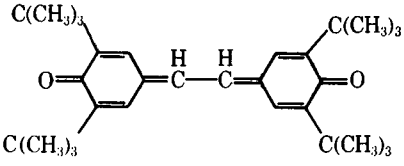
Column chromatography separated the extract into a yellow band and a white polymeric band.

After 10 TLC separations for purification, the yellow substance exhibited an IR spectrum identical to that obtained by Bohn and Campbell (4).

Mass spectra were obtained at various temperatures. Below 110°, poor fragmentation occurred. Above 160°, the compound disintegrated. The ideal conditions for obtaining mass spectra were 110–160° and 70 ev. The mass spectrum obtained was similar to that obtained previously (2).

The NMR spectrum was similar to that reported for II (6). Values of 1.30 and 1.33 ppm resulted from *tert*-butyl groups. Peaks at 6.90 and 7.52 ppm were due to the aromatic CH's. Peaks at 7.08 and 7.18 ppm were due to the olefinic CH's.

Based on the IR, mass, and NMR spectra, the yellow substance was assigned a quinoid structure with empirical formula $C_{30}H_{42}O_2$. Based on these spectral data, the structure of the isolated compound is:



II

For quantification, 334.7895 g of discolored caps was extracted. The amount of I still present after discoloration was 47.7 ppm. After purification, 2.5 mg or 7.46 ppm of II was obtained.

Synthesis of Authentic Yellow Compound—The synthesis of the authentic yellow compound was carried out by a literature method (7). Compound I was put in an oven at 100° for 2 weeks. The yellow material obtained was purified by column chromatography and TLC. The isolation and purification procedures were identical to those used for II extracted from the closures.

IR and mass spectra were recorded. The sample was smeared with mineral oil and sandwiched between two salt blocks to obtain the IR spectrum, which was similar to that obtained for II. The mass spectrum was similar to that obtained for the unknown yellow substance.

The mass spectrum exhibited base peaks at *m/e* 435, 436, and 57. This compound was identical to that isolated from the polyethylene closures.

Synthesis of Yellow Compound by Catalytic Action of Titanium Dioxide—3,3′,5,5′-Tetrabis(*tert*-butyl)stilbenequinone was obtained by adding 1.5 g of rutile titanium dioxide powder to 100 g of I. Yellowing was obtained within 24 hr at 100° in an oven. The yellow compound was purified by the methods used for II and identified by IR and mass spectral analyses. The IR and mass spectra were similar to those reported previously (2, 4, 7). Under identical conditions but without titanium dioxide, discoloration of I to form II only occurred after 2 weeks.

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Dihydroquinidine Contamination of Quinidine Raw Materials and Dosage Forms: Rapid Estimation by High-Performance Liquid Chromatography

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Abstract □ Dihydroquinidine is a commonly encountered contaminant in quinidine raw materials. The USP allows 0–20% dihydroquinidine in quinidine products, but the assays used to quantitate dihydroquinidine have been lengthy or have required sophisticated equipment. The present method separates dihydroquinidine from quinidine and provides rapid, precise quantitation of both dihydroquinidine and quinidine. The clinical importance of dihydroquinidine contamination of quinidine dosage forms remains unanswered.

Keyphrases □ Hydroquinidine—analysis, high-performance liquid chromatography, as contaminant in quinidine raw materials and dosage forms □ Quinidine—analysis, high-performance liquid chromatography, hydroquinidine contamination in raw materials and dosage forms □ High-performance liquid chromatography—analysis, hydroquinidine contamination of quinidine raw materials and dosage forms

Quinidine raw materials and dosage forms routinely contain dihydroquinidine as a contaminant. The amount of the dihydro derivative varies from 0 to 25% (1), although dosage form dihydroquinidine content is limited to 20% by the USP (2). Many methods reported for quinidine quantitation do not separate the dihydroquinidine contaminant from quinidine. Previous methods for dihydroquinidine quantitation in dosage forms and raw materials include TLC (3), NMR (4), chemical-ionization mass spectrometry (5), and normal phase high-performance liquid chromatography (HPLC) (3). These methods are time consuming or tedious or require expensive equipment. The described HPLC method separates the dihydroquinidine contaminant from quinidine and allows rapid, precise quantitation of both compounds.

EXPERIMENTAL

Apparatus—The high-performance liquid chromatograph was equipped with a multiwavelength UV detector[1] and a microparticulate C-18 column[2]. A filter[3] with a 1.2-μm pore size was used for solution filtration prior to injection.

[1] Model 711 solvent delivery system with a Spectromonitor II detector, Laboratory Data Control, Riviera Beach, Fla.
[2] Waters Associates, Milford, Mass.
[3] Millipore Corp., Bedford, Mass.

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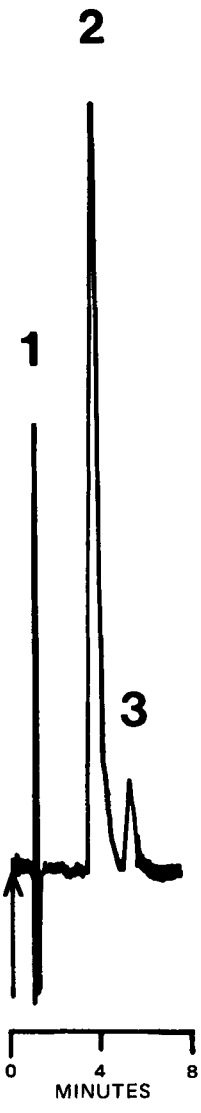

Figure 1—*Typical chromatogram for quinidine and dihydroquinidine in a tablet dosage form. Key: 1, solvent front; 2, quinidine peak; and 3, dihydroquinidine peak. Arrow indicates sample injection time.*

Reagents—All chemicals and reagents were analytical grade unless otherwise indicated. Quinidine sulfate[4,5], quinidine[5,6], and dihydroquinidine[7] were used as obtained. Glass-distilled methanol was used for the mobile phase[8]. Quinidine sulfate tablets from three different suppliers[9] were obtained from a local pharmacy.

Procedure—A quinidine sulfate tablet was weighed accurately, crushed carefully using a mortar and pestle, and added (25 mg) to a 100-ml volumetric flask. Then 5 ml of methanol was added to the powder, and the solution was diluted to 100 ml with acetic acid–water (1:1). This solution was shaken, and an aliquot was diluted 10 times with acetic acid–water (1:1). Then the solution was filtered[3] to remove suspended matter, and a 10-μl aliquot was injected into the chromatograph under the following conditions: flow rate, 2 ml/min; detection, 254 nm; and mobile phase, methanol–acetic acid–water (25:4:71).

The retention times for quinidine and dihydroquinidine under these conditions were 3.83 and 4.86 min, respectively. The assay sensitivity was a function of injection volume; at 10 μl, the dihydroquinidine sensitivity was 0.25 μg/ml, but larger injection volumes or smaller dilutions increased the sensitivity.

Dihydroquinidine standards were prepared in water or acetic acid–water (1:1). Both vehicles gave the same peak height results. Dihydroquinidine concentration was estimated by comparing the peak heights to a standard curve. Theobromine (10 μg/ml) could be added as an internal standard to correct for injection volume errors. Theobromine retention time was 2.25 min under these conditions.

To estimate the dihydroquinidine concentration in various raw materials, quinidine base and quinidine sulfate solutions (20 μg of quinidine/ml) were made as described.

[4] Sample KK, K&K Laboratories, Plainview, N.Y
[5] Sample ME, Merck & Co., Rahway, N.J.
[6] Sample MA, Mallinckrodt Chemical, New York, N.Y.
[7] Pfaltz & Bauer, Stamford, Conn.
[8] Burdick & Jackson Laboratories, Muskegon, Mich.
[9] Product PP, Purepac Pharmaceutical Co., Elizabeth, N.J.; Product PD, Parke-Davis and Co., Detroit, Mich.; and Product PR, Philips Roxane, Columbus, Ohio.

Table I—Dihydroquinidine Content in Three Marketed Quinidine Sulfate Tablet Formulations

Tablet	Dihydroquinidine, %		
	Product PD[a]	Product PP	Product PR
1	9.81[b]	3.19	6.50
2	9.49	3.80	6.57
3	9.09	3.38	6.25
	—	—	—
Mean	9.47 ± 0.52	3.49 ± 0.39	6.44 ± 0.24

[a] For product identification, see *Experimental*. [b] Each value represents the mean of four determinations.

RESULTS

Figure 1 shows a typical chromatogram for quinidine and dihydroquinidine in a tablet. No interfering peaks due to excipients were observed in the quinidine tablets. Slight variations in retention times could be seen from day to day.

The standard quinidine and dihydroquinidine curves for peak height *versus* concentration are linear over a wide concentration range and pass through the origin. Quinidine and dihydroquinidine concentration can be estimated accurately over 0–75 μg/ml, which encompasses the range used in this assay.

All quinidine raw materials contained dihydroquinidine, but no other cinchona alkaloids were detected. Samples from different commercial sources contained 3–29% dihydroquinidine. The mean dihydroquinidine concentration in the two quinidine sulfate raw materials varied from 3 to 16% whereas the content ranged from 25 to 29% in the two quinidine base materials. The percent of dihydroquinidine in the quinidine sulfate samples was (mean ± *SD*): Sample ME, 3.19 ± 0.13%; and Sample KK, 15.7 ± 1%. Similar values for quinidine base samples were (mean ± *SD*): Sample ME, 27.2 ± 1.9%; and Sample MA, 29.3 ± 1.1%. Each reported value is a mean of four determinations from the same sample.

Table I shows the dihydroquinidine content in three commercial quinidine sulfate tablets. Each reported value represents the mean of four determinations from the same solution. The mean dihydroquinidine content varied from 3.19 to 9.81%. Dihydroquinidine content was least in Product PP (3.49%), maximum in Product PD (9.47%), and intermediate in Product PR (6.44%).

DISCUSSION

No significant differences were observed in concentration when estimating dihydroquinidine and quinidine content from filtered or nonfiltered solutions. Thus, there was no significant adsorption to the filter[3]. Filters should be soaked in distilled water for at least 0.5 hr prior to use to remove any surfactant that might interfere with the assay.

The USP monograph for quinidine (2) allows dihydroquinidine concentrations up to 20% of the quinidine concentration, and the content uniformity requirement allows a tablet range of ±15%. Thus, a 200-mg quinidine sulfate tablet could contain as little as 148 mg of quinidine sulfate and 37 mg of dihydroquinidine sulfate (185-mg tablet) or 215 mg of quinidine sulfate with no dihydroquinidine. The three quinidine sulfate tablet formulations tested ranged from 3.4 to 9.5% dihydroquinidine. The sulfate raw materials contained 3.2 and 15.7% dihydroquinidine, and the two base samples contained 27 and 29%.

Quinidine in tablets was quantitated employing the quinidine peak height and the standard curve. All three tablet formulations met the USP content uniformity test; *e.g.*, the quinidine content was 87.5, 94.5, and 95.8% for Products PD, PR, and PP, respectively. The quinidine content of raw materials varied from 84 to 96% for the sulfate and 100–103% for the two base samples.

However, the question of differences between quinidine and dihydroquinidine persists. Previous studies showed that dihydroquinidine has a lower partition coefficient (4) and a slower dissolution rate from formulations (6) than quinidine. In a recent study in rats (7), quinidine and dihydroquinidine had similar efficacy and toxicity when given intravenously, but dihydroquinidine had a 40% higher oral LD_{50}. This

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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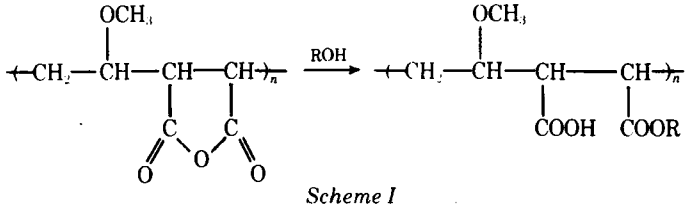
Abstract □ A novel, closed-loop drug delivery system was developed where the presence or absence of an external compound controls drug delivery from a bioerodible polymer. In the described delivery system, hydrocortisone was incorporated into a *n*-hexyl half-ester of a methyl vinyl ether–maleic anhydride 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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