Influence of Formulation, Receptor Fluid, and Occlusion, on *in Vitro* Drug Release from Topical Dosage Forms, Using an Automated Flow-Through Diffusion Cell

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An automated flow-through diffusion cell apparatus was used for comparing the release rates of a naphthoic acid derivative, CD 271, from different topical formulations. The influence of the following parameters on CD 271 release from the formulations was investigated: receptor fluid composition, occlusion, weight of tested formulation, and dosage form type. The amount of tested formulation was shown to have no significant effect on the apparent release constant and lag time for an anionic oil-in-water emulsion and an aqueous gel. Occlusion affected drug release from the different dosage forms. Thus, occlusion increased CD 271 pharmaceutical availability for a lotion and a hydroalcoholic gel containing 0.1% of solubilized drug. The release profile of CD 271 from the formulations was highly dependent on the receptor fluid composition. Drug release was dramatically enhanced with *n*-octanol as compared to an aqueous solution of surfactant. Using occlusive or nonocclusive procedures, CD 271 apparent release constant and lag time were found to be highly dependent on the type of tested formulation. The flow-through diffusion cell proposed in the present study allows an accurate comparison of drug release characteristics from prototype topical formulations and therefore represents a valuable tool for formulation research or quality control process.

KEY WORDS: flow-through diffusion cell; topical drug delivery system; *in vitro* drug release; naphthoic acid derivative; synthetic membrane.

INTRODUCTION

In the development process of new topical drug delivery systems, *in vitro* methods for determining drug release are becoming a major need for pharmaceutical companies (1–6). These *in vitro* tests are designed for comparing drug release characteristics from prototype topical formulations and also as a quality control method for assuring batch-to-batch uniformity (7,8).

Use of an automated diffusion cell apparatus was previously shown to be simple, reliable and reproducible for quantifying pharmaceutical availability of topical dosage forms (9). Therefore, the release of a new modulator of cellular differentiation, the naphthoic acid derivative CD 271, was investigated from different topical formulations using this diffusion cell system.

The objectives of the present work were to determine *in vitro* the influence of various parameters on CD 271 release from topical formulations, e.g., receptor fluid composition, occlusive vs. nonocclusive procedure, weight of applied formulation, and dosage form composition.

MATERIALS AND METHODS

Apparatus

A cyclic system, composed of a flow-through diffusion cell (Fig. 1), a pump, a receptor compartment, a detector, and an integrator, was used for quantifying CD 271 release from the tested formulations (Fig. 2). The flow-through diffusion cell characteristics were described previously (9).

Briefly, the experimental formulations are deposited on an inert membrane which is placed on a sintered glass disk. The receptor fluid is continuously pumped upward through the sintered glass disk and is permanently in contact with the inner side of the artificial membrane. The cell effluent is cycled to the reservoir and the detector at a controlled flow rate (2 ml/min) with an HPLC pump (Waters 590, Millipore-Waters, France). The diffusion cell is thermostated to 35°C with a water jacket connected to a circulating bath (Haake F3, France), so that the membrane–receptor fluid interface is maintained at 32°C.

Artificial Membrane and Receptor Fluid

The membrane characteristics and receptor phase composition greatly influence drug release from topical formulations. The membrane should be chemically inert toward the experimental formulation and permeable to the drug concerned and should not be rate-limiting in the release process. The receptor fluid must be able to solubilize the drug and should not alter the dosage form by back diffusion through the membrane.

CD 271 is a lipophilic drug with a high octanol–water partition coefficient (log $P > 6$); therefore, a nonporous silicone rubber membrane (Silastic 500-1, 130-μm thickness, Dow Corning, France) and two receptor phases, namely, *n*-octanol (Merck, France) and a 0.25% aqueous solution of polysorbate 80 (Tween 80, Fluka, France), were used.

Procedure

The opened system was filled and prewashed for 60 min with the receptor fluid. The Silastic membrane was then introduced into the diffusion cell and the system was allowed to equilibrate for about 30 min. The circuit was finally closed and 50 ml of receptor solution was added to the reservoir so that the final circulating volume was precisely 60 ml.

After a further 30 min in order to stabilize the baseline of the detector (Waters 420 fluorescence detector, Millipore-Waters, France) set at an excitation wavelength of 313 nm and an emission wavelength of 400 nm, either 3 or 6 g of formulation was spread on the membrane surface (10 cm^2). In some experiments, the applied sample was maintained under complete occlusion. The output from the detector was recorded over a 20-hr period on a connected integrator (Waters 740, Millipore-Waters, France) and the signal amplitude

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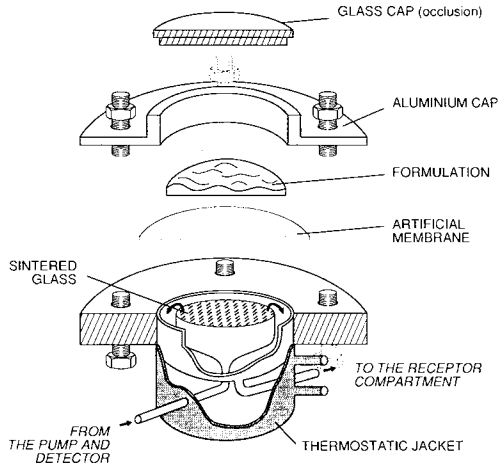

Fig. 1. Flow-through diffusion cell.

was measured every 60 min. After subtracting any placebo effect, and referring to calibration curves, results were finally expressed as cumulative amount of drug released as a function of time. The experiments were performed in triplicate and mean values were calculated.

Individual and mean apparent release constants were obtained by linear regression analysis of plots of cumulative amount of released drug ($\mu g/cm^2$) versus square root of time ($min^{0.5}$). This relationship between drug release and square root of time was previously shown to be valid for topical formulations with either fully dissolved or suspended drug (4,6,7,10–13). The "square root of time law" can be applied to CD 271 release from the various topical formulations because, first, only a single drug diffuses out of the dosage forms with a percentage of drug release less than 30% after a 20-hr period, the membrane being highly permeable to the drug, and, second, sink conditions are ensured by the large receptor volume and the flow-through design.

By linear regression of the curves representing cumulative CD 271 release vs square root of time, an apparent release constant (ARC), corresponding to the measured slopes, was obtained. The lag time, LT, defined by the intercept on the square root of time axis was also used to compare drug release from different formulations. This lag time corresponds to the delay needed for drug molecules to be released from the dosage form and to diffuse across the synthetic membrane, the latter not being rate-limiting.

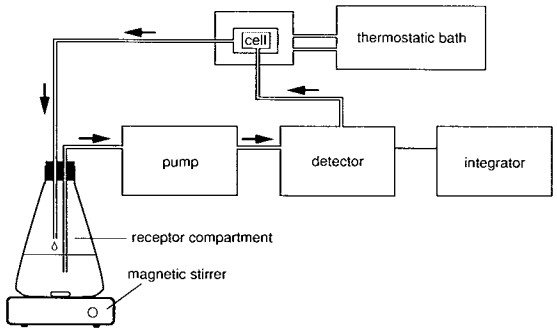

Fig. 2. Schematic representation of the automated diffusion cell apparatus.

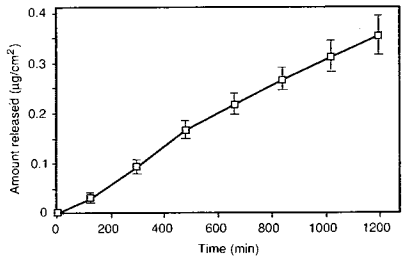

Fig. 3. Mean release profile of CD 271 from an anionic o/w emulsion (E1) (n = 3; mean ± SE).

CD 271 Formulations

CD 271 [6-(3-(1-adamantyl)-4-methoxyphenyl)-2-naphthoic acid] was synthesized at CIRD GALDERMA (14). Different formulations containing 0.1% CD 271 and the corresponding dosage forms were prepared:

- a lotion (L) composed of polyethylene glycol 400 and absolute ethanol (70:30, w/w);
- a hydroalcoholic gel (G1), with 45% ethanol;
- an aqueous gel (G2);
- an anionic oil-in-water emulsion (E1); and
- a nonionic oil-in-water emulsion (E2).

In the formulations L and G1, CD 271 was fully dissolved, whereas the drug was dispersed in G2, E1, and E2.

RESULTS AND DISCUSSION

The *in vitro* dissolution test has become an official requirement for pharmaceutical industries, for determining pharmaceutical availability of solid oral dosage forms (15), and for assuring batch-to-batch uniformity. To date, no such control has been adopted for evaluating the release of drugs from topical formulations, such as lotions, gels, creams, and ointments. However, over the past few years, many investigators have proposed different *in vitro* diffusion cell designs, with specific synthetic membranes and receptor fluids.

In addition to the quality control process for assuring batch-to-batch uniformity, *in vitro* testing of drug release from topical drug delivery systems would represent a valuable tool as initial screening of experimental formulations. In the present work, the influence of different parameters on CD 271 release from topical formulations was investigated: amount of deposited formulation, occlusion, receptor fluid, and dosage form.

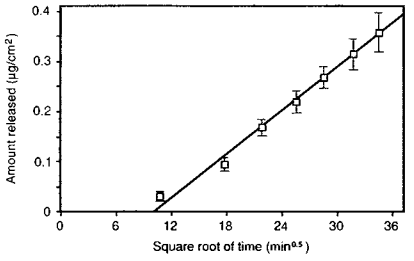

Fig. 4. Linearization of experimental data (Fig. 3) by replotting cumulative amount of released drug versus square root of time (n = 3; mean ± SE).

Table I. Effect of Amount of Tested Formulation (3 or 6 g) on CD 271 Release[a]

Parameter	Formulation			
	E1 (Cream)		G2 (Gel)	
	3 g	6 g	3 g	6 g
Apparent release constant ($\mu g/cm^2/min^{0.5}$)	0.014 ($1.16\ 10^{-3}$)	0.011 ($6.29\ 10^{-4}$)	0.0047 ($9.2\ 10^{-4}$)	0.0039 ($7.57\ 10^{-4}$)
Lag time ($min^{0.5}$)	9.86 (0.26)	10.46 (1.88)	10.31 (0.62)	7.08 (1.29)

[a] Values are means of three assays (±SE).

Amount of Tested Formulation

A typical mean profile of CD 271 release from a topical formulation (E1), using a 0.25% aqueous solution of Tween 80 as receptor phase, a nonocclusive system, and 3 g of tested formulation, is presented in Fig. 3. Linearization of the experimental data is obtained with a good correlation coefficient ($r^2 = 0.997$) by replotting cumulative amount of released drug against square root of time (Fig. 4).

Comparison of the mean release profiles of CD 271 from E1 or G2 did not show any significant effect of application of either 3 or 6 g of formulation to the cell on the apparent release constant (ARC) and lag time (LT) (Table I). Using the same diffusion cell system, the influence of the amount of formulation applied to the membrane on ARC was previously demonstrated and a plateau was reached for deposited volumes of at least 3 ml (8).

Occlusion

In nonocclusive experiments, a glass cover was placed over the formulation in order to avoid any contamination, however, evaporation of some components of the formulations still occurred. Therefore, some assays were also carried out under complete occlusion by hermetically sealing a glass cap to the donor compartment (Fig. 1). Using a 0.25% aqueous solution of Tween 80 as receptor phase and 3 g of tested formulation, occlusion greatly affects drug release from formulations L and G1 (Fig. 5) but has no significant effect in the case of E1 and G2 (Table II). For the cream E1 and the aqueous gel G2, containing dispersed CD 271 crystals, occlusion did not statistically modify ARC and LT, although there was a loss of at least half the weight of each formulation under nonocclusive conditions.

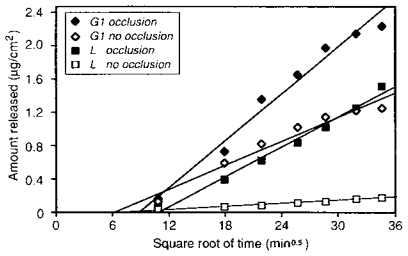

Fig. 5. Comparison of mean release profiles of CD 271 ($n = 3$) from the lotion L and the hydroalcoholic gel G1 under occlusive and nonocclusive conditions.

Regarding the two dosage forms that contained solubilized CD 271, a dramatic effect was observed when the system was used under occlusive conditions. Thus, for the hydroalcoholic gel G1, the mean apparent release constant was increased twofold (Table II). A similar difference was found between the nonocclusive and the occlusive procedure for LT values. For the lotion L, the mean apparent release constant was about 10 times higher under occlusion than without occlusion (Table II). As observed earlier for G1, by placing the lotion L under occlusion, LT was doubled. Under occlusion, no significant weight loss of all formulations, L, G1, G2, and E1, was detected.

It is noteworthy that, in this diffusion cell system, occlusion does not systematically affect drug release from different dosage forms. Comparing CD 271 release from four different formulations, occlusion modifies drug pharmaceutical availability only when the drug is completely solubilized in the dosage form (L1, G1). Since under occlusion alcohol evaporation is avoided, the influence of the solvent on drug release is maintained throughout the experiment.

Receptor Fluid

The receptor fluid composition may greatly influence drug release from topical formulations. The release of hydrocortisone from different vehicles was found to be different when using water or propylene glycol as receptor phase

Table II. Influence of Occlusion on *in Vitro* CD 271 Release from Different Formulations[a]

Formulation	ARC ($\mu g/cm^2/min^{0.5}$)		LT ($min^{0.5}$)	
	Occlusion		Occlusion	
	−	+	−	+
G1	0.048 ($3.5\ 10^{-3}$)	0.092 ($8.7\ 10^{-3}$)	7.17 (0.66)	8.64 (0.43)
L	0.006 ($7.5\ 10^{-4}$)	0.059 ($4.7\ 10^{-3}$)	7.4 (1.16)	10.75 (0.31)
E1	0.014 ($1.2\ 10^{-3}$)	0.013 ($1.2\ 10^{-3}$)	9.86 (0.26)	11.07 (1.33)
G2	0.005 ($9.2\ 10^{-4}$)	0.006 ($3.8\ 10^{-3}$)	10.31 (0.62)	6.92 (0.95)
E2	0.008 ($5.2\ 10^{-4}$)	— —	10.76 (2.26)	— —

[a] Values are means of three assays (±SE).

(4). The effect of receptor fluid on CD 271 release from the formulations E1, L, and G2 was investigated using *n*-octanol and a 0.25% aqueous solution of Tween 80. A nonocclusive procedure was used and 3 g of formulations was deposited on the membrane. The amount of CD 271 released with *n*-octanol as receptor fluid is much higher than that released with a 0.25% aqueous solution of Tween 80 (Table III). However, the rank ordering of the apparent release constant values for a given formulation is independent of the nature of the receptor fluid (E1 > L > G2). The lag time (LT) appears to be independent of the nature of the receptor fluid (Table III).

The difference observed between the two receptor phases is probably the result of *n*-octanol passage through the membrane, and, hence, modification of the formulation. After 10 hr, the cream E1 is completely solubilized by *n*-octanol and the release characteristics become independent of the formulation type. In addition, under nonocclusive conditions with the aqueous surfactant solution, evaporation of formulation components causes 30% weight loss for the cream. The release characteristics of CD 271 from different formulations are highly affected by the receptor phase. Therefore, it is essential to ensure that drug appearance in the receptor phase is controlled only by the formulation, not by the solvent properties and its effects on the dosage form.

Dosage Form

Using a nonocclusive procedure, a 0.25% aqueous solution of Tween 80 as receptor phase and 3 g of formulation deposited on the membrane, major differences in CD 271 release from the tested formulations can be observed (Fig. 6). The ARC of CD 271 from the hydroalcoholic gel G1 is about 10-fold higher than that from the aqueous gel G2, LT being much shorter for G1 than for G2 (Table II). The increased release rate of CD 271 from G1 may be explained by the complete dissolution of the drug by ethanol in the formulation. A similar effect of ethanol on naproxen release from water-washable and hydrophilic bases was observed by Rahman *et al.* (6). CD 271 release rates from the anionic o/w emulsion E1 and from the nonionic o/w emulsion E2 were significantly different (Table II). Although LT values were very similar, ARC for E1 was almost two times higher than for E2. After correction of the CD 271 fluorescence quenching due to PEG 400, ARC from the lotion L was shown to be close to that from E2, LT, however, being two times shorter for L than for E2 (Table II).

Table III. Comparison of CD 271 Release from the Formulations E1, L, and G2, Using *n*-Octanol and a 0.25% Aqueous Solution of Tween 80 as Receptor Phases[a]

	ARC ($\mu g/cm^2/min^{0.5}$)		LT ($min^{0.5}$)	
Formulation	0.25% Tween 80	*n*-Octanol	0.25% Tween 80	*n*-Octanol
E1	0.014 ($1.15\ 10^{-3}$)	0.108 ($4\ 10^{-4}$)	9.86 (0.26)	7.85 (0.06)
L	0.006 ($7.5\ 10^{-4}$)	0.039 ($4.4\ 10^{-3}$)	7.4 (1.16)	7.28 (0.40)
G2	0.005 ($9.2\ 10^{-4}$)	0.011 ($6.4\ 10^{-4}$)	10.3 (0.62)	6.35 (0.72)

[a] Values are means of three assays (±SE).

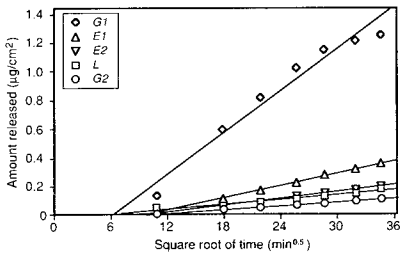

Fig. 6. Effect of dosage forms (G1, G2, E1, E2, L) on *in vitro* CD 271 release ($n = 3$). Nonocclusive procedure, 3 g of tested formulations; 0.25% aqueous solution of Tween 80 as receptor phase.

The influence of the formulation on CD 271 release was also investigated using an occlusive procedure, an aqueous receptor phase, and 3 g of deposited formulation (Table II). CD 271 release from the formulations L and G1 is much higher than from E1 and G2. The release rate of CD 271 from the different formulations increases in the order G2 < E1 ≪ L < G1. Thus, the ARC ratios between G1 and L, L and E1, and E1 and G2 are 1.6, 4.5, and 2.2, respectively. The apparent release constant of CD 271 is about 16 times higher from G1 than from G2.

It appears that CD 271 release is increased from formulations containing solubilized drug (G1, L) as compared to formulations with dispersed drug (E1, G2). Under occlusion, evaporation of alcohol present in formulations G1 and L is avoided, the release rate of CD 271 therefore being increased as compared to E1 and G2. Similarly, the *in vitro* release rate of chlorpheniramine maleate was shown to be higher for o/w and w/o emulsions containing dissolved drug than for ointments with dispersed drug (5).

CONCLUSIONS

For a lipophilic drug such as CD 271, apart from the flow-through diffusion cell design and the nature of the synthetic membrane, parameters such as receptor fluid composition and occlusion may play an important role in drug release rate. Calculation of the apparent release constant and lag time from the cumulative amount of released drug-versus-square root of time curves provides a rational approach for an accurate and meaningful comparison of drug release profiles.

An automated system is presented in this study, with a membrane–receptor phase combination and a standard procedure adapted to the drug's and dosage form's physicochemical characteristics. It represents a method of choice for screening prototype topical formulations and for controlling batch-to-batch uniformity. However, *in vitro* drug release tests should not be regarded as an alternative to *in vivo* experiments for assessing drug bioavailability. Drug percutaneous absorption *in vivo* depends on many parameters that cannot be reproduced *in vitro*, and more work needs to be done in order to assess the relevance of *in vitro* experiments as a predictive test.

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