ADVANCES IN CHEMICAL ENGINEERING

VOLUME 19

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PREFACE

Volume 19 of *Advances in Chemical Engineering* features a variety of articles on chemical engineering, with a special theme on biomedical engineering. Chemical engineers have worked to apply their science to biomedicine since World War II. In the past decade, their impact on the practice of medicine has been unprecedented and useful. Langer writes about pioneering work on using polymer systems for the controlled release of macromolecules, for immobilized enzymes, for medical bioreactors, and for tissue engineering. Linderman *et al.* address receptor binding and signaling. Jain writes about transport phenomena in tumors, a topic that has been recognized as the key to effective treatment with drugs. These three chapters prove that chemical engineering in medicine has advanced from an academic exercise to widespread clinical practice.

Krishna has worked for the Shell Oil Company for many years; his chapter on the selection of multiphase reactors carries the knowledge of a skilled practitioner, which complements his theoretical teachings as a professor at the University of Amsterdam. Allen is one of the early pioneers in the application of engineering design to pollution prevention, and his chapter deals with macro-, meso-, and microscales. Seinfeld *et al.* write on tropospheric chemistry, the arena where a great many of our air pollution problems reside.

Together, these six chapters provide an expanding horizon for the intellectual scope of chemical engineers, in topics from oil refining to biomedicine, in scale from transport in tumors to the troposphere, and in approach from scientific analysis to practical design selections.

James Wei

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POLYMER SYSTEMS FOR CONTROLLED RELEASE OF MACROMOLECULES, IMMOBILIZED ENZYME MEDICAL BIOREACTORS, AND TISSUE ENGINEERING

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Recent advances in biology and medicine have created new challenges and opportunities for engineers. With these advances occurring more and more at a cellular and molecular level, the chemical engineer, in particular, has a unique training to address these challenges in a creative fashion. Research in chemical aspects of biomedical engineering is growing rapidly. Numerous biotechnology and bioengineering companies are being formed; this creates and should continue to create an increasing demand for scientists with interdisciplinary training in biology and engineering. There are numerous efforts involving research in this area. Our research at M.I.T. provides one of many such examples. In this chapter we discuss the following areas of our research: controlled release systems; immobilized

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enzyme medical bioreactors; and tissue engineering using degradable polymers.

I. Controlled Release Systems

Over the past decade there has been increasing attention devoted to the development of controlled release systems for drugs, pesticides, nutrients, agricultural products, and fragrances. However, nearly all of the systems that have been developed have not been capable of slowly releasing drugs of large molecular weight (MW > 600). In fact, up until 1976 it was a fairly common conception in the field of controlled release that effective systems could not be developed for macromolecules (1). However, after several years of effort an approach was discovered that permitted the continuous release of biologically active macromolecules as large as 2,000,000 daltons from normally impermeable, yet biocompatible, polymers for more than 100 days (2). Three areas of our drug delivery research are reviewed here: systems that release large molecules through porous polymer matrices, novel biodegradable polymeric delivery systems; and pulsatile controlled release polymer systems.

A. Porous Delivery Systems for the Release of Proteins and Macromolecules

Our interest in creating controlled release systems for polypeptides and other macromolecules began in 1974 and stemmed from studies on the growth of solid tumors. Most solid tumors require ingrowth of new blood vessels from the host for further tumor development, and we were attempting to isolate a drug that prevents the growth of new blood vessels. This substance is derived from cartilage, a tissue that contains no blood vessels. The bioassay used for this substance involved placing a tumor in the cornea of a rabbit and monitoring the growth of new vessels toward the tumor. It was desired to deliver the drug to the tumor to see if it decreased the rate of blood vessel growth. The assay takes 30 days.

Purified fractions of the cartilage material were highly soluble, so that they disappeared quickly after they were added. Therefore, a small sustained-release system was needed to provide steady diffusion into the tumor. Such a system had to be inert and noninflammatory. In early work (3), polyacrylamide pellets had been tried for this purpose. The test

protein was mixed with acrylamide before polymerization. After polymerization, however, the small pellets were often highly inflammatory. The inflammation could be reduced by extensive washing, but it could never be completely eliminated. Furthermore, washing leached out most of the test protein.

At that time, the only polymer systems reported for administering large molecules were those described by Davis (4), polyacrylamide or polyvinylpyrrolidone. However, these systems damaged the cornea and permitted only brief periods of sustained release (2, 5). Therefore, other polymers and new ways of placing drugs in these polymers were examined. However, one problem was that large molecules would only diffuse through highly porous and permeable membranes (e.g., Millipore filters). In these cases diffusion was too rapid to be of value. A new approach was developed that permitted sustained release of large molecules from biocompatible polymers (2). The polymer was dissolved in an appropriate solvent, and the macromolecule was added in powder form. The resulting mixture can be cast in a mold and dried. When the pellets are placed in water, they release the molecules trapped within the polymer matrix.

A number of polymer systems were tested for tissue biocompatibility and release kinetics. The best long-term release results were obtained with hydrophobic polymers. Examples included non-degradable ethylene–vinyl acetate or biodegradable polylactic acid. Certain hydrogels such as polyhydroxyethylmethacrylate or polyvinylalcohol also worked effectively, but released proteins for shorter time periods. With the hydrophobic polymers, biologically active protein was released for more than 100 days (2). In other tests, larger molecules (2 million MW), such as polysaccharides and polynucleotides, were also successfully released for long time periods (2).

While these initial studies demonstrated the feasibility of releasing macromolecules from biocompatible polymers, the kinetics were often not reproducible; controlled release was not achieved. The irreproducibility results from drug settling and redistribution during casting and drying, caused by the insolubility of the incorporated macromolecule powder in the polymer solvent. At room temperature, the drug migrated vertically, and visible lateral motion was caused by currents (possibly thermal) in the mixture. A low-temperature casting and drying procedure was developed to minimize this drug movement during matrix formation. When the dissolved polymer–solid drug powder mixture was cast in a mold at $-80°C$, the entire matrix froze before any settling could occur. These matrices were then dried at $-20°C$ for 2 days until almost all the solvent was gone. Final drying was conducted under vacuum at room temperature (6).

1. Factors Affecting Release Kinetics

With this reproducible method, factors that regulated release kinetics could now be accurately assessed. Such factors were found to be drug powder particle size and drug loading (drug : polymer ratio) (6). Coating drug-containing polymeric matrices by dropping them into polymer solutions of differing concentrations and drying them, also affected release kinetics. By combining these simple fabrication parameters—drug particle size, loading, and coating—release rates for any drug could be changed several thousandfold (6).

To understand the release mechanism, cryomicrotomy was used to slice 10 μm-thick sections throughout the matrices. Viewed under an optical microscope, polymer films cast without proteins appeared as nonporous sheets. Matrices cast with proteins and sectioned prior to release displayed areas of either polymer or protein. Matrices initially cast with proteins and released to exhaustion (e.g., greater than 5 months) appeared as porous films. Pores with diameters as large as 100 μm, the size of the protein particles, were observed. The structures visualized were also confirmed by Nomarski (differential interference contrast microscopy). It appeared that although pure polymer films were impermeable to macromolecules (2), molecules incorporated in the matrix dissolved once water penetrated the matrix and were then able to diffuse to the surface through pores created as the particles of molecules dissolved. Scanning electron microscopy showed that the pores were interconnected (7).

Next investigated were changes in pore structure over time. Sections were prepared from matrices in the process of release. It was observed that (i) the pore structure changes minimally as a function of time; (ii) after 16–40 h there is no evidence of a receding interface between dissolved and dispersed drug; and (iii) none of the drug remains undissolved at 40 h (30% release). Observations (ii) and (iii) differ from those reported for less soluble low molecular-weight drugs such as certain steroids, and are probably due to the high solubilities of many proteins such as bovine serum albumin (BSA) (solubility > 500 mg/mL). Thus, the widely used moving zone models developed by Higuchi may not be applicable to the situation of macromolecules because of observations (ii) and (iii).

A number of assumptions were made and then verified to develop a model: (i) The rate-limiting step for transport is drug diffusion through pores (other steps such as water penetration into the matrix and drug dissolution occur in less than 40 hours). (ii) The effect of concentration dependence on the drug diffusion coefficient is not significant. This was verified by an analysis of diffusion effects at the concentrations in the

matrix. (iii) No drug diffusion occurs through the polymer backbone (2). (iv) The pores are interconnected, the porosity is uniform, and pore size changes minimally with time. (v) The initial drug distribution is uniform. This was also verified by cryomicrotomy. (vi) No boundary layer effects exist. This was verified by stirring, which would have disrupted boundary layers had they been present. Release rates of matrices stirred in containers at 2000 rpm were identical to unstirred release rates, indicating the lack of boundary layer effect. (vii) Infinite sink conditions exist. The volume of the release medium is approximately 100 times the volume of the polymer/protein matrix. Increasing the volume of the release medium does not alter measured release kinetics. (viii) Minimal effects exist as a result of osmosis due to solutes in the surrounding environment or charge interaction of the drug with the polymer. Consonant with this assumption, no effect on release rate was found to result from increasing the ionic strength of the medium from 0 to 1 M NaCl.

For these assumptions, permitting release from only one side of the slab, the boundary conditions are those of zero flux at the coated edges, and $C = 0$ at the releasing face.

If diffusion through pores occurs, the Fick diffusion equation can be solved:

$$\frac{\partial c}{\partial t} = D_e \frac{\partial^2 c}{\partial x^2}, \qquad 0 < x < L; \tag{1}$$

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[-(2n+1)^2 \pi^2 D_e t / 4L^2\right], \tag{2}$$

where M_t is the cumulative drug mass released, M_∞ is the drug mass originally incorporated in the matrix, t is time (hours), and L is the thickness of the slab (centimeters). In addition, D_e, the effective diffusion coefficient (cm^2/hour) of the drug in the matrix is set equal to $D_0 F$, where D_0 is the bulk diffusion coefficient of the same drug in the release media that has filled the pores, and F is a factor accounting for the geometric effects of the pore structure of the matrix (i.e., tortuosity, dead-end pores, and constrictions between pores). F was determined via a regression analysis for several cases of BSA released from polymer slabs at several porosities (Fig. 1). A log–log plot of F versus porosity was well fitted by the function

$$\log_{10} F = 0.463 + 5.64 \log_{10} \varepsilon, \tag{3}$$

where ε is the porosity. Knowing this equation for F, it can then be

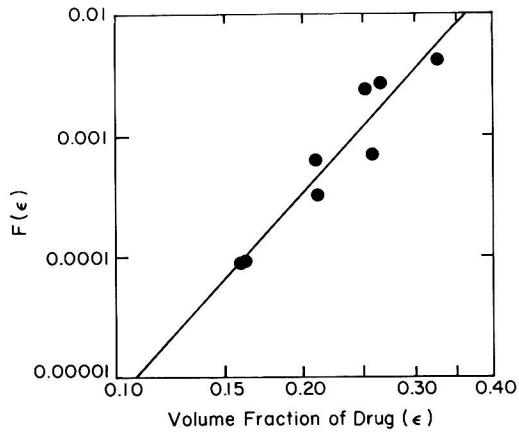

FIG. 1. Log–log plot of factor $F = D_e/D_0$ as a function of porosity for BSA matrices [from Bawa *et al.* (7), with permission of Elsevier Science Publishers BV].

written

$$D_e = D_0(2.904\varepsilon^{5.64}), \tag{4}$$

and this value of D_e can be substituted into Eq. (2).

Both the slab thickness L and the porosity ε were measured. For a given macromolecule, the bulk diffusivity, D_0, is measurable or obtainable from the literature. Thus, a test of the model is to cast slabs using other proteins, measure the parameters L, ε, and D_0, and see whether the release kinetics follow Eq. (2). This has been done for β-lactoglobulin and lysozyme (Fig. 2). The solid lines are predictions based on Eq. (2) which show general agreement with the data (7).

An additional check of the model is to determine if it can predict not only the time-dependent release of the drug, but also the time-dependent position of the drug within the matrix. If Eq. (2) is valid, then the drug distribution within the matrix can be described by

$$c(x,t) = \frac{4C_0}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \exp\left[-(2n+1)^2\pi^2 D_e t/4L^2\right] \cos\frac{(2n+1)\pi x}{2L}, \tag{5}$$

where C_0 is the initial concentration of drug in the matrix (mg/cm^3 matrix), and $C(x,t)$ is the concentration (the concentrations C and C_0 are expressed in terms of the volume of the whole matrix, including both pore and polymer volumes) at time t and distance x (centimeters) into the matrix from the exposed face. To test Eq. (5), cryomicrotomy was used to

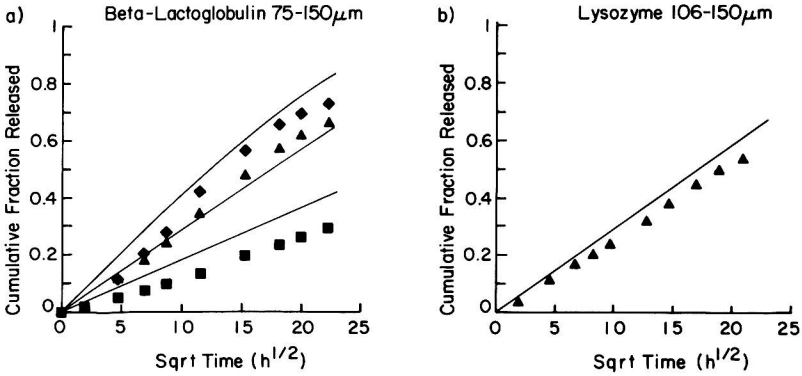

FIG. 2. Release kinetics of β-lactoglobulin and lysozyme [from Bawa *et al.* (7), with permission of Elsevier Science Publishers BV].

determine the drug concentration profiles within the polymer matrix for several cases of loading and release time. Partially released matrices were sectioned at 10 μm intervals, and the remaining protein in each section was assayed and plotted against its normalized (x/L) position within the matrix to yield internal concentration profiles. The data and the predictions from Eq. (5) are within experimental error (7).

The diffusion equations just used are simplifications of more complex processes. The F factor was empirically derived and must take into account those matrix pore geometric factors contributing to decreases in diffusion rates. Such factors may include pore "tortuosity," dead-end pores, and pore constrictions. Initial modeling studies suggest that constrictions, in particular, have large effects in retarding release (8, 9).

2. *In Vivo and in Vitro Release Kinetics and Biocompatibility*

In vitro and *in vivo* release kinetics were compared using two different approaches. In the first approach (the recovery approach) polymer implants containing a radioactively labeled substrate—^{14}C-labeled bovine serum albumin, β-[^{14}C]-lactoglobulin, or [^{3}H]-inulin—were implanted subcutaneously into rates (*in vivo*) or released in phosphate-buffered saline, pH 7.4, at 37°C (*in vitro*). At various time points, the polymer implants were removed from the rats or the saline. They were then lyophilized to remove residual water and dissolved in xylene. When the polymer dissolved, the unreleased macromolecules precipitated to the bottom of the vial. Water was then added to dissolve the macromolecules; scintillation fluid was next added, resulting in a homogeneous translucent emulsion which was counted via liquid scintillation.

Release rates determined in this manner were essentially identical in the *in vivo* and *in vitro* implants. In addition, for the *in vitro* experiments, release was also measured directly by analyzing the radioactivity in the release media. The release rates determined in this way correlated precisely with the *in vitro* and *in vivo* release rates determined by the recovery experiments (last paragraph). Furthermore, they demonstrated that the material balance was completed, showing no material was lost (10).

One limitation of the foregoing approach, however, was that the amount of macromolecules "directly" released *in vivo* could not be assayed. This

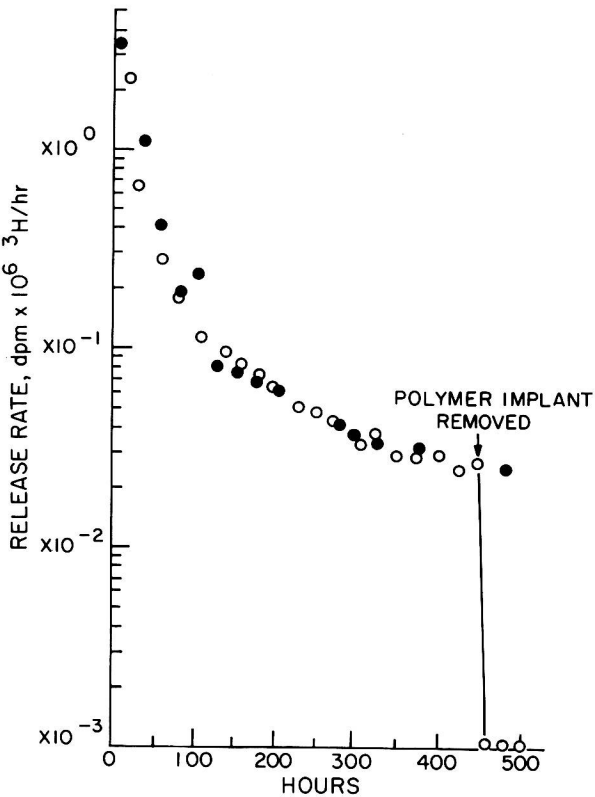

FIG. 3. *In vivo* and *in vitro* comparison of release rates from 44% loaded (w/w) [^{3}H]inulin. Each *in vivo* point (○) represents the average release rate obtained by the collection of urine from five rats. Each *in vitro* point (●) represents the average release rate from four polymer squares in phosphate-buffered saline. The polymer squares were removed from the rats after 450 h [from Brown *et al.* (10), reproduced with permission of the copyright owner, the American Pharmaceutical Association].

is because macromolecules such as proteins are metabolized, making direct *in vivo* release measurements difficult. To solve this problem, [^{3}H]-inulin was used as a model.

Inulin is a polysaccharide of molecular weight 5200. It is one of the very few molecules that is neither metabolized *in vivo* nor reabsorbed or secreted by kidney tubules. Thus, all inulin released from the polymer should be recovered in the urine. An *in vivo–in vitro* comparison was made by making nine identical inulin–polymer pellets. Five pellets were implanted in rats housed in metabolic cages. Four pellets were released into a physiological solution of phosphate-buffered saline (PBS) at pH 7.4 at 37°C. Both urine and PBS were collected daily. The [^{3}H]-inulin was measured by scintillation counting. The experiment was carried out for 500 hours. (Additional experiments have been carried out for 1500 hours with similar results.) Over this period, *in vivo* and *in vitro* release rates agreed to within 1% (10) (Fig. 3).

Furthermore, as an internal control, inulin pellets were removed from several animals at 450 h and the urine analyzed 4.5 h later. Within that time, the inulin recovery rate had dropped by a factor of over 50 (Fig. 3).

The polymer slabs were examined histologically in two different *in vivo* sites at times as long as 7 months after implantation. Nearly no inflammation or fibrous encapsulation was observed (10).

Thus, these experiments show that *in vitro* and *in vivo* release kinetics of macromolecules from ethylene–vinyl acetater copolymer matrices are essentially identical, and they establish a methodology which can be applied to other *in vitro–in vivo* release comparisons.

3. Approaches for Achieving Zero-Order Release

One important goal is the development of a zero-order release device. The difficulty is that the preceding systems contain drug evenly distributed through polymer slabs, and thus, release rates will decrease with time because the drug diffusion distance from the matrix surface increases with time. In order to obtain zero-order release, one could either compensate for the distance-dependent diffusion in a matrix device, or employ a different kind of release system such as a reservoir device (a system in which all drug is centered inside a membrane). Variations of the latter approach have been reported for low molecular-weight drugs in which matrices have been laminated with rate-controlling outer barriers. However, such an approach might prove difficult for macromolecules because the barriers would decrease, if not eliminate, the permeability of these high molecular-weight drugs. Therefore, ways of compensating for the distance-dependent diffusion were developed. The approaches considered