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# A Comparative Study of the Release of Active Ingredients from Semisolid Cosmeceuticals Measured with Franz, Enhancer or Flow-Through Cell Diffusion Apparatus

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## ABSTRACT

This study reports the release properties of the poorly water-soluble drug hydrocortisone, antibacterial agent triclosan, and the water-soluble anti-aging compounds salicylic acid and ascorbic acid from gels, creams, and ointments, alone or combined with triclosan. Drug release was measured with the flow-through, Franz, and Enhancer cell release testing methods. For the 3 methods, a cellulose acetate membrane soaked in lipophilic oleic acid and dissolution mediums composed of a phosphate buffer pH 5.8 or a 50% ethanol: buffer mixture gave more constant and less variable release profiles (similarity factors, $f_2$, above 50). Comparison of the release rates of hydrocortisone, salicylic acid, ascorbic acid, and triclosan from creams and gels showed that the rates measured with the flow-through cells were significantly slower ($p < 0.01$) than those measured with the Franz and Enhancer cells. In all 3 systems, hydrophilic compounds released faster in the buffer solution and from the gels. Less water-soluble compounds and lipophilic compounds released faster in the hydro-alcohol mixture and from creams and ointments. The disadvantages of the flow-through cells were difficult operation and small sample sizes which caused variable results. The advantages of the Enhancer cells were that they used the basic USP dissolution apparatus and had a larger volume range making it easier to adapt the system for studying the release of products containing low concentrations of active ingredients or ingredients that are difficult to analyze.

Key words: cosmeceutical, semisolids, release, dissolution, Franz cell, Enhancer cell, flow-through cell

## INTRODUCTION

The term cosmeceutical is a blend of *cosmetic* and *pharmaceutical* and refers to products which are marketed as cosmetics, but which contain biologically active ingredients that affect the structure or function of the skin, hair or nails. The term was coined by Albert Kligman, the University of Pennsylvania researcher credited with discovering the effectiveness of Retin-A, a vitamin A, in smoothing aging skins. Other examples of cosmeceuticals are anti-wrinkle creams, acne-treatments, baldness treatments, moisturizers, and sunscreens, which contain ingredients such as vitamin E, ascorbic acid, etc. These products are causing problems worldwide for regulatory authorities, such as the U. S. Food and Drug Administration (FDA), which must decide when a product crosses the line between being merely a cosmetic and becoming a drug, the latter having much more stringent controls on its development, testing and supply. For pharmaceutical products, the typical semisolid dosage form quality control tests include identification, assay, homogeneity, viscosity, particle size, and release (dissolution) testing.

Historically, although *in vitro* release rate testing from semisolids could potentially provide valuable information about product performance but it is not an industry wide quality control test requirement as compared to the utility of *in vitro* dissolution testing of oral dosage forms[(1)]. To change this situation the extension of *in vitro* dissolution methodology to semisolid dosage forms has been the subject of substantial effort and debate[(2,3,4)]. Similar to the dissolution testing of oral dosage forms, a simple, reliable and reproducible release rate method can guide formulation development; help to monitor batch-to-batch quality and stability, and control the manufacturing process of cosmeceuticals. It is particularly useful for detecting the effect of product changes including drug substance, excipients, and manufacturing process. This has led to the establishment of the FDA SUPAC-SS guidance requiring the performance of release testing from semisolid dosage forms after certain post approval changes[(5)]. In particular, *in vitro* dissolution of the pre- and post change formulations must be compared whenever changes are made to the product's composition, manufacturing equipment, or process[(5)]. This *in vitro* release requirement is not surrogate for *in vivo* bioequivalency testing, but is intended to assess "sameness" of a product following scale-up or post-approval manufacturing changes.

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Although the FDA SUPAC-SS guidance include general methodology descriptions of diffusion systems, it does not specify a particular test methodology because currently no compendial *in vitro* release test methodology is described for semisolid dosage forms[1,5] Compendial transdermal dosage form release methods include the paddle over disk (apparatus 5), cylinder (apparatus 6), and reciprocating disk (apparatus 7) described in the United States Pharmacopeia[6]. Recently a significant amount of effort, research, innovation, and debate has surrounded the topic of *in vitro* dissolution methodology for semisolid dosage forms[7,8]. From these reports, it is clear that a wide variety of diffusional systems have been utilized and that the current dissolution testing systems for semisolid dosage forms originated from systems used for *in vitro* skin permeation studies[7].

Among these methods, the Franz diffusion cell, Figure 1 (A), has been the standard system used for the study of semisolid drug formulations[9,10,11]. First described by Franz in 1978[12], this cell has a small donor compartment and a cylindrical receptor chamber that allows mixing with a magnetic stir-bar. As an alternative to the Franz-type *in vitro* dissolution testing system the Enhancer Cell, Figure 1 (B), was introduced commercially in the early 1990's by VanKel Industries[7,8]. The system consists of a donor chamber (the cell body) for the dosage form that is covered by a synthetic membrane. The entire assembly is placed in the bottom of a standard USP Apparatus 2 dissolution vessel. A third system that is increasingly used for measuring the release rate of drugs from semisolid dosage forms is a modified version of the flow-through apparatus (USP apparatus 4)[13,14]. For measuring the release of drug from semisolid dosage forms, an 'insertion cell', Figure 1 (C), is placed inside the flow-through cell. The insertion cell offers the advantage that it is readily adaptable for use with the compendial flow-through apparatus and does not suffer from the problem of having to remove air bubbles at the membrane/liquid interface, which commonly occurs when using Franz cells[14].

Although these three methods are used for testing pharmaceutical products, their application for testing cosmeceuticals has not been reported. For pharmaceutical products results obtained by the Franz cell and the Enhancer cell systems have been compared and it is

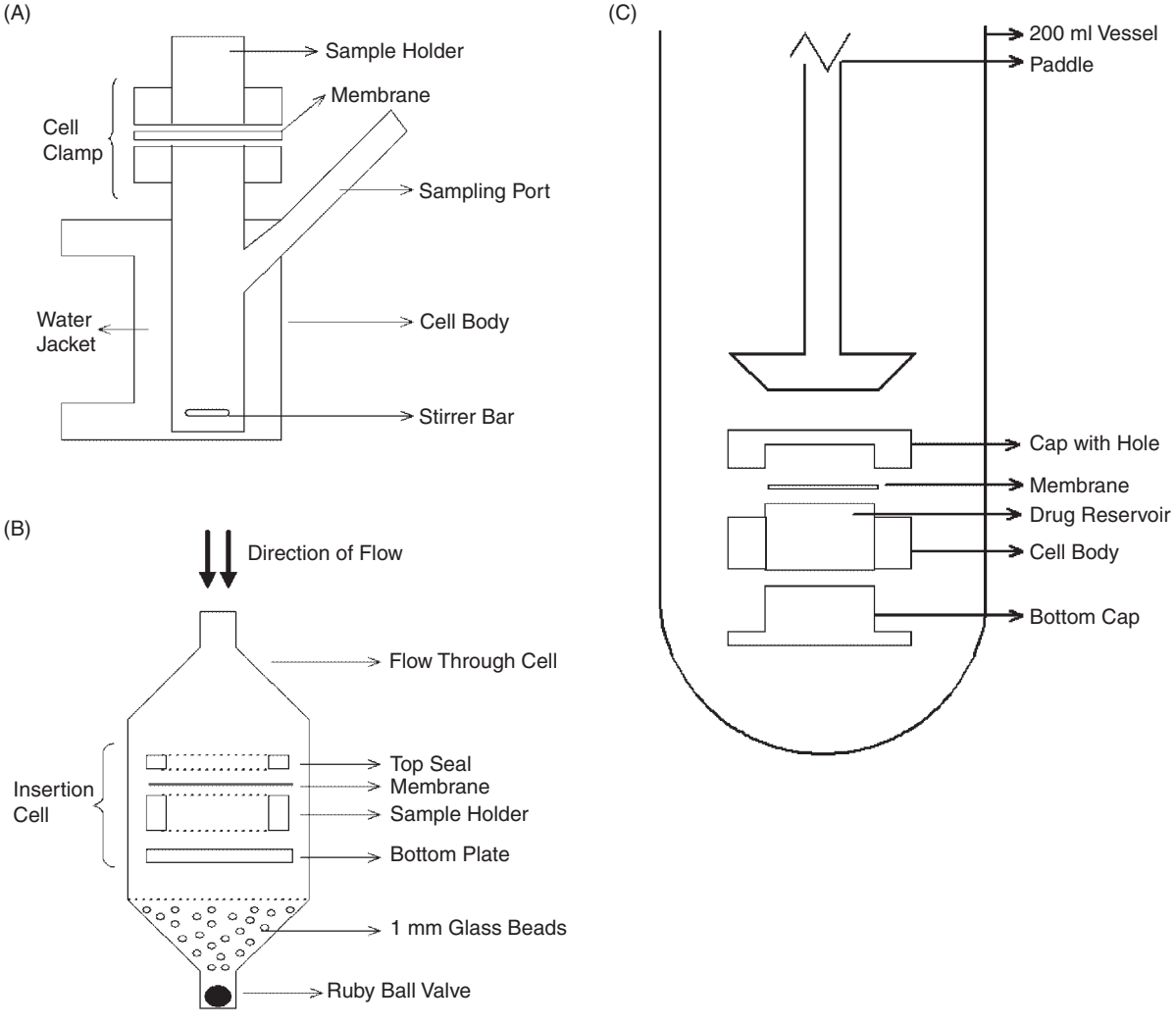


**Figure 1.** Schematic representations of (A) Franz, (B) Enhancer and (C) flow-through cell dissolution apparatuses.

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generally accepted that once the data is corrected for differing surface area, drug release is nearly superimposable[7,8]. However, reports describing comparisons between the 3 methods for products containing compounds with large differences in solubility and multiple active ingredients are not available. This article reports the release properties of the poorly water-soluble drug hydrocortisone and antibacterial agent triclosan, and the water-soluble anti-aging compounds salicylic acid and ascorbic acid from gels, creams, and ointments, alone or combined with triclosan, measured with the flow-through, Franz, and Enhancer cell dissolution testing methods.

## MATERIALS AND METHODS

### I. *Materials*

Two semisolid products containing hydrocortisone acetate Procutan® (1.0% cream and ointment) and Cutaderm (0.5% cream and ointment) were obtained from Scherag Pty. Ltd., Johannesburg, South Africa. The active ingredients incorporated into the anti-aging and acne gels and creams were salicylic acid (Spectrum Chemical Co., USA), triclosan (Irgasan DP 300, Ciba Specialty Chemicals, Basel, Switzerland), and ascorbic acid (Sigma, USA). The excipients used in preparing the gels and creams were ethyl alcohol, propylene glycol (Eastman Chemical Co., USA), Carbopol 934 (BF Goodrich Co., USA), triethanolamine (J.T. Baker Chemical Co., USA), isopropyl myristate, cetearyl octanoate (Croda, USA), cetyl alcohol (Ruger Chemical Co., USA), stearic acid, liquid paraffin, propyl 4-hydroxy benzoate, and methyl 4-hydroxy benzoate (Sigma, USA). Reference standards for analysis and chemicals used to prepare media for release studies were obtained from Sigma Chemical Company (St. Louis, USA) and were used as received.

### II. *Formulation of Creams and Gels*

The gels and creams tested in the study were prepared in small batches of 50-100 g, packaged in glass containers, and kept at 5°C throughout the study. The gels were prepared as follows: Carbopol was slowly added to the ethanolic solution containing salicylic acid, ascorbic acid, or triclosan in a porcelain dish while being stirred with a glass rod continuously. Next, propylene glycol was added drop wise with continuous stirring. The products were kept aside for 30 min and then triethanolamine solution was added slowly until the gel was formed. The creams were prepared as follows: Isopropyl myristate, cetearyl octanoate, cetyl alcohol, stearic acid, liquid paraffin, dimethyl polysiloxane, and glycerol monostearate were mixed together and heated slowly until it liquefies. Propylene glycol, methyl 4-hydroxy benzoate, propyl 4-hydroxy benzoate and water were mixed together, heated and added to the first mixture. The cream was then allowed to cool down. The salicylic acid (0.5, 1, 2 and 3%), ascorbic acid (6%), or triclosan (0.1, 0.5 and 1%) was dissolved in either the oil or the water phase depending on its solubility.

### III. *In Vitro Release Methods*

As stated in the introduction, nearly all of the published work on *in vitro* release from semisolids used vertical Franz-type cells, Figure 1 (A). The system used in this study consisted of 6 cells each with a polyethylene sample ring with a 1 cm diameter hole at the centre, the same size as the opening in the vertical receptor cells, which is placed on top of the membrane and then filled with the semisolid. The membrane with the sample was placed on top of the vertical receptor cell and clamped tightly into place. The receptor cells were filled with the dissolution medium and a small magnetic stirrer placed in each cell was used for mixing.

The Enhancer cell used in this study consisted of a metal load ring, a cap, a membrane or skin, and a drug reservoir, Figure 1 (B). The ointment or semisolid preparation was placed in the drug reservoir (2 cm diameter) on top of the membrane or excised skin. A metal load ring was used to keep the membrane or skin and the washer in place during the cap application. Finally, the bottom screw was tightened to bring the ointment, or semisolid preparation, in complete contact with the membrane or skin making certain that no entrapped air is present at the interface of the ointment, or semisolid preparation, and the membrane or skin. A USP Six Spindle Dissolution Tester (Vanderkamp 600, Van Kel Industries, NJ, USA) with modified flask assemblies consisting of 200 mL flasks instead of the standard 900 mL and smaller sized paddles, was used to measure drug release from the enhancer cell assembly. The paddles were rotated at 100 rpm.

Although the specified official use of flow-through methods is not for semisolid dosage forms, their utility for assessing release rates of drugs from semisolid dosage forms has become a topic of considerable interest[13,14]. In this study a custom-made 'insertion cell' was constructed Figure 1 (C), such that its dimensions permitted this cell to be used with the compendial flow-through cell (USP 4 apparatus, SOTAX CE 70, Sotax, Basel, Switzerland). The upper section of the 'insertion cell' consisted of an oblong block of Plexiglas with a small circular hole (9 mm diameter) cut through the middle and dimensions such that it fitted into the flow-through cell. The middle section consisted of a matching oblong block of Plexiglas with a 9 mm hole that acted as the sample holder. The lower part was a solid block of Plexiglas of compatible dimensions. All 3 sections were screwed together with the aid of 2 bolts on both side of the 3 components. The membrane was placed between the upper part and sample holder sections prior to assembly. A stainless steel spring support was constructed to act as a holder for the 'insertion cell' when used in the 'turbulent flow' mode, whilst a layer of glass beads

(1 mm diameter) placed into the conical section of the flow-through cell acted as a support for the 'insertion cell' when used in the 'laminar flow' mode.

Throughout this study, turbulent flow with the insertion cell positioned at a distance of 10 mm from the conical section of the flow-through cell and orientated down was used to yield the maximum cumulative release. This means that the membrane area was in direct contact with the flow direction. The flow rate was 6 mL/min at a constant temperature of 32˚C and samples (30 mL) were collected in a Sotax C 615 fraction collector at 30 min intervals. The Sotax CE 70 unit controlled the entire system.

The membranes used in this study were either a silicon gel sheet (Silastic® medical grade sheeting, 0.01 mm thickness, Dow Chemical Company, USA) sandwiched between two cellulose acetate membranes (Osmonics Inc., USA) or only cellulose acetate with a pore size of 0.45 $\mu$m soaked in 15% oleic acid in isopropyl myristate. The trilaminar membrane was tested in all three apparatus because Yeung *et al*.[15] found that for water permeation the values obtained for flux (402 $\mu$g/cm$^2$/hr) and permeability coefficient ($6.38 \times 10^{-3}$ cm/hr) were almost identical to those obtained for excised human skin (409 $\mu$g/cm$^2$/hr and $6.36 \times 10^{-3}$ cm/hr). Previously reported release studies generally prescribe the use of single or double cellulose acetate membranes.

For all 3 methods the temperature was controlled at a constant of 32˚C and exact volume samples were withdrawn at 0.5 to 1 hr intervals from each flask up to 6 hr. For the Franz and Enhancer cell methods the volume withdrawn were replaced with an identical volume of fresh medium. A correction factor was included in the calculations to account for the drug loss during sampling. For each sample a set of 6 diffusions were run to obtain cumulative release profiles. The receptor mediums chosen were buffer pH 5.8 and a ethanol/water mixtures. A pH of 5.8 was chosen because the skin is reported to have a slightly acidic pH[4-6] due to secretions from sweat glands, skin oil, and the breakdown of fatty acids by Staphylococcus in the epidermis[15].

IV. *Analysis Methods*

The HPLC methods used in this study complied with specifications for precision, accuracy, selectivity, linearity, and ruggedness as required by the USP XXIV[6]. The analysis of salicylic acid was carried out using an automated high performance liquid chromatograph (Thermo Separation Products, CA, USA) with a UV detector set at 265 nm. Salicylic acid eluted on a Discovery RP C16 HPLC column (250 mm × 4.6 mm, 5 $\mu$m, Phenomenex, USA) after 13 min with a mobile phase of water/methanol/glacial acetic acid (60/40/1); flow rate 1.0 mL/min; injection volume 20 $\mu$L.

The ascorbic acid was analyzed using a C18 column (Macherey-Nagel Lichrospher RP18 5 $\mu$m, 250 × 4 mm, Germany). The apparatus was a Hewlett-Packard 1100 (Agilent Technologies, CA, USA) with a detection wavelength of 254 nm. The mobile phase was methanol/$H_2O$/glacial acetic acid/Triethanolamine (70/900/25/5). The above-mentioned ratio contained 0.005M sodium heptane sulphonate are and 150 mg of EDTA. The final pH was 3.6. A flow rate of 1.0 mL/min was used and the retention time was 5 min.

For the analysis of triclosan the following reagents and equipment were used: Hewlett Packard 1050 HPLC (Agilent Technologies, CA, USA) equipped with a variable wavelength UV detector, pump, injection device and computerized data analysis system; Luna C18 column (2 $\mu$m, 150 × 4.6 mm) from Phenomenex, CA, USA controlled at ±20˚C; mobile phase was a mixture of methanol/water (85/15) containing 0.1% $H_3PO_4$; flow rate 1.0 mL/min; injection volume 10 $\mu$L; UV-detection at 210 nm; retention time of 6 min.

Analysis of hydrocortisone acetate was also by means of HPLC according to the method described in the USP XXIV[6] using a Hewlett Packard 1050 HPLC (Agilent Technologies, CA, USA), a Luna C18 column (2 $\mu$m, 150 × 4.6 mm, Phenomenex, CA, USA); mobile phase was a mixture of methanol/water (60/40); flow rate 1.0 mL/min; injection volume 10 $\mu$L; UV-detection at 254 nm; retention time of 10 min.

V. *Statistical Analysis*

*In vitro* dissolution data from gels and creams was compared by calculating similarity factors. Among several methods used for dissolution profile comparison, the mathematical model using a similarity factor proposed by Moore and Flanner[16] is the most popular and is recommended in FDA guidance documents[5]. The similarity factor between two dissolution profiles is calculated by:

$$f_2 = 50 \times \log\left\{\left[1+\left(\frac{1}{n}\right)\times\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad \text{Eq. 1}$$

Where $R_t$ and $T_t$ are the cumulative percentage dissolved at each of the selected time points (n) of the reference and test products respectively. A $f_2$ value between 50 and 100 ensures sameness or equivalence of the two curves and thus the performance of the two products.

Statistical analysis was performed using SPSS 10.0 for Windows (SPSS Inc., IL, USA). A 99% confidence level ($p < 0.01$) was considered satisfactory for indicating significant differences. The mean values of the release rates were compared for significant differences using one-way or two-way analysis of variance (ANOVA) for single factor and two factor comparisons respectively.

## RESULTS AND DISCUSSION

As a first step, the suitability of the HPLC analytical methods for the active ingredients was determined. Figure

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2 shows representative HPLC chromatograms of the active ingredients. With the aid of these methods, the release rates of the active ingredients from creams, ointments, or gels were measured with the 3 release testing methods. Throughout the study, the release of the compounds was linear when the amount released per square centimeter was plotted as a function of the square root of time, in accordance with Higuchi's model ($r^2 > 0.9$). From these plots, the release rate (flux, $\mu g/cm^2/min^{0.5}$) was calculated and used to compare results. The similarity of release profiles was also estimated from $f_2$ values calculated using equation 1 and $p < 0.01$ was considered satisfactory for indicating significant differences between release rates.

I. *Selection of Conditions for In Vitro Release Testing*

To determine the optimum experimental conditions for comparing the release of the compounds measured with the three diffusion apparatus, the release of salicylic acid and triclosan from creams or gels was determined in different media through cellulose acetate, cellulose acetate soaked in 15% oleic acid, or silicon gel sheet sandwiched between two cellulose acetate membranes. For the purpose of this comparison, salicylic acid represents a hydrophilic and triclosan a lipophilic compound. The media tested were a phosphate buffer pH 5.8 (0.2 M $KH_2PO_4$ and 0.2 M NaOH, USP 24)[6] or mixtures containing the buffer and 30-50% ethanol. Mean release rates are listed in Table 1 and 2.

As expected, the release of the more hydrophilic salicylic acid was faster in the aqueous buffer pH 5.8 while the release of the lipophilic triclosan tended to be faster in media that contained 40-50% ethanol. The release rates

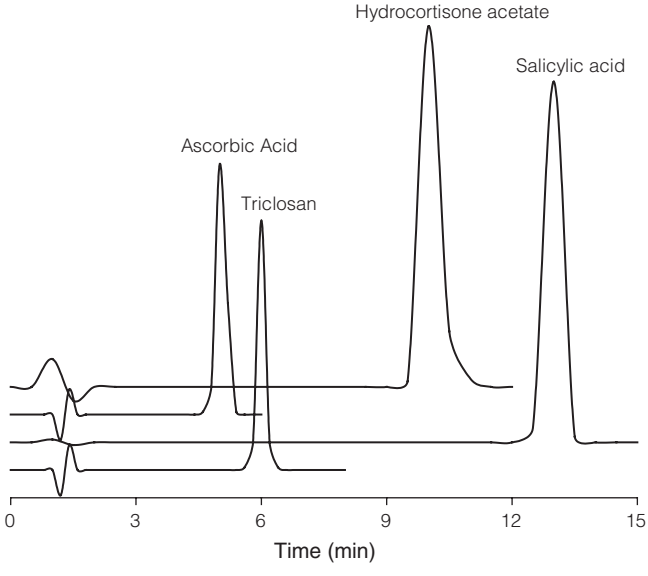


**Figure 2.** HPLC chromatograms of the active ingredients.

**Table 1.** The effect of a change in receptor medium composition and membrane on the release rate of salicylic acid from a 1% gel or cream measured with the Franz, enhancer or flow-through diffusion apparatus

| Dosage Form | Membrane[a] | Medium | Method | | |
|---|---|---|---|---|---|
| | | | Franz ($\mu g/cm^2/min^{0.5}$) | Enhancer ($\mu g/cm^2/min^{0.5}$) | Flow-through ($\mu g/cm^2/min^{0.5}$) |
| Gel | CA | pH 5.8 | 259.08 ± 7.15 | 252.01 ± 9.08 | 217.92 ± 7.64 |
| | | 30% EtOH | 231.34 ± 8.11 | 239.01 ± 7.23 | 203.39 ± 9.97 |
| | | 40% EtOH | 219.44 ± 8.12 | 226.99 ± 8.98 | 196.78 ± 8.89 |
| | | 50% EtOH | 205.78 ± 7.16 | 218.45 ± 7.89 | 187.65 ± 8.23 |
| | CA-OA | pH 5.8 | 211.34 ± 3.78 | 225.67 ± 3.09 | 154.34 ± 3.34 |
| | | 30% EtOH | 208.87 ± 3.22 | 221.34 ± 3.75 | 162.56 ± 3.78 |
| | | 40% EtOH | 215.45 ± 2.72 | 231.43 ± 4.29 | 157.34 ± 4.34 |
| | | 50% EtOH | 220.45 ± 4.21 | 223.56 ± 3.78 | 163.56 ± 3.86 |
| | CA-S-CA | pH 5.8 | 9.08 ± 1.15 | 12.01 ± 1.08 | 17.92 ± 1.64 |
| | | 30% EtOH | —[b] | 3.26 ± 2.78 | — |
| | | 40% EtOH | — | 2.13 ± 3.45 | — |
| | | 50% EtOH | — | — | — |
| Cream | CA | pH 5.8 | 203.78 ± 7.62 | 213.76 ± 8.54 | 164.01 ± 7.89 |
| | | 30% EtOH | 210.32 ± 5.23 | 220.65 ± 7.12 | 172.55 ± 8.46 |
| | | 40% EtOH | 204.56 ± 6.34 | 199.34 ± 8.67 | 167.55 ± 4.76 |
| | | 50% EtOH | 199.45 ± 6.56 | 208.64 ± 6.23 | 158.12 ± 8.22 |
| | CA-OA | pH 5.8 | 177.23 ± 3.41 | 188.76 ± 2.43 | 82.65 ± 3.55 |
| | | 30% EtOH | 185.33 ± 3.44 | 190.34 ± 4.21 | 85.32 ± 2.43 |
| | | 40% EtOH | 182.67 ± 2.89 | 189.52 ± 2.78 | 90.76 ± 2.87 |
| | | 50% EtOH | 180.23 ± 2.34 | 185.56 ± 3.21 | 95.16 ± 2.14 |
| | CA-S-CA | pH 5.8 | — | — | — |
| | | 30% EtOH | — | — | — |
| | | 40% EtOH | 2.44 ± 1.28 | 2.13 ± 0.69 | — |
| | | 50% EtOH | 3.25 ± 1.33 | 3.12 ± 1.21 | 1.61 ± 1.21 |

[a]CA: cellulose acetate; CA-OA: cellulose acetate membrane soaked in oleic acid; CA-S-CA: silicon sheet sandwiched between two cellulose acetate membranes.

[b]Not detectable.

**Table 2.** The effect of a change in receptor medium composition and membrane on the release rate of triclosan from a 1 % cream measured with the 3 diffusion apparatuses

| Membrane[a] | Medium | Method | | |
|---|---|---|---|---|
| | | Franz ($\mu g/cm^2/min^{0.5}$) | Enhancer ($\mu g/cm^2/min^{0.5}$) | Flow-through ($\mu g/cm^2/min^{0.5}$)) |
| CA | pH 5.8 | 2.38 ± 0.61 | 3.11 ± 0.98 | 2.06 ± 1.72 |
| | 30% EtOH | 12.41 ± 2.45 | 17.01 ± 3.09 | 11.30 ± 3.12 |
| | 40% EtOH | 16.15 ± 3.01 | 22.41 ± 2.87 | 14.94 ± 3.65 |
| | 50% EtOH | 20.92 ± 3.22 | 28.13 ± 3.11 | 18.62 ± 3.36 |
| CA-OA | pH 5.8 | 4.12 ± 0.32 | 5.61 ± 0.24 | 2.61 ± 0.72 |
| | 30% EtOH | 6.82 ± 0.41 | 8.01 ± 0.45 | 3.28 ± 0.81 |
| | 40% EtOH | 8.77 ± 0.62 | 10.65 ± 0.71 | 7.57 ± 0.91 |
| | 50% EtOH | 11.45 ± 0.54 | 14.21 ± 0.41 | 11.86 ± 0.89 |
| CA-S-CA | pH 5.8 | —[b] | 0.98 ± 0.56 | — |
| | 30% EtOH | 2.06 ± 0.23 | 2.32 ± 0.31 | 1.72 ± 0.67 |
| | 40% EtOH | 3.12 ± 0.46 | 3.51 ± 0.32 | 2.51 ± 0.52 |
| | 50% EtOH | 4.87 ± 0.54 | 4.65 ± 0.87 | 4.18 ± 1.21 |

[a]CA: cellulose acetate; CA-OA: cellulose acetate membrane soaked in oleic acid; CA-S-CA: silicon sheet sandwiched between 2 cellulose acetate membranes.
[b]Not detectable.

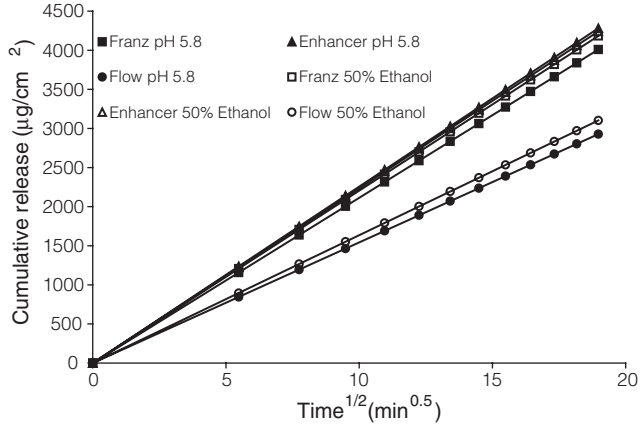


**Figure 3.** Salicylic acid release from a 1% gel measured with a Franz, Enhancer or flow-through diffusion apparatus using an oleic acid soaked cellulose acetate membrane and phosphate buffer pH 5.8 (close symbols) or 50% ethanol/buffer mixture (open symbols) as the dissolution medium.

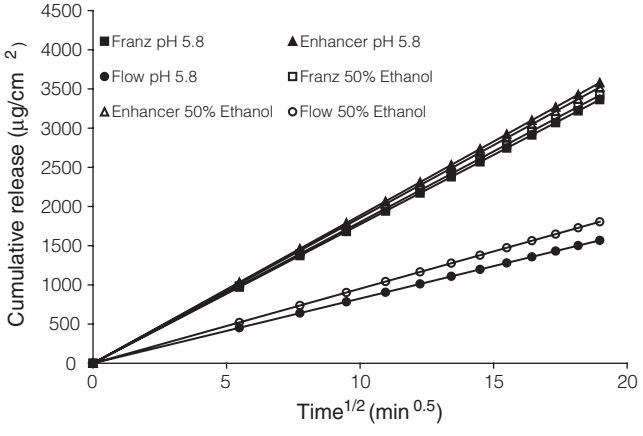


**Figure 4.** Salicylic acid release from a 1% cream measured with a Franz, Enhancer or flow-through diffusion apparatus using an oleic acid soaked cellulose acetate membrane and phosphate buffer pH 5.8 (close symbols) or 50% ethanol/buffer mixture (open symbols) as the dissolution medium.

listed in Table 1 and 2 showed that the cellulose acetate membrane and the cellulose acetate sandwiched silicon gel sheeting had significantly different permeability characteristics. Since these membranes are either hydrophilic or lipophilic in nature, they tend to allow the diffusion of either the salicylic acid or triclosan. Although the release of salicylic acid and triclosan was slower through the cellulose acetate membrane soaked in the lipophilic oleic acid than through just a cellulose acetate membrane the oleic acid soaked membrane gave more constant and less variable release rates for both compounds. The results obtained suggested that although the multi-membrane system was reported to be a good permeability model for excised human skin[15], it might not be suitable for routine quality control evaluation of semisolid products since the percentage drug release for some products was not detectable. Release through this multi-layer membrane also was significantly slower making increasing testing time beyond what would be reasonable for quality control testing.

Under all the conditions tested, the release rate of both salicylic acid and triclosan measured with the flow-through system was significantly slower than that of the other two apparatuses. Although the release rates measured with the Enhancer cell system consistently were slightly higher than that measured with the Franz cell, these differences were not significant ($f_2 > 50$). For each semisolid product, depending on the selected membrane and medium, the release of the compounds measured by the 3 methods was either the same or significantly different. Therefore, it is essential that the right testing conditions are used to make valid comparisons. The results in Table 1 and 2 showed that in this study the oleic acid soaked cellulose acetate membrane and 2 media, the phosphate buffer pH 5.8 and a 50% ethanol/buffer solution, were the best for comparing release from the semisolids. Figures 3-5 show the release

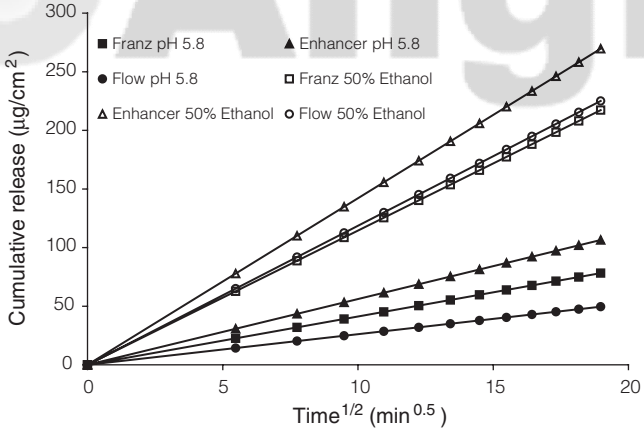


**Figure 5.** Triclosan release from a 1% cream measured with a Franz, Enhancer or flow-through diffusion apparatus using an oleic acid soaked cellulose acetate membrane and phosphate buffer pH 5.8 (close symbols) or 50% ethanol/buffer mixture (open symbols) as the dissolution medium.

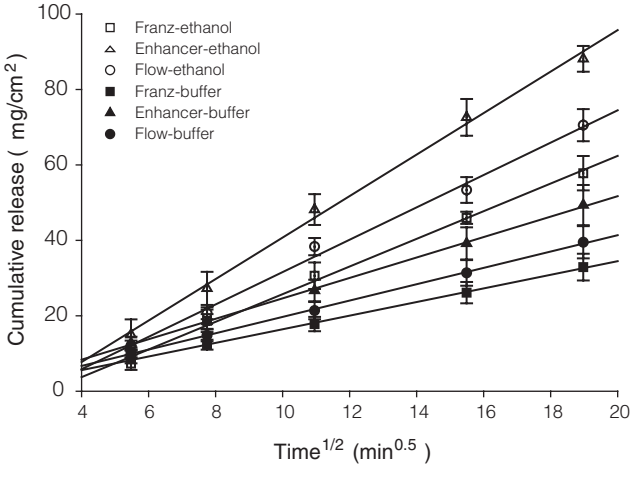


**Figure 6.** Comparison of release of hydrocortisone from a 1% cream measured with the Franz, Enhancer and flow-through methods using phosphate buffer pH 5.8 or 50% ethanol/buffer mixture as the dissolution medium.

of the 2 compounds when these conditions were used. From the release profiles, it is also clear that the release of the water-soluble salicylic acid from the gel was consistently faster than that from the cream, while the release of the very poorly soluble triclosan was significantly slower than that of salicylic acid.

II. *Release from Cortisone Creams and Ointments*

The release rates and profiles for hydrocortisone (Table 3; Figures 6 and 7) measured by the Enhancer, Franz and flow-through cell methods depended on formulation type, concentration of the drug, medium, and apparatus used. For products containing 0.5% hydrocortisone, release was not significantly different from the cream and the ointment, but for both the cream and ointment, the release was significantly faster in the 50% ethanol/buffer mixture

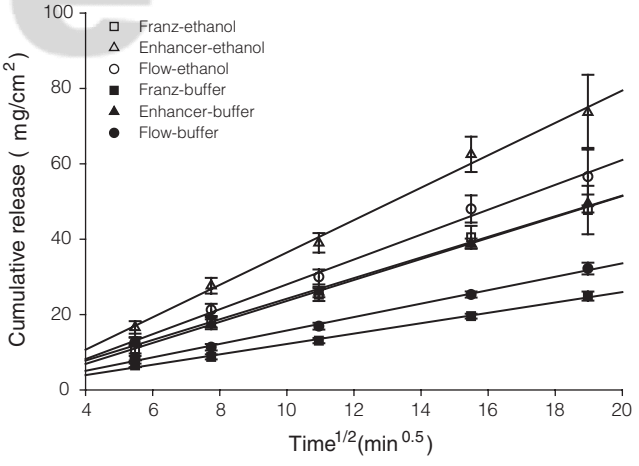


**Figure 7.** Comparison of release of hydrocortisone from a 1% ointment measured with the Franz, Enhancer and flow-through methods using phosphate buffer pH 5.8 or 50% ethanol/buffer mixture as the dissolution medium.

compared to the buffer alone. Increasing the hydrocortisone content from 0.5 to 1% increased the release rate significantly (Figure 6 and 7). In the 50% ethanol/buffer mixture measured with the Franz and Enhancer cells, the release from the 1% cream was also significantly faster than from the ointment. The release rates listed in Table 3 show that the ability of the flow-through cell to measure the release from semisolids was hampered by the small sample it can accommodate. Therefore, it was not possible to measure the release from 0.5% creams and ointments containing hydrocortisone. Comparison of release rates obtained in this study and release rates available in the literature (Table 3) demonstrated that the conditions used gave results similar to that obtained independently by other researchers. Cortisone release results showed that the three diffusion methods, especially the Franz and Enhancer cells (Figures 6 and 7) could be used to guide formulation development, to monitor batch-to-batch quality and stability, to control the manufacturing process, and to demonstrate equivalency between two similar products.

III. *Release from Anti-wrinkle Skin Creams and Gels*

As shown by the release rates listed in Table 1 and the release profiles in Figure 3-5 differences in the dissolution of salicylic acid from gel and cream could be measured with the Franz, Enhancer cell and flow-through cells. For both the cream and the gel $f_2$-values showed that the dissolution rates depended less on the composition of the dissolution medium than on the apparatus used to measure the release. There was no significant difference in the release rates measured with the Franz and Enhancer cells. However, these rates were significantly higher than that measured with the flow-through cell. Since cortisone release results showed that the concentration of the active ingredient in the semisolid can influence the dissolution rate. The dissolution of salicylic acid from a 0.5, 1, 2 and

**Table 3.** Release rates for hydrocortisone from commercially available creams and ointments measured with the Franz, Enhancer, and flow-through cells

| Dosage form | Medium | Method | | |
|---|---|---|---|---|
| | | Franz ($\mu g/cm^2/min^{0.5}$) | Enhancer ($\mu g/cm^2/min^{0.5}$) | Flow-through ($\mu g/cm^2/min^{0.5}$) |
| 0.5 % Cream | pH 5.8 | 1.27 ± 0.43 (1.92)[b] | 1.43 ± 0.42 (1.90) | —[a] |
| | 50% EtOH | 1.53 ± 0.52 (1.93) | 1.89 ± 0.32) (1.91) | — |
| 0.5% Ointment | pH 5.8 | 0.86 ± 0.27 | 1.08 ± 0.28 | — |
| | 50% EtOH | 1.40 ± 0.29 | 1.84 ± 0.31 | — |
| 1.0% Cream | pH 5.8 | 1.81 ± 0.43 | 2.17 ± 0.32 | 2.71 ± 0.31 |
| | 50% EtOH | 3.67 ± 0.62 (5.02) | 5.50 ± 0.28 (6.29) | 4.29 ± 0.35 |
| 1.0% Ointment | pH 5.8 | 1.38 ± 0.23 | 1.78 ± 0.24 | 2.74 ± 0.41 |
| | 50% EtOH | 2.78 ± 0.43 (2.73) | 4.30 ± 0.67 (3.47) | 3.30 ± 0.78 |

[a]Not detectable.
[b]Values listed in brackets were obtained from literature[7,10,12,17,18].

**Table 4.** Ascorbic acid release rates from a 6 % gel and cream measured with the Franz, Enhancer, and flow-through cells

| Dosage form | Medium | Method | | |
|---|---|---|---|---|
| | | Franz ($\mu g/cm^2/min^{0.5}$) | Enhancer ($\mu g/cm^2/min^{0.5}$) | Flow-through ($\mu g/cm^2/min^{0.5}$) |
| Gel | Buffer pH 5.8 | 82.37 ± 2.34 | 83.42 ± 1.89 | 67.86 ± 4.32 |
| | 50% EtOH | 57.45 ± 3.23 | 60.12 ± 1.76 | 48.01 ± 3.32 |
| Cream | Buffer pH 5.8 | 73.45 ± 2.09 | 81.34 ± 2.12 | 56.54 ± 4.32 |
| | 50% EtOH | 66.45 ± 3.11 | 69.82 ± 1.83 | 50.23 ± 3.78 |

3% cream and gel was measured. The differences in the release rates are shown in Figure 8. These results show that release from the gel was significantly faster than from the cream; that the release rate increased with an increase in salicylic acid concentration; that the release rates measured with the Franz and Enhancer cells were significantly faster than that measured with the flow-through cell; and that the release profiles obtained with the Franz and Enhancer cell were similar ($f_2 > 50$).

Another commonly used cosmeceutical ingredient found in many anti-wrinkle creams is ascorbic acid (Vitamin C). The release of ascorbic acid from a 6% cream or gel was measured with the 3 testing methods and the results were compared. The release rates listed in Table 4 showed that the ascorbic acid release from the gel was significantly faster than from the cream. Also faster release rates were measured with the Franz and Enhancer cells than the flow-through cell. Ascorbic acid is very soluble in water and almost 10 times less soluble in alcohol. This difference in solubility could explain why the release in the buffer solution was faster than in the 50% ethanol/buffer mixture.

The results obtained for the water soluble acids showed that once the data is corrected for differing surface area release data obtained with the Franz and Enhancer cells is nearly super-imposable. For these compounds, the release from both creams and gels measured with the flow-through cell was always slower than with the other 2 apparatus. Although the more lipophilic oleic acid soaked cellulose acetate membrane decreased the release rate of these hydrophilic compounds, it increased the consistency of results. This increased the accuracy of results and the ability to compare release data. The solubility of the compounds in the dissolution medium had a significant effect on the release rates; release was faster in media with highly soluble compounds. As expected, the release of

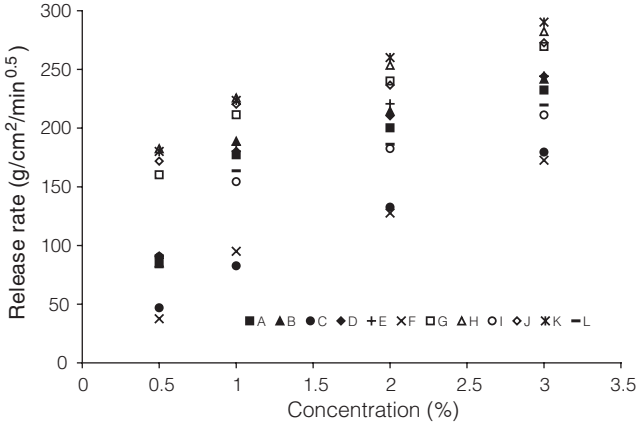


**Figure 8.** Scatter-plots showing the release of salicylic acid from creams containing 0.5 to 3 % salicylic acid, measured with the Franz, Enhancer and flow-through diffusion cells.
A: cream, Franz cell, pH 5.8; B: cream, Enhancer cell, pH 5.8; C: cream, flow-through cell, pH 5.8; D: cream, Franz cell, 50% ethanol; E: cream, Enhancer cell, 50% ethanol; F: cream, flow-through cell, 50% ethanol; G: gel, Franz cell, pH 5.8; H: gel, Enhancer cell, pH 5.8; I: gel, flow-through cell, pH 5.8; J: gel, Franz cell, 50% ethanol; K: gel, Enhancer cell, 50% ethanol; L: gel, flow-through cell, 50% ethanol.

**Table 5.** Release rates for triclosan and salicylic acid from a combination cream measured with the Franz, Enhancer, and flow-through cells

| Ingredient | Medium | Method | | |
|---|---|---|---|---|
| | | Franz ($\mu g/cm^2/min^{0.5}$) | Enhancer ($\mu g/cm^2/min^{0.5}$) | Flow-through ($\mu g/cm^2/min^{0.5}$) |
| Triclosan (0.1%) | pH 5.8 | 2.20 ± 0.87 | 2.75 ± 0.45 | 1.26 ± 0.56 |
| | 50% EtOH | 5.27 ± 0.67 | 4.62 ± 0.43 | 1.49 ± 0.65 |
| Salicylic acid (0.5%) | pH 5.8 | 84.36 ± 2.86 | 85.26 ± 2.56 | 46.93 ± 6.49 |
| | 50% EtOH | 91.06 ± 3.21 | 88.76 ± 2.45 | 37.55 ± 7.83 |

these hydrophilic compounds was faster from gels than creams. This would make it more difficult to distinguish between the release rates from semisolids that differ only slightly in their composition, such as the concentration of the active ingredient or aqueous/alcohol solubility of the formulation.

IV. *Release from Skin Creams Containing a Bacteriostatic Agent*

Many cosmeceutical products contain more than one active ingredient. For example, some products contain multiple alpha hydroxyl acids or alpha hydroxyl acids and a bacteriostatic agent. This can cause problems when measuring the release rate especially when there is a huge difference in the solubility of the 2 compounds. In this study, the release rates from a combination cream containing 0.1% triclosan and 0.5% salicylic acid were measured. The release rates are summarized in Table 5. Calculated $f_2$ values showed that the dissolution of triclosan into the ethanol medium was significantly faster than into the buffer medium and this difference was largest for the Franz cell (3 $\mu g/cm^2/min^{0.5}$) and smallest for the flow-through cell (0.2 $\mu g/cm^2/min^{0.5}$). Due to its higher aqueous solubility the release of salicylic acid from the cream was significantly faster than that of triclosan. Release profiles measured with the Franz and Enhancer cell were similar ($f_2 > 50$) and although the differences in release rates in the buffer and ethanol mixture were much smaller, $f_2$-values indicated that both methods were able to measure this difference. The flow-through cell was not able to distinguish this difference because the variations in release profiles were too big, as shown by the large standard deviations listed in Table 5. Large variations were caused by the complicated operation and small sample size used in the flow-through method. Furthermore, the concentration of the compounds in the dissolution media was very close to the limit of detection for the analytical methods.

## CONCLUSION

Although *in vitro* release tests were never developed for bioequivalence testing this study confirmed they can be used to assess the "sameness" of products during quality testing, formulation development, scale-up or post-marketing formulation and manufacturing changes. The results presented also showed that the Franz, Enhancer, and flow-through cells can be used for these purposes to test cosmeceutical semisolids containing hydrophilic and lipophilic active ingredients if appropriate dissolution medium and membrane are used. The main advantages of the Franz and Enhancer cells over the flow-through cells are the ease of operation and larger sample sizes ensuring more consistent results. Although the Franz and Enhancer cells gave similar release results for the products tested in this study, the advantages of the Enhancer cells are that they used the basic USP dissolution apparatus and had a larger volume range, making it easier to adapt the system to study the release of products containing low concentrations of active ingredients or ingredients that are difficult to analyze.

In the future, if regulatory agencies require more stringent testing of cosmeceutical products, it is hoped that the results presented in this paper will help to show that similar to the dissolution testing of oral dosage forms, *in vitro* release testing with diffusion cells offer a simple, reliable, reproducible quality control test that can be used to monitor the quality and stability of semisolid cosmetic products.

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