Comparison of the Hanson Microette® and the Van Kel Apparatus for *In Vitro* Release Testing of Topical Semisolid Formulations

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Purpose. The major goal of this study was to compare the relative utility of the Hanson Microette® and the Van Kel apparatus, two fully automated devices, as in vitro release tests (IVRT) for semisolids. We attempted to develop methodology that can be used to discriminate formulation changes, and to evaluate the precision, reproducibility and technical complexity of each test apparatus.

Methods. We chose the sunscreen Eusolex®232 (2-Phenylbenzimidazole-5-sulfonic acid) as a model compound, which was incorporated into an emulsion formulation prepared in our laboratory. Test conditions for the two IVRT were made as nearly identical as possible, in order to obtain an accurate comparison.

Results. The formulations were tested and found to be physically stable throughout the entire study. Diffusion coefficients were apparatus-dependent but were independent of the drug concentration in the formulations. The IVRT data were plotted as amount released ($\mu g/cm^2$) vs. square root of time ($s^{0.5}$) and a linear relationship was obtained in each case. Both methods produced similar results and were able to detect changes in drug loading in the formulations.

Conclusions. The linear relationship between the amount released and the square root of time indicates a diffusion-controlled release of drug. Both apparatuses proved to be suitable as tests for formulation "sameness" according to the FDA's SUPAC-SS guidelines, during level 3 changes. However, each apparatus produced a different release profile for the drug. The choice of apparatus will depend upon a number of considerations.

KEY WORDS: Van Kel apparatus; Enhancer cell; Hanson Microette® apparatus; Franz cells; release rate; topical semisolid formulation; Eusolex®232

INTRODUCTION

The use of an in vitro release test (IVRT) to evaluate drug release from semisolid formulations has received increased attention over the last few years (1,2). During this period two apparatuses, i.e., the Van Kel apparatus (Van Kel Industries, Cary, NC) and the Microette® apparatus (Hanson Research, Chatsworth, CA) have evolved as the principle components of the preferred methods (3–6). In spite of this, no reports have appeared in which the preferred, fully automated, apparatuses were compared.

The in vitro release rate reflects the combined effect of the ingredients and rheological properties of a formulation, which makes it suitable for monitoring formulation changes and/or "batch-to-batch" uniformity "in house". This can be a very useful parameter if one intends to change composition, manufacturing processes or equipment. We therefore attempted to follow the guidance published by the Food and Drug Administration for industrial non-sterile semisolid dosage forms (SUPAC-SS) (1). The objective of this study was to compare the ability of these two IVRT devices to monitor changes in semisolid formulations. The same formulation at three different drug concentrations was evaluated by both devices employing test conditions that were as nearly identical as possible. The use of "in house" formulations was chosen in order to mimic more closely the actual process that might occur in industry when reformulating to obtain a different strength of a topical semisolid dosage form. This seemed to be a more robust way of testing the relative ability of the two test apparatuses to detect "product-related" differences and/or product "sameness" than selecting a simpler model formulation or a previously formulated commercial preparation, which might very well have undergone such testing prior to marketing.

MATERIALS AND METHODS

Materials

2-Phenylbenzimidazole-5-sulfonic acid (99.4 %), i.e., Eusolex®232 (E232), was donated by Rona® EM Industries (Hawthorne, NY). Carbopol®Ultrez 10 and Pemulen®TR-2 (Acrylates/$C_{10–30}$ alkyl acrylate crosspolymer) were provided by BF Goodrich (Cleveland, OH). The C_{12}–C_{15} alkyl benzoate (Finsolv TN) was from Finetex, Inc. (Elmwood Park, NJ). Ethomeen O/12 was from Akzo Nobel Chemicals (McCook, IL) and Isopropyl myristate (IPM) was from Alzo Inc. (Sayreville, NJ). 2-Amino-2-methyl-1-propanol (AMP-95) was contributed by Angus Chemical Company (Buffalo Grove, IL). Boric acid and Borax were both purchased from Sigma Chemical Co. (St. Louis, MO). Polysorbate (Tween®80), Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), Acetonitrile, Propylene Glycol and Hydroxypropylmethylcellulose were from standard suppliers. The water utilized was purified by reverse osmosis.

Formulation Procedure

Several formulations were evaluated and the most suitable formulation found was similar to one reported by BF Goodrich. This formulation was then tailored to our desired properties, i.e., composition, viscosity, pH and drug content. The final formulations (Table I) were prepared according to standard protocols and were stored in polypropylene ointment jars until used.

Formulation Stability

The pH of freshly prepared formulations was measured using a Corning pH meter Model 240 (Corning Science Products, Corning, NY) and the viscosity was measured with a Brookfield viscometer, Model DV-I (spindle #5, 100 rpm) (Brookfield Engineering Laboratories Inc., Stoughton, MA), as shown in Table I. Both pH and viscosity were monitored throughout the study. A 10% deviation from the original viscosity in less than a three-month period is a sign of an unstable emulsion. As soon as the formulations were prepared, a freeze and thaw stability test was initiated. The formulations were examined for any evidence of phase separation or precipitation.

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Table I. Summary of Formulations Investigated

Ingredient	Function	Concentration (% w/w)		
Water	Solvent	91.65	90.65	89.65
Methocel® E4M	Thickener	0.1	0.1	0.1
Disodium EDTA	Chelating agent	0.05	0.05	0.05
Carbopol® Ultrez 10™	Thickener	0.25	0.25	0.25
Tween®80	Additive/particle size reducer	0.4	0.4	0.4
Propylene Glycol	Humectant	1	1	1
Eusolex®232	**Model compound**	**1**	**2**	**3**
Finsolv®TN	Emollient	5	5	5
Pemulen®TR-2	Emulsifier	0.25	0.25	0.25
AMP–95	Neutralizer	0.847	1.15	1.59
Viscosity [cps] (n = 3) (± SD)		2057 (28.1)	2041 (59.4)	2052 (30.2)
pH range		6.28–6.42	6.23–6.30	6.24–6.30

Diffusion Coefficient

The viscosity of all formulations was maintained constant in order to ensure that those containing 1%, 2% or 3% of active had as nearly identical properties as possible. The diffusion coefficient was determined using the Higuchi equation (7):

$$D = (\text{slope})^2/(2 \cdot A \cdot C_{sm}), \quad (1)$$

where D is the diffusion coefficient (cm^2/s), the slope is the amount released, i.e, mass per area per square root of time ($\mu g/cm^2/sec^{0.5}$), A is the concentration of drug in the formulation ($\mu g/cm^3$), and C_{sm} the saturation solubility of the drug in the external phase (matrix) of the formulation ($\mu g/cm^3$). In order to calculate D, it was essential to determine C_{sm}, which required that we obtain a sample of the external phase of our formulation. This was achieved by cracking the emulsion by centrifuging (Beckman, Model L8 70M ultracentrifuge, Beckman Instruments Inc., Palo Alto, CA) at 200,000 μg for 15 h.

Light Microscopy

Microscopic examination of the formulation containing 3% E232 was performed using an Olympus 1×70 microscope (Olympus America Inc., Melville, NY), in order to ensure that the release of the active from the formulation was not affected by agglomeration of suspended E232 particles. A magnification of 40× was used and photographs were taken using a COHU 4915 CCD digital camera (Cohu Inc., Electronics Division, San Diego, CA) using IPLab Spectrum software (Signal Analytics, Fairfax, VA). Figure 1 shows the presence of trapezoidal-shaped crystals of E232. The small droplets shown are assumed to be the internal phase of the emulsion.

Volumetric Titration

E232 had to be neutralized to increase its solubility, since the maximum solubility in water is 12 μg/l. The amount of neutralizer (AMP-95™) was adjusted to the E232 content in the formulation in order to make the properties of each formulation as identical as possible. The thickening efficiency of the Carbomer®is highly pH-dependent and therefore changes in viscosity would likely alter the release rate (2). This adjustment enabled us to determine if changes in drug loading in the formulation caused a different release behavior without the possible confounding effect of a change in viscosity. In order to determine the amount of AMP-95™ equivalent to the E232, a potentiometric titration was performed. The E232 was dissolved in the amount of water necessary to produce the desired formulation while AMP-95 was used undiluted as titrant. The pH was measured continuously with a pH meter (Model 240, Corning Science Products, Corning, NY) previously calibrated at pH 4 and 7.

HPLC Analysis

The HPLC system consisted of a Beckman solvent delivery module Model 114M (Beckman Instruments Inc., San Ramon, CA). The samples were eluted on a Supelco Discovery RP-amide C_{16} HPLC column. The drug was determined using a Spectra Physics Model 100 UV/VIS detector (Spectra Physics Analytical, San Jose, CA) and a Spectra Physics Model SP 4290 integrator (Spectra Physics Analytical, San Jose, CA). The absorption maximum (λ_{max}) for E232 was determined to be 324 nm, using a Beckman UV/VIS spectrometer (Beckman Instruments Inc., Fullerton, CA). The absorption maximum (λ_{max}) for E232 is listed as 302 nm. However, we observed absorption of one of the matrix ingredients close to this wavelength, thus we chose the higher wavelength (324 nm), where E232 still exhibits sufficient absorption. The mobile phase chosen was an 80:20 mixture of Boric acid-Borax buffer and Acetonitrile. A flow rate of 0.8 ml/min produced a retentiontime of 3.30 ± 0.2 min. A separate calibration curve was prepared on each day that samples were analyzed. The identification of the peak at 324 nm as E232 was confirmed by LC-MS measurement (Finnigan MAT TSQ/SSQ API System) using APCI (positive atmospheric pressure chemical ionization).

Statistics

A weighted least-squares linear regression model was used to fit the calibration curve data. The assay data were also examined using statistical outlier tests (8, 9), to determine if the data from all runs were from the same population. We also utilized an ANOVA (Analysis of variance) to test the null hypothesis that the means from the samples sets are equal. In addition, the FDA recommend a non-parametric statistical test for comparing the release rates obtained in order to assess batch-to-batch uniformity. According to this

test, the release rates are considered "the same" when the ratio of the median release rate for the postchange (test) product divided by the median release rate for the prechange (reference) product is within the 90% confidence interval limits of 75% to 133.33%. In our study we compared different runs of the same concentration and designated three cells as the reference product and three cells as the test product, as recommended in SUPAC-SS (1).

Choice of Membrane

The membrane chosen should not affect the release rate throughout the entire testing period (6). Cellulose acetate DS0210 membrane filters (Nalge Nunc Corporation, Rochester, NY) with a pore size of 0.45 μm were selected. They were reported to be inert for the medium used in our tests. Drug binding to the membranes was examined by using a stock solution containing E232 as receptor medium. The concentration of E232 was measured before and after the test run. The membranes were pre-soaked for 20 min in a 15% solution of Ethomeen O/12 in IPM, to reduce back diffusion (10).

Release Study

Our primary objective was to compare the Hanson Microette® (Hanson Research, Chatsworth, CA) and the Van Kel enhancer cell™ used in conjunction with a modified USP II dissolution apparatus (Van Kel Ind., Cary, NC) as IVRT. In order to determine how they would respond when the concentration of drug in the formulations was changed, the same formulation was used at three different concentrations of active ingredient. The test conditions were chosen in order for each test to be as nearly identical as possible. The inner diameter of the Van Kel enhancer cell supplied to us was measured and found to be 21 mm, which produces a surface area of 346.4 mm^2, which contrasts with the value provided by the manufacturer (400 mm^2). The modified USP II dissolution device (Van Kel Ind., Cary, NC), includes the VK 7000 with a six spindle apparatus and 200 ml glass flasks (PN: 12-5050). A circulator - pump (VK 650a), was used to circulate the heated water and thereby maintain constant temperature in the system. Samples were removed from the vessel using a bi-directional pump, controlled by the programmable sample collector VK 8000, which was used to set collection times and sample volume. The cell body of the Van Kel enhancer cell was adjusted to carry a sample of 3 g to ensure that an excess of drug was present in the donor compartment. The sample was applied at room temperature. When applying the sample, the membrane was checked to verify the absence of air bubbles underneath the membrane. The exact weight of the formulation in each cell was determined in all runs. The receptor medium consisted of 200 ml of Boric acid-Borax buffer, pH 7.2. The medium was mixed at 100 rpm and the temperature was set at 32°C for each run. The stirrer paddles were adjusted to remain 1 cm from the top of the cells. Samples were withdrawn from the vessels using 10 μm filters at five time points, i.e., 0.5 h, 1 h, 2 h, 3 h and 4 h. The sample volume of 1 ml was not replaced.

The second device evaluated as an IVRT was the Hanson Microette® apparatus (Hanson Research, Chatsworth, CA) which employs six vertical, automated Franz diffusion cells, each of which has a 15 mm diameter and a slightly different volume. The dimensions of the cell disk, into which the sample was placed, were measured and found to have a diameter of 15 mm and a height of 1.7 mm which translates to a volume of 300. 42 mm^3 and a surface area of 176.72 mm^2. The apparatus is composed of a bath circulator (MGW Lauda, RM 20, Koenigshofen, Germany), that was used to maintain constant temperature throughout the entire system. The other components were from Hanson Research. Syringe pumps were used to withdraw the sample and replace it with fresh receptor medium. The mixing speed of the Franz diffusion cells was controlled by the Variomag telemodul 40S. The cell stand with the six automated Franz cells includes a magnetic stirrer to drive the stirring device in the Franz cells. The last component is the control unit Model 89-101-125, e.g., the "sip control", with which one can program the entire apparatus and set such parameters as sample collection times, sample volume and stirring speed, it also includes the automated sampling system Model 57-101-051.

The Franz diffusion cells were set up utilizing conditions as nearly identical as possible to the test conditions used with the Van Kel dissolution apparatus, in order to provide an objective comparison of the results from the two systems. We therefore used the same receptor medium, i.e. Boric acid-Borax buffer, pH 7.2, the identical membrane (cellulose acetate) and treatment conditions, i.e., 20 minutes of presoaking in the 15% Ethomeen O/12 in IPM solution. Sampling time points and the test temperature were identical to those used with the Van Kel apparatus. Unfortunately, it was virtually impossible to have exactly the same degree of agitation of the receptor medium due to the different geometries of the two apparatuses. Thus, for the Hanson Microette®, we chose the lowest possible stirring speed, i.e., 300 rpm. The sample volume, which includes the waste volume (0.5 ml) used to flush the sampling tubes, was 1 ml and was replaced automatically by fresh receptor media. Because of the liquid consistency of the formulation we were not able to determine the applied mass, therefore we calculated the volume of the cavity into which the formula was applied. We then calculated the applied mass of the formula, after removing the excess with a spatula, by assuming that the formula had the same density as water since it contains 90% water. These calculations indicated that approximately 300 mg of formulation was applied. Since the volumes of each Franz cell are slightly different we determined their individual volumes, as these are used in calculating the amount released. The volumes were determined by weighing the cells before and after filling them with a liquid of a known density, i.e., water.

RESULTS AND DISCUSSION

The Formulation

Three test formulations were prepared containing 1%, 2% or 3% of E232, since these concentrations are typical of those used commercially. The results of the continuous measurement of pH and viscosity combined with the results of the freeze and thaw stability tests indicated that the formulations remained stable throughout the entire study. In addition, the freeze/thaw study showed that there was a small amount of precipitation in each of the three formulations following centrifugation at 2,000 × g. This confirms that the formulations are suspensions. The saturation solubilities for E232 in the

external phase (C_{sm}) of the formulation for the three concentrations of active were found to be not significantly different ($p > 0.05$). This substantiates the essential correctness of the volumetric titration, since C_{sm} would have been altered by the addition of excess neutralizing agent, if the amount of E232 used had not been titrated to achieve neutrality. The estimated value of the diffusion coefficient was found to be dependent on the apparatus, with the mean value obtained for the Van Kel apparatus being significantly ($p < 0.05$) higher than that for the Microette apparatus (Table II). This observation is consistent with results reported in a recent publication (5). These results also indicate that the diffusion coefficient determined using either apparatus is not dependent ($p > 0.05$) upon the concentration of active in the formulation. Microscopic examination of the formulation containing the greatest concentration (3%) of active indicated that there was no agglomeration in the formulations, since the trapezoidal crystals of E232 were "uniformly" distributed throughout the formulation (Figure 1). For particles that are not agglomerated and have the same radius r, the Stokes - Einstein equation applies (11):

$$D = (k \cdot T)/(6 \cdot \pi \cdot \eta \cdot r), \quad (2)$$

where D is the diffusion coefficient (cm^2/s), k is the Boltzmann constant (J/°K), T is the absolute temperature (°K), η is the viscosity (Ns/m^2) of the solvent, and r is the radius (m) of the spherical particles. Equation 2 illustrates the relationship between the diffusion coefficient and the viscosity, and underscores the importance of maintaining a constant viscosity in order to obtain an accurate determination of the diffusion coefficient and to compare the release rates of active from different formulations on an equivalent basis.

HPLC Assay

The HPLC analysis showed no extraneous peaks when a sample of the receptor medium was injected, therefore there was no interference that could affect the quantitation of E232. Each sample was filtered (Acrodisc® syringe hydrophilic polyethersulfone filter (0.45 μm pore size), Pall Gelman Laboratory, Ann Arbor, MI) prior to injection onto the HPLC. The results were substantiated using LC-MS, since the identical mass spectrum was obtained for an E232 stock solution and a sample from the IVRT studies. The coefficient of determination (R^2) for each calibration curve was > 0.98 in all cases. We also investigated the individual ingredients of the matrix and observed no interference at a wavelength (λ_{max}) of 324.5 nm. This was confirmed in a run containing 0% E232 in which no peak was observed due to the matrix ingredients. A correction factor (12) was used in the Hanson Microette studies to account for the dilutional effects of sampling. This correction factor was modified slightly for the Van Kel apparatus data since the samples of the receptor fluid removed were not replaced. The minimum detectable concentration of E232 for our method was found to be approximately 0.5 μg per ml in Boric acid-Borax buffer.

Table II. Comparison of Diffusion Coefficients ± SD (cm^2/s) Obtained Using the Hanson Microette®

Apparatus type	Diffusion coefficients (cm^2/s) (SD) for different concentrations of active (% w/w)			Mean (SD)
	1%	2%	3%	
Microette®	4.98E-07[a] (9.82E-08)	8.45E-07[a] (1.41E-07)	6.32E-07[a] (1.86E-07)	6.57E-07[c] (1.42E-07)
Van Kel	11.8E-07[b] (3.00E-07)	18.9E-07[b] (4.14E-07)	18.4E-07[b] (1.85E-07)	16.4E-07[d] (2.99E-07)

Means with identical superscripts (*a* vs. *a; b* vs. *b*) are not significantly different ($P < 0.05$), whereas means with different superscripts (*a* vs. *b; c* vs. *d*) are significantly different.
SD = Standard Deviation.

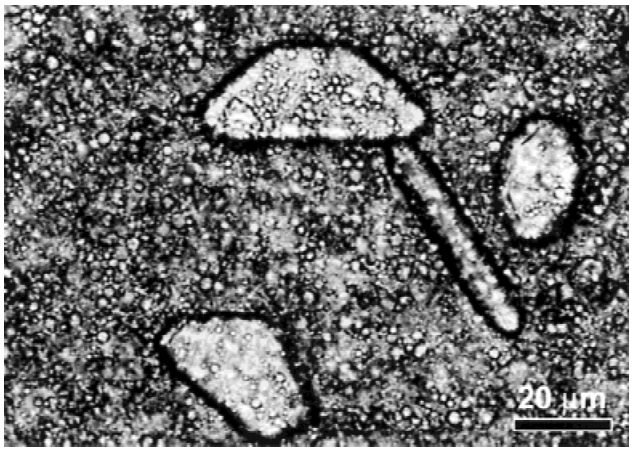

Fig. 1. Microscopic view of the formulation containing 3% Eusolex®232

Membrane

Drug binding to the membrane was determined to be negligible, since the concentration of E232 in the receptor medium was the same before and after the test run. This indicates that the release rate of E232 was controlled by the formulation matrix and not by the membrane. This test also validates that the Franz cells were properly sealed as there would have been an increase in drug concentration if a significant amount of liquid had evaporated. Furthermore, if substantial leakage had occurred, no sample could have been collected. Examination of the membranes after each run indicated that there was no damage to, or slippage of the membrane in any of the runs. Back diffusion of drug was not a significant problem, since all plots of the amount released vs. $(t)^{0.5}$ were found to be linear with a coefficient of determination (R^2) > 0.96 (13). Furthermore, the cells were examined visually after each test and no change in physical consistency of the formulation nor appearance of air bubbles was observed.

In Vitro Release Test

The in vitro release profile of E232 from the formulations was linear in all cases and the coefficient of determination (R^2) was > 0.98 for the Hanson Microette® and > 0.96 for the Van Kel® enhancer cell. The release profile for a suspension system like the emulsion used here can be described by rearranging equation 1 (7):

$$Q = (2 \cdot A \cdot C_{sm} \cdot D \cdot t)^{0.5} \quad (3)$$

where Q is the amount of drug released per unit area ($\mu g/cm^2$), A the amount of drug per unit volume of product ($\mu g/cm^3$), D is the diffusion coefficient (cm^2/s), C_{sm} is the solubility of the drug in the external phase of the vehicle ($\mu g/cm^3$), and t is time in seconds (s). Thus, a plot of the amount released (Q) vs. ($t^{0.5}$) should be linear with a slope (i.e., release rate) of $(2 \cdot A \cdot C_{sm} \cdot D)^{0.5}$.

The reproducibility of the two methods was very high, as shown in Figure 2. The release rate from the formulations was found to be higher in all cases when using the Van Kel enhancer cell (5). This can be confirmed by examining a plot of amount released vs. the square root of time (Figure 3) for the two apparatuses which shows that the ratio of the slopes (i.e., Van Kel/Hanson) for the three different strength formulations tested is not significantly different ($p > 0.05$), which illustrates the close similarity of the two methods.

The values of D and C_{sm} are normally constant in a given system, but A is not. When $A >> C_{sm}$, variation in A leads to different values for $(dQ/d(t)^{0.5})$ and a plot of $(dQ/d(t)^{0.5})$ (the slopes) vs. $(A)^{0.5}$ should be linear and pass through the origin as shown by equation 4,

$$dQ/d(t)^{0.5} = (2 \cdot C_s \cdot D)^{0.5} \cdot d\,(A)^{0.5} \quad (4)$$

Figure 4 shows that a plot of the slopes ($\mu g/cm^2/s^{0.5}$) vs. the square root of active concentration resulted in a linear relationship, in accordance with equation 4. This substantiates that the two methods are able to detect a change in concentration of drug in the formulation. The dashed lines shown in Figure 4 cross each other, which could be due to one or more of the following differences between the devices. First, the thickness of the applied layer of formulation in the Franz cell is very small compared to the thickness of the layer applied for the Van Kel enhancer cell. The thinner layer of sample apparently permits the drug to diffuse more rapidly through the layer into the receptor medium. The net result is that the x-intercept observed for the Franz cells in Figure 4 (dashed line) was shorter than that observed with the enhancer cell. The x-intercept represents the square root of the minimum concentration of drug (E232) in the formulation that is nec-

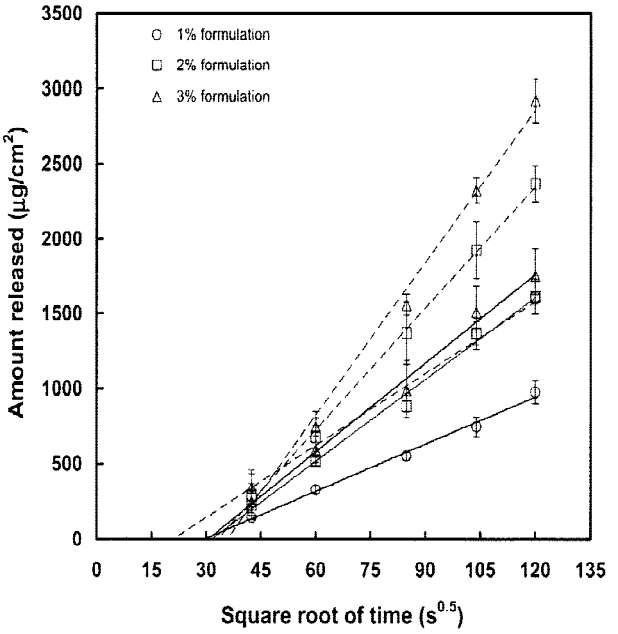

Fig. 3. Comparison of all runs obtained using the Van Kel (- - - -) and Hanson (—) apparatuses. Error bars represent one standard deviation (SD).

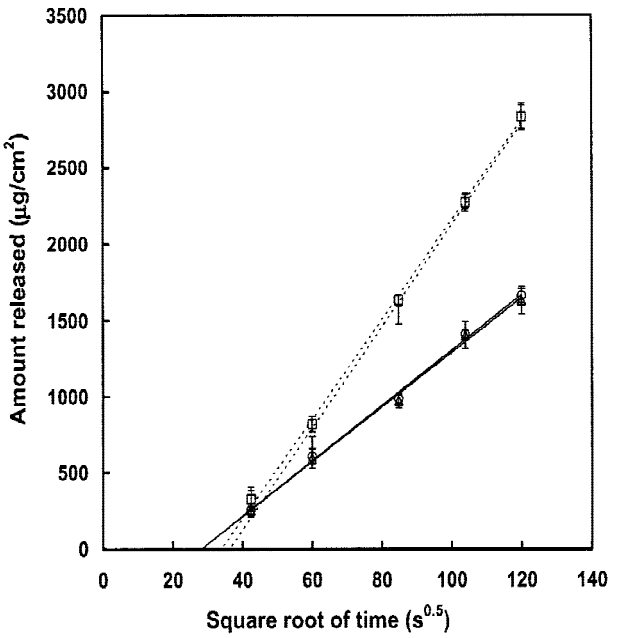

Fig. 2. Reproducibility of method between runs for the same concentration on different days. Release profile obtained with a formulation containing 3% E232 using the Hanson Microette® (—) and the Van Kel apparatus (- - - -). Each data point represents the mean of six replicates (N = 6 cells). Error bars represent one standard deviation (SD).

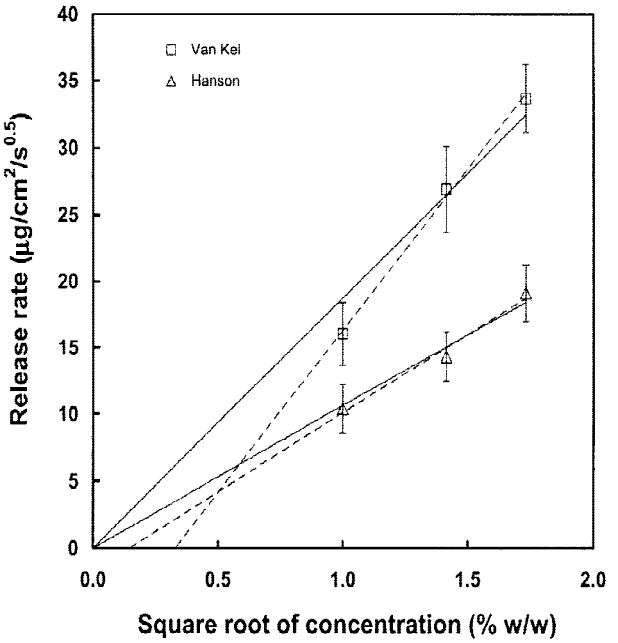

Fig. 4. Comparison of average slopes obtained with formulations containing 1%, 2% and 3% E232 vs. square root of concentration (% w/w), using SD as error bars. Dashed lines (- - - -) show the Y = m*x + b regression fit for the Hanson (R^2 = 0.983) and Van Kel (R^2 = 0.997) apparatus. Continuous lines (—) illustrate the Y = m*x regression fit for the Hanson (R^2 = 0.972) and Van Kel (R^2 = 0.944) apparatus.

essary to produce a measurable release rate. The volume of the receptor medium in the Van Kel enhancer cell was approximately 34 times the volume of the receptor medium in the Franz cells. Thus, when comparing semisolid formulations in which the percentage of a potent active ingredient is much lower, e.g., 0.05%, than the 1%, 2% and 3% formulations compared here, the greater dilution could result in an undetectable concentration of active in the sample withdrawn. The higher dilution of the sample crossing the membrane could also prolong the time for a measurable concentration of drug to appear in the sample, due to the greater assay sensitivity required, which could produce the apparently larger x-intercept. This indicates that it may be possible to collect an earlier timed sample for the Franz cells, since it was shown that the slopes of the two regression lines for the Hanson Microette® were not significantly different ($p > 0.05$). In contrast, the slopes of the regression lines obtained with the Van Kel enhancer cell were shown to be different ($p < 0.05$), thus an earlier timed sample could influence the slope of the release profile.

The SUPAC-SS guidelines list two requirements for an IVRT. First, "sink conditions" must be maintained. This can be achieved by ensuring that the drug has sufficient solubility in the receptor medium such that the receptor medium does not affect the release rate of the drug. This will presumably occur if the concentration of drug in the receptor phase (C_t) $\leq 10\%$ of the saturation solubility of the active in the receptor medium (C_{sr}), i.e., $C_t \leq 0.1C_{sr}$. A second requirement for an IVRT is that the percentage of drug released is less than 30% of the drug placed in the donor compartment (14). Presumably, the relationship between drug release and the square root of time will only be linear for topical formulations as long as these two rules apply (14). Both rules were violated for the formulations containing 1%, 2% and 3% active, while using the Hanson Microette®. The amount of drug released increased as the drug loading of the formulation was increased and reached a maximum at 93% of the saturation solubility (C_{sr}) in a 3% run. The largest amount drug dissolved in the receptor medium observed here was 46%, obtained for a 1% run. In another recent publication addressing this issue the authors observed that about 50% of their drug was dissolved in the receptor medium (13). In contrast, we observed that these two criteria were obeyed when using the Van Kel enhancer cell™.

In all of our plots showing the amount of E232 released vs. square root of time, a x-intercept was observed. This x-intercept is a common characteristic of an IVRT and is referred to as the "lag time" (t_{lag}). This value should normally correspond to only a small fraction of an hour (1). The mean lag times (± SD) were observed to be 16.01 ± 8.36 minutes (Van Kel) and 14.56 ± 2.88 minutes (Hanson). Neither value was significantly different from "0" ($p > 0.05$). The duration of each IVRT was 240 min; thus the mean t_{lag} value obtained was less than 10% of the total run time, which fulfils one requirement for a satisfactory IVRT (1). The lag time indicates that the release of the E232 from the formulation does not occur instantaneously and thus the release pattern may not be linear until after the first sampling time point. In addition, the t_{lag} for each apparatus was shown to be independent of the concentration of active in the formulation. This again illustrates the similarity of the results obtained with both apparatuses. This observation also provides further evidence of the "sameness" of the formulations tested.

Another difference between the two methods was that, when using the Hanson Microette®, the samples withdrawn were replaced by fresh medium. With the Van Kel apparatus the sample of receptor medium was not replaced, since no automatic sample replacement device was available with our apparatus and manual replacement of fluid (for so many samples) would have been associated with a large variation. The Hanson Microette® is a closed system and the concentration in the cell will not be affected by evaporative loss of receptor media. The Van Kel vessels are not fully covered because of the stirring system used. This causes some loss of the receptor medium due to evaporation, which affects the measured concentration of drug present in the receptor fluid samples. Both devices had good temperature control, which was achieved in the vessels of the Van Kel apparatus using a paddle stirrer, and in the Franz cells of the Hanson Microette® with a magnetic helix stirrer. It was not possible to adjust the stirring speed of the Hanson Microette® to the 100 rpm used with the Van Kel apparatus, since the lowest value for the stirrer on the Hanson device was 300 rpm. However, given the different geometries of the two types of cells, and thus different turbulence patterns created for a given stirring speed, this factor was not critical. Sample application was easier with the Van Kel enhancer cells than with the Franz cells of the Hanson Microette®, which might be specific to our method.

The Franz cells are made of glass and thus breakage is possible. In contrast, the Van Kel enhancer cell is made of Teflon® and is not breakable. However, the enhancer cell™ is opaque and thus it was not possible to ensure that there were no air bubbles on the bottom of the enhancer cell because the formulation was opaque and we could not see through to the bottom. When using the transparent glass Franz cells, air bubbles could be seen easily and removed. The mass of formulation added to each cell type could have been adjusted to approximately 300 mg. However, since the surface area of the membranes and the receptor volumes are different for each apparatus, it seemed unnecessary to do this.

IVRT Statistical Comparisons

The slope comparison test recommended by the FDA (1) was performed here and provided evidence of the reproducibility of the methods developed in this study. All of our formulations (1%, 2% and 3%) fell within the 90% confidence interval. This provides statistical evidence of the "sameness" of our formulations, which indicates that the methods used in this study would provide reproducible results when testing complex formulations. The different release rates observed were therefore due solely to the change in drug loading in the formulation and to how the devices vary in their determination of the release rate of the drug from the formulation. We feel confident that our chosen conditions permitted us to make a fair comparison of the relative effectiveness of the two apparatuses as components of an IVRT for topical semisolid formulations.

An example of the results of the slope comparison test is shown in Table III. The data sets obtained consisted of three runs for each E232 concentrations (i.e., 1, 2 and 3%) tested per apparatus. Each run consisted of data from six Franz cells

Table III. Example of the Slope Comparison Test for a 2% E232 Formulation Obtained Using the Hanson Microette®

Formulation	Reference sample	Test sample A	Test sample B
Mean release rate ($\mu g/cm^2/s^{0.5}$) (SD)	18.10 (0.79)	16.61 (1.62)	19.65 (1.75)
Test (T)/Reference (R) ratio	Run #1	Run #2	Run #3
Lower limit	102.26%	84.00%	99.40%
Upper limit	118.99%	97.80%	120.30%
Results of test[a]	Pass	Pass	Pass

[a] To pass the test the lower limit must be >75% and the upper limit <133.3%.
SD = Standard Deviation.

or six Van Kel enhancer cells.™ The slopes of the data plotted according to equation 3 were compared using an ANOVA to determine whether or not differences exist between the observed release rates for E232, i.e., the slopes determined using equation 3. The protocol provided by the FDA for conducting the slope test assumes that the variability for a given cell across runs is less than the variability between different cells within a given run. Our test results verified that the variability when comparing slopes between two runs, using the same cells, is smaller than the variability of the individual cells within a given run. We compared the variability across runs, i.e., the six cells/vessels for three runs were used either as a "reference" run or a "test" run. Using this approach, only two of the six concentrations tested on both apparatuses passed the slope comparison test. The 1% and 2% concentrations tested with the Van Kel apparatus failed the test as did the 1% and 3% concentrations tested with the Hanson Microette indicating that there is no systematic pattern to the runs which failed the test. The slope comparison test also failed using both apparatuses when different concentrations were compared between runs. The latter observation substantiates that the IVRTs are sufficiently discriminating to detect product "differences" as well as product "sameness".

CONCLUSIONS

Both apparatuses produced similar results for the release rate and in their response to a formulation change. However, the behavior of the two apparatuses prior to the first time point (30 min) was different depending on our test conditions. Preference for one of the two apparatuses depends on the use to which they are to be applied. Overall, we obtained linear Higuchi plots and a higher precision with the Hanson Microette®, as illustrated by Figure 2, even though the "sink conditions" and "30% rule" were not obeyed. Despite statements to the contrary (14) it seems clear from our data, and others using drugs in suspension (13, 15), that the "30% rule" is only important when the drug is completely dissolved. This seems logical since the concentration gradient of active (i.e., the driving force for diffusion) would be more rapidly depleted from a solution than a suspension. This would result in a slowing of the release rate as time passes (2). Even for systems where the drug is completely dissolved, the "30% rule" should not be taken literally as noted (15) and implied (13) by others. These observations are consistent with the results of McSpadden and Paul (16) who showed that a linear plot of amount released ($\mu g/cm^2$) vs. the square root of time ($s^{0.5}$) could be obtained over a very wide range of drug concentrations.

If one wishes to have a simpler and somewhat easier to use method to test for product sameness, the Van Kel enhancer cell™ may be favored. However, it should be reiterated that when using an opaque formulation or an opaque membrane one cannot ensure that there are no air bubbles on the bottom of the enhancer cell and the presence of bubbles may seriously influence the release rate. This factor could also vary between different runs or even within a given run, which may be at least partially responsible for the slightly higher variability in the release rates obtained here with the Van Kel apparatus compared to the Hanson Microette®. The Van Kel apparatus uses a larger volume of receptor medium, which makes it easier to achieve the FDA requirements, i.e., the "sink conditions" and "30% release" rule, as observed in our study. However, the larger volume of receptor medium dictates that more of the active must be released in order to achieve a measurable concentration, which might necessitate increased assay sensitivity. Conversely, this implies that a formulation containing a smaller amount of active can be tested using the Hanson Microette® due to the much smaller receptor volume of the Franz cells.

One potential advantage of the Hanson apparatus is that the Franz cells can more readily be used with an animal or human membrane, if so desired. This could permit the data to be compared with in vivo bioavailability data (e.g., the vasoconstrictor assay of Barry (17)) since comparisons with in vivo data require the use of a transport-resistant barrier that can discriminate permeability differences between drugs (18).

The Van Kel enhancer cell system would be preferred if someone already owned the USP II dissolution apparatus, since the cost to modify this apparatus would be much less than to buy a Hanson Microette system. The Hanson Microette® apparatus may require more experience and preliminary experimentation to obtain satisfactory results, but the higher accuracy, the ability to test formulations containing smaller concentrations of active, and the replacement of the receptor media in a closed system may prove to be advantageous when examining semisolid formulations. However, the Van Kel enhancer cell also produced very acceptable results and fulfilled both criteria (i.e., "sink conditions", "30% rule") proposed by the FDA for an IVRT for semisolid preparations, and the correct use of the apparatus requires less experience.

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