lie in the concentration range ($< 1 \times 10^{-5}$ M), where the fluorescence calibration curve of CPT is valid, the fraction (M_t/M_∞) of the amount of drug released at time t can be simply expressed as:

$$\frac{M_t}{M_\infty} = \frac{I_t}{I_\infty} \quad (4-2)$$

This relation will be used to depict the release behavior of CPT from the agar hydrogel under different temperatures.

4.3.3 Profiles of M_t/M_∞ vs. Time

The slow release kinetics of a substance in physical or chemical gels is of scientific significance and applied importance. In order to interpret the kinetics of CPT's release from the agar hydrogel at different temperatures, the fraction (M_t/M_∞) of the amount of

CPT released at time t was plotted in Fig. 4-9 as a function of time.

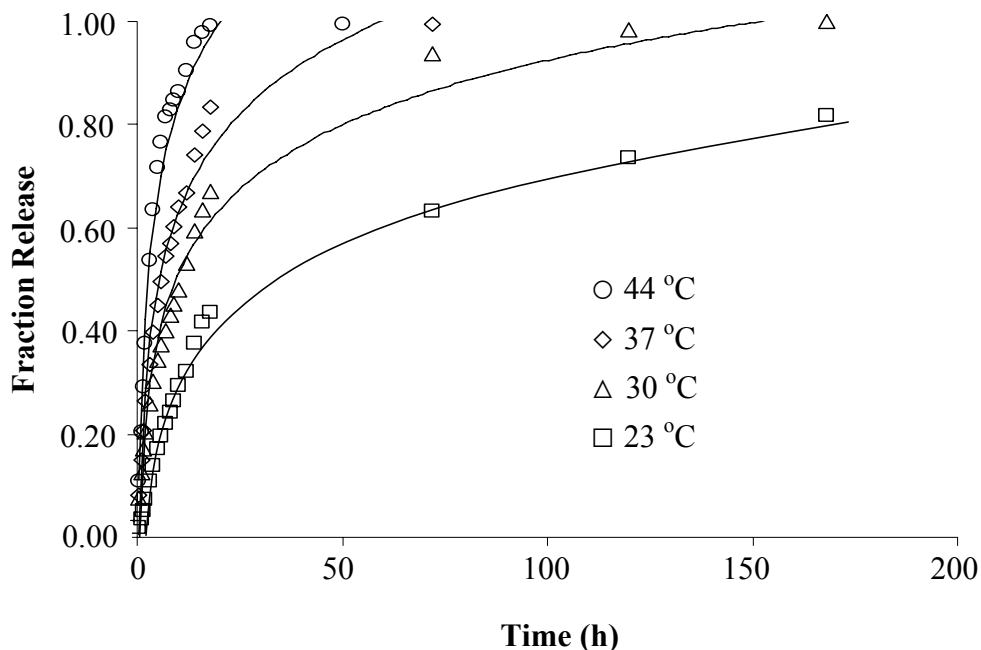


Figure 4-9: Fraction release, M_t / M_∞ of CPT from agar hydrogel at different temperatures as a function of time t .

All experiments showed a fast release of the drug within the first 20 hours; afterwards the release slowed down, and finally tended to level off. It can be clearly seen from Fig. 4-9 that the release of CPT increased with increasing temperature. The sample at 23 °C displayed a prolonged release compared to that at a higher temperature. In these release processes, the drug diffused out from the hydrogel driven by the concentration gradient between the internal and external hydrogel, the increase in temperature could probably enhance the activity of CPT molecules and therefore promote a faster release of drug from the hydrogel. The other reason for the enhanced release would be the thermal expansion of the hydrogel, which may result in a looser network to allow the drug molecules to be released faster.

Table 4-2: Release characteristics for CPT from agar hydrogel at various temperatures

Sample	Temperature (°C)	$t_{50\%}$ (h)	2h%	t_{∞}
#1	23	31.50	13.48	1.5 weeks
#2	30	10.09	20.39	1 week
#3	37	6.02	26.47	6 days
#4	44	3.25	36.67	2 days

The characteristic parameters for CPT's release from agar hydrogel were obtained from the individual experimental curves and summarized in Table 4-2, where 2h%, $t_{50\%}$ and t_{∞} represent the percentage release after 2h of dissolution, the time at which 50 % amount of the drug was released and the time to achieve an equilibrium release, respectively. From Table 4-2, the temperature effect on the release behavior can be clearly observed. At the highest temperature (44 °C) used in this work, it only took 3.2 h to achieve 50% release and 2 days to achieve the equilibrium, while the time needed for the lowest temperature (23 °C) to achieve the same levels of release were 31.5 h and 1.5 weeks, respectively. The other two releases lie between the cases of the lowest and highest temperatures.

4.3.4 Diffusion Coefficients

The release of an active substance from hydrogels is classically assumed to take place by diffusion [Muhr and Blanshard, 1982]. The diffusion coefficient thus appears as a

key parameter if a device has been designed to release a solute at a predetermined rate. Diffusion of drugs is well described by the Fick's equations [Peppas, 1986], which correlate the solute fluxion with its chemical potential gradient in the system. Thus, the structure and pore size of the gel, the polymer composition, the water content, and the nature and size of the solute are all taken into account of the diffusion coefficient of the solute. The Fick's second law describes the solute concentration variation (C) in a flat gel sample as a function of time (t) and distance (x) [Crank, 1975]:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad (4-3)$$

where D is assumed to be constant and the boundary conditions are,

$$\begin{aligned} t = 0 & \quad -l/2 < x < l/2 & \quad C = C_1 \\ t > 0 & \quad x = \pm l/2 & \quad C = C_0 \end{aligned}$$

where l is the thickness of the flat sample.

There are basically two alternative solutions to Eq. (4-3). One is for the early stage of release less than 60%, and the other one is for the later stage of release larger than 40%. The diffusion coefficients of CPT from agar hydrogel will be obtained using these two models respectively.

4.3.4.1 Error Function Series

One of the solutions to Eq. (4-3) that is useful for interpretation of short-time release is given in the form of an error function series [Ritger and Peppas, 1987a].

$$\frac{M_t}{M_\infty} = 4 \left[\frac{Dt}{l^2} \right]^{\frac{1}{2}} \left[\frac{1}{\pi^{\frac{1}{2}}} + 2 \sum_{n=1}^{\infty} (-1)^n \operatorname{ierfc} \frac{nl}{2\sqrt{Dt}} \right] \quad (4-4)$$

where $\text{ierfc } x$ represents the integrated complementary error function of x . For “small times”, i.e., small values of the dimensionless time t , defined as $4Dt/l^2$, Eq. (4-4) can be approximated by

$$\frac{M_t}{M_\infty} = 4 \left[\frac{Dt}{\pi l^2} \right]^{\frac{1}{2}} \quad (4-5)$$

As indicated in Eq. (4-5), Fickian diffusional release from a thin film is characterized by an initial $t^{1/2}$ time dependence of the drug released. The short-time approximation is valid for the first 60% of the total released drug ($M_t / M_\infty \leq 60\%$).

By applying Eq. (4-5), the fitting result of Fig. 4-9 is shown in Fig. 4-10. The slope of these curves can be expressed as $k = 4[D/\pi l^2]^{\frac{1}{2}}$. Diffusion coefficients of CPT from agar hydrogel at different temperatures thus can be obtained by $D = k^2 \pi l^2 / 16$ and they are summarized in Table 4-3.

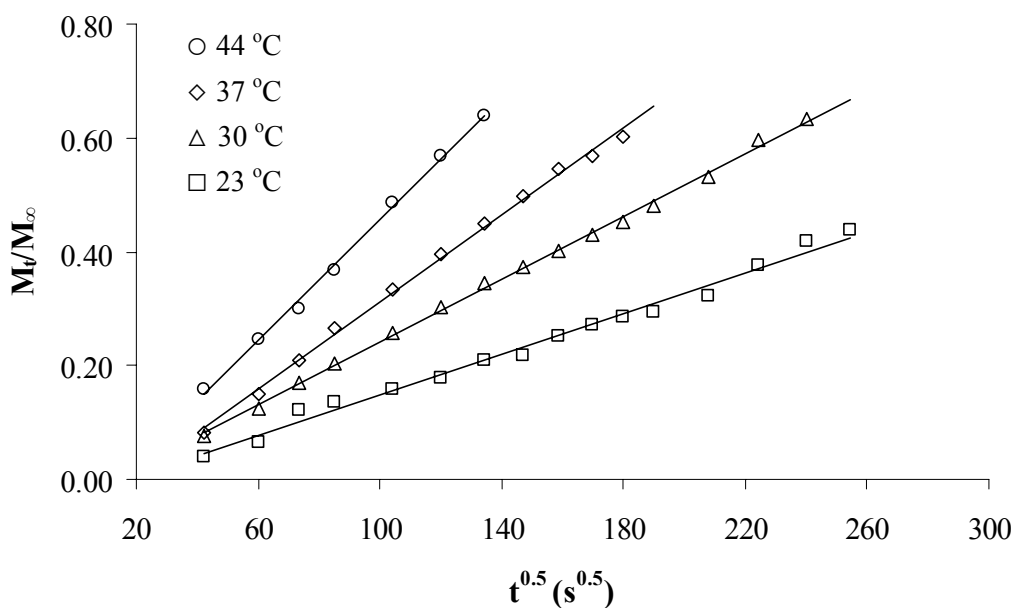


Figure 4-10: Cumulative fraction release of CPT from agar hydrogel at different temperatures as a function of square root of time

Table 4-3: Parameters characterizing the fitting curves shown in Fig. 4-10 and Fig. 4-11, and the diffusion coefficients obtained correspondingly.

Release	y	k	R^2	D (cm ² /s)
$y = kx + \varepsilon$ ($y = M_t / M_\infty$; $x = t^{0.5}$; $k = 4[D/\pi l^2]^{1/2}$)				
23 °C	$y = 1.80 \times 10^{-3}x - 0.032$	1.80×10^{-3}	0.9893	3.97×10^{-8}
30 °C	$y = 2.77 \times 10^{-3}x - 0.035$	2.77×10^{-3}	0.9983	9.38×10^{-8}
37 °C	$y = 3.83 \times 10^{-3}x - 0.071$	3.83×10^{-3}	0.9972	1.80×10^{-7}
44 °C	$y = 5.33 \times 10^{-3}x - 0.076$	5.33×10^{-3}	0.9973	3.48×10^{-7}
$y = kx + b + \varepsilon$ ($y = \ln(1 - M_t / M_\infty)$; $x = t$; $k = -D\pi^2/l^2$; $b = \ln(8/\pi^2)$)				
23 °C	$y = -0.5796x - 0.046$	-0.5796	0.9953	5.04×10^{-8}
30 °C	$y = -0.2533x - 0.106$	-0.2533	0.9935	1.09×10^{-8}
37 °C	$y = -0.1716x - 0.091$	-0.1716	0.9930	1.60×10^{-7}
44 °C	$y = -0.0796x - 0.072$	-0.0796	0.9838	3.67×10^{-7}

4.3.4.2 Trigonometric Series Solution

An alternative solution to the Fick’s law in the form of a trigonometric series under the above-specified boundary conditions is:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left\{-\frac{(2n+1)^2 D\pi^2}{l^2} t\right\} \tag{4-6}$$

By taking only the first term in the summation (Σ) series and performing a logarithmic transformation, Eq. (4-6) can be simplified to Eq. (4-7):

$$\ln\left(1 - \frac{M_t}{M_\infty}\right) = \ln \frac{8}{\pi^2} - \frac{D\pi^2}{l^2}t \tag{4-7}$$

Fig. 4-11 plots $\ln(1 - M_t/M_\infty)$ against t . It can be seen that all the fittings are good and reasonable. Now the diffusion coefficients for CPT can be determined from the slope, $-D\pi^2/l^2$, of the linear regression of Eq. (4-7).

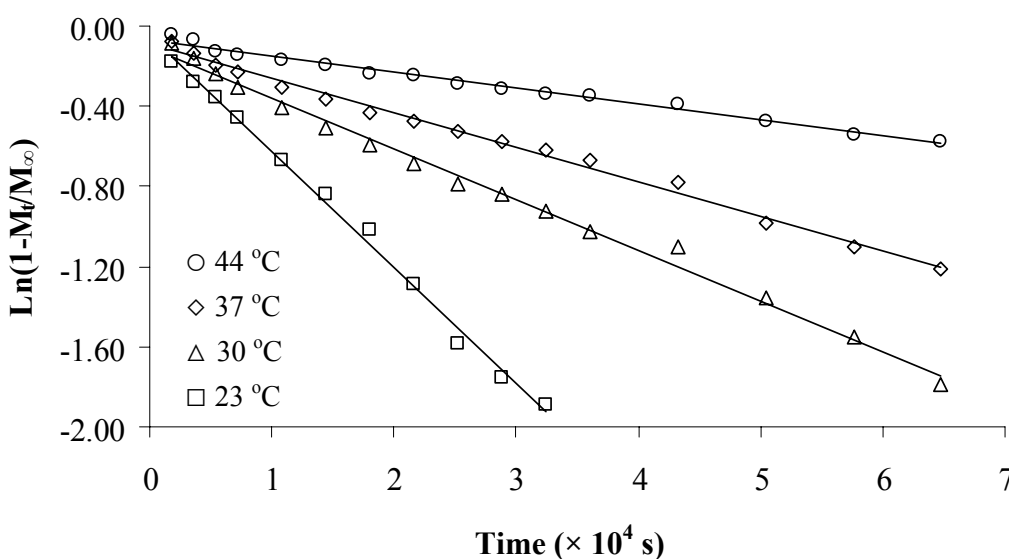


Figure 4-11: Semi-logarithmic plots of the fraction release of CPT from agar hydrogel at different temperatures as a function of time t .

The characteristic parameters for the treadlines in Fig. 4-11 are also summarized in Table 4-3 together with the diffusion coefficients. As anticipated, D values at lower temperatures are found to be smaller than those at higher temperatures indicating a slower release of the drug at low temperatures.

The diffusion coefficients of CPT obtained by fitting the release data to the above two

models are plotted in Fig. 4-12, in which the first columns (the grey ones) represent the diffusion coefficient obtained from Eq. (4-5) and the second columns (the dark ones) represent those obtained from Eq. (4-7). It can be seen that only small variance was caused by using different fitting equations. For example, in the case of 44 °C release, the variance in the diffusion coefficient resulting from different models is only 5.2%. This good consistency in the fitting results actually proves the reliability of the models for the present work. In other words, the Fickian diffusion models can be well applied to the present release results, indicating that the release of CPT from agar hydrogel was controlled by diffusion (Fick's law).

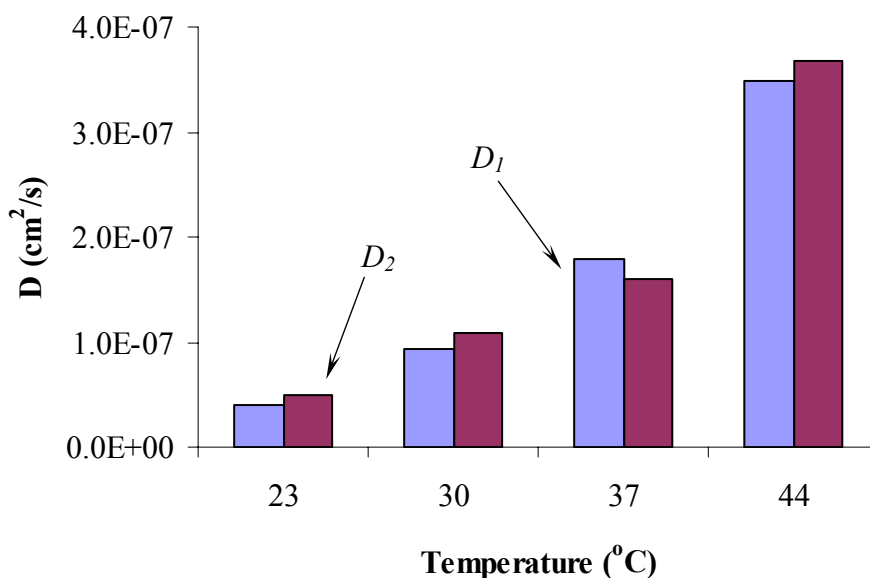


Figure 4-12: The diffusion coefficients (D) of CPT from agar hydrogel at different temperatures. D_1 (grey columns) and D_2 (dark columns) were obtained by fitting to the Eq. (4-5) and Eq. (4-7), respectively.

4.3.5 Activation energy

As seen in Table 4-3, the diffusion coefficient of CPT increases as temperature

increases, indicating that a relationship between D and T may exist. The Arrhenius equation was therefore examined for the D values [Erdoğan and Pekcan, 2001]:

$$D = D_0 \exp\left(-\frac{E_a}{RT}\right) \quad (4-8)$$

where E_a is the activation energy, R the gas constant ($R = 8.31 \cdot \text{J} \cdot \text{mol} \cdot \text{K}^{-1}$), T the absolute temperature, and D_0 the diffusion coefficient at $T = \infty$. The logarithmic form of the D values (the averages of D_1 and D_2) is plotted against T^{-1} in Fig. 4-13, where the slope of the linear curve produces an activation energy E_a of $70.6 \text{ kJ} \cdot \text{mol}^{-1}$ for the release of CPT from the agar hydrogel.

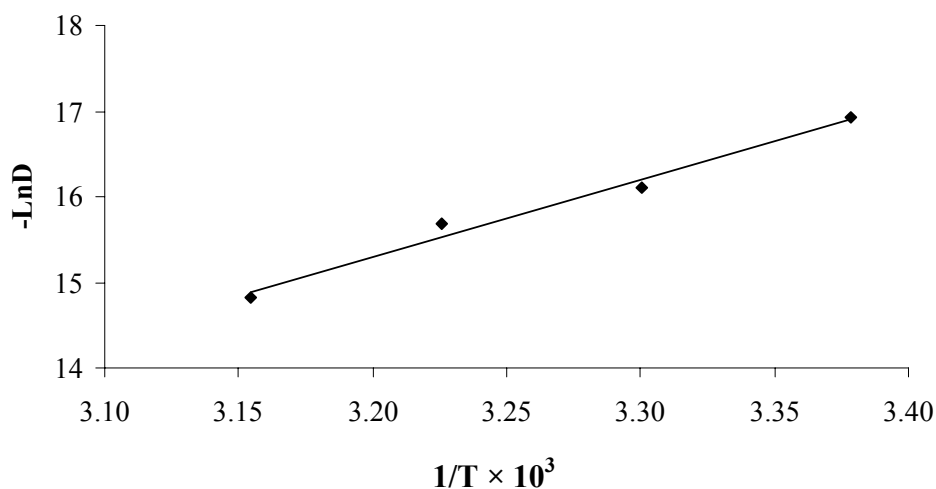


Figure 4-13: The Arrhenius plot of the diffusion coefficients of CPT in agar hydrogel. (D is the mean value of D_1 and D_2 given in Fig. 4-12)

4.4 Summary

The spectral properties of CPT were studied in this chapter in terms of the UV-vis absorption, steady-state fluorescence emission and excitation and time-resolved fluorescence emission. All the above properties of CPT have shown clear pH

dependence, which could be related to the reversible structural conversion of CPT between its lactone closed-ring form and the carboxylate open-ring form, induced by the change of environmental pH.

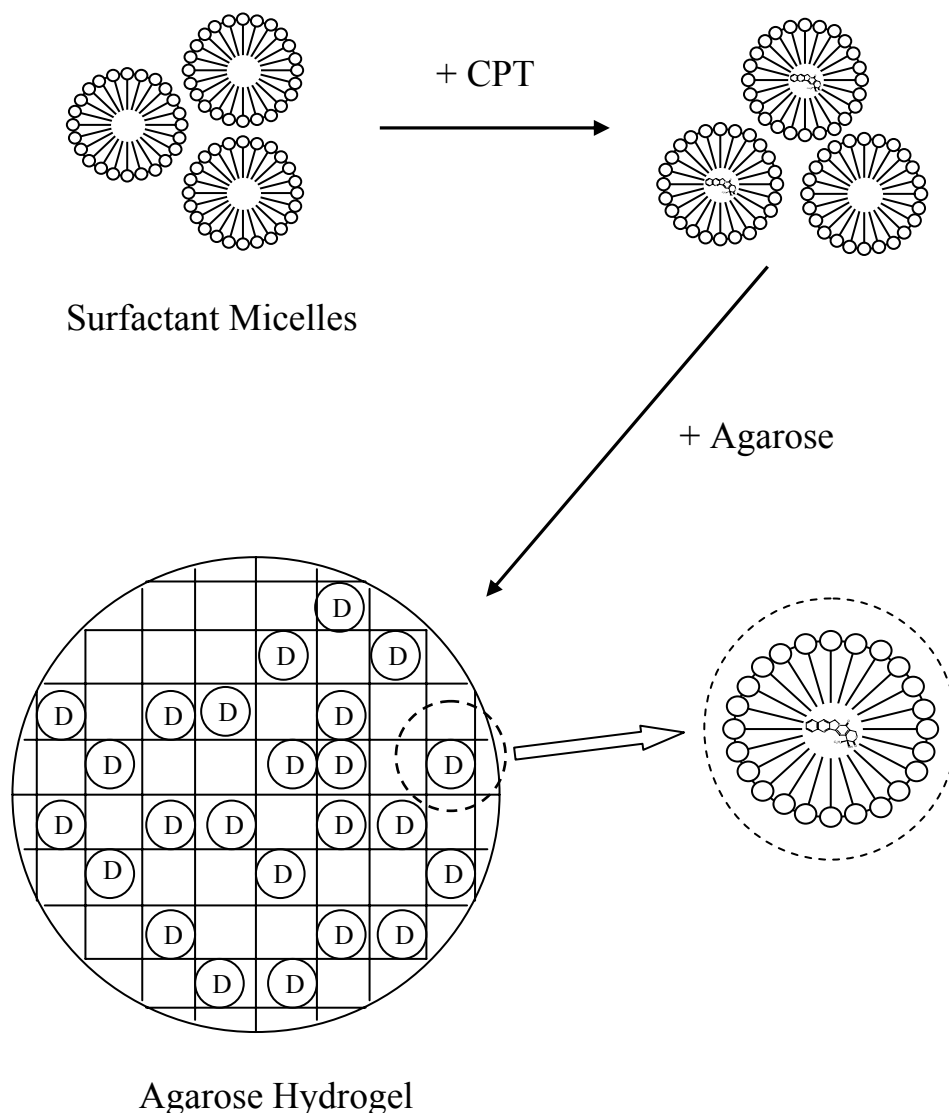
The feasibility of using agar hydrogel as the drug carrier to release CPT was investigated. The drug CPT was loaded to the hydrogel matrix by physical mixing and no other additives such as surfactants were included at this stage of the study. Release of CPT from the hydrogel sample was studied under different temperatures ranging from 23 °C to 44 °C. The study focused on the investigation of the release mechanism and the effect of temperature on release. Steady-state fluorescence spectrometry was used to monitor the slow release process. The diffusion coefficients of the drug were obtained by fitting the release results to the well-known Fickian diffusion equations. Good consistence was observed in the fitting results produced from different mathematic models. The diffusion coefficients of CPT were found to be related to temperature in an Arrhenius relation and the activation energy was obtained to be 70.6 kJ · mol⁻¹. The agar hydrogel thus has shown certain capability to be used as a release system for the drug CPT.

Chapter 5

Anionic Surfactants - Aided Solubilization, Immobilization and Release of Camptothecin

Surfactants play an important role in modern drug delivery, where they may be used to control drug release rate, enhance effective drug solubility, minimize drug degradation, reduce drug toxicity, or facilitate the control of drug uptake [Malmsten, 2002]. They are able to modulate both the pharmacokinetics and the bioavailability of the drug, resulting in an overall increase in the drug therapeutic index [De Oliveira and Chaimovich, 1993; Kreuter, 1994].

A new approach has been made in this chapter to overcome the poor water solubility of CPT, which has greatly limited the therapeutic utility of this drug. Anionic surfactants of sodium dodecyl sulfate (SDS) and sodium dodecylbenzenesulfonate (SDBS) were used. CPT was first solubilized into the micelles formed from these surfactants and then the micellar drug solution was used to prepare the hydrogels for release. It has been found that the presence of surfactants greatly increased the aqueous solubility of CPT. Furthermore, the formulation of a hydrogel using the micellar drug solution has allowed us to obtain a unique and novel drug release system where the drug molecules are incorporated in the micelles and the drug-containing micelles are dispersed in the gel network. The release of CPT from this hydrogel system has been studied. The idea of using surfactants to solubilize drug and the distribution of the drug-micelle system in hydrogel can be illustrated in Scheme 5-1.



Scheme 5-1: Experimental design for preparation of agarose hydrogel containing CPT and SDS. “D” in the gel network stands for “drug”, i.e. CPT, whose structure is shown in the enlarged micellar structure.

5.1 Solubilization of CPT in SDS Solutions

One of the important properties of surfactants is the formation of micelles in aqueous solution, which usually consist of a hydrophobic core and a hydrophilic shell. The

hydrophobic cores can be used to solubilize hydrophobic solutes. In many cases, since the solubilized material (the solubilizate) is in the same phase as the solubilizing solution, the resultant system is consequently thermodynamically stable [Rosen, 2004]. However, it should be noted that the miscibility between the hydrophobic core of a given surfactant and the solute to be solubilized is the key factor to determine the thermodynamic possibility of making a stable system. In other words, hydrophobicity of a solute is not a required condition for a surfactant to be able to immobilize it.

The molecular structure of SDS is given below:

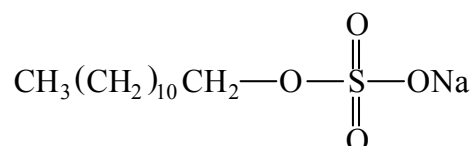


Figure 5-1: Chemical structure of SDS

The dodecyl group ($\text{C}_{12}\text{H}_{25}$ -) serves as the hydrophobic tail while the sulfate group as the hydrophilic head when SDS forms micelles in aqueous solutions. The critical micellization concentration (CMC) of SDS in water was reported to be 8.3 mM at 25 °C [Elworthy and Mysels, 1966].

In order to examine how SDS can help to increase the aqueous solubility of CPT, i.e. to solubilize it, a series of SDS solutions of different concentrations were prepared, and CPT was solubilized in these solutions by vigorous stirring. The amounts of CPT that could be solubilized in each SDS solution were determined by UV-vis absorption and plotted in Fig. 5-2 against SDS concentration.

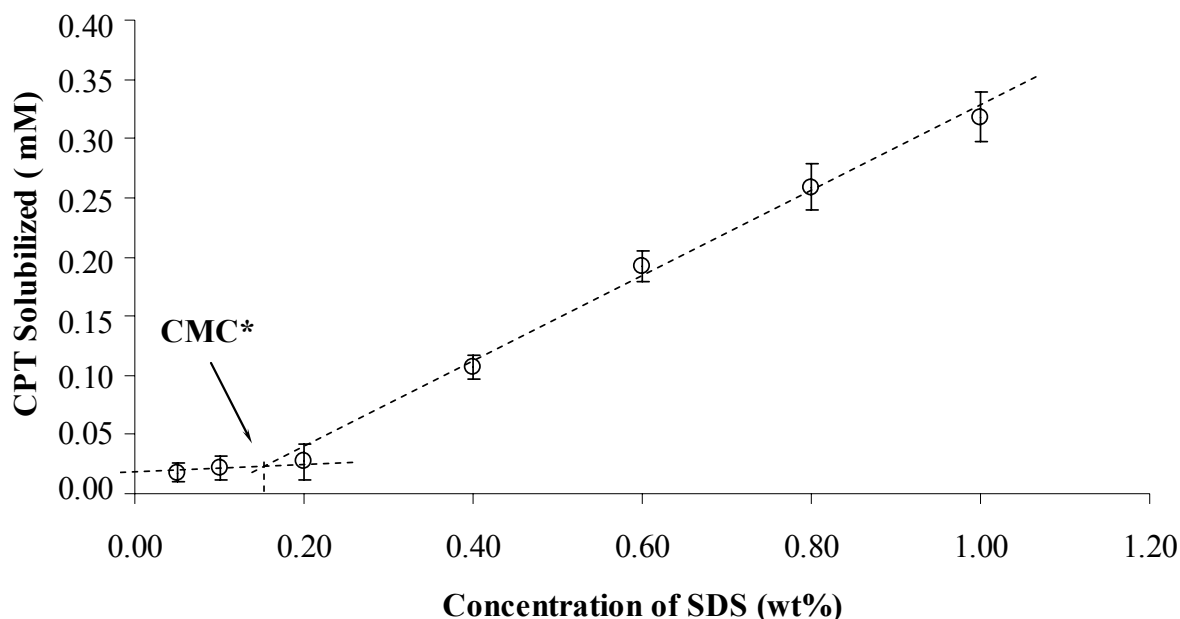


Figure 5-2: CPT solubilized (mean \pm SD, $n = 3$) as a function of SDS concentration. The standard deviations calculated with three replicates are indicated with error bars.

The solubility of CPT in pure water is approximately $3.85 \mu\text{M}$, as reported by Cortesi et al. [1997] and Kang et al. [2002]. From Fig. 5-2, it can be seen that the aqueous solubility of CPT is increased in the presence of SDS. The micelles formed from SDS molecules in aqueous solution provide hydrophobic zones that serve as “rooms” or “containers” for the hydrophobic CPT molecules to reside. This process is called solubilization. However, the SDS-induced increase in the solubility of CPT is nearly negligible until a critical concentration of SDS is reached, after which the CPT concentration increases significantly and linearly with SDS concentrations. This critical concentration is defined as the CMC of the surfactant in the presence of the solubilize, which is about 0.15 wt% obtained from Fig. 5-2. This value is slightly lower than the CMC (~ 0.24 wt%) of SDS in pure water [Elworthy and Mysels, 1966]. The decrease in CMC is considered to be due to the introduction of the hydrophobic drug CPT. It is

generally believed that the presence of a hydrophobic substance may change the CMC of a given surfactant, in most cases reducing it [Rosen, 2004]. When a hydrophobic substance is solubilized into the surfactant micelles, the micelles may be stabilized by the enhanced hydrophobicity. Thus, thermodynamically, SDS tends to form micelles at a lower concentration in the presence of a hydrophobic solute (i.e. CPT) than in pure water.

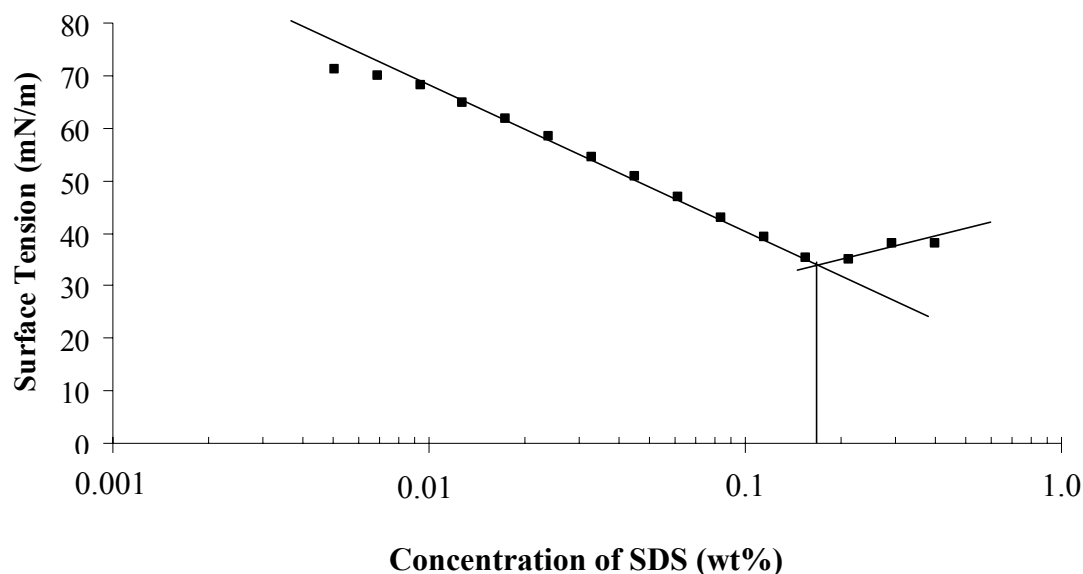


Figure 5-3: Surface tension of SDS aqueous solution in the presence of CPT as a function of SDS concentration.

To further verify the reliability of the lowered CMC observed in Fig. 5-2, the surface tension measurement was conducted. The CMC of SDS in DI water in the presence of CPT was determined by logarithmically plotting the surface tension of the solution against SDS concentration. As shown in Fig. 5-3, the CMC of SDS in presence of CPT was measured to 0.155 wt%, which agrees well with the result obtained from the solubilization experiments shown in Fig. 5-2.

As can be found from Fig. 5-2, after solubilization in SDS solution, the aqueous solubility of CPT was significantly improved. The highest solubility that can be achieved in the studied SDS concentration range is 0.318 mM, which is almost 83 times the solubility of CPT in pure water, when 1.0 wt% SDS was used. This large enhancement in the solubility provides a great flexibility in loading the drug for future controlled release applications. The amount of drug loaded, therefore, can be conveniently controlled by adjusting the concentration of SDS added.

5.2 Partition of CPT in SDS Micelles and Solubilization Number

In the presence of SDS micelles, the CPT molecules in the solution may partition into two parts: some of them are solubilized into the SDS micelles and the rest stay freely in the solution. Paulsson and Edsman [2001a] applied the following equation to express the fraction of a solute in the micellar phase, p_m :

$$p_m = \frac{N_m}{N_{w+m}} = 1 - \frac{N_w}{N_{w+m}} \quad (5-1)$$

where N_{w+m} is the total amount of the solute in the solution, N_w is the amount of the solute in the aqueous phase, and N_m is the amount of solute in surfactant micelles. Since all solutions used in this study were saturated, it is reasonable to consider that the free fraction of CPT in the aqueous phase does not change with the presence of SDS micelles. Therefore, N_w here can be regarded to be equal to the aqueous solubility of the drug, which is 3.85 μM [Cortesi et al. 1997; Kang et al. 2002]. N_{w+m} is the total amount of drug solubilized in each SDS solution as shown in Fig. 5-2. Therefore, we can calculate the amount of CPT staying in the SDS micelles, N_m , to find the partitioning fraction p_m . The values of N_w , N_m and p_m are all given in Table 5-1.

Table 5-1: Aqueous solubility of CPT, partition coefficients (N_w , N_m , p_m), number of SDS micelles, and number of CPT molecules in each SDS micelle. The definitions of N_w , N_m , and p_m are given in Eq. (5-1).

SDS (wt%)	CPT Solubilized (mM)	Increased Solubility	N_w^+ (μM)	N_m^{++} (mM)	p_m	Number of Micelles* (mM)	Number of CPT in Each Micelle
0.05	0.018	4.6	3.85	0.014	0.783	—	—
0.10	0.021	5.5	3.85	0.017	0.819	—	—
0.20	0.027	7.0	3.85	0.023	0.858	0.022	1.0
0.40	0.107	27.9	3.85	0.104	0.964	0.109	1.0
0.60	0.191	49.8	3.85	0.188	0.980	0.196	1.0
0.80	0.258	67.2	3.85	0.255	0.985	0.284	0.9
1.00	0.318	82.8	3.85	0.315	0.988	0.372	0.9

⁺. The amount of CPT that stays freely in aqueous phase is assumed to be equal to its solubility in water

⁺⁺. $N_m = N_{w+m} - N_w$, while N_{w+m} is equal to the total amount of CPT solubilized.

*. The aggregation number of SDS is assumed to be unchanged in the presence of CPT.

In solution, the concentration of surfactant micelles is determined by the relationship:

$$[\text{micelle}] = \frac{[\text{surfactant}] - \text{CMC}}{N_{\text{agg}}} \quad (5-2)$$

In Eq. (5-2), the CMC is referred to as the critical micelle concentration above which surfactant micelles form. When a hydrophobic molecule is present in solution, it is replaced by CMC^* , which is the critical micelle concentration of the surfactant in the presence of the hydrophobic solute. N_{agg} is the aggregation number or the number of

surfactant molecules constituting a surfactant micelle. The N_{agg} of SDS in water at 25 °C was reported to be 80 [Sowada, 1994]. Assuming that it is not changed by the presence of CPT, the total number of SDS micelles in each solution can be estimated. Since the total amount of CPT solubilized in the micelle solution is known, the number of CPT molecule in each SDS-micelle can be calculated. The results are given in Table 5-1. The number of CPT in each micelle was calculated by directly dividing the concentration of CPT molecules in the micelles by the concentration of micelles. For example, at 0.4 wt% SDS solution (corresponding to 14 mM), approximately 0.109 mM SDS-micelles form and 0.104 mM CPT molecules have been solubilized in these micelles. As a result, in average 1.0 (= 104/109) CPT molecule exists in one SDS-micelle. With the increase of the concentration of SDS, the number of CPT molecules in each surfactant micelle undergoes a slight decrease, indicating empty SDS micelles increase in the solution.

It should be noted that in the above discussion, the aggregation number of SDS was assumed as not affected by the presence of CPT. This may not be the fact as the introduction of the hydrophobic solute into the surfactant micellar solution will increase or decrease the aggregation number of the surfactant [Rosen, 2004]. If N_{agg} is not equal to 80 (the value used in the above calculation), the number of CPT in each SDS micelle will be different from those reported in Table 5-1.

The fact that the average solubilization number of CPT in the SDS micelles is no larger than unity means that all CPT molecules are individually dispersed or dissolved in the solution. This condition is important for a homogeneous distribution of the drug in the medium and also particularly important for the validity of the Lambert-Beer's law. If two or more CPT molecules exist in one SDS-micelle, the deviation of the UV-vis

absorption of the surfactant-drug solution from the trend governed by the Lambert-Beer's law would be possible.

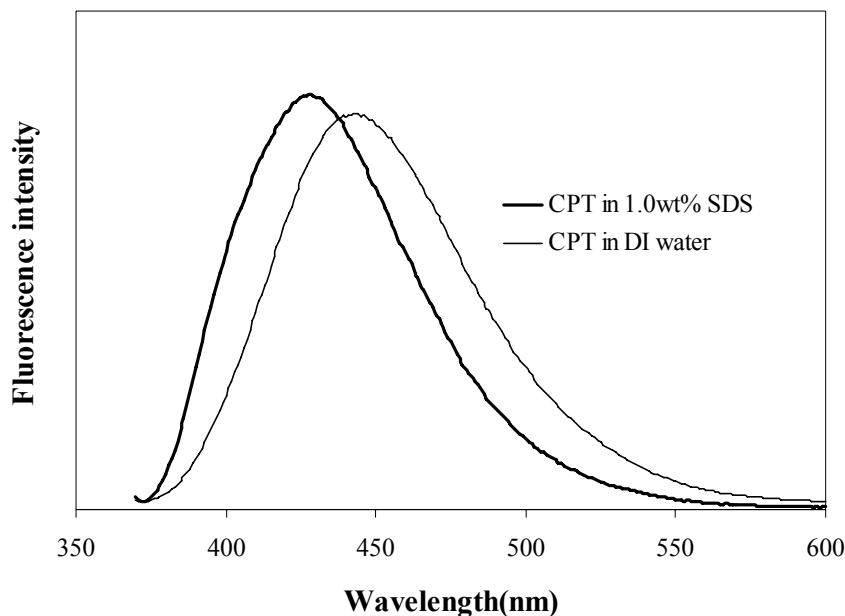


Figure 5-4: Fluorescence emission spectra of CPT before and after solubilized in 1.0 wt% SDS. The excitation wavelength was 369 nm. The slit width is set to 1.5 nm for both excitation and emission.

To make sure that CPT molecules have been partitioned into a hydrophobic environment (i.e. the cores of the SDS micelles), the fluorescence emission spectra were measured at an excitation wavelength of 369 nm for the CPT in DI water and in 1.0 wt% SDS, respectively. The results are shown in Fig. 5-4. A blue shift was observed from the SDS-free solution to the solution with SDS. Such a spectral shift is indicative of a change in the dielectric constant of the medium surrounding the fluorophore [Lakowicz, 1999]. A blue shift is explained by the environmental change from hydrophilic to hydrophobic [Burke et al., 1992]. Since the only hydrophobic environment available in the solution is the cores of the SDS micelles, Fig. 5-4 suggests

that the CPT molecules reside within the SDS micelles in solution.

5.3 Release of CPT from Agarose Hydrogel with SDS

The poor water solubility of CPT limits the utility of this drug in the controlled drug release applications. When a high content of this drug is mixed into a hydrogel matrix, homogeneous distribution of the drug molecules in the release system cannot be assured. Using an amphiphilic surfactant to immobilize CPT into a hydrogel matrix would be one of the simplest and best solutions. By doing so, it will not only help to increase the solubility of the drug, it will also promote a good and stable distribution of the drug molecules in the hydrogel.

5.3.1 Release Processes Illustrated by UV-vis Spectra

By involving various amounts of SDS (0.2 to 1.0 wt%), CPT was successfully immobilized into the hydrogel matrix of agarose, and the release of CPT from those agarose-SDS systems was carried out at 37 °C in DI water. The release process was recorded by UV-vis absorption of the surrounding solution and illustrated in Fig. 5-5. Data in Fig. 5-5 has been collected from the release experiment using the hydrogel sample of 3.0 wt% agarose containing 0.6 wt% SDS at 37 °C, and the release time was from 10 to 180 minutes, which correspond to the lowest and highest UV-vis absorption spectra, respectively. Fig. 5-5 represents a typical example of the release process of CPT from the agarose-SDS systems of various compositions (agarose 3.0 wt%, SDS ranges from 0.2 to 1.0 wt%).

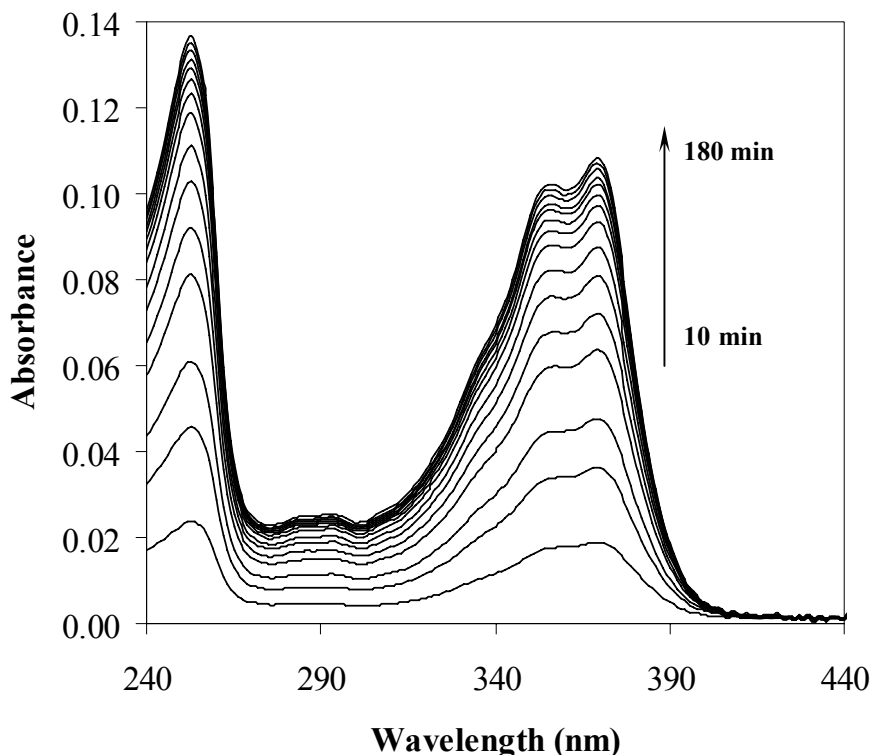


Figure 5-5: Release of CPT from the agarose-SDS hydrogel system at 37°C. SDS concentration in the system is 0.6 wt%. The UV-vis measurements were made at a time interval of 10 minutes. The lowest and highest curves correspond to the release time of 10 and 180 minutes, respectively.

Two conclusions can be drawn from Fig. 5-5:

1. In all the release processes of CPT from the agarose-SDS system, the release of the drug exhibits a well defined pattern: a relatively fast release at the early stage and a slow release at the later stage. The release rate of the drug gradually decreased with time, which can be seen from the regular decrease in the gap between every two neighboring UV-vis spectra, which were measured in a time interval of 10 min in Fig. 5-4. After long time release, the difference between every two measurements becomes negligible and the system reaches equilibrium. The maximum amount of drug that can be released by the system is achieved.

- Throughout the release process, the drug, CPT shows a good stability, which can be reflected by the high-level resemblance in the UV-vis spectra in Fig. 5-5. The stability of the drug is very important in controlled drug release studies. Fig. 5-5 has provided strong evidence on the stability of the drug in the release period as well as the reliability of the agarose-SDS hydrogel drug release system.

5.3.2 Fractional Release Profiles

The cumulative fraction release profiles of CPT from the agarose hydrogel containing SDS were measured and plotted in Fig. 5-6 as a function of release time t . The amount of CPT released at a given time was obtained by measuring the UV-vis absorption of the release medium and then referring to the calibration curve of CPT.

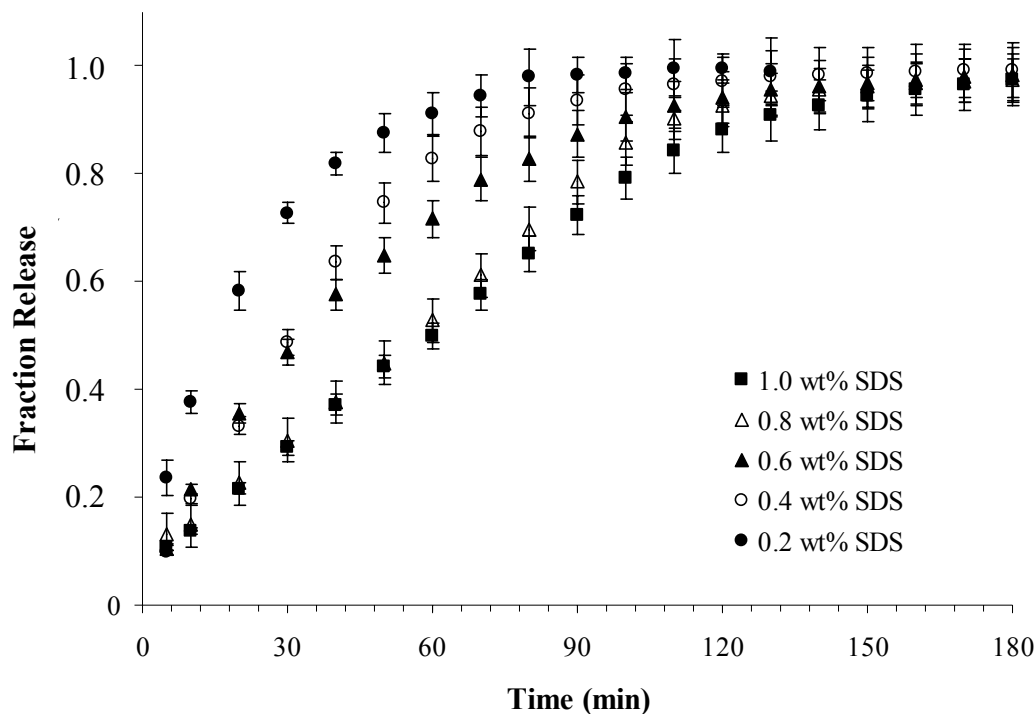


Figure 5-6: Fraction release of CPT from agarose hydrogel at 37 °C in the presence of different concentrations of SDS. The standard deviations calculated with three replicates are shown with the error bars.

From Fig. 5-6, it can be seen that the presence of SDS in the release system resulted in a significant influence on the release behavior of the drug, and this influence could be observed immediately after the release starts. A higher SDS concentration tends to slow down the release of CPT more efficiently, and in the case of 1.0 wt% SDS present in the hydrogel system, the release of the drug took the longest time to reach the equilibrium. All the samples reached the same level of equilibrium, indicating that the presence of SDS in the drug release system did not affect the total amount of drug that could be released, which were theoretically assumed to be equal to the total amounts of drug initially loaded in the hydrogel system.

5.3.3 Diffusion Coefficient

As discussed in the Chapter 4, in diffusion controlled drug release from a hydrogel matrix, the diffusion coefficient of the drug molecules is always an important parameter to be examined. For an assumed constant drug diffusion coefficient, D , with one-dimensional diffusion in the x direction, and for the hydrogel of thickness l , the fractional amount (M_t / M_∞) of the drug released can be written as [Ritger and Peppas, 1987a]:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(n+1)^2} \exp\left\{-\frac{(2n+1)^2 D \pi^2}{l^2} t\right\} \quad (5-3)$$

where M_t is defined as the mass of drug released at time t , and M_∞ is the mass of drug released as time approaches infinity.

By taking only the first term in the summation (\sum) series and performing a logarithmic transformation, Eq. (5-3) can be simplified to Eq. (5-4), which applies only to the later

stages of diffusion [Crank, 1975]:

$$\ln\left(1 - \frac{M_t}{M_\infty}\right) = \ln \frac{8}{\pi^2} - \frac{D\pi^2}{l^2}t \quad (5-4)$$

Using only the experimental data for over 40% of the maximum release ($M_t/M_\infty \geq 0.4$), the diffusion coefficient, D can be determined from the slope, $-D\pi^2/l^2$, of a linear regression of Eq. (5-4), which is Eq. (5-5):

$$Y_i = \beta + k_i x_i + \varepsilon_i \quad (5-5)$$

where Y_i , x_i , β , k_i , and ε_i are $\ln(1 - M_t/M_\infty)$, time, intercept $\ln(8/\pi^2)$, slope ($-D\pi^2/l^2$), and error, respectively. Because Eq. (5-4) is accurate during the later period of release, theoretically Eq. (5-5) will show a good linearity between Y_i and x_i when fitting to the experimental data for over 40% of the maximum release. Eq. (5-5) has been frequently used to interpret drug release result in various studies [Han et al., 2000; Pekcan and Erdoğan, 2002; Yilmaz and Pekcan, 1998].

The release of CPT from the agarose hydrogel in the presence of different concentrations of SDS, after fitting to Eq. (5-5), was shown in Fig. 5-7. Several characteristic parameters of the fitting curves in Fig. 5-7 are summarized in Table 5-2. It is clear that all the fittings are reasonably good and therefore, the diffusion coefficients of CPT can be calculated from the slopes. The absolute slopes of the straight lines in Fig. 5-7 decrease with increasing SDS content, indicating a prolonged release induced by SDS. As can be seen from Table 5-2, D changed with the concentration of surfactant in the hydrogel system. A higher concentration of SDS corresponds to a lower diffusion coefficient and a prolonged release time.

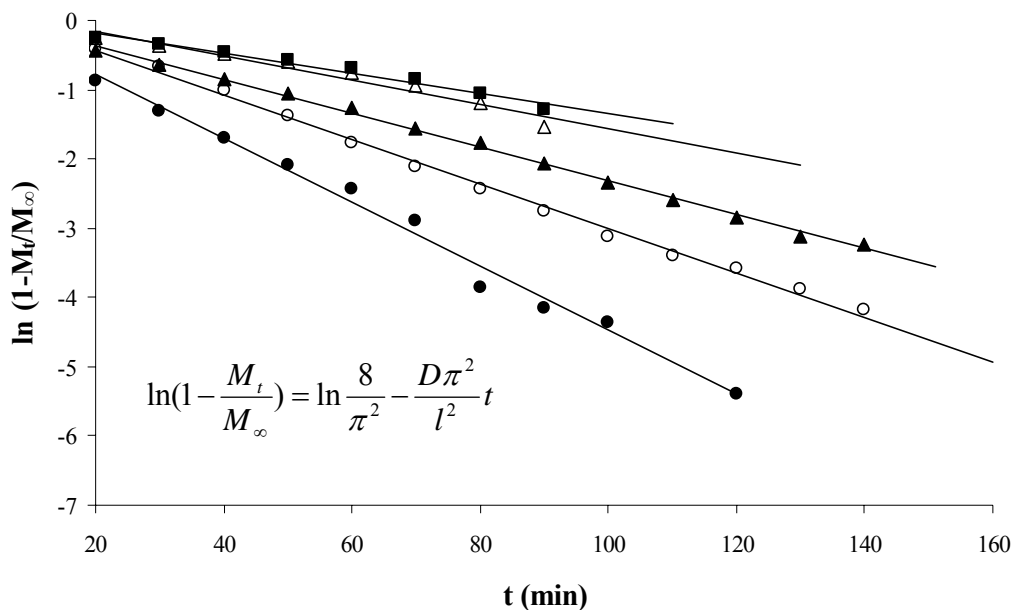


Figure 5-7: Semi-logarithmic plots of the data in Fig. 5-6 as a function of time t . The concentrations of SDS are presented with the same symbols as those in Fig. 5-6. The solid lines are the fitting curves and the fitting equation used is attached.

Table 5-2: Parameters characterizing the fitting curves in Fig. 5-7 and diffusion coefficients of CPT under different SDS concentrations

SDS concentration	Linear regression	R^2	k (slope)	D ($\text{cm}^2 \cdot \text{s}^{-1}$)
1.0 wt%	$Y = -0.01444x + 0.1036$	0.9769	-0.01444	9.37×10^{-7}
0.8 wt%	$Y = -0.01753x + 0.1983$	0.9534	-0.01753	1.13×10^{-6}
0.6 wt%	$Y = -0.02445x + 0.1265$	0.9972	-0.02445	1.58×10^{-6}
0.4 wt%	$Y = -0.03210x + 0.2082$	0.9961	-0.03210	2.08×10^{-6}
0.2 wt%	$Y = -0.04598x + 0.1295$	0.9886	-0.04598	2.98×10^{-6}

5.3.4 Relation between D and SDS Concentration

It is interesting to know that D can be expressed as an exponential function of the SDS concentration, C_{SDS} ,

$$D = 3.84 \times 10^{-6} \cdot \exp(-1.62 \cdot C_{SDS}) \quad (5-6)$$

The relationship between D and the concentration of SDS was plotted in Fig. 5-8. Eq. (5-6) is of practical importance in the design of convenient controlled release systems, in which the release rate of the drug can be predetermined by adjusting the concentration of one of the components, i.e. SDS in the present case.

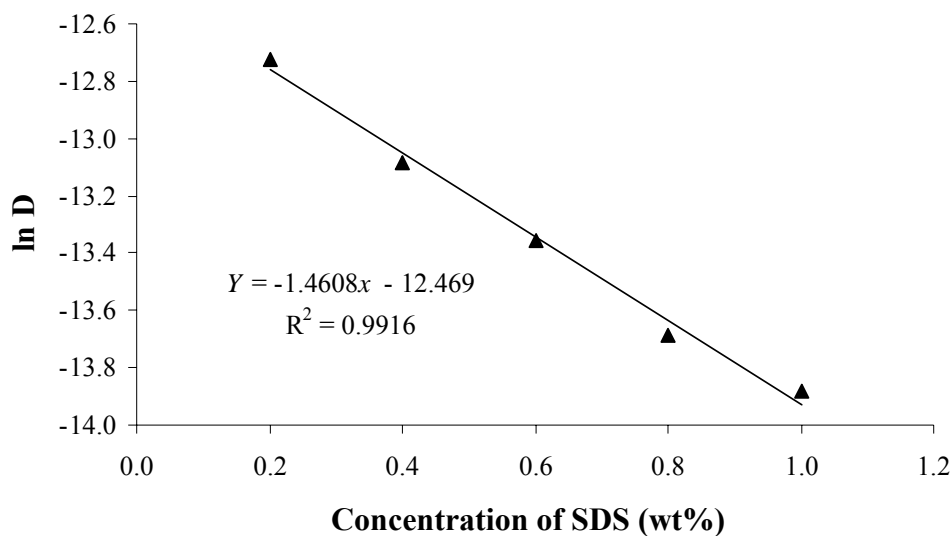


Figure 5-8: Plot of the experimental diffusion coefficient, D , as a function of the concentration of SDS.

When drug molecules are immobilized in surfactant micelles, the diffusion of the drugs may consist of two parts: the diffusion of drug molecule itself and the diffusion of drugs carried by the diffusion of micelles. The diffusion of a drug in solution with micelles present can be described using the following relation [Neogi, 1994]:

$$D = D_f \cdot p_f + D_m \cdot p_m \quad (5-7)$$

where D is the experimentally measured diffusion coefficient, D_f is the diffusion coefficient for the drug free in the aqueous phase, and D_m is the diffusion coefficient for the drug transported by micelles, which is equal to the diffusion of micelles. The parameters p_f and p_m are the fractions of drugs in the aqueous and micellar phases, respectively.

At lower concentrations of SDS, for example from 0 wt% to 0.4 wt%, the first part in Eq. (5-7) shows a big influence on D . With the concentration of SDS increasing, p_f decreases sharply due to the solubilization of drug molecules into micelles, resulting in quantitative shrinkage of the first part in Eq. (5-7). The value of D , therefore, is decreased; however, when the concentration of SDS exceeds 0.6 wt%, especially at 0.8 wt% and 1.0 wt%, p_f shows no further decrease (p_m no further increase), which can be seen from Table 5-1. Now the influence of the second part in Eq. (5-7) on the value of D becomes predominant. The diffusion of the drugs is mainly controlled by the diffusion of the micelles. Upon the further increase in the surfactant concentration in the system, the diffusion of the surfactant micelles might be lowered due to the friction or other possible interaction between themselves as the amounts of micelles increases. The quantitative change in the second part of Eq. (5-7) now dominates the value of the integrated diffusion coefficient of the drug.

5.4 Effect of Temperature

Besides the physiological temperature of 37 °C, the release of CPT from agarose hydrogels containing certain amount of SDS have been also examined at other

temperatures of 23, 30 and 44 °C. The big influence of temperature on the release profiles has been observed. And this influence is even stronger than that in the case when no surfactants are used, which has been reported in Chapter 4.

Fig. 5-9 illustrates the results for the fractional release of CPT from the agarose hydrogel containing 0.6 wt% SDS. Temperature is shown to be an important factor that determines the release property. At 23 °C and 30 °C, the zero-order release (i.e. the case-II transport) [Ritger and Peppas, 1987a; Siepmann and Peppas, 2001] pattern was observed for the CPT's release and the release rates were greatly lower than the releases at 37 and 44 °C. On the other hand, when the temperature was higher than 37 °C, the temperature effect became negligible.

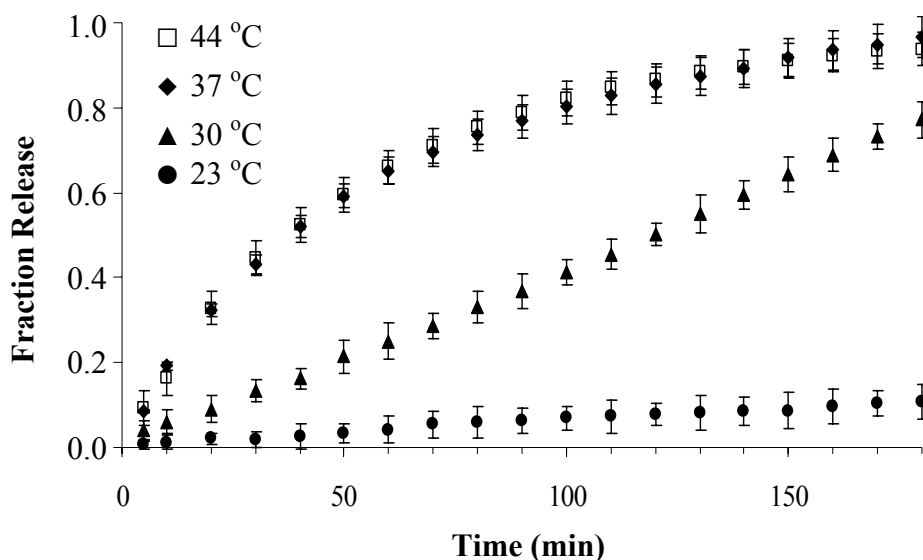


Figure 5-9: Fraction release of CPT from agarose hydrogel at several temperatures containing 0.6 wt% SDS. The standard deviations calculated from three replicates are indicated with the error bars.

5.5 Solubilization and Release of CPT with SDBS

Another frequently used anionic surfactant, sodium dodecyl benzene sulfonate (SDBS), which has the similar functional group with SDS, was also examined for its potential to improve the solubility and release property of CPT. The difference between SDBS and SDS lies in that a benzene group exists in the main chain of the SDBS molecule, which is considered to be highly hydrophobic and therefore endows SDBS with more hydrophobicity than SDS. The molecular structure of SDBS is illustrated in Fig. 5-10. Since the solubilizing ability of one surfactant is immediately related to the hydrophobicity of the core of its micelles, SDBS is expected to be able to give more effective improvement on the solubility of the hydrophobic drug, CPT.

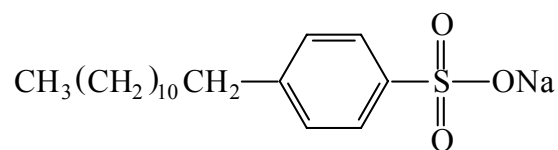


Figure 5-10: Molecular structure of SDBS

5.5.1 Effect of SDBS on Solubility of CPT

The solubility of CPT in SDBS solutions was examined by solubilizing certain amount of CPT in a series of SDBS solutions at different concentrations. The amount of CPT that could be solubilized, i.e. the solubility of CPT under each SDBS concentration was obtained by UV-vis spectrometry measurement and the result was shown in Fig. 5-11 as a function of SDBS concentration.

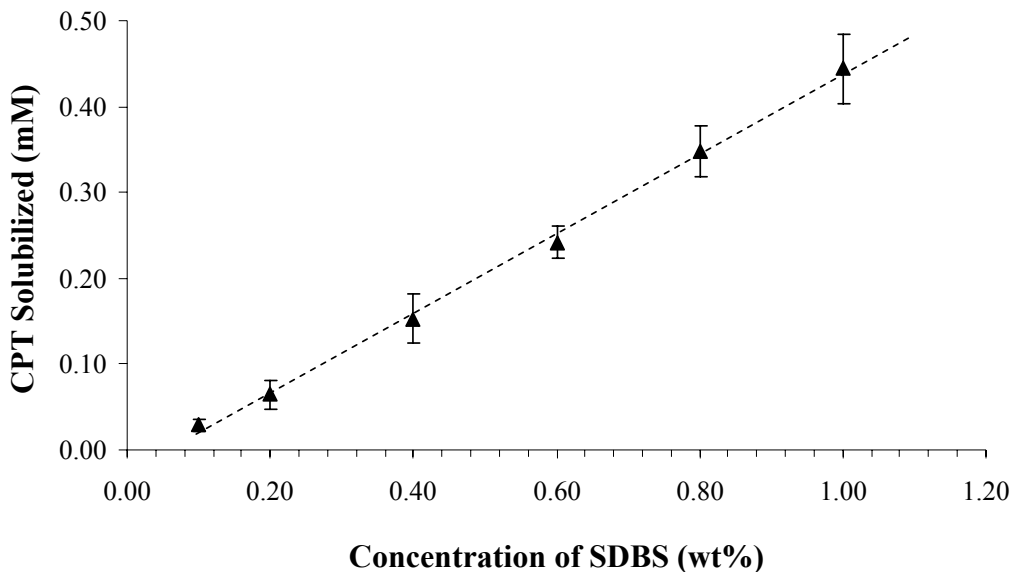


Figure 5-11: CPT solubilized (mean \pm SD, $n = 3$) as a function of SDBS concentration.

As can be seen from Fig. 5-11, in the concentration range of SDBS used in this study, from 0.1 to 1.0 wt%, the solubility of CPT showed a consecutive and linear increase with the surfactant's concentration. No clear turning point was observed as what was seen in the case of SDS, indicating that all the concentrations used here might already exceed the CMC* of SDBS. The CMC* refers to the CMC of the surfactant in the presence of the solubilize (CPT). The solubility of CPT in pure water is approximately 3.85 μM . The highest amount of CPT that could be solubilized in 1 ml 1.0 wt% SDBS solution is 0.155 mg (0.444 mM), which is 115 times the solubility of CPT in pure water and 1.4 times the solubility of CPT in 1.0 wt% SDS. The higher solubilizing ability of SDBS compared to SDS is considered to be related to the stronger hydrophobicity of the core of SDBS micelles. Some important parameters about these SDBS-CPT solutions are summarized in Table 5-3.

Table 5-3: Aqueous solubility of CPT, partition coefficients (N_w , N_m , p_m) in SDBS solutions. The definitions of N_w , N_m , and p_m were given in Eq. (5-1).

SDBS (wt%)	CPT Solubilized (μM)	Increased Solubility *	N_w (μM)	N_m^{++} (μM)	p_m
0.10	28.69	7	3.85	24.84	0.866
0.20	64.91	17	3.85	61.06	0.941
0.40	153.01	40	3.85	149.16	0.975
0.60	241.75	63	3.85	237.90	0.984
0.80	348.18	91	3.85	344.33	0.989
1.00	443.85	115	3.85	440.00	0.991

*. N_w is assumed to be equal to the water solubility of CPT, i.e. $3.85 \mu\text{M}$.

++. $N_m = N_{w+m} - N_w$, while N_{w+m} is equal to the total amount of CPT solubilized.

Fig. 5-12 shows the fluorescence excitation and emission spectra of CPT in 0.1 wt% SDBS solution. Other SDBS-CPT solutions of different concentrations gave similar fluorescent spectra. Compared to the emission spectrum of CPT in DI water, which is also shown in Fig. 5-12, there is a blue shift in CPT's spectrum after it has been solubilized in SDBS solution. Such a blue shift in the fluorescence emission spectrum indicates a change in the dielectric constant of the medium surrounding the fluorophore [Lakowicz, 1999] and it is explained by the environmental change from hydrophilic to hydrophobic [Burke et al., 1992]. This phenomenon suggests that most of the drug molecules were solubilized in the cores of the SDBS micelles instead of other locations in the solution.

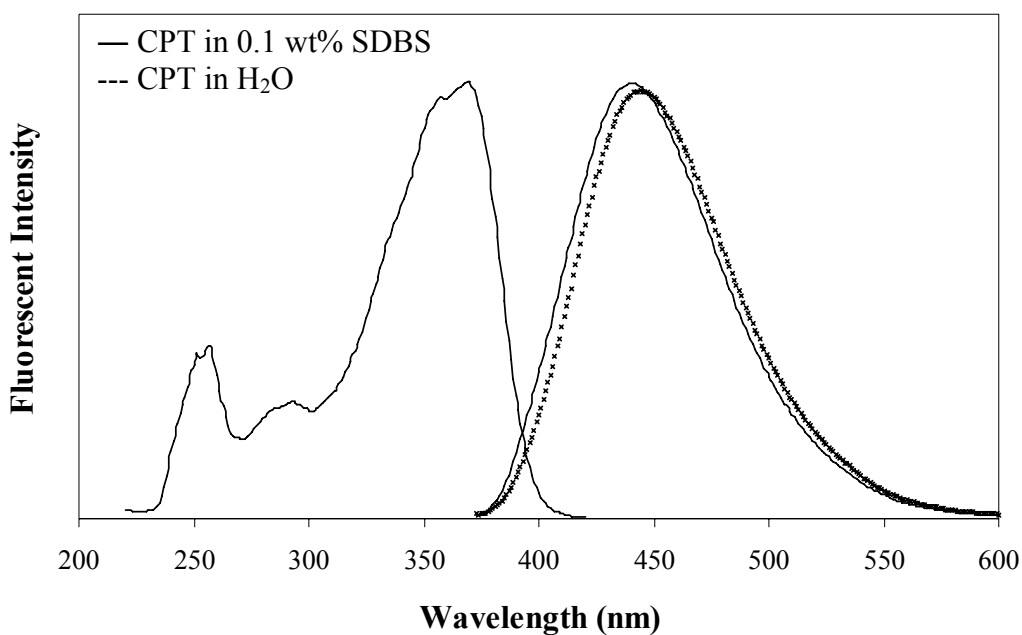


Figure 5-12: Fluorescence excitation and emission spectra of CPT solubilized in 0.10 wt% SDBS (solid line). Also shown is the emission spectrum of CPT in DI water (dashed line, data is amplified for a better comparison).

5.5.2 Release of CPT from Agarose-SDBS Hydrogel

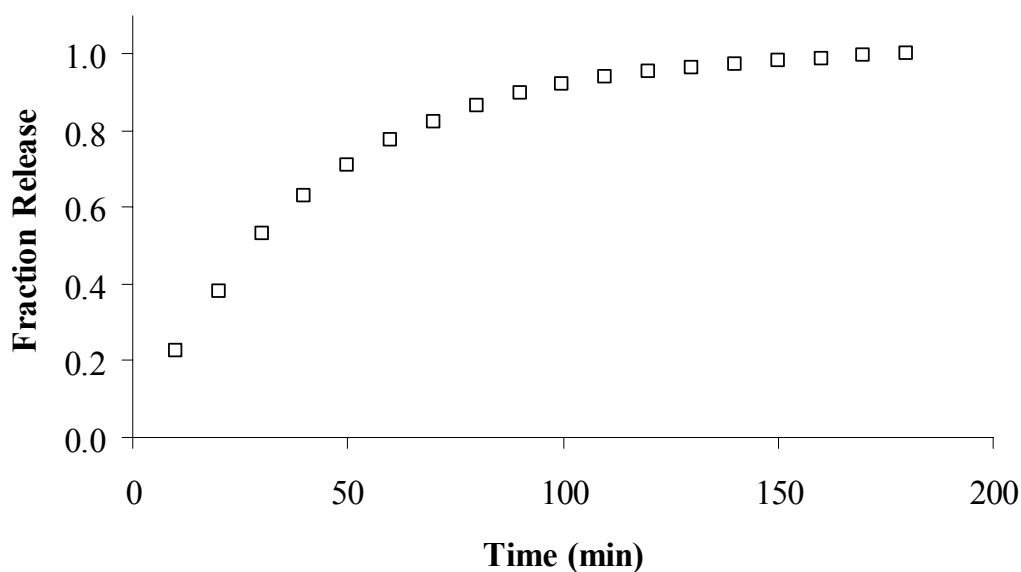


Figure 5-13: Fractional release of CPT from the agarose hydrogel containing 0.40 wt% of SDBS at 37 °C.

The release of CPT molecules from agarose hydrogel containing SDBS was studied at 37 °C. An example of such release is given in Fig. 5-13. The fitting of the release trend using Eq. (5-3) is shown in Fig. 5-14. The diffusion coefficient of CPT under the above conditions was calculated to be $1.69 \times 10^{-6} \text{ cm}^2/\text{s}$.

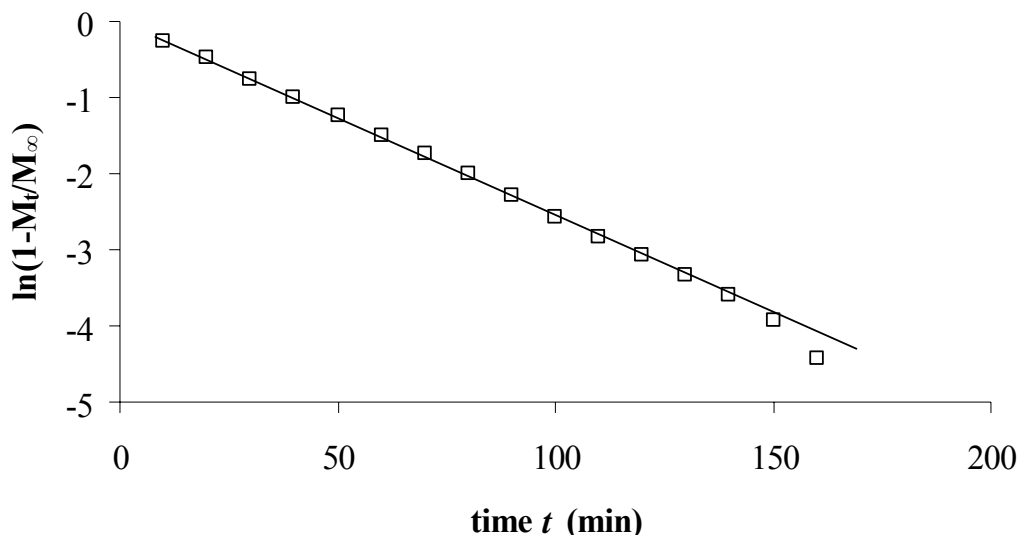


Figure 5-14: Semi-logarithmic plots of the data in Fig. 5-13 as a function of time t .

5.6 Summary

Anionic surfactants were used to improve the poor water solubility of CPT, which results in a limitation of its dosage and effectiveness in potential clinic applications. The hydrophobic drug molecules were first solubilized into micellar solutions of SDS or SDBS, and then immobilized into agarose hydrogel together with the surfactants. Incorporated into the micelles of SDS or SDBS, the aqueous solubility of CPT was greatly increased, for example in 1.0 wt% of SDS and 1.0 wt% SDBS, the solubility of CPT could be improved to 0.318 mM and 0.444 mM, which were respectively 83 and 115 times the solubility of CPT in water. The fluorescence spectra of CPT solutions

with anionic surfactants proved that the drug molecules had been solubilized in the cores of the surfactant micelles.

The release of the drug from the agarose hydrogels containing various contents of anionic SDS or SDBS has been investigated. With the aid of surfactants, homogeneous distribution of the drug in the hydrogel system was believed to be achieved. And most importantly, the presence of surfactant prolonged the release of the drug as proved by the reduced diffusion coefficients by the surfactants. In addition, the temperature effect on the release behaviors has also been examined.

The stability of CPT during the hydrogel sample preparation and during all the release processes is important to be understood. From the UV-vis spectroscopic measurements, it has been observed that there were no considerable changes in the pattern (or shape) and the peak positions (for example, 369 nm) of the UV-vis spectra of CPT after the longest release period at all the release experiments. Thus, it is considered that using such a deliberately fabricated surfactant-hydrogel system will not cause big negative effects on the stability of the drug itself.

This work has proved that the surfactant-aided immobilization of a hydrophobic drug into a hydrogel is a simple and effective way to enhance the drug solubility, facilitate the drug-loading, optimize the drug dispersion in a gel, and control the drug release behavior.

Chapter 6

Cationic Surfactant – Aided Solubilization, Immobilization and Release of Camptothecin

It has been demonstrated in Chapter 5 that the anionic surfactants can be used to solubilize the drug (CPT) and affect the drug release. A big improvement in the solubility of the drug as well as a better control on the drug release process was achieved. In this chapter, the effect of cationic surfactants on the solubilization, immobilization and release of CPT will be studied.

Two commonly used cationic surfactants, dodecyltrimethylammonium bromide (DTAB) and cetyltrimethylammonium bromide (CTAB) were used to solubilize CPT and further immobilize the drug into agarose hydrogels for drug release studies. Both surfactants were found to be able to increase the aqueous solubility of CPT. For example, in 0.01 M CTAB solution, the solubility of the drug could be improved to 4.88×10^{-4} M, which is 127 times the solubility of CPT in pure water. The solubility of the drug in the surfactant micellar solutions were found to be proportional to the concentration of the DTAB after a critical concentration was reached, which was defined as the CMC* of the surfactant in the presence of the drug and lower than the CMC of DTAB in pure water. The big increase in solubility of CPT facilitated the loading of this drug into hydrogels.

Releases of this drug from agarose hydrogel containing cationic surfactant systems were

investigated at physiological temperature of 37 °C. To fully take advantage of the positive electrolyte properties of cationic surfactants, a small amount of another polysaccharide, κ -carrageenan was added to the agarose hydrogel system. κ -Carrageenan is a negatively charged polyelectrolyte. It was found that the presence of κ -carrageenan in the gel system could result in a significant decrease in the drug release rate. This big effect was attributed to the coulombic interaction between the surfactant and the κ -carrageenan. A series of contents of κ -carrageenan was tested and consecutive decrease in the release of CPT was observed. Two mathematical models were employed to fit the release results, and the diffusion coefficients of the drug were found to be between $5.34 \times 10^{-8} \sim 2.28 \times 10^{-7} \text{ cm}^2/\text{s}$, depending on the concentration of κ -carrageenan used.

6.1 Solubilization and Release of CPT with DTAB

The molecular structure of DTAB is shown in Fig. 6-1. The CMC of DTAB in water was reported to be 16 mM [Rosen, 2004].

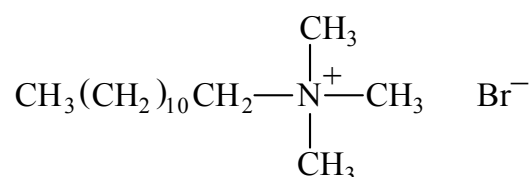


Figure 6-1: Chemical structure of DTAB.

6.1.1 Solubilization of CPT in DTAB Micelles

As demonstrated in the previous chapter, the poor water solubility of CPT could be

improved by using amphiphilic surfactants. To examine how DTAB can help to solubilize CPT, DTAB solutions of various concentrations were used. The concentrations used here were expected to cover the range from unimers to micelles of DTAB, knowing the CMC of DTAB in water is 16 mM. The amounts of CPT solubilized in these solutions were determined by UV-vis absorbance measurements at 369 nm and plotted as a function of DTAB concentration in Fig. 6-2. The inserted graph in Fig. 6-2 shows the corresponding UV-vis absorbance spectra of these CPT-DTAB solutions. Down to up, the DTAB concentration was 5, 10, 20, 30, 40 and 50 mM, respectively. The spectra for 3 and 7 mM DTAB solutions were not shown here in order to avoid a crowded view. Their absorption curves were similar to that of 5 mM DTAB. The spectra for 3 and 7 mM DTAB solutions were not shown here in order to avoid a crowded view. Their absorption curves were similar to that of 5 mM DTAB.

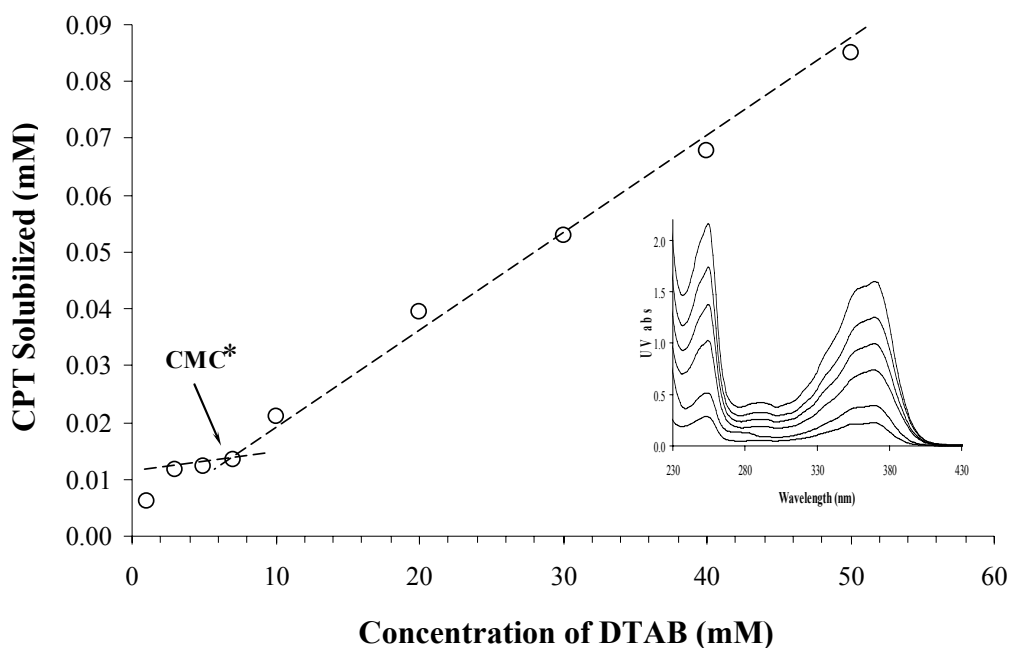


Figure 6-2: Solubilization of CPT in DTAB solutions (the insert is for the corresponding UV-vis absorbance spectra).

The solubility of CPT in pure water is approximately 3.85 μM [Cortesi et al., 1997; Kang et al., 2002]. This value is increased after adding DTAB. However, this increasing

effect is nearly negligible until a critical concentration of DTAB is reached, after which the CPT concentration increases significantly and linearly. As indicated in Fig. 6-2, that critical concentration is defined as the CMC of the surfactant in the presence of CPT, which is about 7.0 mM and lower than the CMC (16 mM) of DTAB in water. This result is quite similar to what was observed in the case of SDS and can be explained by the CMC-reducing effect of the guest hydrophobic substance (CPT) in the surfactant solution [Rosen, 2004].

As shown in Fig. 6-2, the highest solubility of CPT obtained in this study, when 50 mM DTAB is used, is 22 times the solubility in pure water. This significant enhancement provides a great flexibility in drug loading in future controlled release studies. The amount of drug loaded can be conveniently controlled by varying the concentration of DTAB used.

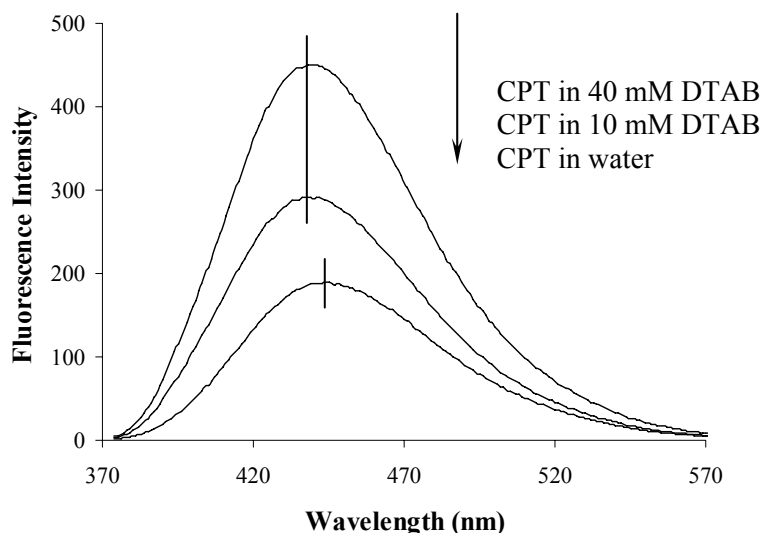


Figure 6-3: Fluorescence emission spectra of CPT before and after solubilized in 10 and 40 mM DTAB solutions.

Evidence was shown in Fig. 6-3 to prove that the drug molecules have been solubilized in the hydrophobic cores of DTAB micelles. The fluorescence emission spectra of CPT in DI water and in 10 and 40 mM DTAB solutions were plotted in Fig. 6-3. A blue shift was observed from the DTAB-free solution to the solutions with DTAB. Such a spectral shift is indicative of a change in the dielectric constant of the medium surrounding the fluorophore [Lakowicz, 1999] and could be explained by the environmental change from hydrophilic to hydrophobic [Burke et al., 1992].

6.1.2 Partition of CPT in DTAB Micelles and Solubilization Number

In the presence of DTAB micelles, the CPT molecules in the solution are partitioned into two parts: some of them are solubilized into the DTAB micelles and the rest stays freely in the solution. Paulsson and Edsman [Paulsson and Edsman, 2001a, b] applied the following equation to describe the fraction of a solute in the micellar phase, p_m :

$$p_m = \frac{N_m}{N_{w+m}} = 1 - \frac{N_w}{N_{w+m}} \quad (6-1)$$

where N_{w+m} is the total amount of the drug in the solution, N_w is the amount in the aqueous phase, and N_m is the amount of drug in the micelles. Since all the solutions studied were saturated with CPT, it is reasonable to consider that the fraction of CPT free in the aqueous phase does not change with the presence of DTAB micelles. Therefore, N_w can be taken as the aqueous solubility of the drug, which is 3.85 μM . N_{w+m} is the total amount of drug solubilized in each solution as shown in Fig. 6-2. Therefore, we can calculate the amount of CPT staying in the DTAB micelles, N_m , to find the partitioning fraction p_m . The values of N_w , N_m and p_m are summarized in Table 6-1.

Table 6-1: The aqueous solubility of CPT, the concentration (N_m) of CPT in the micelles, the fraction (p_m) of CPT in the micellar phase, the number of DTAB micelles in each solution, and the number of CPT molecules in each micelle.

DTAB (mM)	CPT Solubilized (mM)	Increased Solubility	N_m (mM)	p_m	Number of Micelles ⁺⁺ (mM)	Number of CPT Molecules in Each Micelle
3	0.011	3.0	0.008	0.668	—	—
5	0.012	3.2	0.008	0.683	—	—
7	0.013	3.5	0.010	0.712	—	—
10	0.021	5.4	0.017	0.816	0.055	0.3
20	0.039	10.2	0.035	0.902	0.236	0.2
30	0.053	13.7	0.049	0.927	0.418	0.1
40	0.068	17.6	0.064	0.943	0.600	0.1
50	0.085	22.1	0.081	0.954	0.782	0.1

⁺⁺. The aggregation number of DTAB is assumed not to change in the presence of CPT and equal to 55. The number of micelles is determined by the following Equation

$$(\text{CMC}^* \text{ is } 7 \text{ mM}): [\text{micelle}] = \frac{[\text{surfactant}] - \text{CMC}^*}{N_{agg}}$$

The aggregation number of DTAB in water was reported to be 55 [Rosen, 2004]. Assuming this value will not be affected by the presence of CPT, the total number of DTAB micelles in each solution can be estimated. Since the amount of CPT solubilized in the micelles is known, the number of CPT molecule in each DTAB-micelle can be calculated. The results are given in Table 6-1. For example, at 50 mM DTAB, approximately 0.782 mM DTAB-micelles are formed and these micelles help to solubilize 0.081 mM CPT. As a result, in average only 0.1 (= 0.081/0.782) CPT molecules exist in each DTAB-micelle, or in other words, about 10 DTAB micelles share one CPT molecule.

Although all the above calculations are confirmed to be correct, the average number of CPT molecules solubilized in each micelle seems to be too small to be true. In the above calculations, the aggregation number of DTAB micelles was assumed not to change in the presence of CPT. However, when a hydrophobic solute is added to an aqueous solution of a surfactant above its CMC, the water-insoluble solute may be solubilized in the surfactant micelles. This solubilization generally causes an increase in the aggregation number of the micelles, and as the amount of the substance solubilized by the micelles increases, the aggregation number of the surfactant may continue to increase until the solubilization limit is reached [Rosen, 2004]. If the real aggregation number for DTAB in the presence of CPT is higher than 55, the number of micelles will be smaller than that given in Table 6-1, so that the calculated number of CPT molecules will become greater. However at this stage, we have not been able to determine the real aggregation number of DTAB in the presence of CPT, but this could be an interesting future work.

6.1.3 Release of CPT from Hydrogels with DTAB

The efficient loading of CPT into the release system of agarose hydrogel was facilitated by the presence of the DTAB micelles in the system. By incorporating the drug inside the micelles formed by the surfactant, the “property” of the drug was successfully changed from lipophilic to “hydrophilic” and became more compatible with water and the hydrogel network. By doing so, not only the solubility of the drug was increased significantly, but also a good distribution of the drug in the hydrogel matrix could be assured.

6.1.3.1 Effect of DTAB on Release

In order to investigate the effect of DTAB on CPT's release, the DTAB solutions of 10, 30 and 50 mM were used to solubilize CPT and the resultant solutions were further used to make hydrogels after adding 3.0 wt% agarose. The cumulative fraction release profiles of CPT from these gel samples were measured and plotted in Fig. 6-4. It can be seen from Fig. 6-4, DTAB in the hydrogel systems showed a decreasing effect on the release of the drug. With the concentration of DTAB increasing from 10 to 30 and further to 50 mM, the release of CPT has been lowered consecutively.

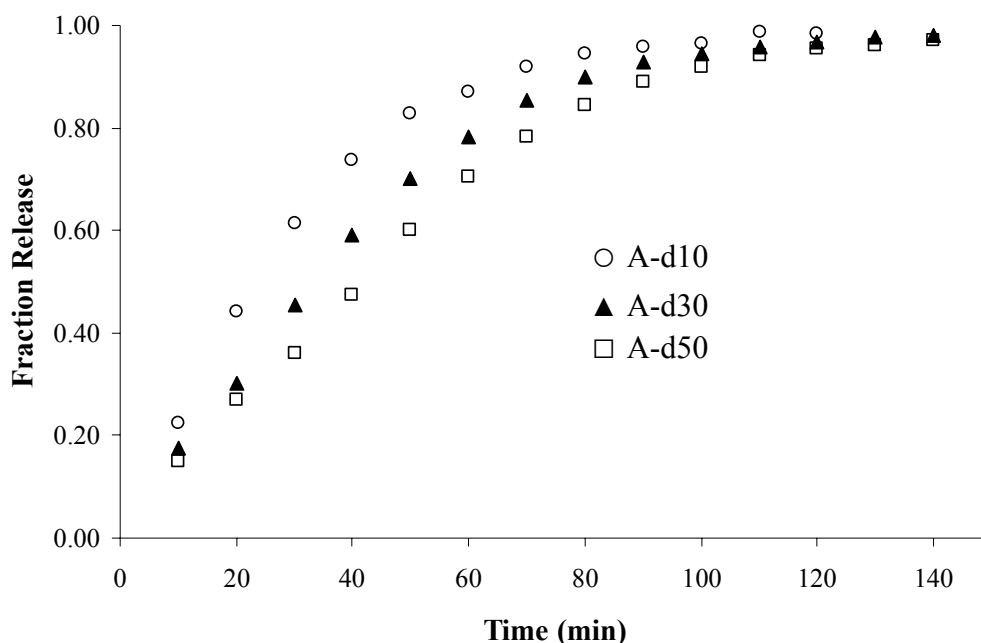


Figure 6-4: Cumulative fraction release of CPT from agarose hydrogel (3.0 wt%) at 37 °C in the presence of different concentrations of DTAB. The composition for each hydrogel sample is given in Table 6-2.

The following equation was used to determine the diffusion coefficients for the above releases of CPT,

$$\ln\left(1 - \frac{M_t}{M_\infty}\right) = \ln \frac{8}{\pi^2} - \frac{D\pi^2}{l^2}t \quad (6-2)$$

Eq. (6-2) is only applied to the latter stage of diffusion for an assumed constant diffusion coefficient with one-dimensional diffusion [Crank, 1975]. M_t is defined as the mass of drug released at time t , M_∞ is the total amount of drug loaded in the system, and l is the thickness of the hydrogel. This equation has been frequently used to interpret drug release data [Han et al., 2000; Pekcan and Erdoğan, 2002; Yilmaz and Pekcan, 1998]. The release results of CPT from agarose-DTAB hydrogels shown in Fig. 6-4 are fitted and plotted in Fig. 6-5. The mathematic expressions for those linear fitting curves are also given.

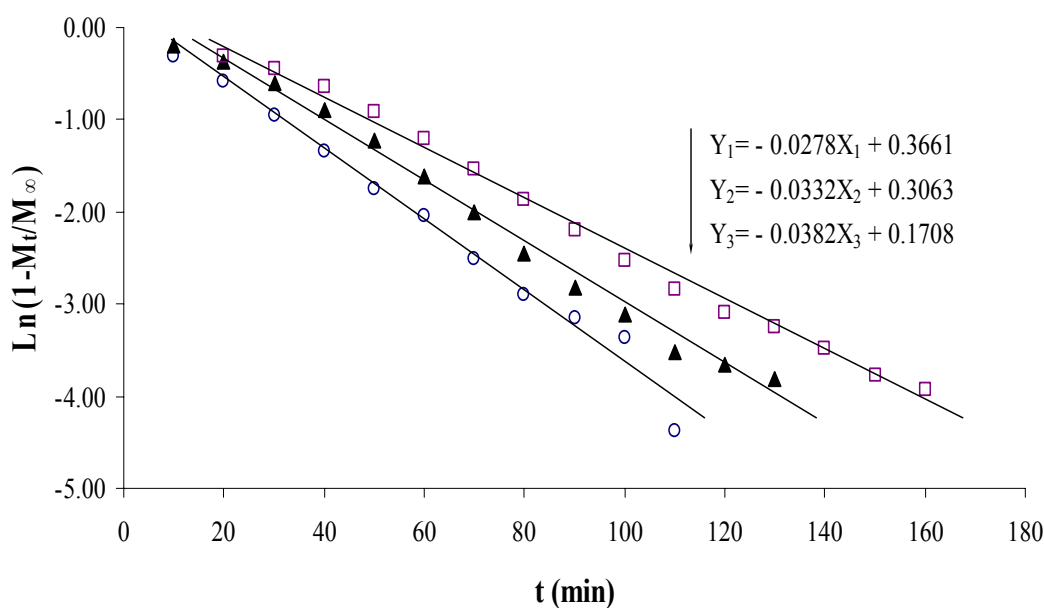


Figure 6-5: Semi-logarithmic plots of the data in Fig. 6-4 as a function of time t .

The experimental diffusion coefficients, D , obtained from Fig. 6-5 are shown in Table 6-2. The decrease in D suggests that a higher concentration of DTAB in the release system can result in a reduced diffusion and a prolonged release of the drug.

Table 6-2: Diffusion coefficients of CPT from agarose-DTAB and agarose-DTAB- κ -carrageenan hydrogel systems at 37 °C (In the designations of hydrogels, “A” stands for agarose, “d” for DTAB and “ κ ” for κ -carrageenan)

Hydrogels	Gel Composition			D (cm ² /s)
	Agarose	κ -Carrageenan	DTAB solution (CPT solubilized)	
<i>for DTAB effect</i>				
A-d01	3 wt%	0	10 mM	2.48×10^{-6}
A-d03	3 wt%	0	30 mM	2.15×10^{-6}
A-d05	3 wt%	0	50 mM	1.80×10^{-6}
<i>for κ-carrageenan effect</i>				
A-d- κ 0	3 wt%	0	40 mM	1.99×10^{-6}
A-d- κ 0.25	3 wt%	0.25 wt%	40 mM	1.03×10^{-6}
A-d- κ 0.50	3 wt%	0.50 wt%	40 mM	0.58×10^{-6}

6.1.3.2 Effect of κ -Carrageenan

After adding certain amount of the negatively charged polymer, κ -carrageenan to the hydrogel of agarose, the release of CPT in the presence of DTAB was greatly changed. Fig. 6-6 is for the cumulative release profiles of CPT from the hydrogels of A-d- κ 0, A-d- κ 0.25 and A-d- κ 0.50. As shown in Fig. 6-6, after adding 0.25 wt% of κ -carrageenan to the agarose (3.0 wt%) hydrogel system, the fraction release of CPT, for example, at the time of 120 minutes was reduced by 20% compared to that without κ -carrageenan, and with 0.50 wt% κ -carrageenan added, another 20% decrease was observed.

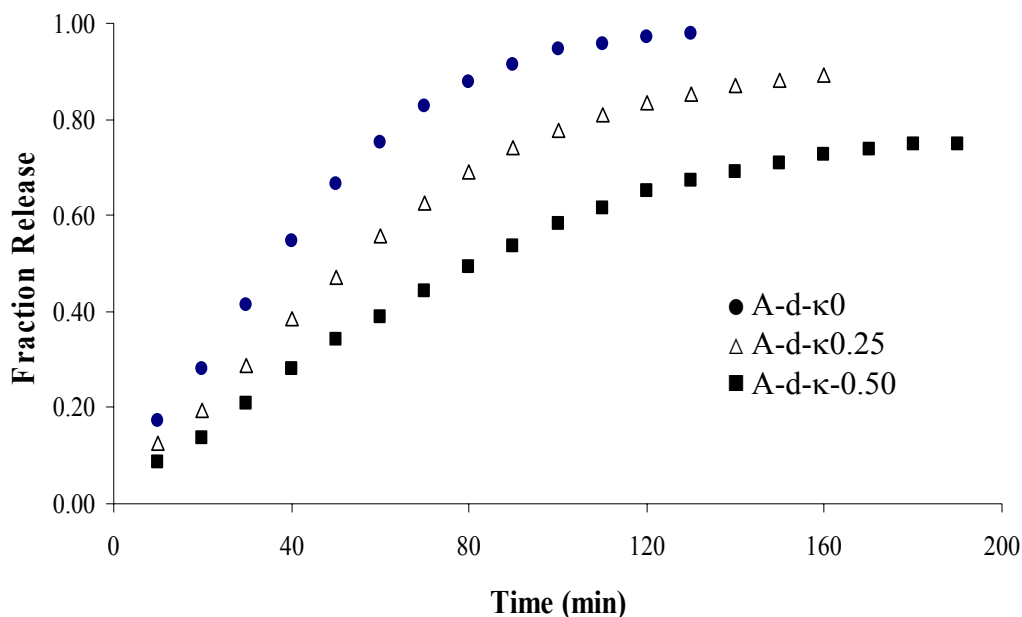


Figure 6-6: Cumulative fraction release of CPT from the agarose hydrogel (3.0 wt%) containing various contents of κ -carrageenan and 40 mM DTAB at 37 °C.

The possible reason for this phenomenon is the coulombic interaction between the oppositely charged surfactant and polymer. Since DTAB is a cationic surfactant, it can form micelles that carry positive charges in the surface. On the contrary, κ -carrageenan is a negatively charged polymer due to the presence of sulphate groups in its molecular chains. It is possible that electrostatic interaction will be developed between DTAB and κ -carrageenan when they are dispersed in one system. Such interaction will greatly affect the movement of DTAB in the gel matrix. Since the diffusion of the drug CPT, which is incorporated in the micellar cores of DTAB, is directly controlled by the diffusion of DTAB micelles, the coulombic interaction between DTAB and κ -carrageenan will also affect the diffusion and release of the drugs. As a part of the gel network, the movement of κ -carrageenan is negligible, so the above interaction tends to

hold the drugs inside the hydrogel and consequently results in a slower release. Increasing the concentration of κ -carrageenan could possibly increase its interaction with DTAB, so the release of the drug will be further lowered.

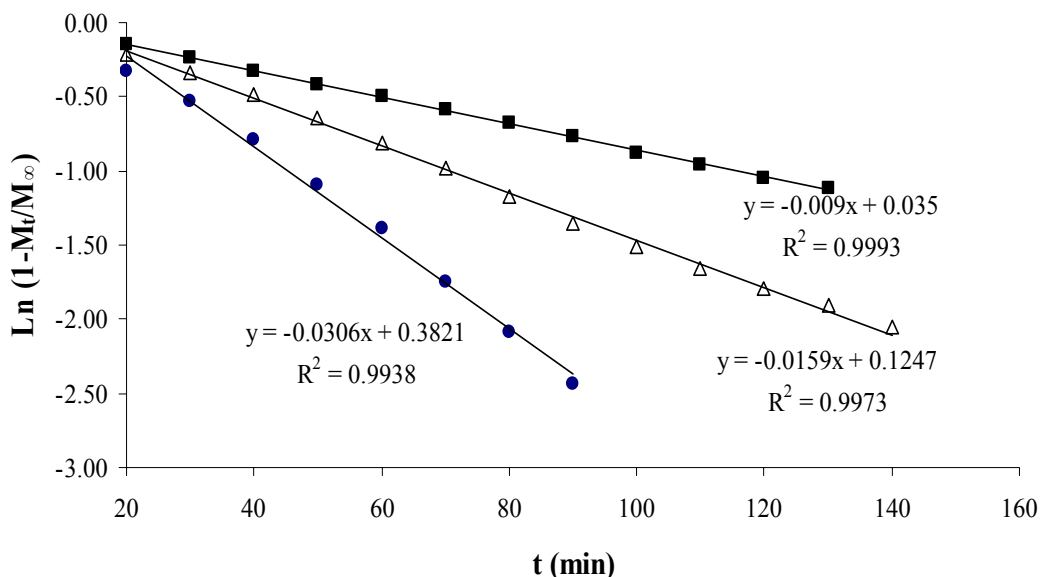


Figure 6-7: Fitting of the data in Fig. 6-6 using the mathematic model shown in Eq. (6-2).

Again, by applying Eq. (6-2), the fitting result of Fig. 6-6 was shown in Fig. 6-7. The decrease in the slope of the fitting curves with increasing contents of κ -carrageenan indicates a decrease in the value of D , which consists well with the conclusions we draw from Fig. 6-6. The diffusion coefficients for the above mentioned releases are obtained given in Table 6-2.

6.1.4 Summary on Effect of DTAB

DTAB was used to improve the water solubility of CPT and subsequently immobilize

the drug into a polysaccharide hydrogel system. Using 50 mM DTAB solution was found to be able to increase the solubility of CPT by 22 times. The DTAB solutions with CPT solubilized in the cores of micelles were used to prepare hydrogels after adding polysaccharide materials. The effect of DTAB as well as the effect of the negatively charged κ -carrageenan on the release of CPT was investigated. It has been found that the presence of DTAB in the gel system contributed to the slow release of the drug and the higher the concentration of DTAB used, the lower the release rate was obtained. The experimental diffusion coefficient of the drug varied from 2.47×10^{-6} to $1.80 \times 10^{-6} \text{ cm}^2/\text{s}$ when the concentration of DTAB increased from 10 to 50 mM. Introduction of κ -carrageenan to the release system greatly changed the release profile and this effect was explained by the possible electrostatic interactions between κ -carrageenan and DTAB micelles. With the content of κ -carrageenan increasing from 0 to 0.50 wt%, the observed diffusion coefficient of CPT was decreased from 1.99×10^{-6} to $0.58 \times 10^{-6} \text{ cm}^2/\text{s}$.

6.2 Solubilization and Release of CPT with CTAB

The molecular structure of CTAB is shown in Fig. 6-8. The CMC of CTAB in water was reported to be $9.2 \times 10^{-4} \text{ M}$ [Holmberg, 2002]. What makes CTAB different from DTAB is the length of the alkyl chain, which may result in different solubilizing ability of the hydrophobic substance.

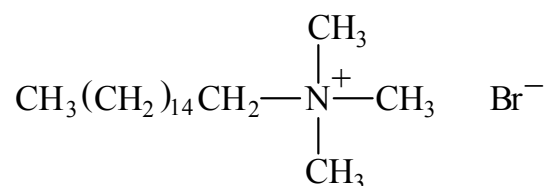


Figure 6-8: Chemical structure of CTAB

6.2.1 Increased Solubility of CPT in CTAB Solution

The saturated solubilization of CPT in CTAB was ensured by adding extra amount of CPT into CTAB aqueous solution. The concentration of CTAB solution used was 10 mM, which far exceeded the CMC of CTAB and therefore, the micelles of CTAB were confirmed to exist in the solution. The subsequent sonication process was believed able to destroy the aggregates of the drug since it tends to aggregate in aqueous solutions, and promote a better dissolution. After 3 days' stirring, centrifugation and filtration, the solution of CPT-CTAB proceeded to UV-vis measurement. Throughout these processes, the CTAB concentrations were assumed to remain unchanged because of two reasons: (1) CTAB has a good solubility in water, and (2) the syringe filter has much bigger pore sizes (0.45 μm) than the micellar sizes of CTAB. The UV-vis absorption measurement suggests that, after solubilized in CTAB solution, the solubility of CPT has been improved to 0.17 ± 0.01 mg/ml (0.488 mM), which is approximately 127 times its solubility in pure water (1.34 $\mu\text{g}/\text{ml}$ [Cortesi et al., 1997; Kang et al., 2002]).

Fig. 6-9 shows the UV-vis spectra of CPT in 10 mM CTAB solution that was used in this study. Also shown are the spectra of CPT in DI water and DMSO, which represent the cases of CPT in highly hydrophilic and highly lipophilic environments, respectively. It can be seen from Fig. 6-9 that the UV-vis properties of CPT is sensitive to the environmental property. In hydrophilic (water) and in hydrophobic (DMSO) environments, CPT gives different spectral shapes and a red shift is observed when the environment changes from hydrophilic to hydrophobic. After solubilization in the CTAB aqueous solution, the UV-vis spectrum of CPT was found to lie between the spectra of CPT in water and in DMSO, and more similar in shape with the latter one.

This character could be ascribed to the change from hydrophilic to hydrophobic of the environment surrounding the CPT molecules, because in the solubilization process, the CPT molecule left the hydrophilic water to the hydrophobic surfactant micelles. Fig. 6-9 is considered to be evidence that the CPT molecules resided in the cores of CTAB micelles, which were the only hydrophobic domain available in solution.

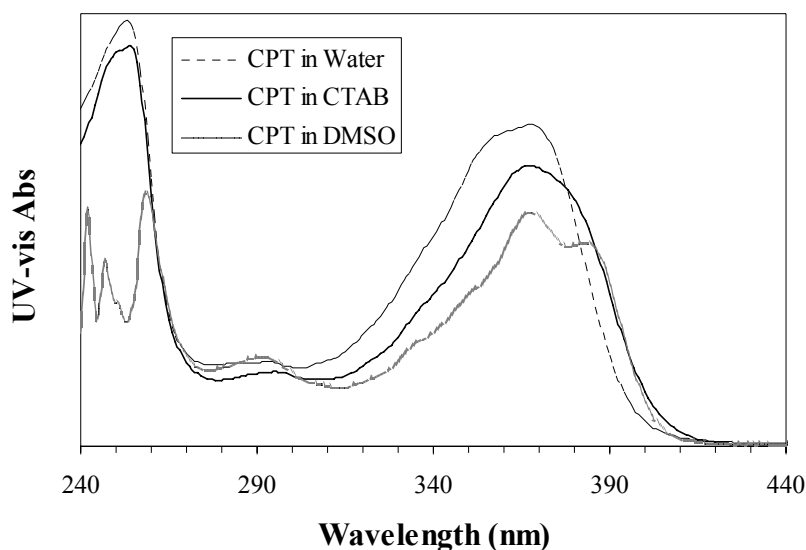


Figure 6-9: UV-vis properties of CPT solubilized in 10 mM CTAB. Also shown are the spectra of unbound CPT in DI water and in DMSO.

6.2.2 Release of CPT from Agarose-CTAB- κ -Carrageenan System

In a gel consisting of polymer, surfactant, and drug, there might be three kinds of interactions affecting the drug release [Paulsson and Edsman, 2001a; b]: (i), the hydrophobic interaction between the drug and the polymer matrix; (ii), the solubilization effect of the surfactant micelles on the drug; (iii), the hydrophobic or electrostatic interactions between surfactants and polymers. In a drug release system

composed of polysaccharide hydrogel matrix, hydrophobic drug molecules and surfactant, interactions of (ii) and (iii) are most possible.

6.2.2.1 Possible Mechanism

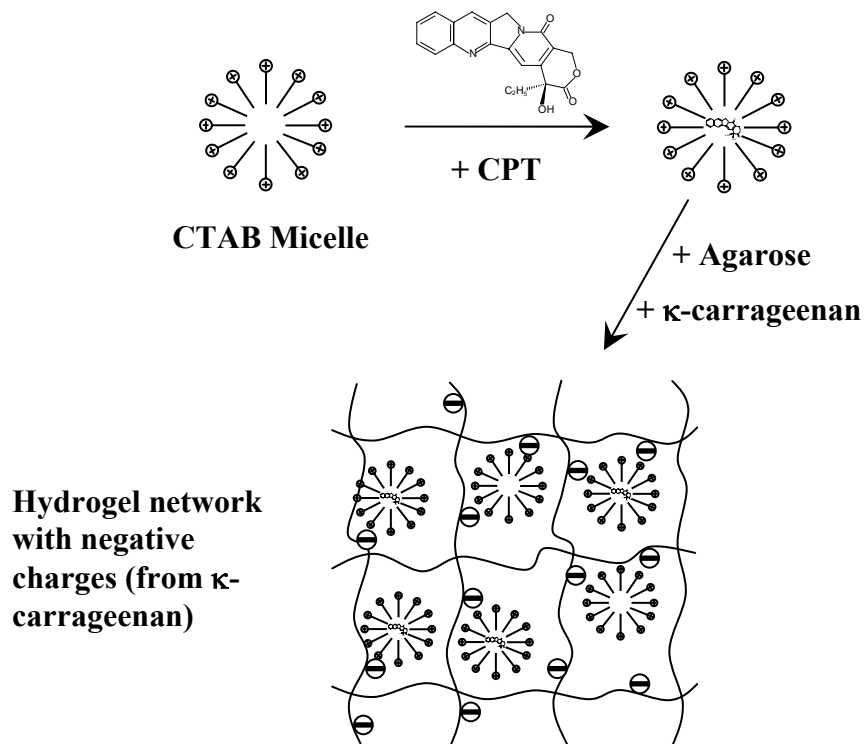
When drug molecules are solubilized in surfactant micelles, the transport of the drug may consist of two parts: the diffusion of drug molecules themselves and the diffusion of drugs carried by the surfactant micelles. The diffusion of a substance in solution with micelles present can be described using the following relation [Neogi, 1994],

$$D = D_f \cdot p_f + D_m \cdot p_m \quad (6-3)$$

where D is the experimentally measured diffusion coefficient, D_f is the diffusion coefficient for the free drugs in the aqueous phase, and D_m is the diffusion coefficient for the drugs transported by micelles, which is equal to the diffusion of micelles. The parameters p_f and p_m are the fractions of drug in the aqueous and micellar phases, respectively.

In most cases, p_f is very small and p_m is infinitely close to 1, which means that the diffusion of the drug is mainly controlled by the diffusion of the surfactant micelles. Any facts that affect the diffusion of micelles will affect the diffusion of the drug. As can be seen in Fig. 6-8, CTAB has a positively charged head and in a hydrophilic environment it can form micelles carrying positive charges in the surface (the shell). When CTAB and the negatively charged κ -carrageenan are dispersed in one system, interaction might be developed between them due to the coulombic attraction. And this interaction may be enhanced with the increase in the concentration of one of them. Once interaction between surfactant micelles and polymers exists, the diffusion of the

micelles will be restricted, and according to Eq. (6-3), the diffusion of the drugs that are carried by the micelles, therefore, will be delayed. The design and preparation of the hydrogel samples used in this chapter are illustrated in Scheme 6-1.



Scheme 6-1: Hydrogel-drug system containing agarose, CTAB (with drugs solubilized), and κ -carrageenan.

6.2.2.2 Release Profiles

The in-vitro release of CPT from the agarose-CTAB (with/without κ -carrageenan) hydrogels was performed at 37 °C. The surrounding solution was subject to UV-vis measurements to determine the amount of drug released at time t . With the time going on, more and more drugs would diffuse into the surrounding solution resulting in a

consecutive increase in its UV-vis absorption intensity. An example of such a release process was shown in Fig. 6-10, which represents the typical spectral change in all the release experiments carried out in this study. This example was taken from the release experiment with the hydrogel sample consisting of 3.0 wt% agarose, 10 mM CTAB and 0.10 wt% κ -carrageenan, and the first and last curves correspond to a release time of 20 and 240 minutes, respectively.

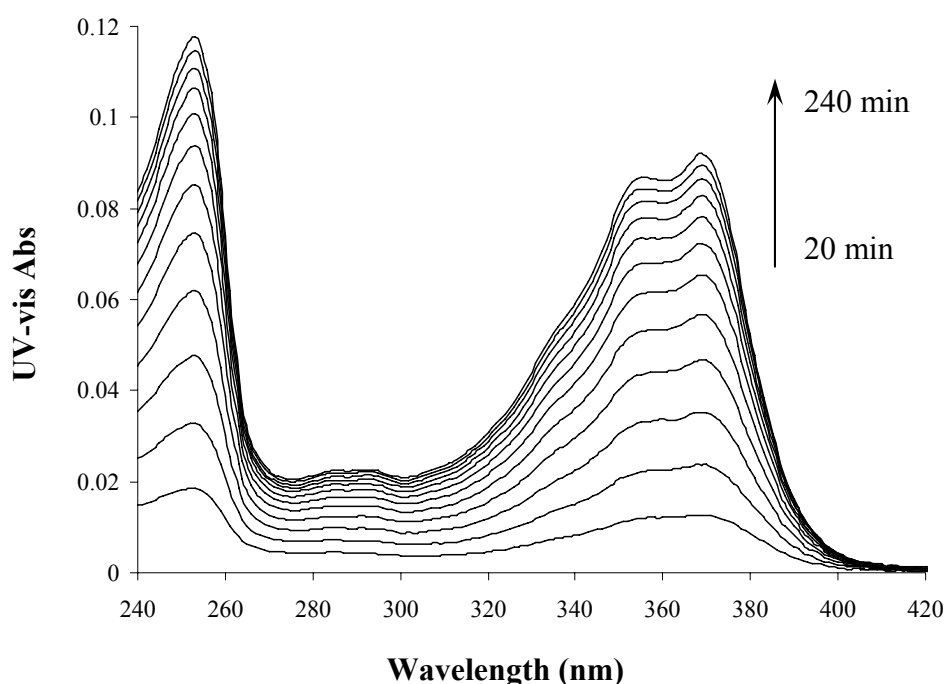


Figure 6-10: Illustrative process of the release of CPT from agarose-CTAB- κ -carrageenan system by UV-vis absorption spectra.

From Fig. 6-10, three conclusions could be made.

1. After diffusing out the hydrogel matrix, the drug molecules stayed unboundly in the target medium solution, rather than residing in the micelles of CTAB.

This is supported by the shape of the UV-vis spectra in Fig. 6-10, which are

identical to the typical spectrum of CPT in water shown in Fig. 6-9. The similarity between them suggests that in the release target solutions the drug molecules stay in a same situation as they are in water. There could be two reasons for the drugs' escaping from the micelles of CTAB: first, some CTAB molecules remained in the hydrogel matrix because of the attraction force from κ -carrageenan, and so the drugs initially solubilized in these CTAB micelles would be freed and diffuse alone to the target solution; second, some CTAB molecules might diffuse along with the drugs targeting to the surrounding solution, but they suffered a sudden decrease in concentration after going out of the hydrogel due to the nearly zero-concentration of surfactants in the target solution. This sudden decrease in concentration would induce the disassociation of micelles, and therefore, drug molecules would be freed.

2. The drug molecules were stable in the long release process, which could be proved by the high resemblance in the UV-vis spectra in Fig. 6-10.
3. The amount of drug in the target solution increased consecutively yet in a decreasing rate.

This could be observed in Fig. 6-10 by the gaps between every two neighboring absorption spectra, which were measured in a fixed time interval of 20 minutes. The value of absorption represents the amount of drug released and the gap represents the amount of drug released between each time interval. The gaps decreased with time, indicating a decreasing release rate of the drug.

The cumulative fraction release profiles of CPT from various types of hydrogels (agarose with/without κ -carrageenan) were plotted in Fig. 6-11. The influence of the negatively charged polymer, κ -carrageenan on the release behavior of the drug was

clearly observed from Fig. 6-11. Even a very low concentration of this polymer, for example 0.05 wt% (1/60 of the concentration of agarose) decreased the release behavior significantly. The concentration of κ -carrageenan now became an important parameter that could be used to control the release of this drug. Increasing the amount of κ -carrageenan would result in the decrease in both the release rate and the total amount of drug that could be released.

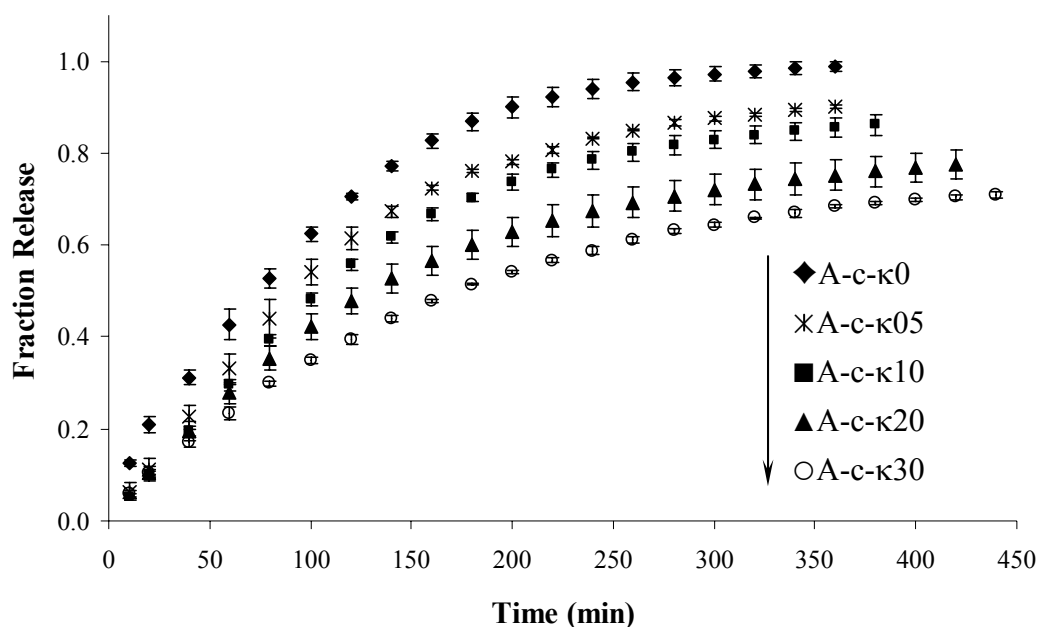


Figure 6-11: Cumulative fraction release of CPT from agarose-CTAB- κ -carrageenan hydrogel systems at 37 °C. The specifications for each hydrogel sample could be found in Chapter 3.

The decreasing effect on the release of CPT from the agarose-CTAB- κ -carrageenan system could be ascribed to the increased interaction between κ -carrageenan and the drug carrier, the surfactant micelles, owing to the coulombic forces between them as explained in Scheme 6-1. Obviously, the decrease was not due to an increase in sterical

hindrance. That is because, first, the amount of κ -carrageenan added was very low. The largest amount used in this study was only 0.3 wt%, 1/10 of the amount of agarose used and at an even lower concentration of 0.05 wt%, the effect was already notable; second and most importantly, it is well-known that the diffusion of small diffusants in a gel with low polymer concentration suffers no big hydrodynamic hindrance [Tarcha, 1990], assuming that no interaction occurs between the matrix and diffusant. This is despite the fact that the macroscopic rheology is very much altered.

The influence of the diffusional transport of κ -carrageenan on the diffusion and release of the drug is considered negligible. It is reasonable to assume that because κ -carrageenan takes part in the formation of the hydrogel network. It will entangle with the agarose (due to its size), and its already slow diffusional characteristic will become even slower, with reference to the drugs. So the consequent effects are thought to be within the margins of error.

6.2.2.3 Diffusion Coefficients

One of the most important and challenging areas in the drug delivery field is to predict the release of the active agent as a function of time, using both simple and sophisticated mathematical models. The importance of such models lies in their utility during both the design stage of a pharmaceutical formulation and the experimental verification of a release mechanism [Narasimhan and Peppas, 1997].

The release of an active agent from a polymeric carrier consists of the movement of the drug through the bulk of the polymer. This phenomenon, known as diffusion, is to a

large degree controlled by the mass transfer limitations at the boundary between the polymer carrier and its surroundings. On a macroscopic level, the diffusion of drug molecules through the polymer carrier can be described by Fick's law of diffusion, which is mathematically stated by Eqs. (6-4) and (6-5) for transport in one dimension [Crank, 1975]:

$$j_i = -D_{ip} \frac{dc_i}{dx} \quad (6-4)$$

$$\frac{\partial c_i}{\partial t} = D_{ip} \frac{\partial^2 c_i}{\partial x^2} \quad (6-5)$$

here, the concentration and mass flux of species, i , are designed as c_i and j_i , respectively; D_{ip} is the diffusion coefficient of species, i , in the polymer matrix, and x and t stand for the independent variables of position and time, respectively. It should be emphasized that in the above written form of Fick's law, the diffusion coefficient is assumed to be independent of concentration. There are basically two alternative solutions to Eqs. (6-4) and (6-5),

1. Early time approximation,

$$\frac{dM_t}{dt} = 2M_\infty \left[\frac{D}{\pi l^2 t} \right]^{1/2} \quad \text{for } 0 \leq M_t/M_\infty \leq 0.6 \quad (6-6)$$

2. Late time approximation,

$$\frac{dM_t}{dt} = \frac{8DM_\infty}{l^2} \exp\left\{-\frac{\pi^2 Dt}{l^2}\right\} \quad \text{for } 0.4 \leq M_t/M_\infty \leq 1.0 \quad (6-7)$$

Based on Eq. (6-6) and Eq. (6-7), the early and late stage of the release results shown in Fig. 6-11 were fitted and plotted respectively in Fig. 6-12 and Fig. 6-13, and the mathematic models used were given correspondingly in the form of M_t/M_∞ . It is clear that all the fittings are reasonably good. The slopes of these straight lines can be expressed as $k_1 = 4(D_1/\pi l^2)^{1/2}$ for Fig. 6-12 and $k_2 = -D_2\pi^2/l^2$ for Fig. 6-13, so that the

diffusion coefficients of the drug can be obtained by $D_1 = k_1^2 \pi l^2 / 16$ for the early time region and $D_2 = -k_2 l^2 / \pi^2$, respectively.

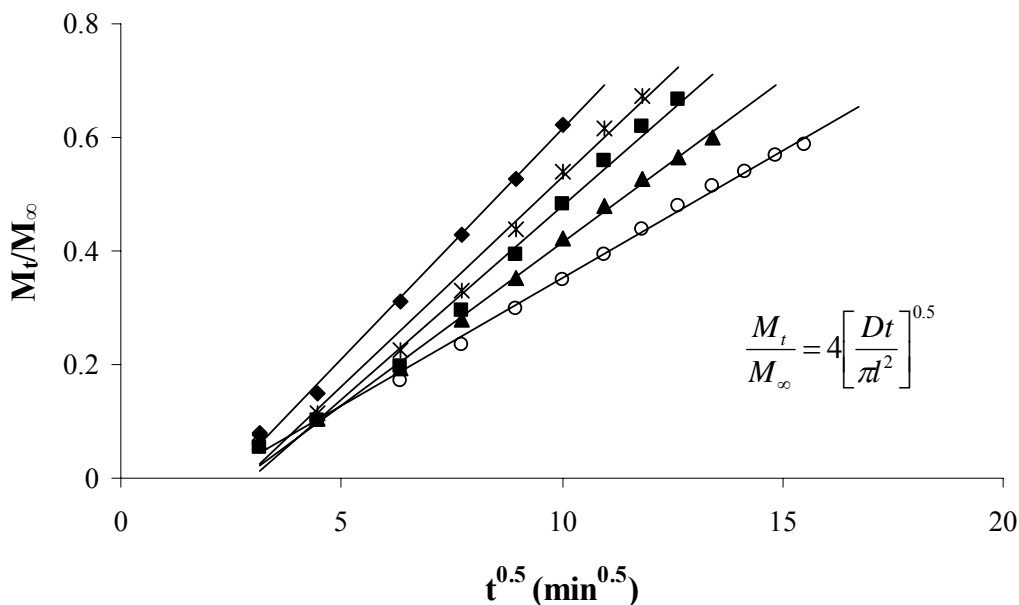


Figure 6-12: Fitting of the release result in Fig. 6-11 by an early time approximation. Inset is the model equation used. Symbols here stand for the same meaning as those in Fig. 6-11.

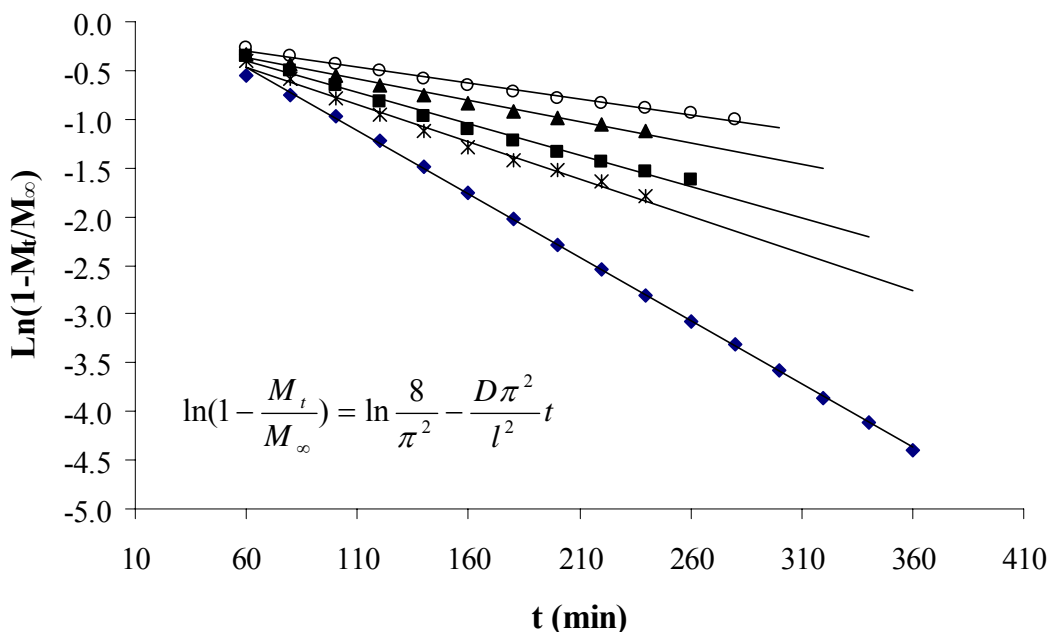


Figure 6-13: Fitting of the release result in Fig. 6-11 by a late time approximation. Inset is the model equation used. Symbols here stand for the same meanings as those in Fig. 6-11.

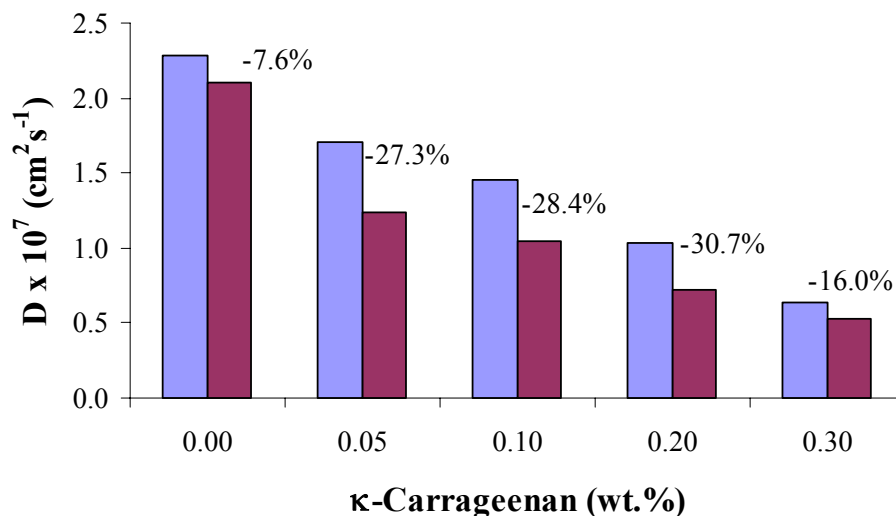


Figure 6-14: Diffusion coefficients of CPT from agarose-CTAB- κ -carrageenan systems. The grey and black columns represent the diffusion coefficients for the early and late stage of release, respectively.

For a better understanding on the change of the diffusion coefficients of CPT at the early and late stages of release from various agarose-CTAB- κ -carrageenan systems, the diffusion coefficients obtained from Fig. 6-12 and Fig. 6-13 were plotted in Fig. 6-14. The decrease in diffusion coefficient from the early time release to the late time release might result from two reasons: (i), as more and more drug molecules diffused into the surrounding solution, the concentration gradient between the inside of the gel matrix and the surroundings decreased, resulting in a slower release rate; (ii), with the release going on, decrease in the concentration of CTAB inside the hydrogel resulted in a relative increase in the total amount of the negative charges of κ -carrageenan that are available to interact with the surfactants, so that the hindrance effect from κ -carrageenan on the diffusion of the drug was enhanced. It is interesting to see in Fig. 6-14, that the smallest variances in D from the early release stage to the late release come from the cases of the hydrogel with zero-content and largest content (0.3 wt%) of κ -

carrageenan, which correspond to a no-interaction situation and probably a saturated interaction situation, respectively. In other words, in both cases, the second influence mentioned in reason (ii) could be margin.

From Fig. 6-11 to 6-14, it can be seen that increasing the amount of κ -carrageenan will result in a prolonged release and a reduced diffusion coefficient of the drug, however, this conclusion might not be able to be applied unboundedly. That is because, when the available negative charges of κ -carrageenan are less than the positive charges of the surfactant present in the gel system, it is possible that increasing the concentration of κ -carrageenan will enforce its interaction with the surfactant, which in turn may result in an enhanced effect on the release of the drug; but when the content of the κ -carrageenan approaches saturation, i.e. all the charges of the surfactant have already been matched, further increasing the concentration of the polymer will not increase its interaction with the surfactant anymore. In addition, at very high concentration of κ -carrageenan, the complete dissolution of this polymer may rise to be a problem.

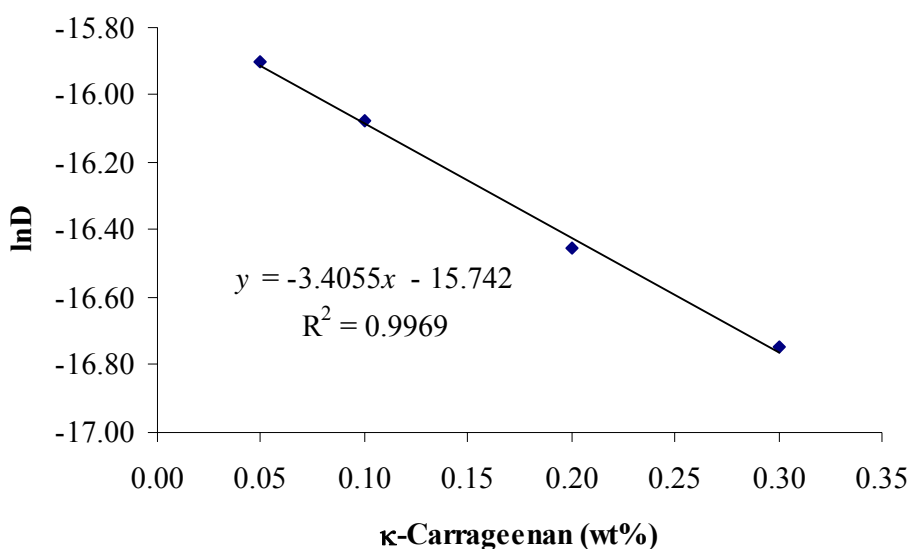


Figure 6-15: Plot of $\ln D$ (diffusion coefficient) as a function of the concentration of κ -carrageenan.

It is interesting to know that D can be approximately expressed as an exponential function of κ -carrageenan concentration, C_{κ} , as shown in Fig. 6-15 (the data of D were obtained from the late stage fitting),

$$D = 1.45 \times 10^{-7} \cdot \exp(-3.41 \cdot C_{\kappa}) \quad (6-6)$$

This is particularly important for the design of new pharmaceutical formations, in which the drug could be released at a predetermined rate by simply adjusting the content of κ -carrageenan used.

6.2.3 Summary on Effect of CTAB

The solubility of CPT was improved by 127 times to 0.488 mM after solubilized in a cationic surfactant, CTAB solution. The resultant solution was further used to prepare hydrogels with agarose and κ -carrageenan. The release of the drug from the agarose-CTAB- κ -carrageenan system was found to be significantly affected by the content of κ -carrageenan. Only 0.05 wt% of this polymer in the gel system could result in a notable decrease in the release rate. This was attributed to the coulombic interaction between the surfactant micelles and the oppositely charged κ -carrageenan. Diffusion coefficients of the drug from the above systems were fitted to be $5.34 \times 10^{-8} \sim 2.28 \times 10^{-7} \text{ cm}^2/\text{s}$ and found to relate to the concentration of κ -carrageenan in an exponential relation. Findings in this study are of particular importance for future design of controlled drug release systems.

Chapter 7

Effects of Other Surfactants and Additives on the Solubilization of Camptothecin

In addition to the surfactants studied in the previous chapters, more surfactants, including some small molecular surfactants and several polymeric surfactants have been examined in this chapter for their abilities to solubilize CPT. Besides surfactant, another type of good solubilizing agent, cyclodextrins, which are widely known for their inclusive complex forming ability [Hirayama and Uekama, 1999], has also been investigated.

Results from this study are believed to be able to provide a useful reference for the selection of appropriate candidates for the fabrication of effective controlled release system for hydrophobic drugs.

7.1 Small Molecular Surfactants - SDeS, Triton X-100

7.1.1 Sodium Decyl Sulfate (SDeS)

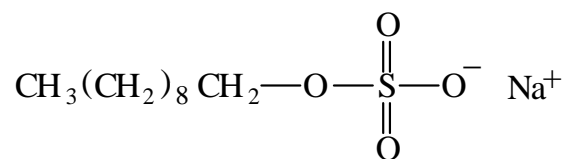


Figure 7-1: Chemical structure of SDeS

Known as one of the commonly used anionic surfactants, SDeS has a chemical structure similar to SDS. The polar sulfate groups of SDeS constitute the hydrophilic shell when forming micelles in the aqueous environment, and the long alkyl chains, $C_{10}H_{21}$ - form the cores of the micelles. The CMC of SDeS was reported to be 33 mM (~ 0.85 wt%) [Rosen, 2004]. The chemical structure of SDeS was given in Fig. 7-1.

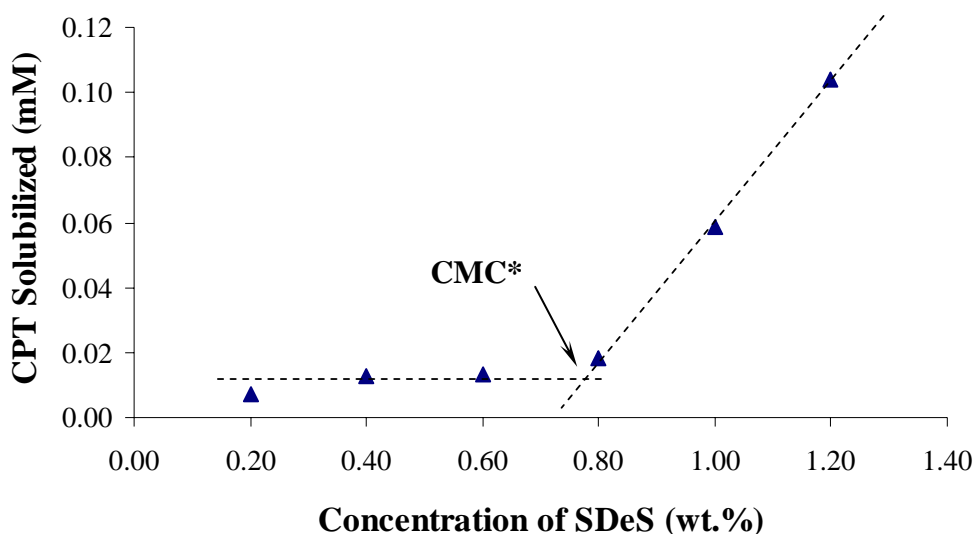


Figure 7-2: Solubilization effect of SDeS on CPT.

The solubilizing capability of SDeS on the hydrophobic drug, CPT was determined and the result was plotted in Fig. 7-2. A sudden increase in the solubility of CPT was observed when the concentration of SDeS exceeded 0.78 wt%, which, as shown in Fig. 7-2, was defined as the CMC* of the surfactant in the presence of CPT. The CMC* was slightly lower than the actual CMC of SDeS in pure water (0.85 wt%). As have been discussed in many cases of other surfactants, the decrease in the CMC of SDeS was due to the presence of the small hydrophobic solute (CPT) in the surfactant solution.

Beyond the CMC*, the effect of SDeS on the improvement of the solubility of CPT was

obvious. For example, at 1.2 wt% SDeS concentration, the solubility of the drug has been improved to 1.04×10^{-4} M, which is 27 times the solubility of CPT in water (3.85 μ M). However, the solubilizing effect of SDeS was found to be lower than that of SDS or SDBS. At 1.0 wt% surfactant concentration, the solubility of CPT in SDeS was 0.59×10^{-4} M, which was much lower than that from SDS (3.18×10^{-4} M) and SDBS (4.43×10^{-4} M). The low solubilizing ability of SDeS might result from the relatively weak hydrophobicity of the alkyl chains of SDeS compared to SDS or SDBS. This work demonstrates that the hydrophobicity of the hydrophobic part of one surfactant plays an important role in determining the solubilizing ability of this surfactant on hydrophobic solutes.

Fig. 7-3 shows the UV-vis absorption spectra of CPT solubilized in SDeS solutions. All the spectra resemble each other, indicating a stable solubilization of CPT in these surfactant solutions.

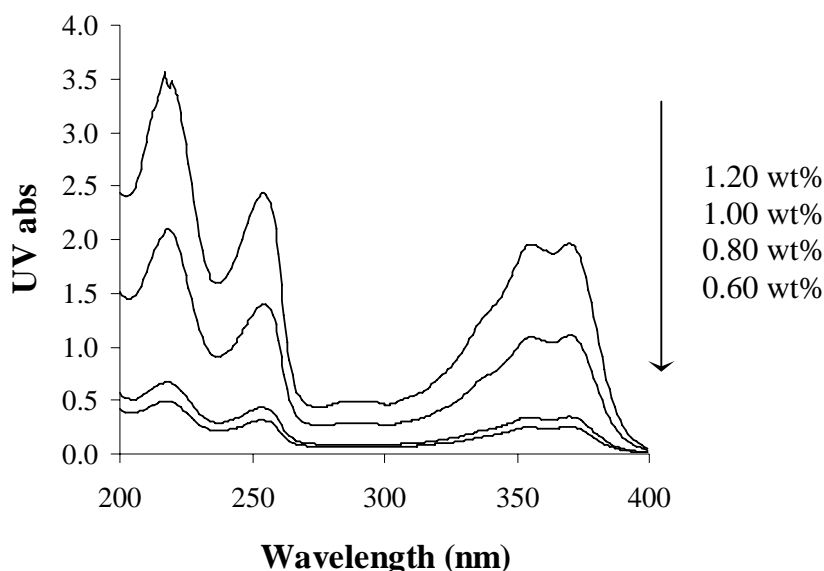


Figure 7-3: UV-vis spectra of CPT solubilized in SDeS solutions.

The fluorescence emission and excitation spectra of CPT before and after being solubilized in 1.0 wt% SDeS were given in Fig. 7-4. Like in the cases of SDS and SDBS, a blue shift in the emission spectra was observed from free CPT to CPT solubilized in SDeS micelles. As have been discussed in Chapter 5-6, the blue shift was considered to be evidence that the drug molecules reside in the cores of the micelles of SDeS. Same blue shifts were also found in the fluorescence spectra of CPT in 0.8 wt% and 1.2 wt% SDeS solutions (data are not shown here).

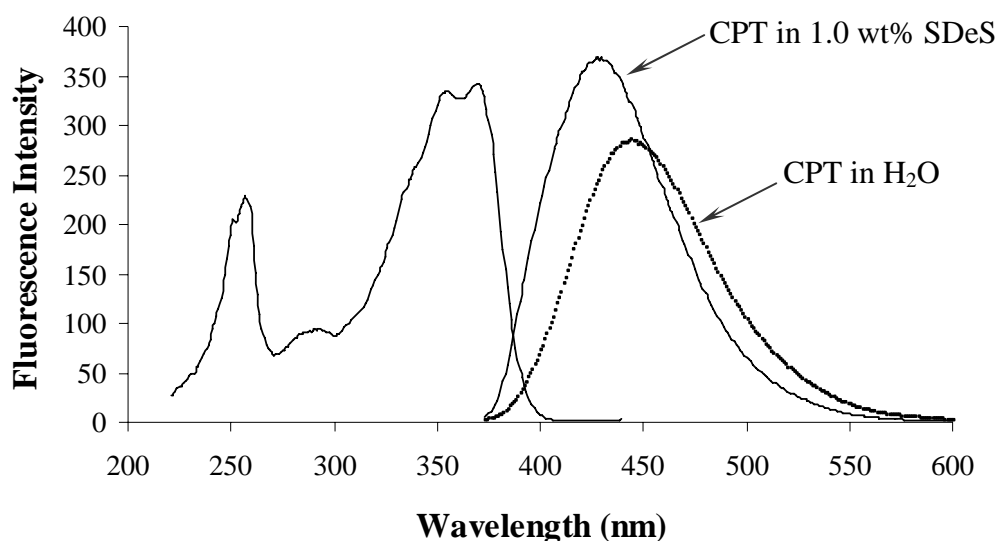


Figure 7-4: Fluorescence emission and excitation spectra of CPT solubilized in SDeS. The dashed line is the fluorescence emission spectrum of CPT in pure water.

7.1.2 Polyoxyethylene (9.5) t-octylphenol (Triton X-100)

Triton X-100 is a well-known nonionic surfactant, about which many research works have been done [Brown et al., 1989; Kushner and Hubbard, 1954; Paradies, 1980]. Triton X-100 is able to form micelles in aqueous solutions, where the hydrophobic alkyl

chain and benzene constitute the interior part of the micelles while the soluble PEO forms the external hydrophilic part. The CMC of Triton X-100 was reported to be 3.1×10^{-4} M [Dai and Tam, 2003].

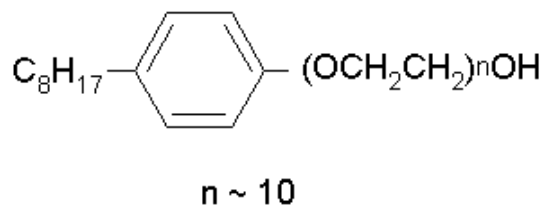


Figure 7-5: Chemical structure of Triton X-100

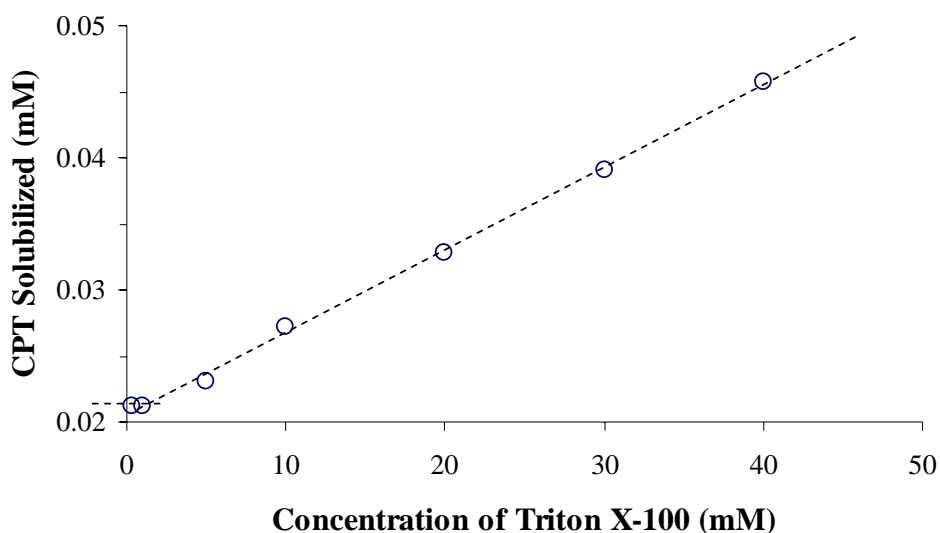


Figure 7-6: Solubilization of CPT in various Triton X-100 solutions

The solubilization result of CPT in solutions of Triton X-100 was plotted in Fig. 7-6 as a function of the concentration of Triton. No clear turning point was observed in Fig. 7-6, which was probably because of the very low CMC of Triton. Considering that the presence of a hydrophobic small molecular substance generally decreases the CMC of a surfactant, the already very low CMC of Triton X-100 will be even lower, which makes

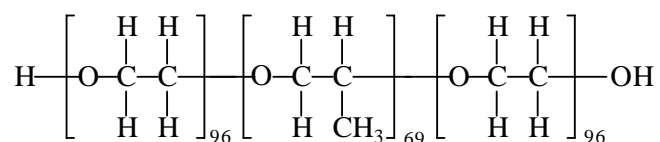
the possibility of micelles forming in all the solutions that have been studied. The improving effect of Triton X-100 on the solubility of CPT was observed. The highest solubility of CPT obtained when using 40 mM Triton X-100 was 4.58×10^{-5} M.

7.2 Polymeric Surfactants – F127, 25R4, PSS

7.2.1 F127

Polymeric surfactants, especially poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock polymers (PEO-PPO-PEO triblock polymers) find broad uses in drug delivery applications [Malmsten, 2002]. Reasons for this include their surface activity, which makes them efficient stabilizers of colloidal drug delivery systems; their gel forming capacity, which allows many opportunities in drug delivery; and their formation of self-assembly structure analogous to those formed by low molecular surfactants.

As a typical representative of many PEO-PPO-PEO triblock polymers, F127 has two 96-unit hydrophilic PEO chains surrounding one 69-unit hydrophobic PPO chain. The chemical structure of F127 is shown below.



The solubilization effect of F127 on CPT was shown in Fig. 7-8. Upon increasing the concentration of F127, the amount of the drug that could be solubilized increased. In a

concentration of 5.0 wt% F127, the solubility of CPT was improved to 3.0×10^{-5} M, which was nearly 8 times the solubility of CPT in water (3.85 μ M).

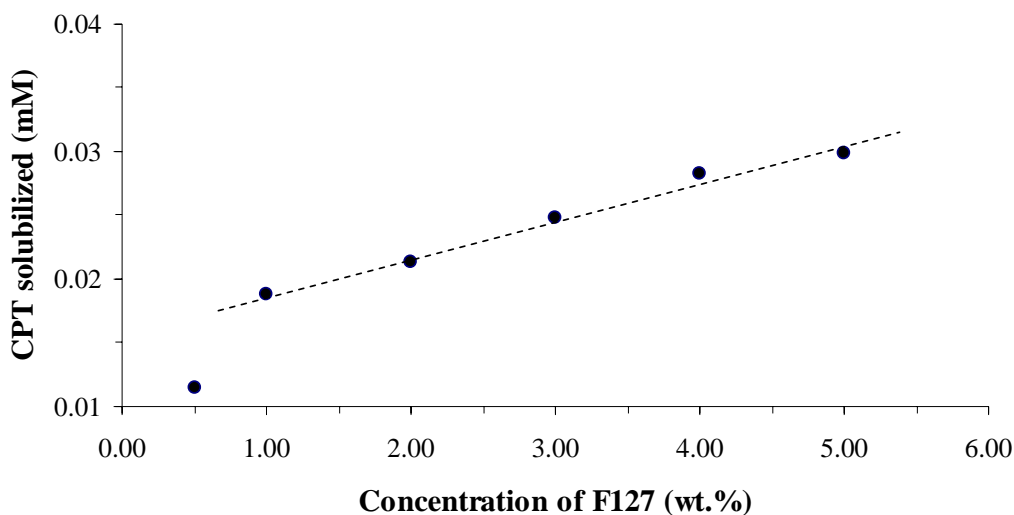
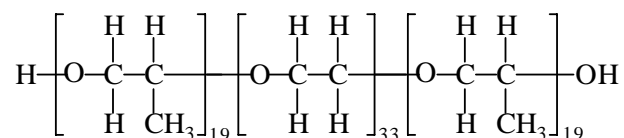


Figure 7-8: Solubilization of CPT in F127 solutions

7.2.2 25R4

Pluronic 25R4 is a difunctional block copolymer surfactant with terminal secondary hydroxyl groups. It is a nonionic surfactant that is 100% active and relatively nontoxic. Unlike F127 or other Pluronic polymers, it has two hydrophobic PPO blocks surrounding one hydrophilic PEO block, as shown below.



The solubilizing capacity of 25R4 as a surfactant on CPT was examined. The result was plotted in Fig. 7-9. 25R4 showed lower solubilizing ability than F127, which might

result from its unique structure. With prominent hydrophobic PPO chains taking up the ends of the molecule, 25R4 does not readily dissolve in aqueous solution to form micelles. As a result, it shows only poor solubilizing ability.

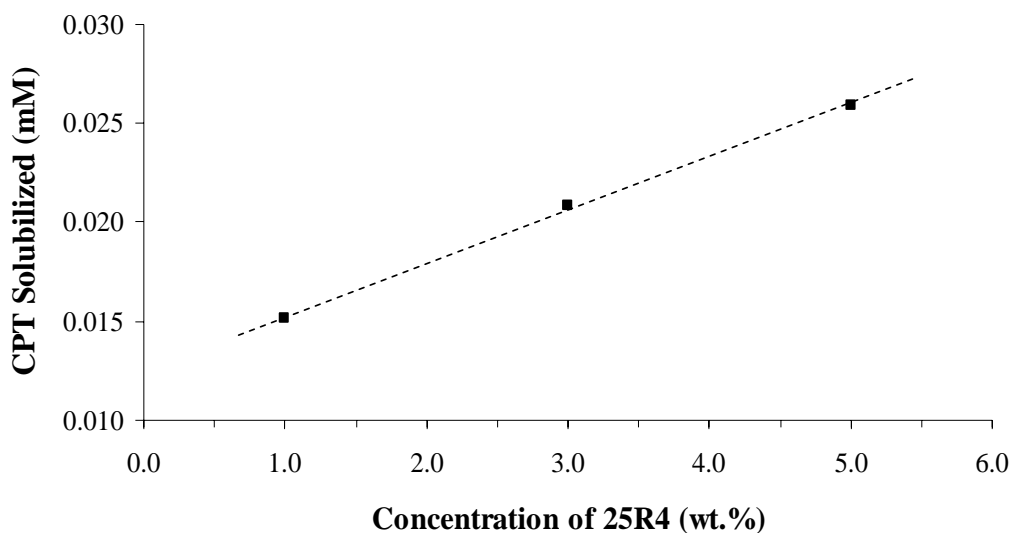


Figure 7-9: Solubilization of CPT in 25R4 solutions

7.2.3 Poly (sodium 4-styrenesulfonate) (PSS)

An anionic polymeric surfactant, PSS was examined. The monomer of the polymer consists of a styrene with sodium sulfonate substitution at the 4th position (Fig. 7-10).

As shown in Fig. 7-10, PSS showed more effective solubilizing result on CPT than F127 and 25R4. The solubility of the drug could be improved as high as 38 times to 1.46×10^{-4} M when a PSS solution of 10 wt% concentration (1.4 mM, which is equal to 0.49 mM monomer concentration) was used.

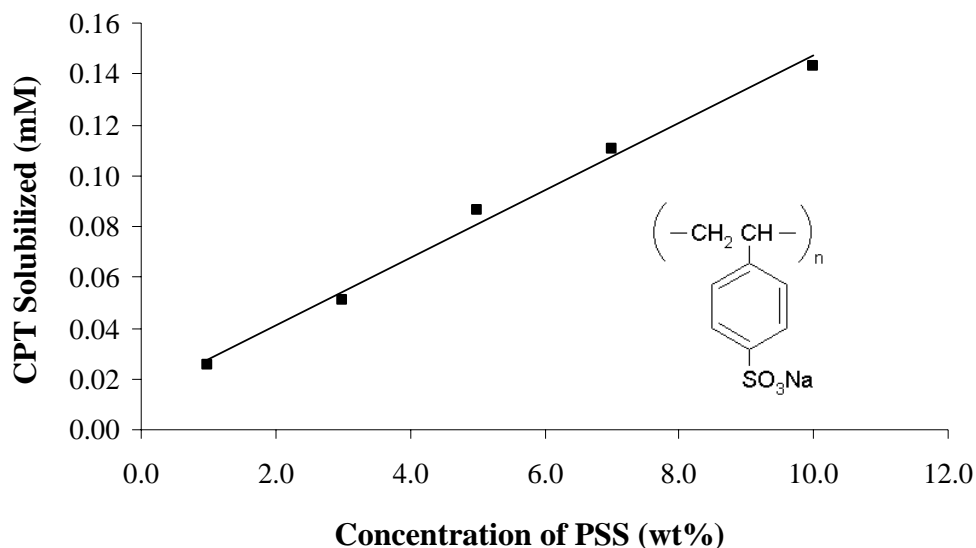


Figure 7-10: Solubilization of CPT in PSS solutions. The chemical structure of PSS is also given.

7.3 Cyclodextrins (CDs)

Cyclodextrins (CDs), the cyclic oligomers of glucose, are some cylinder-shaped molecules that have been extensively used in pharmaceutical and related applications [Hirayama and Uekama, 1999; Li and Purday, 1992]. CDs can be used either for molecular complexation or as functional carrier materials in pharmaceutical formulations [Szejtli, 1982]. One of the important characteristics of CDs is that they form inclusion complexes both in solution and in the solid state, in which each guest molecule is surrounded by the hydrophobic environment of the CD's cavity. This can lead to the alteration of the physical, chemical and biological properties of the guest molecules, and can eventually have considerable pharmaceutical potentials [Hirayama and Uekama, 1999].

Several types of CDs, including α -CD, β -CD, γ -CD, were investigated in this study for their potential as drug release additives. Among them, α -CD showed the most promising result. CPT can be solubilized in α -CD solutions to improve the poor water solubility of the drug and the results are given in Fig. 7-11. At 10 wt% α -CD concentration, the solubility of the drug could be enhanced to 0.976×10^{-4} M, 25 times the solubility in water. Compared to other methods, such as encapsulation within liposomes, lipid complexation, and preparation of soluble derivatives of CPT, the CD-based formulations are simple and easy to prepare and present little or no concerns of instability during storage [Kang et al., 2002].

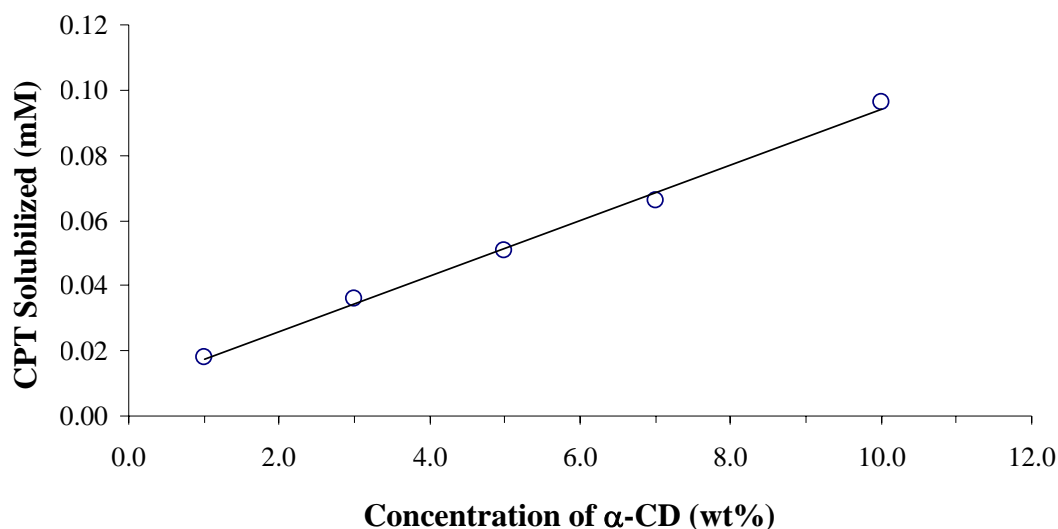


Figure 7-11: Solubilization of CPT in α -CD solutions

The UV-vis absorption and fluorescence emission and excitation spectra of CPT solubilized in α -CD solutions are given in Fig. 7-12 and Fig. 7-13. The stability and the resemblance of the UV-vis absorption spectra of CPT in Fig. 7-12 suggest that the CPT- α -CD system is stable and favorable, while the consecutive increase in the absorption intensity indicates the increased solubility of the drug in the α -CD solutions.

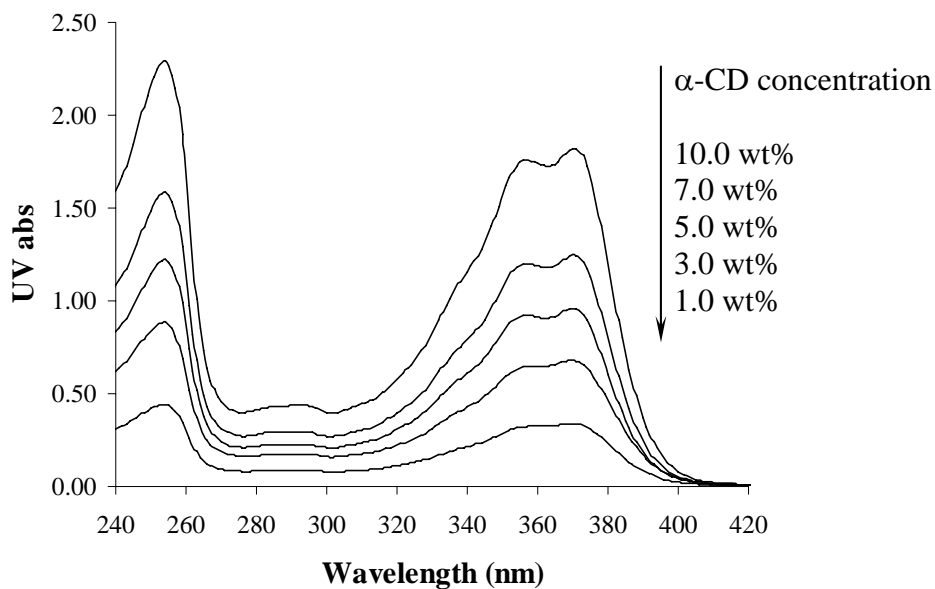


Figure 7-12: UV-vis absorption of CPT in α -CD solutions.

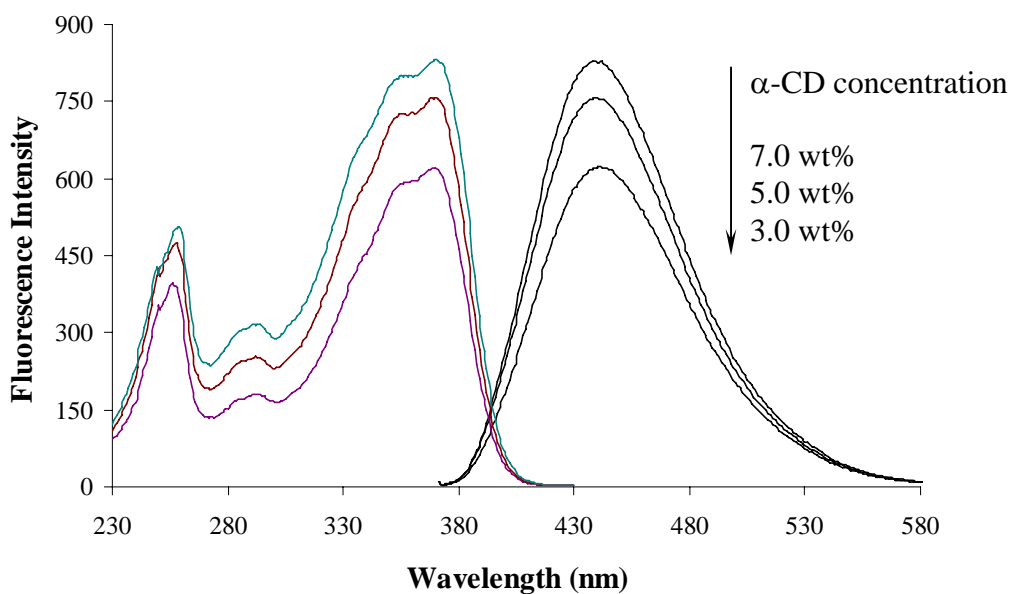


Figure 7-13: Fluorescence emission and excitation spectra of CPT in α -CD solutions.

Compared to the fluorescence emission spectra of CPT in water (not shown here), the fluorescence spectra of CPT in α -CD solutions in Fig. 7-13 were slightly blue shifted

(to short wavelength). As has been demonstrated many times previously, this blue shift indicates the change from a hydrophilic to a less hydrophilic environment surrounding the drug molecules. Since the only hydrophobic environment available in the α -CD solutions are the cavities of the α -CD molecules, Fig. 7-13 suggests that the inclusion complex of CPT and α -CD may form in the solution, where the CPT molecule resides in the cavity of α -CD.

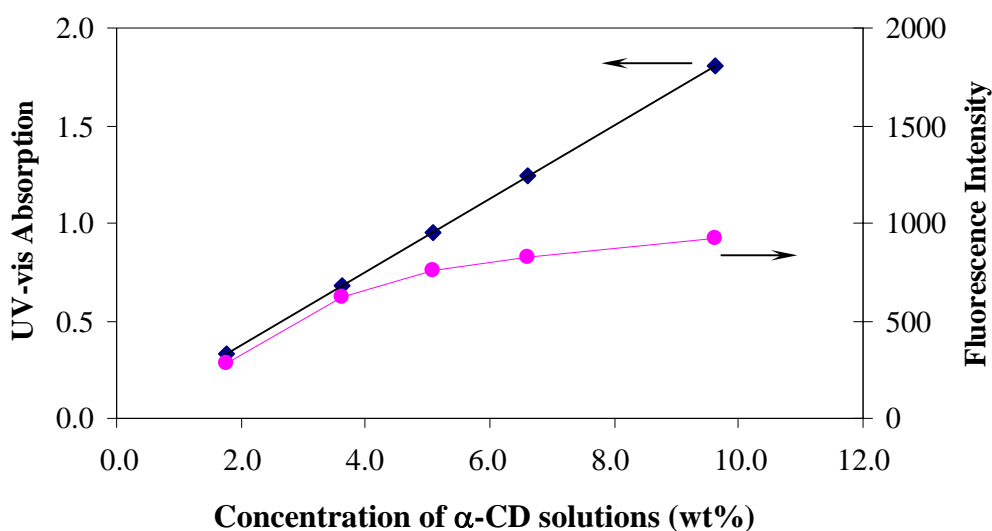


Figure 7-14: The UV-vis absorption (369 nm) and fluorescence emission (440 nm) of α -CD solutions with CPT solubilized (the concentration of α -CD is given in x axis, the corresponding concentration of CPT can be found from Fig. 7-11)

It is interesting to see from Fig. 7-12 and Fig. 7-13 that for the same solutions of CPT- α -CD (3.0, 5.0 and 7.0 wt% of α -CD concentration), their fluorescence emission and UV-vis absorption do not increase at the same pace. The increase in the fluorescence emission falls behind the increase in the UV-vis absorption. It is confirmed that both the fluorescence emission and UV-vis absorption of CPT- α -CD solution are totally from CPT because α -CD shows no emission or absorption in the studied wavelength range. The UV-vis absorption (at 369 nm) and fluorescence emission (440 nm) for all the CPT-

α -CD solutions studied are given in Fig. 7-14. It can be seen from Fig. 7-14 that the UV-vis absorption increase linearly as the concentration (CPT) of the solution increase but the fluorescence does not. At high concentrations, the fluorescence emission will deviate from the linear trend regulated by the Beer's law and not be able to reflect the real concentration of CPT in the solution. This can be ascribed to: (i) the way the fluorescence measurement was made, i.e. using front-face geometry. With the front-face geometry, the fluorescence intensity plateaus at high concentrations; (ii) self-quenching of CPT molecules. It is a common knowledge that aggregation of the fluorescent probes in solution usually leads to severe quenching of fluorescence [Mandal et al., 1999; Verkman et al., 1987]. At high concentrations of a fluorescent probe in solution, the fluorescence intensity might reduce because of the self-quenching between the probe molecules themselves [Lakowicz, 1999].

7.4 Summary

More surfactants, including both small molecular and polymeric surfactants were investigated in this chapter for their potential abilities to improve the poor water solubility of CPT. Effective results were observed for some small molecular surfactants. For example, 0.036 mg CPT could be solubilized in 1 ml SDeS solution (1.2 wt%) to yield a solution of CPT of 1.04×10^{-4} M, which is 27 times the solubility of CPT in water. However, polymeric surfactants such as F127, 25R4 and PSS showed only common abilities in solubilizing the drug, which may be due to their large molecular structures.

α - cyclodextrin (α -CD) and other CD derivatives may be the most promising

candidates for future studies in this area. As demonstrated in Fig. 7-11, α -D showed great potential in solubilizing the drug owing to their inclusive complex forming ability. In 10 wt% α -CD solutions, the solubility of CPT could be improved to 0.96×10^{-4} M and it was proved that the CPT molecules have resided in the cavities of α -CDs. Different from other candidates, CDs have some unique advantages, such as their biocompatibility and bioadaptability.

Results in this chapter could be useful reference for future studies of solubilization of hydrophobic drugs.

Chapter 8

Release of Theophylline from Polymer Blend Hydrogels

In the previous chapters, the release properties of the hydrophobic drug, CPT, as well as its solubilization and immobilization by various surfactants have been studied. One feature of all those studies is that the employed release systems are mainly made of single-polymer hydrogels. In this chapter, blend hydrogels made from different polymers will be examined for their potential utility as drug release systems. Blending is a simple method to combine the advantages of different polymers. The resulting polymer blends may show synergistic properties. Since blend hydrogels usually have more complicated network structures than homo-hydrogels, the practical and expected properties from these heterogeneous hydrogels for drug release application are illustrated as: (1) new release mechanisms for improved release kinetics, (2) biocompatibility, (3) improved mechanical properties, and (4) additional functionality (polymer-polymer interactions) [Bae and Kim, 1993].

Natural polysaccharides or proteins, such as agar/agarose, gelatin and κ -carrageenan have been demonstrated to be good candidates for controlled drug release by our previous work. These polymers have also been extensively used by other researchers as release materials [Picker, 1999a; Kuijpers et al., 2000; Lead et al, 2003; Meilander et al., 2003], but there are only a few reports dealing with the controlled drug release based on their blends [Kandil et al., 2004; Kuijpers et al., 2000; Sjöberg et al., 1999;]. The present work aims at filling this vacancy.

An attempt has been made in this chapter to prepare hydrogel blends from agar, gelatin, and κ -carrageenan. An anti-asthma agent, theophylline (TPH), was used as the model drug to study the release properties of these blended hydrogels. TPH was chosen because of several reasons: (i), TPH is an effective pharmaceutical drug; (ii) it has a simple UV-vis spectrum which is easy to monitor; (iii), TPH has a good solubility in water. For example, 10 mM water solution can be easily prepared. Good water solubility of the drug is essential for release studies, since it will help to achieve a homogeneous distribution of the drug in hydrogel systems.

8.1 UV-vis Spectral Properties of TPH

TPH has been pharmaceutically applied for the treatment of chronic asthma and pulmonary diseases. The most important functions of this drug are to prevent the signs and symptoms of asthma, especially during the night, and to reduce the need for cortisone type medication. It also reduces or prevents symptoms from triggers of asthma such as exercise and allergens. It is a modest bronchodilator relaxing muscles around the airway, allowing air to travel more freely in and out of the lungs [Yu, 1996]. The chemical structure of TPH is shown in Fig. 8-1.

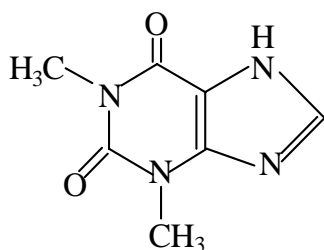


Figure 8-1: Chemical structure of TPH.

8.1.1 UV-vis Spectrum of TPH

The typical UV-vis absorption spectrum of TPH is shown in Fig. 8-2. It peaks at 272 nm. As far as the peak wavelength is concerned, several different values have been reported by various researchers. Miyazaki et al. [Miyazaki, 2000] used the wavelength of 274 nm in their work for the sustained delivery of TPH using in-situ gelation of sodium alginate. Puttipatkhachorn et al. [2001] used 272 nm and Katime et al. [2001] used 270 nm to assay the mass of TPH in their respective studies. However in the present work, as shown in Fig. 8-2, 272 nm is considered to be more suitable as the indicative wavelength for the quantitative assay of TPH and this value has been used throughout the following drug release experiments.

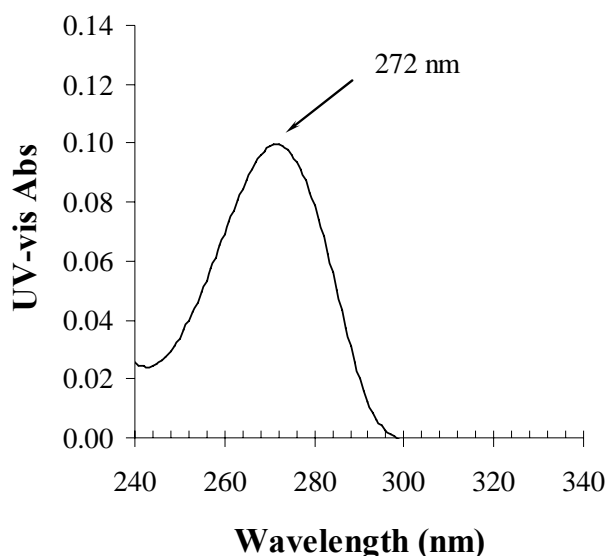


Figure 8-2: UV-vis spectrum of TPH in water (1×10^{-5} M)

8.1.2 Extinction Coefficient of TPH

A specific substance usually can give a particular UV-vis spectrum, which is

characteristic to its unique chemical structure. The UV-vis absorbance of a given solutions proportionally relates to the concentration of the solute in solution according to the Lambert-Beer's law [Knowles and Burgess, 1984]:

$$A = -\log \frac{I_0}{I} = \varepsilon \cdot b \cdot c \quad (8-1)$$

where A is the absorbance at a particular wavelength and is dimensionless, I_0 is the intensity of radiation entering and I the intensity leaving a solution of optical pathlength b (cm) and concentration c (mol/l). The constant of proportionality (ε) is termed the extinction coefficient at the specified wavelength and has a unit of $(\text{mol/l})^{-1} \cdot \text{cm}^{-1}$. The extinction coefficient thus represents the absorbance of a 1 mol/l solution measured in a 1 cm layer. To a specific substance, the extinction coefficient is usually constant if other conditions remain the same.

Eq. (8-1) suggests that by measuring the UV-vis absorbance of a substance, the concentration of its solution could be known. This simple yet important relationship has made UV-vis spectrometry a very useful detective technique in many applications. A number of research works in the controlled drug release area are based upon this technique, in which it is used to monitor the release profiles of drugs [Lu and Anseth, 2000; Qiu et al., 2001; Katime et al., 2001; Coviello et al., 2003].

Before we can use Eq. (8-1) to determine the concentration of an unknown TPH solution, the extinction coefficient (ε) must be known. This is actually why a calibration curve is always needed before any further experiment could be done. The calibration curve based on the Lambert-Beer's law for TPH in water was given in Fig. 8-3. A high linearity was observed up to a concentration of 1.2 mmol/l of TPH. The extinction

coefficient of TPH at 272 nm was obtained from the slope of the trend line to be $1.02 \times 10^4 \text{ (mol/l)}^{-1} \cdot \text{cm}^{-1}$.

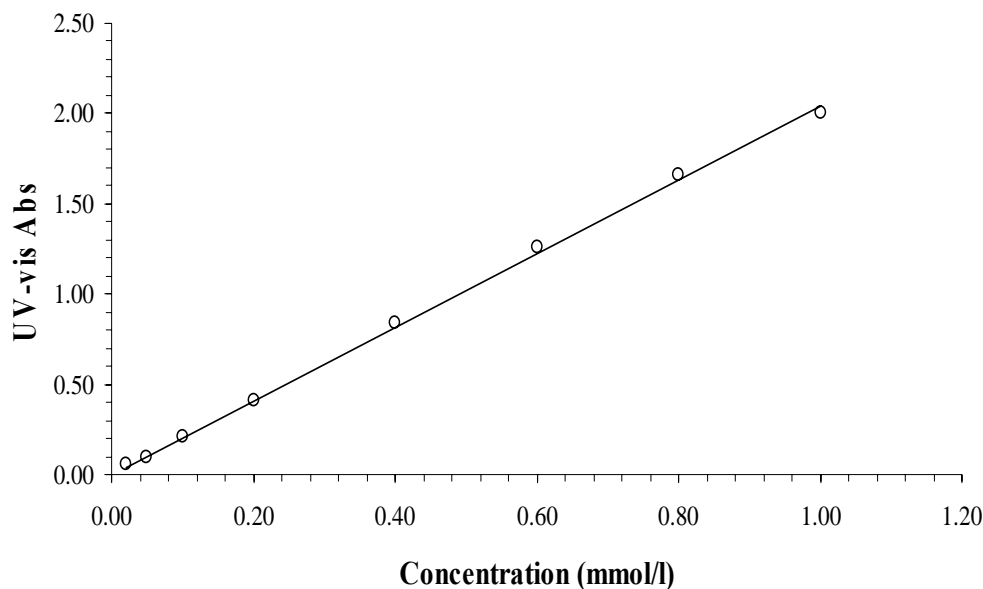


Figure 8-3: Calibration curve for the UV-vis absorbance of TPH at 272 nm in water (measured with $b = 0.2 \text{ cm}$ UV-vis cell).

8.2 Release of TPH from Agar Hydrogel

TPH has been widely used as a model drug in controlled drug release studies. Katime et al. [2001] reported the swelling kinetics and release studies of TPH from acrylic acid/n-alkyl methacrylate hydrogels. They have found the release of TPH from the swollen hydrogel was Fickian and diffusion controlled. Coviello et al. [2003] investigated the release of this drug from the scleroglucan hydrogel under different environmental conditions. The diffusion coefficient of TPH was evaluated to be $(4.2 \pm 0.05) \times 10^{-6} \text{ cm}^2/\text{s}$. TPH's release from the matrices containing κ -carrageenan was studied by Picker et al. [1999b].

In this work, the release of TPH from the agar hydrogel was studied at 37 °C in a cylindrical vessel filled with deionized water (100 ml) under slight stirring. At certain time intervals, sample of the releasing solution was subject to UV-vis measurement. The release profile of TPH recorded by UV-Vis absorbance at 272 nm is given in Fig. 8-4.

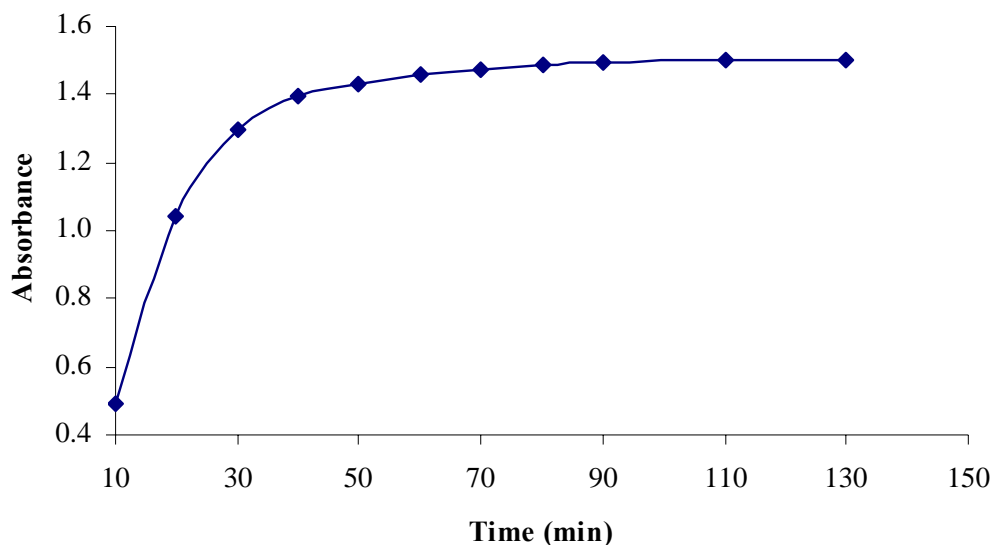


Figure 8-4: Plot of the UV-vis absorbance of TPH released from agar hydrogel in water at 37 °C, as a function of time.

From the extinction coefficient of TPH ($1.02 \times 10^4 \text{ (mol/l)}^{-1} \cdot \text{cm}^{-1}$) and according to Eq. (8-1), the concentration of TPH in the releasing solution at each time could be obtained. Thus, M_t , the amount of drug released at time t could be known. The fraction of the amount of drug released at time t was then calculated by dividing M_t by M_∞ , which is the theoretical concentration corresponding to a complete release of the drug that have been loaded in the release sample. The fractional release profile of TPH was plotted in Fig. 8-5 as a function of release time t .

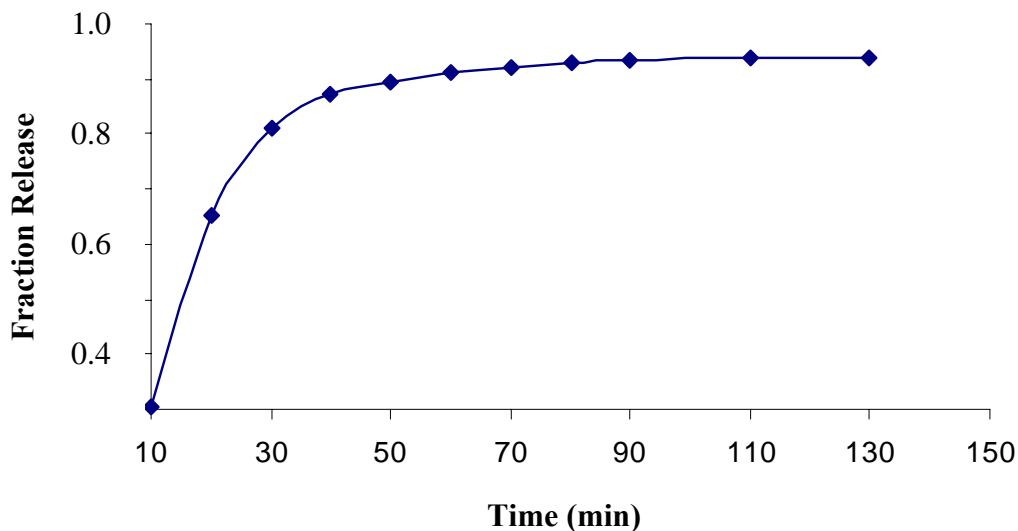


Figure 8-5: Plot of TPH release fraction M_t/M_∞ from agar hydrogel in water at 37 °C, as a function of time.

To determine the feature of TPH release from the agar hydrogel, the following equation was used:

$$\frac{M_t}{M_\infty} = kt^n \quad (8-2)$$

where, M_t and M_∞ represent the amounts of TPH released at time t and at infinite long time, respectively, k is the kinetic release constant and n , the diffusion exponent characteristic for the release mechanism. A value of $n = 0.5$ indicates a Fickian diffusion mechanism, while $0.5 < n < 1$ indicates anomalous or no Fickian diffusion. A special case is $n = 1$, associated with the release kinetics occurring at constant rates [Ritger and Peppas, 1987a]. The logarithm of the fraction, $\frac{M_t}{M_\infty}$, of TPH released was plotted in Fig. 8-6 against $\log t$.

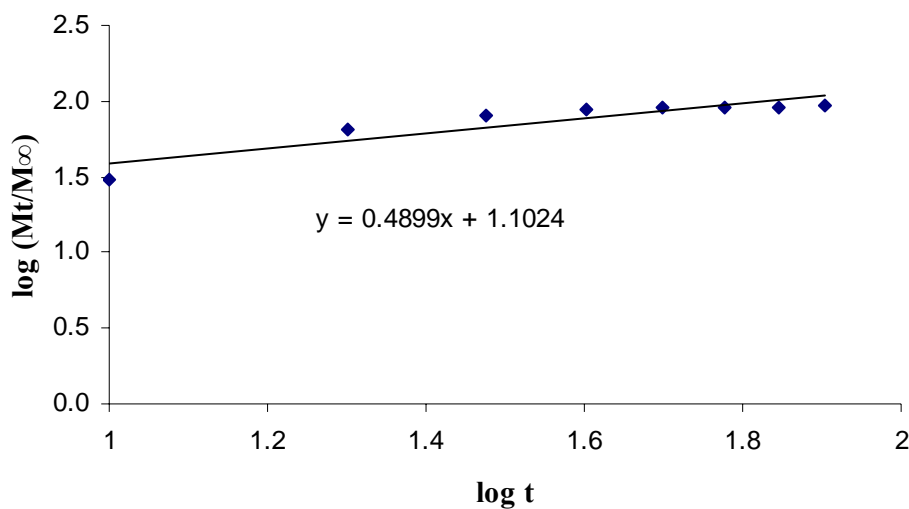


Figure 8-6: Plot of logarithm of TPH release fraction, M_t/M_∞ , as a function of $\text{Log}_{10} t$.

A value of n equal to 0.49 indicated that the release of TPH from agar hydrogel is in Fickian diffusion pattern [Ritger and Peppas, 1987a].

8.3 Release of TPH through Blend Hydrogels of Agar, Gelatin, and κ -Carrageenan

In addition to the agar hydrogel, the release of TPH from hydrogels of gelatin, κ -carrageenan and most importantly, from hydrogels made from two-component blending of gelatin, agar and κ -carrageenan, was also studied.

8.3.1 Effect of Blending

Although agar, gelatin, and κ -carrageenan have shown useful applications in controlled drug release studies [Tabata and Ikada, 1998; Sumathi and Ray, 2002; Lead et al., 2003],

where they are used as the drug carriers in form of hydrogels, drug releases from these single polymer gels are often too fast, especially for the release of hydrophilic drugs. This fact was explained by the negligible hydrodynamic hindrance to the movement of the drug molecules from a hydrogel network [Sjöberg et al., 1999]. Thus, improvement is needed in order to achieve a sustained and controlled release. Use of polymer blend hydrogels might be a good solution, as it has been reported that interpenetrating networks (IPN) may be formed from polymer blends, which may help to slow down the release rate [Clark et al., 1983, 1999; Amici et al., 2000, 2002; Matsuo et al., 2002]. Formation of an IPN structure at the molecular level is able to avoid the problem called “phase separation”. When an IPN hydrogel has been formed from two polymers, a physical phase separation between the component polymers would be nearly impossible because of the infinite zero-viscosity of the gel. IPN is also attractive in producing synergistic properties from the component polymers. For example, when a hydrophilic gelling polymer interpenetrates with a relatively hydrophobic gelling polymer, the resultant IPN hydrogel is expected to have an improved capability at immobilizing a drug. This is actually able to open a new way to use IPN in designing novel drug release systems.

Figs. 8-7-(a), (b) and (c) depict the percentage release profiles of TPH from three two-component blend hydrogels of GA55, Gκ55 and κA55, compared to the releases from single-component hydrogels of G-type, κ-type and A-type, respectively. The composition and designation for the above hydrogels can be found from Table 3-6 in Chapter 3. The release temperature was 37 °C. The blend ratio used here was 1:1 (50%:50%) in weight for all the three blend hydrogels. As can be seen from Fig. 8-7, the release of TPH is decreased significantly when a blend hydrogel is used.

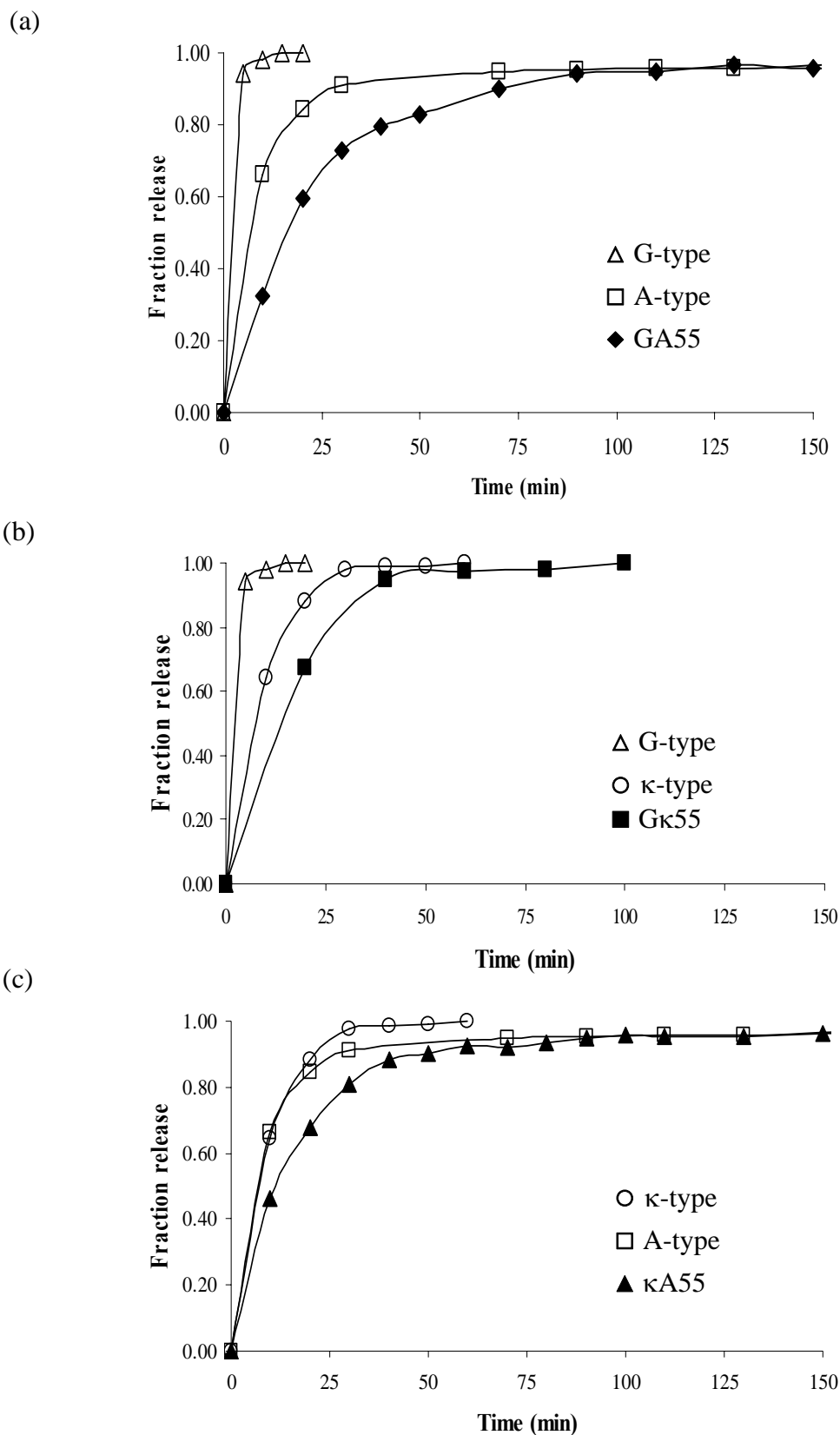


Figure 8-7: Fraction releases of TPH from hydrogels based on (a) gelatin and agar; (b) gelatin and κ-carrageenan; (c) κ-carrageenan and agar at 37 °C.

The release of an active substance from hydrogels is classically assumed to take place by diffusion [Muhr and Blanshard, 1982]. In a diffusion-controlled release, the diffusion of drug molecules within a hydrogel matrix is hindered by the insoluble gel network in which drug molecules have to travel through tortuous pathways to exit the gel matrix. Here, polymer chains in the gel network act as a diffusion barrier [Uhrich et al., 1999]. The slow drug release observed after blending suggests that now the drug has to travel a prolonged pathway through the polymer network. In addition, as a gel is considered as a solid, whose zero-shear viscosity is infinite, the molecular diffusion of the drug is also controlled by the high viscosity of the gel.

Although the molecular interactions between the drug (TPH) and the individual polymer components are not considered to increase by blending to result in the decrease of the release rate, it would be possible for the blending to cause some changes in the gel network structure such as polymer conformation, network density, etc. Particularly, the possibility of formation of an IPN gel from a polymer blend would be an important factor worthy to be investigated. Amici et al. reported the formation of a molecular IPN from agarose and κ -carrageenan [Amici et al., 2002]. When an IPN hydrogel is formed from a polymer blend, the molecular diffusion of a drug immobilized in the gel may be affected if the density or the complexity of the gel network is changed by the IPN structure. However, when the total polymer content in a gel is fixed (as being used in this study), it is difficult to consider a denser network formed from two-component polymers than from a single-component polymer if no additional crosslinks are formed between the two networks. To make a denser network, the formation of inter-network crosslinks (i.e. crosslinks between hetero-polymer networks) would be necessary. In this case, the IPN has a different structure from a normal IPN in which there are no direct

crosslinks between the two networks. It is possible that the inter-network crosslinks are formed through intermolecular hydrogen bonding, ionic bonding, or physical entanglement. In this work, it is considered that all these types of bonds (i.e. hydrogen bond, ionic bond, and physical entanglement) are possible for the blend hydrogels formed from gelatin, agar, and κ -carrageenan. At this moment, however, this hypothesis has not been confirmed due to the technical difficulties in accurately determining the crosslinking densities of the blend hydrogels.

Another interesting finding from Fig. 8-7 is that adding a polysaccharide component (agar or κ -carrageenan) to gelatin has significantly decreased the drug release rate, while this decreasing effect is smaller when two polysaccharide components (agar and κ -carrageenan) are used. This result can be explained by the different IPN structures between the former and the latter. In the case of the latter, the similarity in molecular structure between agar and κ -carrageenan would make it difficult to form the additional crosslinks (hydrogen bonds or ionic bonds) between two networks (i.e. the agar network and the κ -carrageenan network) even though an IPN structure can be formed from agar and κ -carrageenan. In the blend gel formed from gelatin and agar or from gelatin and κ -carrageenan, however, it is possible to form additional crosslinks through hydrogen bonding and/or ionic bonding. For example, gelatin is a protein consisting of amino acid monomer units and it can form a helical structure due to the intramolecular hydrogen bonding. Under acidic conditions, a protein may become a cationic polyelectrolyte so that the formation of ionic bonds between gelatin and κ -carrageenan would be possible because κ -carrageenan is an anionic polyelectrolyte. Similarly to a protein, κ -carrageenan is also able to form double helices upon cooling to result in gelation [Ramakrishnan and Prud'homme, 2000]. None has reported a possibility of forming a

double helix from a gelatin chain and a κ -carrageenan chain. If double helices could be formed from gelatin and κ -carrageenan molecules, additional junctions for the formation of a denser IPN could be expected.

8.3.2 Effect of Composition

In order to investigate the effect of composition of the hydrogels on the release of TPH, three weight ratios of blending have been employed in this study, which were 9:1, 7:3 and 5:5, where gelatin or κ -carrageenan was used as the first polymer (the host polymer) while agar or κ -carrageenan as the second (the guest). The resultant hydrogels were designated as, taking hydrogels from gelatin and agar as an example, GA91, GA73 and GA55. The designation and compositions for other blend hydrogels can be found in Table 3-6 in Chapter 3. The percentage cumulative releases of TPH to water at 37 °C from the three types of hydrogels based on different blending ratios were plotted as a function of time in Figs. 8-8-(a), (b) and (c).

Fig. 8-8-(a) shows the release profiles for the GA hydrogels consisting of gelatin and agar, while Figs. 8-8-(b) and (c) are for the G κ and κ A hydrogels, respectively. From Fig. 8-8, it was observed that with increasing the content of the guest polymer from 10% to 30% and further to 50%, the release profile of TPH was significantly lowered, indicating a reduced release rate. Among the three types of hydrogels, this effect was particularly notable in the GA (gelatin and agar) and G κ (gelatin and κ -carrageenan) hydrogels. The κ A hydrogels did not show a significant effect of blending ratio on the release. This might be due to the molecular similarity between κ -carrageenan and agar, resulting in a similarity in the gel network structure as compared to those formed from

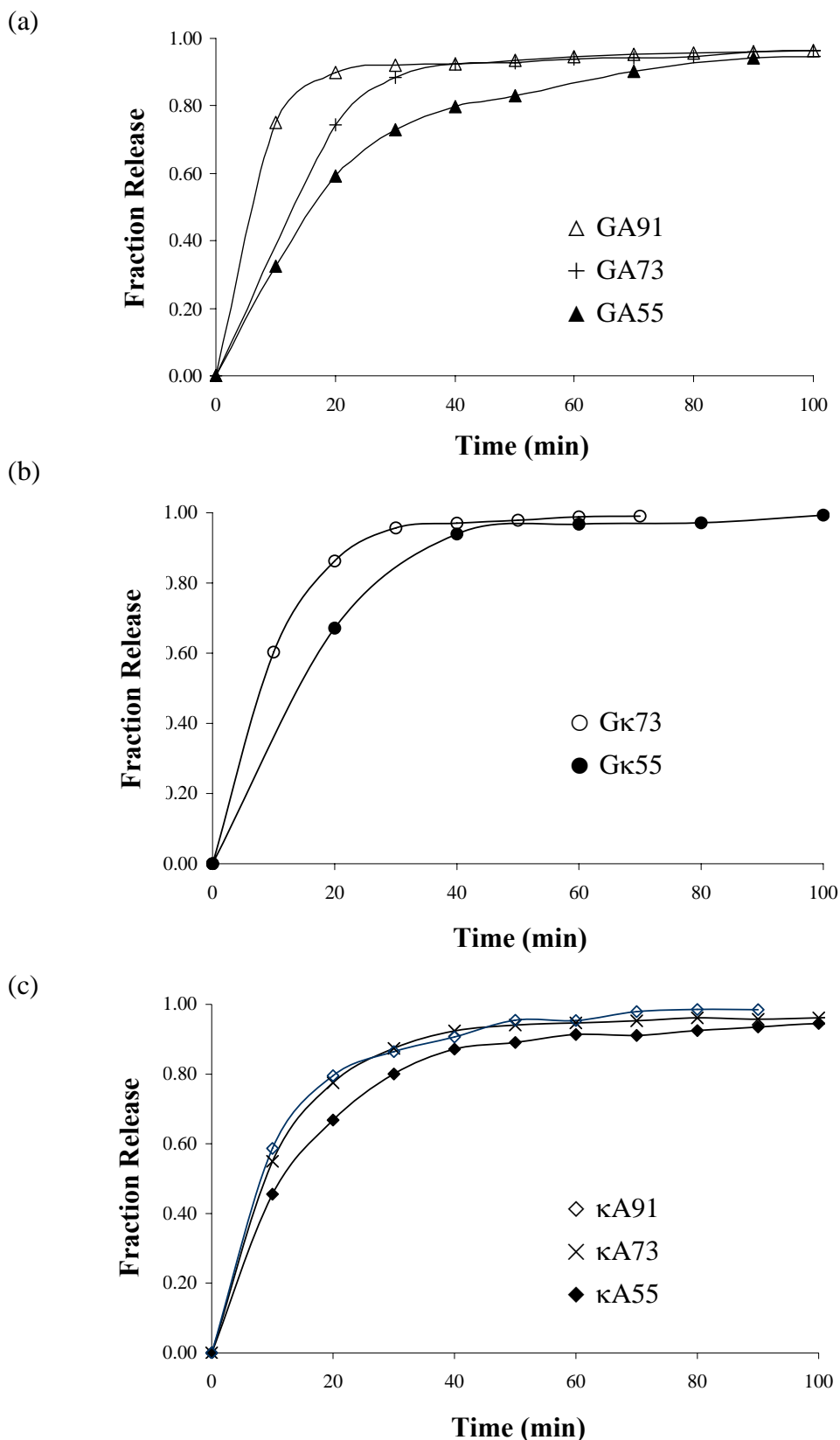


Figure 8-8: Effect of blending compositions of hydrogels based on (a) gelatin and agar; (b) gelatin and κ -carrageenan; (c) κ -carrageenan and agar, on the release of TPH at 37 °C.

individual κ -carrageenan or agar. In any case, the 1:1 ratio always gives the slowest release profile. The reason behind this might be that an optimal formation of an IPN was achieved with a ratio of 1:1. In a two-component hydrogel, when the guest polymer has a fewer amount than the host, the guest polymer would not be able to form a complete network within the network of the host. Moreover, the formation of inter-network crosslinks is reasonably expected to be dependent on the perfection of IPN formation. The inter-network crosslinks would be maximally formed between two complete networks that are interpenetrated physically. The only ratio that satisfies this condition is 1:1. Therefore, the ratio of 1:1 should give the densest gel network to delay the molecular diffusion of TPH through the gel matrix.

As shown above, the different blending composition of hydrogels have resulted in different release properties, which might be due to the different gel network structures controlled by formation of IPN, inter-network crosslinks, etc. Since a gel network structure formed from a polymer blend is more complicated than that from a single polymer, the release mechanism of a drug from a blend hydrogel will be more complicated than that from a single polymer. In this work, it has been proved that there is a clear effect of gel composition.

8.3.3 Effect of Temperature

Thermosensitive hydrogels always show a temperature dependence and we have already observed the effect of temperature on the release of CPT from agar hydrogel in Chapter 4. Different types of hydrogels may have different thermosensitivities. When temperature changes, a gel network expands or shrinks, resulting in a denser or looser

network. A looser gel network may provide more “free volume” for drug molecules to move. Temperature can also affect the mobility of a molecule, and the higher the temperature the higher the molecular mobility in a diffusion process. In addition, a substance may have different water-solubility at different temperatures. Thus, it can be expected that the release of TPH from a hydrogel is temperature-dependent and the effect of temperature on the release is complicated.

In order to investigate the effect of temperature on the release of TPH, three different temperatures 30, 37 and 45 °C were used. Fig. 8-9-(a), (b) and (c) show the release profiles of TPH from the three types of hydrogels with a blending ratio of 1:1 at 30, 37 and 45 °C, respectively. From Fig. 8-9, the temperature effect on the release of TPH was clearly observed. For example, at 30 °C, the time for the Gκ55 release system (Fig. 8-9-b) to reach an equilibrium release was about 200 min. This time has been reduced to 140 min at 37 °C, and further to 60 min at 45 °C. The temperature effect could be attributed to a combined effect from the three factors mentioned above, which were a looser gel network, a higher molecular mobility of the drug in the hydrogel and a higher solubility of the drug in the medium solution at enhanced temperatures. As a result, the rate of the drug molecules diffusing out of the hydrogel matrix would be increased.

The release profiles of TPH from hydrogels with a blending ratio of 7:3 are given in Fig. 8-10 and a similar tendency with what has been discussed in Fig. 8-9 is observed for the temperature effect.

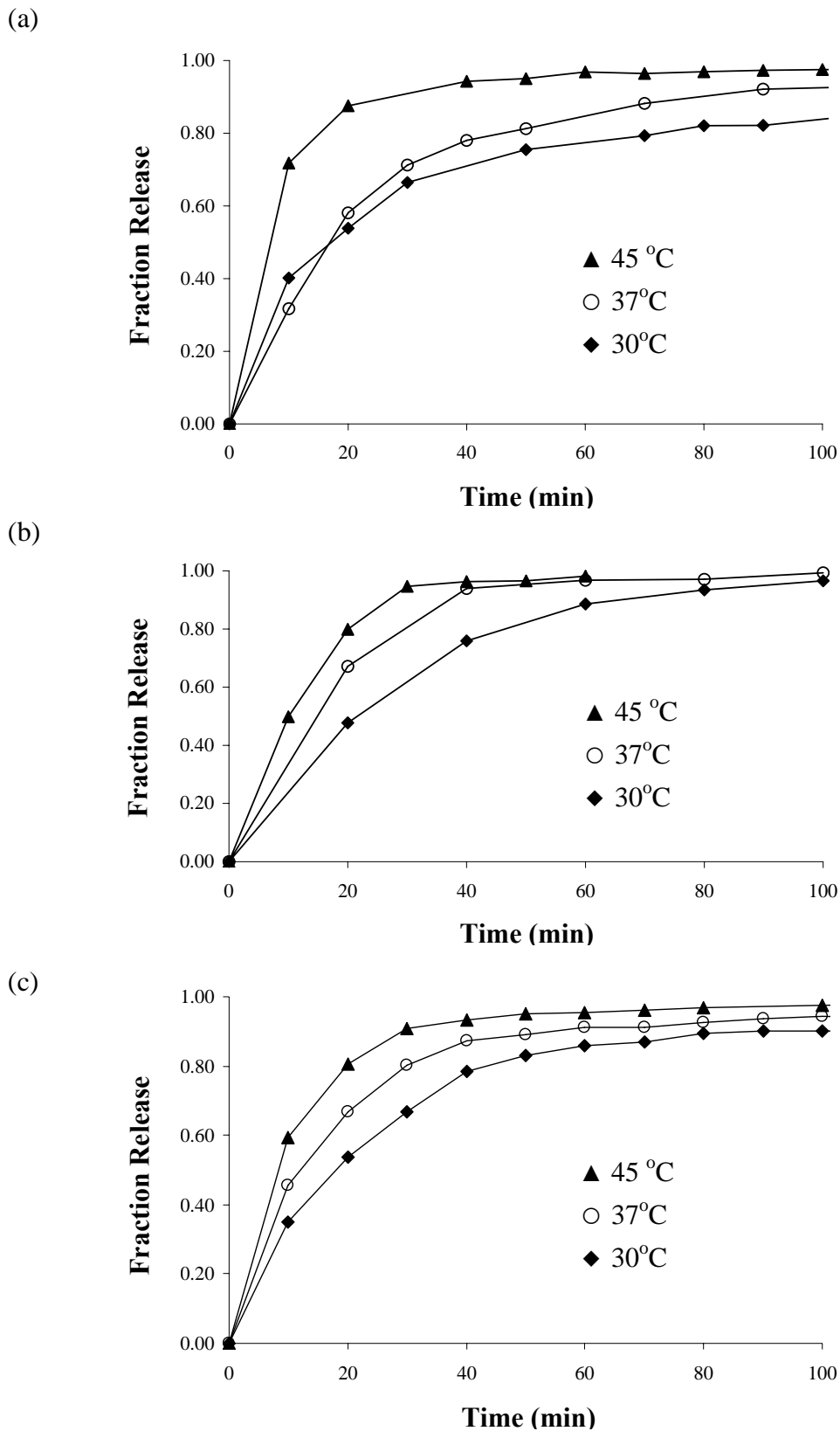


Figure 8-9: Effect of temperature on the release of TPH from hydrogels based on (a) gelatin and agar (5:5); (b) gelatin and κ -carrageenan (5:5); (c) κ -carrageenan and agar (5:5).

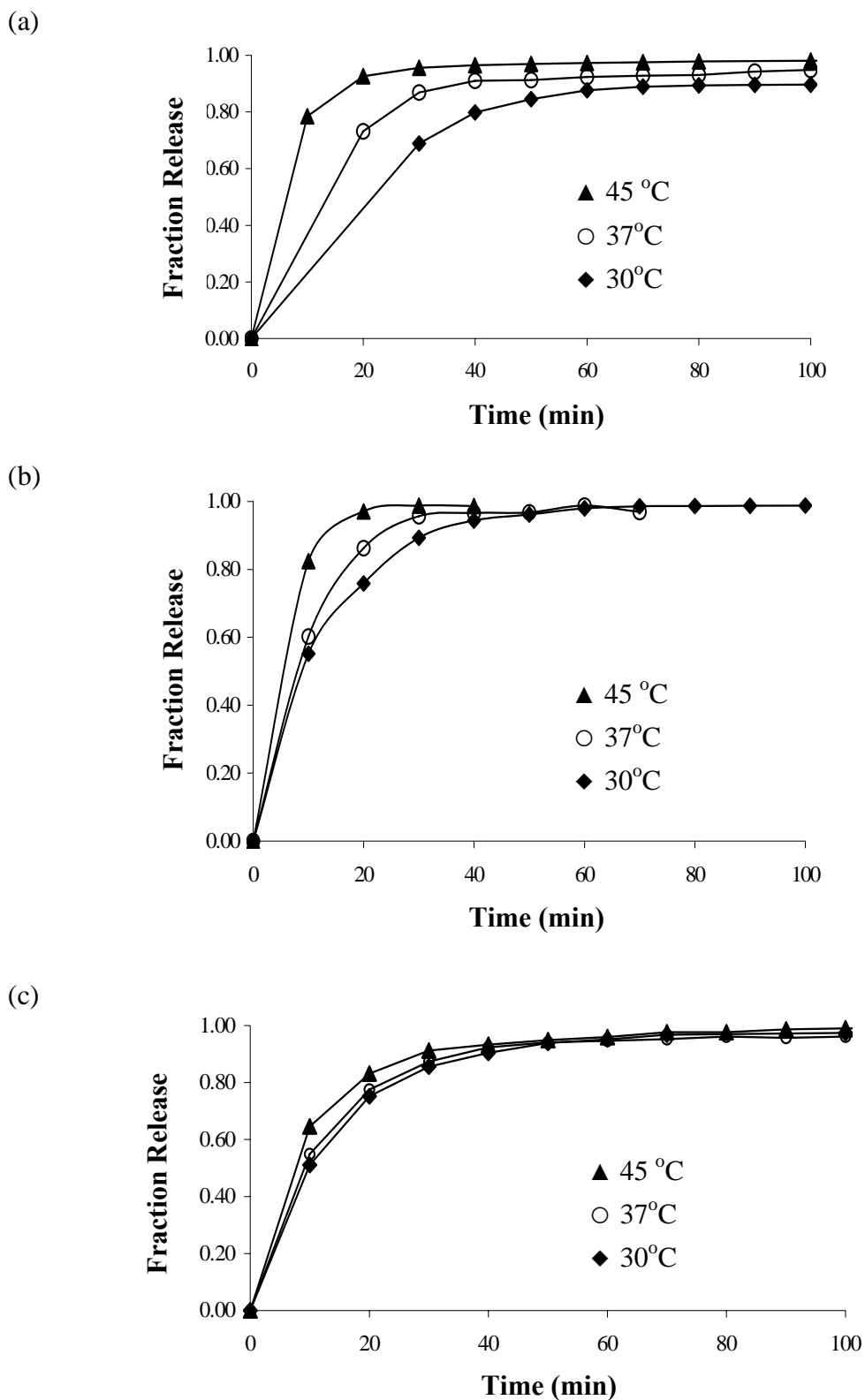


Figure 8-10: Effect of temperature on the release of TPH from hydrogels based on (a) gelatin and agar (7:3); (b) gelatin and κ -carrageenan (7:3); (c) κ -carrageenan and agar (7:3).

8.4 Summary

The molecular properties and the release of a water soluble drug, TPH, were studied in this chapter. TPH is an effective drug, which has been widely used for the treatment of symptoms of chronic asthma. The UV-vis absorption spectral property of TPH was examined. The drug has shown a clear absorption peak at 272 nm in water solution. The calibration curve of TPH for its UV-vis absorption was developed.

Polymer blending is a simple but attractive method to obtain combined physical and mechanical properties of polymers. Blend hydrogels of different compositions have been prepared in this chapter by physically blending every two polymers from gelatin, agar and κ -carrageenan with various blending ratios. The releases of TPH from those blend hydrogels as well as from those single-component hydrogels of agar, gelatin and κ -carrageenan have been studied and compared. These studies revealed the following facts.

1. The release of TPH from hydrogel systems exhibits a typical Fickian diffusion pattern with a characteristic diffusion exponent of 0.49.
2. Using blend hydrogels can always result in an improvement in the release properties of TPH.
3. The blending effect on the improvement of drug release is especially remarkable when blending gelatin with one polysaccharide polymer (agar or κ -carrageenan).
4. Whatever the two original polymers are, a 1:1 in weight blending always results in an optimal release pattern of TPH, which is much slower than that from other possible hydrogel formulations.

5. Temperature shows great influence on the release of TPH from the above hydrogels. The higher the external temperature, the faster the release is.

The possible mechanisms involved in the release experiments have been explored. The formation of IPN as confirmed by Amici et al. [2002] is considered to be a suitable explanation for the release results. However, at fixed polymer content, an IPN itself cannot be used to explain the reduction of the drug release rate if the total gel network density is not increased by the formation of an IPN. Therefore, a new mechanism was proposed that the additional crosslinks called “inter-network crosslinks” should be formed between two interpenetrated gel networks through hydrogen bonding, ionic bonding, co-forming of helical aggregates, or physical entangling.

Chapter 9

Conclusions and Recommendations for Future Work

In this fundamental study, the diffusion and release of two small molecular drugs through hydrogels made from natural polymers were investigated under various conditions. The study mainly focused on the hydrophobic drug of camptothecin (CPT). A new strategy has been developed to overcome the poor water solubility of CPT and the resultant limitations in its controlled release. Various surfactants were used to solubilize CPT and subsequently immobilize the drug into hydrogels. The release of CPT from those hydrogel-surfactant systems was studied. In addition to CPT, the release properties of a water soluble drug, theophylline (TPH), from polymer blend hydrogels were also investigated. Based on the results obtained in the previous chapters, several conclusions about this project can be drawn and some recommendations made for future studies.

9.1 Conclusions

1. Properties of the drugs studied

The molecular structure of CPT changed subject to the environmental pH. All the spectral properties of CPT, which were related to its molecular structure, therefore, showed clear pH dependence. Increasing the environmental pH could result in a structural conversion from the lactone closed-ring form to the carboxylate open-ring

form and the big red shifts in the UV-visible and fluorescent spectra of CPT. The calibration curves for the UV-vis absorption and fluorescence emission of CPT were obtained using an organic solvent, dimethylsulfoxide (DMSO), at 369 nm and 440 nm, respectively.

TPH showed an easily detectable UV-vis absorption spectrum, which peaked at 272 nm. No fluorescence emission could be observed for the aqueous solution of TPH. The calibration for the UV-vis absorption of TPH was done. The extinction coefficient (ϵ) for TPH in water solution at 272 nm was evaluated to be $1.02 \times 10^4 \text{ (mol/l)}^{-1} \cdot \text{cm}^{-1}$.

2. Release of the drugs from hydrogels (without surfactants)

Release of CPT. The drug (in powder form) was loaded to the agar hydrogel by physically mixing it with the gel material. The release was performed in water at different temperatures. Steady-state fluorescence was used to monitor the release profile and the amount of drug that has been released at time t was evaluated using the calibration curve of CPT obtained previously. Temperature has shown to be an important factor affecting the release properties. Higher temperature resulted in a faster release of the drug. The release of CPT exhibited a typical Fickian diffusion pattern and the release results were fitted to two mathematic models derived from the well-known Fickian diffusion equation. The diffusion coefficient (D) was found to range from 3.97×10^{-8} to $3.67 \times 10^{-7} \text{ cm}^2 \cdot \text{s}^{-1}$ and could be related to the releasing temperature in an Arrhenius relation. The activation energy (E_a) for the diffusion and release of CPT through the agar hydrogel was $70.6 \text{ kJ} \cdot \text{mol}^{-1}$.

Release of TPH. The diffusion and release of TPH from single and blend hydrogels made from agar, gelatin, and κ -carrageenan were studied. It was demonstrated that blending was a simple and effective way to improve the network properties and drug release capabilities of the hydrogel materials. The experimental results showed that the releases of TPH from these hydrogels were dependent upon the composition of the hydrogel, the type of component, the possible interactions between two component polymers, as well as the release temperature. Among the three kinds of blend hydrogels, the optimal release was obtained from the blend hydrogel of gelatin and agar with a blending ratio of 1:1 in weight. The drug release patterns and release mechanisms have been discussed by considering the possible molecular interactions and gel network structures. A new mechanism of formation of an IPN structure with “inter-network crosslinks” was proposed.

3. Solubilization of CPT in various surfactant solutions

The solubility of CPT was successfully improved by using surfactants. For example, knowing the solubility of CPT in water is 3.85 μM , it was improved to 53 μM in a 1.2 wt% (0.03 M) dodecyltrimethylammonium bromide (DTAB) solution, 318 μM in a 1.0 wt% sodium dodecyl sulfate (SDS) solution, and 444 μM in a 1.0 wt% sodium dodecylbenzenesulfonate (SDBS) solution, etc. Beyond a critical concentration (CMC^*), which was generally lower than the reported critical micellization concentration (CMC) of the surfactant, the solubility of CPT increased linearly with the concentration of the surfactant used. With CPT molecules in the solution, the CMC^* for SDS and DTAB was 0.15 wt% and 0.22 wt%, respectively.

The CPT molecules, after being solubilized in surfactant solutions, were evidenced to reside in the cores of the micelles of the surfactant. This was supported by the fluorescence or the UV-vis spectrum of the drug-surfactant solution. Both techniques are sensitive to the properties of the environments surrounding the solutes.

Different surfactants gave different solubilizing abilities due to a combined effect from the size of the micelles, the miscibility between the drug and the micellar core and the hydrophobicity of the micellar core. For example, the order of the solubilizing efficiency of several sodium sulfate surfactants is SDBS > SDS > SDeS. Moreover, small molecular surfactants are generally more effective at solubilizing CPT than polymeric surfactants.

4. Release of CPT from hydrogel-surfactant systems

The surfactant induced effects on the drug release profiles of CPT from the hydrogel-surfactant systems were observed. The presence of surfactants in the drug release systems generally resulted in a positive effect on the release behavior, a prolonged release time and a decreased release rate. And the effect of surfactant increased with the concentration of surfactant. This result was considered to be due to the complicated diffusion of the drug in the presence of surfactants, because now the transport of the drug was contributed by two parts: the diffusion of the drug itself and the diffusion of drugs carried by the surfactant micelles, knowing that most drug molecules resided in the micelles of surfactants.

By adding a negatively charged polymer, κ -carrageenan, into the agarose-surfactant

systems, and using a cationic surfactant, such as cetyltrimethylammonium bromide (CTAB) or DTAB, the interaction between the surfactant and the gel network (κ -carrageenan took part in the formation of the gel network) could be improved and this interaction could be used to control the release of the drug. A notable release-delaying effect was observed even when only 0.05 wt% κ -carrageenan was involved in the gel system. The diffusion coefficient of the drug in the presence of κ -carrageenan was found to be related to the concentration of κ -carrageenan in an exponential way.

9.2 The Original Contributions

This PhD research work has:

1. Developed a novel system consisting of surfactant and hydrogel for the controlled release of hydrophobic drugs. In such a system, the drug resides in the cores of micelles of the surfactant, and the drug-containing micelles disperse in the hydrogel network. This kind of study is new and no similar work has been reported before.
2. Systematically studied the effect of various types of surfactants on the solubility and release properties of drugs.
3. Set up an efficient way to improve the solubility of a poorly water-soluble anticancer drug, CPT.
4. Initially explored the feasibility of using blended hydrogels made from natural polymers rather than synthetic polymers in drug release. Significant improvement in the drug release property of the system has been observed.
5. Published four papers in international pharmaceutical journals and other two are under review.

9.3 Recommendations for Future Work

Although a considerable contribution has been made in this study to the area of solubilization and controlled release of drugs, there are still many interesting works that can be continued or extended from the present work. The following topics may be carried out in the future.

1. Study of the solubilization mechanism

The mechanism for solubilization of CPT or other hydrophobic drugs with various types of surfactants are not very clear. For example, why different surfactants give different capabilities of solubilization is an interesting question worthy to be investigated. In order to understand the detailed mechanism of solubilization, the best way is to use a fluorescent probe as the model drug. Pyrene is one of the best candidates because of its well-known fluorescent properties and extensive applications as a probe. When pyrene is added to a surfactant solution, the effects of pyrene on the aggregation number and micellar structure of the surfactant can be conveniently studied using fluorescence. The parallel studies can also be conducted by varying the surfactant type from anionic, cationic to non-ionic.

2. 3D microstructures of surfactant-immobilized drug-loaded hydrogels

A 3D microstructure for the surfactant-immobilized drug-loaded hydrogel network has been proposed in Scheme 5-1, which was useful for the explanation of the immobilization mechanism by the surfactant. However, this proposed microstructure

has not been experimentally proved. To understand the immobilization mechanism and then the controlled release mechanism, it is important to know the microstructures. Thus, the future studies on this aspect can be focused on the experimental elucidation of the 3D microstructures. The fluorescence energy transfer method may be used to estimate the molecular distribution of the fluorescent drug (e.g. pyrene) over the hydrogel network. Other techniques such as TEM and AFM can also be used to directly determine the 3D microstructure. However, the technical difficulty in preparing samples suitable for TEM or AFM observation will be a challenging task. Here, freeze-drying or other similar techniques might be needed.

3. Release mechanisms

When a drug has been immobilized into a gel network through a surfactant, the microstructure is expected to be complicated so that the release of the drug from the system is complicated too. Basically, we can consider that there are possibly three diffusion processes happening at the same time, which are (i) the diffusion of free drug molecules through the gel matrix, (ii) the diffusion of surfactant micelles through the gel matrix, and (iii) the diffusion of the drug molecules initially immobilized in the micelles through the gel matrix after coming out from the micelles. In general, one can only determine an overall or averaged drug release or molecular diffusion. The separation of the three diffusion processes will be of scientific significance in understanding the release mechanisms. The diffusion process (i) can be studied for a simple system (i.e. drug + gel) without surfactant. The diffusion process (ii) may be investigated using fluorescence energy transfer method, where fluorescent-donor micelles (i.e. the micelles are partially made of fluorescent-donor surfactant molecules) are present and their

diffusion out of the gel system will be expected to influence the fluorescence energy transfer between the fluorescent micelles and the gel matrix if the gel matrix can act as the fluorescent acceptor. The diffusion process (iii) is expected to be the slowest one, which will contribute to the long term of drug release. After knowing the diffusion process (i) and (ii), the process (iii) can be deduced from the entire diffusion.

Although good experimental designs may help to explore the details of the diffusion processes and mechanisms, the technical difficulty in separating the three diffusion processes can still be expected. Besides the experimental investigation, theoretical modeling or simulation can be considered. A combination of experimental and theoretical works will be able to provide a whole image of the molecular diffusion through the surfactant-immobilized drug-loaded hydrogel.

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