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Determination of in vitro drug release from hydrocortisone creams

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Summary

A method for the determination of in vitro release of hydrocortisone from creams using Franz diffusion cell system and synthetic membranes has been developed The use of synthetic membrane minimizes the variability observed with skin membranes The method can be employed as a quality control procedure for assuring batch-to-batch uniformity of topical products.

Introduction

The Food and Drug Administration has been the prime driving force in utilizing and gaining acceptance for the employment of dissolution testing as the single most useful in vitro method for assuring batch-to-batch uniformity and bioavailability of solid oral dosage forms. The procedure, which has been successfully applied for nearly two decades for tablets and capsules has additionally been used to provide for the approval of additional strengths of the same product formulation, as well as for the approval of minor formulation changes. At present, no such method exists for determining the in vitro release characteristics of topical products, such as creams, ointments and lotions. The purpose of this investigation was to explore the feasibility of using Franz diffusion cells to study drug diffusion and penetration. This in vitro system which has been employed to study drug penetration through skin, can be employed routinely to characterize the release properties from topical dosage forms. This is the first reported investigation employing Franz diffusion cells for evaluation of cream and ointment dosage forms. Using such a system for the purpose of assuring batch-to-batch uniformity for products such as creams and ointments, however, could be difficult, due to the constraints in availability of viable human skin, skin membrane variability and especially the skin source (AIDS). To obviate such concerns commercially available synthetic membranes have been studied. The objective is to develop a simple, realistic, reliable and reproducible quality control in vitro method for estimating the drug delivery attributes of topical dosage forms and to assure batch to batch uniformity.

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Materials and Methods

Two commercially marketed 2.5% hydrocortisone (HC) creams were used in this study: A, Synacort cream (Syntex) lot no. 24796, and B, Hytone cream (Dermik) lot no. 67514.

Synthetic membranes. Six commercially available synthetic membranes were utilized to study the in-vitro release properties of the two HC creams: pure cellulose acetate, triton-free cellulose, cellulose acetate with wetting agent, polysulfone, glass fiber filter and silastic membrane. The characteristics of these membranes are given in Table 1.

Diffusion cells. A standard open cap, ground glass Franz diffusion cell with 15 mm diameter orifice (1.767 cm^2 area; total diameter of cell = 25 mm) (Fig. 1) was utilized. The receptor phase was stirred by means of a constantly spinning bar magnet (Vari-speed, Side-Bi-Side, Cell Drive Console, Crown Glass Co. Inc, Somerville, NJ).

Receptor phase. Three different media, 0.05 M, pH 5.0 phosphate buffer, 0.09% sodium chloride (normal saline) solution and 0.05 M pH 7.4 phosphate buffer were utilized. The medium was degassed before using.

Procedure. The studies were carried out at 32°C using 6 diffusion cell assemblies. The cell body was filled to overflowing with a degassed receptor phase. Approximately 1 g of sample was added to the open cap and tamped down on the membrane, which had been moistened with the receptor phase and placed over the orifice of each cell cap. The tamping was continued until the view of the underside of the membrane showed no channeling. With the stirring magnet spinning, the cap was placed on the cell and clamped into place. The overflow allowed the cells to be bubble-free.

TABLE 1

Characteristics of membranes

Membranes 25 mm	Pore size (μm)	Thickness (μm)
Pure cellulose acetate [1]	0.5	150 (6 mils)
* Triton-free cellulose [1]	0.45	150 (6 mils)
* Cellulose acetate W/ wetting agent [2]	0.45	150 (6 mils)
Polysulfone [2]	0 45	165 (6.5 mils)
Glassfiber [2]	0.45	450 (18 mils)
Silastic [3]	–	250 (10 mils)

* Mixed cellulose acetate/cellulose nitrate membrane. Source: 1, Microsep Membrane Filters, Honeoye Falls, NY; 2, Gelman Sciences Inc., Ann. Arbor, MI; 3, Dow Corning Corporation, Midland, MI 48640.

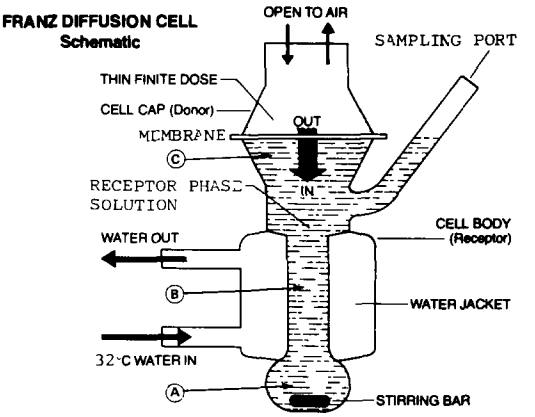

Fig. 1. Franz diffusion cell system, showing areas of sampling the receptor phase

Intervals of 30, 60, 120, 240 and 360 min were chosen as sampling times. In earlier studies, samples were removed simultaneously from the lower, middle and upper region (A, B and C in Fig. 1) of the receptor chamber to determine the mixing efficiency of the diffusion cell system at 30, 60 and 120 min intervals. A 100 μl aliquot was removed using a syringe and 0.5 mm i.d. 4.5 cm flexible teflon tubing and transferred into a low volume insert of an auto-injector. The cell was refilled with the receptor phase. A 20 μl sample was injected into the liquid chromatograph and quantitated by using a standard of known concentration.

HPLC analysis. A liquid chromatograph (Waters) equipped with a μ Bondapak C_{18} column, 3.9 mm i.d. and 30 cm long, an auto-injector (WISP), 254 nm UV detector (Waters – model 440 absorbance detector) and an integrator was used for analysis. The samples were eluted using tetrahydrofuran : acetonitrile : water solvent in ratio of 1 : 3 : 6 at ambient temperature and with a flow of 60 ml/h. When sorbic acid was present in a product (Hytone Cream, as indicated on the label) 1 mg/ml of ammonium phosphate dibasic was

dissolved in water, prior to the addition of the other two solvents. This changed the pH of the mobile phase to 7.5 and forced sorbic acid to elute along with the solvent front. The sensitivity of detector was 0.02 auf. Under these conditions the retention time was 4.9 min. The detection limits were 8 ng per 20 μl injection. Good reproducibility was observed, with percent coefficient of variation ranging from 5 to 10%. The concentration (μg/ml) of HC in the sample was calculated using a standard of known concentration. The total amount of HC released (conc in μg/ml $\times$ volume of the receptor phase) and amount of HC released per unit area (μg/cm^2) were calculated.

Results and Discussion

The HC release data (μg/cm^2) are shown in Tables 2–4 and Figs. 2–5. Franz diffusion cell systems have been used to study the diffusion and penetration properties of chemicals and drugs, from the dosage forms. The system commonly employs skin membranes to study these properties. The constraints in availability of skin membranes and the problems associated with skin variability and the difficulty of its preparation are well recognized by several researchers (Poulsen and Flynn, 1985, Nacht and Young, 1985) and was again emphasized in a recent workshop (Skelly et al., 1987) on in vitro percutaneous penetration studies. Because of these constraints, skin membrane is very difficult to use in routine quality control procedures. The alternative approach is to explore the use of commercially available synthetic membranes for in vitro quality control testing. This minimizes the variability and problems associated with acquiring skin membranes and obviates the risk of employing skin samples from cadavers with AIDS. Synthetic membranes have been used to study drug diffusion kinetics and as a screen for permeability characterization. However, no correlation has been reported between permeability characteristics of such membranes and that of human skin (Garret and Chemburker, 1968; Barry and El Elini, 1976). This obviously is due to significant differences between a structurally highly complex human skin and simple synthetic membrane. Most of these studies were carried out using radioactive chemicals. No studies have been reported where synthetic membranes have been utilized for determining drug release (dissolution) characteristics from marketed cream products.

Six commercially available synthetic membranes each having different composition (with or without wetting agent) and different receptor media were utilized to study the HC release characteristics from two marketed cream formulations (Table 1). Differences in in vitro release characteristics, if observed, were to be studied in in vivo drug penetration/bioavailability studies. No HC was detected in the receptor phase for 6 h after application when the silastic membrane was employed and this was dropped from the study.

In a recent article, Behme et al. (1982) have described a technique for determining the in vitro release rates of drugs from creams. In this method, the cream was applied directly on an 80 mesh screen, immersed in a receptor phase and drug release was determined over a 24-h period. The release rate of the cream was studied in the absence of any rate-influencing barriers. However, this method exposes the cream to the receptor phase on both sides of the screen, and may introduce 'channels' through which the receptor phase could penetrate unnoticed and effect its release characteristics. The method is also limited to water-insoluble or -immiscible cream bases because water-soluble or water-washable base would slough off.

In in vitro release method development the homogeneity of the system should be assured. At the same time, it is important to consider the practical aspects of the procedure. Therefore, during method development, simultaneous samples were collected across different strata of the receptor phase, i.e., from the lower (near the stirring magnet), middle and upper (near the lower surface of the membrane), portion of the receptor, A, B, and C in Fig. 1, at 30, 60, and 120 min and analyzed for HC. The experiments were carried out using Triton-free cellulose acetate, pure cellulose acetate, polysulfone and glass fiber membranes, and HC cream. In all cases, the HC concentration at all 3 levels, lower, middle and upper region of the receptor chamber of the diffusion

TABLE 2

Test for homogeneity in the receptor phase using HC 2 5% cream and synthetic membrane

Membrane	Sample area	30 min		60 min		120 min	
		Mean	RSD	Mean	RSD	Mean	RSD
Glass fiber filter	Top	28 5	4 21	53 8	4.07	97 5	8.01
	Middle	31 0	4.52	56.0	4 30	114 0	8.05
	Bottom	31.0	4.52	53 5	5 03	113 3	7 69
Cellulose acetate	Top	22 8	27.4	53 3	20 3	99.75	12 6
	Middle	22 0	28 4	54.5	20.5	98.5	11 4
	Bottom	22 0	28 9	54 3	19 9	97 3	13 8
Polysulfone	Top	35 0	15 3	72.5	22.1	133	4 58
	Middle	35 8	13.3	70.3	16 7	133 0	4 58
	Bottom	40 0	23 1	82.8	37 1	139 0	7 29
Triton-free cellulose acetate	Top	38 5	5.58	67 8	6.46	112 8	7.50
	Middle	38 5	5.58	67 8	6.34	117 0	6.89
	Bottom	38.5	6 29	67 8	7 54	116.8	7 29

Values in $\mu g/cm^2$, means of 6 cells

cell, at all time intervals, was found to be virtually the same, assuring uniform mixing in the receptor chamber. Table 2 shows data from using triton-free cellulose acetate membrane. Statistical analysis of the mean data (t-statistics comparing top vs middle, top vs bottom and middle vs bottom) indi-

TABLE 3

In vitro release of HC cream, 2 5%, using Franz diffusion cell

Membrane	Receptor phase	30 min	60 min	120 min	240 min	360 min
Synacort cream (Syntex)						
Cellulose acetate	pH 5 0 buffer	18 3 (13.5)	40.5 (7 6)	75.5 (5 4)	123.8 (18.6)	180.5 (3 2)
Cellulose acetate	0.09% NaCl	16.8 (6.1)	39.5 (3.2)	72.3 (3 2)	125 3 (3 6)	160 5 (3.6)
Polysulfone	0 09% NaCl	31 0 (6 2)	52 8 (5 7)	85 5 (5 5)	139 5 (6 4)	177.5 (6 4)
Polysulfone	pH 5 0 buffer	31 0 (4 1)	57.0 (2 7)	91 3 (3 2)	143 8 (2 7)	183.3 (1 9)
Triton-free cellulose	0 09% NaCl	36 3 (8 7)	58 0 (7 9)	89 5 (9 0)	148.3 (10.5)	175 5 (7 0)
Triton-free cellulose	pH 5 0 buffer	33 8 (7.0)	55 3 (5.2)	85 3 (5.8)	131.3 (6 7)	169 8 (7.0)
Cellulose w/w agent	0.09% NaCl	34.8 (4 9)	58 8 (4.6)	93.3 (5.0)	145 5 (5 0)	187 8 (5 8)
Cellulose w/w agent	pH 5 0 buffer	30 0 (12 7)	51 8 (11 8)	83 8 (10 7)	129 8 (10.1)	162 8 (10 6)
Glass fiber	0 09% NaCl	26.5 (5.0)	44.5 (3.7)	71 0 (4.4)	106 0 (5 6)	135 0 (3 8)
Glass fiber	pH 5 0 buffer	29.0 (7 3)	45 5 (4 6)	70 8 (5 1)	105 5 (5 0)	131 0 (6 4)
Hytone Cream (Dermik)						
Cellulose acetate	0 09% NaCl	17 5 (12.2)	43.5 (7 7)	87 0 (5 4)	158 3 (3 7)	215 3 (2 5)
Cellulose acetate	pH 5.0 buffer	18 5 (16.6)	46 8 (11.8)	94.8 (9 8)	168.5 (8.5)	226.8 (8 0)
Polysulfone	0 09% NaCl	32 3 (10 4)	59 9 (9 4)	96 3 (22 4)	167 8 (8 9)	221 0 (8 8)
Polysulfone	pH 5 0 buffer	36 0 (9 6)	65.3 (7.6)	115 0 (4 8)	188.8 (3 5)	247 0 (3.4)
Triton-free cellulose	0 09% NaCl	35 8 (13.8)	64 3 (11 4)	108.0 (10.5)	176.5 (10 7)	221 3 (10.5)
Triton-free cellulose	pH 5 0 buffer	37 0 (7 1)	62 3 (13.9)	109 0 (5.3)	181.3 (5 1)	232.3 (5.3)
Cellulose w/w agent	0.09% NaCl	38 3 (8 8)	68 0 (6.1)	112 8 (6 5)	184 0 (5 7)	241 0 (5 9)
Cellulose w/w agent	pH 5 0 buffer	36 0 (9 7)	64 0 (7 6)	107 3 (6 2)	175.3 (5.3)	227 8 (6.6)
Glass fiber	0 09% NaCl	30.8 (13.1)	54.3 (14.8)	92.5 (16.1)	147 3 (18.1)	195 5 (17 6)
Glass fiber	pH 5 0 buffer	32.5 (4 3)	56 8 (2 5)	93.8 (2 3)	153 0 (2 5)	190.3 (2 5)

Values in $\mu g/cm^2$, mean (RSD)

cated no significant difference between the means from aliquots taken from the 3 levels. These findings contradict the conclusions of Grummer et al. (1987) who had concluded that mixing in the Franz diffusion cell was not uniform. A close examination of their experimental procedure reveals that Grummer et al. carried out their experiment using a crystal of potassium permanganate, and began sampling after 30 s. Rarely (probably never) should samples be taken in such a short time when studying the diffusion properties, since drug penetration is a relatively slow process. Moreover, the purpose of our experiment was to determine the drug release properties from topical dosage forms over several hours. In this case, we find complete mixing and homogeneity in the receptor phase. There was no need to modify the commercially available Franz diffusion cells. Since our findings in all cases indicated no significant difference in the concentration of HC from all areas of the receptor compartment, we decided to take all further samples from the middle area of the receptor.

The HC release rate data using synthetic membranes and two receptor phases, pH 5 phosphate buffer and 0.09% sodium chloride (normal saline) are shown in Table 3 and Figs. 2 and 3. In some instances, the release rate was also determined using pH 7.4 phosphate buffer. The amount of drug that penetrated through the membrane per unit area was plotted against time, and the slope, which represents the steady state flux was calculated by linear regression of the last 3 time points.

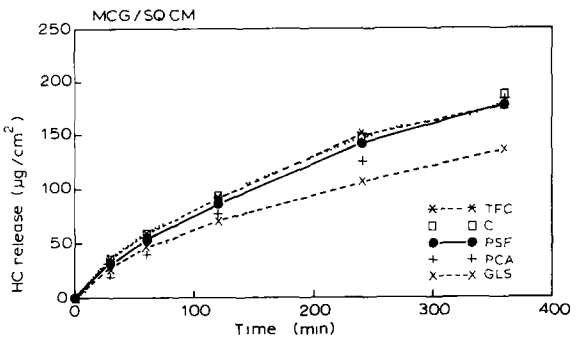

Fig 2 In vitro release profile of HC cream (Syntex Synacort) using Franz diffusion cell, different synthetic membranes and normal saline as receptor phase. Values and means of 6 cells

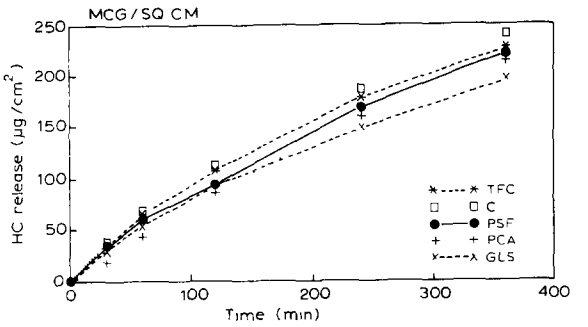

Fig 3 In vitro release profile of HC (Dermik Hytone) using Franz diffusion cell, different synthetic membranes and normal saline Values are means of 6 cells

The majority of the regression coefficient of these lines were 0.99. The slope was then substituted into the following equation for the determination of the diffusion coefficient.

$$dM/dt = D \cdot C/h$$

where dM/dt = slope of steady state flux, D = diffusion coefficient, C = concentration of the drug, h = thickness of the membrane. Rearranging the above gives

$$D = dM/dt \cdot h/C$$

The results of these calculations are shown in Table 4. The 5 membranes studied are hydrophilic in nature. The diffusion (partition) coefficient discussed here relates to the portion between the vehicle of the cream formulation and the membrane, and between the membrane and the receptor phase. But since the synthetic membranes are saturated with receptor phase, the diffusion (partition) coefficient in this case can be considered primarily between the vehicle of the cream formulation and the receptor phase. The diffusion (partition) coefficient value may vary with different formulations, due to different vehicle properties. The average diffusion coefficient was 2.8×10^{-4} and 4.1×10^{-4} cm^2/h, and the average flux was 0.354 and 0.511 μg/cm^2/h for Synacort and Hytone 2.5% HC cream respectively. Thus Hytone cream released the drug slightly faster than Synacort cream. The rate (flux) was not influenced by the type of synthetic membrane used except the slowing with glass fiber filter. The HC flux mea-

TABLE 4

Average flux ($\mu g/cm^2/h$) of HC creams

Membrane	pH 5 0 buffer	0.09% NaCl
Synacort cream		
Polysulfone	0.379	0.383
Pure cellulose acetate	0 368	0.438
Triton-free cellulose	0.352	0.358
Cellulose with wetting agent	0 329	0.394
Glass fiber	0.270	0 267
Average	0.340	0.368
Mean average	0.354	
Hytone cream		
Polysulfone	0 550	0.520
Pure cellulose acetate	0.550	0.534
Triton free cellulose	0.514	0 472
Cellulose with wetting agent	0 503	0.534
Glass fiber	0 402	0 429
Average	0 504	0 518
Mean average	0 511	

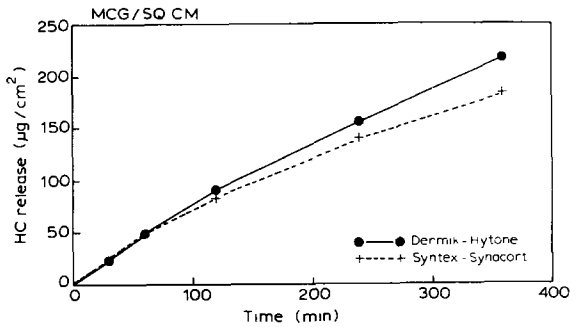

Fig 4 In vitro release profile of two HC creams using Franz diffusion cell, cellulose acetate membrane and pH 7 4 phosphate buffer. Values are means of 6 cells

sured through synthetic membranes is much larger than that observed with excised human skin. But in order to measure the HC without radioactivity from marketed formulations, this large flux is essential.

Figs. 2 and 3 show the comparative release profile of the two creams in normal saline using different synthetic membranes. Fig. 4 shows the release profile of two HC creams using a cellulose acetate membrane and pH 7.4 phosphate buffer. The release profile between the two creams under these test conditions was not significantly different ($P = 0.05$). The flux was 0.498 and 0.598 $\mu g/cm^2/h$ for Synacort and Hytone 2.5% HC creams respectively. This difference in flux is attributed to the difference in formulation of the two products. From these data, it is clear that the method developed can be used to determine the release rate profile of the creams, using appropriate synthetic membranes and receptor phases.

Using this procedure, the release profile of additional batches was also studied. The results, shown in Fig. 5, demonstrate that the release profiles between the batches from a given manufacturer were not significantly different ($p = 0.05$), and had very reproducible release profiles. The method developed shows promise as a tool for comparison of in vitro release profiles of creams with possible future applications to ointments, lotions, ophthalmics and otic products. The use of synthetic membranes not only minimizes the variability observed with animal or human skin, the method shows promise as a control procedure for

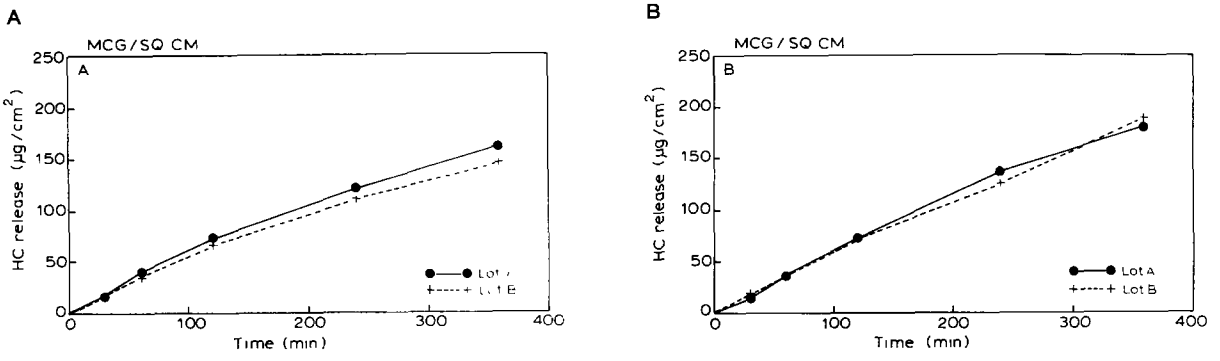

Fig. 5 In vitro release profile of two lots of hydrocortisone creams A Syntex-Synacort cream using cellulose acetate membrane and pH 5.0 buffer, B, Dermik Hytone cream using cellulose acetate membrane and normal saline solutions. Values are means of 6 cells

assuring batch-to-batch uniformity of topical products.

Conclusions

The method developed for determining the in vitro release rate profile of the drug from the creams using a Franz diffusion cell system and a synthetic membrane is simple and reproducible and can be easily adopted to assure batch-to-batch uniformity and quality of the product.

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