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In vitro study of the release of drugs impregnated by supercritical technology in polylactic acid for biomedical applications

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The manufacture of functionalized bioabsorbable polymeric implants is gaining attention in recent years. These devices administer gradually and locally drugs or biomolecules to treat several diseases and they disappear once they perform their function.
In this work, ketoprofen, an anti-inflammatory drug with analgesic and antipyretic effects, was loaded by supercritical solvent impregnation (SSI) in polylactic acid (PLA) filaments, and the initial stage of its *in vitro* release was explored. Impregnation experiments were carried out in a range of pressure and temperature of 25-40 MPa and 328-348 K.
Different drug loading values were obtained (up to 9 % respecting the polymer mass) depending on the operating conditions.The ketoprofen release profile and the mathematical modelling (Korsmeyer-Peppas and Peppas-Sahlin models) showed an initial release governed by diffusion and with different kinetics depending on the impregnation conditions.
Thus, this study reveals that SSI could be used to manufacture personalized drug-delivery implants based on the final dose and required dosing rate.

1. Introduction

Drug dosing has undergone a radical change in recent decades through the entrapment or encapsulation of active pharmacological compounds in biocompatible polymeric systems. These systems (micro or nanoparticles for oral administration, implants, and patches for transdermal application, among others) ideally have a high load of the drug, which is released gradually and transported to the site of action, improving efficacy and safety of the drug action. Furthermore, in the case of systems made with bioabsorbable polymers, there is the advantage that the device disappears once its function has been fulfilled without the need for new surgical interventions.
Conventionally, the incorporation of drugs or other bioactive species in polymeric matrices is carried out by addition prior to the synthesis of the polymer or by immersion and soaking of the polymer in a solution containing the drug. Although these methods are relatively simple, they have several disadvantages, such as the use of toxic organic solvents (Dias et al, 2011). In recent years, new impregnation techniques have been implanted, aimed to be more environmentally friendly and more efficient in overcoming some of the inconveniences posed by the conventional methods. One of these procedures is supercritical solvent impregnation (SSI).
This technique is based on dissolving the active substance in the supercritical fluid and the increasing of its internal diffusion rates into a polymeric matrix, taking advantage of the capacity of supercritical fluids to penetrate inside polymers, plasticizing and swelling them (Kazarian, 2000). In this way, the solute loading is not limited to the outer surface of the polymer but is also retained within the matrix (Kikic and Vecchione, 2003).

Ketoprofen (KET) is a non-steroidal anti-inflammatory drug (NSAID) derived from phenylpropanoic acid, which is commonly used in treating diseases. It is a pure compound soluble in CO_2, a condition that points to it as a good candidate for polymer impregnation. In fact, it has been satisfactory loaded by SSI in several polymers, such as PLA, polypropylene (PP), polyethylene terephthalate (PET), polyethylene oxide (PEO) or polyvinyl pyrrolidone (PVP) (Champeau et al, 2015a; Champeau et al, 2015b; Marizzaa et al, 2016).
On the other hand, one of the most widely used polymers for controlled drug release is polylactic acid (PLA), a biobased, biocompatible, and bioabsorbable polymer (Pawar et al, 2014; Tyler et al, 2016; Lopes, Jardini and Filho, 2014). This polymer has been impregnated by this technique both with drugs and bioactive compounds such as indomethacin, 5-FU or thymol (Cabezas et al., 2012; Cabezas et al., 2014; Milovanovic et al, 2019) for biomedical purpose.
In this work, the supercritical impregnation of ketoprofen in PLA filaments and the subsequent *in vitro* drug delivery during the initial time of the release process was analysed. Mathematical models (Korsmeyer-Peppas and Peppas-Sahlin) were applied to define the mechanism of drug release. This study was aimed to generate a material with possible use in biomedical implants.

2. Methods

2.1 Raw materials and reactants

PLA filament was provided by Mundo Reader, S.L. (Madrid, Spain). Ketoprofen ($C_{16}H_{14}O_3$) greater than 98% purity was supplied by Sigma Aldrich (Steinheim, Germany). Carbon dioxide (99,99%) was supplied by Abelló Linde (Barcelona, Spain). Other reactants used were: NaOH, Na_2HPO_4, KH_2PO_4, NaCl, KCl and HCl 37% all of them by Panreac AppliChem (Darmstadt, Germany).

2.2 Supercritical solvent impregnation method

Experiments were carried out in a lab scale high pressure unit (SFE100 model, by Thar Technologies Inc,Pittsburgh, PA, USA) which mainly includes a cool bath, a high-pressure pump and a pre-heater for CO_2, a thermostatic jacketed vessel (100 cm^3) and a back-pressure regulator (BPR) to control the system pressure (Figure 1).

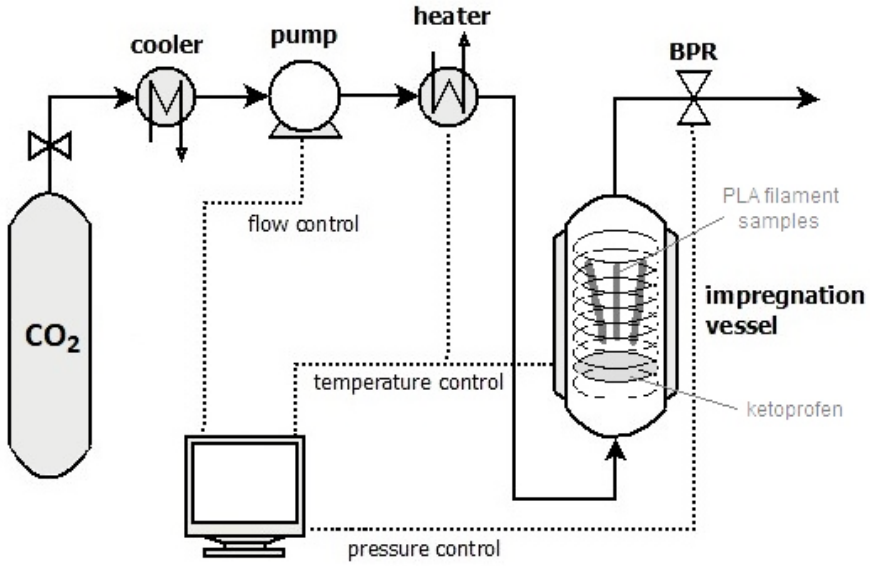

Figure 1: Flowchart of the SSI methodology

The PLA filament samples and the ketoprofen were inserted into the impregnation vessel into a steel support. Then the vessel was hermetically sealed and pre-heated to the operational temperature by an electrical aluminium jacket. Three thermocouples, situated on the heater and on the internal and external sides of the vessel, controlled the temperature of the system. CO_2 was pumped until suitable pressure condition had been achieved and keeping it constant during the impregnation process by the action of the back-pressure regulator (BPR). Once the appropriate time has passed, the CO_2 flow and the heating were switched off and depressurization was started. Samples obtained were stored in cold prior to analysis, to avoid their possible degradation.

2.3 Operational parameters

Different operational pressure and temperature conditions were studied based in our previously experience, *viz.* 348 K, 40 MPa; 348 K; 25 MPa and 328 K, 40 MPa. An operation time of 2 h and a fast depressurization rate were employed in all experiments.

2.4 Study of the drug loading

The estimation of the amount of ketoprofen impregnated was carried out by spectrophotometric analysis. Two analyses were carried out, *i.e.* the estimation of the amount of solute in the surface of the polymer and the total amount of ketoprofen impregnated in the polymer.

To calculate the amount of ketoprofen deposited on the surface of the polymer, 0.1 g of impregnated PLA filament was introduced into 15 cm^3 of 3 M NaOH for 10 s shaking vigorously. After that, the filament was removed and the resulting solution was measured at 260 nm using a *Cary 60* UV-Vis spectrophotometer (*Agilent Technologies*, *Santa Clara, CA, USA*), due to that wavelength corresponds to the highest absorbance of the compound. The amount of ketoprofen was calculated based on a previous calibration curve using different concentrations of ketoprofen in the NaOH solution.

To calculate the global drug loading of the polymer, the filament extracted from the previous test was introduced into 25 cm^3 of the NaOH solution until completely dissolved. The solution was also analysed by spectrophotometry to quantify the amount of impregnated ketoprofen.

The drug loading was expressed in mass of impregnated ketoprofen (m_{KET}) with respect to the mass of a sample of PLA (m_{PLA}), according to equation 1, and the superficial drug was evaluated as a percentage of the total drug loading (equation 2).

$$Drug\ loading\ \% = \frac{m_{KET}}{m_{PLA}} \cdot 100 \quad (1)$$

$$Superficial\ drug\ \% = \frac{m_{SUP-KET}}{m_{KET}} \cdot 100 \quad (2)$$

2.5 *In vitro* drug release assay procedure

To study the drug release kinetics, phosphate buffered saline solution (PBS) was used as releasing medium. This solution was prepared in the laboratory according to this final composition: 100 mM Na_2HPO_4, 18 mM KH_2PO_4, 1.37 M of NaCl, 27 mM KCl with adjusted pH to 7.4 with NaOH or HCl.

In each test, 0.05 g of impregnated PLA filament were immersed into 10 cm^3 of PBS contained into a hermetically closed vessel and kept at a constant temperature of 310 K in a laboratory incubator in a static mode. Regularly, an aliquot was taken and analysed by spectrophotometry at 260 nm, calculating the concentration of ketoprofen based on a calibration curve made with different concentrations of KET in PBS. After each analysis, the aliquot was returned to the container to maintain the initial volume. All the tests were performed in triplicate.

3. Results and discussion

3.1 Study of the drug loading

The results of the ketoprofen loaded are shown in table 1. Operating pressure and temperature play a very important role in impregnation yield since they determine the density of the supercritical CO_2 and thus its ability to diffuse into the polymer. For the PLA-KET system, an increase in pressure and temperature resulted in an increase of the impregnated drug in the range studied, being the temperature a more influential parameter. In our experience, more extreme conditions than those used in these experiments produce structural damage to the polymeric material.

Table 1: Impregnation yield of KET into PLA filaments

Conditions	Drug loading %	Superficial drug %
348 K, 40 MPa	9.0 ± 0.1	1.0 ± 0.3
348 K, 25 MPa	8.9 ± 0.1	1.4 ± 0.1
328 K, 40 MPa	1.8 ± 0.1	4.5 ± 1.7

3.2. Analysis of the release profile and kinetics models

The drug release process is affected by multiple complex factors such as the structure, geometry or the swelling and degradation capacity of the polymer, the solubility and charge of the drug, the pH, temperature, or the action

of enzymes (Fu and Kao, 2010). The different porous structures achieved by PLA filaments after SSI at different pressure and temperature conditions, as well as the amount of ketoprofen loaded, defined the drug release.
It was interesting to modelling the drug release during the first days, when an anti-inflammatory action is most necessary for an implant. Figure 2 shows the release profile of the three experiments, expressed as ketoprofen mass released (M_t) relative to the total mass loaded (M_∞) during the initial nine days.
Biodegradable drug delivery systems have a three phases release profile: a first stage of diffusion, a second period driven by polymer degradation and a third phase where the polymer has a bulk erosion and a total drug dissolution occurs (Fu and Kao, 2010). Examining the experimental data in figure 2, the first seven days would be included in the first phase, which is governed by diffusion. From the seventh day on, the delivery rate becomes higher, which evidence the second phase would begin and the degradation of the polymer becomes the governing mechanism in the drug release.
Mathematical modelling aims to provide information about the release profile of a solute in a specific system simplifying this complex process. The experimental data until day seven were fitted to two of the most widely used models for drug release: Korsmeyer-Peppas and Peppas-Sahlin. Several authors have previously applied these models successfully to drug dosage systems made with polymers similar to the one discussed in this work (Manna et al, 2018; Moorkoth et al, 2021; Champeau et al, 2020).
One of the first models developed was the power law or Korsmeyer-Peppas model (Korsmeyer and Peppas, 1981), since these authors were the first to give indications about the use and limitations for films or thin layers. This model is represented by equation 3, where M_t and M_∞ are the accumulated amounts of drug released at a time *t* and at an infinite time respectively, *k* is a constant that incorporates the structural and geometric characteristics of the device and n is an exponent that indicates the release mechanism.

$$\frac{M_t}{M_\infty} = k\ t^n \tag{3}$$

For cylinders, as is this case, Ritger and Peppas (1987) gave indications about the values adopted by the exponent depending on the releasing mechanism. When this exponent is inferior to 0.45, the drug release is governed by diffusion and Fick´s law. For values up to 0.89 the released mass of drug has a practically linear dependence relationship with time, indicating that drug release occurs simultaneously with polymer degradation, corresponding to a zero-order kinetics or case II transport. Finally, intermediate values suggest an anomalous transport that can be considered as a superposition of both phenomena, diffusion and polymer degradation.
Another interesting modelling equation for anomalous transport is considered in equation 4 and it was given by Peppas-Sahlin (Peppas and Sahlin, 1989). In this mathematical expression k_1, k_2 and m are constant. The first term of this equation ($k_1\ t^m$) represent the Fickian diffusion contribution to release mechanism, while the second term ($k_2\ t^{2m}$) includes case II transport. Equation 5 represents the relationship between both contributions.

$$\frac{M_t}{M_\infty} = k_1\ t^m + k_2\ t^{2m} \tag{4}$$

$$\frac{R}{F} = \frac{k_2\ t^m}{k_1} \tag{5}$$

Table 2 records the parameters adjusted to the Korsmeyer-Peppas model. Experimental values at 40 MPa and 328 K correctly fit a diffusion release kinetic, since they are expressed with an exponential profile with an exponent (*n*) lower than 0.45. The kinetic constant (*k*) will depend on the amount of drug impregnated in the outermost zone of the polymer, that is the amount of drug that can easily diffuse. At 328 K and 40 MPa, *k* constant is much higher than those of the other experiments, which is consistent with the determination of the superficial ketoprofen (see table 1).
Both experiments at 348 K have an *n* value between 0.45 and 0.89, consequently, they correspond to an anomalous type of transport where both the diffusion of the drug and the degradation of the polymer are present. The drug loading is directly related to the porosity achieved by the polymer after the process, which implies that the polymer will be eventually degraded faster, and the drug can delivery quicker too.

Table 2: Adjusted Korsmeyer-Peppas model parameters

Conditions	Model parameters	Goodness-of-fit	Release mechanism
348 K, 40 MPa	$n = 0.58$; $k = 0.06$	$R^2 = 0.99$	Anomalous transport
348 K, 25 MPa	$n = 0.47$; $k = 0.07$	$R^2 = 0.97$	Anomalous transport
328 K, 40 MPa	$n = 0.11$; $k = 0.38$	$R^2 = 0.97$	Diffusion

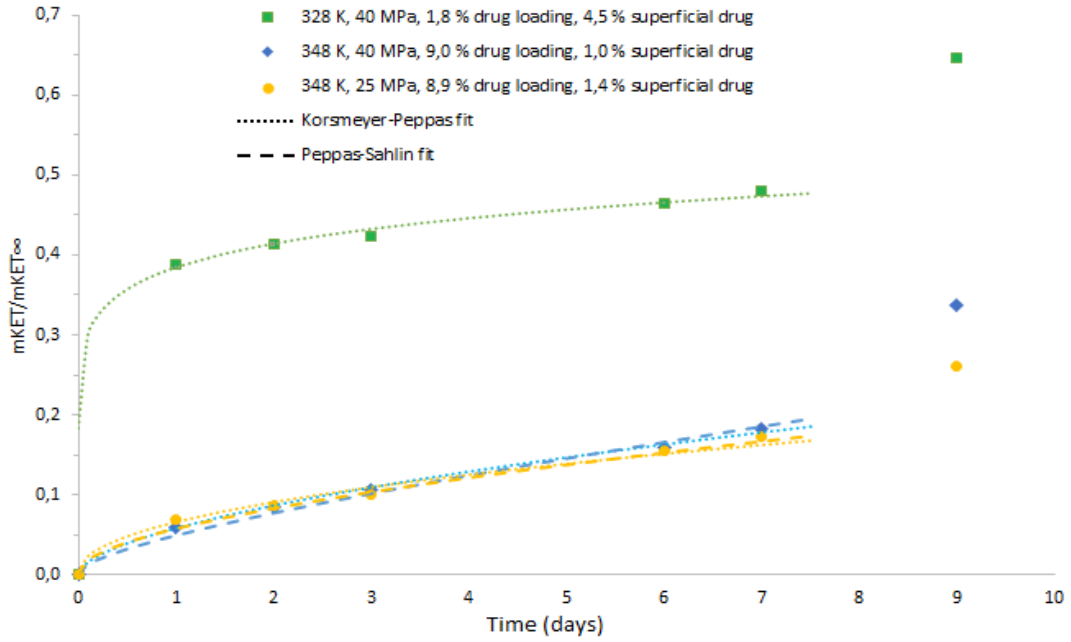

Figure 2: Ketoprofen release profile from impregnated PLA filaments and mathematical model fit

Experiments presenting an anomalous transport were adjusted to Peppas-Sahlin model, with an exponent $m = 0.45$ (pure diffusion for cylindrical geometry). Coefficients fitted are showed in Table 3.

Table 3: Adjusted Peppas-Sahlin model parameters

Conditions	Diffusion parameter ($days^{-m}$)	Degradation parameter ($days^{-2m}$)	k_2/k_1	Goodness-of-fit
348 K, 40 MPa	$k_1 = 0.029$	$k_2 = 0.020$	0.69	$R^2 = 0.99$
348 K, 25 MPa	$k_1 = 0.050$	$k_2 = 0.008$	0.16	$R^2 = 0.85$

According to equation 5, the polymer degradation becomes a more influential factor along time, which is in detriment to the Fickian diffusion. Thus, the ratio k_2/k_1 is related to the degradation rate of the polymer. In concordance, samples impregnated at 40 MPa and 348 K have a faster degradation than those impregnated at 25 MPa and 348 K.

As can be seen in figure 2, the adjustment lines of both models for these two samples notably fit, which evidence the diffusion as the main releasing mechanism in the first days of the experiment, where the degradation of the polymer did not influence overmuch the delivery of the drug.

4. Conclusions

This work reveals the feasibility of the supercritical impregnation of ketoprofen on polylactic acid for its use in the manufacture of biodegradable drug-releasing devices.

The study of the process shows that the operating pressure and temperature modify the drug loading. In general, an increase in both pressure and temperature favours the diffusion of CO_2 within the polymer, causing an increase in the porosity and that facilitates the drug loading, improving the impregnation yield.

The release studies show a kinetic governed by diffusion in the first days of contact with the releasing medium, with a higher earlier delivery in the samples whose operating conditions bring a more superficial drug impregnation.

These results are very promising since it seems the impregnation conditions would determine both the dosage of the drug and the response time, and therefore the devices obtained through this technology could be customized according to the patient's needs.

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