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UMI
Combination of hydrogel and liposomes as a responsive drug delivery system

by

Erik Ho Yan Cheng

A thesis submitted in conformity with the requirements for the degree of Master of Applied Science and Engineering
Graduate Department of Chemical Engineering and Applied Chemistry
University of Toronto

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Combination of hydrogel and liposomes as a responsive drug delivery system

Master of Applied Science and Engineering

1997

Erik Ho Yan Cheng

Department of Chemical Engineering and Applied Chemistry

University of Toronto

The idea of combining hydrogel and liposome as a responsive drug delivery system was investigated. Two hydrogels, one as a static network and the other as a gellable solution, were formulated. Poly(vinyl alcohol) was chosen as the static network and gellan gum as the gellable solution. Results from light microscopy and DSC measurements had shown that liposomes were formed within the PVA hydrogel. The release of different solutes from PVA hydrogels with or without liposomes were examined. It was found that the existence of liposomes was able to extend the release period and increase the loading capacity of lipophilic solutes. The release rate of the solutes were responsive to temperature variation. The vitreous was selected as a model site for injection of the gellable solution. Simulation of intravitreal injection had shown that the gellable solution was able to gel within the injected site and encapsulate liposomes inside the gel.
I would like to thank Professor Yu-Ling Cheng for her patient guidance, advice and encouragement throughout the performance of this work. I would like to thank all of my lab co-workers for their useful suggestions and accompanied me throughout the years of research. Thanks to Professor Wu for her generosity in allowing me to use the DSC machine. Finally, I would like to thank the Natural Sciences and Engineering Research Council of Canada for its financial support.
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In this thesis, the idea of combining a hydrogel and liposomes as a responsive drug delivery system (DDS) is tested. Liposomes are membrane vesicles capable of encapsulating drugs. The release rate of drugs from liposomes could be responsive to different external stimuli. It has been increasingly recognized that responsive delivery is needed for some drugs/diseases. The application of liposomes as a DDS has been limited by problems such as biological and mechanical stability. One possible solution to improve the stability of liposomes is to encapsulate them within a hydrogel. Moreover, the hydrogel can be in the form of a static network or a gellable solution. A static hydrogel is suitable for implantation, while a gellable solution may be suitable for injection subcutaneously, intramuscularly, etc. and thus removes the need for surgical implantation of device. In particular, a gellable solution can be injected intravitreally. It has been demonstrated that current intravitreal injection methods are sub-optimal and a need exists for improved injection methods. The focus of the present work is to develop a DDS suitable for sustained drug delivery in the vitreous.

1.1 Background

Successful drug therapy requires that the applied drug be present at the target site at an effective concentration for a sustained period of time. Traditionally, drugs are administered to the body orally, topically or by direct injection. Although oral intake has the advantage of being simple, it remains unsuccessful for the delivery of large molecules such as peptides and proteins. This is due to slow absorption and fast degradation of these molecules in the gastrointestinal (GI) tract. Moreover, there is extensive drug metabolism in the liver.

Topical formulations can be in the form of ointments, pastes, creams, gels, rigid foams and eyedrops (Banker, 1996). Dermal and transdermal drug delivery are usually limited to highly lipophilic drugs. The thick lipophilic keratin layer of the skin forms a very effective barrier for the penetration of hydrophilic drugs without modification
clearance of hgs by tear drainage and poor penetration through the crystalline lens render this method inefficient. To ensure effective drug concentration at the required therapeutic site, higher initial concentration raises the possibility of systemic toxicity.

In systemic administration, frequent injections are required because of rapid elimination and degradation of the drugs from the blood stream by the kidneys and the liver. In addition, accessibility of drugs to poorly perfused sites in the body is limited. For example, the supply of drugs to the eye from the blood vessels in the retina or from the choroid is unsatisfactory due to the pigment epithelium.

By far, the most successful form of drug delivery to the posterior segment of the eye is by intravitreal injection. The advantage of performing intravitreal injection is that a known quantity of drug is introduced directly into the affected area. Nevertheless, it is not advisable to routinely perform intravitreal injections because of the danger of complications.

As the therapeutic concentration range for some drug may be quite narrow, it is desirable if some kind of DDS could be utilized to improve the bioavailability at the target site, reduce toxicity as a whole and improve patient compliance by reducing the required dosing frequency.

Different DDS can accompany different routes of administration for drug delivery. The most common route of entry is the gastrointestinal route. Other routes such as the pulmonary route, the dermal and transdermal route, the nasal route and the ocular pathway are also selected effective applications. Moreover, a DDS can be implanted at the target site for sustained drug release and drug confinement at the target site.

Historically, there are two main groups of DDS. The first type of system contains drug dispersed in a polymer matrix. The second type of system contains drug in reservoir confined by polymer. Drug release via diffusion from the polymer matrix devices typically follows square root time kinetics. Drug release from the reservoir devices follows zero order kinetics (Tyle, 1988, Junginger, 1992, Banker, 1996). Drug release from both configurations may occur by diffusion, rupturing, or polymer degradation.
particulate systems (Johnson, 1987, Rosoff, 1989). These systems can act as parenteral drug carriers that circulate in the vascular system upon injection and deliver the drugs to the appropriate site(s) for a certain period of time.

Of the many studies based on this approach, polymeric beads and liposomes have attracted the most interest. The permeability of liposomes can respond to variations in temperature due to changes in membrane fluidity (New, 1990). However, because of limitations in their size, physical and chemical properties and stability, the applications of liposomes as drug delivery systems are limited (Senior, 1983, Mayer, 1989, Gabizon, 1990, Fielding, 1991, Gregoriadis, 1993, Lasic, 1996).

With the explosion in the development of potentially potent drugs, the requirements for complex drug release profiles and more sophisticated site specificity are apparent. The recent development of better DDS to fulfill these needs has focused on responsive drug delivery. The drug release rate from these systems could be responsive to pH, temperature, ionic strength and photoirradiation.

Liposomes are vesicles composed of concentric bilayer lipid membranes. At temperatures below a specific temperature known as the phase transition temperature \( T_m \), the lipid bilayers remain intact in a relatively ordered gel state. As the temperature increases to above \( T_m \), the lipid bilayers transform to a more disordered, fluid-like liquid crystalline state. In the gel state, liposomal membranes are more stable, less permeable to solutes and less likely to interact with destabilizing macromolecules than in the liquid crystalline state. Due to this specific property, the release rate of drugs entrapped inside a liposome could be responsive to temperature variation due to changes in membrane fluidity.

Sometimes, to confine a drug at the specific target site, a DDS is implanted. However, this could lead to more complications due to the requirement of surgery. Consequently, it is highly desirable if an injection medium could be responsive to the physiological environment of gel at the target site. Poloxamer 407 solution, a viscous solution at room temperature, transforms to a viscous gel once the temperature is raised to that of the eye (Zignani, 1995). Cellulose acetophalate (CAP) latex, a solution at pH 4.5,
Gellan gum, a polysaccharide consisting of tetrasaccharide repeating units, gels when the concentration of gellan gum and/or cation reach a specific level (Rozier, 1989, Zignani, 1995, Nakajima, 1996). All these systems could accompany liposomes as a DDS for responsive drug delivery.

1.2 Rationale and Scope of Thesis

In the present study, hydrophilic-hydrophobic systems composed of hydrogels and liposomes were examined. Two different approaches were taken. The first approach is the development of a solid drug delivery system that could be implanted at the target site for controlled drug-delivery. This approach was characterized by the dispersion of liposomes in a hydrogel polymer matrix. The second approach was the design of a liquid drug delivery system that could be injected into the target site and gel at the target site. This approach was characterized by the containment of a liposome-drug reservoir surrounded by gellan gum in gel state after injection.

Given the responsive properties of liposomes and gellan gum, the objectives are to examine the capability of the systems to sequester high concentrations of lipophilic solute, prolong the release period of the solute and investigate the possibility of temperature-responsive release. More specifically, given problems related to intravitreal delivery, a specific application of gellan gum-liposome combination may be as an intravitreal injection dosage form.
2.1 Liposomes

2.1.1 General Characteristics of Liposomes

A liposome is defined as any structure composed of membrane-like lipid bilayers surrounding an aqueous compartment. Phospholipids are the most commonly used lipid component. When phospholipids are dispersed mechanically in an aqueous phase, a heterogeneous mixture of vesicular structures is usually formed. These were the liposomes first prepared and now called multilamellar vesicles (MLVs) (Figure 2.1)

![Diagram of Liposomes](image-url)
The size of a MLV liposome could range from 20 nm to 100 μm (Lasic, 1996). If a lipid dispersion is sonicated, the MLVs are reduced to much smaller structures in the size range 25-100 nm diameter. These are called small unilamellar vesicles (SUVs) since they contain only a single bilayer (Ostro, 1983, New, 1990, Lasic, 1996). Larger liposomes with a single bilayer and with diameter greater than 100 nm are known as large unilamellar vesicles (LUVs) and require special techniques such as reverse-phase evaporation and detergent dilution (Ostro, 1983, Machy, 1987) for production.

The composition of the lipid bilayer is critically important in determining the pharmaceutical properties of liposomes, mainly through influences on membrane fluidity, permeability, surface properties and stability. As temperature increases, phospholipid bilayers change from a relatively ordered gel state to a more disordered, fluid-like liquid crystalline state at a specific temperature known as the phase transition temperature (T_m). Liposome membranes in the gel state are more stable and less permeable to solutes. Moreover, they are less likely to interact with destabilizing macromolecules than in the liquid crystalline state.

The permeability of liposome membranes varies greatly from compound to compound. Small molecules such as water, protons and hydroxyl ions have the fastest diffusion rate, while molecules with a high solubility in both lipophilic and hydrophilic media can permeate through the membrane fairly quickly. Conversely, polar with high molecular weight permeate across the membrane at a much slower rate. Metal ions, similar to polar solutes, diffuse through the membrane very slowly (New, 1990).

Figure 2.2 (New, 1990) illustrates the difference in permeability of neutral liposome membranes for different solutes above and below T_m. At T_m, the gel and liquid-crystalline phases co-exist. Consequently, there is a large increase in packing defects of the membranes as compared to temperatures above and below T_m. As a result, the permeability of metal ions and small molecules show a maximum at T_m. In contrast, the permeability of protons, hydroxyl ions and water increase monotonically as temperature increases. New (1990) suggested that the difference in permeability of the different
solutes is due to existence of water channels across the membrane accessible to protons, hydroxyl ions and water only.

While most liposomes have no net charge, negatively or positively charged lipids are frequently added to reduce liposome aggregation (Fielding, 1991). However, charged liposomes are generally more leaky than uncharged ones (Fielding, 1991).

2.1.2 Limitations of Liposomes

During the last few decades, considerable interest has been raised concerning the potential of liposomes as an in vivo drug-carrier system. Although such ideas have been in existence for over thirty years, their applications are limited by several factors. First is the fast and dominant uptake of liposomes by the mononuclear phagocytic system (MPS) or the reticulo-endothelial system (RES) upon injection into the vascular system. Second is the interaction of high density lipoproteins (HDL’s) with liposomes after intravenous injection. Third is the mechanical stability of the liposomes.

After intravenous injection, opsonin, a plasma protein, adsorbs onto the surface of the liposome and mediates the endocytosis by the macrophages of the RES (Senior, 1983, Gabizon, 1990, Fielding, 1991, Gregoriadis, 1993, Lasic, 1996). Accumulation of liposomes with drugs in the RES could impair the defense system and prevent them from functioning properly. Rate of elimination could vary greatly with vesicle size and charge. SUV liposomes (<100 nm diameter), especially those composed of lipids in the rigid, gel state, are less efficiently taken up by the RES and may have a considerably longer residence time than larger liposomes (half-life, 80 minutes) (Machy, 1987, Fielding, 1991). Very large liposomes (>1000 nm) (Abra, 1984) may lodge in the capillaries, and are observed to localize in the lungs after intravenous injection (half-life,
with a positive charge. Neutral liposomes have an intermediate rate of elimination.

Another problem that limits the potential of liposomes as in vivo drug-carryers is the reaction of HDL’s with liposomes following intravenous injection, which remove lipids from the membrane bilayer. The rate of drug release is related to the degree of vesicle disintegration and thus are relatively uncontrolled when attacked by HDL.

The third problem is the mechanical stability of the liposomes. Free liposomes in circulation were fragile and could break up easily. Early release of drug from liposomes in the circulatory system could result in drug dilution due to blood volume and fast removal of drug by the kidneys and the liver.

2.1.3 Liposome Stabilization

2.1.3.1 Prevention of RES uptake

Recently, many reports have demonstrated that surface modification of liposomes can decrease the rate of rapid uptake of liposomes by the RES. Most approaches have attempted to decrease liposome interaction with biological components, thereby increase the blood circulation time. One of the earliest attempts is coating liposomes with natural products: proteins, polysaccharides, and glycoproteins. Although much work was done, no experiment shows improvement in liposome stability against macrophage uptake (Ringsdorf, 1988, Woodle, 1992).

Somewhat different from coating, doping methods involve co-mixing of amphipathic molecules with natural glycolipids at low ratios in the lipid bilayer. Findings have shown that incorporation of a few specific natural glycolipids in liposomes result in prolonged circulation as compared with control liposome compositions. Nevertheless, the need for expensive natural glycolipids for incorporation has limited their potential use in the pharmaceutical industry (Woodle, 1992).

The successes with a few natural glycolipids have led to the focus of developing better alternatives. Most of these studies show that incorporation of the hydrophilic
circulation time and reduced RES uptake. These results indicate that the pharmaceutical use of liposomes can be improved by synthetic lipids with polymeric head groups, preparable in a standardized way (Woodle, 1992).

Similar methods using PEG to improve the bilayer properties are not limited to liposomes alone. Warriner et al. (1996) have shown that incorporation of a small amount of PEG-lipid to a phospholipid lamellar membrane could vary the permeability of the bilayers by creating defects at higher temperature. Nevertheless, the bilayers are not stable enough to withstand the in vivo environment, thus rendering them theoretical tools for drug delivery.

2.1.3.2 Mechanical Stabilization

In the last decade, numerous methods using polymeric systems to stabilize liposomes have been developed. Increased stability of these liposomes is achieved either by polymerizing the polar head groups or the hydrophobic tails of the amphiphiles. In addition, membrane-spanning lipids, insertion of hydrophobic anchor groups and polymerizable counterions are used to stabilize the liposomes. Figure 2.3 (Ringsdorf, 1988) summarizes the possible molecular architectures of stabilized liposomes. Although all approaches have increased the stability of the liposomes, their applications are still limited by rapid uptake of the MPS and toxicity effects of the degradation products.

Another novel design developed by Jin et al. (1995) is a hybrid particulate system between liposomes and polymeric beads known as "Lipobeads". Such beads are created by coating a layer of phospholipids around a solid bead that acts as the skeleton of the lipobead. Advantages of these beads are their increase drug loading capacity, enhanced mechanical stability over liposomes, controllable size and long term storage. However, their application is still limited by the rapid uptake of the MPS.

A third possible solution to increase the stability of liposome is to load them within a vehicle consisting of inert materials to implant at the diseased site for sustained periods. Takagi et al. (1996) reported the examination of alginate gel as a possible
Preliminary results show that alginate gel released the drug by a water-soluble drug incorporated in the liposomes. Moreover, calcium ions, an inducer for gelation of alginate, also stimulated the leakage of the drug from the liposomes. Reduced encapsulation efficiency and undesired drug leakage would limit the application of this system in drug delivery.

Morimoto et al. (1996) studied drug release for PVA containing dispersed phospholipid. Although it is possible that the dispersed phospholipid formed liposomes in the PVA hydrogel, the authors did not present any evidence nor suggest liposomes were formed. The authors showed that poly(vinyl alcohol) (PVA) hydrogels containing dispersed phospholipids could prolong the drug concentration in vivo by approximately 2.5 times compared with oral administration. Furthermore, the bioavailability for rectal administration of the phospholipid dispersed hydrogel increased by more than a factor of two relative to rectal suppositories.

Figure 2.3 Strategies for the stabilization of lipid bilayer membranes

2.1.4 Phospholipids
polar hydrocarbon tails. The most common type of phospholipid used in biological research is glycerophospholipid. Several parameters can affect the T_m of a phospholipid. For example, T_m for a homologous series of phosphatidyl cholines increases as the fatty acid chain length increases; also T_m is higher for saturated lipids than for homologous unsaturated lipids. The nature of the polar head group is also important; thus, the critical temperature for phosphatidylethanolamines is about 20°C higher than for the homologous phosphatidyl cholines. Another important parameter is the pKa of a phospholipid, which has a direct effect on the stability of a liposome. Abrupt changes in surrounding pH could cause destabilization of liposomes. Table 2.1 lists some phospholipids with T_m around 37°C (Marsh, 1990, Huang, 1991).

Table 2.1 Relevant Physical Data of some Phospholipids

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>T_m (°C)</th>
<th>Lipid Chain Length *</th>
<th>pK_a</th>
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<tbody>
<tr>
<td>Phosphatidylcholine</td>
<td>33.9</td>
<td>15</td>
<td>&lt;1 (PO_4^{3-})</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>41.9</td>
<td>16</td>
<td>&lt;1 (PO_4^{3-})</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>30.2</td>
<td>12</td>
<td>1.7 (PO_4^{3-}) 9.8 (NH_3^+)</td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>32.5</td>
<td>15</td>
<td>3.1 (PO_4^{3-})</td>
</tr>
<tr>
<td>Phosphatidic acid</td>
<td>33.5</td>
<td>12</td>
<td>4.0 (PO_4^{3-})</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>32.5</td>
<td>12</td>
<td>2.6 (PO_4^{3-}) 4-5(COO^-) 12(NH_3^+)</td>
</tr>
</tbody>
</table>

* Both chains in the same phospholipid are saturated and have the same number of carbons

2.2 Hydrogel

2.2.1 General description of hydrogels
A hydrogel is a polymer that exhibits the ability to absorb water and retain a significant fraction of water within its structure. Their ability to swell in water is due to the presence of hydrophilic groups such as -OH, -CONH-, -CONH₂, -COOH, and -SO₃H (Peppas, 1993). Hydrogel is chosen in this project because it is desirable that the hydrophilic matrix allows easy diffusion of the drug, but be practically impermeable to larger particles such as liposomes.

Hydrogels are characterized by their weight percentage of water content \( W \), the number average molecular weight between cross-links \( M_c \), and mesh size \( \xi \) (Canal, 1989). \( W \) is the easiest to determine:

\[
W = \frac{(M_{A,s} - M_D)}{M_D} \times 100\% \tag{1}
\]

where \( M_{A,s} \) is the swollen weight of the hydrogel and \( M_D \) is the dry weight of the hydrogel.

To determine limitations on the size of drug that can be delivered, it is necessary to calculate the maximum size of solutes that can pass through the networks. From the theory of solute diffusion in polymers the maximum size is known as the mesh size \( \xi \) (Figure 2.4) (Canal, 1989). Canal et al. (1989) showed, using scaling laws, that the polymer volume fraction after swelling, \( \psi_{2,s} \), is the only parameter needed to determine \( \xi \):

\[
\psi_{2,s} = \frac{M_D \cdot \rho_v}{\rho_v \cdot (M_{A,s} - M_{c,s})} \tag{2}
\]
where \( \rho_c \) is the density of cyclohexane, \( \rho_b \) is the bulk density of PVA and \( M_w \) is the weight of the hydrogel in cyclohexane after swelling in water.

\[
\zeta = k_1 + k_2 \omega_{2,s}^n
\]  

(3)

where \( k_1, k_2 \) and \( n \) are constants specific to each hydrogel system. For PVA at 37°C, Canal et al. (1989) determined that

\[
\begin{align*}
  k_1 &= \begin{cases} 
  -310.1 \text{ Å} & \text{if } \omega_{2,s} < 0.1 \\
  -31.6 \text{ Å} & \text{if } \omega_{2,s} > 0.1 
  \end{cases} \\
  k_2 &= \begin{cases} 
  117.0 \text{ Å} & \text{if } \omega_{2,s} < 0.1 \\
  12.6 \text{ Å} & \text{if } \omega_{2,s} > 0.1 
  \end{cases} \\
  n &= \begin{cases} 
  -1/2 & \text{if } \omega_{2,s} < 0.1 \\
  -1 & \text{if } \omega_{2,s} > 0.1 
  \end{cases}
\]

Hydrogels are either ionic or neutral in nature (Gould, 1976, Peppas, 1993). The swelling properties of hydrogels can be responsive to stimuli such as pH, ionic strength, temperature and photoirradiation. In networks that contain weakly acidic (anionic) or basic (cationic) pendant groups, the degree of ionization and ion-ion interactions will depend on the solution pH and ionic composition. As a result, the swelling ratio can become a function of solution pH and ionic concentration.

A negligible or small positive enthalpy of mixing is usually associated with the swelling process of a polymer hydrogel. Therefore, swelling is driven by the large gain in entropy. For hydrogels with strong bonding between the solvent and the polymer, polymer solubility decreases when temperature increases. This unusual behaviour is associated with the large increase in enthalpy of mixing as the temperature approaches a critical value, known as the 'lower critical solution temperature' (LCST) (Peppas, 1993). Networks with LCST shrink when the temperature is raised above LCST and swell below it. Consequently, the swelling ratio is a function of temperature for these hydrogels.

Properties such as conformation, shape, and degree of swelling of photoresponsive gels may be sensitive to irradiation. A photoresponsive polymer has a photoreceptor and a functional part. The photoreceptor captures optical signals and convert them to chemical signals. The latter signals are then transferred to the functional
2.2.2 Hydrogels in Medicine

Non-responsive hydrogels such as cross-linked poly(vinyl alcohol) (PVA), poly(2-hydroxyethyl methacrylate) (PHEMA) and poly(vinyl pyrrolidone) (PVP) are widely used in the biomaterials industry (Gould, 1976, Peppas, 1986, Suzuki, 1991, Knuth, 1993, Peppas, 1993). Their wide applications range from artificial organs to drug delivery systems.

Poly(vinyl acetate) is converted to PVA upon hydrolysis. A highly porous sponge of PVA was commercially available under the name Ivalon®. It had been extensively used in hernia treatment, duct replacement, cardiac-vascular surgery, plastic surgery, and reconstructive surgery (Gould, 1976). Other potential applications of PVA include preparation of sustained-release drug delivery systems for transdermal, rectal, vaginal and oral administration (Morimoto, 1989, 1990; Knuth, 1993).

The primary clinical use for PHEMA hydrogels has been for flexible, hydrophilic contact lenses. PHEMA hydrogels are best known for their high degree of chemical stability. Studies of disks of PHEMA subcutaneously implanted in rats and pigs had shown that most of them were well tolerated and did not induce unfavourable reaction (Gould, 1976).

PVP solutions had been used as a plasma expander in the past (Gould, 1976). When injected intravenously PVP is non-toxic and non-thrombogenic. PVP has also been used extensively as a tablet binder, a tablet coating and for the solubilization and stabilization of drugs.

Recently, a number of phase transition systems have been tested for in-situ phase transition. Poloxamer 407 solution, a viscous solution at room temperature, transforms to a viscous gel once the temperature is raised to that of the eye (Zignani, 1995). Cellulose acetophthalate (CAP) latex, a solution at pH 4.5, gels if the pH is raised to pH 7.4
Although these systems seem to meet the requirement of gelation in situ, the necessity of a high polymer concentration (25% Poloxamer, 30% CAP) prevents them from actual application.

Gellan gum, a polysaccharide consisting of tetrasaccharide repeating units (Figure 2.5) (Nakajima, 1996), is believed to possess the required property of gelation while only a small amount of the polysaccharide is needed. It is known that gellan molecules, without the presence of cations, dissolve in water forming disordered coils at low concentration, while taking on double helical conformations and forming weak gels at high concentrations (Figure 2.6) (Nakajima, 1996). However, if moderate amounts of cations such as sodium (> 60 mM) are present, gellan molecules will form a much stronger gel with cation-mediated aggregates acting as cross-linking points (Nakajima, 1996). Another interesting feature of gellan gum is its sol-gel transition temperature ($T_m$). $T_m$ of gellan gum solution depends on the concentration of gellan gum, the concentration of cation and the average molecular weight of the gellan gum in use (Rozier, 1989, Zignani, 1995, Nakajima, 1996). Thus, it is necessary to characterize the $T_m$ of a new gellan gum supply before any further experiment is carried out.
2.3 The Eye

2.3.1 Anatomy of the Eye (Sebag, 1989, Edman, 1993)

The eye is divided into two sections, the anterior segment and the posterior segment. A cross-section through the eye is shown in Figure 2.7 (Vale and Cox, 1985). The anterior segment of the eye includes the cornea, conjunctiva, iris, ciliary body, anterior and posterior chambers, and the lens. The posterior segment of the eye refers to the parts of the eye which are situated behind the lens. The posterior segment includes the sclera, the choroid, the retina and the vitreous body. The vitreous body is surrounded by three layers of tissues. The sclera is the outermost layer, which surrounds the choroid, the retina and the vitreous body (Figure 2.7). The middle layer is the choroid and is highly vascularized. Blood vessels in the choroid provide most of the nutrition to the rest of the posterior segment. The retina is the innermost layer that surrounds the vitreous.
body, which consists of two major functional parts: the neural layer and the pigment epithelium. The pigment epithelium is located between the choroid and the retina. It forms a very tight barrier and selectively transfers nutrients to the retina from the choroid. The pigment epithelium also prevents the direct contact of blood with the rest of the retina. The remaining inner layers form the neural portion of the retina. Within these inner layers are retinal vessels that supply nutrients to the rest of the retina. These vessels have an endothelial layer of tightly joined cells which forms a second barrier between the blood and the retina. Lastly, the vitreous body is a transparent gel which holds the globe in shape and allows the unhindered transmission of light between the lens and the retina.

2.3.2 The Vitreous
The vitreous humour is a gel located between the retina and the lens. Its volume is about 4 ml in adult humans and makes up 80% of the volume of the globe. The water content of the vitreous is about 98 to 99.7% and the pH is about 7.5 (Edman, 1993). The rest of the vitreous is composed of collagen, hyaluronic acid, a high molecular weight polymer made of disaccharide units (Figure 2.8) and other constituents as shown in Table 2.2 (Sebag, 1992). Collagen and hyaluronic acid are the major structural molecules of the vitreous. These molecules form interpenetrating networks (Figure 2.9) (Sebag, 1992) and function as a structural support for the vitreous. The collagen fibrils provide a solid structure for the vitreous, and are "inflated" by the hydrophilic contribution of hyaluronic acid. The viscoelastic behaviour of the vitreous in vivo is not simply the arithmetic summation of the properties of these two molecular elements in independent forms. If the collagen is removed from the vitreous, the remaining hyaluronic acid will form a viscous solution. If hyaluronic acid is removed, the gel will shrink. It is believed that there is

Figure 2.8 Repeating unit of hyaluronic acid
strong interaction between the collagen and the hyaluronic acid that maintains the structure of the vitreous (Schepens, 1987, Sebag, 1989, Edman, 1993).

**Table 2.2 Chemical Composition of the human Vitreous**

<table>
<thead>
<tr>
<th>Inorganic Constituents (mmol/kg H₂O)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>137.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>3.8</td>
</tr>
<tr>
<td>Chloride</td>
<td>112.8</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>19.6 to 32.4 mEq/kg H₂O</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water and Organic Constituents (mg/100 ml H₂O)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>99,000</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>23.5</td>
</tr>
<tr>
<td>Protein</td>
<td>40</td>
</tr>
<tr>
<td>Glucose</td>
<td>30 to 70</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>70</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>7.3</td>
</tr>
<tr>
<td>Citrate</td>
<td>1.9</td>
</tr>
</tbody>
</table>
During childhood, there is significant growth of the vitreous. With aging, substantial rheologic, biochemical and structural alternations occur in the vitreous. One important aspect of such alternation is vitreous liquefaction. As shown in Figure 2.10 (Schepens, 1987, Sebag, 1989), liquid vitreous appears after the age of 4 and increases in volume steadily with the growth of the eye. By the time the human eye reaches adult size (age 14-18), liquid vitreous constitutes about 20% of the total volume (Schepens, 1987, Sebag, 1989). Volume of the gel vitreous remains steady until about the age of 40, when a continued increase in the volume of liquid vitreous results in a significant decrease in the volume of gel vitreous. By the age of 80 to 90, the volume of liquid vitreous surpasses that of gel vitreous (Schepens, 1987, Sebag, 1989). The central vitreous is the first region to undergo liquefaction. During liquefaction, macroscopic fiber formation at

Figure 2.10 Liquefaction of the human vitreous
Vitreous liquefaction is observed with transfer of HA from the gel vitreous to the liquid vitreous. This finding is consistent with the concept that vitreous liquefaction is associated with the redistribution of HA molecules and aggregation of collagen filaments into bundles of parallel fibrils seen as large fibers (Schepens, 1987, Peyman, 1994).

Researchers had suggested that the vitreous serves as a metabolic repository for the hyalocytes and neighboring tissues (Sebag, 1989). This suggestion is supported by the fact that substantial amount of galactose, glucose, mannose, fructose, and the hyaluronic acid precursors glucuronic acid and glucosamine were found in the vitreous. Further studies have shown that the vitreous may also serve as a repository for metabolic wastes, such as lactic acid (Schepens, 1987, Sebag, 1989). This property is critical for the surrounding tissues because high levels of this compound could be toxic.

The movement of solutes and solvents within the vitreous may also influence the metabolic activity of surrounding tissues. Early studies showed that 50% of water in the vitreous turned over every 10-15 minutes. Movement of molecules inside the vitreous is affected by diffusion, hydrostatic pressure, osmotic pressure, convection, and active transport by surrounding tissues (Sebag, 1989, Edman, 1993). Moreover, whereas both diffusion and bulk flow occur in the vitreous, diffusion is eight times greater than bulk flow for small molecules such as glucose and lactic acid (Sebag, 1989, Edman, 1993). Thus, small molecules diffuse because of concentration gradient while large molecules travel as a front with convective flow. However, researchers have found that once inside the vitreous, the diffusion of a large molecule is faster than would be expected in water, due to the “excluded-volume” effect (Schepens, 1987, Sebag, 1989). Because of the existence of the HA-collagen network, part of the vitreous is inaccessible to large molecules like albumin. As a result, the effective concentration of the solute is higher, resulting in a faster rate of diffusion. This effect is, of course, proportional to the network densities and may vary considerably during aging. Moreover, as over 98 wt% of the vitreous is water, the effect is significant with large molecules only, as small molecules can permeate through the HA-collagen network freely. Such physiologic properties within the vitreous are important for drug delivery to the posterior segment because the delivery pattern could be altered by changes in vitreous composition and/or structure.
2.3.3 Diseases of the Posterior Segment

Common diseases and disorders found in the eye are endophthalmitis, viral retinitis and proliferative vitreoretinopathy (PVR). Endophthalmitis is due to bacterial, fungal, or parasitic invasion of the eye. It is often associated with cataract extraction, filtering procedures, and traumatic eye injuries as a complication (Peyman, 1994). During the time from 1944 to 1955, the percentage of endophthalmitis occur after cataract operations was 0.08%, where there was no use of preoperative antibiotics. In the subsequent period from 1955 to 1968, the percentage was 0.12% with the use of topical preoperative antibiotics (Peyman, 1994). These findings indicate that the use of preoperative antibiotics has no positive effect on the prevalence of endophthalmitis after surgery. From the world literature prior to 1950, researchers found the rate of endophthalmitis was 1.14% after cataract operation. In the subsequent period from 1945 to 1960, the percentage was 0.21%. Although the infection percentage is small, the problem is that once it occurs, it’s extremely difficult to cure and often results in loss of vision.

Cytomegalovirus (CMV) is the most common cause of viral retinitis. Almost all patients with CMV infection have immunologic deficiencies (Peyman, 1994). As the number of acquired immunodeficiency syndrome (AIDS) patients spread to an epidemic scale, CMV retinitis has assumed an more important role in ophthalmologic practice. Sometimes, individuals with cancer or other debilitating diseases causing lowered defence to infection may be infected by CMV. It is estimated that 1.8% of AIDS patients have CMV retinitis as their first display of AIDS and eventually 15-40% of AIDS patients will have this disorder (Peyman, 1995).

Proliferative vitreoretinopathy (PVR) is a disease process which occurs as a complication of rhegmatogenous retinal detachment. Membranes characteristic to PVR are grown on both surfaces of the detached retina and on the posterior surface of the detached vitreous gel. Contraction of these membranes can cause severe failure in retinal reattachment surgery. Approximately 5-10% of all rhegmatogenous retinal detachments
27% of failures in retinal detachment surgery were caused by PVR (Peyman, 1994).

Ocular diseases may be developmental or inherited, including persistence of the hyloid vascular system, dominant exudative vitreoretinopathy, retinopathy of prematurity (ROP), Wagner’s disease and vitreoretinal dystrophy of Goldmann-Favre (Sebag, 1989). Connective-tissue disorders like diabetes mellitus, Marfan’s syndrome, Ehlers-Danlos syndrome and Stickler syndrome will also attack the vitreous and connective tissues elsewhere. Since the primary function of the vitreous is to transmit light, it must be mostly acellular to remain transparent. However, problems such as cell proliferation at the vitreoretinal interface, ocular trauma, severe chorioretinal or ciliary-body inflammation and neoplasia could invade the vitreous and alter the transparency of the vitreous. Other similar problems causing opaqueness or even blindness of the eye are vascular invasion and vitreous hemorrhage (Kanski, 1983, Schepens, 1987, Sebag, 1989, Peyman, 1994, Peyman, 1995).

2.3.4 Treatment of Posterior Segment Diseases

As many diseases and disorders occur in the posterior segment, systemic administration is ineffective because transport of drug from the bloodstream to the posterior segment of the eye is limited by the blood-ocular barrier in the retina. Other treatments such as eyedrop instillation and subconjunctival injections are ineffective because negligible amounts of drug can reach the vitreous humour. Consequently, most vitreous related illnesses are treated by intravitreal injection. There are many problems associated with intravitreal injection. Since the therapeutic window for some drugs is very narrow, only small doses of drugs could be injected before exceeding the toxicity level. As a result, frequent injections are required to maintain the drug concentration within the therapeutic range. As the number of injection increases, it increases the chance of complications such as risks of retinal detachment, lens damage and infection by other diseases. Moreover, the problem of patient compliance remains unsolved. The drug is usually prepared as an aqueous solution and injected into the vitreous by hand. The
bioavailability and possible toxicity (Lin, 1996, Friedrich, 1996). Nevertheless, it is the best form of drug administered clinically at the present time.

To overcome the problems with intravitreal injection, recent researchers have focused on developing better drug delivery systems. One possible way to achieve such a result is by altering the injection medium to extend the drug delivery period. As of today, the two most promising approaches under investigation are the encapsulation of drugs in liposomes made of lipids, and microspheres made of polymers (Khoobehi, 1988, Meisner, 1995, Peyman, 1994, Peyman, 1995, Zimmer, 1995). In most circumstances, experimental results showed that the drug’s toxicity to other uninfected sites was reduced and the clearance time was extended. Nevertheless, these formulations could spread diffusely within the vitreous cavity and cause cloudiness, block the patient’s optic path and interfere with the ophthalmologist’s ability to examine the eye until complete resorption of the formulation after administration. The drug delivery period for these systems was about one month.

Due to difficulties encountered when working with intravitreal injection, investigators have developed another class of drug delivery systems that does not require intravitreal injection. Devices similar to the one shown in Figure 2.11 (Peyman, 1995) have been investigated for implantation in the eye as sustained-release drug delivery systems. It was prepared by coating a 6 mg pellet of ganciclovir (a drug for the treatment of CMV retinitis) with PVA and ethylene vinyl acetate. Prolonged release of ganciclovir up to 4-5 months after implantation was shown with implants sewn into the pars plana. However, several complications such as vitreous hemorrhage, astigmatism, and suprachoroidal placement of the device have been observed. In addition, surgery is required to remove or replace the device after drug depletion, which could cause further complications.

To avoid the necessity of repeated surgery, different investigators have reported the development of various types of biodegradable carriers, including drug matrices and porous reservoir systems. A type of drug matrix is prepared by combining high- and low-molecular weight poly-(caprolactone) (PCL) (Peyman, 1994, Peyman, 1995). Different
types of drugs can be placed within the matrix through solution casting and melting. In an experiment with 5-FU (a drug for the treatment of PVR), results from a rabbit model has shown substantial improvement of PVR suppression with PCL carrier as compared to free drug application. Another type of drug matrix is the scleral plug, which consists of poly(lactic-glycolic) acid and contains 1% doxorubicin (Peyman, 1994, Peyman, 1995). The copolymer matrix was shown to be completely metabolized in the rabbit eye without any adverse or toxic reactions. Apart from drug matrix, researchers have used PCL to prepare porous drug reservoir implants. Pores were created by first mixing non-toxic water-soluble inorganic salts such as NaCl or KCl with various composites of PCL. Experimental drugs were then loaded into the devices. The composites were then placed in water to dissolve the salts, leaving pores in the PCL device. Experiments showed a constant rate of release with zero-order kinetics, and the release lasted for over a year (Peyman, 1994, 1995).

Figure 2.11 Schematic drawing of ganciclovir delivery device in the vitreal cavity
3.1 Rationale

3.1.1 General Objective

The focus of this research is to develop a drug reservoir for sustained drug delivery. The idea is to disperse liposomes within hydrogels that could either be implanted into the body through surgery or injected directly to the target site and gel inside the site without the necessity of implantation. The device was proposed as a means of extending the delivery period of a drug in the body in a controllable fashion. Liposomes are membrane vesicles capable of encapsulating drugs. They are known to possess responsive drug release characteristics. Hydrogels are hydrophilic networks that allow fast diffusion of small molecules, and can entrap large particles such as liposomes within their networks. As an illustration of the idea of a gellable solution for injection, the vitreous was chosen as a model target site.

3.1.2 Rationale for Material Selection

The reasons for selecting MLV liposomes are two-fold. First, it is the easiest type of liposome to be fabricated. As mentioned in the previous chapter, the preparation of SUV or LUV liposomes require special techniques not readily available in the laboratory. Second, it has the highest lipophilic solute encapsulation capacity among the three types of liposomes. As many drugs are lipophilic, MLV liposomes can store more of them than others with the same volume.

Three criteria were used for the selection of a suitable phospholipid. First, the gel to liquid-crystalline phase transition temperature $T_m$ should be close to body temperature (37°C). Second, the fatty acid chain length of the phospholipid should be as long as possible. It is known that the longer the chain length, the better the lipid bilayer can prevent content leakage. Also, the ratio of hydrophobic region to hydrophilic region is
The storage of lipophilic drug. Third, the pKa of the phospholipid should be well below a certain pH depending on the application site. The best choice is to have a pKa as low as possible. Based on the selection criteria mentioned above, 1,2-dipentadecanoyl-sn-glycero-3-phosphocholine and 1,2 dihexadecanoyl-sn-glycero-3-phosphocholine are chosen as the building blocks for the liposomes.

The selection of hydrogel for implantation, or solid insertion, was based on several criteria. First, the hydrogel should be insensitive to temperature and pH variations. Second, the hydrogel should be neutral to minimize its effect on the stability of the liposomes. Third, the hydrogel should be durable, while maintaining a high percentage of water inside the network. Fourth, the hydrogel should be easy to fabricate at mild conditions to prevent destruction of the liposomes. Fifth, the hydrogel should have well-documented biocompatibility. Based on the above selection criteria, PVA was chosen due to its ease of fabrication while satisfying all the requirements. The study of Peppas et al. (1983) showed that the cross-linked structure of swollen PVA acts as a molecular screen for the diffusion of large solutes. They also established that the mesh size of the cross-linked polymer network affects the rate of diffusion: with decreasing mesh size, the solute diffusion was slowed or even stopped, depending on the size of the solute. Furthermore, the high content of hydroxyl groups of PVA acts as a co-surfactant to stabilize the liposomes in dispersion.

Three different methods can be used to produce PVA hydrogels: chemical-crosslinking, radiation crosslinking, and physical crosslinking by the repeated freeze-thaw cycles. Chemical-crosslinking was selected in the present study. Radiation crosslinking was not used because radiation could increase the rate of degradation of the phospholipid. The requirement for low temperature (-20°C) in the freeze-thaw method could destroy the liposomes.

To avoid the necessity of surgery, injection is the only fast and easy alternative for direct drug delivery to the target site. As mentioned in the previous chapter, different approaches have been considered to improve the efficacy of a drug at the infected site. Whereas examples such as liposomes and microspheres have shown improvements in
be an issue for further application. Hence, it would be desirable if the injection medium could gel inside the target site and trap all the liposomes or microspheres within the gel. Moreover, such injections could give a better controlled release profile at the target site since the shape and release rate of the drug in the injection medium can be predicted beforehand. Gellan gum was chosen as the gelling medium because of its low requirements in gum and cation concentration.

As drugs have a wide range of properties, it is necessary to employ a wide variety of solutes to test the limitations of a newly developed drug delivery system. Solutes of a wide range of molecular weight and octanol-water partition coefficient were selected. Octanol-water partition coefficient is defined as the ratio of the equilibrium concentration which the chemical adopts in octanol to that in water (Mackay, 1991). Table 3.1 shows the solutes used in this project and their molecular weight, octanol-water partition coefficient, $K_{\text{ow}}$, and solubility in water (Schoenwald, 1983, Wang, 1991, Pimsuwan, 1995, Herbert, 1995).

Table 3.1 Physical data of selected solutes

<table>
<thead>
<tr>
<th>Name of solute</th>
<th>Molecular weight (Daltons)</th>
<th>$K_{\text{ow}}$</th>
<th>Water solubility (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>192.6</td>
<td>1.02</td>
<td>21.7</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>1355.4</td>
<td>&lt; 1</td>
<td>12.5</td>
</tr>
<tr>
<td>Benzamide</td>
<td>107.15</td>
<td>6.92</td>
<td>13.5</td>
</tr>
<tr>
<td>m-cresol</td>
<td>110.07</td>
<td>91.2</td>
<td>23.0</td>
</tr>
<tr>
<td>p-Chlorophenol</td>
<td>128.56</td>
<td>245.5</td>
<td>27.0</td>
</tr>
<tr>
<td>Propranolol(\text{HCl})</td>
<td>295.8</td>
<td>1621.8</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Although the molecular weight of a solute is readily available, it cannot be directly compared to hydrogel mesh size in diffusional consideration. The Stokes hydrodynamic radius ($r_s$) provide a better measurement of diffusional size. For reference, Table 3.2 shows $r_s$ for a number of solutes with different molecular weights (Peppas &
Table 3.2: Molecular weight and Stokes hydrodynamic radius of various solutes

<table>
<thead>
<tr>
<th>Solute</th>
<th>Molecular weight (Daltons)</th>
<th>$r_s$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Chloride</td>
<td>58.44</td>
<td>2.4</td>
</tr>
<tr>
<td>Urea</td>
<td>60.06</td>
<td>2.5</td>
</tr>
<tr>
<td>Creatinine</td>
<td>113.12</td>
<td>3.1</td>
</tr>
<tr>
<td>Uric acid</td>
<td>168.11</td>
<td>3.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>342.3</td>
<td>4.7</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>1355.4</td>
<td>8.5</td>
</tr>
</tbody>
</table>

### 3.2 Scope of Thesis

This research is divided into five sections. The first section describes the synthesis and characterization of PVA hydrogel. The second section is about release studies of solutes with different lipophilicity and molecular weight from PVA or PVA-liposome hydrogels. The third section presents the characterization of the phase transition temperature of gellan gum. The fourth section is about release studies of solutes from gellan gum solution, aqueous solution, liposome solution or gellan gum and liposome mixture. The last section illustrates the dispersion shape of gellan gum and/or liposome solution in model solutions simulating the vitreous as an example for in-situ gelation.
Liposomes possess physical properties suitable for drug encapsulation. However, they are not stable enough as an in vivo drug carrier on their own. To improve stability, a possible solution is to load liposomes within a vehicle for local drug delivery. In this chapter, the procedures for the synthesis and characterization of PVA hydrogel as a liposome carrier for implantation will be described. PVA was selected as a liposome carrier because of its inertness to changes in physiological conditions and its low toxicity (Takamura, 1992). Synthesis and characterization of PVA hydrogel are described in Section 4.1. Section 4.2 describes the preparation of PVA hydrogels containing liposomes. Section 4.3 explains how the release studies of different solutes from the PVA hydrogels and PVA-liposome hydrogels were conducted.

Gellan gum was also considered as a liposome carrier for injection to gel within the injected site. Gellan gum was chosen because of its low concentration requirement to induce gelation. Section 4.4 shows how the phase transition temperature of gellan gum is characterized. Release studies of propranolol•HCl from different gellan gum/liposome mixtures are described in Section 4.5. A simulation of intravitreal injection with gellan gum and liposomes as injected solutions is discussed in Section 4.6.

### 4.1 Synthesis and Characterization of PVA Hydrogel

#### 4.1.1 Materials

PVA (99+% hydrolyzed, m.w. = 124,000 ~186,000 g/mol), glutaric dialdehyde (50 wt% aqueous solution) and cyclohexane (99% purity) were purchased from Sigma Chemical Co. They were used without further purification.
4.1.2 Synthesis

A stock solution of 8 wt% PVA solution was prepared by mixing 8 g of PVA with 92 g of deionized water. The mixture was heated at 95°C for 8h. To 0.48 g of the 8 wt% PVA solution, 0.42 g of water and 0.035 g of 2M hydrochloric acid were added. pH of the mixture was 2.5. The 50 wt% glutaric dialdehyde was diluted to 10 wt% with deionized water for further use. 0.035 g of the 10 wt% glutaric dialdehyde aqueous solution was added as the cross-linking agent. The final cross-linking ratio, as mol glutaric dialdehyde/mol PVA repeating unit was 0.04. The cross-linking reaction was carried out in 5 ml test tubes at 30°C for 24h. Both inter- and intra- molecular acetalization reactions are possible (Figure 4.1) (Jacobs, 1993). After the reaction,

![Chemical structures](image)

Figure 4.1 PVA cross-linked by formation of acetal linkages
for two days. They were then equilibrated in 0.01M pH 3 citric buffer solution, 0.01M pH 7.4 phosphate buffer solution or deionized water for 7 days at 30°C, 37°C or 42°C.

4.1.3 Characterization

The weight percentage and mesh size of cross-linked PVA hydrogels were determined. Weight percentage was determined gravimetrically. Hydrogels pre-equilibrated at various temperatures were weighed in air to determine the swollen weight, \( M_{A,S} \). Then they were dried at 90°C for 3 days to constant weight, \( M_D \). All samples were blotted gently to remove any excess water on the surface before weighing. The weight percentage was then calculated with Equation (1) in Section 2.2.1.

Swelling studies were performed at 37°C using the buoyancy method to determine the hydrogel mesh size. Hydrogel samples pre-equilibrated at 37°C were blotted gently. They were then placed in cyclohexane to obtain the weight of the hydrogel in cyclohexane, \( M_{C,S} \), by measuring the weight of cyclohexane displaced. The polymer volume fraction after swelling was calculated with Equation (2) in Section 2.2.1. The density of PVA is assumed as the polymer bulk density. \( M_D \) and \( M_{A,S} \) used in Equation (2) were the same as those determined in the weight percentage calculation. \( \rho_{2,3} \) is then substituted into equation (3) in Section 2.2.1 for the determination of mesh size \( \zeta \). As stated in Chapter 2, equation (3) is valid at 37°C only.

4.2 Synthesis and Characterization of PVA Hydrogels Containing Liposomes Dispersion

4.2.1 Materials

In addition to the materials listed in 4.1.1, 1,2-dipentadecanoyl-sn-glycero-3-phosphocholine (DPPC) (m.w. = 706 g/mol, \( T_m = 33.9°C \) (99.9 wt% purity), 1,2-
(99.9 wt% purity), Oil Red O and chloroform of reagent grade were also purchased from Sigma Chemical Co. All were used without further purification.

4.2.2 Synthesis

As previously documented (Ostro, 1983, Machy, 1987, Gregoriadis, 1993), synthetic multilamellar liposomes (MLV) were first formed and described by Bangham et al. (1965). The procedure reported by Bangham et al. has not been changed since it was reported and remains the simplest method for the preparation of liposomes (Machy, 1987). This procedure was followed in this project. 0.042 g of phospholipid was dissolved in 2 ml of chloroform. It was heated above T_m and the chloroform was evaporated to form a thin film of dry lipid on the wall of a 5 ml test tube. To this thin film of lipid, 0.38 g of deionized water was added and heated above T_m of the phospholipid for 24h with occasional vortexing, producing MLV liposomes.

To produce a PVA-liposome hydrogel, 0.48 g of 8 wt% PVA solution and 0.035 g 2M HCl were added to the MLV liposome solution and vortexed vigorously for 3 minutes. Following that, 0.035 g of 10 wt% glutaric dialdehyde aqueous solution was added to the solution and vortexed vigorously for another 3 minutes. As the initial wt% of phospholipid was relatively low (4.3 wt%), the procedure should produce PVA-liposome hydrogels with similar swelling properties as in Section 4.1.2.

4.2.3 Characterization of PVA-liposome hydrogel with differential scanning calorimetry (DSC) and light microscopy

DSC was used to examine the effect of PVA hydrogel synthesis on the phase transition temperature of the liposomes. DSC measurements were carried out with Perkin Elmer DSC-2C. The heating rate was set at 0.5°C/min. Two samples were being tested. The first sample was a PVA-liposome hydrogel with 15wt% of liposomes. The second sample was a 15wt% liposome solution.
To determine if liposomes exist in PVA hydrogel, pictures of liposomes stained with Oil Red O and dispersed in PVA hydrogel were taken with a light microscope at 400X magnification. The wt% of liposomes in the hydrogel was 2%.

4.3 Solute Release studies from PVA Hydrogels and PVA/Liposome Composites

4.3.1 Materials

Caffeine, vitamin B₁₂, benzamide, 4-chlorophenol, m-cresol and propranolol•HCl, all used as solute for release study, were purchased from Sigma Chemical Co. Each solute was selected according to their molecular weight and lipophilicity (Schoenwald, 1983, Windholz, 1983, Wang, 1991, Pinsuwan, 1995, Herbert, 1995).

4.3.2 Procedure

All individual solute solutions except propranolol•HCl were prepared with deionized water. Solute concentrations ranged from 1000 to 5000 ppm. Propranolol•HCl was dissolved in NaOH solution at pH 9.3 so the major species in the solution would be in non-ionized form to increase its lipophilicity. To load solute into the hydrogels, a cylindrical PVA hydrogel or PVA-liposome hydrogel with diameter = 0.5±0.02 cm, length = 1.1±0.1 cm and mass = 0.30±0.03 g was placed in 20 ml of solute solution for at least 1 week at room temperature to reach solute equilibrium.
After solute imbibition, each solute loaded hydrogel was hung from a needle and placed at the center of a 250 ml dissolution vessel. The hydrogel was fully submerged in 200 ml of release solution (Figure 4.2). The release solution used was 0.01M pH 7.4 phosphate buffer solution with 0.02 wt% sodium azide as a bacteriostatic agent. The release solution was stirred at 300 rpm and the dissolution vessel was placed in a temperature-controlled water bath. The temperature of each water bath was maintained at 30°C, 37°C or 42°C respectively.

At each sampling period, 5 ml of the release solution was removed and replaced with 5 ml of fresh solution. The solute concentration of the release solution was measured using UV absorption at appropriate wavelengths. The total amount of solute loaded was calculated from the accumulated amount of solute released after at least 100 h. DSC was used to examine the effect of propranolol, the most lipophilic solute in this study, on the phase transition temperature of the liposomes. A sample with 15wt% liposomes and 1wt% propranolol in deionized water was tested and compared with the results from Section 4.2.3.

4.4 Transition temperature characterization of Gellan Gum

4.4.1 Materials

Gellan gum, under the trademark name Phytagele™, was purchased from Sigma Chemical Co., and used without purification.
4.4.2 Procedure

To investigate the effect of sodium ion concentration on the transition temperature at which gellan gum solutions turn from liquid to gel, an array of mixtures with different gellan gum and sodium chloride concentrations were made. Gellan gum concentrations ranging from 0.2-0.8 wt% and sodium chloride concentrations ranging from 0-105 mM were used. All solutions were made with deionized water. Each mixture (13-15 g) was placed in a 20 ml vial, sealed and heated at 95°C in a 1000 ml beaker with water for 10 minutes or until a clear solution was formed. Subsequently, the mixture was cooled to 0°C for gelation to occur. After gelation, the temperature of the gel-filled vial was raised at a rate of 1°C per minute in a 1000 ml beaker with water. As the temperature approached the transition temperature, the heating rate was decreased to 0.33°C per minute.

A thermometer was placed at the center of the vial to determine the temperature difference between the center of the gel and the bath temperature during the heating process.

4.5 Release Studies of Propranolol from Gellan Gum/Liposome Mixtures

4.5.1 Materials

Dialysis membranes with a molecular weight cut-off of approximately 10,000 Daltons were purchased from Fisher Scientific, Toronto, Canada. All other chemicals were listed in previous sections.

4.5.2 Procedure
1 wt% gellan gum in deionized water. The mixture was heated at 95°C for 10 minutes. After dissolution, a known amount of solid propranolol•HCl was added to the solution and stirred vigorously for at least 1h. To produce a mixture with liposomes, a 0.9 wt% gellan gum solution was added to a known amount of solute-containing liposome solution to give a final 15 wt% liposome concentration.

After the mixing period, propranolol•HCl loaded solutions, which can be water, gellan gum solution, liposomes or gellan gum plus liposomes solution, were injected into dialysis membranes and sealed with clamps. These membranes were then placed in 200 ml of 130 mM sodium chloride solution and stirred at 300 rpm in a water bath. A sodium ion concentration of 130 mM was chosen to simulate the vitreous. The temperature was maintained at 37°C or 42°C. At each sampling period, 5 ml of the release solution was removed and replaced with 5 ml of fresh solution. The propranolol concentration of the release solution was measured using UV absorption at 290 nm.

4.6 Vitreous body and drug dispersion simulation

The objective of these experiments is to show how the gellan gum solution could form a sphere, seal the liposome solution inside the sphere, and prevent it from leakage or external contact. Hyaluronic acid solution is the ideal model system for vitreous body simulation; however, due to its high cost, PVA solutions were chosen as model systems. Fluorescein was chosen as a model solute to facilitate visualization.

4.6.1 Materials

PVA, gellan gum and sodium chloride were the same as described previously. Fluorescein was purchased from Sigma Chemical Co., and used without further purification.

4.6.2 Procedure
4.6.2.1 PVA solutions

To show how gellan gum prevents the leakage of liposomes in the “vitreous”, three PVA solutions with different viscosities were prepared. Briefly, suitable amounts of PVA powder were added to 10 ml Florence flasks with 0.8 wt% sodium chloride solution to give a final weight percentage of 13%, 17% and 23% respectively. The mixtures were sealed and heated to 105°C for 24 hours before being cooled to room temperature. The viscosities of the solutions, determined using a rotational viscometer (model ERV 8 from Viscometer UK Ltd.), were 40000 cP, 75000 cP and 140000 cP, respectively. All viscosities were determined with spindle 7, which has a measuring range of 1000 cP to 1000 kCP, a shear rate of 0.5 rpm and at room temperature.

4.6.2.2 Injected Solutions

Four injection media were prepared. The first was a 1500 ppm (by mass) fluorescein solution dissolved in deionized water. The second was a 1500 ppm fluorescein solution with 0.6 wt% gellan gum dissolved in deionized water. The third was a 15 wt% liposome solution in deionized water. The fourth consisted of two layers. The upper layer was a 15 wt% liposome solution in deionized water. The lower layer was a 0.6 wt% gellan gum dissolved in deionized water.

4.6.2.3 Simulation and Visualization of Intravitreal Injections

Injection speed, needle size, needle shape and viscosity of the drug carriers are the four most important factors which affect the early stage drug distribution in the vitreous body. Since the focus of this work is to examine the effect of gellan gum on liposome distribution, injection speed, needle size and shape were held constant. A syringe pump (SAGE INSTRUMENT MODEL 341B) was used to control the injection speed. The operating speed was set at 0.1 ml/min. Each injection lasted for 0.5 minutes with a total
injection of 0.05 ml per sample. Hand injection was used to examine the possibility of injecting a two phase solution with 0.6 wt% gellan gum solution as the bottom phase and 15 wt% liposomes solution as the top phase. A 30G needle, typically used in intravitreal injection, was used for these experiments.

The experimental setup to take dispersion pattern pictures consists of seven components. A light source, a diffuser, a sample-holding stand, a 10 ml Florence flask, a special syringe described below, a syringe pump and a camera. A simplified diagram is shown in Figure 4.4. The light source, a 150 W GE lamp, was used in combination with a diffuser to provide a stable white light background. The flask was filled with simulated vitreous and held upright with the sample-holding stand. The special syringe was made by connecting two syringes with a plastic tube (1 mm inside diameter). One side of the syringe was inserted vertically into the flask and the other side connected to a syringe pump. Pictures were taken accordingly with the camera.

![Diagram of experimental setup for intravitreal injection simulation](image)

Figure 4.3 Experimental setup for intravitreal injection simulation
5.1 Characterization of PVA Hydrogels

5.1.1 Swelling Ratio

PVA hydrogels were prepared using PVA of molecular weight 124,000 to 186,000 Daltons dissolved to 4.0 wt% in water, and crosslinked with glutaric dialdehyde as the crosslinking agent and HCl as the catalyst. The water content of PVA hydrogel after crosslinking, as prepared by the methods outlined in 4.1.1, was determined as described in 4.1.2, and found to be:

Table 5.1 Weight percentage of water in PVA hydrogels at different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Wt % of water ± sample SD (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30°C</td>
<td>84.1 ± 0.2%</td>
</tr>
<tr>
<td>37°C</td>
<td>83.1 ± 0.2%</td>
</tr>
<tr>
<td>42°C</td>
<td>83.0 ± 0.2%</td>
</tr>
</tbody>
</table>

All samples were tested in 0.01 M pH 7.4 phosphate buffer solution. Table 5.2 summarizes results from the experiment of water content’s sensitivity to pH changes. All samples were tested at 37°C.

Table 5.2 Weight percentage of water in PVA hydrogel at different pH

<table>
<thead>
<tr>
<th>pH</th>
<th>Weight percentage of water ± SD (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>83.1 ± 0.1 %</td>
</tr>
<tr>
<td>7.4</td>
<td>83.1 ± 0.2 %</td>
</tr>
<tr>
<td>deionized water (pH 6.1)</td>
<td>83.1 ± 0.1 %</td>
</tr>
</tbody>
</table>

As seen from Table 5.1, PVA hydrogel water content at 30°C was only slightly different from the other two temperatures. Since PVA is highly soluble in water, its difference in solubility over a narrow temperature range (30°C-42°C) is too small to have any large effect on the water content of its hydrogel.
within the range tested. As PVA contains hydroxyl groups with a pKa higher than 9, the OH groups should remain un-ionized at any pH below 9.

Although the sensitivity of PVA hydrogel water content to ionic strength was not tested, it is believed that water content is insensitive to ionic strength variation because PVA hydrogel is virtually an un-ionizable hydrogel at normal conditions (i.e. physiological conditions). Previous work (Gould, 1976) has shown that only ionizable hydrogels are sensitive to ionic strength changes in the swelling medium.

Since PVA is just a mechanical scaffold to hold liposomes for drug delivery, it should be insensitive to any external medium variation. Any sensitivity could change the diffusive characteristics of the hydrogel and in turn, affect the release profile of the drug. Therefore, the insensitivity of PVA to variation in temperature, pH and ionic strength makes it a suitable inert scaffold for the present study.

5.1.2 Mesh Size

Using the methods described in Section 4.1.2, it was found that the average mesh size of PVA hydrogels at 37°C is 32.3 ± 2.1 Å. From the work of Burczak et al. (1994), the anticipated mesh size of PVA hydrogel with initial concentration of 4 wt% PVA and 0.04 GA concentration (mol/mol) should be less than 100 Å. Similarly, Canal et al. (1989) had shown that a PVA hydrogel with glutaric dialdehyde concentration of 0.04 mol GA/mol PVA repeating unit have a mesh size between 37-63 Å, which is close to the present finding. From Section 5.1.1, it was shown that PVA hydrogel water content is insensitive to variations in temperature and pH. As mesh size is related to water content, it should also be insensitive to variation in temperature and pH.

As mentioned in Section 2.3.1, mesh size is an important parameter because it determines the maximum size of solute that could diffuse into or out of a hydrogel. Moreover, it determines the minimum size of a solute that could be entrapped inside a hydrogel without leakage. Since the drug diffusion coefficient through hydrogels depends on the mesh size (Reinhart, 1983), a consistent mesh size is important for control
over drug transport. There were two reasons for producing a network with such a mesh size. First, hydrogels with higher water content, i.e., larger mesh size, were not mechanically strong enough for repetitive experiments. Second, hydrogels with larger mesh size are leakier than smaller ones. Leakage of liposomes from hydrogels was observed in hydrogels with a high water content (> 90 wt%) in preliminary studies.

It had been demonstrated by Reinhart et al. (1983) that with a mesh size of 38 Å, the diffusion of albumin (r_s = 22.5 Å) was significantly retarded. The major drawback of having a small mesh size is that it could become another limiting barrier for any solute to diffuse out from the hydrogel, and decreases the responsiveness of PVA-liposome hybrid drug delivery system to temperature variation. However, with a mesh size of 32 Å, relatively large molecules such as Vitamin B_{12} (MW = 1355.4 g/mol, r_s = 8.5 Å) can still diffuse freely through the hydrogel while liposomes with the smallest diameter (100–200 Å) are entrapped inside the hydrogel.

5.2 Effect of PVA and propranolol on phase transition temperature of liposomes

Figure 5.1 shows the picture of liposomes dispersed in PVA hydrogel. Each square represents 1 μm². This picture verifies the hypothesis that liposomes, not individual phospholipids, exist within the hydrogel. Results from DSC experiments show that the presence of PVA and propranolol had no effect on the position of the phase transition temperature of the liposomes. T_m remains between 41.5–42°C. Such result is in accord with the conclusion from Figure 5.1. Any destabilization of liposomes should accompany a shift in the position of the phase transition temperature. As PVA is a hydrophilic polymer, the major interaction between PVA and liposomes is H-bonding. Such bonding is between the hydroxyl groups of PVA and the polar head groups on the surface of the liposomes. Consequently, such interaction has little effect on T_m of MLV liposomes, which are composed of many bilayers.

New et al. (1990) had shown that at low concentrations of cholesterol (20 mol% or less) incorporated into liposomes, it has little effect on the position of T_m. In the
New et al. (1990) had shown that at low concentrations of cholesterol (20 mol% or less) incorporated into liposomes, it has little effect on the position of $T_m$. In the present study, the mol% of propranolol incorporated was 15%. As propranolol is a lipophilic solute, most of it will reside within the bilayer membrane of liposomes. Although it is clear that propranolol interacts with liposomes, 15 mol% propranolol was not concentrated enough to affect $T_m$. 

**Figure 5.1** Liposomes dispersed in PVA hydrogel
5.3.1 Solute Release from PVA

In this experiment, six solutes were used. All solutes are listed in Table 5.3 with their molecular weights and estimated $r_s$.

Table 5.3 Molecular weight and estimated $r_s$ of various solutes

<table>
<thead>
<tr>
<th>Name of solute</th>
<th>Molecular weight (Daltons)</th>
<th>$r_s$ (Å, estimated value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzamide</td>
<td>107.15</td>
<td>3.1</td>
</tr>
<tr>
<td>m-cresol</td>
<td>110.07</td>
<td>3.1</td>
</tr>
<tr>
<td>p-Chlorophenol</td>
<td>128.56</td>
<td>3.2</td>
</tr>
<tr>
<td>Caffeine</td>
<td>192.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Propranolol•HCl</td>
<td>295.8</td>
<td>4.5</td>
</tr>
<tr>
<td>Vitamin B$_{12}$</td>
<td>1355.4</td>
<td>8.5</td>
</tr>
</tbody>
</table>

5.3.1.1 Effect of Temperature

Figures 5.2, 5.3, 5.4, 5.5 and 5.6 show the release profiles at 37 and 42°C for benzamide, m-cresol, p-chlorophenol, propranolol•HCl and Vitamin B$_{12}$ from PVA hydrogels respectively. Figure 5.7 shows the release profile of caffeine from PVA hydrogels at 30 and 35°C.

As expected from the discussion in Section 5.1, the release rate of various solutes from the PVA hydrogels was not affected by a small temperature difference. This result is in agreement with earlier results that PVA water content and mesh size are
Benzamide Release from PVA hydrogels at 37 & 42°C

Propanolol Release from PVA hydrogels at 37 & 42°C

m-cresol Release from PVA hydrogels at 37 & 42°C

Vitamin B12 Release from PVA hydrogels at 37 & 42°C

p-chlorophenol Release from PVA hydrogels at 37 & 42°C

Caffeine release from PVA hydrogel at 30 & 35°C
5.3.1.2 Effect of Solute Size

Figures 5.8 and 5.9 show the release profile of various solutes at 37 and 42°C respectively. Results from both temperatures show that solute size had no effect on the release rate. This result contradicts the fact that the release profile of solutes should depend on their size. However, as the difference between the diffusion coefficients of the solutes (Colton, 1971) is not large, errors in measurements could be larger than the difference in release rate.

5.3.2 Solute Release from PVA-Liposome Systems Compared to PVA Systems

Figure 5.10a shows the release profile of caffeine from PVA and PVA-liposome hydrogels at 30°C. Figure 5.10b is the release profile at 35°C. Liposomes were prepared using DPPC, a phospholipid with T_m = 33.9°C. Other relevant data are included in the figures.

Figures 5.11a, 5.12a, 5.13a, 5.14a and 5.15a are the release profiles of vitamin B_{12}, benzamide, p-chlorophenol, m-cresol and propranolol at 37°C respectively. Figures 5.11b, 5.12b, 5.13b, 5.14b and 5.15b are the release profiles of vitamin B_{12}, benzamide, p-chlorophenol, m-cresol and propranolol at 42°C respectively. Liposomes were prepared using DHPC, a phospholipid with T_m = 41.9°C. Other relevant data are included in the figures.

As Figures 5.10a and 5.10b show, the presence of liposomes had no effect on caffeine’s release rate from the PVA hydrogels. It is believed that as caffeine is a hydrophilic compound, most of the solute loaded in the PVA-liposome hydrogel resides in the hydrophilic phase (>90%) and to a much lesser extent, inside the liposomes. Consequently, caffeine release rates from PVA and PVA-liposome hydrogels are comparable.
Different solutes release from PVA hydrogels at 37°C

Different solutes release from PVA hydrogels at 42°C
Caffeine release from PVA hydrogels with or without liposomes at 30°C

Figure 5.19

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>0.44</td>
<td>0.44</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Caffeine release from PVA hydrogels with or without liposomes at 35°C

Figure 5.10b

Vitamin B12 release from PVA hydrogel with or without liposomes at 37°C

Figure 5.11a

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12</td>
<td>0.52</td>
<td>0.51</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Vitamin B12 release from PVA hydrogel with or without liposomes at 42°C

Figure 5.11b

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12</td>
<td>0.5</td>
<td>0.51</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>9.4</td>
</tr>
</tbody>
</table>
Figures 5.12a & b, 5.13a & b, 5.14a & b and 5.15a & b show that the release rate of lipophilic solutes from the PVA hydrogels were affected by the presence of liposomes. It is believed that most lipophilic solutes were encapsulated within the bilayer membrane of liposomes. Therefore, the permeation rate of the solutes from the liposomes becomes a limiting step for the release of the solutes from the hydrogels.

Figures 5.11a and 5.11b show that for vitamin B₁₂, the presence of liposomes affected its release rate from the PVA hydrogels. As vitamin B₁₂ is a hydrophilic solute, most of the solute should reside in the hydrophilic phase of the PVA-liposome hydrogel. It is hypothesized that paths inaccessible to vitamin B₁₂ but available to caffeine were formed within gaps between the liposomes and the PVA hydrogel. As a result, the release rate of vitamin B₁₂ from PVA-liposome hydrogel was decreased significantly as compared to that of PVA hydrogel.

5.3.3 Effect of Temperature on Solute Release from PVA-Liposome Systems

Figure 5.16 shows the release profiles of caffeine from PVA-liposome hydrogels at 30°C and 35°C. Figures 5.17, 5.18, 5.19, 5.20 and 5.21 are the release profiles at 37°C and 42°C for vitamin B₁₂, benzamide, m-cresol, p-chlorophenol and propranolol·HCl respectively. Other relevant data are included in the figures.

As seen from Figures 5.16 and 5.17, for hydrophilic molecules, temperature variation had no effect on the release rate of these solutes. Results from Figure 5.18 indicate that for benzamide, a slightly lipophilic solute (Kₗₒₕ = 6.92), the release rate from the PVA-liposome hydrogel is more dependent on temperature than the hydrophilic solutes. A possible reason is that more benzamide was loaded inside the liposomes than the hydrophilic solutes. Similar situations are observed for m-cresol and p-chlorophenol (Figures 5.19 and 5.20 respectively), solutes more lipophilic (Kₗₒₕ = 91.2 and 245.5 respectively) than benzamide. The dependence of solute release rate from PVA-liposome hydrogel on temperature is most apparent with propranolol (Figure 5.21), a solute larger and more lipophilic (Kₗₒₕ = 1621.8) than other lipophilic solutes.
Benzamide release from PVA hydrogels with or without liposomes at 37°C

![Graph](image1)

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzamide</td>
<td>1.17</td>
<td>0.82</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Benzamide release from PVA hydrogels with or without liposomes at 42°C

![Graph](image2)

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzamide</td>
<td>1.17</td>
<td>0.83</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>13.8</td>
</tr>
</tbody>
</table>

Chlorophenol release from PVA hydrogels with or without liposomes at 37°C

![Graph](image3)

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophenol</td>
<td>0.75</td>
<td>1.03</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Chlorophenol release from PVA hydrogels with or without liposomes at 42°C

![Graph](image4)

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophenol</td>
<td>0.76</td>
<td>1.06</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>14.1</td>
</tr>
</tbody>
</table>
m-cresol release from PVA hydrogels with or without liposomes at 37°C

Figure 5.14a

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-cresol</td>
<td>1.15</td>
<td>1.01</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>13.7</td>
</tr>
</tbody>
</table>

Figure 5.14b

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-cresol</td>
<td>1.12</td>
<td>1.00</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>14.0</td>
</tr>
</tbody>
</table>

Propranolol release from PVA hydrogels with or without liposomes at 37°C

Figure 5.15a

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>propranolol</td>
<td>0.86</td>
<td>1.11</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Figure 5.15b

<table>
<thead>
<tr>
<th>Wt%</th>
<th>PVA</th>
<th>PVA-lipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>propranolol</td>
<td>0.88</td>
<td>1.11</td>
</tr>
<tr>
<td>DPPC</td>
<td>0</td>
<td>14.3</td>
</tr>
</tbody>
</table>
Caffeine release from PVA-liposome hydrogels at 30 & 35°C

Vitamin B12 release from PVA-liposome hydrogels at 30°C, 37°C & 42°C

Benzamide release from PVA-liposome hydrogels at 37°C & 42°C

<table>
<thead>
<tr>
<th>Temperature</th>
<th>wt% caffeine</th>
<th>wt% DPPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>30°C</td>
<td>0.44</td>
<td>7.9</td>
</tr>
<tr>
<td>35°C</td>
<td>0.44</td>
<td>8.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature</th>
<th>wt% Vitamin B12</th>
<th>wt% DHPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>0.51</td>
<td>9.3</td>
</tr>
<tr>
<td>42°C</td>
<td>0.51</td>
<td>9.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature</th>
<th>wt% benzamide</th>
<th>wt% DHPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>0.82</td>
<td>13.6</td>
</tr>
<tr>
<td>42°C</td>
<td>0.83</td>
<td>13.8</td>
</tr>
</tbody>
</table>
m-cresol release from PVA-liposome hydrogels at 37C & 42C

Chlorophenol release from PVA-liposome hydrogels at 37C & 42C

Propranolol release from PVA-liposome hydrogels at 37C & 42C

n = 3

<table>
<thead>
<tr>
<th>Temperature</th>
<th>wt% m-cresol</th>
<th>wt% DHPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>37C</td>
<td>1.01</td>
<td>13.7</td>
</tr>
<tr>
<td>42C</td>
<td>1.01</td>
<td>14.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature</th>
<th>wt% chlorophenol</th>
<th>wt% DHPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>37C</td>
<td>1.03</td>
<td>14.3</td>
</tr>
<tr>
<td>42C</td>
<td>1.06</td>
<td>14.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature</th>
<th>wt% propranolol</th>
<th>wt% DHPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>37C</td>
<td>1.11</td>
<td>14.2</td>
</tr>
<tr>
<td>42C</td>
<td>1.12</td>
<td>14.3</td>
</tr>
</tbody>
</table>
From the release studies of different solutes from PVA-liposome hydrogels at different temperatures, the dependence of release rate on temperature is demonstrated. However, the effect is probably too small for most practical applications.

5.3.4 Effect of Solute Properties

Figure 5.22 is a re-graph of the release profiles of all solutes at 37°C, except caffeine. Similar to Figure 5.22, Figure 5.23 is a re-graph of the release profiles of all solutes at 42°C, except caffeine. Other relevant information is shown in the figures.

As Figure 5.22 and 5.23 show, vitamin B₁₂, a hydrophilic solute, requires a high soaking concentration (5000 ppm) to load a significant amount of solute into the PVA-liposome hydrogels. For benzamide, which is slightly lipophilic, a smaller soaking concentration (2000 ppm) could load 60–90% more drug than vitamin B₁₂. The effect of solute’s lipophilicity on PVA-liposome hydrogel’s loading capacity is most apparent with the more lipophilic compounds. For solutes with $K_{ow} > 90$, a soaking concentration one-fifth of that of vitamin B₁₂ could load a higher wt% in final concentration. Nevertheless, the loading capacity does not increase significantly with an increase in $K_{ow}$, which indicates that the partition of solute between liposome and water is different from $K_{ow}$.

As seen from Figure 5.22, the release profiles of benzamide, chlorophenol and m-cresol, three lipophilic solutes with similar molecular weight, are almost the same. Release rate of these solutes are significantly higher than propranolol, a larger molecule. As the release rate of these solutes from PVA hydrogels were similar (Section 5.3.1.2), the slower release rate of propranolol showed that a large molecule is less permeable than smaller ones in the liposomes. Vitamin B₁₂, the solute with the largest molecular weight in this work, has the highest release rate. As vitamin B₁₂ is hydrophilic, it is hypothesized that most of the solute was outside the liposomes; therefore, its release rate is higher than the rest of the solutes.

Figure 5.23 shows that as the temperature increased to 42°C, the release rate of all solutes except vitamin B₁₂ increased significantly as compared to Figure 5.22. The major difference between vitamin B₁₂ and other lipophilic solutes is that while most of the
Different solutes release from PVA-liposome hydrogels at 37°C

Different solutes release from PVA-liposome hydrogels at 42°C

<table>
<thead>
<tr>
<th>Solute name</th>
<th>wt% solute</th>
<th>wt% DHPC</th>
<th>Conc. of soaking sol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12</td>
<td>0.51</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>Benzamide</td>
<td>0.82</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>Chlorophenol</td>
<td>1.03</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>m-cresol</td>
<td>1.01</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>1.11</td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>0.51</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Benzamide</td>
<td>0.83</td>
<td>13.8</td>
<td></td>
</tr>
<tr>
<td>Chlorophenol</td>
<td>1.06</td>
<td>14.1</td>
<td></td>
</tr>
<tr>
<td>m-cresol</td>
<td>1</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>1.12</td>
<td>14.3</td>
<td></td>
</tr>
</tbody>
</table>
molecules were entrapped inside the liposomes. As a result, the release rate of these lipophilic solutes were more dependent on the permeability of the liposomes.

**5.4 Gellan Gum Solution Transition Temperature Characterization**

Table 5.4 summarizes all results from the mechanical experiments as described in Section 4.4. The results are shown graphically in Figures 5.24 and 5.25. Figure 5.24 shows the effect of sodium concentration on transition temperature of gellan gum at constant gellan gum concentration. Each curve represents a phase diagram of a specific gellan gum concentration. Figure 5.25 shows the effect of gellan gum concentration on transition temperature at constant sodium concentration. Each curve defines a boundary in the phase diagram of a gellan gum solution. The region to the left of each curve denotes the liquid region, and to the right, the gel region.

As seen from Figure 5.24, the transition temperature increased as sodium concentration increased. Similarly, Figure 5.25 shows that as the gellan gum concentration increased, the transition temperature increased. For comparison, the phase diagram of a 0.5 wt% gellan gum solution prepared by Izumi et al. (1996) is shown in Figure 5.26. Izumi et al. (1996) used gellan gum supplied by the Kelco Division of Merck & Co. Inc., a different supplier from the present work. As seen from Figure 5.24 (0.5 wt% curve), the locations of the liquid region and the gel region were different from that of Figure 5.26. It is believed that the major difference between the two gellan gum products is the molecular weight of the gellan molecules, since other experimental conditions are the same. Unfortunately, this hypothesis cannot be verified since the molecular weights of both products are not known.

Figure 5.27 shows the phase transition temperature of the gellan gum used by Izumi et al. (1996) at different gellan gum concentrations without salt. The solid line of Figure 5.27 represents the transition curve determined from mechanical experiments similar to the present work. As seen from Figure 5.25, the relationship between transition temperature and gellan gum concentration is not linear. Such a result differs
Table 5.4  Phase Transition Temperature of Gellan Gum Solution with concentrations ranging from 0.2~1.6 wt%

<table>
<thead>
<tr>
<th>Sodium Conc. (mM)</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
<th>1.0</th>
<th>1.1</th>
<th>1.2</th>
<th>1.3</th>
<th>1.4</th>
<th>1.5</th>
<th>1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt; 0°C</td>
<td>&lt; 0°C</td>
<td>&lt; 0°C</td>
<td>&lt; 0°C</td>
<td>&lt; 0°C</td>
<td>&lt; 0°C</td>
<td>1~2°C</td>
<td>31~32°C</td>
<td>50~51°C</td>
<td>59~60°C</td>
<td>64~65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>&lt; 0°C</td>
<td>&lt; 0°C</td>
<td>&lt; 0°C</td>
<td>&lt; 0°C</td>
<td>0~1°C</td>
<td>7~8°C</td>
<td>59~60°C</td>
<td>64~65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>&lt; 0°C</td>
<td>&lt; 0°C</td>
<td>0~1°C</td>
<td>31-32°C</td>
<td>36~37°C</td>
<td>50~51°C</td>
<td>63~64°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
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<td></td>
</tr>
<tr>
<td>45</td>
<td>&lt; 0°C</td>
<td>2~3°C</td>
<td>34~35°C</td>
<td>53~54°C</td>
<td>59~60°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1~2°C</td>
<td>6~7°C</td>
<td>43~44°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
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<td>&gt; 65°C</td>
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<td>&gt; 65°C</td>
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</tr>
<tr>
<td>75</td>
<td>5~6°C</td>
<td>53~54°C</td>
<td>60~61°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
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<tr>
<td>90</td>
<td>49~50°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
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</tr>
<tr>
<td>105</td>
<td>54~55°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
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<td>&gt; 65°C</td>
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<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td>&gt; 65°C</td>
<td></td>
</tr>
</tbody>
</table>
Phase Diagram of gellan gum solution (Gellan gum concentration holding constant)

Figure 5.24

Phase Diagram of gellan gum solution (sodium concentration holding constant)

Figure 5.25
major reason, as pointed out before, could be the difference in the molecular weight of the products.

In conclusion, these results provide a foundation for the selection in the range of gellan gum concentration that should be used and how the concentration of a cation (sodium) can affect the phase transition temperature. Nevertheless, this is only the first step for a better designed experiment since the human body contains other cations such as potassium, calcium and magnesium, etc. Although these cations exist at a smaller amount compared to sodium, recent studies (Izumi, 1996) have shown that divalent cations have a more profound effect on the phase transition temperature of gellan gum than the effect of monovalent cations. Future work with gellan gum should include these ions to give a more realistic picture. Moreover, as the final goal of this work is to utilize gellan gum as a drug carrier, the effect of a specific drug on the phase transition temperature should also be examined in order to include every possible factor.

5.5 Release studies of Propranolol from solutions of water, gellan gum, liposomes and gellan gum & liposomes
As discussed in Sections 5.3.3 and 5.3.4, the release rate of propranolol is most responsive to temperature variation. It is anticipated that the same should apply with gellan gum. Hence, only propranolol was tested as the model solute in this section.

5.5.1 Effect of temperature on Propranolol release from dialysis membrane filled with water, gellan gum, liposomes or gellan gum & liposomes

Figures 5.28, 5.29, 5.30 and 5.31 show the release profiles of propranolol at 37°C and 42°C from dialysis membranes filled with water, 0.6 wt% gellan gum aqueous solution, 15 wt% DHPC liposome aqueous solution and a mixture of 0.6 wt% gellan gum and 15 wt% DHPC liposome aqueous solution respectively. Each curve represents the average of three samples. All other relevant data are summarized in Table 5.5.

Table 5.5 Relevant data of Propranolol release from dialysis membrane filled with different solutions

<table>
<thead>
<tr>
<th>Type of solution injected</th>
<th>Concentration of propranolol</th>
<th>Average mass injected ±SD (g) (n=3)</th>
<th>Release temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>1000 ppm</td>
<td>0.475 ± 0.008</td>
<td>37°C</td>
</tr>
<tr>
<td>water</td>
<td>1000 ppm</td>
<td>0.475 ± 0.005</td>
<td>42°C</td>
</tr>
<tr>
<td>0.6 wt% Gellan Gum solution</td>
<td>1000 ppm</td>
<td>0.466 ± 0.006</td>
<td>37°C</td>
</tr>
<tr>
<td>0.6 wt% Gellan Gum solution</td>
<td>1000 ppm</td>
<td>0.477 ± 0.008</td>
<td>42°C</td>
</tr>
<tr>
<td>15 wt% Lipo. solution</td>
<td>1000 ppm</td>
<td>0.472 ± 0.006</td>
<td>37°C</td>
</tr>
<tr>
<td>15 wt% Lipo. solution</td>
<td>1000 ppm</td>
<td>0.476 ± 0.008</td>
<td>42°C</td>
</tr>
<tr>
<td>0.6 wt% GG &amp; 15 wt% lipo.</td>
<td>1000 ppm</td>
<td>0.472 ± 0.003</td>
<td>37°C</td>
</tr>
<tr>
<td>0.6 wt% GG &amp; 15 wt% lipo.</td>
<td>1000 ppm</td>
<td>0.476 ± 0.010</td>
<td>42°C</td>
</tr>
</tbody>
</table>
Propranolol release from water medium in dialysis membrane at 37°C & 42°C

Figure 5.28

Propranolol release from 0.6 wt% GG solution in dialysis membrane at 37°C & 42°C

Figure 5.29
Propranolol release from 15 wt% liposome solution in dialysis membrane at 37°C & 42°C

![Graph showing propranolol release from 15 wt% liposome solution in dialysis membrane at 37°C & 42°C.]

n = 3

Propranolol release from 0.6 wt% GG & 15 wt% liposome solution in dialysis membrane at 37°C & 42°C

![Graph showing propranolol release from 0.6 wt% GG & 15 wt% liposome solution in dialysis membrane at 37°C & 42°C.]

n = 3
As seen from Figure 5.28, the release rates of propranolol in water at 37°C and 42°C show no difference. The result shows that the permeability of the dialysis membrane is not temperature dependent within the narrow temperature range tested. The permeability of the membrane should remain constant because it is not a part of the drug delivery system; any variation in permeability could affect the actual results. A similar trend is observed in Figure 5.29, which shows that the release rate of propranolol from gellan gum solution is also not temperature dependent within the range tested. Similar to the discussion for PVA in Section 5.1.1, the lack of sensitivity of propranolol release from gellan gum solution over temperature variation is important. As the function of gellan gum solution is to gel at the injected site, any sensitivity to external medium variation could trigger undesirable drug release patterns or even leakage of liposomes. Therefore, the results have shown that gellan gum is a suitable candidate for the present study. Figure 5.30 shows that with the addition of liposomes, there is a small difference in the release rate of propranolol at 37°C and 42°C. Nevertheless, the difference is too small for any useful application. A similar trend in Figure 5.31 is observed with the addition of gellan gum. These results, along with those discussed in Section 5.3, have shown that liposomes with the present composition are not suitable for temperature control release of lipophilic solutes.

5.5.2 Effect of difference in Propranolol-loaded medium

Figures 5.32 and 5.33 show the release profiles of propranolol from dialysis membranes filled with water, 0.6 wt% gellan gum aqueous solution, 15 wt% liposome aqueous solution and a mixture of 0.6 wt% gellan gum and 15 wt% liposome aqueous solution at 37°C and 42°C respectively. Each curve represents the average of three samples. All other relevant data are summarized in Table 5.5.

As shown in Figures 5.32 and 5.33, the release rate of propranolol from the gellan gum solution is comparable to that from water at both temperatures. Such observation is in accord with another preliminary experiment with gellan gum. In that experiment, 0.5 g of 0.6 wt% gellan gum solution was injected into the cellulose membrane mentioned in
Propranolol release from dialysis membrane filled with water, gellan gum solution with or without liposome at 37°C

Figure 5.32

Propranolol release from dialysis membrane filled with water, gellan gum solution with or without liposome at 42°C

Figure 5.33
that the gellan gum solution took 20 minutes to completely gel inside the membrane. As the diffusivity of a 0.6 wt% gellan gum solution before gelation is nearly the same as that in water, it is reasonable that the release rate of propranolol from the gellan gum solution is close to that of water.

As seen from both Figures, the addition of liposomes causes a decrease in the release rate of propranolol at both temperatures. This observation is in agreement with the hypothesis that most of the propranolol molecules reside inside the liposomes, which act as effective barriers for propranolol diffusion.

Lastly, a closer look at both figures shows that the difference in release rate of propranolol from water and from gellan gum solution is about 10% after 60 minutes. However, the difference is only about 5% only for the release rate of propranolol from liposome solution and from gellan gum plus liposome solution. It is believed that as the diffusion rate of propranolol from the liposomes phase is slower than from the gellan gum phase, the overall release rate is controlled by the release rate from the liposomes, not from the gellan gum solution.

5.6 Intravitreal Drug Injection Simulation

5.6.1 Effect of Gellan Gum Injected into Low Viscosity Medium (Liquid Vitreous)

Figures 5.34a and 5.34b show the distribution of 1500 ppm fluorescein injected into a 40000 cP medium at 30 seconds and 10 minutes after injection. Figures 5.35a, 5.35b and 5.35c show the distribution of 1500 ppm fluorescein with 0.6 wt% gellan gum into the same 40000 cP medium at 30 seconds, 10 minutes and 45 minutes after injection.

As seen from Figure 5.34a, a slow speed injection of fluorescein solution into a low viscosity medium enhances spherical formation of injected solution. However, as Figure 5.34b shows, due to the difference in density of the injected solution and the medium, most of the solution floats to the top 10 minutes after the injection. Similar to Figure 5.34a, Figure 5.35a shows the same shape characteristic of the injected solution to
the same medium as in Figure 5.34a. The only difference between Figure 5.34a & 5.35a is that the injected solution of Figure 5.35a contains 0.6 wt% gellan gum. As seen in Figure 5.35b, the presence of gellan gum prevents the injected solution from floating to the top 10 minutes after the injection, which is observed in Figure 5.34b. Figure 5.35c shows the injected solution takes 45 minutes to float to the top after injection. This is more than 3 times the time needed for a solution without gellan gum to float to the top. Studies from this section have shown that an in situ gel-forming solution could slow down the mixing speed of an injected solution with the surrounding medium. Moreover, the addition of gellan gum aids the injected solution to maintain its initial shape. This helps to generate a more predictable release profile.

5.6.2 Effect of Gellan Gum Injected into High Viscosity Medium (Gel Vitreous)

Figures 5.36a, 5.36b and 5.36c show the distribution of 1500 ppm fluorescein injected into a 140000 cP medium at 30 seconds, 3 hours and 24 hours after injection. Figures 5.37a, 5.37b and 5.37c show the distribution of 1500 ppm fluorescein with 0.6 wt% gellan gum into the same 140000 cP medium at 30 seconds, 3 hours and 24 hours after injection. The 140000 cP medium represents a gel vitreous model without flowing motion.

Although gellan gum has shown a favourable effect on intravitreal injection into the low viscosity model, the same effect is not observed in the high viscosity model. Comparison of Figures 5.36a & 5.37a (Time = 30 s), 5.36b & 5.37b (Time = 3 hr), and 5.36c & 5.37c (Time = 24 hr) show that there is no difference in the mixing rate of both solutions with the viscous medium. From the above results, it is concluded that for a highly viscous solution such as a gel solution, gellan gum does not have a profound effect on the extent of drug release. Consequently, it is believed that such technique should be most beneficial to old age people where a high portion of the vitreous had liquefied due to the aging process.
Two injected solutions, one with gellan gum and the other without it, were examined. The viscosity of the viscous medium was 75000 cP. The first solution was a 15 wt% liposome solution. Figure 5.38a shows the shape of the injected solution immediately after injection. Figures 5.38b, 5.38c and 5.38d show the distribution of the injected solution at 10 minutes, 30 minutes and 40 minutes after the injection respectively. Figure 5.38e shows the shape of the injected solution 45 minutes after injection from another angle.

Figure 5.39a shows the syringe filled with the second solution. The lower level of the syringe was filled with 0.6 wt% gellan gum solution while the upper part was filled with 15 wt% liposome solution. Figure 5.39b shows the formation of a gellan gum sphere enclosing the liposome solution. Figure 5.39c shows the increase in size of the sphere after all the liposome solution was injected. Figures 5.39d and 5.39e show the distribution of the injected solution 3 h and 24 h after the injection.

From the result in Section 5.5.1, it is observed that there was not enough time for gelation of gellan gum to complete before the solution reached the top and dispersed horizontally. From the result in Section 5.5.2, it is shown that the viscosity of the viscous medium was too high to allow any movement at all. Based on the results, a viscous medium with an intermediate viscosity of the two was produced.

As seen from Figures 5.38a to 5.38e, a higher viscosity medium does not prevent the liposome solution from floating and disperse horizontally at the top. As the density of the liposome solution was less than the surrounding medium, the observation was reasonable because the viscosity of the liposome solution is close to that of water.

As for the experiment with gellan gum, it is intended to mix the liposome solution and gellan gum solution homogeneously before injection. However, preliminary results have shown that once after mixing, the solution gels as a heterogeneous mixture. To circumvent the problem, a new method, named here as the "two phase injection method", is employed. Briefly, the lower part of the syringe is filled with gellan gum solution and
Figure 5.39b, one advantage of using such method is the containment of a high concentration of liposome/drug within the injected solution. As the drug diffuse out, the gellan gum solution will have gelled already. This is due to the faster diffusion rate of smaller cations like sodium ions in the vitreous. In addition, liposomes will remain inside the injected sphere. It has been shown that injection of free liposomes into the vitreous had caused cloudiness (Peyman, 1995). The present formulation could prevent the occurrence of cloudiness by entrapping the liposomes within the gelled solution. As seen from Figures 5.39c, 5.39d and 5.39e, the injected solution remained intact after 24 h. This result shows that gellan gum is an effective vehicle for the containment of liposomes.

The major problem connected with this technique is how the gelled solution could be removed from the vitreous body after depletion of the drug from the gelled solution. One possible solution is to prepare a gel that has a phase transition temperature slightly above the temperature of the eye. With such a formulation in hand, the gel could dissolve in the eye by slightly increasing the temperature of the eye using microwave (Khoobehi, 1988). A trade-off in the above suggestion is that the delivery system cannot be temperature responsive because the gellan gum layer will erode away quickly at higher temperature. Early studies showed that water movement within the vitreous is very active, with 50% of labeled water turning over every 10-15 minutes (Sebag, 1989). At such a turnover rate, it might be possible that the water will dilute the dissolved gellan gum solution and decrease its phase transition temperature to below that of the eye. Further experiments should be performed to examine the above hypothesis. In conclusion, this technique suggests an alternative way to deliver a high dose of drug through intravitreal injection while maintaining therapeutic safety level.
Figure 5.34a  In vitro distribution of 1500 ppm fluorescein solution 30 seconds after injection into a 40000 cP medium

Figure 5.34b  In vitro distribution of 1500 ppm fluorescein solution 10 minutes after injection into a 40000 cP medium
Figure 5.35a  In vitro distribution of 1500 ppm fluorescein solution with 0.6 wt% gellan gum 30 seconds after injection into a 40000 cP medium

Figure 5.35b  In vitro distribution of 1500 ppm fluorescein solution with 0.6 wt% gellan gum 10 minutes after injection into a 40000 cP medium
Figure 5.35c  In vitro distribution of 1500 ppm fluorescein solution with 0.6 wt% gellan gum 45 minutes after injection into a 40000 cP medium

Figure 5.36a  In vitro distribution of 1500 ppm fluorescein solution 30 seconds after injection into a 140000 cP medium
Figure 5.36b In vitro distribution of 1500 ppm fluorescein solution 3 hours after injection into a 140000 cP medium

Figure 5.36c In vitro distribution of 1500 ppm fluorescein solution 24 hours after injection into a 140000 cP medium
Figure 5.37a  In vitro distribution of 1500 ppm fluorescein solution with 0.6 wt% gellan gum 30 seconds after injection into a 140000 cP medium

Figure 5.37b  In vitro distribution of 1500 ppm fluorescein solution with 0.6 wt% gellan gum 3 hours after injection into a 140000 cP medium
Figure 5.37c  In vitro distribution of 1500 ppm fluorescein solution with 0.6 wt% gellan gum 24 hours after injection into a 140000 cP medium

Figure 5.38a  In vitro distribution of 15 wt% liposome solution 30 seconds after injection into a 75000 cP medium
Figure 5.38b In vitro distribution of 15 wt% liposome solution 10 minutes after injection into a 75000 cP medium

Figure 5.38c In vitro distribution of 15 wt% liposome solution 30 minutes after injection into a 75000 cP medium
Figure 5.38d In vitro distribution of 15 wt% liposome solution 40 minutes after injection into a 75000 cP medium

Figure 5.38e In vitro distribution of 15 wt% liposome solution 45 minutes after injection into a 75000 cP medium
Figure 5.39a  Syringe filled with solution for injection

Figure 5.39b  In vitro distribution of 15 wt% liposome solution and 0.6 wt% gellan gum solution 30 seconds after injection into a 75000 cP medium
Figure 5.39c In vitro distribution of 15 wt% liposome solution and 0.6 wt% gellan gum solution 35 seconds after injection into a 75000 cP medium.

Figure 5.39d In vitro distribution of 15 wt% liposome solution and 0.6 wt% gellan gum solution 3 hours after injection into a 75000 cP medium.
Figure 5.39e In vitro distribution of 15 wt% liposome solution and 0.6 wt% gellan gum solution 24 hours after injection into a 75000 cP medium
It has been shown that a liposome embedded hydrogel system can act as a lipophilic drug reservoir. Such a delivery system has certain advantages over delivery systems using just a hydrogel alone. The liposome embedded hydrogel can prolong the release period, increase the drug loading capacity of lipophilic drugs and respond to temperature variations in drug delivery. Moreover, it is possible to prepare the system in solution form and gel within the injected site. The vitreous body was selected as a model site for illustration of a gellable solution for injection. In vitro experiments have shown the gellable solution is able to gel in the injected site and encapsulate liposomes within the gelled solution.

The applicability of this gellable solution as an intravitreal drug delivery system is as yet unproven. Indeed, the problem of removing the system after drug depletion may hinder the application of the formulation. However, the technology may apply to other drug delivery sites where localization of drug is required.
1. The gellable solution should be tested in vivo to ascertain its ability to gel within the injected site.

2. The use of other phospholipids and temperature responsive materials suitable for encapsulation within hydrogels should be investigated.

3. LUV liposomes encapsulated in hydrogel should be tested for temperature responsive delivery of hydrophilic solutes.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>CAP</td>
<td>cellulose acetophthalate</td>
</tr>
<tr>
<td>CMV</td>
<td>cytomegalovirus</td>
</tr>
<tr>
<td>DDS</td>
<td>drug delivery system</td>
</tr>
<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>LCST</td>
<td>lower critical solution temperature</td>
</tr>
<tr>
<td>LUV</td>
<td>large unilamellar vesicles</td>
</tr>
<tr>
<td>$M_{AS}$</td>
<td>swollen weight of hydrogel in air</td>
</tr>
<tr>
<td>$M_D$</td>
<td>dry weight of hydrogel</td>
</tr>
<tr>
<td>MLV</td>
<td>multilamellar vesicles</td>
</tr>
<tr>
<td>PCL</td>
<td>poly(caprolactone)</td>
</tr>
<tr>
<td>PEG</td>
<td>poly(ethylene glycol)</td>
</tr>
<tr>
<td>PHEMA</td>
<td>poly(2-hydroxyethyl methacrylate)</td>
</tr>
<tr>
<td>PVA</td>
<td>poly(vinyl alcohol)</td>
</tr>
<tr>
<td>PVP</td>
<td>poly(vinyl pyrrolidone)</td>
</tr>
<tr>
<td>PVR</td>
<td>proliferative vitreoretinopathy</td>
</tr>
<tr>
<td>RES</td>
<td>reticulo-endothelial system</td>
</tr>
<tr>
<td>ROP</td>
<td>retinopathy of prematurity</td>
</tr>
<tr>
<td>SUV</td>
<td>small unilamellar vesicles</td>
</tr>
<tr>
<td>$T_m$</td>
<td>phase transition temperature</td>
</tr>
<tr>
<td>W</td>
<td>weight percentage of water in hydrogel</td>
</tr>
</tbody>
</table>
\( \rho_c \)  
\hspace{1cm} \text{density of cyclohexane}  

\( \rho_p \)  
\hspace{1cm} \text{bulk density of PVA}  

\( \psi_{2,S} \)  
\hspace{1cm} \text{polymer volume fraction after swelling}  

\( \xi \)  
\hspace{1cm} \text{mesh size of hydrogel}
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