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Validation of a release diffusion cell for topical dosage forms

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Abstract

The methodology followed to choose the agitation speed and posterior validation of a new design of diffusion cell, used in studies of in-vitro release of naproxen in the classical topical dosage forms is described. It was found that the methodology is repeatable, reproducible and robust for the chosen speed (600 rev./min) providing that the test temperature is carefully controlled.

Keywords: Naproxen; Topical dosage forms; Validation; Diffusion cell; In-vitro release

1. Introduction

The success of percutaneous therapy depends, in addition to the absorption characteristics of the drug, on its access to the skin with sufficient speed and in suitable quantities from the vehicle or dosage system employed. Once the first requirement has been met, and for classical dosage forms, it is necessary to make the correct choice of the quantity of the drug and of the components of the excipient (Barry, 1983). Of the methods used for testing, in-vitro release studies in diffusion cells have many advantages; among others, they permit the study of each of the factors that affect the process, and of the compatibility of components, both with each other and with the drug. The test conditions may easily be established and maintained, and the resulting data are derived both quickly and at low cost (Zuber and Chemtob, 1977; Wepierre and Marty, 1979). Furthermore, they are suitable for the control of the production of various batches of a preparation, and may be a useful indication in bioequivalence tests for different preparations. Due to the interest aroused by these studies, the FDA and AAPS (Skelly et al., 1987) proposed that their development should be encouraged.

As there are no general directives in the pharmacopeia regarding which cells should be employed, researchers have designed their own systems (Martin et al., 1989; Amerongen et al., 1992), although there does exist a general series of considerations which must be taken into account to guarantee the reliability of the release tests. The speed of liberation depends, among other factors, on the temperature at which the test is performed, on the type of membrane employed, and on its integrity over time and thickness. It is, moreover,

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absolutely essential for the preparation to be in direct contact with the membrane. The receptor solution must also be maintained in sink conditions with respect to the drug (Aïache et al., 1978), and it is advisable for it to be stirred at an adequate speed in order not to distort the results (Olson et al., 1969; Smith and Haigh, 1992).

This paper presents a diffusion cell of original design, intended to be used in posterior release studies of drugs using classical topical dosage forms, and also includes the methodology employed for the optimization of the stirring rate in the receptor phase and its validation.

2. Materials and methods

2.1. Release cell

This is a static diffusion cell, with a horizontal membrane, with the receptor solution being stirred in the receptor chamber (Fig. 1). It is made entirely of methacrylate except for the four stainless steel screws which, in addition to their supportive function, also make it possible to obtain a perfect match of the membrane with the equipment and to control its height. The horizontal perforation provides a means to eliminate air bubbles that might remain in the chamber–receptor liquid interface and distort the results, which is the main disadvantage of this type of cell (Friend, 1992). The apparatus is placed in a hermetically sealed truncated cone container. The distance between the cell and the base of the apparatus means that the level of the receptor phase (200 ml) remains at the same height as that of the preparation, and its composition (phosphate regulating solution, pH = 7.5) fulfils the 'sink' condition for the naproxen.

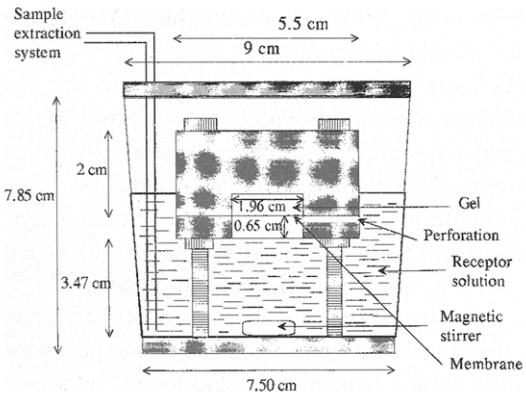

Fig. 1. Diffusion cell.

In our trial, we used six diffusion cells with identical characteristics, within a bath at a constant temperature of 25°C (SELECTA). The stirring system of the receptor phase was controlled by a tachometer (KANE-MAY Limited, accuracy ± 1 rev./min).

2.2. Sample composition and preparation

The sample under study constituted 6.75% (P/P) naproxen (Elmu, S.A.), 74.71% (P/P) dibutyl adipate (Henkel–Iberica), 8.53% (P/P) ethanol and 10% (P/P) ethylcellulose (Ethocel 10 cps, Dow Chemical). It was obtained by dissolving the naproxen at 90°C in dibutyl adipate, followed by gelling, cooling and the addition of ethanol. The drug remained in solution (Contreras et al., 1993).

The solubility of naproxen in the receptor phase ($PO_4HNa_2 \cdot 2H_2O$ = 2.05 g; PO_4H_2K = 0.28 g; distilled water c.s.p. 100 cc; pH = 7.4) at 25°C was experimentally found to be 0.74% (P/P).

A cellulose acetate (Sartorius, SM 11106) porous (pore diameter 0.45 mm) membrane was used, previously soaked for 24 h in the receptor solution. This membrane was chosen for its compatibility with the components of the gel and of the receptor phase.

2.3. Evaluation of the naproxen release

In every case, aliquots (10 ml) were taken of the receptor phase, at specified time intervals, and spectrophotometrically evaluated at a wavelength (273 nm) that was unaffected by other components of the gel that might be released together with the drug. The samples extracted were replaced with an equal volume of the receptor solution. Each test had a total duration of 5.5 h, and the cumulative amount of naxopren released per unit of surface area was established.

Table 1
Mean value, coefficient of variation (CV) and naproxen release kinetics versu time and stirring rate

Time (h)	Stirring rate (rev./min)											
	0		200		400		600		800		1000	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
0.5	0.181	16.80	1.65	9.91	1.79	1.09	1.88	2.55	1.85	2.28	2.13	9.91
1	0.3	32.21	2.69	7.09	2.9	0.22	2.98	2.12	2.88	2.98	3.16	6.94
1.5	0.51	17.36	3.57	6.76	3.78	2.59	3.89	2.13	3.82	5.38	4.13	6.41
2.5	1.23	36.32	4.92	5.68	5.15	1.11	5.34	3.89	5.31	1.46	5.49	6.12
3.5	1.79	17.37	6.12	6.44	6.36	3.23	6.68	2.98	6.4	1.17	6.69	5.28
4.5	2.19	8.68	7.14	3.31	7.4	0.98	7.48	0.48	7.74	2.49	7.92	5.59
5.5	2.78	13.66	8.08	6.59	7.87	0.5	8.71	0.68	8.57	4.39	9.00	8.90
Model of naproxen release kinetics for $t > 0.5$ h, $y = A + B \cdot t^{1/2}$												
A	−1.55						−1.08					
B	1.76						3.97					
r^2	0.93						1					
t_r (lag time)	46 min						4 min					

Mean in $g \cdot cm^{-2}$ ($n = 3$), coefficient of variation in percentage.

2.4. Selection of the number of revolutions

To decide upon the shaking rate for the receptor solution, we set up, simultaneously, six diffusion cells functioning at different speeds (0, 200, 400, 600, 800, and 1000 rev./min), at a temperature of 25°C, for sample weights of 2.1 g ± 0.3 g. The test was repeated over 3 consecutive days, with a random distribution of the apparatus.

2.5. Validation tests

Tests for repeatability, reproducibility and robustness were performed. These are all included in the U.S.P. XXII directives for the validation of analytical methods used to characterize dosage forms (Category III) (United States Pharmacopoeia, 1990).

To determine the degree of repeatability and reproducibility of the method, we simultaneously set up five diffusion cells with an identical sample quantity (2.15 g), shaking speed and temperature (25°C). The procedure was repeated six times. By analysing the results of each diffusion cell individually, the degree of repeatability was established; by comparing the results of the five diffusion cells, the reproducibility was determined.

The robustness of the process was verified by the study of small variations in the main factors that might directly affect the results. These factors were: temperature (25 and 28°C), rev./min (550 and 600), quantity of gel (2.1 and 1.9 g), extraction height of the sample from the base of the apparatus (0 and 5 cm), volume of receptor solution (200 and 210 cc), soaking time of the membranes (20 and 24 h) and operator ($n = 2$). The quantitative variation of each of the factors is based on those alterations that might inadvertently occur during the test. The trial was carried out during two consecutive days in accordance with a 2^7 factorial design developed by the statistical program Statgraphics V. 6 (1992).

3. Results and discussion

3.1. Selection of stirring rate

Table 1 shows the mean value ($n = 3$) and the coefficient of variation (CV %) of the naproxen released per unit of surface area for each of the times and stirring rates analysed. It may be seen that there is a difference between the groups that were stirred and those that were not. Within the former group, the differences are slight.

Table 2
Analysis of variance by Statagraphics V 6.0

Type	Sum of squares	*d.f.*	Mean square	*F*-ratio	Significance level
Stirring rate (rev./min) (A)	271.00205	5	54.20041	10.470	0.0000
Date test (B)	2.44251	2	1.22125	0.236	0.7903
A·B	2.90162	10	0.29016	0.056	1.0000
Residual	559.08956	108	5.17676		
Total	835.44	125			

The precision of the tests, determined by CV, depends on the number of revolutions. With no stirring, the tests are less precise; the precision increases considerably at 400 and 600 rev./min, but falls again at 1000 rev./min, although not to the levels produced at 0 rev./min. This phenomenon is probably due to the greater turbulence caused in the receptor medium, which produces alterations to the membrane–receptor solution interface that are not visually appreciable. Independently of the stirring rate, and for each test, precision generally improved with time.

The fact that a variation in the shaking speed altered the total quantity of naproxen released, though sometimes only to a slight degree, led us to perform a multifactor analysis of variance between the stirring rate, and the test date and the interaction of the latter two (Table 2). It was noted that, for $P < 0.05$, there were only significant differences in the 'number of revolutions' factor. There were no significant differences between the tests performed on different days, or in the number of revolutions–day interaction. There is only a significant difference ($P < 0.05$) between the means of the drug released at 0 rev./min and that released at any other stirring speed (Fig. 2). The residuals (with normal distribution in every case) showed that dispersion is less for intermediate rates (400 and 600 rev./min) and increased at extreme rates (0 and 1000 rev./min). In other words, the precision of the cells is greater at moderate stirring rates. Similar results were obtained on analysing the coefficient of variation.

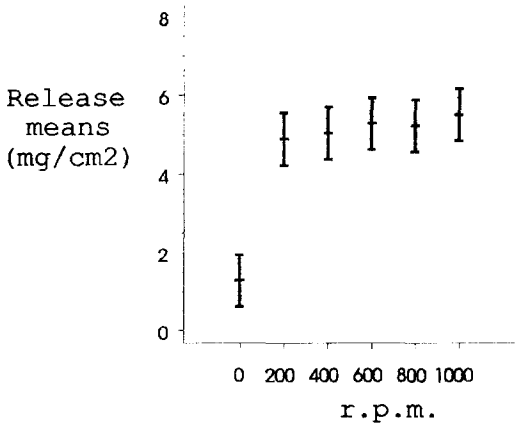

Fig. 2. Mean homogeneity analysis for naproxen released at different stirring rates.

As there were no significant differences in the quantity of solute released in the 200–1000 rev./min interval, and the precision of the tests increased with intermediate stirring rates, 600 rev./min was chosen as the optimum working rate.

The purpose of the diffusion cell is its posterior use in preformulation studies of topical forms. To determine the influence of the membrane on the release of naproxen, the results obtained were submitted to the kinetic study proposed by Higuchi (1962): linearity (influence) or absence of linearity (no influence) between the cumulative release of the drug and the square root of the time. It was found, under all conditions (both with and without stirring), that the release of naproxen fits this kinetic model for experimental times superior to 0.5 h. The chosen membrane (cellulose acetate), in addition to maintaining its integrity during the test, did not affect the release of naproxen, which was controlled by its relation to the components of the excipient.

Applying the test proposed by Snedecor and Cochran (1982) for the comparison of different straight-line regressions, to the individual kinetic equations, no significant differences were observed

($P < 0.05$) for those derived from within the 200–1000 rev./min interval. Thus they may be considered to proceed from a same population, and a common equation may be used for all of these. On the other hand, differences were observed for 0 rev./min. Its equation is distinct from the above (Table 1). When the two release kinetics are compared, it can be seen that stirring doubles the release rate and reduces the time required to establish an equilibrium between the membrane and the contiguous phases (gel and receptor liquid), quantified as the time lag. Both these effects seem to be due to the conjunction of the differences in polarity between the naproxen (lipophilic) (Contreras et al., 1993), the receptor phase (aqueous) and the type of membrane used (porous). The latter, in permanent contact with the receptor medium, impregnates and permeates the pores through which the diffusion of the naproxen is probably affected. Under these experimental conditions, when there is no stirring, the diffusion of the naproxen both through the 'membrane' and through the adjacent layer of receptor solution is slow, obstructing the free diffusion of the solute in the gel. When there is stirring, the constant renewal of the diffusion layer in the receptor phase avoids this phenomenon.

Similar behaviour has been observed by other authors (Smith and Haigh, 1992), who stressed the need to individually select the stirring speed of the receptor phase for each diffusion cell.

3.2. Validation

After selecting the stirring rate (600 rev./min), the validity of the release test was established by determining the behaviour of each of the diffusion cells used in consecutive tests (repeatability, $n = 6$) and between each other (five diffusion cells, reproducibility), together with the possible influence of small variations in some experimental factors (robustness).

The degrees of repeatability and reproducibility were quantified by the coefficient of variation (%) of the cumulative release of the drug for each of the times analysed (Figs. 3 and 4). In both cases the C.V. values were notably higher for times of less than 1 h from the start of the test, a period in which it is considered that the stable state of the release system has not yet been achieved.

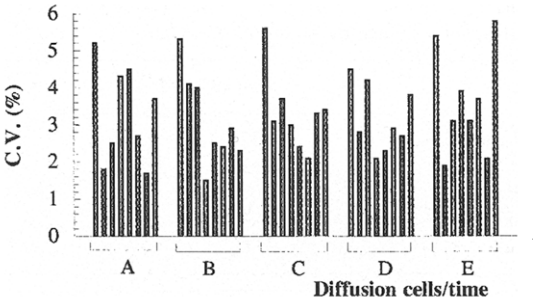

Fig. 3. Repeatability ($n = 6$) of naproxen released versus time (0.5, 1, 1.5, 2, 2.5, 3.5, 4.5, 5.5 h) for different diffusion cells.

In the stable state ($t > 0.5$ h) and in a situation of repeatability (Fig. 3), CVs were between 1.7 and 5.5%, sufficiently precise for this type of test, with a confidence interval of 94–104% ($n = 6$) (Sachs, 1978). The higher values may be due to the methodology of the test (mainly the extraction and replacement of the receptor solution), and could be improved by reducing the total number of extractions of the receptor phase or by evaluating the released solute by continuous methods.

In the stable state ($t > 0.5$ h) and in conditions of reproducibility (Fig. 4) CVs are homogeneous (3.21–4.58), with regular, reproducible behaviour of all the cells employed. These coefficients are within the interval considered to be acceptable for situations of reproducibility for analytical methods (Sachs, 1978).

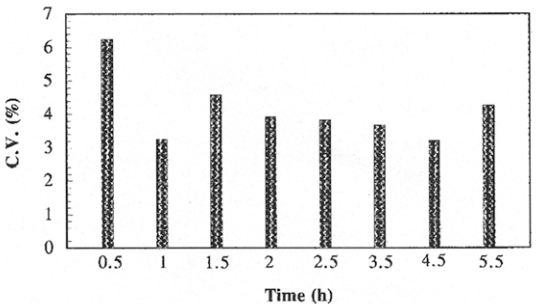

Fig. 4. Reproducibility: coefficient of variation of the naproxen released in five diffusion cells ($n = 6$) versus time.

Table 3
Analysis of variance by Statgraphics V 6.0

Type	Sum of squares	*d.f.*	Mean square	*F*-ratio	Significance level
Diffusion cell (A)	2.27644	4	0.56911	0.126	0.9729
Test (B)	0.61713	5	0.12343	0.027	0.9996
A·B	2.00496	20	0.10025	0.022	1.0000
Residual	948.30512	210	4.51574		
Total	953.2	239			

The repeatability and reproducibility of the method were confirmed by the multifactor analysis of variance of the relation between the cumulative naproxen released between the different diffusion cells and tests; there was no significant difference ($P < 0.05$) between the mean of the two parameters or of their interaction (Table 3).

The release kinetics of the solute per unit of surface area (for $t > 0.5$ h), for the different tests and apparatus, fitted the kinetics established by Higuchi (1962), with no significant differences ($P < 0.05$) between their linear equations. They are considered to derive from a same population, and are quantitatively analogous to the equation included in Table 1. Thus, it was confirmed that the release of naproxen is determined by its relation to the excipient.

3.3. Robustness

An analysis of the results obtained from the above described factorial design shows that, at 0.5 h from the start of the test, none of the studied factors (quantity of gel, operator, temperature, etc.) influences the release of naproxen. In previous tests, it has been shown that this time is too short to establish the stable state conditions for release, and that the precision of the method is reduced.

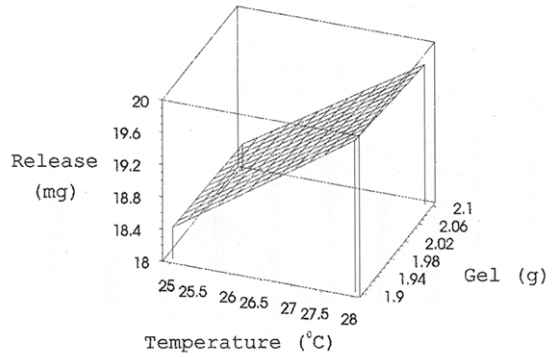

Fig. 5. Robustness: response surface of the variation of the release of naproxen versus temperature and sample quantity.

At a time of 1 h or more (in the stable state), the method is robust for all the experimental factors analysed except for that of temperature (Fig. 5); a difference of 3°C is sufficient to increase the quantity of the drug released. There are various possible causes of this increase: an increase in the solubility and diffusibility of the naproxen in the receptor solution; a reduction in the thickness of the membrane/receptor liquid interface; a reduction in the viscosity of the excipient, etc. Identification of the factor remains to be performed.

The selected stirring speed (600 rev./min) was suitable to obtain the homogenisation of the receptor phase, confirmed by the fact that its extraction level is not influenced by this rate.

From this study, we conclude that the apparatus designed and the methodology employed are acceptable for naproxen release studies with classical topical dosage forms. The method is repeatable, reproducible and robust for a stirring rate of 600 rev./min, provided that the test temperature, which is influential, is carefully controlled.

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