finding is in agreement with a study conducted in humans (8) where quinidine gluconate gave higher blood levels than dihydroquinidine gluconate after single and maintenance doses. These studies suggest that the cardiovascular activities of quinidine and dihydroquinidine are similar but that differences may exist when these two components are incorporated into dosage forms. For these reasons, monitoring of the dihydroquinidine content in quinidine raw materials and dosage forms may have practical importance.

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Controlled Drug Release by Polymer Dissolution II: Enzyme-Mediated Delivery Device

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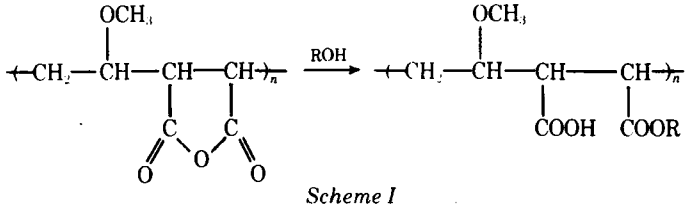
Abstract □ A novel, closed-loop drug delivery system was developed where the presence or absence of an external compound controls drug delivery from a bioerodible polymer. In the described delivery system, hydrocortisone was incorporated into a *n*-hexyl half-ester of a methyl vinyl ether–maleic anhydride copolymer, and the polymer–drug mixture was fabricated into disks. These disks were then coated with a hydrogel containing immobilized urease. In a medium of constant pH and in the absence of external urea, the hydrocortisone release was that normally expected for that polymer at the given pH. With external urea, ammonium bicarbonate and ammonium hydroxide were generated within the hydrogel, which accelerated polymer erosion and drug release. The drug delivery rate increase was proportional to the amount of external urea and was reversible; that is, when external urea was removed, the drug release rate gradually returned to its original value.

Keyphrases □ Dosage forms—controlled-release delivery devices, *n*-hexyl half-ester of a methyl vinyl ether–maleic anhydride copolymer, urease, pH-controlled hydrocortisone release □ Hydrocortisone—controlled-release delivery, pH controlled, *n*-hexyl half-ester of a methyl vinyl ether–maleic anhydride copolymer, urease □ Copolymers—controlled-release delivery devices, *n*-hexyl half-ester of a methyl vinyl ether–maleic anhydride, urease, pH-controlled hydrocortisone release □ Urease—controlled-release delivery devices, pH-controlled hydrocortisone release □ Corticosteroids—hydrocortisone, controlled-release delivery device

Drug formulations that deliver an active agent to a specific body site in precisely regulated amounts are superior to those that indiscriminately flood the whole body with a therapeutic agent. Consequently, sustained drug release is receiving great attention (1).

However, even precisely controlled sustained delivery is not always the optimum therapeutic regimen. In many applications, a better delivery system is one that delivers the active agent only when needed. The essential ingredients of such a system are a sensing mechanism that can detect minute amounts of a specific compound in a complex mixture such as blood and some means of transferring this information to a delivery device that can then modify therapeutic agent delivery. While electromechanical devices that use microelectronics and enzyme probes to control miniaturized pumps are possible, this study centers on purely chemical methods.



Scheme I

BACKGROUND

A previous paper (2) described the dissolution and concomitant drug release from partially esterified copolymers of methyl vinyl ether and maleic anhydride prepared as shown in Scheme I. Two notable features of these polymer systems were: (*a*) their ability to undergo surface erosion and, hence, to release an incorporated drug by zero-order kinetics, and (*b*) an extraordinary sensitivity of the erosion rate to the surrounding aqueous environment pH. These systems also exhibited a characteristic pH above which they were completely soluble and below which they were completely insoluble. This pH was very sharp and depended on the size of the alkyl group in the copolymer ester. Consequently, polymer erosion behavior can be tailored to fit any desired pH environment; even very small pH variations will have a major effect on the erosion rate and, thus, on drug release.

Any sensing mechanism that can convert the presence of a specific compound in the external environment to a pH change can be used to control polymer dissolution and therapeutic agent delivery. Enzymes almost ideally fit this requirement because their mode of action is highly specific; in many cases, enzyme–substrate reaction products are acidic or alkaline compounds.

For this study, an enzyme was needed that, after reaction with a substrate, liberated an alkaline product so that the net effect of the enzyme–substrate reaction would be a pH increase at the polymer–water interface. The enzyme urease, which interacts with urea as shown (3), was selected (Scheme II).

$$(NH_2)_2CO + 3H_2O \xrightarrow{\text{urease}} HCO_3^- + 2NH_4^+ + OH^-$$

Scheme II

The purpose of this study was to develop a system in which urea in an external environment would affect the release of hydrocortisone incor-

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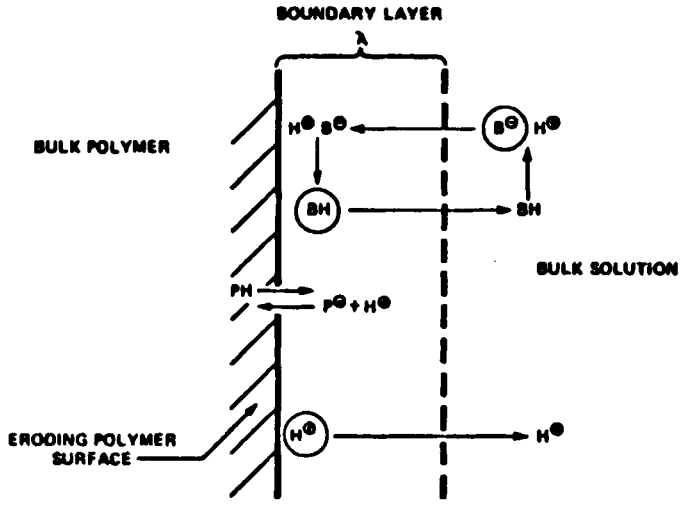


Figure 1—*Model for polymer erosion mechanism.*

porated into a polymer matrix. Such a system serves as a model; it has no obvious therapeutic application. More useful systems are being developed.

EXPERIMENTAL

***n*-Hexyl Half-Ester of a Methyl Vinyl Ether–Maleic Anhydride[1] Copolymer (50:50)**—A three-necked 2000-ml, round-bottom flask equipped with a mechanical stirrer, a heating oil bath, a condenser, and a nitrogen inlet and exit was charged with 109.30 g of the copolymer (1.400 equivalent) and 794.82 g of 1-hexanol (7.779 moles). The alcohol, present in an 11:1 molar excess, was sufficient to produce a 20% (w/w) solution of the half-ester product.

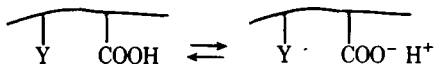
After the flask was purged with nitrogen, the reactants were vigorously stirred and heated over 0.5 hr to 145°. The reaction was followed by IR analysis of the carbonyl peaks associated with residual cyclic anhydride in the copolymer and with the formed ester linkage in the half-ester product. The reaction was judged complete after 2.5 hr at 145°. The solution was cooled to room temperature with an ice bath and precipitated in 8 liters of methanol–water (1:1 v/v). The precipitated polymer was dissolved in 2 liters of acetone and precipitated in 8 liters of methanol–water (1:2 v/v). This step was repeated twice. The product was dried in a forced-air oven for 3 days at 50°. A tough, clear-to-slightly-hazy material (148.10 g) was collected in an 82% yield.

Test Specimens—*Film Casting*—The polymer and micronized hydrocortisone[2] were added to 2-ethoxyethyl acetate–isopropylacetone (7:3 w/w) to produce a 9% polymer solution containing 10 parts of dispersed hydrocortisone/100 parts of resin. A homogeneous solution was obtained by using a jar mill. Films were cast by pouring this solution into level polytef-lined molds. The molds were then partially covered and, to prevent bubble formation in the film, were dried slowly for 8 days. They were further dried in a forced-air oven at 35° for 1 day and then *in vacuo* for 1 day.

Disks, 9.5 mm in diameter, were cut from the film using a drill press and hole cutter. The disks were weighed after being placed in a vacuum oven for 48 hr at room temperature and then being equilibrated to a constant weight in air. The disks selected for release-rate testing weighed 47.5–52.5 mg and were ~0.75 mm thick.

Enzyme Coating—Initial attempts to incorporate the enzyme urease into the polymer matrix involved dissolving the enzyme and polymer in acetone, dispersing micronized hydrocortisone in the solution, casting films, and gently drying at room temperature. However, considerable enzyme denaturation took place.

Subsequent successful urease coupling with the polymer matrix was based on the work of Mascini and Guibault (4). A small locking forceps was affixed to the edge of each polymer disk so that it could be manipulated without touching the surfaces during the immobilized enzyme-coating procedure. A 30% aqueous solution of bovine serum albumin was prepared, and 1 g of urease was added to 10 ml of this solution. After quick stirring until the urease had dissolved, the solution was chilled in an ice



water insoluble water soluble

Scheme III

bath. Each disk was held horizontally by the attached forceps, and 1 drop of the albumin–urease solution was added to the upper disk face. The disk was quickly rotated, and a drop was added to the opposite face. Similarly, 1 drop of 25% aqueous glutaraldehyde was added to each face. One minute after the glutaraldehyde addition, the coating had gelled sufficiently to allow the disks to be hung vertically.

After standing in air for 15 min, the coated disks were immersed in cold, deionized water for 15 min, in 0.1 *M* glycine for 15 min, and in pH 5.75 phosphate buffer for 2 hr. Finally, they were immersed in fresh pH 5.75 phosphate buffer for 4 hr.

Release Rate Measurements—The coated disks were heat sealed in polypropylene mesh bags having 0.5-mm openings. Care was taken to apply heat only to the edges of the bags and to avoid applying heat to the disks. The mesh bags were attached to 10-gauge stainless steel wires and moved vertically up and down at ~140 cm/min in test tubes[3], as described previously (2). Hydrocortisone release was followed spectrophotometrically at 242 nm.

RESULTS AND DISCUSSION

A previous paper (2) described the erosion behavior of a hydrophobic polymer system where dissolution occurred by carboxyl group ionization. This solubilization behavior has been represented generally as shown in Scheme III (COOH is a solubilizing group and Y is a hydrophobic substituent). The dissolution behavior also was rationalized in terms of the model shown in Fig. 1. In this model, the erosion rate is principally determined by the rate at which hydrogen ions are neutralized within the boundary layer, so the induced pH change in that layer will significantly affect polymer erosion and, hence, drug release.

In the system described, a hydrophobic *n*-hexyl half-ester of a methyl vinyl ether–maleic anhydride copolymer containing hydrocortisone was surrounded by a hydrogel consisting of the enzyme urease immobilized in bovine serum albumin by crosslinking with glutaraldehyde. In the absence of external urea, the polymer dissolution and hydrocortisone release occur by the mechanism shown in Fig. 1, *i.e.*, hydrogen-ion neutralization by external buffer and diffusion of hydrogen ions away from the eroding polymer.

When urea is in the external environment, it diffuses into the hydrogel layer and is converted to ammonium bicarbonate and ammonium hydroxide by the immobilized urease. These basic species neutralize hy-

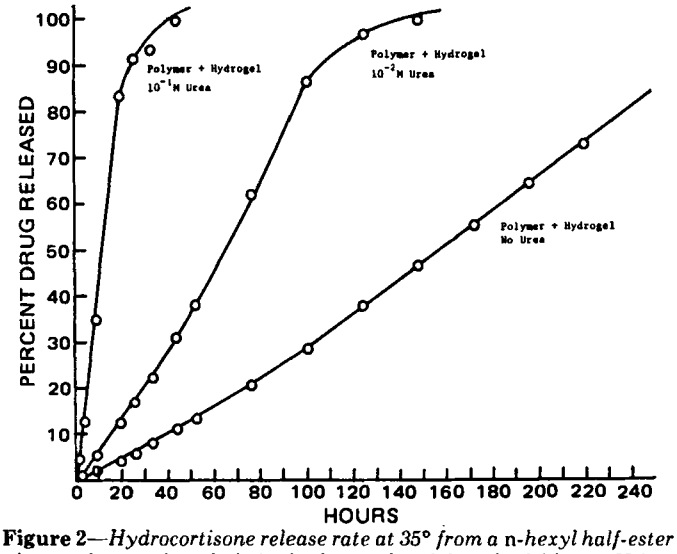


Figure 2—*Hydrocortisone release rate at 35° from a* n*-hexyl half-ester of a copolymer of methyl vinyl ether and maleic anhydride at pH 6.25 in the absence and presence of external urea. The stirring rate was ~140 cm/min*[3].

[1] Gantrez AN-169, GAF Corp., New York, N.Y.
[2] The Upjohn Co., Kalamazoo, Mich.

[3] Approximately 18.4 cycles/min at a total sample travel distance of 7.62 cm/cycle.

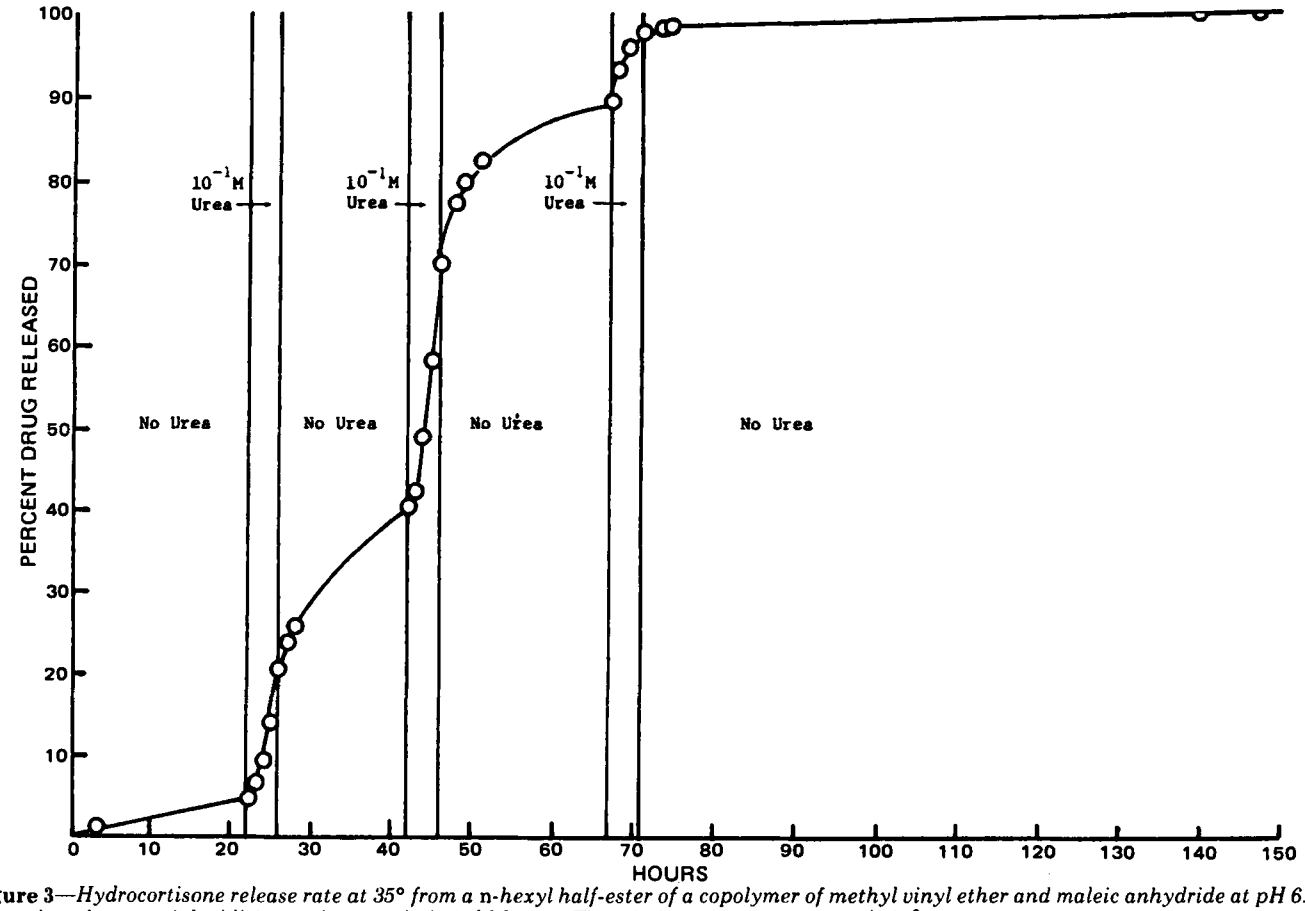


Figure 3—*Hydrocortisone release rate at 35° from a* n*-hexyl half-ester of a copolymer of methyl vinyl ether and maleic anhydride at pH 6.25 as a function of sequential addition and removal of* 10^{-1} M *urea. The stirring rate was ~140 cm/min*[3].

drogen ions generated by the eroding polymer, thus accelerating the process. Consequently, urea in the external environment should lead to accelerated drug release, and this acceleration should be directly proportional to the external urea concentration. Data in Fig. 2 corroborate these expectations.

While care was taken to maintain a constant pH in the external environment, this goal was accomplished only with 10^{-2} *M* urea. In the presence of 10^{-1} *M* urea, basic species generation was so rapid that the external medium buffering capacity was exceeded and a pH increase of ~0.25 occurred. Furthermore, the polymer dissolution rate was such that when the solubilized polymer chains diffused through the hydrogel, presumably through open pores, they precipitated again when exposed to the lower external pH. Because of the considerable difference in polymer dissolution rates between the hydrogel and the bulk solution, the devices gradually acquired a significant thickness of precipitated gelatinous polymer. Nevertheless, even though a rigorous comparison between no urea and urea is possible only in the 10^{-2} *M* case, the device definitely delivers hydrocortisone significantly faster in the presence of 10^{-1} *M* urea than in the presence of 10^{-2} *M* urea.

Data in Fig. 2 illustrate a situation where a device had a fixed and constant drug delivery rate that increased in response to the presence and concentration of external urea. The next question was whether this situation was reversible; that is, whether the drug delivery rate would return to its original value when urea was removed from the external environment.

Results illustrating this situation are shown in Fig. 3. As in the previous experiments, polymer erosion in the presence of 10^{-1} *M* urea was so rapid that the polymer precipitated around the device. Nevertheless, when the device was removed from a buffer solution without urea and placed in a buffer solution with 10^{-1} *M* urea, a significant increase in delivery immediately took place. When the device was next removed from that solution and again placed in a solution without urea, a delivery rate decrease took place.

The decrease in delivery was not abrupt but showed a first-order dependence. This first-order dependence was a consequence of the relatively water-insoluble hydrocortisone being trapped in the hydrogel layer and in the precipitated polymer around the device from which it was slowly released by a diffusional mechanism when the device was in the low rate erosion regimen. In the high rate delivery regimen, a zero-order delivery was noted because the slow diffusional release was masked by the rapid erosional release.

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