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Automatic multicommmutated flow system for diffusion studies of pharmaceuticals through artificial enteric membrane

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Abstract

An automatic flow procedure with spectrophotometric detection was developed for the study of pharmaceuticals diffusion through an artificial enteric membrane. The manifold comprised two independent flow pathways, gathered by a diffusion unit with two compartments and an enteric lipophilic membrane. The pathways were automatically filled with solutions simulating digestive and plasmatic conditions by means of four solenoid valves. The diffusion of pharmaceuticals from the enteric to the plasmatic compartment was performed in closed loop pathways, and was continuously monitored by a flow cell coupled to the acceptor solution pathway. The volumes of the digestive and plasmatic solutions were 6.0 and 3.6 ml, respectively, which comprised filling unit compartment, pumping tubing and connecting flow lines. Pumping flow rates of donor and acceptor solutions were maintained at 6.0 and 2.5 ml min^{-1}, respectively. The proposed system was employed in diffusion studies of caffeine and aminophylline, and in the evaluation of the influence of tensioactive agents on the diffusion process. After continuous solutions circulation for 60 min, caffeine concentration in the acceptor stream was ca. 18% of its initial concentration at the digestive compartment. The system could be programmed to perform several replicates, stopping them with different degrees of diffusion without operator assistance. The data generated by the spectrophotometer was read by the microcomputer as a time function, and stored for further mathematical treatment.

Keywords: Flow system; Multicommutation; Artificial enteric membrane; Pharmaceutical diffusion

1. Introduction

The evaluation of the bioavailability of active ingredients in pharmaceutical oral dosage forms based on the time of disintegration [1] is in many

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situations insufficient. The disintegration process does not guarantee the pharmacological activity, for which a previous dissolution of the active ingredient in the gastrointestinal fluids is required [2].

Mostly for pharmaceuticals of low aqueous solubility, studies indicate that the therapeutical activity of a formulated product is greatly dependent on the rate of dissolution of active ingredients [2,3]. In this sense, the dissolution study of oral dosage forms became a mandatory requirement for quality control in many commercial pharmaceutical preparations [4].

However, studies concerning dissolution rate still do not provide a realistic information about the therapeutic activity because not all available pharmaceuticals at the gastrointestinal tract is absorbed to the blood stream. This second stage in the development of the therapeutical activity is dependent of physicochemical characteristics of the compound and of physiological factors inherent to each person [5]. For a more realistic evaluation, in vivo experiments should be performed, but these involve humans and are very expensive and difficult to carry out in routine studies.

Thus, in vitro experiments, simulating the conditions existing in vivo have been proposed [5]. In general, a conventional system for in vitro studies comprises two compartments, one containing an aqueous solution simulating gastrointestinal medium, and the other an aqueous solution that simulates plasmatic medium [5]. The difference in concentrations in both compartments promotes diffusion of pharmaceuticals through a porous or lipidic membrane. These experiments are therefore a useful tool for conception of new pharmaceutical preparations.

Nevertheless, for recording the diffusion profile of a specific pharmaceutical through an artificial membrane, an operator must collect and analyze several aliquots of sample, at predetermined periods of time. These trials are therefore of slow execution and data is not processed within real time. Furthermore, as the pharmaceutical amount that usually diffuses through the membrane is very small, results obtained are usually of low precision.

Automatic equipment set-up with the ability to carry out all steps involved in this type of assay should be than appreciated. The only automated system found in literature is dedicated to the study of diffusion of pharmaceuticals through skin, reporting several designs of Kelder cells [6] as an alternative to the Franz diffusion cells, this last running under static conditions and requiring periodic withdraw of aliquots [6]. Even though these cells might be closest to an in vivo trial, since the receptor stream at kelder cells mimics the blood beneath the skin, the automation of typical static diffusion cells has not been yet performed, most particularly concerning enteric diffusion studies.

Among the automatic techniques that would allow achieving that purpose, flow analysis is one of the most attractive [7], associating low cost instrumentation and low reagent consumption. Furthermore, when the multicommutation approach is considered it is also possible to handle solutions automatically and to process them within real time [8,9]. In this sense, it is proposed here the establishment of an automatic flow procedure that enables both the continuous monitoring of pharmaceutical diffusion through a lipidic membrane and the automatic data acquisition and processing in real time. The system feasibility will be ascertained by performing diffusion assays for caffeine and aminophylline.

2. Experimental

2.1. Solutions and diffusing membrane

All chemicals were of analytical grade and deionized water was used throughout.

The digestive phase was simulated by a buffer solution of pH 8.0 and ionic strength (I) of 0.06 mol l^{-1}, obtained by adding 15 ml of NaH_2PO_4 solution to 200 ml of Na_2HPO_4 solution. This buffer was used as enteric phase (EP). The plasmatic phase (PP) was a buffer solution of pH 7.3 ($I = 0.05$ M), prepared by adding 150 ml of NaH_2PO_4 solution to 450 ml of Na_2HPO_4 solution.

A 1.5 mg ml^{-1} caffeine (Sigma) stock solution was prepared by weighing 0.15 g of the solid and dissolving it in 80 ml of the EP buffer solution. After complete dissolution, the volume was made up to 100 ml with the same buffer. A 1.5 mg ml^{-1} aminophylline (Sigma) stock solution was prepared as described for caffeine. The working solutions were obtained by appropriate dilution of the standard stock solution with EP buffer. A 5% (w/v) solution of Polysorbate 80™ (a tensioactive compound) was obtained by weighing the corresponding liquid, and diluting it in the same buffer. This solution was used to prepare 20 μg ml^{-1} caffeine or aminophylline solutions, doped with 0.05, 0.1 or 1% of Polysorbate 80™.

For calibration curves, a set of caffeine or aminophylline standard solutions in PP buffer with concentrations ranging 0.4 to 20 μg ml^{-1}, was also prepared. These solutions were obtained by accurate dilution of a stock solution of 1.5 mg ml^{-1}, prepared in the same buffer.

The synthetic membrane was made of a cellulose nitrate support (Sartorius, SM 15702) impregnated with lipids assigned as type N or type S_2 (Sartorius, RS). The membrane was prepared immediately before use by immerging it for 30 min into a mixture of 0.92 g of type N lipids with 4 g of type S_2 lipids, melted at 30°C. After the preparing step, the membrane was dried with absorbent paper and assembled in the diffusion unit depicted in Fig. 1.

2.2. Apparatus

The equipment set-up consisted of a 482 Fento spectrophotometer (São Paulo, Brazil) furnished with a quartz flow cell (10 mm optic path length, 100 μl inner volume). An IPC-4 Ismatec peristaltic pump with Tygon pumping tube was used to propel the solutions. The system was controlled by a 486 microcomputer equipped with a PCL7-711S Advantech (American Advantech Corp., San Jose, CA) electronic interface card and running a software written in Quick Basic 4.5. A homemade electronic interface [8] was used to drive the solenoid valves of the flow system.

The flow manifold consisted of four 161T031 NResearch (Stow, MA) three-way solenoid valves, a home-made diffusion unit (DU) made in acrylic (Fig. 1) and flow lines of polyethylene tubing (i.d. = 0.8 mm). When the lipidic membrane was assembled in the DU, the diffusion area was of 6 cm^2 and each compartment had a volume of 600 μl (Fig. 1). While experiments were carried out, the DU was maintained immersed into a NT245 Nova Técnica water bath (São Paulo, Brazil), with temperature control adjusted to 37°C ($\pm$ 1.0°C).

2.3. Procedure

The UV spectra for caffeine or aminophylline stock solutions in PP buffer with a concentration of 15 μg ml^{-1} indicated a maximum absorbency at 272 nm. At this wavelength, the linear response concentration range was determined by introducing in the system eight standards of caffeine or aminophylline prepared in PP buffer solution as C_1 carrier (Fig. 2), with concentrations ranging 0.4 to 20 μg ml^{-1}. For these experiments, only the II flow pathway of the system (Fig. 2) was

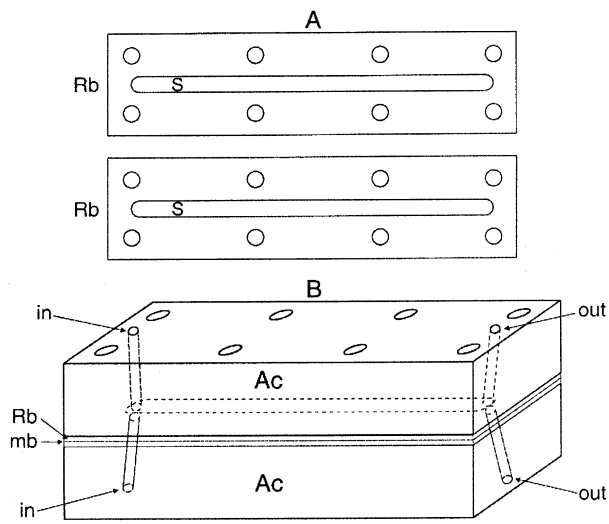

Fig. 1. Diffusion unit. Rb = rubber sheet, 1.0 mm tick; S = channel, 6 cm^2; B = overview of the diffusion unit; Ac = acrylic blocks; mb = membrane; in = solution input; out = solution output; O = lead hole. The acrylic blocks, the rubber sheets, and the membrane were attached tightly together by means of a screw through the lead hole to avoid fluid leakage.

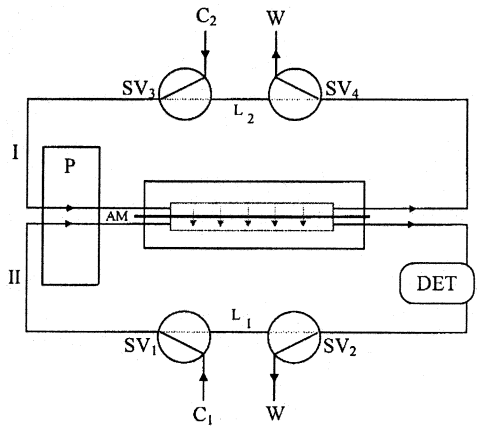

Fig. 2. Flow diagram of the system. DU = diffusion unit; P = peristaltic pump; SV_1, SV_2, SV_3 and SV_4 = three-way solenoid valves; DET = spectrophotometer at 272 nm; C_1 = PP solution; C_2 = caffeine or aminophylline solutions prepared in EP buffer; $L_1 = L_2 = 4$ cm polyethylene tubing, 0.8 mm i.d.; AM = artificial membrane; W = waste; I = donor stream circuit; and II = acceptor stream circuit. Under the selected conditions, I and II have 200 and 150 cm, respectively, of 0.8 mm i.d. polyethylene tubing.

employed, for which the DU was replaced by a 20 cm long polyethylene tube. With SV_1 and SV_2 valves switched on, standard solutions were directed towards the flow cell of the detector. That set of standard solutions was processed every day in order to obtain the analytical curve required to determine the pharmaceutical concentration at the acceptor stream.

Before performing diffusion experiments, the two flow pathways had to be filled with the required solution, which was achieved by having appropriate solutions flowing at 4 ml min^{-1} for 150 s with the solenoid vales switched on. The C_2 stream (Fig. 2) was either a caffeine or aminophylline standard solution prepared in EP buffer and flowed through the SV_3 valve, passed over the donor compartment of the DU, and was directed towards the waste (W) by the SV_4 valve. Simultaneously, the PP buffer (C_1 stream) was pumped through SV_1 valve, the acceptor compartment of the DU, and the flow cell of the detector (DET), and was further conducted to waste (W) through SV_2 valve. At this stage, conduits L_1 and L_2 connecting SV_1 to SV_2 and SV_3 to SV_4, respectively, were still to be filled. To perform this, the valves were switched off for 5 s and solutions were pumped towards the opposite direction at 4 ml min^{-1}. After restoring the pumping direction, having the solenoid valves opened for 10 s, the compartments were completely filled with the corresponding solutions, guarantying also air disposal. All changes connected to pumping direction and opening/closing of solenoid valves were computer controlled.

Diffusion through the membrane from donor to acceptor solution was investigated pumping C_2 and C_1 streams into a closed loop pathway, at which all valves were switched off. As shown in the Fig. 2, the flow cell of the spectrophotometer was coupled in the circuit of the acceptor solution, enabling a continuous measurement of analyte diffusing to this stream. The acquisition of the signal was made by the microcomputer through the analog input of the PLC711S that was connected to the analog output of the spectrophotometer by mean of a shielded cable. Experiments were programmed with a time duration of one and two hours and the signal generated was continuously read and stored as a time function for further processing. Since the analog to digital converting rate of the PCL711S interface card was very high, each datum stored was the average of 200 consecutive measurements.

3. Results and discussion

Under batch conditions, the pharmaceutical passage through the lipidic membrane is mainly governed by a passive diffusion process [3], for which after several hours the amount of compound diffused to the plasmatic solution is still very small. However, with the proposed system it is expected an increase of the usually attained concentrations, promoted by the pressure applied in the artificial enteric membrane by the donor and acceptor streams, which is a consequence of the continuous circulation of EP and PP solutions. Nevertheless, the diffusion rate through the lipidic membrane under flow conditions could still be very small to enable both to decrease the time involved and the precision of the results, for which optimization steps of the experimental con-

ditions were carried out. On the other hand, the increase of the precision also demanded a high number of readings within time, which was guaranteed by software designed to perform data acquisition during 0.1 s and waiting a time interval of 19.9 s to begin the next reading step. Under these conditions, when experiments were run for 2 h, about 340 measurements were collected. For each trial, a plot of the analytical signal given by the spectrophotometer was simultaneously displayed on the screen as time function, and stored for further analysis based on the system calibration.

3.1. System calibration

As described in the experimental section, a set of standard solutions was processed in order to obtain the analytical curve that would allow determining the pharmaceutical concentration in the acceptor solution. For sample concentrations ranging from 0.4 up to 20 μg ml^{-1}, the linear responses of caffeine and aminophylline were expressed by $Abs = 0.051C + 0.001$ and $Abs = 0.046C + 0.002$, respectively, where 'Abs' is absorbance and 'C' the concentration (μg ml^{-1}).

3.2. Flow rate optimization

The effect of the flow rate on the pharmaceutical diffusion was studied employing an EP solution with 20 μg ml^{-1} of caffeine. For pumping flow rates of 3 ml min^{-1} for both EP and PP solutions, the caffeine concentration in the PP solution after 2 h was of 4.7 μg ml^{-1}. When flow rates were both increased to 6 and 9 ml min^{-1}, the caffeine concentrations obtained in the acceptor stream were of 4.9 and 5.2 g ml^{-1}, respectively, which corresponded to an addition of 5 and 10% of the pharmaceutical diffused at the smallest flow rate. Even though the highest increase was recorded for 9 ml min^{-1}, the plotting the signals as a time function showed an irregular profile for the diffusion process. Fluid leakage through the joint of the conduits was another difficulty that could occur at that flow rate. These reasons indicated that further experiments should be performed with the flow rate of 6 ml min^{-1}.

Changing the ratio of acceptor/donor stream flow rate (pathway II/pathway I, in Fig. 2), other set of experiments was carried out. Pumping the solutions in the same direction and using ratios of 6.0/2.5 and 2.5/6.0, the caffeine concentrations in the PP solutions were of 4.4 and 5.6 g ml^{-1}, respectively. Comparing these results with that obtained when flow rates were both 6 ml min^{-1} (4.9 μg ml^{-1}), an 11% decrease in the first case and a 24% increase in the last one were observed. These results indicated that for donor and acceptor solutions pumped with different flow rates, the pharmaceutical diffusion was greatly affected by the difference of pressure exerted by the EP and PP solutions at the enteric membrane. This effect was further magnified having solutions flowing with opposite directions; when a ratio of 2.5/6.0 was pumped in reversed direction a concentration of 6.1 μg ml^{-1} of caffeine was obtained.

3.3. Effect of the solution volume

Each compartment of the diffusing unit had a volume of 600 μl and the overall volumes of the stream pathways I and II, flowing at equal flow rates (6 ml min^{-1}), were varied by changing the length of the tubes therein (Fig. 2). The results of caffeine diffusion after 2 h for volume ratios of 0.57, 1.00, and 1.28 are shown in Table 1. The highest degree of diffusion achieved (36.3%) was for the 0.57 ratio, which was a result of the higher volume of the PP solution, making it difficult to reach the equilibrium in concentrations of both streams. Nevertheless, the concentration here recorded was the smallest, due to the dilution effect. Actually, that concentration increased in 20 and 66% when 1.00 and 1.28 ratios were used. Since a high concentration value at the acceptor stream could improve the quality of detection, the ratio of 1.28 was selected, corresponding to EP and PP solution volumes of 4.6 and 3.6 ml, respectively.

3.4. Applications

Considering the results discussed in the previous sections, the following operational conditions were established: 4.6 ml of donor solution flowing

Table 1
Effect of the volume of EP and PP solutions on the amount of caffeine diffused after 2 h

Volume ratio (EP/PP, ml)	EP[a]		PP[b]		Diffusion
	Conc.[c]	μg[d]	Conc.[c]	μg[d]	(%, w/w)
2.6/4.6	20.0	52.0	4.1	18.9	36.3
3.6/3.6	20.0	72.0	4.9	17.6	24.4
4.6/3.6	20.0	92.0	6.8	24.5	26.6

[a] Values at the beginning of diffusion experiments.
[b] Values found after 2 h diffusion experiments.
[c] Concentration in μg ml^{-1}.
[d] Total amount of caffeine in the flowing stream.

at 6 ml min^{-1} (flow pathway I), and 3.6 ml of acceptor solution flowing at 2.5 ml min^{-1} (flow pathway II). Donor and acceptor solutions were pumped in opposite direction. This flow set-up was used to obtain typical diffusion profiles of both caffeine and aminophylline as well as to study the effect of excipients on the diffusion processes.

3.4.1. Diffusion of caffeine and aminophylline

The diffusion of caffeine and aminophylline solutions was studied for a C_2 stream (Fig. 2) comprising an EP solution with 20 μg ml^{-1} of pharmaceutical. One of the profiles recorded for both pharmaceuticals is shown in Fig. 3. After 1 h, the mean pharmaceutical concentrations in the PP solution were of 4.68 ± 0.17 μg ml^{-1} caffeine and 2.44 ± 0.51 μg ml^{-1} aminophylline ($n = 4$), corresponding to pharmaceutical diffusions of 18.3 and 9.5% (w/w), respectively. The diffusion rate was not evaluated, as it was not the purpose of the present work.

3.4.2. Effect of excipients

Some of excipients present in oral dosage forms can influence the pharmaceutical bioavailability, consequently affecting its therapeutic effect. In this sense, the in vitro study concerning the influence of those compounds on the pharmaceutical diffusion through artificial membranes may gather important information for the selection of a more appropriate composition of the pharmaceutical formulation.

To confirm the applicability of the proposed system in this area, some experiments were carried out to evaluate the influence of Polysorbate 80™, a tensioactive agent, on the characteristic diffusion of caffeine and aminophyline. Under certain conditions, that compound has the ability of increasing the amount of pharmaceutical absorbed to the blood stream [5]. For these experiments, solutions containing 20 μg ml^{-1} pharmaceutical were doped with 0.05, 0.50 or 1.00% (w/v) of Polysorbate 80™. Results obtained with solutions containing 0.50% of Polysorbate 80™ and circulating for 1 h are shown in the Fig. 4 The maximum pharmaceutical concentrations in PP solution were of 5.24 ± 0.43 μg ml^{-1} for caffeine and 4.28 ± 0.43 μg ml^{-1} for aminophyline. Comparing these results with those depicted Fig. 3, an increasing of 12% for caffeine and 75% for aminophyline concentrations were recorded here.

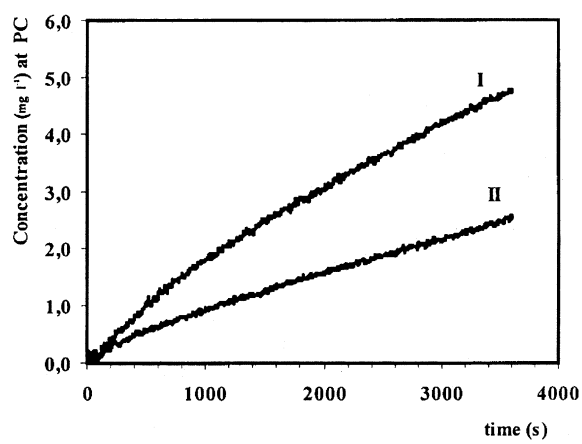

Fig. 3. Diffusion profiles of caffeine (I) and aminophylline (II) during 1-h experiments, recorded under the selected conditions.

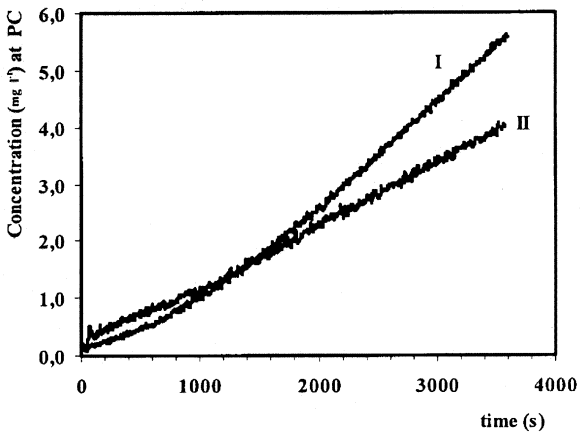

Fig. 4. Effect of 0.5% Polysorbate 80™ on the diffusion profiles of caffeine (I) and aminophylline (II).

It cannot be neglected, however, that the effect of osmotic pressure within the membrane may also contribute to this increase. It is interesting to state that the characteristic profile of caffeine diffusion (Fig. 3) was altered in the presence of that excipient, at that particular concentration (Fig. 4). This effect would probably be difficult to state by the manual procedure. With the 0.05% Polysorbate 80™ solution, no significant difference in the results was observed for both pharmaceuticals, which was a result of a concentration of excipient lower to its critical micelle value. With a 1.0% Polysorbate 80™ concentration, results presented a high variability, which was probably related to some 'disorders' at the lipidic membrane caused by a high concentration value of excipient.

4. Conclusions

The established system was able to perform automatically in vitro diffusion profiles of pharmaceuticals, thus significantly reducing the workload time. This was made possible because the flow network, based in the multicommutation concept, was controlled by software specifically designed for this purpose.

The results obtained performing experiments with caffeine and aminophyline were more precise than those usually achieved by employing manual procedure and results were recorded in real time, thus suggesting the feasibility of the system to carry out in vitro assay.

The large number of measurements accumulated for each experiment (ca, 340) can ease studies concerning both kinetics of pharmaceutical diffusion through artificial membranes and influence of excipients in the diffusion process.

Furthermore, each experiment was carried out using 4.6 and 3.6 ml of EP and PP solutions, respectively, resulting in a low volume of effluent to be discarded.

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