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Films based on the biopolymer poly(3-hydroxybutyrate) as platforms for the controlled release of dexamethasone



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ABSTRACT

Controlled drug delivery aims to achieve an effective drug concentration in the action site for a desired period of time, while minimizing side effects. In this contribution, biodegradable poly(3-hydroxybutyrate) films were evaluated as a reservoir platform for dexamethasone controlled release. These systems were morphological and physicochemically characterized. *In vitro* release assays were performed for five different percentages of drug in the films and data were fitted by a mathematical model developed and validated by our research group. When the profiles were normalized, a single curve properly fitted all the experimental data. Using this unique curve, the dissolution efficiency (*DE*), the time to release a given amount of drug ($t_{x\%}$), and the mean dissolution time were calculated. Furthermore, the dissolution rate, the initial dissolution rate (*a%*) and the intrinsic dissolution rate were determined. The *a%* mean value was $1.968 \times 10^{-2}\%$ released/min, $t_{80\%}$ was about 14 days, and the *DE* was 59.6% at 14 days and 66.5% at 20 days. After 2 days, when approximately 40% of the drug was released, the dissolution rate decreased about 60% respect to the initial value. The poly(3-hydroxybutyrate) platforms behaved as an appropriate system to release and control the dexamethasone delivery, suggesting that they could be an alternative to improve drug therapy.

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1. Introduction

One of the major challenges of pharmacotherapy is the attainment of an effective drug concentration at the intended site of action for a desired period of time. Controlled drug delivery systems aim to achieve the efficient administration of a drug while minimizing its systemic and/or local side effect for a successful treatment (Anselmo and Mitragotri, 2014). These systems provide other advantages, since they reduce the frequency of administration, increase patient compliance, and may add commercial value to marketed drugs by extending patent protection.

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The usefulness of polymers in drug delivery systems has been well established (Sosnik et al., 2014; Gandhi et al., 2015). Research on polymeric materials has played a vital role in the development of controlled release technologies. Especially, in the area of pharmaceutical applications, the intense interest in the development of new drug delivery systems has driven the research of polymeric materials. The advances in drug release systems also serve to solve another great challenge for pharmaceutical researchers, since they allow to deliver poorly soluble drugs (Sharma, 2016; Simonazzi et al., 2018). In this regard, one of the possible strategies is the use of films as platforms to modulate the release rate of the therapeutic agent towards the site of action (Luo and Wang, 2014; Tartara et al., 2014). Different systems or devices can be designed for this purpose (Pundir et al., 2017). The simplest consists of an inert membrane that encapsulates the drug to be released. The membrane controls the drug diffusion from the reservoir to achieve the required release rate. Another type of controlled diffusion device is a monolithic system, in which the agent to be released is uniformly incorporated into the polymeric matrix controlling the release rate.

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Currently, there is a growing trend in the use of biodegradable polymers for the design and preparation of these systems. Biodegradable polymers can be defined as polymers that are degradable *in vivo*, either enzymatically or non-enzymatically, to produce biocompatible or nontoxic by-products. These polymers can be metabolized and excreted via normal physiological pathways.

Among the biodegradable polymers used to prepare these systems, those belonging to the polyhydroxyalkanoate family stand out, being also biocompatible. They are linear polyesters that have proven to be excellent candidates for medical and pharmaceutical applications (Lizarraga-Valderrama et al., 2016; Koller, 2018). In particular, a member of this family called poly(3-hydroxybutyrate) (PHB) is synthesized by bacterial fermentation (Vieyra et al., 2018) and, unlike other members of the polyhydroxyalkanoate family such as polyhydroxyvalerate and polyhydroxyhexanoate, is produced in large amounts by bacteria of different genus (El-Hadi et al., 2002). It presents several characteristics that made it widely studied for medical purposes, both in human and veterinary applications (Chen et al., 2018; Sabarinathan et al., 2018).

In the present contribution, technological research is focused on the design of platforms for the controlled release of dexamethasone (DX), a synthetic corticosteroid with mainly glucocorticoid activity, using biodegradable polymeric films. Dexamethasone (9-fluoro-16-methyl-substituted hydrocortisone) is almost insoluble in water, barely soluble in alcohol, in acetone, in dioxan, and in methyl alcohol. It is slightly soluble in chloroform and very slightly soluble in ether. When applied topically, particularly to large areas, corticosteroids may be absorbed in sufficient quantity to cause systemic effects. Its biological half-life in plasma is around 190 min and its binding to plasma proteins is about 77%, less than most other corticosteroids. On the other hand, it presents a high anti-inflammatory activity, being 0.75 mg of DX equivalent to about 5 mg of prednisolone (Sweetman, 2009; Cholkar et al., 2013; Abaya et al., 2018). It has been used when corticosteroid therapy is recommended, either in the form of free alcohol or in one of the esterified forms. It is commonly indicated for ophthalmic disorders or for topical application in the treatment of various skin disorders. DX concentration is usually 0.05 to 0.1% in eye or ear drops and ointments (Torkildsen et al., 2011; Awwad et al., 2017; Pepose et al., 2018) and 0.1% in topical skin preparations (Dammeier et al., 1998; Mukherjee et al., 2005; Sweetman, 2009). DX anti-inflammatory properties have also been explored for bioresorbable systems in vascular applications for stenosis prevention (Zilberman, 2005).

The purpose of this research was to design and characterize DX loaded PHB films, and to evaluate the DX release from these matrices. Kinetic and transport phenomena involved in the release of the drug were considered by the “lumped model” developed and validated by our research group (Fernández-Colino et al., 2016; Romero et al., 2016; Romero et al., 2017). Pharmaceutical parameters were calculated and the influence of different DX loads on the release profiles was evaluated through similarity and difference factors.

2. Materials and methods

2.1. Materials

PHB, as powder with a purity of 99.5%, a moisture content below 0.3%, and a molecular weight of 524,000 g/mol approximately, was kindly provided by BIOCYCLE®, PHB Industrial S.A. (Brazil). DX was purchased from Todo Droga (Argentina) and chloroform was from Biopack® (Argentina). All chemicals were of analytical grade and used without further purification.

2.2. Films preparation

PHB was dissolved at 6 %w/v in chloroform at 60 °C for 4 h under reflux and 15 ml of the solution were casted in glass Petri dishes (90 mm diameter). Solvent evaporation was completed after 24 h at room temperature. To load the DX, the drug was added to the polymeric solution by direct dispersion at a w/w ratio of 6% (DX1), 17% (DX2), 21% (DX3), 25% (DX4), and 29% (DX5) with regard to PHB weight, stirring constantly at room temperature, following the same casting procedure mentioned. Films' thicknesses were measured using an Electronic Outside Micrometer 0–1", 54–850-001 (Fowler, China).

2.3. Physicochemical characterizations

2.3.1. Differential scanning calorimetry (DSC)

DSC thermograms were obtained using a DSC TA Q200, TA Instruments (Delaware, USA). Samples were accurately weighed into hermetic aluminum pans and heated from 25 °C to 300 °C at a heating rate of 10 °C/min, under nitrogen atmosphere (20 ml/min). Melting temperatures of DX, DX5 loaded PHB film and physical mixture of both components (50/50 %w/w) were determined.

2.3.2. Scanning electron microscopy (SEM)

Cross section images of PHB and DX5 loaded PHB films were taken by SEM (JEOL JSM-6480 LV, Tokyo, Japan) in LASEM laboratory (UNSa – CONICET). The preparation of the samples included their fracture in liquid nitrogen to avoid deformations, and subsequent metallization by gold deposition (Denton Vacuum LLC Desk IV Sputter, Tokyo, Japan). Images of gold metallized DX powder were also taken.

2.3.3. Fourier transform infrared spectroscopy (FTIR)

Spectrum data were recorded on a GX FTIR Perkin Elmer Spectrometer (Shelton, USA). Transmission spectra of PHB and DX powders were obtained from the samples diluted with KBr. PHB and DX5 loaded PHB films were casted over silicon wafers, whose bands do not interfere in the analysis, to perform IR by transmission.

2.4. *In vitro* drug release assays

Release profiles were obtained from *in vitro* assays using DX loaded PHB films ($1 \times 1 \text{ cm}^2$), with the 5 different drug loads, immersed in 3 ml of normal saline (NS) as release medium in glass vessels, at 37 °C and under continuous stirring in an orbital shaker at 90 rpm, for 41 days. At pre-set time intervals, the entire release medium was removed and replaced by an equal volume of fresh medium. The amount of DX released was determined by UV-visible spectrophotometry (JENWAY 7315 spectrophotometer, Bibby Scientific, Staffordshire, UK) at 244 nm, using the corresponding calibration curves. All assays were performed by triplicate. In order to check any polymer interference, a complete UV scan (200 to 400 nm) of the release medium in contact with PHB membranes without drug was performed, and no absorption was observed.

The proposed and validated mathematical model used (Fernández-Colino et al., 2016; Romero et al., 2017) follows a lumped second-order kinetics. It describes satisfactorily processes where diffusion and transfer phenomena are present, or when there is only an external transfer into a fluid medium where the drug concentration increases steadily. The equations and assumptions made were already described and they will be retaken in the results section, including other parameters relevant to the analysis.

To compare the release profiles, the similarity and difference factors were determined, according to the model independent statistical analysis methods (Costa and Lobo, 2001). Different

parameters of pharmaceutical relevance, such as the initial dissolution rate, dissolution efficiency, mean dissolution time, among others, were also calculated.

2.5. Data analysis

Dissolution assays were performed by triplicate and data are presented as the mean \pm the standard deviation (*s*). Statistical analysis was performed using Polymath 6.0 software. For statistical comparisons, a *p*-value less than 0.05 ($p < 0.05$) was considered significant.

3. Results and discussion

3.1. Physicochemical characterizations

Films' average thickness was $174 \pm 35 \mu\text{m}$. SEM images of DX powder (Fig. 1a) show that it presented a variable granulometry and structure. PHB film (Fig. 1b) was dense (non-porous), characteristic that remained after DX incorporation (Fig. 1c). The drug was homogeneously distributed throughout the film thickness. DX particles were observed in the film, suggesting that its content was above the solubility limit in the polymer. This information is relevant for the assumptions made and the boundary conditions assumed in the proposed release model.

DSC of DX, PHB film without the drug, PHB-DX powder physical mixture and DX loaded PHB film was used to determine the thermal behavior of the samples. PHB film melted at 174°C , which is consistent with the temperature reported by Pradhan et al. (2017), while DX powder melted at 262°C (Fig. 2). When both powder components were physically mixed, these values remained approximately unchanged, but when the DX was incorporated in the PHB matrix, its melting peak disappeared, probably due to the high polymer proportion, despite the fact that the film with the highest drug load was analyzed.

DX, PHB and DX5 loaded PHB films FTIR spectra in the wavelengths of 3800 to 3100 cm^{-1} and 1700 to 1500 cm^{-1} are shown in Fig. 3. This zone was selected since the characteristic peaks of DX are evident in these wavelength ranges, without overlapping with those of PHB. The complete PHB spectrum was described in a previous paper (Romero et al., 2016). In the DX spectrum, there were four sharp and well resolved bands. The peak at 3473 cm^{-1} corresponds to O–H stretching vibration, the one at 1662 cm^{-1} is assigned to carbonyl-stretching bands ($\nu \text{ C=O}$), and less intensive bands at 1618 cm^{-1} and 1604 cm^{-1} correspond to the stretching vibration of C=C. The FTIR spectrum of the DX loaded PHB film showed the characteristics bands of DX, suggesting that the chemical structure of DX did not undergo changes (without shifting or disappearance of peaks) when it was introduced into the PHB film.

This behavior is completely desirable since the drug must remain unchanged in the release platform. The presence of chloroform, used to dissolve the polymer during the film preparation, was not detected (with a characteristic peak in 760 cm^{-1} - not shown in the figure), indicating that this would not produce an incompatibility for a possible medical application.

3.2. In vitro drug release experiments

Dissolution tests are critical for evaluating the performance of a product and they should be simple, reliable and reproducible. The *in vitro* assays can serve not only as a quality control specification for the manufacturing process but also as an indicator of *in vivo* product performance. In this context, dissolution tests of the films with the five different drug loads were performed during 41 days. The experimental data of the cumulative amount of DX released (M_t , mg) vs. time are shown in Fig. 4.

When designing controlled release systems, it is important to understand the particular mechanism involved in the release process. Often, more than one mechanism is involved at a given time or different mechanisms may predominate at different stages of the drug delivery process. Once the variables affecting the drug release from the platform are identified, it is possible to modulate the release rate. The DX loaded PHB system is a monolithic dispersion, where a fraction of the DX is dissolved in the PHB matrix and the remainder is dispersed in the form of particles (not dissolved drug). In the monolithic system, the matrix acts not only as a storage medium but also as a diffusion mediator. At the beginning, particles at the surface dissolve quickly, leading to a burst release. Then, drug release occurs by dissolution followed by diffusion through the matrix. While the dissolved drug diffuses through the matrix, it can be replaced by dissolution of neighboring solid drug, when available. Particles further inside dissolve more slowly, since dissolution rate depends on diffusion through the matrix. During the process a mobile front is observed, separating the central core containing solid drug from the periphery that contains the completely dissolved drug (Fernández-Colino et al., 2016). Since the diffusion distance from the core to the surface increases with time, the advance of this mobile front slows down as the release process proceeds and the release rate decreases.

The mathematical model used to fit the experimental values (Eq. (1)) considers both the lumped effect of the diffusion inside 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 amount of drug released at time t , and a (mg/min) and b (min^{-1}) are parameters of the model. This model was validated by comparing it with Higuchi equation, Ritger & Peppas model, Peppas-Sahlin equation, Siepmann & Peppas model, first

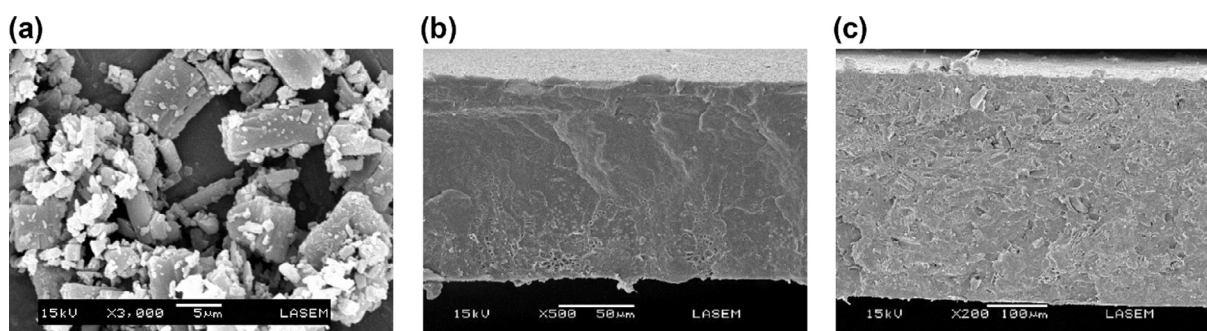


Fig. 1. SEM images of: (a) DX powder, (b) PHB film cross section, and (c) DX 5 loaded PHB film cross section.

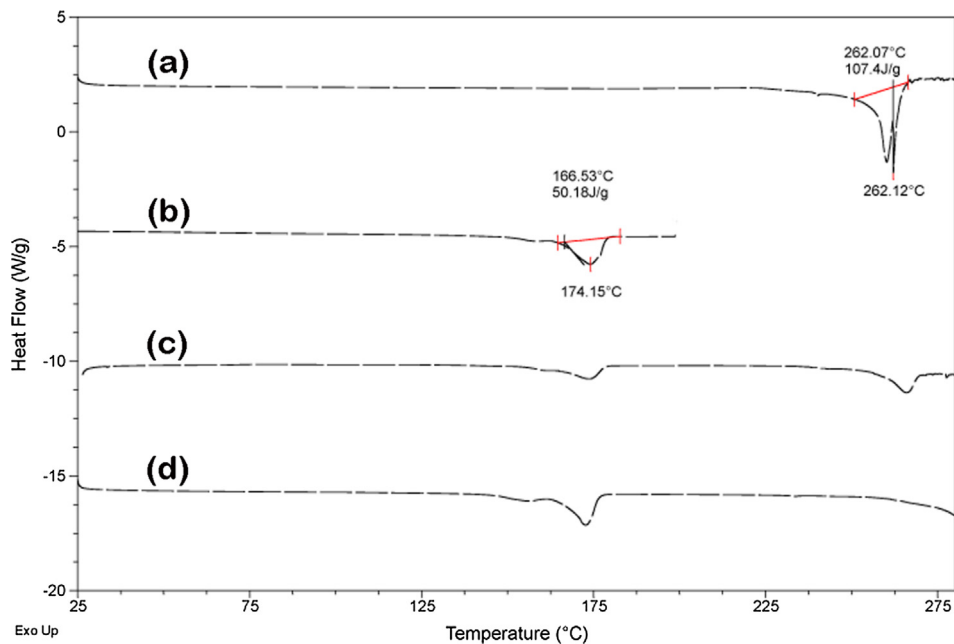


Fig. 2. DSC of (a) DX, (b) PHB polymer, (c) PHB-DX (50%w/w) physical mixture, and (d) DX5 loaded PHB film.

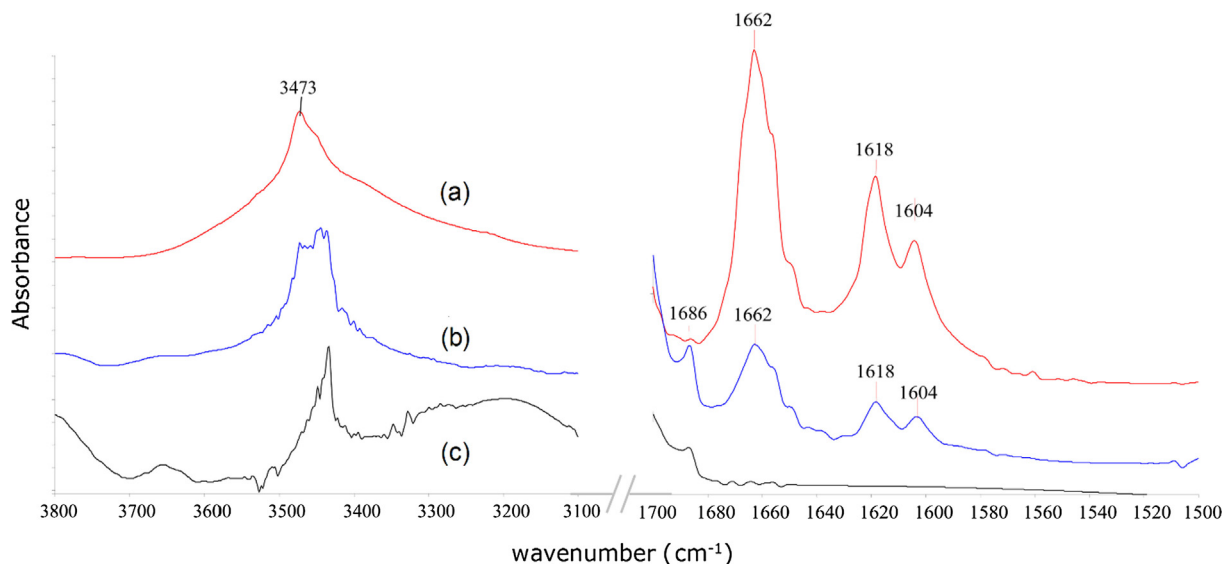


Fig. 3. FTIR spectra of: (a) DX, (b) DX5 loaded PHB film, and (c) PHB polymer.

order kinetic equation, Lecompte-Siepmann-Walther equation and Siepmann-Siegel-Rathbone model (Romero et al., 2017).

The model fitted properly the experimental data (Fig. 4), and the values of the parameters a and b , the correlation coefficient (R^2), and the standard deviation ($s\%$) for the five different drug loaded films are shown in Table 1, as well as M_∞ (mg), which is the maximum amount of drug available in the film to be released. The M_∞ values were determined from Eq. (1) at $t \rightarrow \infty$.

This model also allows to calculate the dissolution rate (DR) (mg/min) (Eq. (2)):

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

Initial DR is obtained from Eq. (2) when $t = 0$ (Eq. (3)) and it results to be the model parameter a :

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

By normalizing the experimental data of the DX loaded PHB films with respect to M_∞ , the release profiles of the percentages of drug released as a function of time ($M_t\%$) are obtained (Fig. 5), and Eq. (1) results in the Eq. (4). The $s\%$ for each curve was approximately 6%.

$$M_t\% = \frac{a\% \times t}{(1 + b\% \times t)} \quad (4)$$

Interestingly, the normalized profiles obtained for all the films with the different drug loads were almost the same and all the data could be fitted by a unique curve with $s\%$ of 10% (Fig. 5). The mean values of the normalized parameters $a\%$ and $b\%$ were 1.968×10^{-2}

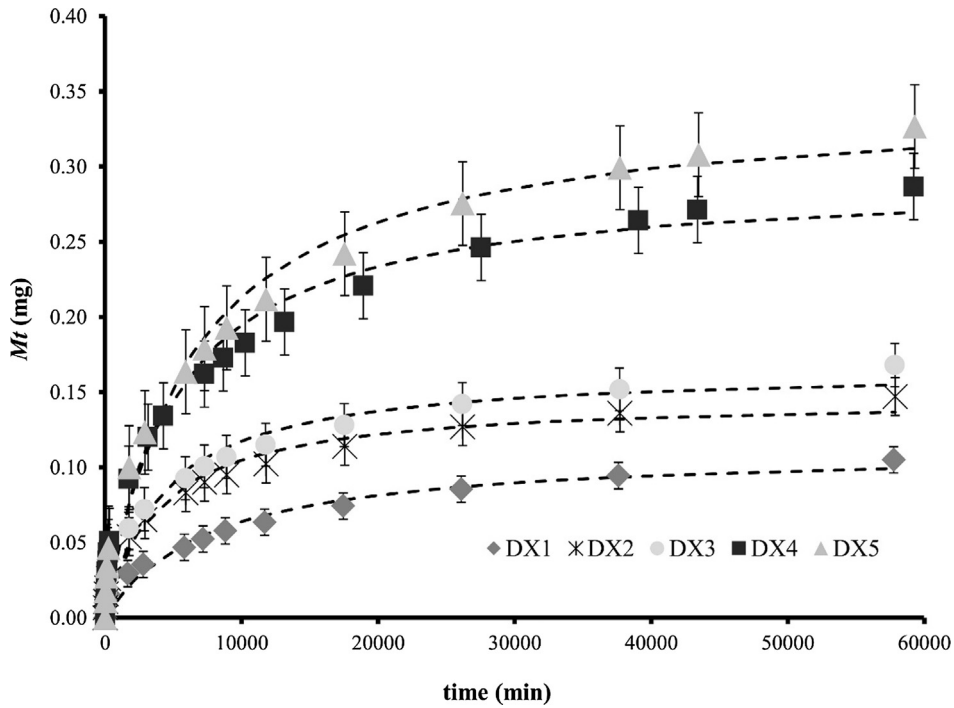


Fig. 4. Cumulative amount of DX released (M_t) from DX loaded PHB films vs. time. Symbols are the mean value of the experimental data and dotted lines represent the theoretical release predictions with the non-linear regression adjustment.

Table 1
Model parameters, correlation coefficients, standard deviations and M_∞ .

DX loaded PHB film	a (mg/min) $\times 10^5$	b (min ⁻¹) $\times 10^4$	R^2	s%	M_∞ (mg)
DX1	1.48	1.31	0.962	0.659	0.112
DX2	3.69	2.53	0.968	0.891	0.146
DX3	4.00	2.41	0.973	0.939	0.166
DX4	5.79	1.98	0.970	1.717	0.293
DX5	5.56	1.61	0.980	1.629	0.345

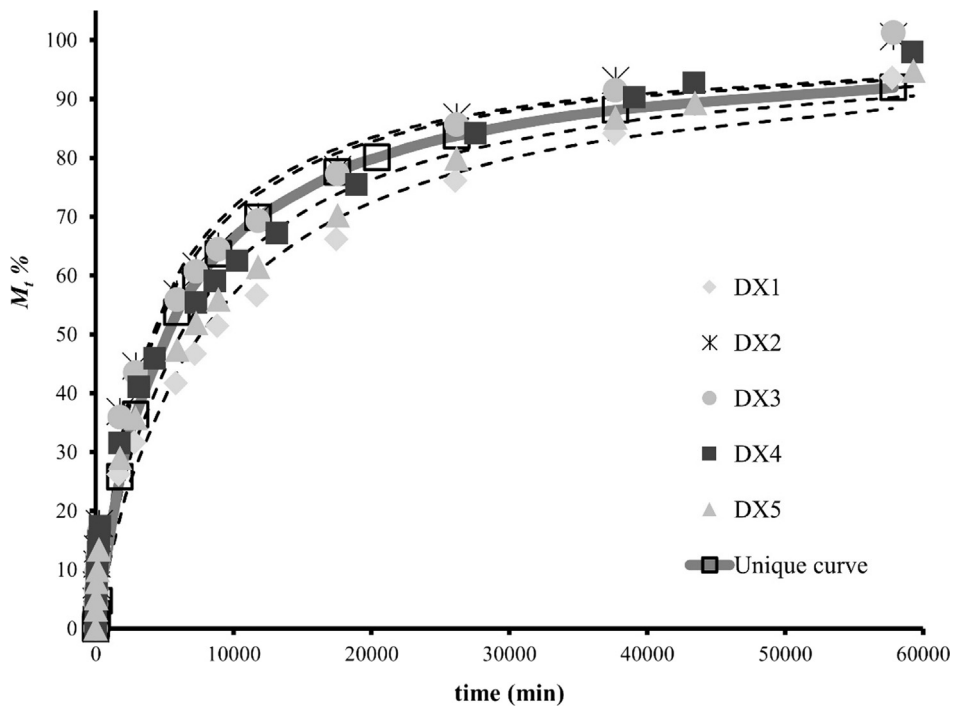


Fig. 5. Percentage of DX released ($M_t\%$) vs. time. Symbols are the mean value of the experimental data and dotted lines represent the theoretical release predictions with the nonlinear regression adjustment. The unique curve adjusting all the data is represented by the thick line.

(%/min) and 1.968×10^{-4} (min^{-1}), respectively, for a 95% confidence level. The independence of $M_t\%$ with respect to the DX load in the PHB films means that neither the solubility nor the dissolution rate of the drug in NS medium are affected by the different loads of DX in PHB.

Among the model independent methods, the pair-wise procedures include the difference (f_1) and similarity (f_2) factors, adopted by the Center for Drug Evaluation and Research of the Food and Drug Administration (FDA) and by the European Medicines Agency (EMA) to compare release profiles. The first describes the relative percent error between two dissolution curves in the whole time range studied, and is defined in Eq. (5).

$$f_1 = \frac{\sum_{i=1}^n |R_i - D_i|}{\sum_{i=1}^n R_i} \times 100 \quad (5)$$

where R_i and D_i are the percentage of drug dissolved in the release medium of the reference and test samples at time i , respectively, and n is the number of experimental samples taken during the release test. The similarity factor is defined in Eq. (6).

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{i=1}^n (R_i - D_i)^2 \right]^{-0.5} \times 100 \right\} \quad (6)$$

The FDA and the EMA have established as criterion that two dissolution profiles are similar when f_1 is lower than 15 and f_2 is higher than 50. Taking the profile of the unique curve as reference, values of 14.3 and 57.0 (DX1), 13.4 and 57.8 (DX2), 11.7 and 60.0 (DX3), 11.4 and 61.8 (DX4), and 10.4 and 62.9 (DX 5) were obtained for f_1 and f_2 , respectively. According to the criterion established, it can be concluded that the profiles of the different DX loaded PHB films are indeed similar to the profile obtained fitting all the data together.

3.3. Pharmaceutical parameters

Other parameters of pharmaceutical relevance are the dissolution efficiency (DE), the time needed to release a determined percentage of drug ($t_{x\%}$), and the mean dissolution time ($MDT_{x\%}$). The DE is defined by the FDA and the EMA as the ratio between the area under the release profile up to a certain final time (t_f), and the area of the rectangle described by a 100% release at the same t_f (Eq. (7)). In addition, when limits are set for the DE , this parameter can be used for quality control instead of the conventional dissolution test. Since a unique curve fit properly all the data of the samples, these parameters (DE , $t_{x\%}$ and $MDT_{x\%}$) will be the same for the five different loaded films.

$$DE = \frac{\int_0^{t_f} M_{t\%} dt}{100 \times t_f} \times 100 = \frac{\int_0^{t_f} M_{t\%} dt}{t_f} \quad (7)$$

Considering the model equation (Eq. (5)), DE can be determined from Eq. (8).

$$DE = \left(\frac{a\%}{b\%^2} \right) \frac{[b\% \times t_f - \ln(1 + b\% \times t_f)]}{t_f} \quad (8)$$

The DE was 59.6% at 14 days and 66.5% at 20 days. Since this parameter is a ratio of areas, the dispersions of experimental points does not significantly affect the results. On the other hand, the time needed to release the 80% of the drug ($t_{80\%}$) was 20000 min (about 14 days) (Fig. 5). The pharmacopeia states that if this parameter takes a value lower than 45 min, the release can be considered immediate. The results obtained in this work indicate that the PHB films are capable of regulating the DX release.

The $MDT_{x\%}$ is defined by Eq. (9) (Dugar et al., 2016):

$$MDT_{x\%} = \frac{\int_0^{M_{t\%}} t \times dM_{t\%}}{\int_0^{M_{t\%}} dM_{t\%}} \quad (9)$$

According to the model equation, $MDT_{x\%}$ can be calculated from Eq. (10):

$$MDT_{x\%} = \frac{a\%}{b\%^2} * \frac{[\ln(1 + b\% \times t_{x\%}) - \frac{b\% \times t_{x\%}}{(1 + b\% \times t_{x\%})}]}{t_{x\%}} \quad (10)$$

$MDT_{x\%}$ indicates the ability of the polymer to retard drug release. Values of 2679 and 5142 min were calculated for $MDT_{60\%}$ and $MDT_{80\%}$, respectively, from the unique curve for the DX loaded films. The high values obtained indicate a great drug-retarding capacity of the polymer, fulfilling the objective of DX controlled release.

3.4. Intrinsic dissolution rate

The rate of drug dissolution in a liquid to form a solution is governed by several physical parameters, such as the surface area at a given time, the characteristics of the solid/liquid interface, and the solubility of the drug in the medium. As DR and a were calculated from Eq. (1), $\%DR$ (Eq. (11)) and a (Eq. (12)) can be obtained from Eq. (4), being the percent of drug dissolved per unit of time (%/min), and the percentage initial rate, respectively. Since the experimental data were adjusted reasonably well by a unique curve from Eq. (4), the $\%DR$ will be the same regardless of the amount of drug in the film.

$$\%DR = \frac{dM\%}{dt} = \frac{a\%}{(1 + b\% * t)^2} \quad (11)$$

$$\left. \frac{dM\%}{dt} \right|_{t=0} = a\% \quad (12)$$

The dissolution rate per unit of surface area is known as the “intrinsic dissolution rate” (IDR), and is defined as the dissolution rate of the drug under constant conditions (i.e., identical surface area, temperature, agitation rate, pH, and ionic strength of the dissolution media). It is used to demonstrate equivalency of raw components (i.e., comparison of salt vs. free base) and physical mixtures of drugs and excipients or final formulations (Lee et al., 2011; Shekunov and Montgomery, 2016). Information on IDR is important in early drug product development, since it allows the screening of possible drugs and helps to understand their behavior in solution in various biophysiological conditions. Since squared films of 1×1 cm with both faces in contact with the NS solution were used, and considering Eq. (11), $\%IDR$ ($\% / (\text{min} \times \text{cm}^2)$) can be estimated from Eq. (13), where A (cm^2) is the film area in contact with the medium. Fig. 6 shows the variation of $\%IDR$ over time.

$$\%IDR = \frac{\%DR}{A} \quad (13)$$

To obtain the absolute intrinsic dissolution rate ($AIDR$) ($\text{mg} / (\text{min} \times \text{ml} \times \text{cm}^2)$) for each DX loaded PHB film, $\%IDR$ must be multiplied by $M_{\infty} / (100 \times 3)$, where M_{∞} corresponds to each film (Table 1). Considering the PHB film with the highest DX load (DX5), the $AIDR$ ($\text{mg} / (\text{min} \times \text{cm}^2)$) was 1.13×10^{-5} at the beginning, and 4.49×10^{-7} after 14 days. Both the $\%IDR$ and the $AIDR$ are useful values for the researcher who carries out *in vivo* experiments, since from Eq. (11) it is easy to estimate the $\%DR$ and the time needed to release a given amount of drug.

Interesting information is obtained comparing the percentage amount of drug released and the percentage ratio between the $\%DR$ and the initial one ($\%DR \times 100/a\%$) as a function of time (Fig. 7). It can be observed that at 2900 min (curves crossover point), when near the 40% of the drug was released, the dissolution rate decreased about 60% respect to the initial value.

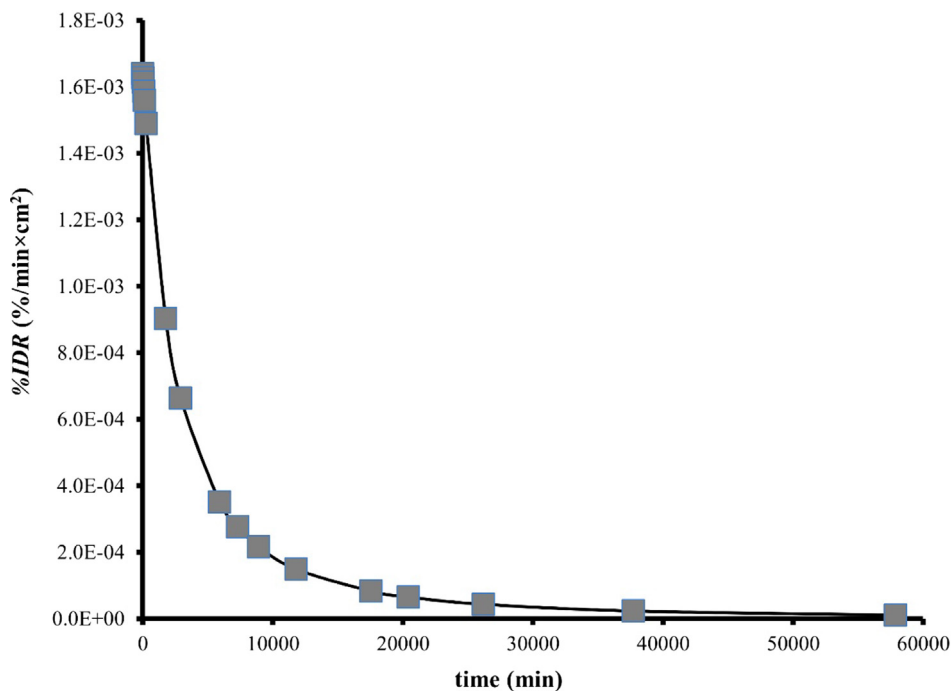


Fig. 6. Intrinsic Dissolution Rate (IDR) of DX loaded PHB films in normal saline solution vs. time.

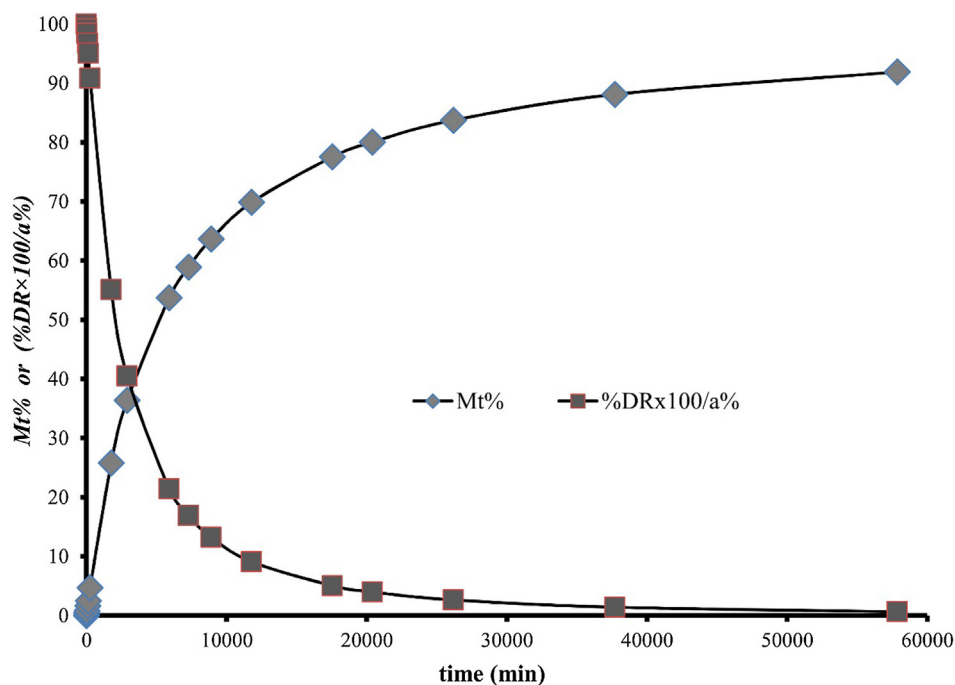


Fig. 7. Percentage of DX released with time ($M_t\%$) from DX loaded PHB films and the percentage reduction of %DR respect to $a\%$ (% initial rate).

4. Conclusions

The designed platforms based on PHB films allowed to efficiently modulate DX release, reaching an effective drug concentration for a prolonged period of time, possibly reducing systemic side effects. The simple mathematical model developed by our research group was successfully used to describe the release phenomena. It provides a first approach for the evaluation of release platforms, before carrying out complete *in vitro* and *in vivo* experiments.

Besides, mathematical modelling is also important for future intellectual property prosecution and quality assurance/quality control. Results suggest that DX loaded PHB films could be an alternative to improve the drug performance compared to conventional formulations, when a bioresorbable device is required, for example, in vascular applications to prevent restenosis.

Combination of mathematical prediction and reliable *in vitro* data may provide the information needed to determine the feasibility of delivering a drug. The determination of the dissolution

rate (especially regarding to the intrinsic dissolution procedure) allows a better *in vitro-in vivo* correlation, since it sometimes helps in the prediction of potential bioavailability problems.

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Declaration of interest

The authors declare no conflict of interest.

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