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Transport properties of hydrophilic compounds in PLGA microspheres

Propriedades de transporte de compostos hidrofílicos em microesferas de PLGA

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Abstract
Biodegradable polyesters, such as the poly(lactic-co-glycolic acid) (PLGA), have been extensively used as a polymer matrix for entrapping a variety of active compounds. In this study, the physicochemical phenomena that control the mass transport mechanism of hydrophilic compounds released from PLGA microspheres were identified. This study aims to produce and characterize PLGA microspheres loaded with metformin hydrochloride (MH) and perform a case study using the literature data of PLGA microspheres loaded with fluorescein isothiocyanate-dextran (FITC-dextran). The MH is a low molecular weight compound that was easily and rapidly transported by diffusion mechanism through the microsphere pores. The FITC-dextran, as a high molecular weight compound, depended on the mechanism of polymer erosion and mesopore formation, with 18 days of duration, before its release by diffusion mass transfer. Values of the effective diffusion coefficient of MH and FITC-dextran, both in PLGA, were 2.4×10^{-13} and 5.3×10^{-18} m^2 s^{-1}, respectively, with a difference of five orders of magnitude attributed to the molecular weight of these hydrophilic compounds and the main mass transport that governed their release. This study provides important insights into the mechanisms of mass transfer and their correlation with the physicochemical properties of both hydrophilic compounds and the PLGA matrix, contributing to the development of biodegradable controlled delivery systems for a variety of applications in chemical, biotechnological, and pharmaceutical industries.
Keywords: Biodegradable polymer; Controlled release; Mathematical modeling; Diffusion; Erosion.

Resumo
Poliésteres biodegradáveis, como o poli(ácido láctico-co-glicólico) (PLGA), têm sido amplamente utilizados como matriz polimérica para encapsular uma variedade de compostos ativos. Neste estudo, os fenômenos físico-químicos que controlam o mecanismo de transporte de massa de compostos hidrofílicos liberados de microesferas PLGA foram identificados. Este estudo visa produzir e caracterizar microesferas de PLGA carregadas com cloridrato de metformina (CM) e realizar um estudo de caso utilizando dados da literatura de microesferas de PLGA carregadas com isotiocianato de fluoresceína (FITC)-dextrano. O CM é um composto de baixa massa molecular que foi rapidamente transportado por mecanismo difusivo através dos poros da microesfera. O FITC-dextrano, por ser um composto de alta massa molecular, dependeu do mecanismo de erosão do polímero e formação dos mesoporos, com 18 dias de duração, antes da sua liberação por transferência de massa por difusão. Os valores do coeficiente de difusão efetivo do CM e do FITC-dextrano, ambos em PLGA, foram iguais a $2{,}4 \times 10^{-13}$ e $5{,}3 \times 10^{-18}$ m^2 s^{-1}, respectivamente, com uma diferença de cinco ordens de grandeza atribuídas às diferentes massas moleculares desses compostos hidrofílicos e ao principal mecanismo

de transporte de massa durante a liberação. Este estudo fornece informações importantes sobre os mecanismos de transferência de massa e sua correlação com as propriedades físico-químicas tanto dos compostos hidrofílicos quanto da matriz de PLGA, contribuindo com o desenvolvimento de sistemas de liberação controlada biodegradáveis para uma variedade de aplicações nas indústrias químicas, biotecnológicas e farmacêuticas.
Palavras-chave: Polímero biodegradável; Liberação controlada; Modelagem matemática; Difusão; Erosão.

Resumen
Los poliésteres biodegradables, como el poli(ácido láctico-co-glicólico) (PLGA), se han utilizado ampliamente como matriz polimérica para encapsular una variedad de compuestos activos. En este estudio, se identificaron los fenómenos fisicoquímicos que controlan el mecanismo de transporte de masa de los compuestos hidrofílicos liberados de microesferas de PLGA. Este estudio tiene como objetivo producir y caracterizar microesferas de PLGA cargadas con clorhidrato de metformina (CM) y realizar un estudio de caso utilizando datos de la literatura de microesferas de PLGA cargadas con isotiocianato de fluoresceína (FITC)-dextrano. El CM es un compuesto de baja masa molecular que fue transportado rápidamente por el mecanismo de difusión a través de los poros de la microesfera. El FITC-dextrano, como compuesto de alta masa molecular, dependió del mecanismo de erosión del polímero y de la formación de mesoporos, durante 18 días, antes de su liberación por transferencia de masa por difusión. Los valores del coeficiente de difusión efectivo del CM y del FITC-dextrano, ambos en PLGA, fueron iguales a $2,4 \times 10^{-13}$ y $5,3 \times 10^{-18}$ m^2 s^{-1}, respectivamente, con una diferencia de cinco órdenes de magnitud atribuida a las diferentes masas moleculares de estos compuestos hidrofílicos y al principal mecanismo de transporte de masa durante la liberación. Este estudio proporciona importantes conocimientos sobre los mecanismos de transferencia de masa y su correlación con las propiedades fisicoquímicas tanto de los compuestos hidrofílicos como de la matriz de PLGA, contribuyendo al desarrollo de sistemas biodegradables de liberación controlada para diversas aplicaciones en la industria química, biotecnológica y farmacéutica.
Palabras clave: Polímero biodegradable; Liberación controlada; Modelización matemática; Difusión; Erosión.

1. Introduction

Biodegradable polymeric microspheres have been extensively investigated as delivery systems for a variety of active compounds. The benefits of sustained delivery systems are well-known in terms of improved safety, convenience, compliance, and therapeutic effects (Ghitman et al., 2020). Polyesters are among the most used class of biodegradable and biocompatible polymers for biotechnological and pharmaceutical purposes, including the poly(lactic-co-glycolic acid) (PLGA), which is approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for clinical applications (Martins et al., 2018). Also, PLGA is metabolized into its constituent monomers, i.e., lactic acid and glycolic acids, which are biocompatible and toxically safe by-products (Ding & Zhu, 2018). The commercial availability and high solubility in several organic solvents are other reasons for the wide use of PLGA.

PLGA microspheres can be prepared by various techniques. Among them, the solvent extraction/evaporation technique is commonly employed because of its simplicity and control of particle size (Han et al., 2016). This technique consists of four major steps: (i) PLGA is dissolved in a water-immiscible, volatile organic solvent; (ii) the organic phase is emulsified in a large volume of water phase by using high-speed homogenizers or ultrasound in the presence of emulsifier or surfactant; (iii) the organic solvent is removed to convert the droplets into solid particles, either by evaporation or extraction with a large quantity of water or another quenching medium to diffuse the solvent out; (iv) the resultant solid particles are washed to remove the surfactant and freeze-dried to achieve the final formulation (Ding & Zhu, 2018). The single emulsion or oil-in-water (o/w) emulsification processes are employed to encapsulate hydrophobic compounds, whereas the double emulsion or water-in-oil-in-water (w/o/w) emulsification is best suited for encapsulating hydrophilic compounds (Makadia & Siegel, 2011).

The use of mathematical modeling of delivery systems has increased from academic to industrial levels because of the predictability of release profiles, also providing insights into the main mass transfer phenomena. The analysis of physicochemical phenomena that control the release defines system-specific parameters used to design novel dosage forms, saving time, and reducing the costs of experimental studies. This knowledge is a pre-requisite for improving the safety of pharmaceutical treatments (Siepmann & Siepmann, 2012). Numerous mathematical models have been proposed in the literature, including empirical and mechanistic ones. Empirical or correlative mathematical models are purely descriptive and have limited predictive

capability outside the experimental conditions used to fit the model parameters. In contrast, mechanistic mathematical models are based on real phenomena (diffusion, dissolution, erosion and/or degradation) that contribute to the mass transfer mechanism, allowing a theoretical prediction of the effects of formulation and processing parameters on the resulting release kinetics (Versypt et al., 2013).

This work aims to identify the mass transport mechanisms that control the release of hydrophilic compounds from PLGA microspheres based on the ability of mathematical models in describing their release behavior. PLGA microspheres loaded with metformin hydrochloride (MH), a hydrophilic model compound with low molecular weight, were produced and characterized before the *in vitro* release studies and mathematical modeling of diffusion-controlled systems. A case study using the experimental data taken from the literature (Mao et al., 2007) of fluorescein isothiocyanate dextran (FITC-dextran), a hydrophilic model compound with high molecular weight, loaded in PLGA microspheres was used for mathematical modeling of erosion-controlled systems.

2. Methodology

2.1 Materials

PLGA (Resomer® RG503H, LA:GA 50:50, inherent viscosity 0.32-0.44 dL g^{-1}, M_w = 24-38 kg mol^{-1}, end group: free carboxylic acid) was supplied by Evonik Degussa (Brazil). MH was obtained from Aarti Drugs (India). Poly(vinyl alcohol) (PVA) (Mowiol® 40-88, M_w = 205 kg mol^{-1}, 86.7-88.7 % hydrolyzed) was purchased from Sigma-Aldrich (Brazil). Dichloromethane (purity 99.5 %) was supplied by Cromoline Química Fina (Brazil). Other chemicals were of analytical grade. All materials were used as received.

2.2 Preparation of PLGA-MH microspheres

PLGA-MH microspheres were prepared using a modified double emulsion ($w_1/o/w_2$) solvent extraction/evaporation technique (dos Santos et al., 2018). Briefly, 150 mg of MH were solubilized in 500 μL of distilled water (internal aqueous phase, w_1), and added to 5 mL of dichloromethane containing 500 mg of PLGA (oil phase, o). The water-in-oil (w_1/o) emulsion was homogenized in an Ultra-Turrax® (T25-basic, IKA-Werke, Germany) at 17500 rpm for 2 min. This primary emulsion was dispersed into 75 mL of 0.5 % (w/v) PVA aqueous solution (external aqueous phase, w_2), and emulsified in a magnetic stirrer (DI-06, Dist, Brazil) at 900 rpm for 5 min to obtain the double emulsion ($w_1/o/w_2$). For solvent extraction/evaporation, 100 mL of 0.1 % (w/v) PVA aqueous solution was added to the double emulsion that was maintained under magnetic stirring (DI-06, Dist, Brazil) at 800 rpm and 40 °C for 1 h. The solid microspheres were recovered by centrifugation (4K15, Sigma, Germany) at 4500 rpm for 5 min, washed three times with distilled water to remove residual PVA and non-entrapped MH. The microspheres were lyophilized (LD 1500, Terroni, Brazil) at -50 °C under vacuum (133.3 mbar) for 24 h and stored in a desiccator at 25 °C.

2.3 Physicochemical characterization of PLGA-MH microspheres

The process yield was calculated based on the mass of lyophilized microspheres relative to the initial sum of masses of PLGA and MH used in the process. The entrapment efficiency and loading capacity were determined by dissolving 20 mg of microspheres in 1 mL of dichloromethane. This mixture was maintained in an ultrasonic water bath (USC 700, Unique, Brazil) until complete polymer dissolution. Then, 5 mL of distilled water was added, and the suspension was centrifuged (4K15, Sigma, Germany) at 2500 rpm for 5 min. The MH concentration in the aqueous phase was quantified using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) at 232 nm in triplicate. The entrapment efficiency was calculated as the percentage ratio between the mass of MH in microspheres and the initial mass of MH used in the formulation. The loading capacity was expressed as mg of MH/100 mg of microparticles.

Particle size and size distribution of microspheres were obtained by laser diffraction using a Mastersizer 2000 (Malvern Instruments, UK). The microspheres were dispersed in distilled water during measurements. Particle size results were reported as the volume mean diameter and the particle size distribution was expressed by the span index according to Equation 1, in which $d_{0.1}$, $d_{0.5}$, and $d_{0.9}$ are the equivalent diameters at 10, 50 and 90 % of the cumulative volume. Measurements were performed in triplicate.

$$\text{Span index} = \frac{d_{0.9} - d_{0.1}}{d_{0.5}} \quad (1)$$

The internal and surface morphologies of microspheres before and after release studies were analyzed by Scanning Electron Microscopy (SEM) in a JSM-6390LV microscope (Jeol, Japan) at 10 kV. For internal morphology, the microspheres were included in an embedding medium (Jung Tissue Freezing Medium, Leica Instruments, Germany) and frozen at -20 °C for 2 hours. A cryostat microtome (CM1850 UV, Leica Instruments, Germany) was used to slice thin sections (5 μm) of the frozen block containing the microspheres. Samples were mounted on metal stubs using double-sided adhesive carbon tape and coated with gold under vacuum.

Thermal properties of PLGA-MH microspheres, MH, PLGA, and their physical mixture were evaluated by Differential Scanning Calorimetry (DSC) in a Perkin-Elmer calorimeter (Jade DSC, USA) previously calibrated with indium and zinc. Samples of approximately 5 mg were sealed in aluminum pans and heated at 10 °C min^{-1} from -20 to 300 °C under a nitrogen flow of 20 mL min^{-1}. The glass transition temperature (T_g) and melting temperature (T_m) of samples were obtained from the second heating curve, according to the ASTM method (ASTM D3418 - 15, 2015).

2.4 *In vitro* MH release from PLGA microspheres

A dialysis membrane (70 mm length and 30 mm diameter) of regenerated cellulose (MWCO 10 kDa, Viscofan, Spain) was used to immerse microspheres in the release medium. The membrane was washed in running water and soaked overnight in distilled water before use. Then, it was filled with approximately 200 mg of microspheres, suspended in 50 mL of phosphate buffer pH 6.8 (release medium), and maintained in a thermostatic water bath with magnetic stirring (DI-06, Dist, Brazil) at 37 °C and 150 rpm. At predetermined time intervals, 1 mL of the release medium was withdrawn and replaced with the same volume of fresh medium (dos Santos et al., 2018). The MH concentration in each aliquot was quantified in a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) at 232 nm. All experiments were performed in triplicate. Results were expressed as the cumulative mass fraction of MH released as a function of time. Also, pure MH was placed in the dialysis bag (50 μg mL^{-1} in phosphate buffer pH 6.8) following the same experimental procedure to verify the membrane influence in diffusion. The MH concentration in release assays was maintained below 10 % of the saturation value, calculated by the MH solubility in water (> 300 mg mL^{-1} at 25 °C) (Barot et al., 2010). The *in vitro* release data of MH from PLGA microspheres was used for mathematical modeling of diffusion-controlled systems.

2.5 Case study

A case study using literature data was proposed to evaluate the mass transfer based on erosion mechanisms that govern the release of hydrophilic compounds from PLGA microspheres. The literature data selection was based on the following criteria: (i) use of PLGA Resomer® RG503H, LA:GA 50:50, end group: free carboxylic acid, also used in the experimental data (Section 2.1); (ii) entrapment of a hydrophilic compound with high molecular weight; (iii) microspheres produced by the double emulsion solvent extraction/evaporation technique. The work of Mao et al. (2007) met the selection criteria and the *in vitro* release data of

FITC-dextran from PLGA microspheres was used for mathematical modeling of erosion-controlled systems.

2.6 Mathematical models for diffusion-controlled systems

The release kinetics of hydrophilic compounds from PLGA microspheres can be described by analogy to the Fick's law of diffusion in dilute solutions combined with the equation of continuity for the component. This mechanistic mathematical model, Equations 2 to 5, is applied for diffusion-controlled systems considering unidirectional (radial) gradients of concentration, absence of chemical reaction and macroscopic flux only by diffusion at unsteady state,

$$\frac{\partial \rho}{\partial t} = D\left(\frac{\partial^2 \rho}{\partial r^2} + \frac{2}{r}\frac{\partial \rho}{\partial r}\right) \quad (2)$$

in which ρ is the mass concentration of the hydrophilic compound (kg m^{-3}), r is the radial coordinate (m), t accounts for time (s), and D represents the diffusion coefficient (m^2 s^{-1}) of the active compound in the polymer matrix, a constant parameter independent of distance, time, and concentration. Equation 2 can be solved by employing an initial condition of uniform active compound distribution throughout the microsphere (Equation 3) and boundary conditions of spherical symmetry (Equation 4), and constant active compound concentration at the microsphere surface (Equation 5), assuming negligible mass transfer resistance:

$$t = 0;\ 0<r<R;\ \rho = \rho_i \quad (3)$$

$$t > 0;\ r = 0;\ \frac{\partial \rho}{\partial r} = 0 \quad (4)$$

$$t > 0;\ r = R;\ \rho = \rho_s \quad (5)$$

in which ρ_i is the initial mass concentration of the active compound (kg m^{-3}) obtained from the loading capacity, ρ_s is the mass concentration of the active compound at the surface in equilibrium with the release medium (kg m^{-3}), and R is the microsphere radius (m), determined in particle size measurements. It was assumed: (i) absence of active compound in the release medium ($t = 0$; $\rho_s = 0$) and as the active compound is released, its concentration is instantaneously and homogeneously increased in the bulk (perfectly agitated medium); (ii) no polymer swelling making the parameter R to be constant; and (iii) the active compound concentration in the release medium remains below its saturation value during the entire period of release, as described in Section 2.4. The analytical solution of Equations 2-5 is given by Equation 6 (Crank, 1975), in which M_t and M_∞ represent the cumulative mass of the active compound released from PLGA microspheres at any instant t and at a sufficiently long time to reach equilibrium, respectively. The diffusion coefficient D is the adjustable parameter.

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\sum_{i=1}^{\infty}\frac{1}{i^2}\exp\left(-i^2\pi^2\frac{Dt}{R^2}\right) \quad (6)$$

The power-law model (Equation 7) is derived from the analytical solution of Fick's law of diffusion for constant diffusivity and one-dimensional diffusion. It was first applied by Peppas and co-workers to describe the release behavior of active agents from polymer systems and it is valid for the first 60 % of the total release (Ritger & Peppas, 1987). The simple and empirical exponential relation can describe the diffusion behavior of non-swellable polymeric delivery systems, such as the PLGA.

$$\frac{M_t}{M_\infty} = kt^n \quad (7)$$

in which k is a constant incorporating characteristics of the polymer and the active agent. The diffusional exponent n depends on the system geometry and is indicative of the transport mechanism. The constant k and the exponent n are the adjustable parameters of this model.

2.7 Mathematical models for erosion-controlled systems

Batycky et al. (1997) proposed a mechanistic mathematical model for predicting the release profile of spherical bulk eroding PLGA microspheres prepared by the double emulsion technique containing a hydrophilic compound. This model, Equation 8, considers that the active compound is released by desorption and diffusion during microsphere degradation. Desorption originates with the active agent being released from the microsphere surface (initial burst release), whereas diffusion is delayed until an induction time is reached, allowing the coalescence of micropores and diffusion of the remaining macromolecular compound.

$$\frac{M_t}{M_\infty} = 1 - \Phi_b[1 - \exp(-k_d t)] - (1 - \Phi_b)\left[1 - \frac{6}{\pi^2}\sum_{i=1}^{\infty}\frac{1}{i^2}\exp\left(-i^2\pi^2\frac{D(t-t_d)}{R^2}\right)\right] \quad (8)$$

in which Φ_b is the mass fraction of the compound released during the initial burst, k_d represents the rate of active compound desorption (s^{-1}) and t_d is the induction time (s). The last term of Equation 8 is the analytical solution of Fick's law of diffusion (Equation 6), except for the inclusion of the parameter that represents the induction time required for active compound diffusion. The adjustable parameters are D, k_d, and t_d.

Several empirical approaches have been employed to model polymer erosion. One of the most employed mathematical models for PLGA eroding microspheres was proposed by Hopfenberg (1976). In this model, the release of the active agent is directly proportional to the matrix erosion rate, i.e., it depends on the continuously changing area of the microsphere due to erosion. The Hopfenberg model, Equation 9, is valid for spherical geometries with one-dimensional diffusion and constant diffusion coefficients,

$$\frac{M_t}{M_\infty} = 1 - \left(1 - \frac{k_e t}{c_0 R}\right)^3 \quad (9)$$

in which k_e is the adjustable parameter that represents the erosion rate of microsphere ($m\ s^{-1}$) and c_0 is the initial mass fraction of the active agent obtained from the loading capacity.

2.8 Numerical solution

Mechanistic and empirical models for diffusion-controlled systems (Equations 6 and 7) were fitted to the release data of MH from PLGA microspheres, whereas mechanistic and empirical models for erosion-controlled systems (Equations 8 and 9) were fitted to the release data of FITC-dextran from PLGA microspheres. A computational routine was written in the Matlab® software (version R2013a, Mathworks, USA) using the nonlinear least-squares method and the trust-region reflective Newton algorithm (Longhi et al., 2017; Medeiros et al., 2018). The values of the initial try for each parameter were based on the observation of experimental data. The following statistical indexes were used to explain the performance of mathematical models: the adjustable coefficient of determination (R^2_{adj}) and the root-mean-square error ($RMSE$), Equations 10 and 11, respectively.

$$R^2_{adj} = 1 - \left[\frac{d-1}{d-(p+1)}\right]\left\{1 - \left[\frac{\Sigma(y_{exp}-y_{pred})^2}{\Sigma(y_{exp}-\bar{y})^2}\right]\right\} \quad (10)$$

$$RMSE = \sqrt{\frac{\Sigma(y_{exp}-y_{pred})^2}{d-p}} \quad (11)$$

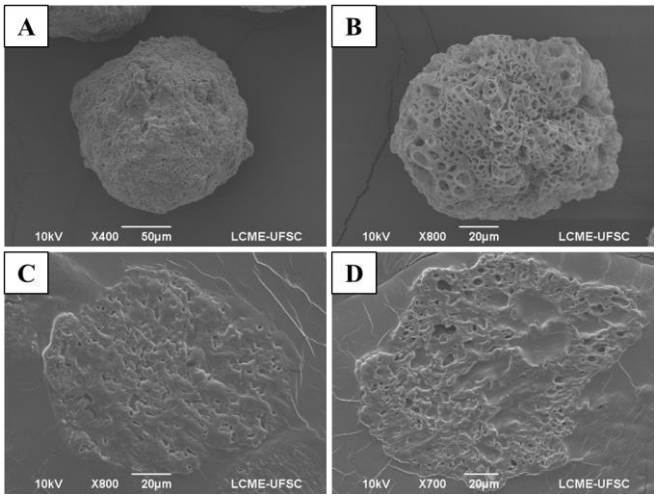
in which d is the number of experimental data, p is the number of model parameters, y_{exp}, y_{pred}, and $\bar{y}$ represent the experimental values, predicted values, and average experimental values, respectively.

3. Results and Discussion

3.1 Characterization of PLGA-MH microspheres

The PLGA-MH microspheres were produced by the double emulsion solvent extraction/evaporation technique. The yield was 72 ± 2 % with an entrapment efficiency of 1.4 ± 0.1 % and a loading capacity of 0.33 ± 0.02 %. Similar results were also reported for the microencapsulation of hydrophilic agents in PLGA with end group of carboxylic acid using the double emulsion technique, with values of entrapment efficiency and loading capacity at about 1 % (dos Santos et al., 2018; Kashi et al., 2012; Mao et al., 2007). The literature reports that low values of loading capacities can be achieved for hydrophilic agents entrapped in PLGA when using the double emulsion technique because such hydrophilic molecules tend to diffuse out of the primary emulsion droplets to the external aqueous phase during the solvent removal step (Regnier-Delplace et al., 2013). Also, PLGA with an uncapped carboxylic acid end group makes the polymer more hydrophilic, facilitating the leakage of hydrophilic compounds into the external aqueous phase while the polymer phase stays in a transitional, semi-solid state just before the complete microsphere hardening. The obtained microspheres presented an average diameter of 243 ± 12 μm with a monodisperse particle size distribution, resulting in a span index of 1.02 ± 0.05. Before exposure to the release medium, the PLGA-MH microspheres were spherical with a uniform internal structure and several pores distributed on their surface (Figure 1). The internal and surface porosity of microspheres can be attributed to the presence of MH in primary emulsion that generates an osmotic gradient and a water influx in microspheres, creating channels that are converted into pores during microsphere hardening (Han et al., 2016). The absence of hollow cores before the MH release is due to the use of PVA as a stabilizer agent that prevented the coalescence of emulsion droplets during the homogenization step. Moreover, the high porosity of PLGA microspheres can lead to a faster MH release (Gaignaux et al., 2012).

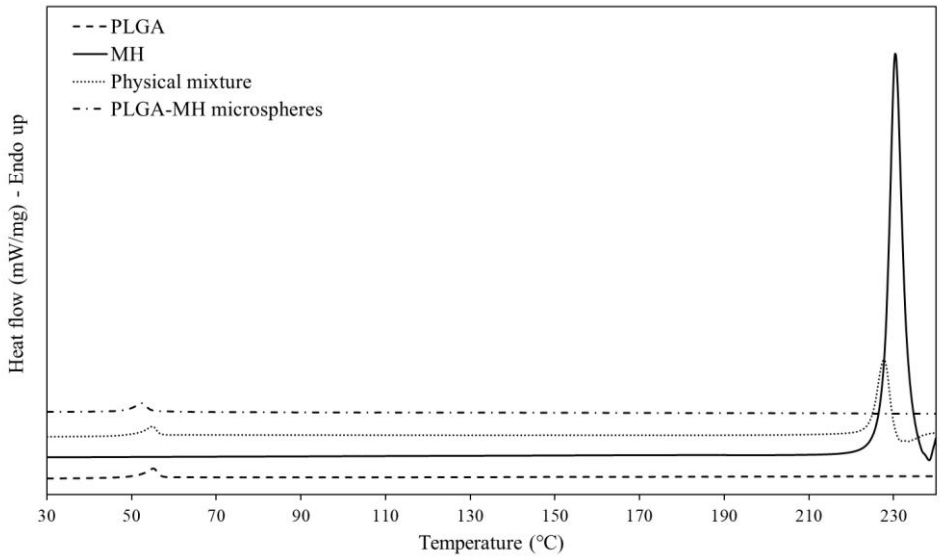
Figure 1 - SEM images of surface and cross-sections of PLGA-MH microspheres before (A and C) and after (B and D) *in vitro* release.



Source: Authors.

The thermal properties of PLGA-MH microspheres as well as that of the PLGA, MH, and their physical mixture are shown in Figure 2. The physical mixture presented two thermal events relative to the T_g of PLGA, at around 50 °C, and the T_m of MH, at 228 °C, whereas the microspheres had one thermal event corresponding to the T_g of PLGA. The disappearance of the T_m peak of MH suggests that the active agent was molecularly or amorphously dispersed in the PLGA microsphere (Chen et al., 2017). Also, the T_g of PLGA and that of the physical mixture occurred at 55 °C, while the T_g of PLGA-MH microspheres was recorded at 52 °C. This temperature reduction indicates that PLGA underwent a transition from glassy to the rubbery state after microsphere formation, suggesting a plasticizing effect of MH in the polymer. The physical state of the polymer matrix is an important parameter to provide information on the underlying mass transfer mechanisms because diffusion tends to be faster in rubbery polymers than in glassy ones (Kaunisto et al., 2011).

Figure 2 - DSC thermograms of PLGA-MH microspheres, PLGA, MH, and their physical mixture.

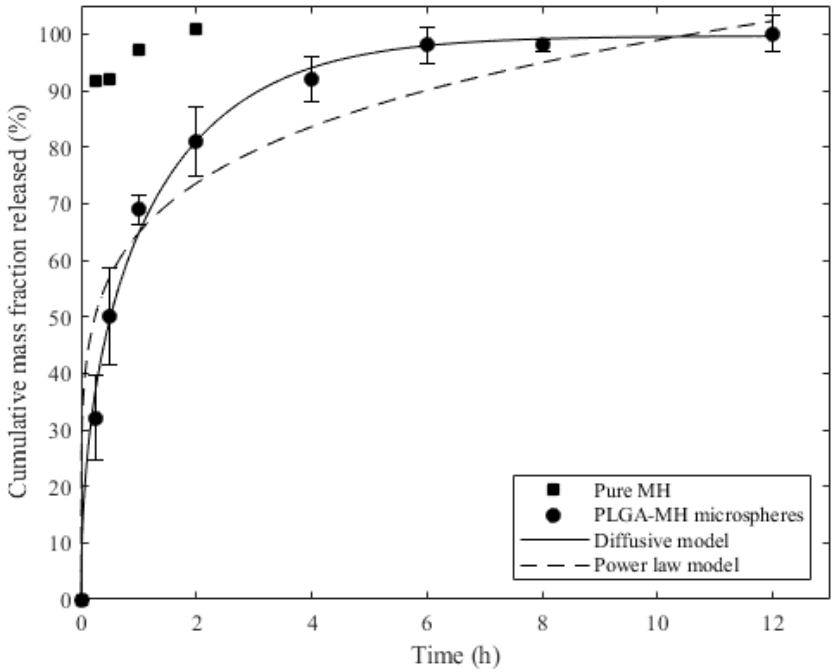


Source: Authors.

3.2 MH release from PLGA microspheres and mathematical modeling of diffusion

The cumulative percent release of pure MH from the dialysis membrane (control) and MH from PLGA microspheres are shown in Figure 3. The membrane used in release assays was not a barrier against MH diffusion, evidenced by a complete release of the active agent in both assays. A fast release was observed for the control assay with 92 % of the MH being released in the first 15 minutes. On the contrary, 32 % of the MH was released from the PLGA microspheres in the same period, suggesting a sustained release profile compared to the control assay with a 60 % reduction in the amount of MH released. The complete release of the active agent from PLGA microspheres was achieved in 12 hours.

Figure 3 - *In vitro* release of MH contained in the dialysis membrane (■) and MH from PLGA microspheres (●). Symbols: experimental data. Solid line: diffusive model. Dashed line: power-law model.



Source: Authors.

Table 1 presents the values of the adjusted parameters of diffusive and power-law models fitted to the experimental data of MH release from PLGA microspheres. The effective diffusion coefficient of MH in PLGA was 2.4 x 10^{-13} m^2 s^{-1}. The exponent n in the power-law model, indicative of the mass transport mechanism, was equal to 0.18. For spherical geometries, *n*-values lower than 0.43 are attributed to diffusion-controlled systems if the following assumptions are fulfilled: a polymer matrix with negligible edge effects, time- and position-independent diffusion coefficients, and a non-swellable and insoluble matrix (Lao et al., 2011), as in the case of the PLGA-MH microspheres.

Table 1 - Adjusted parameters of mathematical models for diffusion-controlled systems (diffusive and power-law) and erosion-controlled systems (Batycky and Hopfenberg).

Mathematical model[1]	Parameter (unit)	Value
Diffusive	D ($m^2\ s^{-1}$)	2.4×10^{-13}
Power-law	n (dimensionless)	0.18
	k (s^{-1})	1.8×10^{-4}
Batycky	D ($m^2\ s^{-1}$)	5.3×10^{-18}
	k_d (s^{-1})	4.5×10^{-4}
	t_d (h)	444
Hopfenberg	k_e ($m\ s^{-1}$)	1.0×10^{-14}

[1] Diffusive and power-law models adjusted to MH release from PLGA microspheres; Batycky and Hopfenberg models adjusted to FITC-dextran release from PLGA microspheres. Source: Authors.

The release profile can be used as the basis for evaluating the mass transport mechanisms in polymeric systems (Fredenberg et al., 2011). The good fit of the mathematical models for diffusion-controlled systems to the experimental data indicates that the diffusion mechanism controlled the MH release from PLGA microparticles. When comparing the two mathematical models, the diffusive model presented a better description of the *in vitro* release data than the power-law model, as best viewed in Figure 3 and considering the values of statistical indexes (Table 2).

Table 2 - Statistical indexes of mathematical models for diffusion-controlled systems (diffusive and power-law) and erosion-controlled systems (Batycky and Hopfenberg).

Mathematical model	R^2_{adj}	$RMSE$
Diffusive	0.995	0.022
Power-law	0.914	0.102
Batycky	0.990	0.024
Hopfenberg	0.957	0.056

Source: Authors.

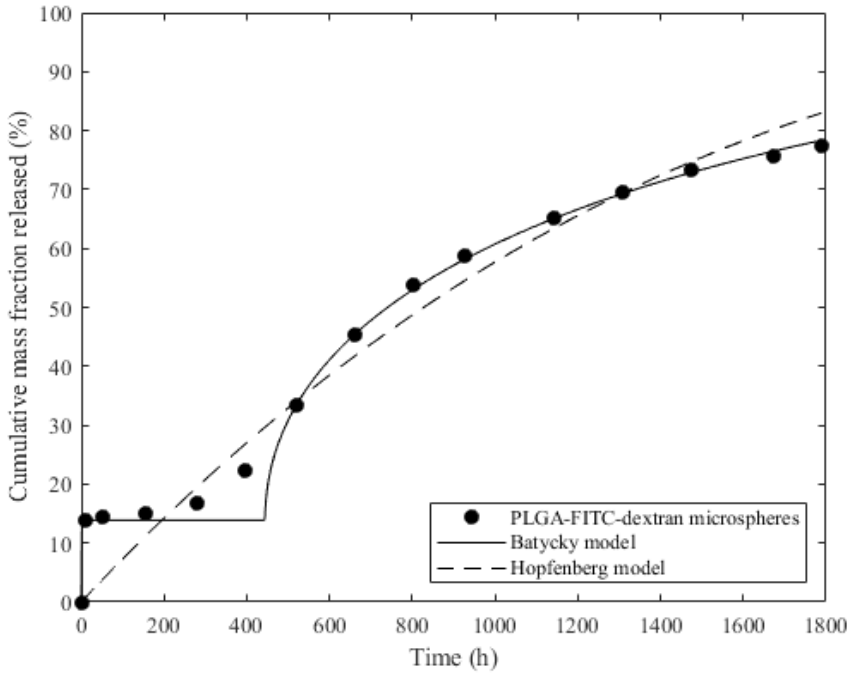
The diffusion mechanism of MH from PLGA microspheres can be explained by two main steps: an initial release caused by the active agent dissolution from superficial pores followed by its diffusion from inner regions to the microsphere surface through water-filled pores. For microspheres produced by the solvent extraction/evaporation technique, the step of solvent removal contributes to the initial release, as some molecules of the active agent diffuse from the inner regions of the semi-solid polymer and accumulate on the particle surface during its hardening (Ye et al., 2010). In this context, the presence of pores facilitates the water absorption, promoting the dissolution and release of MH from the PLGA microsphere.

3.3 Case study and mathematical modeling of erosion

The case study with literature data was proposed to help to understand the mass transfer mechanisms during the release of hydrophilic compounds with high molecular weight, such as the FITC-dextran, from PLGA microspheres. The cumulative percent release of FITC-dextran from PLGA microspheres is presented in Figure 4, suggesting the occurrence of erosion mechanism coupled with diffusion mass transfer. Some biodegradable polyesters, such as PLGA, can be classified as erosion-controlled systems. For these devices, the combination of polymer degradation and bulk erosion favors the active compound

release, especially for release periods longer than 3 days, evidenced by an increased internal and external porosity of the system (Versypt et al., 2013). The release profile of FITC-dextran consisted of an initial burst of 13 % in 4 hours followed by a slow and steady release until reaching an erosion-controlled phase corresponding to the polymer degradation and diffusion of the remaining active agent. The FITC-dextran reached 77 % of release in 1800 hours (75 days). The adjusted parameters of Batycky and Hopfenberg models fitted to these experimental data are listed in Table 1.

Figure 4 - *In vitro* release of FITC-dextran from PLGA microspheres. Symbols: experimental data from Mao et al. (2007). Solid line: Batycky model. Dashed line: Hopfenberg model.



Source: Authors.

The Batycky model was developed to predict the release profile of hydrophilic glycoprotein from bulk eroding PLGA microspheres by considering the initial burst and the emergence of mesopores. Since the glycoprotein is a water-soluble component with a high molecular weight, and thus a relatively large size, the equation incorporates an induction phase (represented by the parameter t_d) that takes place when the initial porosity is low, preventing the protein release by diffusion until some mesopores are available. The induction time observed for the FITC-dextran release from PLGA microspheres was 444 hours (18 days). After this period, the release rate of the active compound increases by diffusion mechanism due to pore formation and polymer erosion. In this context, the erosion, i.e., the polymer mass loss, starts when the polymer degradation products (oligomers and monomers) diffuse into the release medium. The presence of ester bonds in the PLGA backbone contributes to this bulk erosion because these functional groups are rapidly hydrated in the release medium and become more susceptible to hydrolysis. The dissolution of polymer erosion products also carries the active agent out of the matrix, contributing to the overall release. However, it is important to notice that some PLGA microspheres do not show this induction phase, especially when incorporating hydrophilic agents of low molecular weight, as discussed in the case of PLGA-MH microspheres. These low molecular weight compounds rapidly diffuse through the micropores leading to a substantial or even a complete release during the hydration phase in the early stages of the release.

The Hopfenberg model considers that the surface detachment caused by erosion mechanisms is the main step of release (Sackett & Narasimhan, 2011). This phenomenon explains the predicted continuous release of the active compound from the

beginning of the release period (Figure 4). However, this continuous release was not able to describe the initial burst release experimentally observed, also influencing the values of statistical indexes in Table 2. The good fit of the mathematical models for erosion-controlled systems to the experimental points suggests an erosion-controlled release of FITC-dextran from PLGA microspheres. The *in vitro* release data were better described by the Batycky model than the Hopfenberg model, based on the statistical values listed in Table 2 and illustrated in Figure 4.

The effective diffusion coefficient of FITC-dextran in PLGA was 5.3 x 10^{-18} m^2 s^{-1} (Table 1), five orders of magnitude higher than that of MH in PLGA (2.4 x 10^{-13} m^2 s^{-1}). The effective diffusion coefficient is a parameter dependent on the molecule size and other properties of the diffusing substances, besides the temperature and pressure of the system (Welty et al., 2014). The lower the diffusivity of one compound relative to the other, the slower they diffuse into the same material. Considering that both the polymer and the method of particle preparation are the same, the different values of the effective diffusion coefficient are related to the characteristics of the diffusing hydrophilic agents. The MH has a low molecular weight of 165.62 g mol^{-1} (Brasil, 2019), while the molecular weight of FITC-dextran is 40000 g mol^{-1} (Mao et al., 2007), approximately 240 times higher than that of MH. The molecular weight of hydrophilic agents played an important role in the release profile and main mass transfer mechanism. The MH, as a low molecular weight compound, was easily and rapidly transported by diffusion mechanisms through the microsphere pores. On the contrary, the FITC-dextran is a high molecular weight compound that depended on the mechanism of polymer erosion to increase its diffusion rate through the particle mesopores.

In general, empirical models were helpful to describe the release profile, but the detailed mechanism regarding the release process was difficult to be fully elucidated. Conversely, mechanistic mathematical models showed a better description of the release profile, also giving indications of the main mass transport mechanism that governed the release.

4. Conclusion

The physicochemical phenomena that control the mass transport mechanism of hydrophilic compounds released from PLGA microspheres were investigated. The MH is a low molecular weight compound that was rapidly transported by diffusion mechanism through particle micropores. The FITC-dextran, as a high molecular weight compound, depended on polymer erosion and mesopore formation, with 18 days of induction time duration, before its release by diffusion mass transfer. The effective diffusion coefficient of the systems PLGA-MH and PLGA-FITC-dextran were 2.4 x 10^{-13} m^2 s^{-1} and 5.3 x 10^{-18} m^2 s^{-1}, respectively. The difference of five orders of magnitude can be attributed to the molecular weight of these hydrophilic compounds and the governing mass transport mechanism during their release. This study provides important insights into the correlation between the mass transfer mechanism and the physicochemical properties of hydrophilic compounds entrapped in PLGA microspheres, allowing the design of controlled delivery systems for biotechnological, pharmaceutical, and chemical applications. Parameters that incorporate particle porosity in empirical mathematical models are suggested for future research.

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