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UMI®
PULSATILE RELEASE OF AN IONIZABLE DRUG FROM MICROCAPSULES
WITH ELECTROCHEMOMECHANICAL WALLS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Jessica Miller, B.A.

****
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2001

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ABSTRACT

An electric field has been used to alter and control the release of an ionizable compound from microcapsules with electrochemomechanical (ECM) walls. The electric current was applied to the system to cause changes in pH which affects not only the solubility and charge of the drug, but the physical properties of the gel wall matrix as well. In this manner, by simply altering the electric current in on/off cycles, in vitro electrically-facilitated drug release in controlled pulsatile patterns can be achieved. The system consisted of four components: an ionizable drug core, an electrochemomechanical hydrogel wall, an aqueous electrolytic suspension medium, and an external power supply. Sulfadiazine is an ionizable drug and was used as the model compound for these studies. Gelatin, Type B, was the model ECM hydrogel wall.

The experimental protocol first involves microencapsulation of drug with an ECM hydrogel. In vitro drug release from microcapsules has been carried out under passive conditions and under the influence of an electric current. In most cases, platinum wire electrodes were used for the electrically-facilitated release studies. These electrodes are considered to be chemically inert since they do not participate in electrode reactions. However, platinum electrodes cause pH fluctuations at their surfaces due to water hydrolysis. Release studies were carried out for 70 min and samples were collected in a fraction collector. Currents of 0, 0.5, and 1.0 mA DC were applied to the microcapsules.
from $t = 20$ to $t = 50$ min. Sulfadiazine release was dependent on the magnitude of the applied current in that there were 5.20 and 11.0 fold increases in the amounts of sulfadiazine released after 70 min compared to that by passive diffusion. The ionic strength of both the eluant and the suspending medium in the donor compartment did not appear to have an effect on drug release. In order to test the effect of pH on sulfadiazine release, passive and electrically-facilitated release studies were carried out using sulfadiazine suspensions, suspensions of gelatin/sulfadiazine microcapsules and suspensions of PVP/sulfadiazine microcapsules using four different pH protocols (Protocols 1 - 4). Sulfadiazine suspensions served as controls to determine the effect of current on drug release alone, whereas PVP/sulfadiazine microcapsules were used to determine the effect of current on a nonionizable polymer wall since unlike the case with gelatin, its porosity should not be affected by pH. Sulfadiazine release was found to increase with the application of 0.5 mA DC using Protocols 2 - 4, in which the donor compartment medium pH was 4.5, 6.0 and 7.0, respectively. For Protocol 1 (donor pH 2.0), no increase occurred, suggesting that pH change at the electrodes was not sufficient to cause any noticeable effects on drug release. The gelatin wall became a partial barrier to drug release when current was applied using Protocol 3. This may be due to the closing of pores in the gelatin matrix at pH values near its isoelectric point (pH ~5). It was determined that the electrically-facilitated release of sulfadiazine can occur by one or a combination of the following mechanisms:

1.) passive diffusion through the microcapsule hydrogel wall,

2.) diffusion through pores in the wall created by or closed by the electrically induced pH change in the donor compartment,
3.) an increase in the solubility of sulfadiazine in the donor compartment caused by pH fluctuations induced by the reactions at the electrodes, 

4.) iontophoresis of charged drug through the microcapsule hydrogel wall and/or SpectraPor® membrane, and 

5.) solvent flow or electroosmosis of drug solution through the wall. 

Although the five mechanisms listed may all need to be considered, mechanisms 2 and 3 appear to be the most prominent contributors to the electrically-facilitated release of sulfadiazine from the gelatin-walled microcapsules.
Dedicated to my mother and father
ACKNOWLEDGMENTS

I would like to give special thanks to:

My adviser, Dr. Sylvan G. Frank for his ideas, mentorship and patience, for his enthusiasm for learning, and especially for his support and dedication to not only my professional growth, but my spiritual growth as well.

Dr. Arne Brodin for his insightful ideas on my project and invaluable discussions.

Members of my committee, Dr. William Hayton, Dr. Alfred Staubus, and Dr. Robert Curley for their suggestions and time.

My labmates: Pammi, Wen, Marie, Chao and Carmen for their help and friendship; especially Chao for listening, laughing and for her faith and support.

Chris, for his friendship, support and for consistently being there for me. His friendship is unforgettable and irreplaceable.

Ms. Karen Lawler and Ms. Kathy Brooks for their help and encouragement; especially Karen for listening and for her friendship.

Julie Masura and Kathy Wolken; Julie for statistical interpretation of my data, and Kathy for all her help with microscopy.

Astra Pain Control and AstraZeneca Inc., for financial support.

My family, especially my mother and father for their love, support, generosity, patience and as always, undying faith in me and my dreams.

My good friends Mike and Michelle; Mike, for listening and caring. Michelle, for all our interesting and inspirational conversations, and for sharing with me in my life’s little adventures.

For all my friends and family who are not mentioned here, you do not go unrecognized by me. My cup runneth over.
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CHAPTER 1

INTRODUCTION

CONCEPT MAP

Perspective

- The application of an electric current to microcapsules consisting of an ionizable drug core and an electrochemomechanical gel wall to achieve and control pulsatile drug release has been studied.

Background

- Pulsatile drug delivery is a type of controlled release in which drug is released in pulses at specific time intervals. This type of release may be important for mimicking natural hormonal cycles or circadian dependencies of disease states, or to provide drug on demand such as in pain control.

- Electrochemomechanical gels can expand and contract in response to an electric field, and this behavior can be applied to controlled drug release systems.

Novel Drug Delivery System

- An electric current, when applied to microcapsules with electrochemomechanical walls may affect drug release from the system by altering drug solubility and/or by hydrogel swelling/deswelling. Hydrogel swelling may cause opening of aqueous channels which facilitate diffusion of drug and permeation of dissolution medium through the wall. Conversely, hydrogel deswelling or contraction may close aqueous channels thereby decreasing its permeability.
1.1 Perspective

The application of an electric current to microcapsules with electrochemomechanical walls to achieve and control a pulsatile drug release pattern has been studied. For potent drugs, with small therapeutic windows, low, but controlled periodic doses of medication may be necessary to attain a suitable therapeutic outcome. In this context, traditional methods of administration including intravenous bolus and oral formulations may not be appropriate due to factors such as increased patient distress associated with invasive techniques, or to reduced patient compliance, respectively. Such systems may be more appropriate for therapies involving relatively few pulses. The application of an electric current to microcapsules consisting of an ionizable drug core and an electrochemomechanical gel wall may provide a novel alternative for the development of a controlled pulsatile drug release system. The present work involves model systems designed to test the in vitro feasibility of such a strategy.

1.2 Background

1.2.1 Pulsatile release

Controlled release drug delivery systems have become increasingly important in the last few decades as new drugs and therapeutic modalities have evolved with more demanding requirements for administration (1-4). Not only can the rate of drug release be controlled, but also the site of administration. Originally, the term controlled release
was commonly associated with sustained drug release which is the type of controlled release when there is a gradual release of drug at therapeutic levels for an extended period of time. Although such constant plasma concentrations of drug may be desirable in some cases, it has been shown that a pulsatile or oscillatory release may be necessary to achieve an optimal therapeutic effect (5, 6). For example, many endogenous functions of the body show variations throughout the day (7). These variations can lead to changes in pharmacokinetic parameters which can affect a drug's therapeutic outcome. Therefore, the concept that “not only must the right amount of the right substance be at the right place, but also at the right time” should be applied (5).

Pulsatile drug delivery is a type of controlled release system in which the drug is released in pulses at specific time intervals. Ideally, the drug should be released rapidly within a short period of time, preceded or followed by a period in which no drug, or a minimal amount of drug is released. As mentioned above, in the case of pulsatile delivery, the maintenance of constant plasma levels of drug is not necessarily preferred. It is assumed rather, that the optimal therapy would be one that consists of oscillating drug levels. These oscillating drug levels can provide drug on demand from a biosensor or upon patient need, to follow circadian rhythms, to decrease the development of drug tolerance, or to minimize tissue damage such as in iontophoresis. For example, some disease states (including asthma, cancer, and hypertension) show dependency on circadian rhythms (7-9). Asthma is a disease in which attacks occur more frequently in the night time hours. Therefore, it was found that theophylline should be dosed higher at night than in the daytime hours to accommodate for such disease fluctuations (5, 7). Circadian timing of drug delivery is also an important consideration in the treatment of
cancer. For example, studies show that antitumor drug toxicity and dose intensity, tumor control, and patient survival were dependent on the timing of a doxorubicin/cisplatin treatment (9). In the same context, the blood pressure of hypertensive patients is dependent on the time of day. This dependency is influenced by the type and severity of the hypertension, and hence appropriate periodic dosing is advantageous for achieving an optimal therapeutic effect (7).

Certain hormones have been shown to be released in a pulsatile fashion in the body (6, 8, 10, 11). Examples include gonadotropin releasing hormone, insulin, growth hormone, testosterone, and luteinizing hormone. When considering the examples of gonadotropin releasing hormone for the treatment of infertility and testosterone therapy, pulsatile release at specific times is necessary to more closely follow natural endogenous hormonal release patterns (6, 12).

In addition, sustained release of some drugs may also lead to drug tolerance in which the therapeutic effect can decrease with time at constant plasma levels (6, 11). A common example of drug tolerance is seen with chronic sustained transdermal delivery of the cardiovascular drug, nitroglycerin. It was found that the beneficial effects of the drug were reduced after 12 h of administration from such a transdermal patch (6). However, it was also found that drug tolerance was reversible since after a period of time in which no drug was administered, the normal therapeutic effect of nitroglycerin returned (6). In this case, and the cases mentioned above, a pulsatile drug delivery system may be a better therapeutic modality.
1.2.2 Methods of pulsatile release

There are several different approaches for achieving pulsatile drug release. For example, the administration of multiple traditional i.v. bolus doses may lead to increased patient distress inherent in this invasive technique. Oral dosing may be ineffective for some drugs which are unstable in the gastrointestinal tract or undergo extensive first pass effects. In this context, it has been found that there is an inverse relationship between the number of doses per day and compliance rate for oral dosing (13). Lastly, in order to accomplish intermittent drug release from a transdermal system, the original devices needed to be removed at a specific time, and a new patch applied later also at a specified time (14). The potential problem with such a sequential method is again, a failure of patient compliance.

More recently, pulsatile release has been achieved by different routes of administration and delivery system technologies that tend to minimize problems associated with the more traditional methods of administration (15). The use of electronics and microelectronics has had an impact on pulsatile drug delivery systems (16). These technologies include iontophoresis, sonophoresis, electroporation, and implantable infusion pumps (17-23). The use of polymers for pulsatile delivery has also become popular. Some polymers have been shown to be “intelligent” or “smart” systems since they can respond to their environment (see Section 1.2.4). An alteration of the environment can provide control of drug release from the polymer by changing polymer properties such as porosity and hydration. Responsive polymers can be open-loop (externally controlled) systems, or closed-loop (self-regulating) systems (24). Externally
modulated polymer drug release systems consist of magnetically-triggered, ultrasonically-triggered, electrically-triggered, and temperature responsive formulations (25). Self-regulating systems are also responsive to the environment and consist of pH, ionic strength, chemical and enzyme responsive polymers (24-26). These systems can be designed for single pulses or multiple pulses (27). For example, pH, temperature, and electrically activated polymer systems can be reversible and therefore multiple pulses are feasible. Conversely, polymer dissolution and osmotically controlled systems may be more appropriate for a single pulse (27).

1.2.3 Gels

As mentioned above, along with growing interest in controlled drug release, there has been considerable research into the applications of hydrogels and polymers in such delivery systems. Most of the interest appears to lie in the utilization of biocompatible and biodegradable polymers for implants or transdermal systems (28-30). In such cases, the bulk and surface properties of the gels need to be considered. For example, the diffusivity of solutes through a polymer network is related to the pore size of the gel and to possible ionic interactions that can occur (31-33). As such, polymers are well-suited for drug delivery systems since their permeabilities can be modified and controlled to meet specific requirements.

Hydrogels consist of networks of hydrophilic polymer chains swollen with water held together by covalent or other cohesive forces. Hydrogels which are bonded by noncovalent forces are called physical gels, whereas gels covalently crosslinked are
called chemical gels (34). In physical gels, the polymer chains form a network by hydrogen bonding, ionic associations, van der Waals forces and/or hydrophobic interactions (34, 35). The contacts between the chains are termed “junction zones,” and serve to hold the chains together to maintain structural order.

1.2.4 Chemomechanical gels

Chemomechanical systems are those which are capable of transforming chemical energy directly into mechanical work (36-38). Certain hydrogels are considered to be chemomechanical systems since their expansion and contraction in response to an external stimulus show this type of energy conversion. Such gels can swell or contract due to changes in the temperature, ionic strength and pH of the solvent, and/or to the presence of electric or magnetic fields (24-27). This behavior has been utilized experimentally in gel-based actuators, valves, sensors, artificial muscles for robotics, and controlled release drug delivery systems (36, 38-41).

Chemomechanical systems are of interest since the conversion of chemical into mechanical work is performed in living organisms, such as in the movement of muscles and flagella. Such chemomechanical systems are extremely efficient and can perform work without causing noise or exhaustion (36). Gels which have these chemomechanical properties are sometimes called “smart” or “intelligent” systems since they can respond to external stimuli (25, 35, 42).
1.2.5 Hydrogel swelling

As mentioned above, hydrogels are of interest because they can adapt to environmental changes. Successful application of environmentally responsive hydrogels for drug delivery requires that the magnitude of the swelling and deswelling be characterized and controllable. In general, most hydrogels can absorb at least 10 to 20 percent of their original weight of water (43). The swelling and deswelling behavior of hydrogels is most commonly described by thermodynamic analysis. In this way, the hydrogel can be thought of as a system involving two components: the gel network and the aqueous phase. At equilibrium, the chemical potentials of water inside the gel and outside the gel are equal. Therefore, any change in the chemical potential of the water surrounding the gel will cause a change in the degree of swelling of the gel. The magnitude of swelling at equilibrium is then determined by the nature and concentration of solutes in the surrounding water (44). For example, if solutes which cannot enter the gel network are added into the surrounding medium, the chemical potential of water in the medium would decrease. This in turn would cause the hydrogel to contract, i.e., deswell, to drive water out to reduce the chemical potential of water within the gel. This will occur until the two aqueous systems are in equilibration. Conversely, an increase in the chemical potential of water in the surrounding medium would cause the gel to swell. These properties can be affected however, by the addition of solutes which permeate the hydrogel network. The solutes in turn can then interact with the polymer chains, thereby changing the swelling properties of the system. In general, when considering hydrogel swelling, the degree to which the gel will swell is determined by factors such as the
hydrophilicity of the gel monomers, the degree of crosslinking and the ionic strength, pH and degree of dilution of the surrounding medium (27, 33, 45).

1.2.6 Electrochemomechanical (ECM) gels

If a hydrogel can expand and contract in response to an electric field, then it is considered to be an electrochemomechanical, electrosensitive or electroresponsive gel. These gels usually consist of polymers and co-polymers containing ionizable groups (46). Table 1.1 lists examples of some electrochemomechanical gels (ECM). The swelling and shrinking (or deswelling) properties of such ECM gels appear to be simple in nature, however the mechanisms have not been fully defined or understood. Such behavior has been attributed to an electrokinetic process occurring within the gel; that is, the electroosmosis of water and the electrophoretic migration of charged species toward the opposite electrode (47). The amount and rate of swelling and contraction depends on the precise structure of the gel, temperature, pH of the solvent, and the magnitude of an applied electric field (27, 33, 45, 48). The type of deformation, i.e., shrinking, swelling or bending, has been found to be dependent on variables such as the shape of the gel, the pH of the solution, the concentration of salt in the solution and the gel, and on the position (i.e., in contact or not in contact) of the gel with respect to the electrodes (45, 48-50).

An example of such systems are microparticles consisting of the sodium salt of polyacrylic acid, (Na-PAA), which contract in aqueous solutions under the influence of
- Polymers and co-polymers which contain ionizable groups (46)
- Lightly crosslinked sodium salt of poly(acrylic acid) (51)
- Sodium alginate/poly(acrylic acid), and calcium salt of alginic acid (52)
- Polymer/polymer complexes prepared from poly(methacrylic acid), poly(acrylic acid), poly(vinyl alcohol) (54, 51)
- Collagen fibers (56, 60)
- Poly(methacrylic acid) (38, 46)
- Partially hydrolyzed poly(acrylamide) (53)
- Proteins, e.g., gelatin (59)
- Biopolymers, e.g., agar-agar and gum arabic (46)
- Hyaluronic acid (32)

Table 1.1 Examples of electrochemomechanical gels cited in the literature
an electric current (Figure 1.1). Kishi, et al., (51) explained the electrochemomechanical behavior of these gels in terms of the following sequence:

a) The microparticles move to the anode; at the anode, contraction takes place;

b) Sodium ions move to the cathode due to electrophoretic migration;

c) Electrodiffusion of mobile cations carries sodium ions away from the carboxylate groups within the gel;

d) Carboxylate anions undergo substitution with hydrogen ions produced at the anode due to water hydrolysis; and,

e) Carboxylates become largely undissociated and the gel contracts at the anode.

This mechanism partially accounts for the contraction and expansion behavior of negatively charged gels in certain experimental designs. For example, in the study reported by Kishi, et al., described above, the gels were able to come close to, or be in direct contact, with the electrodes. The local pH change near the electrodes due to water hydrolysis caused a change in the ionic state of the gels, and consequently the microparticles contracted. In order to test the contribution of local pH change on the rate of contraction of the microparticles, a salt bridge was used instead of platinum electrodes. In this case, the microparticles underwent contraction even though the pH of the surrounding medium did not change. The rate of microparticle contraction using a salt bridge however, was slower than the rate of contraction using platinum electrodes alone.
Figure 1.1  Na-Polyacrylic acid microparticles in aqueous solution (51).

a. Before the application of current, (0.22 mA).
b. After 3 minutes of current.
c. After 5 minutes of current.
d. 12 minutes after the current was turned off.
It was found that if a 90% volume change due to contraction was achieved in one minute with platinum electrodes, only a 47% volume change occurred under the same conditions using a salt bridge (51).

This 47% volume change may be explained alternatively by an electroosmotic or ionic strength mechanism (48, 49). That is, in the case of negatively charged gels, the movement of mobile cations takes sodium ions away from the gel, causing a change in the ionic strength of the solution near the electrodes. Increasing the ionic strength of the solution locally may in turn then cause deswelling of the gel by one or both of the two following possible mechanisms:

a) An increase in osmotic pressure caused by a change in ionic strength may result in a loss of water from the gel; and/or,

b) The local change in ionic strength of the solution may shield charges on the gel network, which then in turn reduces repulsion between the fixed charges on the polymer chains, thereby reducing the degree of hydration of the gel.

The osmotic pressure gradient mechanism, described by a above, has also been used to explain the swelling behavior of nonionizable gels (50, 52, 53).

1.2.7 Electrochemomechanical gels as drug delivery systems

The electrochemomechanical properties of gels may be applied to potential controlled release drug delivery systems. In such systems, the electric field could serve as a means of controlling drug release from the gel by controlling the degree of gel
swelling and deswelling. For example, when an electric current is applied to an electrochemomechanical hydrogel, the gel may shrink and force out water droplets. If the gel is loaded with drug solution, the application of current may then cause the gel to release the drug as well. Figure 1.2 demonstrates this "squeezing" out effect caused by contraction of the gel. The amount of drug released is then dependent on the degree of hydration and the nature of the gel, as well as the magnitude of the applied current. In this manner, drug release may be controlled by simply altering the magnitude of the applied current, and delivery systems based on this concept have been developed (48, 52, 54). In particular, studies of Na-PAA microparticles loaded with pilocarpine or glucose indicated that the amount of drug released increased with the magnitude of the current (54).

Another potential application of electroresponsive gels for controlled drug delivery is in membrane-type devices. For example, a hydrogel membrane can act as a gate to control drug release (41, 42, 55-58). Figure 1.3 shows an electroresponsive hydrogel membrane placed isometrically between two electrodes. When current is applied to the planar membrane, the gel matrix shrinks, thereby opening pores in its network for solvent to flow through. Differences in water permeation through such membranes due to the passage of current are illustrated in Figure 1.4. In some cases, the swelling of the membrane will open up aqueous channels allowing the drug to more easily diffuse. For example, Eisenberg et al., showed that the thickness (swelling) of a collagen membrane could be increased as the pH or the NaCl concentration in the surrounding medium decreased (56). Sucrose was used as a tracer in their experiments,
Figure 1.2  A schematic diagram of drug release by a “squeezing” mechanism (38).
Figure 1.3  Schematic diagram of an electrochemomechanical membrane and experimental apparatus (38).
Figure 1.4  Water permeation through an electrochemomechanical hydrogel membrane controlled by on and off cycles of current (38).
and the permeability of the membrane to this marker increased with increasing proton
ccentration. They concluded that the permeability of a solute through the swollen
collagen membrane was dependent on the size of the solute molecule and also on its
diffusion path through the membrane.

1.3 A novel drug delivery system for pulstite release

1.3.1 Introduction

In the present work, microcapsules consisting of an electrochemomechanical wall
and an ionizable drug core are proposed as a novel pulsatile drug delivery system. By
combining the electrochemomechanical properties of the gel with an ionizable drug, the
rate of release can be controlled with an electric field. An electric current applied to the
system would cause changes in pH around the electrodes which would in turn affect not
only the solubility and charge of the drug, but the physical properties of the gel wall
matrix as well. In this manner, by simply altering the electric current in on/off cycles,
controlled pulsatile drug release can be achieved (see Section 1.3.4).

1.3.2 Strategy

The proposed system consists of four components:

1) electrochemomechanical gel wall

2) ionizable drug core
3) electrodes with an external power supply
4) an aqueous electrolytic suspension medium

The electrochemomechanical walls of this pulsatile dosage form would function as a membrane or "gate" controlling drug release (Section 1.2.7). For this type of system, it is proposed that the encapsulating wall around the drug particles be an electrochemomechanical hydrogel which contracts or swells under an applied electric current (Section 1.3.4.2.3). As described above, such deformation can alter the permeability of the membrane to solutes. By alternating the current at different levels, swelling of the gel can be controlled, thereby directly controlling the amount of solute released from the system.

The ionizable drug component would be responsive to pH fluctuations rendering it charged or uncharged. The charge of the drug affects its solubility as well as its ability to partition through body tissues (Section 1.3.4.2.4). By selecting appropriate electrodes (Section 1.3.4.2.2), the pH in the locale of the electrodes would be altered upon the application of current.

The electric current would be applied through an external power supply such as a battery, or in the case of the present work, a computerized power supply (which could be miniaturized for future prototypes). Both the anode and cathode would be placed in an electrolyte solution containing the microcapsules (Section 2.2.3). In this case, the microcapsules are allowed to come into direct contact with the surface of the electrodes, and therefore be directly affected by any change in local pH.
1.3.3 Choice of materials

1.3.3.1 Sulfadiazine

Sulfadiazine is a good model for this purpose because it is an ionizable compound and its pKas can represent the pKas of more potent compounds of interest. Sulfadiazine can also be encapsulated by a coacervation process. The properties of sulfadiazine which are significant to this system are:

Molecular Formula: $\text{C}_{10}\text{H}_{10}\text{N}_4\text{O}_2\text{S}$

Molecular Weight: 250.27

Structure:

\[
\begin{array}{c}
\text{H}_2\text{N}\quad \text{S}\quad \text{O} \quad \text{H} \\
\text{N} \quad \text{N}
\end{array}
\]

Melting Point: 251 - 254 °C

pKa: 2.1 and 6.3 (62)

Solubility: Insoluble in water, sparingly soluble in alcohol and acetone. Soluble in dilute mineral acids. The Na salt is freely soluble in water (62, 63).

Stability: In its solid form, sulfadiazine is stable in air, humidity, and temperature up to 100 °C for two weeks (62).
1.3.3.2 Gelatin

Although other compounds may be used (see Table 1.1), gelatin was the initial model for the electrosensitive hydrogel to form the microcapsule wall. Gelatin is good for this purpose because of its long history of use in microencapsulation and in hydrogel systems, its cost effectiveness, and ready availability. Gelatin is an ionizable compound consisting of a mixture of water soluble proteins derived from water insoluble collagen fibers (64). It contains both carboxyl and amine groups. This polymer shows electrochemomechanical behavior and can also be used in various encapsulation techniques (59, 65, 66). There are two types of gelatin: acid processed, Type A; and base processed, Type B (67-69). Gelatin, Type B was used in the present work.

1.3.3.3 Polyvinylpyrrolidone

Polyvinylpyrrolidone, PVP, is a nonionizable polymer which was also used to microencapsulate sulfadiazine. PVP is relatively chemically inert and is stable in solution or powder form (70, 71). Because of the nonionic characteristics of the polymer chains, the porosity of the PVP wall of a microcapsule should not be pH dependent, as is the case with gelatin.
1.3.3.4 Polyacrylic acid

Polyacrylic acid, (PAA) gels also have been found to show electrochemomechanical behavior (51). PAA has a pKa of ~4, above which its carboxylate groups are mostly disassociated (72, 73). Above pH 4, PAA would be expected to swell due to repulsion between like groups. PAA is a hydrophilic polymer which can be coacervated, thereby making it a candidate wall material for encapsulating sulfadiazine (74).

1.3.4 Theoretical considerations

When no current is applied to the microcapsules, sulfadiazine will passively diffuse at a steady rate through the microcapsule walls. Any lag period that occurs would be due sequentially to:

- the time for equilibration of the microcapsule suspension to appropriate temperature,
- the rate of diffusion of dissolution medium into the core of the microcapsules,
- the rate of dissolution of drug in the core, and diffusion of drug through the microcapsule wall,
- diffusion of drug through the aqueous medium in the donor compartment,
- diffusion of drug through the Spectra/Por® membrane, and
- the rate of flow of eluant through the receiver compartment.
If sulfadiazine release from a microcapsule is thought of simply in terms of the diffusion of a small molecule through a spherical shell, Fick's second law for spheres can be applied to drug release from the microcapsules as long as the following assumptions are made: 1) the microcapsules are hollow spheres of equal diameter with a wall of uniform thickness, 2) the diffusion coefficient of drug through the wall is independent of drug concentration, and 3) there is no adsorption of drug to the microcapsule wall (75). Fick's second law is:

\[
\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial r^2}
\]  

Where, \( c \), is the concentration of drug in the microcapsule; \( D \), the diffusion coefficient of the drug in the hydrogel; and, \( r \), the position of drug in the microcapsule wall.

When no current is applied to the microcapsules, sulfadiazine would be expected to be released passively at a steady rate as described by Eqn. 1 above. Conversely, when current is applied to the microcapsules, a change in the rate of release should occur. Therefore, the rate of sulfadiazine release from microcapsules exhibiting electrochemomechanical properties induced by an electric current cannot be described solely by Eqn. 1. The reason for this is that electrically induced release of sulfadiazine can occur by one or a combination of five mechanisms:

- passive diffusion through the microcapsule hydrogel wall,
- diffusion through pores in the wall created by, or closed by, pH and/or ionic strength changes at the electrode surfaces,
an increase in the solubility of sulfadiazine in the donor compartment caused by pH fluctuations induced by reactions at the electrodes.

- iontophoresis of charged drug through the microcapsule hydrogel wall, and/or Spectra/Por® membrane, and

- solvent flow or electroosmosis of drug solution through the wall.

Eqn. 2 which follows can then be used to describe the total flux from this system, if the following assumptions are made: 1) the microcapsules are hollow spheres of equal diameter with uniform walls, 2) there is no adsorption of drug on the wall matrix, 3) the diffusion coefficient of the drug is not concentration dependent, 4) the hydrogel response time to the current is insignificant, and 5) the current causes the formation of uniformly sized pores. Eqn. 2 is:

\[
J_t = J_p + J_{E1} + J_{E2} + J_{E3} + J_{E4} \tag{2}
\]

where \( J_t \) is the total drug flux; \( J_p \), passive flux; \( J_{E1} \), flux through pores in the hydrogel caused by current; \( J_{E2} \), flux caused by a change in solubility of drug due to pH changes in the donor compartment, \( J_{E3} \), electrically-facilitated flux due to iontophoresis through the hydrogel wall and/or Spectra/Por® membrane; and \( J_{E4} \), electrically-facilitated flux due to electroosmosis and/or solvent flow.

Two situations can therefore be defined:

- For passive diffusion (in the absence of a current), Equation (2) reduces to \( J_t = J_p \)

- For electrically-facilitated release \( J_t = J_p + J_{E1} + J_{E2} + J_{E3} + J_{E4} \). It should be kept in mind that \( J_p \) can remain unchanged, be increased or decreased depending on the effect of current on the drug and ECM wall.
1.3.4.1 Passive diffusion ($J_p$)

Diffusion is defined as "a process of mass transfer of individual molecules of a substance, brought about by random molecular motion and associated with a concentration gradient" (76). Diffusion of a drug in solution occurs from a region of higher concentration to a region of lower concentration, and is therefore a spontaneous process. In the case of a microcapsule, the diffusion rate is dependent on the rate of solvent penetration into the microcapsule core, the dissolution rate of the core, and the diffusion rate of drug solution through the microcapsule wall (77). The polymer coating around the drug core can be used to control the diffusion of the active drug in two ways:

1) by controlling how quickly the drug solution moves through the polymer matrix

2) by controlling the movement of drug solution through pores in the matrix (76, 78, 79).

Both mechanisms can contribute to the diffusion of drug solution out of the microcapsules into the surrounding medium. The extent to which each mechanism contributes is dependent on the properties of the drug, the polymer and the dissolution medium (78). The permeability of the polymer can be altered by different factors including the type of polymer, its molecular weight and degree of crosslinking, thickness and porosity of the membrane (76, 78, 80).

There are two types of microcapsules: reservoir and matrix. The reservoir type is defined as having a solid core surrounded by a polymer wall, whereas the matrix type is defined as having the drug material distributed within a matrix formed by polymer (78,
For the purposes of this project, the reservoir type is considered since electron microscopy indicated a radial-like or star-like structure for the drug core (see Figures 3.24 and 3.32).

Passive diffusion of sulfadiazine is also expected to occur when current is applied to the microcapsules since current can cause a change in drug solubility (Section 1.3.4.2.4) and/or the porosity of the gelatin wall (Section 1.3.4.2.3). For example, if the solubility of sulfadiazine increases, the rate of release of sulfadiazine will also increase. If the hydrogel wall contracts under the influence of an electric field, drug release may be slower since the gel network is more compressed in this form and therefore functions as a greater barrier to diffusion. However if the current causes an opening of pores in the polymer matrix, the rate of drug diffusion may be increased. The chaotic physical nature of the pores and the changing of pore structure due to the application of the current can render the mathematical modeling of drug release more difficult than when no current is applied. Therefore when current is applied to the microcapsules, drug release will be defined by Equation 2 (Section 1.3.4).

1.3.4.2 Electrical factors ($J_{E1}$-$J_{E4}$)

1.3.4.2.1 Current

An electric current is a flow of electric charge through a conducting circuit due to the presence of a potential difference (82). The electric charge refers to electrons in conducting materials or ions in electrolyte solutions (83). When considering electrical
flow through an electrolytic solution, the amount of substance liberated at the electrodes is related to the amount of electricity supplied. This concept is shown in Faraday's First Law of Electrolysis that the amount of substance oxidized or reduced at an electrode is proportional to the quantity of the electricity passing through the electrolyte solution (84, 85). As discussed in the next section, these changes in ion composition occurring at the electrode surfaces can affect sulfadiazine release from gelatin-walled microcapsules.

In addition to the magnitude and duration of the current, the type of current applied to the system can also affect drug release (see Chapter 3). For example, current can be applied in a direct mode (DC) in which the current flow is in one direction, or in an alternating mode (AC) in which the flow of current changes (83). In the DC mode, reactions occurring at the electrodes may cause pH changes at the electrode surfaces which in turn can alter ionization state of the drug and polymer wall. Conversely, in the AC mode, local pH changes at the electrodes are minimal since the cathode and anode alternate. In this case, there may not be any noticeable change in sulfadiazine release from gelatin-walled microcapsules (see Section 3.3.2.1.1).

The use of an electric current to enhance drug delivery through a membrane is most recognized in iontophoresis. Iontophoresis can increase the range of drugs which can penetrate the skin, both ionized and unionized (86, 87). The interaction between the ionic charge and the electric field gives a greater driving force for penetration into the skin than does passive diffusion (84) since the ions are attracted to the opposite electrodes. In the present work, iontophoresis of sulfadiazine through the Spectra/Por® membrane or microcapsule wall may also be a factor influencing drug release (Section 3.2.3.2.1).
1.3.4.2.2 Electrodes

There are two types of electrodes: inert and active (85). Ag/AgCl electrodes produce the reaction \( \text{Ag} + \text{Cl}^- \leftrightarrow \text{AgCl} + \text{e}^- \) at the electrode surfaces, and are considered to be “active” since they participate in the electrochemical reaction. This reaction limits water hydrolysis, thereby reducing the effect of local pH change, which in itself could affect the properties of the formulation (see Sections 1.3.4.2.3 and 1.3.4.2.4). Platinum electrodes, however, are considered by comparison to be “inert”. Under neutral conditions, the reaction at the anode is \( 2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^- \), while \( 4\text{H}_2\text{O} + 4\text{e}^- \rightarrow 2\text{H}_2 + 4\text{OH}^- \) occurs at the cathode. As such, the local pH may be affected in the donor compartment which can alter the charge and solubility of the drug as well as the electrochemomechanical properties of the hydrogel (see Sections 1.3.4.2.4 and 1.3.4.2.3).

1.3.4.2.3 Gel wall matrix considerations \((J_{\varepsilon_1})\)

The application of current to the microcapsules may affect drug release in two ways:

1) opening of pores in the hydrogel matrix through which drug can more easily pass (see Figure 1.5 A).

2) closing of pores in the hydrogel matrix which act as a barrier to drug release (see Figure 1.5 B)
Figure 1.5  Schematic diagrams of the effect of electrical current on drug release from microcapsules with electrochemomechanical walls.

A. When current is turned on, pores open in the gel allowing drug and solvent to more easily pass.
B. When current is turned on, pores close in the gel providing a barrier to drug release.
If the current causes a closing off of pores, $J_{E1}$ would take on a negative value since drug release may be inhibited (Section 1.3.4, Eqn. 2). By contrast, $J_{E1}$ is positive if pores are opened since the permeability of the wall to the solutes increases.

Augmentation of drug release due to pore formation can also arise for two possible reasons:

1) opening of pores allowing dissolution medium to enter into the microcapsule core more rapidly to dissolve drug, and

2) the same opening of the pores allows outward diffusion of drug from the microcapsule.

Whether the hydrogel expands (opens pores) or contracts (closes pores) will depend on the pKas of the ionizable groups contained in the polymer chains, as well as the pH of the donor solution. Gelatin contains two ionizable groups, the carboxyl and amine groups. At its isoelectric point (pKa ~5) gelatin has an equal amount of negative and positive groups which render the membrane neutral at this pH (64, 88). If the pH is increased above its isoelectric point, gelatin becomes mostly negatively charged, and if the pH is decreased to below the isoelectric point, the fixed positive charge density of the membrane increases. If the gelatin membrane has an overall positive or negative charge, swelling and hydration will occur since the like charges on the molecules will repel each other. Conversely, if the overall charge on the gel wall is zero (at the isoelectric point), the gel is expected to contract or deswell due to attraction of the oppositely charged molecules which make up the matrix. Figure 1.6 shows these mechanisms.

If inert electrodes are used, the application of an electric current to the donor compartment can be used to change the local pH of the medium (see Section 1.3.4.2.2).
Figure 1.6 Schematic representation of the effect of pH on the charge of the gelatin chains of the microcapsule wall.
A. pH below 5: the gelatin wall swells due to repulsion between like charges
B. pH at 5: gelatin deswells at its isoelectric point
C. pH above 5: the gelatin wall swells due to the repulsion between like charges
This change in pH can change the spacing between the polymer chains of a charged membrane and therefore alter the permeability of the membrane to solutes. For example, if platinum wire electrodes are used, the pH around the anode may decrease due to proton (i.e., hydronium ion) production. If the pH drops to below 5, the gelatin chains in this region will become more positively charged, and consequently the gel will swell, allowing the formation of more aqueous channels or pores in the matrix.

Correspondingly, the pH around the cathode will increase with hydroxyl ion production. If the pH increases above 5, gelatin walls in this local area will have a net negative charge, and will swell because of the repulsion between like charges.

In the present system, the ionizable properties of the drug and the electrochemomechanical properties of the gelatin wall were used to control sulfadiazine release. For example, when current was applied under certain experimental conditions (see Chapter 3), sulfadiazine release increased. This increase resulted from the ionization or deprotonation of the drug at the cathode. Deprotonation of sulfadiazine as well as the production of protons at the anode in turn resulted in a net decrease in the pH of the donor compartment medium. Again the pH will then affect the barrier effects (swelling and deswelling) of the gelatin wall to sulfadiazine release.

As discussed in Section 1.2.6, the salt concentration or ionic strength of the medium surrounding the gel also affects its swelling properties. An electric field can alter the local ionic strength of the medium and therefore alter the degree of hydration of the gel. When an electric current is applied in the donor compartment of the cell, the mobile ions will move toward the electrode of opposite charge, whereas any charge in the membrane is fixed and cannot move. This will set up an ionic strength gradient and
therefore an osmotic pressure gradient between the inside and the outside of the gel. If
the ionic strength is higher in the donor medium than in the gel, the gel will contract as
water flows into the surrounding medium to reduce the gradient. Figure 1.7 shows an
example of such a concept. The change in ionic strength may also lead to gel contraction
by shielding the charges on the polymer chains thereby reducing repulsive forces. Again,
contraction of the gel will close pores allowing the wall to be an increasing barrier to
drug release.

1.3.4.2.4 Drug charge and solubility (J_E_2)

Solutions of solids in liquids are important in pharmaceutical practice (89). Most
sulfa drugs, including sulfadiazine have poor water solubility (62). However, by altering
the pH of the dissolution medium, the solubility of sulfadiazine can be increased or
decreased (see Section 3.2.2). For the purposes of this study, pH is a main consideration
since the electrodes produce local pH changes in the donor compartment (Section
1.3.4.2.2). For example, if the pH of the dissolution medium is such that the
concentration of the ionized form of the drug is reduced, precipitation may occur since
the solubility of the nonionized form is less than that of the ionized (salt) form.
Sulfadiazine is an ionizable compound and therefore its charge is pH dependent. Figure
1.8 shows the dissociation of sulfadiazine into its two ionized forms.
Figure 1.7  The effect of current on the ionic strength gradient and water flow inside and outside a cube of gelatin. The increase in ionic strength around the cathode causes the gel to deswell in this region.
Figure 1.8  Dissociation of sulfadiazine

\[
\begin{align*}
\text{pKa} &= 2.1 \\
\text{pKa} &= 6.3
\end{align*}
\]
The amount of ionized drug available for a weak acidic drug can be defined by the Henderson-Hasselbalch Equation:

$$\log \frac{[A^-]}{[HA]} = pH - pKa \quad (3)$$

Whereas the amount of ionized drug available for a weak basic drug can be defined by the following form of Equation 3:

$$\log \frac{[BH^+]}{[B]} = pKa - pH \quad (4)$$

The charge on a drug is not only important for solubility and dissolution rate, but also for drug absorption in the body (90, 91). According to the pH partition hypothesis, only the unionized form of a drug will pass readily through lipid membranes (91). As for gastrointestinal and percutaneous absorption, the gastrointestinal/blood barrier and the skin/blood barrier function as lipid barriers. In both cases these barriers are considered to be impermeable to the ionized form of the drug. If penetration through the membrane is desired, the nonionized form of the drug is favored. Conversely, if penetration through the membrane is not desirable, the ionized form would be favored. Therefore, by manipulating the pH of the formulation, the degree of drug absorption can be affected.
1.3.4.2.5 Iontophoresis ($J_{E3}$)

Iontophoresis is commonly defined as “the introduction of therapeutic agents into the tissues of the body by means of an electric current” (84). However, in the present system, iontophoresis refers to the movement of charged drug through a membrane. The membrane would be the gel wall of the microcapsule and/or the Spectra/Por® membrane which separates the donor and receiver compartments of the diffusion cell. The drug available for iontophoretic delivery is the amount of ionized species in the donor compartment of the cell which is dependent on the pH of the donor medium, as well as the pKas of sulfadiazine (see Equations 3 and 4). For example, if the pH of the system is above 6.3, some iontophoresis of sulfadiazine, which has a negative charge at this pH, may increase its apparent rate of release from the microcapsules and/or through the Spectra/Por® membrane. On the other hand, if the pH of the system is decreased to the point that sulfadiazine is uncharged ($2.1 < \text{pH} < 6.3$), then the release of sulfadiazine due to iontophoresis should be reduced.

1.3.4.2.6 Electroosmosis and solvent flow ($J_{E4}$)

Electroosmosis is defined as “the movement of liquid relative to a stationary charged surface by an applied electric field” (92), and occurs in the direction opposite to the membrane charge (93). In traditional iontophoresis, as described above, electroosmosis is considered to be a secondary effect, but one which can help or hinder drug delivery into the body depending on the pKas of the drug and the pH of the donor
vehicle (94). In the current system, the hydrogel wall is the membrane in which the drug diffuses, and the charge of the membrane may therefore affect osmotic flow into and out of the microcapsule. The movement of water can affect drug release in two ways:

1) by increasing water flux through the microcapsule wall into the core resulting in an enhanced rate of dissolution of drug.

2) by increasing water flux (i.e., drug solution) out of the microcapsule, resulting in a faster apparent rate of drug diffusion through the wall.

Although the direction of water flux into and out of the microcapsule is complex and not clearly defined, solvent flow in the donor compartment is thought to play a role in the enhancement of drug release when current is applied.

Mobile hydrated ions in the donor compartment may also create a net movement of solvent when current is applied (93), and this movement may lead to a displacement of microcapsules in the diffusion cell. For example, microcapsules can surround the anode or the cathode, or be equally distributed between the two electrodes, and the extent to which they migrate in the cell is dependent on the molecular weight of the polymer used for the microcapsule wall, and to the duration of the current (Section 3.5.2.2.1). Because both of the electrodes are placed directly into the donor compartment of the diffusion cell in this system, there will be ionic strength and pH gradients located between the two electrodes when the current is turned on. If the microcapsules surround the anode, it would be expected that current would cause a decrease in local pH. This in turn can affect microcapsule wall porosity and therefore affect the rate of drug release.
1.4 Objectives

The overall goal of this research is to develop a means for safe and effective controlled pulsatile release of drug. More specifically, the following will be considered:

- Development of a microcapsule formulation from which drug release can be controlled externally by an electric field.

- Determination of the mechanisms of drug release from the microcapsules under passive and electrically-facilitated conditions, and other variables which may alter release.
CHAPTER 2

EXPERIMENTAL

CONCEPT MAP

Materials and Equipment

- Sulfadiazine was the model ionizable drug for the microcapsule core and gelatin the electrochemomechanical gel wall. In order to study the release of drug from the microcapsules, release studies were performed using a thermostatted two-compartment diffusion cell with a Spectra/Por® membrane separating the donor and flow-through receiver compartments. Both passive diffusion and electrically-facilitated release of sulfadiazine were measured in this cell.

- An electric current (DC or AC) was supplied by a Scepter™ Power Supply system. Platinum wire, Ag/AgCl wire or Ag/AgCl electrodes were placed directly into the donor compartment to carry the current.

Experimental Methods

- Microcapsules were prepared using a coacervation encapsulation technique in which simultaneous drug precipitation and coacervation of the polymer occurred.

- Both passive diffusion and electrically-facilitated drug release studies were performed and sample concentrations were analyzed by HPLC.

- Various microscopy techniques were used to study the gross structural appearance of the microcapsules.
2.1 Materials and equipment

2.1.1 Materials

Materials and suppliers are listed in Table 2.1.

2.1.2 Equipment

A list of equipment and manufacturers is given in Table 2.2.

2.1.3 Experimental apparatus

A schematic diagram of the experimental apparatus used for drug release studies is shown in Figure 2.1. The apparatus consists of two-compartment diffusion cells mounted on a cell warmer, a constant temperature waterbath to control the temperature of the eluant, a pump to maintain flow of the eluant at a constant rate, and a fraction collector for the eluant.

2.1.4 Diffusion cell

The diffusion cell is made of Teflon® and contains a donor and a receiver compartment (Figure 2.2). The donor compartment is a cylinder with a diameter of
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<td>Sulfadiazine</td>
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Table 2.1 List of materials Cont.
Table 2.1 cont.

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Table 2.2 List of equipment
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<tr>
<td>Optical microscope</td>
<td>Ernst Leitz Wetzlar, Germany</td>
</tr>
</tbody>
</table>
Figure 2.1  Experimental apparatus for drug release studies.
Figure 2.2  The diffusion cell with donor compartment and flow-through receiver compartment.
1.10 cm and a working volume of 1.05 mL. The receiver compartment is flow-through in design so that sink conditions a flow-rate can be maintained.

2.1.5 Spectra/Por® membranes

The Spectra/Por® membrane is an amorphous material made from regenerated cellulose (95). In this system, Spectra/Por® 1 membrane tubing (MWCO 6000-8000) and Spectra/Por® 2 membrane tubing, (MWCO 12000-14000), were cut into 2 x 2 cm pieces and placed in the diffusion cell to separate the donor and receiver compartments. New membranes were used for each experiment and were soaked for at least 1 h in the appropriate eluant prior to use. Unless otherwise noted, two layers of membrane were used in all experiments.

2.1.6 Electrodes

Ag/AgCl wire electrodes, commercially available Ag/AgCl electrodes (In Vivo Metric), and platinum wire electrodes were used. The Ag/AgCl wire was 0.5 mm in diameter and the platinum wire electrodes were 1 mm in diameter. The In Vivo Metric Ag/AgCl electrode segment was 0.8 mm in diameter and 8 mm in length.
2.1.7 Electrode holder apparatus

For the initial experiments, the wire electrodes were held in the donor compartment by a wrapping of Parafilm®. Since this method allowed for variation of electrode placement within the cell, a new device was fabricated consisting of a rubber stopper in which the electrodes were spaced 0.5 cm apart and extended 0.7 cm into the donor compartment (see Figure 2.3). An airhole was provided to vent gas produced at the electrode surfaces.

2.1.8 External power supply

A Scepter™ Programmable Power Supply was the controller/power source and data logger for this model system (96). The Scepter™ software system runs on an IBM-compatible PC and has twelve programmable channels which allow up to twelve diffusion cells to be operated simultaneously. With this system, direct current can be altered or held constant with an accuracy of ~ 0.1%, over a maximum voltage range of up to 10 v. Alternating current can also be applied at a frequency of 60 Hz ± 1%, with a maximum voltage range of up to 10 v.

2.2 Experimental methods

The experimental protocol involved first the microencapsulation of sulfadiazine. Following this, in vitro release of sulfadiazine from the microcapsules was determined by
Figure 2.3 The donor compartment holder for platinum wire electrodes with an airhole for venting hydrogen gas and air pressure.
passive diffusion and under the influence of an electric current. Unless otherwise noted, all drug release and pH and solubility studies were performed three times (n = 3).

2.2.1 Preparation of microcapsules

Microcapsules with electrochemomechanical walls were prepared from a suitable polymer and sulfadiazine using a coacervation method (97,98). The coacervation method uses pH change in aqueous solutions of drug and polymer to simultaneously form and encapsulate small particles. Since the solubility of sulfadiazine is pH dependent, it is a suitable compound for such a microencapsulation technique. With sulfadiazine, the pH change is from at least two pH units above its pKa to at least two pH units below its pKa. Therefore, upon the addition of a suitable acid, simultaneous precipitation of drug and coacervation of the polymer will form the microcapsules. The sequence of steps is as follows:

1) Sodium sulfadiazine is dissolved in water and Na₂SO₄, at least two pH units above the upper pKa of sulfadiazine.

2) A coacervation and wetting agent, such as alcohol or sodium lauryl sulfate, is added and the solution is stirred.

3) Polymer is added to the solution and stirred until dissolved.

4) Hydrochloric acid is then added as a bolus under stirring to decrease the pH of the mixture to at least two pH units below the pKa of sulfadiazine.

5) The mixture is diluted with cold sodium sulfate solution to gel or “harden” the encapsulating material.
2.2.2 HPLC assay for sulfadiazine

HPLC was used to determine the concentrations of sulfadiazine released from the microcapsules under passive and electrically-facilitated conditions.

Column: Reverse Phase, Hamilton PRP-1, 4.1 mm x 25 cm
Mobile Phase: 11:1, 0.1 N Sodium Borate: Acetonitrile
Flow Rate: 1.0 mL min\(^{-1}\)
Wavelength: 254 nm

2.2.3 Passive diffusion and electrically-facilitated in vitro release studies of sulfadiazine from microcapsules

Microcapsules with electrochemomechanical walls were prepared from polymer (gelatin, polyvinylpyrrolidone, or polyacrylic acid), and sulfadiazine using the coacervation method described above (Section 2.2.1). The resulting suspension was centrifuged at 25 °C at 1000 rpm for 8 min in a Beckman, Model GS-6KR centrifuge, the supernate decanted, and the microcapsules resuspended in an appropriate medium. In most cases, the resuspension of gelatin microcapsules was in 2 mL of normal saline solution or appropriate eluant. In the case of the PVP microcapsules, resuspension was in ~1.1 mL of eluant. The resulting microcapsule suspension (0.7 mL) was then placed in the donor compartment of the diffusion cells. Some of the PAA microcapsules did not sediment upon centrifugation apparently due to variations in size in that the smallest microcapsules sediment more slowly. Therefore an estimated amount, comparable to the
concentrations of the gelatin and PVP-walled microcapsules (≈ 24 mg/mL) was placed into the donor compartment and release studies were performed.

Passive diffusion of sulfadiazine was studied using the two-compartment diffusion cell with a Spectra/Por® membrane separating the donor and receiver compartments. Unless otherwise noted, a Spectra/Por® 2 membrane was used for both passive diffusion and electrically-facilitated release studies. The receiver compartment was flow-through in design and samples of eluant were collected periodically in the fraction collector at a constant rate of 1 mL min⁻¹. For most studies, a normal saline solution of appropriate pH was used as the eluant. The temperature of the cell was maintained at ~ 37 °C using a cell warmer, and the temperature of the bulk eluant was maintained at ~ 32 °C in a thermostatted waterbath. Samples were collected every 5 or 6 min and analyzed by HPLC (Section 2.2.2).

Electrically-facilitated release of sulfadiazine from the microcapsules was determined using the same diffusion cell and fraction collector as described above. The microcapsule suspension (0.7 mL) was placed directly into the donor compartment of a diffusion cell and the anode and the cathode were inserted (see Figure 2.4). In the initial studies, Ag/AgCl wire and Ag/AgCl electrodes (In Vivo Metric) were held in place by layers of Parafilm®. Various other methods of supporting the electrodes were tried to more consistently secure and place them in the donor compartment, with the ultimate resolution being a rubber stopper with holes for electrode placement (see Section 2.1.6). Pulsatile release was tested by cycling the current on and off for varying periods of time. Unless otherwise noted, a 0.5 mA DC was applied from t = 20 to t = 50 min of a 70 min release study. Drug concentrations in the eluant were also analyzed by HPLC.
Figure 2.4 Schematic diagram of the diffusion cell donor compartment containing electrodes and microcapsules.
2.2.3.1 Preparation of gelatin/sulfadiazine microcapsules

Gelatin/sulfadiazine microcapsules were prepared as described in Section 2.2.1. The gelatin walls of the microcapsules were gelled upon the addition of cold sodium sulfate solution to give a more compacted or “hardened” wall due to partial desolvation. For comparison purposes, unhardened microcapsules were prepared by omitting the final gelling step. Drug release studies were carried out for 70 minutes with samples collected every 5 minutes. For studies of electrically-facilitated release, platinum wire electrodes were used, and a 0.5 mA direct current was applied from \( t = 20 \) to \( t = 50 \) min.

2.2.3.2 Effect of current on the temperature of the donor compartment contents

A suspension of gelatin/sulfadiazine microcapsules (0.7 mL) was placed into the donor compartment of the diffusion and platinum wire electrodes were used in all release studies. The temperature of the suspension in the donor compartment was measured using a Fisher Scientific Traceable\textsuperscript{®} thermometer. Temperature was recorded before and after (at \( t = 0 \) and \( t = 30 \) min) 0.5 mA and 1.0 mA direct current were applied for 30 min.

2.2.4 Passive and electrically-facilitated in vitro release studies from sulfadiazine suspensions

Solutions of sodium sulfadiazine and suspensions sulfadiazine were used for studies of passive and electrically-facilitated release. Sodium sulfadiazine or sulfadiazine
was added to the appropriate suspending medium (i.e., normal saline or phosphate buffer) resulting in \( \sim 9.6 \times 10^{-5} \) mol/mL of the drug. Suspensions or solutions of sodium sulfadiazine and sulfadizine (0.7 mL) were placed into separate cells for the release studies. For electrically-facilitated release studies, platinum wire electrodes were used, and a 0.5 mA direct current was applied from \( t = 20 \) to \( t = 50 \) min.

### 2.2.5 Effect of pH on sulfadiazine release from gelatin-walled microcapsules

All pH readings were taken with a MI-410 combination microelectrode, (Microelectronics, Inc., Bedford, NH) by inserting the electrode directly into the donor compartment medium or into the eluant during release studies. The experimental protocols for release studies on sulfadiazine suspensions, gelatin/sulfadiazine microcapsules and PVP/sulfadiazine microcapsules are as follows:

- **Protocol 1**
  Normal saline solution was adjusted to pH 1.7 with HCl, and was used as the eluant and suspending medium for the microcapsules or drug.

  Average pH of Eluant: 1.7  
  Average pH of Donor medium: 2.0*

- **Protocol 2**
  Normal saline solution was adjusted to pH 3.7 with HCl, and was used as the eluant and suspending medium for the microcapsules or drug.

  Average pH of Eluant: 3.7  
  Average pH of Donor medium: 4.5*

- **Protocol 3**
  The pH of normal saline solution was approximately 5.8. This solution was used as the eluant and suspending medium for the microcapsules or drug.
Average pH of Eluant: 5.8
Average pH of Donor medium: 6.0*

- Protocol 4
  Normal saline solution was adjusted to pH 10.9 with NaOH, and was used as the eluant and suspending medium for the microcapsules or drug.
  
  Average pH of Eluant: 10.9
  Average pH of Donor medium: 7.0*

  * after the addition of drug or microcapsules

2.2.6 Studies of an isolated donor compartment

A scaled-up model of the donor compartment of the diffusion cell was made in a glass beaker. Suspensions of gelatin microcapsules were prepared and then were centrifuged. The resulting concentrates were suspended in 25 mL of normal saline, and placed in this model donor compartment. Both platinum electrodes were also placed in the beaker and appropriate current was applied. Samples were taken every 10 min, filtered or centrifuged as necessary, and analyzed by HPLC.

2.2.7 Solubility of sulfadiazine

The solubilities of sulfadiazine in water and in normal saline solution (pH 1.7, 3.7, 5.8 and 10.9) were determined at room temperature (~ 22 °C) and 32 °C. Samples consisting of 0.1 g of sulfadiazine were suspended in 40 mL of water or normal saline and placed in vials with screw-caps which were then over-wrapped with Parafilm®. The
vials were rotated end-over-end at 41 rpm at room temperature or at 32 °C in a Vankel sustained release apparatus. After 24 h, the vials were allowed to stand at room temperature or 32 °C for 24 h. 2 mL of the water or normal saline suspension was filtered, and 1.0 mL of the filtered saturated solution was diluted to 10 mL with the appropriate solution. The concentration of sulfadiazine in the resulting sample was analyzed by HPLC.

2.2.8 Gelatin swelling and deswelling

A 5% gelatin (Type B)/water (w/v) solution was prepared. This concentration of gelatin was used since a 5% gelatin/water solution is used in the encapsulation method. The resulting gel was refrigerated overnight (~ 20 h) after which it was allowed to stand at room temperature. Small cubes (~2 x 1.5 x 1 cm) were cut, weighed, and placed in 50 mL water of appropriate pH (1.7, 3.7, 5.8, 10.9). After 20 h, the gels were removed from the water and reweighed to determine the amounts of water contained in the hydrogels.

2.2.9 Gelatin porosity

A 5% (w/v) gelatin gel was prepared from Type B gelatin and water. The resulting gel was refrigerated overnight (~ 20 h) after which it was allowed to stand at room temperature. The gel was frozen in liquid nitrogen. The frozen pieces were broken so that a clean surface was exposed. The resulting sample was dried in a desiccator for SEM analysis.
2.2.10 Optical microscopy

Optical microscopy, both brightfield and polarized light, was used to determine the sizes and morphologies of the microcapsules. Microcapsules were prepared as indicated in Section 2.2.1. Samples of the microcapsule suspension (25 mL) were placed into 50 mL centrifuge tubes and centrifuged at 1000 rpm for 8 min at 25 °C. The supernate was decanted and the microcapsules resuspended in 2 mL of normal saline solution. Samples were observed before and after studies of passive diffusion and electrically-facilitated release of sulfadiazine.

2.2.11 Scanning electron microscopy

Scanning electron microscopy (SEM), was used for further determinations of the sizes and morphologic characteristics of the microcapsules. Microcapsules were centrifuged at 1000 rpm for 8 min at 25 °C, the supernate decanted, and the microcapsules allowed to air-dry in a glass petri dish. The petri dish was then placed in a glass desiccator filled with Drierite® and the microcapsules were allowed to dry for at least 48 h.

In the case of the gelatin microcapsules, samples were also prepared using the contents of the donor compartment after release studies were performed. More specifically, a microcapsule suspension (0.7 mL) was placed in the donor compartment of a diffusion cell, and release studies were performed as described in Section 2.2.3 with normal saline as the eluant. Studies of passive diffusion and electrically-facilitated
release were carried out for 70 min, 4 h, and 5 h. In the case of electrically-facilitated release, 0.5 mA DC was applied using platinum wire electrodes from \( t = 20 \) to \( t = 50 \) min for the 70 min study, from \( t = 30 \) to \( t = 60 \), 90 to 120, 150 to 180 and 210 to 240 min for the 4 h study, and from \( t = 30 \) to \( t = 60 \), 90 to 120, 150 to 180, 210 to 240, and 270 to 300 min for the 5 h study. After the release studies were performed, the microcapsules were collected from the donor compartments, placed in a petri dish in a desiccator, allowed to dry for at least 48 h, and examined by scanning electron microscopy.

SEM was also used to examine the porosity of a gelatin gel. A 5% gelatin/water w/v gel was prepared, frozen in liquid nitrogen, and dried in a desiccator. Dry gelatin samples were mounted on stubs with silver paint for SEM studies (see Section 2.2.9).

2.2.12 Transmission electron microscopy

Transmission electron microscopy (TEM), was used to study the morphology and ultrastructure of the microcapsules. Gelatin/sulfadiazine microcapsules were prepared and centrifuged at 1000 rpm for 8 min at 25 °C. The supernate was decanted and the remaining concentrated suspension of microcapsules was frozen in liquid nitrogen for TEM.
2.2.13 Environmental electron scanning microscopy

Sulfadiazine/gelatin microcapsules and 25 mL of the microcapsules suspension was centrifuged for 8 min at 1000 rpm at 25 °C. The supernate was decanted and the microcapsule sediment was used for the ESEM studies.
CHAPTER 3

RESULTS AND DISCUSSION

CONCEPT MAP

Sulfadiazine Suspensions

- The rate of precipitation of sodium sulfadiazine from solution can influence drug particle size and structure.
- Due to the 2 pK_{a}s of sulfadiazine, its solubility is dependent on pH.
- Passive diffusion studies using sulfadiazine suspensions also show that sulfadiazine release is dependent on pH.
- The application of an electric current to sulfadiazine suspensions can alter drug solubility and thereby affect the amounts of sulfadiazine released. This is most likely due to pH changes occurring at the electrode surfaces.
- Iontophoresis of the sulfadiazine ion does not appear to be a major factor contributing to electrically-enhanced drug release.

Gelatin/Sulfadiazine Microcapsules

- Gelatin can swell or deswell depending on the pH of the surrounding medium.
- Microscopy shows a radial structure consisting of sulfadiazine crystals which are presumed to be coated with gelatin.
- Studies of the release of sulfadiazine were carried out in order to evaluate the roles of the following factors: the procedure for microcapsule preparation; ionic strength, pH and temperature of the donor medium and eluant; and the type of electrode on sulfadiazine release.
Electrically-facilitated release of sulfadiazine was dependent on the magnitude, duration, and type of current; and on the pH of the donor compartment medium. Pulsatile drug release was achieved using on/off current cycles.

PVP/sulfadiazine Microcapsules

- Microscopy shows a radial structure of sulfadiazine crystals which are presumed to be coated by a PVP wall.
- Passive diffusion and electrically-facilitated release of sulfadiazine was dependent on the pH of the dissolution medium.
- The sizes and masses of the microcapsules may affect their rate of migration within the donor compartment when current is applied.

Comparison of sulfadiazine release from sulfadiazine suspensions and from gelatin-walled and PVP-walled microcapsules

- Passive diffusion and electrically-facilitated release of sulfadiazine from all three formulations was dependent on the pH of the suspending medium.
- The gelatin wall becomes a barrier to release when the pH nears the isoelectric point of gelatin due to the closing of aqueous pores; there is less of a barrier effect below and above this pH since aqueous pores should open.
3.1 **Introduction: Properties of formulations: sulfadiazine suspensions, gelatin/sulfadiazine, PAA/sulfadiazine and PVP/sulfadiazine microcapsules**

The purpose of this project was to develop a controlled pulsatile microcapsule drug delivery system which utilizes the ionizable properties of a drug core and the electrochemomechanical properties of a hydrogel wall. By taking advantage of these two properties, electrical control of drug release was achieved. In Chapter 1, several factors were discussed which may contribute to this electrically-facilitated drug release. These factors are:

- passive diffusion through the microcapsule hydrogel wall,
- diffusion through pores in the microcapsules wall created by, or closed by, pH and/or ionic strength changes at the electrode surfaces,
- increased solubility of sulfadiazine in the donor compartment due to pH fluctuations induced by reactions at the electrodes.
- iontophoresis of charged drug through the microcapsule hydrogel wall, and/or Spectra/Por® membrane, and
- solvent flow or electroosmosis of drug solution through the microcapsule wall.

Experiments described in the following sections were intended to elucidate the mechanisms responsible for such electrically controlled pulsatile release. For this purpose, the following three formulations were compared to evaluate properties of the wall materials:

- sulfadiazine suspensions
Specifically, all three systems showed reversible increases in sulfadiazine release when current was applied. This type of reversible behavior is important to allow reproducible pulsatile release patterns for drug delivery. Sulfadiazine suspensions were used as the non-microencapsulated “control” to evaluate the effects of an electric current on the properties of an ionizable drug. Gelatin/sulfadiazine microcapsules were a model system in which the pH sensitive electrochemomechanical gelatin wall should swell and/or deswell in response to a current. And finally, PVP/sulfadiazine microcapsules were also used as a model system since PVP is a nonionizable polymer and therefore should not be affected by pH changes induced by an electric current.

Sulfadiazine solubility and the swelling and deswelling properties of gelatin were dependent on the pH of the donor medium due to changes in the ionic state of the drug and in the gelatin chains. In order to determine the effects of pH on sulfadiazine release from gelatin-walled microcapsules the following four experimental protocols were developed as described in Chapter 2, Section 2.2.5:

- Protocol 1 (Average pH of eluant: 1.7; Average pH of donor medium: 2.0)
- Protocol 2 (Average pH of eluant: 3.7; Average pH of donor medium: 4.5)
- Protocol 3 (Average pH of eluant: 5.8; Average pH of donor medium: 6.0)
- Protocol 4 (Average pH of eluant: 10.9; Average pH of donor medium: 7.0)

It is recognized that the pH values of the eluants do not represent physiologic conditions (pH ~7.4), however the conditions for the protocols were chosen so the
mechanism of electrical-facilitation of the release of sulfadiazine could be evaluated as a function of pH. Since the pH of the suspending medium influences the degree of ionization of sulfadiazine and of gelatin with these four protocols, the different ionization states of the drug (pKₐ 2.1 and 6.3) and of gelatin (isoelectric point ~5) are represented. In this context, sulfadiazine is positively charged below pH 2.1, uncharged between pH 2.1 and 6.3, and negatively charged above pH 6.3. Similarly, gelatin swells above and below pH 5, and deswells at its isoelectric point of pH 5 due to changes in its ionization state. Further explanations of these properties can be found in Chapter 1, Sections 1.3.4.2.3 and 1.3.4.2.4).

3.2 Sulfadiazine suspensions (the control)

3.2.1 Morphology

Sulfadiazine is a white crystalline material that forms needle-like structures (see Figures 3.1 and 3.2), which precipitate from a solution of sodium sulfadiazine upon the addition of a suitable acid. The rate at which acid is added can determine the shape and size of the crystals. For example, when the acid is added slowly, large, needle-shaped crystals form, whereas faster additions of acid will result in smaller star- or radial-shaped structures of crystals (see Figures 3.3-3.5). Precipitation of sulfadiazine in this manner, i.e., by the addition of a suitable acid, but from solutions also containing a suitable wall material, was the method used in this work to form microcapsules of sulfadiazine. This process resulted in the simultaneous precipitation of sulfadiazine and coacervation of the
Figure 3.1  Photomicrograph of sulfadiazine crystals as received. Brightfield, 788x.
Figure 3.2  Photomicrograph of sodium sulfadiazine crystals as received. Brightfield, 788x.
Figure 3.3  Photomicrograph of sulfadiazine crystals formed by slow precipitation from an aqueous solution of sodium sulfadiazine upon the addition of HCl. Brightfield, 788x.
Figure 3.4 Photomicrograph of sulfadiazine crystals formed by rapid precipitation from an aqueous solution of sodium sulfadiazine upon the addition of HCl. Brightfield, 788x.
Figure 3.5  Photomicrograph of sulfadiazine crystals formed by rapid precipitation from an aqueous solution of sodium sulfadiazine upon the addition of HCl. Brightfield, 788x.
polymer to form the microcapsules. As in the case of the crystals, the rates of precipitation can also affect the ultimate size of the microcapsules (98). This is an important consideration since the size and shape of the sulfadiazine crystals forming the core of the microcapsules may affect its rate of release due to proportionally higher or lower surface areas available to the dissolution medium.

3.2.2 Solubility

The solubility of sulfadiazine was studied to determine the degree to which it is affected by temperature, pH and ionic strength. Sulfadiazine is an ionizable compound with pKas at 2.1 and 6.3 (see Section 1.3.4.2.4). Below pH 2.1 it is positively charged, while above pH 6.3 it is negatively charged; and mostly uncharged between these two pH values. Due to the different ionization states of sulfadiazine, its solubility is therefore dependent on pH. As already mentioned, four pH values of 1.7, 3.8, 5.8 and 10.9 were chosen for the solubility studies to represent the different ionization states of sulfadiazine. Table 3.1 gives the solubilities of sulfadiazine at 32 °C in normal saline solution at pH 1.7, 3.7, 5.8 and 10.9. The higher solubilities shown in normal saline at pH 1.7 and 10.9 represent higher concentrations of sulfadiazine due to its more soluble salt forms (> 50 % and > 80% ionized, respectively), whereas the lower solubilities in normal saline at pH 3.7 and 5.8 represent proportionally higher concentrations of its uncharged form, i.e., 184 μg/mL at pH 1.7, 283 μg/mL at pH 10.9, 90.6 μg/mL at pH 3.7 and 82.0 μg/mL at pH 5.8.
<table>
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<th>pH</th>
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</tr>
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<td>5.8</td>
<td>82.0</td>
</tr>
<tr>
<td>10.9</td>
<td>283</td>
</tr>
</tbody>
</table>

Table 3.1 Solubilities of sulfadiazine in normal saline solution at 32 °C
Sulfadiazine solubility also varies with temperature. In normal saline solution at room temperature (22-24 °C), its solubility is lower than its solubility in normal saline at 32 °C (i.e., 55.4 μg/mL vs. 82.0 μg/mL). Ionic strength appears however, to have only a slight affect on sulfadiazine solubility at 32 °C, i.e., its solubility is 82.0 μg/mL in NaCl solution (ionic strength 0.154 M) compared with 74.5 μg/mL in NaCl solution (ionic strength 0.75 M).

3.2.3 *In vitro* release of sulfadiazine from sulfadiazine suspensions

In Section 3.2.2 it was shown that the aqueous solubility of sulfadiazine is dependent on pH. In this context, an electric current applied directly in the donor compartment of a diffusion cell can be used to change the pH in the vicinity of the electrodes, as was discussed in Section 1.3.4.2.2. This electrically-induced pH change can be used therefore to control sulfadiazine solubility within the diffusion cell and in turn the release of drug into the receiver compartment.

Studies of sulfadiazine release by passive diffusion and electrical-facilitation were first performed with sulfadiazine suspensions in order to determine the effect of an electrical current on sulfadiazine release in the absence of the microcapsule wall. Figures 3.6-3.10 show sulfadiazine release under passive diffusion and electrically-facilitated conditions as functions of pH using Protocols 1 - 4, and are described in the following sections.
Figure 3.6 Release of sulfadiazine by passive diffusion from sulfadiazine suspensions as functions of donor medium pH. Donor medium: normal saline solution at pH 2.0, 4.5, 6.0 and 7.0.
Release of sulfadiazine from sulfadiazine suspensions. Protocol 1; donor medium: normal saline solution (pH 2.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.8 Release of sulfadiazine from sulfadiazine suspensions. Protocol 2; donor medium: normal saline solution (pH 4.5); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.9 Release of sulfadiazine from sulfadiazine suspensions. Protocol 3; donor medium: normal saline solution (pH 6.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.10  Release of sulfadiazine from sulfadiazine suspensions. Protocol 4; donor medium: normal saline solution (pH 7.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
3.2.3.1 Passive diffusion

When Protocol 1 (donor pH 2.0) was used for studies of release by passive diffusion, it was found that sulfadiazine solubility, and hence the amounts of sulfadiazine released after 70 min were higher than those when Protocols 2 and 3 were employed, i.e., 37.8 ± 2.23 µg for Protocol 1 vs. 15.1 ± 0.58 µg and 14.5 ± 0.40 µg for Protocols 2 and 3, respectively. This would be expected since the pH of the donor medium was close to the lower pKa of sulfadiazine (2.1), leaving the drug partially ionized (>50% ionized). As discussed in Section 3.2.2, the solubilities of sulfadiazine in normal saline solution at pH 3.7 and 5.8 are low, and this is reflected in the lower amounts released by passive diffusion under the conditions of Protocols 2 and 3. When Protocol 4 (donor pH 7.0) was used instead, sulfadiazine solubility was high due to ionization (>80%) and the amount released (253 ± 33.8 µg) was the highest in this study (Figure 3.6). Therefore by altering the pH of the donor compartment medium different rates of release can be achieved.

3.2.3.2 Electrically-facilitated release

The effect of current on the pH of the donor medium is more difficult to define since the application of current causes local pH fluctuations to occur at the electrodes. When current is applied to platinum wire electrodes, protons are produced at the anode due to water hydrolysis, while hydroxyl ions are produced at the cathode (see discussion in Section 1.3.4.2.2). The extent to which these ions are produced depends on the duration and magnitude of the current (Section 1.3.4.2.1). For these studies, a single
pulse of 0.5 mA DC was applied to the donor compartment suspension for a period of 30 minutes. Sample t-tests were run on the total amounts of sulfadiazine released after 70 min for the passive diffusion and electrically-facilitated release portions (see Table 3.2), using the four protocols outlined in the previous section.

For Protocol 1, sulfadiazine release was not affected by the application of current ($p = 0.884$), indicating that any pH changes at the electrode surfaces were not sufficient to cause changes in drug release (see Figure 3.7). Conversely, Figures 3.8-3.10 and Table 3.2 show that when Protocols 2, 3 and 4 were used there were significant increases in the amounts of sulfadiazine released compared with that by passive diffusion, i.e., 7.68, 10.8, and 1.38 fold increases.

The outcome of these release studies can be explained by the ionization of sulfadiazine due to pH changes in the donor compartment induced by the electric current. As already discussed, sulfadiazine has 2 $pK_a$s, and therefore its solubilities and rates of release are dependent on pH. In other words, the increases in release are most likely due to increases in the solubility of sulfadiazine caused by its degree of ionization resulting from electrically-induced pH changes. Under these conditions, (i.e., an increase in the ionization of sulfadiazine), iontophoresis of charged drug through the Spectra/Por® membrane is also a possibility, however, its effects may be negligible in these experiments (see Section 3.2.3.2.1).

For Protocols 2 and 3, the initial pH values of the donor compartment media were approximately 4.5 and 6.0, respectively. A decrease in pH below 2.1 at the anode will ionize sulfadiazine and an increase in release should occur. However, if the pH is maintained between 2.1 and 6.3, no appreciable change in solubility and amounts
<table>
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<td>Std. Dev.</td>
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<tr>
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Table 3.2  Statistical t-test comparisons of the release of sulfadiazine from sulfadiazine suspensions, by passive diffusion and electrical-facilitation after 70 min.

^a^ indicates the Satterthwaite p-value for unequal variances was used
^b^ indicates marginal significance
^c^ indicates statistically significant
released should occur since sulfadiazine is uncharged in this range. The most likely explanation for the electrically-induced increase in release is that an increase in pH at the cathode will cause an increase in sulfadiazine solubility when pH exceeds 6.3 from ionization or deprotonation of the drug, and consequently there will be an increase in the release of drug.

For Protocol 4, the initial pH of the donor compartment medium was approximately 7.0. At this pH, sulfadiazine is approximately 83% ionized, and its solubility and release are high. When current is applied, the ionization of sulfadiazine at the cathode may be masked in terms of the amounts of drug released due to the initial high passive diffusional flux of the drug. Therefore, the application of an electric current (i.e., 0.5 mA DC) to the microcapsules under these conditions should cause a slight increase in release compared to that by passive diffusion. This was indeed found with a 1.38 fold increase in release.

3.2.3.2.1 Iontophoresis

In order to determine if iontophoresis of sulfadiazine ion occurs through the Spectra/Por® membrane, phosphate buffer (pH 6.0) was used as both the eluant and the suspending medium for sulfadiazine suspensions. Although, only ~33% of sulfadiazine is ionized at pH 6.0, it was chosen for these experiments since it approximately matches the pH of normal saline solution (Protocol 3), the system in which the highest increases in electrically-facilitated release of sulfadiazine occurred (~10.8 fold). Phosphate buffer was also used to eliminate pH fluctuations from electrode reactions in the donor
compartment. If iontophoresis occurred, an increase in drug release would be expected when current is applied due to electrically-induced migration of charged drug through the donor compartment and membrane. Accordingly, Figure 3.11 shows that there is no apparent increase in the release of sulfadiazine when 0.5 mA DC was applied for 30 min, suggesting that iontophoresis of the drug ion through the Spectra/Por® membrane was negligible under these conditions.

To further study the possibility of iontophoresis, sodium sulfadiazine, which has a high aqueous solubility, was used for release studies conducted under the same conditions as described above, i.e., using phosphate buffer. The salt form of the drug is ionized and therefore may be more susceptible to electrically-induced migration toward the oppositely charged electrode. In fact, the application of current to suspensions of sodium sulfadiazine resulted in a 2.3 fold increase in the amount of drug released compared with that by passive diffusion (Figure 3.12). This suggests that iontophoresis may occur in this system. However, when normal saline solution was used as both the eluant and the suspending medium, there was no increase over that from passive diffusion when current was applied (see Figure 3.13); rather, precipitation of sulfadiazine occurred in the donor compartment (Figure 3.14). The amount of sodium sulfadiazine used in these experiments (~26 mg/mL) was in solution. Precipitation of drug occurred at the anode, where pH would be reduced sufficiently to decrease its solubility. Iontophoresis did not occur in this system since current is most likely carried by the smaller, more mobile ions, i.e., Na⁺, Cl⁻, hydronium and hydroxyl ions, rather than the larger
Figure 3.11  Release of sulfadiazine from sulfadiazine suspensions. Donor medium: phosphate buffer (pH 6.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.12  Release of sulfadiazine from sodium sulfadiazine suspensions. Donor medium: phosphate buffer (pH 6.0); current: 0.5 mA DC from \( t = 20 \) to \( t = 50 \) min; and electrodes: platinum wire.
Figure 3.13  Release of sulfadiazine from a solution of sodium sulfadiazine. Donor medium: normal saline solution (pH 6.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.14  Photomicrograph of sulfadiazine precipitate from a solution of sodium sulfadiazine in the donor compartment of the diffusion cell. Brightfield, (788x). Donor medium, normal saline (pH 6.0); current, 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes, platinum wire.
sulfadiazine ion. On the other hand, when phosphate buffer was used, which consists of larger ions than does normal saline solution, the sulfadiazine ion may carry part of the current, resulting in some iontophoresis.

3.3 Gelatin/sulfadiazine microcapsules (the model ionizable drug and ECM wall)

3.3.1 Morphology

Gelatin is a water-soluble product of denatured collagen. Collagen in turn, is a proteinaceous compound composed of amino acid linkages joined by peptide bonds to form a polymer. The collagen monomer unit, tropocollagen, consists of a triple helix of three polypeptide chains each having a helically coiled configuration (60). The ordered arrangement of the collagen molecules appears to be due to the side chains on the amino acid groups which interact with other groups in adjacent molecules through hydrogen bonds, ionic bonds, hydrophobic interactions and some covalent crosslinks (64).

Gelatin can be obtained from collagen by partial acid hydrolysis (Type A gelatin) or by a partial alkaline liming process (Type B gelatin) (68, 69). For example, the triple helical structure of tropocollagen can be destroyed by breaking the hydrogen bonds and hydrophobic interactions that stabilize the collagen helix. Next, there is a further detanglement of the chains into smaller components in random configurations. Therefore, unlike collagen which takes on a very ordered structure, gelatin usually consists of a random arrangement of amino acids joined by peptide linkages.
3.3.1.1 Gelatin-gel porosity

Gelatin is soluble in warm water (above 40 °C), but forms a gel upon decreasing temperature or upon the addition of a salt such as sodium sulfate which can coacervate the gelatin chains. When a gelatin gel network forms, there is a partial reordering of gelatin molecules into the collagen helical structure (67). The gelatin gel can therefore be considered to be a three-dimensional gel network containing entrapped water.

A microcapsule wall can be obtained by coacervation of gelatin onto sulfadiazine crystals (see Section 2.2.1). Unfortunately, the structure and porosity of the gelatin microcapsule wall was not detectable using optical or scanning electron microscopy (SEM), perhaps due to its thinness. Therefore, a 5% (w/v) gelatin gel was prepared from Type B gelatin for SEM studies as a means of evaluating the porosity of the gel. The gel was cut, frozen in liquid nitrogen, and the frozen pieces broken to produce clean surfaces for study, following which they were dried. Figures 3.15 and 3.16 show pores in the gel, which have a random structural organization, but which vary considerably in diameter. This could be due to the partial disorganization of the gelatin chains inherent in the structure of the gel, or to effects from drying the matrix.

3.3.1.2 Gelatin swelling and deswelling

Gelatin is amphoteric and therefore reacts with both acids and bases. The amino acid residues in the chains may contain carboxyl and amino groups which exchange protons and other ions with a surrounding aqueous medium. As indicated in Chapter 1, the
Figure 3.15  Scanning electron photomicrograph of a gelatin gel (5252x)
Figure 3.16    Scanning electron photomicrograph of a gelatin gel (1260x)
isoelectric point of Type B gelatin used for the microcapsule walls is \( \sim \text{pH 5} \). Below this pH, gelatin is positively charged, whereas above, it is negatively charged. The ionization of the chains can cause swelling of the gel, thereby opening aqueous channels within the gel matrix. This type of ECM behavior is essential for achieving a controlled pulsatile drug release system (see Chapter 1, Section 1.2.7).

A 5% w/v gel was prepared from Type B gelatin and water. The gel was allowed to cool for 24 h after which rectangular pieces were cut and weighed. The samples were placed in 50 mL of water at pH 1.7, 3.7, 5.8 or 10.9. After 20 hours, the samples were dried and weighed. Table 3.3 shows the weights of the gelatin samples before and after placement into water. At pH 1.7 and 10.9, the gelatin gel showed considerable swelling, and was fragile in that breakage occurred easily during handling for weighing. At pH 10.9 there was about a five fold increase in weight from the original sample due to the uptake of water. Conversely, at pH 3.7 and 5.8, there were minimal to no weight changes or swelling of the gels. This can be explained by the proximity of these two pH values to the isoelectric point of gelatin. The net charge of gelatin should be neutral at pH 5 and therefore, no swelling would be seen. In this context, Figures 3.17-3.20 show samples of gelatin before and after placement into water at pH 5.8 and 10.9. Swelling is evident at pH 10.9 (see Figures 3.19 and 3.20), since the gel appears larger in size due to increased amounts of water held in the gelatin matrix. Conversely, after the gel equilibrates in water at pH 5.8 (Figure 3.18), little swelling is evident. Although the gel was photographed in a different orientation than Figure 3.17, the gel appears to be the same size as it was before placement in water, again indicating little swelling occurs at pH values close to the isoelectric point of gelatin (pH \( \sim 5 \)).
Table 3.3  Weights of gelatin gels as functions of pH before and after placement in water for 20 hours.

<table>
<thead>
<tr>
<th>pH</th>
<th>Weight (g)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before placement in water</td>
<td>After 20 h. in water</td>
</tr>
<tr>
<td>1.7</td>
<td>2.24</td>
<td>Breakage occurred</td>
</tr>
<tr>
<td>3.7</td>
<td>1.86</td>
<td>3.50</td>
</tr>
<tr>
<td>5.8</td>
<td>1.59</td>
<td>1.77</td>
</tr>
<tr>
<td>10.9</td>
<td>2.11</td>
<td>10.8</td>
</tr>
</tbody>
</table>
Figure 3.17  Gelatin gel *before* placement in water at pH 5.8.
Figure 3.18  Gelatin gel (from Figure 3.17) 20 hours after placement in water at pH 5.8.
Figure 3.19  Gelatin gel *before* placement in water at pH 10.9.
Figure 3.20  Gelatin gel (from Figure 3.19) 20 hours after placement in water at pH 10.9.
3.3.1.3 Gelatin/sulfadiazine microcapsules

Gelatin/sulfadiazine microcapsules were prepared using a simple coacervation technique as described in Section 2.2.1. Simple coacervation involves the removal of water from the gelatin upon the addition of a salt, in this case, sodium sulfate. The salt competes with the gelatin for water and as a result, gelatin is forced to coacervate and adsorbs at the liquid/sulfadiazine interface. The microcapsules ranged in size from 7-12 μm, with the gelatin wall comprising ~20% of the microcapsule (98).

The application of an electric current to the microcapsules would be expected to cause pH changes that may alter the porosity of the gelatin wall. In order to examine the effect of an electric current on the gelatin wall, optical observations of gelatin/sulfadiazine microcapsules were made before and after passive diffusion and electrically-facilitated release studies. The objective was to detect possible size or morphologic changes occurring due to the passage of current. Drug release studies were performed for 70 min, or for 4 h or 5 h (as described in Section 2.2.11), after which samples of the contents of the donor compartment were examined first by visual observation and then by SEM. In all three studies, the microcapsules appeared to have sedimented at least in part onto the Spectra/Por® membrane. In addition, many of the microcapsules were found to have migrated toward the positive electrode (anode), creating a space virtually devoid of microcapsules surrounding the negative electrode (cathode). This is to be expected because both Type B gelatin and sulfadiazine are anionic in normal saline solution at a pH values greater than 5 and 6.3, respectively. The
degree of migration appeared to be dependent on the duration of the current in that
migration was more evident in the 4 and 5 h studies when compared to that of the 70 min
study.

3.3.1.3.1 Optical microscopy

The microcapsules were examined by optical microscopy (788x) under brightfield
and polarized light (Figures 3.21), the latter of which allowed detection of sulfadiazine
crystals within the gelatin walls. As shown in Figures 3.22 and 3.23, there were no
apparent changes in the sizes and morphologies of the microcapsules or in their apparent
wall structure after passive diffusion and electrically-facilitated release studies were
carried out for 70 minutes.

3.3.1.3.2 Scanning electron microscopy

Gelatin/sulfadiazine microcapsules were also examined by scanning electron
microscopy to determine if there were any changes in microcapsule size and/or
morphology that could not be detected by optical microscopy. Figure 3.24 shows a
microcapsule before release studies had been carried out. The star-like structure of the
sulfadiazine crystals is presumed to be surrounded by gelatin.

In order to determine if electric current has an affect on the structure of the
microcapsules, passive and electrically-facilitated release studies were carried out for 70
min, 4 h and 5 h, as described in Section 2.2.11). Figures 3.25-3.30 indicate that no
Figure 3.21  Gelatin/sulfadiazine microcapsules before a release study (788x):
A. Brightfield 
B. Polarized light
Figure 3.22  Gelatin/sulfadiazine microcapsules after a 70 minute release study (788x). No current was applied.
A. Brightfield  
B. Polarized light
Figure 3.23  Gelatin/sulfadiazine microcapsules after a 70 minute release study (788x). 0.5 mA DC was applied from $t = 20$ to $t = 50$ minutes.
A. Brightfield
B. Polarized light
Figure 3.24  Scanning electron photomicrograph of a gelatin/sulfadiazine microcapsule before a release study (5000x).
Figure 3.25 Scanning electron photomicrograph of gelatin/sulfadiazine microcapsules after a 70 minute release study (5000x). No current was applied.
Figure 3.26  Scanning electron photomicrograph of gelatin/sulfadiazine microcapsules after a 4 hour release study (5000x). No current was applied.
Figure 3.27  Scanning electron photomicrograph of gelatin/sulfadiazine microcapsules *after* a 5 hour release study (5000x). No current was applied.
Figure 3.28  Scanning electron photomicrograph of gelatin/sulfadiazine microcapsules after a 70 minute release study (5000x). 0.5 mA DC was applied from $t = 20$ to $t = 50$ minutes.
Figure 3.29 Scanning electron photomicrograph of gelatin/sulfadiazine microcapsules after a 4 hour release study (5000x). 0.5 mA DC was applied from $t = 30$ to $t = 60$, 90 to 120, 150 to 180 and 210 to 240 minutes.
Figure 3.30  Scanning electron photomicrograph of gelatin/sulfadiazine microcapsules after a 5 hour release study (5000x). 0.5 mA DC was applied from $t = 30$ to $t = 60$, 90 to 120, 150 to 180, 210 to 240 minutes and 270 to 300 minutes.
apparent changes in microcapsule morphology appear after application of current since they appear to maintain their size and the gelatin wall appears to remain intact.

3.3.1.3.3 Environmental scanning electron microscopy

Environmental scanning electron microscopy (ESEM) was also used to study the size and morphology of the sulfadiazine/gelatin microcapsules. ESEM differs from SEM in that it is able to image water in a sample. By selecting appropriate temperatures and pressures, the sample can be dehydrated and then rehydrated. This technique was chosen to determine if there are changes in microcapsule size and morphology due to drying which may affect the porosity of the gelatin wall.

Sulfadiazine/gelatin microcapsules were prepared and centrifuged as discussed in Section 2.2.13. The supernate was decanted and the microcapsule sediment used for the ESEM studies. Figure 3.31 shows these microcapsules at different levels of hydration, and no apparent changes in microcapsule size or morphology can be seen. This is contrary to what would be expected since the hydrophilic gelatin wall should swell in the presence of water and deswell upon drying. In this regard, there are probable limitations to this method since the small size of the microcapsules may not allow sufficient dimensional changes in wall morphology to be noticeable, and/or to the presence of water which camouflages the wall structure (see Figure 3.31 B).
Figure 3.31  ESEM photomicrographs of two different sulfadiazine/gelatin microcapsules at different vapor pressures:
A.  5.4 Torr
B.  4.9 Torr
3.3.1.3.4 Transmission electron microscopy

Microcapsule suspensions were also prepared and centrifuged to concentrate them for transmission electron microscopy (TEM) studies. The TEM photomicrograph in Figure 3.32 shows that the microcapsule consists of sulfadiazine crystals oriented in a radial structure around which would be the gelatin wall. The structure of the microcapsule forms upon rapid precipitation of drug and simultaneous coacervation of gelatin (see Section 3.2.1). The radial structure would have an inherently higher surface area of drug available to the dissolution medium than that of a more compact structure, generally resulting in a faster dissolution of drug.

3.3.2 In vitro release of sulfadiazine from gelatin-walled microcapsules

3.3.2.1 Passive diffusion

3.3.2.1.1 pH dependency

The influence of pH on sulfadiazine release by passive diffusion was evaluated by measuring the pH of the donor and receiver compartments during release studies. Measurements of pH in the donor compartment were taken at the beginning, \( t = 0 \) min and at the end, \( t = 70 \) min. All other readings were measured in the receiver compartment every 5 min.
Figure 3.32  Freeze fracture electron photomicrograph of a gelatin/sulfadiazine microcapsule (20,000x)
Figure 3.33 shows passive diffusional release of sulfadiazine as functions of pH using Protocols 1 - 4. As with the sulfadiazine suspensions, sulfadiazine release from the microcapsules was dependent on pH despite the presence of the gelatin wall. The total amounts of sulfadiazine released were lower when using Protocols 2 and 3 systems than for 1 and 4, i.e., 20.5 ± 1.06 µg and 22.7 ± 0.86 µg vs. 46.4 ± 3.71 µg and 257 ± 19.8µg, respectively. Such changes in the amounts released would be expected since the solubilities of sulfadiazine in normal saline solution at pH 1.7 and 10.9 (Protocols 1 and 4) are higher than in normal saline solution at pH 3.7 and 5.8 (Protocols 2 and 3; see Section 3.2.2).

Furthermore, the pH in the donor compartment decreased slightly after 70 min when Protocols 1 and 2 were used, i.e., 2.0 vs. 1.7 and 4.5 vs. 4.4, (see Table 3.4); whereas for Protocol 4, there was an increase in pH (7.0 vs. 7.5). Such changes may be due to the back diffusion of eluant from the receiver to the donor compartment. For example, when using Protocol 4, the initial donor compartment pH was ~7.0. After 70 min, the pH increased to ~7.5, indicating that some of the eluant at pH ~10.9 may have diffused into the donor compartment. Conversely, there was no change in the pH of the donor compartment medium over the course of the 70 min release study using Protocol 3. The initial and final pH values of the donor medium were approximately 6.0, and the initial and final pH in the eluant ranged from 5.8 to 6.1 (see Table 3.4). This can be explained by the similar pH values in the donor and receiver compartments, i.e., if the eluant at pH 5.8 diffuses into the donor compartment media at pH ~6.0, then the effective change in pH may be negligible. For all four systems therefore, the pH of the eluant
Figure 3.33  Release of sulfadiazine by passive diffusion from gelatin-walled microcapsules as functions of donor medium pH. Donor medium: normal saline solution at pH 2.0, 4.5, 6.0 and 7.0.
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>pH (No Current)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>0 (Donor)</td>
<td>2.03</td>
</tr>
<tr>
<td>0 (Eluant)</td>
<td>1.49</td>
</tr>
<tr>
<td>5</td>
<td>1.51</td>
</tr>
<tr>
<td>10</td>
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</tr>
<tr>
<td>15</td>
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</tr>
<tr>
<td>70 (Donor)</td>
<td>1.67</td>
</tr>
</tbody>
</table>

Table 3.4 pH of the donor and receiver compartment media (normal saline solution).
essentially does not change over the 70 min experiment as would be expected since the amounts of sulfadiazine released are also constant as shown in Figure 3.33.

The gelatin wall was also affected by the pH of the eluant and the suspending medium. When using Protocol 1, in which the donor pH is approximately 2.0, gelatin is positively charged, and therefore swells and creates open aqueous channels due to repulsion between like-charged groups (see Section 1.3.4.2.3). These aqueous channels can increase drug release by allowing dissolution medium and drug solution to more easily pass through the wall, albeit in opposite directions. Similarly, when using Protocol 4, in which the donor medium pH was ~7.0, gelatin is negatively charged, and as before, pores should open resulting in an increase in drug diffusion through the wall.

Since the isoelectric point of gelatin is ~ 5, at this pH gelatin chains will instead attract each other thereby closing down pores. In these release studies, pores may begin to shrink when Protocols 2 and 3 (donor media pHs of 4.5 and 6.0, respectively) are used, due to the proximity of the donor media pH to that of the isoelectric point of gelatin. The closing of pores in the wall will decrease drug release from the microcapsules by forming an increasingly resistant barrier to drug diffusion. However, the barrier effects of the gelatin wall are not apparent in these cases, most likely due to the slow dissolution of unionized sulfadiazine.

Table 3.5 summarizes the pH dependency of sulfadiazine release from gelatin-walled microcapsules. In order to determine the extent that the gelatin wall porosity has on sulfadiazine release, this data is compared in Section 3.6 with studies on sulfadiazine suspensions and PVP/sulfadiazine microcapsules in which the effects of the electrochemomechanical properties of the gelatin wall can be seen.
Formulation | Protocol 1  
|---|---|
| Donor pH: 2.0 | Sulfadiazine solubility is low at this pH since it is in its uncharged form.  
|  | Sulfadiazine release is slightly higher than that when it is uncharged (2.1 < pH < 6.3).  
| Sulfadiazine | Since the pH of the donor and receiver compartments are close to the pKa of sulfadiazine, sulfadiazine release is slightly higher than that when it is uncharged (2.1 < pH < 6.3).  
| Gelatin | Gelatin is positively charged at this pH. Swelling of the gel wall occurs due to repulsive forces between like charges. This should result in a more hydrated and porous wall.  
| Combined effects | Sulfadiazine release is not hindered by the gelatin wall.  
|  Protocol 2  
| Donor pH: 4.5 | Sulfadiazine solubility is low at this pH since it is in its uncharged form.  
|  | Gelatin is neutral and pores close creating a barrier to release.  
|  Protocol 3  
| Donor pH: 6.0 | Sulfadiazine solubility is low at this pH since it is in its uncharged form.  
|  | Gelatin is neutral and pores close creating a barrier to release.  
| Protocol 4  
| Donor pH: 7.0 | Sulfadiazine is ionized and therefore its solubility is high at this pH  
|  | Gelatin is negatively charged and swells, and is therefore not a significant barrier to release of sulfadiazine  
|  | Sulfadiazine release is high. The wall is not a significant barrier due to the opening of aqueous pores.

Table 3.5 Summary of the effects of pH on the passive diffusion of sulfadiazine from gelatin/sulfadiazine microcapsules. See text for details.
3.3.2.2 Electrically-facilitated release

3.3.2.2.1 Effect of current and electrode parameters

3.3.2.2.1.1 Alternating vs. direct current

The Scepter™ Power Supply system can be set for direct or alternating current on/off cycles. In the direct current mode, the direction of current flow remains constant, whereas in the alternating mode, the direction of the current changes at a specific frequency of 60 Hz (see Section 1.3.4.2.1). In all release studies, direct current varied from 0 to 1 mA. If inert electrodes consisting of platinum wire are used with a direct current, there should be a decrease in pH at the anode and an increase at the cathode due to water hydrolysis. On the other hand, alternating current reduces pH fluctuations in the donor compartment since the polarity of the cathode and anode constantly change. Therefore, when alternating current is applied to electrodes in a gelatin/sulfadiazine microcapsule suspension, any increase in drug release due to local pH effects should be negligible. For this study, 0.5 mA AC was applied from $t = 20$ to $t = 50$ min using platinum wire electrodes. Figure 3.34 shows that sulfadiazine release was indeed not increased when alternating current was used under these conditions. This suggests that local pH change at the electrodes is an important mechanism for electrically-facilitated release of sulfadiazine from these gelatin-walled microcapsules (see Section 3.3.2.2.5).
Figure 3.34 Release of sulfadiazine from gelatin-walled microcapsules. Donor medium: normal saline solution; current: 0.5 mA AC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
3.3.2.1.2 Ag/AgCl vs. platinum electrodes

Ag/AgCl wire electrodes produce the reaction \( Ag + Cl^- \leftrightarrow AgCl + e^- \) at the electrode surfaces. This reaction limits water hydrolysis, thereby reducing pH fluctuations in the donor compartment of the diffusion cell. These electrodes were used along with a phosphate buffer suspending medium to eliminate the effect of pH on sulfadiazine release from the gelatin-walled microcapsules, thereby helping to elucidate other mechanisms which may influence electrically-facilitated drug release. However, when phosphate buffer was used, the voltage increased above the maximum limit of the Scepter™ System of 10 volts during the application of 1.0 mA DC for 30 min. This occurred because the AgCl coating on the wire electrodes had sufficiently eroded away during the 30 min of applied current. If enough of the AgCl coating is removed, the electrodes will behave as inert electrodes and hydrolysis of water will occur. In this context also, the phosphate buffer may not allow enough water to be available for hydrolysis and an increase in resistance results. Normal saline solution was used therefore as the suspending medium instead of phosphate buffer to promote water hydrolysis and thereby increase the duration of the current.

Drug release studies were carried out for the usual 70 min period, and a Spectra/Por® 1 membrane was used to separate the donor and receiver compartments. Table 3.6 shows that the voltage stays below 10 over the 30 min period, suggesting that sufficient hydrolysis occurs under these conditions. As can be seen in Figure 3.35, there was a ~3.6 fold increase in the amount of sulfadiazine released compared to that from
<table>
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</tr>
<tr>
<td>24.0</td>
<td>3.75</td>
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</tr>
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<tr>
<td>30.0</td>
<td>3.81</td>
<td>2.03</td>
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</tbody>
</table>

Table 3.6  Range of voltages when 1.0 mA DC is applied to suspensions of gelatin/sulfadiazine microcapsules. Donor medium: normal saline solution.
Figure 3.35  Release of sulfadiazine from gelatin-walled microcapsules. Donor medium: normal saline solution; current: 1.0 mA DC from $t = 20$ to $t = 50$ min; and electrodes: Ag/AgCl wire.
passive diffusion when 1.0 mA DC was applied. As discussed in Section 3.1, this increase in sulfadiazine occurs from an increase in sulfadiazine solubility due to a change in pH induced by the current.

The Ag/AgCl wire mentioned in the previous paragraph was initially used for studies of the release of sulfadiazine from the gelatin-walled microcapsules. However, new wire was needed for each experiment since the thickness and durability of the AgCl coating when carrying an electric current was rather variable. In order to reduce this variability and to test the effect of "active" electrodes on drug release, commercially prepared Ag/AgCl electrodes (In Vivo Metric, Inc.) were used in subsequent studies. The pH remained constant with these electrodes since the surface area of the coating was maintained during the experiments. Figure 3.36 indicates that the extent of drug release is not altered when current was applied from these electrodes. As before, it appears that a local change in pH was necessary to achieve an increase in the amounts of sulfadiazine released.

The application of current by itself does not appear to have a noticeable effect on the permeability of the gelatin wall matrix. Instead, a pH change may be needed for gelatin to exhibit electrochemomechanical behavior. Due to the slow dissolution rate of sulfadiazine, it is even possible that the effects of such parameters may be hard to detect. It is also possible that there is a minimum voltage threshold that must be exceeded for the hydrogel to be responsive to the current. For example, in these experiments the current was 1.0 mA DC, and the voltage stayed in the range of 0.25 to 2.03 v as shown in Table 3.6. In previous experiments however, using the Ag/AgCl wire, the voltage varied between 2.53 and 4.08 under the same conditions (Table 3.6).
Figure 3.36 Release of sulfadiazine from gelatin-walled microcapsules. Donor medium: normal saline solution; current: 1.0 mA DC from $t = 20$ to $t = 50$ min; and electrodes: commercial Ag/AgCl electrodes (In Vivo Metric).
Platinum wire electrodes were also used for the drug release studies since they are inert and pH changes occur at the electrode surfaces as described in Section 1.3.4.2.2. The amounts of sulfadiazine released were enhanced ~11.0 fold from that of passive diffusion when 1.0 mA DC was applied from $t = 20$ to $t = 50$ min (Figure 3.37). Table 3.6 shows that the voltage, and hence the electrical resistance, was lower and more consistent with platinum wire electrodes than with Ag/AgCl wire electrodes ($2.57$-$2.94$ v vs. $2.53$-$4.08$ v, respectively) in the 70 min period. This would be due to the greater extent of hydrolysis occurring with the platinum electrodes which produces more ions to carry the current. The platinum wire electrodes used for these studies also have a larger surface area per wire for hydrolysis compared to that of the Ag/AgCl wire ($0.23$ cm$^2$ vs. $0.11$ cm$^2$). Therefore, the magnitudes of pH change and of the amounts of drug released using platinum wire electrodes were higher than was drug release using the Ag/AgCl wire electrodes (~11.0 vs. 3.6 fold increases from that by passive diffusion).

### 3.3.2.2.1.3 Current magnitude (single pulse)

The initial studies of sulfadiazine release were performed using Ag/AgCl wire electrodes to carry currents of 0.25, 0.5, 0.75 and 1.0 mA DC. Figure 3.38 shows a comparison of the passive diffusional and electrically-facilitated release of sulfadiazine with a Spectra/Por® 1 membrane separating the donor and receiver compartments. It can be seen that there was an increase in the amounts of drug released at current magnitudes equal to, or above, 0.75 mA DC. For example, there was only about a 1.1 fold increase at 60 min from that of passive diffusion when 0.25 mA DC was applied, no apparent
Figure 3.37  Release of sulfadiazine from gelatin-walled microcapsules. Donor medium: normal saline solution; current: 1.0 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.38  Release of sulfadiazine from gelatin-walled microcapsules as functions of current magnitude. Donor medium: normal saline solution; current: from $t = 0$ to $t = 15$ and $t = 30$ to $t = 45$ min; and electrodes: Ag/AgCl wire.
increase after 60 min when 0.5 mA DC was applied, but 1.4 and 1.9 fold increases from that of passive diffusion after 60 min when 0.75 or 1.0 mA DC were applied, respectively. This suggests that drug release may be directly related to, and proportional to, the magnitude of the current, but only above 0.5 mA DC when using Ag/AgCl wire electrodes.

The effect of current magnitude on sulfadiazaine release from gelatin-walled microcapsules was also studied using platinum wire electrodes and a Spectra/Por® 1 membrane. Currents of 0, 0.5 and 1.0 mA DC were applied to the microcapsules, and the data are compared in Figure 3.39. With these electrodes, increases in the amounts of drug released were ~5.2 and 11.0 fold, respectively, compared to that from passive diffusion. As discussed above, increases in sulfadiazine release can be attributed to the degree of ionization of the drug into its more soluble salt form due to an increase in pH at the cathode. Therefore, the release of sulfadiazine under these conditions appears to be directly proportional to the magnitude of the current.

It should also be noted that when an electric current is applied in the donor compartment, there may be increases in temperature due to the liberation of heat created by the passage of the current. An increase in temperature may in turn increase sulfadiazine solubility and its rates of release since its aqueous solubility is temperature dependent (see Section 3.2.2). The temperature of the donor compartment medium was measured before and after 0.5 and 1.0 mA DC were applied for 30 min and no temperature change occurred. Therefore, it appears that any temperature change caused by the application of current is not detectable and is therefore not likely to affect sulfadiazine release.
Figure 3.39  Release of sulfadiazine from gelatin-walled microcapsules as functions current magnitude. Donor medium: normal saline solution; current from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
3.3.2.2.1.4 Duration of current (single pulse)

The amounts of drug released from gelatin/sulfadiazine microcapsules was also dependent on the duration of the current. A 0.5 mA direct current was applied for 30 min from $t = 20$ to $t = 50$ min, and for 60 min from $t = 20$ to $t = 80$ min. Figure 3.40 shows a comparison of the amounts of sulfadiazine released in both experiments, and it is apparent that higher amounts of sulfadiazine were released when current was applied for a longer duration. This is to be expected since the effect of current on drug release is a reversible process. In other words, when current is turned on, there is an increase in sulfadiazine release which will continue at a steady rate as long as the current is on. When the current is turned off, however, the rate of sulfadiazine release decreases and the repetition on/off cycles should continue until drug is depleted from the microcapsules. Section 3.3.2.2.1.5 discusses this type of reversible cyclic or pulsatile release from the microcapsules.

3.3.2.2.1.5 Periodic cycles (multiple pulsing)

As discussed in Chapter 1, the electrochemomechanical properties of a hydrogel wall in combination with the ionizable properties of a drug core were used to develop a pulsatile controlled drug delivery system. Studies comparing the effect of current magnitude on drug release as discussed above show that when the current is applied, sulfadiazine release should increase, whereas when current is turned off, release should decrease. Section 1.3.4 focuses on the various mechanisms which could contribute to
Figure 3.40  Release of sulfadiazine from gelatin-walled microcapsules as functions of current duration. Donor medium: normal saline solution; electrodes: platinum wire.
sulfadiazine release and it appears that pH change occurring at the electrode surfaces is the primary mechanism. It is responsible for changing both sulfadiazine solubility and the porosity of the gelatin wall. Since both of these changes depend on the ionization state of the material (drug or polymer chains), the effect should be not only reversible, but also repeatable thereby forming a periodic or pulsatile release system.

In order to test the feasibility of pulsatile release from gelatin-walled microcapsules, release studies were performed by varying current in 30 min on/off cycles as shown in Figure 3.41. Currents of 0, 0.25, 0.5 and 1.0 mA DC were compared, and again it was found that the amounts of drug released from the microcapsules were dependent on the magnitude of the current in that there were 62.9 ± 3.6 µg, 201 ± 22.4 µg, 364 ± 17.6 µg and 687 ± 111 µg of sulfadiazine released after 180 min, respectively. The rates of sulfadiazine release from these four studies were taken from the slopes of the curves in Figure 3.41; and the slopes of the on/off cycles were calculated separately.

Under passive diffusion conditions, i.e., when no current was applied to the system, sulfadiazine was released at a steady rate of 0.355 µg/min. However, when current was applied, the rate of release of sulfadiazine increased proportionally with currents of 0.25, 0.5, and 1.0 mA DC (see Table 3.7). At all three magnitudes of current, the rates of release of sulfadiazine were higher for the last two off cycles (60-90 and 120-150 min) compared to that of the first off cycle (0-30 min). For example, the rate of release of sulfadiazine in the first cycle is comparable to passive diffusion for all three currents, i.e., 0.349, 0.353, and 0.376 µg/min vs. 0.355 µg/min for passive diffusion. However, after the current was turned off, the rate of passive diffusion remained higher than it was before current was applied (see Table 3.7). For example, after 1.0 mA DC has been applied
Figure 3.41 Release of sulfadiazine from gelatin-walled microcapsules as functions of current magnitude. Donor medium: normal saline solution; current: from $t = 30$ to $t = 60$ min, 90-120 min, and 150-180 min; and electrodes: platinum wire.
<table>
<thead>
<tr>
<th>Current (mA)</th>
<th>Rate (µg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Off 0-30 min</td>
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<tr>
<td>0</td>
<td>0.355</td>
</tr>
<tr>
<td>0.25</td>
<td>0.345</td>
</tr>
<tr>
<td>0.50</td>
<td>0.353</td>
</tr>
<tr>
<td>1.00</td>
<td>0.376</td>
</tr>
</tbody>
</table>

Table 3.7 Rates of sulfadiazine as functions of current magnitudes. Current was applied from $t = 30-60$ min, 90-120 min and 150-180 min. Electrodes: platinum wire; donor medium: normal saline solution.
from 30 to 60 min, the passive diffusion rate from 60 to 90 min was 1.67 µg/min compared to 0.376 µg/min for the first off cycle (0-30 min). This is most likely due to a slow diffusion of sulfadiazine which was ionized by the formation of hydroxyl ions at the cathode (see Section 3.3.2.2.5). Although the rates of passive diffusion from each cycle differ, the rates of release of sulfadiazine when current was applied, i.e., electrically-facilitated release, were essentially the same for all three on cycles. For sulfadiazine, electrically-facilitated release is controlled by the magnitude of the current, rather than from diffusion of drug into the receiver compartment. Figure 3.42 shows that the rates of release during the second cycle and the magnitudes of the current are directly proportional by the straight line ($r^2 = 0.994$). Similar effects are seen with the plots of the first and third on cycles ($r^2 = 0.992$ and 0.987, respectively). Therefore, by applying an electric current in on/off cycles, a controlled, pulsatile pattern of release of sulfadiazine can be achieved in its in vitro model.

3.3.2.2 Effect of the method preparation of the microcapsules

As previously described, the degree of compaction of the gelatin wall can influence drug release. When the gel contracts, pores or aqueous channels will close, and the wall will become an increasing barrier to drug release. Conversely, when the gel expands, pores open, and release can be enhanced.

Microcapsules were prepared by a coacervation method and were “gelled” or “hardened” with sodium sulfate at approximately 5°C. If the final gelling step is eliminated, an “unhardened” microcapsule with a less compact and more hydrated wall
Figure 3.42 Rates of release of sulfadiazine from gelatin-walled microcapsules as functions of current magnitude. Current: from $t = 30$ to $t = 60$ min, 90-120 min, and 150-180 min. Second cycle rates are shown (90-120 min). Donor medium: normal saline solution; and electrodes: platinum wire.
will result. The hydrated wall may allow easier passage of drug and dissolution medium which may in turn contribute to a faster rate of solution of sulfadiazine.

In order to determine the effect of the degree of microcapsule wall hydration on sulfadiazine release, both hardened and unhardened forms of the microcapsules were studied. Figure 3.43 shows that when current was applied, there were 3.24 and 4.10 fold increases at the end of 70 min in the amounts of drug released from the hardened and unhardened microcapsules, respectively compared to that by passive diffusion. Both passive diffusion and electrically-facilitated release of sulfadiazine from the unhardened microcapsules were slightly higher than that from the hardened microcapsules. However for the unhardened microcapsules, there was a high variability (cv >40%) for the electrically-facilitated release studies. This variation can be explained in part by an apparent sedimentation of the microcapsules onto the Spectra/Por® membrane. By visual observation, it appeared that most of the microcapsules, whether hardened or unhardened, tended to sediment onto the membrane during the 70 min release studies. However, the unhardened microcapsules appeared to stick together and to form a gel-like matrix on the membrane, possibly hindering drug transport into the receiver compartment (see Section 3.3.2.2.3). The hardened microcapsules also formed a layer on the membrane, but did not appear to form a continuous plug or gel and were easily resuspended. This latter type of sedimentation does not appear to interfere with drug release (see Section 3.3.2.2.3).

The differences in the sedimentation behavior of the two types of microcapsules can be attributed to their differences in the method of preparation. For example, both were prepared using a coacervation method in which a solution of gelatin was coacervated upon the addition of a salt. The encapsulation procedure takes place at 37-45 °C, which
Figure 3.43  Release of sulfadiazine from hardened and unhardened gelatin-walled microcapsules. Donor medium: normal saline solution; current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
is above the melting point of gelatin (~37 °C). This temperature range produces unhardened microcapsules. To form the hardened microcapsules, the unhardened microcapsules were gelled by cooling the microcapsule suspension below the melting point of gelatin with sodium sulfate at approximately 5 °C.

Although there was high variation in amounts of sulfadiazine released from the unhardened microcapsules when current was applied, the trend is that the more hydrated wall, apparently functions as less of a barrier to drug diffusion through the microcapsule wall. The effect of sedimentation of the microcapsules onto the Spectra/Por® membrane is discussed further in the following section.

### 3.3.2.2.3 Effect of microcapsule sedimentation on the membrane

With this model, a suspension of microcapsules was placed directly into the donor compartment that was separated from the receiver compartment by a Spectra/Por® membrane. The microcapsules were found to sediment onto the Spectra/Por® membrane throughout the duration of the release study. Stirring of the microcapsule suspension to prevent settling was not incorporated into the design of the experimental model since stirring in drug delivery devices such as for transdermal administration or for surgical implantation may not be practical due to size and power considerations.

In order to determine if sedimentation of the microcapsules influences sulfadiazine release, suspensions of hardened microcapsules and suspensions of unhardened microcapsules containing two different amounts of microcapsules were used.
If microcapsule sedimentation does partially hinder drug release, then a larger number of microcapsules would form a greater barrier with a corresponding decrease in sulfadiazine release.

In this context, twice the number of hardened microcapsules, which provided ~48 mg/mL of sulfadiazine as opposed to ~24 mg/mL used in the original system, was added to the donor compartment. Under passive diffusional conditions, the total amount released after 70 min was 20.8 ± 1.07 µg compared to 22.7 ± 0.86 µg for the system containing the smaller number of microcapsules. Doubling the number of microcapsules did not appear, therefore, to inhibit sulfadiazine release.

Similarly, when unhardened microcapsules were used in double the original concentration, the total amount of sulfadiazine released was 24.3 ± 1.99 µg which was comparable to 24.1 ± 2.12 µg using the smaller number of microcapsules. This suggests that the “barrier” effects of the sedimented unhardened microcapsules are not dependent on the numbers of microcapsules present.

Furthermore, when current was now applied to the hardened microcapsules, the total amount released was 80.6 ± 12.1 µg compared to 73.6 ± 7.39 µg for the system containing half as many microcapsules, suggesting, as before, that these microcapsules do not have a significant effect on the release of sulfadiazine. Conversely, when unhardened microcapsules were used under the same conditions, there were approximately 98.9 ± 40.1 µg and 28.9 ± 0.24 µg of sulfadiazine released from the 24 mg/mL and 48 mg/mL samples, respectively. This indicates that sedimented unhardened microcapsules form a layer which may inhibit the electrically-facilitated release of sulfadiazine. As already mentioned, these unhardened microcapsules apparently stick...
together and form a continuous gel on the Spectra/Por® membrane. If such a gel forms, the release of sulfadiazine would be expected to be dependent on the viscosity and thickness of the gel. However, under passive diffusion conditions, as discussed earlier, this result was not apparent, most likely due to slow dissolution of the drug. When current was applied, pH changes in the donor compartment would cause increases in dissolution rate by increasing the solubilities of the drug. Therefore the properties of the "gel" membrane (consisting of the sedimented unhardened microcapsules) become rate-limiting in release.

With regard to hardened microcapsules which do not form a "gel" layer, increasing the viscosity of the donor compartment medium by changing to a 1:1 polyethylene glycol (8000)/normal saline solution reduces the rate of release of sulfadiazine. Therefore, the overall result is essentially the same for both the hardened and unhardened microcapsules in that drug release is dependent on the rheological properties of the donor system.

3.3.2.2.4 Ionic strength of the donor medium and eluant

Ionic strengths of the donor media over the range of 0 – 0.75 M do not affect the solubility of sulfadiazine (Section 3.2.2), but may affect the porosity, e.g., expansion or contraction, of the hydrogel wall (see Section 1.3.4.2.3). In addition, an electric current can change the ionic strength in the vicinity of the electrodes since charged ions migrate to the oppositely charged electrodes. Ions are also produced at the electrodes during electrode reactions. An increase in ionic strength near the hydrogel wall could result in
an osmotic gradient between the wall and the surrounding medium, resulting in a net loss of water from the hydrogel as the wall contracts and closes completely or partially. However, as shown in Figure 3.44, there are no apparent differences in the passive diffusion of sulfadiazine as functions of ionic strength. Since the solubility of sulfadiazine is not dependent on ionic strength at these concentrations, it appears that the gelatin wall is also essentially unaffected by the ionic strength as well. When current was applied, there was also no dependency of sulfadiazine release on ionic strength. These studies suggest that any ionic strength change occurring in the system when current was on, was not sufficient to affect drug release through the gelatin wall. This conclusion further supports the hypothesis that local pH changes may be the predominant mechanism for electrical-facilitation of drug release from the microcapsules.

3.3.2.2.5 pH dependency

As discussed in Chapter 1 and elsewhere, when direct current is applied to inert electrodes in an electrolytic solution, changes in the pH in the vicinity the electrodes occurs which in turn may alter drug solubility and release. These pH changes may also affect the gelatin wall by changing its ionic state, porosity, and thereby its permeability to a drug. It has been concluded therefore that electrically-induced pH changes in the donor media are the main mechanism for controlling sulfadiazine release. More specifically, it has been shown that the pH can be altered by the use of inert electrodes, or held constant with the use of active electrodes. In addition, a phosphate buffer eluant can also be employed to reduce pH fluctuations caused by electrode reactions. By comparing the use
Figure 3.44  Release of sulfadiazine from gelatin-walled microcapsules as functions of the molarity of the donor compartment medium. Donor medium: sodium chloride solution; current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
of active and inert electrodes, and the type and pH of the donor compartment media, the
effects of pH on sulfadiazine release from gelatin-walled microcapsules can be evaluated.

Studies of electrically-facilitated drug release were performed following Protocols
1 - 4 as described in Section 2.2.5. Measurements of pH were taken in the donor
compartment at $t = 0$ min and $t = 70$ min. It should be noted however, that the final pH
was measured 20 minutes after the current had been turned off in order to eliminate
measurement fluctuations caused by electrically-induced gas production at the electrode
surfaces. As such, the final pH of the donor medium does not necessarily represent the
pH at each electrode, but was measured to help provide in elucidating the mechanisms to
sulfadiazine release. All eluant samples were measured in the receiver compartment
every five min.

When a 0.5 mA direct current was applied to the donor media for 30 min, Figures
3.45 - 3.48 show it had no apparent effect on sulfadiazine release when Protocol 1 was
used, whereas increases were seen for Protocols 2, 3 and 4. In addition, for Protocols, 2,
3 and 4, there appeared to be a net migration of microcapsules toward the anode where a
decrease in pH occurs. Statistical comparisons of the total amounts of sulfadiazine
released under each of the four protocols by passive diffusion and upon the application of
an electrical current were made. A series of sample t-tests were performed along with
Bonferroni correction for multiple testing (Table 3.8). The t-tests suggest that when the
donor medium pH is 4.5 and above (Protocols 2 - 4), the application of a 0.5 mA current
for 30 min to a suspension of gelatin/sulfadiazine microcapsules will cause an increase in
sulfadiazine release compared to that by passive diffusion.
Figure 3.45  Release of sulfadiazine from gelatin-walled microcapsules. Protocol 1; donor medium: normal saline solution (pH 2.0); current: 0.5 mA DC from \( t = 20 \) to \( t = 50 \) min; and electrodes: platinum wire.
Figure 3.46  Release of sulfadiazine from gelatin-walled microcapsules. Protocol 2; donor medium: normal saline solution (pH 4.5); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.47 Release of sulfadiazine from gelatin-walled microcapsules. Protocol 3; donor medium: normal saline (pH 6.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.48  Release of sulfadiazine from gelatin-walled microcapsules. Protocol 4; donor medium: normal saline solution (pH 7.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Table 3.8 Statistical t-test comparisons of the release of sulfadiazine from gelatin-walled microcapsules, by passive diffusion and electrical-facilitation after 70 min.

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<th>p-value</th>
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<td>Mean Amt (μg)</td>
<td>Std. Dev.</td>
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</tr>
<tr>
<td>4</td>
<td>257</td>
<td>19.8</td>
<td>303</td>
</tr>
</tbody>
</table>

<sup>a</sup> Indicates the Satterthwaite p-value for unequal variances was used
<sup>b</sup> Indicates marginal significance
<sup>c</sup> Indicates statistically significant
As with the case for sulfadiazine suspensions (Section 3.2.3.2), these increases can be explained by analyzing the pH effects brought on by electrode reactions. For example, when current is applied to the donor compartment, the pH at the cathode should increase, whereas at the anode it should decrease. It is apparent in Figure 3.45, that there was no increase in the amounts of sulfadiazine released from passive diffusion when Protocol 1 (donor pH 2.0) was used. This was due to the minimal pH change occurring in the donor compartment when current was applied. For example, when Protocol 1 was used, the pH in the donor compartment decreased after 70 min only slightly for both passive and electrically-facilitated release (pH 2.0 vs. 1.6; see Tables 3.4 and 3.9). This decrease is most likely due to the diffusion of eluant from the receiver into the donor compartment. In other words, 0.5 mA DC applied for 30 min is not sufficient to affect the pH of the donor compartment medium. At the lower pH values, there is a high concentration of protons in the system and therefore the addition of protons and hydroxyl ions from the electrode reactions would be insufficient to produce any noticeable change in the pH, and therefore in the degree of ionization of sulfadiazine. The gelatin wall should also remain unaffected; that is, pores would remain open, creating less of a barrier to drug release.

Figures 3.46-3.48 show that there were increases in release when current was applied according to Protocols 2, 3 and 4, i.e., 4.43, 3.24, and 1.18 fold increases, respectively. These increases were most likely due to increases in pH at the cathode. As already mentioned, by increasing pH above 6.3, sulfadiazine will deprotonate, ionize, become more soluble and the amounts released will increase. For example, for Protocol 3 in which the donor medium pH is approximately 6.0, a slight increase in pH at the
<table>
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<th>Time (min)</th>
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<th>Protocol 2</th>
<th>Protocol 3</th>
<th>Protocol 4</th>
</tr>
</thead>
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<td>70 (Eluant)</td>
<td>1.49</td>
<td>3.61</td>
<td>6.09</td>
<td>10.5</td>
</tr>
<tr>
<td>70 (Donor)</td>
<td>1.63</td>
<td>4.17</td>
<td>5.60</td>
<td>6.64</td>
</tr>
</tbody>
</table>

Table 3.9  pH of the donor and receiver compartment media. Donor and receiver medium: normal saline; current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
cathode will increase the ionization of sulfadiazine thereby enhancing its release. Conversely, a decrease in pH between 2.1 and 6.3 at the anode may not result in any sufficient change in solubility and hence release of drug. Since sulfadiazine release was enhanced under these conditions, the greater pH at the cathode is most likely responsible by increasing sulfadiazine solubility. However, the pH of the donor compartment medium 20 min after current was turned off, shows that it was less than the initial pH (6.0 vs. 5.6; see Table 3.9). This may appear to be a contradiction, but ionization of drug at the cathode results in a loss of protons from sulfadiazine which in turn results in a decrease in pH of the donor medium. These increases in sulfadiazine release were also apparent in the eluant using Protocol 3 since there was an increase in the pH of the receiver medium over the period of $t = 30$ to $t = 50$ min (current was applied from $t = 20$ to $t = 50$ min). The change in pH apparently results from increases in the amounts of ionized drug in the eluant.

Changes in pH of the donor medium can also affect the gelatin wall of the microcapsules in that if it becomes neutral (~pH 5), aqueous channels will close, and if it is below or above pH 5, aqueous channels will open (see Section 1.3.4.2.3). For example, when Protocol 3 (donor pH 6.0) is used, a decrease in the pH of the donor media occurs due to the deprotonation of sulfadiazine at the electrodes as well as the production of protons at the anode. The hydrogen ions will encounter the gelatin wall and attach themselves to it causing it to contract due to attractive forces between chains at its isoelectric point (~pH 5). This closing of pores would be expected to decrease drug permeability through the wall (see Section 3.6 for further discussion).
The same rationale holds for Protocol 2 (donor pH 4.5). When 0.5 mA DC is applied, there was a 4.43 fold increase in sulfadiazine release over that from passive diffusion. In this case, the initial pH of the donor compartment was approximately 4.5, whereas the final pH was ~4.2, also indicating that ionization of sulfadiazine is caused by the passage of an electric current (see Table 3.9). Therefore, the gelatin wall may also act as a barrier to release due to the proximity of the donor media pH to the isoelectric point of gelatin (pH ~5).

In the case of studies performed under Protocol 4 (donor pH 7.0), drug release was also enhanced by the application of current, although the increase was not as significant as with Protocol 3 (see Figure 3.48). This can be explained by the presence of high concentrations of ionized drug (>80%), and therefore the effect of current on the further ionization of sulfadiazine may not be as significant in terms of drug release. In this case, the donor media pH was approximately 7.0, and the gelatin wall would become negatively charged and pores open. A decrease in pH of the donor compartment may cause pores to close however, given the effect of this pH on wall porosity (increased) along with the higher solubility of sulfadiazine at this pH, the barrier effects of the gelatin wall are not as significant when current is applied (see Section 3.6). Table 3.10 summarizes the effects of current and pH as discussed above on both gelatin and sulfadiazine.
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Protocol 1</th>
<th>Protocol 2</th>
<th>Protocol 3</th>
<th>Protocol 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfadiazine</td>
<td>Donor pH: 2.0</td>
<td>Donor pH: 4.5</td>
<td>Donor pH: 6.0</td>
<td>Donor pH: 7.0</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>The application of current does not cause any noticeable change in pH due to the high number of protons present. Therefore drug release is not altered.</td>
<td>The application of current causes an ionization of sulfadiazine at the cathode which in turn results in a net increase in sulfadiazine release.</td>
<td>The application of current causes an ionization of sulfadiazine at the cathode which in turn results in a net increase in sulfadiazine release.</td>
<td>The application of current causes an ionization of sulfadiazine at the cathode which in turn results in a net increase in sulfadiazine release.</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Opening of aqueous channels resulting in higher drug release.</td>
<td>Deswelling of the gelatin wall due to a net decrease in pH results in closing of the aqueous channels, therefore decreasing drug release.</td>
<td>Deswelling of the gelatin wall due to a net decrease in pH results in closing of the aqueous channels, therefore decreasing drug release.</td>
<td>Opening of aqueous channels.</td>
</tr>
<tr>
<td>Combined effects</td>
<td>There is no effect on drug solubility. The gel wall is not a major hindrance to release due to the opening of aqueous channels.</td>
<td>Drug release increases, however the gelatin wall becomes more of a barrier to release due to the closing of aqueous channels in the wall.</td>
<td>Drug release increases, however the gelatin wall becomes more of a barrier to release due to the closing of aqueous channels in the wall.</td>
<td>Increase in solubility. The gel wall is not a major hindrance to release due to the opening of aqueous channels.</td>
</tr>
</tbody>
</table>

Table 3.10 Summary of the effects of pH on the electrically-facilitated release of sulfadiazine from gelatin/sulfadiazine microcapsules. See text for details.
3.3.2.2.5.1  Studies of an isolated donor compartment

As discussed earlier, the diffusion cell used in the release studies has a donor compartment and a flow-through receiver compartment, the latter of which allows virtual sink conditions to be maintained. The microcapsule suspensions were placed directly into the donor compartment. In order to isolate the mechanisms occurring in the donor compartment, a scaled-up model of this compartment was assembled in a glass beaker as described in Section 2.2.6. Platinum wire electrodes were placed in the beaker and pH was measured every 10 min for 70 min. Table 3.11 shows that when the microcapsules were suspended in normal saline solution, pH values did not change significantly upon the application of current.

Samples of the suspension were taken every 10 min, filtered or centrifuged as necessary, and the supernate analyzed by HPLC. Figure 3.49 indicates that there was an increase in drug release when current was applied. In Table 3.11, it can be seen that the concentrations of sulfadiazine in this model donor compartment when current was applied were higher than when the current was off i.e., passive diffusion. However, the application of current did not change the amounts of drug released when the microcapsules were suspended in phosphate buffer (pH 5.4 or 7.6; see Figure 3.50). An increase in drug solubility in normal saline solution must therefore be due to undetectable local pH fluctuations at the electrode surfaces.
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Current (mA DC)</th>
<th>pH</th>
<th>Concentration of sulfadiazine (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>5.5</td>
<td>75.7</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>5.5</td>
<td>79.3</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>5.4</td>
<td>84.8</td>
</tr>
<tr>
<td>30</td>
<td>2.0</td>
<td>5.5</td>
<td>108</td>
</tr>
<tr>
<td>40</td>
<td>2.0</td>
<td>5.4</td>
<td>121</td>
</tr>
<tr>
<td>50</td>
<td>2.0</td>
<td>5.4</td>
<td>134</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>5.4</td>
<td>116</td>
</tr>
<tr>
<td>70</td>
<td>0</td>
<td>5.4</td>
<td>112</td>
</tr>
</tbody>
</table>

Table 3.11 Average concentrations of sulfadiazine and pH of a microcapsule suspension in a scaled-up donor compartment model. Suspending medium: normal saline solution; current: 2.0 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.49  Release of sulfadiazine from gelatin-walled microcapsules in a scaled-up donor compartment. Suspending medium: normal saline solution; current: 2.0 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.50  Release of sulfadiazine from gelatin-walled microcapsules in a scaled-up donor compartment. Suspending medium: phosphate buffer; current: 2.0 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
3.4 Polyacrylic acid as an electrochemomechanical hydrogel wall

3.4.1 Introduction

Hydrogels which display electrochemomechanical behavior are usually those which contain some ionic character. Gelatin, used as the model ECM microcapsule wall, swells and contracts depending on the pH of the surrounding medium (see Section 3.3.2.2.5. Other polymers have also shown ECM behavior and examples are listed in Table 1.1. Of these, polyacrylic acid (PAA) was used to microencapsulate sulfadiazine to study the effect of current on microcapsules with a different ECM hydrogel wall. As discussed in Chapter 1, Section 1.2.6, the sodium salt of PAA showed deswelling behavior when current was applied. Unlike gelatin, which is a biodegradable hydrogel, PAA is a synthetic polymer and is not biodegradable. When considering a pulsatile drug delivery system in which multiple pulses are preferred, a reproducible ECM behavior would be favored. When current is applied, the microcapsule hydrogel wall should expand or contract to the same degree for each cycle, and when the current is off, the hydrogel wall should return to its original state. In the case of a biodegradable material for the wall, such as gelatin, its ECM behavior may not be consistent over long times since there may be changes in the wall structure due to biodegradation. By microencapsulating sulfadiazine with PAA, or another non-biodegradable polymer, this possibility is eliminated.
3.4.2 Morphology

PAA is a hydrophilic polymer, but would not form a coacervate in the presence of sodium sulfate, as had the PVP/sulfadiazine and gelatin/sulfadiazine systems. Therefore, a divalent salt, calcium chloride, which has a greater dehydrating effect, was used to coacervate PAA. This resulted in the formation of distinct microcapsules along with accumulations of larger drug/polymer gel “formations” in which polarized light microscopy indicated that sulfadiazine crystals were present in both types of structures. Figure 3.51 shows PAA/sulfadiazine microcapsules.

3.4.3 In vitro release of sulfadiazine from PAA-walled microcapsules

Passive and electrically-facilitated release studies were carried out with water as the suspending medium and eluant. Polyacrylic acid has an isoelectric point of ~4 and therefore is negatively charged at pH 6.0, the pH of the donor compartment medium. Repulsion between the negatively charged groups on the polymer chains should cause the gel wall to swell, opening aqueous channels in the gel matrix allowing greater diffusion of drug and dissolution medium. However, unlike the gelatin/sulfadiazine microcapsules, no increase in release occurred when 0.5 mA DC was applied for 30 min (see Figure 3.52 and Section 3.3.2.2.5). This suggests that the PAA wall may inhibit the electrical-facilitation of sulfadiazine release. This is contrary to what was expected since PAA should swell under these conditions thereby opening aqueous pores. However, like the flocculation that occurs in polyelectrolyte/di- or tri-valent systems, coacervation of PAA
Figure 3.51  Photomicrograph of polyacrylic acid/sulfadiazine microcapsules. Brightfield, 788x.
Figure 3.52 Release of sulfadiazine from PAA-walled microcapsules. Donor medium: normal saline solution (pH 6.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
by divalent calcium ion may result in a tightly linked hydrogel which does not respond to electrical currents leading to a lower release of sulfadiazine when current is applied.

3.5 PVP/sulfadiazine microcapsules (ionizable drug and nonionizable polymer wall)

3.5.1 Morphology

Polyvinylpyrrolidone (PVP), molecular weights 10,000 and 1,300,000, was used to encapsulate sulfadiazine by a coacervation method similar to that described in Section 2.2.1. The microcapsules were examined by optical microscopy. Differences in the appearances of the walls of the microcapsules prepared with PVP are evident in Figures 3.53 and 3.54. For MW 10,000, the radial structure of sulfadiazine crystals within the apparent PVP wall can be clearly seen by brightfield microscopy at 788x. However, for the PVP (MW 1,300,000), the structure of the sulfadiazine within the PVP wall cannot be easily determined at this magnification, perhaps due to a thicker wall. Scanning electron microscopy was used instead for these microcapsules. Figures 3.55 and 3.56 show the radial-like structure of sulfadiazine crystals apparently surrounded by the PVP wall, as was found for gelatin/sulfadiazine microcapsules and PVP (MW 10,000)/sulfadiazine microcapsules. The PVP (MW 1,300,000)/sulfadiazine microcapsules appear to have a "pinwheel" type organization, which would have a high surface area of drug and polymer available for dissolution thereby contributing to a higher release of drug compared to larger needle-like sulfadiazine crystals.
Figure 3.53  Photomicrograph of PVP (MW 10,000)/sulfadiazine microcapsules. Brightfield, 788x.
Figure 3.54  Photomicrograph of PVP (MW 1,300,000)/sulfadiazine microcapsules. Brightfield 788x.
Figure 3.55  Scanning electron photomicrograph of a PVP (MW 1,300,00)/sulfadiazine microcapsule (5000x).
Figure 3.56  Scanning electron photomicrograph of PVP (MW 1,300,000)/sulfadiazine microcapsules (1500x).
3.5.2  *In vitro* release of sulfadiazine from PVP-walled microcapsules

PVP, a nonionizable polymer, was used as an alternative to gelatin to microencapsulate sulfadiazine since the permeability or porosity of a PVP wall should not be affected by pH as would gelatin. Sulfadiazine release from the PVP-walled microcapsules was determined by passive diffusion and electrical-facilitation in order to test the effect of current on sulfadiazine release from microcapsules having an uncharged hydrogel wall. Figures 3.57-3.61 show sulfadiazine release under passive diffusion and electrically-facilitated conditions as functions of pH using Protocols 1 - 4, and is described in the following sections.

3.5.2.1 Passive diffusion

In Figure 3.57 it can be seen that sulfadiazine release from PVP-walled microcapsules is dependent on pH since there was a higher release from Protocols 1 and 4 (30.1 ± 3.56µg and 257 ± 17.4µg, respectively) than from 2 and 3 (17.4 ± 1.09 µg and 21.1 ± 0.46 µg, respectively) due to the effect of pH on sulfadiazine solubility. As mentioned above, PVP is a nonionizable polymer and therefore the permeability of the wall should remain unchanged at different pH values. In order to determine the effect of current on the microcapsule wall, these release studies are compared with that for sulfadiazine suspensions, gelatin/sulfadiazine microcapsules and PVP/sulfadiazine microcapsules in Section 3.6.
Figure 3.57  Release of sulfadiazine by passive diffusion from PVP-walled microcapsules as functions of donor medium pH. Donor medium: normal saline solution at pH 2.0, 4.5, 6.0 and 7.0.
Figure 3.58  Release of sulfadiazine from PVP-walled microcapsules. Protocol 1; donor medium: normal saline solution (pH 2.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.59 Release of sulfadiazine from PVP-walled microcapsules. Protocol 2; donor medium: normal saline solution (pH 4.5); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.60  Release of sulfadiazine from PVP-walled microcapsules. Protocol 3; donor medium: normal saline solution (pH 6.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
Figure 3.61 Release of sulfadiazine from PVP-walled microcapsules. Protocol 4; donor medium: normal saline solution (pH 7.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
3.5.2.2 Electrically-facilitated release

When 0.5 mA DC was applied in the donor compartment using Protocol 1, there was no apparent increase in sulfadiazine release from that by passive diffusion. As discussed in Sections 3.2.3.2 and 3.3.2.2.5, there were no significant pH changes occurring in the donor compartments under these conditions, and therefore the solubilities and release of sulfadiazine remained the same as that by passive diffusion. For Protocols 2 and 3 however, there were increases in the amounts of sulfadiazine released compared to that by passive diffusion (112 ± 7.15 µg vs. 17.4 ± 1.09 µg and 107 ± 14.6 µg vs. 21.1 ± 0.46 µg, respectively), most likely due to increases in pH at the cathode resulting in increases in the degrees of ionization of the drug. The ionized or salt form is more soluble than the uncharged form, and therefore the rate of release of sulfadiazine will increase. In this context, the properties of the PVP wall should remain unaffected by changes in pH occurring upon the application of current. Lastly, it should be noted that for the Protocol 4 system, drug solubility is high and therefore there was only a 1.34 fold increase from that by passive diffusion due to current-induced ionization of sulfadiazine.

Sample t-tests were performed using Bonferroni correction for multiple testing on the total amounts of sulfadiazine released after 70 min by passive diffusion and electrically-facilitated release for the four experimental protocols (see Table 3.12). These statistical tests suggest that when the initial pH of the donor compartment is 4.5 and above, i.e., Protocols 2, 3 and 4, the application of 0.5 mA DC for 30 min to a suspension
<table>
<thead>
<tr>
<th>Protocol</th>
<th>No Current</th>
<th>0.5 mA DC (20-50 min.)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Amt (µg)</td>
<td>Std. Dev.</td>
<td>Mean Amt (µg)</td>
</tr>
<tr>
<td>1</td>
<td>30.1</td>
<td>3.56</td>
<td>28.3</td>
</tr>
<tr>
<td>2</td>
<td>17.4</td>
<td>1.09</td>
<td>112</td>
</tr>
<tr>
<td>3</td>
<td>21.1</td>
<td>0.46</td>
<td>107</td>
</tr>
<tr>
<td>4</td>
<td>257</td>
<td>17.4</td>
<td>344</td>
</tr>
</tbody>
</table>

Table 3.12 Statistical t-test comparisons of the release of sulfadiazine from PVP-walled microcapsules, by passive diffusion and electrical-facilitation after 70 min.

<sup>a</sup> indicates the Satterthwaite p-value for unequal variances was used
<sup>b</sup> indicates marginal significance
<sup>c</sup> indicates statistically significant
of PVP/sulfadiazine microcapsules will cause an increase in sulfadiazine release when
compared to that under passive diffusion conditions apparently due to the ionization of
sulfadiazine, as discussed above.

3.5.2.2.1 Electroosmosis and/or solvent flow

As discussed in Chapter 1, one of the factors that can influence the electrical
facilitation of sulfadiazine release from microcapsules with gelatin walls is an increase in
solvent flow within the donor compartment. The application of an electric current to an
electrolyte solution containing microcapsules may affect drug release by:

- electroosmosis of solvent into and out of the microcapsule wall thereby
  increasing drug dissolution and release, and
- migration of hydrated ions toward the opposite electrodes leading to the
  movement of microcapsules within the donor compartment.

In the first case, for electroosmosis to occur, the microcapsule hydrogel wall must
be charged, such as in the case of gelatin. Increases in the flux of dissolution medium
into and out of the hydrogel wall may cause increases in drug dissolution and hence drug
release. However, increases in the amounts of sulfadiazine released from
gelatin/sulfadiazine microcapsules when current was applied were less than or
comparable to that from sulfadiazine suspensions (see Section 3.6). This suggests that
the effects of electroosmosis on sulfadiazine release from gelatin-walled microcapsules
may not be a primary mechanism in the electrical facilitation of sulfadiazine release
under the conditions tested.
For the second case above, an electrically-induced solvent flux caused by mobile hydrated ions can affect the location of microcapsules within the donor compartment. For example, when current was applied for periods 30 min and longer to suspensions of gelatin/sulfadiazine microcapsules, a region devoid of microcapsules formed around the cathode and an apparent increased number of microcapsules appeared around the anode. This movement could be a due to:

- an attraction of negatively charged chains of the gelatin to the anode,
- an attraction of hydroxyl ions and/or other ions adsorbed onto the gel wall to the anode, and/or to
- a migration of hydrated ions toward the oppositely charged electrode.

The result of these three factors is that the microcapsules appear to move and because of the necessity to displace solvent, there will be a net flow of solvent within the donor compartment. In this context, the physical location of microcapsules in the donor compartment can be an important consideration for those with an ionizable wall since their position within the cell may affect the porosities of their walls. For example, if gelatin/sulfadiazine microcapsules are located near the anode where there is a decrease in pH, the ionization state of gelatin may change and cause the walls to swell as discussed previously in Section 3.3.1.2. Similarly, if the microcapsules are situated near the cathode where there is a local increase in pH, permeability of the gelatin wall to sulfadiazine may also be affected due to swelling of the gel.

In this context, the release of sulfadiazine from microcapsules with a nonionizable PVP wall was determined. The uncharged wall of these microcapsules eliminates the possibility of wall permeability changes caused by electrically-induced pH fluctuations.
When a 0.5 mA direct current was applied to a suspension of PVP (MW 1,300,000)/sulfadiazine microcapsules for 30 min there was no noticeable change in their physical location in the donor compartment. Instead, PVP (MW 10,000) was used and the release experiments were repeated. After 30 min of 0.5 mA DC, these PVP/sulfadiazine microcapsules migrated toward the anode with a region devoid of microcapsules appearing around the cathode. Apparently the lower molecular weight microcapsule wall made them more susceptible to electrically-induced migration than the higher molecular weight wall. Therefore the location of the microcapsules within the donor compartment is dependent on the duration of the current as well as the molecular weight of the polymer used for the microcapsule wall.

3.6 Comparison of sulfadiazine release from sulfadiazine suspensions, gelatin-walled and PVP-walled microcapsules

3.6.1 pH effects

Sections 3.2, 3.3 and 3.5 indicate that changes in pH whether caused by the addition of HCl or NaOH, or by electrode reactions, can be used to control drug release from microcapsules as well as from suspensions of sulfadiazine crystals. The effect of an electric current on sulfadiazine release was evident in the studies of sulfadiazine suspensions (see Section 3.2), however the effects of current (i.e., pH changes) on the microcapsule wall are not as well defined. In order to more clearly determine the affect of current on the microcapsule wall, side-by-side comparisons have been made between
the release of sulfadiazine from suspensions of sulfadiazine, gelatin/sulfadiazine microcapsules, and PVP (MW 1,300,000)/sulfadiazine microcapsules. Specifically, the sulfadiazine suspensions were used to eliminate the effects of the microcapsule wall, gelatin was used because it is charged and shows ECM behavior, and PVP was used because it is uncharged and its porosity should not be affected by electrically-induced pH changes. Release studies were carried out for 70 min using Protocols 1 - 4 (see Section 2.2.5).

3.6.1.1 Passive Diffusion

When considering sulfadiazine suspensions, the degree of ionization of sulfadiazine is a crucial parameter in determining the extent of its release. As shown in Section 3.2.2, due to the two pKas of sulfadiazine, its solubility and release changes with pH. On the other hand, in the case of the microcapsules, there are two components that must be considered: the drug and the hydrogel wall.

3.6.1.1.1 Protocol 1 (donor pH 2.0)

When Protocol 1 is followed, sulfadiazine is ionized and its solubility is higher than when it is in its uncharged form (2.1 < pH < 6.3). This was also seen for all three formulations in the passive diffusion studies where total sulfadiazine release was lower for Protocols 2 and 3 (see Figure 3.62). This data also shows that sulfadiazine release from gelatin/sulfadiazine microcapsules was higher than from either the PVP/sulfadiazine
Figure 3.62  Total amounts of sulfadiazine released after 70 min from sulfadiazine suspensions, gelatin/sulfadiazine and PVP/sulfadiazine microcapsules. Donor medium: normal saline solution at pH 2.0, 4.5, 6.0 and 7.0. Current: 0.5 mA DC applied from $t = 20$ to $t = 50$ min. Electrodes: platinum wire.
microcapsules or the sulfadiazine suspensions (46.4 µg, 30.1 µg, and 37.8 µg, respectively). Tukey's method of multiple comparisons was used to adjust for multiple testing between the three formulations using an overall α-level of 0.05. This test also shows that passive diffusional release of sulfadiazine from gelatin/sulfadiazine microcapsules was higher than from the other two formulations. An explanation for this is that gelatin is positively charged at this pH (donor pH ~2) and it should therefore swell as aqueous channels open due to repulsion between like-charged groups. These aqueous channels can then increase drug release by allowing dissolution medium to enter the microcapsule core, as well as allowing drug in solution to more easily diffuse through the wall. Because of this swelling, the gelatin wall becomes a less efficient barrier under these conditions. In fact, this more hydrated wall may actually facilitate dissolution of drug by increasing the amount of dissolution medium in contact with sulfadiazine crystals in the microcapsule core. Therefore, the more hydrated wall, together with an increase in drug surface area (drug particles vs. radially aligned needle-like crystals in the microcapsules), may explain the higher release of sulfadiazine from the gelatin-walled microcapsules compared to that from sulfadiazine suspensions.

When considering PVP/sulfadiazine microcapsules, Tukey's Comparison testing also shows that there is no difference between passive diffusional release of sulfadiazine from both PVP/sulfadiazine microcapsules and sulfadiazine suspensions, suggesting that the PVP wall does not hinder or aid sulfadiazine release under these conditions.
3.6.1.1.2 Protocols 2 and 3 (donor pH 4.5 and 6.0)

The release of sulfadiazine using Protocols 2 and 3 was low from all three formulations, undoubtedly due to the low solubility of the drug in this range. In this context there were 15.1 ± 0.58 µg, 20.5 ± 1.06 µg and 17.4 ± 1.09 µg from sulfadiazine suspensions, and gelatin-walled and PVP-walled microcapsules, respectively when Protocol 2 was used; and there were 14.5 ± 0.4 µg, 22.7 ± 0.86 µg, 21.1 ± 0.46 µg from sulfadiazine suspensions, gelatin-walled and PVP-walled microcapsules, respectively, when Protocol 3 was used. It appears that sulfadiazine release from the both the gelatin-walled and PVP-walled microcapsules was higher than that from the sulfadiazine suspensions. This would be contrary to what would be expected for the gelatin/sulfadiazine microcapsules as the gelatin wall should resist release, since the pH of the donor media was close to the isoelectric point of gelatin, i.e., donor media pH was approximately 4.5 and 6.0 using Protocols 2 and 3, respectively. At the isoelectric point, very little or no swelling should occur (see Section 3.3.1.2), however, smaller aqueous channels should still be present in the gelatin matrix. The small size and number of these aqueous channels will limit drug transport in essence creating a "barrier" at this pH. However, under passive diffusion conditions in which drug dissolution is slow, the large surface area of the hydrated walls may facilitate drug dissolution by allowing water to come into contact with sulfadiazine.

In order to test the effect of surface area on sulfadiazine release, sodium sulfadiazine was precipitated in the same manner as the microcapsules. This rapid precipitation also forms clusters of needle-like sulfadiazine crystals organized in a radial
array as described in Section 3.1.1 and Figures 3.4-3.5. Release of sulfadiazine by passive diffusion from suspensions of the precipitated drug was determined using Protocol 3. The amounts of sulfadiazine released at 70 min were lower than that from microcapsules under the same conditions, i.e., 14.4 μg vs. 14.5 μg vs. 22.7 μg vs. 21.1 μg from precipitated sulfadiazine suspensions, sulfadiazine suspensions, gelatin/sulfadiazine microcapsules and PVP/sulfadiazine microcapsules, respectively, as shown in Figure 3.63. Therefore, hydration of the hydrogel wall which in turn promotes contact of the donor medium with the microencapsulated drug, is most likely responsible for the higher amounts released from the microcapsules under these conditions.

3.6.1.1.3 Protocol 4 (donor pH 7.0)

Passive diffusional release of sulfadiazine was similar from all three formulations when Protocol 4 was used, i.e., 253 ± 33.8 μg vs. 257 ± 19.8 μg vs. 257 ± 17.4 μg, for the sulfadiazine suspensions, gelatin/sulfadiazine microcapsules, and PVP/sulfadiazine microcapsules, respectively. This similarity in release can be explained by the higher solubility of sulfadiazine at this pH (donor pH ~7) since sulfadiazine is over 80% ionized (see Section 3.2.2). Under these conditions, the hydrated gel wall does not have a noticeable effect on the dissolution and release of sulfadiazine.
Figure 3.63  Release of sulfadiazine by passive diffusion as functions of the type of formulation. Protocol 3; Donor medium: normal saline solution.
3.6.1.2 Electrically-facilitated release

As indicated earlier, when current is applied to the donor compartment, pH fluctuations caused by the ionization of the drug and the reactions at the electrodes may affect the porosity of a gelatin wall and thereby drug release. Figure 3.62 shows both the passive diffusional and electrically-facilitated release of sulfadiazine from the three formulations. When the pH of the donor compartment was 4.5 or above, the amounts of sulfadiazine released from the three formulations increased when an electric current was applied. When considering the top three curves in the figure, those representing electrically-facilitated release from all three formulations, are not parallel, suggesting that the electric current affects the three formulations differently as functions of pH. In order to more easily see these effects, the ratios of the amounts released when current was applied to that from passive diffusional release are plotted in Figure 3.64. From this figure, it appears that the barrier effects of the gelatin wall to sulfadiazine release increased when Protocols 2 and 3 were used. Statistical analysis was performed using a series of contrasts that compares the difference in passive diffusion and electrically-facilitated release from the three formulations using experimental Protocols 1 - 4. Table 3.13 shows the results of the contrasts from the ANOVA model. All testing was done using an $\alpha$ level of 0.05, and the comparisons are discussed in the following sections.
Figure 3.64 The magnitude of increase in the amounts of sulfadiazine released by passive diffusion and electrical facilitation from sulfadiazine suspensions, gelatin/sulfadiazine and PVP/sulfadiazine microcapsules. The pH values are those of the donor compartment medium. See text for details.
<table>
<thead>
<tr>
<th>Protocol</th>
<th>Source of Variation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Formulations 1 and 2</td>
<td>0.797</td>
</tr>
<tr>
<td></td>
<td>Formulations 1 and 3</td>
<td>0.709</td>
</tr>
<tr>
<td></td>
<td>Formulations 2 and 3</td>
<td>0.530</td>
</tr>
<tr>
<td>2</td>
<td>Formulations 1 and 2</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td>Formulations 1 and 3</td>
<td>0.697</td>
</tr>
<tr>
<td></td>
<td>Formulations 2 and 3</td>
<td>0.106</td>
</tr>
<tr>
<td>3</td>
<td>Formulations 1 and 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Formulations 1 and 3</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Formulations 2 and 3</td>
<td>0.053</td>
</tr>
<tr>
<td>4</td>
<td>Formulations 1 and 2</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>Formulations 1 and 3</td>
<td>0.707</td>
</tr>
<tr>
<td></td>
<td>Formulations 2 and 3</td>
<td>0.109</td>
</tr>
</tbody>
</table>

Table 3.13  Contrast from the ANOVA model comparing the difference between passive diffusion and electrically-facilitated release of sulfadiazine using a normal saline suspending medium. Formulation 1: sulfadiazine suspensions. Formulation 2: gelatin/sulfadiazine microcapsules. Formulation 3: PVP/sulfadiazine microcapsules.
3.6.1.2.1 Protocol 1 (donor pH 2.0)

When Protocol 1 was used, there was no change in release from passive diffusion. As previously discussed, this suggests that any pH change in the donor compartment is not sufficient to cause an increase in sulfadiazine solubility. Statistical analysis also supports this result in that there was no difference (p > 0.05), in the effects of current on the release of sulfadiazine from sulfadiazine suspensions, gelatin/sulfadiazine and PVP/sulfadiazine microcapsules. As mentioned in Section 3.6.1.1.1, in this case, release from the gelatin-walled microcapsules was highest due to the opening of aqueous channels.

3.6.1.2.2 Protocols 2 and 3 (donor pH 4.5 and 6.0)

When Protocols 2 and 3 were used, there was an increase in sulfadiazine release from all three formulations upon application of a current. As previously discussed, this increase was most likely due to ionization of sulfadiaizine at the cathode (see Sections 3.2.3.2 and 3.3.2.2.5).

When comparing sulfadiazine release from the three formulations for Protocols 2 and 3, as shown in Figure 3.64, it was highest from the sulfadiazine suspensions. Statistical analysis of Protocol 3 shows that current has the most significant effect on the gelatin wall when it is compared to the sulfadiazine suspensions (p < 0.001). In this case, the decrease in pH at the anode (the electrode to which the microcapsules migrate towards under the influence of current) and the resulting ionization of sulfadiazine may
affect the porosity of the wall when the pH reaches the isoelectric point of gelatin (~5). In other words, the production of hydroxyl ions at the cathode will deprotonate the drug leading to a net increase in the concentration of protons in the donor compartment, and therefore an overall decrease in pH. The protons produced at the anode and by the ionization of sulfadiazine will in turn, encounter the gelatin walls of the microcapsules and attach themselves to them. The closing of pores by the contraction of the gelatin wall would block sulfadiazine release and the passage of dissolution medium through the wall, causing a significant decrease in release from the system. The PVP wall may also prove to be a barrier to sulfadiazine release when Protocol 3 was used, compared to that for sulfadiazine suspensions. However its effects may not be as significant as that of the gelatin wall (p = 0.004) since pores in the PVP wall should be unaffected by electrically-induced local pH changes. Although the differences in the amounts of sulfadiazine released from the three formulations are not statistically significant for Protocol 2, a similar explanation as for Protocol 3 can be used to describe the trends seen in Figure 3.64.

3.6.1.2.3 Protocol 4 (donor pH 7.0)

When using experimental Protocol 4, there were no significant differences in the amounts of drug released between the three systems after 70 min when current was applied. The gelatin wall is negatively charged at this pH and therefore is not a significant barrier to release. As was the case with Protocol 1, the PVP wall also does not appear to inhibit sulfadiazine release when compared with sulfadiazine suspensions.
3.6.2 Rates of hydrolysis

Rates of release were calculated from the slopes of plots of the total amounts of sulfadiazine released as functions of time, i.e., from \( t = 35 \text{ min} \) to \( t = 55 \text{ min} \), in which all three formulations showed a constant slope. Estimates of the degrees of ionization of sulfadiazine in the presence of the current were then calculated. Platinum wire electrodes were used in these release studies. The rate of the electrochemical hydrolysis reaction occurring at the inert platinum electrodes can be estimated from the following equation:

\[
v = \frac{I}{nF}
\]

where: \( v \), is rate of hydrolysis; \( I \), the current density; \( n \), the number of electrons, and \( F \), Faraday's constant.

Equation 5 can be used to calculate the rate at which hydroxyl ions are produced at the cathode representing the maximum rate of ionization of sulfadiazine when current is applied. Since the change in local pH could not be measured directly due to unstable readings, Equation 5 was used to determine the effect of current on the ionization of sulfadiazine in sulfadiazine suspensions, and for gelatin/sulfadiazine and PVP/sulfadiazine microcapsules. The estimations of the rates of hydrolysis are for Protocols 2, 3 and 4, in which the most significant effects in electrically-facilitated release were found (see Section 3.6.1.2).

When 0.5 mA DC is applied, the rate of hydroxyl ion production is \( 1.36 \times 10^{-6} \text{ mol/min} \) according to Equation 5. As already mentioned, this number should represent the maximum rate of ionization of sulfadiazine at the cathode. Table 3.14
<table>
<thead>
<tr>
<th>Protocol</th>
<th>Formulation</th>
<th>Rate (mol/min)</th>
<th>% of hydrolysis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Passive diffusion</td>
<td>Electrically-facilitated release</td>
</tr>
<tr>
<td>2</td>
<td>SS</td>
<td>$8.25 \times 10^{-10}$</td>
<td>$1.11 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>G/S</td>
<td>$1.15 \times 10^{-9}$</td>
<td>$1.09 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>P/S</td>
<td>$9.67 \times 10^{-10}$</td>
<td>$1.08 \times 10^{-8}$</td>
</tr>
<tr>
<td>3</td>
<td>SS</td>
<td>$8.12 \times 10^{-10}$</td>
<td>$1.59 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>G/S</td>
<td>$1.26 \times 10^{-9}$</td>
<td>$9.21 \times 10^{-9}$</td>
</tr>
<tr>
<td></td>
<td>P/S</td>
<td>$1.18 \times 10^{-9}$</td>
<td>$9.42 \times 10^{-9}$</td>
</tr>
<tr>
<td>4</td>
<td>SS</td>
<td>$1.50 \times 10^{-8}$</td>
<td>$2.67 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>G/S</td>
<td>$1.49 \times 10^{-8}$</td>
<td>$2.45 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>P/S</td>
<td>$1.46 \times 10^{-8}$</td>
<td>$2.32 \times 10^{-8}$</td>
</tr>
</tbody>
</table>

Table 3.14  Estimations of the rates of release of sulfadiazine and the percent ionized when current was applied.

SS = Sulfadiazine suspensions
G/S = Gelatin/sulfadiazine microcapsules
P/S = PVP/sulfadiazine microcapsules
shows the actual rates of electrically-facilitated release following Protocols 2, 3 and 4 for sulfadiazine suspensions, and for gelatin-walled and PVP-walled microcapsules. The most significant differences in sulfadiazine release occurred when Protocol 3 was used (donor pH ~6). The rate of release of sulfadiazine from sulfadiazine suspensions was approximately 1.5 x 10^{-8} \text{ mol/min} which is the largest rate of the three formulations. This suggests that ~0.01 mole of sulfadiazine was ionized at the cathode (assuming that no ionization was occurring at the anode) per mole of hydroxyl ion produced by hydrolysis (1.36 x 10^{-6} \text{ mol/min}). Conversely, the rate of sulfadiazine release from gelatin-walled microcapsules was 0.58\% of the rate of hydrolysis, suggesting again that the gelatin wall partially hindered electrical-facilitated release of sulfadiazine. The PVP wall also hindered sulfadiazine release under these conditions since the rate of sulfadiazine release in this case was ~0.61\% of the rate of hydrolysis.

When Protocol 2 was used, the rates of release were 0.76\%, 0.72\% and 0.72\% of the rate of hydrolysis from sulfadiazine suspensions, gelatin-walled microcapsules and PVP-walled microcapsules, respectively. Although the differences between these estimations are not as significant as with Protocol 3, they support the results shown in Figure 3.66, the conclusion that the barrier properties of the gelatin increase when current is applied under appropriate conditions.

As discussed in Section 3.6.1, the release of sulfadiazine was high when Protocol 4 (donor pH ~7.0) was used. It was found that no significant differences existed between the amounts of drug released from the three formulations when current was applied. The microcapsule walls consisting of PVP or gelatin did not appear to hinder the electrical-facilitation of sulfadiazine release, as seen with Protocols 2 and 3.
Since the passive diffusional release of sulfadiazine using Protocol 4 was already high, the effects of current on the gelatin walls may have been masked. Therefore, the rates of ionization of sulfadiazine were calculated to help define the barrier effects of the polymer walls. The rates of release of sulfadiazine were 0.86%, 0.71% and 0.63% of the calculated rates of hydrolysis (see Table 3.14) from sulfadiazine suspensions, gelatin-walled and PVP-walled microcapsules, respectively. This suggests that the PVP and gelatin walls may still have been a barrier to sulfadiazine release when current was applied. However, the gelatin wall was less of a barrier to sulfadiazine release when compared to that of the PVP-walled microcapsules (0.71% vs. 0.63%, respectively).

Conversely, when Protocols 2 and 3 were used, the gelatin wall was more of a barrier to sulfadiazine release when under the influence of an electric field, again indicating that at pH values higher than the isoelectric point of gelatin, aqueous pores open.

3.6.3 Lag-time effects

Sections 3.6.1 and 3.6.2 indicate that under the four protocols and an electric current, the release of sulfadiazine is dependent on the type of system being studied, i.e., sulfadiazine suspensions or gelatin-walled and PVP-walled microcapsules. It was also found that the rates of response of the formulations to the electric current differed, as was seen in the lag-times required before increases in sulfadiazine were detectable above that of passive diffusion. The rate of response of a gel to an electric current is an important consideration for a potential pulsatile drug delivery system in which the current can be applied "on demand" according to the patients needs or in response to a biosensor. In
these cases, a quick response, or a short lag time, would be preferred. On the other hand, if a preprogrammed release pattern is preferred, such as in circadian rhythm dependent disease states, then the response time of the gel to the current could be accounted for in programming the device.

In the present systems, 0.5 mA DC was applied to the suspensions in the donor compartments from $t = 20$ min to $t = 50$ min of a 70 min release study. When Protocols 2, 3 and 4 were used, significant increases in sulfadiazine release from that by passive diffusion were seen for sulfadiazine suspensions, gelatin/sulfadiazine microcapsules and PVP/sulfadiazine microcapsules. The lag times for each system were determined from the data in Figures 3.8-3.10, 3.46-3.48 and 3.59-3.61, and shown in Table 3.15. These lag times are due to constant instrumentation parameters and also to variable parameters due to the differences in the formulations.

The lag time occurring from instrumentation parameters are constant for all three formulations. For example, transport of sulfadiazine through the Spectra/Por® membrane and receiver compartment is affected by the rate of flow of the eluant (1 mL/min) combined with the volume of the receiver compartment (~2 mL). Therefore, at least hypothetically, if each system responded immediately to the electric current, a lag time of ~2-3 min would still be expected. However, as shown in Table 3.15, the lag times for all three formulations at each of the three protocols were greater than 3 min (i.e., ranging from ~5 min to ~15 min). Therefore, the differences between the formulations in response to an electric current need to be considered. For sulfadiazine suspensions, the lag times may be affected by the surface area and wettability of the large needle-like crystals. When considering the gelatin-walled and PVP-walled microcapsules, both of
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Protocol 1</th>
<th>Protocol 2</th>
<th>Protocol 3</th>
<th>Protocol 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfadiazine suspensions</td>
<td>---</td>
<td>~14</td>
<td>~13</td>
<td>~13</td>
</tr>
<tr>
<td>Gelatin/sulfadiazine microcapsules</td>
<td>---</td>
<td>~11</td>
<td>~15</td>
<td>~8</td>
</tr>
<tr>
<td>PVP/sulfadiazine microcapsules</td>
<td>---</td>
<td>~5</td>
<td>~5</td>
<td>~5</td>
</tr>
</tbody>
</table>

Table 3.15 Lag times for sulfadiazine suspensions, gelatin/sulfadiazine microcapsules and PVP/sulfadiazine microcapsules. Current: 0.5 mA DC from \( t = 20 \) to \( t = 50 \) min; and electrodes: platinum wire.
the microcapsules contained a similar star-like sulfadiazine core, however only the gelatin-walled microcapsules had a functional electrochemomechanical wall. In this context, the electrochemomechanical behavior of the gelatin walls functioned as a “gate” controlling sulfadiazine release. This gate-type of behavior was dependent on electrically-induced pH changes occurring at the electrode surfaces, in that it swelled and opened aqueous channels allowing for easier passage of drug and dissolution medium, or contracted and closed aqueous channels essentially decreasing its permeability to drug and dissolution medium, depending on the pH of the surrounding medium. Therefore, the lag times for the gelatin-walled microcapsules are expected to change with pH whereas the lag times for the non-ECM PVP-walled microcapsules should not be pH dependent.

Table 3.15 shows the lags times for all three formulations for Protocols 2, 3 and 4. The lag times for sulfadiazine suspensions were approximately 13 to 14 minutes for the three protocols. The consistency between the three protocols is expected since there is no functional wall material. Again, as expected, the lag times for the PVP/sulfadiazine microcapsules also remained constant at ~5 min for each protocol, due to the non-functional wall material. In contrast, the lag times for the gelatin/sulfadiazine microcapsules differed between protocols, i.e., ~11 min, 15 min, and 8 min for Protocols 2, 3 and 4, respectively. This is consistent with the ECM behavior of the gelatin wall. For example, at pH 4.5 (Protocol 2) and pH 6.0 (Protocol 3), it was found that the gelatin wall deswelled and closed aqueous channels thereby decreasing the permeability of the wall to sulfadiazine. This is shown in the longer lag times of approximately 11 and 15 min. Conversely, at pH 7.0 (Protocol 4), the gelatin wall should swell and open up
channels allowing for easier passage of drug and dissolution medium, and this is represented by the shorter 8 min lag time in which more drug is available to the effects of the current.

More specifically, when considering the composition of a potential pulsatile drug delivery system, e.g., for transdermal delivery, Protocol 3 appears to be the best model since the pH of the eluant best matches that of physiologic conditions. Under conditions of this protocol the greatest differences in electrically-facilitated release of sulfadiazine were seen among the three formulations. In fact, the model gelatin/sulfadiazine microcapsules had the longest lag time of ~15 min, whereas the PVP/sulfadiazine microcapsules had the shortest response time, ~5 min. Therefore as already discussed, the 10 minute differences in the lag times between the PVP-walled microcapsules and the gelatin-walled microcapsules was apparently due to the deswelling of the wall at pH values close to the isoelectric point of gelatin thereby hindering the effects of current on the release of sulfadiazine. On the other hand, PVP is a nonionizable polymer and should not show pH dependent swelling behavior. Therefore, the PVP wall should not act as a gate controlling sulfadiazine release, and as such, its response time to electrically-facilitated release of sulfadiazine should be shorter than that of the gelatin wall.

As with the PVP/sulfadiazine microcapsules, the lag time for the rate of response of the sulfadiazine suspensions was also shorter than that of the gelatin/sulfadiazine microcapsules due to the absence of a wall (~13 min vs. 15 min, respectively). However, the lag time for the sulfadiazine suspensions was approximately 8 minutes longer than that for the PVP-walled microcapsules (~13 vs. 5 min, respectively). This difference may be due to the apparently larger sizes of the sulfadiazine particles, or to reduced wetting of
the insoluble drug in its uncharged form and therefore more hydrophobic in nature. For example, the sulfadiazine suspensions consisted of large needle-like sulfadiazine crystals are essentially uncharged in the pH range of 2.1 > pH > 6.3, and therefore have low aqueous solubilities. By contrast, sulfadiazine encapsulated within PVP walls takes on a star-like structure consisting of small individual crystals resulting in an apparent greater surface area available to the dissolution medium than the sulfadiazine needle-like crystals. In addition, the hydrophilicity of the PVP walls would attract water to the sulfadiazine, thereby increasing its rate of dissolution.

A noticeable lag time also occurred in all three formulations when current was turned off (see Figure 3.65). In the present systems, when Protocol 3 was used for example, the rates of electrically-facilitated release of sulfadiazine was highest from the sulfadiazine suspensions (see Table 3.16). In Figure 3.65 it can be seen that when the current was turned off at 50 min, release did not appear to decrease until approximately $t = 65$ min, whereas a decrease was evident at $\sim 57$ min for the gelatin/sulfadiazine microcapsules and at $\sim 55$ min for the PVP/sulfadiazine microcapsules. These differences were apparently due to lower amounts of microencapsulated sulfadiazine being ionized at the electrode surfaces (see previous Section). In other words, after the current had been turned off, some of the drug, which had been ionized at the electrode surfaces, must continue to diffuse into the receiver compartment. In this context, Table 3.16 shows the rates of release of sulfadiazine before, during, and after the current was turned on, under which the differences in slopes between these three off/on/off cycles are apparent. When considering the model gelatin/sulfadiazine microcapsules, the rates were $0.33 \mu g/min$, $2.40 \mu g/min$ and $0.72 \mu g/min$, respectively. The differences between
Figure 3.65 Release of sulfadiazine from sulfadiazine suspensions, gelatin/sulfadiazine microcapsules and PVP/sulfadiazine microcapsules. Protocol 3. Donor medium: normal saline solution (pH 6.0); current: 0.5 mA DC from $t = 20$ to $t = 50$ min; and electrodes: platinum wire.
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Lag time (min)</th>
<th>Rate (μg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Current on (t = 20 min)</td>
<td>Current off (t = 50 min)</td>
</tr>
<tr>
<td>Sulfadiazine suspensions</td>
<td>~13</td>
<td>~15</td>
</tr>
<tr>
<td>Gelatin/ sulfadiazine microcapsules</td>
<td>~15</td>
<td>~7</td>
</tr>
<tr>
<td>PVP/ sulfadiazine microcapsules</td>
<td>~5</td>
<td>~5</td>
</tr>
</tbody>
</table>

Table 3.16 Lag times and rates of release of sulfadiazine from sulfadiazine suspensions, gelatin/sulfadiazine microcapsules and PVP/sulfadiazine microcapsules. Protocol 3; donor medium: normal saline (pH 6.0); current: 0.5 mA DC from t = 20 to t = 50 min; and electrodes: platinum wire.
passive diffusion, i.e., during the off cycles (0.33 µg/min vs. 0.72 µg/min), are apparently due to diffusion of residual sulfadiazine which had been ionized by the current. With time (~35 min) in the case of the gelatin/sulfadiazine microcapsules under these conditions, most of the ionized form of the drug will be depleted and drug release will return to the original rate of release by passive diffusion. These lag times were therefore dependent on the extent of drug ionization as determined by the magnitude and duration of the current, and by the type of formulation used.
CHAPTER 4

GENERAL SUMMARY

CONCEPT MAP

Summary

- Pulsatile drug release from microcapsules with an electrochemomechanical wall has been achieved upon the application of an electrical current in on/off cycles.
- Changes in pH occurring at the electrode surfaces upon application of current appear to be the mechanism responsible for such electrically-facilitated pulsatile release of sulfadiazine.
It has been shown that pulsatile release of an ionizable drug from microcapsules with electrochemomechanical (ECM) walls can be achieved and controlled by the application of an electric current. The electric current causes pH fluctuations in the vicinities of the electrodes. These pH changes affect drug solubility, and therefore the dissolution rate of drug, as well as the porosity of the hydrogel wall which controls transport processes into and out of the microcapsule. Since the current causes reversible changes in the ionization states of both the wall and the core materials of the microcapsules, drug release can be controlled by simply altering the current in on/off cycles. In order to evaluate the effect of current on the structure of the microcapsule wall and the release of drug through it, the following three formulations were studied: sulfadiazine suspensions (the control system with no wall), gelatin/sulfadiazine microcapsules (the model system with an ECM wall) and PVP/sulfadiazine microcapsules (another model system, but not with an ECM wall).

Sulfadiazine suspensions:

The solubility of sulfadiazine was dependent on the pH of the dissolution medium and on the two pKas of the drug. When sulfadiazine is ionized (2.1 > pH > 6.3), its aqueous solubility increases when compared to that of its uncharged form. Therefore, passive diffusion of sulfadiazine from sulfadiazine suspensions using Protocols 1 and 4 (i.e., donor compartment pH 2.0 and 7.0, respectively) was higher than for those by Protocols 2 and 3, where the donor pH was 4.5 and 6.0, respectively. In this context, the application of an electric current to the suspension resulted in pH fluctuations which
altered the solubility of the drug and therefore its rate of release. When Protocols 2 - 4 were used, there were significant increases in sulfadiazine release from that by passive diffusion upon application of 0.5 mA DC for 30 minutes (7.68, 10.8 and 1.38 fold, for Protocols 2, 3 and 4, respectively). For Protocol 1, such changes were not as apparent due to the relatively small change of pH in the donor compartment.

The solubility of sulfadiazine appears to be the main contributor to its electrically-facilitated release when normal saline solutions were used as both the eluant and donor media. In some cases, iontophoresis of charged drug may also occur; and this was seen as an increase in the release of sodium sulfadiazine when phosphate buffer was used as the suspending medium in the donor compartment and as the eluant.

**Gelatin/sulfadiazine microcapsules:**

Gelatin/sulfadiazine microcapsules were prepared by a pH change method in which the drug was precipitated simultaneously with coacervation of the wall material. Passive and electrically-facilitated *in vitro* release studies were performed under various conditions in order to determine the mechanism for sulfadiazine release when current was applied. Ag/AgCl wire, Ag/AgCl commercially prepared electrodes, and platinum wire electrodes were all evaluated for use in the drug release studies. When current was applied to platinum and Ag/AgCl wire electrodes, sulfadiazine release was enhanced from that by passive diffusion (no current), i.e., ~11.0 vs. 3.60 fold, respectively. Platinum wire electrodes were eventually selected for use since AgCl was rapidly depleted from the Ag/AgCl wire electrodes resulting in changes in voltage. These
electrodes (Ag/AgCl after the AgCl has been removed, and platinum wire) behaved as inert electrodes producing local pH fluctuations in the donor compartment due to the hydrolysis of water. These pH changes would in turn lead to changes in the degree of drug and gelatin ionization which thereby increase the amounts and rates of drug release from the microcapsules. Conversely, no increase in sulfadiazine release occurred using the commercial Ag/AgCl electrodes. The commercial Ag/AgCl electrodes are considered to be active since they participate directly in the electrode reactions thereby limiting local pH changes in the donor compartment. Studies with a scaled-up, but isolated donor compartment also showed that drug solubility increased under the influence of current when platinum wire electrodes were used.

To further study the effect of pH on drug release, Protocols 1 - 4 were developed (see Section 2.2.5). Both passive diffusion and electrically-facilitated drug release studies were performed for 70 min with current in the latter study applied from $t = 20$ to $t = 50$ min. Significant increases in the amounts of sulfadiazine released occurred when current was applied according to Protocols 2 and 3, resulting in 4.30 and 3.24 fold increases, respectively, compared to that by passive diffusion. With the platinum wire electrodes, there is an increase in pH at the cathode due to water hydrolysis which will ionize sulfadiazine making it more soluble. However, it should be noted that the gelatin wall may not be as permeable under these conditions, due to contraction of the wall occurring at its isoelectric point, pH ~5. In addition, there were changes in donor compartment pH when Protocols 2 and 3 were used with current applied, i.e., pH 4.5 vs.
4.2 and 6.0 vs. 5.6 respectively. There were no apparent changes in the pH of the eluant after 70 min for the four protocols except for Protocol 3, where the presence of drug may have caused a slight increase.

The ionic strength (0 - 0.75 M) of the suspending medium and eluant did not cause a noticeable effect on the permeability of the gelatin wall to sulfadiazine under passive diffusion conditions or upon application of current.

The increases in the amounts and rates of sulfadiazine release due to the presence of an electric current was dependent on the magnitude and duration of the current. By controlling direct current in on/off cycles, periodic release or pulsatile delivery of sulfadiazine from the gelatin-walled microcapsules was achieved.

From various microscopy techniques, the microcapsules did not appear to be affected by the presence of an electric field. Therefore the electrically enhanced release of sulfadiazine from gelatin-walled microcapsules was most likely due to changes in pH at the electrode surfaces which result in changes in drug solubilities and in the porosities of the walls of the microcapsules in the vicinity of the electrodes. Tables 4.1 and 4.2 summarize the factors and mechanisms contributing to sulfadiazine release from the gelatin-walled microcapsules.

PVP/sulfadiazine microcapsules:

Polyvinylpyrrolidone, molecular weights 10,000 and 1,300,000, were used to microencapsulate sulfadiazine. Microscopy of these microcapsules indicated a radial
<table>
<thead>
<tr>
<th>VARIABLE TESTED</th>
<th>RESULT</th>
</tr>
</thead>
</table>
| Alternating vs. Direct current  
*Section 3.3.2.2.1.1*                                                           | Sulfadiazine release is affected by a direct current depending on pH, but not an alternating current.                                                                                               |
| Current magnitude  
*Section 3.3.2.2.1.3*                                                          | Sulfadiazine release increases with increasing magnitude of current over the range tested (0-1 mA DC).                                                                                                      |
| Duration of current  
*Section 3.3.2.2.1.4*                                                          | Sulfadiazine release increases as the duration of current increases.                                                                                                                                 |
| Effect of the method of preparation of the microcapsules on the release of sulfadiazine  
*Section 3.3.2.2.2*                                                           | The more hydrated the gelatin wall, the less of a barrier it is to drug release.                                                                                                                        |
| Effect of microcapsule sedimentation on the Spectra/Por® membrane on the release of sulfadiazine  
*Section 3.3.2.2.3*                                                           | Reversible sedimentation of hardened microcapsules does not appear to affect release; however irreversible sedimentation of unhardened microcapsules hinders drug release when current is applied. |

Table 4.1  Summary of the factors affecting drug release from gelatin/sulfadiazine microcapsules. *See text citations for details.*
<table>
<thead>
<tr>
<th>MECHANISM</th>
<th>RESULT</th>
</tr>
</thead>
</table>
| Iontophoresis of sulfadiazine  
Section 3.2.3.2.1                                                            | Iontophoresis of sulfadiazine does not appear to be a major contributor to sulfadiazine release when current is applied.                                                                                                                                                                                                                           |
| Effect of electroosmosis/solvent flow on sulfadiazine release  
Section 3.5.2.2.1                                                         | Electroosmosis does not appear to be a major factor in the electrically-facilitated release of sulfadiazine. However, a net solvent flow may be produced in the donor compartment due to the movement of hydrated ions and microcapsules to the opposite electrodes.                                                                                           |
| Effect of ionic strength of the donor medium  
Section 3.3.2.2.4                                                            | Ionic strength (0-0.75 M) does not appear to affect the solubility of sulfadiazine. It also has no apparent effect on the gelatin wall when current is applied.                                                                                                                                                                            |
| pH dependency  
Section 3.3.2.2.5                                                                 | The pH of the donor compartment affects sulfadiazine solubility and release, and also the permeability of the gelatin wall. pH change is the primary mechanism for the electrically-facilitated release of sulfadiazine from gelatin-walled microcapsules.                                                                                       |

Table 4.2  
Summary of possible mechanisms for electrically-facilitated release of sulfadiazine from microcapsules with electrochemomechanical walls. See text citations for details.
orientation of sulfadiazine crystals that were presumably surrounded by or coated with polymer. Studies of drug release by passive diffusion and electrical-facilitation were performed for 70 min using Protocols 1 - 4. As with the sulfadiazine suspensions and the gelatin/sulfadiazine microcapsules, passive diffusion of drug was dependent on the pH of the donor medium in that higher release occurred with Protocols 1 and 4. When 0.5 mA DC was applied for 30 min, there were increases in sulfadiazine release for studies following Protocols 2 - 4. These increases in release also appeared to occur from local increases in pH at the cathode resulting in ionization of sulfadiazine. The neutral characteristics of PVP would make the porosity of its hydrogel wall unaffected by changes in pH and therefore it would not be a factor in control of drug release. It also appears that when the current was applied to the PVP (MW 10,000)/sulfadiazine microcapsules, there was a net movement of microcapsules toward the positive electrode. Migration of the microcapsules was dependent on the molecular weight of the wall material and to the duration of the current.

Conclusions:

By comparing the passive and electrically-facilitated release of sulfadiazine from sulfadiazine suspensions, gelatin/sulfadiazine microcapsules and PVP/sulfadiazine microcapsules, it can be concluded that the gelatin wall of the microcapsule acts essentially as a gate to control sulfadiazine release. Swelling causes the opening of aqueous channels at pH values less than 5 (the isoelectric point of gelatin), allowing easier passage of drug and dissolution medium. Conversely, deswelling at pH 5 causes
closing of the aqueous channels thereby decreasing the permeability of the wall to sulfadiazine. Table 4.3 summarizes the effect of current on sulfadiazine release from the three model formulations.

Overall, the original hypothesis that pulsatile drug release from microcapsules with electrochemomechanical walls can be controlled with a direct electric current has been supported. While several factors can influence sulfadiazine release from gelatin-walled microcapsules, it appears that electrically induced pH changes in the vicinity of the platinum electrodes in the donor compartment is the primary mechanism for this form of controlled release. In this context, the use of an electric current to create a pulsatile drug release profile from microcapsules with electrochemomechanical walls may be promising for a transdermal system, for example, in which drug can be released "on demand" initiated by the patient or a biosensor, or programmed to release drug in accordance to natural hormonal cycles or the circadian rhythms of certain disease states.
<table>
<thead>
<tr>
<th>Protocol</th>
<th>Formulation</th>
<th>Passive Diffusion</th>
<th>Electrically–facilitated release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Conclusions</td>
<td>Conclusions</td>
</tr>
<tr>
<td>1</td>
<td>SS</td>
<td>Release is higher compared to that by Protocols 2 and 3 due to the higher solubility of drug.</td>
<td>No electrically-induced pH change due to high proton concentration. Release is unchanged.</td>
</tr>
<tr>
<td></td>
<td>G/S</td>
<td>Release depends on the solubility of drug and opening of aqueous channels in the gelatin wall.</td>
<td>No electrically-induced pH change due to high proton concentration. Release is unchanged.</td>
</tr>
<tr>
<td></td>
<td>P/S</td>
<td>Release depends of the solubility of drug. PVP wall does not appear to affect drug release.</td>
<td>No electrically-induced pH change due to high proton concentration. Release is unchanged.</td>
</tr>
<tr>
<td>2</td>
<td>SS</td>
<td>Low release due to low drug solubility.</td>
<td>Increase in release due to electrically-induced pH changes which increase the solubility of drug.</td>
</tr>
<tr>
<td></td>
<td>G/S</td>
<td>Low release due to low drug solubility. The gelatin wall helps drug transport due to partial hydration of the wall in contact with the drug.</td>
<td>Increase in release due to the increase in solubility of drug, but release is inhibited by the closing of pores in the gelatin matrix.</td>
</tr>
<tr>
<td></td>
<td>P/S</td>
<td>Low release due to low drug solubility. The PVP wall may help drug transport by increasing hydration of the drug core.</td>
<td>Increase in release due to the increase in solubility of drug. The PVP wall may act as a partial barrier to drug release.</td>
</tr>
<tr>
<td>3</td>
<td>SS</td>
<td>Same as for Protocol 2</td>
<td>Same as for Protocol 2</td>
</tr>
<tr>
<td></td>
<td>G/S</td>
<td>Same as for Protocol 2</td>
<td>Same as for Protocol 2</td>
</tr>
<tr>
<td></td>
<td>P/S</td>
<td>Same as for Protocol 2</td>
<td>Same as for Protocol 2</td>
</tr>
<tr>
<td>4</td>
<td>SS</td>
<td>High drug solubility leads to an increase in release.</td>
<td>Moderate increase is drug release due to ionization of drug caused by current-induced pH change.</td>
</tr>
<tr>
<td></td>
<td>G/S</td>
<td>High release due to high drug solubility. The well hydrated wall does not appear to hinder drug release.</td>
<td>Increase in drug release. The gelatin wall does not act as a major barrier to release due to the opening of pores in the matrix.</td>
</tr>
<tr>
<td></td>
<td>P/S</td>
<td>High release due to high drug solubility. The wall does not appear to affect release.</td>
<td>Increase in drug release. The PVP wall does not act as a barrier to release. However, it is more of a barrier than the gelatin wall.</td>
</tr>
</tbody>
</table>

Table 4.3  Summary of the effects of pH on electrically-facilitated release of sulfadiazine from sulfadiazine suspensions, gelatin-walled and PVP-walled microcapsules. See text for details.
SS = Sulfadiazine suspensions
G/S = Gelatin/sulfadiazine microcapsules
P/S = PVP/sulfadiazine microcapsules
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