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# PLGA nanoparticles containing praziquantel: effect of formulation variables on size distribution

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**Abstract**

Praziquantel has been shown to be highly effective against all known species of Schistosoma infecting humans. Spherical nanoparticulate drug carriers made of poly(D,L-lactide-*co*-glycolide) acid with controlled size were designed. Praziquantel, a hydrophobic molecule, was entrapped into the nanoparticles with theoretical loading varying from 10 to 30% (w/w). This study investigates the effects of some process variables on the size distribution of nanoparticles prepared by emulsion–solvent evaporation method. The results show that sonication time, PLGA and drug amounts, PVA concentration, ratio between aqueous and organic phases, and the method of solvent evaporation have a significant influence on size distribution of the nanoparticles.


*Keywords:* Nanoparticles; Controlled release; PLGA; Praziquantel; Emulsification evaporation method

## 1. Introduction

Schistosomiasis is a serious public health problem in tropical countries. Praziquantel is a broad-spectrum anti-helmintic drug. The drug has proved to be especially useful in the treatment of schistosomiasis (Meier and Blaschke, 2001). The failure of mass treatment to control schistosomiasis has been attributed to the fact that therapy is not sufficiently long lasting (Akbarieh et al., 1992). This effect can occur because of the low bioavailability of praziquantel due to its low hydrosolubility (El-Arini and Leuenberger, 1998). Another factor that influences the effectiveness of the treatment is the fast drug metabolism, evidenced by the low effectiveness against younger forms, which, being in systemic circulation, are less exposed to praziquantel (Xiao et al., 1985; El-Arini and Leuenberger, 1998). Furthermore, high oral doses are necessary to overcome first pass metabolism and thereby achieve sufficient drug concentrations at the larval tissue (Becket et al., 1999).

Praziquantel is administered to humans only by the oral route. If alternative routes of delivery, such as

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parenteral formulations, could be considered, drugs administered by such routes will achieve direct systemic delivery, thereby avoiding first pass hepatic metabolism and reaching a reduction in the dose delivered (El-Arini and Leuenberger, 1998; Becket et al., 1999).

Nanoparticles is a collective name for nanospheres and nanocapsules. Nanospheres have a matrix type structure, where active compounds can be adsorbed at their surface, entrapped or dissolved in the matrix. Nanocapsules have a polymeric shell and an inner core. In this case, the active substances are usually dissolved in the core, but may also be adsorbed at their surface (Allemann et al., 1998; Nishioka and Yoshino, 2001; Soppimath et al., 2001; Panyam and Labhasetwar, 2003).

Nanoparticles or colloidal carriers have been extensively investigated in biomedical and biotechnological areas, especially in drug delivery systems for drug targeting because their particle size (ranging from 10 to 1000 nm) is acceptable for intravenous injection (Allemann et al., 1998; Jeon et al., 2000; Soppimath et al., 2001).

Depending on the desired administration way, the size of the carriers should be optimized. Thus, if the carrier size is under 1 μm, an intravenous injection (the diameter of the smallest blood capillaries is 4 μm) is enabled and this carrier size is also desirable for intramuscular and subcutaneous administration, minimizing any possible irritant reactions (Görner et al., 1999; Hans and Lowman, 2002).

Although a number of different polymers have been investigated for formulating biodegradable nanoparticles, poly(L-lactic-acid) (PLA) and its copolymers with glycolic acid (PLGA) have been extensively used for controlled drug delivery systems (Park, 1995; Vert et al., 1998; Uhrich et al., 1999; Jain, 2000; Soppimath et al., 2001; Hans and Lowman, 2002; Sahoo et al., 2002). The lactide/glycolide polymers chains are cleaved by hydrolysis into natural metabolites (lactic and glycolic acids), which are eliminated from the body by the citric acid cycle. PLGA provides a wide range of degradation rates, from months to years, depending on its composition and molecular weight (Brannon-Peppas, 1995; Anderson and Shive, 1997; Görner et al., 1999; Uhrich et al., 1999; Jain, 2000; Burkersroda et al., 2002; Panyam and Labhasetwar, 2003).

Thus, the goal of our study was to design a nanoparticulate drug system with a drug controlled delivery, based on the biodegradable polymer PLGA. The active substance used was praziquantel, a hydrophobic molecule and ideal model drug for incorporation in systems prepared by emulsion–solvent evaporation technique.

## 2. Materials and methods

### 2.1. Materials

The polymer studied was poly(D,L-lactide-*co*-glycolide acid) (PLGA), with a copolymer ratio of DL-lactide to glycolide of 50:50 ($M_w$ 40,000–100,000 g/mol as indicated by the supplier, Sigma Chemical CO, USA). The surfactant used in the emulsification process was poly(vinylalcohol) (PVA) (87–89% hydrolysis degree and molecular mass 12,000–13,000 g/mol, Sigma Chemical CO, USA). The organic solvent was methylene chloride (Labsynth Ltd., Brazil). As suspending medium, purified water (Milli-Q, Millipore Corporation, Billerica, MA) was used. The encapsulated drug was praziquantel (Henrifarma, Brazil). Acetonitrile (Mallinkrodt, HPLC grade) was used in the analytical method.

### 2.2. Preparation of nanoparticles

The nanoparticles, loaded or not with praziquantel, were prepared by an emulsion–solvent evaporation method. Typically, a solution of 25 mg of PLGA in 1 mL of methylene chloride containing or not praziquantel (10–30% w/w), was mixed with 10 mL of 0.3% PVA aqueous solution. This mixture was homogenized for 1 min by vortex and then sonicated using a microtip probe sonicator set at 55 W of energy output (XL 2002 Sonicator® ultrasonic liquid processor) during 1 min to produce the oil-in-water emulsion. The organic phase was evaporated during 20 min using a rotative evaporator under partial vacuum. The nanoparticles were recovered by ultracentrifugation (21,000 rpm, 25 min, Hitachi). The amount of non-entrapped praziquantel in the supernatant was determined by HPLC, as described later. The nanoparticles were washed twice with water in order to remove the adsorbed praziquantel. The washing solutions were eliminated by a further centrifugation as described above. The purified nanoparticles were freeze-dried.

### 2.3. Nanoparticles characterization

The nanoparticles size distribution was determined in bidistilled water at 30 °C by photon correlation spectroscopy (PCS) using a particle size analyzer (Brookhaven Instruments Corp.). For the measurements, 1 mL of the nanoparticles suspension was dispersed in 5 mL of distilled water and sonicated during 1 min. The analyses were performed at a scattering angle of 90° and at a temperature of 25 °C. For each sample, the mean diameter and the standard deviation of ten determinations were calculated using multimodal analysis.

The morphology of nanoparticles was observed by scanning electron microscopy (SEM) (JEOL JSM T330A). A drop of the nanoparticles suspension was placed on a metallic surface. After drying under vacuum, the sample was coated with a gold layer. Observations were performed at 10 and 20 kV.

### 2.4. Determination of praziquantel entrapment

The amount of non-entrapped praziquantel was determined by HPLC by UV detection set at 262 nm (Varian ProStar 330). The mobile phase consisted of acetonitrile:water (3:2) and the flow rate was set at 1 ml/min. Separation was achieved using a Lichrospher C18 column (240 mm × 4 mm, 5 μm). This method was also used to determine the non-incorporated praziquantel in the supernatant after the nanoparticles formation (indirect method). The supernatant containing free praziquantel was separated from solid nanoparticles by ultracentrifugation as described in Section 2.2.

The amount of praziquantel entrapped in the nanoparticles was determined after their dissolution in methylene chloride (direct method). The solutions were passed through a membrane filter (pore size 0.22 μm, Millipore) before HPLC measurements.

### 2.5. In vitro release profiles

For the in vitro release of PRZ, the diffusion cell model was used. A spectrophotometer cell, with 1 cm of optical way and 2.5 mL of volume, was used as diffusion cell. A cellulose acetate membrane was adapted to the terminal portion of a glass cylinder. The cylinder was coupled to the diffusion cell containing the receptor phase (2 mg/mL sodium lauril sulphate, aqueous solution) (USP XXVI) at 37 °C. Amounts of nanoparticles containing PRZ, sufficient for establishing sink conditions were weighed and put over the membrane. At different time intervals, aliquots of 100 μL were withdrawn, filtered and the PRZ concentration was assessed by HPLC. The chromatographical conditions were: mobile phase comprised of a mixture of acetonitrile:water (3:2), flow rate of 1 mL/min, UV–vis detector set at 262 nm and injection volume of 100 μL. The measurements were performed twice for each batch.

## 3. Results and discussion

### 3.1. Effect of preparation variables on formulation characteristics

By using the emulsion–solvent evaporation technique, several process parameters were assessed in order to achieve optimal preparation conditions, including time of sonication, PLGA content in the formulation, surfactant content in the formulation, evaporation rate of organic solvents, aqueous to organic phase volume ratio and praziquantel content. Only one parameter was changed in each series of experiments.

#### 3.1.1. Sonication time

In order to obtain emulsified systems, the addition of energy is a fundamental step. To verify the influence of this factor on nanoparticles shape and size distribution, sonication time was varied between 1 and 20 min. The results are presented in Table 1. The preparation procedure gave spherical particles in all cases (according to SEM experiments, Fig. 1A). From the results obtained, it can be concluded that the increase in the sonication

Table 1
Influence of sonication time on nanoparticles mean diameter and granulometric distribution

| Sonication time (min) | Mean diameter (nm) | Polydispersity | Size distribution |
|---|---|---|---|
| 1 | 380 ± 23 | 0.23 | 67% (245–349 nm)<br>43% (932–1324 nm) |
| 5 | 335 ± 16 | 0.19 | 20% (90–200 nm)<br>80% (480–550 nm) |
| 10 | 298 ± 25 | 0.24 | 19% (151–202 nm)<br>81% (511–645 nm) |
| 20 | 255 ± 10 | 0.22 | 100% (256–265 nm) |

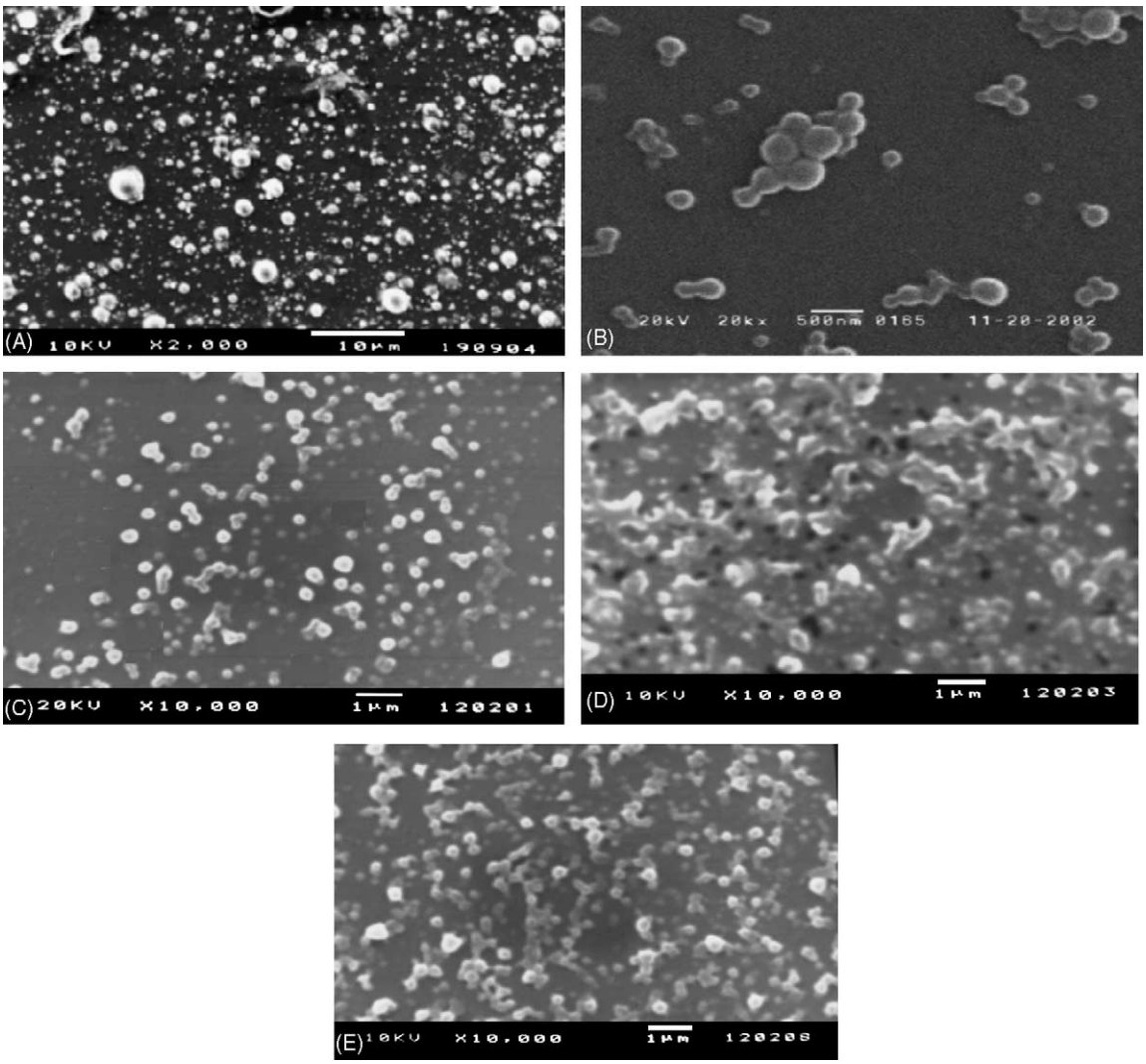


Fig. 1. (A) Nanoparticles prepared with 1 min of sonication. (B) Nanoparticles prepared with 12.5 mg de PLGA. (C) Nanoparticles prepared with 50 mg de PLGA. (D) Nanoparticles prepared with 0.7% de PVA. (E) Nanoparticles prepared with 20% of praziquantel.

time leads to a reduction in the nanoparticles mean diameter. Another important factor observed was that an increase in time sonication leads to a narrower granulometric distribution. The particles prepared with 20 min of sonication showed a monomodal distribution profile, while the nanoparticles prepared with 1, 5 and 10 min showed two families of particles with different sizes. With the larger time of sonication (20 min), the high energy released in the process leads to a rapid dispersion of polymeric organic phase as nanodroplets of small size and monomodal distribution profile. The emulsification can be considered one of the most important steps of the process, because an insufficient dispersion of phases results in large particles with wide size distribution. The final size of the nanoparticles depends on the globule size throughout the emulsification process. A reduction of the emulsion globule size allows the formation of smaller nanoparticles. Our results are in accordance with those observed by other authors (Quintanar-Guerrero et al., 1996; Kwon et al., 2001).

Table 2
Influence of PLGA content on nanoparticles mean diameter and granulometric distribution

| PLGA content (mg) | Mean diameter (nm) | Polydispersity | Size distribution |
|---|---|---|---|
| 12.5 | 243 ± 12 | 0.05 | 5% (114–125 nm)<br>95% (212–276 nm) |
| 25 | 255 ± 10 | 0.22 | 100% (256–265 nm) |
| 50 | 360 ± 25 | 0.08 | 85% (280–375 nm)<br>15% (895–1129 nm) |

### 3.1.2. PLGA content

PLGA content was varied between 12.5 and 50 mg, and the influence of the initial mass of polymer on the particles morphology and size distribution was studied. The results are listed in Table 2. According to SEM experiments (Fig. 1B and C), nanoparticles prepared with 12.5 mg of PLGA presented spherical shape and absence of both agglomeration and amorphous polymer. However, nanoparticles prepared with 50 mg of PLGA presented a non-spherical shape, presence of agglomerates and some amorphous polymer. When the amount of PLGA was doubled from 25 to 50 mg, the particle diameter increased from 260 to about 359 nm, a monomodal profile remaining only when 25 mg of PLGA were used. The increase on the particle size with an increasing polymer concentration was observed by other authors for PLA and PLGA polymers (Quintanar-Guerrero et al., 1996; Murakami et al., 1999; Kwon et al., 2001; Chorny et al., 2002). The polymer concentration in the internal phase of the emulsion was another important factor, since the size of nanoparticles increased as polymer concentration was also increased. This was probably caused by the increasing viscosity of dispersed phase (polymer solution), resulting a poorer dispersability of the PLGA solution into the aqueous phase. There is a high viscous resistance against the shear forces during the emulsification. Coarse emulsions are obtained at higher polymer concentrations, which lead to the build of bigger particles during the diffusion process. This fact is explained by the greater probability that the desolvated macromolecules (or small aggregates formed from these molecules) coalesce in a more concentrated solution, thereby forming larger coacervates or particles (Quintanar-Guerrero et al., 1996).

Table 3
Influence of PVA content on nanoparticles mean diameter and granulometric distribution

| PVA content (% w/v) | Mean diameter (nm) | Polydispersity | Size distribution |
|---|---|---|---|
| 0.15 | 335 ± 31 | 0.087 | 100% (280–390 nm) |
| 0.30 | 260 ± 10 | 0.22 | 100% (256–265 nm) |
| 0.70 | 242 ± 15 | 0.19 | 100% (239–265 nm) |

### 3.1.3. Surfactant content

In order to study the influence of PVA content on the nanoparticles properties, some batches were prepared by using an external aqueous phase consisting of PVA at different concentrations. The results are shown in Table 3. It can be observed, that there was a decrease in particle size (345–242 nm) when the PVA concentration in the external aqueous phase was increased from 0.15 to 0.7% (w/v). A high concentration of emulsifier leads to a reduced size of the nanoparticles produced. It was also observed, that the granulometric distribution became narrower as the amount of PVA was increased. This phenomenon can be expected from the stabilizing function of an emulsifier. It is easy to understand that an insufficient amount of emulsifier would fail in stabilizing all the nanoparticles and thus some of them would tend to aggregate. As a result, nanoparticles with larger size would be produced (Feng and Huang, 2001). In the emulsion–solvent evaporation method, the emulsification and stabilization of the globules are crucial factors. The amount of surfactant plays an important role in the emulsification process and in the protection of the droplets, because it can avoid the coalescence of globules (Quintanar-Guerrero et al., 1996; Murakami et al., 1999; Kwon et al., 2001). SEM experiments (Fig. 1D) showed that the nanoparticles prepared with 0.15% PLGA presented spherical shape, but some agglomeration was present where some particles fuse together one by one. This phenomenon is perfectly predictable since the process of globules stabilization was made difficult at low surfactant contents. The nanoparticles obtained with greater surfactant concentrations showed spherical shape and absence of agglomerates (SEM images not shown).

### 3.1.4. Organic solvent evaporation rate

In order to verify the influence of organic solvent evaporation rate on the mean diameter and size

Table 4
Influence of solvent evaporation method on nanoparticles mean diameter and granulometric distribution

| Internal phase volume (mL) | Mean diameter (nm) | Polydispersity | Size distribution |
|---|---|---|---|
| 1 | 298 ± 23 | 0.23 | 19% (159–200 nm)<br>81% (510–640 nm) |
| 2 | 275 ± 16 | 0.11 | 100% (271–281 nm) |
| 3 | 248 ± 25 | 0.08 | 5% (105–114 nm)<br>95% (224–278 nm) |

distribution, two methods of solvent evaporation were tested: one of them using a vacuum rotative evaporator and the other using magnetic stirring under normal pressure. The results are listed in Table 4. It was observed that when the former method was used the nanoparticles presented smaller diameter than the particles obtained by the latter method. Besides, it was possible to perform the solvent evaporation in a shorter time with the aid of reduced pressure, what is extremely important in the drug entrapment, because minimizes its diffusion to the aqueous external phase. The reason for the formation of smaller particles is the higher solvent front kinetic energy. A critical parameter determining the particle size seems to be the rate of diffusion of the organic solvent through the interface. At higher diffusion rates smaller particles are obtained. Increased solvent front kinetic energy causes a higher degree of the droplet dispersion in the aqueous phase. Therefore, the local concentration of the oil droplets in the aqueous phase is decreased and the diffusion rate is higher, thus resulting in smaller particles (Jung et al., 2000). During the solvent evaporation process, there is a gradual decrease of the dispersion volume and consequently an increase of the viscosity of the dispersed droplets. This affects the droplet size equilibrium, involving the processes of droplet coalescence and agglomeration during the early step of the solvent removal (Lamprecht et al., 2001). Taken it into account, it is extremely important that the solvent evaporation occurs in the shorter possible time.

The residual solvent present in nanoparticles was assessed by HPLC and for all batches it remained below 5 ppm.

### 3.1.5. Aqueous to organic phase volume ratio

The ratio between external and internal phases of emulsion is of great importance to its stability and influences the size of dispersed globules. The organic internal volume was varied between 1 and 3 mL, and its influence on mean diameter and size distribution of nanoparticles was observed. The results are presented in Table 5. It can be seen that an increase in the internal/external ratio leads to a slight decrease of the nanoparticles' average size for a given polymer concentration. This occurs because the coalescence of droplets can be prevented by a large amount of organic solvent available for diffusion in the O/W emulsion.

### 3.1.6. Praziquantel content

In this step the incorporation of praziquantel into PLGA nanoparticles was examined. Maintaining a constant initial mass of polymer (25 mg), the mass of praziquantel used was varied between 10 and 30% in relation to polymer mass. Table 6 shows the results of the influence of drug amount on nanoparticles mean diameter and size distribution. It can be observed that the increase in the initial loading of praziquantel increases the nanoparticles mean diameter and the granulometric distribution became wider. This can be explained by the fact that a greater amount of drug results in a more viscous dispersed phase, making difficult the mutual dispersion of the phases and originating larger particles. SEM experiments (Fig. 1E) showed that the particles remained with a spherical shape in all cases. The encapsulation efficiency of the nanoparticles containing 10, 20 and 30% of praziquantel (theoretical loading) is illustrated in Fig. 2. The direct and indirect

Table 5
Influence of internal phase volume on nanoparticles mean diameter and granulometric distribution

| Solvent evaporation method | Mean diameter (nm) | Polydispersity | Size distribution |
|---|---|---|---|
| Vacuum rotative evaporator (20 min) | 298 ± 35 | 0.24 | 19% (151–202 nm)<br>81% (511–645 nm) |
| Magnetic stirring (3 h) | 390 ± 27 | 0.15 | 33% (261–309 nm)<br>67%(606–747 nm) |

Table 6
Influence of theoretical praziquantel content on nanoparticles mean diameter and granulometric distribution

| Theoretical praziquantel content (% w/w) | Mean diameter (nm) | Polydispersity | Size distribution |
|---|---|---|---|
| 10 | 250 ± 23 | 0.067 | 100% (230–295 nm) |
| 20 | 280 ± 16 | 0.081 | 100% (273–370 nm) |
| 30 | 340 ± 25 | 0.115 | 100% (283–486 nm) |

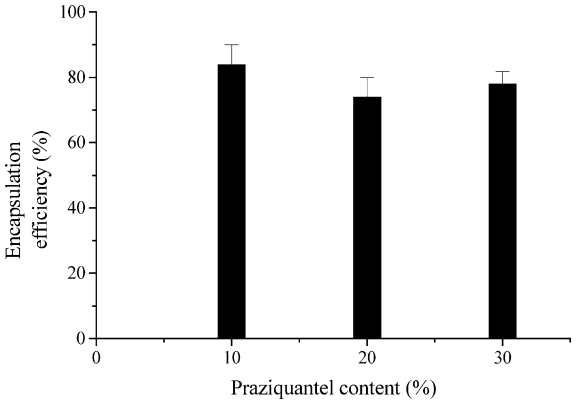


Fig. 2. Encapsulation efficiency (%) by different praziquantel contents.

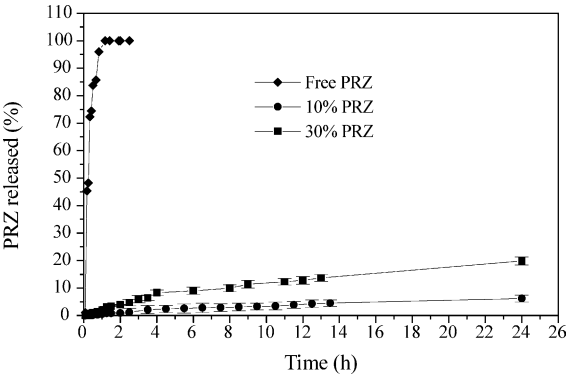


Fig. 3. In vitro release kinetics of PRZ nanoparticles.

methods of drug assay showed similar results. It was observed that the encapsulation efficiency was practically the same for different praziquantel contents (82 ± 5%).

### 3.2. In vitro release profiles

The PRZ released was studied as a function of time. Nanoparticles containing the minimum (10%) and maximum PRZ loading (30%) (theoretical loadings) were studied. The results over 24 h are shown in Fig. 3.

The results of the assay show that there was a pronounced time prolongation of drug release from nanoparticles in relation to the non-encapsulated drug. While about 100% of non-encapsulated drug were found after approximately 2 h, only 6 and 20% of PRZ were released from nanoparticles after 24 h from batches containing, respectively, 10 and 30% of drug. An important phenomenon observed here is that as bigger the amount of drug present in nanoparticles then more quickly the release occurred, and the particles with smaller PRZ amounts exhibited a release in a more sustained fashion.

## 4. Conclusions

The emulsion–solvent evaporation method allowed the preparation of spherical drug-loaded systems of biodegradable PLGA carrier containing an anti-schistosoma drug, praziquantel, incorporated in the polymeric matrix. The process of nanoparticles formation was related to the interfacial area generated by emulsion formation and reduction of globule size due to the fast solvent diffusion. Both the emulsification process and the stability of emulsion globules were the most important factors to control the particles size. The homogeneity of size and shape are important characteristics, because some behaviors can be foreseen with more security. Preparative variables such as concentration of stabilizer and polymer, time of sonication, diffusion rate of organic solvent, and ratio between external and internal phases, showed to be important factors for the formation of PLGA nanoparticles. Release kinetics of PRZ was governed by the initial drug loading, higher initial drug loadings resulting in faster drug release.

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