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Release of acyclovir from semi-solid dosage forms: a semi-automated procedure using a simple plexiglass flow-through cell

S.C. Chattaraj, I. Kanfer *

School of Pharmaceutical Sciences, Rhodes University, Grahamstown, 6140, South Africa

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Abstract

A simple plexiglass flow-through cell (PFTC) was designed and utilized to monitor the release of acyclovir from two different semi-solid preparations. The influence of membrane type, flow rate and temperature was investigated. A small difference in temperature (32–37°C) had a significant effect on the release rate as did increasing flow rates from 2.9 to 10.0 ml/min. Cellulose acetate membranes showed a greater degree of release when compared with polytetrafluoroethylene and polyvinylidine difluoride membranes. The rank order of release of acyclovir between the two different semi-solid preparations remained the same irrespective of the membrane type, flow rate or temperature used during these studies. Data obtained from these experiments indicate that the plexiglass flow-through cell is a simple, reproducible and reliable tool which can be readily applied to assess the pharmaceutical availability of drugs from semi-solid dosage forms.

Keywords: Acylcovir; Semi-solid dosage form; Plexiglass flow-through cell; Drug release; Temperature effect; flow rate

1. Introduction

The utility and value of an in vitro method to assess the release of active ingredient(s) from semi-solid dosage forms has been the subject of much discussion (Behme et al., 1982; Skelly et al., 1987; Shah et al., 1989; Tuomi et al., 1989; Velissaratou and Papaioannou, 1989; Guy and Hadgraft, 1990; Rahman et al., 1990; Jamoulle et al., 1990; Kundu et al., 1993; Sanghvi and Collins, 1993).

Automation of such in vitro systems has obvious advantages and successful automated systems have been reported in the literature (Akhter et al., 1984; Cooper, 1984; Liron and Cohen, 1984; Bronaugh and Stewart, 1985; Hawkins and Reifenrath, 1986; Okamoto et al., 1986; Addicks et al., 1987; Skelly et al., 1987; Tiemessen et al., 1988; Akazawa et al., 1989; Martin et al., 1989; Shah et al., 1991; Rolland et al., 1992; Delgado et al., 1994; Reifenrath et al., 1994).

A simple plexiglass diffusion cell to monitor drug release from semi-solid dosage forms has recently been described (Chattaraj et al., 1995). This system consisted of a receptor reservoir sep-

* Corresponding author.

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arated from the sample reservoir by a semi-permeable membrane and sequential manual sampling of receptor fluid at predetermined time intervals. The system was shown to be highly reproducible, precise and extremely reliable.

Although Franz cells appear to be a popular method for in vitro drug diffusion testing (Delgado et al., 1994), studies using the plexiglass cells described by Chattaraj et al. (1995) indicated that better precision was obtained using the latter system as reflected by the lower degree of variability obtained between replicate experiments when compared with the data obtained using Franz cells.

In view of the above, a study was undertaken to develop and validate a flow-through version of the plexiglass cell in order to obviate the tedium of manual sampling.

2. Materials and methods

2.1. Apparatus

(1) The previously described plexiglass diffusion cell (Chattaraj et al., 1995) was modified as follows:

The receptor fluid reservoir was divided into

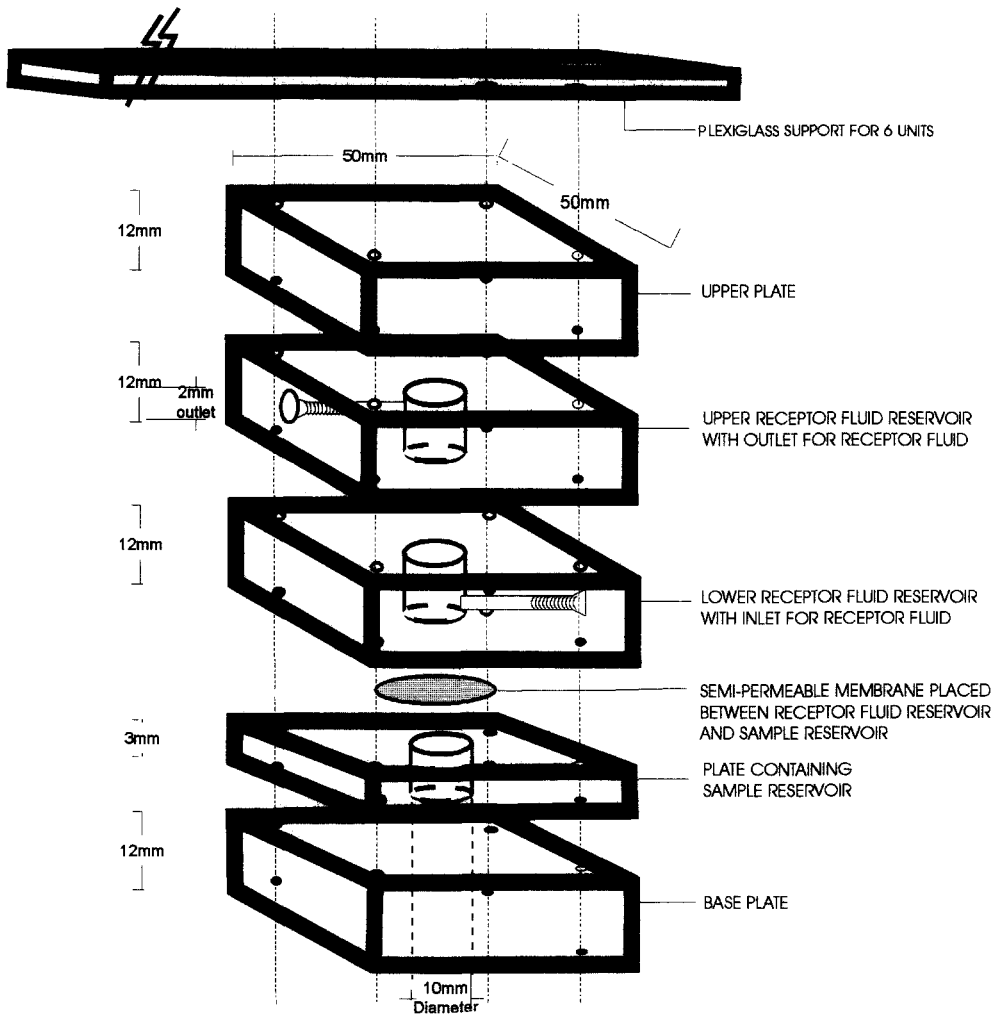

Fig. 1. Plexiglass flow-through cell.

two equal sections such that when placed together, the central reservoir volume (height 2.4 cm and diameter 1 cm) was kept constant. A small hole (diameter 2 mm) was drilled in the centre of one side of the lower section and another small hole of the same diameter was similarly drilled in the centre of the upper section. Both sections were placed together in such a way that the holes were positioned directly opposite each other to provide an inlet and outlet for the receptor fluid. A 5 cm × 5 cm solid block of plexiglass (thickness of 1.2 cm) was used to seal the top of the receptor fluid reservoir. Extended bolts and an additional block of solid plexiglass was attached to the flow-through cell to act as a support for clamping the cell in position (Fig. 1). The entire cell was immersed into a constant temperature water bath (Colora Ultra-thermostat, Model NB 34980, Germany) after connecting teflon tubing into each of the respective inlets and outlets.

(2) Components of a Sotax Dissotest Flow Through Dissolution Apparatus (Sotax AG, Basel, Switzerland) were used and the flow-through plexiglass cell positioned in-line by connecting the tubing from the Sotax CY 7-50 peristaltic pump to the inlet of the plexiglass flow-through cell. Tubing from the outlet of the plexiglass flow-through cell was connected to a Sotax MS 70 medium splitter and samples collected with the aid of a Sotax C 615 fraction collector. The microprocessor of the Sotax CE 70 unit together with a personal computer were used to control the system (Fig. 2). The receptor fluid was pumped into the reservoir (bottom inlet) at various flow rates ranging from 2.9 to 10.0 ml/min and samples were automatically collected in the fraction collector at 10 min intervals.

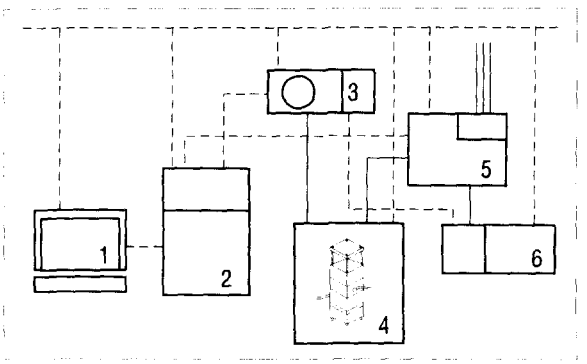

Fig. 2. Diagrammatic representation of plexiglass flow-through cell in a Dissotest CE 70 system. (————) Tubing, (---) control cable, (1) personal computer, (2) Sotax CE 70, (3) Sotax CY 7-50, (4) plexiglass flow-through cell, (5) medium splitter Sotax MS 70, (6) fraction collector C 615.

2.2. Membranes / receptor fluid

Five synthetic membranes were used in separate experiments. These consisted of cellulose acetate (CA, 502500, pore size = 0.45 μm, Lida Manufacturing Corp., USA), polyvinylidene difluoride (HVLP, 02500, pore size = 0.45 μm, Millipore Corp., Bedford, MA, USA), polytetrafluoroethylene (PTFE, 504700, pore size = 0.45 μm, Lida Manufacturing Corp., USA), Spectrapore dialysis membrane (Mol. Wt cutoff = 12 000–14 000, Spectrum Medical Industries, INC., USA), and mixed esters of cellulose (HAWP, 02500, pore size = 0.45 μm, Millipore Corp., Bedford, MA, USA). All membranes were pre-conditioned by immersing in receptor fluid for a period of 30 min and blot-dried prior to use. The receptor fluid consisted of a degassed solution of normal saline (0.9% sodium chloride).

2.3. Drug formulations

Commercially available Zovirax cream (Acyclovir 5% cream, Wellcome, Pty Ltd, South Africa) and an extemporaneously prepared cream (cream A) containing 5% acyclovir were assessed.

2.4. Procedure

Prior to assembly of the flow-through plexiglass cell, a sample of the relevant semi-solid preparation was accurately weighed directly into the sample reservoir (approx. 265 mg) and the preconditioned membrane placed on top. In each of the experiments, a uniform thickness of semi-solid was achieved by levelling with a spatula. The cell was then assembled by firmly bolting all the components together. The inlet and outlet

openings were then connected by tubing to the relevant components of a Sotax Dissotest Flow-Through Dissolution Apparatus as described above and the flow-through cell immersed into the water bath and allowed to equilibrate to constant temperature. Separate experiments were performed at 32 and 37°C in order to investigate the effects of temperature. Dissolution medium was pumped at flow rates of either 2.9, 5.8, or 10 ml/min in separate experiments and 29, 58 or 100 ml sample volumes separately collected at each time interval using the fraction collector. Each determination was performed in triplicate. The influence of membrane type on the release of acyclovir was also investigated using triplicate determinations.

2.5. Analysis of samples

Samples were analysed by a validated HPLC procedure using a system consisting of a Beckman 114 constant flow pump (Beckman Instruments Inc., Fullerton, CA, USA), a WISP Model 710B auto-sampler (Waters Associates, Milford, MA, USA), a 481 Waters Lambda Max UV detector (Waters associates, Milford, MA, USA) and a model 3390 A Hewlett Packard recorder/integrator (Hewlett Packard, Palo Alto, CA, USA). Analyses were performed on a custom-packed 20 cm × 3.9 mm. i.d. stainless-steel column containing 10 μm C_{18} reversed-phase packing material (Techsil, HPLC Technology, Macclesfield, UK) at ambient temperature. The mobile phase consisted of methanol/water (10:90) and was pumped at a flow rate of 1 ml/min. Uracil (5 μg/ml) was used as the internal standard and the eluent monitored at a wavelength of 254 nm. A 20 μl sample was injected into the chromatographic system. Peak areas were automatically measured and concentrations were automatically computed. Calibration solutions consisting of a range of concentrations of acyclovir (5–100 μg/ml) were prepared and four replicate injections from each of five different standard solutions injected. Percent RSD values ranged between 1.00 and 8.42.

3. Results and discussion

The effect of a number of physical parameters of the experimental system on the release characteristics of acyclovir was investigated. In particular, flow rate of dissolution medium, temperature effects and influence of membrane type were studied. In all instances the release of acyclovir from both Zovirax and cream A were compared.

3.1. Influence of flow rate and temperature

3.1.1. Flow rate

The influence of flow rate on the release of acyclovir at 32°C using an HVLP synthetic membrane was investigated at flow rates of 2.9, 5.8 and 10.0 ml/min. The results are depicted in Table 1 and Fig. 1. Increasing flow rates resulted in an increase of release of acyclovir from both Zovirax and cream A (Fig. 3). In all cases, plots

Table 1
In vitro release of acyclovir at different flow rates (HVLP membrane at 32°C)

Formulation	Flow rate (ml/min)	30 min	60 min	120 min	180 min	Release rate [b]	R^2
Cream A	2.9	128.8 (9.8) [a]	211.3 (10.9)	334.7 (7.5)	426.0 (6.2)	36.62	0.998
Cream A	5.8	135.3 (11.9)	238.8 (7.8)	386.8 (5.1)	491.4 (4.2)	44.97	0.999
Cream A	10	257.0 (11.3)	393.3 (9.5)	556.4 (7.8)	681.1 (6.5)	54.03	0.997
Zovirax	2.9	65.7 (10.7)	102.9 (17.6)	159.7 (12.8)	208.4 (9.6)	17.10	0.997
Zovirax	5.8	79.6 (5.6)	122.5 (4.3)	180.9 (4.9)	231.5 (4.9)	19.03	0.994
Zovirax	10	121.1 (9.7)	171.5 (10.7)	246.3 (8.7)	319.3 (7.9)	24.43	0.997

[a] Mean of three; values in parentheses %RSD.
[b] Slope = release rate (μg/cm^2 per min$^{0.5}$).
HVLP, polyvinylidine difluoride; R^2, regression correlation coefficient.

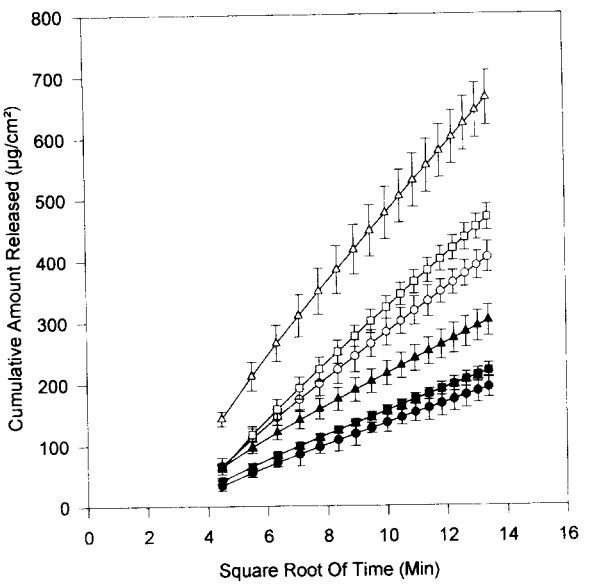

Fig. 3. Influence of flow rate on in vitro release of acyclovir. (○) Flow rate 2.9 ml/min (cream A, HVLP membrane), (●) flow rate 2.9 ml/min (Zovirax cream, HVLP membrane), (□) flow rate 5.8 ml/min (cream A, HVLP membrane), (■) flow rate 5.8 ml/min (Zovirax cream and HVLP membrane), (△) flow rate 10 ml/min (cream A, HVLP membrane), (▲) flow rate 10 ml/min (Zovirax cream, HVLP membrane). * Replicate of three experiments.

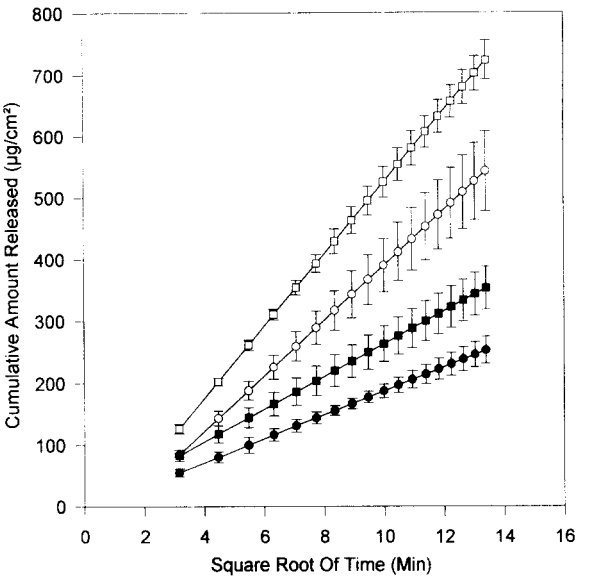

Fig. 4. Influence of temperature on in vitro release of acyclovir. (○) Cream A (32°C, cellulose acetate membrane, 0.45 μm), (●) Zovirax cream (32°C, cellulose acetate membrane, 0.45 μm), (□) cream A (37°C, cellulose acetate membrane, 0.45 μm), (■) Zovirax cream (37°C, cellulose acetate membrane, 0.45 μm). * Replicate of three experiments.

of cumulative amounts released vs the square root of time were found to be linear and the values of the relevant slopes of these plots as well as the cumulative amounts released at 30, 60, 120 and 180 min are depicted in Table 1.

3.1.2. Temperature

The release of acyclovir from Zovirax and cream A using a cellulose acetate membrane was measured at 32 and 37°C at a flow rate of 5.8 ml/min. These results indicate that the relatively small difference in temperature appeared to have a significant effect on the release of acyclovir from both preparations (Fig. 4). This temperature effect is probably due to the nature of the semi-solid vehicles which would account for the difference in release seen as a result of the heat sensitivity of the components of the vehicles. Unlike a tablet formulation, vehicles used to formulate semi-solid dosage forms would become less viscous with increasing temperature and thereby facilitate the release of drug. Furthermore, this flow-through system clearly indicates the utility of

Table 2
Influence of temperature on in vitro release of acyclovir (flow rate = 5.8 ml/min; CE membrane)

Formulation	Temperature (°C)	30 min	60 min	120 min	180 min	Release rate [b]	R^2
Cream A	32	174.9 (8.5) [a]	274.3 (9.6)	433.2 (11.7)	561.4 (11.5)	48.08	0.998
Cream A	37	274.5 (3.4)	385.3 (3.2)	582.5 (4.8)	731.3 (4.3)	61.87	0.999
Zovirax	32	94.8 (13.6)	145.9 (6.8)	206.2 (6.7)	252.0 (8.6)	19.62	0.999
Zovirax	37	144.8 (11.3)	208.6 (11.5)	290.5 (10.7)	344.9 (10.1)	26.38	0.994

[a] Mean of three; values in parentheses %RSD.
[b] Slope = release rate ($\mu g/cm^2$ per $min^{0.5}$).
CE, cellulose membrane; R^2, regression correlation coefficient.

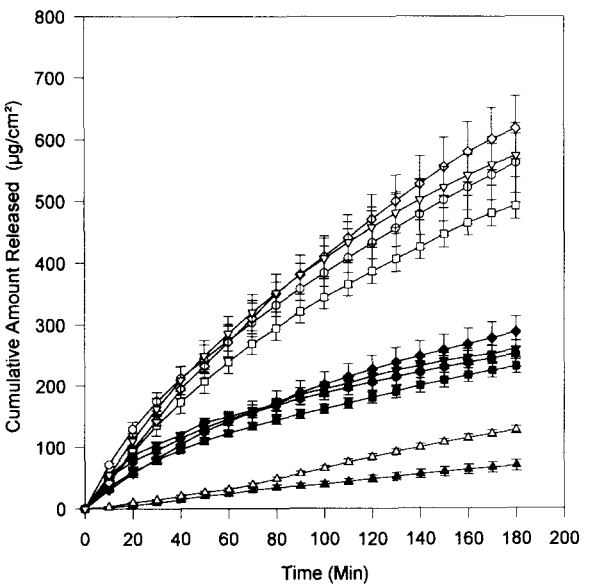

Fig. 5. Influence of membrane on in vitro release of acyclovir. (○) Cream A (cellulose acetate, 0.45 μm), (□) cream A (HVLP, 0.45 μm), (△) cream A (PTFE, 0.45 μm), (◇) cream A (dialysis membrane), () cream A (HAWP, 0.45 μm), (●) Zovirax cream (cellulose acetate, 0.45 μm), (■) Zovirax cream (HVLP, 0.45 μm), (▲) Zovirax cream (PTFE, 0.45 μm), (◆) Zovirax cream (dialysis membrane), (▼) Zovirax cream (HAWP, 0.45 μm). * Replicate of three experiments.

the method to discern such a phenomenon. Plots of the square root of time vs cumulative amount released were also linear in all instances and the relevant data depicted in Table 2.

3.2. *Influence of membrane*

Irrespective of type of membrane used, the rank order of release of acyclovir between Zovirax and cream A remained the same as clearly seen in the plot of cumulative amount released vs time (Fig. 5). Once again, plots of square root of time vs cumulative amount released were linear in all instances and the relevant data are shown in Table 3. Release of acyclovir was found to be the slowest when a PTFE membrane was used. This is probably due to the hydrophobic nature of this membrane. Acyclovir had greater permeability through the cellulose membranes, including the dialysis membrane whereas a lower degree of permeability was seen through the polyvinylidene difluoride. This effect is probably due to the physico-chemical properties of the drug whose permeation is facilitated by the nature of the membrane used. This dependence has been discussed in a previous report (Guy and Hadgraft, 1990) and thus re-emphasizes the importance of the correct choice of membrane. This is particularly important when undertaking in vitro experiments to compare two semi-solid products on a comparative basis. A pre-requisite for such comparative assessment is to ensure that the release of active ingredient into the receptor phase is controlled by the semi-solid vehicle and not by the properties of the membrane.

The effects of membrane properties on the release of acyclovir during the present studies have thus been clearly demonstrated.

4. Conclusions

The rank order of release of acyclovir from two different semi-solid preparations remained the same irrespective of the nature of the membrane used in this study. However, the use of cellulose acetate membranes resulted in better discrimination between the two formulations. The

Table 3
Influence of membrane on in vitro release of acyclovir (flow rate = 5.8 ml/min at 32°C)

Formulation	Membrane	Release rate [a] ($\mu g/cm^2$ per $min^{0.5}$)	R^2
Cream A	CE	48.08	0.998
Cream A	HVLP	44.97	0.999
Cream A	PTFE	13.14	0.951
Cream A	dialysis	58.58	0.997
Cream A	HAWP	52.10	0.999
Zovirax	CE	19.62	0.999
Zovirax	HVLP	19.03	0.994
Zovirax	PTFE	6.99	0.984
Zovirax	dialysis	25.71	0.999
Zovirax	HAWP	19.95	0.998

[a] Mean of three.
R^2, regression correlation coefficient; CE, cellulose acetate; PTFE, polytetrafluoroethylene; dialysis (dialysis membrane); HAWP, mixed esters of cellulose; HVLP, polyvinylidene difluoride.

results from these experiments indicated that the order of release of acyclovir was better from cream A than from Zovirax cream which are in agreement with results of a previous study (Chattaraj et al., 1995) using the same plexiglass cell with manual sampling when the same two products were assessed.

A small difference in temperature was shown to have a significant effect on the release of acyclovir and thus emphasizes the necessity of temperature control during in vitro experiments of semi-solid formulations.

Whereas a flow rate of 10.0 ml/min resulted in the greatest discrimination between the two formulations, plots of square root of time vs cumulative amount released were linear when either flow rates of 2.9, 5.8 or 10.0 ml/min were used.

The flow-through plexiglass cell presented here has thus been shown to be a simple, reliable method to assess the pharmaceutical availability of acyclovir from semi-solid preparations and should be readily applicable to monitor drug release from semi-solid dosage forms.

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