Performance evaluation of automated static Franz cell equipment for *in vitro* release and skin penetration testing

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PURPOSE

Franz cell testing methodology is frequently used for tests evaluating either the in vitro release of actives from topical formulations or the penetration of the drug across the skin. Several manual and automatic equipments are commercially available.

The objective was to evaluate the performance of an automated static Franz-cell equipment system S912-SCT-S (Logan Instruments, Somerset, NJ) for skin penetration testing of (trans)dermal dosage forms.

Using the model permeant caffeine, the objective was to evaluate the following criteria:

1) sampling accuracy and reproducibility from receptor,

2) test accuracy and reproducibility under conditions of "skin" permeation testing (in silico) versus manual cells.

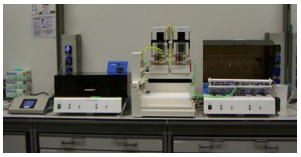

Figure 1: Logan automatic Franz cell equipment

EQUIPMENT & METHODOLOGY

1.Sampling procedure of the Logan equipment

The Logan system employs static-type Franz diffusion cells with computer controlled, automated sampling. A sampling sequence is composed of the following steps:

1) Flush –line rinse between cells, syringes and autosampler with receptor medium from cells

2) Sampling from the cell: - 5 ml of receptor fluid is withdrawn from the cells. Of these 5 ml, a defined volume is used for

2.1. Waste – additional rinse of the lines

2.2. Sample – receptor fluid is sampled into HPLC vials

2.3. Return to waste/return to cell – the remaining volume is sent either to the waste or returned to the Franz cells.

3) Replacement – the total volume withdrawn, i.e. the flush volume (step 1)+ 5 ml (step 2) + 0.2 ml is replaced with fresh receptor medium

Table 1: Instrument settings for sampling from receptor

Parameter	5 ml cells (n=12)		12 ml cells (n=6)	
	Program 1	Program 2	Program 1	Program 2
Flush	0.1 ml	0.1 ml	5.0 ml	1.0 ml
Waste	2.0 ml	2.0 ml	1.0 ml	2.0 ml
Sample	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Replacement volume	7.0 ml (overfill, theoretical refill 5.2 ml)	3.2 ml	10.2 ml	6.2 ml
Return to	Waste (2.0ml)	Cell (2.0ml)	Waste (3.0ml)	Waste (2.0 ml)

2. Test methodology

Testing was carried out in two steps using two cell sizes of 0.38 cm^2/ 5 ml and 1.77 cm^2/ 12ml and two different modes of sampling:

1) 100 ppm caffeine in water was injected into the receptor and successively sampled (Table 1) over five intervals between 0.5 and 6 h. The actual caffeine concentration profiles were compared to the theoretical prediction.

2) Full *in vitro* "skin" permeation test over 24 hours:

Donor: 4 mg/ml caffeine solution in EtOH/water 50:50 (v/v), 1 ml/cm^2

Receptor: 0.9% saline, pH 7.4

Membrane: semipermeable *Dow Corning*® 7-4107 Silicone Elastomer Membrane (7-4107)

Sampling: Hourly between 0-8 h, then every 2 h until 24 h, as per Table 2

Caffeine diffusion was tested against manual Franz cells of 4.52 cm^2/12ml size

Caffeine was quantified via a validated HLPC separation technique with PDA detection.

Table 2: Instrument settings for caffeine diffusion test across 7-4107 membrane (n=6)

Parameter	5 ml cells	12 ml cells
Flush	0.5 ml	1.0 ml
Waste	1.0 ml	2.0 ml
Sample	1.0 ml	1.0 ml
Replacement volume	2.7 ml	6.2 ml
Return to	Cell (2.5 ml)	Waste (2.0ml)

RESULTS & DISCUSSION

1. Sampling from receptor

Successive sampling of caffeine from the receptor using 0.38cm^2/5ml cells led to an over-estimation of 10 % of the concentration of the active (Figure 2). This is due to the combination of available instrument settings and the small cell volume.

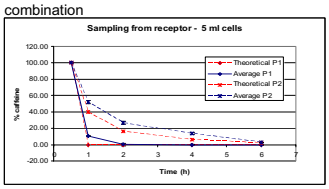

Figure 2: Sampling accuracy from receptor – 5 ml cells

Sampling accuracy was improved using 1.77cm^2/12ml cell size, leading to an overestimation of less than 5 % depending on the sampling mode (Figure 3). Excellent precision between cells.

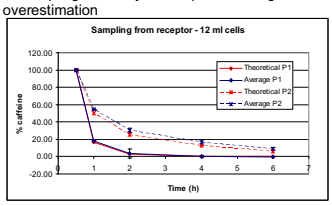

Figure 3: Sampling accuracy from receptor – 12ml cells

2. In vitro "skin" penetration test

Average caffeine fluxes at 24 hours were 1.23 ± 0.50 mg/(cm^2*h) with 0.38 cm^2/ 5 ml cells, 5.56 ± 0.64 mg/(cm^2*h) using the 1.77cm^2/ 12ml cells, and 8.48 ± 1.47 mg/(cm^2*h) with the 4.52cm^2/ 12 ml cells, respectively. No steady-state was reached after 24 hours.

Although the absorbed dose was normalized by the penetration surface, caffeine diffusion was increased with raising cell surface (Figures 4 and 5). It is likely that Ethanol evaporation within the donor chamber led to different caffeine activity in the remaining donor fluid. The size of the headspace in the donor increased with raising cell size.

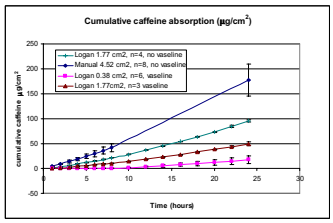

Figure 4: Cumulative caffeine diffused across Dow Corning® 7-4107 Silicone Elastomer Membrane

The use of a sealing agent (vaseline = petrolatum jelly) to prevent leaks across the flatground joints of the Logan diffusion cells decreased caffeine fluxes by a factor of 2 (Figure 4 and 5).

Vaseline spread across the silicone membrane when sealing the cells, thus decreasing the active penetration surface. No sealing agent was required for the manual cells which had o-ring joints.

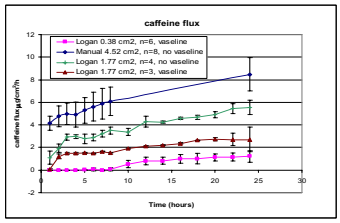

Figure 5: Caffeine flux across Dow Corning® 7-4107 Silicone Elastomer Membrane

The use of the automated Logan system versus manual diffusion cells led to superior precision between individual cells.

The data using the Logan 1.77 cm^2/ 12 ml cells without sealing agent were in agreement with other laboratories using similar conditions and cell types in an inter-laboratory trial.

CONCLUSION

The automated Franz cell equipment can be employed for skin penetration testing.

Diffusion rates from a liquid, partly volatile donor varied with the cell size employed.

Superior accuracy and precision were obtained with the 1.77 cm^2/1 2ml cells size, both in sampling tests from the receptor and in a simulated skin penetration test.

Diffusion tests should always be carried out against a reference formulation in order to minimize bias originating from the sampling procedure and test conditions.