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Development in release testing of topical dosage forms: use of the Enhancer Cell™ with automated sampling

Pankaj R. Rege, Vinod D. Vilivalam, Charles C. Collins *

Department of Pharmaceutics, Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh PA 15282, USA

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Abstract

The aim of this study was to evaluate an automated method using the Enhancer Cell™ and compare the release of the corticosteroid triamcinolone acetonide (TA) from commercial semisolid formulations. The method used a modified USP Apparatus 2 using the Enhancer Cell™ in 200 ml capacity flasks instead of the standard 900 ml flasks. The additional equipment included an adapter plate to position the flasks in the center, a cover to reduce the receptor phase evaporation and smaller sized (1/4 in.) shaft and collets. All products were evaluated prior to their expiration date. Effects of system variables such as the temperature and composition of the receptor medium, stirring speed, and the choice of membrane on the drug release were evaluated. Statistical analysis was carried out using SAS Ver. 6.07 and the slopes and intercepts (of the cumulative release/unit area versus square root of time plots) were compared. TA release was a linear function of the square root of time ($P \leq 0.0001$), in accordance with Higuchi's model ($r^2 \geq 0.9$ in most cases). Temperature (32 and 37°C) did not affect the drug release ($P > 0.32$) but a significantly higher release rate was observed ($P \leq 0.0001$) at 50°C. Stirring speed (50, 100, 200 rpm) ($P > 0.26$) and receptor media composition (38 and 76% ethanol) ($P > 0.68$) did not significantly alter the release rates. Membrane selection (regenerated cellulose, polyethylene, and rat skin) was found to be a significant variable ($P \leq 0.004$). This study demonstrates the use of the Enhancer Cell™ as an automated quality control tool in the in vitro release testing procedure for semisolid drug formulations.

Keywords: Enhancer cell; Triamcinolone acetonide; In vitro release studies; Diffusion cell; Method variables; Automation

1. Introduction

In the last two decades the Food and Drug Administration (FDA) has successfully used in vitro dissolution evaluation procedures to access batch-to-batch bioequivalence of solid oral drug products [1,2]. Several automated procedures, including the use of robotics, are available for dissolution testing of oral drug products. Until recently, in vitro release methods did not exist for topical drug products [3]. Present quality control tests for topical dermatologic preparations include identification, assay, homogeneity, and in some

* Corresponding author. Tel.: +1 412 3965007; fax: +1 412 3965130; e-mail: collinsc@duq3.cc.duq.edu

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cases viscosity, specific gravity and particle size determination. These tests provide little information about the drug release properties of the product or the effect of processing and manufacturing variables on the performance of the finished dosage forms. Official methods have been developed to study drug release from transdermal delivery patch systems which utilizes USP Apparatus 5, 6 and 7 [4].

Dissolution testing has served as a simple routine indicator of batch-to-batch uniformity for solid dosage forms. However, since the method is an oversimplified version of the exceedingly complex processes involved in drug application and absorption, it is important to avoid inappropriate extrapolation of the results to in vivo conditions [5]. In 1986, a joint FDA and AAPS (American Association of Pharmaceutical Scientists) workshop recommended that procedures be developed to assure batch-to-batch drug release equivalence for topical dosage forms [6].

Recently VanKel Industries (Edison, NJ), in conjunction with our laboratory, introduced the Enhancer Cell™ [7], a device which can be used to study the drug release profiles of topical formulations. It is made of Teflon®, an inert and non-reactive material. Earlier work [8] demonstrated the ease of use of the Enhancer Cell™ in comparison with the Franz cell. Recently, another group [9] compared the Enhancer Cell™ with the Franz cell using 'in-house' gel formulations. This work describes the evaluation of commercially available semisolid preparations of triamcinolone acetonide (TA) using the Enhancer Cell™ method. TA was selected as the model drug because of its availability in various commercial formulations and different concentrations (0.025, 0.1 and 0.5%). The main objective was to develop a simple, affordable, reliable and reproducible quality control method which could be used to discriminate variations in the release characteristics of topical dosage forms.

Table 1
Formulations

Creams		
Triacet™ 0.1%	Lot no. 4613; expiry date: Aug 96	Lemmon, Sellersville PA
Aristocort® LP 0.025%	Lot no. 388–303; expiry date: Oct 96	Fujisawa Pharmaceutical, IL
Aristocort® RP 0.1%	Lot no. 390–320; expiry date: Nov 96	Fujisawa Pharmaceutical, IL
Aristocort® HP 0.5%	Lot no. 388–312; expiry date: Oct 96	Fujisawa Pharmaceutical, IL
Aristocort A® 0.025%	Lot no. 368–304; expiry date: Oct 96	Fujisawa Pharmaceutical, IL
Aristocort A® 0.1%	Lot no. 368–334; expiry date: Sep 96	Fujisawa Pharmaceutical, IL
Aristocort A® 0.5%	Lot no. 376–303; expiry date: Apr 96	Fujisawa Pharmaceutical, IL
Ointments		
Aristocort® RP 0.1%	Lot no. 382–322; expiry date: Jul 96	Fujisawa Pharmaceutical, IL
Aristocort® HP 0.5%	Lot no. 388–305; expiry date: Oct 96	Fujisawa Pharmaceutical, IL
Aristocort A® 0.1%	Lot no. 376–304; expiry date: Apr 96	Fujisawa Pharmaceutical, IL

2. Materials and methods

2.1. Materials

TA USP/NF (Lot no. JE449) and fluoxymesterone USP/NF (Lot no. KB123) were purchased from Spectrum Chemical, Gardena, CA. Acetonitrile HPLC grade (Lot no. 953704) and methanol HPLC grade (Lot no. 943704) were purchased from Fisher Scientific, Fairlawns, NJ. Ethyl alcohol 95%-190 proof (Lot no. 95K27–38) was purchased from McCormick Distilling, Industrial Alcohol Division, MI. Tables 1 and 2 lists the commercial formulations and the membranes used in the study.

Table 2
Membranes

Regenerated cellulose membrane	Cat # H40299	Bel-Art Product, Pequannock, NJ
Microporous polyethylene film, No. 9711 Co-Tran™ membrane	Lot # 031157-5	3M Pharmaceuticals, St. Paul, MN
Male Sprague Dawley rats (animal membrane)	—	Zivic Miller, Pittsburgh, PA

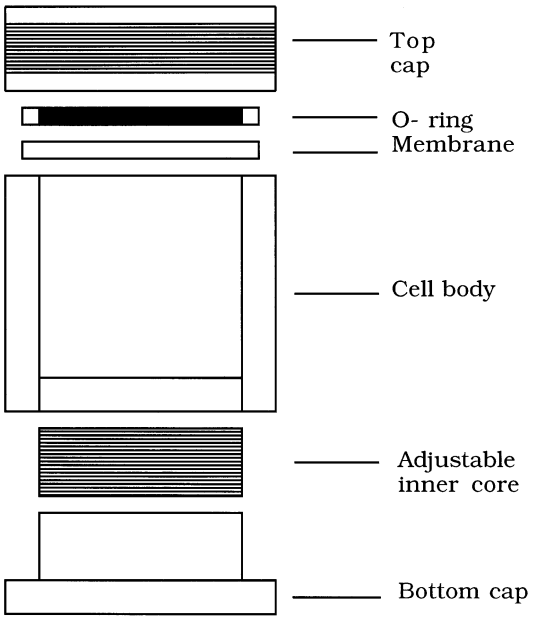

Fig. 1. The Enhancer Cell™.

2.2. In vitro release apparatus and methodology

(a) Enhancer Cell™ (PN-12-4000, VanKel Industries, NJ) (Fig. 1) consisted of a cap, a washer, membrane, an O-ring, and a drug reservoir. The outer diameter of the body and the solid ring are identical to the inner diameter of the cap, which aids in keeping the membrane in place while tightening the cell.

(b) An USP six spindle dissolution tester (Vanderkamp® VK 6010, VanKel Industries, NJ) was used. The USP Appatatus 2 was modified (Fig. 2) with 200 ml capacity flasks (PN-12-0305, Vankel Industries, NJ) instead of the standard 900 ml flasks. It was essential to use smaller receptor volumes to obtain samples with detectable concentration of drug for HPLC analysis. The additional equipment included an adapter plate to position the flasks in the center, a cover to reduce the receptor phase evaporation and smaller sized (1/4 in.) shaft and collets. Sample collection was carried out using an automated sample collector (Intelligent Fraction Collector, VK 3000®, VanKel Industries, NJ).

A 1500 mg sample was placed in the reservoir of the Enhancer Cell™. The membrane (previously wetted with the receptor medium for 15 min and then patted dry) was placed on the top of the reservoir and the screw cap secured in place. Care was taken to make certain that no entrapped air was present at the interface of the formulation and the membrane. The Enhancer Cell™ was then placed in the 200 ml vessels. Ethanol:water (38:62) was used as the receptor media to maintain sink conditions. The hydro-alcoholic receptor medium was used to improve the solubility of the steroid [10]. The medium was mixed using a magnetic stirrer (Cat. # 11-500-4SH, Fisher Scientific, NJ) and degassed in a sonicator (Bransonic®3200 Ultrasonic Cleaner, Branson Cleaning Equipment, CT) for 30 min. The solubility of TA in this media was found to be 0.91 ± 0.08 mg ml^{-1}. The assembly was completed and 200 ml of the receptor medium was added to each vessel. The paddles

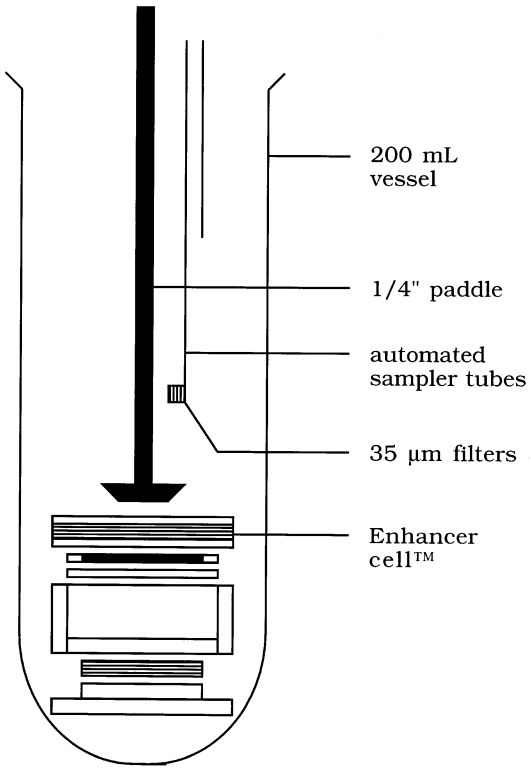

Fig. 2. The Enhancer Cell™ assembly.

were rotated at 50 rpm and were adjusted to remain 1 cm from the top of the membrane throughout the study. The temperature of the receptor medium was maintained at 32°C ± 0.1°C. Samples (3 ml) were filtered through 35 μm filters (Full Flow™ Filters, PN-17-4010, VanKel Industries, NJ). Sample collection was carried out at 0.25, 0.5, 1.0, 2.0, 4.0 and 6.0 h. The sample withdrawn from the flasks was not replaced but a correction factor was used to account for the lost volume. Preliminary studies indicated that the dissolution medium lost due to evaporation over 6 h at 32°C was $< 2\%$. All experiments were run in sets of three to six replicates to obtain the cumulative release profiles.

2.3. Sample preparation using animal membrane

The skin from the abdominal area of male Sprague Dawley rats were used. The animal was sacrificed and the skin from the dorsal area was excised. The hair was removed using a clipper fitted with a Size 40 (0.1 mm) blade (Golden A-5® Clipper, Oyster Professional Products, TN) and the skin samples were placed in a 0.9% sodium chloride solution. After the removal of the excess subcutaneous fat the skin samples were cut into the appropriate size. The thickness of the skin samples was found out to be 1427 ± 127 μm (Lymann® Electronic Digital Caliper, Lymann Products, CT). The samples were then frozen in a 0.9% NaCl solution. These samples were placed in the Enhancer Cell™ such that the stratum corneum was in contact with the drug formulation and the dermis in contact with the receptor medium.

2.4. Evaluation of method variables

Experiments were designed to determine the effect of the method variables such as the temperature of the receptor medium, receptor phase concentration, stirring speed, and the choice of the membranes. Release profiles were studied at three temperatures of the receptor medium, i.e. 32, 37 and 50°C. Three stirring speeds of 50, 100 and 200 rpm were used. Release profiles using two receptor phase concentrations (38 and 76% ethanol) were evaluated. Release as a function of membrane selection was evaluated using regenerated cellulose, polyethylene, and animal skin.

2.5. Sample preparation and data analysis

HPLC analysis was used to determine the drug concentration in all samples in the study. A 712 WISP autoinjector and a M-45 Solvent Delivery System (Waters Associates, Milford, MA), Spectroflow 738 UV detector (Kratos Analytical, Ramsey, NJ) with a Hewlett Packard 3392A Integrator (Hewlett Packard, Avondale, PA) were used. Fluoxymesterone USP/NF (250 ng ml^{-1}) was used as the internal standard. A Phenomenex C-18 column, 3.9 mm i.d. × 300 mm long, was used. The limit of detection of this method was 0.05 μg ml^{-1} with an injection volume of 60 μl. An acetonitrile:water (40:60) mobile phase was used to analyze the 0.025 and 0.1% creams and ointments while a mobile phase containing acetonitrile:water (35:65) was used to analyze the 0.5% creams and ointments (where a higher amount of drug was expected to be released). This was done to improve the separation between the internal standard and drug peak for better resolution. The flow rate in both cases was maintained at 1.0 ml min^{-1} and both peaks were measured at 254 nm. The mobile phase was filtered through 0.22 μm filters (Filtrate™ hydrophilic filters, Cat # 7DF009, US Medicare, NC) and degassed in a sonicator (Bransonic® 3200 Ultrasonic Cleaner, Branson Cleaning Equipment, CT) for 45 min. and prior to use. Data were recorded and evaluated using Microsoft Excel, Ver. 4.0 (Microsoft), and Cricket Graph, Ver. 1.5.1 (Computer Associates). The statistical analysis was carried out using SAS, Ver. 6.07 (SAS Institute, NC).

3. Results and discussion

The in vitro drug release studies were evaluated for 6 h. Data were linearized using the square root of time transformation and linear plots were obtained by plotting the cumulative amounts released (μg) per square root of time ($min^{1/2}$). The coefficient of determination in most cases was > 0.9 ($P \leq 0.0001$). This was in accordance with

Higuchi's model [11]. Higuchi proposed two models, one in which the drug exists as a suspension in the base (Model I) and one where the drug exists as a solution in the base (Model II). Irrespective of whether the drug is suspended or solubilized, the release should be linear with the square root of time, if the release is governed via a diffusion process.

Model I

$$Q = \sqrt{2AC_sDt}$$

Model II

$$Q = 2A\sqrt{Dt/\pi}$$

where Q is the amount of drug released per unit area; A is the amount of drug per unit volume of product; D is the diffusion coefficient and C_s is the solubility of the drug in the external phase of the vehicle. Statistical analysis was carried out using SAS [Ver. 6.07] and the slopes and intercepts of the cumulative release/unit area versus square root of time plots were compared.

The drug release studies were carried out using the regenerated cellulose membrane as the barrier. All the commercial formulations released TA except the 0.1 and 0.5% Aristocort® ointments, where no drug was found in the receptor phase even after the experiments were expanded to 48 h. It was concluded that these formulation did not release the drug or the amount of drug released was less than the limit of detection of this method. This emphasizes the importance of the test conditions. The cumulative amount of TA released from a cream formulation was more than the amount released from the ointment formulation in all cases (Fig. 3). The hydrophobic nature of TA and its affinity for an oleaginous base explains the slower release from the ointment formulation. In the comparison of the 0.1% creams the Triacet™ formulation released the most drug (192.29 ± 20.93 μg) followed by the Aristocort A® formulation (95.57 ± 9.24 μg) and the Aristocort® formulation (70.10 ± 4.42 μg). The differences in the amounts released could be attributed to the differences in the formulation or differences in the method of manufacture. Triacet™ cream has a vanishing cream base, whereas Aristocort A® has a hydrophilic base containing Aquatin™. Aristocort® has a vanishing cream base containing mono- and diglycerides. The greater the hydrophobicity of the base the greater would be the affinity of the drug for the base environment. Statistical analysis showed that the release profiles were significantly different ($P \leq 0.0001$). These differences in amount of drug released could be representative of the differences in the compositions of the creams evaluated.

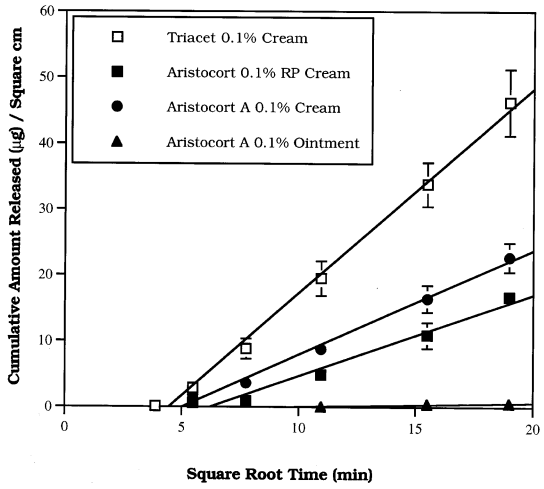

Fig. 3. Release profile from different formulations (creams and ointments) containing the same strength (0.1% TA) (Mean (SD); $n = 6$; {temp. = 32°C, rpm = 50, medium = 38% ethanol, and membrane = regenerated cellulose}).

The release was found to be directly proportional to the concentration of the drug in the base. As the concentration of drug in the formulation increased, it resulted in a greater amount released in all cases (Figs. 4 and 5). Similar observations have been reported by Kundu et al. [12]. All experiments were repeated to check the reproducibility of the method. Statistical analysis using SAS showed that the slopes and the intercepts of the profiles obtained in the replicate run were similar to the previous run (data not shown). Triacet™ 0.1% cream was selected as the model formulation to study the effect of the method variables. A total of 192.29 ± 20.93 μg was released from Triacet™ 0.1% cream at 32°C, 192.95 ± 10.41 and 449.93 ± 68.25 μg at 37 and 50°C, respectively. A higher amount of TA release would be expected at a higher temperature of the

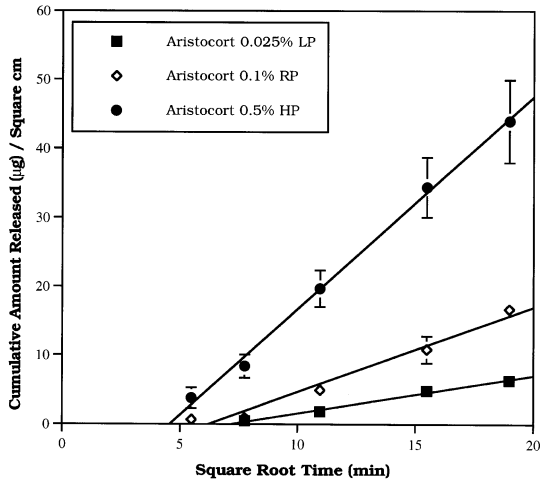

Fig. 4. Release profile from Aristocort® cream formulations (0.025, 0.% and, 0.5% TA) (Mean (SD); $n = 6$; {temp. = 32°C, rpm = 50, medium = 38% ethanol, and membrane = regenerated cellulose}).

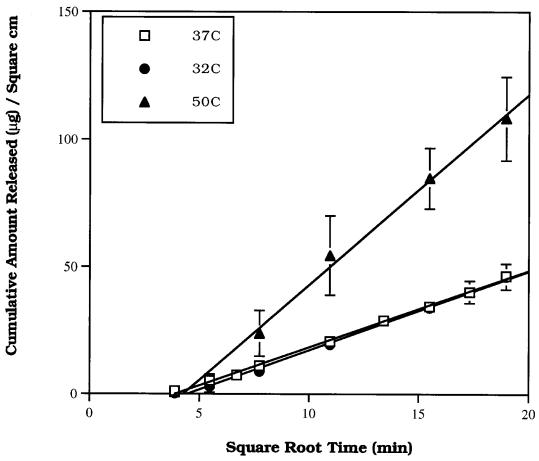

Fig. 6. Release profile from Triacet™ 0.1% cream formulation at different temperatures of the receptor phase (Mean (SD); {$n = 6$: 32 and 50°C; $n = 3$: 37°C}; {other conditions rpm = 50, medium = 38% ethanol, and membrane = regenerated cellulose}).

receptor medium. Temperature of the receptor phase could alter the viscosity (both micro- and macroscopic) of the base and change the resistance to drug diffusion into the receptor medium. Temperature may also affect the solubility of the drug and thus its diffusion rate. It was seen that the release profile at 32 and 37°C was not significantly different ($P > 0.32$) but a significantly higher rate of release was seen ($P \leq 0.0001$) at 50°C (Fig. 6). However, upon visual examination of the drug reservoir of the Enhancer Cell™ after the experiment, no change in the consistency of the formulation was observed indicating that the temperature did not affect the physical stability of the formulation. This increase in the amount of drug release at elevated temperature could be due to a significant increase in the diffusion coefficient at that temperature [13]. This was not observed in the temperature range of 32–37°C.

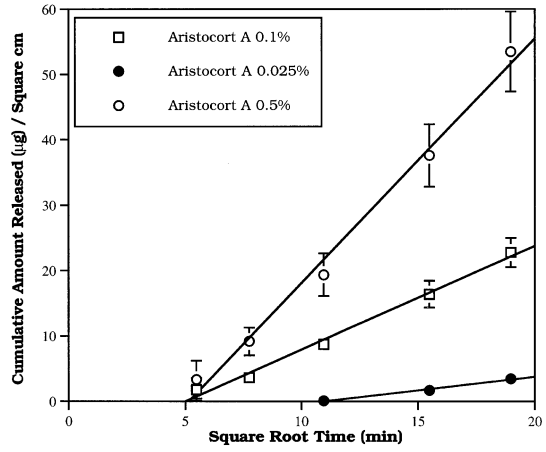

Fig. 5. Release profile from Aristocort A® cream formulations (0.025, 0.1 and, 0.5% TA) (Mean (SD); $n = 6$; {temp. = 32°C, rpm = 50, medium = 38% ethanol, and membrane = regenerated cellulose}).

Generally an increase in agitation leads to a reduction in the thickness of the diffusion layer (at the interface between the receptor phase and the membrane) and provides better mixing. A total of 110.45 ± 14.23, 89.38 ± 10.97 and 98.47 ± 5.50 µg was released using stirring speeds of 50, 100 and 200 rpm, respectively (Fig. 7). The P value (> 0.26) determined from the differences in the slopes and intercepts shows that these profiles are not significantly different from one another. This would mean that the system was well mixed and the stagnant layer above the membrane had a minimal effect. The release profiles obtained using different concentrations of the receptor phase (38 and 76% ethanol) were not significantly different ($P > 0.68$) (Fig. 8).

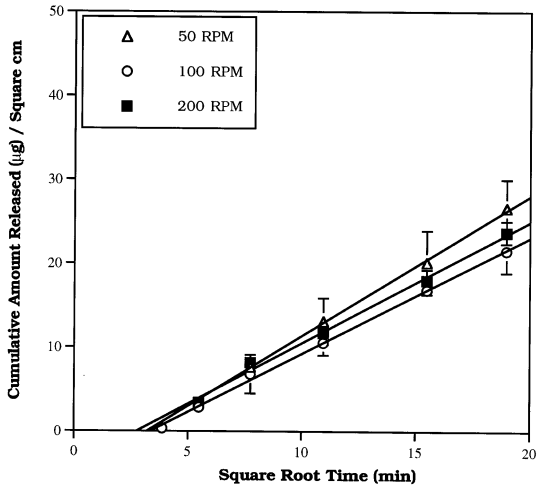

Fig. 7. Release profile from Triacet™ 0.1% cream formulation at different stirring speeds (Mean (SD); {$n = 6$: 50 rpm; $n = 3$: 100 and 200 rpm}; {other conditions temp. = 32°C, medium = 38% ethanol, and membrane = regenerated cellulose}).

Artificial membranes (synthetic and semi-synthetic) have been recommended for in vitro quality control testing due to the variability involved with biological membranes. Selection of the artificial membrane is important. The membrane should be permeable to the drug and its pore size and thickness should not affect drug release. We evaluated the release profiles using regenerated cellulose, polyethylene, and rat skin. The characteristics of the membranes are listed in Table 3. No physical changes were observed for the membranes tested at the conclusion of each evaluation upon exposure to an hydro-alcoholic receptor phase.

Table 3
Characteristics of the membranes used in the study

Membrane	Pore size (µm) & molecular weight cut-off	Thickness (µm)
Regenerated cellulose	<0.01 µm, 6000 mol. wt. cut-off	73
Microporous polyethylene film	0.18 µm, >1000 000 mol. wt. cut-off	51
Rat skin	—	1427 ± 127 ($n = 36$)

The nature of the membrane affected the release ($P \leq 0.004$), with the greatest amount released (193.87 ± 4.14 µg) from the polyethylene membrane (Fig. 9). These differences in the

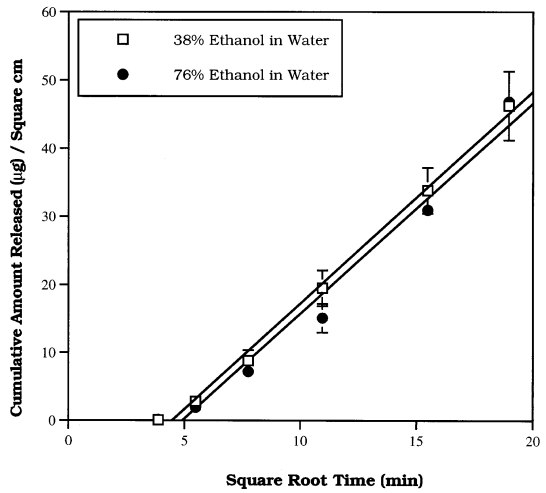

Fig. 8. Release profile from Triacet™ 0.1% cream formulation using different composition of the receptor medium (Mean (SD); {$n = 6$: 38% ethanol; $n = 3$: 76% ethanol}; {other conditions temp. = 32°C, rpm = 50, and membrane = regenerated cellulose}).

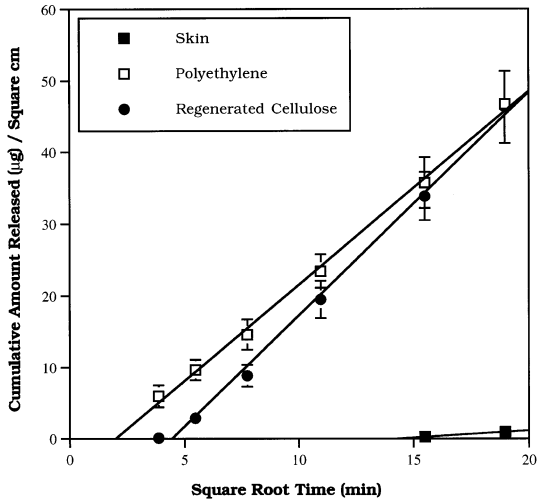

Fig. 9. Release profile from Triacet™ 0.1% cream formulation using different membranes as a barrier (Mean (SD); {$n = 6$: regenerated cellulose; $n = 3$: polyethylene and rat skin}; {other conditions temp. = 32°C, rpm = 50, and medium = 38% ethanol).

amounts released can be explained in part as differences in the pore size and thickness of the membranes. This also shows that the use of biological membranes is not recommended in the development of an in-vitro technique, especially when the method is not being developed for an in-vitro in-vivo correlation. The membrane type influenced the TA delivery due to the reasons mentioned above. This identifies the choice of membranes as a significant variable and it would be inappropriate to compare data obtained using different membranes.

4. Conclusion

Currently no official procedure exists for the evaluation of in vitro drug release from semisolid dosage forms. The main objective of this study was to develop an in vitro quality control procedure, analogous to the dissolution test for oral solid dosage form, and evaluate the drug release from topical semisolid formulations using the Enhancer cell™ method. With appropriate conditions, this could then be used to assess the batch-to-batch uniformity for these semisolid drug products.

The advantages of the Enhancer cell™ method is that it uses a modification of the existing and easily available apparatus (USP Apparatus 2) which is universal to most researchers and manufacturers. It requires less accessories and hence reduces the time and cost required for equipment setup. This method can be automated with relative ease whereby the sample can be collected and transferred to the HPLC. The Enhancer cell™ is made of Teflon® which is an inert material and thus has no problems of interaction of the formulation with the cell (these problems can arise while using glass diffusion cells). The problem of breakage, common with most glass diffusion cells, is also avoided.

The disadvantage of the Enhancer cell™ is that being made of Teflon® (a poor conductor of heat with a small heat transfer coefficient), the temperature equilibrium between the formulation and the receptor phase could take a finite time, requiring the cells and the formulation be stored at the study temperature before use. This temperature control would be especially important during the evaluation of drug release from ointments which would take a longer time to equilibrate.

This method has the ability to distinguish between formulations. The method could detect products of different strengths (as shown by the different release profiles). Earlier work [8,9] has shown that data obtained using the Enhancer cell™ method were more consistent as compared to the data obtained from other diffusion cells. This study supplements the work and characterizes the Enhancer cell™ method with respect to its variables. This is a simple method, reliable and reproducible and could also be used as a screening device in preformulation and product development.

Acknowledgements

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