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DEVELOPMENT AND VALIDATION OF METHOD FOR DETERMINATION OF *IN VITRO* RELEASE OF RETINOIC ACID FROM CREAMS*

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ABSTRACT

The objective of this study was to develop and validate an *in vitro* test method that can be used as a tool for accessing batch-to-batch uniformity of finished topical products. The studies were performed by utilizing topical creams containing the 13-*cis* isomer of retinoic acid. Various physicochemical factors which may affect drug release from topical cream formulations were evaluated including drug concentration, internal phase droplet size distribution, viscosity and the composition of the emulsion internal and external phases. Utilizing a modified Franz diffusion cell with a cellulose membrane, the *in vitro* drug release profile from various cream formulations was established.

The effect of varying the concentrations of various key ingredients by 30% does affect the viscosities and release rates compared to a standard formulation. However, there is no correlation between the viscosities and the release rates. No significant differences in pH and droplet size distribution were observed in these cream formulations compared to a standard formulation. The oil phase volume ratio appears to have the largest influence on the *in vitro* release of the drug. Intra- and inter-batch comparisons

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of the finished cream products show reproducible release profiles. Based on the results obtained in this study, when used together, both *in vitro* release and viscosity measurements may be useful as tools to assess batch-to-batch uniformity and consistent manufacturing of the finished cream product.

INTRODUCTION

There is a need for a simple, reliable and reproducible *in vitro* method that can measure the batch-to-batch uniformity of topical dosage forms (ointments, creams and lotions). In a recent article, Shah et al.[1] described a technique for determining the *in vitro* release rates of drugs from creams utilizing a Franz[2] diffusion cell system and a synthetic membrane. The advantages of using synthetic membranes *in vitro* studies have been reported by Garret et al.[3] and Barry et al.[4], but basically provide for a more consistent performance of the membrane relative to animal or human skin. Other investigators[5,6] have reported automated methods for measuring the in vitro release of drugs from topical dosage forms. Although these investigators have shown the utility of these methods for measuring the release characteristics of the drug from topical dosage forms, these methods were not rigorously tested for sensitivity and reproducibility. The object of this study was to develop and validate an *in vitro* test method that maybe used as a tool for accessing the batch-to-batch uniformity of finished topical products. Intra- and inter-batch comparisons of the *in vitro* release rate from topical dosage forms would provide a measure of homogeneity and the reproducibility of the manufacturing process, respectively.

The studies were performed by utilizing topical creams containing isotretinoin (Ro 4-3780). Various physicochemical factors which may affect drug release from topical cream formulations were evaluated including drug concentration, droplet size, pH, viscosity, and the composition of the emulsion internal and external phases.

MATERIALS AND METHODS

Materials

Ro 4-3780 was obtained from Hoffmann-La Roche Inc., Nutley, NJ. Cetyl alcohol, stearyl alcohol, glyceryl monostearate, primary and secondary emulsifiers, glycerin, fatty

oil (medium chain), preservatives and all other chemicals were obtained commercially and were either pharmacopeial or analytical reagent grade.

Methods

Preparation of Formulations

The formulations were prepared by adding a heated solution of isotretinoin in the molten wax base (glyceryl monostearate, fatty oil, primary and secondary emulsifiers and propylparaben) to a heated aqueous solution containing glycerin and methylparaben while stirring constantly. The mixture was homogenized (Greerco Co., Hudson, NH) for a few minutes and then allowed to cool and congeal.

Microscopic Examination

Immediately after preparation of the samples, microscopic observation was made with an optical microscope (Nikon, Garden City, NY) at 400 x magnification. The samples were diluted about 10 times with water and a drop of the diluted sample was then placed on a glass slide with a small depression in the center.

Determination of Emulsion Type

The emulsion type, O/W or W/O, was determined by adding water soluble dye (FDC red #4, Pylam, Garden City, NY) to the emulsion and observing the location of the dye with an optical microscope at 400 x magnification.

Determination of pH

The pH of the product was determined by dispersing a portion of the cream (1 gm) in a given volume of distilled water (4 ml) and recording the pH (Orion, Boston, MA) of the resulting dispersion.

Measurement of Viscosity

The viscosity was measured by a cone and plate viscometer (Digital DV-II, Brookfield, Stoughton, MA) connected to a personal computer (IBM, PS-2, Paramus, NJ) at various shear rates (Spindle No. 42) with the temperature maintained at 25°C.

In Vitro Drug Release

To obtain the *in vitro* release profiles of the drug under investigation from different formulations, a standard open cap, ground glass Franz diffusion cell was utilized with some minor modifications. Uniform samples of the cream (approximately 0.45 gm) were

applied to a cellulose membrane (Spectrum Medical, Los Angeles, CA) using a 0.2 cm thick circular, 1.2 cm diameter, teflon ring (2.2 cm^2 area). The receptor phase was stirred by means of a constantly spinning bar magnet (vari-speed, side-by-side, cell drive console, Crown Glass, Somerville, NJ). The cell body was filled to overflowing with a degassed receptor phase. The composition of the receptor phase was 50% methanol/phosphate buffer (0.1 M, pH 7.4) containing 0.1% butylated hydroxyanisole as an antioxidant. The studies were carried out at 32°C using 9 diffusion cell assemblies. A 0.5 ml aliquot of the receptor phase was withdrawn at predetermined time intervals and transferred into a low volume insert of an auto-injector. The cell was refilled with the receptor phase. A 100 μl aliquot of this solution was injected into the liquid chromatograph and quantitated by using an external standard method.

HPLC Analysis

A high performance liquid chromatograph (Waters, Milford, MA) equipped with a Ultrasphere ODS (Beckman, Fullerton, CA) column; 4.6 mm i.d. and 25 cm long, maintained at ambient temperature, an auto-injector (WISP-Model 712, Waters, Milford, MA), a UV variable wavelength detector (Applied Biosystems-Model 783A, Foster City, CA) operated at 365nm and an integrator (Perkin-Elmer Nelson, Cupertino, CA) were used for the quantitation of drug. The samples were eluted using methanol:2-propanol:acetonitrile:water:glacial acetic acid solvent in a ratio of 25:30:25:20:1 at a flow rate of 1 ml/min. The detector range was set at 0.001 AUF. Under these conditions the retention time of isotretinoin was 12.5 min. The detection limit was 20 ng/ml. Good reproducibility was observed, with the percent coefficient of variation less than 2%.

Determination of Release Rate

The release of the drug from an emulsion in which the drug is initially uniformly dissolved, generally follows the "square-root" approximation[7,8]:

$$Q=2C_o\sqrt{\frac{D_e t}{\pi}}$$

where

Q is the amount of drug released per unit area of application ($\mu g/cm^2$)

C_o is the initial concentration of drug in the emulsion ($\mu g/cm^3$)

D_e is the effective diffusion coefficient of the drug in the emulsion (cm^2/sec), and,

t is the time after application (sec).

A graph of the amount of drug released versus the square root of time is plotted. The intermediate points were used for the regression analysis. The initial points, considered to be in the lag-time period, were not included. Similarly, the final points, which indicate that the rate of diffusion decreases as the drug in the applied formulation is depleted, were ignored. The slopes of the linear portion of the plots (between 1 to 4 hours) give the release rates. Each point is the average of at least three separate experiments.

RESULTS AND DISCUSSION

The evaluation and validation of the described *in vitro* release method was accomplished by measuring the release rate of the drug from various cream formulations. The first set of experiments evaluated the effect of drug concentration on release rate, followed by experiments to evaluate the effect of emulsion type (oil-in-water (O/W) versus water-in-oil (W/O) on release rate, and droplet size on the rate of release of isotretinoin from various cream formulations. The second set of experiments were designed to investigate the effect of varying the concentration of various key ingredients ($\pm$30 wt.%) from the standard cream formulation. In the third experiment the effect of varying the oil phase volume ratio was evaluated. Finally, the method and apparatus were used to compare intra- and inter-batch variation of drug release from the standard formulation.

Effect of Drug Concentration, Emulsion Type and Droplet Size

Figure 1 shows the drug release profiles from O/W creams containing 0.01, 0.05, and 0.1 wt.% isotretinoin. The correlation coefficients of these linear regression lines are greater than 0.99. The data are reported in Table 1.

A seven fold increase in the release rate was observed as the concentration of isotretinoin was increased from 0.01 to 0.1 wt.% in the O/W emulsion system. The release rate from 0.1 wt.% W/O cream prepared by modifying formulation composition decreased

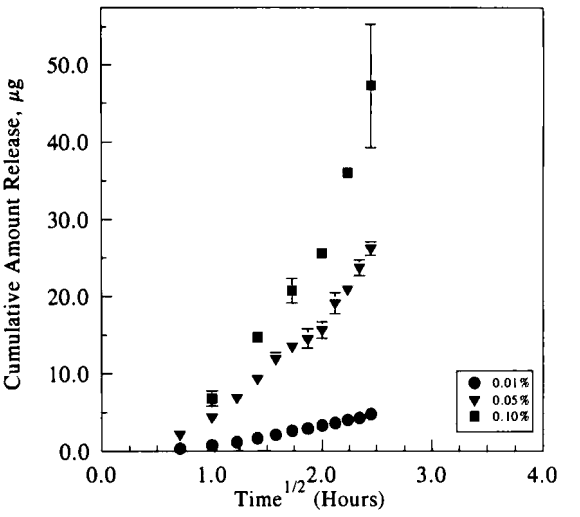

FIGURE 1
Effect of drug concentration on the *in vitro* release of Isotretinoin from O/W creams. Each point represents the mean ± s.d of at least three experiments.

TABLE 1

Viscosity, Droplet Size, and Release Rates of Isotretinoin from Topical Cream Formulations.

Emulsion Type	Drug Concentration % w/w	Viscosity Centipoise	pH	Droplet Size microns	Release Rate $\mu g/cm^2/hr^{1/2}$
O/W	0.01	5300	5.7	5	1.2
O/W	0.05	6450	5.6	5	5.4
O/W	0.10	4925	5.6	5	9.0
O/W	0.10[a]	3925	5.4	20	12.1
W/O[b]	0.10	30,000	-	5	3.6

[a] This cream contains larger oil droplets (Range 10-40 microns)
[b] Formulation composition is different than the O/W emulsion

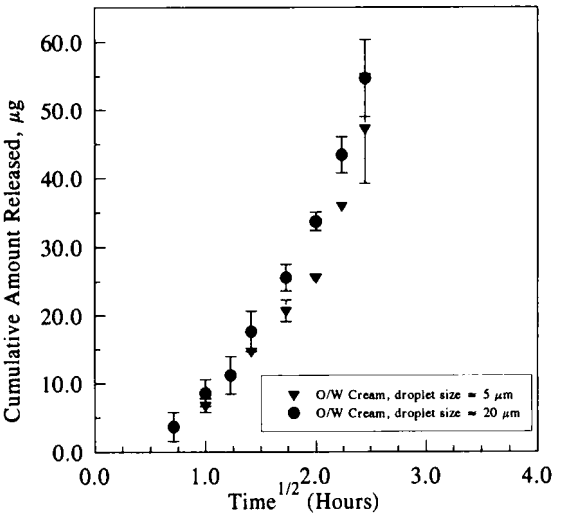

FIGURE 2
Effect of droplet size on the *in vitro* release of Isotretinoin from creams. Each point represents the mean ± s.d of at least three experiments.

2.5 times when compared to the O/W cream of the same drug concentration. This may be due to higher viscosity of W/O cream than O/W cream or to the different phase volume ratio or a change in the activity of the drug in the formulation due to increased solubility. Figure 2 shows the effect of droplet size on the release profiles from 0.1% O/W cream, one containing smaller oil droplets (approx. 5μ) and the other containing larger oil droplets (approx. 20μ). There was no significant difference in release rate ($p=0.05$) and hence the droplet size did not effect the release rates of the drug from the O/W cream.

Microscopic examination shows that water soluble solid dye tints only O/W emulsions and *vice versa*.

Effect of Variation of the Level of Key Ingredients

The effect of a ±30 wt.% variation of the level of key ingredients from the standard formulation is shown in Table 2. The key ingredients include the primary emulsifiers, the secondary emulsifier, the lipophilic solvent, and water.

TABLE 2

The effect of concentration of primary emulsifiers, secondary emulsifier, and water on the viscosity and release rates of isotretinoin from O/W cream formulations.

Ingredients	% Change in Concentration	Viscosity (10 rpm, 25°C) Centipoise	Release Rate $\mu g/cm^2/hr^{1/2}$
Primary Emulsifier I	−30	3850	7.0
	0	2900	7.0
	+30	3830	6.8
Primary Emulsifier II	−30	4420	4.3
	0	2900	7.0
	+30	2900	5.4
Secondary Emulsifier	−30	3720	9.2
	0	2900	7.0
	+30	5300	8.3
Water	−30	4670	8.2
	0	2900	7.0
	+30	3275	6.6

This variation of the primary emulsifier I concentration results in an increase of viscosity by 25%, but had little effect on the release rates. The primary emulsifier II variation results in an increase of viscosity by 34%, however the release rate decreased by 39%. The increase in viscosity and decrease in release rate may be due to greater lipophilicity of emulsifier II relative to emulsifier I.

The secondary emulsifier concentration variation results in an increase of viscosity ranging from 22-45%. This variation also leads to a decrease in the release rates ranging from 13-16%.

The variation of the level of lipophilic solvent (Table 3) results in an increase in viscosity ranging from 22-29% and the release rates decreased by 30-46%. Likewise the variation of the level of water affected the viscosity ranging from 11-38% which decreased the release rates ranging from 6-15%. There was no correlation between the viscosities and the release rates (Figure 3).

TABLE 3

Effect of phase-volume ratio on the viscosity, droplet size, and release rate of Isotretinoin from 0.1% O/W creams.

Neutral Oil (% w/w)	Oil Phase Volume Ratio*	Viscosity (10 rpm, 25°C) centipoise	Droplet Size microns	Release Rate $\mu g/cm^2/hr^{1/2}$
0.0	0.09	2368	-	1.05
6.0	0.16	3700	1.4	4.88
9.0	0.21	2900	2.3	6.93
12.0	0.25	4075	4.7	3.75
19.0	0.37	5725	5.5	0.05
24.0	0.47	5000	8.2	0.06
29.0	0.59	5475	8.6	5.45
34.0	0.72	5675	10.5	6.36

* Phase-volume ratios were calculated by dividing the total volume of oil phase by the total volume of water phase, assuming the density is equal to 1.

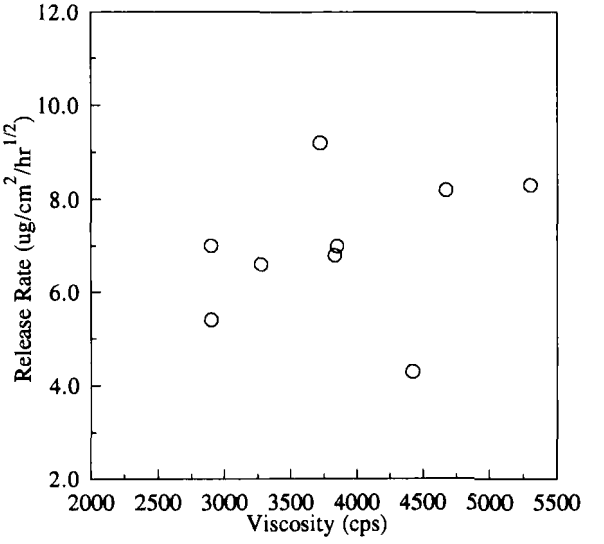

FIGURE 3
Effect of viscosity on the release rates of Isotretinoin from O/W creams.

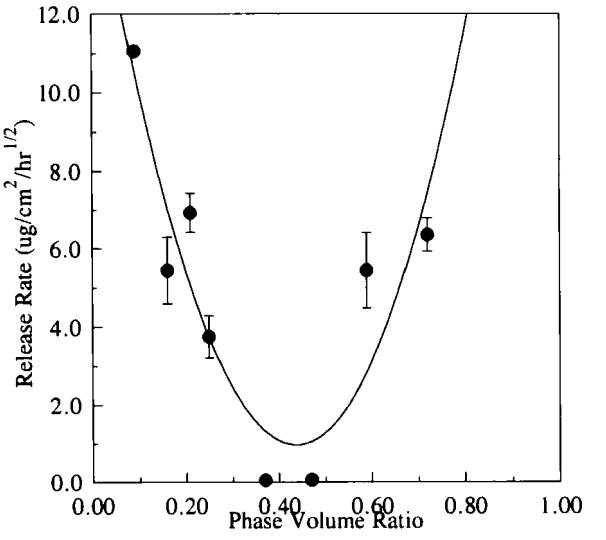

FIGURE 4
Correlation of the release rate constants and phase-volume ratios of the O/W emulsions. Each point represents the mean ± s.d of at least three experiments.

There also were no significant differences in pH and mean droplet size of these cream formulations compared to the standard formulation ($p=0.05$). Although these changes do not statistically affect the release rate of the drug when compared to a standard formulation ($p=0.05$), the variation of the emulsifier II concentration had the most profound effect on the release rate next to lipophilic solvent. The decrease in the release rates associated with these variations is likely due to an increase in the liphophilicity of the formulation with the drug having a lower partition coefficient between the formulation and the receptor phase.

Effect of Phase-Volume Ratio

Increasing the oil phase-volume effected the rheological behavior of the formulations. The mean droplet size and the viscosity of the emulsions increased moderately but significantly with oil phase volume ratio increase from 0.09 to 0.72. The viscosity, droplet size, and the release rates are listed in Table 3.

Figure 4 depicts the relationship between the release rate constants and a range of phase-volume ratios of O/W creams.

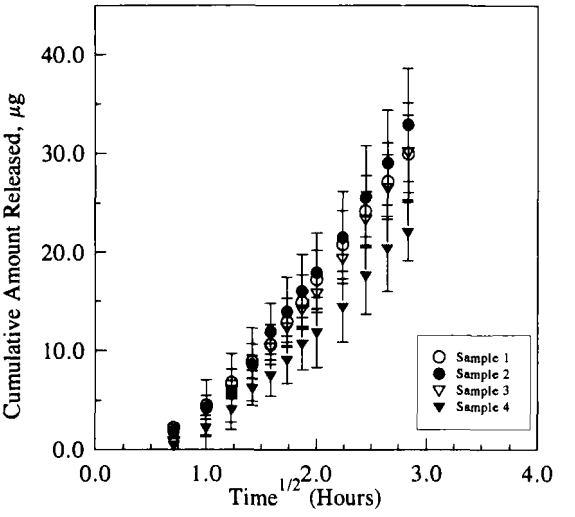

FIGURE 5
Intra-batch comparison of release profiles
Each point represents the mean ± s.d of at least three experiments.

The increase in release rate observed when the neutral oil concentration is zero may be due to the high concentration of drug in the emulsifiers and fatty alcohols where the drug is highly soluble and which may help solubilize the drug in the receptor phase. As the phase volume ratio is increased, there is a decrease in the release rate. A minimum is reached which may be explained by surpassing the optimal neutral oil surface ratio (which occurs at a phase volume ratio of 0.21) leading to only a partial coverage of the oil by the emulsifier, decreasing the effect of the emulsifier on enhancing drug release. In addition, the diffusion pathway may become longer due to increased droplet size as well as partitioning. As the phase volume further increases, more and more oil remains unemulsified. The increased surface area of the oil, where the drug is dissolved, decreases the diffusion pathway, leading to an increase in the release rates (9).

Intra- and Inter-batch Comparisons

Figure 5 shows the intra-batch comparison of the release profile of the standard cream formulation. The intra-batch variation was less than 6% (n=4) and had very reproducible release rates (Table 4). Figure 6 shows the comparative release profile of

TABLE 4

Intra-batch comparison of release rates.

Sample Number	Release Rate $\mu g/cm^2/hr^{1/2}$
1	4.5
	4.8
	4.8
2	4.5
	4.7
	4.9
3	4.5
	5.7
	3.8
Mean ± s.d	4.7 ± 0.5

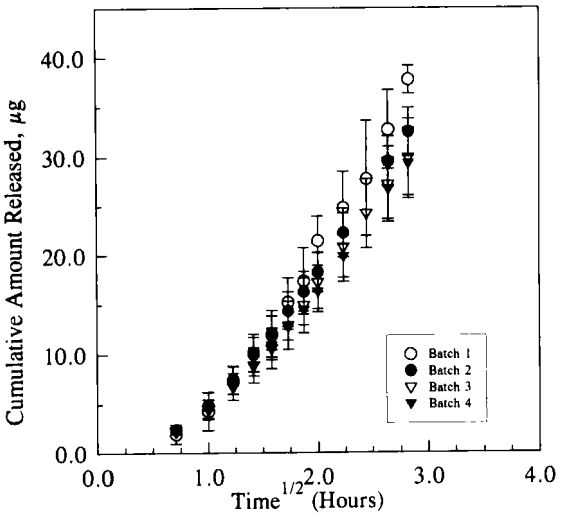

FIGURE 6

Inter-batch comparison of release profiles.
Each point represents the mean ± s.d of at least three experiments.

TABLE 5

Batch-to-batch comparison of release rates.

Batch Number	Viscosity (10 rpm, 25°C) cps	Mean Release Rate* $\mu g/cm^2/hr^{1/2}$	S.D
1	3100	6.93	0.51
2	3400	6.03	0.46
3	3517	5.02	0.64
4	3908	5.20	0.56

* $n = 4$

4 different batches of the standard cream formulation and the data are listed in Table 5. The release profile between these batches of the cream was not significantly different ($p=0.05$). These results indicate that the method developed shows promise as a tool for comparison of *in vitro* release profiles of creams.

CONCLUSION

The *in vitro* release method presented here has been used successfully to distinguish among a number of topical cream formulations containing isotretinoin. Monitoring only parameters such as pH, droplet size, may not be sensitive enough to distinguish the intra- and inter-batch variation. The *in vitro* release method is simple and reproducible. *In vitro* release parameters measurement is sensitive to formulation changes. When used together, both *in vitro* release and viscosity measurements may be useful as tools to assess batch-to-batch uniformity and consistent manufacturing of the finished cream product.

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