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Journal of Controlled Release 69 (2000) 357–367

journal of controlled release

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Validation protocol of an automated in-line flow-through diffusion equipment for in vitro permeation studies

M. Córdoba-Díaz[a], M. Nova[a], B. Elorza[b], D. Córdoba-Díaz[a], J.R. Chantres[b], M. Córdoba-Borrego[a,*]

[a]*Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Complutense University of Madrid, Avda. Complutense s/n, E-28040 Madrid, Spain*

[b]*Department of Physical Chemistry II, Faculty of Pharmacy, Complutense University of Madrid, Madrid, Spain*

Received 18 April 2000; accepted 18 July 2000

Abstract

Transdermal drug delivery experiments are often tedious and time consuming in terms of sampling, labor, etc. In this way, the automation of such experiments has increased in the last few years. A protocol suitable to validate an automated diffusion equipment with seven in-line flow-through cells is described. The proposed protocol was divided into two parts. First, validation of each component which makes up the whole equipment, including the study of the statistical variability of the internal volumes between the cells, the temperature into the different chambers, the time and sample volumes, etc. In the second part, a series of permeation studies were carried out comparing the performance of the system against a classical Franz-type diffusion cell. Ketoprofen was used as a model drug. It was proved the low variability of the replicates obtained with the automated flow-through diffusion cells. The best work conditions as flow rate into the receptor chamber, temperature, etc., as well as the best mathematical approach for the diffusion data, were determined. The advantages in terms of time saved and easiness of validation of the flow-through cell design in comparison to the Franz-type cell were evidenced.

Keywords: Diffusion; Franz-cell; Flow-through-cell; Validation; Automation

1. Introduction

Transdermal drug delivery provides several potential advantages for some drugs in comparison to other administration routes, such as the avoidance of first-pass gut and hepatic metabolism, fewer side effects with a decrease in the risks of toxicity and relative ease of drug input [1]. In vitro diffusion experiments have become one of the most important kinds of studies of transdermal forms for drug administration, not only in the development of new formulations but also for quality control of the final preparations. The use of the Franz-type diffusion cell to perform such experiments was quickly widespread but it presented some inconvenience in comparison to the flow-through cells that were firstly described by different authors [2,3].

*Corresponding author. Tel.: +34-91-394-1727; fax: +34-91-394-1736.

E-mail address: mcordoba@eucmax.sim.ucm.es (M. Córdoba-Borrego).

0168-3659/00/$ – see front matter
PII: S0168-3659(00)00306-0

In the flow-through design, the continuous flow through the receptor chamber helps to maintain sink conditions throughout the course of an experiment, what is an important feature to be taken into account, particularly for those drugs having large permeability coefficients through a certain membrane [4]. On the other hand, this kind of cell is more suitable for the simulation of in vivo conditions, in comparison to the classical static Franz-type cells.

Transdermal penetration studies are often tedious, time consuming and sometimes require complicated sample schedules; besides, the implementation of such in vitro methods is a question of an increasing importance, not only because these studies are being used to assess the effects on drug delivery through the skin (preformulation studies) but also to compare generic products with an innovator as well as for batch-to-batch variations testing at industrial scale. Considering all these points it is clear that automation of the equipment used in the frame of this kind of studies is highly recommended and the full validation of the system is mandatory. In this way, some automated permeation equipments based on the conventional Franz cell can be found but, since this kind of cell was not originally designed for its possible adaptation to automatic sampling, these equipment require big complexity mainly because it is necessary to maintain a constant volume into the receptor compartment to assure a certain level of receptor medium in order to keep it in contact with the surface of the membrane. This requires a complex system of level probes and pumps to provide an accurate volume of sample withdrawal with a simultaneous media replacing [5]. In contrast, the use of flow-through cells offers several advantages over the static cells in terms of automation since only a pump to accurately assure a certain flow rate of receptor medium and a programmable fraction collector are necessary. The use of this kind of cells in permeation studies has augmented dramatically in the last few years and there is an increasing body of information in the scientific literature about this [6–10]. In the present paper, a whole protocol to validate an automated diffusion equipment based on the flow-through cells is reported.

2. Materials and methods

A PermeGear® ILC-07 automated system (PermeGear, Riegelsville, PA) was used in the present study. The equipment incorporated seven in-line flow-through diffusion cells, made of Kel-F, in which the donor and receptor chambers and the diffusion membrane were clamped by threaded rods with adjustable locking nuts (see Fig. 1). The membrane was placed over a support with an orifice of 1 cm in diameter (diffusional area, 0.785 cm^2). The receptor compartment was equipped with a glass-viewing window that could be disabled with a

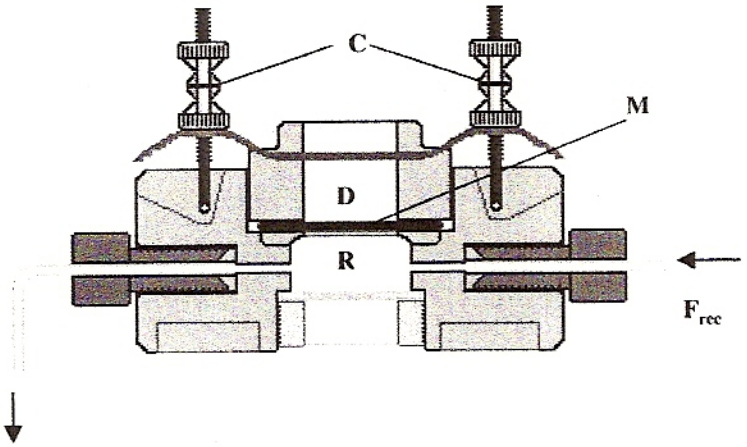

Fig. 1. General description of a flow-through diffusion cell: D, donor compartment; R, receptor compartment; M, diffusion membrane; C, clamping system for the donor chamber; F_{rec}, flow of receptor fluid.

locknut. The inlet and outlet ports of the lower chamber were connected to the Tygon tubing with 1/4-28 HPLC fittings and all the cells were placed in a cell warmer connected with a Haake®-DC10 circulating bath (Gebrüder Haake, Karlsruhe, Germany) to permit the system to be brought up to a certain work temperature. The cells were connected to a 16-channel peristaltic pump Ismatec® IPC-16 (Ismatec, Zürich, Switzerland) and the receptor fluid was pumped continuously through each cell to be collected in the receptor tubes/vials of an Isco® Retriever IV fraction collector (Isco, Lincoln, NE, USA). An Indexing Controller (also available from PermeGear) was used to program independently the duration of each shuttle in the retriever so that 19 samples could be collected simultaneously from each cell.

2.1. Validation of the mechanical elements

A number of variables related to the mechanical parts and the performance of each component were chosen to be validated. The following variables have a dramatic influence on the development of diffusion experiment with this kind of equipment and a modification of the values of such variables could be decisive in the interpretation of the resulting data.

2.1.1. Volumes of donor and receptor chambers for each cell

All volumes were measured by gravimetric methods by filling the chambers with Milli-Q water and assuming a density of 1 g/ml. All the determinations were made in triplicate for each cell.

2.1.2. Temperature control in the different chambers

The whole equipment was assembled and a laboratory film (Parafilm™) was placed instead of a diffusion membrane in each cell. The system was filled with distilled water and a flow rate of receptor fluid of about 4 ml/h was selected; 0.5 ml of water was placed in each donor compartment and the temperature was measured at 30 and 60 min in each chamber using a calibrated thermometer. The experiment was carried out programming two temperatures: 35 and 37°C.

2.1.3. Pumping system and flow rates

The equipment was assembled as in Section 2.1.2 and a series of pump speeds were checked in terms of amount of fluid dispensed in a previously weighed tubes during 5 min measured with a chronograph so that the flow rate into the receptor chamber F_{rec} (ml/h) could be easily calculated. This test was made with two different kinds of Tygon® pumping tubes: 1.52 and 0.64 mm internal diameter (ID). Linear regression analysis was used to establish the relation, pumping speed versus F_{rec}. The reproducibility of the pumping system was also determined between two days and selecting three different flow rates (F_{rec}): 1, 5 and 10 ml/h.

2.1.4. Variability of sampling volumes

Forty-two previously weighed 4-ml HPLC vials were placed in the fraction collector. The experiment was conducted at 37°C and the pump was adjusted to obtain a F_{rec} of 10 ml/h. The indexing controller was programmed to change each shuttle every 15 min. The theoretical amount of liquid collected in each vial should be 2.5 ml. Six samples were collected from each cell at 0, 0.25, 0.50, 0.75, 1.00 and 1.25 h, respectively, and four tests were carried out in consecutive days. The difference between the weight before and after the experiment was considered to be equivalent to the volume of water collected. The variability of sampling volume was statistically analyzed by a two-way multi-factorial ANOVA using two variables: sample (or sampling time) and test; 95% confidence was applied to search for significant differences.

2.1.5. Evaporation of receptor fluid from the collected samples

Due to the fact that in a hypothetical long-time experiment the samples collected during the night cannot be sealed automatically with a cap, this test is essential and must be done with the receptor fluid that is going to be used. In the present work, evaporation was studied with aqueous solvents such as a phosphate-buffered saline solution (PBS) having a pH of 7.2 and previously described by many authors [11]. Seven HPLC vials (4 ml) and seven tubes (10 ml) were partially filled with receptor fluid from the cells under the same conditions as in a real permeation experiment. Both tubes and vials were

accurately weighed to calculate the exact amount of fluid collected and were kept in the fraction collector and re-weighed at 1, 2, 4, 6 and 24 h. Data corresponding to the remaining amount of liquid were analyzed by linear regression.

2.2. Validation of work conditions in permeation experiments

In order to evaluate the in-line flow-through cells, and to establish the most appropriate work conditions, a direct comparison against with a modified Franz-type diffusion cell was performed. This vertical cell was made in glass and was designed to have a volume into the donor compartment of 100 ml, to assure sink conditions. The diffusional area of this cell was 12.56 cm^2. In both kinds of cells (flow-through and Franz), the permeation experiments were conducted at 37°C and unreinforced 0.0127-cm thick polydimethylsiloxane (PDMS) sheets (Prolastic Sheeting™, Pillar Surgical, La Jolla, CA, USA) were chosen as nonporous diffusion membranes. The use of that kind of membrane in permeation experiments has been reported by many authors, in validation studies [12], because is one of the most widely used artificial membrane in many types of diffusion experiments. PDMS is a non-polar, non-porous elastomer that exhibits solubility characteristics that closely parallel those of hexane [13]. Moreover, PDMS membranes have been described as a useful skin surrogate for comparing the availability of certain compounds for absorption from different formulations [14]. The use of this kind of artificial membrane is more recommended in a validation study due to the lower variability exhibited in comparison to those data resulting when any kind of 'natural skin' is selected.

In all the experiments, ketoprofen was used as a model drug. A solution of such drug in phosphate-buffered saline solution (PBS) having a concentration of C_0=2 mg/ml was placed into the donor compartment using an amount suitable to obtain a relation of about 0.5 ml per cm^2 of diffusional area (6.5 ml with the Franz cells and 0.5 ml in the flow-through cells). The amount of drug in the resulting samples was spectrophotometrically assayed at 259 nm, using a previously validated analytical method.

Some variables were evaluated: variability among the different cells in terms of diffusion profiles in both systems and influence of the kind of cell and of the flow rate of the receptor fluid (also PBS) on the intrinsic flux of drug through the membrane.

2.3. Data analysis: mathematical approach

Due to the fact that all compounds are thought to transfer by a passive diffusion mechanism, it is then possible to apply Fick's laws of diffusion to establish a model to study the data obtained from in vitro permeation experiments [15].

The simplest way of modeling the absorption process is to assume that Fick's first law is applicable. In this way, many authors studied the data simply by plotting the cumulative amount of drug permeated versus time. In such plots, the steady-state rate of absorption or flux (J) of the drug could be directly calculated from the slope obtained by a linear regression analysis. Such treatments are well known and widely used. However, some considerations related to the experimental variables that are present in the flow-through systems must be taken into account. Sclafani et al. [16] claimed that fraction collector tube volume, receiver cell volume, flow rate and sampling interval could modify the apparent flux data. On the other hand, both finite-dose flux profiles and infinite-dose diffusional lag times could be modified by those parameters. Some mathematical approaches have been proposed to estimate the intrinsic flux in such conditions. Many authors [4,17] studied the influence of the volume of the receptor chamber using different kind of cells. They found a good agreement could be obtained between drug fluxes measured in a low volume flow-through cell and those measured in a static cell. Due to the fact that the flux must be calculated from the concentration versus time data, proper analysis of the flow-through diffusion cell is critically important in the accuracy of the calculation [18]. In general lines, the evolution of the concentration of drug into the receiver chamber (C_{rec}) of a flow-through diffusion cell versus time (t) can be studied by using the following equation:

$$V_{rec} \cdot \frac{dC_{rec}}{dt} = J \cdot A - F_{rec} \cdot C_{rec} \quad (1)$$

where V_{rec} is the volume of the receiver chamber, J the flux of drug out the skin, A the diffusional area and F_{rec} the flow rate of receptor fluid. Assuming that the accumulation of drug into the receiver chamber is negligible because of the low volume of that chamber, Eq. (1) could be directly used to calculate the intrinsic flux J at a certain time t, considering the solution for this expression described by many authors [18,19]. In this way the term dC_{rec}/dt could be easily estimated from the concentration versus time raw data. Taking into account that the normal situation corresponds to a finite dose system, it means that both the concentration into the receptor chamber and the calculated intrinsic flux increase rapidly to reach maximums and then decrease. In this way, some authors [11,19] reported previously that the apparent flux profiles could be fitted to a bi-exponential function as follows:

$$J = A \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t}) \quad (2)$$

This equation is derived from an assumed exponential decaying donor concentration for a drug with a short diffusional lag time. In such expression, A is a constant related to the amount of drug permeated from zero to infinite and K_1 and K_2 are the apparent increasing and decreasing rate constants of J. The first derivative of Eq. (2) yields the value of the maximum J, corresponding to the flux obtained in the steady state. In our study, the parameters A, K_1 and K_2 were estimated from the experimental flux profiles by unconstrained weighted least-squares non-linear regression by using the SIMFIT computer package (W. G. Bardsley, 1998).

3. Results and discussion

3.1. Validation of the mechanical elements

The volumes of both receptor and donor compartments (V_{rec} and V_{don}) were found to be homogeneous among the seven diffusion cells. The resulting values were (mean±confidence interval, $P=0.05$):

$V_{rec} = 0.855 \pm 0.030$ ml; R.S.D. = 3.46%
$V_{don} = 1.096 \pm 0.023$ ml; R.S.D. = 2.12%.

About the temperature control, no statistically significant differences between the values obtained in both chambers at 30 and 60 min were found. From these results, 30 min was adopted as the reference time necessary to make the equipment reach the work temperature. A difference of 5°C between the programmed temperature in the heater and those obtained into the donor chambers (at the diffusion membrane surface) was evidenced at both 35 and 37°C. A difference of about 1.5°C was also found between donor and receptor compartments due to the flow rate and renovation of medium into the second chamber. This ensured that the membrane surface temperature (t_{don}) was maintained at 32.53±0.50°C (R.S.D. = 1.29%) along the experiments when a temperature of 37°C was programmed, obtaining also a temperature t_{rec} = 30.79±0.51°C (R.S.D. = 1.36%).

The flow rates of medium into the receptor chambers were also studied as a function of pumping speed and the internal diameter of the Tygon™ tubes installed inside the peristaltic pump. A good linear relation was found as can be seen in Fig. 2. The reproducibility of the pumping system was also validated between two days and the resulting values are shown in Table 1. As can be seen, good repeatability was obtained at the three flow rates programmed. The mean percentage of concordance between real and predicted values was 99.44±1.65%.

The variability of sampling volumes was checked as previously described. The amount of liquid collected in each vial along four different tests is shown in Table 2 and the results corresponding to the two-way multi-factorial ANOVA of such data are shown in Table 3. It was statistically proved that no significant differences exist between the studied variables: cell, sample number (or sampling interval) and test number, with a significance level of 95%. The homogeneity in terms of interaction of variables was also confirmed.

The degree of evaporation of fluid from the collected samples was determined as previously described. The evolution of the amount of remaining liquid versus time for both 10-ml tubes and 4-ml HPLC vials is plotted in Fig. 3. Linear regression analysis of such data revealed that, in all cases, the loss of liquid was less than 2% in 8 h, what is not relevant in terms of critical modification of the drug

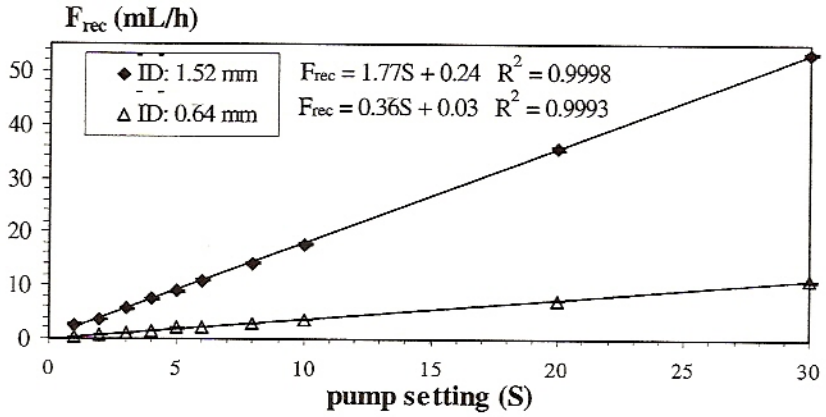

Fig. 2. Flow rate values (F_{rec}) obtained at each pumping speed for the two kinds of Tygon tubes (error bars denote standard deviation between cells).

concentrations in the automatically collected samples during the night in long-term experiments. Nevertheless, this evaporation could be relevant when working with other receptor fluid (i.e., organic solvents) and should be studied for each medium.

3.2. Influence of work conditions on the results obtained from permeation studies

Fig. 4 shows the evolution of the ketoprofen concentration into the receptor chamber (C_{rec}) with the different flow rates: 0 (Franz cell), 1.1, 5.5 and 10.8 ml/h. As can be seen, totally different behavior of the concentration was obtained with the first experiment, due to the characteristics of that cell in which there was no renovation of receptor fluid and the permeated drug was being accumulated inside the receptor chamber. In contrast, when a flow-through diffusion cell was used, the concentration values raised to a maximum and then decreased progressively to zero at long times. As expected, the higher the F_{rec}, the lower the maximum concentration resulted because the elimination of the permeated drug from the receptor chamber was faster. A curve fitting was carried out from such data using a bi-exponential function like $C_{rec} = Z \cdot [\exp(-X_1 \cdot t) - \exp(-X_2 \cdot t)]$. The calculated maximum concentration (C_{max}) and the time necessary to reach that maximum (t_{max}) as well as those factors inherent to the fitting are shown in Table 4. From those data, a clear concordance between the maximum concentration obtained and the flow rate was observed: C_{max} was approximately 5 and 10 times less when the F_{rec} was increased 5 and 10 times, respectively, whereas the t_{max} values were not significantly affected by the flow rate.

Table 1
F_{rec} (ml/h) obtained at two different days (mean of seven cells±S.D.) with different theoretical programmed flow rates (TPF)

TPF	Day 1	Day 2	Mean	R.S.D. (%)
1	1.122±0.035	1.090±0.038	1.105	1.92
5	4.840±0.063	4.910±0.086	4.875	1.02
10	10.015±0.311	10.030±0.209	10.022	0.11

The amount of drug permeated at each time was calculated as previously described. Fig. 5 shows the resulting permeation profiles in terms of cumulative percent of drug permeated versus time. The accumulation of the drug into the receptor chamber was real only in the static cell, whereas in the flow-through system, the data were mathematically accumulated for comparison purposes. A clear relation between the slope of the permeation profile and flow rate could not be found. Nevertheless, the plot obtained revealed a remarkable similitude between the results obtained with both kind of cells, taking into account the big differences among the design of the two systems. Apart from this, lower variability was found

Table 2
Amount of liquid collected in each vial: F_{rec} = 10 ml/h; sampling interval, 0.25 h

Test	Sample	Cell 1 (ml)	Cell 2 (ml)	Cell 3 (ml)	Cell 4 (ml)	Cell 5 (ml)	Cell 6 (ml)	Cell 7 (ml)	Mean (ml)	R.S.D. (%)
1	1	2.609	2.543	2.577	2.391	2.575	2.640	2.611	2.563	3.20
	2	2.601	2.569	2.585	2.473	2.568	2.637	2.639	2.582	2.18
	3	2.594	2.558	2.580	2.480	2.535	2.619	2.645	2.573	2.13
	4	2.604	2.588	2.593	2.540	2.569	2.627	2.671	2.599	1.61
	5	2.602	2.566	2.586	2.493	2.550	2.630	2.644	2.582	1.99
	6	2.606	2.568	2.587	2.506	2.592	2.627	2.675	2.594	2.,00
2	1	2.591	2.534	2.612	2.661	2.590	2.620	2.672	2.611	1.78
	2	2.593	2.531	2.601	2.644	2.597	2.631	2.658	2.608	1.61
	3	2.581	2.529	2.596	2.628	2.567	2.613	2.651	2.595	1.56
	4	2.568	2.525	2.583	2.620	2.569	2.595	2.626	2.585	1.43
	5	2.546	2.522	2.579	2.616	2.550	2.600	2.638	2.579	1.62
	6	2.549	2.515	2.577	2.654	2.558	2.585	2.633	2.582	1.87
3	1	2.584	2.571	2.589	2.572	2.578	2.596	2.655	2.592	1.12
	2	2.550	2.577	2.564	2.582	2.563	2.584	2.633	2.579	1.04
	3	2.545	2.567	2.578	2.573	2.561	2.581	2.629	2.576	1.01
	4	2.560	2.554	2.557	2.559	2.559	2.576	2.625	2.570	0.99
	5	2.560	2.569	2.576	2.586	2.560	2.583	2.629	2.580	0.93
	6	2.565	2.591	2.591	2.594	2.564	2.600	2.651	2.594	1.12
4	1	2.595	2.585	2.595	2.564	2.619	2.612	2.677	2.607	1.37
	2	2.551	2.530	2.562	2.580	2.608	2.570	2.639	2.577	1.41
	3	2.538	2.533	2.573	2.554	2.610	2.589	2.638	2.576	1.50
	4	2.507	2.555	2.552	2.557	2.607	2.592	2.640	2.573	1.69
	5	2.515	2.550	2.561	2.555	2.612	2.618	2.648	2.580	1.81
	6	2.540	2.569	2.602	2.570	2.620	2.610	2.741	2.608	2.50

in the profiles obtained with the flow-through cells, due to the higher complexity of the sampling in the static cells.

The intrinsic flux of ketoprofen through the artificial membrane (J in $\mu g/cm^2$ h) at each time was calculated from Eq. (1) and the resulting values were fitted to a bi-exponential model as previously described (Eq. (2)). The estimated values for A, K_1 and K_2 are reported in Table 5 and the plot corresponding of those equations are shown in Fig. 6, where a big increase in the J values can be observed in the beginning of the experiment in all cases, to reach to a maximum value within the first 2 h. Since this part of the plot is described by K_2, the comparison of such parameter revealed that there were no significant differences among the results obtained at each flow rate. In contrast, some differences were found in the second part of the plot: the decrease of J, which

Table 3
Two-way multi-factorial analysis of variance of sampling volumes

Source of variation	Sum of squares	d.f.	Mean squares	F ratio	Sig. level	Critical F
Sample no.	0.004599	5	0.0009198	0.49603	0.778824	2.27704
Test no.	0.003448	3	0.0011492	0.61973	0.603326	2.66744
Interaction	0.017474	15	0.0011649	0.62823	0.847992	1.73636
Residual	0.267026	144	0.0018543			
Total	0.292547	167				

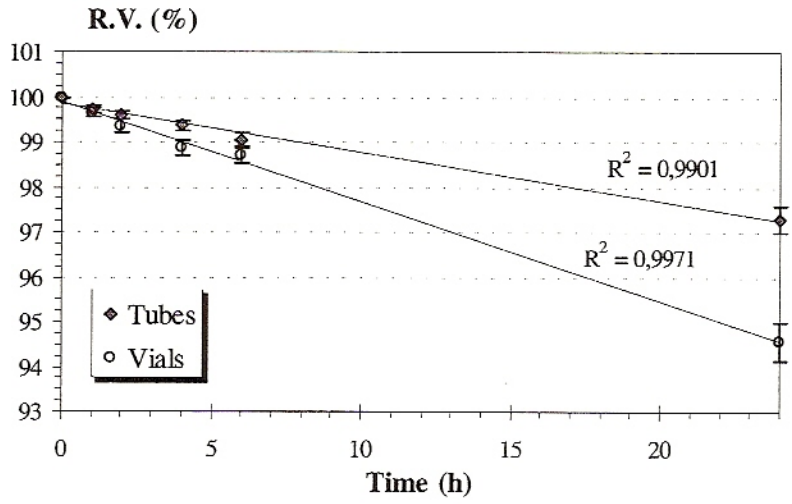

Fig. 3. Plot showing the evolution of the remaining volume (R.V.) of liquid (expressed in percentages) in the collected samples (mean±S.D.).

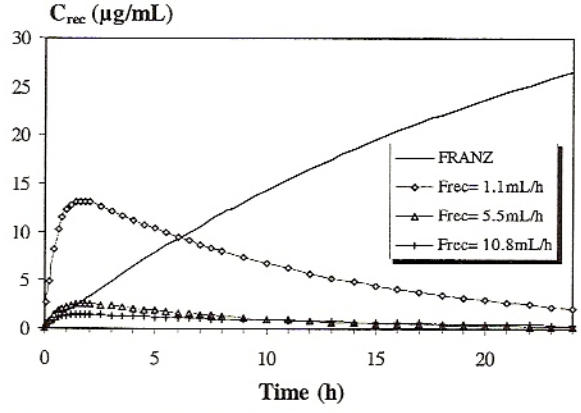

Fig. 4. Concentration into the receptor chamber (C_{rec}) versus time profiles fitted to a bi-exponential model for both Franz-type and flow-through diffusion cell at different flow rates.

is described by K_1. It was observed that K_1 was significantly higher with a $F_{rec}=0$ ml/h (Franz type cell) in comparison to those obtained with a flow-through cell. That indicated that the flux of drug to the receptor compartment after reaching a maximum value decreased more rapidly with the static cell, what can be due to the lack of renovation of receptor medium in that design with an increase in the concentration in such chamber as previously commented (see Fig. 4).

Fig. 7 shows the calculated values for maximum intrinsic flux, J_{max} (µg/cm^2 h) and the time in which this flux is maximum, t_{Jmax} (h). The permeability coefficient (K_p in cm/h) was also determined by dividing the J_{max} that was considered to be the flux in the steady state, by the initial concentration of ketoprofen into the donor compartment. As can be seen, the J and K_p obtained values with the Franz-type cell were not significantly different to those obtained with the flow-through cells, taking into account the variability of such determinations. Nevertheless, a progressive increase in the $t_{J_{max}}$ was detected when increasing the flow rates. A good linear relationship between those parameters (t_{Jmax} and F_{rec}) was also found ($R=0.9887$). Those results

Table 4
Calculated values for C_{max} and t_{max} from a curve fitting to a bi-exponential function

Conditions	Z	X_1	X_2	r^2	t_{max} (h)	C_{max} (µg/ml)
$F_{rec}=1.1$ ml/h	15.76	0.0840	0.4978	0.613	1.654	13.138
$F_{rec}=5.5$ ml/h	3.36	0.1214	1.1760	0.921	1.759	2.501
$F_{rec}=10.8$ ml/h	1.60	0.0610	2.0150	0.921	1.790	1.392

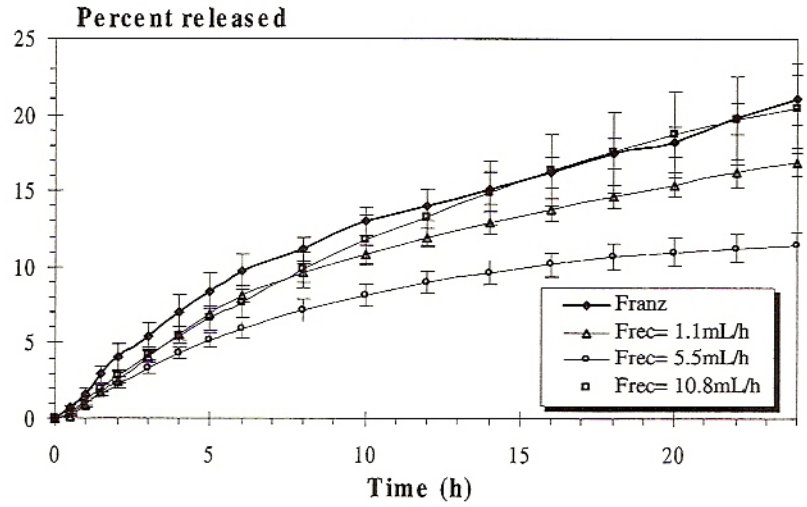

Fig. 5. Cumulative amount (in percentage) of ketoprofen released at each time trough the membrane from the donor to the receptor compartment at each work condition (mean of seven experiments±S.D.).

Table 5
Curve fitting of the intrinsic flux versus time data to a bi-exponential function

Conditions	A	K_1	K_2	r^2	Fit[a]
F_{rec}=0 (Franz)	24.22±2.41	0.225±0.031	3.822±0.949	0.868	****
F_{rec}=1.1 ml/h	26.14±3.86	0.098±0.014	3.801±2.190	0.867	*
F_{rec}=5.5 ml/h	20.43±1.20	0.120±0.005	1.747±0.327	0.940	****
F_{rec}=10.8 ml/h	21.84±1.70	0.069±0.010	2.296±0.693	0.920	**

[a] Asterisks denote the verdict on goodness of fit.

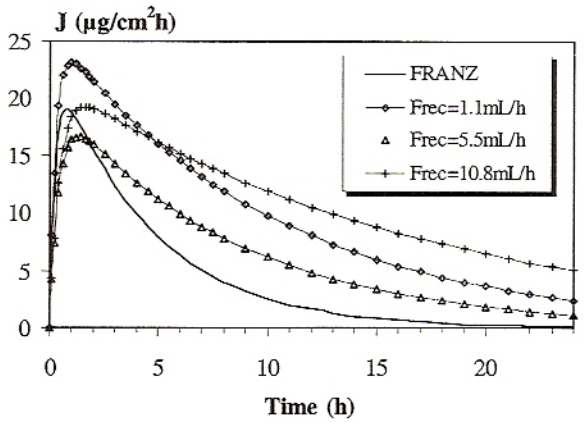

Fig. 6. Bi-exponential curves resulting from the fitting of intrinsic flux data at the different flow rates studied and in comparison to the obtained with the Franz type cell.

confirmed that the flow rate into the receptor chamber did not affected the numerical value of flux of drug though the membrane but could modify the time in which the steady-state is reached.

4. Conclusions

The results obtained in the present paper confirmed that the studied flow-through diffusion equipment provided good reproducibility in terms of temperatures obtained at each point, flow rate into the receptor chambers, programmed sampling intervals and collected volumes.

The resulting permeation profiles obtained were not substantially different with the static and the flow-through diffusion cells, even taking into account the big differences among the design of both systems. It was demonstrated that the variability

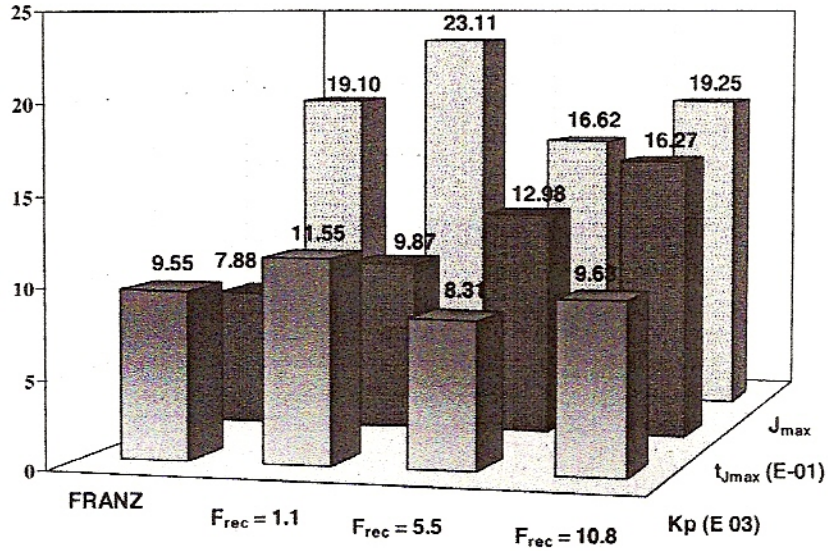

Fig. 7. Comparative plot showing the resulting values of intrinsic flux (J in $\mu g/cm^2h$), time in which the maximum flux was reached ($\times 10$, in h) and permeability coefficient ($\times 10^3$, K_p in cm/h).

among the different experiments was lower with the flow through equipment.

About the influence of the flow rate on the permeation rate of ketoprofen trough the artificial membrane, it was observed that the lack of renovation of receptor medium in the static cell, provoked a faster decrease of the intrinsic flux after a maximum value had been reached. Besides, an increase in the F_{rec} increased significantly the time in which the steady state was reached.

No significant differences were found between the results obtained with the PermeGear® equipment and those obtained by other authors [20] for ketoprofen through human skin with a Franz type cell. Under this conditions, an intrinsic flux of $J = 16.00 \pm 9.00$ $\mu g/cm^2$ h was obtained.

From the above-mentioned results, we are able to conclude that the reliability of the Perme-Gear® in-line flow through diffusion equipment has been demonstrated and validated for diffusion experiments.

Acknowledgements

Authors would like to express their gratitude to ITALFARMACO S.A. for its financial support for the development of the present work.

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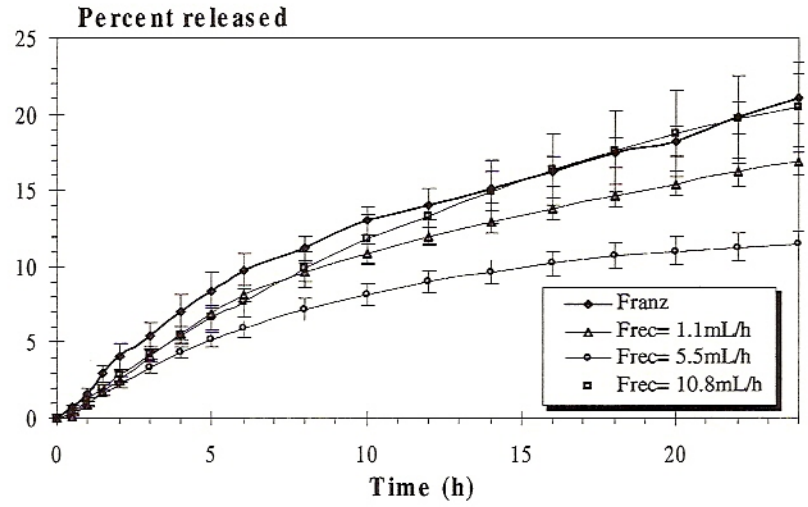

Fig. 5. Cumulative amount (in percentage) of ketoprofen released at each time trough the membrane from the donor to the receptor compartment at each work condition (mean of seven experiments±S.D.).

Table 5
Curve fitting of the intrinsic flux versus time data to a bi-exponential function

Conditions	A	K_1	K_2	r^2	Fit[a]
$F_{rec}=0$ (Franz)	24.22±2.41	0.225±0.031	3.822±0.949	0.868	****
$F_{rec}=1.1$ ml/h	26.14±3.86	0.098±0.014	3.801±2.190	0.867	*
$F_{rec}=5.5$ ml/h	20.43±1.20	0.120±0.005	1.747±0.327	0.940	****
$F_{rec}=10.8$ ml/h	21.84±1.70	0.069±0.010	2.296±0.693	0.920	**

[a] Asterisks denote the verdict on goodness of fit.

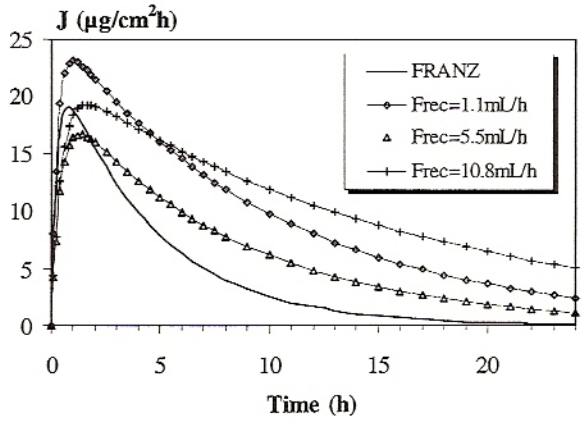

Fig. 6. Bi-exponential curves resulting from the fitting of intrinsic flux data at the different flow rates studied and in comparison to the obtained with the Franz type cell.

confirmed that the flow rate into the receptor chamber did not affected the numerical value of flux of drug though the membrane but could modify the time in which the steady-state is reached.

4. Conclusions

The results obtained in the present paper confirmed that the studied flow-through diffusion equipment provided good reproducibility in terms of temperatures obtained at each point, flow rate into the receptor chambers, programmed sampling intervals and collected volumes.

The resulting permeation profiles obtained were not substantially different with the static and the flow-through diffusion cells, even taking into account the big differences among the design of both systems. It was demonstrated that the variability

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