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# Nimesulide PLA microspheres as a potential sustained release system for the treatment of inflammatory diseases

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**Abstract**

Polylactic acid (PLA-L) microspheres were prepared as a biodegradable polymeric carrier for a non-steroidal anti-inflammatory drug, nimesulide. The preparation of this system was performed by the classical emulsion solvent-evaporation method. Size analysis of the microparticulate system showed that unloaded and loaded nimesulide-PLA microspheres had average diameters of about 42.9 nm and 2.1 μm, respectively. Scanning electron microscopy (SEM) of loaded and unloaded microsphere samples showed that the particles shape were perfectly spherical, the loading efficiency of nimesulide in PLA microspheres was 70%; Thus, the microparticle system evaluated in this work showed the potential to act as a sustained release system for nimesulide: in vitro dissolution profiles showed the PLA-L microparticles were able to sustain the release of the drug for a considerable period of time (28.7% within 108 h).


*Keywords:* Polylactic acid; Nimesulide; Microspheres; Sustained release system; Emulsion solvent-evaporation method

## 1. Introduction

Nimesulide (4 nitro-2 phenoximethanosulfonil-amid) is a potent and widely used nonsteroidal anti-inflammatory, antipyretic and anesthetic drug (NSAID) showing local effects. This drug is five to sixteen times more selective for the inhibition of cyclooxygenase-2 (COX-2), than other drugs of the same class; it has also been employed by peripheral administration, for regional management of acute and chronic major pain (Tognella, 1993). Nimesulide's usefulness is limited by a short duration of action and the loss of its inhibitory COX-2 selectivity, when administrated orally. Repeated injections have been required to achieve long lasting and constant patient pain relief. Complications may result from these techniques, which in certain cases are contra indicated. Long-acting formulations acting by single-dose injection would be a clinically important means of extending the time of action of nimesulide. The preparation of nimesulide aqueous

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formulations is rather difficult; thus, a sustained release microparticle system could be an interesting and suitable way for its administration by promising to extend the release and period of action of this drug.

Polymeric micro/nanoparticles are being increasingly investigated for sustained release effects and to achieve target drug delivery. Colloidal drug carriers are interesting in this field because their small particle size, allows their permeation through biological barriers (Couvreur et al., 1995).

A wide variety of polymeric particulate carriers have been devised to protect active molecules from inactivation by the host and to control drug release in body fluids.

Special attention has been paid to the biodegradability of polymers in order to prevent chronic toxicity encountered in special after parenteral administration of non-biodegradable polymers. Microparticles made from biodegradable polymers, such as poly lactide-co-glicolic (PLGA) (O'Hagan et al., 1991), poly D-,L-lactide (PLA) (Wada et al., 1990) and poly glicolide (PGA) (Redmon et al., 1989) have been studied as delivery systems for controlled-release vaccines, cytostatics and insulin.

Polylactic acid (PLA) microspheres have been investigated for the past 15 years as a long-acting, injectable drug delivery system. Since the properties of polymers can be controlled by their molecular weight and monomer composition, the in vitro releasing behavior of drugs from microsphere systems may differ, many products have been investigated. A wide range of synthetic, as well as of biological drugs (enzymes, hormones and proteins), have been microencapsulated. Microspheres have been manufactured by various techniques, including solvent evaporation (Mortada, 1982; Uchida et al., 1986) and phase separation (Jalsenjak and Kondo, 1981; Deasy et al., 1980), using non-solvent addition or solvent partition. The simplest and most commonly employed method, oil-in-water (O/W) emulsion solvent-evaporation, showed a good encapsulation rate of water insoluble compounds. For this reason, it was employed in the present study.

The potenciality of site specific drug delivery for the optimization of drug therapy (Douglas et al., 1987) has given impetus to significant advances in the engineering of novel dosage forms like nano/microparticles, i.e. solid colloidal polymeric carriers. A nano/microparticle system with maximal drug loading and a high entrapping efficiency should reduce the quantity of carrier required for the administration of therapeutically sufficient amounts of active compounds (Thirumala et al., 1999).

Biodegradable nano/microparticles have shown promise in controlling the biodistribution and elimination pattern of drugs following parenteral administration; preparation techniques for injectable nano/microparticles formulations have been developed and improved over the past two decades (Michael et al., 2002). The major aim of the present study was to prepare and characterize a parenterally administrable nimesulide microparticle system, evaluating its loaded and unloaded microparticle dispersion parameters, mean diameter, encapsulation efficiency, morphology, drug releasing profile and kinetic properties.

## 2. Materials and methods

### 2.1. Materials

Polylactic acid (PLA-L) was purchased from Boehringer Ingelheim, Germany. Nimesulide (MW 308.31, mp 143–144.5, oral $LD_{50}$ in rats 324 mg/kg, solubility in water of 10 μg/ml at 25 °C) was purchased from Galena, a chemical products distributor from Campinas, São Paulo, Brazil. Polyvinil alcohol (PVA, 99–100% hydrolyzed, MW 85,000–146,000) was purchased from Mallinckrodt Chemical®; dialysis tubing (12,000 Da) was purchased from Sigma-Chemicals, St. Louis, USA. Solvents like acetonitrile (HPLC grade), methanol (HPLC grade), chloroform (HPLC grade), ethanol (HPLC grade) were used as delivered by Sigma, St. Louis, MO. Deionized water was used in the preparation of aqueous phase and potassium phosphate buffer used in the mobile phase.

Equipment: Ultra Turrax T 25 basic IKA–Works; Column $C_{18}$ Cat. 1.50943 Lichrospher 100 RP-18 (5 μm), Lot 1497117; scanning electron microscope, Leica Stereoscan 440; light scattering autosizer 4700, Malvern, USA and dissolution equipment SR8 Plus Q-Pak, Hanson Corporation, Chatsworth, CA, USA.

### 2.2. Methods

#### 2.2.1. Microsphere preparation

The preparation of PLA-L microparticles was performed by the classical emulsion solvent-evaporation

method (Niwa et al., 1993; Birnbaum et al., 2000). A 1%, 3% or 8% (w/v, 50 ml) of PVA aqueous solution was prepared by heating and stirring during PVA addition. The organic phase containing different amounts of PLA-L (0.05; 0.10 or 0.15%) dissolved in chloroform (5 ml) was then slowly added to the aqueous phase during around five minutes under stirring at about 11,000 rpm using the Ultra Turrax equipment. The microparticle suspension was subsequently left under magnetic stirring at a controlled temperature of 25 °C for 4 h; thus, all the chloroform had evaporated, the spherical microparticle were produced.

Samples produced were identified as (A) PVA 1% and PLA-L 0.10%, (B) PVA 3% and PLA-L 0.10%, (C) PVA 8% and PLA-L 0.10%, (D) PVA 3% and PLA-L 0.05% and (E) PVA 3% and PLA-L 0.15%. For the first three samples, we could choose the adequate amount of surfactant and for the other two samples the suitable amount of polymer was determined.

After the determination of these adequate amounts of polymer and surfactant, the formulation was prepared with nimesulide (7.5 mg) dissolved in the organic phase (sample F). The separation of the nimesulide microparticles was performed by centrifugation (3000 rpm; 15 min), thus, washing with water for three times to remove PVA and final sample lyophilization.

### 2.2.2. Characterization of the PLA-L microspheres

The shape and surface topography of the dry microparticles were observed under the scanning electron microscope. Particle diameters and distribution of microspheres dispersed in an aqueous system (1 ml of microparticle suspension in 9 ml of water) were measured by means of a dynamic light scattering method.

### 2.2.3. Determination of the drug encapsulation rate

Nimesulide was assayed by high performance liquid chromatography (HPLC) as described by Ptacek et al. (2001).

The system consisted of an SPD-10A Shimadzu detector, LC-10AD, pump, and a $C_{18}$ Cat. 1.50943 Licrhospher 100 RP-18 (5 μm) column. The mobile phase was acetonitrile/methanol/potassium phosphate buffer, 15 Mm (30/5/65, v/v/v) adjusted to pH 7.3 with KOH (0.1N). Total sample volume was 20 μl, nimesulide was detected at 404 nm, with a retention time of about 3.7 min at 30 °C.

Nimesulide PLA-L microparticles in powdered form were obtained by lyophilization after the microparticles washed with water, and the surfactant PVA and the nonencapsulated drug had been removed following repeated centrifugation at 3000 rpm. A known amount of weighed lyophilized microparticles (5 mg) was dissolved in chloroform; ethanol was added to preferentially precipitate the polymer. The suspension was filtered through a membrane filter to remove the polymer, dried and was properly diluted with the mobile phase to be analyzed by HPLC as described below. The percentage of drug entrapped in the PLA-L microparticles is represented by Eq. (1) and the scheme of its determination is shown in Fig. 1.

$$\text{Drug entrapment (\%)} = \frac{\text{total amount of drug determinate in microparticles} \times 100}{\text{total amount of drug theoretically associated with microspheres}} \quad (1)$$

### 2.2.4. In vitro drug release studies and its kinetic evaluation

Drug-loaded microparticle suspensions (5 mg corresponding to 0.318 mg of nimesulide in 200 μl of phosphate buffer, pH 7.4), were placed in a dialysis membrane bag having a molecular weight cut-off at 12,000 g/mol, closed by tying, and dropped into 100 ml of a 0.1 M, pH 7.4, (sink condition), phosphate buffer. The entire system was kept at 37 °C under continuous magnetic stirring. At selected time intervals, during 108 h, 200 μl of the aqueous solution were withdrawn from the release medium, and replaced by the same volume of fresh medium. The absorbance of the phosphate buffer solution was negligible at 404 nm when measured at the nimesulide concentration in this relatively dilute release medium. The solutions were assayed by HPLC under the conditions described above. The drug released in each sample was determined using a calibration curve; the reported values are averages of three replicates.

The profile and kinetic dissolution studies are important because they correlate the in vitro–in vivo drug responses by comparing results of pharmacokinetics and dissolution profile patterns (Khan, 1996).

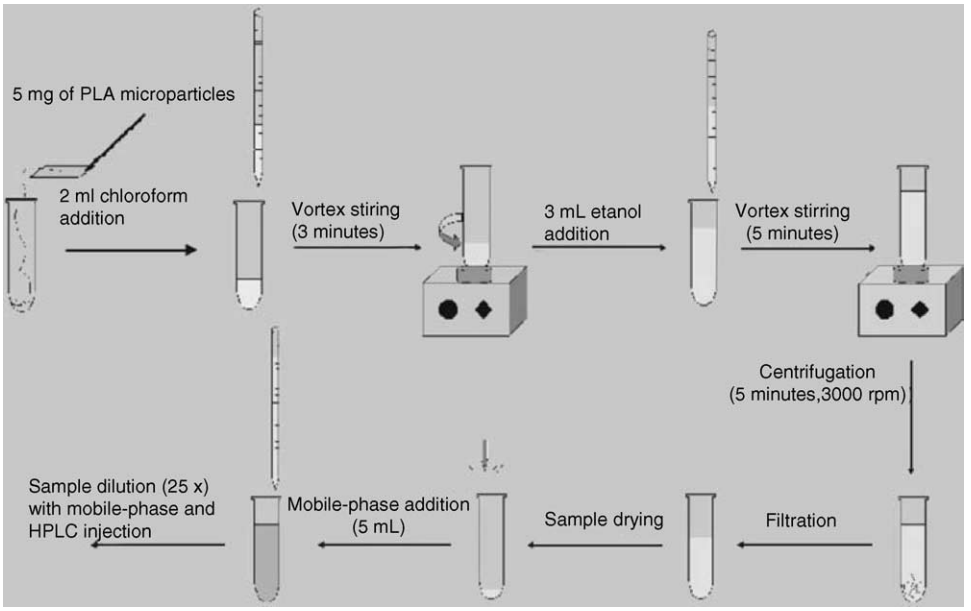


Fig. 1. Scheme of the determination of the drug encapsulation efficiency.

The kinetic parameter determinations permit one know more about the formulation dissolution process because one will be able to foresee its velocity, the maximal dissolvable drug amounts, and the points of significant dissolution changes (Ferraz, 1997).

The interpretation of values obtained in dissolution assays is eased by the use of mathematical equations describing the release profiles as functions of some parameters related to the pharmaceutical dosage forms. Some of the most relevant and commonly used mathematical models describing the dissolution curves are of zero order, first order, Higuchi, Baker–Lonsdale, Hixon–Crowell and Korsmeyer–Peppas. These models best describe drug release from pharmaceutical systems resulting from a simple phenomenon, or when this phenomenon, by being the rate-limiting step, conditions all the other processes occurring in the system (Costa and Souza Lobo, 2001).

In this work, the Higuchi, zero and Baker–Lonsdale mathematical models were applied to study the dissolution profile of nimesulide from micro spheres.

Higuchi (1961, 1963) developed several theoretical models to study release of high and low water soluble drugs incorporated in semi-solid and/or solid matrices; drug release was described as a square root time dependent diffusion process based in Fick's law. This relation can be used to describe drug dissolution from several types of modified release pharmaceutical dosage forms, as of some transdermal systems (Costa et al., 1996) and from matrix tablets of water soluble drugs (Desai et al., 1966a,b; Schwartz et al., 1968a,b).

The zero order model describes the drug dissolution from several types of modified release pharmaceutical dosage forms, like some transdermal systems, as well as matrix tablets of little soluble drugs (Varelas et al., 1995), coated forms, osmotic systems, etc.

The Higuchi and zero order models represent two limiting cases of drug transport and release phenomena. Higuchi's model has a large application in polymeric matrix systems and the zero order model is ideal to describe coated dosage forms or membrane controlled dosage forms.

Table 1
Mathematical models used to describe the kinetics of the drug dissolution curves

| | |
|---|---|
| Zero order | $Q_t = Q_0 + K_0 t$ |
| Higuchi | $Q_t = K_H \sqrt{t}$ |
| Baker–Lonsdale | $(3/2)[1 - (1 - (Md/Mt))^{2/3}] - (Md/Mt) = Kt$ |

The model developed by Baker and Lonsdale (1974) derives from the Higuchi model and describes drug controlled release from a spherical matrix represented by the equation (Table 1) that has been used for the linearization of release data from several microcapsules or microspheres formulations (Seki et al., 1980; Jun and Lai, 1983; Chang et al., 1986; Shukla and Price, 1991; Branja and Pal, 1994).

## 3. Results

### 3.1. Microsphere preparation

The formulations produced showed differences in aspect and stability at 25 °C. To confirm PLA-L microsphere formation and to detect the presence of aggregates, samples were diluted with filtered water (1:3, v/v) and observed by optical microscopy.

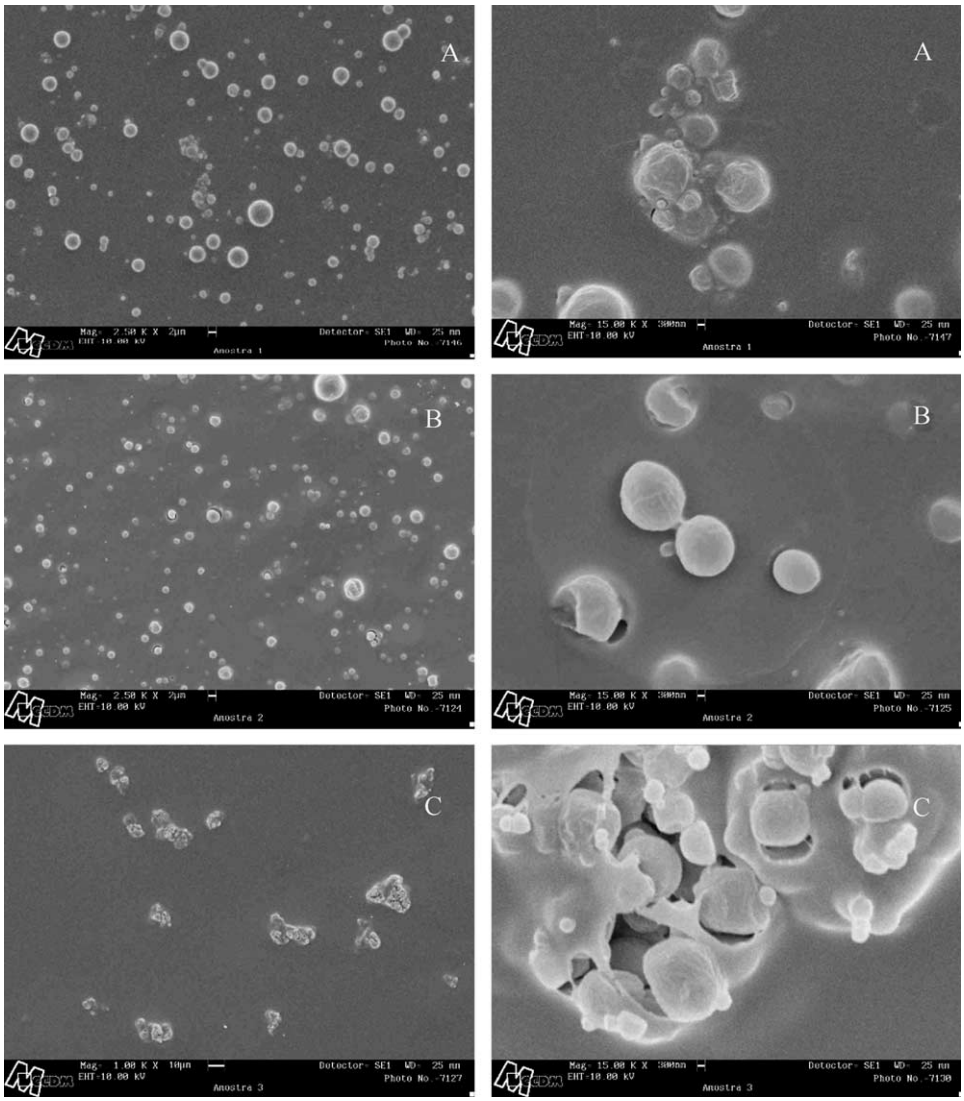


Fig. 2. Scanning electron microphotographs of microspheres formulations at different magnification (×): (A) 2.50 K and 15.00 K, EHT 10.00 kV; (B) 2.50 K and 15.00 K, EHT 10.00 kV; (C) 1.00 K and 15.00 K, EHT 10.00 kV; (D) 1.87 K and 15.00 K, EHT 10.00 kV; (E) 2.50 K and 15.00 K, EHT 10.00 kV; (F) 5.00 K and 45.00 K, EHT 20.00 kV.

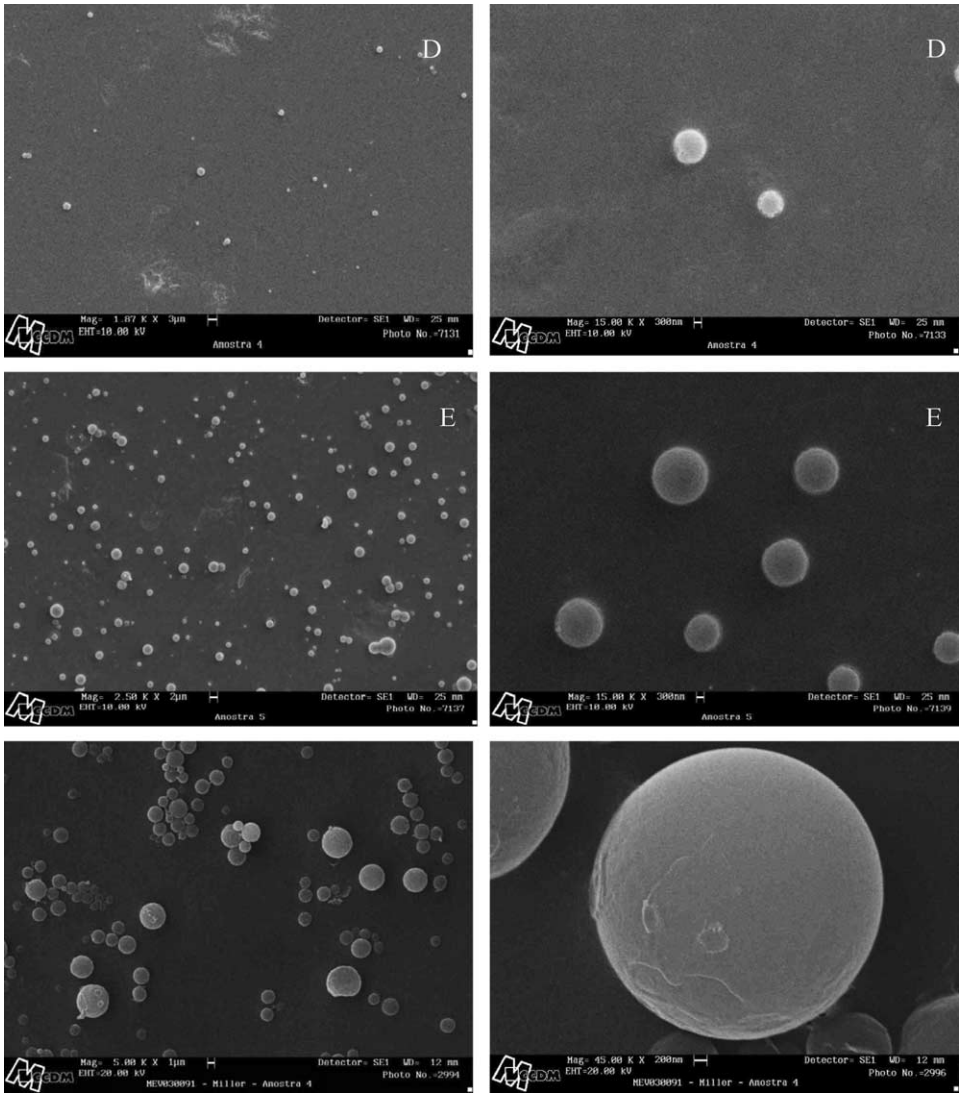


Fig. 2. (*Continued*).

Only formulation (E) presented a significant amount of spherical vesicular microspheres, without aggregated structures confirmed by scanning electron microscopy (SEM) analysis. Although formulation (D) did no aggregate presence, it contained only very low number of microparticulated structures. Therefore, most suitable formulation chosen to associate with the drug was (E) using 3% PVA and 0.15% PLA-L.

### 3.2. Characterization of PLA-L microspheres

Scanning electron micrographies of formulations A to F are shown in Fig. 2. Spherical microspheres having average diameters of 42.9 nm (E) and 2.1 μm (F), respectively, were produced as shown by dynamic light scattering (Table 2 and Fig. 3). Microparticles without the drug had a smaller size than that of nimesulide-loaded PLA-L microparticles presenting

Table 2
The diameter distribution determinated by light scattering method

| Sample | Diameter (nm) | Intensity (%) |
|---|---|---|
| PLA-L microespheres, unloaded | 42.9 | 63.7 |
| | 54 | 8.10 |
| | 271.0 | 8.7 |
| | 341.2 | 4.7 |
| | 857.5 | 0.9 |
| | 1079.7 | 9.2 |
| | 1359.4 | 4.5 |
| | 8584.1 | 0.3 |
| Nimesulide PLA-L microespheres | 159.7 | 25.6 |
| | 195.1 | 33.3 |
| | 2156.1 | 41.1 |

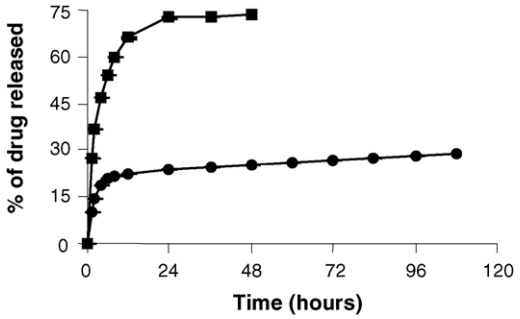


Fig. 4. In vitro drug release profiles of Nimesulide PLA microspheres (●), and nimesulide powder (■).

the drug dispersed in and adsorbed on the PLA-L polymer matrix according to the model described by Schaffazick et al. (2003). These results agree with the observations obtained by SEM.

### 3.3. Determination of the rate of drug encapsulation

The nimesulide PLA-L microparticle entrapment efficiency was 70% and depended mainly on the polymer's composition. This relationship is a rather complicated one, and can be affected by many factors, such as molecular weight, the ratio between hydrophobic to hydrophilic segments, crystallinity, etc. (Haixiong et al., 2000).

### 3.4. Studies on the kinetic evaluation of in vitro drug release

Fig. 4 shows the in vitro release profile of nimesulide from PLA-L microparticles. The main values are shown in Table 3; the coefficient of variation at each point, measured in triplicate, was less than 10%. In this study, a characteristic initial burst of release was followed by a slower continuous rate of drug release. The burst release effect was observed for just the first hours of the experiment.

The burst effect is normally attributed to the presence of the included drug near the surface of the particle, which is known to be permeable to water. As time progress, these microparticles are degraded, leading to water diffusion into their core and nimesulide diffusing into the water. In a biodegradable system, drug release from the polymer matrix is very complex, and follows erosion-diffusion kinetics. The results obtained in our dissolution studies show that 22.65% of the drug was released during the first 12 h, and 28.67% during a total of 108 h.

The results obtained by the kinetic evaluation (Figs. 5–7) show that the release of nimesulide from PLA-L microspheres followed a Higuchi model, while Table 4 shows some kinetic parameters for this model according to the equation: percentage of drug released = $0.9813\sqrt{t}$ (h) + 18.511. The burst effect was not considerate in the kinetic analysis.

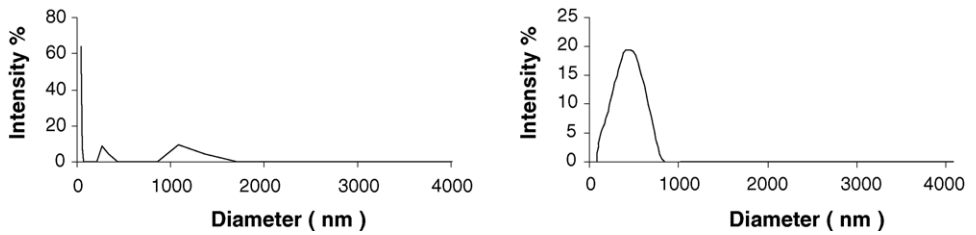


Fig. 3. Loaded (2) and unloaded (1) microsphere dispersion and shape analysis by dynamic light scattering of samples (E) and (F), respectively.

Table 3
Percentage of drug released from PLA-L microspheres comparing with its own dissolution. The variation between three replicates was less than 10%

| Time (h) | Percentage of drug released | | | |
|---|---|---|---|---|
| | Nimesulide from PLA-L microspheres | ±S.D. | Nimesulide | ±S.D. |
| 0 | 0 | 0 | 0 | 0 |
| 1 | 10.41807 | 0.00687 | 27.34882 | 0.045873 |
| 2 | 14.22597 | 0.02841 | 36.89017 | 0.149608 |
| 4 | 19.08050 | 0.04690 | 46.8141 | 0.052612 |
| 6 | 20.81400 | 0.03301 | 54.35167 | 0.030386 |
| 8 | 21.80519 | 0.01369 | 59.89283 | 0.056518 |
| 12 | 22.65257 | 0.02861 | 66.00545 | 0.133054 |
| 24 | 24.06907 | 0.03432 | 72.49919 | 0.216407 |
| 36 | 24.56440 | 0.03931 | 72.84133 | 0.220528 |
| 48 | 25.11325 | 0.04268 | 73.28542 | 0.211345 |
| 60 | 26.13579 | 0.04362 | – | – |
| 72 | 26.53489 | 0.06441 | – | – |
| 84 | 27.29595 | 0.06488 | – | – |
| 96 | 28.15776 | 0.06444 | – | – |
| 108 | 28.67520 | 0.07416 | – | – |

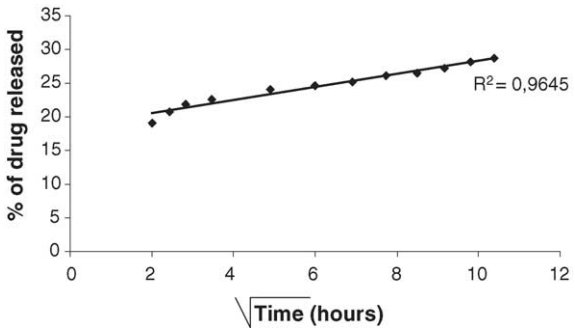


Fig. 5. The Higuchi mathematical model applied to the dissolution profile of nimesulide from PLA-L micro spheres.

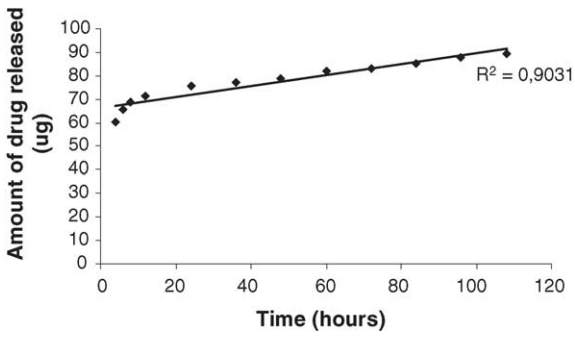


Fig. 6. The zero order mathematical model applied to the dissolution profile of nimesulide from PLA-L micro spheres.

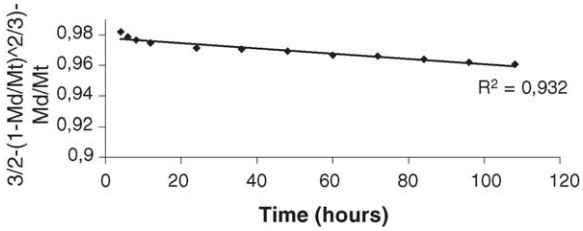


Fig. 7. The Baker–Lonsdale model applied to the dissolution profile of nimesulide from PLA-L microspheres. Md: amount of drug dissolved into the medium, Mt: Theoretical amount of drug associated to the microsphere mass used.

Table 4
Kinetics parameters determined by the Higuchi mathematical model

| PLA microspheres | PLA-L |
|---|---|
| $P_{(12)}$ (%) | 21.910 |
| $\lvert K_H \rvert$ ($h^{-1}$) | 0.9813 |

$P_{(12)}$ (%): percentage of drug released in 12 h according to the equation obtained (item 3.4) and $K_H$ is the kinetic constant.

## 4. Discussion and conclusion

The preparation of PLA-L microspheres was based on the emulsion evaporation method and for this preparation the relationship between the amounts of polymer and surfactant seemed to be related to the volume of the aqueous phase. The aqueous volume of

50 ml and the stirring velocity of 11,000 rpm were used because we observed in the preliminary studies that these parameters affect particles size distribution. Thus, when the volume of the aqueous phase was increased, the particles size increased as well, possibly because it is much difficult to remove the organic from the formulation, and resulted in microdroplet's coalescence.

This technique for preparation of microparticles is usually unsuitable because the poor incorporation of water-soluble drugs. Another problem related to this method is the possibility of the emulsion stabilizer binding to microparticle surfaces (Verrecchia et al., 1993; Shakessheff et al., 1997). In this method, PLA dissolved in an organic solvent was emulsified in an aqueous phase that contained the emulsion stabilizer, and the evaporation of the organic solvent led to the formation of PLA microparticles. Since the emulsion stabilizer remains at the oil/water (O/W) interface during solvent evaporation, there was a possibility of adsorption of the stabilizers. Lee et al. (1999) demonstrated the existence of PVA layers on PLG microparticle surfaces and examined their quantitative behavior by direct measurement. The mechanism of PVA binding seemed to be by interpenetration of PVA and PLG molecules, since a rheological study demonstrated that hydrophobic segments of PVA penetrated into organic phase and interacted with PLG molecules during the preparation of microparticles (Boury et al., 1995). The irreversible binding of PVA on the particle surface is likely to occur when the organic solvent is removed from the interface in which interpenetration of PVA and PLG molecules had occurred. According to Boury's study, the surface PVA density was independent of the PVA concentration in the aqueous phase and it would have been important in the emulsion droplet formation, however, it did not prevent the droplets coagulation.

Moreover, we observed that the surfactant amount that was in the continuous phase could interfere with the microparticles. As showed in Fig. 2, the lower and higher concentration of PVA in the continuous phase promoted the coagulation of the microparticles. Thus, the adequate amount of PVA in the aqueous phase for our formulation was 3%. This surfactant concentration was selected to be used for the preparation of microparticles loaded with the drug.

The choice of organic solvent constitutes another important parameter to obtain a successful formulation. The choice should take into account the solvent's miscibility with water (if an oil water emulsion is to be used); the ability to dissolve the polymer and drug; and its toxicity. In general, less solubility of the organic solvent in water results in a more stable emulsion that leads to spherical particles with nonporous surface and a better size distribution (Thies, 1992; O'Donnel, 1997). The ability of the solvent to dissolve large amounts of polymer renders it easier to control particle size distribution and drug encapsulation efficiency. The evaporation step must be optimized to eliminate the organic phase in the formulation.

Chloroform was the organic phase used in this work because of the ability to dissolve large amounts of PLA-L as well as the low solubility in water.

Both the loaded and unloaded microparticles prepared by emulsion evaporation method were studied by SEM and light scattering. SEM revealed that both microparticles had a spherical shape and that surface pores were not perceptible. Dynamic light scattering measurements showed, not unexpectedly, that following drug encapsulation, the average microparticle diameter increased from 42.9 nm to 2.1 μm. The modifications in polymer amount were in agreement with the findings of Jeffery et al. (1991), that suggested that the higher concentration of polymer in the sample led to an increased frequency of collisions, resulting in fusion of the droplets and producing an overall increase in the size of the microspheres. The microparticles size obtained is suitable for intra muscle administration.

The emulsion solvent-evaporation method used in this work showed to lead a good encapsulation (70%) dependent on the solubility of the drug in the organic solvent and the continuous phase. An increase in the concentration of polymer in a fixed volume of organic solvent could result in increased encapsulation efficiency (Youan et al., 2001). However, other methods such as interfacial polymer deposition followed by solvent evaporation, described by Fessi et al. (1989) were able to produce better results for other drugs reaching an encapsulation above 95%.

The profile of the release of nimesulide from PLA-L microparticles was sustained. In vitro dissolution tests results showed that 28.67% of the drug was released from microparticles within 108 h. The drug release from microparticles seems to consist of two phases: an

initial rapid release followed by a slower exponential stage.

The results obtained until 4 h for the in vitro drug release study were not considered because the burst effect that do not correspond to the real mechanism of the drug release from microspheres. Although the significance of burst release in controlled delivery systems has not been entirely considered, no successful theories have described the phenomenon yet. Despite the fast release of drug in a burst stage is used strategically in certain drug administration, the negative effects caused the burst may be pharmacologically dangerous and non-viable economically. Therefore a through understanding of the burst effect in controlled release systems is undoubtedly necessary. Some authors as Huang and Brazel (2001), described some experimental observations of burst release in monolithic systems, and theories of the physical mechanisms that cause it. These researchers were the first to think about burst prevention as well as of the treatment of the burst release in controlled release models.

Calculating and comparing the coefficient of determination for each kinetic model proposed, Higuchi model presented the higher $R^2$, thus, it was selected to describe the kinetics release of nimesulide from microspheres.

According to Higuchi model theory, the nimesulide released from the microspheres studied in this work is mainly controlled by micropores diffusion. Thus, with the porous size decrease we would observe an increase of nimesulide time release or dissolution from PLA-L microspheres

According to our experimental obtained results we could concluded that the developed formulation demonstrated a potential use for intra muscle sustained release system to vehicle nimesulide. Further studies must be conducted to check stability, toxicity and the correlation of the in vitro–in vivo release profile, specially regarding burst release effect.

## Acknowledgement

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## References

Baker, R.W., Lonsdale, H.S., 1974. Controlled Release of Biologically Active Agents. Plenum Press, New York, pp. 15–71.

Birnbaum, T.D., Kosmala, D.J., Henthorn, B.D., Peppas-Brannon, L., 2000. Controlled release of 17-β-estradiol from PLGA microparticles: the effect of organic phase solvent on encapsulation and release. J. Control. Release 65, 375–387.

Boury, F., Ivanova, T., Panaiotov, I., Proust, J.E., Bois, A., Richou, J., 1995. Dynamical properties of poly(D-L-lactide) monolayers at the air/water and at the dichloromethane/water interfaces. J. Colloid Interface Sci. 169, 380–392.

Branja, R.S., Pal, T.K., 1994. In vitro release kinetics of salbutamol sulphate microcapsules coated with both Eudragit RS 100 and Eudragit RL 100. Drug Dev. Ind. Pharm. 20, 375–386.

Chang, R.K., Price, J.C., Whithworth, C.W., 1986. Control of drug release rates through the use of mixtures of policaprolactone and cellulose propionate polymers. Pharm. Technol. 10, 24–33.

Costa, P., Ferreira, D.C., Souza Lobo, J.M., 1996. Nitroglicerina em sistemas de liberação transdérmica-determinação da velocidade de libertação. Rev. Port. Farm. 46, 4–8.

Costa, P., Souza Lobo, J.M., 2001. Modeling and comparison of dissolution profiles. Eur. J. Pharm. Sci. Rev. 13, 123–133.

Couvreur, P., Dubernet, C., Puisideux, P., 1995. Eur. J. Pharm. Biopharm. 41, 2.

Deasy, P.B., Brophy, M.R., Ecanow, B., Joy, M., 1980. J. Pharm. Pharmacol. 32, 15–20.

Desai, S.J., Singh, P., Simonelli, A.P., Higuchi, W.I., 1966a. Investigation of factors influencing release of solid drug dispersed in inert matrices. III. Quantitative studies involving the polyethylene plastic matrix. J. Pharm. Sci. 55, 1230–1234.

Desai, S.J., Singh, P., Simonelli, A.P., Higuchi, W.I., 1966b. Investigation of factors influencing release of solid drug dispersed in inert matrices. IV. Some studies involving the polyvinyl choride matrix. J. Pharm. Sci. 55, 1235–1239.

Douglas, S.J., Davis, S.S., Davis, L., Illum, L., 1987. Nanoparticles in drug delivery, CRC. Crit. Rev. Ther. Drug Carrier Syst. 3, 233–261.

Ferraz, H.G., 1997. Avaliação biofarmacêutica in vitro in vivo (bioequivalência) de comprimidos de ampicilina 500 mg comercializados no Brazil (Tese de doutoramento, Faculdade de Ciências Farmacêuticas da Universidade de São Paulo). São Paulo, p. 135.

Fessi, H., Puisieux, F., Devissaguet, J.PH., Ammony, N., Benita, S., 1989. Nanocapsulde formation by interfacial deposition following solvent displcement. Int. J. Pharm. 55, R1-R4.

Haixiong, G., Yong, H., Shicheng, Y., Xiqun, J., Changzheng, Y., 2000. Preparation, characterization, and drug release behaviours of drug-loaded e-caprolactone/L-lactide copolymer nanoparticles. J. Appl. Polym. Sci. 75, 874–882.

Higuchi, T., 1961. Rate of release of medicaments from ointment bases containing drugs in suspension. J. Pharm. Sci. 50, 874–875.

Higuchi, T., 1963. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J. Pharm. Sci. 51, 802–804.

Huang, X., Brazel, C.S., 2001. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. J. Control. Release 73, 121–136.

Jalsenjak, I., Kondo, T., 1981. Effect of capsule size on permeability of gelatin-acacia microcapsules toward sodium chloride. J. Pharm. Sci. 70, 456–457.

Jeffery, H., Davis, S.S., O'Hagan, D.T., 1991. The preparation and characterization of poly(lactide-co-glycolide) microparticles. I. Oil-in-water emulsion solvent evaporation. Int. J. Pharm. 77, 169–175.

Jun, H.W., Lai, J.W., 1983. Preparation and in vitro dissolution tests of egg albumin microcapsules of nitrofurantion. Int. J. Pharm. 16, 65–77.

Khan, M.Z.I., 1996. Dissolution testing for sustained or controlled release oral dosage forms and correlation with in vivo data: challenges and opportunities. Int. J. Pharm. (Amsterdam) 140, 131–143.

Lee, S.C., Oh, J.T., Jang, H.M., Chung, L.S., 1999. Quantitative analysis of polyvinyl alcohol on the surface of poly(D-,L-lactide-co-glycolide) microparticles prepared by solvent evaporation method: effect of particle size and PVA concentration. J. Control. Release 59, 123–132.

Michael, C., Ilia Fishbein, M.D., Haim, D.D., Gershon, G., 2002. Lipophilic drug nanospheres prepared by nanoprecipitaion: effect of formulation variables on size, drug recovery and release kinetics. J. Control. Release 83, 389–400.

Mortada, M., 1982. Preparation of ethyl cellulose microcapsules using the complex emulsion method. Pharmazie 37, 427–429.

Niwa, T., Takeuchi, H., Hino, T., Kunou, N., Kawashima, Y., 1993. Preparation of biodegradable nanospheres of water-soluble and insoluble drugs with D-,L-lactide/glycolide copolymer by a novel emulsification solvent diffusion method, and drug release behavior. J. Control. Release 25, 89–98.

O'Donnel, P.B., 1997. Mcginity, preparation of microspheres by the solvent evaporation technique. Adv. Drug Del. Rev. 228, 25–42.

O'Hagan, D.T., Rahman, D., McGee, J.P., Jeffery, H., Davies, M.C., Williams, P., Davis, S.S., Challacombe, S.J., 1991. Biodegradable microparticles as controlled release antigen delivery systems. Imunology 73, 239–242.

Ptacek, P., Macek, J., Klima, J., 2001. Rapid and simple high performance liquid chromatograpic determination of nimesulide in human plasma. J. Chromatogr. B 758, 183–188.

Redmon, M.P., Hickey, A.J., DeLuca, P.P., 1989. Predinizolone-21-acetate poly(glycolic acid) microspheres; influence of matrix characteristics on release. J. Control. Release 9, 99–109.

Schaffazick, R.S., Guterres, S.S., Freitas, L.L., Pohjmann, A.R., 2003. Characterization and physico-chemical stability of polymeric nanoparticulated systems for drug administration. Química Nova 26 (No. 5), 726–737.

Schwartz, B.J., Simonelli, A.P., Higuchi, W.I., 1968a. Drug release from wax matrices. I. Analysis of data with first order kinetics and with the diffusion-controlled model. J. Pharm. Sci. 57, 274–277.

Schwartz, B.J., Simonelli, A.P., Higuchi, W.I., 1968b. Drug release from wax matrices. II. Application of a mixture theory to the sulfanilamide-wax system. J. Pharm. Sci. 57, 278–282.

Seki, T., Kawaguchi, T., Endoh, H., Ishikawa, K., Juni, K., Nakano, M., 1980. Controle release of 3,5-diester prodrugs of 5-fluoro-2-deoxyuridine from poly-L-lactic acid microspheres. J. Pharm. Sci. 79, 985–987.

Shakessheff, K.M., Evora, C., Soriano, I., Langer, R., 1997. The absorption of poly(vinyl alcohol) to biodegradable micorparticles studied by X-ray photoelectron spectroscopy (XPS). J. Colloid Interface Sci. 185, 538–547.

Shukla, A.J., Price, J.C., 1991. Effect of drug loading and molecular weight of cellulose acetate propionate on the release characteristics of theophyline microspheres. Pharm. Res. 8, 1369–1400.

Thies, C., 1992. Formulation of degradable drug-loaded microparticles by in in-liquid drying processes. In: Dunbrow, M. (Ed.), Microcapsules and Nanoparticles in Medicine and Pharmacy. CRC Press, Boca Raton, FL, pp. 47–71.

Thirumala, G., Snjezana, S., Martin, C.G., Lisbeth, I., Stanley, S.D., 1999. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drugs. J. Control. Release 57, 171–185.

Tognella, S., 1993. Nimesulide: new clinical opportunities. Drugs 46, 275–276.

Uchida, T., Kawata, M., Goto, S.J., 1986. In vivo evaluation of ethyl cellulose microcapsules containing ampicillin using rabbits, beagle dogs and humans. Pharmacobio-Dyn. 9, 631–637.

Varelas, C.G., Dixon, D.G., Steiner, C., 1995. Zero order release from biphasic polymer hydrogels. J. Control. Release 34, 185–192.

Verrecchia, T., Huve, P., Brazile, D.V., Veillard, M., Spenlehauer, G., Couvreur, P., 1993. Adsorption/desorption human serum albumin at the surface of poly(lactid acid) nanoparticles prepared by a solvent evaporation process. J. Biomed. Mater. Res. 27, 1019–1028.

Wada, R., Hyon, S.H., Ikada, Y., 1990. Lactic acid oligomer microspheres containing hydrophilic drugs. J. Pharm. Sci. 79, 919–924.

Youan, B.B.C., Jacson, T.L., Dickens, L., Hernandez, C., Ababio, G.O., 2001. Protein release pro®les and morphology of biodegradable microcapsules containing an oily core. J. Control. Release 76, 313–326.