Study of the Fosfosal Controlled Permeation through Glutaraldehyde Crosslinked Chitosan Membranes

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Abstract. Fosfosal, a phosphate derivative of salicylic acid, which presents both analgesic and anti-inflammatory properties, was used as a model drug to study the potential of recently developed chitosan membranes (with different crosslinking degrees) to be used as drug release rate-controlling membranes. The fosfosal permeation across these membranes was studied using an in-house built developed diffusion cell with online automatic monitoring. Experiments were performed using phosphate buffer saline (PBS) solution at 37ºC. Different flow properties of the detection set up were determined in order to estimate the errors introduced by the automatic online monitoring system. For increasing crosslinking degrees the permeability initially decreased, and then increased, likely as a consequence of the crosslinking influence on a variety of properties like crystallinity and hydrophilicity that have opposite influence on permeability. In summary, it was possible to control the drug release profile by means of changing the degree of crosslinking of chitosan membranes and to follow the respective release kinetics by means of using the developed diffusion cell.

Introduction Chitosan is the *N*-deacetylated derivative of chitin, which is the second most abundant natural polymer. The amine groups of this polysaccharide can react with glutaraldehyde, if two Schiff bases are formed between each crosslinker bi-functional molecule and two different polymer chains [1, 2]. In this way, by means of controlling the crosslink density it should be possible to control the drug release properties of polymeric devices. On the other hand, crystallinity decreases as the crosslinking degree increases [2], which can play an opposite effect on the permeation of drug molecules. Moreover, the overall chemical character of the materials should be also affected by the amount of the more hydrophobic crosslinker moieties, likely changing the water uptake capability and the interactions between the polymeric matrix and studied drug. Chitosan has been proposed for several biomedical applications, ranging from implantable materials such as guided bone regeneration (GBR) membranes [3,4] and skin tissue engineering [5,6], to external devices like transdermal drug delivery patches [7-9]. In this way, and referring to the controlled drug release applications, chitosan membranes can be both used as rate-controlling membranes and as matrix/monolith release systems. Furthermore, if using chitosan membranes for GBR or skin tissue engineering applications, the release of growth factors or other bioactive agents can be a desirable feature in such type of formulations. In any case, the knowledge of the membranes permeability physical constants, which can be studied using a diffusion cell, is essential to define strategies both for drug loading or to attain the desired drug release profile.

Many attempts have been made to modify classic Franz type diffusion cells in order to automatically monitor the released drug [10-12]. Among several configurations that have been tried, many of those make use of a peripheral detection technique, which is accessed by circulating the drug receiving solution within an external pumping system and through the respective detector.

In fact, the use of this type of external detection methods facilitates the use of temperature control (by simply immersing the diffusion cell on a thermostatic bath) and simultaneously to register other properties values like pH or conductivity, since one is not limited to the space available inside the detector working area. In addiction, this type of experimental set up is very flexible, since if different detection methods are required for different drugs, the same diffusion cell is readily available and results are easily comparable.
However, depicting the amount of studies that made use of external sensors [10,11], the potential error introduced by the external measuring circuit has often been neglected. In this work, a diffusion cell was built and a validation method was used together with mathematical criteria to evaluate the error introduced by the monitoring system. This inaccuracy can be due to the obvious measuring delay, but can also result from the dispersion effects resulting from the flowing profile throughout the external detection circuit.
In this work, fosfosal, an anionic model drug, has been used to study the chitosan membranes resistance to small molecular weight water soluble molecules. The release experiments were undertaken at pH 7.4, when chitosan is expected to be essentially neutral, given that the pH value is higher than the intrinsic pKa, 6.0, of chitosan [13]. The interactions between the anionic drug and the protonated crosslinked polymer at lower pH will be addressed elsewhere.

Materials and Methods

Membranes preparation. Chitosan with a *N*-deacetylation degree of 65.0% was purchased from Sigma-Aldrich and purified prior to use by means of dissolving in an acetic acid (AcOH) solution, followed by filtration of the insoluble fraction, re-precipitation with sodium hydroxide (NaOH), washing with ethanol and drying at 50ºC. Purified chitosan powder was dissolve in 1% wt. AcOH aqueous solution and glutaraldehyde was added at a ratio of 1% and 10% (mol) (or none) related to the chitosan amine groups. The solutions were carefully stirred in order to avoid the formation of any air bubble, poured on Petri Dishes (5 mg of chitosan/cm^2) and dried at room temperature in a dust free environment. The obtained chitosan membranes were immersed in a 0.1M NaOH solution, washed thoroughly with distilled water, hold in a frame and dipped in ethanol. The obtained membranes presented fairly smooth surface and homogeneous thickness, without the typical ripples derived from the material shrinking during consecutive drying processes.

The Diffusion cell with online monitoring. The diffusion cell comprises (see Fig. 1): a donor compartment (c); a receptor compartment (d) that is separated from the previous compartment by the permeable membrane (a); and a magnetic stirrer (b). The diffusion cell was immersed in a thermostatic bath at 37ºC. The drug diffusion is detected by means of UV spectroscopy (UV-1610 Shimadzu). Besides the spectrophotometer, a peristaltic pump, the respective inlet and outlet tubes, which are attached to a quartz cell, complete the detection system. The drug accumulates on the receptor container, since the reception solution returns to the respective diffusion cell compartment. The absorbance was recorded at 279 nm and its relationship with the fosfosal concentration was determined performing a calibration curve using 7 standards with different concentrations.

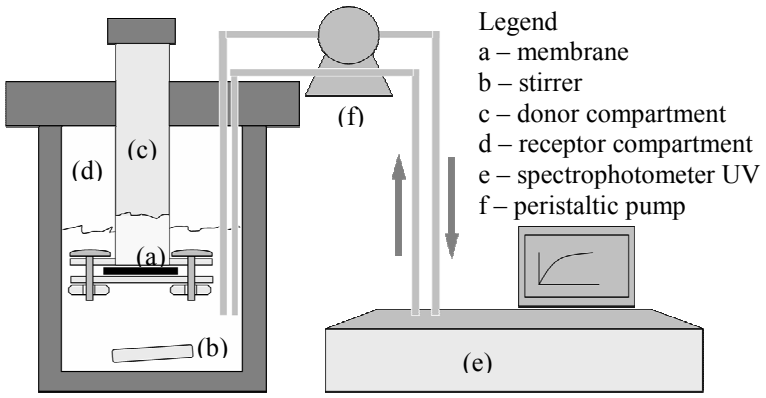

Fig 1. Schematic representation of the in-house built diffusion cell and of the respective automatic detection system.

The online monitoring validation. When analysing the cumulative drug release curves, one should take into consideration that the drug detection is not immediate. In fact, considering that the receptor compartment is perfectly stirred, the average time that a concentration variation step will ideally take to be detected (average residence time) is the ratio between the flow rate and the detection system volume. Moreover, when the concentration step takes place, the interface travels through the tube inlet till the light beam where this higher concentration is detected. However, during this journey, it is expected that the concentration values dispersion will occur due to both the drug diffusion at this different concentration interface and resulting from liquid mixture. In order to assess the detection system effect on the drug release curves, a concentration step was simulated by removing the diffusion cell and pumping a phosphate buffer saline (PBS) solution (blank) through the detector. The peristaltic pump was set at 20 rpm. At a certain time (t = 0 s), the blank solution was substituted by another PBS solution with a known concentration (C_0) of drug (5 and 200 mg/l) and the absorbance was recorded. This procedure simulates what takes place on the receptor compartment in the real experiment, using the same experimental conditions, but without any membrane between the donor and receptor compartments. The concentration ($C(t)$) was determined in function of the time. The detection set up (namely, tubes diameter, length, spectroscopy detection cell, etc) was kept constant throughout the experiments. The cumulative distribution of residence times ($F(t)$) was calculated as the normalized concentration in function of time, according to the equation 1. The residence times distribution ($E(t)$) was achieved by differentiating the previous function according to equation 2. The average residence time (τ) was determined using the equation 3.

$$F(t) = [C(t)/C_0]_{step} \quad (1)$$

$$E(t) = \mathrm{d}[F(t)] / \mathrm{d}t \sim \Delta C/(\Delta t \mathrm{x} C_0) \quad (2)$$

$$\tau = \int_0^\infty t \cdot E(t)dt \sim \frac{1}{c_0}\sum_0^\infty t.\Delta C \quad (3)$$

where, ΔC is the concentration variation between two different measures, which are recorded each Δt. On the other hand, the time for a step response of 95% ($\Delta t^{95\%}$) was determined using the following equations:

$$\int_0^{t_0} E(t)dt = 0.025 \text{ and } \int_0^{t_1} E(t)dt = 0.975 \quad (4)$$

where ($\Delta t^{95\%}$) = t_1-t_0 is the time required to distinguish 95% of a concentration increase of C_0 (note that $\Delta t^{95\%}$ is independent of C_0). One is now capable of defining the criteria that ensures that the detection system does not distort the real drug permeation data. First, the time lag (t_{lag}), which is the time required for the drug to appear on the receptor compartment, should be much higher than the average residence time (equation 5). On the other hand, the time considered to determine the changes within time periods (Δt), such as [$\Delta C/\Delta t$], should be high enough when compared with $\Delta t^{95\%}$. This means that the sensor should be much faster in reading two different concentrations, than the time required for these concentrations variation to take place within the diffusion cell (equation 6).

$$t_{lag} >> \tau \quad (5)$$

$$\Delta t >> \Delta t^{95\%} \quad (6)$$

Fosfosal permeation experiments. The membrane was first placed and sealed between the diffusion cell compartments. The receptor compartment was filled with 30 ml of PBS solution and the membrane was visually checked for the presence of air bubbles, which were removed if needed. The temperature was left to equilibrate at 37ºC and the receiving PBS solution was pumped through the detection system, keeping the same detection set up as studied before. At time t = 0 s, 1 ml of 5 mg/ml of fosfosal solution was added to the donor compartment and absorbance values at 279 nm

were started to be recorded each 1 second. The donor cell was sealed in order to avoid water evaporation.

Results and Discussion

The online monitoring validation. A typical detector normalized response to a concentration step of C_0 is shown in fig. 2a. The distribution of the residence times determined by differentiating the former curve is shown in fig 2b. From these results, the residence time (τ) and the time required to distinguish 95% of a concentration increase ($\Delta t^{95\%}$) were found to be 27 s and 28 s, respectively.

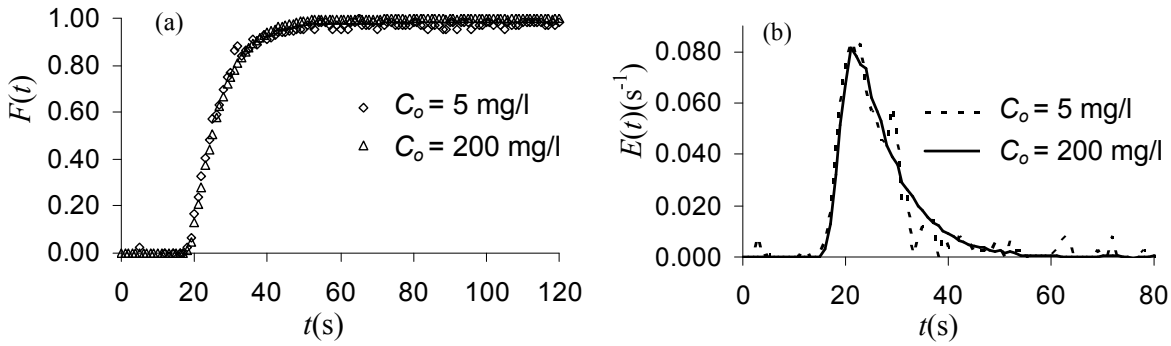

Fig. 2. Cumulative distribution $F(t)$ (a) and distribution $E(t)$ (b) of the residence times inside the detection system.

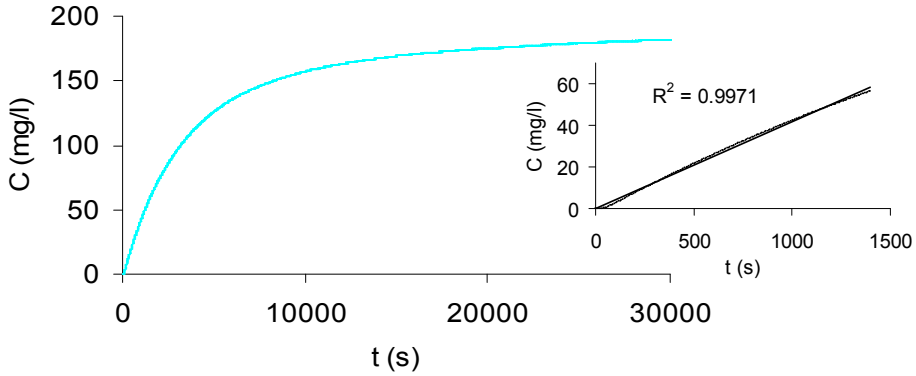

Fig. 3. Typical curve of the fosfosal permeation through a non-crosslinked chitosan membrane (the inset graphic is a magnification for short times, showing the linearity achieved at the early stage of the permeation experiment that was long enough to be considered valid).

Fosfosal permeation experiments. A typical permeation curve for the studied drug and membranes is shown in fig. 3. The permeability was estimated by means of determining the slope of the permeation curves by linear regression at the release early stage $(dC/dt)_{rc}$. Simultaneously, the time interval used to determine the accumulating concentration rate was high enough to agree with the validation criterion defined by equation 6, but still the linear correlation factor was superior than 0.99, what means that the sink conditions are maintained. The permeability, P, was then calculated as follows:

$$\frac{V_{RC}}{A}\left(\frac{dC}{dt}\right)_{RC} = P \times \frac{C_{DC}}{l} \qquad (7)$$

where V_{rc} is the receptor compartment solution volume, A is the membrane useful mass transfer area, l the membrane thickness and C_{dc} is the initial drug concentration at the donor compartment. The boundary layers resistance was not considered. The lag time was not used in the calculations, since it did not pass the first validation criteria (equation 5). Table 1 shows the permeability obtained in these preliminary results. It can be seen that the membrane properties can be highly dependent upon the crosslinking degree, which will also change the crystallinity degree of the material. Therefore it may be concluded that, crosslinking chitosan membranes with glutaraldehyde is a suitable method to tailor the fosfosal permeability and, therefore, it should be also adequate to

control the release of low molecular weight bioactive agents.

Table 1. Permeability of fosfosal through chitosan membranes presenting different degrees of crosslinking

glutaraldehyde to chitosan amine groups ratio	permeability [cm^2/s] ± standard deviation of three different experiments
0 %	$(7.75 \pm 0.78) \times 10^{-07}$ (*)
1 %	$(2.90 \pm 0.66) \times 10^{-07}$ (*) (**)
10 %	$(6.38 \pm 0.35) \times 10^{-07}$ (**)

(*)(**) statistically different ($p<0.005$)

Conclusions

A new rather useful device was developed to monitor at real time the concentration of a model drug, fosfosal, which permeates through chitosan based membranes. Special attention was paid to consider the diffusion of the drug in the flow that could smear the measurements of relevant parameters, such as permeability. In this context, the residence time, as well as the time required to detect 95% of a drug concentration variation step, were calculated, and two criteria were defined to ensure accurate measurements. The proposed experimental procedure was successfully validated by means of preliminary measurements of the diffusion of fosfosal throughout chitosan membranes with different crosslinking degrees. It was concluded that this chitosan modification could have a strong influence on the permeability of the anionic model drug.

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