Drug Release from Hydroxypropyl Cellulose–Polyvinyl Acetate Films

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Abstract □ The release of methapyrilene, pentobarbital, and salicylic acid dispersed in films composed of different ratios of hydroxypropyl cellulose and polyvinyl acetate was investigated. The results indicate that drug release follows a diffusion-controlled matrix model, where the quantity released per unit area is proportional to the square root of time. The release rate was found to increase with increasing hydroxypropyl cellulose–polyvinyl acetate ratios, with the logarithm of the rate constant proportional to the fraction of hydroxypropyl cellulose in the matrix. The release rates were also shown to be proportional to drug concentration but independent of film thickness.

Keyphrases □ Drug release (methapyrilene, pentobarbital, salicylic acid) from hydroxypropyl cellulose–polyvinyl acetate films—diffusion-controlled matrix model, effects of polymer ratio and drug concentration on release kinetics □ Polymer films, hydroxypropyl cellulose–polyvinyl acetate—effects of polymer ratio and drug concentration on drug (methapyrilene, pentobarbital, salicylic acid) release kinetics

The use of drugs dispersed in inert polymer matrixes to achieve controlled release by diffusion has received considerable attention. This method has long been favored for the preparation of sustained-release tablets for oral ingestion (1–3). More recently, the concept has been suggested for other uses including catheters coated with antibiotic-impregnated polymers (4), prolongation of the release of pilocarpine in eye administration (5), long-term buccal absorption of drugs (6), dermatological applications (7, 8), and long-acting implants (9, 10).

Higuchi (11) suggested that release from a planar system having dispersed drug in a homogeneous matrix should follow the relationship:

$$Q = [D(2A - C_s)C_s t]^{1/2} \quad \text{(Eq. 1)}$$

where Q is the amount of drug released after time t per unit exposed area, D is the diffusivity of the drug in the matrix, A is the initial drug concentration, and C_s is the drug solubility in the matrix. He later derived a similar relationship for planar release from a granular matrix system in which diffusion occurs through channels (12):

$$Q = \left[\frac{D\epsilon}{\tau}(2A - \epsilon C_s)C_s t\right]^{1/2} \quad \text{(Eq. 2)}$$

where D and C_s refer to diffusivity and solubility in the permeating fluid, respectively; τ is the tortuosity of the matrix; and ϵ is the porosity of the matrix. Although the two equations are for different mechanisms, they both describe drug release as being linear with the square root of time:

$$Q = k_H t^{1/2} \quad \text{(Eq. 3)}$$

where for the homogeneous matrix system:

$$k_H = [D(2A - C_s)C_s]^{1/2} \quad \text{(Eq. 4)}$$

and for the granular matrix system:

$$k_H = \left[\frac{D\epsilon}{\tau}(2A - \epsilon C_s)C_s\right]^{1/2} \quad \text{(Eq. 5)}$$

The validity of the relationships has since been confirmed experimentally by a number of workers using various systems (13–25).

The release rate of drugs from a polymer matrix may be controlled by the concentration, surface area, leaching solvent, and polymer system. However, where a specific dose, size, and solvent are defined by required use, the release rate in a specific polymer is limited by the physicochemical properties of the drug. It would, therefore, be desirable to achieve a wide range of release rates for a drug by simple modification of a polymer system. Shah and Sheth (26) demonstrated that modifying the hydroxypropyl methylcellulose–ethylcellulose ratio in barrier films could drastically alter the permeability of dye.

This paper describes studies of drug release from thin films containing drug dispersed in physical mixtures of hydroxypropyl cellulose and polyvinyl acetate. The polymers were selected based on film-forming characteristics, compatibility, demonstrated pharmaceutical applicability, and differing solubility and hydrophilicity characteristics. Pentobarbital, methapyrilene, and salicylic acid were selected as model drugs to study the effect of polymer ratio modifications on drug release kinetics.

EXPERIMENTAL

Chemicals—The hydroxypropyl cellulose[1] used in these studies was a food or pharmaceutical grade, with an average molecular weight of 100,000, which gives a viscosity of 75–150 cps as a 5% water solution. The polyvinyl acetate[2] used had an average molecular weight of 500,000 and a viscosity of 90–110 cps as an 8.6% solution in benzene. Sodium pentobarbital and salicylic acid were USP grade, while methapyrilene hydrochloride was NF grade. The pH 7.0 sodium phosphate buffer was made 0.5 N in phosphate (0.274 mEq/ml Na^+) by dissolving 28.9 g $Na_2HPO_4 \cdot 7H_2O$ and 8.05 g $NaH_2PO_4 \cdot H_2O$/liter of solution (27).

Film Preparation—Generally the films were cast from a solution containing 10% solids (drug plus polymer), using a methylene chloride–methanol mixture (9:1) as the solvent. The polymers were

[1] Klucel LF, Hercules, Inc., Wilmington, DE 19899
[2] Gelva V-100, Monsanto Co., St. Louis, MO 63166

Table I—Comparison between First-Order and Q *versus* $t^{1/2}$ Treatments of Pentobarbital Release Rate Data

Concentration[a], %	Hydroxypropyl Cellulose–Polyvinyl Acetate Ratio	Number of Runs	First Order		Q *versus* $t^{1/2}$	
			t_{lag}, min[b]	Correlation Coefficient[b]	t_{lag}, min[b]	Correlation Coefficient[b]
36.4	10:0	4	−3.2	0.996	3.1	0.997
18.2	10:0	4	−14.0	0.957	2.6	0.993
18.2	9:1	2	−9.8	0.986	2.8	0.996
18.2	8:2	2	−6.5	0.986	2.3	0.993
18.2	6:4	2	−84.0	0.982	1.4	0.996
18.2	4:6	4	−142.0	0.983	0.3	0.998
18.2	2:8	4	−176.0	0.981	0.6	0.998
18.2	1:9	4	−176.0	0.982	0.7	0.998
18.2	0:10	3	−214.0	0.979	1.0	0.997
9.1	0:10	4	−199.0	0.981	1.3	0.990

[a] Weight of drug per weight of dry film. [b] All t_{lag} and correlation coefficient values expressed are mean values.

Table II—Effect of Film Thickness on Pentobarbital Release Rate Constant and Half-Life

Drug Concentration[a], %	Hydroxypropyl Cellulose–Polyvinyl Acetate Ratio	Wet Film Thickness Setting, mm	Dry Film Thickness[b], μm	k_H, mg cm^{-2} min$^{-1/2}$	Correlation Coefficient	$t_{1/2}$, min
36.4	10:0	0.64	44.0 ± 1.1	0.33	0.996	14.8
		1.27	56.2 ± 2.1	0.29	0.996	25.2
		1.91	100.2 ± 4.6	0.35	0.998	49.8
		2.54	109.3 ± 3.3	0.32	0.998	60.2
18.2	4:6	0.64	61.4 ± 4.9	0.032	0.999	360
		1.27	113.2 ± 9.9	0.034	0.999	1,280
		1.91	145.0 ± 8.0	0.034	0.999	2,280
		2.54	204.2 ± 3.1	0.037	0.997	3,850
18.2	1:9	0.64	76.8 ± 1.2	0.0101	0.996	8,520
		1.27	118.4 ± 2.6	0.0102	0.999	19,200
		1.91	202.8 ± 2.2	0.0101	0.998	50,900
		2.54	268.0 ± 2.4	0.0102	0.998	83,600

[a] Weight of drug per weight of dry film. [b] Thickness = mean ± standard deviation of five measurements.

added as dry powders by slow addition to the vigorously stirring solution. Salicylic acid was added as the dry powder, and pentobarbital and methapyrilene were incorporated from stock methylene chloride solutions prepared from the drug salts. The pentobarbital solution containing 60.8 mg/ml was prepared by dissolving 12.0 g sodium pentobarbital in 50 ml water, adding 5 ml concentrated hydrochloric acid, extracting three times with 50-ml portions of methylene chloride, and adjusting the volume of the combined extract to 180 ml. The methapyrilene base solution containing 58.5 mg/ml was prepared by dissolving 12.0 g methapyrilene hydrochloride in 60 ml water, adding 10 ml 5 *N* NaOH, extracting three times with methylene chloride, and adjusting the volume of the combined extract to 180 ml.

Films were cast from the solutions at various wet thicknesses (0.64–2.54 mm) using a knife[3] on Teflon-coated plate glass. The films were allowed to air dry at least 48 hr before evaluation. The percent drug in the dry film was calculated from the ratio of drug and polymer weights used.

Release Rate Determination—Rectangular films measuring 2.2 × 4.0 cm (8.8 cm^2) were obtained by cutting a selected portion of the cast film with a razor blade, using a microscope cover glass as a template. The film was weighed on an analytical balance, and the thickness was measured at the four corners and center with a micrometer[4]. A thin coating of high vacuum silicone lubricant[5] was applied to a 2.54 × 7.62-cm microscope slide; the film was carefully pressed into the slide, making sure that all edges adhered and no lubricant touched the exposed surface. Silicone lubricant was found superior to solvent-based adhesives by virtue of its noninteracting compatibility with the film. In addition to its capacity to maintain adhesion of the film to the slide, its water repellency provided secondary assurance of only single surface release. When the exposed surface of film was coated with silicone lubricant, only insignificant drug release was obtained. The slide was placed at an angle into a 250-ml beaker in a 37° water bath containing 200 ml of pH 7.0 buffer preheated to 37°. A nonagitated system was selected to eliminate any effect of turbulence on the release rate as well as to assure that no disruption of the film occurred. Periodic assay samples (approximately 10/run) were obtained by removing the slide, stirring the solution, and pipeting a 5-ml sample. The slide was quickly reinserted, making sure that the film remained completely immersed throughout the release study. The beaker was kept covered[6] throughout the run to prevent evaporation. The run was continued for at least 7 hr or until the assays indicated complete release had occurred. With many extremely slow releasing systems, the runs were continued for 31 hr.

All samples were assayed by UV spectrophotometry[7]. Pentobarbital was assayed at 240 nm in 0.1 *N* NH_4OH, methapyrilene at 312 nm in 0.1 *N* HCl, and salicylic acid at 297 nm in 0.1 *N* NaOH.

RESULTS AND DISCUSSION

Kinetics of Drug Release—Adherence of drug release to the Q *versus* $t^{1/2}$ relationship discussed in Eqs. 1–5 requires that the drug concentration in the undepleted zone far exceeds the solution concentration at the interface ($A \gg C_s$ or ϵC_s). A first-order release relationship, with the release rate proportional to the drug concentration remaining within the film, might also be considered possible if this condition does not exist:

$$\ln (Q_\infty - Q) = -k_1 t + \ln Q_\infty \qquad \text{(Eq. 6)}$$

Sciarra and Gidwani (7, 8) reported that gentian violet, cetylpyridinium chloride, and benzalkonium chloride were released from

[3] Gardner Laboratories, Bethesda, MD 20014
[4] L. S. Starrett Co., Athol, Mass.
[5] Dow Corning Corp., Midland, MI 48640
[6] Parafilm M, American Can Co., Neenah, Wis.
[7] Coleman model 124, Perkin-Elmer Co., Norwalk, Conn.

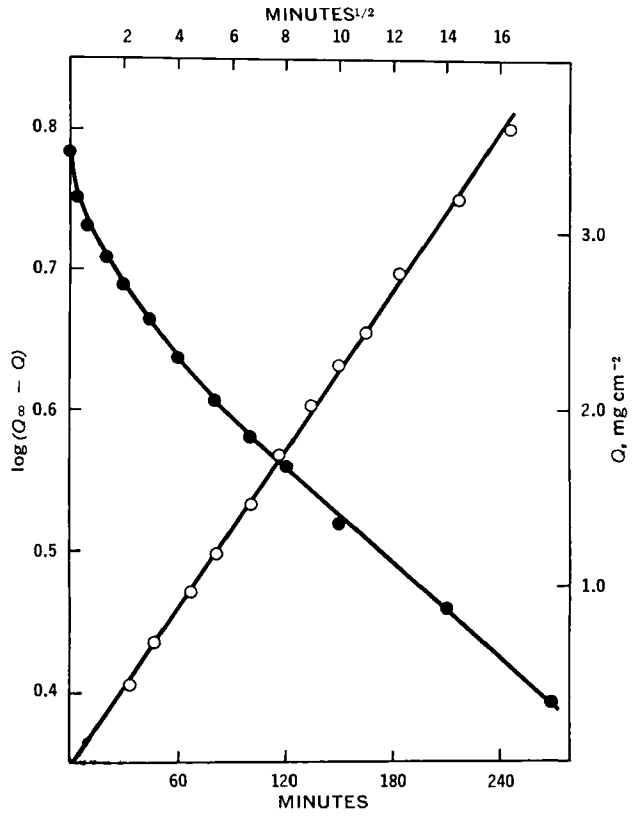

Figure 1—*Comparison between first-order release treatment and* Q versus $t^{1/2}$ *treatment of data from a film containing 26.3% methapyrilene at a 5:5 ratio of hydroxypropyl cellulose–polyvinyl acetate. Key:* ●, *log* (Q_∞ − Q) versus t; *and* ○, Q versus $t^{1/2}$.

various films by this first-order relationship. Schwartz *et al.* (22) rigorously examined the release data from wax matrixes by both first-order and the *Q versus* $t^{1/2}$ treatments before concluding that the latter was most applicable.

The release data obtained in this study were treated by both methods to ascertain which relationship gave the best fit. The correlation coefficients for the best statistical lines and the lag times (time intercept extrapolated to $Q = 0$) were used as the principal criteria for evaluation. The data comparing the two treatments for a number of pentobarbital systems where multiple runs were made are shown in Table I. Although the relatively high correlation coefficients obtained with first-order treatment indicate that this type of release can easily be misconstrued as following such a relationship after an initial surge of drug, the data continually showed the *Q versus* $t^{1/2}$ relationship to be clearly superior. The curvature with first-order treatment is shown in Fig. 1, a representative graph obtained when the data for a methapyrilene release experiment are plotted by both equations.

The linear relationships obtained by plotting *Q versus* $t^{1/2}$ generally gave correlation coefficients greater than 0.995. When plotted, deviation appeared to be random rather than due to any curvature. In experiments run through drug exhaustion, linearity generally held through greater than 75–80% of drug release, with negative deviation occurring in the last 10–20%. This latter deviation may be attributed to exhaustion of the drug suspension phase when the diffusion gradient distance in the film equals the film thickness. Unevenness in film thickness, where depletion of the suspension phase would be nonuniform, could also cause this negative deviation.

The lag times necessary to establish the release mechanism were generally no greater than several minutes. However, they appeared to become more significant with slower release rates, and some early assay points were observed to indicate deviation from linearity in the initial release stages.

The derivation of the *Q versus* $t^{1/2}$ relationship requires a constant concentration gradient throughout release. Therefore, the drug concentration in the buffer solution must remain insignificant compared to its solubility. This condition was easily met with salicylic acid and methapyrilene, where the maximum concentration that could be encountered with complete drug release was well below 1% of its solubility. In the case of pentobarbital, this value could rise to as high as 10% of solubility. However, the experimental results gave no indication of this causing deviation from the linear relationship.

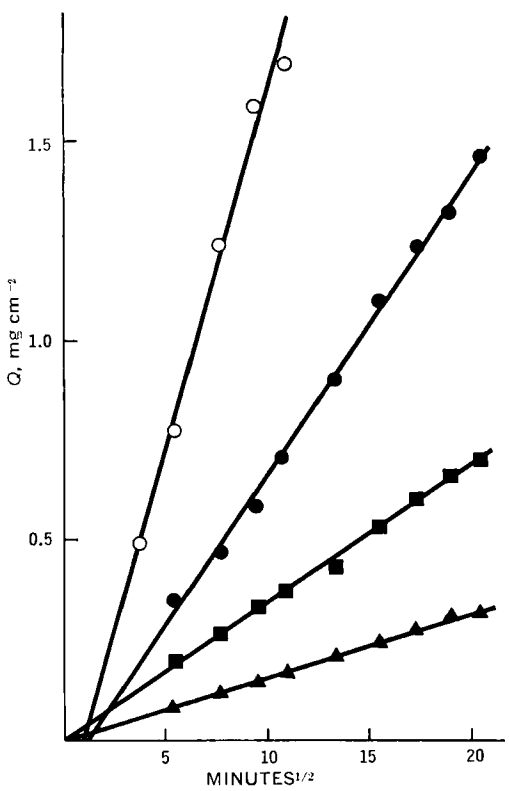

Figure 2—*Drug release from films containing 18.2% pentobarbital at different hydroxypropyl cellulose–polyvinyl acetate ratios. Key:* ○, *8:2;* ●, *6:4;* ■, *4:6; and* ▲, *2:8.*

Effect of Film Thickness—The release rate constant, k_H, has the dimensions of weight per area per square root of time and is, therefore, independent of film thickness. The constancy of k_H with varied film thickness is shown in Table II, where the results obtained with several pentobarbital systems are compiled.

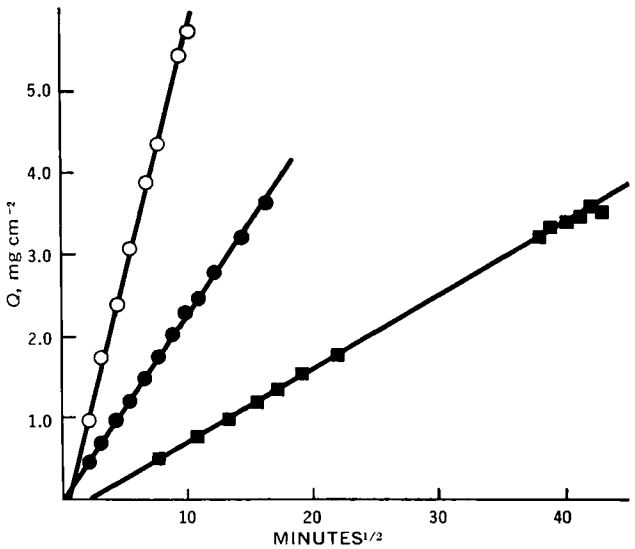

Figure 3—*Drug release from films containing 26.3% methapyrilene at different hydroxypropyl cellulose–polyvinyl acetate ratios. Key:* ○, *8:2;* ●, *5:5; and* ■, *2:8.*

Table III—Effect of Hydroxypropyl Cellulose–Polyvinyl Acetate Ratio on the Release Rate from Films Containing 26.3% Methapyrilene

Hydroxypropyl Cellulose–Polyvinyl Acetate Ratio	Dry Film Thickness[a,b], μm	k_H, mg cm^{-2} min$^{-1/2}$	Correlation Coefficient	$t_{1/2}$, min
10:0	210 ± 7.4	0.549	0.999	33
9:1	210 ± 6.5	0.593	0.997	28
8:2	216 ± 9.1	0.497	1.000	42
7:3	181 ± 7.8	0.272	0.995	99
6:4	218 ± 4.4	0.217	0.998	226
5:5	208 ± 4.6	0.225	0.999	190
4:6	142 ± 4.5	0.191	0.997	124
3:7	157 ± 3.3	0.094	0.998	630
2:8	136 ± 3.3	0.089	0.999	523
1:9	131 ± 6.0	0.075	0.998	681
0:10	185 ± 0.9	0.074	0.996	1404

[a] Thickness = mean ± standard deviation of five measurements. [b] All films cast using a wet thickness setting of 2.54 mm.

Although film thickness does not affect the rate constant, it determines the duration of drug release. By utilizing Eq. 3, the time, t_F, required for release of any fraction, F, of the drug in the film may be expressed as:

$$t_F = \left[\frac{Q_\infty F}{k_H}\right]^2 \quad \text{(Eq. 7)}$$

Additionally, the initial amount of drug in the film, $Q_\infty \times$ area, equals the concentration, A, times the volume. Dividing both sides by area, Q_∞ may be equated to Ah, where h is the film thickness. By substituting in Eq. 7, t_F is shown to be proportional to the square of thickness:

$$t_F = \left[\frac{AF}{k_H}\right]^2 h^2 \quad \text{(Eq. 8)}$$

The calculated values of 50% drug release ($t_{1/2}$) are included in Table II. Since the fractional time is proportional to the square of the fraction released, the calculated times for complete drug release would be four times the half-lives. However, since negative deviation from linearity occurs in the last stages of release, essentially complete depletion would be expected to take somewhat longer.

Effect of Polymer Ratio—Altering the hydroxypropyl cellulose–polyvinyl acetate ratio in the polymer composite mixture markedly affected the release rate (Figs. 2 and 3); the results obtained with the three drugs tested are compiled in Tables III–V. The results, with some exceptions, showed an acceleration of the release rate with an increasing proportion of hydroxypropyl cellulose in the polymer matrix. Pure hydroxypropyl cellulose gave k_H values seven to eight times those obtained with pure polyvinyl acetate. Based on Eq. 8, this would correspond to half-lives 50–75 times longer with polyvinyl acetate films than with hydroxypropyl cellulose films of identical thickness and concentration. The variability from this range indicated in Tables III–V, with half-lives being 42, 37, and 348 times greater for polyvinyl acetate films, is due to differences in the film thicknesses. Although the release rates differed substantially, there appeared to be little effect on the release mechanism as shown by the constancy of the *Q versus* $t^{1/2}$ relationship.

Table IV—Effect of Hydroxypropyl Cellulose–Polyvinyl Acetate Ratio on the Release Rate from Films Containing 20.0% Salicylic Acid

Hydroxypropyl Cellulose–Polyvinyl Acetate Ratio	Dry Film Thickness[a,b], μm	k_H, mg cm^{-2} min$^{-1/2}$	Correlation Coefficient	$t_{1/2}$, min
10:0	189 ± 2.8	0.403	0.997	28
9:1	223 ± 5.6	0.336	0.997	57
8:2	210 ± 0.9	0.328	0.993	53
7:3	229 ± 2.5	0.241	0.998	116
6:4	209 ± 6.6	0.216	0.984	120
5:5	204 ± 7.9	0.175	0.997	175
4:6	145 ± 0.5	0.115	0.997	206
3:7	156 ± 3.4	0.122	0.997	210
2:8	181 ± 7.9	0.112	0.998	338
1:9	222 ± 4.3	0.073	0.998	1190
0:10	162 ± 1.8	0.057	0.996	1040

[a] Thickness = mean ± standard deviation of five measurements. [b] All films cast using a wet thickness setting of 2.54 mm.

Table V—Effect of Hydroxypropyl Cellulose–Polyvinyl Acetate Ratio on the Release Rate from Films Containing 18.2% Pentobarbital

Hydroxypropyl Cellulose–Polyvinyl Acetate Ratio	Wet Thickness Setting, mm	Dry Film Thickness[a], μm	k_H, mg cm^{-2} min$^{-1/2}$	Correlation Coefficient	$t_{1/2}$, min
10:0	1.27	98 ± 5.4	0.225	0.995	20
9:1	2.54	163 ± 3.4	0.224	0.995	57
8:2	1.27	87 ± 6.4	0.178	0.987	25
6:4	2.54	236 ± 7.1	0.0767	0.997	1,010
4:6	1.91	145 ± 8.0	0.0342	0.999	2,280
2:8	1.27	94 ± 1.9	0.0161	0.999	3,630
1:9	1.27	118 ± 2.6	0.0102	0.999	19,200
0:10	1.91	210 ± 15.5	0.0260	0.998	6.960

[a] Thickness = mean ± standard deviation of five measurements.

Attempts to relate mathematically the release rate constant, k_H, to the polymer ratio yielded the best results when log k_H was plotted against the fraction of hydroxypropyl cellulose in the composite matrix. The relationship between the rate constant and polymer ratio may be empirically depicted by the following equation:

$$\log k_H = k_R F_H + \log k_p \quad \text{(Eq. 9)}$$

where k_R is a constant specific for each drug and concentration, F_H is the fraction of hydroxypropyl cellulose in the matrix, and k_p approximates k_H in pure polyvinyl acetate ($F_H = 0$). Table VI shows the results obtained when applying this relationship to the three drugs tested, and Fig. 4 graphically depicts the results with salicylic acid and pentobarbital.

The value for k_H obtained in the pentobarbital system with the pure polyvinyl acetate matrix deviated considerably from the relationship. Repeated determinations of k_H in pure polyvinyl acetate gave reproducible results, indicating the validity of this value. The most likely cause of this deviation would appear to be the plasticizing effect of hydroxypropyl cellulose on polyvinyl acetate. With total elimination of hydroxypropyl cellulose, mechanical changes in the film matrix alter the release rate from that otherwise expected. This deviation may not occur with methapyrilene and salicylic acid because the drugs themselves provide sufficient plasticity.

Although hydroxypropyl cellulose is water soluble, even with this pure polymer as the matrix no visual dissolution of the films was observed until all of the drug was released. Since the proposed diffusion mechanism requires maintenance of the matrix, adherence to the *Q versus* $t^{1/2}$ relationship confirms the observations. Lapidus and Lordi (14, 21) likewise observed adherence to the diffusion-controlled matrix model with drug release from water-soluble hydroxypropyl methylcellulose.

The best explanation to account for the large k_H changes with

Table VI—Linear Relationship of Log k_H to Hydroxypropyl Cellulose Fraction in the Polymer Matrix

Drug	Concentration, %	Number of Ratios Tested	k_R[a]	Log k_p[a]	Correlation Coefficient
Salicylic acid	20.0	11	0.83	−1.196	0.973
Methapyrilene	27.3	11	1.01	−1.209	0.950
Pentobarbital	18.2	7[b]	1.58	−2.110	0.985

[a] k_R and log k_p are defined by Eq. 9. [b] Covers hydroxypropyl cellulose–polyvinyl acetate ratio range of 10:0–1:9.

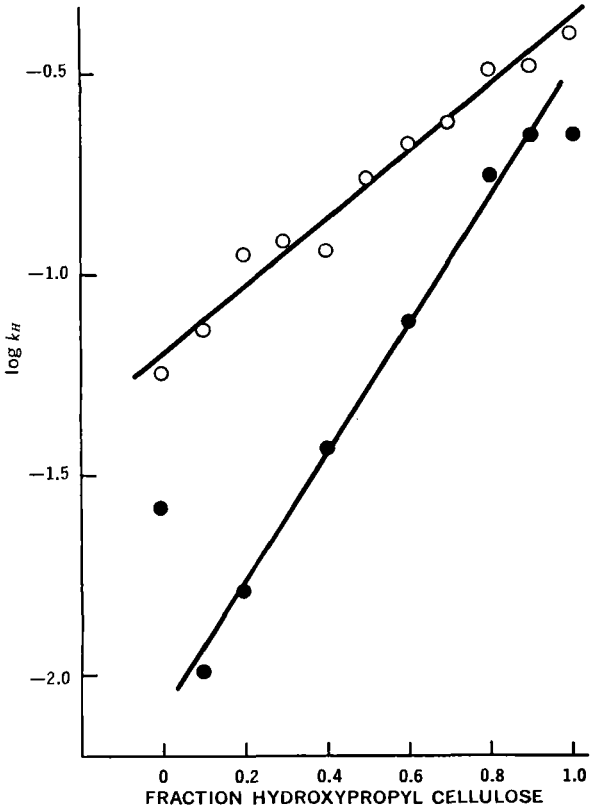

Figure 4—*Relationship of log* k_H *to fraction of hydroxypropyl cellulose in the polymer matrix. Key:* ○, *20.0% salicylic acid films; and* ●, *18.2% pentobarbital films.*

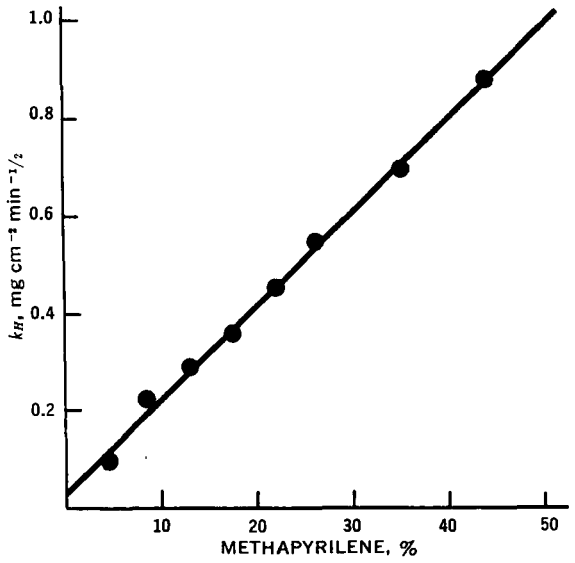

Figure 5—*Relationship of* k_H *to percent methapyrilene in 100% hydroxypropyl cellulose films.*

polymer ratio in terms of the parameters shown in Eqs. 4 and 5 would be through consideration of the hydrated film obtained upon immersion in the buffer as the actual diffusion matrix. Weight gain studies with films suspended in a 100% relative humidity atmosphere at 37° showed very rapid equilibration, with the percentage moisture absorbed increasing with hydroxypropyl cellulose content from about 4% with pure polyvinyl acetate to 32% with pure hydroxypropyl cellulose. In terms of the homogeneous matrix system (Eq. 4), the increasing k_H values obtained with increasing hydroxypropyl cellulose may occur primarily through higher solubility of the drug, C_s, in the more hydrophilic matrix. Alternatively, in terms of the granular matrix system (Eq. 5), increased porosity, ϵ, and decreased tortuosity, τ, would accompany the greater hydration of films containing increasing ratios of hydroxypropyl cellulose.

When the release rate constants of the different drugs were compared, after adjustment for drug concentration, methapyrilene and salicylic acid were found to give similar results. This may be attributed to their similar high solubilities of greater than 600 mg/ml in pH 7.0 buffer. Pentobarbital, with a solubility of only about 3 mg/ml at pH 7.0, gave much lower concentration-adjusted k_H values in comparative polymer systems. However, when release from several pentobarbital films was studied in 1 *N* ammonium hydroxide, where its solubility is comparable to methapyrilene and salicylic acid at pH 7.0, the relative rate constants were quite similar to those obtained with these latter two drugs in pH 7.0 buffer. Although solubility in the release medium appears to be an important parameter of the k_H value, it did not quantitatively fit a linear k_H *versus* $C_s^{1/2}$ relationship which might be expected from Eq. 5. The use of the undissociated drug forms in the films could cause the effective value of C_s at the dispersed drug–solvent interface to be substantially less than the drug solubility in the buffer.

Drug Concentration—The effect of drug concentration on the release rate constant was tested using eight different methapyrilene concentrations, from 4.39 to 43.9%, in a pure hydroxypropyl cellulose matrix. The results showed a linear relationship (correlation coefficient = 0.997) between k_H and methapyrilene concentration (Fig. 5). Similar relationships were observed in other systems where only two or three different concentrations were tested. This implies that the fraction of drug released from a given film composition is independent of drug concentration.

This relationship is difficult to explain in terms of Eq. 4, where k_H might be expected to be linear with the square root of drug concentration. However, it may be rationalized on the basis of drug concentration effect on D and C_s through film hydration. A more plausible explanation may be made on the basis of Eq. 5 for a granular matrix system. By assuming all matrix porosity necessary for the diffusion pathway is due to that vacated by the dispersed drug, ϵ may be equated to A. Assuming also that $2A \gg \epsilon C_s$, Eq. 5 may be simplified to:

$$k_H = \left(\frac{DC_s}{\tau}\right)^{1/2} A \qquad \text{(Eq. 10)}$$

Since D, C_s, and τ should be constants for this system, the release rate constant would be proportional to the drug concentration, the relationship obtained experimentally. Huguchi (12) hypothesized just such a concentration relationship in his development of Eq. 2.

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Microultrafiltration Technique for Drug–Protein Binding Determination in Plasma

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Abstract □ A rapid microultrafiltration technique for determining plasma protein binding of drugs in small volumes of plasma was developed. The reliability of the method was tested by determining protein binding of acetazolamide, diphenylhydantoin, and salicylic acid in undiluted human plasma. The results are compared to literature values.

Keyphrases □ Drug–protein binding determination—microultrafiltration technique, plasma □ Plasma protein binding—microultrafiltration technique □ Protein binding, plasma—microultrafiltration technique □ Ultrafiltration, micro—technique for drug–protein binding determination in plasma

Plasma protein binding of drugs can affect their distribution, elimination, and thus the intensity and duration of their pharmacological action. The drug binding characteristics of plasma proteins may be altered in disease states by changes in the concentration of plasma proteins and by structural changes in the protein molecules themselves. Such structural perturbations may influence the affinity of drug–protein binding and/or the number of binding sites available on the protein molecule. Since the fraction of drug bound is frequently a function of drug concentration, determination of binding at a single concentration is not sufficient. Unfortunately, existing methods require relatively large volumes of plasma (up to 30 ml for measurements at six drug concentrations) and prolonged equilibration. Yet, one is limited by the volume of blood that may be obtained from a single patient. Therefore, a method is needed that allows characterization of an individual patient's drug–protein binding capacity using only a small volume of blood. This paper reports a simple, rapid, ultrafiltration method capable of defining the binding characteristics of a drug in sufficiently small volumes of plasma to make the method practicable for use with patients. The validity of the method was tested using salicylic acid, diphenylhydantoin, and acetazolamide in undiluted human plasma at 37°.

EXPERIMENTAL

Materials—Acetazolamide[1], diphenylhydantoin[2], and salicylic acid[3] were used as obtained from standard sources without further purification. ^{14}C-Diphenylhydantoin[4] (54 mg/mCi) and ^{14}C-salicylic acid[4] (28.7 mg/mCi) were appropriately diluted with nonradioactive drug to yield counts at least four to five times greater than background in the plasma water samples.

Microultrafiltration membranes were cut to size (13 and 25 mm diameter circles) from ultrafiltration membrane cones[5].

Apparatus—The binding of salicylic acid, diphenylhydantoin, and acetazolamide to plasma proteins was determined in small volumes (0.4 ml) of undiluted human plasma by a modified ultrafiltration technique. A circular membrane (13 mm in diameter) cut from ultrafiltration cones was secured inside an adapter[6]. A disposable needle was attached to the lower end of the adapter to deliver the filtrate to the glass tube below, and the whole assembly was inserted in a plastic tube (Fig. 1).

Procedure—Plasma (0.4 ml) containing known concentrations of drug (eight to 10 concentrations for each drug) was equilibrated for 2 hr at 37°. After a 20-μl aliquot was removed for analysis of total drug, the remaining plasma was introduced into the reservoir above the membrane using a Pasteur pipet. Centrifugation[7] of the apparatus for 10 min at 2200 rpm yielded 25–30 μl of plasma water in the lower glass tube (less than 10% of the total plasma volume). A 20-μl aliquot of plasma water was analyzed for free drug concentration. If the sensitivity of the analytical procedure requires a larger sample of plasma water, an adapter[8] may be used. This adapter accommodates approximately 1.5 ml of plasma and yields 125 μl of plasma water. The extent of drug–membrane binding was assessed using the same apparatus by substituting water for plasma. The protein concentration in plasma water determined by the method of Lowry *et al.* (1) was between 0.25 and 0.75% of the total plasma proteins.

Diphenylhydantoin and Salicylic Acid Radioisotope Measurements—Aliquots (20 μl) of whole plasma and plasma water obtained by microultrafiltration were added to 10.0 ml of scintillator solution[9], and the radioactivity was determined in a liquid scin-

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Eastman Organic Chemicals, Rochester, N.Y.
[3] Aldrich Chemical Co., San Leandro, Calif.
[4] New England Nuclear, Boston, Mass.
[5] Centriflo, 2100 CF-50, Amicon Corp., Lexington, Mass.
[6] Millipore Swinnex 13, Millipore Filter Corp., Bedford, Mass.
[7] International centrifuge model UV, International Equipment Co., Needham, Mass.
[8] Swinnex-25.
[9] Aquasol, New England Nuclear, Boston, Mass.