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Nanoparticle infiltration to prepare solvent-free controlled drug delivery systems

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ABSTRACT

The purpose of this work was to propose a drug delivery system based on a biodegradable porous membrane, whose surface is covered by a nanoparticle film, thus achieving a controlled drug release rate. Furthermore, due to the fact that the assembly of the system is performed in aqueous medium, contact with organic solvents is avoided. The method is performed in two steps: (i) preparation of biodegradable porous membranes (by a solvent casting and particulate leaching technique) and biodegradable nanoparticles (by the emulsification–diffusion method), extensively eliminating the solvent in both of them; (ii) infiltration into membranes of an aqueous solution of a model drug (carbamazepine) and a nanoparticle dispersion. In both cases, poly(DL-lactic-*co*-glycolic acid) (PLGA 50:50) was used as a biodegradable polymer. Carbamazepine adsorbed onto biodegradable porous membranes shows an immediate release behavior (95% released in <15 min). Infiltration of different amounts of nanoparticles (50, 100, 400 and 600 mg of nanoparticles/0.625 g of membrane) into biodegradable porous membranes shows a Fickian diffusion according to Peppas model, and fits Higuchi's model. This behavior was attributed to the diffusional barrier constituted by the nanoparticle film. As expected, the carbamazepine release rate was dependent on the amount of infiltrated/adsorbed nanoparticles into biodegradable porous membrane. DSC studies show molecular dispersion of the drug throughout the membrane.

1. Introduction

Sensitive drugs face different problems during their formulation processes due to contact with organic solvents, particularly if biodegradable polymers (e.g., poly(lactic acid), poly(glycolic acid), poly(lactide-*co*-glycolide acid), poly-ε-caprolactone, etc.) are involved. As it is known, organic solvents are very often used in the manufacturing of pharmaceutical products. However, an important drawback of organic solvents is their effect on the physicochemical properties of the drug, as well as the potential risk to human health owing to their toxicity and undesirable effects (Witschi and Doelker, 1997). In general, sensitive drugs need to be protected during formulation against chemical attack, and require gentle manufacturing processes (Cui et al., 2005).

Biodegradable porous scaffolds have been extensively used in medicine as temporary templates for tissues regeneration, and have recently been subject of study for the pharmaceutical industry because they have been shown to be excellent drug devices due to the high porosity and interconnectivity throughout the system (Sato et al., 1988; Mikos et al., 1993, 1994; Kim et al., 2006). However, their behavior is characterized by a high initial burst effect and an incomplete release (Kim et al., 2006).

Nanotechnology is currently undergoing advancements in different areas, such as biomedical applications, molecular imaging, biomarkers, biosensors, and drug delivery. Nanoparticles have been studied for the pharmaceutical industry due to their effective drug delivery and carrier properties; hence they are considered as one of the most promising dosage forms for controlled release and drug targeting (Lane and Heath, 2004).

The aim of the present study is to propose a new controlled drug delivery system combining the properties of biodegradable porous membranes and biodegradable nanoparticles. The preparation process of these systems consists of: (a) production of biodegradable porous membranes, (b) preparation of biodegradable nanoparticles; and once these systems are available, (c) incorporation of an aqueous solution of a model drug (carbamazepine) and a nanoparticle dispersion, into biodegradable porous membranes, in order to form a nanoparticle film on the surface of the membrane, thus retaining the drug and controlling

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its release rate. It is hypothesized that the drug release rate will be dependent on the amount of nanoparticles infiltrated into the system.

In this study, the drug is not exposed to any stress process (i.e. heat, stirring, etc.), and contact with the solvent is avoided since the systems (biodegradable nanoparticles and biodegradable porous membranes) are prepared by independent methods, in which the model drug was not yet included, and solvents are extensively eliminated. System assembly is then performed in an aqueous medium.

Nanoparticles were prepared according to the well-known emulsification-diffusion technique (Quintanar-Guerrero et al., 1996), while biodegradable porous membranes were prepared by solvent casting and particulate leaching technique (Mikos et al., 1994). In both systems poly(DL-lactic-*co*-glycolic acid) (PLGA 50:50) was used as a biodegradable polymer.

Carbamazepine (chosen as a model drug) is a polymorphic drug used in the treatment of epilepsy, characterized by a slow and irregular gastrointestinal absorption (Bertilsson, 1978). Carbamazepine polymorphs can be interconverted by phase transformations or by a solvent-mediated process; phase transformations can also be induced by heat or by mechanical stress (Byrn, 1982).

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-*co*-glycolide) (PLGA 50:50, Medisorb®, MW 31 kDa), was obtained from Lakeshore Biomaterials, USA. Poly(vinyl alcohol) (PVAL, MW 58 kDa) was purchased from Glomarza, Mexico. Carbamazepine was supplied by Sigma–Aldrich, China. Granular sodium chloride and methylene chloride were provided by J.T. Baker, Mexico. Distilled water was obtained from a RiOs Millipore® distiller, USA. Ground particles of sodium chloride were sieved with USA Standard Testing Sieves (ASTM-E11 Specification, W.S. Tyler), with 53 μm (No. 270), 106 μm (No. 140) and 150 μm (No. 100) openings, placed on a sieve shaker (Octagon model 200, England).

2.2. Preparation of biodegradable porous membranes

Biodegradable porous membranes were prepared by a solvent casting and particulate leaching technique (Mikos et al., 1994). Briefly, 0.625 g of polymer (PLGA 50:50) were dissolved in methylene chloride, and 0.625 g of sodium chloride particles (size ~63 μm) were scattered into this organic solution. Sodium chloride was used with the purpose of creating pores within the system. The solution was casted in Teflon moulds with a diameter of 3.0 cm. The solvent was allowed to evaporate from the moulds at room temperature for 48 h. Residual amounts of solvent were removed by vacuum. Once dried, the membranes were immersed in 250 ml of distilled water during 72 h at room temperature, at 100 rpm (water was changed every 6 h) to leach out the sodium chloride. Sodium chloride-free membranes were dried at room temperature in a desiccator (RH = 56%).

2.3. Nanoparticle preparation

Nanoparticles were prepared using the emulsification–diffusion technique (Quintanar-Guerrero et al., 1996). Briefly, the organic solvent (ethyl acetate) and water were mutually saturated for at least 20 min before use, in order to ensure initial thermodynamic equilibrium of both liquids. 400 mg of PLGA 50:50 were dissolved in 20 ml of water-saturated organic solvent, and this organic solution was emulsified with 40 ml of an organic solvent-saturated aqueous solution of PVAL (5%, w/v) using a stirrer (Heidolph RZR-1) at 2200 rpm for 10 min. 160 ml of distilled water were subsequently added to the emulsion to induce diffusion of the organic solvent into the continuous phase, leading to the formation of nanoparticles. The organic solvent was eliminated from the raw nanoparticle suspension by vacuum steam distillation at 35 °C. The nanoparticle suspension was centrifuged (Optima® LE-80K, Beckman, USA) at 20,000 rpm for 20 min. Nanoparticles were finally frozen at −40 °C for 20 min and freeze-dried for 24 h at 60×10^{-3} mbar (Labconco®, USA).

2.4. Scanning electron microscopy (SEM) studies

Cross-sections of biodegradable porous membranes dried samples were coated with a thick gold layer (~20 nm) (Fine Coat Ion Sputter JFC-1100, JEOL, Japan) and observed by SEM using a JSM-25 S II scanning electron microscope (JEOL, Japan) before and after nanoparticle infiltration.

In the same way, the surface morphology of nanoparticles was also observed. To do this, an aqueous nanoparticle dispersion (0.2 mg/ml) was spread over a slab and dried under vacuum at room temperature.

2.5. Particle size analysis

The average size and the polydispersity index of nanoparticles were determined by the laser light scattering technique (Coulter N4 Plus, FL, USA). The laser light wavelength was 678 nm (He/Ne 10 mW). Measurements were obtained at a 90 °C fixed-angle for 180 s, at a temperature of 25 °C. The scattering intensity data were analyzed by a digital correlator under an unimodal analysis mode. Dispersions were diluted with water to ensure that the light scattering signal, as indicated by the particle counts per second, was within the instrument's sensitivity range. Measurements were made in triplicate for all batches prepared.

2.6. Specific surface area characterization

The specific surface area was calculated in terms of the standard Brunnauer–Emmett–Teller (BET) method with an Autosorb 1MP analyzer (Quantachrome Instruments), equipped with a 1 mTorr pressure transducer. The sample was vacuum degassed at 22 °C for 8 h following a standard protocol for the Autosorb-1 series. Adsorption isotherms were constructed with a nitrogen adsorbate. The temperature transpiration effect was accounted for automatically by the instrument.

2.7. Drug loading and infiltration of nanoparticles into biodegradable porous membranes

Biodegradable porous membranes were dipped into 10 ml of a saturated aqueous solution of carbamazepine (~180 μg/ml) for 12 h, so that the drug was adsorbed into the system. This system is the control (without nanoparticles, system 1). Biodegradable porous membranes were vacuum-dried at room temperature.

The systems with nanoparticles were prepared by dipping biodegradable porous membranes into 10 ml of a saturated aqueous solution of carbamazepine containing different amounts of lyophilized PLGA-nanoparticles: 50, 100, 400 or 600 mg (designated as system 2, 3, 4 and 5, respectively) for 12 h. They were vacuum-dried at room temperature.

2.8. Differential scanning calorimetry (DSC) studies

DSC analyses were carried out on samples of individual substances such as carbamazepine and PLGA 50:50, as well as on biodegradable porous membranes with and without nanoparticle infiltration. The dried samples were weighed directly in aluminum pans (4–6 mg) and scanned between 25 and 230 °C at a 10 °C heating

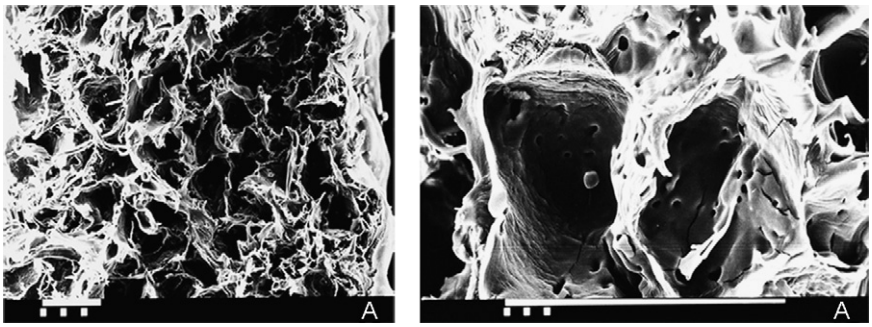

Fig. 1. Scanning electron micrographs of biodegradable porous membrane cross-sections before film formation with nanoparticles; (A) system 1 (bar = 100 μm).

rate under nitrogen, using a DSC Q10 differential scanning calorimeter (TA Instruments, USA).

2.9. In vitro release kinetics

Carbamazepine's release profiles from biodegradable porous membranes were performed in 10 ml of simulated intestinal fluid, according to the US Pharmacopeia XXIV, ($n = 3$). Sink conditions (carbamazepine concentration <60 μg/ml) were maintained throughout the study. The systems were shaken at 25 rpm, at 37 °C with a water bath shaker (Gyromax™, model 939, USA). At selected time intervals, 2 ml of medium were withdrawn and replaced with fresh medium. Sample absorbance was measured spectrophotometrically at 285 nm and interpolated in a calibration curve (ranging from 5 to 40 μg/ml, $r^2 = 0.9993$). The amount of drug released at each time point was corrected for the dilution effect. Release experiments were carried out in triplicate.

3. Results and discussion

Scanning electron microscopy (SEM) showed that biodegradable porous membranes had a high porosity with an average diameter of the exposed pore of ~44.5 ± 3.0 μm (Fig. 1). A large surface area with an interconnected porous structure throughout the matrix was observed in cross-sectional cuts. B.E.T. studies for biodegradable porous membranes showed a specific surface area of 10.07 ± 0.21 m^2/g and a total pore volume of 0.03 cm^3/g.

Regarding nanoparticle preparation, the emulsification–diffusion technique allowed the obtention of spherical submicronic polymeric particles up to 300 nm in size for all batches prepared, with a polydispersity index lower than 0.03. A representative micrograph of the nanoparticles is shown in Fig. 2. The process efficiency was higher than 99.0%. The drug and nanoparticles were infiltrated into biodegradable porous membranes in order to form a film on the membrane surface. Nanoparticles can adsorb the drug and they can form films on the surface of biodegradable porous membranes due to an effective attraction between the nanoparticle surface and the membrane surface, since both of them have the same chemical nature (Israelachvili, 1995). This film can act as an additional diffusional barrier, thereby obtaining a controlled drug delivery system.

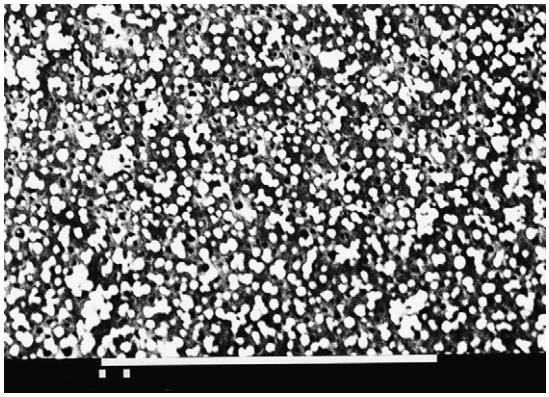

Fig. 2. Scanning electron micrograph of PLGA-nanoparticles (bar = 10 μm).

The infiltration effect of different nanoparticle concentrations within biodegradable porous membranes is evidenced in Fig. 3. As shown, a thin nanoparticle coating covers the biodegradable porous membrane surface. As expected, film continuity was dependent on the amount of infiltrated nanoparticles. In this sense, system 2, with the smallest amount of infiltrated nanoparticles, did not show a continuous film on the biodegradable porous membrane surface (Fig. 3B). The systems showed a continuous film as the amount of infiltrated nanoparticles increased (Fig. 3C–E). Higher concentrations of infiltrated nanoparticles may build up an interface promoting the formation of a continuous film adsorbed on a solid surface, a process similar to that observed for the film coating technology.

The release profiles of carbamazepine with and without nanoparticles are shown in Fig. 4. System 1 (without nanoparticles) showed an immediate release behavior (90% released in <15 min). This burst effect is attributed to the drug adsorbed on the surface of the system's pores, as well as to the high porosity with interconnected channels that were present in the system. The drug had a high contact with the dissolution medium, allowing a rapid desorption. It is expected that the drug substance near the surface will diffuse out of the system quickly, causing burst release (Sato et al., 1988; Mao et al., 2008). Obviously, porosity has an important effect on drug release characteristics, and it is related with this rapid release (Mao et al., 2008; Chung et al., 2001).

However, when carbamazepine was loaded in biodegradable porous membranes in the presence of nanoparticles (systems 2–5), a controlled release behavior was obtained. As the amount of nanoparticles infiltrated in the systems was increased, the release rates were significantly slower than that for system 1. As expected, the release rate of carbamazepine was dependent on the amount of nanoparticles infiltrated into the system. This behavior can be explained by the fact that the drug remains occluded by nanoparticle deposition, which forms a film on the biodegradable porous membrane surface (Table 1). Therefore, this film acts as an additional diffusion barrier for the drug on the surface of the membrane entrapping the drug (Fig. 3B–E). Thus, if the amount of nanoparticles infiltrated is higher, the diffusion barrier will be thicker and the release rate of the drug will be slower.

Drug loading within biodegradable porous membranes was calculated for each system (Table 1). As the amount of nanoparticles infiltrated was increased, the amount of nanoparticles adsorbed into the system increased, and, consequently, the amount of drug adsorbed in biodegradable porous membranes was also increased.

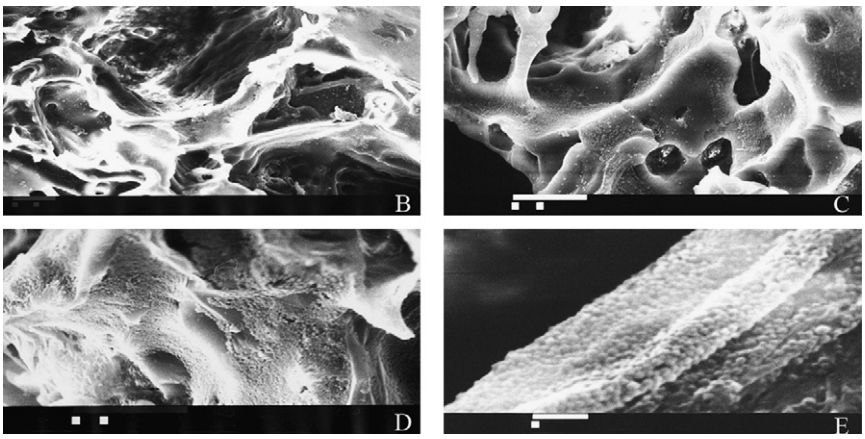

Fig. 3. Scanning electron micrographs of biodegradable porous membranes with film formation with different concentrations of PLGA-nanoparticles; (B) system 2, (C) system 3, (D) system 4 and (E) system 5; (bar: ■ 1 μm, ■■ 10 μm, ■■■ 100 μm).

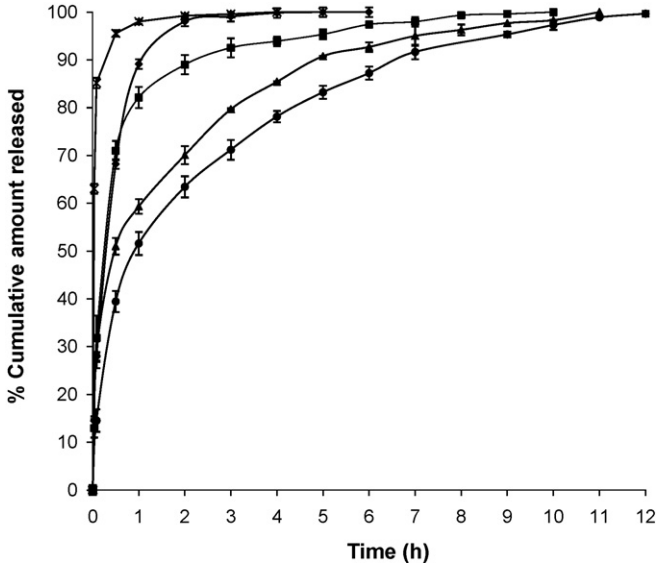

Fig. 4. Release profiles of carbamazepine from biodegradable porous membranes without and with film formation with different concentrations of infiltrated nanoparticles; system 1 (*), system 2 (♦), system 3 (■), system 4 (▲) and system 5 (●).

This effect was attributed to the greater surface area exposed, able to adsorb the drug on both membrane and nanoparticle surface. For this reason, system 5 had a greater amount of carbamazepine adsorbed into the biodegradable porous membrane than systems 1–4.

Systems 2–5 showed a Fickian diffusion according to Peppas model (Table 2), which was dependent on time ($t^{0.5}$) and fitted Higuchi's model (Table 3, Fig. 5). Therefore, carbamazepine release occurred by diffusion throughout the matrix. If drug release is faster than matrix erosion, as seen in this work, the mechanism of drug release occurs mainly by diffusion (Niwa et al., 1993; Soppimath et al., 2001).

Table 1
Carbamazepine load within biodegradable porous membranes for each system.

System number	Carbamazepine adsorbed (μg)
1	139
2	172
3	292
4	493
5	625

Table 2
Release rate constants according to the Higuchi's model from biodegradable porous membranes with infiltrated nanoparticles.

System number	K_H (μg/min)$^{1/2}$	Correlation coefficient
2	0.09	0.996
3	62.91	0.965
4	3.18	0.974
5	1.27	0.968

Table 3
Diffusion exponents (n) according to Peppas model from biodegradable porous membranes with infiltrated nanoparticles.

System number	n	Correlation coefficient
2	0.59	0.998
3	0.59	0.950
4	0.52	0.998
5	0.44	0.987

DSC thermograms of carbamazepine, PLGA 50:50, biodegradable porous membranes without and with nanoparticle infiltration were carried out in order to determine the physical state of both carbamazepine and the polymer, to detect any drug–polymer interactions and to determine if carbamazepine was either totally dispersed or was present in its crystalline form. Fig. 6 shows the DSC thermograms of carbamazepine, PLGA 50:50, and biodegradable porous membranes with and without film formation. Pure

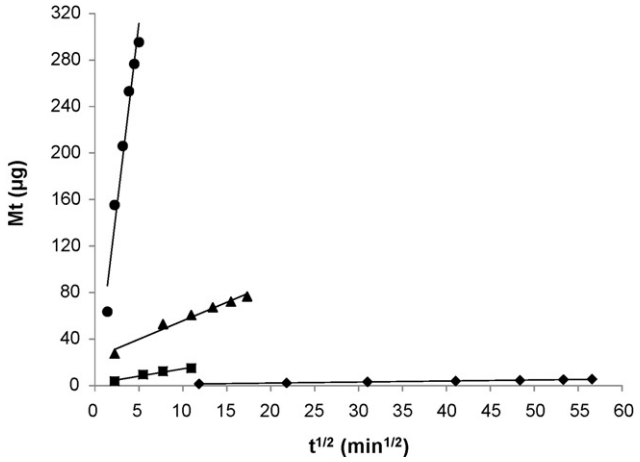

Fig. 5. Higuchi's model for carbamazepine released from biodegradable porous membranes with different concentrations of infiltrated nanoparticles; system 2 (♦), system 3 (■), system 4 (▲) and system 5 (●).

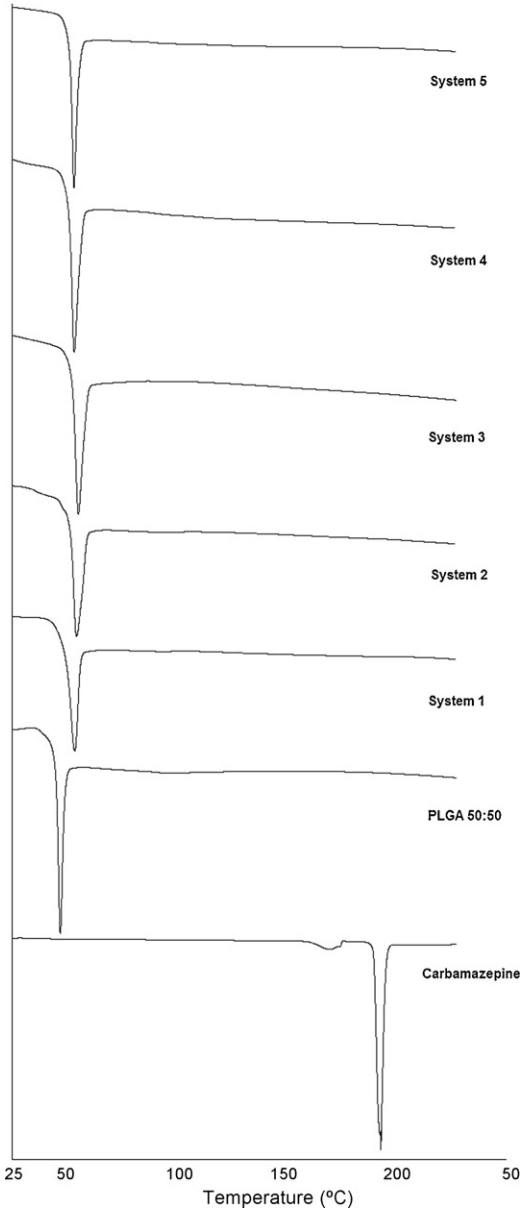

Fig. 6. DSC thermograms of free-carbamazepine, PLGA 50:50 and systems 1–5.

carbamazepine exhibited a small endothermic peak at 170 °C, which is characteristic of the transition of the β form to the α form (Katzhendler et al., 1998), as well as an endothermic peak of the melting point at 192 °C. The theoretical peak of carbamazepine ranges from 189 to 193 °C (Moffat, 2005). PLGA showed a glass transition temperature (Tg) at 47 °C (Tg reported 43–48 °C). In the case of systems 1–5, the Tg for PLGA increased to 53, 54, 54, 53 and 53 °C, respectively. Okada et al. (1994) reported that the Tg of a PLGA matrix raised as loading with a basic drug increased. This was due to the electrostatic interaction between carbamazepine (a basic drug) and the terminal carboxylic acids of the polymer. DSC studies did not detect any crystalline drug material in membrane samples. Taking this into consideration, this behavior suggests a molecular dispersion of carbamazepine throughout the membrane.

4. Conclusions

The present study has shown a possible method to obtain controlled drug delivery systems by film formation with nanoparticles onto biodegradable porous membranes, avoiding drug contact with organic solvents, since drug loading was in aqueous medium using preformed systems as platforms, which is a suitable option to develop drug delivery systems for sensitive drugs. None film formation on biodegradable porous membranes showed an immediate release, nevertheless, film formation allowed a controlled release, dependent on the amount of nanoparticles infiltrated into the systems. This behavior was attributed to drug adsorption on the pores and on the surface of the nanoparticles contained in the system, which remained occluded by the film formed on the biodegradable porous membrane surface, having as a result a film which acts as a diffusion barrier.

The concept proposed in this work could provide a useful approach to solve the problems during the formulation process for sensitive drugs such as peptides, proteins, etc., combining porous and submicronic systems. Actually, we are working with different porous platforms and submicronic systems in order to encapsulate proteins and other sensible molecules.

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