The correlation coefficient for the standard curves over the concentration range used in this study was >0.997 (n = 22 or 25). The analysis of the concentrations of I–VI in the experimental samples compared favorably with the theoretical values of the drug formulations (Table II) with <1% error.

The described HPLC assay has been successfully applied as a stability-indicating method for determining the rate and percentage of autoxidation of commercially supplied injectable solutions of I–VI which had been diluted in 5% dextrose in water injection, with and without aminophylline (500 μg/ml) (14). The chromatographic separation of I–VI from their autoxidation products is illustrated in Fig. 2, with III, which has undergone 70% decomposition after being exposed to 17 hr of fluorescent light. Peak 1 represents the principal degradation product and Peak 3 a minor product. The unidentified Peak 2 is present in unexposed samples and does not appear to change after exposure to light. Similarly, the degradation products of I, II, and IV–VI elude within 2–3 min following injection. Figure 1B represents III before exposure to fluorescent light and decomposition has already commenced. This method has demonstrated the capability of separating degradation products from the analysis of pure I–VI.

The application of ion-pair HPLC for the analysis of formulations containing VI or IV has not been previously described. This reported method is applicable for the accurate determination of these drugs, using either UV or fluorescence detectors.

DISCUSSION

Various analytical methods have been used in stability studies for catecholamines but suffered from their inability to quantitate or separate degradation products from the parent substance. The combination of fluorescence detection with reversed-phase ion-pair HPLC yields a rapid and selective method for the quantitative separation and determination of I–VI in the presence of their autoxidation products and pharmaceutical adjuvants. The lowest concentration at which the sympathomimetic amines used in this study could be detected was 0.1 μg/ml.

The described method is applicable for the content uniformity and quality control of products containing VI and IV.

Despite the widespread use of UV detection in HPLC assays, the sensitive fluorescence measurement for low concentrations of sympathomimetic amines in pharmaceutical preparations has not been extensively reported except for their clinical analysis in biological fluids. The sensitive measurement of I–VI will allow studies of the purity of dosage forms and investigation of their degradation kinetics and pharmacokinetics. Without the use of a fluorescence detector, II, III, V, or VI could not be quantitatively measured in the presence of aminophylline. Also, the presence of pharmaceutical adjuvants should not interfere, because of their poor fluorescent properties.

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Enhanced Entrapment of a Quaternary Ammonium Compound in Liposomes by Ion-Pairing

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Received September 9, 1981, from the *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Kentucky, Lexington, KY 40536.* Accepted for publication November 10, 1981.

Abstract □ The encapsulation of a quaternary ammonium compound by multilamellar liposomes was enhanced by formation of ion-pairs with a counterion. Thus, [^{14}C]methantheline bromide was synthesized and paired with a 25 M excess of trichloroacetate. Under these conditions, the amount of radioactivity entrapped by phosphatidylcholine liposomes was three times greater than when no trichloroacetate was present. The increased liposomal loading was probably due to the solubilization of the ion-pair in the lipid membrane of the liposome.

Keyphrases □ Liposomes—quaternary ammonium compound, entrapment, ion pairing □ Ion-pairing—quaternary ammonium compound, entrapment, liposomes □ Ammonium—quaternary compound, entrapment in liposomes, ion-pairing

Liposomes are microscopic lipid vesicles originally used to study the structure and function of biological membranes (1). In recent years, liposomes have demonstrated potential as carriers and transporters of biologically active compounds (2). A wide variety of compounds have been encapsulated in liposomes, ranging from RNA (3) and insulin (4) to small molecules like histamine (5). Liposomes have been administered intravenously and orally (6), and a recent report describes the delivery of drugs *via* liposomes by the topical route (7). Liposomes elicit no immunological or toxicological responses and are completely biodegradable. For these reasons, liposomes have been viewed as an attractive mechanism for drug delivery, especially for biologically unstable compounds, but not without certain drawbacks. Special techniques have been developed in an attempt to direct liposomes to specific targets or organ systems by immunological methods (8) and by producing pH-sensitive liposomes (9). Polymerized

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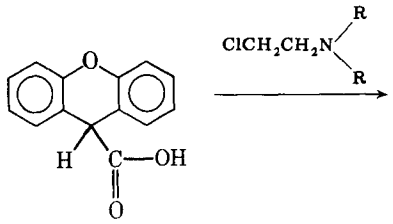
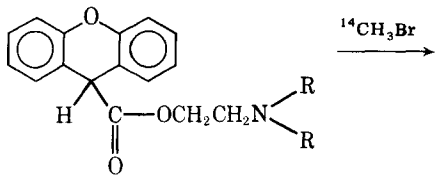
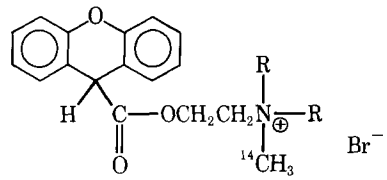


I



II



III

R = C_2H_5

vesicles have been produced to increase their stability (10), but one major problem still exists, *i.e.*, many drugs are very poorly encapsulated by lipsomes, especially water-soluble compounds (11).

Some progress in this case has been reported. The degree of encapsulation of the chemotherapeutic agents 6-mercaptopurine and 8-azaguanine in single compartment liposomes has been improved by the formation of charge-transfer complexes with chloranil (2,3,5,6-tetrachloro-*p*-benzoquinone) and cyanocobalamin (12, 13). In this report, the enhanced entrapment of a quaternary ammonium compound in multilamellar liposomes by the formation of ion-pairs is described. More specifically the entrapment of the antimuscarinic drug, methantheline bromide, in the presence and absence of trichloroacetate was compared. A relatively simple procedure for the synthesis of [^{14}C]methantheline bromide, based on the method of Cusic and Robinson (14) is reported. This differs from the product synthesized in a previous study in the position of the carbon 14 label (15).

EXPERIMENTAL[1]

***N,N*-Diethylaminoethyl Xanthene-9-carboxylate (II)**—2-Diethylaminoethyl chloride hydrochloride[2] (3.0 g, 0.017 mole) was added to 30 ml of 1.0 *N* NaOH and extracted three times with 30 ml of ether. The combined ether extracts were dried over sodium sulfate, filtered, and evaporated under reduced pressure yielding a viscous oil. To this oil was added 4.0 g (0.019 mole) of xanthene-9-carboxylic acid[3] (I) in 15 ml of isopropyl alcohol and the mixture was refluxed overnight. After filtering, the solvent was evaporated, yielding an oily residue which crystallized upon further drying. Recrystallization from ethyl acetate afforded 4.277 g (0.012 mole) of analytically pure material which melted at 145–147° [lit. (15) 143–145°], IR (KBr): 1735 (C=O) cm^{-1}, with a 68% yield.

[^{14}C]Methantheline Bromide (*N,N*-Diethylaminoethyl xanthene-9-carboxylate[^{14}C]methobromide) (III)—A 0.3-g aliquot of II was made alkaline and extracted into ether as described previously. After drying, the solvent was evaporated and the resultant oily residue was dissolved in 4 ml of absolute ethanol. The solution was transferred to a vial containing 0.25 mCi (4.75 mg) of [^{14}C]methyl bromide[4] (5 mCi/mmole) which was cooled to 0–4° in an ice bath. The vial was sealed and allowed to stand for 48 hr at room temperature. The addition of dry ether to the ethanolic solution resulted in the formation of a precipitate which, upon recrystallization from benzene, gave 12.1 mg (0.029 mmole, 0.144 mCi) of fine, white needles (58% yield). The product had an IR spectrum identical to the authentic material[5] and melted at 173–175° in close agreement with the literature value of 175° (6). A radiochromatogram of the product [(silica gel) ethanol–water–acetic acid (3:2:1), R_f = 0.56] verified its radiochemical purity.

For the experiments with liposome entrapment of III, unlabeled methantheline bromide was added to III resulting in a final specific activity of 1 μCi/μmole.

Preparation of Multilamellar Liposomes—Unsonicated multilamellar liposomes were prepared according to the method of Maghew *et al.* (16). Phosphatidylcholine[6] (10 μmoles) and cholesterol[6] (3 μmoles) were dissolved in 2 ml of chloroform and placed in a cylindrical test tube. The chloroform was evaporated leaving a thin film in the bottom of the tube. To this was added the aqueous phase which consisted of 1 μmole of III (1 μCi in 2 ml of phosphate buffer at pH 7.4). In the ion-pairing experiments, 25 μmoles of trichloroacetate[7] was added as well. The mixture was mechanically agitated for 10 min after which time the liposome-entrapped radioactivity (arising from III) was separated from the free radioactivity (*i.e.*, nonliposome-bound activity) by molecular sieves[8]. The extent of entrapment by liposomes of III in the presence and absence of trichloroacetate was calculated by comparing the total number of counts arising from liposome-bound radioactivity with the free radioactivity.

RESULTS AND DISCUSSION

The partitioning of charged species into a nonaqueous medium results from the masking of the hydrophilic site by a counterion. Some counterions are more effective than others in increasing the lipophilicity of the charged species. The use of ion-pairing techniques has been utilized to enhance the lipid solubility of certain ionic compounds (17) and, in this connection, has improved the GI absorption of large cationic drugs (18). The chloroform–water partition coefficient of methantheline bromide was shown to increase over 200-fold when ion-paired with a 25 *M* excess of trichloroacetate (19). Higher concentrations of trichloroacetate did not improve the partitioning of methantheline into the chloroform layer (20). The entrapment of this drug by multilamellar liposomes would presumably be enhanced by the formation of ion-pairs with trichloroacetate, which would allow methantheline to be solubilized in the lipid membrane of the liposome. Multilamellar liposomes have a high lipid content relative to the aqueous phase compared to sonicated unilamellar liposomes. As a result, the effects of ion pairing on increasing lipid solubility should be most pronounced in the multilamellar liposomes. Solubilization of methantheline in the liposomal membrane should significantly increase the total amount of drug carried by the liposome. In the absence of trichloroacetate, the drug would only be carried in the small aqueous core of the liposome and the thin aqueous layers between the lipid bilayers of the multilamellar liposome. In the presence of trichloroacetate, the drug would be carried in these aqueous compartments as well as in the membrane itself.

The addition of a 25 *M* excess of trichloroacetate (with respect to III) in the aqueous phase during liposome production resulted in a threefold increase in the amount of radioactivity, which was entrapped by the multilamellar liposomes (1005–3027 dpm). Varying the cholesterol content of the liposomal membrane from 0 to 5 μmoles per 10 μmoles of phosphatidylcholine, did not significantly alter the entrapment of III. The increase of entrapped radioactivity arising from [^{14}C]methantheline from 0.44–1.2% by ion-pairing could be increased further by utilizing phospholipid membranes with physical properties differing from phos-

[1] Infrared spectra (KBr) were recorded on a Perkin-Elmer 567 spectrophotometer. Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. Radiochemical purity was determined with a Packard 7201 radiochromatogram scanner. Radioactive samples were counted in a Packard 3375 Tri-Carb Liquid Scintillation Spectrometer. ACS (Amersham) was the cocktail used for radioactive samples.

[2] Aldrich Chemical Co., Milwaukee, Wis.

[3] K and K Laboratories, Plainview, N.Y.

[4] New England Nuclear, Boston, Mass.

[5] Searle and Co., San Juan, P.R.

[6] Sigma Chemical Co., St. Louis, Mo.

[7] Sodium trichloroacetate, Fisher Scientific Co., Pittsburg, Pa.

[8] Sephadex G-50, Pharmacia Fine Chemicals, Piscataway, N.J.

phatidylcholine. The use of a charged membrane as well as the addition of other membrane adjuvants may affect the entrapment of methantheline and other compounds by liposomes.

These ion-pairing techniques can be used to increase liposomal loading of a large number of ionic compounds. The liposome-entrapped compounds can be used to overcome some traditional problems in the oral administration of drugs such as acid lability, inadequate intestinal absorption, and poor palatability.

The administration of methantheline bromide *via* liposomes is particularly desirable considering the poor absorption observed for quaternary ammonium compounds. In addition, ion-pairing of quaternary ammonium compounds increases the loading of these poorly entrapped drugs in liposomal membranes.

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pH-Sensitive Microcapsules for Drug Release

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Abstract □ Microcapsules were designed for a sustained drug release, where the external medium controls the rate of release of the drug. As a model, secretin was encapsulated in acryloyl chloride–lysine capsules, and the conditions of formation are described. The scanning electron micrographs show the formation of good spherical microcapsules in the size range of 5–10 μm. The release of secretin was studied in four media having different pH. Polymer dissolution was pH sensitive, and the capsules placed in different media eroded at a constant rate, depending on the pH of the medium. Dissolution of the microcapsules was limited to the polymer buffer media and the drug was released by zero-order kinetics. The possible use of such a system is in the treatment of duodenal ulcers and the diagnosis of pancreatic diseases.

Keyphrases □ Microcapsules—pH sensitive, drug release □ Drug release—pH sensitive microcapsules, polymerization □ Polymerization—pH sensitive microcapsules for drug release □ Dosage forms—pH-sensitive microcapsules for drug release

Microencapsulation is a process by which individual entities of a solid, liquid, or gas are discretely enclosed in a shell of inert polymeric materials. These inert shells may be designed to release their ingredients at a specific rate and/or under a specific set of conditions. Microencapsulation of a material may permit the alteration of its physical properties so that the desired availability is achieved and at the same time the encapsulated material is protected from its environment. Release of drug may be achieved *via* erosion, dissociation, or diffusion. Dosage forms have become more complex and now include such forms as sustained release, prolonged action, and repeat action. The technique of microencapsulation is one of the newer methods for sustained delivery which is receiving increasing attention (1, 2).

Precisely controlled sustained delivery does not always correspond to the optimum therapeutic regimen, however. In many applications a better delivery system is the one that delivers the active agent only when needed. In the present study secretin was microencapsulated in acryloyl chloride–lysine microcapsules, and the rate of release was studied in different media of varying pH.

BACKGROUND

Secretin (3), a small polypeptide (molecular weight ~3400), is present in the mucosa of the upper small intestine in the inactive form of prosecretin. When chyme enters the intestine, it causes the release and activation of secretin, which is subsequently absorbed into the blood. The constituent that causes greatest secretin release is hydrochloric acid, although almost any type of food will cause at least some release. Secretin is released any time the pH of the duodenal contents falls below ~4.0. This immediately causes large quantities of pancreatic juice containing abundant amounts of sodium bicarbonate to be secreted. Carbonic acid is formed by reaction of sodium bicarbonate with hydrogen chloride. The carbonic acid is immediately dissociated into carbon dioxide and water, and the carbon dioxide is absorbed into the body fluids, thus, leaving a neutral solution of sodium chloride in the duodenum. In this way, the acid contents emptied into the duodenum from the stomach become neutralized and the peptic activity of the gastric juices is immediately blocked. Since the mucosa of the small intestine cannot withstand the intense digestive properties of gastric juice, this is a highly important protective mechanism against the development of duodenal ulcers (4). A second function of hydrolytic secretin by the pancreas is to provide an appropriate pH for action of pancreatic enzymes. All such enzymes

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