RESEARCH ARTICLE

A Modified Franz Diffusion Cell for Simultaneous Assessment of Drug Release and Washability of Mucoadhesive Gels

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ABSTRACT

A modified Franz cell is proposed to simultaneously measure the amount of drug diffused from semi-solid preparations into the receptor chamber and the amount washed away by a tangential buffer stream . Four gels containing acyclovir as model drug and based on hydrophilic polymers (sodium carboxymethylcellulose, methylvinyl ether/maleic anhydride copolymer, methacrylic acid/methacrylic acid methylester copolymer, and polyacrylic acid) were tested. The drug release profiles to the receptor chamber of a standard Franz cell apparatus were obtained and compared to the profiles obtained with the modified apparatus at two buffer stream rates (1.0 and 0.3 ml/min). Some significant differences were observed between the wash-away profiles obtained with the two buffer stream rates. At both flux rates the amount of drug washed away was quite high, and in turn, the drug release profiles to the receptor chamber were lower with respect to those obtained with the standard Franz cell test. The importance of this phenomenon was not the same for all of the polymers: the polyacrylic acid sample, because of the presence of slight crosslinking, was less sensitive to wash away. For all of the other samples, when 1.0 ml/min tangential stream was used, the amount of drug released to the receptor chamber was significantly lower with respect to the standard method. With 0.3 ml/min buffer stream, some significant reduction in release amounts could be observed for the methacrylic acid/methacrylic acid methylester copolymer sample only, which was also the most erodible sample. The method proposed appears suitable to differentiate the examined samples for sensitivity to the washing effect.

***KEY WORDS**: Acyclovir; In vitro release; Semi-solid dosage forms; Simultaneous release and wash-away test.*

INTRODUCTION

Semisolid dosage forms, such as hydrogels (in a broad sense, both true and pseudohydrogels), ointments, creams, and gels, which are intended for the administration to mucosal membranes, provide good patient compliance because of good feel and low irritancy. Adaptability to biological surfaces, which is related to the

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rheological properties of the formulation, allows the preparation to penetrate into the crevices of the tissue and to release the drug close to the site of action (1). For these reasons, some semisolid formulations have been proposed for oral (1–3), vaginal (4), and ocular administration (5). More examples can be found if tablets based on hydrophilic polymers are considered (1–5); also in these cases a gelified erodible layer forms at the surface of the solid formulation in contact with the biological fluids. For semisolids designed to deliver drugs locally (not protected by backing membranes) the residence time at the administration site can be difficult to predict and is usually quite short because of the wash-away effect of physiological secretions and mechanical abrasion. In the oral cavity, for example, a complex pattern of salivary flux can affect the residence time of semisolids to a different extent, depending on the site of placement and the tongue movements (6). For ocular administration, the mechanical effect of blinking must be considered together with the lacrimal drainage (5). As a consequence, not only the availability of the drug is reduced, but these losses can also lead to side effects resulting from ingestion and peroral absorption of the drug. Longer contact with the site of application can be obtained through employment of mucoadhesive polymers (3–5). These interact by chemical or physical bonds with the mucus lining the biological tissue, to produce an interface layer having higher rheomechanical strength (7–9). Because the interface layer is likely to be quite thin, the cohesion properties of the bulk formulation are important for its sensitivity to erosion or dissolution into the surrounding environment.

The amount of drug available at the site of application depends, therefore, on the release (usually diffusional) from the formulation toward the underlying tissues and on the loss (because of diffusion of the drug and erosion of the formulation) toward the external environment.

To quantify drug diffusion, a variety of in vitro diffusion cell designs have been proposed (10–13). However, a measure of the diffusion alone does not take into account the influence of the drug ''washed away'' by the physiological secretions; this can in turn affect the actual amount of drug released by the gel to the application site.

Although it clearly is not possible to reproduce in this respect the complexity of the in vivo conditions, it seems useful to develop in vitro tests that at the very early stages of the formulation development allow excipients and formulations to be compared for their sensitivity to erosion and wash away.

A rheological characterization of the consistency of the formulation can give very useful information (14). However, although it is commonly believed that the

higher the consistency, the lower the erosion–dissolution rate, this is not always true, especially when polymeric excipients having different chemical nature are compared.

The aim of the present work was to modify a Franz diffusion cell in order to simultaneously measure the amount of drug released by diffusion from a semisolid formulation to the site of application and the amount washed away by a tangential stream. Such a stream was obtained by fluxing a buffer above the sample at a predefined rate: in the present work, 1.0 ml/min and 0.3 ml/min were used. These values were chosen to check the suitability of the apparatus in discriminating between the tested formulations under different stress conditions. There was therefore no attempt to reproduce particular in vivo situations.

Four gels containing acyclovir as model drug and based on hydrophilic polymers (sodium carboxymethylcellulose [NaCMC], methylvinyl ether/maleic anhydride copolymer [MVE/MA], methacrylic acid/methacrylic acid methylester copolymer [MAA/MAAME], and polyacrylic acid [PAA]) were tested. The fraction of the drug diffused into the receptor chamber and the fraction washed away by the fluxing buffer were measured simultaneously.

EXPERIMENTAL

Materials

The following polymers were used: NaCMC high-viscosity grade (Prodotti Gianni, Milano, Italy); PAA (Carbopol® 934P, B.F. Goodrich Co., Breksville, OH); and MAA/MAAME copolymer high-viscosity grade (Eudispert®) and MVE/MA copolymer (Gantrez® AN 169, gifts from Bichema, Milan, Italy). As active principle, acyclovir powder (batch 138/93C, kindly donated by Sintopharm, Milan, Italy) was employed. The particle size analysis, effected by means of Coulter Counter Multisizer (Coulter Electronics Ltd., Luton, UK), yielded a surface-weighted mean diameter (d_{vs}) value equal to 11.0 µm.

Gel Preparation

The polymers were hydrated in pH 5.5 KH_2PO_4/Na_2HPO_4 phosphate buffer (BP1993) to obtain the following concentrations: 4.2% (w/w) for NaCMC, 4% (w/w) for PAA, 8% (w/w) for MAA/MAAME, and 8% (w/w) for MVE/MA. Different concentrations of polymers were employed in order to obtain approximately

isoviscous gels: the apparent viscosities (100 s^{-1}, 37°C) ranged between 8.4 and 9.9 Pa·sec.

PAA and MAA/MAAME gels were adjusted at pH 5.5 and 6.0 with triethanolamine, respectively. The MVE/MA gel was prepared by heating at 90°C and was adjusted at pH 5.5 with 6 N NaOH.

An exact amount of acyclovir was added in order to obtain a final drug concentration equal to 5% (w/w). *p*-Hydroxybenzoic acid methyl ester and *p*-hydroxybenzoic acid propyl ester were used as preservatives at 0.08 and 0.02% w/w concentrations, respectively.

Rheological Analysis

All rheological measurements were carried out using a Bohlin CS Rheometer (Bohlin Instruments Division, Metric Group Ltd., Cirencester, UK) connected to a personal computer for setting analysis parameters, and processing and recording data. A cone/plate combination (Cp4/20) was employed as measuring system. All measurements were performed at 37°C after a rest time of 3 min.

The viscoelastic properties of formulations were studied. A constant shear stress, chosen in the linear viscoelastic region, was applied to the sample at increasing frequency values (ranging from 0.1 to 4 Hz) and the viscoelastic parameters (storage modulus (G') and loss modulus (G'') were measured (oscillation test). Loss tangent (tgδ), which represents the ratio between the viscous (G'') and the elastic (G') properties was also calculated.

Three replicates were performed on each sample.

Standard Release Test

A standard Franz diffusion cell (FDC40020FF, Crown BioScientific, Inc., Clinton, NJ) with a 20-mm-diameter orifice (3.14 cm^2 area) was used [Fig. 1(a) and (b)]. It consists of a receptor chamber (a) (14.3 ml volume), thermostated to 37°C by means of a water jacket, and a donor chamber (b), equipped with a cover lid.

The two chambers were separated by 12,000–14,000 MW cut-off dialysis membrane, previously boiled for 15 min in distilled water and extensively washed. As receptor phase, a pH 7.0 0.1 M (KH$_2$PO$_4$/NaOH) phosphate buffer was used. The dissolved gas was removed from the receptor phase prior to testing; the receptor phase was stirred by means of a spin bar magnet during the test.

The gel (100 mg) was layered on the paper filter disk, which was then placed on the dialysis membrane. Care was taken to avoid the formation of air bubbles at the filter disk–membrane and membrane–liquid interfaces.
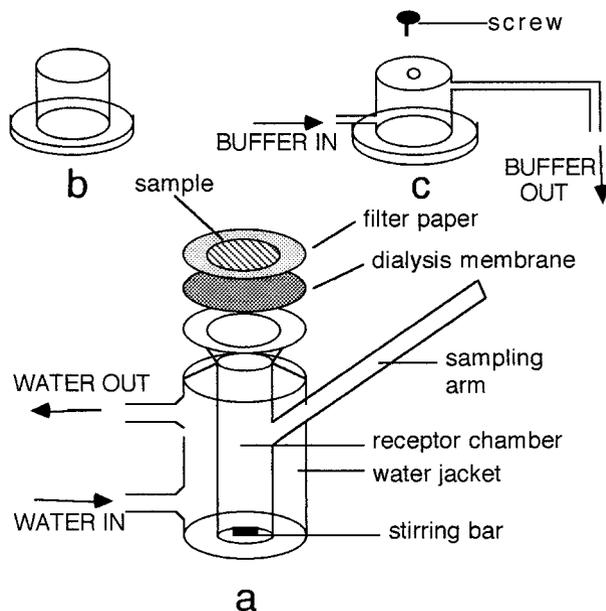
Figure 1. Schematic illustration of the Franz cell assembly in the standard [cover lid (b)] and in the modified [cover lid (c)] version.

Then the donor chamber was clamped to the receptor chamber. Every 15 min, 0.5-ml samples were taken from the middle area of the receptor phase through the sampling arm and replaced by fresh buffer. The replacement of the buffer was taken into account when the cumulative drug release was calculated.

Drug in the receptor phase was spectrophotometrically determined at 252 nm. Three replicates were performed on each sample.

Simultaneous Release and Wash-Away Test

The Franz diffusion cell was modified [Fig. 1(a) and (c)]: the donor chamber (c) was closed, with the exception of two side arms which allowed the buffer to stream over the gel layer. A hole was present in the upper part of the cover lid; this hole was closed by a screw after the air in the filling phase of the chamber was released.

The procedure employed was the same as that described for the release test with the exception that buffer, thermostated at 37°C, was fluxed over the gel at a constant rate in order to mimic the effect of physiological secretions on the formulation. The buffer was fluxed from a reservoir and was regulated by means of a floating ball flowmeter.

The buffer employed was pH 7.0 phosphate buffer at two stream rates: 1.0 ml/min and 0.3 ml/min. The buffer that flowed through the donor chamber was collected in a beaker and stirred by means of a spin bar magnet.

The drug was spectrophotometrically (λ = 252 nm) quantified both in the receptor phase and in the buffer that flowed from the donor chamber. Every 15 min, 0.5-ml samples of receptor phase were collected. At the same times, 2-ml samples of the buffer that flowed through the donor chamber were taken from the beaker. Both of the amounts were replaced by fresh buffer. The replacement of the buffer was taken into account when the cumulative drug profiles were calculated.

Drug in the receptor phase was spectrophotometrically determined at 252 nm. Three replicates were performed on each sample.

RESULTS AND DISCUSSION

Rheological Characterization of the Samples

Figure 2 illustrates the viscoelastic parameters of the tested formulations at increasing frequency values. The storage modulus G' and the loss modulus G'' are given in Fig. 2(a) and (b), respectively, whereas Fig. 2(c) gives the ratio between G'' and G', which is the tangent of the loss angle (tgδ). This parameter is often used to describe the consistency of a sample: low tgδ values indicate that elastic properties are predominant over the viscous properties (15,16).

Although the samples were approximately isoviscous, they substantially differed in their viscoelastic behavior. PAA shows the highest G' and the lowest G'' values, and therefore a very low tgδ value at all of the frequencies considered, obeying the typical behavior of a true gel with a tridimensional network. For the other three polymers, the dependence of G' and G'' on the frequency is characteristic of polymeric entangled solutions; consistency, as expressed by tgδ, follows the rank order MVE/MA < NaCMC < MAA/MAAME.

Standard Release Test

Figure 3 shows diffusion profiles of acyclovir obtained by means of a standard Franz cell. In Table 1, the drug percentages released at 60, 90, 120, and 150 min are given together with the variability, expressed both as standard deviation (SD) and as relative standard deviation (CV%). Diffusion rates appear to be inversely related
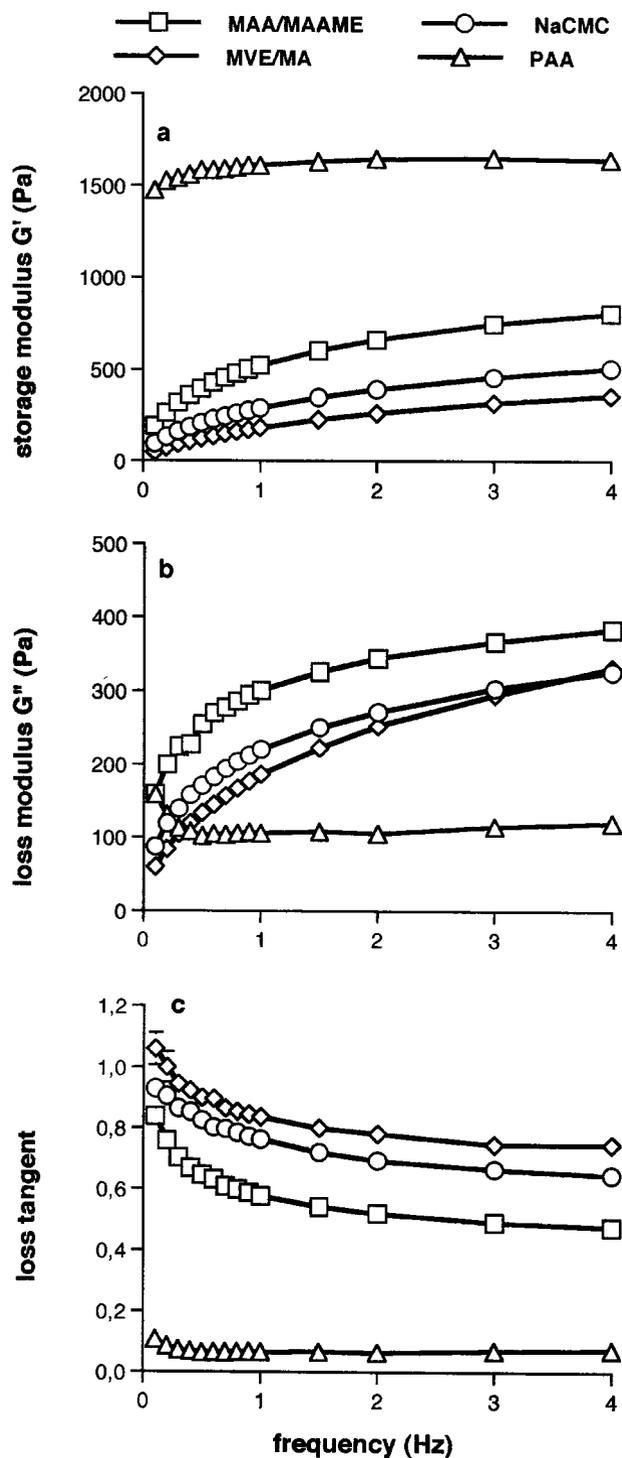
Figure 2. Rheological characterization of the samples: viscoelastic parameters.
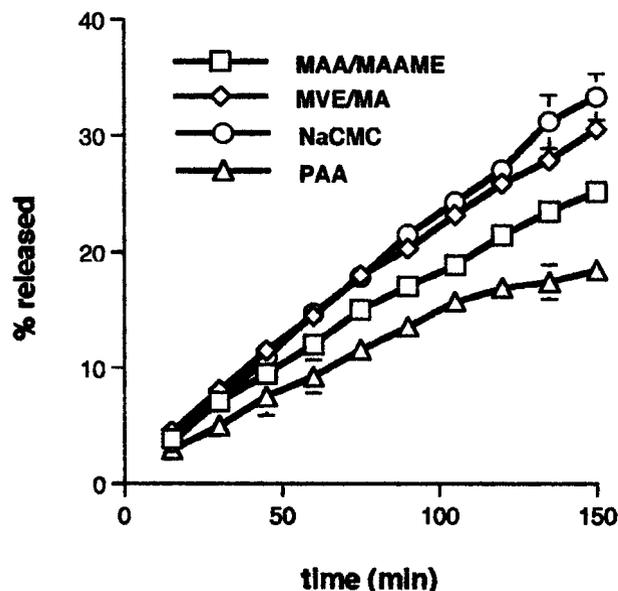
Figure 3. Release profiles of acyclovir in the standard Franz cell apparatus (mean ± SE; $n = 3$).

to sample consistency, suggesting that the internal structure described by viscoelastic parameters is to some extent relevant to drug release. Variability of results was reasonably low with respect to the variability reported in the literature for this kind of test (11,12).

Simultaneous Release and Washability Test

Figure 4 illustrates the results of the simultaneous measurement of the amount of drug washed away [Fig. 4(a)] and the amount that at the same time is released
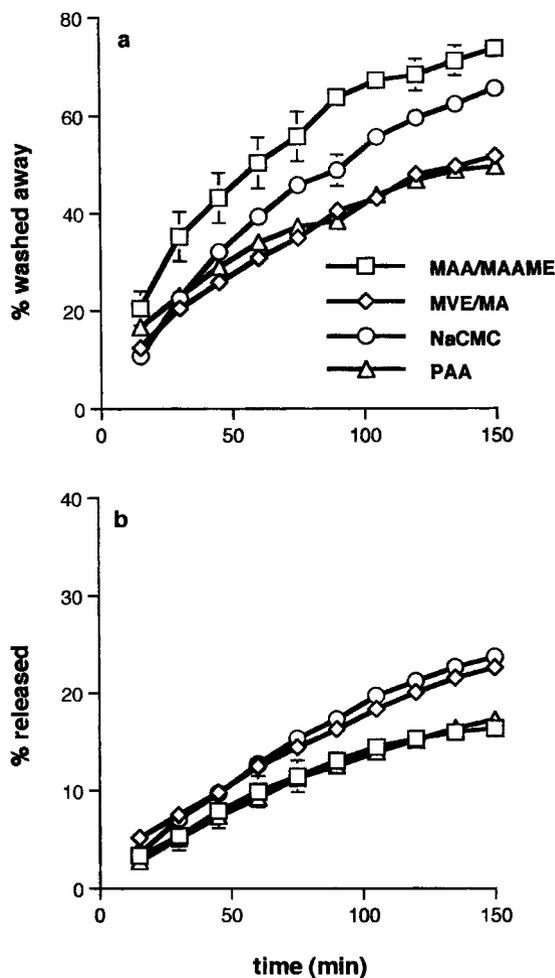
Figure 4. Percentage of drug washed away (a) and released to the receptor chamber (b) with the modified apparatus. Buffer stream 1.0 ml/min (mean ± SE; $n = 3$).

Table 1

Percentages of Drug Released in a Diffusion Test Performed with the Standard Franz Cell[a]

Formulation	Time (min)			
	60	90	120	150
PAA	9.26	13.52	16.88	18.41
	(2.42; 26.1%)	(0.91; 6.7%)	(2.08; 12.3%)	(1.57; 8.5%)
NaCMC	14.70	21.48	27.03	33.27
	(0.24; 1.6%)	(0.50; 2.3%)	(1.80; 6.7%)	(3.46; 10.4%)
MAA/MAAME	12.00	17.05	21.43	25.12
	(0.32; 2.7%)	(0.44; 2.6%)	(1.58; 7.3%)	(2.10; 8.4%)
MVE/MA	14.46	20.30	25.86	30.49
	(1.45; 10.0%)	(1.55; 7.7%)	(2.03; 7.9%)	(1.96; 6.4%)

[a] Data in parentheses are SD and CV%; $n = 3$.

Table 2

*Percentages of Drug Washed Away and Released in a Simultaneous Test
with 1.0 ml/min Buffer Stream*[a]

	Time (min)			
	60	90	120	150
Percent of drug washed away from formulation				
PAA	34.09	38.44	46.70	49.52
	(4.38; 12.8%)	(2.71; 7.1%)	(4.81; 10.3%)	(1.39; 2.8%)
NaCMC	39.34	48.81	59.54	65.51
	(1.08; 2.7%)	(5.55; 11.4%)	(1.26; 2.1%)	(1.83; 2.8%)
MAA/MAAME	50.35	63.74	68.36	73.67
	(9.04; 18.0%)	(3.74; 5.9%)	(5.66; 8.3%)	(4.26; 5.8%)
MVE/MA	30.90	40.50	47.91	51.62
	(3.08; 10.0%)	(2.59; 6.4%)	(3.19; 6.7%)	(1.02; 2.0%)
Percent of drug released from formulation				
PAA	9.28	12.58	15.19	17.30
	(0.83; 9.0%)	(1.70; 13.5%)	(1.56; 10.3%)	(1.82; 10.5%)
NaCMC	12.70[b]	17.34[b]	21.21[b]	23.69[b]
	(0.36; 2.90%)	(0.35; 2.0%)	(0.34; 1.6%)	(0.02; 0.07%)
MAA/MAAME	9.91	13.08	15.33[b]	16.39[b]
	(2.73; 27.6%)	(2.33; 17.8%)	(0.47; 3.1%)	(0.32; 2.0%)
MVE/MA	12.52	16.33[b]	20.07[b]	22.61[b]
	(0.74; 5.9%)	(0.69; 4.2%)	(0.65; 3.2%)	(1.89; 8.4%)

[a] Data in parentheses are SD and CV%; $n = 3$.
[b] Statistically different (Student's *t*-test) with respect to the corresponding values obtained by a standard Franz cell test.

to the receptor chamber [Fig. 4(b)] when a 1.0 ml/min tangential stream was used. In Tables 2 and 3, the data of percentages of drug washed away and released to the receptor chamber after 60, 90, 120, and 150 min are given when 1.0 ml/min and 0.3 ml/min tangential streams were used, respectively. The variability of the measurements, expressed both as SD and as CV% is also given.

If one examines the results of the test performed at 1.0 ml/min buffer stream, it is possible to see that the rank order of the wash-away profiles [Fig 4(a)] is not in line with the consistency of the samples, as described by means of the rheological parameters. In particular, MAA/MAAME formulation, despite a relatively high consistency, is subject to the fastest erosion. For all of the formulations considered, the amount of acyclovir washed away is quite high, also with respect to the amount diffused to the receptor chamber. This is conceivably because the amount of drug washed away is the result of two phenomena: the diffusion of the drug toward the streaming buffer and the erosion of the formulation. This

erosion occurred mainly at the interface between the formulation and the tangential stream, whereas the bulk remained in contact with the holder, as verified by visual observations during and at the end of the test. The importance of the wash-away phenomenon confirms the sensitivity of semisolid formulations to losses because of biological fluids and mechanical abrasion. However, the test showed evidence of quite strong differences in this respect among the formulations considered: the wash-away rate for MAA/MAAME is considerably higher than that for PAA and MVE/MA.

From the data shown in Table 2, it appears that in the simultaneous test at 1.0 ml/min buffer stream, the SDs of the measurements are higher for drug washed away than those for drug released. This is probably because of intrinsic variability in erosion phenomenon. The CV% values are, however, comparable to those observed for the standard Franz cell measurements (Table 1).

The wash-away phenomenon at 0.3 ml/min tangential stream (Table 3) is clearly lower than that at 1.0 ml/min, especially at the early times of the test; the rank order

Table 3

Percentages of Drug Washed Away and Released in a Simultaneous Test with 0.3 ml/min Buffer Stream[a]

	Time (min)			
	60	90	120	150
Percent of drug washed away from formulation				
PAA	21.26	29.85	36.18	41.66
	(2.94; 13.8%)	(3.74; 12.5%)	(4.30; 11.9%)	(4.59; 11.0%)
NaCMC	26.49[b]	38.93	48.40	56.06
	(5.24; 19.8%)	(6.67; 17.1%)	(7.33; 15.1%)	(7.88; 14.1%)
MAA/MAAME	28.36[b]	44.65[b]	59.32	70.46
	(5.52; 19.5%)	(6.75; 15.1%)	(4.56; 7.7%)	(5.51; 7.8%)
MVE/MA	20.27[b]	30.12[b]	37.71[b]	42.77[b]
	(2.28; 11.2%)	(1.96; 6.5%)	(2.90; 7.7%)	(1.73; 4.0%)
Percent of drug released from formulation				
PAA	8.97	12.21	15.01	17.52
	(1.49; 16.6%)	(1.26; 10.3%)	(1.46; 9.8%)	(2.49; 14.2%)
NaCMC	13.63	19.65	23.82	26.37
	(3.15; 23.1%)	(4.17; 21.2%)	(4.17; 17.5%)	(3.55; 13.4%)
MAA/MAAME	10.87	14.68	16.68	17.45[c]
	(3.31; 30.5%)	(4.97; 33.9%)	(3.81; 22.8%)	(1.33; 7.6%)
MVE/MA	13.56	17.82	23.61	28.94
	(0.11; 0.8%)	(1.01; 5.7%)	(0.88; 3.7%)	(0.45; 1.6%)

[a] Data in parentheses are SD and CV%; $n = 3$.
[b] Statistically different (Student's *t*-test) with respect to the corresponding values obtained in the simultaneous test performed at 1.0 ml/min buffer stream.
[c] Statistically different (Student's *t*-test) with respect to the corresponding values obtained by a standard Franz cell test.

between the formulations is, however, maintained. The variability is slightly higher than at 1.0 ml/min, probably because of less precise control of the low stream rate. This can be responsible, in turn, for the variability observed in the release data to the receptor chamber, especially for MAA/MAAME and NaCMC, which are the two more erodible formulations.

Sometimes, the relatively high variability in the measurements made it difficult to determine statistical differences between the results obtained under different experimental conditions. In Tables 2 and 3, the percentage values of drug released that were found to be significantly different (by means of a Student's *t*-test) from those obtained at the corresponding times in the standard test, are marked. At 1.0 ml/min (Table 2), despite variability, only PAA release shows no statistical differences in the two tests. For 0.3 ml/min flux rate, only the fastest eroding MAA/MAAME shows a statistical difference (at 150

min) with respect to the standard test. It is envisaged that a better control of the buffer stream rate (as can be provided, for example, by an HPLC pump) should improve the sensitivity and make the simultaneous test useful to illustrate the effect of low buffer streams, particularly for slowly eroding samples. In this perspective, the number of replicates also can be increased (from three to six) to reduce the uncertainty of the measurements.

Figure 5 compares the rates of release to the receptor chamber in the standard and simultaneous release tests at the two buffer stream rates.

Release rates observed in simultaneous tests were always lower than the corresponding release rates observed with the standard Franz cell, and decrease with the increase of buffer stream rate. This result is expected, considering that in the simultaneous test a relevant amount of drug is lost into the fluxing buffer. It is, however, possible to observe that the influence of the wash away is
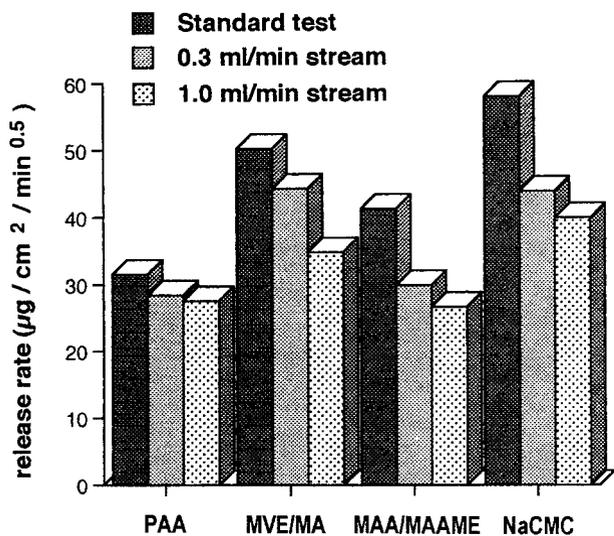
Figure 5. Comparison of the drug release rates to the receptor chamber measured in the standard Franz cell and in the modified apparatus at the two buffer stream rates.

not the same for all of the formulations: for PAA, the rate of drug release in the simultaneous test is only slightly lower than in the case of the standard test, whereas the decrease is more evident for the other formulations. This result is in line with the different nature of PAA with respect to the other samples. PAA is, in fact, slightly crosslinked, with a rheological behavior (Figure 2) close to that of a true gel with a quite rigid network; the prevalent elastic character exhibited by PAA indicates that the movement of chain segments is restricted. Irreversible slippage of the chains past one another is hindered by crosslinking, and this is reflected also in a reduced sensitivity to erosion.

The different sensitivity of the formulations to wash away, in turn, can bring about variations in the rank order of the release profiles. In the present case, for example, the gap observed between MAA/MAAME and PAA release curves disappears under the new experimental conditions, because of the high sensitivity to erosion of the MAA/MAAME sample.

CONCLUSIONS

The results obtained show that the amount of the formulation washed away under the employed experimental conditions is quite important in all the cases examined. In spite of the variability, the apparatus described showed

the different sensitivity of the considered formulations to this phenomenon. This result suggests that adequate studies in the early stages of formulation development can help in the selection of the most promising excipients. The rheological characterization gives useful information, but the direct relationship between the consistency of the samples and their erosion behavior is not immediate.

Although the apparatus proposed in this study, like any other in vitro apparatus, cannot be considered a close model of the in vivo behavior, it allows us to directly measure, in a reproducible way, a phenomenon relevant to in vivo performance such as washability of the formulation, and to determine the influence that it can have on the drug availability at the site of action.

Given the complexity of the in vivo conditions, only in vitro/in vivo correlation studies will help to make the test proposed in this study more relevant to actual in vivo behavior.

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