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Drug release from membranes of hyaluronic acid and its esters

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Drug release from three types of hyaluronate based (sodium hyaluronate and ethyl and benzyl esters of hyaluronic acid) membranes was investigated. In the membranes, drug was either: 1) physically incorporated, 2) physically incorporated in the membrane, then laminated with a second polymer or 3) covalently bound to the polymer. The release of model compounds was found to be rapid when the compounds were physically incorporated; the release could be slowed by laminating the core membranes. Permeability and partition coefficient values were used to explain the release profiles. The amount of drug released was linearly related to the square root of time for both "physically incorporated" and "laminated" systems. When drug was covalently bound to the polymer, the release was slow and near zero-order. The solubility of the polymer and/or the hydrolysis of ester bonds are thought to be some of the important processes involved in drug release. The results suggest that a range of release rates can be achieved with hyaluronate based membrane systems.

Key words: Esters of hyaluronic acid; Glycosaminoglycans; Polymeric prodrugs; Permeability coefficient

Introduction

Hyaluronic acid (HA) is an endogenous glycosaminoglycan found in various tissues, including connective tissue, the synovial fluid of joints and the aqueous humor of the eye [1]. This report presents various procedures for preparing drug-loaded membranes of esters of HA and the resulting in vitro release profiles. Model compounds were either physically incorporated in the membranes or covalently bound to HA through ester linkages. These model compounds were selected based on their charge and on their applicability for dosage forms. Among these, hydrocortisone and benzyl alcohol are neutral, mafenide acetate is positively charged, and fluorescein sodium and hydrocortisone hemisuccinate are negatively charged at neutral pH. These model compounds are currently used as active agents in dosage forms (hydrocortisone, hydrocortisone hemisuccinate, and mafenide acetate) [2], as a pharmaceutical aid (benzyl alcohol) [3] and as a diagnostic aid (fluorescein sodium) [4].

An objective of this project is to ascertain whether the membrane drug release profiles can be explained using physical constants. The permeabilities and partition coefficients for these compounds in ethyl, benzyl and partial benzyl ester membranes of HA have been studied. Many previous articles have reported transport properties of membrane systems [5–7] and using the data, the release profiles often can be predicted.

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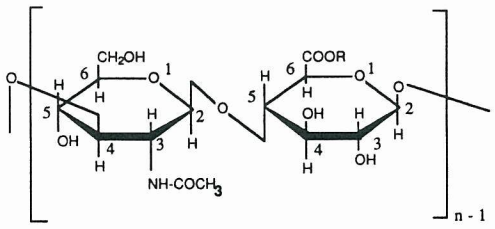
This prediction is rather straightforward if diffusion of drug is the only rate limiting step in drug release. However, for systems in which more than one process is rate determining (e.g. erosion and swelling of the matrix), prediction is more complicated. For example, for a poly (lactic acid) device, drug release was found to be controlled by diffusion and erosion. The relative contributions of these two factors were difficult to determine, as was the mechanism of drug release [8].

Therefore, the specific objectives of this study were: (i) to fabricate drug-loaded membranes of sodium hyaluronate (Na-HA) and esters of HA, (ii) to study drug-release from these membranes, (iii) to understand the release profiles on the basis of transport properties and other physico-chemical constants, and (iv) to propose a mechanism of drug release from the membranes.

Materials

The structure of HA and its esters is shown in Fig. 1; the accompanying table lists structures of various esters of HA. For convenience, the ester membranes will be referred to as ethyl, 100% benzyl or partial benzyl esters throughout this manuscript. Na-HA powder and membranes of ethyl and benzyl esters were supplied by Fidia, S.p.A. (Abano Terme, Italy) and were used as received.

Partial benzyl ester membranes were prepared from the 100% benzyl ester membranes. Pieces of 100% benzyl ester membranes were exposed to pH 9.0 phosphate buffer maintained at 32°C in a VanderKamp® Sustained Release Apparatus (model #103906, VanKel Industries, Inc., Edison, NY). The rotation speed was 25 r.p.m. The benzyl ester groups undergo hydrolysis, releasing benzyl alcohol; the amount of benzyl alcohol released was determined by HPLC assay. From the amount of benzyl alcohol released versus time profiles, the times required to release 25% and 50% of the bound benzyl alcohol groups were determined (8.9 and 19.1 h, respectively). The membranes were removed from the rotating bottles after the desired partial hydrolysis,



R *=	name in the text
H	Hyaluronic acid, (HA)
Na^+	Sodium hyaluronate, (NA-HA)
Ethyl	Ethyl ester
Benzyl	Benzyl ester
75% benzyl and 25% Na^+	75% benzyl ester
50% benzyl and 50% Na^+	50% benzyl ester
25% benzyl and 75% Na^+	25% benzyl ester
25% hydrocortisone and 75% ethyl	hydrocortisone/ethyl ester
25% hydrocortisone and 75% Na^+	25% hydrocortisone ester

* = For various esters of HA, the percent values in the left hand column (R) indicate the percent esterification by the corresponding alcohol or it is in the sodium salt form, e.g., 75% benzyl and 25% Na+ means 75% of the total number of carboxylic acid groups on HA have been esterified with benzyl alcohol and the remaining 25% carboxylic acid groups are present as the sodium salt.

Fig. 1. Structure of hyaluronic acid (HA) and its esters. HA is a linear polysaccharide made up of alternating units of glucuronic acid and N-acetyl glucosamine. Various esters of HA used in the study and their name in the text are listed in the accompanying table. In the case of ethyl and benzyl esters, all the carboxylic groups were esterified with ethyl alcohol and benzyl alcohol, respectively.

washed with distilled water and stored in phosphate buffer, pH 4.0, prior to use.

Hydrocortisone, hydrocortisone hemisuccinate (sodium salt), fluorescein sodium and 1,1,1,3,3,3-hexafluoro 2-propanol (HFIP) were purchased from Sigma Chemical Company (St. Louis, MO). The sample of mafenide acetate was supplied by Sterling-Winthrop Research Institute (Rensselaer, NY). 1-Octanesulfonic acid sodium salt and 1-pentanesulfonic acid sodium salt, 1-hydrate were purchased from Eastman Kodak Company (Rochester, NY). All other chemicals were obtained from Fisher Scientific (Pittsburgh, PA) and were used as received.

Methods

Fabrication of drug-loaded membranes

In the 'unilayered' membranes, drugs were physically incorporated into Na-HA, ethyl or

100% benzyl ester membranes; in the 'laminated' membranes, the drug-loaded 'unilayered' membranes were sandwiched between either ethyl or 100% benzyl ester membranes.

'Unilayered' membranes

'Unilayered' membranes of Na-HA, ethyl or 100% benzyl esters, loaded with the model compounds, were fabricated in our laboratory by modifying a technique described previously [9]. The solubilities of Na-HA and esters of HA are very different: Na-HA is highly water soluble, whereas the esters of HA are insoluble in water but soluble in HFIP. The procedures for formulating solutions for drug/Na-HA or drug/ester membranes differed only in the drying procedures. The drug-loaded membranes of Na-HA were prepared from an aqueous solution, while the membranes of HA esters were prepared using HFIP solutions. In both procedures, 10 mg of the drug were dissolved in 4 ml of the appropriate solvent, to which 100 mg of the polymer was added. Complete dissolution of the polymer required about 5–6 h. The membranes were then cast on glass plates by pouring the solution onto 5 cm × 5 cm squares etched on the surface of the glass.

The Na-HA membranes were dried in a microwave oven according to a previously reported procedure [10] (total drying time ≈1 h). The membranes of ethyl and 100% benzyl esters of HA were dried in a desiccator surrounded by an ice/sodium chloride mixture (temperature ≈8°C). A slight vacuum was applied to remove the evaporated HFIP in the desiccator (total drying time: 6–8 h). Residual HFIP in the membranes was removed by air-drying the membranes in a fume hood for 24 h. The dried membranes were removed from the glass-plates with a sharp blade and stored between two glass plates for a day to avoid wrinkling of the membranes. The membranes were stored in plastic zip-lock bags prior to use. The thickness of each membrane was measured using a micrometer (Ames, Waltham, MA). Five replicate measurements were made for each membrane. Membranes containing the model compounds were shiny and transparent, with the exception of the membranes containing sodium fluorescein, which were orange colored and opaque.

'Laminated' membranes

'Laminated' membranes were prepared by sandwiching the drug-loaded 'unilayered' membranes between ethyl or 100% benzyl ester membranes. The drug-loaded 'unilayered' membranes were cut into discs using a cork borer (#9, diameter 11.5 mm) as were the membranes (cork borer #6, diameter 16 mm) (Fisher Scientific, Pittsburgh, PA). A small amount (≈0.1 ml) of HFIP was applied to the laminating membranes with a paint-brush in order to dissolve some of the surface polymer. The dissolved polymer formed a glue-like substance onto which the drug-loaded membrane was adhered. The membranes were exposed to air in the fume-hood for about 24 h to evaporate traces of HFIP.

Membranes with covalently bound drugs

Polymers in which benzyl alcohol and hydrocortisone were covalently bound to HA through ester linkages (Fig. 1) were supplied by Fidia, S.p.A. as powders. Before use, the powders of these polymers were washed several times with methanol to remove any traces of free drug; no significant quantities of free drug were found in the methanol extracts. The powders were air-dried to evaporate the methanol and then used to prepare membranes. The polymers were dissolved in HFIP. Membranes were then cast on glass-plates and dried at low temperature under vacuum using the procedure described above.

Release studies

Drugs physically incorporated in the membranes

Franz diffusion cells were used to study the release of drugs physically incorporated in various membrane formulations. The composition of different membrane formulations ('unilayered' or 'laminated' membranes) evaluated are listed in Tables 1 and 2. The membranes were placed

TABLE 1

The $t_{50\%}$ values (time to release 50% of the drug) for the release of model drugs from sodium hyaluronate, ethyl ester, and benzyl ester membranes. Dissolution medium used: 0.05 M phosphate buffer, pH 7.00, μ=0.3 M, temperature: 32°C, n=5

Model drug	Time to release 50% of the drug (min) ± SD		
	Sodium hyaluronate membranes	Ethyl ester membranes	Benzyl ester membranes
Hydrocortisone	9.3 ± 2.2	4.3 ± 0.3	61.5 ± 9.7
Hydrocortisone hemisuccinate, sodium	4.7 ± 0.5	3.1 ± 0.5	–
Fluorescein, sodium	3.7 ± 0.9	4.6 ± 1.0	–
Benzyl alcohol	–	< 2.0	< 2.0
Mafenide acetate	–	< 2.0	–

Experiments were performed in the Franz diffusion cells.

on a nylon mesh (#8 mesh size, thickness 0.3 mm) which was assumed to offer no diffusional resistance to the drug transport. Phosphate buffer (0.05 M, pH 7.00, μ=0.3 M) was used as the medium in the receiver compartment. The volume of the receiver compartment was 5.5 ml and the temperature was maintained at 32.0 ± 0.1 °C by circulating water (VWR Scientific, Inc., CA, model #80 T) in the water-jacket of the cells. The receptor fluid was stirred with a magnetic stir-bar rotating at 600 r.p.m. Samples (250 µl) were withdrawn with a syringe through the side-arm of the cells; the volume removed was immediately replaced by phosphate buffer, also maintained at 32.0 °C. The cells were covered with a piece of Parafilm® (American Can Company, Greenwich, CT) to prevent any evaporation of the medium. Necessary dilutions of the samples were made prior to analysis. Graphs of cumulative amount released versus time were plotted and the $t_{50\%}$ (time to release 50% of the drug) values were read directly.

In preliminary experiments, the presence of an aqueous boundary layer under the membranes was detected when the release was studied at different stirring rates. Boundary layer thicknesses for the Franz diffusion cells were determined by the method of Keshary and Chien [11] using hydrocortisone pellets. Pellets of hydrocortisone weighing 20–25 mg were made by using compressing the powder in a Carver Laboratory Press (model 2512, Fred S. Carver, Inc., Summit, New Jersey) using 5000 pounds pressure for 1 min. The pellets were then placed on the nylon mesh support in the Franz cells. Samples were withdrawn at various times and analyzed for hydrocortisone using HPLC. A graph of ln (C_s/C_s-C_t)

TABLE 2

The $t_{50\%}$ values (time to release 50% of the drug) for the release of model drugs from sodium hyaluronate or ethyl ester core membranes laminated with either ethyl or benzyl ester membranes, dissolution medium used: 0.05 M phosphate buffer, pH 7.0, μ=0.3 M, temperature=32°C, n=5

Model drug	Core membrane	Time to release 50% of the drug ± SD deviation, min	
		Ethyl ester membrane lamination	Benzyl ester membrane lamination (lag time* in parentheses)
Hydrocortisone	Ethyl ester	12.5 ± 3.7	312.4 ± 62.0 (48.9 ± 17.5)
	Sodium hyaluronate		492.8 ± 76.5 (108.3 ± 21.2)
Hydrocortisone hemisuccinate, sodium	Ethyl ester	–	718.1 ± 74.9 (170.4 ± 25.2)
	Sodium hyaluronate		573.9 ± 40.8 (162.5 ± 17.3)
Fluorescein, sodium	Sodium hyaluronate	8.3 ± 1.7	101.5 ± 16.1
Benzyl alcohol	Ethyl ester	–	11.7 ± 4.2
Mafenide acetate	Ethyl ester	–	11.8 ± 3.0

Experiments were performed in the Franz diffusion cells.
*Lag times were calculated by extrapolation of the initial leading lines of the release profiles.

versus $S \cdot t/V$ yielded a straight line, where C_s is the saturation solubility of the drug, C_t is drug concentration at time 't', S is the surface area, and V is the volume of the medium. The slope of this profile is related to the boundary layer thickness (slope $= D/h$, where D is the diffusivity of drug in the boundary layer and h is the boundary layer thickness). Using the diffusivity of benzoic acid (5.5×10^{-6} cm^2/s) reported by Keshary and Chien [11], the diffusivity of hydrocortisone was estimated using the relationship $D_a/D_b = (M_b/M_a)^{0.5}$, where D_a and D_b are the diffusivities of substances a and b, and M_a and M_b are their molecular weights. The boundary layer thickness was calculated using the slope and D values obtained. Using the relationship between the molecular weight and diffusivity, the diffusivities of the other model compounds were estimated.

The resistance (R) offered by a layer for the mass transport of a drug is equal to the reciprocal of the permeability coefficient ($R = 1/P = h/D \cdot K$, where K is the partition coefficient) [9]. A detailed procedure to determine h, D, K for the membranes in this study is discussed in the "Physical Constants" section below. For the resistance of the boundary layer, the same relationship exists; where K (boundary layer surface:bulk solution) was assumed to be one. Assuming the resistance offered by different layers to drug diffusion to be additive, the total resistance (s/cm) and the contribution of the boundary layer resistance to the total resistance for various drugs were calculated.

Drugs covalently bound to HA

Due to the slow release of covalently bound drugs, significant error was introduced by the evaporation of buffer during the experiments in the Franz diffusion cells. The VanderKamp® sustained release apparatus (model #103906, Vankel Industries, Inc., Edison, NJ) was therefore used to study the release of drugs from these membranes. Phosphate buffer (0.05 M, pH 7.00, $\mu = 0.3$ M) with 0.2% sodium azide (antibacterial agent) was used as the dissolution medium. The volume of the medium was 100 ml and the bottles were rotated at 25 r.p.m. The temperature was maintained at 32.0° ± 0.1 °C by a Vankel circulator (model #VK-6, Vankel Industries, NJ). Samples (1 ml) were collected at various times and analyzed for the drug contents. In the case of benzyl alcohol, the release of benzyl alcohol from 100% benzyl ester membranes was compared with the release from 25%, 75% and 100% esterified benzyl ester powders.

Physical constants

The permeability coefficients of ester membranes of HA were determined using Side-Bi-Side® glass diffusion cells (model #DC100-B, Crown Glass Co., Inc., Somerville, NJ) according to a previously reported procedure [9]. The temperature in both the half-cells was maintained at 32.0° ± 0.1 °C by a constant temperature circulating water bath (VWR Scientific, Inc., San Francisco, CA). In all experiments, 0.05 M phosphate buffer, pH 7.0 was used as the donor and receptor phase fluid.

Partition coefficients (membrane:buffer) of model drugs in ethyl, benzyl, and partial benzyl ester membranes were determined using the 'solution depletion' method as described in a previous report [9] with the following minor differences. The temperature of the study was 32.0 ± 0.1 °C. Since it was noted that the radial expansion of the membranes was not significant, the volume of the hydrated membrane was calculated from the dry membrane diameter and the hydrated membrane thickness using a micrometer. The partition coefficient values were then calculated as the ratio of the concentration of drug (mass/volume) in the membrane to the concentration in the buffer [9].

Apparent diffusion coefficients values (D_{app}) of the model drugs in various ester membranes of HA were calculated using the relationship: $D_{app} = P \cdot h/K$, where P is the permeability coefficient, h is the hydrated membrane thickness, and K is the membrane:buffer partition coefficient value. Since the true diffusion path length is unknown, it was assumed that the diffusion path length is equal to the hydrated membrane thickness. The diffusion coefficients thus obtained are

effective values valid for the stated experimental conditions.

Permeability and partition coefficient values were compared statistically, using the computer program NESTAN [12]. In most cases, sample sizes were not equal and therefore, when the data showed significance, a multiple comparison was performed using the GT-2 method [12].

Assay procedures

Sodium fluorescein

A reversed phase HPLC assay was developed to determine the concentration of fluorescein sodium in partition coefficient experiments. A C_{18} column (ODS Hypersil, 5 μm, 15 cm$\times$4.6 mm) and a C_{18} guard cartridge (Rainin Instrument Co., Inc., Woburn, MA) were used. The mobile phase consisted of 0.05 M phosphate buffer (pH 7.0, μ=0.3 M), acetonitrile (buffer: acetonitrile=80:20 v/v) and 5 mM tetramethyl ammonium bromide; the pH was adjusted to 7.0 using a few drops of concentrated phosphoric acid. The chromatographic system consisted of a Waters Associates' chromatographic pump (model 6000A), a fluorescence HPLC monitor (Shimadzu, model RF-530), and an integrator (Shimadzu, Chromatopak, model C-R3A). At a flow rate of 1 ml/min, the retention volume was 3.1 ml. Linearity was observed in the concentration range 100 ng/ml to 3 μg/ml.

The samples from the release-studies had relatively high concentrations of fluorescein and therefore a simpler but less sensitive assay was used. After suitable dilution with buffer, the concentration of fluorescein sodium was determined by using a fluorescence spectrophotometer (Perkin Elmer, model 650-40, Norwalk, CT).

Mafenide acetate

A reversed phase HPLC assay was developed using a C_{18} column (MOS-1, Hypersil, 5 μm, 15 cm$\times$4.6 mm). The chromatographic system consisted of a pump (model LC-6A), UV spectrophotometric detector (model SPD-6A), integrator (Chromatopak C-R4A), auto-injector (model SCL-6A), and a system controller (model SCL-6A) (Shimadzu Corporation, Kyoto, Japan). The mobile phase consisted of 0.04 M phosphate buffer (pH 7.0, μ=0.3 M), 5% acetonitrile (v/v), 0.2% tetrahydrofuran (v/v), 4.5 mM 1-pentanesulfonic acid, and 0.5 mM 1-octanesulfonic acid. The final pH of the mobile phase was adjusted to 7.0 using a few drops of concentrated phosphoric acid. The wavelength of detection for mafenide acetate was 268 nm. The flow rate was 1 ml/min and the retention volume was found to be 6.0 ml under these conditions. Linearity was observed in the concentration range of 50 ng/ml to 20 μg/ml.

Hydrocortisone

Hydrocortisone was assayed by reversed phase HPLC, using the system described for mafenide acetate. The mobile phase consisted of 0.05 M phosphate buffer (pH 7.0, μ=0.3 M) and acetonitrile (72:28 v/v). The final pH was adjusted to 7.0 using phosphoric acid. Hydrocortisone was detected at 242 nm and the retention volume was 5.0 ml. Linearity was observed in the concentration range of 200 ng/ml to 50 μg/ml.

Hydrocortisone hemisuccinate

The same reversed phase HPLC system was used as described above for hydrocortisone. However, in this case the mobile phase was made up of 0.05 M acetate buffer (pH 4.6) and acetonitrile (70:30 v/v); the final pH was adjusted to 5.1 using glacial acetic acid. The wavelength of detection was 242 nm and the retention volume was 4.0 ml. Linearity was observed in the concentration range of 1 μg/ml to 25 μg/ml.

Benzyl alcohol

The same reversed phase HPLC system was used as described above. Benzyl alcohol was analyzed using 0.05 M phosphate buffer and acetonitrile (70:30 v/v) as the mobile phase. The wavelength of detection of benzyl alcohol was 258

nm. Linearity was observed in the concentration range of 1 μg/ml to 50 μg/ml.

During release studies involving covalently bound benzyl alcohol, it was found that benzyl alcohol oxidized to benzaldehyde and benzoic acid. The assay procedure was modified to separate benzyl alcohol and its degradation products. The mobile phase consisted of 0.15 M acetate buffer (pH 3.00) and acetonitrile (80:20 v/v). In these studies, the degradation products of benzyl alcohol were not detected.

Results and Discussion

Drug release: physical incorporation of drugs

The release of physically incorporated drugs from membranes may involve the following steps: dissolution of drug in the membrane, diffusion through the core polymer matrix, partitioning of drug from core membrane into the laminating membrane (for 'laminated' membranes), diffusion through the laminating membrane, and finally partitioning of drug from the 'unilayered' or laminating membrane into the dissolution medium.

Release from 'unilayered' membranes

Table 1 lists the $t_{50\%}$ values for drug release from 'unilayered' membranes. The release of model drugs was very rapid from Na-HA, ethyl and 100% benzyl ester membranes; the values were comparable to those obtained previously for the drug release from the microspheres of esters of HA [13]. In general, the profiles showed a non-zero order release of the drugs, with no appreciable lag time. A burst effect was not observed, suggesting minimal drug residing on the surface of the membranes. Note also that the release of hydrocortisone from 100% benzyl ester membranes was slower than that from ethyl ester membranes.

Fig. 2 is a bar diagram depicting the permeability coefficient values of all the model compounds for the various membranes used in the study. All the compounds followed the same general trend – the permeability coefficients in the

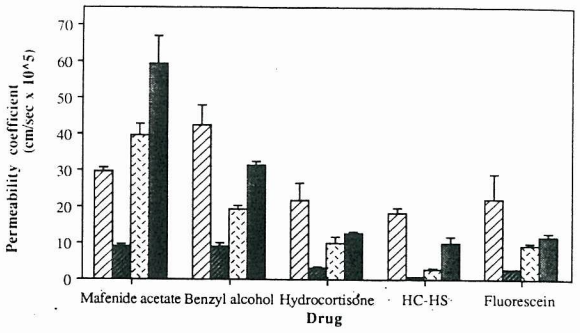


Fig. 2. Permeability coefficient values (cm/s) of model drugs for various membranes used in the study. Experiments were performed using Side-Bi-Side® diffusion cells. Medium used: 0.05 M phosphate buffer, pH 7.00, μ=0.3 M, temperature=32 °C, n=5, ☐ = ethyl ester, ☐ = benzyl ester, ☐ = 75% benzyl ester, ☐ = 50% benzyl ester of HA. Model compounds used: hydrocortisone, hydrocortisone hemisuccinate, benzyl alcohol, mafenide acetate, fluorescein sodium.

ethyl ester membranes were significantly greater ($P<0.05$) than those in 100% benzyl ester membranes. For example, the mean permeability coefficient of hydrocortisone in the ethyl ester membrane ($21.9\pm4.6\times10^{-5}$ cm/s) was significantly greater than that in 100% benzyl ester membranes ($3.1\pm0.4\times10^{-5}$ cm/s). This difference in the permeability coefficient values was probably due to the difference in the degree of hydration of these membranes (ethyl ester membrane=258% and 100% benzyl ester membrane=48%, [9]). Similarly, a comparison of benzyl ester and partial benzyl ester membranes showed that, in general, de-esterification was associated with increases in the degree of hydration and permeability coefficient values. The effect of the difference in permeability coefficient values was reflected in the $t_{50\%}$ values; for example the $t_{50\%}$ value for hydrocortisone was 61.5 ± 9.7 min in the 100% benzyl ester membrane, much larger than the 4.3 ± 0.3 min in the ethyl ester membrane. Interestingly, although a significant difference in permeability coefficients was found for benzyl alcohol in the ethyl and 100% benzyl ester membranes, no difference in the $t_{50\%}$ values was observed. The very rapid release of benzyl alcohol in the Franz diffusion cell experiments may prohibit an accurate measurement of release rate differences.

Fig. 3 shows the partition coefficients between membranes and buffer solutions for various drugs. The partition coefficients of mafenide acetate, benzyl alcohol and fluorescein sodium for all the membranes (ethyl ester, 100% benzyl ester, and partial benzyl esters) were small (less than 5). Slightly larger values were observed for hydrocortisone and hydrocortisone hemisuccinate in the ethyl ester membranes ($K=3.1\pm0.7$ and 3.5 ± 0.9, respectively). The largest partition coefficient values were those for hydrocortisone and hydrocortisone hemisuccinate in 100% benzyl ester membranes ($K=43.4\pm3.6$ and 16.3 ± 3.0, respectively). These values may suggest a partial hydrophobic nature of the 100% benzyl ester membranes as reported earlier [9]. The partition coefficients of these two drugs decreased with a decrease in the degree of esterification of the benzyl ester membranes. In these experiments, it is assumed that partitioning is an equilibrium process, not a kinetic one. Thus, the partition coefficient per se does not affect the kinetics of release. Large partition coefficient values (membrane:buffer) indicate that the drug concentration in solution at the membrane surface is small relative to that for a drug with a low partition coefficient with the same loading. This smaller concentration, in turn, is expected to provide a smaller driving force for drug release and hence a smaller rate of release if all other factors (e.g. diffusion coefficient) are equal.

Prior to release from these membranes, the drug must dissolve in the medium present in the hydrated polymer; the solubility of the drug in this medium therefore can be one of the factors governing drug release. The percent hydration values for the ethyl and the 100% benzyl ester membranes were 258 and 48%, respectively [9]. Thus, since hydration is rapid, more water is available in the ethyl ester membranes for the dissolution of the physically incorporated drugs. With the exception of hydrocortisone, all the model compounds have adequate aqueous solubility. The combined effects of large membrane:buffer partition coefficient and low capacity of the hydrated membrane to solvate the drug may explain the relatively slow release of hydrocortisone from 'unilayered' 100% benzyl ester membranes (Table 1).

For the set of Franz diffusion cells in our laboratory and the experimental conditions mentioned above, the effective boundary layer or stagnant layer thickness was calculated using hydrocortisone pellets. The boundary layer thickness was found to be 136.5 ± 19.9 μ. The hydrated thicknesses of the membranes were on the

TABLE 3

Values for total resistance (s/cm) and for the percent contribution to the total resistance# offered by membrane and boundary layer for drug release from ethyl and benzyl ester membranes; the thickness of the boundary layer was 136.5μ*

Model compounds	Ethyl ester membrane			Benzyl ester membrane		
	Total resistance 10^{-3}	Percent contribution to total resistance by		Total resistance 10^{-3}	Percent contribution to total resistance by	
		Membrane	Boundary layer		Membrane	Boundary layer
FLU	8.9	50.6	49.4	32.7	86.6	13.4
HCHS	10.4	52.4	47.6	150.4	96.7	3.3
Benzyl alcohol	4.7	49.7	50.3	13.4	82.6	17.4
Mafenide acetate	6.9	48.4	51.6	14.6	75.7	24.3
Hydrocortisone	8.9	51.6	48.4	36.4	88.2	11.8

FLU = fluorescein sodium; HCHS = hydrocortisone hemisuccinate sodium salt.

*Boundary layer thickness was determined by using the method of Keshary and Chien [11].

#Resistance by a layer for drug transport is equal to the reciprocal of the permeability [2], and it was assumed that total resistance is additive for successive layers.

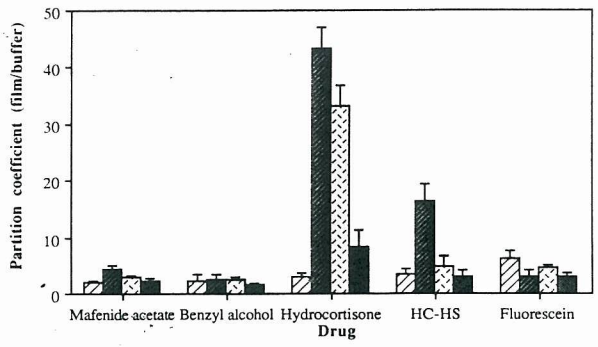


Fig. 3. Partition coefficient values (membrane:buffer) of various membranes to model compounds. Model drugs used: hydrocortisone, hydrocortisone hemisuccinate, benzyl alcohol, mafenide acetate, fluorescein sodium. Experiments were performed using the 'solution depletion' method, in which the hydrated membrane was equilibrated in the drug solution and the partition coefficient values were determined by measuring drug concentrations in the solution before and after partitioning. Medium used: 0.05 M phosphate buffer, pH 7.00, $\mu=0.3$, temperature = 32 °C, $n=4$, ▨ = ethyl ester, ▨ = benzyl ester, ▨ = 75% benzyl ester, ▨ = 50% benzyl ester.

order of 40 μ to 80 μ. Thus, the relative magnitudes of the membrane and boundary layer thicknesses alone suggest that the boundary layer could not be neglected in drug transport. Table 3 lists values of total resistance (s/cm) and the values of percent contribution to the total resistance offered by the boundary layer for the diffusion of various drugs. For ethyl ester membranes, the boundary layer resistance was approximately 50% of the total resistance for all the compounds studied. This suggests that differences in release rates in ethyl ester membranes may be masked by the boundary layer effect. In contrast, the boundary layer provided only 3 to 25% of the total resistance in benzyl ester membranes, suggesting that membrane resistance is controlling the overall release.

Release from 'laminated' membranes

The 'laminated' membranes were designed with the objective of slowing drug release, as required in various applications. Table 2 lists the $t_{50\%}$ values for the release of the model compounds from ethyl ester or Na-HA core membranes laminated with ethyl or benzyl ester membranes. In general, ethyl ester lamination did not slow the release of any drug to a great extent, as expected due to the high permeability of drugs through these membranes. For example, the $t_{50\%}$ value for hydrocortisone release was 4.3 ± 0.3 min from 'unilayered' ethyl ester membrane; this value increased to 12.5 ± 3.7 min after lamination with ethyl ester membrane.

Due to relatively low permeability values in 100% benzyl ester membranes, lamination with 100% benzyl ester membranes increased the $t_{50\%}$ values (Table 2). The effect of 100% benzyl ester lamination was greatest for hydrocortisone, hydrocortisone hemisuccinate and fluorescein sodium, with the $t_{50\%}$ values increasing from 28 to 180 times. As expected, lamination with the 100% benzyl ester membrane was more effective in slowing the release of fluorescein sodium; the $t_{50\%}$ value increased from 3.7 ± 0.9 min to only 8.3 ± 1.7 min with ethyl ester lamination, but to 101.5 ± 16.1 min with 100% benzyl ester lamination. The permeability values of all the drugs in 100% benzyl ester membranes are significantly less than in ethyl ester membranes (3 to 26 times for different drugs). However, lamination with 100% benzyl or ethyl ester membranes showed greater differences than could be predicted by permeabilities alone. The results of release studies can be explained only qualitatively using the permeability coefficients, but there are quantitative discrepancies.

Two of the steps involved in the release of drugs from laminated membranes are partitioning from the core membrane to the laminating membrane and from the laminating membrane to the buffer solution. Low membrane:buffer partition coefficients for mafenide acetate, fluorescein sodium and benzyl alcohol suggest that membrane:buffer partitioning was insignificant in drug release. However, for hydrocortisone and hydrocortisone hemisuccinate, large partition coefficient values suggested that the partitioning effect significantly reduced the release rate. For these two drugs, the partition coefficient values for the drug between ethyl and benzyl (ethyl:benzyl) ester membranes were estimated by dividing the aqueous partition coefficient for

the ethyl ester membrane by the value for the 100% benzyl ester membrane. Thus, the expected partition coefficient values for hydrocortisone and hydrocortisone hemisuccinate between the ethyl and 100% benzyl ester membranes (ethyl:benzyl) were 0.07 and 0.2, respectively. These drugs incorporated in the ethyl ester core membrane would therefore preferentially partition into the laminating 100% benzyl ester membranes. The $t_{50\%}$ values for these two drugs in the 100% benzyl ester laminated membranes were 312.4 ± 62.0 min and 718.1 ± 74.9 min, respectively. The rapid release of hydrocortisone can be partially explained by the greater partitioning from the core ethyl ester membrane into the laminating 100% benzyl ester membrane. However, the preferential partitioning from the core ethyl ester membrane into the laminating 100% benzyl ester membrane would be expected to increase the release rate. Thus, the partitioning results may not explain slow release with 100% benzyl ester lamination.

Other factors may hinder the process of quantitating the effect of lamination. It may be true that the method of estimation of ethyl:benzyl partition coefficient is faulty, yielding erroneous results. Also, the core and laminating membranes are not homogeneous. This may create a thin barrier layer between these membranes. The assumption that the partitioning is an equilibrium process may also be incorrect; if so, the rate of partitioning may contribute to the release rates.

Graphs of percent released versus square root of time yielded linearity for all the drugs with 'unilayered' or 'laminated' systems. Fig. 4 is a representative graph for hydrocortisone and hydrocortisone hemisuccinate release from the various membranes. A square root of time relationship is observed in two models for drug release: a moving boundary model [14] and a model with semi-infinite domain [15]. In the case of the moving boundary model [14], drug is assumed to be suspended in the matrix and two regions are proposed – a region with dissolved drug and a region with suspended drug. The dissolution of solid particles is assumed to be instantaneous; the release of drug depends on the movement of a boundary between these two regions. The semi-infinite domain model assumes that drug release occurs from a region that is bounded only at the drug/medium interface. The concentration at the surface ($x=0$) is assumed to be zero at all times and the initial concentration of the substance in the membrane is assumed to be constant. As with the moving boundary model, the amount of substance diffusing in the medium is related to the square root of time and the diffusion coefficient [15]. In both models, the diffusion of drug in the matrix is the rate-limiting process in drug release. The linearity observed in the square root of time relationship (Figure 4) in the present study suggests that diffusion of drugs through the membrane matrix is rate-limiting.

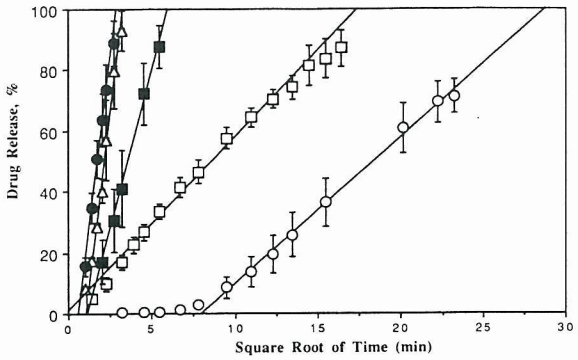


Fig. 4. Square root of time relationship for hydrocortisone and hydrocortisone hemisuccinate release from various ester membranes of hyaluronic acid. Experiments were performed in Franz diffusion cells. Dissolution medium used: 0.05 M phosphate buffer, pH 7.00, $\mu=0.3$ M, $n=5$, temperature $=32\,^{\circ}C$, r=coefficient of correlation for the linear regression, –●– = hydrocortisone hemisuccinate in ethyl ester membrane ($r=0.995$), –△– = hydrocortisone hemisuccinate in sodium hyaluronate membrane ($r=0.995$), –■– = hydrocortisone in ethyl ester core membrane laminated with ethyl ester membrane ($r=0.997$), –○– = hydrocortisone in ethyl ester core membrane laminated with benzyl ester membrane ($r=0.998$).

Drug release: covalently bound drugs

Fig. 5 shows the release of hydrocortisone from hydrocortisone/ethyl ester membranes in which 25% of the carboxylic acid groups are esterified

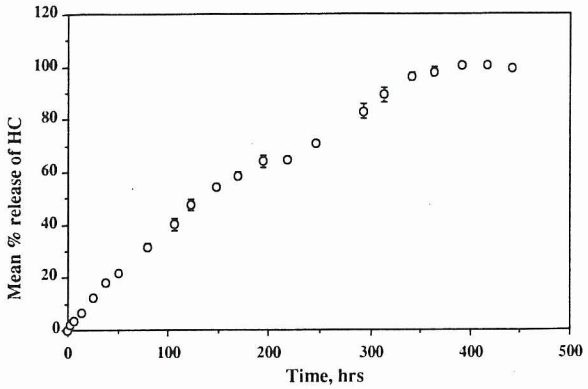


Fig. 5. Release of hydrocortisone (HC) from hydrocortisone ester membranes (25% hydrocortisone and 75% ethyl ester). Experiments were performed in a rotating bottle apparatus, 25 r.p.m., dissolution medium used: 0.05 M phosphate buffer, pH 7.00, μ=0.3 M, temperature=32°C, n=7. Change in the release rate at 200–250 h may be due to the radial expansion of membrane disks during this period.

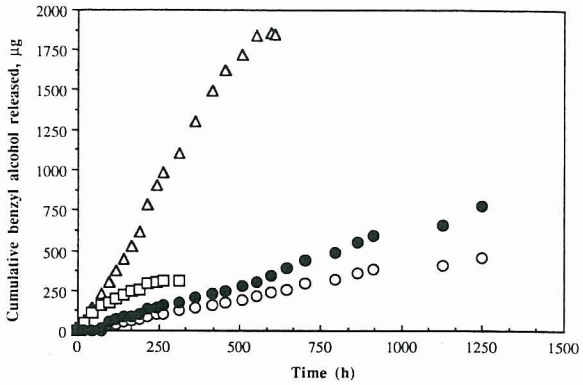


Fig. 6. Release of benzyl alcohol from benzyl ester membranes/powders, ○ = 100% benzyl ester membranes (n=3), ● = 100% benzyl ester powder (n=2), △ = 75% benzyl ester powder (n=1), □ = 25% benzyl ester powder (n=2). Experiments were performed using rotating bottle apparatus, 25 r.p.m. Dissolution medium used: 0.05 M phosphate buffer, pH 7.00, μ=0.3 M, temperature=32°C.

with hydrocortisone and the remaining groups are esterified with ethyl alcohol. Drug release was found to be very slow relative to the release of physically incorporated hydrocortisone. The time to release 50% hydrocortisone was 137 h; in contrast, the longest $t_{50\%}$ value achieved with physical incorporation of the drug was only 8 h (Table 2). The 137 h value is comparable to the value 110–115 h obtained for hydrocortisone release from microspheres of the same polymer [13]. At 200–250 h, the release-profile showed a change in the release rate (Fig. 5), which may be due to the sudden radial expansion of membranes that occurred during that period.

Fig. 6 shows the release of benzyl alcohol from benzyl ester membranes with different degrees of esterification. For lower degrees of esterification, the release was more rapid and was complete in a significantly shorter time. The $t_{50\%}$ values were approximately 3 days and 10 days for 25% esterified and 75% esterified benzyl ester powders, respectively. In contrast, release from fully esterified benzyl ester membranes and powders was very slow; only about 25% of the benzyl alcohol was released in approximately 38 days. The curves for the 25% and 75% esterified membranes are nearly superimposible for early time points. The curve for the 100% esterified membrane is not coincident with the others at any time, suggesting that the mechanism of release in these membranes is different, perhaps due to their greater hydrophobicity or lack of polymer charge.

Hydrolysis studies on water soluble benzyl (25% benzyl ester and 75% sodium) and hydrocortisone (25% hydrocortisone ester and 75% sodium) esters of HA were performed by modifying the procedure reported by Goei et al. [16]. In the present studies, the temperature was maintained at 32.0° ± 0.1°C and the pH of the phosphate buffer used as the medium was 7.00. The apparent first-order rate constants for the hydrolysis of hydrocortisone and benzyl esters were 0.029 ± 0.002 h^{-1} and 0.016 ± 0.001 h^{-1}, respectively. The corresponding half-lives for hydrolysis were 23.9 ± 1.6 h and 43.1 ± 3.9 h, respectively. The slow release of these covalently bound drugs can be partially explained by these hydrolysis half-lives. The half-lives for hydrolysis are far longer than the total time for release of physically incorporated drugs. Not surprisingly, the release of covalently bound drug is slower. In addition, the estimated half-life for release of covalently bound benzyl alcohol (approximately 1800 h assuming zero order release) is much greater than the value for hydrocortisone (137

h); this is consistent only qualitatively with the greater hydrolysis half-life for benzyl alcohol. It is important to remember that during the hydrolysis kinetics experiments, the polymers are completely dissolved in the medium, and all the ester groups are exposed for drug hydrolysis. In contrast, during the release studies the polymer membranes/powders are hydrated, but not completely dissolved. Thus, only a fraction of the ester groups is 'available' for hydrolysis.

For this system, a classical definition of the solubility of a substance may not be of value because the molecular dispersion (solvation) of even a portion of the polymer chain will allow ester hydrolysis. The effective solubility of this polymer may be defined as an exposure of ester linkages on the polymer backbone to the aqueous environment on a molecular level so as to allow the hydrolysis of ester bonds. The partial hydrophobic nature of the 100% benzyl ester may be the cause of its smaller value of percent hydration and of its lower "solubility" that is, fewer ester linkages per unit volume of water available for hydrolysis. Partial benzyl esters have greater water "solubility"; that is, more ester groups per unit volume of water are exposed for hydrolysis. This also may explain partially the more rapid release of benzyl alcohol from the partial benzyl ester membranes. Therefore, in addition to the rate of hydrolysis, the "solubility" of the polymer may be another factor controlling the release of drugs covalently bound to HA. Hume et al. [17] also observed that the release of methyl prednisolone covalently bound to HA was faster from a soluble polymer than from sparingly soluble polymers.

The half-lives of permeation of hydrocortisone and benzyl alcohol were calculated from the permeation rate (permeation rate = permeability coefficient/membrane thickness); the half-lives of permeation were found to be 0.019 h and 0.006 h in benzyl ester membranes. The half-lives of hydrolysis were found to be 23.9 and 43.1 h, respectively. The comparison of these values suggests that the diffusion process is rapid compared to hydrolysis and does not limit the release of drugs when covalently bound to HA.

The results also suggest that some other process or processes are involved in the release of drugs covalently bound to HA. An altered microclimate pH within the membrane may affect the rate of hydrolysis of ester linkages. The pKa value of the carboxylic acid groups in HA was found to be about 3.3; therefore at pH 7.0, essentially all the carboxylic acid groups must be ionized. The microclimate pH therefore may decrease with the release of covalently bound drugs. Sodium, phosphate, chloride, hydroxyl and other ions are known to have very high diffusivity in aqueous media and are expected to buffer the environment inside the membrane, keeping the internal microclimate pH near that of the bulk dissolution medium. Without experimental verification, however, the effect of de-esterification on the microclimate pH in the polymer matrix is unknown.

In summary, the release of model compounds was found to be rapid when the compounds were physically incorporated. Lamination with 100% benzyl ester membranes seemed to slow the release of drugs. These laminated membranes can release drugs over a period of 2–3 days and could find application in wound healing [18], ocular [19] and transdermal [20] drug delivery systems. Drugs covalently bound to HA were released over a long period (time for 25% release of benzyl alcohol: 900 h). These polymers may have applications in sustained release. In these applications, the polymer offers the advantages of probable biocompatibility and bioerodibility [21]; the hyaluronic acid skeleton remaining after drug release is expected to be degraded by endogenous hyaluronidases and absorbed by the body. Thus, the results of this study suggest that depending upon the end use and length of treatment required, membranes of a suitable ester of hyaluronic acid can be selected to achieve desired drug release. Since in vitro and in vivo release rates can differ significantly, confirmation of the utility of hyaluronate ester membranes awaits future in vivo testing.

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