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Ciprofloxacin Release from Polymeric Films. Modeling and Pharmaceutical Parameters Determination †

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Abstract: Ciprofloxacin (Cipro) is a broad-spectrum antibiotic used against both Gram (+) and Gram (−) bacteria. Its biological half-life is very short (4–5 h) and its conventional administration forms present a limited absorption efficiency. For this reason, the aim of this work was to study other administration strategies based on topical films. Sodium alginate (SA), a naturally occurring polymer, and a recombinant elastin-like polymer (rELP) produced by advanced genetic engineering techniques were evaluated as potential carrier systems. The films were obtained by the casting technique, adding the Cipro by direct dispersion in the polymer solution using 16.6% *w*/*w* rELP or 1.5% *w*/*w* SA. The in vitro release assays were performed at 37 °C in physiological solution and with orbital shaking at 90 rpm. Cipro concentration was determined by ultraviolet (UV) spectrophotometry at 276 nm. The release profiles were analyzed and adjusted using the Lumped model developed and validated by our research group. Pharmaceutical interest parameters were calculated and compared for both polymer-Cipro systems: the time required to reach 80% of the drug dissolved ($t_{80\%}$), the dissolution efficiency (*DE*) and the mean dissolution time (*MDT*). The SA-Cipro platform released the 80% of the drug in 35 min, while this parameter was 209 min for the rELP-Cipro system. The $MDT_{80\%}$ was 8.9 and 53 min for the SA-Cipro and rELP-Cipro, respectively, while the *DE*, evaluated at 200 min, was 66.6 and 58.8 for each platform, respectively. These parameter values demonstrate that the rELP films were able to modulate the drug release rate and for the SA ones, release can be considered immediate. Therefore, both systems are promising strategies for the topical application of Cipro.

Keywords: ciprofloxacin; topical films; sodium alginate; recombinant elastin-like polymer; mathematical modeling

1. Introduction

Ciprofloxacin (Cipro) is a pale yellow crystalline powder. It responds to the chemical structure of 1-cyclopropyl-6-fluoro-1,4-dihydro 4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid (Figure 1) which belongs to the family of quinolones. The central structural unit is a quinolone ring with a fluorine atom in position 6, a piperazine group in position 7, a cyclopropyl ring in position 1 and a carboxyl group in position 3 [1].

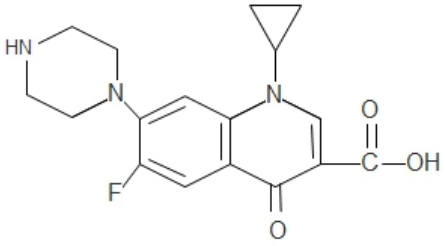

Figure 1. Molecule of Ciprofloxacin.

The Cipro molecule has amphoteric characteristics due to the presence of the carboxyl and amino groups (Figure 2). It is well known that Cipro can form complexes with certain multivalent cations, according to the degree of ionization and the prevalence of certain chemical variants present in the aqueous solution [2]. The antibacterial effects of Cipro are due to the inhibition of bacterial topoisomerase IV and DNA gyrase, preventing the replication and transcription of bacterial DNA [3].

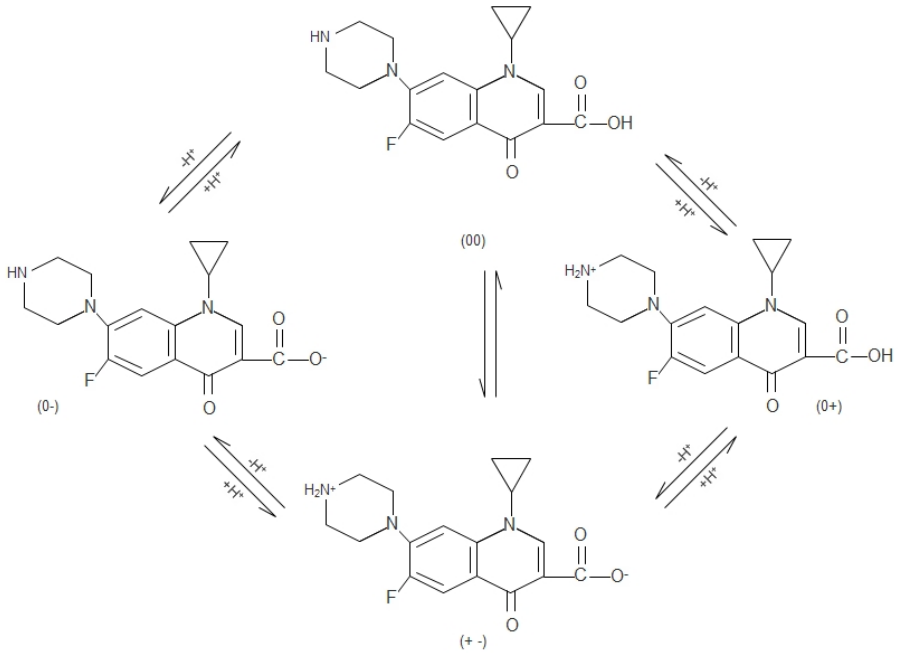

Figure 2. Ionization equilibria of Ciprofloxacin molecule.

Cipro belongs to the group of synthetic fluoroquinolone antibiotics with broad antimicrobial activity. It is commonly used for infections of the urinary tract, intestinal, among others, but it has a very short biological half-life of approximately 4 to 5 h [4]. On the other hand, the limited absorption efficiency of the drug in conventional form prompted the development of new delivery systems. Among them, we can mention the transdermal systems which have numerous advantages, such as their application in a specific site, painless application, less frequent replacement, and greater dosage flexibility.

Among the materials used to prepare these systems, polymers stand out, particularly those that are biodegradable, biocompatible and from natural sources such as chitosan, sodium alginate (SA) and cellulose, among others. SA is an anionic polysaccharide, derived mainly from brown algae and bacteria. This polymer represents an outstanding class of materials for its biocompatibility, biodegradability, low toxicity and low relative cost [5]. On the other hand, rELPs are self-gelling, biodegradable, and biocompatible polymers, tailored designed for different applications in human medicine. This material was synthesized from recombinant elastin-type protein polymers (rELPs) [6]. rELPs are a class of protein polymers that have promising applications in the fields of biomedicine and nano-biotechnology. These are synthesized by recombinant DNA technology produced by fermentation of *Escherichia coli* and purified by thermo-dependent reversible segregation cycles. This polymer is capable of self-assembling and forming nanoparticles at low concentrations and hydrogels at high concentrations with a very strong and irreversible stability. The molecular mass of this polymer is 101.12 kDa [7].

The aim of this work was to evaluate the feasibility of developing films based on rELP and SA for the controlled release of Cipro. With this framework, films loaded with Cipro were prepared and characterized by evaluating drug release profiles. The data obtained were analyzed using the "Lumped" model, which allowed determining the initial release rate and parameters of pharmaceutical relevance, such as the mean dissolution time (*MDT*), the time to release 80% of the drug ($t_{80\%}$) and dissolution efficiency (*DE*) [8,9] to compare the different formulations developed.

2. Experiments

2.1. Materials

The rELP was designed and synthesized by the BIOFORGE group—University of Valladolid (Spain), SA was purchased from Tododroga (Córdoba, Argentina) and the Cipro hydrochloride from Parafarm® (Buenos Aires, Argentina).

2.2. Cuantification of Ciprofloxacin

The Cipro concentration was determined by UV spectrophotometry (JENWAY) at 276 nm. These tests were performed in triplicate.

2.3. rELP Films Preparation

To obtain the films, 10 g of aqueous solutions were prepared with concentrations of 16.6% *w*/*w* of rELP, to which 10 mg of Cipro was added. They were subsequently subjected to 0 °C for 10 min to ensure complete dissolution of the polymer. These solutions were placed in glass plates covered with non-stick material and placed in an oven at 37 °C for 24 h.

2.4. Sodium Alginate Films Preparation

For these films, 0.45 g of SA and 100 mg of Cipro were weighed, and 30 mL of distilled water was added. This solution was homogenized under magnetic stirring (150 rpm) at room temperature and then poured into Petri dishes and oven-dried at 37 °C for 24 h. Finally, crosslinking was carried out for 7 min in a 0.2 M calcium chloride solution.

2.5. In Vitro Drug Release Tests

To carry out the release tests, 1 × 1 cm^2 samples were cut from the different films and their weights and thicknesses were determined (Table 1). The samples were carefully placed in test tubes containing 3 mL of physiological solution, used as a release medium, at 37 °C and with orbital shaking at 90 rpm. At pre-established time intervals, samples were taken by complete removal of the release medium, replacing the same volume with fresh medium to re-establish the maximum driving force at each sampling point. The

Cipro concentration was quantified for each sample. The tests were carried out in triplicate.

Table 1. Thicknesses and weights of the film samples used in the drug release tests.

Polymer	**rELP**			**SA**		
Thickness (μm)	342	465	261	47	44	59
Weights (g)	0.0244	0.0234	0.0199	0.0033	0.0039	0.0052

2.6. Data Analysis

The data obtained from the release tests were analyzed using a second-order kinetic model, called the Lumped model, developed and validated by our research group [8,9]. To compare the release profiles, the experimental data were adjusted using the Polymath 6.0 program and parameters of pharmaceutical relevance were calculated: the MDT, the time to release 80% of the drug ($t_{80\%}$) and the DE. Furthermore, the model makes it possible to determine the initial release rate (a).

3. Results and Discussion

In Vitro Drug Release Tests

In vitro drug release tests are used to characterize the release of a drug from a certain pharmaceutical form, the dissolution test being the most relevant. According to the United States Pharmacopeia (USP), drug dissolution and release tests are required for dosage forms in which absorption of the drug is necessary for the product to exert the desired therapeutic effect [10].

A central goal in the modified release of the drugs is to establish the desired release kinetics of a given drug for a specific application.

The experimental data obtained from the Cipro release tests of the 2 systems are shown in Figure 3.

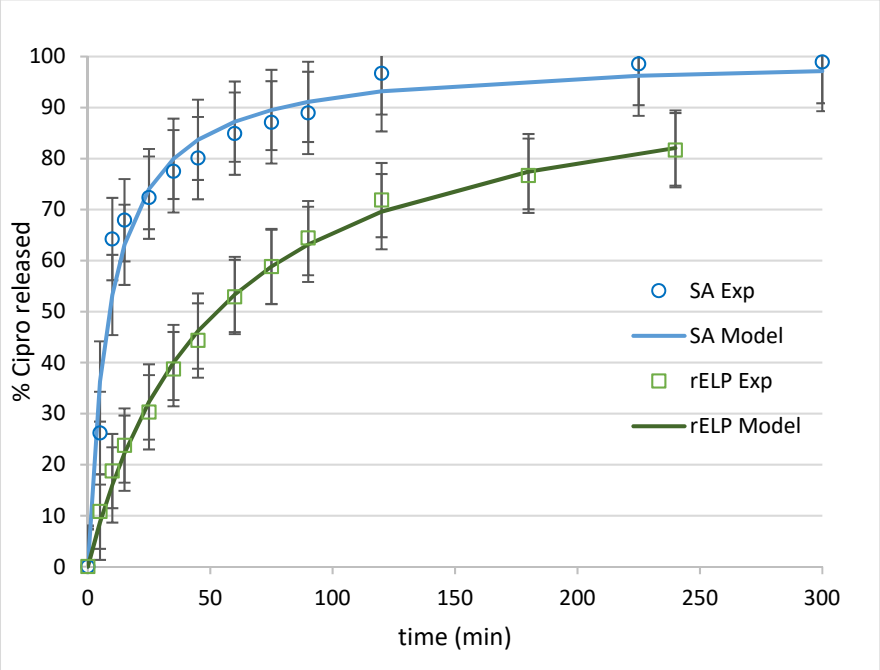

Figure 3. Cipro release profiles from rELP and SA films. The solid line represents the fit made using the Lumped mathematical model.

In the platforms developed, the drug is distributed homogeneously in a continuous matrix formed by the polymeric network that controls the release rate, forming what is called a monolithic system. In this type of devices, release is usually controlled by diffusion through the monolith matrix material or through aqueous pores, and an initial burst of drug release from the surface is often observed. Over time, the rate of drug release

decreases as the distance of drug diffusing to the surface increases. This can be seen in Figure 3, where a high initial rate of drug release from both polymer platforms is observed due to the transfer phenomenon that occurs as a consequence of the presence of drug on the surface of the films. Then, a moving front of solvent advances through the polymeric film, allowing the Cipro to diffuse to the surface face so that it is available for dissolution in the release medium. As the distance between the surface and the advance of the moving front increases with time, the release rate decreases.

The object of any mathematical model is: (i) to be able to accurately represent the processes associated with drug release, (ii) to be able to describe/summarize experimental data with parametric equations or moments, and (iii) to predict processes under varying conditions. However, when describing the processes involved, some models developed often suffer from being too complex to be useful in practice. Under these premises, the mathematical model used to adjust the experimental values was the Lumped model developed by our research group in pharmaceutical technology. (Equation (1)). It considers the grouped effect of diffusion within the film and the transfer to the physiological solution.

$$M_t(\%) = \frac{a \times t}{[1 + b \times t]} \quad (1)$$

where $M_t(\%)$ is the cumulative percentage amount of drug released at the moment t. Equation parameters a (%/min) and b (min^{-1}) can be obtained graphically. However, the best procedure to fit the model with the experimental data is through a nonlinear regression analysis using the values a and b found graphically as a first approximation. Nonlinear regression analysis was performed using Polymath 6.0 program. The model adjusted well to the experimental values for the 2 polymers evaluated (Figure 3).

The model also allows us to determine the initial dissolution rate, since the rate at any time will be:

$$\frac{dM\%}{dt} = \frac{a}{(1 + b \times t)^2} \quad (2)$$

Therefore, when $t = 0$, the initial rate of dissolution will be:

$$\left.\frac{dM\%}{dt}\right|_{t=0} = a \quad (3)$$

Table 2 shows the values of the parameters a and b of the equation, as well as other parameters of pharmaceutical relevance, such as $t_{80\%}$, DE and MDT and $M\infty$, which are calculated from the following equations:

$$t_{80\%} = \frac{80}{a - b \times 80} \quad (4)$$

$$DE = \left(\frac{a}{b^2}\right)\frac{[b \times t_F - ln(1 + b \times t_F)]}{t_F} \quad (5)$$

$$MDT_{80\%} = \frac{a}{b^2}\,\frac{\left[ln(1 + b \times t_{80\%}) - \frac{b \times t_{80\%}}{1 + b \times t_{80\%}}\right]}{M\%(t_{80\%})} \quad (6)$$

Table 2. Lumped model parameters and parameters $t_{80\%}$, MDT, and DE for the release curves of films with different polymer.

	Model Lumped Parameters			Pharmaceutical Relevant Parameters		
Platform Polymer	**a ± sd ***	**b ± sd ***	**M∞**	**$t_{80\%}$ (min)**	**$MDT_{80\%}$ (min)**	**DE_{200min} (%)**
SA	11.396 ± 3.245	0.114 ± 0.0375	0.2709	35.10	8.88	66.63
rELP	1.907 ± 0.165	0.019 ± 0.002	0.5605	209.76	53.06	58.79

* sd: standard deviation.

It is observed that SA systems have an initial rate of about 6 times higher than that of rELP systems. Furthermore, the $t_{80\%}$ parameter, which is the time required to reach the dissolution of 80% of the drug available for dissolution, is 35 min for the SA films. The pharmacopeia states that if this parameter is lower than 45 min, the release can be considered immediate [11], as in the case of SA platforms. In a system that modulates the dissolution of a drug, it is desirable a $t_{80\%}$ high value, as is the case with the rELP films, which would indicate a delay or control in the release process. The *DE* is defined as the area under the dissolution profile up to a certain time (t_F), expressed as the percentage of the area of the rectangle described by 100% dissolution at the same time. These *DE* values are very close for both systems.

Finally, the *MDT* value is a widely used pharmaceutical parameter to characterize the release rate of drugs from a specific dosage form that provides information about the ability to delay the release of the active ingredient from the polymer platform. A high *MDT* value indicates a greater ability to delay release. In this case, the behavior of $MDT_{80\%}$ is correlated with that presented by $t_{80\%}$, showing a value about 6 times higher for rELP systems than for SA ones.

It is known that for topical products, understanding of safety and efficacy is based on the release of the active drug from its dosage form to the surface of the skin. Once the drug is in contact with the surface of the skin, it penetrates through the stratum corneum to achieve its pharmacological action. For this reason, the determination of the release rate through in vitro studies is a relevant parameter to control the quality of a topical product, in the same way that dissolution tests are important for solid dosage forms administered via the oral route.

4. Conclusions

Platforms based on rELP polymer have a greater ability to release the drug gradually, while the systems based on SA would be useful for topical applications where rapid delivery of the drug is needed, providing high concentrations for a short time. Therefore, it can be concluded that it is possible to modulate the release rate of Cipro through the films developed based on both polymers, with characteristics suitable for different applications. Consequently, both systems are promising strategies for the topical application of Cipro.

Finally, managing the ability to modulate drug release through various polymeric platforms can reduce the variability of the performance of pharmaceuticals. This last aspect is increasingly important given the current emphasis on "quality by design" by regulatory agencies such as the FDA.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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