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The development of crosslinked gelatin thin films as controlled release matrices

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THE DEVELOPMENT OF CROSSLINKED GELATIN THIN FILMS AS CONTROLLED RELEASE MATRICES

by

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Bachelor of Engineering – Chemical Engineering

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Tehran, Iran, 1994

A thesis

presented to Ryerson University

in partial fulfillment of the
thesis requirement for the degree of

Master of Applied Science

in

Chemical Engineering

Toronto, Ontario, Canada

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ABSTRACT

THE DEVELOPMENT OF CROSSLINKED GELATIN THIN FILMS AS CONTROLLED RELEASE MATRICES

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Master of Applied Science in Chemical engineering (2007)

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The aim of this thesis was to develop gelatin-based thin films that may be used as matrices for the controlled release of bioactive compounds. There were three objectives to this research: i) develop a method to generate the thin films; ii) experimentally quantify the release of a fluorescent marker from these films, and iii) implement a mathematical model to characterize the release of the fluorescent marker. To achieve the first objective, a novel method of developing thin films was implemented where sub-micron thickness films affixed to standard glass slides were crosslinked with genipin, a naturally-occurring fixative. Gel thickness measured using atomic force microscopy (AFM) varied from 350 to 650 nm irrespective of the concentration of genipin added. AFM-based surface roughness decreased with increasing genipin concentration. Release behavior of a fluorescent marker from the thin films demonstrated a strong influence of genipin concentration on release kinetics, with greater genipin leading to slower release. A mathematical model for the water transport into, and fluorescent marker release from, the genipin-crosslinked gelatin films was developed and successfully implemented with both the water ingress (swelling) and marker release being effectively characterized by the model.

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To My Family

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LIST OF SYMBOLS

Symbols

A	Constant for probability of finding holes of a certain size in free volume
b	Numerical factor of probability of holes of a certain size in free volume
B	Dimensionless front-fixing thickness
C_0	DSBP concentration at initial conditions in matrix (mole/mL)
$C_{l,eq}$	Equilibrium water concentration in the fully swollen matrix (mole/mL)
C_l	Water concentration in the matrix (mole/mL)
C_2	DSBP concentration in the matrix (mole/mL)
$\overline{C}_l$	Dimensionless water concentration in the matrix
$\overline{C}_2$	Dimensionless DSBP concentration in the matrix
D	Solute diffusion coefficient in the hydrogel (cm ² /s)
D_l	Water diffusion coefficient in the matrix (cm ² /s)
D_2	DSBP diffusion coefficient in the matrix (cm ² /s)
$D_{l,eq}$	Diffusion coefficient of DSBP in the fully swollen matrix (cm ² /s)
$D_{2,eq}$	Diffusion coefficient of water in the fully swollen matrix (cm ² /s)
D_0	Solute diffusion coefficient in water (cm ² /s)
G	Amplification factor
ΔG	Change in free energy (J)

ΔG_{mix}	Change of free energy of mixing (J)
ΔG_{gel}	Change of the elastic free energy (J)
i	DSBP and water component
j	Point step
k	Constant for absorption of solvent
M	Number of negative sign in run test
$\overline{M}_c$	Molecular weight of polymer chains (gr/mole)
$\overline{M}_n$	Average molecular weight of polymer before crosslinking (gr/mole)
M_t	The amount of solvent absorbed per unit area (gr/cm ²)
n	Time interval
n^*	Parameter related to the diffusion mechanism
n_i	Swelling agent concentration in gel
N^*	Number of the RMS samples
N	Number of positive sign in run test
P	Probability level
$P(v^*)$	Probability of finding holes of a certain size in the free volume
P_ξ	Probability of moving through the gel with certain mesh size
$P^!_0$	Probability of finding the required free volume in the gel
P^+_0	Probability of finding the required free volume in the solution

q	Crosslinking density (mole/mL)
R	Universal gas constant (J/ K · mol)
R^2	Coefficient of multiple determinations
SI	Swelling index
t	Time (s)
T	Temperature (K)
T_g	Glass transition temperature (K)
V_p	Dry matrix volume (cm ³)
V_l	Molar volume of solvent (mL/mol)
$\hat{U}^n$	Amplitude of Fourier wave
W_1	Hydrogel initial weight (gr)
W_2	Hydrogel interval weight (gr)
X	Diffusant displacement
x_n	Thickness of gel film (nm)
$\bar{x}$	Average thickness of gel film (nm)
y_i	Experimental values in regression
$\hat{y}_i$	Theoretical values in regression
$\bar{y}$	Mean value of experimental values in regression
z	Axial coordinate in the disk matrix (cm)

Z_0	Initial half-height of the matrix (cm)
$\overline{Z}$	Dimensionless half-height of the matrix
Z_N	New half height of matrix (cm)
Z_i	(New Height half-height of the disk matrix)/(initial half-height of the disk matrix)

Creek Letters

β_1	Dimensionless constant for the diffusion of water
β_2	Dimensionless constant for the diffusion of incorporated molecule
μ_1	Chemical potential of the swelling agent in the gel (J/mol)
μ_1^0	Chemical potential of the solvent in the bulk (J/mol)
ρ_1	Density of water (gr/cm ³)
ρ_2	Density of DSBP (gr/cm ³)
Φ	Phase angle in Fourier wave
τ	Dimensionless time
V^*	Holes of a given size in the free volume (Å ³)
$V_{2,s}$	The volume fraction of the polymer in the swollen gel
$\overline{V}$	Specific volume of the polymer (cm ³ /gr)
χ	Polymer - solvent interaction parameter

1 INTRODUCTION

A hydrogel is a network of polymer chains that are water-soluble in which water is the dispersion medium (Swarbrick *et al.*, 1988). When a hydrogel is exposed to a compatible fluid (*e.g.*, water), an influx of the solution will occur causing the biopolymer chains to relax and network volume to expand (Kim *et al.*, 2003). Recently, hydrogel research has focused on the development of suitable alternatives to synthetic materials that are more biocompatible, such as food-grade biopolymers (*e.g.*, gelatin). In the absence of crosslinking agents, most biopolymer networks have poor mechanical strength and dissolve rapidly within an aqueous medium, limiting usage in biomedical and pharmaceutical applications (Masaro and Zhu, 1999). Thus, crosslinking agents are added to form non-soluble structures with greater mechanical strength. Typically, toxic crosslinking agents (*e.g.*, formaldehyde or glutaraldehyde) are used, which presents a problem when striving for fully biocompatible systems (Sung *et al.*, 1998). Crosslinking can occur *via* the following bonds: covalent, ionic bonds, van der Waals and hydrophobic interactions between non-polar zones along the biopolymer chains (Nishinari and Doi, 1994).

Controlled release occurs when an agent, typically a drug or bioactive compound, is released from a (bio)polymer network in a predesigned manner. There are three ways in which release can occur: *via* i) diffusion, ii) degradation and, iii) swelling followed by diffusion (Wise, 2000). Diffusion occurs when an incorporated compound passes from the (bio)polymer matrix into the external environment due to a difference in chemical potential between the gel matrix and external (fluid) environment. Release *via*

degradation occurs due to the dissolution of the gel network within the body in the presence of acids, salts or mechanical breakdown. Finally, swelling-controlled release occurs when a gel placed in a fluid absorbs that fluid and swells, which leads to subsequent release (Wise, 2000). In diffusion-limited systems, free volume is the paramount factor in controlling the release rate of molecules (Masaro and Zhu, 1999). Free volume is generally defined as the volume of a system at a given temperature minus its volume at absolute zero. It is essentially the volume of a system not occupied by matter and all components of the system contribute to this free volume (solute, solvent and polymer). Movement of free volume through the system allows for pores or voids through which diffusing species may pass.

As will be discussed later, several constructs have been designed to deliver drugs or bioactive compounds into the human body for various purposes and in variety of ways. One frequent means of delivery is passage through the skin, directly into the blood stream. A commonplace example of such a construct is a nicotine hydrogel patch (Pi *et al.*, 1991). Within this context, in this thesis, a fully-biocompatible gelatin-based patch-style film for the controlled release of bioactive ingredients was developed. The addition of genipin, a naturally-occurring fixative in lieu of toxic crosslinkers, was used to modulate network strength and release behavior.

More often than not, swelling and controlled release studies will involve experimentation and modeling in two or three dimensions, which can complicate proper characterization of a given construct. The present thesis focused on such behavior in only one dimension.

There are two methods of studying swelling and release kinetics in one dimension. One is to expose only one surface of a hydrogel to the release solution whereas another method is to prepare the gel such that the exposed surface area is so large that its thickness is considered negligible.

In this work, a novel method of making sub-micron films with a large surface area was developed, using the well-known spin-coating technique as inspiration. The developed films were then used for release and swelling studies of a fluorescent marker. The acquired experimental results were the basis for the implementation of a predictive model that effectively simulated the release of a fluorescent marker as a function of the available free volume. This model incorporated the effect of swelling on the hydrogel's aspect ratio and dimensions and permitted delineation of the mass transport mechanism at play (*i.e.*, Fickian vs. non-Fickian).

1.1 Overall Goal and Objectives

The overall goal of this thesis was to evaluate and model the ability of gelatin-based sub-micron thin films to act as controlled release matrices. Specific objectives included the following:

- Generation of a thin film based on food-grade materials (gelatin and genipin)
- Investigation of the surface morphology and structure of the films
- Examination of the release behavior of a model fluorescent marker from the films
- Investigation of water uptake behaviour in the films
- Modeling and simulation of water uptake and compound release from the films

Through these experiments, it was possible to understand the release of an entrapped marker molecule into, and from, the hydrogel matrix. The outcome of this thesis was the development of a sub-micron thickness hydrogel platform suitable for controllably releasing bioactive agents.

1.2 Hypotheses

- It is possible to develop gelatin-based thin films for controlled release.
- The application of genipin as crosslinking agent would not only improve gel strength and reduce solubility, but would also serve as an effective means of modulating swelling and release behavior of an embedded fluorescent marker.
- Furthermore, it was hypothesized that the free volume theory could be used to characterize the observed behavior and that this could be used to mathematically model in an effective manner the swelling and release behavior of these sub-micron gelatin films.

1.3 Thesis Organization

The thesis is broken down into eight chapters and organized in the following manner:

Chapter 1: Introduction and brief background.

Chapter 2: Literature review.

Chapter 3: Materials and methods developed to generate, understand and model film generation and controlled release.

Chapter 4: Results and discussion on the physical properties of the thin films (morphology and crosslinking density) of the genipin-fixed gelatin films

as well as the swelling and release behavior of a marker molecule [4,4'-Bis(2-sulfoxytolyl)biphenyl (DSBP)] from these films.

- Chapter 5: Mathematical modeling of water uptake and DSBP release in genipin-fixed gelatin matrices.
- Chapter 6: General Conclusion.
- Chapter 7: References.
- Chapter 8: Appendices.

2 LITERATURE REVIEW

Controlled delivery of bioactive molecules (*e.g.*, drugs) occurs when an active agent trapped within a natural or synthetic matrix is released in a predetermined manner. The release of the active agent may be constant or cyclic over a long time, or it may be triggered by the environment or other external events. Regardless of the mode of release, the purpose behind controlled delivery is to achieve a more effective therapy while eliminating the potential for both an under- and overdose (Figure 2.1). The design of a delivery system is usually based on the physicochemical properties and interactions of the matrix and active ingredient. Conventional delivery systems are limited by the lack of harmony between the required time to administer therapeutic doses and the actual release profile from the delivery system (Gupta *et al.*, 2002). An ideal drug delivery system is inert, biocompatible, strong, capable of achieving high drug loading, safe from accidental release, simple to administer and remove, and easy to fabricate and sterilize (Brannon and Peppas, 1999). Hydrogels are emerging as a promising alternative to conventional systems. Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. Hydrogels can be designed for swelling-controlled drug release or stimuli-responsive changes (Gupta *et al.*, 2002).

Figure 2.1: Drug levels in the blood following (a) traditional drug treatment and (b) controlled delivery.

(Adapted from Brannon and Peppas, 1999)

2.1 Controlled-Released Mechanisms

There are three primary mechanisms by which active agents can be released from a delivery system: diffusion (Figure 2.2), swelling followed by diffusion (Figure 2.3) or degradation (Figure 2.4). Any or all of these mechanisms may occur in a given release system. Diffusion occurs when an active agent passes through the polymer that forms the controlled-release matrix (Brannon and Peppas, 1999). For diffusion-controlled systems,

the drug delivery matrix is fundamentally stable in the biological environment and does not change its size either through swelling or degradation. The combination of polymer matrices and bioactive agents chosen must allow for diffusion through the pores or macromolecular structure of the polymer upon introduction of the delivery system into the biological environment without inducing any change in the polymer itself (Brannon and Peppas, 1999).

Figure 2.2: Drug delivery from a typical matrix drug delivery system

(Adapted from Brannon and Peppas, 1999)

It is also possible for a delivery system to be designed so that it is incapable of releasing its agent until it is placed in an appropriate environment. Swelling-mediated controlled release systems are initially dry, but when placed in the release environment will swell after absorbing water or other fluids. Swelling increases the aqueous solvent content within the formulation as well as the polymer mesh size, enabling the active component to diffuse through the swollen network into the external environment. Most of the materials used in swelling-mediated controlled release systems are based on hydrogels,

which are polymers that will swell without dissolving when placed in water or other biological fluids. These hydrogels can absorb a great deal of fluid and, at equilibrium, typically comprise 60–90% fluid and only 10–30% polymer (Brannon and Peppas, 1999).

Figure 2.3: Release and delivery from environmentally-sensitive release systems

All of the previously-described systems are based on polymers that do not change their chemical structure beyond what occurs during swelling. However, a great deal of attention and research effort is being concentrated on biodegradable polymers. These materials degrade in the presence of a given environment, thus eliminating the need to remove the delivery system after release of the active agent has been completed.

Most biodegradable polymers are designed to degrade as a result of hydrolysis of the (bio)polymer chains into smaller, non-toxic compounds. Degradation may take place through bulk hydrolysis, in which the (bio)polymer degrades in a fairly uniform manner throughout the matrix, as shown schematically in Figure 2.4a. For some polymers (*e.g.*,

polyanhydrides and polyorthoesters), degradation occurs only at the surface, which results in a release rate that is proportional to the surface area of the delivery system.

Figure 2.4: Drug delivery from (a) bulk-eroding and (b) surface-eroding systems

(Adapted from Brannon and Peppas, 1999)

2.2 Hydrogel Structure

The term hydrogel describe a physical state of matter, not a particular chemical entity (Swarbrick *et al.*, 1988). A hydrogel is a water-insoluble (bio)polymer which, when combined with water, yields a rubber-like colloid. Specifically, a hydrogel is a system comprised of a three-dimensional network of a water-plasticized (bio)polymer in which

water fills the space between the polymer chains. The suitability of a hydrogel as a delivery system for bioactive ingredients and its performance in a particular application depends on many factors, including (bio)polymer composition, microstructure, rheological properties (*e.g.*, viscosity), temperature and presence/type of crosslinking agent (Samchenko *et al.*, 2003). The most important parameters used to characterize the network structure of a hydrogel are the polymer volume fraction in the swollen state, molecular weight of the polymer chain between two neighboring crosslinking points (crosslinking is described in detail later), and the mesh size. The polymer volume fraction in the swollen state is a measure of the amount of fluid imbibed and retained by the hydrogel (Peppas *et al.*, 2000). The molecular weight between two consecutive crosslinks, which can be either of a chemical or physical nature, is a measure of the degree of crosslinking of the polymer. It is important to note that due to the random nature of the polymerization process itself, only average values of the molecular weight between two consecutive crosslinks can be calculated. The correlation distance between two adjacent crosslinks provides a measure of the space available between the macromolecular chains available for diffusion. These parameters, which are related to one another, can be determined theoretically or through the use of a variety of experimental techniques (Peppas *et al.*, 2000).

2.3 Hydrogel-Based Delivery Systems

Polymers from natural or synthetic sources can be used for synthesizing hydrogels. Usually, polymers containing hydroxyls, amines, amides, ethers, carboxylates or sulfonates as functional groups in their side chains are used (Gupta *et al.*, 2002). This is

done in order to vary the polarity of the polymer so as to modulate the water holding capacity of the gel. The physical design of a hydrogel-based delivery system depends on the route of administration. The synthesis of hydrogels usually involves crosslinking of the polymers within a mold to impart the rigidity and shape suitable for administration into the body. Hydrogels of different shapes developed for various routes of administration include:

- Oral route – spherical beads, cylinders and discs
- Implants – drum-shaped, disc-shaped and cylindrical preparations
- Rectal route – cylinders
- Vaginal administration – cylindrical and torpedo-shaped devices.

The function of a hydrogel is central to its properties, namely equilibrium swelling, swelling kinetics, permeability and biocompatibility, which are characteristic for a particular polymeric network (Gupta *et al.*, 2002).

2.4 Diffusion in Hydrogels

Diffusion is one of the three primary mechanisms by which active agents can be released from a delivery system. Diffusion is the movement of particles from a higher chemical to a lower chemical potential (chemical potential can in most cases of diffusion be represented by a change in concentration) (Askeland and Phule, 2004). Diffusion in polymers is complex and the diffusion rates should lie between those in liquids and in solids (Masaro and Zhu, 1999). It depends strongly on the concentration and degree of swelling of polymers. Consequently, it remains a challenge to understand, predict and control the diffusion of small and large molecules in polymer systems.

Fickian and non-Fickian diffusion are the major mechanisms that may be used to characterize transport in hydrogels. Fickian diffusion (Case I) is often observed in polymer networks when the temperature is well above the glass transition temperature of the polymer. The glass transition temperature is the temperature below which the physical properties of amorphous materials vary in a manner similar to those of a crystalline phase (glassy state), and above which amorphous materials behave as liquids (rubbery state) (Vitkup *et al.*, 2000). When a (bio)polymer is in the rubbery state, the polymer chains have a higher mobility that allows an easier penetration of the solvent (Grinsted *et al.*, 1992). Therefore, Fickian diffusion is characterized by a solvent diffusion rate that is slower than the polymer relaxation rate. A large gradient of solvent penetration is observed in the system when the polymer is exposed to solvent. The solvent concentration profile shows an exponential decrease from the completely swollen region to the core of the polymer as it is only function of solvent concentration. The diffusion distance is proportional to the square-root of time (Ritger and Peppas, 1987):

$$M_t = kt^{0.5} \quad (2.1)$$

where k is the kinetic constant, which increases with an increase in diffusivity.

Non-Fickian diffusion processes are mainly observed in glassy polymers. At temperatures below T_g , the polymer chains are not sufficiently mobile to permit

immediate penetration of the solvent in the polymer core (Grinsted *et al.*, 1992). Three kinds of non-Fickian diffusion have been defined: Case II diffusion, anomalous diffusion and super Case II. The main difference between these three diffusion categories concerns the solvent diffusion rate. Contrary to Case I diffusion, in Case II and super Case II diffusion, the solvent diffusion rate is faster than the polymer relaxation process whereas in anomalous diffusion, the solvent diffusion rate and the polymer relaxation are about the same order of magnitude (Alfrey *et al.*, 1966). In general, Case II diffusion is observed when solvents have high activity or chemical potential, for example when an external pressure is applied to promote solution diffusion (Weisenberger and Koenig, 1990). The characteristics of Case II diffusion are the following: (1) a rapid increase in the solvent concentration in the swollen region leads to a sharp solvent penetration front between the swollen region and the inner polymer core such that as solvent penetrates the polymer, a sharp advancing boundary separates the inner glassy core from the outer swollen and rubbery shell. This is contrary to Case I transport where there is no glassy core and thus no sharp solvent concentration boundary; (2) the solvent concentration is quite constant in the swollen region behind the solvent penetration front; whereas in Case I, it increases up to equilibrium concentration; (3) the solvent penetration front is sharp and advances at a constant rate, thus the diffusion distance is directly proportional to time:

$$M_t = kt \quad (2.2)$$

(4) There is an induction time which precedes the solvent penetration front into the glassy polymer core (Thomas and Windle, 1977).

Fickian and Case II diffusions are considered as limiting types of transport processes. Anomalous diffusion lies in between and is characterized by the following equation:

$$M_t = kt^{n^*} \quad \text{and} \quad \frac{1}{2} < n^* < 1 \quad (2.3)$$

2.5 Free Volume Theory

Diffusivity of solvent in either Fickian or non-Fickian models depends on the available space in polymer network as molecules (e.g, solvent and embedded molecules) need free space within the (bio)polymer network to reposition themselves. This may be explained by the free volume theory.

Free volume is the paramount factor in controlling diffusivity of molecules in polymers (Masaro and Zhu, 1999). The free volume is defined as the volume not occupied by solvent, solute(s) and polymer. More generally, the free volume can be specified as the volume of a given system at the temperature of study minus the volume of the same system at absolute zero. Thus, rearrangement of the free volume due to stretching or relaxation creates holes through which diffusing particles are able to pass. The free volume theories are based on the assumption that the free volume is the major factor controlling the diffusion rate of molecules. The following models are described herein:

Fujita (1967), Yasuda *et al.*(1969), Vrentas and Duda (1977), and Peppas and Reinhart (1983).

2.5.1 Fujita's Model (1967)

The first diffusion model based on the free volume theory was proposed by Fujita (1967). The measurements were carried out in a ternary system including a solvent, a polymer and a penetrating molecule (a plasticizer). The concentration of the plasticizer was kept low compared to the polymer concentration, thus the system could be approximated as a pseudo-binary system, and thus, the average free volume in such a system was contributed by the polymer and the solvent. In order to estimate the free volume, Fujita used the concept of Cohen and Turnbull (1959) which defines the probability $P(v^*)$ to find holes of size v^* in a liquid of identical molecules:

$$P(v^*) = A \exp\left(-\frac{bv^*}{f_v}\right) \quad (2.4)$$

where A is a constant, b is a numerical factor of the order of unity and f_v is the average free volume per molecule. The product bv^* is interpreted as the measure of the minimum hole size required for diffusant displacement X . The diffusion model is based on several assumptions: (1) diffusion occurs because of the redistribution of the free volume within the matrix; (2) the redistribution of the free volume does not require an energy change; (3) the diffusion process is enabled when the free volume exceeds holes

of size V^* ; (4) the diffusion is directly proportional to the probability $P(v^*)$ of finding a hole of volume V^* or larger adjacent to the diffusant molecule (Cohen and Turnbull, 1959). The application of Fujita's free volume theory has shown successful correlations between the model and data in the case of the diffusion of small molecules in semi-crystalline polymers (Hedenqvist and Gedde, 1996). Zhu and co-workers (1992; 1993) showed that self-diffusion of ketone and ester solvents of various sizes and shapes in polymer solutions could be well-described by Fujita's free volume model.

2.5.2 The Model of Yasuda *et al.* (1969)

Yasuda *et al.* (1969) examined the free volume theory of diffusion assuming that the free volume of a binary system, as proposed by Fujita (1967), mostly depended on the volume fraction of the solvent as opposed to that of the solvent and polymer. This assumption was based on the fact that: (1) the polymer was less mobile than the solvent; (2) the solvent was the main contributor to the effective free volume; and (3) in practice, the solvent diffusion decreased with increasing polymer concentration. Yasuda *et al.* (1969) used the free volume theory to examine electrolyte (NaCl) diffusion in polymers in which the dependence of the diffusion coefficient on the volume fraction of water can be described by this model. More recently, Shirlynn and Lostritto (1996) studied the diffusion of benzocaine (a drug whose size was smaller than the gel mesh size examined) in highly swollen poly(ethylene-co-vinyl acetate) membranes, and found that their results were in good agreement with the model of Yasuda *et al.* (1969).

2.5.3 The Model of Vrentas and Duda (1977)

A major contribution to the development of the free volume theory was made by Vrentas *et al.* (1975; 1977; 1979; 1980; 1984; 1985; and 1989) who re-examined and improved upon the free volume model of Fujita and others. They extended the free volume theory to a wide range of temperatures and polymer concentrations (Vrentas *et al.*, 1993). As with previous models, the free volume contributions from both the solvent and the polymer were taken into account. However, Fujita's free volume model (Fujita, 1967) was considered as only a special case of the newer model of Vrentas and Duda (1993). Contrary to Fujita and others, the free volume theory of Vrentas and Duda took account of several physical parameters such as temperature, activation energy, polymer concentration, solvent molecular size, and the molecular weight of the diffusant. The Vrentas and Duda model was based on the following assumptions: (1) polymer and solvent mixing does not lead to a volume change; (2) the polymer thermal expansion is approximated as the average expansion across the experimental temperature range; (3) the total free volume of the system is computed by using free volume parameters determined from the pure individual components (solvent and polymer); and (4) the solvent diffusional activation energy depends on the polymer concentration. This model has been used to characterize diffusion in polymer systems. For example, Lodge *et al.* (1990) measured tracer diffusion in a poly(vinyl acetate)–toluene system and found agreement between the model of Vrentas and Duda and their experimental data.

2.5.4 The Model of Peppas and Reinhart (1983)

The model of Peppas and Reinhart (1983) was also based on the free volume concept. Diffusion was said to occur through the gel space not occupied by polymer chains. Thus, the self-diffusion coefficient of a diffusant was considered proportional to the probability, P_ξ , of moving through the gel with a given mesh size, but also proportional to the probability of finding the required free volume in the gel and solution, P'_o / P^+_o , as given by:

$$\frac{D}{D_o} = P_\xi \frac{P'}{P^+_o} \quad (2.5)$$

where D is the solute diffusion coefficient in the hydrogel and D_o is the solute diffusion coefficient in water. These authors further assumed that water, and not the polymer, was the major contributor to the free volume available for solute diffusion. Peppas and coworkers have published several papers with diffusants of various sizes in various hydrogels which showed agreement with their model (Lustig and Peppas, 1988; Bell and Peppas, 1996; Peppas and Wright, 1996).

2.5.5 Summary of Free Volume Theories

The free volume models have generally found success in describing diffusion in polymer systems. The advantages and constraints for each model described in this section are summarized in Table 2.1. The model of Vrentas and Duda (1977) is the most useful as it

is applicable over a large range of polymer concentrations and temperatures. However, obtaining the numerous parameters required represents quite a task since these parameters are not always available in the literature. When these parameters are known, it is possible to predict solvent diffusion in certain binary systems. The model of Peppas and Reinhart (1983) is specifically applicable to diffusion in crosslinked gels. Problems may arise when the size of the diffusant is close to, or larger than, the network mesh size, and when the diffusant is bound to the polymer network by ionic (or other) interactions. This model also fails to describe diffusion in polymer solutions where there is no cross-linking.

In summary, each of these theories is based on the modelling of the space available for diffusants to diffuse within a (bio)polymer network. Within this context, a key parameter is the role of crosslinking density and the resultant effect on the (bio)polymer gel mesh size.

Table 2.1: Summary of the diffusion models based on the free volume theory
(Masaro and Zhu, 1999)

Author	Application	Limitation
Fujita	Solvents and small-sized diffusants; semi-dilute polymer solutions	Large diffusants; concentrated polymer solutions
Yasuda <i>et al.</i>	Solvents and small-sized diffusants; semi-dilute polymer solutions	Large diffusants; concentrated polymer solutions
Vrentas and Duda	Various solutes and solvents; both semi-dilute and concentrated polymer solutions	Determination of the numerous parameters; dilute polymer solutions
Peppas and Reinhart	Various solutes and solvents; chemically cross-linked gels and hydrogels	Diffusants with size closer to or greater than the mesh size of the network; diffusion in non-cross-linked polymers

2.6 Crosslinking Density

Knowledge of the crosslinking density, or the number of average molecular weight between crosslinks, is of great importance because of its effect on the mechanical and physical properties of hydrogels (Peppas, 1986). Characterization of the hydrogel network structure may be complex given that many types of crosslinked network are known to exist, including those that are crosslinked in a regular or irregular manner as

well as those that are loosely or highly-crosslinked. Given these variations, only average values for the crosslinking density and the molecular weight between crosslinks may be obtained experimentally and theoretically.

In order to determine the crosslinking density of a hydrogel, two main theories, the equilibrium swelling theory and the rubber elasticity theory have been implemented. When evaluating the crosslinking density of a swollen hydrogel, the free energy of mixing, ΔG_{mix} , and the elastic free energy, ΔG_{gel} , which is a function of the expansion of the network structure, must be considered. The overall change in free energy may be expressed by the combination of the free energy of mixing and the elastic energy (Peppas, 1986):

$$\Delta G = \Delta G_{mix} + \Delta G_{gel} \quad (2.6)$$

At equilibrium, the chemical potential of the swelling agent in the gel equals the chemical potential of the solvent in the bulk:

$$\mu_1 - \mu_1^0 = \frac{\partial \Delta G_{mix}}{\partial n_1} + \frac{\partial \Delta G_{gel}}{\partial n_1} = 0 \quad (2.7)$$

where n_1 is the concentration of the swelling agent in the gel.

The quantity $\frac{\partial \Delta G_{mix}}{\partial n_1}$ can be defined as:

$$\frac{\partial \Delta G_{mix}}{\partial n_1} = RT \left(\ln(1 - v_{2,s}) + v_{2,s} + \chi v_{2,s}^2 \right) \quad (2.8)$$

where $v_{2,s}$ is the volume fraction of polymer in the swollen gel, R is universal gas constant, χ is the polymer - solvent interaction parameter, and T is the temperature of the study.

The quantity $\frac{\partial \Delta G_{gel}}{\partial n_1}$ can be defined as:

$$\frac{\partial \Delta G_{gel}}{\partial n_1} = \frac{RTV_1}{\bar{v}} \left(\frac{1}{\bar{M}_c} - \frac{2}{\bar{M}_n} \right) v_{2,r} \left[\left(\frac{v_{2,s}}{v_{2,r}} \right)^{\frac{1}{3}} - \frac{1}{2} \left(\frac{v_{2,s}}{v_{2,r}} \right) \right] \quad (2.9)$$

where V_1 is the molar volume of the solvent, $\bar{v}$ is specific volume of the polymer, $\bar{M}_c$ is the molecular weight of polymer chains between two successive crosslinks, $\bar{M}_n$ is the number-average molecular weight of the polymer before crosslinking, and $v_{2,r}$ is the volume fraction of polymer in the relaxed gel.

Substitution of these relationships into Eq (2.7) yields Eq (2.10):

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{\left(\frac{\bar{v}}{V_1}\right) \left(\ln(1 - v_{2,s}) + v_{2,s} + \chi v_{2,s}^2 \right)}{v_{2,r} \left[\left(\frac{v_{2,s}}{v_{2,r}} \right)^{\frac{1}{3}} - \frac{1}{2} \left(\frac{v_{2,s}}{v_{2,r}} \right) \right]} \quad (2.10)$$

Finally, crosslinking density may be defined as:

$$q = \frac{1}{v \overline{M}_c} \quad (2.11)$$

2.7 Gelatin Hydrogels

Gelatin is a heterogeneous mixture of single or multi-stranded polypeptides, each with extended left-handed proline helix conformations (Oakenfull and Scott, 2003). The key amino acids in gelatin are glycine (21 %), proline (12 %), hydroxyproline (12 %), glutamic acid (10 %), alanine (9 %), arginine (8 %), aspartic acid (6 %), lysine (4 %), serine (4 %), leucine (3 %), valine (2 %), phenylalanine (2 %), threonine (2 %), isoleucine (1 %), hydroxylysine (1 %), methionine and histidine (< 1 %) and tyrosine (< 0.5 %). These values vary, especially the minor constituents, depending on the source of the raw material and processing technique.

zones stabilized by hydrogen bonds. Perhaps the main disadvantage of gelatin as a material is due to its poor mechanical properties, which limit its possible applications as a biomaterial. Noticeable improvement of the mechanical properties of gelatin films in the direction of deformation can be obtained by inducing segmental orientation in gelatin films (Bigi *et al.*, 2002). The improvement of the mechanical properties of drawn gelatin films has been related to the renaturation level of the protein. This can be improved by crosslinking gelatin to maintain the thermal and the mechanical stability of the material (Fakirov *et al.*, 1996; Fakirov *et al.*, 1997).

2.8 Genipin as a Crosslinking Agent

Since gelatin is soluble in aqueous solutions, materials for long-term applications must be submitted to crosslinking, which improves both the thermal and the mechanical stability of the material. Physical crosslinking methods include hydrothermal treatment and ultraviolet and γ -irradiation. As well, due to the large number of functional side groups it contains, gelatin readily undergoes chemical crosslinking. This is usually performed using bifunctional reagents such as glutaraldehyde (GTA) and diisocyanates, as well as carbodiimides, polyepoxy compounds and acyl azide methods (Bigi *et al.*, 2002). GTA is by far the most widely used agent, due to its efficiency of stabilization of collagen-based biomaterials, though it has shown some local cytotoxicity and calcification of long-term implants (Khor, 1997). GTA is easily available, inexpensive and its aqueous solutions can effectively crosslink collagenous tissues in a relatively short period (Khor, 1997). However, if released into the host due to biodegradation, GTA is toxic. This is the reason

for increasing demand of crosslinking agents able to form stable and biocompatible crosslinked products, without added cytotoxicity problems (Bigi *et al.*, 2002).

Genipin is a promising naturally-occurring crosslinking agent. Genipin can be obtained from an iridoid glucoside, geniposide, which is abundant in gardenia fruits. Genipin has been widely used in herbal medicine, and the dark blue pigments obtained by its spontaneous reaction with amino acids or proteins have been used in the fabrication of food dyes. It has also been used in the preparation of gelatin capsules and the immobilization of enzymes (Bigi *et al.*, 2002). The results of a series of studies carried out on porcine pericardia crosslinked with genipin indicated that this agent can form stable crosslinked products. In particular, it was found that genipin-fixed tissue has a resistance against enzymatic degradation comparable to GTA-fixed tissue (Sung *et al.*, 1998), and that genipin is less cytotoxic than GTA (Tsai *et al.*, 2000). Furthermore, gelatin-derived bioadhesives display higher biocompatibility and less cytotoxicity when crosslinked with genipin rather than with other agents, such as formaldehyde, GTA and epoxy compounds (Sung *et al.*, 1998).

Genipin can crosslink materials containing primary amine groups via two proposed mechanisms, shown in Figure 2.2. One is a nucleophilic substitution (Scheme 1) and the second is a nucleophilic attack (scheme 2). Scheme 1 outlines the slowest reaction of the two in which the ester group is substituted and a secondary amide link with gelatin is formed. Scheme 2, the faster reaction, is a nucleophilic attack on genipin by a primary

amine group of gelatin to form a heterocyclic compound of genipin that is linked with the basic residues in gelatin (Butler *et al.*, 2003).

First Scheme

Second Scheme

Figure 2.6: Crosslinking reactions involving genipin

(Adapted from Butler *et al.*, 2003)

Nickerson *et al.* (2006) investigated the kinetics and mechanisms of gelation of genipin-crosslinked gelatin, and found that the storage modulus of gel samples decreased with increasing temperature until a minimum was reached. Temperature increases beyond this minimum led to an increase in storage modulus. Results demonstrated that gelatin gel strength increased with increasing levels of genipin, irrespective of the temperatures studied. It was concluded that the temperature of gelation in the low temperature regime was consistent with a gel that forms primarily due to stabilizing van der Waals interactions while increasing temperature resulted in a more disordered structure, which

led to weaker gel strengths. By increasing the temperature through the low-temperature regime, gel strength decreased. Furthermore, the rate of formation of chemical crosslinks was slow in this regime, due to a kinetic reaction barrier. Above 40 °C, a chemically-crosslinked gelatin network was evident, given the decrease in gelation time and increase in gel strength with an increase in temperature and/or genipin concentration (Nickerson *et al.*, 2006).

2.9 Gelatin-Based Matrices for Controlled Release

Applications

The following section highlights some recent examples where gelatin has been evaluated as a carrier for controlled release applications. Tabata *et al.* (1999) prepared acidic and basic GTA-crosslinked gelatin microspheres for the release of a basic fibroblast growth factor (BFGF). Approximately 30% of the incorporated BFGF was released from the acidic gelatin microspheres within 3 hr, followed by no substantial release. In the basic gelatin microspheres, the incorporated BFGF was almost completely released within 1 day. Differences were ascribed to the BFGF molecules binding to the acidic gelatin through polyion complexation, which limited its release. Overall, it was concluded that the gelatin microspheres were a promising carrier matrix of BFGF. Liang *et al.* (2003) prepared GTA- and genipin-crosslinked gelatin microspheres as drug release matrices. The water transport mechanism in these microspheres was anomalous, ranging from Fickian to Case-II extremes. The increase of the swelling-induced diameter for the genipin-crosslinked microspheres was significantly less than that observed for the GTA-crosslinked microspheres. Dong *et al.* (2006) made films of alginate and gelatin, cross-

linked with Ca^{2+} , with ciprofloxacin hydrochloride as a model drug incorporated in different concentrations. Chemical, morphological and mechanical property characterization was carried out, as well as studies on the factors that influenced the drug release, including the component ratio of alginate and gelatin, ciprofloxacin hydrochloride loading, the pH and ionic strength of the release solution, the thickness of the drug-loaded films and the cross-linking time with Ca^{2+} . Results showed that the amount of ciprofloxacin hydrochloride released decreased with an increase in gelatin present in the film. Moreover, the release rate of drug decreased as drug load in the film increased. Rokhade *et al.* (2006) prepared gelatin and sodium carboxymethyl cellulose (NaCMC) microspheres, using GTA as a crosslinker. Ketorolac tromethamine (KT), an anti-inflammatory and analgesic agent, was encapsulated in the microspheres. Various formulations were prepared by varying the ratio of gelatin and NaCMC, % drug loading, and amount of GTA. The diffusion coefficient of water transport through the microspheres was determined using an empirical equation. Values of diffusion coefficients decreased with increasing GTA as well as increasing content of NaCMC in the matrix. The results indicated a dependence of slow release rate on both the extent of crosslinking and the amount of NaCMC in the microspheres. Cumulative release data indicated a non-Fickian trend. More recently, the swelling and release characteristics of caffeine from genipin-crosslinked gelatin and phase-separated gelatin-maltodextrin hydrogels were studied as a function of gelatin concentration (Khan *et al.*, 2006). The release mechanism was Fickian in nature for all hydrogel constructs. The system showed adjustable swelling behavior based on its composition that suggested the mechanism of release could also be modified beyond a Fickian mode. A mathematical model that

describes the transport behavior within a cylindrical hydrogel based on composition was also employed (Abbasi *et al.*, 2006). In this model, diffusivity of water ingress and bioactive compound release was predicted for a wide range of bioactive compounds and hydrogels with different cylindrical dimensions.

3 MATERIALS AND METHODS

3.1 Materials

A 500 mL gelatin (Type A, Bloom 300, Sigma–Aldrich Co., Oakville, ON, Canada) stock solution was prepared by dispersing the gelatin powder in distilled de-ionized water (DDW) at 50 °C with continual stirring for 30 min. The pH was adjusted to 5 using HCl (1 M). A 200 mL genipin (Challenge Bioproducts Co., Taichung, Taiwan) stock solution was prepared by dissolving the genipin powder in 60% (v/v) ethanol, as described by Yao *et al.* (2004).

The marker molecule used, 4,4'-Bis(2-sulfoxyryl)biphenyl (DSBP) (Ciba Co., Mississauga, ON, Canada) (Figure 3.1), is a highly-hydrophobic fluorescent whitening agent used in laundry detergents. DSBP absorbs UV light and fluoresces in the visible blue region of the spectrum (K esselman *et al.*, 1999).

Figure 3.1: Molecular structure of 4,4'-Bis(2-sulfoxyryl)biphenyl (DSBP)

(Adapted from Kesselman *et al.*, 1999)

A 500 mL DSBP stock solution with concentration of $1.75\text{E-}7$ mol/L, was prepared by dilution of DSBP in 0.01 M acetate buffer pH 5 with NaCl (0.5 M).

3.2 Thin Film Preparation

3.2.1 Genipin-Crosslinked Gelatin Films

Figure 3.2 outlines the preparation of the thin films. In order to make genipin-crosslinked gelatin films, 20 mL volumes of a 10 wt% gelatin solution at 70 °C were prepared and mixed with 0.125, 0.25, 0.5, 0.75, 1 or 2 wt% of genipin at 5 °C while gently stirring the solution to avoid foam formation. Solutions were then poured into 2 x 2 x 8 cm slide containers (Fisher Scientific, Ottawa, ON, Canada). Clean 25 x 76 mm slides (Fisher Scientific, Ottawa, ON, Canada) were placed inside for 1 min to let the solution adsorb to the slide's surface. Slides were then taken out and placed upside down in 50 mL centrifuge tubes (Fischer Scientific, Ottawa, ON, Canada) to be centrifuged at 1600 RPM for 2.5 minutes. The orientation of slides in centrifuge tubes was kept constant to avoid an unequal centrifugal force acting along the width and length of slide. Following centrifugation, crosslinking was allowed to proceed for 24 hr at room temperature (25 °C). All gels slides were prepared in triplicate.

3.2.2 Genipin-Crosslinked Gelatin Films Loaded with the Marker

A 13 mL aliquot of DSBP ($1.75\text{E-}7$ mol/L) stock solution was poured in slides container where the crosslinked thin films at different concentrations of genipin (0.25, 0.5, 0.75, 1 or 2 wt% where housed (Figure 3.3). Contact between the thin films and DSBP was

maintained for 50 min to permit the DSBP to diffuse into the gel and reach equilibrium. Subsequently, the slides were rinsed with DDW and left at room temperature (25 °C) for 30 min to permit water evaporation from the surface of the slides. All gel slides were prepared in triplicate.

Figure 3.2: Preparation of genipin-crosslinked gelatin films

Absorption of DSBP in Genipin Crosslinked Gelatin films

Figure 3.3: Preparation of genipin-crosslinked gelatin DSBP-loaded films

3.2.3 Genipin-Crosslinked Gelatin Disks

In order to accurately measure swelling behaviour and relate this to the swelling observed in the films, genipin-crosslinked gelatin disks were prepared. A 20mL solution of 10 wt% gelatin solution at 60°C was mixed with 0.125, 0.25, 0.5, 0.75, 1 or 2 wt% of genipin at 25°C while stirring the solution very slowly to avoid foam formation. The mixed solutions were poured into plastic screw-capped tubes (molds), pre-lubricated with a thin layer of cooking spray, and then allowed to set for 24 h at room temperature. Once set, gels were prepared in disk form (3 cm diameter and 2 mm height). All gel disks were prepared in triplicate.

3.3 Thin Film Surface Morphology

The morphology of the genipin-crosslinked gelatin films as well as their thicknesses and roughnesses at different concentrations of genipin (0.25, 0.5, 0.75, 1 or 2 wt%) were examined using an atomic force microscope (AFM) (Bioscope, Digital Instruments, Veeco Metrology, CA, USA) (Figure 3.4). The thickness of the films was measured at nine different positions on the film by scoring the films with a stainless steel razor blade at distances of 0.2, 1.0, and 1.8 cm from the dipped end of the film. All gels slides tested in triplicate.

Figure 3.4: Thickness measurement of genipin-crosslinked gelatin films

3.4 DSBP Incorporation into Films

A 13mL aliquot of DSBP (1.75×10^{-7} mol/L) stock solution was poured into slides container where the crosslinked thin films at different concentrations of genipin (0.25, 0.5, 0.75, 1 or 2 wt%) were housed. Contact was maintained for 50 min. In order to find how much DSBP was absorbed in each slide, the films were dissolved in 8 M urea. Urea breaks hydrogen bond and substantially weakens hydrophobic interactions (Jana and Moulik,

1993). The fluorescence intensity emitted by the DSBP was measured at a wavelength of 430 nm for each of the withdrawn samples by a luminescence spectrometer (model LS 50B, Perkin-Elmer Life and Analytical Sciences, Inc., Waltham, MA, USA).

3.5 DSBP Release Experiments

DSBP release experiments were carried out in triplicate in a beaker containing 40 mL of buffer (acetate 0.01 M, pH 5, 0.5 M NaCl) at room temperature. The gel slides loaded with DSBP were immersed in the buffer solution while stirring continuously in order to avoid boundary layers over the slide surface. During the release experiments, samples of the buffer solution were withdrawn from the beaker at specific times, and the fluorescence intensity generated by the released DSBP at 430 nm was measured using a luminescence spectrometer (Model LS 50B, Perkin-Elmer Life and Analytical Sciences, Inc., Waltham, MA, USA). The measured sample was poured back into the buffer solution. All release measurements were in triplicate.

Figure 3.5: DSBP release from genipin-crosslinked gelatin films

3.6 Swelling Index

Genipin-crosslinked gelatin disks samples were initially weighed, W_1 , then immersed in 15mL of acetate buffer (0.01 M, pH 5, 0.5M NaCl) at ambient temperatures for the same duration as the release experiments. Samples were blotted dry and weighed, W_2 , after each time interval. The swelling index (SI) was determined as described by (Yao *et al.*, 2004):

$$SI = \frac{(W_2 - W_1)}{W_1} \quad (3.1)$$

All measurements were made in triplicate.

3.7 Computer Modeling

Simulation of entrapped DSBP release from the genipin-crosslinked gelatin films and the ingress of water into genipin-crosslinked gelatin disks and films were implemented by Fick's second law of diffusion based on the free volume theory. An explicit finite-difference scheme was applied for solving the equations at each time step. The Von Neumann stability analysis was implemented in order to have a stable explicit method.

3.8 Statistical Analyses

A one-way analysis of variance (ANOVA) was used to assess statistically significant differences between the means of the results. Following the one-way ANOVA, Fisher's

least significant difference (LSD) test was used to determine differences between the means (D'Urso and Gastaldi, 2003). The Von Neumann stability analysis was implemented in order to have a stable explicit method (Verwer and Sommeijer, 1997). For fitting the experimental data with theoretical results, the Wald-Wolfowitz run test was applied (D'Urso and Gastaldi, 2003). The goodness of fit was determined by the calculating adjusted the coefficient of multiple determinations , R^2 , as described by (Härdle, 1992):

$$R^2 = \frac{\text{regression sum of squares}}{\text{total sum of squares}} = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \quad (3.2)$$

where y_i and $\hat{y}_i$ are the experimental and theoretical values at time i and $\bar{y}$ is the mean value of experimental values.

4 PHYSICAL PROPERTIES AND RELEASE BEHAVIOR OF GENIPIN-CROSSLINKED GELATIN-BASED THIN FILMS

4.1 A Novel Approach to the Development of Sol-Gel Films

One of the primary goals of this thesis was to develop a novel means of developing thin films based on gelatin using commonly-available laboratory equipment. There already exists a number of means to develop thin films. The two most common techniques are the evaporation method and the spin-coating method. The former method is widely used in the fabrication of optical systems such as antireflection coatings on lenses (Athey, 1996). In practice, film deposition of high-melting polymers may be difficult unless the substrate is first heated, as substrate temperature during the deposition is key in determining film structure (Heavens, 2007). Any method where this factor cannot be accurately controlled should be avoided if a reproducible structure is critical.

One of the most important methods of sol-gel processing is the spinning-coating method. Compared to the evaporation method, this method of thin film formation requires less equipment and is potentially less expensive (Heavens, 2007). The most important advantage of the technique over other coating techniques is the control of deposited film structure. However, this method does lead to non-uniformity near the film edges (Pham *et al.*, 2005), this being caused by the centrifugal force when spinning.

The method developed in this thesis is called the dip-centrifuge method. This method permits control of the deposited film microstructure as well as and film edge uniformity. Furthermore, thin film generation does not need expensive equipment. However, film thickness within a given film may vary along the length of the substrate, due to the effect of gravity and centrifugal force. This method consists of two stages - dipping of the substrate in the deposition solution followed by centrifugation of the substrate. Film thickness will depend on centrifugal force and time, as well as deposition solution viscosity.

4.2 Materials and Methods

Genipin-crosslinked gelatin films were prepared by gelatin solution 10 wt% mixed with 0.125, 0.25, 0.5, 0.75, 1 or 2 wt% of genipin at 25 °C. The experimental techniques are described in detail in Chapter 3.

4.3 Results and Discussion

4.3.1 Film Thickness

The height of the freshly-made genipin-crosslinked gelatin films was measured via AFM (Figure 4.1). Using a stainless steel razor blade, the gels were scored at distances of 0.2, 1.0 and 1.8 cm from the base of the slide. Each slide was divided in three zones along its length. In each zone, the thickness was measured across the film at three locations. At each distance from the base of the slide, gel thickness for all genipin concentrations did

not differ. With an increase in distance from the bottom edge, film thickness increased from 350 to 630 nm ($P < 0.05$). One-way ANOVA followed by Fisher's least significant difference (LSD) showed significant effect of distance from the bottom edge on film thickness ($P < 0.05$). The output shows the results of fitting a multiple linear regression model to describe the relationship between film thickness and distance from bottom edge. Goodness of the fit shows followed by t-test showed a significant relationship between the variables at the 95% confidence. The level coefficient of multiple determinations, R^2 , value is > 0.99 .

Figure 4.1: Thickness profile of genipin-crosslinked gelatin films as a function of distance from bottom edge of slide (15 measurements for each point)

4.3.2 Film Morphology

The surface morphology of the gelatin thin films was measured at surface scan of 2500 x 2500 x 50 nm (Figure 4.2). Generally speaking, the presence of genipin altered the surface morphology of the films. The scanning area of AFM shows peaks and valleys over the gel.

Figure 4.2: Surface of genipin-crosslinked gelatin films via AFM. The scale is 2500 x 2500 x 50 nm.

4.3.3 Surface Roughness of the Thin Films

A common means of calculating the average roughness of a surface is based on the root mean square (RMS) analysis (Graham, 2002). An RMS value is given by the standard deviation of the height profiles on the surface, and is determined as follows:

$$RMS = \sqrt{\frac{\sum_{n=1}^{N^*} (x_n - \bar{x})^2}{N^* - 1}} \quad (4.1)$$

where x_n represents the thickness of the nth datum, $\bar{x}$ is equal to the mean thickness of x_n based on AFM topography, and N^* is the number of the data points.

RMS roughness profile is shown (Figure 4.3). Analyses showed a significant difference in roughness for gels with genipin concentrations below 1 %wt ($P < 0.05$) and no significant difference on roughness for gel with genipin concentration over 1 %wt ($P > 0.05$). The roughness decreased in an exponential fashion until a concentration of 1 wt% genipin was reached. Above this concentration, there was no subsequent decrease in surface roughness. The reason to explain this phenomenon is not completely understood.

Figure 4.3: Surface roughness (RMS) profile of genipin-crosslinked gelatin films via AFM . Scale is 2500 x 2500 x 50 nm. (3 measurement for each point)

4.3.4 Crosslinking Density

Crosslink density is expressed as the average polymer mass between crosslinks. It is a major parameter that will strongly affect the mechanical and physical properties of hydrogels. As the concentration of genipin increases in fixed gelatin gels, the molecular weight between crosslinks will decrease, limiting the available space between molecules. This is important as the swelling index is a major factor for calculating the polymer fraction in relaxed and swelled hydrogels. The polymer fraction in swelled and relaxed hydrogels, $V_{2,s}$ and $V_{2,r}$ can be calculated by using this relationship: $1/(1 + \text{Swelling}$

Ratio) (Rajvaidya *et al.*, 2005). The molar volume of the water, V_l , is 18 mL/mol. χ , the gelatin - water interaction parameter is 0.495 (Ofner and Bubnis, 1996). The number-average molecular weight of the hydrogels and the crosslinking density, $\overline{M}_c$ and q , have been calculated for different genipin compositions using Eq (2.10) and Eq (2.11) and are summarized in Table 4.1. One-way ANOVA followed by Fisher's least significant difference (LSD) analysis showed a significant effect of genipin concentration on crosslinking density ($P < 0.05$). By increasing the genipin concentration in the gel, the crosslinking density increased whereas the molecular weight of the biopolymer chains between two successive crosslinks decreased. Figure 4.4 shows the crosslinking density profile versus the genipin concentration.

Table 4.1: Crosslinking density of genipin-crosslinked gelatin gel

Genipin Concentration %wt	$V_{2,s}$ polymer fraction (Swelled polymer)	$V_{2,r}$ polymer fraction (Relaxed polymer)	$\overline{M}_c$ molecular weight of polymer chains between two successive crosslinks (gr/mole)	q Crosslinking Density (mole/mL)
0.25	0.0650	0.103	9753.405	0.000102
0.5	0.0687	0.104	8358.137	0.000119
0.75	0.0718	0.105	7386.777	0.000135
1	0.0744	0.111	6959.772	0.000143
2	0.0804	0.116	5783.641	0.000172

Figure 4.4: Crosslinking density profile of genipin-crosslinked gelatin gels
(3 measurements for each point)

4.3.5 DSBP Absorption, Release and Water Uptake

Diffusion is the net action of particle, heat, momentum, or light in order to minimize a concentration gradient. Absorption and release of a matter into a (bio)polymer is equal to a diffusivity multiplied by a gradient concentration. Diffusivity of molecules in (bio)polymers strongly depends on the (bio)polymer structure which can be adequately described by the free volume theory (Masaro and Zhu, 1999). The free volume is defined as the volume not occupied by solvent, solute(s) and (bio)polymer (Masaro and Zhu, 1999). By altering crosslinking density, the probability of finding a hole of a sufficiently

large volume adjacent to diffusant molecules will alter the diffusivity of those diffusants. In this study, the diffusivity of a model marker through genipin-crosslinked films and water uptake in genipin-crosslinked gels were investigated by changing the amount of genipin as the crosslinking agent in hydrogel.

4.3.6 DSBP Absorption in Gel Films

Table 4.2 shows the total amount of absorbed DSBP in gel slides respect to different concentrations of genipin. The total amount of absorbed DSBP was dependent on the genipin concentration in the gel. One-way ANOVA followed by Fisher's least significant difference (LSD) analysis showed a significant effect of genipin concentration on DSBP absorption ($P < 0.05$).

Table 4.2: Total absorbed DSBP in genipin-crosslinked gelatin gel films

Genipin %wt	Absorbed DSBP (mol/L x 10⁻⁹)
0.25	1.35 ± 0.046
0.5	1.98 ± 0.069
0.75	2.29 ± 0.011
1	2.46 ± 0.021
2	2.81 ± 0.033

These results may be interpreted as there being substantial interaction between DSBP and gelatin. DSBP is a negatively-charged molecule with a sulfate group at each end. If a

positively-charged molecule is present in the vicinity, DSBP will be electrostatically attracted towards it.

Gel formation in gelatin is the result of helix formation of parts of the macromolecules, where hydrogen bonding plays an important role. Gelation of gelatin solutions is primarily determined by the amount of the amino acids proline (PRO) and hydroxyproline (HYP), whereas also the amino acid glycine (GLY) plays an important role (Veis, 1967). Genipin crosslinks break open the alpha helices inside gelatin exposing additional positively charged amino acid R-groups (Grinsted *et al.*, 1992; Butler *et al.*, 2003). In a neutral to acidic environment these amino acids are positively charged, therefore DSBP will bind to gelatin due to electrostatic interactions. The exposure of additional amino acids increases the electrostatic attraction of DSBP towards gelatin.

4.3.7 DSBP Release from the Gel Films

Figure 4.5 shows the DSBP released from the gel films. The total amount of DSBP released in the gel films increased with genipin concentration. In order to compare the diffusivity of the model marker in the gel systems, released DSBP amounts were normalized (Figure 4.6). According to the free volume theory, diffusivity of a molecule through a hydrogel will depend on the structure of the hydrogel. In this case, the size of the DSBP molecule as well as the hydrogel structure controls the diffusion rate. The diffusion rate of small molecules within the (bio)polymer system will be less affected by the mobility of (bio)polymer chains than in the case of large molecules. For diffusing molecules smaller than the average void size in a (bio)polymer, diffusion occurs by

localized activated jumps from one pre-existing void to another (Siepmann and Peppas, 2001). With larger diffusing species, pre-existing voids may be unable to accommodate the diffusing molecules. In this study, the size of the diffusant molecule was constant, but the structure of the gel system changed. By adding more crosslinking agent in the gel, the free volume available for diffusion of DSBP was reduced. A one-way ANOVA followed by Fisher's least significant difference (LSD) analysis showed significant differences in DSBP release through the film with genipin concentration below 1 %wt ($P < 0.05$) and no significant differences with a genipin concentration over 1 %wt ($P > 0.05$). These results may show that the voids in the hydrogel with a genipin concentration > 1 %wt was unable to permit DSBP passage.

Figure 4.5: Released DSBP from genipin-crosslinked gelatin gel films

(3 measurements for each point)

Figure 4.6: Relative release of DSBP from genipin-crosslinked gelatin gel films
(3 measurements for each point)

4.3.8 Swelling of Gel Disks

In order to accurately measure the swelling behavior of crosslinked-genipin gelatin gel and relate this back to the swelling observed in the films, genipin-crosslinked gelatin disks were prepared and evaluated. One-way ANOVA followed by Fisher's least significant difference (LSD) analysis showed a significant effect of genipin concentration on water swelling index ($P < 0.05$). Figure 4.7 shows the swelling index of gelatin samples with different genipin concentrations. It is obvious that as the concentration of genipin increases in the sample, the total water uptake decreases. These results confirm

that diffusivity of molecules increases with an increase free volume in the biopolymer structure. When the biopolymer is placed into solution, it absorbs water, and consequently swells. The absorbed water stretches the polymer chains, resulting in a larger pore size. The absorbed water, along with the genipin concentration used will control the free volume in the system, which in turn will govern the release kinetics of the model marker.

Table 4.3: Swelling index in genipin-crosslinked gelatin gel disks

Genipin % wt	Maximum Swelling Index
0.25	0.57 ± 0.0170
0.5	0.28 ± 0.0110
0.75	0.19 ± 0.0050
1	0.13 ± 0.0048
2	0.070 ± 0.0011

Figure 4.7: Swelling index of genipin-crosslinked gelatin gel disks
(3 measurements for each point)

4.4 Conclusion

Crosslinking density has a major impact on swelling rate of the gel and DSPB diffusivity. The reason is that the molecular weight between crosslinks decreases proportionally with addition of genipin, which restricts the diffusivity of DSPB and water molecule in the gelatin gel matrix.

5 MATHEMATICAL MODELING

Modeling of controlled release may be classified according to the controlling physical mechanism of release of an incorporated molecule. These mechanisms are classified as diffusion release, degradation release and swelling followed by diffusion release (Wise, 2000). The objective of this chapter was to develop a mathematical model that describes the release behavior of incorporated molecules and water uptake from the crosslinked gelatin films that were developed. The major advantages of this models are: (1) the explanation of the fundamental mass transport mechanisms; (2) the possibility of predicting the effect of the matrix design parameters (*e.g.*, shape, size and composition of the gelatin-based matrix) on the resulting release rate; and (3) predicting diffusion coefficients of incorporated molecules (*e.g.*, drugs or bioactive ingredients) and water in the system.

The development of new matrices for controlled release strongly depends on the development of predictive models that can accurately model release behavior of incorporated molecules. In many cases, the use of a simple empirical or semi-empirical model is fully sufficient. However, when reliable, detailed information is required, more complex, mechanistic theories must be applied. Mathematical modeling of diffusivity of a molecule in hydrogels is often based on the free volume theory. The free volume theory is based on the assumption that the free volume is the major factor controlling the diffusion rate of molecules. The diffusion model is based on the probability of finding a hole of a specific volume or larger adjacent to the diffusant molecule (Cohen and Turnbull, 1959).

5.1 Mathematical Model and Assumptions

There are a number of initial conditions and assumptions made to implement the model. The DSBP loaded genipin-crosslinked gelatin matrix was a film that swelled uniformly in the axial direction, and was assumed to be ideal. Release and swelling were considered in one direction only. Thus, the matrix swelled during water uptake, changing the dimension of the matrix from the initial thickness (Z_0) to new thickness (Z_N).

Figure 5.1: Swelling of genipin-crosslinked gelatin film

The system was considered Fickian as in rubbery systems, stresses during swelling have little effect on diffusion (Ju *et al.*, 1995). Fickian diffusion is often observed in

(bio)polymer networks when the temperature is well above the glass transition temperature (Vitkup *et al.*, 2000) where non-Fickian diffusion processes are mainly observed in glassy polymers. Fick's second law for Cartesian coordinate systems (Crank, 1975a), where we consider axial mass transfer with concentration-dependent diffusivities, was used to describe water and DSBP transport, the reduced form being:

$$\frac{\partial C_i}{\partial t} = \frac{\partial}{\partial z} \left(D_i \frac{\partial C_i}{\partial z} \right) \quad (5.1)$$

where for water $i=1$ and for DSBP $i=2$. C_i is the mass concentration of component i within the matrix. z is the axial coordinate and t represents time.

A Fujita-type model (Fujita, 1967) for the exponential dependence of the diffusivities of water and DSBP was used:

$$D_i = D_{i,eq} \exp \left(-\beta_i \left(1 - \frac{C_i}{C_{i,eq}} \right) \right) \quad (5.2)$$

Here, $D_{i,eq}$ is the diffusion coefficient of component i (water or DSBP compound) in the fully swollen biopolymer matrix system at equilibrium. β_i is a dimensionless constant that characterizes the concentration dependence of water or DSBP diffusivity and $C_{i,eq}$ is the equilibrium water concentration in the fully swollen biopolymeric

matrix. The values of $D_{1,eq}$, $D_{2,eq}$, β_1 , β_2 were determined by fitting the obtained experimental data in Chapter 4. The values for $D_{1,eq}$ and β_1 were determined by fitting the experimental data in the biopolymer-water system in the absence of any incorporated molecule, during swelling and dissolution studies. Values of $D_{2,eq}$ and β_2 were obtained by fitting the DSBP release data. With the system considered ideal, the total volume of the matrix at any time was calculated by sum of the biopolymer, the DSBP and water. The surface of the biopolymer matrix was considered a perfect sink for the DSBP. As the gelatin was crosslinked, dissolution was considered negligible.

5.2 Initial and Boundary Conditions

Prior to considering the initial boundary conditions, Eq (5.1) was written in dimensionless form:

$$\frac{\partial \bar{C}_i}{\partial \tau} = \frac{\partial}{\partial \bar{z}} \left(\bar{D}_i \frac{\partial \bar{C}_i}{\partial \bar{z}} \right) \quad (5.3)$$

where

$$\bar{C}_1 = \frac{C_1}{C_{1,eq}}, \quad \bar{C}_2 = \frac{C_2}{C_0}, \quad \bar{D}_1 = \frac{D_1}{D_{1,eq}}, \quad \bar{D}_2 = \frac{D_2}{D_{1,eq}}, \quad \bar{z} = \frac{z}{z_0}, \quad \tau = \frac{t D_{1,eq}}{z_0^2} \quad (5.4)$$

Initially ($t=0$), the biopolymer was considered waterless and the DSBP was assumed to be uniformly distributed throughout the matrix. C_0 was defined as the DSBP concentration at $t=0$ whereas $C_{1,eq}$ was the equilibrium water concentration in the fully swollen biopolymeric matrix. These conditions can be written as:

$$\tau = 0 \quad \bar{C}_1 = 0 \quad \bar{C}_2 = 1 \quad 0 \leq \bar{z} \leq 1 \quad (5.5)$$

The boundary conditions may then be written as follows:

$$\tau \succ 0 \quad \bar{z} = z_i \quad \bar{C}_1 = 1 \quad \bar{C}_2 = 0 \quad (5.6)$$

where $z_i = \frac{z_N}{z_0}$

To simplify calculations, the origin of the coordinate system was placed at the interface of slide and biopolymer and only one side of the film was considered in the model. The diffusion flux at the slide-biopolymer interface was considered to be zero. Thus,

$$\tau \succ 0 \quad \bar{z} = 0 \quad \frac{\partial \bar{C}_1}{\partial \bar{z}} = 0 \quad \frac{\partial \bar{C}_2}{\partial \bar{z}} = 0 \quad (5.7)$$

Given that the model was a moving boundary problem, a front-fixing method was used

(Crank, 1975b) where $B = \frac{\bar{Z}}{Z_i}$, and introduced in the model:

$$\frac{\partial \bar{C}_i}{\partial \bar{z}} = \frac{1}{z_i} \frac{\partial \bar{C}_i}{\partial B} \quad (5.8)$$

$$\left. \frac{\partial \bar{C}_i}{\partial \tau} \right|_{\bar{z}} = \left. \frac{\partial \bar{C}_i}{\partial \tau} \right|_B + \frac{\partial \bar{C}_i}{\partial B} \frac{\partial B}{\partial \tau} \quad (5.9)$$

As a result, Eq (5.3) was rewritten as:

$$\frac{\partial \bar{C}_i}{\partial \tau} = \frac{\partial}{\partial B} \left(\bar{D}_i \frac{\partial \bar{C}_i}{\partial B} \right) \frac{1}{z_i^2} + \frac{1}{2} \frac{\partial \bar{C}_i}{\partial B} \frac{B}{z_i^2} \frac{\partial (z_i^2)}{\partial \tau} \quad (5.10)$$

To solve Eq (5.10), Z_i requires calculation. This may be found by assuming that the total volume of the system is the sum of the biopolymer, water and DSBP. Thus,

$$2yxz_N = V_p + \frac{2}{\rho_1} \int_0^{z_N} C_1 yx dz_N + \frac{2}{\rho_2} \int_0^{z_N} C_2 yx dz_N \quad (5.11)$$

where V_p is volume of the dry biopolymer, y and x are the width and length of the film, and ρ_1 and ρ_2 are the density of water and of the DSBP, respectively. Eq (5.11) is first differentiated with respect to time and to the boundary conditions and substituted by Eq (5.1), yielding:

$$\left(1 - \frac{C_{1,eq}}{\rho_1}\right) \frac{dz_N}{dt} = \frac{1}{\rho_1} D_1 \frac{\partial C_1}{\partial z} \Big|_{z=z_N} + \frac{1}{\rho_2} D_2 \frac{\partial C_2}{\partial z} \Big|_{z=z_N} \quad (5.12)$$

Rewriting Eq (5.12) in dimensionless form, Z_t can be calculated as follows:

$$\frac{C_{1,eq} \bar{D}_1}{\rho_1} \frac{\partial \bar{C}_1}{\partial B_{B=1}} + \frac{C_{2,eq} \bar{D}_2}{\rho_2} \frac{\partial \bar{C}_2}{\partial B_{B=1}} - \frac{1}{2} \left(1 - \frac{C_{1,eq}}{\rho_1}\right) \frac{\partial Z_t^2}{\partial \tau} = 0 \quad (5.13)$$

Finally, Eq (5.10) may be solved numerically with respect to Eq (5.13) using the initial boundary conditions in Eq (5.5) - Eq (5.7). An explicit finite-difference scheme was applied for solving the equations at each time step where B was divided into 50 intervals with 51 points axially. The time interval was calculated, 0.0001, from the Von Neumann stability analysis in order to have a stable explicit method (Verwer and Sommeijer, 1997).

5.3 Result and Discussion

5.3.1 Water Uptake in Genipin-Crosslinked Gelatin Disks

In order to accurately measure swelling behaviour and relate this to the swelling observed in the films, genipin-crosslinked gelatin disks were prepared. The diffusivity coefficients of water in the system, $D_{l,eq}$ and β_l , were obtained once the model was fitted to the experimental swelling data of genipin-crosslinked gelatin disks.

Figure 5.2: Swelling of genipin-crosslinked gelatin disk

Figure 5.3 shows that the mass transfer-based model for swelling is in good agreement with experimental data for different genipin concentration. Run tests were used to check whether a function fitted well to a data set, by marking with a plus sign the data above the function and with minus sign the data below the function (Miller and Miller, 2007). It showed that the probability of rejecting the model for swelling of genipin-crosslinked gelatin disks was $< 5 \%$ (Appendix). $D_{l,eq}$ and β_l values calculated for systems with different genipin concentrations are shown in Table 5.1. All R^2 values were > 0.99 .

Inside the biopolymer, water concentration plays a crucial role in expanding the pore size. When the biopolymer is placed into water, it absorbs water, and consequently swells. The molecular reorganization due to the water stresses the biopolymer in axial direction, which ‘stretches’ the polymer chains, resulting in wider pore sizes. With more genipin, the pores will not stretch as much and water uptake will be limited by the total available space. Consequently, the gel matrix will come to equilibrium more rapidly. However, the water concentration inside the biopolymer matrix dictates the free volume in the system. Thus, the free volume available for diffusion will be a function of the water concentration, resulting in concentration-dependent diffusivities. Consequently, increasing the concentration of genipin in the gelatin hydrogels restricts the free volume in the system.

Figure 5.3: Water uptake fitting for the genipin-crosslinked gelatin disk matrices

Table 5.1: $D_{1,eq}$ and β_1 values for genipin-crosslinked gelatin gels

Genipin concentration (wt %)	$D_{1,eq} \times 10^{-6}$ Water diffusion coefficient at equilibrium (cm ² /s)	β_1 Diffusivity dimensionless constant
0.25	11.50	1.9
0.5	3.25	1.9
0.75	1.98	2.9
1	1.18	2.9
2	0.737	2.9
Water diffusion coefficient at equilibrium in gelatin measured by pulsed-gradient N.M.R. spectroscopy 1.318 x 10 ⁻⁵ (cm ² /s) at 15.2°C and 2.511 x 10 ⁻⁵ (cm ² /s) at 40.2°C		

The results are comparable with water diffusion coefficients at equilibrium in gelatin measured by pulsed-gradient N.M.R. spectroscopy (Yapel *et al.*, 1994).

5.3.2 Swelling Kinetics in Genipin-Crosslinked Gelatin Disks

Swelling due to water diffusion occurred axially. Furthermore, it was assumed that no volume contraction occurred during swelling. Figure 5.4 shows the swelling of the genipin-crosslinked gelatin gels with $Z_0 = 0.2$ cm. According to free volume theory, the voids in the gel matrix can become larger with less crosslinked agent, and so more water can enter the gel matrix, which lead to increased swelling.

Figure 5.4: Swelling of the genipin-crosslinked gelatin disk matrices

5.3.3 Water Concentration Profiles in Genipin-Crosslinked Gelatin

Disks

Using this model, further insights into the mass transfer mechanisms were achieved. The concentration profiles of water were calculated at any instant for all genipin concentration gel. Figures 5.5-5.9 show the water uptake from the start of imbibing to equilibrium, which correlated well with experimental results. The time for water uptake decreased from 1200 to 40 minutes for hydrogels upon increasing genipin concentration from 0.25 to 2 wt%.

Figure 5.5: Water uptake profile of the 0.25 wt% genipin-10 wt% gelatine disk matrix

Figure 5.6: Water uptake profile of the 0.5 wt% genipin-10 wt% gelatin disk matrix

Figure 5.7: Water uptake profile of the 0.75 wt% genipin-10 wt% gelatin disk matrix

Figure 5.8: Water uptake profile of the 1 wt% genipin-10 wt% gelatin disk matrix

Figure 5.9: Water uptake profile of the 2 wt% genipin-10 wt% gelatin disk matrix

5.3.4 Water Uptake in Genipin-Crosslinked Gelatin Films

$D_{l,eq}$ and β_l values calculated for genipin-crosslinked gelatin disks were used to predict the swelling time and swelling kinetics of genipin-crosslinked gelatin films. Table 5.2 shows the swelling time and swelled thickness of the films. The results show the time for film swelling was much shorter than that for releasing DSBP molecules. These results confirm that the genipin-crosslinked gelatin films swelled completely before starting to release DSBP through gel films. The initial thickness of gel films used for the modeling was 475 nm.

Table 5.2: Swelling time and swelled thickness of genipin-crosslinked gelatin films

Genipin concentration (wt %)	Swelled thickness Z_s (nm)	Swelling time $t \times 10^{-4}$ (s)
0.25	745	3.000
0.50	608	1.520
0.75	566	0.800
1.00	535	0.412
2.00	508	0.108

5.3.5 DSBP Release from Genipin-Crosslinked Gelatin Films

The rate of gel swelling is much faster than DSBP release. In any given system, the transfer kinetics of diffusing molecules will depend greatly on molecular size and shape as well as pore size. Small pore sizes will normally lead to lower diffusion coefficients (Masaro and Zhu, 1999). In this context, ‘small’ may be defined as the perimeter of the average pore size within the matrix. Pores that are larger than the diffusing molecule will permit diffusion with little or no resistance whereas diffusing species larger than the pores will encounter resistance against their flow as they entangle within the matrix mesh. The jump from one pore in the biopolymer structure to another for a given pore size will be easier for smaller than for larger molecules (Fan and Singh, 1989). Hence, the diffusion coefficient of DSBP in gel matrices with more genipin was lower than those with less genipin (Table 5.3). Figure 5.10 shows that the DSBP mass transfer-based

model is in good agreement with experimental data for different genipin concentrations. Run tests checked whether a function fitted well to a data set (Miller and Miller, 2007). It showed that the probability of rejecting the model for all genipin-crosslinked gelatin films was less than 5% (Appendix).

Table 5.3: DSBP diffusion coefficients in fully-swelled genipin-crosslinked gelatin films.

Genipin concentration (wt%)	DSBP diffusion coefficient $D_{2,eq} \times 10^{-12} \text{ (cm}^2\text{/s)}$
0.25	7.70
0.50	3.05
0.75	1.85
1.00	1.30
2.00	1.10

Figure 5.10: Modeling DSBP release from genipin-crosslinked gelatin films

Comparing the diffusion coefficient results for different molecules in gelatin shows the release of DSBP is very slow through genipin-crosslinked gelatin films. Caffeine molecule diffusion coefficient through agar gels containing a hyaluronic acid–protein complex was measured at $6.79 \times 10^{-6} \text{ cm}^2/\text{s}$ (McCabe, 1972; Abbasi *et al.*, 2006). Comparing caffeine and DSBP shows that molecular charge has a significant effect of the diffusion coefficient, however both molecules have almost the same size. The diffusion coefficient of benzalkonium chloride, which is charged, released from poly(2-hydroxyethyl methyl methacrylate) hydrogel was $5.7 \times 10^{-10} \text{ cm}^2/\text{s}$ (Smith *et al.*, 2003), which is comparable with the DSBP diffusion seen here.

5.4 Conclusion

In this work, a mathematical model was developed based on experimental data carried out for the controlled release of DSBP from genipin-crosslinked gelatin films. The swelling of the hydrophilic matrix due to water penetration, and DSBP release, were taken into account simultaneously in this model. The moving boundary conditions for the swelling was derived by using a front-fixing method, and the resulting coupled partial differential equations were solved numerically by an explicit method. The predicted swelling and release profiles were in very good agreement with the observed experimental data.

6 GENERAL CONCLUSION

The hypotheses laid out at the beginning of this thesis were successfully verified. A novel method of developing sub-micron films for controlled release purposes was executed. Gelatin thin films crosslinked with genipin at different concentrations were used to examine the release of a fluorescent marker, DSBP. The concentration of genipin effectively modulated DSBP release. The release mechanism of DSBP from the crosslinked gelatin films depended on the following phenomena. Initially, the large water concentration gradient between the film and its aqueous environment resulted in water ingress into the film. As a result, the biopolymer chains expanded, leading to swelling. The swelling altered the dimensions of the matrix, which continued until the chemical potential within the film was equal to that of the surroundings. This event happened quickly such that the diffusivity of DSBP was independent of water concentration in the film. The release of DSBP was dependent on the available free volume being large enough to permit diffusion of the DSBP. The diffusivity of DSBP continued to rise until the available free volume exceeded the minimum required free volume for diffusion. Finally, a free volume-based model was developed and implemented. It effectively predicted the experimentally-observed release behavior of DSBP.

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8 APPENDICES

8.1 Von Neumann Stability Analysis

The Von Neumann stability (Verwer and Sommeijer, 1997) analysis applies to linear systems of finite difference equations (FDE). The method applies a Fourier-like solution to the finite difference operator, and stability is determined by the change in magnitude of the Fourier waves as time is advanced one step. Representing a 1-D solution as,

$$U^n_{ij} = \hat{U}^n e^{I\Phi j} = \hat{U}(\cos(\Phi j) + I \sin(\Phi j)) \quad (8.1)$$

where $\hat{U}^n$ is the amplitude of Fourier wave at time n , and $I = \sqrt{-1}$

At time level $n + 1$,

$$U^{n+1}_{ij} = \hat{U}^{n+1} e^{I\Phi j} \quad (8.2)$$

At the point $j + 1$,

$$U^{n+1}_{ij+1} = \hat{U}^{n+1} e^{I\Phi(j+1)} \quad (8.3)$$

In order to investigate stability analysis, all the Fourier transformed U^n_{ij} terms in a finite difference equation should be substituted and solved for the amplification factor G , where

$$G = \frac{\hat{U}^{n+1}}{\hat{U}^n} \quad (8.4)$$

So, the general stability criterion is defined by:

$$|G| \leq 1 \quad (8.5)$$

8.1.1 Stability analysis of explicit method

Two main equations that describe the model are Eq(5.10) and Eq(5.13):

$$\frac{\partial \bar{C}_i}{\partial \tau} = \frac{\partial}{\partial B} \left(D_i \frac{\partial \bar{C}_i}{\partial B} \right) \frac{1}{z_i^2} + \frac{1}{2} \frac{\partial \bar{C}_i}{\partial B} \frac{B}{z_i^2} \frac{\partial (z_i^2)}{\partial \tau} \quad (5.10)$$

$$\frac{C_{1,eq} \bar{D}_1}{\rho_1} \frac{\partial \bar{C}_1}{\partial B_{B=1}} + \frac{C_{0,eq} \bar{D}_2}{\rho_2} \frac{\partial \bar{C}_2}{\partial B_{B=1}} - \frac{1}{2} \left(1 - \frac{C_{1,eq}}{\rho_1} \right) \frac{\partial z_i^2}{\partial \tau} = 0 \quad (5.13)$$

A Neumann stability analysis was implemented to analyze the stability of the explicit method in solving Eq(5.10). Eq(5.10) can be differentiated with respect to time as follows:

$$\begin{aligned}
\frac{\overline{C}_{i,j}^{n+1} - \overline{C}_{i,j}^n}{\Delta \tau} = & \frac{1}{z_i^2 \Delta B} \left(\frac{\overline{D}_{i,j+1}^n + \overline{D}_{i,j}^n}{2} \right) \left(\frac{\overline{C}_{i,j+1}^n - \overline{C}_{i,j}^n}{\Delta B} \right) \\
& - \frac{1}{z_i^2 \Delta B} \left(\frac{\overline{D}_{i,j}^n + \overline{D}_{i,j-1}^n}{2} \right) \left(\frac{\overline{C}_{i,j}^n - \overline{C}_{i,j-1}^n}{\Delta B} \right) \\
& + \frac{1}{2} \left(\frac{\overline{C}_{i,j+1}^n - \overline{C}_{i,j}^n}{\Delta B} \right) \left(\frac{B}{z_i^2} \right) \left(\frac{2}{1 - \frac{C_{1,eq}}{\rho_i}} \right) \\
& \left(\frac{C_{1,eq} (1 - \overline{C}_{i,nj-1}^n)}{\Delta B \rho_i} - \frac{C_0 D_{2,eq} (0 - \overline{C}_{2,nj-1}^n)}{\Delta B \rho_2 D_{1,eq}} \right) \quad (8.5)
\end{aligned}$$

Replacing all the $\overline{C}_{ij}^n, \overline{D}_{ij}^n$ terms in Eq(8.5) with the Fourier representation yields:

$$\begin{aligned}
\frac{\widehat{C}_i^{n+1} e^{l\varphi\varphi} - \widehat{C}_i^n e^{l\varphi\varphi}}{\Delta \tau} = & \frac{1}{z_i^2 \Delta B} \left(\frac{\widehat{D}_i^n e^{l\varphi(j+1)} + \widehat{D}_i^n e^{l\varphi\varphi}}{2} \right) \left(\frac{\widehat{C}^n e^{l\varphi(j+1)} - \widehat{C}^n e^{l\varphi\varphi}}{\Delta B} \right) \\
& - \frac{1}{z_i^2 \Delta B} \left(\frac{\widehat{D}_i^n e^{l\varphi\varphi} + \widehat{D}_i^n e^{l\varphi(j-1)}}{2} \right) \left(\frac{\widehat{C}_i^n e^{l\varphi\varphi} - \widehat{C}_i^n e^{l\varphi(j-1)}}{\Delta B} \right) \\
& + \frac{1}{2} \left(\frac{\widehat{C}_i^n e^{l\varphi(j+1)} - \widehat{C}_i^n e^{l\varphi\varphi}}{\Delta B} \right) \left(\frac{B}{z_i^2} \right) \left(\frac{2}{1 - \frac{C_{1,eq}}{\rho_i}} \right) \\
& \left(\frac{C_{1,eq} D_{1,eq} (1 - \widehat{C}_i^n e^{l\varphi\varphi(n-1)})}{\Delta B \rho_i} - \frac{C_0 D_{2,eq} (0 - \widehat{C}_2^n e^{l\varphi\varphi(n-1)})}{\Delta B \rho_2 D_{1,eq}} \right) \quad (8.6)
\end{aligned}$$

Simplifying Eq(8.6) yields:

$$\begin{aligned}
 \frac{\bar{\hat{C}}_{i^{n+1}} - \bar{\hat{C}}_i^n}{\Delta \tau} = & \frac{\bar{\hat{D}}_i^n \bar{\hat{C}}_i^n e^{l_{\varphi\varphi}}}{z_i^2 \Delta B^2} \left(\frac{e^{2l_{\varphi}} + e^{-2l_{\varphi}} - 2}{2} \right) + \\
 & \frac{1}{2} \frac{B \bar{\hat{C}}_i^n}{z_i^2} \left(\frac{e^{l_{\varphi}} - 1}{\Delta B} \right) \left(\frac{2}{1 - \frac{C_{l,eq}}{\rho_i}} \right) \\
 & \left(\frac{C_{l,eq} D_{l,eq} \left(1 - \bar{\hat{C}}_i^n e^{l_{\varphi\varphi}(n-1)} \right)}{\Delta B \rho_i} - \frac{C_0 D_{2,eq} \left(0 - \bar{\hat{C}}_2^n e^{l_{\varphi\varphi}(n-1)} \right)}{\Delta B \rho_i D_{l,eq}} \right)
 \end{aligned} \tag{8.7}$$

Substitution of Eq(8.1) in Eq(8.7) gives:

$$\begin{aligned}
 \frac{\bar{\hat{C}}_{i^{n+1}} - \bar{\hat{C}}_i^n}{\Delta \tau} = & \frac{\bar{\hat{D}}_i^n \bar{\hat{C}}_i^n e^{l_{\varphi\varphi}}}{z_i^2 \Delta B^2} \left(\frac{2 \cos(2\varphi) - 2}{2} \right) + \\
 & \frac{B \bar{\hat{C}}_i^n}{\Delta B^2 z_i^2} \left(\frac{\cos(\varphi) + I \sin(\varphi) - 1}{1 - \frac{C_{l,eq}}{\rho_i}} \right) \\
 & \left(\frac{C_{l,eq} D_{l,eq} \left(1 - \bar{\hat{C}}_i^n e^{l_{\varphi\varphi}(n-1)} \right)}{\Delta B \rho_i} - \frac{C_0 D_{2,eq} \left(0 - \bar{\hat{C}}_2^n e^{l_{\varphi\varphi}(n-1)} \right)}{\Delta B \rho_i D_{l,eq}} \right)
 \end{aligned} \tag{8.8}$$

The stability limit is $|G \leq I|$. To achieve this condition, $\bar{\hat{C}}_1^n e^{I\phi(nj-1)}$ and $\bar{\hat{C}}_2^n e^{I\phi(nj-1)}$ should be 0 and 1.

$$\left| \frac{\bar{\hat{C}}_1^{n+1}}{\bar{\hat{C}}_1^n} \right|_{\max} = \left| \frac{\Delta \tau}{z_1^2 \Delta B^2} \bar{D}_{1,j} (\cos(2\phi) - 1) \right| + \left| \frac{B \Delta \tau}{z_1^2 \Delta B^2} \left(\frac{C_{1,eq}}{\rho_1} + \frac{D_{2,eq} C_0}{D_{1,eq} \rho_2} \right) \left(\frac{\cos(\phi) + I \sin(\phi) - 1}{1 - \frac{C_{1,eq}}{\rho_1}} \right) \right| \quad (8.9)$$

The maximum value of $\left| \frac{\bar{\hat{C}}_1^{n+1}}{\bar{\hat{C}}_1^n} \right|$ is obtained when z_1 , B and $\bar{D}_{1,j}$ are 1:

$$\left| \frac{\bar{\hat{C}}_1^{n+1}}{\bar{\hat{C}}_1^n} \right|_{\max} = \left| \frac{\Delta \tau}{\Delta B^2} \left(\cos(2\phi) + B \left(\frac{C_{1,eq}}{\rho_1} - \frac{D_{2,eq} C_0}{D_{1,eq} \rho_2} \right) \frac{(\cos(\phi) - 1)}{\left(1 - \frac{C_{1,eq}}{\rho_1} \right)} - 1 \right) \right| + \left| \frac{B \Delta \tau}{\Delta B^2} \left(\frac{C_{1,eq}}{\rho_1} - \frac{D_{2,eq} C_0}{D_{1,eq} \rho_2} \right) \frac{I \sin(\phi)}{\left(1 - \frac{C_{1,eq}}{\rho_1} \right)} \right| \quad (8.10)$$

$\frac{C_{l,eq}}{\rho_1}$ is the volume fraction of water in equilibrium and $\frac{C_0}{\rho_2}$ is the volume fraction of

the incorporated molecule at initial conditions. The swelling index is investigated in pure

genipin-crosslinked gelatin where $\frac{C_0}{\rho_2}$ is zero. By drawing the real value and imaginary

value of $\left| \frac{\hat{C}_i^{n+1}}{\hat{C}_i^n} \right|_{max}$, the optimum value for $\Delta\tau$ and ΔB can be found to yield the

stability limit. By considering $\Delta\tau = 0.0001$, $\Delta B = .02$, the condition for stability is

obtained. Figure 8.1 shows the value of $\left| \frac{\hat{C}_i^{n+1}}{\hat{C}_i^n} \right|_{max}$ for 0 to 2π phase angles, which for

$\Delta\tau = 0.0001$, $\Delta B = .02$, the condition is always less than 1.

Figure 8.1: Von Neumann Stability Analysis of Eq(8.10)

8.2 Run Test

The run test or Wald-Wolfowitz test is a non-parametric test that checks the randomness hypothesis of a data sequence (D'Urso and Gastaldi, 2003). In curve fitting, the positive or negative residue is the difference between the actual value and the model value. A sequence of positive signs followed by a sequence of negative signs, and is then followed by a sequence of positive signs is called a run. The Wald-Wofowitz method tests whether the number of runs is small enough for the null hypothesis of a random distribution of signs to be rejected (Miller and Miller, 2007). The number of runs in the experimental data is compared with the numbers in Table 8.1, which refers to the $P=0.05$ probability level. The table is completed by using the appropriate value for N , the number of positive signs and M , the number of negative signs. If the experimental number of runs is smaller than the tabulated value, then the null hypothesis can be rejected. Runs tests result for water uptake simulation in genipin-crosslinked gelatin disks and DSBP release from genipin-crosslinked gelatin films are shown in table 8.2. The table confirms that the probability of rejecting the model for all genipin-crosslinked gelatin films is less then 5%.

Table 8.1: The Wald-Wolfowitz run test

N	M	At $P=0.05$, the number of runs are significant, if they are:	
		Less than	Greater than
2	12-20	3	NA
3	6-14	3	NA
3	15-20	4	NA
4	5-6	3	8
4	7	3	NA
4	8-15	4	NA
4	16-20	5	NA
5	5	3	9
5	6	4	9
5	7-8	4	10
5	9-10	4	NA
5	11-17	5	NA
6	6	4	10
6	7-8	4	11
6	9-12	5	12
6	13-18	6	NA
7	7	4	12
7	8	5	12
7	9	5	13
7	10-12	6	13
8	8	5	13
8	9	6	13
8	10-11	6	14
8	12-15	7	14

Table 8.2: Run test result

The Wald-Wolfowitz runs test	<i>N</i>	<i>M</i>	Run Test
Water uptake fitting model for 0.25 wt % genipin-10 wt % gelatin disk	8	13	8
Water uptake fitting model for 0.50 wt % genipin-10 wt % gelatin disk	4	11	5
Water uptake fitting model for 0.75 wt % genipin-10 wt % gelatin disk	7	8	5
Water uptake fitting model for 1.00 wt % genipin-10 wt % gelatin disk	4	5	5
Water uptake fitting model for 2.00 wt % genipin-10 wt % gelatin disk	5	8	4
DSBP release fitting model for 0.25 wt % genipin- 10 wt % gelatin film	3	6	4
DSBP release fitting model for 0.50 wt % genipin-10 wt % gelatin film	3	6	4
DSBP release fitting model for 0.75 wt % genipin-10 wt % gelatin film	4	5	4
DSBP release fitting model for 1.00 wt % genipin-10 wt % gelatin film	5	5	4
DSBP release fitting model for 2.00 wt % genipin-10 wt % gelatin film	5	8	4

DL-40-50