# STRUCTURAL AND DYNAMIC RESPONSE OF NEUTRAL AND INTELLIGENT NETWORKS IN BIOMEDICAL ENVIRONMENTS

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## I. Introduction

As biomedical materials are becoming more advanced and sophisticated, advanced techniques of combinatorial chemistry and molecular design are becoming of utmost importance in order to achieve better design and



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desirable response to the biological environment. In particular, hydrophilic polymer networks have attained new importance in this field as they are particularly prone to molecular or structural changes. Indeed, their structure can be modified by copolymerization to achieve hydrophilicity or hydrophobicity. In addition, the presence of selected functional groups can make these networks responsive to environmental changes. In this chapter, a detailed analysis of the structural and biological properties of polymer networks, both neutral and responsive, is given with an emphasis placed upon the significance of these properties in biomedical applications. As such, an overview of currently pursued hydrogels technologies is also provided at the end of the chapter.

## II. Structure of Three-dimensional Polymeric Networks as Biomaterials

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids (Brannon-Peppas and Harland, 1990; Peppas and Mikos, 1986). The networks are composed of hydrophilic homopolymers or copolymers, which are rendered insoluble by the presence of chemical crosslinks (tie-points, junctions) or physical crosslinks such as entanglements or crystallites (Hickey and Peppas, 1995; Peppas, 1986a; Peppas and Merrill, 1976a,b; Peppas and Mongia, 1997; Stauffer and Peppas, 1992). These crosslinks define the network structure and the physical integrity of the hydrogel. Due to the hydrophilic nature of the polymer chains, hydrogels exhibit a thermodynamic compatibility with water which allows them to swell in aqueous media (Brannon-Peppas and Harland, 1990; Flory, 1953; Flory and Rehner 1943a,b; Peppas and Mikos, 1986).

There are numerous applications of these hydrogels, in particular in the medical and pharmaceutical sectors (Peppas, 1986b; Peppas and Langer, 1994; Ratner and Hoffman, 1976). Since they possess high water content and a soft, elastic consistency, hydrogels more closely resemble natural living tissue than any other class of synthetic biomaterial (Ratner and Hoffman, 1976). As a result of their high water content, hydrogels are typically very biocompatible. Thus, hydrogels can be used as contact lenses, membranes for biosensors, linings for artificial hearts, materials for artificial skin, and drug delivery devices (Park, 1997; Peppas, 1986b, 1997; Peppas and Langer, 1994; Ratner and Hoffman, 1976).

## A. Hydrogel Classification

Hydrogels (Brannon-Peppas and Harland, 1990; Peppas and Mikos, 1986) can be neutral or ionic based on the nature of the side groups. They can also be classified based on the network morphology as amorphous, semicrystalline, hydrogen-bonded structures, supermolecular structures, and hydrocolloidal aggregates. Additionally, in terms of their network structures, hydrogels can be classified as macroporous, microporous, or nonporous (Brannon-Peppas and Harland, 1990; Peppas and Merrill, 1976a; Peppas and Mikos, 1986). A convenient way to classify hydrogels is based on the nature of the pendent groups, which can be either neutral or ionic. The chemical nature and number of these pendent groups can be precisely controlled by the choice of the monomers used in the polymer synthesis. Depending on the type and organization of the monomers in the polymer network, they can be further classified as neutral, biodegradable, complexing, and/or responsive hydrogels.

The literature on synthesis of new polymer materials has exploded since the discovery of poly( hydroxyethyl methacrylate) (PHEMA) by Wichterle and Lim (1960). Instead of utilizing ''off-the-shelf'' polymeric materials designed for use in consumer applications and adapting them for medical purposes, researchers are attempting to molecularly control material properties in order to elicit desired cellular and biological interactions. An extension of such molecular design is in the field of molecular imprinting, where specific substrate recognition sites are incorporated into a network by polymerization in the presence of the desired solute. An illustration of this process is shown in Fig. 1.

## B. Network Structure of Hydrogels

One of the primary features of a hydrogel is its solute permeability properties, which is of key importance in drug delivery, controlled release, and tissue engineering. The performance of a hydrogel in any of these applications depends largely on its bulk structure. A number of excellent reviews discuss this topic in great detail. The most important parameters used to characterize the network structure of hydrogels are the polymer volume fraction in the swollen state, $\upsilon_{2,s}$, molecular weight of the polymer chain between two neighboring crosslinking points, $\overline{M}_c$ and the corresponding mesh size, $\xi$ (Peppas and Barr-Howell, 1986).

The polymer volume in the swollen state, $\upsilon_{2,s}$, is a measure of the amount of fluid imbibed and retained by the hydrogel. The molecular weight between two consecutive crosslinks, which can be either of chemical

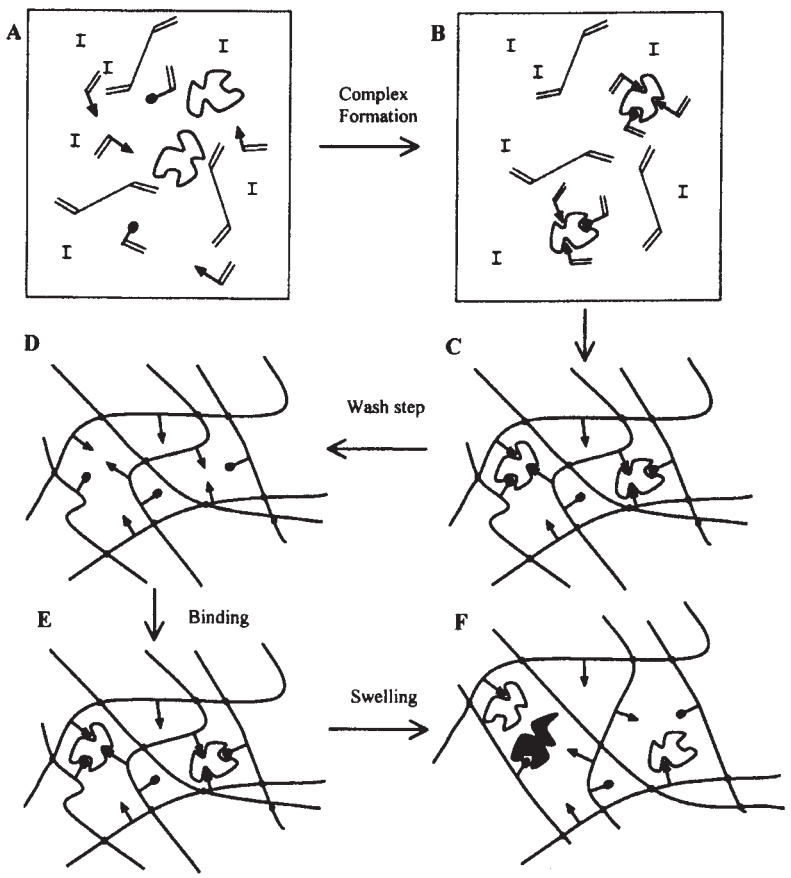



FIG. 1. Preparation of configurational biomimetic imprinted networks for molecular recognition of biological substrates. **A**: Solution mixture of template, functional monomer(s) (triangles and circles), crosslinking monomer, solvent, and initiator (I). **B**: The prepolymerization complex is formed via covalent or noncovalent chemistry. **C**: The formation of the network. **D**: Wash step where original template is removed. **E**: Rebinding of template. **F**: In less crosslinked systems, movement of the macromolecular chains will produce areas of differing affinity and specificity (filled molecule is isomer of template).

or physical nature, is a measure of the degree of crosslinking of the polymer. It is important to note that due to the random nature of the polymerization process itself only average values of $\overline{M}_c$ can be calculated. The correlation distance between two adjacent crosslinks, $\xi$, provides measure of the space available between the macromolecular chains available for the drug diffusion; again, it can be reported only as an average value. These parameters, which are related to one another, can be

determined theoretically or through the use of variety of experimental techniques. The two most prominently used methods among the growing number of techniques utilized to elucidate the structure of hydrogels are the equilibrium swelling theory and the rubber elasticity theory.

*1. Equilibrium Swelling Theory*

The structure of hydrogels that do not contain ionic moieties can be analyzed by the Flory–Rehner theory (Flory and Rehner 1943a). This combination of thermodynamic and elasticity theories states that a crosslinked polymer gel which is immersed in a fluid and allowed to reach equilibrium with its surroundings is subject only to two opposing forces, the thermodynamic force of mixing and the retractive force of the polymer chains. At equilibrium, these two forces are equal. Equation (1) describes the physical situation in terms of the Gibbs free energy.

$$\Delta G_{\text{total}} = \Delta G_{\text{elastic}} + \Delta G_{\text{mixing}} \tag{1}$$

Here, $\Delta G_{\text{elastic}}$ is the contribution due to the elastic retractive forces developed inside the gel and $\Delta G_{\text{mixing}}$ is the result of the spontaneous mixing of the fluid molecules with the polymer chains. The term $\Delta G_{\text{mixing}}$ is a measure of the compatibility of the polymer with the molecules of the surrounding fluid. This compatibility is usually expressed by the polymer–solvent interaction parameter, $\chi_1$ (Flory, 1953).

Differentiation of Eq. (1) with respect to the number of solvent molecules while keeping temperature and pressure constant, results in Eq. (2):

$$\mu_1 - \mu_{1,\text{o}} = \Delta\mu_{\text{elastic}} + \Delta\mu_{\text{mixing}} \tag{2}$$

In Eq. (2), $\mu_1$ is the chemical potential of the solvent in the polymer gel and $\mu_{1,\text{o}}$ is the chemical potential of the pure solvent. At equilibrium, the difference between the chemical potentials of the solvent outside and inside the gel must be zero. Therefore, changes of the chemical potential due to mixing and elastic forces must balance each other. The change of chemical potential due to mixing can be expressed using heat and entropy of mixing.

The change of chemical potential due to the elastic retractive forces of the polymer chains can be determined from the theory of rubber elasticity (Flory, 1953; Treloar, 1958). Upon equaling these two contributions an expression for determining the molecular weight between two adjacent crosslinks of a neutral hydrogel prepared in the absence of

a solvent can be written:

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} \frac{(\overline{v}/V_1)\left[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1 \upsilon_{2,s}^2\right]}{\left(\upsilon_{2,s}^{1/3} - \frac{\upsilon_{2,s}}{2}\right)} \tag{3}$$

Here, $\overline{M}_n$, is the molecular weight of the polymer chains prepared under identical conditions but in the absence of the crosslinking agent, $\overline{v}$ is the specific volume of the polymer and $V_1$ is the molar volume of water.

Peppas and Merrill (1977) modified the original Flory–Rehner theory for hydrogels prepared in the presence of water. The presence of water effectively modifies the change of chemical potential due to the elastic forces. This term must now account for the volume fraction density of the chains during crosslinking. Equation (4) predicts the molecular weight between crosslinks in a neutral hydrogel prepared in the presence of water.

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} \frac{(\overline{v}/V_1)\left[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1 \upsilon_{2,s}^2\right]}{\upsilon_{2,r}\left[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right) - \left(\frac{\upsilon_{2,s}}{2\upsilon_{2,r}}\right)\right]} \tag{4}$$

Here, $\upsilon_{2,r}$ is the polymer volume fraction in the relaxed state, which is defined as the state of the polymer immediately after crosslinking but before swelling.

The presence of ionic moieties in hydrogels makes the theoretical treatment of swelling much more complex. In addition to the $\Delta G_{mixing}$ and $\Delta G_{elastic}$ in Eq. (1), there is an additional contribution to the total change in Gibbs free energy due to the ionic nature of the polymer network, $\Delta G_{ionic}$.

$$\Delta G_{total} = \Delta G_{elastic} + \Delta G_{mixing} + \Delta G_{ionic} \tag{5}$$

Upon differentiating Eq. (5) with respect to the number of moles of solvent keeping T and P constant, expression similar to Eq. (2) for the chemical potential can be derived.

$$\mu_1 - \mu_{1,o} = \Delta\mu_{elastic} + \Delta\mu_{mixing} + \Delta\mu_{ionic} \tag{6}$$

Here, the $\Delta\mu_{ionic}$ is the change of chemical potential due to the ionic character of the hydrogel. Expressions for the ionic contribution to the chemical potential have been also developed (Brannon-Peppas and Peppas,

1991a; Katchalsky and Michaeli, 1955; Ricka and Tanaka, 1984). They exhibit strong dependencies on the ionic strength of the surrounding media and on the nature of the ions present in the solvent. Equations (7) and (8) are expressions that have been derived for swelling of anionic and cationic hydrogels, respectively, prepared in the presence of a solvent.

$$\frac{V_1}{4IM_r}\left(\frac{\upsilon_{2,s}^2}{\upsilon}\right)\left(\frac{K_a}{10^{-pH}-K_a}\right)^2 = \left[\ln(1-\upsilon_{2,s})+\upsilon_{2,s}+\chi_1\upsilon_{2,s}^2\right]+\left(\frac{V_1}{\upsilon\overline{M}_c}\right)\left(1-\frac{2\overline{M}_c}{\overline{M}_n}\right)\upsilon_{2,r}\left[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3}-\left(\frac{\upsilon_{2,s}}{2\upsilon_{2,r}}\right)\right] \tag{7}$$

$$\frac{V_1}{4IM_r}\left(\frac{\upsilon_{2,s}^2}{\upsilon}\right)\left(\frac{K_b}{10^{pH-14}-K_a}\right)^2 = \left[\ln(1-\upsilon_{2,s})+\upsilon_{2,s}+\chi_1\upsilon_{2,s}^2\right]+\left(\frac{V_1}{\upsilon\overline{M}_c}\right)\left(1-\frac{2\overline{M}_c}{\overline{M}_n}\right)\upsilon_{2,r}\left[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3}-\left(\frac{\upsilon_{2,s}}{2\upsilon_{2,r}}\right)\right] \tag{8}$$

In these expressions, $I$ is the ionic strength, $K_a$ and $K_b$ are the dissociation constants for the acid and base, respectively, $M_r$ is the molecular weight of the repeating unit.

### 2. *Rubber Elasticity Theory*

Hydrogels resemble natural rubbers in their remarkable property to elastically respond to applied stresses. A hydrogel subjected to a relatively small deformation, less than 20%, will fully recover to its original dimension in a rapid fashion. This elastic behavior of hydrogels can be used to elucidate their structure by utilizing the rubber elasticity theory originally developed by Treloar (1958) and Flory (Peppas and Moynihan 1985; Peppas and Reinhart 1983) for vulcanized rubbers and modified to polymers by Flory (1953). However, the original theory or rubber elasticity does not apply to hydrogels prepared in the presence of a solvent. Such expressions were developed by Silliman (1972) and later modified by Peppas and Merrill (1977).

Here, only the form of rubber elasticity theory used to analyze the structure of hydrogels prepared in the presence of a solvent is presented

and it is left up to the reader to consult the original reference for detailed derivations.

$$\tau = \frac{\rho RT}{\overline{M}_c}\left(1 - \frac{2\overline{M}_c}{\overline{M}_n}\right)\left(\alpha - \frac{1}{\alpha^2}\right)\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} \tag{9}$$

Here, $\tau$ is the stress applied to the polymer sample, $\rho$ is the density of the polymer, R is the universal gas constant, T is the absolute experimental temperature, and $\overline{M}_c$ is the desired molecular weight between crosslinks.

In order to perform analysis of the structure of hydrogels using the rubber elasticity theory, experiments need to be performed using a tensile testing system. Interestingly, the rubber elasticity theory has not been used to analyze only chemically but also physically crosslinked hydrogels (Anseth *et al.*, 1996; Mark, 1982; Patterson *et al.*, 1982), as well as hydrogels exhibiting temporary crosslinks due to hydrogen bonding (Lowman and Peppas, 1997).

### *3. Analysis of Structural Characteristics of Networks*

The primary mechanism of release of many drugs from hydrogels is diffusion occurring through the space available between macromolecular chains. This space is often regarded as the "pore." Depending upon the size of these pores, hydrogels can be conveniently classified as (i) macroporous; (ii) microporous; and (iii) nonporous. A structural parameter that is often used in describing the size of the pores is the correlation length, $\xi$, which is defined as the linear distance between two adjacent crosslinks and can be calculated using the following equation:

$$\xi = \alpha\left(\overline{r}_o^2\right)^{1/2} \tag{10}$$

Here, $\alpha$ is the elongation ratio of the polymer chains in any direction and $\left(\overline{r}_o^2\right)^{1/2}$ is the root-mean-square, unperturbed, end-to-end distance of the polymer chains between two neighboring crosslinks (Canal and Peppas, 1989). For isotropically swollen hydrogel, the elongation ratio, $\alpha$, can be related to the swollen polymer volume fraction, $\upsilon_{2,s}$, using Eq. (11).

$$\alpha = \upsilon_{2,s}^{-1/3} \tag{11}$$

The unperturbed end-to-end distance of the polymer chain between two adjacent crosslinks can be calculated using Eq. (12) where $C_n$ is the Flory

characteristic ratio, $l$ is the length of the bond along the polymer backbone (for vinyl polymers 1.54 Å) and $N$ is the number of links per chain that can be calculated by Eq. (13).

$$\left(\bar{r}_{\mathrm{o}}^{2}\right)^{1/2} = l(C_n N)^{1/2} \tag{12}$$

$$N = \frac{2\overline{M}_{\mathrm{c}}}{M_{\mathrm{r}}} \tag{13}$$

In Eq. (13) $M_{\mathrm{r}}$ is the molecular weight of the repeating units from which the polymer chain is composed. Finally, when one combines Eqs. (10) through (13), the correlation distance between two adjacent crosslinks in a swollen hydrogel can be obtained:

$$\xi = \upsilon_{2,s}^{-1/3}\left(\frac{2C_n\overline{M}_{\mathrm{c}}}{M_{\mathrm{r}}}\right)^{1/2} l \tag{14}$$

A detailed theoretical characterization of the network structure of the polymer carrier in terms of the correlation length, $\xi$, in combination with diffusion studies of model drugs and proteins provide an invaluable insight into the very complex structure of polymer networks and aid in the design of drug delivery carriers (Narasimhan and Peppas, 1997a).

## C. Solute Transport in Hydrogels

One of the most important and challenging areas of use of such biomaterials is as carriers in drug delivery. In this field it is important to predict the release of the active agent as a function of time using physical and mathematical models. The importance of such models lies in their utility during both the design stage of a pharmaceutical formulation and the experimental verification of a release mechanism (Narasimhan and Peppas, 1997b).

In order to design a particular release mechanism, experimental data of statistical significance are compared to a solution of the theoretical model. It is therefore clear that only a combination of accurate and precise data with models accurately depicting the physical situation will provide an insight into the actual mechanism of release.

The vast majority of theoretical models are based on diffusion equations. The phenomenon of diffusion is intimately connected to the structure of the

material through which the diffusion takes place thus the morphology of the polymeric materials should be accounted for in a successful model. There have been a limited number of reviews that have addressed these aspects of controlled release formulations (Langer and Peppas, 1983; Narasimhan and Peppas, 1997b; Narasimhan *et al.*, 1999). The mechanisms of drug release offer a convenient way to categorize controlled release systems into (i) diffusion-controlled; (ii) chemically-controlled; and (iii) swelling-controlled (Hennink *et al.*, 1997). Due to the fact that ordinary diffusion takes place in each one of these mechanisms to a certain degree and since most of the models used are based on diffusion equations, the next two sections are devoted to the fundamentals of diffusion in matrix systems and how they pertain to solute transport.

### *1. Fundamentals of Ordinary Diffusion*

The release of an active agent from a polymeric carrier consists of the movement of the drug through the bulk of the polymer. This phenomenon known as diffusion is to a large degree controlled by the mass transfer limitations at the boundary between the polymer carrier and its surroundings. On a macroscopic level the diffusion of drug molecules through the polymer carrier can be described by Fick's law of diffusion, which is mathematically stated by Eqs. (15) and (16) for transport in one dimension (Crank and Park, 1968):

$$j_i = -D_{ip}\frac{\mathrm{d}c_i}{\mathrm{d}x} \tag{15}$$

$$\frac{\partial c_i}{\partial t} = D_{ip}\frac{\partial^2 c_i}{\partial x^2} \tag{16}$$

Here, the concentration and mass flux of species $i$ are designated $c_i$, and $j_i$, respectively; $D_{ip}$ is the diffusion coefficient of species $i$ in the polymer matrix, and $x$ and $t$ stand for the independent variables of position and time, respectively.

Several important assumptions have been implicitly incorporated in Eqs. (15) and (16). First, these equations describe the release of a drug from a carrier of a thin planar geometry, equivalent equations for release from thick slabs, cylinders, and spheres have been derived (Crank and Park, 1968). It should also be emphasized that in the above written form of Fick's law, the diffusion coefficient is assumed to be independent of concentration. This assumption, while not conceptually correct, has been

largely accepted due to the computational simplicity. Finally, $j_i$ is a flux with respect to the mass average velocity $v$ of the system.

Initial and boundary conditions, which are necessary for solving Eq. (15) and (16), allow for the appropriate description of the experimental conditions imposed upon the drug release device. The solutions of Eqs. (15) and (16) subject to a number of boundary conditions that can be applied to various *in vitro* and *ex vivo* experiments have been obtained (Crank and Park, 1968).

In order to improve the predictive power of the Fickian diffusion theory, a concentration dependent diffusion coefficient is used in Eqs. (15) and (16). Equation (16) is then rewritten and solved with the appropriate boundary conditions:

$$\frac{\partial c_i}{\partial t} = \frac{\partial}{\partial x}\left(D_{ip}(c_i)\frac{\partial c_i}{\partial x}\right) \tag{17}$$

In Eq. (17), $D_{ip}(c_i)$ is the concentration-dependent diffusion coefficient; its form of concentration dependence is affected by the structural characteristics of the polymer carrier. A selective summary of the various forms of the diffusion coefficient is provided in Table I.

One of earliest approaches of estimating the diffusion coefficient through a polymer carrier is that of Eyring (1936). In this theory, diffusion of a solute through a medium is presented as a series of jumps instead of a continuous process. Therefore, in Eq. (18) in Table I, which comes from the Eyring analysis, $\lambda$ is the diffusional jump of the drug in the polymer and $v$ is the frequency of jumping.

TABLE I
A Selective Summary of Drug Diffusion Co-efficients

| Type of carrier | Form of $D_{ip}$ | Eq. | Ref. |
|---|---|---|---|
| Porous | $D_{ip} = \frac{\lambda^2 v}{6}$ | (18) | Eyring, (1936) |
| Porous | $D_{\text{eff}} = D_{iw} K_p K_r \frac{\varepsilon}{\tau}$ | (19) | Lightfoot, (1973) |
| Microporous | $\frac{D_{ip}}{D_b} = (1-\lambda)^2\left(1 + \alpha\lambda + \beta\lambda^3 + \gamma\lambda^5\right)$ | (20) | Faxén, (1923) |
| Nonporous | $D_{ip} = D_o \exp\left\{-\frac{k}{v_f}\right\}$ | (21) | Fujita, (1961) |
| Nonporous | $\frac{D_{2,13}}{D_{2,1}} = \varphi(q_s)\exp\left[-B\left(\frac{q_s}{V_{f,1}}\right)\left(\frac{1}{H} - 1\right)\right]$ | (22) | Yasuda and Lamaze, (1971) |
| Nonporous (highly swollen) | $\frac{D_{2,13}}{D_{2,1}} = k_1\left(\frac{\overline{M}_c - \overline{M}_c^*}{\overline{M}_n - \overline{M}_c^*}\right)\exp\left(-\frac{k_2 r_s^2}{Q-1}\right)$ | (23) | Peppas and Reinhart, (1983) |

Fugita (1961) utilized the idea of free volume in polymers to estimate the drug diffusion coefficient and arrived at an exponential dependence of the drug diffusion coefficient on the free volume, $\upsilon_f$, which is given by Eq. (21) in Table I. Yasuda and Lamaze (1971) refined the Fujita's theory and presented molecularly based theory, which predicts the diffusion coefficients of drugs through a polymer matrix rather accurately [Eq. (22)]. In their treatment the normalized diffusion coefficient, the ratio of the diffusion coefficient of the solute in the polymer, $D_{2,13}$, to the diffusion coefficient of the solute in the pure solvent, $D_{2,1}$, is related to the degree of hydration, $H$, and free-volume occupied by the swelling medium, $V_{f,1}$. In addition, $\varphi$ is a sieving factor which provides a limiting mesh size impermeable to drugs with cross-sectional area $q_s$, and $B$ is a parameter characteristic of the polymer. In Eq. (22), the subscripts 1, 2, and 3 refer to the swelling medium, drug, and polymer, respectively.

Peppas and Reinhart (1983) also developed a theoretical model based on a free volume of the polymer matrix. In their theory they assumed the free volume of the polymer to be the same as the free volume of the solvent and they arrived at Eq. (23) in Table I. They related the normalized diffusion coefficient to the degree of swelling, $Q$, the solute radius, $r_s$, and the molecular weight of the polymer chains. More specifically, $\overline{M}_c$ is the average molecular weight of the polymer chains between adjacent crosslinks, $\overline{M}_n$ is the average molecular weight of the linear polymer chains prepared under identical conditions in the absence of the crosslinking agent, and $\overline{M}_c^*$ is the critical molecular weight between crosslinks below which a drug of size $r_s$ could not diffuse through the polymer network. In addition, $k_1$ and $k_2$ are constants related to the polymer structure. This theory is applicable to drug transport in highly swollen, nonporous hydrogels. Equations for moderately or poorly swollen (Peppas and Moynihan, 1985) and semicrystalline (Harland and Peppas, 1989) hydrogels were also developed.

Yet another approach for the prediction of the diffusion coefficient of a drug in a controlled-release device has been adopted from the chemical engineering field (Lightfoot, 1973). More specifically, the transport phenomena in porous rocks, ion-exchange resins, and catalysis are of very similar nature to a drug diffusing through a macro- or micro-porous polymer. In these types of polymers the diffusion is assumed to be taking place predominantly through the water, or bodily fluids, filled pores. The diffusion coefficient of a drug in a polymer, $D_{ip}$, in Eq. (15) and (16) is replaced by an effective diffusive coefficient, $D_{\text{eff}}$, which is defined by Eq. (19) in Table I. In Eq. (19), $\varepsilon$ is the porosity, or void fraction, of the polymer, which is a measure of the volume of the pores available for diffusion and $\tau$ is the tortuosity, which describes the geometric characteristics of

the pores. The term $K_p$ is the equilibrium-partitioning coefficient, which is a parameter needed when the drug is soluble in the polymer matrix. It is the ratio of the concentration inside of the pore to the concentration outside of the pore. The term $K_r$ describes the fractional reduction in diffusivity within the pore when the solute diameter, $d_s$, is comparable in size to the pore diameter $d_r$. Equation (20) in Table I is a semiempirical relation proposed by Faxen (1923) for diffusion of spheres through porous media. In this equation, $\lambda$ is the ratio of the drug radius, $r_s$, to the pore average radius, $r_p$, $D$ and $D_b$ are the diffusion coefficients of the sphere through the pore and in bulk, respectively; and $\alpha$, $\beta$, and $\gamma$ are constants. It is clear to see that as the size of the drug gets smaller with respect to the size of the pore, the ratio of $D/D_b$ approaches the limit of one.

### 2. *Solute Transport in Matrix-Based Systems*

The drug can be either dissolved or dispersed throughout the network of the hydrogels. The drug release from these systems is modeled using Eq. (17) with concentration-dependent coefficient given by either of Eq. (21) through Eq. (23). It is clear from solutions to Eq. (17) that the fractional drug release obtained form these systems is proportional to $t^{1/2}$. This is significant in that it is impossible to obtain time independent or zero-order release in this type of system with simple geometries.

Drug can be incorporated into the gels by equilibrium partitioning, where the gel is swollen to equilibrium in concentrated drug solution, or during the polymerization reaction. Equilibrium partitioning is the favorable loading method for drug/polymer systems with large partition coefficients or for sensitive macromolecular drugs such as peptides or proteins that could be degraded during the polymerization.

In swelling-controlled release systems, the drug is dispersed within a glassy polymer. Upon contact with biological fluid, the polymer begins to swell. No drug diffusion occurs through the polymer phase. As the penetrant enters the glassy polymer, the glass transition of the polymer is lowered allowing for relaxations of the macromolecular chains. The drug is able to diffuse out of the swollen, rubbery area of the polymers. This type of system is characterized by two moving fronts; the front separating the swollen (rubbery) portion and the glassy regions which moves with velocity, $v$, and the polymer/fluid interface (Fig. 2). The rate of drug release is controlled by the velocity and position of the front dividing the glassy and rubbery portions of the polymer. A very important phenomenon of macromolecular relaxation takes place at the glass–rubbery interface and significantly affects the drug release.

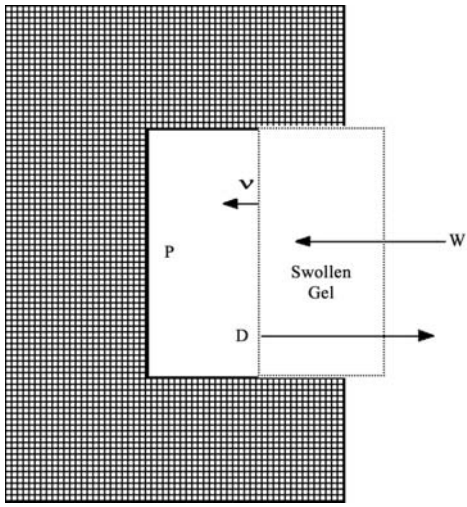



FIG. 2. Schematic representation of the behavior of a one-dimensional swelling controlled release system. The water (W) penetrates the glassy polymer (P) to form a gel. The drug (D) is released through the swollen layer.

## D. ENVIRONMENTALLY RESPONSIVE HYDROGELS

Hydrogels may exhibit swelling behavior which is dependent on the conditions of the external environment. Over the last thirty years there has been a significant interest in the development and analysis of environmentally or physiologically responsive hydrogels (Peppas, 1991). Environmentally responsive materials show drastic changes in their swelling ratio due to changes in their external pH, temperature, ionic strength, nature and composition of the swelling agent, enzymatic or chemical reaction, and electrical or magnetic stimulus (Peppas, 1993). In most responsive networks, a critical point exists at which this transition occurs.

Responsive hydrogels are unique in that there are many different mechanisms for drug release and many different types of release systems based on these materials. For instance, in most cases drug release occurs when the gel is highly swollen or swelling and is typically controlled by rate of swelling, drug diffusion, or a coupling of swelling and diffusion. However, in a few instances, drug release occurs during gel syneresis by a squeezing mechanism. Also, drug release can occur due to erosion of the polymer caused by environmentally responsive swelling.

Another interesting characteristic about many responsive gels is that the mechanism causing the network structural changes can be entirely reversible in nature. This behavior is depicted in Fig. 3 for a pH- or

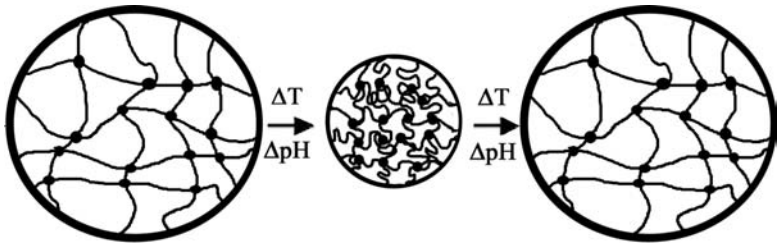



FIG. 3. Swollen temperature- and pH-sensitive hydrogels may exhibit an abrupt change from the expanded (left) to the collapsed (syneresed) state (center) and then back to the expanded state (right) as temperature and pH change.

temperature-responsive gel. The ability of these materials to exhibit rapid changes in their swelling behavior and pore structure in response to changes in environmental conditions lend these materials favorable characteristics as carriers for bioactive agents, including peptides and proteins. This type of behavior may allow these materials to serve as self-regulated, pulsatile drug delivery systems. This type of behavior is shown in Fig. 4 for pH- or temperature-responsive gels. Initially, the gel is in an environment in which no swelling occurs. As a result, very little drug release occurs. However, when the environment changes and the gel swells, rapid drug release occurs (either by Fickian diffusion, anamolous transport or case II transport). When the gel collapses as the environment changes, the release can be turned off again. This can be repeated over numerous cycles. Such systems could be of extreme importance in the treatment of chronic diseases such as diabetes. Peppas (1993) and Siegel (1997) have presented detailed analyses of this type of behavior.

### *1. pH-Sensitive Hydrogels*

One of the most widely studied types of physiologically responsive hydrogels is pH-responsive hydrogels. These hydrogels are swollen from ionic networks. These ionic networks contain either acidic or basic pendant groups. In aqueous media of appropriate pH and ionic strength, the pendant groups can ionize developing fixed charges on the gel as shown in Fig. 5 for an ionic gel. The swelling behavior of these materials has been analyzed in a previous section.

There are many advantages to using ionic materials over neutral networks. All ionic materials exhibit a pH and ionic strength sensitivity. The swelling forces developed in these systems will be increased over the nonionic materials. This increase in swelling force is due to the localization of fixed charges on the pendant groups. As a result, the mesh size of the polymeric networks can change significantly with small pH changes.

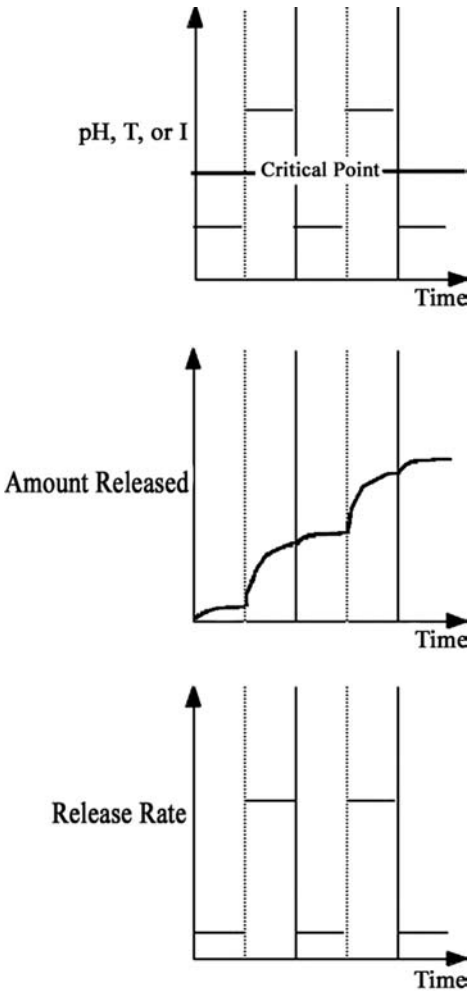



FIG. 4. Cyclic change of pH, $T$, or ionic strength ($I$) leads to abrupt changes in the drug release rates at certain time intervals in some environmentally responsive polymers.

In these materials, the drug diffusion coefficients and release rates will vary greatly with environmental pH.

### *2. Temperature-Sensitive Hydrogels*

Another class of environmentally sensitive materials that are being targeted for use in drug delivery applications is thermally sensitive polymers. This type of hydrogel exhibits temperature-sensitive swelling behavior

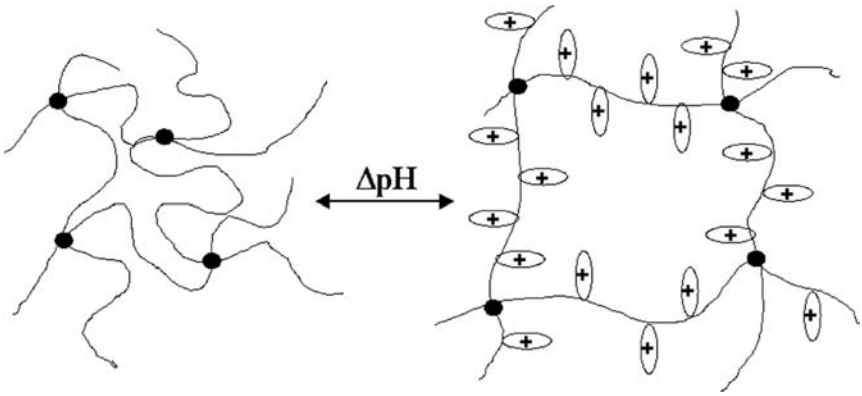



FIG. 5. Expansion (swelling) of a cationic hydrogel due to ionization of pendent groups, at specific pH values.

due to a change in the polymer/swelling agent compatibility over the temperature range of interest. Temperature-sensitive polymers typically exhibit a lower critical solution temperature (LCST), below which the polymer is soluble. Above this temperature, the polymers are typically hydrophobic and do not swell significantly in water (Kim, 1996). However, below the LCST, the crosslinked gel swell to significantly higher degrees because of the increased compatibility with water. For polymers that exhibit this sort of swelling behavior, the rate of drug release would be dependent on the temperature. The highest release rates would occur when the temperature of the environment is below the LCST of the gel.

### 3. *Complexing Hydrogels*

Some hydrogels may exhibit environmental sensitivity due to the formation of polymer complexes. Polymer complexes are insoluble, macromolecular structures formed by the noncovalent association of polymers with the affinity for one another. In this type of gel, the polymer complex behaves as a physical crosslink in the gel. As the degree of effective crosslinking is increased, the network mesh size and degree of swelling is significantly reduced. As a result, the rate of drug release in these gels will decrease dramatically upon the formation of interpolymer complexes. Since some of the more novel concepts immerging in hydrogel research involve these types of networks, the next section is devoted to a more detailed discussion on the formation and characterization of polymer complexation with an emphasis on pH sensitive complexes.

## E. Complexation in Polymers

### *1. Overview of Complexation*

Interpolymer complexes possess unique physical and chemical properties which are different from those of the initial components and have found applications in technology, medicine, and other fields (Bekturov and Bimendina, 1981). The unique properties of the complexes arise due to a higher degree of molecular ordering that is a result of secondary binding forces. The resulting secondary structures are dictated primarily by the primary structure (monomer sequence), solvent, and temperature of the system. Interpolymer complexes can be classified based on the nature of the secondary binding forces as:

1. Polyelectrolyte complexes
2. Hydrogen-bonding complexes,
3. Stereocomplexes
4. Charge transfer complexes.

Polyelectrolyte complexes form due to Coulomb forces when two oppositely charged polyelectrolytes are mixed together. Mixing Lewis acids (proton donating macromolecules) with Lewis bases (proton accepting macromolecules) results in the formation of hydrogen-bonding complexes. The formation of stereocomplexes is the result of weak dispersive interactions between oriented polymer chains; for example, the van der Waals forces between isotactic and syndiotactic poly(methyl methacrylate). Charge transfer complexes arise due to charge-transfer interactions between electron-accepting and electron-donating polymers (Tsuchida and Abe, 1982). A number of experimental techniques have been applied to study interpolymer complexes: potentiometry (Bailey *et al*., 1964), conductometry (Bimendina *et al*., 1974), turbidimetry (Sato and Nakajima, 1975), viscometry (Antipina *et al*., 1972), calorimetry (Biros *et al*., 1974), sedimentation (Bimendina *et al*., 1977), light scattering (Liquori *et al*., 1966), high resolution H-nuclear magnetic resonance (NMR) spectroscopy (Spevacek and Schneider, 1974, infrared (Philippova and Starodubtzev, 1995), and electron spectroscopy (Bakeev *et al*., 1959).

It is the sensitivity of hydrogen bonds to their external environment that provide the pH-dependence of physical properties of hydrogen-bonding interpolymer complexes that are of interest to us.

### *2. Effect of PEG Chain Length on Interpolymer Complexation*

Antipina *et al*. (1972) also investigated the complexation of poly(ethylene glycols) with linear polymethacrylic and acrylic acids of molecular

weights of 100,000 and 120,000, respectively. They used potentiometry and viscometry to examine the effect of molecular weight of the PEG chain, concentration of the polymers in solution, and pH and temperature of the medium on the complex formation. By monitoring the pH levels of solutions of the polymeric acids to which PEG chains of molecular weights of 1000, 2000, 3000, 6000, 15,000 and 40,000 were added, they found that there is a critical PEG chain length that is necessary for the complexation reaction to occur. The addition of 3000 molecular weight PEG to 0.1 g/l solution of PMAA resulted in gradual increase in the solution pH. A similar effect was observed for the PAA solutions, however, the rise in the solution pH took place upon the addition of PEG 6000, and it was less profound than for the PMAA solution. The addition of higher molecular weight PEGs was accompanied by steeper increase in the solution pHs which leveled off at the polyacid/PEG molar ratio of one. These results were in complete agreement with the results obtained by viscometry. Therefore, it was concluded that the critical molecular weight of PEG needed to promote the complexation reaction with PMAA and PAA is 2000 and 6000, respectively. Additionally, the stability of the complex was suggested to be dependent on the chemical structure of the polyacid which in turn would promote hydrophobic interactions contributing to the stability of the formed complexes.

Papisov *et al.* (1974) performed calorimetric and potentiometric experiments to determine the thermodynamic parameters of the complex formation of PMAA and PAA with PEG. They investigated how temperature and the nature of the solvent affected the complex stability. They found that in aqueous media the enthalpy and entropy associated with the formation of the PMAA/PEG complex are positive while in an aqueous mixture of methanol both of the thermodynamic quantities become negative. The exact values are shown in Table II. The viscosities of aqueous solutions containing complexes of PMAA and PEG increase with decreasing temperature as a result of a breakdown of the complexes.

The temperature stability of the complexes seems to be dependent on the molecular weight of the PEG chain, i.e., the larger the PEG the lower the temperature at which the complex dissociates. An important observation was that the complexation/decomplexation phenomenon was reversible by changing the temperature of the system. The positive values of the thermodynamic parameters as well as the experimental observations clearly indicate the important role of hydrophobic interactions in the stabilization of the PMAA/PEG complexes. Since PAA is considerably more hydrophilic than PMAA, hydrophobic interactions do not play an important role in stabilizing the PAA/PEG complexes. This is represented by the much

TABLE II
ENTHALPY VALUES ASSOCIATED WITH THE FORMATION OF PMAA/PEG AND PAA/PEG COMPLEXES IN WATER AND WATER/METHANOL MIXTURES

| Complex | $M_{PEG}$ | Solvent | $\Delta H$ [kcal/mol] |
|---|---|---|---|
| PMAA–PEG | 15,000 | Water | $0.30 \pm 0.04$ |
| | 40,000 | | |
| | 6000 | Water | $0.26 \pm 0.04$ |
| | 20,000 | Methanol:water (30:70 vol%) | $-0.17 \pm 0.04$ |
| PAA–PEG | 40,000 | Water | $0.13 \pm 0.04$ |
| | 40,000 | Methanol:water (30:70 vol%) | $-0.18 \pm 0.04$ |

lower value of $\Delta H$ of the PAA/PEG complex and the almost nonexisting effect of temperature on the stability of the PAA/PEG complex.

Miyoshi *et al.* (1996, 1997) investigated interpolymer interactions, morphology and chain dynamics of the poly(acrylic acid)/poly(ethylene oxide) complex in the solid state using high-resolution solid state $^{13}$C-NMR. In their study, they utilized PEO and PAA of molecular weight of 20,000 and 90,000, respectively. They concluded that there exists three hydrogen bonding forms of the carboxyl group in the PAA, namely: (1) the complex form, groups actively participating in the interpolymer hydrogen bonding with PEO chains; (2) the dimeric form, groups that form intramolecular hydrogen bonding complexes among PAA molecules; and (3) the free form, groups that are not a part of the complex nor the dimeric form.

Philippova and Starodubtzev have also extensively studied the complexation behavior of polyacids and PEG, especially, the system of crosslinked of poly(methacrylic acid) and linear poly(ethylene glycol) (Philippova and Starodubtzev, 1995; Philippova *et al.*, 1994). They observed that decreasing the molecular weight of PEG from 6000 to 1500 resulted in its slower diffusion into the swollen network of PMAA, and a drastic decrease in both the stability and equilibrium composition of the intermacromolecular complex. Analysis of dried polymer networks of PMAA with absorbed PEG chains by FT-IR spectroscopy revealed the presence of two types of hydrogen bonded structures: (1) dimers of methacrylic acid at absorption frequency of 1700 $cm^{-1}$ and (2) interpolymer complexes of PMAA and PEG at 1733 $cm^{-1}$. In addition, they also suggested as a result of their studies, that the hydrogen bonded dimer of PMAA forms preferentially to the intermacromolecular complex between the PMAA network and PEG chains.

In a more recent study, Philippova *et al*. (Skirda *et al*., 1999) utilized the pulse field gradient (PFG)-NMR method to investigate the translational mobility of linear PEG macromolecules absorbed in loosely crosslinked PMAA hydrogels mentioned above. The goal of this study was to also explain why hydrogels of PMAA collapse when exposed to relatively low concentration of PEG ($<5$ wt%) and collapse and then reswell when immersed in solutions of PEG of rather high concentrations (ca. 5–10 wt%). The results of the PFG-NMR technique showed the existence of two fractions of PEG macromolecules with different chain mobilities inside the collapsed gel: (1) some PEG molecules had self-diffusion characteristics similar to those of chains of the crosslinked network, and (2) some PEG molecules exhibited free diffusion properties. In contrast, the PEG chains inside of the reswollen gels had self-diffusion coefficient independent of time indicating their absence in participating in interpolymer complexation with the PMAA network.

The formation of inter- and intrapolymer complexes has also been shown to affect the polymerization kinetics. For example, Ferguson and Shah (1968) investigated the influence of intrapolymer complexation on the kinetics of AA in the presence of copolymer matrices composed of either *N*-vinylpyrrolidone and acrylamide or *N*-vinylpyrrolidone and styrene. The polymerization rate reaches a maximum in the vicinity of AA to VP ratio equal to one for the VP/AAm matrix. This maximum in the polymerization rate is most pronounced in the presence of copolymer with the highest content of VP. When the hydrophilic acrylamide is replaced with the more hydrophobic styrene monomer in the copolymer matrix, the observed maximum in AA polymerization rate occurred at a lower than equimolar ratio of AA to VP. The hydrophilic groups of VP were interacting with the hydrophobic nucleus consisting of the styrene units in the VP/St copolymer, and were thus unable to participate in the formation of the complex unlike in the case of VP/AAm copolymer matrix.

Bajoras and Makuska investigated the effect of hydrogen bonding complexes on the reactivities of (meth)acrylic and isotonic acids in a binary mixture of dimethyl sulfoxide and water using IR spectroscopy (Bajoras and Makuska, 1986). They demonstrated that by altering the solvent composition it was possible to carry out copolymerization in the azeotropic which resulted in the production of homogeneous copolymers of definite compositions at high conversions. Furthermore, it was shown that water solvent fraction determines the rate of copolymerization and the reactivity ratios of the comonomers. This in turn determines the copolymer composition.

Verhoeven *et al*. (1989) addressed the possible effect of manufacturing conditions such as the presence of additives, namely poly(ethylene glycol),

on the polymerization of 2-hydroxyethyl methacrylate (HEMA) and methacrylic acid (MAA). The suspected complexation between the additive and the methacrylic acid monomer did not have a significant effect on the reaction ratios nor the copolymer composition and tacticity as demonstrated by $^{13}$C-NMR studies.

The effect of solvent on the polymerization kinetics of a system of poly(ethylene glycol) monomethacrylate and methacrylic acid was investigated by Smith and Klier (1998). In this work, a parameter of copolymer structure, namely the sequence distribution in the copolymer, was estimated by determining the reactivity ratios of the monomers using $^{1}$H-NMR spectroscopy for two different solvents, $D_2O$ and a mixture of ethanol and water. The paramount effect of the solvent on the polymerization process was exhibited by a profound change of $r_1$ and $r_2$ from 1.03 and 1.02 in the case of the $D_2O$ solvent to 2.0 and 3.6 in the 50/50 wt% mixture of EtOH and $H_2O$, respectively. In the context of polymer structure, this means that while the resulting polymer would have a random structure when synthesized in $D_2O$ it would have significantly large blocks of each co-monomer in its chains when manufactured in the EtOH/$H_2O$ mixture. Clearly, this would significantly affect the final properties of the polymer system. Due to their potential industrial use, these polymers were the subject of a more recent publication where their molecular weight distributions and compositions were characterized (Drescher *et al.*, 2001).

### 3. *Infrared Spectroscopy*

Infrared (IR) spectroscopy is one of the most commonly used techniques for the study and characterization of polymers (Koenig, 1992). The goal of such characterization is to relate the structure of polymers to their performance properties. IR has been used to characterize not only the resulting polymers but also the polymerization processes leading to the production of polymer systems (Scranton *et al.*, 2003). The aim of the following sections is to summarize the use of IR in the characterization of polymer network structure with particular attention paid to intermolecular hydrogen bonding that occurs in such systems.

There are several excellent review articles and books (Coleman *et al.*, 1991; Koenig, 1992; Scranton *et al.*, 2003) on the use of IR in polymer systems that stand out from the rather voluminous literature on this topic. In addition to the above-mentioned monographs, there is also a number of exceptional series and articles such as the classic series by Castillo *et al.* (1984a,b; 1985, 1986a,b; Deng *et al.*, 1986). IR was also employed by Ratner as one of the primary techniques to study the anomalous swelling

behavior of PHEMA in urea solutions using deuterium oxide as the solvent (Ratner and Miller, 1972). In a more recent work, Perova *et al.* (1997) alluded to the role of and existence of different types of water present in PHEMA hydrogels using FT-IR. It was confirmed that depending upon the water concentration, there exists up to three types of water in a swollen hydrogel. At water concentrations greater than 18 wt%, loosely bound water was observed in addition to the tightly bound water molecules. When concentration of water was increased above 30 wt% some of the excess loosely bound water was shown to behave more like bulk water.

There are two major experimental techniques that can be used to analyze hydrogen bonding in noncrystalline polymer systems. The first is based on thermodynamic measurements which can be related to molecular properties by using statistical mechanics. The second, and much more powerful, way to elucidate the presence and nature of hydrogen bonds in amorphous polymers is by using spectroscopy (Coleman *et al.*, 1991). From the present repertoire of spectroscopic techniques which includes IR, Raman, electronic absorption, fluorescence, and magnetic resonance spectroscopy, the IR is by far the most sensitive to the presence of hydrogen bonds (Coleman *et al.*, 1991).

### *4. Infrared Spectroscopy and Polymer Complexation*

Before discussing the details of hydrogen bonding in different polymer systems and how it affects their spectra it will be useful to review the definition of hydrogen bond. In polymer systems hydrogen bonds can be classified as:

1. self-associating
2. inter-associating

An example of self-associating hydrogen bond is a dimer of carboxylic acids while hydrogen bonding between a carboxylic acid and a nonself-associating functional group, such as ether or ester, is an example of the latter type.

Another type of classification of hydrogen bonds is based upon the relative strength of the interaction:

1. weak (PVC–Polyesters)
2. medium (self-association of –OH, Amides, Urethanes)
3. intermediate (self-association of –COOH)
4. strong (acid salts such as –COOH/NHP)

The strength of the hydrogen bonding interaction has a very profound effect on the appearance of the IR spectra and has been discussed in

great detail by a number of authors (Coleman *et al.*, 1991; Hadzi, 1965, 1976; Lee *et al.*, 1988; Odinokov *et al.*, 1976). In summary, weak hydrogen bonds exhibit very broad, structureless band with many submaxima centered around 3300 $cm^{-1}$. In spectra of hydrogen bonds of intermediate strength, such as in carboxylic acid dimers, the –OH stretching frequency is shifted lower to 3100–2800 $cm^{-1}$ and, at the same time, "satellite" bands are observed on the lower frequency side of the broad fundamental profile. The origin of these bands was a subject of several publications and the explanation put forth by Bratoz, Hadzi, and Sheppard has been widely accepted since it satisfactorily accounts for all the observable peaks in the 3000–2500 $cm^{-1}$ range of the spectra (Bratoz *et al.*, 1956). The peaks are thought to arise from overtones and combinations that are intensity enhanced by Fermi resonance with the OH fundamental (Bratoz *et al.*, 1956). IR spectra of polymer systems undergoing strong hydrogen bonding exhibit a number of peculiarities and are not commonly observed in polymer systems. One exception is that of a polyaminic model compound that complex with *N*-methyl pyrrolidinone (NMP). As a result of the strong hydrogen bonds formed between carboxylic acid groups present on the model compound and NMR, three broad bands are observed at 2900, 2400, and 1900 $cm^{-1}$ in addition to various "satellite" bands (Coleman *et al.*, 1991). These bands, typical of strong hydrogen bonds, are labeled by Hadzi as the "A," "B," and "C" bands (Hadzi, 1965, 1976).

## F. Tissue Engineering Aspects of Neutral Networks

When considering the similarity of hydrogels to soft tissue, it should come as no surprise that in the late 1960s cellularly invasive porous networks consisting of PHEMA were being designed as soft tissue replacements, such as breast augmentation and nasal cartilage replacement (Kliment *et al.*, 1968; Simpson, 1969; Voldrich *et al.*, 1975). However, complications with long-term calcification hindered further development. Then in the 1980s, work was done with pancreatic islet sequestering using PHEMA sponges (Klomp *et al.*, 1983; Ronel *et al.*, 1983). While the hydrogel sponge performed well as an immunoisolation device, long-term viability of the islets was not achieved. Although, with the rather recent advancements in scaffolding for supporting the formation of new tissue and a more developed understanding of the implant tissue response, these networks have found a revival in their utility and application. The following sections will discuss some of the key aspects of macroporous

network formation, and the tissue response to these networks which is critical to the proper development of tissue supporting scaffolds.

### *1. Macroporous Structure of Neutral PHEMA Containing Networks*

There is a significant difference in aqueous solubility of HEMA and PHEMA; the HEMA monomer is infinitely soluble in water while the polymer exhibits limited water compatibility. This dissimilar solution behavior allows for the formation of a macroporous, cellularly invasive sponge structure when reacted in dilute monomer solutions. As such, PHEMA hydrogel sponge formation is controlled by the thermodynamic phase behavior between the polymer-rich phase, and the aqueous-rich phase during polymerization. Chirila noted that the formation of the porous structure is dependant upon a kinetic competition between gel point and phase separation (Chirila *et al*., 1998). If gelation occurs first, the resulting material is a hydrogel with little to no macropores, but will still contain the typical hydrogel mesh size on the angstrom level. If phase separation occurs first, the resulting material contains water filled spaces that can vary in size from submicron up to 20 microns in size. The presence of the two different pore sizes present in macroporous PHEMA sponges is schematically shown in Fig. 6. Since the sponge formation is dependant upon both polymerization kinetics and solution thermodynamics, there are many variables that can be altered in order to control the pore morphology of the resulting hydrogel sponge. The following is a selection of methods that can be used to tailor PHEMA porous networks.

The amount of water added to the reaction mixture produces the most dramatic effect upon the size of the pores in a PHEMA sponge (Chirila *et al*., 1998; Ronel *et al*., 1983; Simpson, 1969). When the water content is below 45–50%, the PHEMA polymer chains remain soluble and do not form a two-phase system. When the reaction solution's water content is increased, phase separation occurs with excess water acting as the pore forming agent. Hence, as we further increase the water content, the number of water molecules excluded from the polymer phase increases creating larger voids between the polymer droplets. It is well established that networks containing 85% water or greater possess pore sizes that are large enough for cellular invasion. Unfortunately, these high water solutions result in materials with characteristically weak mechanical properties and large pore size distributions.

Since different crosslinking agents possess different solubilities in water, it was hypothesized that by altering the crosslinking agent used it should be possible to alter the networks pore morphology. Chirlia *et al.* (Clayton *et al*., 1997a,b; Lou *et al*., 2000) performed a rather extensive evaluation of

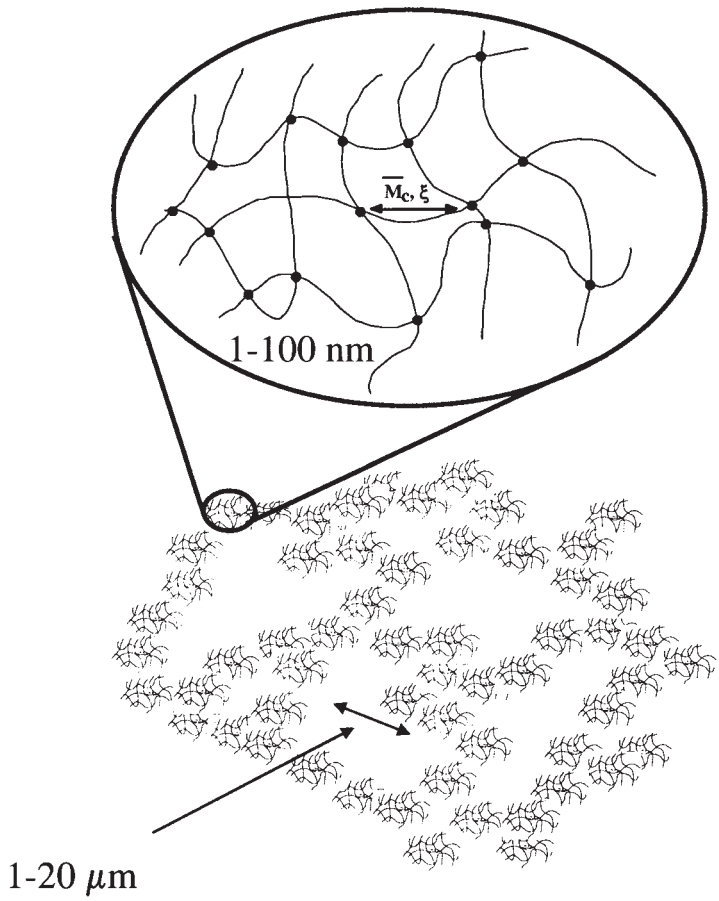



FIG. 6. Schematic representation of macroporous PHEMA hydrogel sponges. Interstitial spaces between polymer droplets create a macroporous structure 1–20 μm in size, whereas the polymer network creates a 1–100 nm mesh size in the polymer phase.

crosslinkers to determine their relative impact upon the networks ability to form large macropores. They determined that using typical concentrations of crosslinker content (0.1–2 mol%) had very little effect of the ultimate morphology and mechanical strength of the networks formed. While many studies on crosslinker selection have been performed, little work has been done on the effect of more/less hydrophilic comonomers on the formation of the macropores. The comonomers that have been attempted were more hydrophobic monomers such as methyl methacrylate (Dalton *et al.*, 2002). This is most likely due to the commonly used hydrophilic comonomers,

acrylic acid and 2,2-diethylaminomethacrylate result in transparent, homogeneous gels.

The presence of nonreacting, inert, components (porogens) can also affect the pore size of the resulting polymer sponge. A porogen is a space filling particulate that prevents polymerization in specific locations through physical hindrances (Badiger *et al*., 1993). Sucrose, glucose, and ice crystals have all been used as void fillers to create macroporous PHEMA hydrogels (Kang *et al*., 1999; Oxley *et al*., 1993). The porogen must be selected based on its ability to remain suspended in the reaction mixture, and provide some mechanism of being leached from the network after the sponge is formed (Carenza and Veronese, 1994).

PHEMA solubility decreases with increasing ion concentration. As a result, Mikos *et al*. used salt solutions of varying ionic strength to dilute the reaction mixtures (Liu *et al*., 2000). It was noted that increasing the ion content of the aqueous solution to 0.7$M$, interconnected macropores were obtained at 60 vol% water. Surfactants may also be used to control the network pore structure. However, not much work has been done in this area, since surfactants typically work to reduce the surface repulsions between the two phases and form a uniform emulsion. These smaller emulsion droplets when gelled will create a network with an even smaller porous structure. Yet, this is still a promising area of exploration, since it may be possible to form alternate phase structures such as bicontinuous phases, which would be ideal for cellular invasion.

Isotactic PHEMA was found to possess negative temperature dependence in water (Oh and Jhon, 1989). While atactic PHEMA is not expected to have a strong negative temperature dependence, the mechanisms of this behavior can still exist over short ranges and may effect the phase behavior. As such, increased temperatures may also function to control the pore morphology by allowing the polymer to phase separate early on in the reaction.

Temperature not only plays a critical role with the thermodynamics, but also with the kinetics of the polymerization. Once phase separation occurs, the polymer phase will start to settle out of solution since it is denser than the aqueous phase. Chirlia noted this phenomenon by stating that in some reactions, a water layer was evident over the polymer sponge layer (Chirila *et al*., 1993). Temperature can reduce this settle-out by ramping up polymerization rate, and forcing gelation to occur sooner.

### 2. *Tissue–Implant Interactions*

*a. Classic foreign body response.* Implants are foreign bodies that will invoke the natural defense mechanism against such intrusions; the

inflammatory response. Typically, the inflammatory response is split into two categories, acute and chronic inflammation (Anderson, 1988, 1993). During the acute phase, an influx of fluid, plasma proteins, and neutrophils enter the wound/implant site (Malech and Gallin, 1987). These neutrophils accumulate at the site of implantation and start to phagocytize any small debris/bacteria that are present. Phagocytosis is activated when the neutrophils comes into contact with activating factors called opsonins (Anderson, 1993). If an implant surface absorbs opsonins, such as the antibody immunoglobulin G (IgG), the neutrophil will try to engulf the implant. But since there is a large size disparity between the implant and neutrophils, phagocytosis cannot occur. This leads to an event known as frustrated phagocytosis, where the neutrophils dump the contents of lysosomes into the ECM (Henson, 1980). This process is highly unfavorable since it is very irritating to the surrounding tissue and leads to chronic inflammation. After the neutrophils have entered the area and cleared away any debris, granulation tissue (highly vascularized tissue) begins to form, and the natural wound healing response continues. At this point the response can split into either a chronic inflammatory response or a foreign body reaction of the acute type (Anderson, 1988). If there is a constant chemical or physical irritation (as in free movement of the implant), the chronic inflammatory response will occur (Gallin *et al.*, 1988). If there are no negative chemical or physical signals, then classic foreign body response occurs. Typically, the foreign body response results in three characteristic layers (Anderson, 1988). A primary layer of macrophages and/or foreign body giant cell formations surrounds the implant. These cells secrete the second layer composed of dense fibrous tissue 30–100 μm in thickness. A third layer of granulation tissue surrounds this fibrous wall. This response is indefinitely stable except for a decrease in cellularity of the primary layer. The dense nature of the fibrous layer greatly impedes the diffusion of most chemical species, as a result prevents any implanted drug delivery device from functioning effectively (Scharp *et al.*, 1984).

*b. Tissue response to porous materials.* The tissue response changes greatly when the implanted material has a porous morphology. Brauker *et al.* (1995) published a paper demonstrating the ability of porous materials to remodel the tissue response, and support vasculature up to one year postimplantation. They subcutaneously implanted several hydrophobic materials (PTFE, cellulose acetate, cellulose esters, and acrylic copolymers) with pore sizes ranging from 0.02–15 μm. It was found that materials with pores greater 5 μm were surrounded by highly vascular loose connective tissue. When the pore sizes increased further, evidence of vascular penetration was evident. The astounding part of their study was that this

vasculature persisted for the entire duration of the study, one year. Shwarkawy *et al.* studied acetylized PVA with pore sizes 5, 60, and 700 μm in size (Shwarkawy *et al.*, 1997, 1998a,b). Their 5-micron pore size corroborated the results obtained by Brauker *et al.* (1995). However, they noted a very high degree of vascularization of implants with the 60 μm pore size, and when this pore size increased beyond 100 μm, the vascularity of the materials actually decreased.

Shwarkawy *et al.* (1997, 1998a,b) also demonstrated that changes in pore size not only effected vascular density but also the response to systemic uptake of drug through a vascularized implant. It was demonstrated that the 60 μm pore material delivered the drug in almost half the time it took for a subcutaneous injection to be taken up systemically. This is due to the increased vascular density as well as increased vascular permeability at these pore sizes (Shwarkawy *et al.*, 1997, 1998a,b).

There are two main theories that have been proposed to describe the dependence of vascular penetration on implant pore size. Padera and Colton have suggested that it is the macrophages degree of attachment onto the material surface that dictates the signals that they send out (Padera and Colton, 1996). When the macrophages are able to spread onto the surface of the material, they release signals that call for the deposition of the tight collagen layer. When these macrophages penetrate into a porous sample, and cannot spread fully on the surface, this signal is not released or released to a lesser extent. However, due to the macrophages being farther from a nutrient source, they release signals that initiate angiogenesis. When the macrophages penetrate into the very large pores, they are able to once again release the collagen deposition signals, and the pores become filled with the avascular collagen layer that typically surrounds a nonporous implant.

Rosengren has stated that it may be implant mobility that controls the degree of implant vascularity (Rosengren *et al.*, 1999). They suggest that smooth implants are capable of high relative motion. This motion shears the adjacent cells inducing necrosis. The degree of necrosis is the cause of the severity of the inflammatory response, hence the thickness of the fibrous capsule. They further suggest that porous materials possess little to no fibrous capsule, because the tissue that penetrates works to stabilize the relative motion. While it is still not known whether or not these hypotheses are correct or to what degree they are important, it is evident that simple morphological changes have a great effect upon the vascularization of implants.

*c. Chemical and physical determinants of tissue attachment and in growth.* Many of the porous implant studies compared the results of

materials with varying surface chemistries. These studies looked at materials of varied hydrophilicity, such as hydrophobic PTFE, and acetylized PVA, to the more hydrophilic cellulose esters and acetates and poly(vinyl alcohol)s (Brauker *et al.*, 1995; Lipsky, 1989; Sieminski and Gooch, 2000a; Shwarkawy *et al.*, 1997, 1998a,b; Wake *et al.*, 1995). It was found that the ingrowth of vascularized and loose connective tissue was dictated primarily by the pore size rather than chemical properties of the material. However, it would be wrong to assume that no control could be obtained through modifications of the implant surface chemistry.

Endothelial cells interact with the ECM through adhesion moieties called integrins (Saltzman, 1997). It is believed that cells attach onto synthetic materials through intermediary proteins, such as fibrin, which absorb onto polymer surfaces. Hence, by changing the protein absorption properties of surfaces, it is possible to alter the adhesion of endothelial cells. Moreover, it is also possible to bind specific adhesion ligands onto surfaces for a more direct control of the cellular attachment (Cook, 1997; Hubbel, 1992). Endothelial cells are able to adhere to the common attachment sequences that are found on fibrin, such as RGD and YISGR. It was found, however, that another adhesion peptide sequence, the RDEV ligand, preferentially bound endothelial cells over fibroblasts, smooth muscle cells, or activated platelets (Hubbel, 1992). Through this ligand, it may be possible to control explicitly the formation of capillaries into the implant.

Tube formation of the endothelial cells is an essential characteristic for the formation of capillaries, and is controlled by both chemical and physical properties of the material. There has been a significant lack of *in vitro* research showing the effects of synthetic biomaterials on endothelial cell's ability for tube formation (Sieminski and Gooch, 2000a). One study coated fibronectin in 10 and 30 μm stripes. They noted that tube formation occurred on the 10 μm stripes but not on 30. This study demonstrates the general trend of tube formation that the more adherent the cells are to a surface, the more they spread and the less likely they are to express phenotypes like tube formation. Also, that cells with greater spreading (attachment) exhibited increased proliferation, yet a decrease in cellular mobility. Moreover, tube formation was most prominent in surfaces that exhibited moderate adhesive characteristics (Matsuda and Kurumantani, 1990). More recently, Dziubla and Lowman (2003) demonstrated that 3D scaffolds of PEG-grafted PHEMA hydrogels were able to support EC tubule formation regardless of the adhesive characteristics. This is believed to be a result of the network pores to trap and contain the secreted ECM components of the migrating endothelial cells. There is also evidence that material stiffness also plays

a part on tube formation. Ingber *et al.* showed that softer, more malleable materials exhibited an increase in cell tube formation (Ingber and Folkman, 1989).

## III. Applications of Hydrogels

Hydrogels have been most extensively studied for their use in the field of controlled drug delivery. While this work is of primary importance, it is not the only biomedical application available for hydrogels. They have also been finding interesting utility in areas such as tissue engineering, biosensors, microfabrication, and cell-culturing. In this section, a more detailed summary of hydrogel applications from drug delivery to newer, novel innovations is provided.

### A. Neutral Hydrogels

A major goal in drug delivery is to develop systems that deliver therapeutic agents at a constant rate over an extended period. This can be achieved by using release systems in which gel swelling is the controlling mechanism for drug release. Researchers have also attempted to develop constant-release systems by alteration of device geometry and polymer composition. One of the first researchers to use hydrogels for swelling-controlled release was Good (1976). In this work, glassy poly(2-hydroxyethyl methacrylate) containing tripelennamine-hydrochloride was swollen in water. The release rate of the solute was non-Fickian, but zero-order release was not obtained. The first such system in which zero-order release was observed was developed by Hopfenberg and Hsu (1978). In these systems, crosslinked polystyrene was used to release red dyes into hexane. Other polymers that have been used extensively in controlled release systems include poly(vinyl alcohol) (PVA), poly(*N*-vinyl-2-pyrollidone) (PNVP), poly(ethylene glycol) (PEG), poly(ethylene oxide) (PEO), and poly(ethylene vinyl *co*-acetate) (PEVAc) or copolymers thereof. In this section, we will discuss some of the applications of some of these materials.

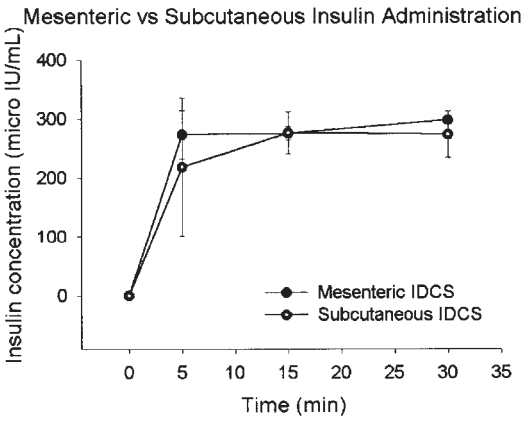
#### *1. Poly(2-Hydroxyethyl Methacrylate)*

Poly(2-hydroxyethyl methacrylate) (PHEMA) has been the most widely used polymer in drug delivery applications. It is an extremely hydrophilic

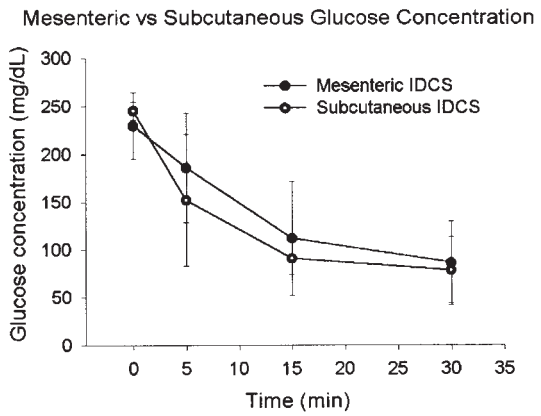
polymer and is highly stable. The permeability of these membranes is easily controlled based on the degree of crosslinking used. Researchers have studied the swelling behavior, morphology and diffusional behavior of PHEMA gels and copolymers thereof. The release of a wide range of drugs from these gels has also been studied. For example, Anderson *et al.* (1976) studied the release behavior of hydrocortisone from PHEMA gels. The release behavior was non-Fickian, but true zero order release was not achieved with these gels. Another significant study was performed by Sefton and Nishimura (1980). They investigated the diffusional behavior of insulin in PHEMA-based hydrogels. Song *et al.* (1981) developed one of the first pharmaceutically relevant zero-order release systems. They used a reservoir device consisting of a crosslinked PHEMA cylinder encapsulating a solution silicon oil and progesterone. Zero-order release was obtained with these devices for up to 10 days. Lee (1984; 1986) was able to use PHEMA to achieve zero-order release for short periods of time. In his work, oxprenolol was released from highly crosslinked PHEMA gels at constant rates for up to 3 h.

Peppas and co-workers contributed greatly to the understanding of the underlying phenomena of macromolecular relaxations in swelling controlled release systems. In particular, they studied hydrogels prepared from PHEMA and hydrophobic poly(methyl methacrylate) (PMMA). Franson and Peppas (1983) prepared crosslinked copolymer gels of P(HEMA-*co*-MAA) of varying compositions. Theophylline release was studied and it was found that near zero-order release could be achieved using copolymers containing 90% PHEMA. Further studies by Davidson and Peppas (1986) studied the effects of hydrophobicity and crosslink density on the release kinetics and diffusional properties of P(HEMA-*co*-MMA) membranes. Additionally, Korsmeyer and Peppas (1984) examined the behavior of copolymer gels consisting of PHEMA and hydrophilic PNVP (PHEMA-*co*-NVP). In this work, zero-order release of theophylline was observed for up to 5 h.

Macroporous networks of PHEMA containing hydrogels have also shown utility in long term implantable delivery devices, such as the implantable insulin pump. Typically, the functional life of the insulin pump is limited by the eventual occlusion of the catheter port. In the studies of Dziubla *et al.* (1999, 2002) it was shown that when the catheter port was coated in a hydrogel capable of supporting vascular tissue ingrowth, port occlusion is prevented even at 5 months postimplantation (typically cellular occlusion occurs after 8 weeks). Moreover, a rapid insulin uptake and systemic glucose response was noted (Fig. 7). This was assumed to be a result of the higher vascular density surrounding the catheter port.




(a)




(b)

FIG. 7. Systemic (a) human insulin concentration and (b) glucose response following infusion of human insulin from external pump, 5 months postimplantation (Dziubla *et al.*, 2002).

## 2. *Poly(Vinyl Alcohol)*

Another hydrophilic polymer that has received attention is poly(vinyl alcohol) (PVA). This material holds tremendous promise as a biological drug delivery device because it is nontoxic, hydrophilic and exhibits good mucoadhesive properties (Peppas, 1987). In one of the first applications of this material, Langer and Folkman (1976) investigated the use of copolymers of PHEMA (Hydron®) and PVA as delivery vehicles for polypeptide drugs.

Two methods exist for the preparation of PVA gels. In the first method, linear PVA chains are crosslinked using glyoxal, gluteraldehyde, or borate. In the second method, pioneered by Peppas (1975), semi-crystalline gels were prepared by exposing aqueous solutions of PVA to repeating freezing and thawing. The freezing and thawing induced crystal formation in the materials and allowed for the formation of a network structure crosslinked with the quasi-permanent crystallites. The latter method is the preferred method for preparation as it allows for the formation of an "ultrapure" network without the use of toxic crosslinking agents.

Korsmeyer and Peppas (1981) prepared PVA gels by crosslinking with borate. They studied the swelling behavior and mechanical properties of these gels. In this work, the release rate of theophylline was dependent on the degree of crosslinking. Future studies by Morimoto *et al.* (1989) examined the release behavior of a wide range of drugs. In this work, they were able to release indomethacin, glucose, insulin, heparin, and albumin from chemically crosslinked PVA gels.

Since the development of the semi-crystalline PVA gels by Peppas, significant work has been done in characterizing of these systems. Peppas and Hansen (1982) studied the kinetics of crystal formation during the freezing and thawing process. Subsequent work by Urushizaki *et al.* (1990) evaluated the effects of the number of freezing and thawing cycles on the networks properties. The gels became more rigid with increasing number of cycles. Peppas and Stauffer (1991) have also investigated the effects of crystallization conditions such as freezing temperature, number of cycles and freezing time on the structure and properties of the PVA networks.

Studies on the use of PVA prepared by the freezing/thawing technique as controlled release devices have recently been reported. Ficek and Peppas (1993) and Peppas and Scott (1992) used PVA gels for the release of bovine serum albumin. In the work of Peppas and Scott, drug release occurred by classical Fickian diffusion. Ficek and Peppas (1993) developed a method to prepare novel PVA microparticles containing BSA by a freezing/thawing technique. Here, they were able to release bovine serum albumin using these microparticles.

Other researchers have investigated the use of PVA gels as mucoadhesive delivery devices. Nagai and co-workers reported novel buccal delivery systems for ergotamine tartrate (Tsutsumi *et al.*, 1994). Peppas and Mongia (1997) have also considered PVA for mucoadhesive drug delivery applications. In their work, they investigated the mucoadhesive behavior of PVA gels prepared by the freezing/thawing technique. Additionally, they studied the release behavior of theophylline and oxprenolol from these materials. Additionally, the group of Peppas reported on the

release behavior of ketanserin (Mongia *et al.*, 1996) and metronidazole (Mallapragada and Peppas, 1997a) from these systems.

New phase erosion controlled release systems based on semicrystalline have been reported by Mallapragada and Peppas (1997a,b). These systems exhibited an unusual molecular control of the drug or protein delivery by a simple dissolution of the carrier. Hydrophilic carriers pass through a process of chain unfolding from the semicrystalline phase to the amorphous one, eventually leading to complete chain disentanglement. It has been shown that PVA and PEG are the best systems for such release behavior, and that such devices have the potential to be used for a wide range of drug delivery applications release. A detailed mathematical analysis has been developed to analyze such swellable systems (Mallapragada and Peppas, 1997b; Narasimhan and Peppas, 1997a; Peppas and Colombo, 1997).

### 3. *Poly(Ethylene Oxide)/Poly(Ethylene Glycol)*

Hydrogels of poly(ethylene oxide) (PEO) and poly(ethylene glycol) (PEG) have received significant attention in the last few years in biological drug delivery applications, especially because of their associated stealth characteristics and their protein resistance (Graham, 1992). Three major preparation techniques exist for the preparation of crosslinked PEG networks: (1) chemical crosslinking between PEG chains, (2) radiation crosslinking of PEG chains, and (3) chemical reaction of mono- and difunctional PEGs.

Some of the first chemically crosslinked PEG networks were prepared by McNeill and Graham (1984). The crosslinked linear PEG chains using diisocyanates. These gels were used as resevoir devices for the controlled delivery of smaller molecular weight drugs. McNeill and Graham (1996) have investigated the release behavior of small molecular weight solutes from PEG crosslinked with 1,2,6-hexanetriol. The release of proxyphylline from PEG spheres, slabs, and cylinders was studied. For each of the matrix devices, they observed non-Fickian release kinetics. Bromberg (1996) also studied the release of chemically crosslinked PEG networks. In this work, PEG networks were crosslinked using tris(6-isocyanatohexyl)isocyanurate. The kinetics of insulin release from PEG gels obeyed non-Fickian release kinetics.

The advantage of using radiation crosslinked PEO networks is that no toxic crosslinking agents are required. However, it is difficult to control the network structure of these materials. Stringer and Peppas (1996) have recently prepared PEO hydrogels by radiation crosslinking. The network structure was analyzed in detail, and the diffusional behavior of smaller molecular weight drugs, such as theophylline, in these gels was investigated.

Kofinas *et al.* (1996) have prepared PEO hydrogels by a similar technique. In this work, they studied the diffusional behavior of two macromolecules, cytochrome C and hemoglobin, in these gels. They noted an interesting, yet previously unreported dependence between the crosslink density and protein diffusion coefficient and the initial molecular weight of the linear PEGs.

Lowman *et al.* (Dziubla *et al.*, 1999; Lowman *et al.*, 1997) have presented an exciting new method for the preparation of PEG gels with controllable structures. Here, highly crosslinked and tethered PEG gels were prepared from PEG-dimethacrylates and -monomethacrylates. The diffusional behavior of diltiazem and theophylline in these networks was studied. The technique presented in this work is promising for the development of a new class of functionalized PEG-containing gels that may be of use in a wide variety of drug delivery applications.

## B. Responsive Networks

### *1. pH-Sensitive Hydrogels*

Hydrogels that have the ability to respond to pH changes have been studied extensively over the years. These gels typically contain side ionizable side groups such as carboxylic acids or amine groups (Oppermann, 1992; Scranton *et al.*, 1995). The most commonly studied ionic polymers include polyacrlyamide (PAAm), poly(acrylic acid) (PAA), poly(methacrylic acid) (PMAA), poly (diethylaminoethyl methacrylate) (PDEAEMA), and poly(dimethylaminoethyl methacrylate) (PDMAEMA).

Cationic copolymers based on PDEAEMA and PDMAEMA have been studied by the groups of Peppas and Siegel. Siegel and co-workers has focused on the swelling and transport behavior of hydrophobic cationic gels. Siegel and Firestone (Firestone and Siegel, 1988; Siegel and Firestone, 1988) studied the swelling behavior of hydrophobic hydrogels of PDMAEMA and poly(methyl methacrylate). Such systems were collapsed in solutions of pH greater than 6.6. However, in solutions of pH less than 6.6, such systems swelled due to protonation of the tertiary amine groups. The release of caffeine from these gels was studied (Siegel *et al.*, 1988). No caffeine was released in basic solutions, however, in neutral or slightly acidic solutions steady release of caffeine was observed for 10 days. Cornejo-Bravo and Siegel (1996) have investigated the swelling behavior of hydrophobic copolymers of PDEAEMA and PMMA. Additionally, Siegel (1990) has presented an excellent model of the dynamic behavior of ionic gels.

Peppas and co-workers has studied the swelling behavior of more hydrophilic, cationic copolymers of P(DEAEMA-*co*-HEMA) and P(DMAEMA-*co*-HEMA) Hariharan and Peppas (1996). These gels swell in solutions of pH less than 7 and collapse in basic solutions. These materials swelled to a greater degree than those prepared by Siegel (1990). Schwarte and Peppas (1997, 1998); (Schwarte *et al.*, 1998) studied the swelling behavior of copolymers of PDEAEMA grafted with PEG. The permeability of dextrans of molecular weight 4400 and 9400 was studied. The membrane permeabilities in the swollen membranes (pH = 4.6) were two-orders of magnitude greater than permeabilities of the collapsed membranes.

Anionic copolymers have received significant attention as well. The swelling and release characteristics of anionic copolymers of PMAA and PHEMA (PHEMA-*co*-MAA) have been investigated. In acidic media, the gels did not swell significantly, however, in neutral or basic media, the gels swelled to a high degree due to ionization of the pendant acid group (Brannon-Peppas and Peppas, 1990; Kou *et al.*, 1988). Brannon-Peppas and Peppas (1991b) have also studied the oscillatory swelling behavior of these gels. Copolymer gels were transferred between acidic and basic solutions at specified time intervals. In acidic solutions, the polymer swelled due to the ionization of the pendant groups. In basic solutions, rapid gel syneresis occurred. Brannon-Peppas and Peppas (1991c) modeled the time-dependent swelling response to pH changes using a Boltzman superposition-based model. The pH-dependent release behavior of theophylline and proxyphylline from these anionic gels was also studied (Bettini *et al.*, 1995; Brannon-Peppas and Peppas, 1989). Khare and Peppas (1993, studied the pH-modulated release behavior of oxprenolol and theophyllne from copolymers of PHEMA-*co*-MAA and PHEMA-*co*-AA. In neutral or basic media, the drug release occurred rapidly by a non-Fickian mechanism. The release rate was slowed significantly in acidic media. In another study, Am Ende and Peppas (1997) examined the transport of ionic drugs of varying molecular weight in PHEMA-*co*-AA. They compared experimental results to a free-volume based theory and found that deviations occurred due to interactions between the ionized backbone chains and pendant acid groups. The swelling and release behavior of interpenetrating polymer networks of PVA and PAA was also investigated (Gudeman and Peppas, 1995; Peppas and Wright, 1996). These materials also exhibit strong pH-responsive swelling behavior. The permeability of these membranes was strongly dependent on the environmental pH and the size and ionic nature of the solute. New studies have used ATR–FTIR spectroscopy to characterize the interactions between

polyelectrolytes and solutes (Am Ende and Peppas, 1995; Peppas and Wright, 1996).

Heller *et al.* (1990) studied the behavior of another type of pH-responsive hydrogel. Here, they evaluated the pH-dependent release of insulin from degradable poly(ortho esters). Other researchers have used chitosan (CS) membranes for drug delivery applications. These materials exhibited pH-dependent swelling behavior due to gelation of CS upon contact with anions (Bodmeier and Paeratakul, 1989). Interpenetrating networks of CS and PEO have been proposed as drug delivery devices due to their pH-dependent swelling behavior (Shiraishi *et al.*, 1993; Yao *et al.*, 1993). Calvo *et al.* (1997) prepared novel CS–PEO microspheres. These systems were to provide a continuous release of entrapped bovine serum albumin for one week. In another study, methotrexate, an anti-cancer drug, was encapsulated in microspheres of pH-sensitve CS and alginate (Narayani and Rao, 1995). Zero-order release of the drug was observed from the microspheres in pH = 1.2 buffer for greater than one week. Okano and co-workers (Kikuchi *et al.*, 1997) studied pH-responsive calcium–alginate gel beads. In such systems, modulated release of dextran was achieved by varying the pH and ionic strength of the environmental solution. Such systems may be promising for use in protein and peptide delivery applications.

### 2. *Temperature-Sensitive Hydrogels*

Some of the earliest work with temperature-sensitve hydrogels was done by the group of Tanaka (1979). They synthesized with crosslinked poly(*N*-isopropylacrylimide) (PNIPAAm) and determined that the LCST of the PNIPAAm gels was 34.3°C. Below this temperature, significant gel swelling occurred. The transition about this point was reversible. They discovered that the transition temperature was raised by copolymerizing PNIPAAm with small amounts of ionic monomers. Beltran *et al.* (1991) also worked with PNIPAAm gels containing ionic comonomers. They observed results similar to those achieved by Tanaka (1979).

The earliest investigators studying PNIPAAm gels discovered that the response time of the materials in response to temperature changes was rather slow. Future studies focused on developing newer materials that had the ability to collapse/expand in a more rapid fashion. Dong and Hoffman (1990) prepared heterogeneous gels containing PNIPAAm that collapsed at significantly faster rates than homopolymers of PNIPAAm. Kabra and Gehrke (1991) developed new method to prepare PNIPAAM gels that resulted in significant increases in the swelling kinetics of the gels. They

prepared gels below the LCST to produce a permanent phase separated microstructure in the gels. These gels expanded at rates 120 faster and collapsed at rates 3000 times faster than homogeneous PNIPAAm gels. Okano and co-workers (Kaneko *et al.*, 1996; Yoshida *et al.*, 1995) developed an ingenious method to prepare comb-type graft hydrogels of PNIPAAm. The main chain of the crosslinked PNIPAAm contained small molecular weight grafts of PNIPAAm. Under conditions of gel collapse (above the LCST), hydrophobic regions were developed in the pores of the gel resulting in a rapid collapse. These materials had the ability to collapse from a fully swollen conformation in less than 20 min while comparable gels that did not contain graft chains required up to a month to fully collapse. Such systems show major promise for rapid and abrupt or oscillatory release of drugs, peptides, or proteins.

Thermo-responsive polymers may be particularly useful for a wide variety of drug delivery applications (Hoffman, 1987; Kim, 1996). Okano *et al.* (1990) studied the temperature dependent permeability of PNIPAAm gels. They were able to use these gels as "on–off" delivery devices in response to temperature fluctuations. This type of behavior was useful for controlling the release of insulin (Bae *et al.*, 1989) and heparin (Gutowska *et al.*, 1992). These materials were used to modulate the release behavior of protein and peptide drugs (Gutowska *et al.*, 1992), and were also used as "squeezing" systems (Yoshida *et al.*, 1994). Okano *et al.* (Yamato *et al.*, 2000, 2001) has pursued a novel temperature-sensitive cell culturing plate. By coating tissue culture plates with a uniformly thin coat of PNIPAAm, they have been able to create cell culture flasks with a switchable cellularly adhesive surface. This allows the growth of confluent layers of cells, which can be removed and either stacked to form multi-layered cells for *in vitro* study or *in vivo* tissue replacement (Nandkumar *et al.*, 2002; Shimizu *et al.*, 2003; von Recum *et al.*, 1998).

Another promising application of these systems was explored by the Vernon *et al.* (1996). In this study, islets of Langerhans were entrapped by thermal gelation of PNIPAAm for use as a rechargeable artificial pancreas. Also, Fukumori *et al.* (Ichikawa and Fukumori, 2000; Ichikawa *et al.*, 1998) worked with microcapsules coated in a thermosensitive layer comprised of either hydroxypropyl cellulose (HPC) or ethyl cellulose embedded with nano-particles of PNIPAAm hydrogels. These two systems result in two entirely different temperature sensitive drug release schemes. Drug release occurs readily below the LCST for the HPC coating, due to the more open mesh size present. However, above this temperature, the network collapses hindering solute transport. In the other system, the drug release occurs only at temperatures above the LCST. At these temperatures the nano-hydrogels are collapsed, leaving voids

that allow for rapid release of solute. These systems were found to be fully reversible resulting in an "on/off" release behavior.

Other materials possessing a LCST near physiological conditions have also been persued. Yuk *et al.* (1997a,b) have proposed another temperature sensitive comonomer system comprising of DMAEMA and acrylamide (Aam). By changing the comonomer feed ratio, the LCST of these systems varied from 28°C to 50°C. They also developed a mathematical model to describe solute transport as a function of temperature and network swelling kenetics (Grassi *et al.*, 1999). Ogata has also worked on hydrogels composed of the nucleic acid, uracil (Ogata, 1996). These hydrogels have been show to possess rapid volume changes at 35°C.

Another application of thermally sensitive hydrogels is in injectable, localized drug delivery systems. Cui and Messersmith (1998) used an aqueous solution of sodium alginate and temperature sensitive liposomes containing $Ca^{2+}$ and drug. Once the solution is injected, the temperature of the body causes the liposomes to release the calcium ions and drug. These calcium ions then crosslink the alginate forming a hydrogel, and controlled release of the drug to the surrounding tissue is possible. Hoffman *et al.* (1997) created networks of chitosan grafted with Pluronic side chains. This copolymer system remains a solution until the temperature is raised to 37°C, and the side chains for hydrophobic domains that act to crosslink the network forming a hydrogel.

### *3. pH- and Temperature-Sensitive Hydrogels*

Over the last decade, researchers have developed a novel class of hydrogels that exhibit both pH- and temperature-sensitive swelling behavior. These materials may prove to be extremely useful in enzymatic or protein drug delivery applications. Hydrogels were prepared from PNIPAAm and PAA that exhibited dual sensitivities (Dong *et al.*, 1992; Feil *et al.*, 1992). These gels were able to respond rapidly to both temperature and pH changes. Kim and co-workers investigated the use of such systems for carriers for insulin (Kim *et al.*, 1994) and calcitonin (Serres *et al.*, 1996). In general, these hydrogels only exhibited strong temperature sensitive swelling behavior with large amounts of PNIPAAm in the gel. Cationic pH- and temperature-sensitive gels were prepared using poly(amines) and PNIPAAm (Nabeshima *et al.*, 1996). These systems were evaluated for local delivery of heparin.

Chen and Hoffman prepared new graft copolymers of PAA and PNIPAAm that responded more rapidly to external stimulus than previously studied materials (Chen and Hoffman, 1995). These materials

exhibited increased temperature sensitivity due to the presence of the PNIPAAm grafts. Such systems were evaluated for use in prolonged mucosal delivery of bioactive agents, specifically peptide drugs (Hoffman *et al*., 1996).

Brazel and Peppas (1995) studied the pH- and temperature-responsive swelling behavior of gels containing PNIPAAm and PMAA. These materials were used to modulate the release behavior of streptokinase and heparin in response to pH and temperature changes (Brazel and Peppas, 1996). Baker and Siegel (1996) used similar hydrogels to modulate the glucose permeability. However, large amounts of PNIPAAm were needed to observe large temperature sensitivities. The group of Peppas developed novel pH- and temperature-sensitive terpolymers of PHEMA, PMAA, and PNIPAAm (Vakkalanka and Peppas, 1996). These systems were prepared to contain PNIPAAm-rich blocks and as a result, these materials were able to exhibit strong temperature sensitivity with only 10% PNIPAAm in the gel. Using these materials, they were effectively able to modulate the release kinetics of streptokinase (Vakkalanka *et al*., 1996).

A novel approach to pH and temperature sensitive drug release has been developed by the Ron *et al*. (1998). They developed a system which consists of two compartments, one which is a drug reservoir and the other contains a pH–temperature sensitive hydrogel comprised of carboxylic acid functionalized hydroxypropyl cellulose. During periods of neutral pH and low temperature, the gel is swollen preventing release of the drug from the reservoir. However, when the temperature increases, or pH decreases, the hydrogel barrier collapses, thus allowing drug release. Kaetsu *et al*. (1991, 1999a,b, 2000) also developed a similar technology on a silicon wafer chip. They photo etched pits into the wafer, and these pits were subsequently covered in a layer of polyethylene teraphthalate mesh filled with a polyelectrolytes gel. These gels covered the holes in the silicone wafer. Enzymes can be immobilized onto the gel, and act as the sensing mechanism. When the internal pH of the gel changes, it collapes, thus releasing the drug contained in the hole on the silicone wafer. The advantage to such a system is that a multitude of drug reservoirs can be placed onto a single chip, thus allowing for a localized complex drug delivery scheme.

### 4. *Complexing Hydrogels*

Another promising class of hydrogels that exhibit responsive behavior is complexing hydrogels. Osada studied complex formation in PMAA hydrogels (Osada, 1980). In acidic media, the PMAA membranes collapsed in the presence of linear PEG chains due to the formation of interpolymer complexes between the PMAA and PEG. The gels swelled when placed in

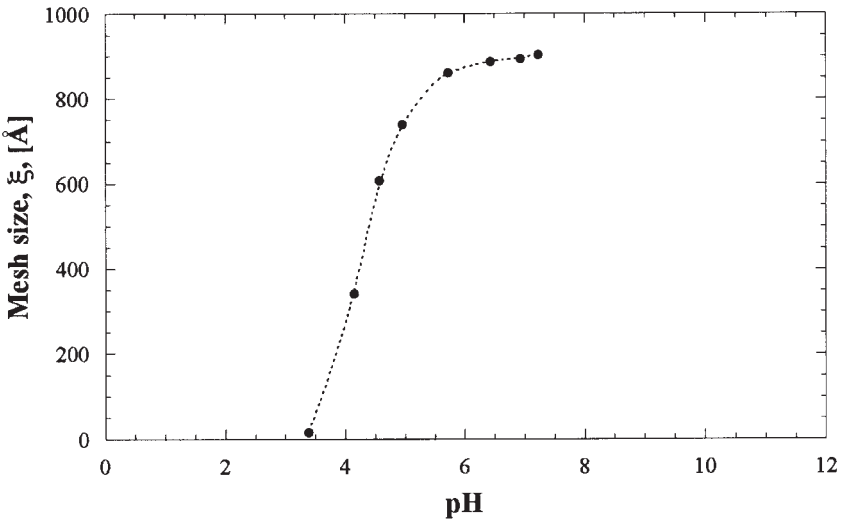



FIG. 8. Effect of the environmental pH on the mesh size, $\xi$, of PEG-grafted PAA polymer networks P(AA-*g*-PEG) of PEG molecular weight of 2000.

neutral or basic media. The permeability of these membranes was strongly dependent on the environmental pH and PEG concentration (Osada *et al.*, 1986). Similar results were observed with hydrogels of PAA and linear PEG (Nishi and Kotaka, 1986). The significant change in permeability is directly related to the disruption of the polymer complexes, which results in gross changes in polymer mesh size (Fig. 8). Polymer complexation was also achieved in interpenetrating polymer networks of PVA and PAA (Byun *et al.*, 1996; Shin *et al.*, 1997). These systems, which exhibit pH and weak temperature sensitive behavior, were studied for their release behavior of indomethacin.

Peppas and co-workers has developed a class graft copolymer gels of PMAA grafted with PEG (P(MAA-*g*-EG) (Bell and Peppas, 1995, 1996a,b,c; Klier *et al.*, 1990; Lowman and Peppas, 1999a,b; Lowman *et al.*, 1998; Peppas and Klier, 1991). These gels exhibited pH dependent swelling behavior due to the presence of acidic pendant groups and the formation of interpolymer complexes between the ether groups on the graft chains and protonated pendant groups. In these covalently crosslinked, complexing P(MAA-*g*-EG) hydrogels, complexation resulted in the formation of temporary physical crosslinks due to hydrogen bonding between the PEG grafts and the PMAA pendant groups. The physical crosslinks were reversible in nature and dependent on the pH and ionic strength of the environment. As a result, complexing hydrogels exhibit drastic changes in their mesh size over small changes of pH as shown in Fig. 9. One

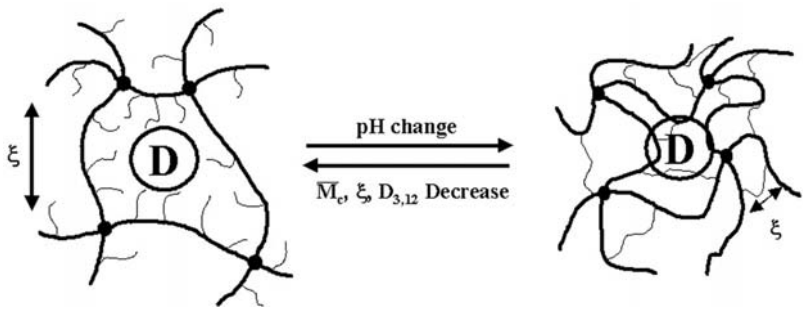



FIG. 9. The effect of interpolymer complexation on the correlation length, $\xi$, and the effective molecular weight between crosslinks, $\overline{M}_c$, in P(MAA-*g*-EG) graft copolymer networks with permanent, chemical crosslinks (●).

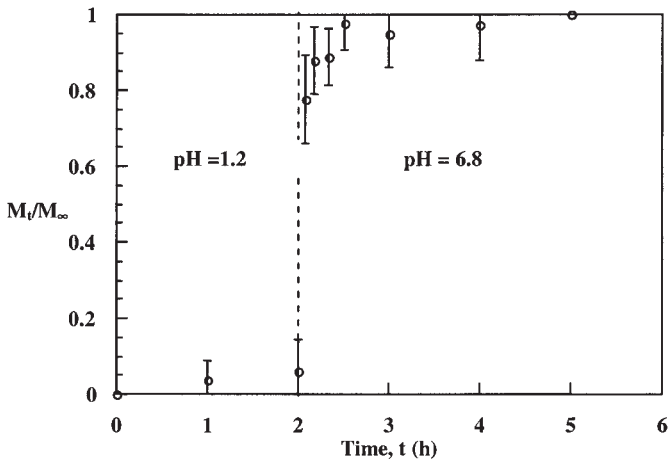



FIG. 10. Controlled release of insulin *in vitro* from P(MAA-*g*-EG) microparticles simulated gastric fluid (pH = 1.2) for the first two hours and phosphate buffered saline solutions (pH = 6.8) for the remaining three hours at 37°C (243).

particularly promising application for these systems is the oral delivery of protein and peptide drugs (Lowman *et al.*, 1998, 1999). As shown in Fig. 10, these copolymers severely limited the release of insulin in acidic environments like those found in the stomach. However, in conditions similar to those found in the intestines, insulin release occurred rapidly.

### 5. *Glucose-sensitive Systems*

Major developments have been reported in the utilization of environmentally responsive hydrogels as glucose-sensitive systems that

could serve as self-regulated delivery devices for the treatment of diabetes. Typically, these systems have have been prepared by incorporating glucose oxidase into the hydrogel structure during the polymerization. In the presence of glucose, the glucose oxidase catalyzed the reaction between water and glucose to form gluconic acid. The gluconic acid lowered the pH of the microenvironment of the gel.

The first such systems developed by Kost *et al.* (1984) consisted of glucose oxidase immobilized in hydrogels based on PHEMA and PDMAEMA. These systems exhibited glucose sensitive swelling behavior. In the presence of glucose, gluconic acid was formed resulting in a decrease in the local pH. As a result, the cationic-based gel swelled to larger degrees in the presence of glucose due to the production of gluconic acid. The glucose responsive swelling behavior allowed for control over insulin permeation in these membranes by adjusting the environmental glucose concentrations (Albin *et al.*, 1985; Ishihara *et al.*, 1984). The kinetics of gel swelling and insulin release from cationic, glucose sensitive hydrogels was also studied (Goldraich and Kost, 1993).

Glucose responsive systems were proposed that were based on anionic hydrogels (Hassan *et al.*, 1997; Ito *et al.*, 1989). Ito *et al.* (1989) prepared systems of porous cellulose membranes containing an insulin reservoir. The pores of these devices were grafted with PAA chains functionalized with glucose oxidase. In the presence of glucose, the decrease in environmental pH caused the PAA chains to collapse opening the pores allowing for insulin release. More recently, glucose-sensitive complexation gels of P(MAA-*g*-EG) were developed by the group of Peppas (Hassan *et al.*, 1997). In these gels, as the pH decreased in response to elevated glucose concentrations, interpolymer complexes formed resulting in rapid gel syneresis. The rapid collapse resulted in insulin release due to a ''squeezing'' phenomenon.

Other glucose responsive systems have been developed that take advantage of the formation of complexes between glucose molecules and polymeric pendant groups. Lee and Park (1994) prepared erodible hydrogels containing allyl glucose and poly(vinyl pyrrolidone). These systems were crosslinked by the noncovalent associations between concanavalin-A (Con-A) and the glucose pendant groups. In the presence of free glucose, the Con-A was bound to the free glucose and the gels dissolved due to disruption of the physical crosslinks. Newer materials developed by the group of Okano exhibited glucose responsive swelling behavior and insulin release (Aoki *et al.*, 1996; Shiino *et al.*, 1995, 1996). These gels were based on phenylboronic acid (PBA) and acrylamides. Another class of glucose-sensitive gels was prepared containing PBA, PNIPAAm, and PVA (Hisamitsu *et al.*, 1997). These gels were

designed to allow for the release of insulin at physiological pH and temperature.

### C. Oral Insulin Delivery Systems

One of the major objectives of researchers working in the controlled release field is to design an effective, oral insulin delivery system. However, this is a difficult task due to the degradation of the drug in the upper gastrointestinal (GI) system barrier and the slow transport of insulin across the lining of the colon into the blood stream (Lee *et al*., 1991; Saffran, 1992, 1997). Numerous attempts have been made by researchers to use hydrogels as carriers for oral delivery of insulin in order to protect the drug in the stomach and release it into more favorable regions of the GI tract.

Touitou and Rubinstein (1986) designed a reservoir system consisting of insulin encapsulated by a polyacrylate gel. The coating was designed to dissolve only in the colon. In this work, weak hypoglycemic effects were observed only with very high insulin doses and the addition of absorption enhancers. Saffran *et al*. (1986) developed a biodegradable hydrogel containing insulin. The device consisted of insulin dispersed in a terpolymer of styrene and PHEMA crosslinked with a difunctional azo-containing compound. The azo bond was cleaved by microflora present in the colon, and the polymer degraded allowing for release of insulin into the colon. In this work, a hypoglycemic effect was obtained only with addition of absorption enhancers and protease inhibitors. However, the hypoglycemic effect obtained was not affected by the initial dosing.

Morishita *et al*. (1992) administered insulin contained within Eudragit 100 gels. In these systems, the pH-responsive Eudragit® degraded in the upper small intestine allowing for insulin release. They observed strong hypoglycemic effects in healthy and diabetic rats after the addition of absorption enhancers. Platé *et al*. (1994) developed a hydrogel system containing immobilized insulin and protease inhibitors that was effective in lowering the blood glucose levels in rabbits. Mathiowitz *et al*. (1997) have developed insulin containing poly(anhydride) microspheres. These materials adhered to the walls of the small intestine and released insulin based on degradation of the polymeric carrier. They observed a 30% decrease in the blood glucose levels of healthy rats. Lowman *et al*. (1999) have developed a bioadhesive, complexation hydrogel system for oral delivery of insulin. This delivery system consisted of insulin-containing microparticles of crosslinked copolymers of P(MAA-*g*-EG). The P(MAA-*g*-EG) were more effective in delivering biologically active insulin than traditional enteric coating-type carriers because of the presence of the PEG-grafts.

The addition of PEG to the gels was critical because the PEG chains participate in the macromolecular complexes, function as a peptide stabilizer and enhance the mucoadhesive characteristics of the gels. In this work, strong dose-dependent hypoglycemic effects were observed in healthy and diabetic rats following oral administration of these gels.

### D. Protein Based Hydrogels

Due to the overwhelming similarities of hydrogels and soft tissue extracellular matrices, it is not surprising to find many of its constituents being used to generate networks known as natural hydrogels. Collagen networks can be used as typical neutral hydrogels for sustained, local drug release. Moreover, collagen networks can be reabsorbed/remodeled by fibroblasts and endothelial cells, removing the need for explanting the hydrogel posttreatment (Sieminski and Gooch, 2000b). One interesting application of these networks has been the treatment of hair growth (Ozeki and Yasuhiko, 2002, 2003). In this work, they used collagen loaded with vascular endothelial growth factor or fibroblast growth factor in subcutaneous injections in order to stimulate follicle formation. In another system, Gooch *et al.* (Sieminski *et al.*, 2002) used collagen gels for the support of endothelial cells genetically engineered to release human growth hormone. Here, the collagen networks allowed for the *in vitro* tubule formation of the modified EC. Then the collagen nework could be implanted, where the attachment of existing vasculature with the engineered cellular vasculature could occur.

Muzykantov *et al.* (Muzykantov *et al.*, 1996, 1999; Shuvaev *et al.*, in press) have developed a unique nanoparticle hydrogel composed entirely of crosslinked proteins. In their system, biotin–streptavidin conjugation chemistry, a strongly associating recognition pair, was used to link targeting groups, immunoglobulins (IgG), with drug. Dziubla *et al.* found that a simple Carathor's equation modified for nonequimolar monomer ratios and functionalities greater than 2 described the formation of the super-macromolecular structure of the conjugate (Shuvaev *et al.*, 2003). These conjugates were capable of targeting organs with high vascular beds such as the lungs, when anti-PECAM (a unique endothelial cell adhesion molecule) was conjugated to antioxidants, such as catalase and super oxide dismutase. These conjugates were found to have a high utility in organ transplantation, where oxidative stress is one of the primary factors limiting organ storage prior to reimplantation (Kozower *et al.*, 2003).

## E. Other Promising Applications

Promising new methods for the delivery of chemotherapeutic agents using hydrogels have been recently reported. Novel biorecognizable sugar-containing copolymers have been investigated for the use in targeted delivery of anti-cancer drugs (Peterson *et al.*, 1996; Putnam *et al.*, 1996; Rathi *et al.*, 1997). Kopecek and associates have used poly(*N*-2-hydroxypropyl methacrylamide) carriers for the treatment of ovarian cancer (Peterson *et al.*, 1996).

In the last few years there have been new creative methods of preparation of novel hydrophilic polymers and hydrogels that may represent the future in drug delivery applications. The focus in these studies has been the development of polymeric structures with precise molecular architectures. Stupp *et al.* (1997) synthesized self-assembled triblock copolymer, nanostructures that may have very promising applications in controlled drug delivery. Novel biodegradable polymers, such as polyrotaxanes, have been developed that have particularly exciting molecular assemblies for drug delivery (Ooya and Yui, 1997).

Dendrimers and star polymers (Dvornik and Tomalia, 1996) are exciting new materials because of the large number of functional groups available in a very small volume. Such systems could have tremendous promise in drug targeting applications. Merrill (1993) has offered an exceptional review of PEO star polymers and applications of such systems in the biomedical and pharmaceutical fields. Griffith and Lopina (1995) have prepared gels of controlled structure and large biological functionality by irradiation of PEO star polymers. Such new structures discussed in this section could have particularly promising delivery applications when combined with emerging new technologies such as molecular imprinting (Cheong *et al.*, 1997; Mosbach and Ramström, 1996).

A number of investigators have concentrated on the development of environmentally responsive gels that exhibit biodegradability. This can be achieved by a number of synthetic methods. Kopecek and co-workers (Ghandehari *et al.*, 1996) have developed biodegradable hydrogels by incorporating azo-compounds. Bae and co-workers (1989) have synthesized very promising biodegradable carriers by preparing 8-arm, star-shaped, block copolymers containing PLA and PEO. Another potentially useful biodegradable system is a photo-crosslinked polymer based on poly(L-lactic acid-*co*-L-aspartic acid) (Elisseeff *et al.*, 1996) which could be prepared *in situ* for delivery of anti-inflammatory drugs following surgery.

One of the most recent developments in the application of hydrogels have been in the field of microfluidics and microsensors. Peppas *et al.* (Bashir *et al.*, 2002; Hilt *et al.*, 2002) have successfully composed a

microcantilever coated on one side with a PMAA hydrogel. Under pH changes, the equilibrium swelling of the coating changes, resulting in the deflection of the cantilever. Since this process is fully reversible, this has great implications for a rapid local pH and ionic devices. In another microfabrication application, DNA sequences have been included into poly acrylamide gels, which were photopolymerized as plugs within microfluidic channels (Seong *et al*., 2002). These plugs can be used as micro-electrophoretic gels which can hybridize with complementary DNA. Such systems may provide an enhancement over existing microchip assaying technologies due to increased mass transfer, lower sample volumes, and a potentially reusable substrate. Finally, microbioreactors have also been developed using hydrogels as the entrapment mechanism (Heo *et al*., 2003). Here, *E. coli* were trapped within a hydrogel inside a microchannel. A nonfluorescent substrate (BCECF–am) that is converted into the fluorescent (BCECF) product by the esterase enzymes in the bacteria. Their work showed that viable bacteria can be immobilized within microchannels, and be used as either microbioreactors or sensors for the fluid flow.

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