PLA-Starch Microparticles Containing Clays Focusing Controlled Release of Rifampicin

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Abstract

Polymer nanocomposites have been successfully used as excipients in pharmaceutical technology because of the convenient features that make them suitable for many applications, especially in controlled drug delivery therapies. The objective of this work was to prepare and characterize PLA and starch microparticles containing Viscogel B8 organophilic clay as a matrix for controlled release of rifampicin, one of the most spread drugs for tuberculosis treatment. The systems obtained by the spray drying technique were characterized by DRX, FTIR, SEM and low-field NMR. In addition, dissolution tests were performed on simulated gastric fluid. The micrographs indicate that the presence of the drug caused a small change in the shape and size of the particles. In the presence of the drug, the particles were spherical and presented wider size distribution. The XRD assays didn't show crystalline peaks of rifampicin in the microparticles, which suggests that rifampicin exists in an amorphous solid solution inside the starch-PLA particles. The mathematical model of Baker-Lonsdale was chosen for the treatment of the dissolution data, describing the controlled release of the drug from the matrix by a spherical diffusion process. The results suggest that the proposed release system may be used for the delivery of orally administered drugs, such as rifampicin.

Keywords

PLA, Starch, Drug Release, Nanotechnology

1. Introduction

Due to its various applications and functionality, especially in therapies for controlled drug release, polymers are among the most commonly used excipients in pharmaceutical technology. The choice of a polymer for a drug release system

depends on several factors that correlate to the properties of the active principle of polymer and other excipients in the formulation [1] [2] [3].

Starch, a polysaccharide of vegetable origin, is one of the most frequently used excipients in the manufacture of solid dosage forms, and it may be formulated as a filler and disintegrating agent or a coating agent. The starch is of great interest for the controlled release formulation due to its water swelling and gel barrier formation [4] [5] [6] [7].

Poly (lactic acid) (PLA) belongs to a type of polymer having hydrolysable groups in its chains, which are susceptible to biodegradation. It is a material of great interest in science as a biodegradable and bioabsorbable polymer with extensive use in biomedicine [8] [9] [10].

Starch and PLA nanocomposites with different nanoparticles are promising materials in the area of pharmaceutical technology, acting as a matrix in the modified drug release. The modified drug release has advantages such as: reducing the frequency of administration, with consequent improvement in patient adherence to therapy, reducing fluctuations of the drug in blood levels, reducing the adverse effects and reduction of treatment price [11]

Current treatment of tuberculosis involves oral administration and high long-term doses of various drugs. This treatment can lead to serious side effects such as liver damage. Moreover, it has a low patient acceptance [12].

Rifampicin (RIF) is the first-line drug for use in tuberculosis therapy and is included in the list of treatments recommended for the treatment of latent *Mycobacterium tuberculosis* infection in adults. Mainly due to the low solubility, rifampicin presents problems as its bioavailability [13].

The objective of this study was to develop and characterize a polymeric nanocomposite for controlled release of rifampicin to improve adherence to tuberculosis treatment, reducing the required doses and systemic side effects.

2. Materials and Methods

2.1. Samples

NatureWorks®PLA Polymer 2002D sample was supplied by NatureWorks LLC; potato starch was supplied by Yoki®. The organophilic clay Viscogel B8 was supplied by Bentec and the sodic clay NT25 by BentonitUnião Nord. Ind. e Com. Ltda.

2.2. Methods

2.2.1. Preparation of Polymer-Clay Suspensions Drug
First of all, rifampicin was solubilized with PLA (3% w/v) in chloroform, then slowly poured into gelled starch solution (2.5% w/v) with 0.5% PVAL. From that, an emulsion was obtained with high dispersing power (Ultra Turrax, model T18 Basic, IKA) for 20 min. Then, this emulsion was subjected to spray drying (Spray Dryer Bench—Labmaq, 0.5 MSD model).

Emulsions Viscogel B8 clays 1% concentration at 10 and 100 mg of rifampicin

was obtained. The particles obtained were immediately placed in a desiccator and stored at room temperature.

2.2.2. Drying Conditions

The process yield in percentages was calculated for each sample. The operating conditions maintained during the assays are summarized in Table 1.

2.2.3. Scanning Electron Microscopy

The SEM analysis was performed in order to identify the morphology (surface and shape) of particles obtained by spray drying using SEM Jeol Microscope JSM-5610 LV. The photomicrographs were obtained using a 20 Kv potential and zoom from 1000× to 8000×.

2.2.4. Preparation of Tablets

Tablets were prepared using a 300 psi manual press-molding compressor. Tablets with mixtures of starch and PLA have been prepared with Viscogel B8 containing 10 and 100 mg of rifampicin.

2.2.5. Dissolution in Simulated Gastric Fluid

The simulated gastric fluid (SGF) was prepared according to the specifications of U.S. Pharmacopeia 30 (USP, 2007). NaCl (2.0 g) was solubilized in a sufficient amount of distilled water. 7.0 ml of HCl were added to the NaCl solution and the volume was adjusted to 1.0 liter with distilled water. The pH of the solution was monitored during the addition of calibrated potentiometer acid and adjusted to 1.2 ± 0.1.

For the test, simulated gastric fluid in each vat was added to one tablet; 10 ml aliquots were taken every 1 h for each of the vats, replenishing the volume of gastric medium removed at a temperature of 37˚C. Assays were performed in a total time of 24 h [14].

2.2.6. Evaluation of Release Kinetics

The release kinetics of a formulation is an important parameter and should be assessed during the development phase, because from this analysis it is possible to evaluate the influence of certain parameters, such as crystallinity and solubility of active [15]. The data obtained from the release profile were placed on mathematical models according to different equations that describe drug release kinetics: Zero-order, First order, Higuchi, Hixon-Crowell, Baker-Lonsdale.

Table 1. Spray dryer operating conditions.

Operating conditions	
Flow rate (L/h)	0.3
Inlet temperature (˚C)	100
Outlet temperature (˚C)	65
Drying gas (L/h)	4

2.2.7. X-Ray Diffraction

X-ray analyses were carried out in a Rigaku D/Max 2400 diffractometer, with nickel-filtered CuK*α* radiation of wavelength 1.54 Å, at room temperature. The 2θ scanning range was varied from 2˚ to 30˚, with 0.02˚ steps, operated at 40 KV and 30 mA.

2.2.8. FTIR

The infrared spectra were recorded with a Varian 3100 FTIR spectrometer at room temperature. A zinc selenide (ZnSe) internal reflection element (IRE) with a fixed incidence angle of 45˚ was used for attenuated total reflection (ATR) measurements.

3. Results

After spray drying, the yields were calculated and the particles were characterized by scanning electron microscopy. Table 2 shows the yield of the material obtained after drying.

The main advantage of spray drying is that small droplets provide a high surface area which promotes mass and heat transfer, facilitating rapid evaporation. The drying time of a droplet is only a fraction of a second and the total time inside the dryer is only a few seconds. The resulting powder has a uniform particle size, which can control production costs; spray drying is a process that provides a post-dry and free-flowing, with spherical particles that are especially appropriate for preparing tablets due to their excellent flow properties and compression.

However, there are drawbacks to this technique, such as the size of the equipment and low thermal efficiency, since the air exiting the dryer should be hot enough to prevent moisture from condensing; similarly, large volumes of hot air, pass through the chamber without contact with the particles and thus, this hot air does not contribute directly to the drying process [16].

Another disadvantage that should be mentioned is the loss of material, due to the adhesion in the drying tower and the increased capillary adhesion. Nevertheless, the disadvantages can be minimized by adding a mannitol solution or by adding silica particles before atomization of the material or by proper adjustment of the drying parameters for the type of material studied [17] [18]. However, according to the literature, it can be seen that the yields are in agreement with results obtained by other authors.

Figure 1 shows micrographs of rifampicin (powder). Note the irregular size and shape of drug crystals.

Table 2. Yield of particles obtained by spray drying.

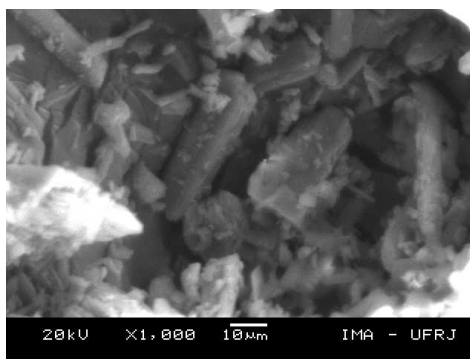| Microparticles	Start weight (g)	Final weight (g)	Yield (%)
PLA + Starch Rifampicin 10 mg	2.4	1.0	41.6
PLA + Starch + B8 1% Rifampicin 10 mg	3.4	1.0	29.4
PLA + Starch + B8 1% Rifampicin 100 mg	3.4	0.7	20.6

Figure 1. Micrograph of rifampicin (1000×).

Many drugs exist in crystalline form and are classified as polymorphs, solvates or hydrates, or amorphous. Such forms can undergo transitions during their production steps, which result in changes in Physico-chemical parameters, such as the rate of dissolution and the permeability of the molecule. The amorphous substances have convenient properties, such as increased solubility, dissolution rate, and improved compression characteristics with respect to their respective crystals, whereas amorphous solids are usually less physically and chemically stable [16] [19].

It is well known that rifampicin has variable solubility, mainly due to its particle size, purity level of crystals, electrostatic interactions and pH of the medium. At pH 6.8 the amorphous form has higher intrinsic dissolution of the isoforms I and II, at pH 2.0 isoform II contains less than the intrinsic dissolution and this amorphous form is slightly smaller than isoform I [20].

The reduction of particle size leads to an increase in the specific surface of the powder. The dissolution and absorption of the drug, content uniformity, and the stability of the pharmaceutical form are dependent. In many cases, it is necessary to reduce the particle size of both the drug and the adjuvants, trying to obtain the desired physical and chemical characteristics.

Poorly water-soluble drugs, whose absorption stage is limited by the efficiency of the dissolution process, will present better bioavailability when administered as finely divided particles, because of their improved contact area. This permits to overcome the issue of low solubility, increasing thus the drug release efficiency of poorly water-soluble drugs.

Figures 2-4 show micrographs of starch-PLA particles, starch-PLA with rifampicin and starch-PLA particles with rifampicin and Clay Viscogel B8 1 % obtained by spray drying with an increase of 1000× and 3000×, respectively.

The presence of the drug caused a small change in the shape and size of the particles, because without the drug, particles showed a more irregular appearance. In the presence of the drug, particles appear to be more spherical and presented wider size distribution; however, most of them were under 10 microns. This suggests the possibility of drug encapsulation; thus it does not observe the presence of irregular crystals of the drug.
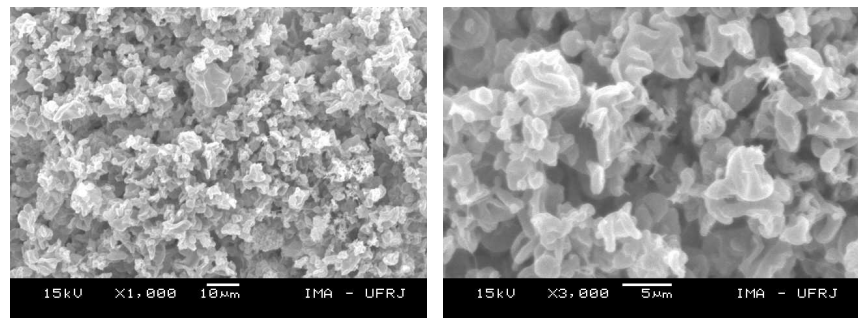
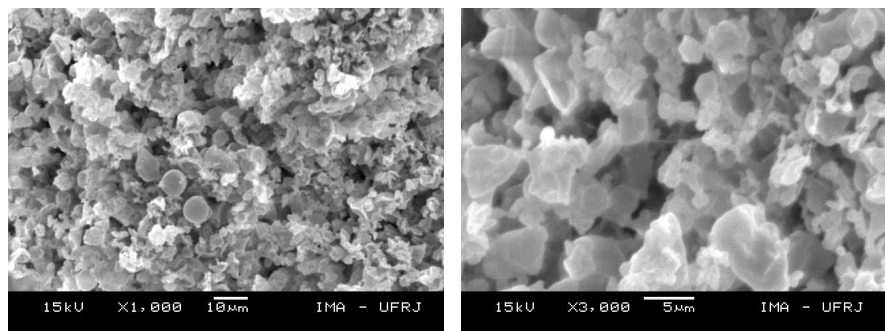**Figure 2.** Micrograph of starch-PLA particles obtained by spray drying (magnification of 1000× and 3000×).

Figure 3. Micrographs of the starch-PLA particles with rifampicin obtained by spray drying (magnification of 1000× and 3000×).
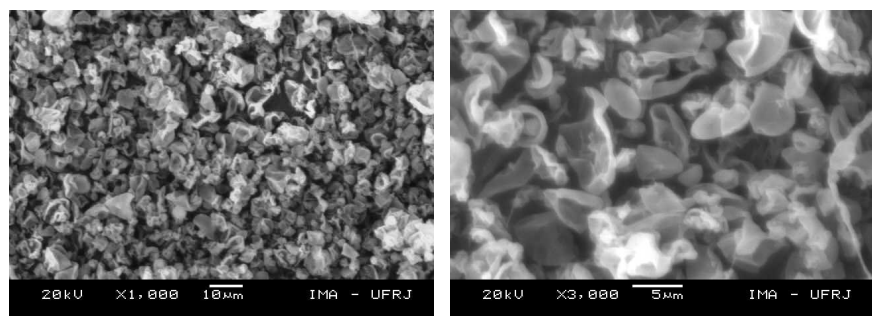

Figure 4. Micrographs of starch-PLA particles with clay Viscogel B8 1% and rifampicin obtained by spray drying (magnification of 1000× and 3000×).

In general, it was observed that the particles agglomerated and the presence of clays did not cause a significant difference in the physical appearance of the particles when observed at higher magnification.

The spray dried products are most often uniform. The particles have a characteristic form of hollow spheres, sometimes with a small orifice that results from the drying process [21].

X-ray diffraction

Regarding the mixture, it was observed the appearance of the characteristic peak of PLA at approximately 17˚ and the appearance of an amorphous halo related to the structure of the starch.

X-ray diffraction (XRD) analysis was used to determine the crystalline content

of rifampicina in the starch-PLA nanoparticles for all samples. Initially, the XRD diffractograms for the pure active substances were recorded. Rifampicin characteristic peaks can be identified in Figure 5.

No crystalline rifampicin was detected according to Figure 6. This suggests that rifampicin exists as amorphous solid solution in the starch-PLA particles.

FTIR

Since the starch-PLA particles were obtained by a spray-drying method using chloroform and water as solvents, solvent elimination was accompanied through the FTIR technique by monitoring chloroform band [22]. The peaks at about 1756 cm^{-1} and 1180 cm^{-1}, belonging to the CO stretching and C-O-C stretching of PLA, were visible in all the IR spectra [9] [15]. Drying process was stopped when the solvent peak was not detected in the FTIR spectrum (Figure 7).

Dissolution tests

The dissolution of a drug is the phenomenon by which a solid drug is released in its pharmaceutical form and then solubilized in the medium, which consequently causes its absorption.
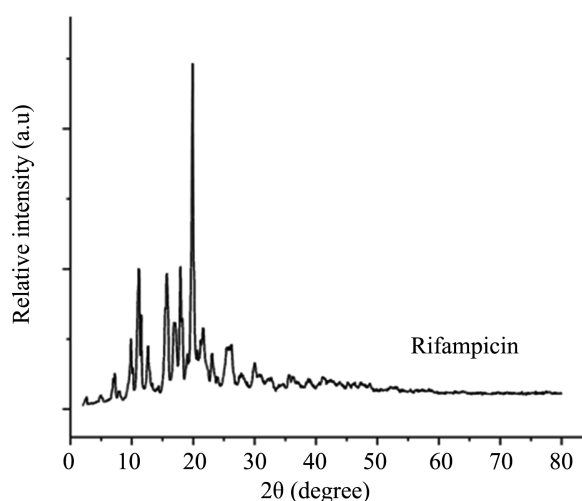

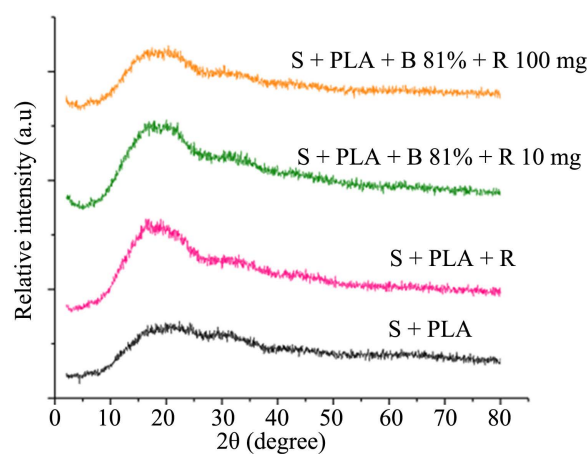Figure 5. XRD diffractograms for the pure rifampicin.

Figure 6. XRD diffraction of starch-PLA microparticles with organoclay and rifampicin.
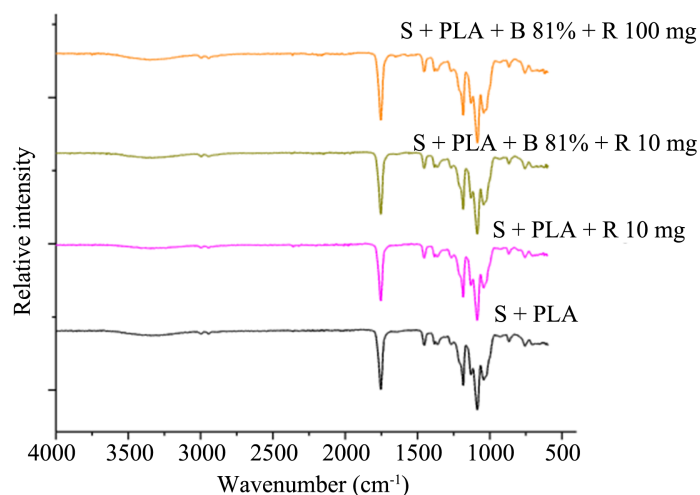
Figure 7. FTIR spectra of starch-PLA microparticles with and without rifampicin.

Thus, after administration by oral route in a solid dosage form, it occurs the disintegration process, in which the drug is released from the pharmaceutical form and remains as smaller particles. After this, the disintegration step occurs, where there is a size diminution of particles and finally the drug dissolution in the medium.

In the pharmaceutical industry, dissolution testing has become increasingly important in the development of solid and semi-solid dosage forms, because it predicts the release behavior of the active, as well as it is a quality control tool, since tests such as identification, purity, content and stability are not sufficient to ensure the clinical efficacy of the drug, among other applications.

In this study, the dissolution tests of compressed tablets were performed in simulated gastric fluid.

First, the calibration curve of rifampicin in simulated gastric fluid was obtained. The linear equation obtained was $y = 17.182x + 0.1376$ with linear correlation coefficient $r^2 = 0.9903$.

With the results from the linear regression analysis of the calibration curve, the rates of withdrawals could be quantified. This information permitted us to plot the amount of released drug versus time for the three systems (Figure 8).

Quantitative interpretation of values obtained during dissolution tests, is facilitated by using a general equation that mathematically reflects the dissolution curve as a function of some parameters related to the dosage form. In some cases, this equation can be derived via a theoretical analysis of the process as zero-order kinetics.

In most cases, however, there is no theoretical foundation, and it is used a more appropriate empirical equation. The type of drug, its polymorphous form, crystallinity, particle size, solubility and amount incorporated into the dosage form can influence the release kinetics [15]. Many mathematical models aiming to describe the drug release from the dosage form containing it have been developed.
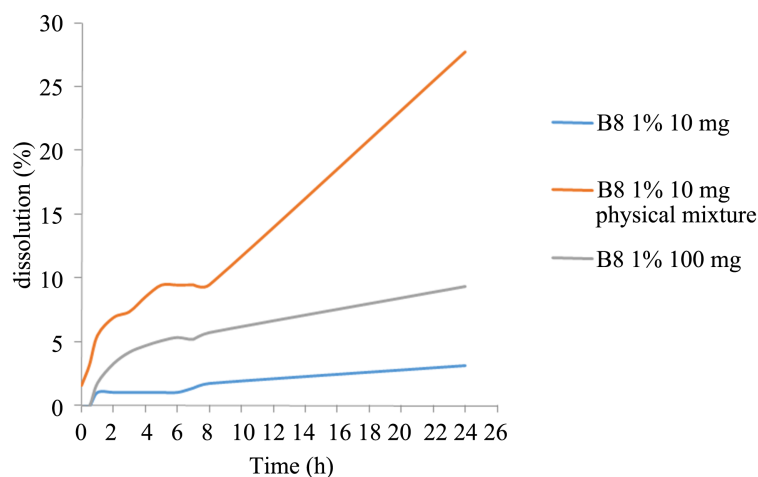
Figure 8. Percentage dissolution of rifampicin in simulated gastric fluid.

The models used in this work were: Zero-order, Higuchi, First order, Hixon Crowell and Baker Lonsdale in order to check the profile of the release kinetics of the systems studied. The results can be observed in **Table 3**.

The parameter to select the best model is the value of r^2. According to this parameter, the model chosen was a Zero-order for the system where the physical mixture of the microparticles without rifampicin ($r^2 = 0.97$) and the Baker and Lonsdale model for the systems 10 and 100 mg of rifampicin respectively were performed.

Dosage forms, which exhibit the release profile of zero-order, release the same amount of drug per unit time. This is ideal for the extended-release dosage forms model. The following expression represents this model:

$$Q_t = Q_0 + K_{0t} \tag{1}$$

where:

Q_t = is the amount of dissolved drug at time t;

Q_0 = is the initial amount of drug dissolved in the solution (most often $Q_0 = 0$);

K_0 = is the zero order release constant.

In this regard, the model developed by Baker and Lonsdale (1974) from the Higuchi model, describes the controlled release of the drug from its matrix by a spherical diffusion process. This equation has been used for linearizing release data for several microcapsules and microsphere formulations. The description of the model is shown in Equation (2).

$$\frac{3}{2}\left[1-\left(1-\frac{M_t}{M_\infty}\right)^{2/3}\right]-\frac{M_t}{M_\infty} = kt \tag{2}$$

where:

k = corresponds to the constant release;

to = time t;

M_t and M_∞ = the amount of drug released at time "t" and at infinity, respectively time.

Table 3. Applied mathematical models and r^2 values for systems with rifampicin.

Mathematic model	r^2 values		
	Starch + PLA + B8 1% rif. 10 mg	Starch + PLA + B8 1% rif 10 mg (physical mixture)	Starch + PLA + B8 1% 100 mg de rif.
Zero order	0.85	0.97	0.75
Higuchi	0.89	0.88	0.94
First order	0.85	0.96	0.77
Hixon-Crowell	0.85	0.95	0.76
Baker-Lonsdale	0.95	0.92	0.98

This model is applicable if there is a linear correlation with the first term of the equation as a function of time. Linear correlation coefficients of 0.95 and 0.98 were obtained for tablets with 10 and 100 mg rifampicin, confirming that diffusion is the main phenomenon involved in drug release.

The solid oral dosage forms of modified release are acquiring increasing importance due to patient acceptance and the therapeutic advantages are compared to the corresponding conventional solid dosage forms release.

Because of this fact, and following the developments with regard to formulations, it is necessary to develop dissolution studies, which reproduce *in vivo* the results obtained *in vitro*.

4. Conclusion

The present study obtained promising results in relation to the formulation obtained; the micrographs showed the crystallinity and irregularity of rifampicin, which was not observed in the starch and PLA particles, suggesting a possible encapsulation of the drug. The XRD diffractograms suggest that rifampicin exists as an amorphous solid solution in the starch-PLA particles. To explain the dissolution test results, the model chosen was a Zero-order for the system where the physical mixture of the microparticles without rifampicin and the Baker and Lonsdale model for the systems with 10 and 100 mg of rifampicin, respectively. The results suggest that the proposed release system (tablets containing the drug and incorporated into microspheres) may be used for the delivery of orally administered drugs, such as rifampicin. However, the formulation proposed in this work needs to be optimized to obtain a dosage suitable for the oral administration of rifampicin.

Acknowledgements

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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