A polymeric film responding in diffusion properties to environmental pH stimuli: a model for a self-regulating drug delivery system

F. ALHAIQUE†, M. MARCHETTI, F. M. RICCIERI*, E. SANTUCCI

*Cattedra di Tecnica Farmaceutica and † Centro di Chimica del Farmaco
Instituto di Chimica Farmaceutica e Tossicologica, Università di Roma, Rome, Italy*

A new polymeric film, ethylene-vinyl-*NN*-diethylglycinate random copolymer, has been studied to evaluate possible applications of such a material to produce controlled release dosage forms. Protonation of the substituted amino groups took place to different extents according to environmental pH conditions. The polymeric film proved to be permeable only to uncharged species, the rate of diffusion of which increased with the increasing charge of the membrane. This effect might be related to strong polymer-proton interactions affecting structure and transport properties of the film and enhancing the solvation process. The charged form of a number of barbituric acid derivatives did not diffuse through the membrane. The uncharged forms permeated the polymeric sheeting at rates consistent with their partition coefficients and which increased with the increasing charge of the polymer.

Synthetic polymeric films are used in pharmaceutical technology to produce controlled release dosage forms which moderate the toxicity and adverse effects of drugs (Rahman et al 1974), to give better drug utilization and consequently a more uniform blood concentration, and to allow the rate and site of drug release to be predicted and the intervals of administration to patient to be extended (Harding 1971). The variety of structures and forms displayed by synthetic polymers, the versatility of their physicochemical properties, and the degree of control that can be exercised over those properties during synthesis, justify the increasing use of such polymers in pharmaceutical technology (Paul & Harris 1976; Robinson 1978).

Much attention has been given to the design of specialized drug delivery systems that are self-regulating in that they respond in conformation and physicochemical properties to chemical stimuli (Henkin & Bradley 1969a; Henkin et al 1969b; Kamo et al 1974; Soeldner 1975; Alhaique et al 1975). The pH range of fluids in various segments of the gastrointestinal tract may provide environmental stimuli that alter the permeation of drugs through a film acting as a barrier to free diffusion, by protonation of basic groups attached to the polymer chain.

We report a preliminary study of the diffusion of ionic and non-ionic compounds at different pH values and from different solvent systems through a new polymeric material, with the aim of evaluating possible applications of such a material to controlled release formulations.

MATERIALS AND METHODS

Materials

The polymeric sheeting, an ethylene-vinyl-*NN*-diethylglycinate copolymer, was obtained from A.N.I.C. S.p.A., S. Donato Milanese, Milan (Pending Italian Patent n. 29235 A/76). It was available in labelled thicknesses of 0·05, 0·1, 0·2 and 0·35 mm. The actual thicknesses were measured to within 0·01 mm at 10 equally spaced sites on five different 4·30 × 4·30 cm square patches by micrometer. The film was a non-porous dense membrane with no added filling, made of a random copolymer with the following general structure:

$$-[CH_2-CH_2]_n-[CH_2-CH]_m$$
$$|$$
$$O-CO-CH_2-N = (C_2H_5)_2$$
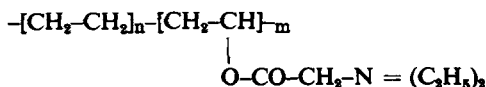
(labelled average molecular weight $= 4·0 \times 10^4$)

A 2·70% nitrogen content indicated that n/m ratio was the same (10·8/1·0) in all patches. Scanning electron photomicrograph of the surface of this membrane showed an amorphous and homogeneous structure. 4'-Aminopropiophenone (PAPP), m.p. 138–141 °C, and benzyl alcohol were purchased from Merck-Schuchardt, Hohenbrunn. The following compounds were supplied by the Carlo Erba Co., Milan: benzoic acid m.p. 122–123 °C, pK_a

---

* Correspondence.

4·19; barbitone, $\epsilon = 10\,310$ (238 nm), pK_a' 7·45; cyclobarbitone, $\epsilon = 9\,880$ (238 nm), pK_a' 7·27; diallylbarbituric acid, $\epsilon = 9\,690$ (238 nm), pK_a' 7·30; phenobarbitone, $\epsilon = 10\,880$ (238 nm), pK_a' 7·40; secbutobarbitone, $\epsilon = 10\,410$ (238 nm), pK_a' 7·63; pentobarbitone, ϵ 11 070 (238 nm), pK_a' 7·65. The molar absorptivities, ϵ, are given for the designated wavelengths at which the absorbance was measured. The peanut oil was U.S.P. All compounds were purified by repeated crystallization or distillation. Other reagents were of analytical grade.

Analytical methods
Spectrophotometric measurements of absorbance at $25 \pm 1{\cdot}0\,°C$ were used for the quantitative estimation of barbituric acid derivatives, benzoic acid, PAPP and benzyl alcohol in a Perkin Elmer-Hitachi 200 spectrophotometer using 10 mm quartz cells and 0·1 mm slit width at the pertinent wavelengths. As molar absorptivities of the charged form are higher than those of the uncharged ones, the absorbances of barbituric acids were measured in pH 10·1 borate buffer. The linear relationship between absorbance and concentration was verified in all cases. Conductivity measurements were carried out by means of a WTW model LF3 bridge in a thermostatic bath at $25 \pm 0{\cdot}05\,°C$ (Lorimer et al 1956).

Partition coefficients
Aqueous pH 4·7 acetate buffer and chloroform were saturated with respect to each other. 1·0 ml of an approximately 5×10^{-4} M solution of the barbituric acid derivatives in the acetate buffer was diluted with 9·0 ml of pH 10·1 borate buffer. The absorbance of these solutions was measured using appropriate blanks. The partition coefficients of the drugs in the chloroform–acetate buffer system were determined according to Garrett & Chemburkar (1968b).

Diffusion studies
The diffusion cell used in permeation experiments was previously described by Alhaique et al (1972, 1977). The area available for the diffusion was 5·30 cm² and each compartment occupied about 8·5 ml. The cell was initially equilibrated for 48 h at $25 \pm 0{\cdot}1\,°C$ with appropriate buffers and no added permeating species. After equilibration, the solutions were replaced by a test solution containing the permeating species in one compartment and a fresh buffer solution in the other, both previously warmed to the same temperature. Stirring was maintained throughout the experiment and samples were taken from the receptor compartment at set

times and the volume made up with buffer at the same temperature. The volume and pH of the solutions in the diffusion cell did not show significant changes at the end of any experiment. Sampling in the desorbing solution did not yield a precipitate or a coloured solution in the presence of ammonium molybdate reagent, when phosphate buffer was used in one of the compartments of the diffusion cell. Thus, phosphate buffer salts do not diffuse significantly through the membrane over the period of time required for measurements. Spectrophotometric determinations were sensitive to 10^{-5} M concentration in all cases. The membrane was considered to be impermeable to a given diffusing species when that species was not detected in the receptor compartment at that sensitivity after 72 h. Elemental and i.r. analysis of the membrane did not show any significant variation after its use in diffusion experiments. Thus, it can be assumed that no hydrolysis of the ester group of the polymer occurred.

Solubility studies
Saturated solutions of both PAPP and benzyl alcohol were prepared in a pH 6·8 phosphate buffer and equilibrated at $25 \pm 0{\cdot}1\,°C$ in a thermostatic bath for 48 h. The same procedure was used to prepare saturated solutions containing 10, 20, 30, and 40% ethanol by volume. The solutions were filtered or centrifuged accordingly, appropriately diluted with pH 6·8 phosphate buffer, and spectrophotometrically analysed against a blank.

RESULTS AND DISCUSSION
The effect of pH on the apparent diffusion constant (Lueck et al 1957; Nogami et al 1970) of an uncharged species, i.e., benzyl alcohol, through the substituted polyethylene membrane in quasi-steady state conditions is illustrated in Fig. 1. The plot shows a sharp discontinuity indicating a non-linear decrease of the apparent diffusion constant as pH increased in the bathing solutions. This suggests a positive effect of hydrogen ion concentration on membrane permeability to an uncharged species. Anomalies in the shape of the permeation curve at different pH values can be explained by the presence of basic residues in the polymer chain.

The i.r. spectrum of the membrane after immersion for 24 h in a 10^{-2} M HCl solution, showed a large absorption band at 2600–2700 cm⁻¹, which corresponds to the protonated form of the polyelectrolyte. This absorption band could not be detected when the membrane was equilibrated in a HCl solution at pH 3·5 or above, even over 9 days.
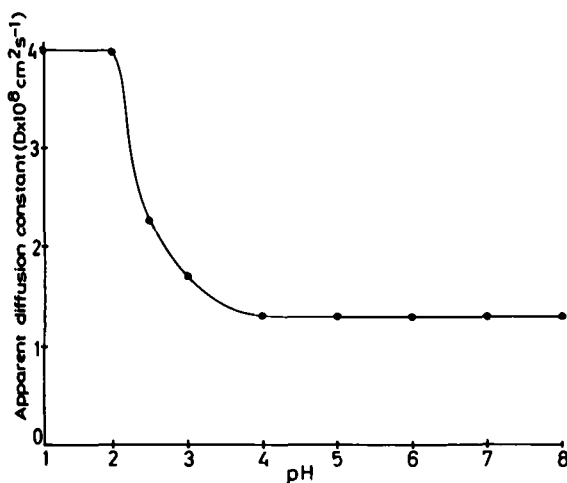
FIG. 1. Apparent diffusion constants (D) for 10^{-2} M benzyl alcohol through a 0·1 mm membrane at 25 ± 0·1 °C are plotted versus the pH of desorbing and diffusing solutions. D was calculated according to Nogami et al (1970).

FIG. 2. Specific conductivity plotted against time for membranes of different thickness (X) at 25·0 ± 0·05 °C. Bathing solutions in both compartments of the conductivity cell were 10^{-2} or 10^{-3} M HCl.

Conductivity measurements, carried out in different experimental conditions, indicated that HCl diffused through the polymeric film at a rate consistent with membrane thickness, providing that the pH values of the bathing solutions were below pH 3—a critical threshold (Fig. 2). Thin membranes exhibited a lower degree of permselectivity than thicker ones because defects in the form of large permeation sites have a high probability of completely penetrating a thin structure (Statton & Geil 1960).

I.r. spectra and conductivity measurements suggested that membranes with measured thickness 0·35 and 0·1 mm were protonated when the HCl concentration in the bathing solutions was not lower than 10^{-3} M. Conversely, these membranes could be assumed impermeable to HCl over the period of time required for our measurements (72 h) when pH of the bathing solution was above 3.0.

The sharp discontinuity in membrane permeability that followed proton-polymer interactions, as shown in Fig. 1, may be ascribed to different degrees of polymer protonation arising from changes in the environmental pH conditions. The plot in Fig. 1 can be interpreted as the titration curve of the basic residues in the polymeric chain. In this sense, it was assumed that the pK_a of the polyelectrolyte fell within the pH interval corresponding to the sharp change in membrane permeability. The increasing permeability exhibited by the membrane at pH values below the assumed pK_a is most
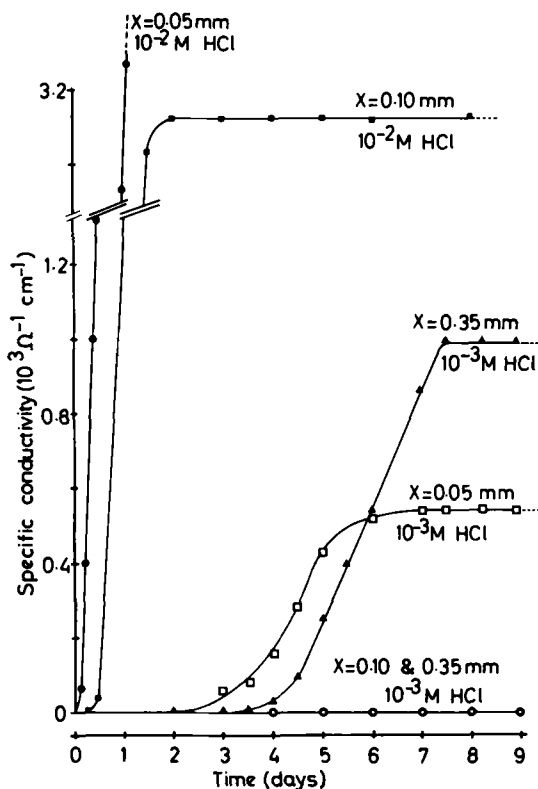
probably accompanied by structural transitions of the polymer substrate, which enhances the solvation process (Michaels et al 1962).

The diffusion rate of an ionizable species, i.e., benzoic acid, from a pH 2·0 solution, through various thicknesses of polymeric membrane can be obtained from quasi-steady state diffusion plots of the concentration of phosphate buffered desorbing solutions (pH 8·0) versus time (Fig. 3). When the ratio of the diffusion constant to the thickness is plotted against the reciprocal of the thickness, a linear plot is obtained (Fig. 3, inset), the slope of which represents the apparent diffusion constant of undissociated benzoic acid through the membrane (Garrett & Chemburkar 1968a). The permeation of benzoic acid through a 0·1 mm thick membrane into a pH 8·0 phosphate buffer from solutions at various pH values with constant concentrations (Fig. 4, I), takes place with an apparent diffusion constant that decreases as pH increases in the diffusing compartment, and approaches zero for pH values above 8·0. As the estimated apparent diffusitivities of benzoic
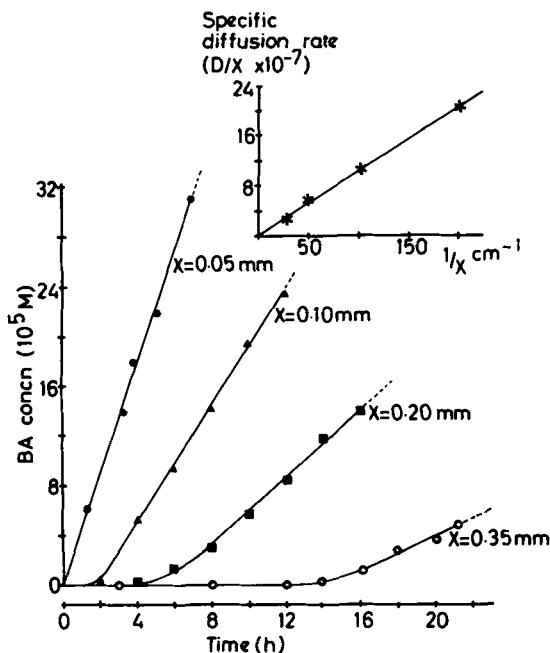
FIG. 3. Effect of membrane thickness (X) on the diffusion of 10^{-3} M benzoic acid from pH 2·0 (HCl) diffusing solution into pH 8·0 (phosphate buffer) desorbing solution at 25 ± 0·1 °C. Inset. Specific diffusion rates (D/X) of 10^{-2} M benzoic acid (BA) in the same experimental conditions against the reciprocal of membrane thickness (1/X).

acid decrease with increasing ionization, it may be concluded that this membrane acts as a lipid-like barrier permeable only to uncharged species. Thus, sink conditions can be assumed for appropriate pH values in the desorbing compartment of the cell. When PAPP was used as a diffusing species (Fig. 4, II), a similar effect was observed consistent with the pK_a of that compound. The increase in permeability of an uncharged species through a charged membrane denotes that permeants are transferred by mechanisms other than solubility or partitioning with the polymer.

In an attempt to differentiate the effect of the charge of the membrane from that arising from ionization of the permeant, the diffusion of benzoic acid from peanut oil solution into aqueous solutions buffered at different pH values was examined. Results in Fig. 4, III, show that the diffusion rate of un-dissociated benzoic acid sharply decreased when the pH of the desorbing solution was above the pK_a of the polymer. Thus, the degree of association of an uncharged species to the membrane changes according to the charge of the polymer and it is at
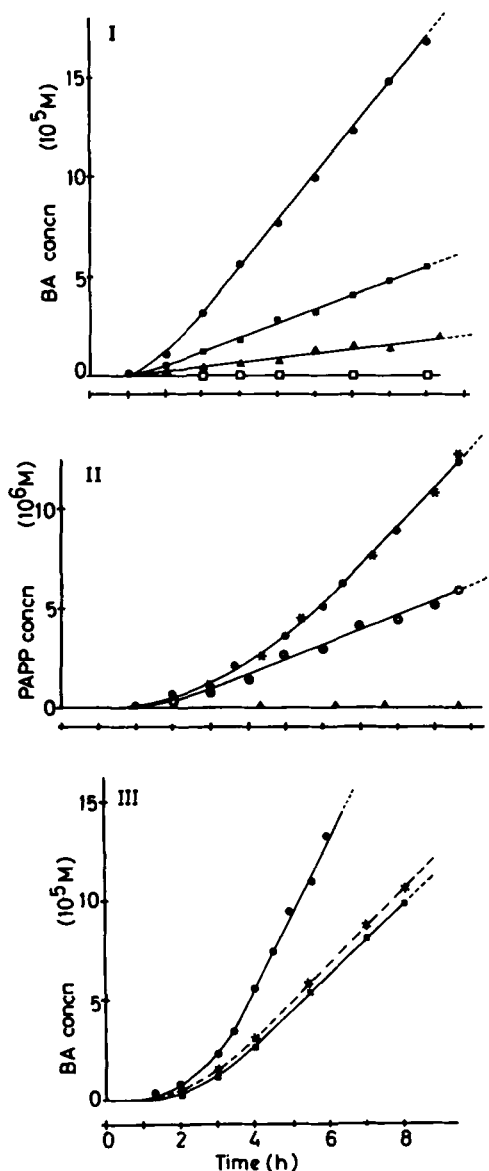

FIG. 4. Effect of pH on the permeation of benzoic acid (BA) and 4'-aminopropiophenone (PAPP) through a 0·1 mm membrane at 25 ± 0·1 °C in different experimental conditions. The absorbance of the desorbing solution at 230 nm (BA) and 307 nm (PAPP) was measured at set times against a blank. The corresponding concentrations were determined from a calibration curve obtained in the same experimental conditions. (I). Effect of pH on the permeation of 10^{-2} M BA diffusing into pH 8·0 (phosphate buffer) desorbing solution. ● pH 2·0, ■ pH 4·2, ▲ pH 6·7, □ pH 8·0. (II). Effect of pH on the permeation of 5×10^{-3} M PAPP diffusing into PH 0 (HCl) desorbing solution. ★ pH 7·0, ● pH 5·0, ○ pH 1·0, ▲ pH 0. (III). Effect of pH on the permeation of 10^{-2} M BA peanut oil solution diffusing into an aqueous solution buffered at different pH values. ● pH 3·0, ★ pH 4·2, ■ pH 8·0.

its maximum for the protonated form of the macro-molecule. In the same way, the charged forms of a number of barbituric acid derivatives do not permeate the membrane. On the other hand, the uncharged forms diffuse at rates which show a linear relation with the i.r. partition coefficients (Garrett & Chemburkar 1968b; Kaneniwa et al 1979) and are consistent with the increase in permeability that a charged membrane always displays (Fig. 5).
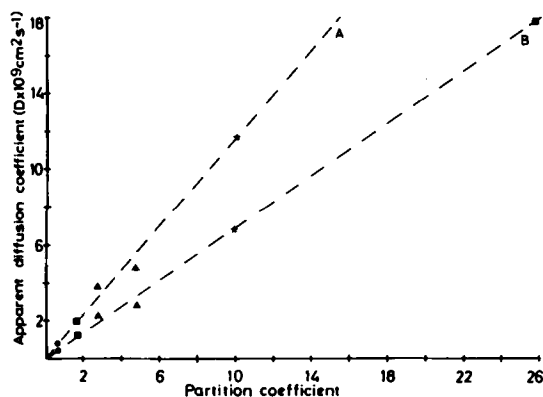

FIG. 5. Apparent diffusion constants (D) of several barbituric acid derivatives (2×10^{-3} M) diffusing through a 0·1 mm membrane at $25 \pm 0·1$ °C as a function of their partition coefficient between pH 4·7 acetate buffer and chloroform. Diffusing solutions were buffered at pH 4·7 (acetate buffer) while desorbing solutions were buffered at pH 2·0 (HCl, plot A) and pH 10·1 (borate buffer, plot B) respectively. ● barbitone; ■ cyclobarbitone; △ diallylbarbituric acid; ▲ phenobarbitone; ★ secbutobarbitone; □ pentobarbitone.

When a cosolvent, like ethanol, is added to both solutions bathing the membrane, a decrease in the diffusion rate is predictable following an increase in solubility of the permeants and a parallel decrease of their partitioning to the membrane (Rodel et al 1966; Garrett & Chemburkar 1968a). Substituted polyethylene membranes pretreated with ethanol-water mixtures are permeated by benzyl alcohol at a rate that increases with increasing ethanol concentration of the bathing solutions (Fig. 6). This indicates that the diffusing properties of the membrane are altered by ethanol. Furthermore, after exhaustive washing with water, the resultant membranes exhibited the same permeability as before ethanol treatment. If it is assumed that strong interactions between ethanol and the membrane occur, the solubility of the permeant within the membrane is modified by solvent molecules that are bound or otherwise restricted within the polymer.
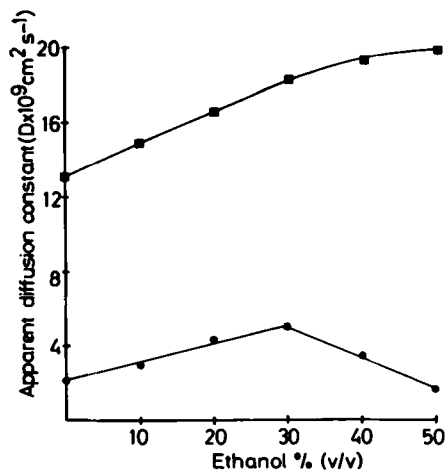

FIG. 6. Diffusion through a 0·1 mm membrane at $25 \pm 0·1$ °C of 10^{-2} M benzyl alcohol (■) and 5×10^{-3} M PAPP (●), from various percentages (v/v) of ethanol–water phosphate buffer (pH 6·8, aqueous) mixtures into desorbing solutions of the same ethanol–water phosphate buffer composition. Apparent diffusion constants (D) are plotted as a function of ethanol percentage (v/v) in the mixture.

When PAPP was used as a permeating species, a similar effect was observed up to 30% of ethanol concentration. A further addition of ethanol caused a sharp decline in permeability and PAPP diffused through the membrane at a rate that decreased with increasing ethanol percentage (Fig. 6). This effect can be explained by comparing the solubility of both benzyl alcohol and PAPP in different water–ethanol mixtures. Table 1 shows that PAPP solubility increases over ten fold if compared with that of benzyl alcohol in the presence of the same percentage of ethanol. These data allow us to conclude that the effect of ethanol on the permeability properties of the membrane is balanced or overcome by the significant increase in solubility of PAPP in water–ethanol mixtures.

Table 1. Effect of different percentages (v/v) of ethanol-phosphate buffer (pH 6·8, aqueous) mixtures on the solubility of PAPP and benzyl alcohol at $25 \pm 0·1$ °C.

Percentage of ethanol (v/v)	Solubility ratio $(Cm/Cw)^1$ PAPP	Solubility ratio $(Cm/Cw)^1$ Benzyl alcohol
0	1·0	1·0
10	2·2	1·2
20	4·4	1·4
30	7·9	1·9
40	16·0	2·3

[1] Cm and Cw are the solubilities in ethanol–water mixtures and water respectively.

The present investigation of the permeability of a substituted polyethylene membrane, and the effect on the diffusion rate of environmental pH, emphasize that conformational transitions occurring in a macromolecule represent a general approach to self-regulating drug delivery systems and that changes in the mechanical properties of the polymer must be taken into account.

REFERENCES

Alhaique, F., Marchetti, M., Riccieri, F. M., Santucci, E. (1972) Il Farmaco Ed. Sci. 27: 145–155

Alhaique, F., Giacchetti, D., Marchetti, M., Riccieri, F. M. (1975) J. Pharm. Pharmacol. 27: 811–817

Alhaique, F., Giacchetti, D., Marchetti, M., Riccieri, F. M. (1977) Ibid. 29: 401–406

Garrett, E. R., Chemburkar, P. B. (1968a) J. Pharm. Sci. 57: 949–959

Garrett, E. R., Chemburkar, P. B. (1968b) Ibid. 57: 1401–1409

Harding, N. G. L. (1971) Ann. N.Y. Acad. Sci. 186: 270–283

Henkin, R. I., Bradley, D. F. (1969a) Proc. Nat. Acad. Sci. U.S. 62: 30–37

Henkin, R. I., Graziadei, P. P. G., Bradley, D. F. (1969b) Ann. Int. Med. 71: 791–821

Kamo, N., Miyake, M., Kurihara, K., Kobatake, Y. (1974) Biochim. Biophys. Acta 367: 1–11

Kaneniwa, N., Hiura, M., Nakayawa, S. (1979) Chem. Pharm. Bull. 27: 1501–1509

Lorimer, J. W., Boterenbrood, E. I., Hermans, J. J. (1956) Discussion Faraday Soc. 21: 141–149

Lueck, L. M., Wurster, D. E., Higuchi, T., Lemberger, A. P., Busse, L. W. (1957) J. Am. Pharm. Ass. 46: 694–698

Michaels, A. S., Baddour, R. F., Bixler, H. J., Choo, C. Y. (1962) Ind. Eng. Chem. Process Design Develop 1: 14–25

Nogami, H., Nagai, T., Sonobe, T. (1970) Chem. Pharm. Bull. 18: 2101–2111

Paul, D. R., Harris, F. W. (1976) American Chemical Society Symposium Series 33, Controlled Release Polymeric Formulations. American Chemical Society, Washington D.C.

Rahman, Y. E., Cerny, E. A., Tollaksen, S. L., Wright, B. J., Nance, S. L., Thomson, J. F. (1974) Proc. Soc. Exp. Biol. Med. 146: 1173–1176

Robinson, R. J. (1978) Drugs and the Pharmaceutical Sciences, Vol 6 Sustained and Controlled Release Drug Delivery Systems. Marcel Dekker Inc. New York

Rodell, M. B., Guess, W. L., Autian, J. (1966) J. Pharm. Sci. 55: 1429–1434

Soeldner, J. S. (1975) in: Urquhart, J., Yates, F. E. (eds) Temporal Aspects of Therapeutics. Plenum Press, New York

Statton, W. O., Geil, P. H. (1960) J. Appl. Polymer Sci. 3: 357–361