Controlled Release of Tetracycline I: *In Vitro* Studies with a Trilaminate 2-Hydroxyethyl Methacrylate–Methyl Methacrylate System

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Abstract □ A membrane-controlled drug delivery device was developed to release tetracycline at zero-order rates. The tetracycline delivery vehicle is a trilaminate disk consisting of core and coating membranes fabricated from a series of 2-hydroxyethyl methacrylate and methyl methacrylate copolymers. Appropriate adjustment of the monomer composition ratio imparts a hydrophobic nature to the copolymer outer coating membrane (relative to the core material), which serves as the rate-limiting membrane in drug diffusion. The trilaminate disks demonstrated a zero-order tetracycline release over 4 months *in vitro*. The zero-order release rate was a function of the general device geometry, coating membrane thickness, disk surface area, level of core reservoir drug loading, and membrane coating copolymer composition. Permeability parameters of tetracycline diffusion through a series of 2-hydroxyethyl methacrylate–methyl methacrylate copolymer membranes were determined by a flux–lag time method. Equilibrium hydration values of these membranes also were determined. The ability of trilaminate 2-hydroxyethyl methacrylate–methyl methacrylate devices to release tetracycline at constant rates over a prolonged period offers unique therapeutic and investigational possibilities.

Keyphrases □ Tetracycline—controlled-release dosage form, pharmacokinetics, hydroxyethyl methacrylate–methyl methacrylate copolymer, *in vitro* □ Antibacterial agents—tetracycline, controlled-release dosage form, pharmacokinetics, hydroxyethyl methacrylate–methyl methacrylate copolymer, *in vitro* □ Models, pharmacokinetic—tetracycline, controlled-release dosage form, *in vitro* □ Dosage forms, controlled release—tetracycline, hydroxyethyl methacrylate–methyl methacrylate copolymer, *in vitro*

Numerous drug–polymer delivery systems have been proposed with the expressed purpose of releasing a biologically active agent into the surrounding medium at a constant (zero-order) release rate. Attempts to achieve zero-order release rates have included infusion pumps (1), erodable matrixes (2), controlled geometries (3), and various membrane-enclosed reservoir devices (4–6). Unfortunately, many of these systems fall far short of their goal, suffering from prolonged burst effects, device breakdown, and constraints in design, fabrication, or material properties. The result is usually an exponential release pattern with time and a relatively limited period of constant release.

Previous experience with a hydrogel matrix–drug delivery system (7) led to the investigation of a membrane-controlled drug delivery device in which a hydrophilic polymer core is supersaturated with a given pharmaceutical. The core–drug mixture is coated with a drug-free polymer layer which, by its hydrophobic nature (relative to the core material), functions as the rate-limiting membrane in drug diffusion from the device. Membrane-enclosed drug delivery systems maintain a constant activity source of the permeating drug at the interface between the core and membrane-coating material. The result is a constant drug release rate that is proportional to the concentration gradient established over the diffusion rate-limiting outer membrane.

The described membrane-controlled drug delivery system is a trilaminate device consisting of core and outer membranes fabricated from a series of hydroxyethyl methacrylate and methyl methacrylate copolymers. Yasuda *et al.* (8, 9) reported on the permeability properties of sodium chloride and a number of water-soluble organic solutes through 2-hydroxyethyl methacrylate–methyl methacrylate copolymers of varying composition. Diffusive permeabilities were related to the equilibrium water content of the copolymer films, which increased as the 2-hydroxyethyl methacrylate content was increased. Thus, by varying the relative percentage of the hydrophilic and hydrophobic monomer components (2-hydroxyethyl methacrylate and methyl methacrylate, respectively) of a copolymer, the diffusivity of a given drug through the copolymer coating membrane can be varied over a sizable range. In addition, the drug release rate from a trilaminate delivery vehicle can be controlled by altering the coating membrane thickness and/or the device geometry. The control of diffusion properties in a similar trilaminate delivery device for the constant release of sodium fluoride was described previously (10).

Tetracycline, a middle molecular weight bacteriostatic antibiotic, was chosen as the diffusant species to be incorporated within the delivery device core. Previous (11, 12) controlled-release tetracycline preparations incorporated the drug in a cross-linked hydrogel matrix or in an erodable complex of polyvinylpyrrolidine, shellac, and a low solubility organic acid.

Tetracycline in an implantable controlled delivery form with long-term constant release rates offers numerous therapeutic and investigational applications. The purposes of this report are to evaluate the *in vitro* performance of the trilaminate device and to describe the effects of copolymer composition and device geometry on tetracycline permeability through various 2-hydroxyethyl methacrylate–methyl methacrylate copolymers.

EXPERIMENTAL

Copolymer Synthesis—Random copolymers of 2-hydroxyethyl methacrylate[1] and methyl methacrylate[2] were prepared by mixing varying molar weight percentages of the monomers (total of 25 g) in 475 ml of a 3:2 ethanol–water mixture. The reaction mixture was purged of oxygen by bubbling with nitrogen, and polymerization was initiated by the addition of 0.125 g of potassium persulfate and 0.25 g of sodium persulfate/liter of reaction mixture.

Ten days was allowed to carry the copolymerization reaction to completion, after which the copolymer was precipitated in an excess of water. After separation by filtration, the resultant copolymer was subjected to multiple washings with water and dried at 50° *in vacuo*. Reaction yields

[1] Hydron Labs, New Brunswick, N.J.
[2] Eastman Kodak, Rochester, N.Y.

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Table I—Polymer Composition and Diffusional Properties for 2-Hydroxyethyl Methacrylate–Methyl Methacrylate Copolymers

Feed Composition, mole ratio[a]	Copolymer Composition[a,b], mole ratio	Diffusion[c] Coefficient, D, $cm^2/sec \times 10^{-9}$	Partition[c] Coefficient, K_p, $\times 10^{-3}$
10:90	2:98	8.0 ± 4.7	6.8 ± 5.9
20:80	14:86	12.0 ± 2.1	16.4 ± 6.6
32:68	22:78	25.1 ± 11.0	30.7 ± 16.8
75:25	63:37	43.2 ± 8.9	126.7 ± 35.9

[a] 2-Hydroxyethyl methacrylate–methyl methacrylate. [b] As determined by NMR analysis. [c] Mean values ± SD.

were 72–95%. The 2-hydroxyethyl methacrylate–methyl methacrylate copolymers were prepared in the following molar feed ratios: 10:90, 20:80, 32:68, and 75:25.

Chemicals—[7-^{3}H]Tetracycline[3] was mixed with unlabeled tetracycline[4] to the desired specific activity by dissolving both drug forms in methanol (~100 mg of mixture/5 ml of solvent). The solvent was evaporated slowly at a temperature not exceeding 42° until a constant crystalline tetracycline weight was obtained. The drug mixture was stored at −4° until needed.

Copolymer Composition—Determination of the respective copolymer composition was performed by NMR analysis[5]. Copolymer specimens were analyzed in deuterochloroform–methanol with increasing amounts of d-4-methanol for copolymers of higher 2-hydroxyethyl methacrylate content. To determine the molar composition of the individual components, the area under the peak due to the methyl methacrylate methyl ester was compared to the area under the combined peaks attributable to the α-methyl hydrogens (representing total methacrylate content).

Tetracycline–Copolymer Device Fabrication—Disk-shaped trilaminate tetracycline devices were prepared by the following process. Copolymer material was dissolved in a cosolvent of 3:2 (v/v) acetone–dioxane (0.5 g/5 ml of solvent). Films were cast by pouring the copolymer solution onto a clean glass plate and adjusting the wet film thickness with a Gardner knife. The film was covered and allowed to dry for 15–30 min under ambient conditions. Subsequent films were cast and allowed to dry until the desired outer membrane-coating thickness was obtained.

After the total bottom coating film was cast, the core material was constructed by casting additional individual films of a copolymer–tetracycline mixture (either a solution or suspension, depending on the percent drug loading) over the coating membrane. When the core layer was complete, the top coating membrane was film cast in individual layers directly over the core layer. The completed trilaminate film was dried overnight in a 37° oven. The film was slightly swollen with an aerosol application of 95% ethanol, and disks were punched out with a cork borer. Residual alcohol was allowed to evaporate, and the disks were edge coated by brushing on two or three layers of the coating copolymer solution. The completed disks were stored in a desiccant jar at 4°.

Permeability Parameters and Hydration Studies—Drug-free copolymer films were cast as described for use in diffusion characterization and hydration studies. Permeability parameters were determined with a diffusion cell apparatus described in detail elsewhere (13). The permeation studies were designed to measure the steady-state flux of the penetrant molecule ([^{3}H]tetracycline) through the copolymer membrane according to the following principle:

$$Q = \frac{K_p D C_0}{l}\left(t - \frac{l^2}{6D}\right) \qquad \text{(Eq. 1)}$$

where:

Q = total amount of permeant species diffused through a unit area of membrane as a function of time
K_p = membrane–diffusant system partition coefficient
D = diffusion coefficient
C_0 = permeant concentration in reservoir solution
l = membrane thickness
t = time

Measurements were made on the cumulative diffusive transfer of tetracycline from the saturated reservoir solution through the copolymer film and into the effluent stream on the flow side of the diffusion cell. Cumulative drug transfer was plotted against time to determine the steady-state flux, Q/t, equal to the slope and the lag time, t_L, equal to the time intercept on the abscissa. Slope and intercept values were determined by linear regression techniques.

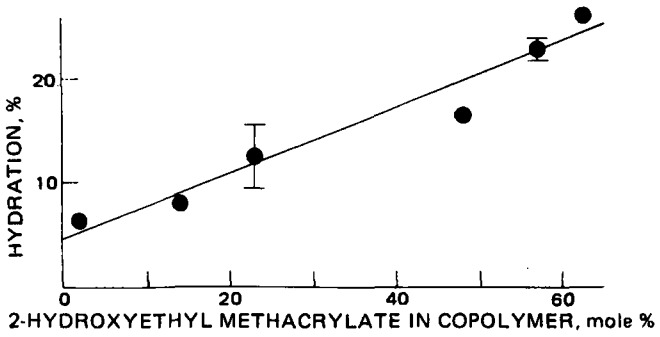

Figure 1—*Variation in percent hydration with copolymer composition (mean values ± SD). Copolymer membrane samples swelled in distilled water.*

By rearranging Eq. 1 and applying it to the region of steady-state mass transfer, the permeability parameters may be calculated:

$$K_p D = Ql/tC_0 \qquad \text{(Eq. 2)}$$

where:

$$D = l^2/6t_L \qquad \text{(Eq. 3)}$$

Hydration studies were performed by swelling films of predetermined dry weights in both Ringer's solution and distilled water at 37°. The wet films were quickly blotted to remove excess surface water and weighed. Percent hydration was expressed as:

$$\%\ \text{hydration} = (\text{wet weight} - \text{dry weight})/\text{wet weight} \qquad \text{(Eq. 4)}$$

***In Vitro* Diffusion Studies**—*In vitro* [^{3}H]tetracycline diffusion was followed by placing the trilaminate disks on a cellulose Millipore filter (0.65-μm pore diameter) sealed with acetone to the ground flat top of a 25-ml erlenmeyer flask with a glass side arm for sampling. The flask was filled with Ringer's solution, which served as the desorbing medium. A glass cap was fitted over the polymer disk and filter to provide a constant temperature and humidity environment. The diffusion flasks were maintained at 37°. At sampling, 1-ml aliquots were removed from the flask through the side arm. Volume was replaced by the addition of fresh Ringer's solution to keep the fluid level continuous with the bottom of the filter. All *in vitro* diffusion experiments were run in triplicate.

Tetracycline diffusion was measured in the permeation studies and *in vitro* diffusion experiments by analyzing for [^{3}H]tetracycline. One milliliter of the aqueous [^{3}H]tetracycline sample was added to 5 ml of a 1:1 nonionic surfactant[6]–toluene scintillation fluor (0.14 g of p-bis[2-(5-phenyloxazolyl)]benzene[7] and 5.34 g of 2,5-diphenyloxazole[7]/liter). The samples were counted in glass vials[8].

In vitro diffusion flask samples were obtained at the termination of the procedure and analyzed for pure tetracycline content and tetracycline breakdown products by a previously reported UV spectroscopic technique (14). The percent pure tetracycline content was calculated from the absorbance ratio of the solution read at 357 and 391 nm (Q:357:391) according to:

$$\%\ \text{tetracycline} = \frac{Q{:}357{:}391 - 0.23}{0.0367} \qquad \text{(Eq. 5)}$$

The pure tetracycline level in the diffusion samples (after 28 days) was 81.3% (original unlabeled drug = 100%).

RESULTS AND DISCUSSION

NMR Analysis—For the series of 2-hydroxyethyl methacrylate–methyl methacrylate copolymer membranes (Table I), NMR studies revealed that the actual mole percentage of methyl methacrylate in the polymer was higher (by 6–12%) than in the original feed. This result was

[3] New England Nuclear, Boston, Mass.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Dr. Donald Gregonis, Department of Pharmaceutics, University of Utah, Salt Lake City, Utah, personal communication.
[6] Triton X-100, Research Products International Corp., Elk Grove Village, Ill.
[7] Research Products International Corp., Elk Grove Village, Ill.
[8] Isocap liquid scintillation counter, Searle Analytic, Des Plaines, Ill.

Table II—2-Hydroxyethyl Methacrylate–Methyl Methacrylate Trilaminate Tetracycline Disks: *In Vitro* Diffusion Results

Disk Composition[a]					
Coating Co-polymer	Core Co-polymer	Surface Area, cm^2	Core Loading, mg of drug/ mg of core	Coating Thickness, cm × 10^{-2}	Steady-State Release Rate, μg of tetracycline/day
2:98	63:37	0.709	0.02	1.40	0.54
2:98	63:37	0.709	0.02	0.81	0.77
2:98	63:37	1.33	0.02	1.47	0.97
2:98	63:37	0.709	0.2	0.54	18.5
2:98	63:37	0.950	0.2	0.53	23.9
22:78	63:37	0.709	0.2	0.59	28.9

[a] 2-Hydroxyethyl methacrylate–methyl methacrylate.

due to the higher reactivity ratio of the methyl methacrylate monomer with respect to the 2-hydroxyethyl methacrylate monomer. The result of such a difference in reactivity ratios would lead to a skewed distribution of random copolymers in a particular polymerization batch.

Copolymer chains polymerizing during the initiation of the synthesis process would be expected to contain larger proportions of the more reactive methyl methacrylate monomer, depleting the methyl methacrylate monomer pool. Those copolymers synthesized later in the reaction would have increasing proportions of the remaining 2-hydroxyethyl methacrylate monomer in the chain. Multiple washings with water during the preparative procedure would lead to a greater relative loss of those copolymers with higher 2-hydroxyethyl methacrylate content (of associated higher water solubilities), effectively fractionating the copolymer batch and producing a copolymer whose final composition contains a higher mole percentage of the methyl methacrylate monomer than is reflected in the original monomer feed.

Hydration Studies—As expected, copolymer samples of increasing mole percent 2-hydroxyethyl methacrylate composition were more hydrophilic in nature, demonstrating greater degrees of equilibrium swelling (Fig. 1). There was no significant difference in hydration values obtained when the copolymer samples were swelled in distilled water or in Ringer's solution. In the 2-hydroxyethyl methacrylate composition range below 50 mole %, there was good general agreement in equilibrium swelling values between these samples and similar experimental 2-hydroxyethyl methacrylate–methyl methacrylate copolymers described by Cowsar *et al.* (10).

For samples of >50 mole % 2-hydroxyethyl methacrylate content, Cowsar *et al.* (10) reported no further increase in equilibrium water content, whereas the copolymers in the present study continued to increase in percent hydration with increased 2-hydroxyethyl methacrylate content. Cowsar *et al.* (10) failed to provide confirmation of their assumption that the final composition of their experimental copolymers was equal to the molar ratio of monomers in the original feed. Falsely high estimates of 2-hydroxyethyl methacrylate content in samples reported to be of 50 mole % 2-hydroxyethyl methacrylate or greater would explain the observed discrepancy in hydration values.

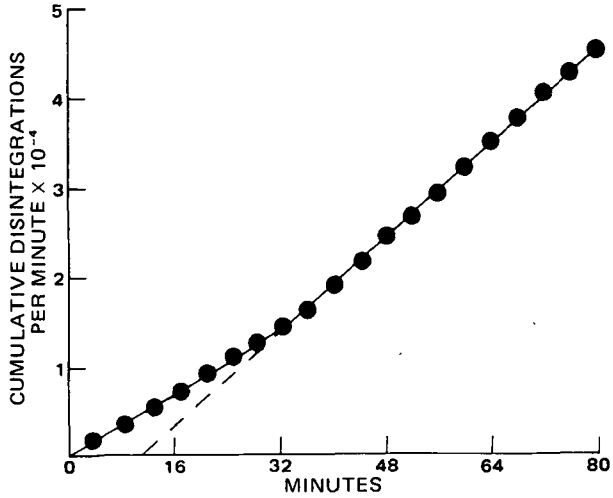

Figure 2—*Lag time method for determination of steady-state flux and diffusion coefficient of a 63:37 2-hydroxyethyl methacrylate–methyl methacrylate copolymer membrane. Flux (Q) is expressed as cumulative disintegrations per minute of labeled drug permeating through the membrane sample per unit time during steady-state period of diffusion. Lag time value (t_L) was taken at the intercept of the steady-state flux line (dashed line) with the time axis.*

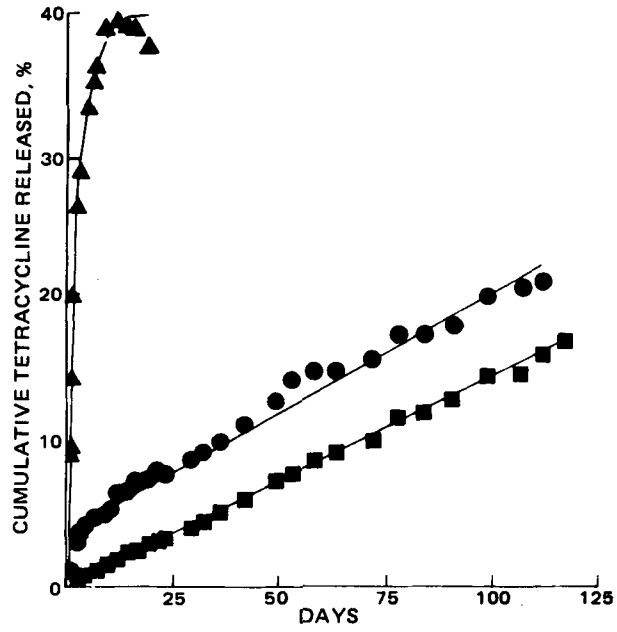

Figure 3—*Zero-order tetracycline release rates maintained by trilaminate membrane device over 4 months of testing* in vitro, *showing effect of copolymer coating composition on constant release rate. Key:* ■, *2:98 2-hydroxyethyl methacrylate–methyl methacrylate coating polymer;* ●, *14:86 2-hydroxyethyl methacrylate–methyl methacrylate coating copolymer; and* ▲, *rapid tetracycline release from 63:37 2-hydroxyethyl methacrylate–methyl methacrylate core material alone. Total average loading of trilaminate device equals 0.155 mg of tetracycline.*

Trilaminate disks, constructed of a core of 63:37 copolymer and coated with an equal total thickness of 22:78 copolymer, achieved 90% of their equilibrium water content after ~3 hr of swelling in Ringer's solution. In general, all copolymers exhibited rapid hydration, and equilibrium swelling was achieved in <6 hr.

Permeability Studies—Diffusion parameters for copolymer membranes of varying composition are presented in Table I. Each set of permeability parameters (D and K_p) represents an average of results obtained in at least five separate experiments, and an example of the data obtained using the flux–lag time experimental method is presented in Fig. 2. The slope of the line determined by linear regression techniques through the steady-state region of the curve provides an accurate measurement of the tetracycline flux through the copolymer film. The diffusion coefficient, D, was determined from the lag time measurement, t_L, equal to the intercept value on the abscissa.

The tetracycline permeability ($P = DK_p$) of the various copolymers increased in relationship to their increase in equilibrium water content. Plotting the log of the diffusion coefficients, D, *versus* the reciprocal of the equilibrium hydration values, $1/H$, for the series of copolymers produced a straight line. This result is consistent with an analysis of the free volume of diffusion theory presented by Yasuda *et al.* (8) who stated that a linear relationship would exist between the logarithm of the diffusion coefficient and the reciprocal hydration value. This relation assumes a linear variation of the free polymer volume with the diluent volume fraction (as expressed by the hydration value, H). The free polymer volume would consist of thermally transient random holes or voids in the polymer matrix, which would serve as the passage for diffusing molecules. This discussion provides an explanation of the tetracycline diffusion process in the membranes. The free volume available for tetracycline permeation in the copolymer would be directly related to the hydration volume of the membrane.

***In Vitro* Diffusion Studies**—Figure 3 demonstrates the ability of the trilaminate device to release tetracycline at constant (zero-order) rates over a 4-month period. The results of several diffusion studies with the trilaminate tetracycline disks are detailed in Table II. The effect of

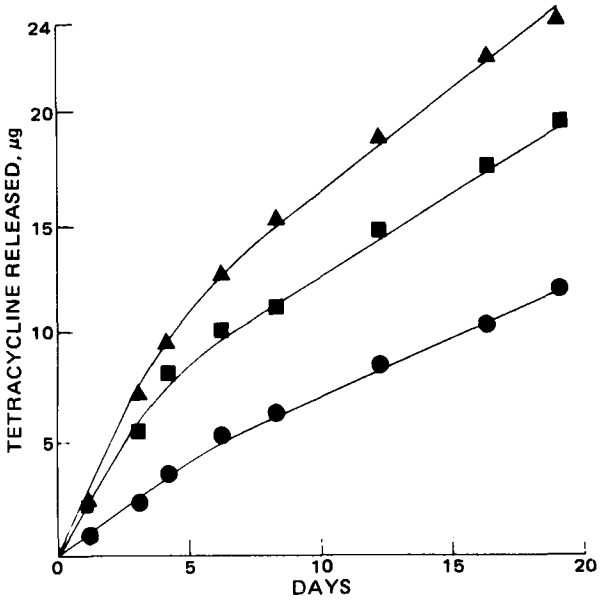

Figure 4—*Effect of trilaminate device geometry on steady-state tetracycline release rates. The 2:98 copolymer coating membrane had a thickness and surface area available for diffusion of 0.14 mm and 0.709 cm^2 (●), 0.081 mm and 0.709 cm^2 (■), and 0.14 mm and 1.33 cm^2 (▲), respectively.*

varying device geometry on the steady-state release is presented graphically in Fig. 4. Increasing surface areas available for diffusion by 1.88 times (0.709–1.33 cm^2) produced a proportionate increase in the release rate by 1.80 times (0.54–0.97 μg/day). Correspondingly, increasing the coating membrane thickness by 1.73 times (0.81–0.14 mm) while holding the surface area constant had an inverse effect on the release rate, decreasing the rate by 1.43 times.

Increasing the drug loading in the core material (expressed as milligrams of drug per milligram of total core weight) by an order of magnitude (0.02–0.2) increased the tetracycline release rate by 16.0 times when corrected for differences in membrane thickness. This higher-than-predicted increase in the release rate is probably the result of osmotic effects that become significant for core materials of higher drug loading. The effects of varying the coating membrane composition are also evident in Table II and Fig. 3.

These results are consistent with steady-state diffusion theory as presented in Eq. 2. In conclusion, the described membrane-controlled trilaminate drug delivery system demonstrated the ability to release tetracycline at constant rates over a prolonged period. The trilaminate device is constructed of a series of 2-hydroxyethyl methacrylate–methyl methacrylate copolymers, which control the tetracycline diffusion rate from the system by their characteristic properties of equilibrium hydration and solute permeability. Tetracycline release rates from the trilaminate device also are dependent on device geometry. The ability of the trilaminate device to deliver tetracycline at zero-order rates offers potential therapeutic and investigational applications. The *in vivo* release characteristics of the tetracycline trilaminate delivery system will be discussed in a separate article (15).

REFERENCES

(1) F. Theeuwes, *J. Pharm. Sci.*, **64**, 1987 (1975).

(2) D. L. Wise and H. Rosenkrantz, in "Drug Delivery Systems," H. L. Gabelnick, Ed., DHEW Publication (NIH)77-1238, 1976, p. 252.

(3) D. Brooke and R. J. Washkuhn, *J. Pharm. Sci.*, **66**, 159 (1977).

(4) S. Borodkin and F. E. Tucker, *ibid.*, **64**, 1289 (1975).

(5) J. W. Shell and R. W. Baker, *Ann. Ophthamol.*, **6**, 1037 (1974).

(6) A. Zaffaroni, *Acta Endocrinol., Suppl.*, **185**, 423 (1974).

(7) J. M. Anderson, T. Koinis, T. Nelson, M. Horst, and D. S. Love, in "Hydrogels for Medical and Related Applications," J. D. Andrade, Ed., American Chemical Society, Washington, D.C., 1976, p. 167.

(8) H. Yasuda, C. E. Lamaze, and L. D. Ikenberry, *Die Makromol. Chemie*, **118**, 19 (1968).

(9) H. Yasuda, L. D. Ikenberry, and C. E. Lamaze, *ibid.*, **125**, 108 (1969).

(10) D. R. Cowsar, O. R. Tarwater, and A. C. Tanquary, in "Hydrogels for Medical and Related Applications," J. D. Andrade, Ed., American Chemical Society, Washington, D.C., 1976, p. 180.

(11) K. Kliment, J. Vacik, Z. Ott, V. Majkus, M. Stol, V. Stoy, and O. Wichterle, U.S. pat. 3,689,634 (Sept. 5, 1972) and 3,551,556 (Dec. 29, 1970).

(12) M. E. Corn, U.S. pat. 3,499,959 (Mar. 10, 1970).

(13) L. Sogor and J. M. Anderson, in "Coatings and Plastic Preprints, ACS San Francisco Meeting," American Chemical Society, Washington, D.C., 1976, p. 1.

(14) M. Pernarowski, R. O. Searl, and J. Naylor, *J. Pharm. Sci.*, **58**, 470 (1969).

(15) L. Olanoff and J. M. Anderson, *ibid.*, **68**, 1151 (1979).

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