Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 294 (2005) 217–232
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Preparation and characterization of triclosan nanoparticles for periodontal treatment

E. Piñón-Segundo, A. Ganem-Quintanar, V. Alonso-Pérez, D. Quintanar-Guerrero *

Departamento de Posgrado en Farmacia, Facultad de Estudios Superiores Cuautitlán, UNAM. Av. 1° de Mayo s/n, Campo 1, Cuautitlán Izcalli, C.P. 54704, Estado de México, México

Received 16 July 2004; received in revised form 12 November 2004; accepted 12 November 2004

Abstract

The aim of this work was to produce and characterize triclosan-loaded nanoparticles (NPs) by the emulsification–diffusion process, in an attempt to obtain a novel delivery system adequate for the treatment of periodontal disease. The NPs were prepared using poly(D,L-lactide-co-glycolide) (PLGA), poly(D,L-lactide) (PLA) and cellulose acetate phthalate (CAP). Poly(vinyl alcohol) (PVAL) was used as stabilizer. Batches were prepared with different amounts of triclosan (TCS) in order to evaluate the influence of drug on NP properties. Solid NPs of less than 500 nm in diameter were obtained. Entrapment efficiencies were higher than 63.8%. The characterization by scanning electron microscopy and light scattering indicated that high concentrations of TCS seemingly caused the increase of NP mean size. A decrease in the PLGA glass transition temperature was observed by differential scanning calorimetry. This could indicate that TCS in PLGA-NPs behaves as a non-conventional plasticizer.

Subsequently, in vitro release studies were carried out under sink conditions using a device designed in our laboratory to allow a direct contact between the particles and the dissolution medium. A fast release of TCS from NPs was detected. A preliminary in vivo study in dogs with induced periodontal defects suggested that TCS-loaded NPs penetrate through the junctional epithelium.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Nanoparticles; Emulsification–diffusion; Triclosan; Periodontal diseases; Gingivitis; Periodontitis

1. Introduction

According to the World Oral Health Report 2003 (World Health Organization, 2004), most children show signs of gingivitis (bleeding gums), and among adults, the initial stages of periodontal disease are widespread. Severe periodontitis, which may result in tooth loss, is found in 5–15% of most populations.

* Corresponding author. Present address: Bolognia 4-28, Bosques del Lago, Cuautitlán Izcalli, Estado de México, C.P. 54766, México. Tel.: +52 55 58 77 19 07; fax: +52 55 58 93 86 75.
E-mail address: quintana@servidor.unam.mx (D. Quintanar-Guerrero).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.11.010

Although there is no official information in Mexico concerning the number of people suffering from periodontal disease, it is well known that oral diseases are a health problem in developing countries, especially amongst poorer communities (Carrillo et al., 2000).

The most common diseases of tooth-supporting structures are plaque-induced inflammatory alterations in the gingiva and the periodontum. It has been established that periodontal diseases are caused by bacterial infection. Periodontitis usually develops from a more or less pronounced gingivitis and is characterized by the presence of periodontal pockets, which form as the gingiva detaches from the tooth. Current periodontal therapy focuses on the removal of the bacterial plaque by mechanical scaling and root planning as antimicrobial measures. However, the use of systemic antibiotics or a localized delivery system incorporating antibacterial agents is also required (Baker et al., 1988; Friedman and Steinberg, 1990; Medlicott et al., 1994; Esposito et al., 1997).

To date, a great number of local drug-delivering devices have been proposed, including fibers, strips, films, gels, sponges, microparticles, etc. Unfortunately, the difficulty accessing the periodontal pocket has rendered both of these suggested methods only partially successful. On the other hand, systemic administration can achieve therapeutic concentrations in the gingival crevicular fluid only with high doses (Medlicott et al., 1994; Drisko, 1996; Schwach-Abdellaoui et al., 2000; Piñón-Segundo, 2000).

In the last few years, several studies have shown that an effective approach to optimizing the pharmacological action of drugs is to associate the active molecule with a carrier system. Drug carrier systems may take many forms: liposomes, niosomes, NPs and microemulsions. The purpose of these colloidal vectors is to enhance the therapeutic effect by targeting the active molecule to its action site and by creating a high local concentration. Efficiency is increased, while drug dosage and the risk of side effects are decreased. Evidently, these carriers have several advantages and disadvantages. The greatest advantage of liposomes, for instance, is that their main components are materials that are present in the body and thus have a good bioacceptability. On the other hand, they have some limitations, such as poor stability and low drug entrapment efficiency (Kreuter, 1994). Alternatively, NPs have a better stability than liposomes in biological fluids and during storage, and their preparation is more suitable for scaling up (Quintanar-Guerrero et al., 1996, 1998a, 1998b; Colombo et al., 2001). Moreover, microemulsions offer several advantages; for example, their solubilization capacity, transparency, high stability, and simplicity of manufacture (they require no mechanical work in their preparation). In view of the advantages and disadvantages of these carriers, the most suitable system will have to be chosen depending on drug properties and the therapeutic effect sought (Kreuter, 1994).

It has been demonstrated that the transport of substances through the junctional epithelium, which forms the epithelial attachment of the gingiva to the tooth surface, can occur in both directions. (1) From the underlying connective tissue outward into the gingival sulcus, as was reported after intravenous administration of carbon particles (Ratcliff, 1966) and fluorescein (Brill and Krasse, 1958), and when fluorescein was orally administered (Brill and Björn, 1959). (2) From the gingival sulcus inward to the underlying connective tissue, as was observed with proteins such as albumin (Tolo, 1971) and carbon particles (Fine et al., 1969). More recently, using confocal laser scanning microscopy (Ganem-Quintanar, 1997), established that biodegradable NPs, when gently applied to the porcine gingival sulcular space, are able to penetrate into the junctional epithelium. These results suggest that NPs can provide a potential intrapocket carrier system for the delivery of active substances to the periodontal pocket. Ideally, NPs may establish some physical bonding with the tissue, avoiding their being flushed out by the crevicular fluid that fills the periodontal pockets. Additionally, NPs could be a drug-delivery system used to maintain an effective drug release rate in the periodontal pocket.

For the purposes of this work, triclosan was chosen as the drug to be incorporated into NPs. Triclosan (2,4,4′-trichloro-hydroxydiphenylether) is a non-cationic antimicrobial agent that has a recognized efficacy against several plaque-forming bacteria (Rosling et al., 1997a, 1997b). TCS, is a lipid-soluble compound, which can penetrate skin and mucous membranes (Waaler et al., 1993). In an experimental gingivitis study, Ramberg et al. (1995) demonstrated that triclosan, used in a mouthrinse, inhibited or retarded the gingival inflammation. Other researchers (García-

Godoy et al., 1990; Ramberg et al., 1995; Furuichi et al., 1997) have demonstrated that TCS reduced the already established supragingival plaque and resolved gingival lesions.

We have used the emulsification–diffusion technique (Quintanar-Guerrero et al., 1996) in order to obtain NPs that could be used as drug carriers for the treatment of periodontal disease. Several polymers (Eudragit® E 100, Eudragit® RS 100, Resomer® RG 502, Resomer® R 104 and cellulose acetate phthalate) and different stabilizers (Pluronic®F-127, Epikuron®200 and poly(vinyl alcohol)) have been used in our laboratory. No NPs were obtained when Epikuron®200 was used as stabilizer. Pluronic®F-127 allowed preparation of NPs with some of the above-mentioned polymers, but after freeze-dried aggregates were detected. The best results were obtained using poly(vinyl alcohol) as stabilizer. (Piñón-Segundo, 2000; Piñón-Segundo et al., 2000; Piñón et al., 2001). In addition, we have reported that Eudragit® RS 100 could be solubilized in surfactant aqueous solutions, for instance, Brij® 58 (polyoxyethylene (20) cetyl ether) and poly(vinyl alcohol) (Fuentes et al., 2001). Considering these previous results, three polymers were chosen for the preparation of TCS-loaded NPs (TCS-NPs): poly(D,L-lactide-co-glycolide) (PLGA, Resomer® RG 502), poly(D,L-lactide) (PLA, Resomer® R 104) and cellulose acetate phthalate (CAP).

The present study had two principal aims. The first one was the preparation and characterization of TCS-NPs obtained by the emulsification–diffusion technique. Different TCS/polymer ratios were used in order to analyze the TCS effect on NP properties. Furthermore, a system to evaluate the TCS release from prepared NPs, avoiding the use of membrane diffusion techniques, was also proposed. Additionally, as a second aim, a preliminary in vivo study to assess the effects of TCS-NPs in induced periodontitis in dogs was set forth.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA, Resomer® RG 502, MW 8000) and poly(D,L-lactide) (PLA, Resomer® R 104, MW 2000) were obtained from Boehringer Ingelheim, Germany. Cellulose acetate phthalate (CAP, MW 2534.12) was purchased from Vita Drug, Mexico. Poly(vinyl alcohol) (PVAL, MW 26,000) was purchased from Glomarza, Mexico. Triclosan (TCS) was supplied by Multiquim, Mexico. HPLC-grade ethyl acetate and methyl ethyl ketone were provided by J.T. Baker, Mexico. Distilled water was obtained from a RiOs Millipore® distiller, USA. All the other reagents were of analytical grade and used without further purification.

2.2. Nanoparticle preparation

NPs were prepared using the emulsification–diffusion technique (Quintanar-Guerrero et al., 1996). Briefly, the organic solvent (ethyl acetate or methyl ethyl ketone) and water were mutually saturated for at least 20 min before use, in order to ensure initial thermodynamic equilibrium of both liquids. Polymer and TCS were dissolved in water-saturated organic solvent, and this organic solution was emulsified with a 5% (w/v) PVAL organic solvent-saturated aqueous solution using a stirrer (Caframo® RZR-1, Germany) at 1700 rpm for 10 min. Distilled water was subsequently added to the emulsion to induce diffusion of organic solvent into the continuous phase, leading to the formation of NPs. Organic solvent was eliminated from the raw NP suspension by vacuum steam distillation at 35 °C. NP suspension was centrifuged (Optima® LE-80K, Beckman, USA) at 20,000 rpm for 20 min. The NPs were finally frozen at −40 °C for 10 min and freeze-dried for 24 h (Labconco®, USA). Typically, 20 ml of organic solvent, 40 ml of PVAL solution, and 160 ml of distilled water were used per 400 mg of polymer. Table 1 shows the materials used in each batch.

2.3. Particle size analysis

Mean particle size was determined by photon correlation spectroscopy (PCS) using a Coulter N4 Plus submicron particle analyzer (Beckman, USA). Each suspension was diluted with MilliQ water (Millipore®, USA) until the appropriate particle concentration was achieved. The analysis was performed at a scattering angle of 90° and a temperature of 25 °C. Val-

Table 1
Composition of the Batches of NPs produced by the emulsification–diffusion technique

Batch number (%, w/w)	Polymer	Organic solvent	Initial TCS content
1	PLGA	Ethyl acetate	0.00
2	PLGA	Ethyl acetate	1.23
3	PLGA	Ethyl acetate	4.76
4	PLGA	Ethyl acetate	9.09
5	PLGA	Ethyl acetate	16.67
6	PLGA	Ethyl acetate	23.08
7	PLGA	Ethyl acetate	33.33
8	PLA	Ethyl acetate	0.00
9	PLA	Ethyl acetate	1.23
10	PLA	Ethyl acetate	4.76
11	PLA	Ethyl acetate	9.09
12	CAP	Methyl ethyl ketone	0.00
13	CAP	Methyl ethyl ketone	1.23
14	CAP	Methyl ethyl ketone	4.76
15	CAP	Methyl ethyl ketone	9.09

External phase: PVAL 5%.

ues given in Table 2 are means of three measurements.

2.4. Scanning electron microscopy (SEM) studies

An aqueous dispersion of the NPs was spread over a slab and dried under vacuum at room temperature. A thick gold layer (∼20 nm) was applied on the dried samples (Fine Coat Ion Sputter JFC-1100, JEOL, Japan). The surface morphology of NPs was then observed by SEM using a JSM-25 S II scanning electron microscope (JEOL, Japan).

2.5. Stabilizer quantification

The residual amount of PVAL in NPs was determined in triplicate by a colorimetric method, considering that PVAL in solution forms stable complexes with iodine in the presence of boric acid (Allémann et al., 1993). About 10 mg of the lyophilized cake (PLGA-NPs or PLA-NPs) were digested in 5 ml of 0.1 M NaOH for 48 h; the solution was then neutralized and filtered. The volume was adjusted to 10 ml with water (solution A). Two milliliters of a 0.65 M boric acid solution and 1 ml of an iodine solution (0.05 M iodine and 0.15 M

Table 2
NP properties: mean size, drug loading (DL), residual PVAL, entrapment efficiency (EE) and process efficiency (PE).

Batch number	Mean size (nm) ± S.D.	DL (%) ± S.D.	Residual PVAL (%) ± S.D.	EE (%)	PE (%)
1	176 ± 4.4	–	2.14 ± 0.05	–	99.0
2	219 ± 4.7	0.84 ± 0.06	2.85 ± 0.06	70.04	99.1
3	246 ± 5.1	3.93 ± 0.08	3.04 ± 0.08	85.12	99.0
4	272 ± 7.1	5.62 ± 0.09	3.22 ± 0.09	63.88	99.0
5	308 ± 4.4	11.46 ± 0.10	3.70 ± 0.10	71.40	99.1
6	354 ± 13.3	16.18 ± 0.06	2.93 ± 0.06	72.23	99.1
7	458 ± 11.1	23.75 ± 0.06	2.45 ± 0.06	73.04	99.2
8	219 ± 3.5	–	2.74 ± 0.07	–	99.1
9	207 ± 1.5	1.06 ± 0.06	2.99 ± 0.06	88.51	99.0
10	215 ± 3.5	4.10 ± 0.08	3.69 ± 0.08	89.40	99.1
11	286 ± 2.5	7.03 ± 0.07	2.76 ± 0.07	79.52	99.0
12	222 ± 9.6	–	2.35 ± 0.06	–	99.0
13	219 ± 5.2	0.98 ± 0.09	3.52 ± 0.09	82.28	99.2
14	192 ± 3.2	4.07 ± 0.07	2.77 ± 0.07	87.90	99.1
15	235 ± 14.5	7.92 ± 0.07	2.34 ± 0.07	89.21	99.0

S.D.: standard deviation, $n = 3$.

potassium iodide in water) were added to 5 ml of solution A. The absorbance of the resulting samples was measured at 640 nm (DU® 64, Beckman, USA), using as blank a solution of 5 ml of water with 2 ml of boric acid solution (0.65 M) and 1 ml of iodine solution (0.05 M iodine and 0.15 M potassium iodide). The calibration curve for PVAL quantification was linear over the 2–40 μg/ml range, $R^2 = 0.9999$.

For the residual stabilizer of CAP-NPs, ∼10 mg of lyophilized NPs were dissolved in methyl ethyl ketone; the solvent was evaporated, and water was added. The suspension was then filtered and adjusted to 10 ml with water; this solution replaced solution A, following the same process to form the complexes.

2.6. Drug loading, entrapment efficiency, and process efficiency

Approximately 10 mg of lyophilized (PLGA-NPs or PLA-NPs) were digested in 5 ml of 0.1 M NaOH for 48 h and then filtered. The volume was adjusted to 10 ml with 0.1 M NaOH. For CAP-NPs, ∼10 mg of lyophilized NPs were dissolved in methyl ethyl ketone. The solvent was evaporated and ∼5 ml of 0.1 M NaOH were added. This suspension was filtered and the volume was adjusted to 10 ml with 0.1 M NaOH. TCS content was assayed spectrophotometrically at 290 nm (DU® 64, Beckman, USA). Samples of unloaded NPs of each polymer were tried in the same way and used as blanks.

The calibration curve for the quantification of TCS in NaOH was linear ($R^2 = 0.9998$, range of 4–80 μg/ml). Additionally, amounts of TCS and polymer, equivalent to those included in NPs, were processed as described earlier for PLGA-NPs, PLA-NPs and CAP-NPs, in order to determine the TCS recovery efficiency. As a result, approximately 100% of the original amount of TCS was detected in the assays.

Drug loading (DL) and entrapment efficiency (EE) were calculated according to Eqs. (1) and (2), respectively:

$$\text{DL}\,(\%) = \frac{\text{amount of TCS in NPs}}{\text{amount of NPs}} \times 100 \quad (1)$$

$$\text{EE}\,(\%) = \frac{\text{DL}\,(\%)}{\text{percent of the initial TCS content} \times (1 - \text{fraction of residual PVAL})} \times 100 \quad (2)$$

Because of the adsorption of a certain amount of PVAL onto the NPs during the manufacturing process, the correction factor 1/(1 − fraction of residual PVAL) was introduced in Eq. (2) to avoid an underestimation of the entrapment efficiency. The initial TCS content corresponds to the theoretical TCS loading in the batch. These quantities are displayed in Table 1. Determinations of DL was done in triplicate. EE was calculated from the DL and residual PVAL averages.

The process efficiency was calculated from Eq. (3) after the preparation and freeze-drying of NPs.

$$\text{PE}\,(\%) = \frac{\text{weight of recovered NPs}}{\text{weight of TCS} + \text{weight of polymer} - \text{weight of residual PVAL}} \times 100 \quad (3)$$

The weight of TCS included in Eq. (3) corresponds to the loaded TCS into the NPs and the weight of the polymer is the amount of polymer used in the NP preparation. The weight of residual PVAL was determined from the average of residual PVAL assays.

2.7. Differential scanning calorimetry (DSC) studies

DSC analyses were carried out on samples of individual substances as well as on unloaded NPs, and TCS-NPs. The dried samples were weighed directly in aluminum pans (3–5 mg) and scanned between 0 and 200 °C at a heating rate of 10 °C/min or between 0 and 250 °C at the same heating rate, under nitrogen, using a DSC Q10 (TA Instruments, USA).

2.8. In vitro release kinetics

The release properties of NPs were studied in a 2% (w/v) Brij® 58 (polyoxyethylene (20) cetyl ether) aque-

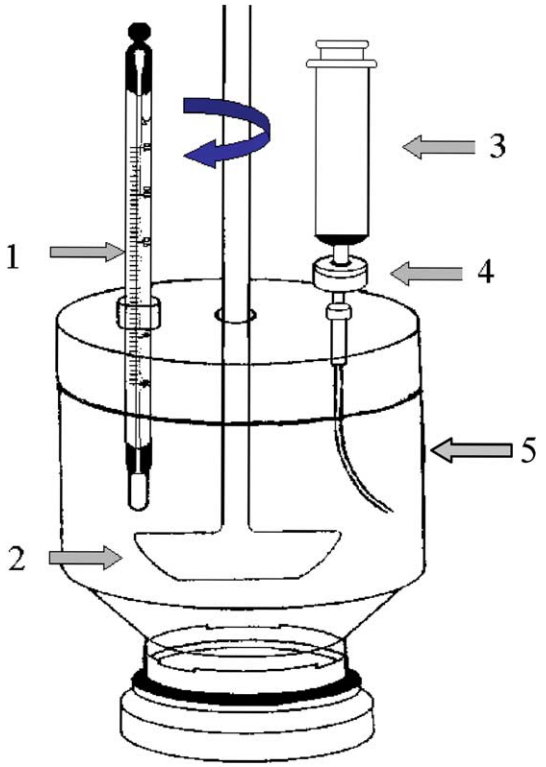

Fig. 1. Schematic representation of the device used in the in vitro release experiments. (1) Thermometer, (2) paddle, (3) syringe, (4) filter with membrane of 0.1 μm, and (5) container.

ous solution as dissolution medium to keep sink conditions. An accurate weight of lyophilized NPs was suspended in 500 ml of this solution using the device shown in Fig. 1. This device was especially designed to be easily adapted to a paddle dissolution apparatus (Apparatus 2, USP). The suspension was paddle-stirred at 25 rpm. The temperature was maintained at 37 °C using a water bath. At selected time intervals, 3 ml of the suspension were withdrawn using a syringe adapted to a 0.10 μm-filter and replaced by fresh medium. The replaced volume was added through the filter with the purpose of rinsing and cleaning it. The absorbance of the samples was measured spectrophotometrically at 282 nm and interpolated in a previously prepared calibration curve (ranging from 5 to 100 μg/ml, $R^2 = 0.9999$). The amount of drug released at each time point was corrected for the dilution effect. Release experiments were carried out in triplicate.

2.9. Preliminary in vivo study

This study used beagle dogs (15–20 kg) with surgically created intrabony periodontal defects. The experiment was conducted in compliance with the Mexican Regulations for Animal Care and Maintenance (NOM-062-ZOO-, 1999).

Periodontitis was induced in three healthy mature male dogs by surgically created intrabony periodontal defects (Lawter et al., 1990). Bone immediately adjacent to the interproximal surfaces of the teeth was removed by a slow speed dental hand piece, under sterile saline irrigation. The exposed root surfaces were encircled using a stainless steel wire. Later than, the flaps were replaced onto their original sites. The wire was left in place for 6 weeks, in order to suit plaque accumulation. Meanwhile, the dogs were fed with a soft diet. Two weeks after removing the ligatures, the defects were measured with a periodontal probe to confirm the presence of defects with a minimum depth of 5 mm. Before NP administration, mechanical scaling and root planning were applied in control and experimental sites.

Approximately 100 mg of lyophilized TCS-loaded PLGA-NPs (Batch 4, 9.09% of TCS) were suspended in sterilized water (1 ml). The dogs were put under general anesthesia and the suspensions of TCS-loaded PLGA-NPs were injected into the bottom of the experimental pockets with a syringe (29-gauge needle). Only sterilized water was applied to the control periodontal pockets. The dogs were observed at days 1, 7 and 15 after the treatment. Gingival index (GI) and bleeding on probing (BOP) were determined for experimental and control sites.

3. Results and discussion

The emulsification–diffusion technique allowed the preparation of polymeric submicron particles. All NPs obtained were less than 500 nm in size. Process efficiency was higher than 99.0% and entrapment efficiency (EE) was between 63.8 and 89.4% (Table 2). Fig. 2 shows the influence of TCS on NP mean size. As shown, the higher the quantity of TCS in the PLGA-NPs, the greater the NP mean size. A similar relationship was not clearly found for PLA-NPs and CAP-NPs because only small quantities of TCS were used.

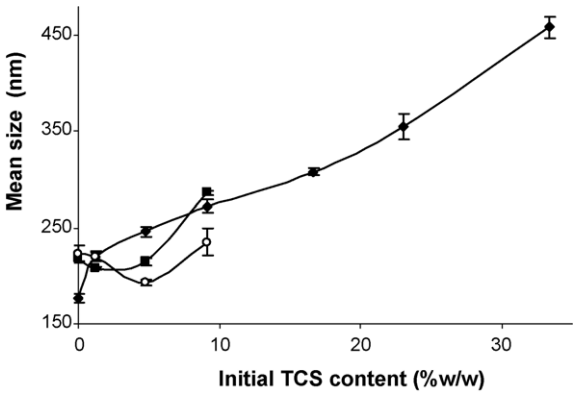

Fig. 2. Influence of the percentage of TCS on NP mean size ($n = 3$). PLGA-NPs (♦); PLA-NPs (■) and CAP-NPs (◯).

Using SEM, it was shown that all NPs had a homogeneous solid matrix structure, with no evidence of crystals on the surface. Fig. 3 shows the PLGA-NPs loaded with different quantities of TCS. Fig. 3A corresponds to the batch with the lowest theoretical content of TCS (1.23%) and Fig. 3F shows PLGA-NPs with the highest theoretical TCS content (33.33%). These results supported the increase of NP mean size as the TCS/polymer ratio is increased. An interesting aspect is that a high TCS concentration (Fig. 3E and F) causes the PLGA-NPs fusion and film formation. This behavior suggests that TCS works as a plasticizer.

DSC thermograms of free TCS, polymers, stabilizer, unloaded NPs and loaded NPs were obtained to define the physical state of the drug and the polymer in the NPs and to detect any drug-polymer interactions within the polymeric network of the NPs. Figs. 4–6 show the DSC thermograms of PLGA-NPs, PLA-NPs and CAP-NPs, respectively, with different TCS/polymer ratios, and the thermograms of stabilizer and polymers used in each formulation. Pure TCS showed an endothermic peak of melting at 63 °C. There was no peak observed at this temperature for TCS-NPs. DSC studies did not detect any crystalline drug material in the nanosphere samples. This confirmed the molecular level dispersion of TCS. After the process, TCS could be in an amorphous or disordered-crystalline phase of a molecular dispersion or a solid solution state in the polymer matrix (Mu and Feng, 2002).

The increase of TCS concentration in PLGA-NPs caused a shift of the glass transition temperature (T_g) to lower temperatures. That is why PLGA-NPs with high content of TCS tended to form films. On the other hand, the stabilizer did not have a significant influence on the thermal properties of polymeric material. DSC thermograms also showed that the T_g of pure PLGA was 47.27 °C. When this polymer was formulated in NPs containing 9.09, 16.67, and 23.08% of theoretical TCS loading, the T_g decreased to 41.72, 35.31, and 28.75 °C, respectively. In Fig. 4 (Batch 1), when the PLGA was in nanospheres, the T_g temperature was slightly higher (50.00 °C), but after the addition of different amounts of TCS (Batches 2–7) to the PLGA-NPs, the T_g falls around 23.03 °C in NPs with 33.33% of TCS. Fitzgerald and Corrigan (1996) prepared NPs with PLGA and levamisole. They found an endotherm associated with the glass transition of the co-polymer at 51 °C; this value is similar to that obtained in this work. In contrast, results in Fig. 4 differed with data obtained by Okada et al. (1994), who reported that the T_g of the PLGA matrix increased, as the loading of a basic drug increased, due to the electrostatic interaction between the basic drug and the carboxylic acids at the polymer terminal.

Fig. 5 shows the DSC thermograms of unloaded PLA-NPs and PLA-NPs loaded with TCS. The thermograms of pure TCS, PLA, and PVAL are also included. As previously mentioned, no peaks of TCS melting were detected in thermograms of PLA-NPs. Thermograms of unloaded and loaded PLA-NPs (Batches 8–11) showed an exotherm that was not detected for pure PLA. This exotherm could be related to a crystallization process. It has been reported that PLA can be obtained at the amorphous state by abrupt cooling of the melted product; nevertheless, crystallites of the polymer could appear after 24–72 h when the polymer is placed in a humid environment (Doelker, 1985). During the NP formation process, a rapid diffusion of solvent from the globules of the emulsion carries molecules into the aqueous phase, forming local regions of supersaturation, from which new globules or polymer aggregates (not totally desolvated) are formed (Quintanar-Guerrero et al., 1998b). It is possible for this process to cause a different accommodation of the polymer chains, and crystallites could be detected after PLA-NP formation. The thermograms of pure PLA and PLA-NPs also showed an endothermic process that began around 100 °C, with the maximum around 140 °C, which could be related to PLA decomposition.

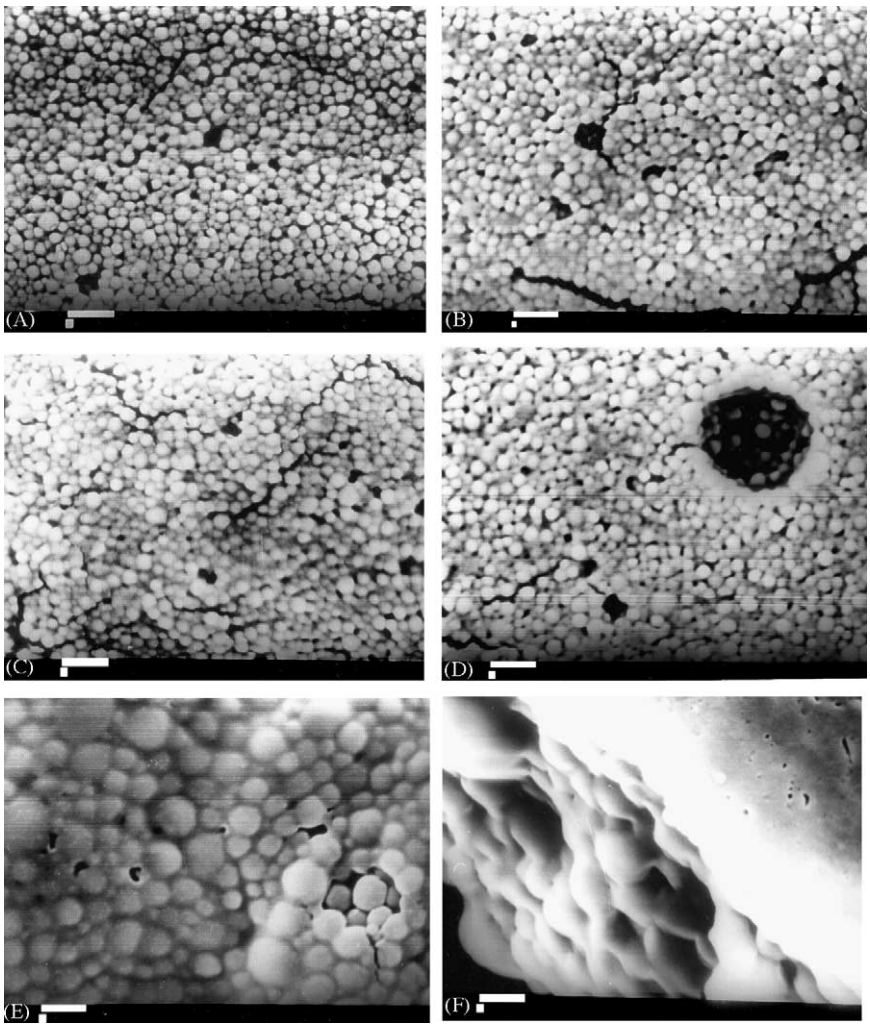

Fig. 3. Scanning electron micrographs of loaded PLGA-NPs with different theoretical loadings (bar = 1 μm). (A) Batch 2 (1.23% of TCS), (B) Batch 3 (4.76% of TCS), (C) Batch 4 (9.09% of TCS), (D) Batch 5 (16.67% of TCS), (E) Batch 6 (23.08% of TCS) and (F) Batch 7 (33.33% of TCS).

In Fig. 6, thermograms for CAP, unloaded CAP-NPs and TCS-loaded CAP-NPs are shown. Theoretical CAP glass transition temperature ranges from 160 to 170 °C. It was not possible to clearly detect the T_g of the polymer. Thermograms of pure CAP and CAP-NPs did not show well-defined transitions or peaks. The results only confirmed that TCS was dispersed molecularly into NPs since no endothermic peak of TCS was detected.

The characterization of in vitro drug release from a colloidal carrier, especially under sink conditions is technically difficult to achieve. This could be attributed to the inability to separate successfully the particles from the dissolved or released drug in the sink solution due to the very small size of the particles. Attempting to evaluate the drug release from colloidal carriers, diverse techniques have been used; for example, diffusion cells, where the undiluted colloidal drug carrier suspension is separated from the sink solution by a porous membrane or a dialysis membrane. Several release studies using the dialysis bag technique under either dynamic or static

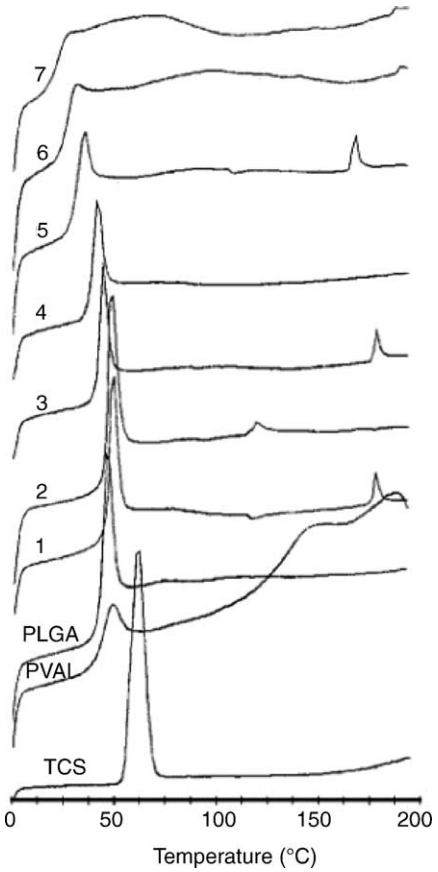

Fig. 4. DSC thermograms of TCS; PVAL; PLGA; unloaded PLGA-NPs (1) and TCS-loaded PLGA-NPs, Batches 2–7 (2–7).

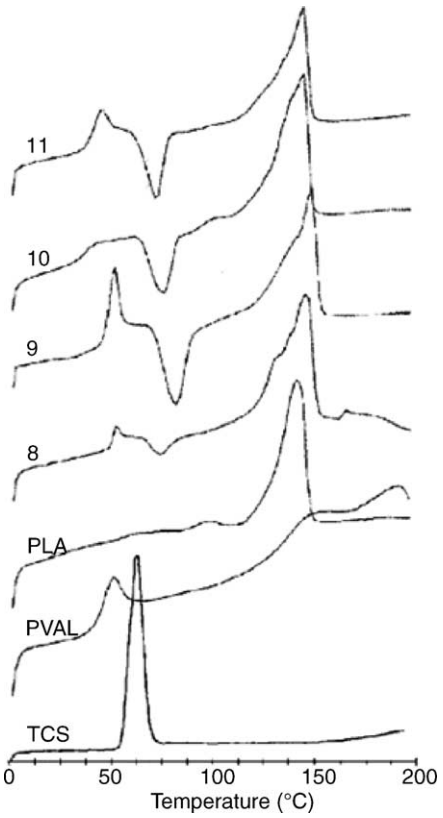

Fig. 5. DSC thermograms of TCS; PVAL; PLA; unloaded PLA-NPs (8) and TCS-loaded PLA-NPs, Batches 9–11 (9–11).

conditions have been reported (Levy and Benita, 1990).

It has been recommended as a rule of thumb that drug concentration in the sink phase in dissolution experiments be kept below 10% of saturation. If the drug is poorly soluble in water, it may be permissible to add nonaqueous solvents or solubilizing agents to the dissolution medium (Washington, 1990). In this study, we proposed a system to evaluate the in vitro release of TCS from NPs adapted to a conventional dissolution apparatus. An amount of lyophilized NPs equivalent to ≈30 mg of TCS was suspended in a 2% (w/v) Brij® 58 aqueous solution, allowing the experiments to be carried out under sink conditions (TCS solubility in dissolution medium = 9.7 mg/ml). The release rate was not controlled by a membrane or dialysis bag and the NPs were in direct contact with the dissolution medium. It is important to point out that the selection of the sink medium and its volume were optimized in a previous work (Piñón-Segundo, 2003). The kinetic release process is very sensitive to the prevalence of sink condi-

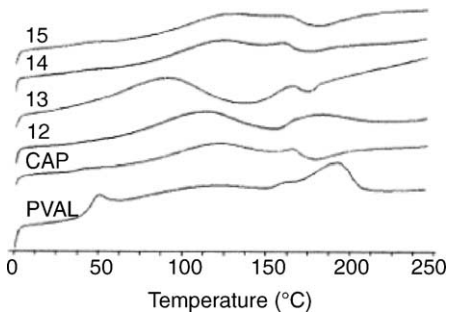

Fig. 6. DSC thermograms of PVAL; CAP; unloaded CAP-NPs (12) and TCS-loaded CAP-NPs, Batches 13–15 (13–15).

tions. Any factor able to perturb sink conditions will alter the release profile (Levy and Benita, 1990).

Calvo et al. (1996) analyzed the in vitro release of several colloidal systems (nanospheres, nanocapsules and nanoemulsions) as indomethacin carriers. These studies were carried out by the bulk equilibrium reverse dialysis bag technique, at 37 °C. The release profiles obtained were similar for all formulations and 85% of drug diffused out from the colloidal systems within 2 h and practically all the drug was released within 4 h. Indomethacin was rapidly and completely released only when sink conditions were maintained. Therefore, they concluded that with higher volume of the dissolution media, a faster and complete release of the drug takes place. Considering the above-mentioned results and that a high volume of the dissolution medium (500 ml) was used during our experiments, a fast release of TCS from NPs could be expected.

The mean values of the in vitro release studies are shown in Figs. 7–9, where M_t is the cumulative amount of TCS at predetermined time intervals and M_α is the maximum amount of TCS released during the dissolution experiment. In all release studies, the maximum amount of TCS released was higher than 97% of the drug loaded into NPs, according to the DL. The residual amount in NPs was not determined; it was impossible to recover all NPs after dissolution. Fig. 7 shows the release of TCS from PLGA-NPs. A burst effect was observed in all Batches. The profiles of Batches 2–5 (1.23–16.67%, w/w theoretical loading) showed that at least 75% of the TCS was released from PLGA-NPs at 30 min and, approximately 90% of TCS was released from NPs of Batch 6 (23.08% of theoretical TCS) in the same time. It was observed that NPs with a greater content of TCS released more quickly than Batch 2, which contained only 1.23% of TCS. It has been reported that an increase in the amount of drug in the nanospheres not only increases the porosity of the system as the drug dissolves, but also, as in TCS-NPs, reduces the relative amount of polymeric material acting as a diffusional barrier (Radwan, 1995).

With regard to PLA-NPs and CAP-NPs, TCS was also quickly released. In fact, more than 90% of TCS was released from CAP-NPs (Batches 13 and 14) at

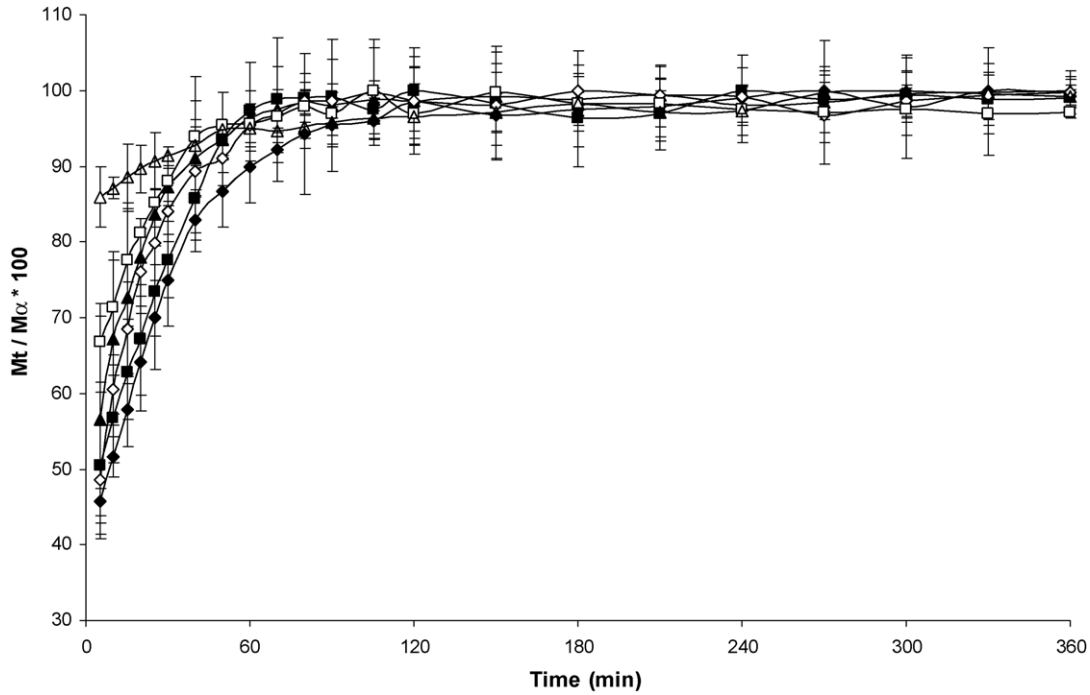

Fig. 7. Release profiles of TCS from PLGA-NPs. Mean values and their standard deviations (bars), $n = 3$. Percentages of TCS: Batch 2, 1.23% (♦); Batch 3, 4.76% (■); Batch 4, 9.09% (▲); Batch 5, 16.67% (◊); Batch 6, 23.08% (□) and Batch 7, 33.33% (△).

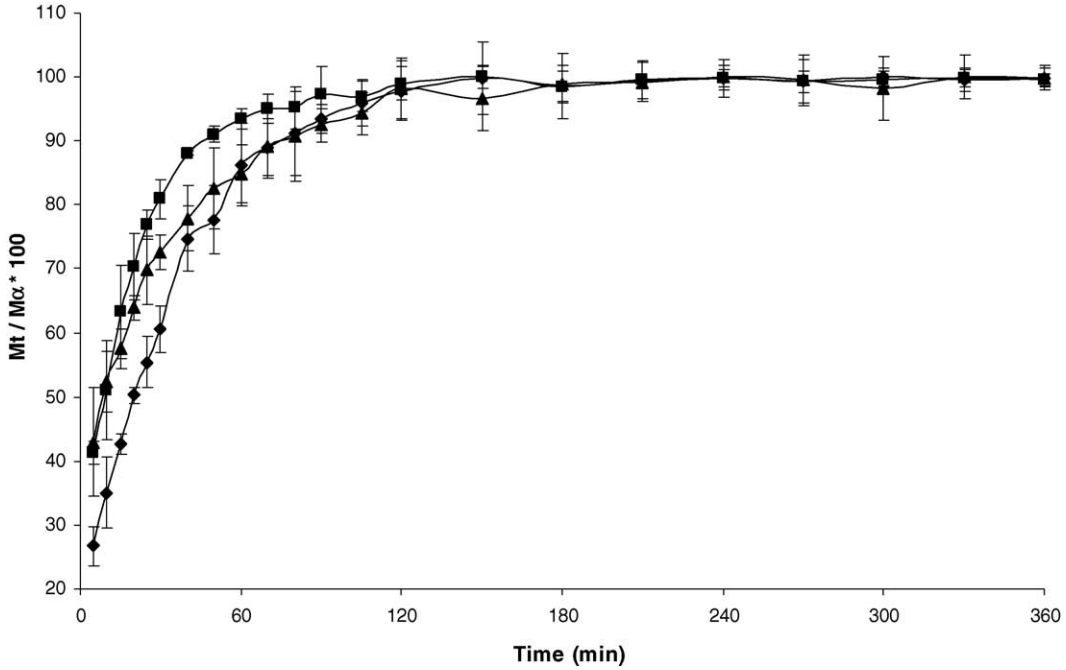

Fig. 8. Release profiles of TCS from PLA-NPs. Mean values and their standard deviations (bars), $n = 3$. Percentages of TCS: Batch 9, 1.23% (♦); Batch 10, 4.76% (■) and Batch 11, 9.09% (▲).

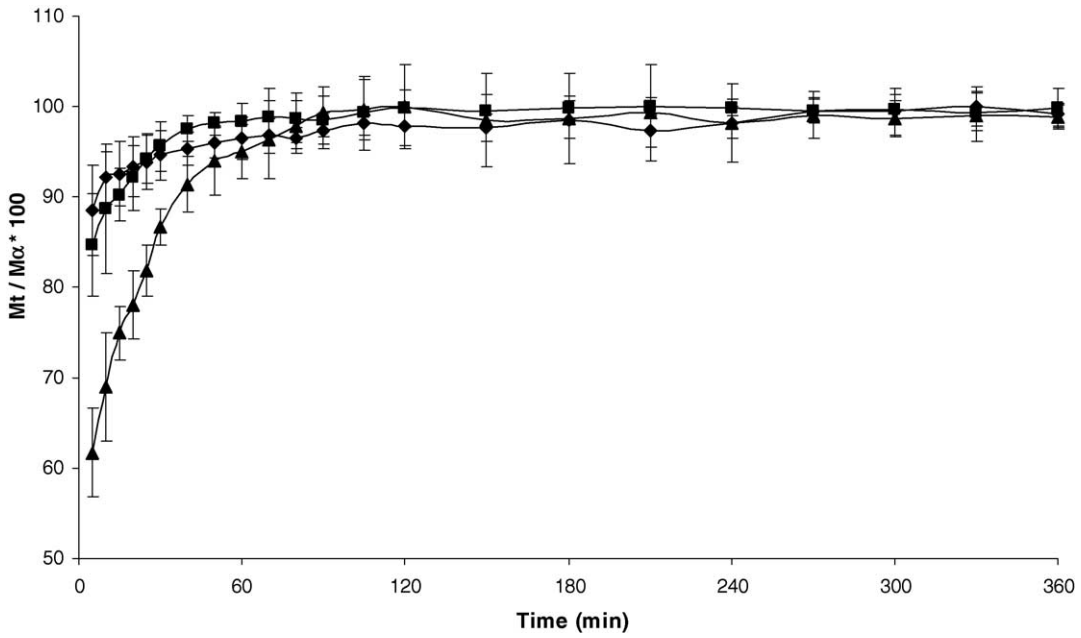

Fig. 9. Release profiles of TCS from CAP-NPs. Mean values and their standard deviations (bars), $n = 3$. Percentages of TCS: Batch 13, 1.23% (♦); Batch 14, 4.76% (■) and Batch 15, 9.09% (▲).

20 min. Apparently, PLA-NPs released the drug at a slightly slower rate than PLGA-NPs with similar proportions of TCS. Actually, analysis of variance showed no significant differences during the first 30 min. The cumulative amounts of TCS released were not significantly different among TCS-NPs with theoretical loads of 1.23, 4.76 and 4.09% ($F = 0.84$; $F_{0.05;2,4} = 6.94$); independently of the polymer with which they have been obtained ($F = 5.58$; $F_{0.05;2,4} = 6.94$).

Although no statistical differences in the release profiles were detected, it is necessary to explain the observed differences in the percentages of TCS released. Poly-glycolide acid is highly crystalline because it lacks the methyl side groups of the PLA (Jain, 2000). In contrast, PLA is amorphous. Therefore, the addition of increasing proportions of glycolide to PLA lowers glass transition temperature and generally increases polymer hydrophilicity (Markland and Yang, 2002). With this in mind, we can say that the release of TCS from PLA-NPs was slower than the release of TCS from PLGA-NPs because water access throughout PLGA-NPs is easier than throughout PLA-NPs. Furthermore, DSC studies suggested that TCS could behave as a plasticizer, so that the relaxation of the PLGA chains favors the TCS release from PLGA-NPs.

The rapid initial release of TCS can be attributed to the large surface to volume ratio of the NP geometry. Due to their size, a burst effect was observed for drug molecules entrapped into all of the NPs prepared. Taking this into consideration, in the case of a matrix device, drug is uniformly distributed/dissolved in the matrix and the release occurs by drug diffusion or erosion of the matrix. If drug diffusion is faster than matrix degradation (as in this work), the mechanism of drug release occurs mainly by diffusion (Niwa et al., 1993; Soppimath et al., 2001).

Higuchi's equation (Higuchi, 1961) was originally developed to explain the drug release from an ointment base.

In view of the fact that prepared TCS-NPs can be considered as a homogeneous polymer matrix-type delivery system, with the drug (TCS) molecularly dispersed, Higuchi's equation can be extensive for these matrix systems, considering that the depletion zone moves to the center of the NPs as the drug is released. The equation: $Q = (2ADC_s t)^{1/2}$ indicates that the cumulative amount of drug released (M_t) per unit surface (S) is proportional to the square roots of A, the total amount of drug in a unit volume of the matrix; D, the diffusion coefficient of the drug in the matrix; C_s, the solubil-

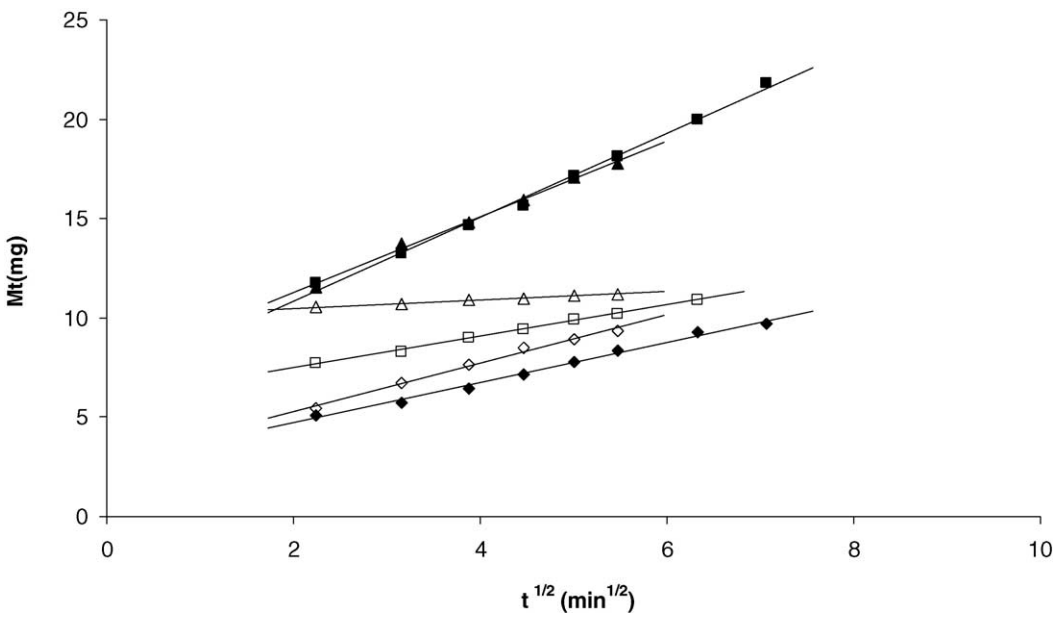

Fig. 10. Higuchi's model for TCS released from PLGA-NPs. Percentages of TCS: Batch 2, 1.23% (♦); Batch 3, 4.76% (■); Batch 4, 9.09% (▲); Batch 5, 16.67% (◊); Batch 6, 23.08% (□) and Batch 7, 33.33% (△).

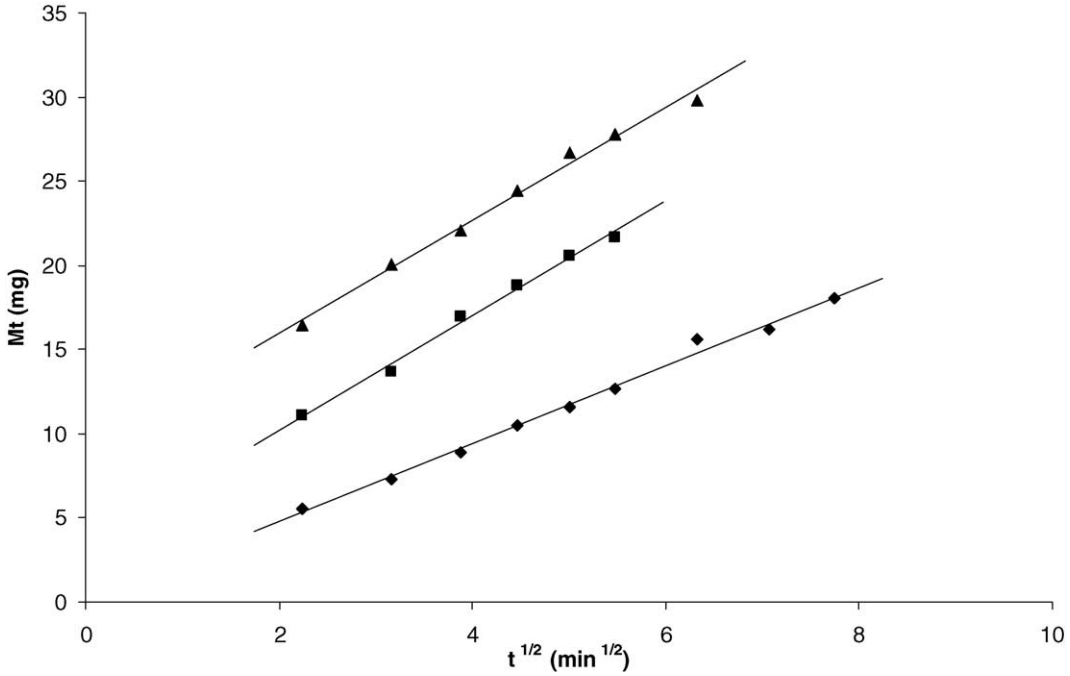

Fig. 11. Higuchi's model for TCS released from PLA-NPs. Percentages of TCS: Batch 9, 1.23% (♦); Batch 10, 4.76% (■) and Batch 11, 9.09% (▲).

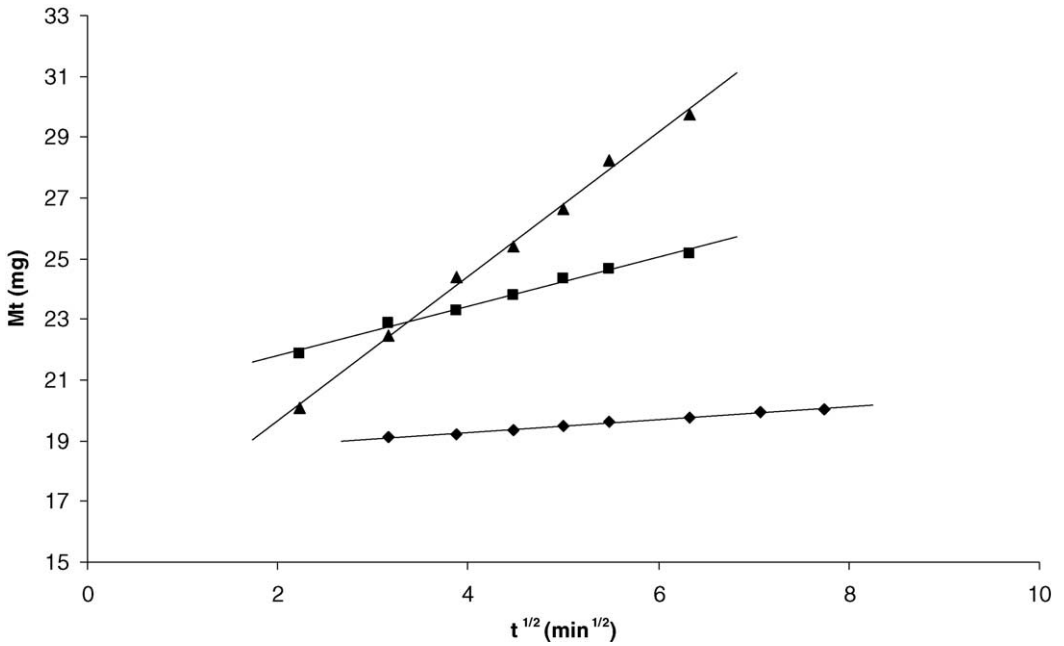

Fig. 12. Higuchi's model for TCS released from CAP-NPs. Percentages of TCS: Batch 13, 1.23% (♦); Batch 14, 4.76% (■) and Batch 15, 9.09% (▲).

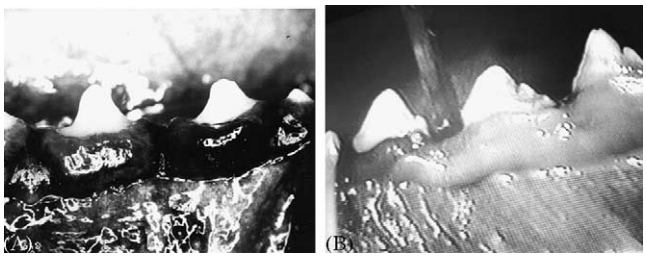

Fig. 13. Experimental sites after the administration of TCS-loaded PLGA-NPs (9.09% of TCS) in dogs; (A) 8 days and (B) 15 days.

ity of drug in the polymeric matrix; and t, the time. The factor $(2ADC_s)^{1/2}S$ is the drug release rate constant (K_H). When the Higuchi's equation was applied (M_t versus $t^{1/2}$) to the TCS-NPs (Figs. 10–12), straight lines ($R^2 > 0.99$) were always obtained, suggesting that the release rate can be satisfactorily described by this model. The ensuing rate constants and their correlation coefficients are shown in Table 3. This behavior suggests that the diffusion is the controlling factor of the release.

The in vivo study was performed in dogs according to the Mexican Regulations for Animal Care and Maintenance (NOM-062-ZOO-, 1999). In this preliminary study, only the gingival index (GI) and bleeding on probing (BOP) were determined. GI is a measure of gingival inflammation and is evaluated on a scale: 0 (no inflammation, healthy gingiva) to 3 (severe inflammation) at four sites of each tooth. A positive BOP indicates sites that showed bleeding within 10–30 s after probing (Schwach-Abdellaoui et al., 2000). In this study, all control and experimental sites bled after probing at all time periods. With respect to GI, at days 1 and 8, a severe inflammation was detected in control and experimental sites (GI = 3). Gingival structures had clearly visible redness, edema ulceration, and a tendency to spontaneous hemorrhage. At day 15, a clear difference between control and experimental sites was detected (Fig. 13). All the experimental sites showed only a moderate inflammation (GI = 2), with less redness than control sites, although BOP was positive. Thus, it could be possible that NPs have a certain effect over the reduction of the inflammation in the experimental sites. However, a complete study is necessary to find statistical differences that could support that idea. In vivo experiments are in progress in order to confirm the therapeutic value of this delivery system as a new option in periodontal treatment. Parameter measurements of plaque score, gingival bleeding, gingival inflammation and pocket depth should also be recorded. Besides comparisons between TCS-NPs, TCS solutions and TCS suspensions should be evaluated.

Table 3
Release rate constants of TCS released from NPs according to the Higuchi's model

Batch number	K_H (mg/min$^{1/2}$)	Correlation coefficient
2	1.010	0.992
3	2.106	0.994
4	1.908	0.996
5	1.224	0.992
6	0.790	0.997
7	0.212	0.997
9	2.317	0.993
10	3.400	0.993
11	3.335	0.991
13	0.204	0.981
14	0.810	0.991
15	2.374	0.996

4. Conclusion

The emulsification–diffusion process allowed the obtainment of submicron polymeric particles charged with TCS. The entrapment efficiencies were higher than 63.8% for all Batches. The TCS incorporated into the PLGA-NPs increased the mean size diameter and decreased the glass transition temperature of the polymer. The in vitro release studies were carried out using a device designed in our laboratory, which allows a direct contact between medium and NPs. TCS released quickly from all NP Batches. This behavior was mainly attributed to the large surface area of NPs. The release data followed the model proposed

by Higuchi for matrix systems. A preliminary in vivo study suggested that TCS-NPs could help decrease gingival inflammation, but it is necessary to conduct several studies to evaluate the capacity of these carriers as drug delivery systems for periodontal treatment.

Acknowledgments

This work was supported by CONACYT, Mexico (ref. J 27741-M). The authors are grateful to Mr. Rodolfo Robles for his technical assistance during the SEM experiments. Elizabeth Piñón-Segundo acknowledges a grant from CONACYT, Mexico (ref. 145133) and DGEP, UNAM.

References

Allémann, E., Doelker, E., Gurny, R., 1993. Drug loaded poly(lactic acid) nanoparticles produced by a reversible salting-out process, purification of an injectable dosage form. Eur. J. Pharm. Biopharm. 39, 13–18.

Baker, R.W., Krisko, E.A., Kochinke, F., Grassi, M., Armitage, G., Robertson, P., 1988. A controlled release drug delivery system for the periodontal pocket. In: Proceedings of the 15th International Symposium on Controlled Release of Bioactive Materials, Controlled Release Society Inc., Basel, Switzerland, 15–19 August, pp. 238a–238b, 140.

Brill, N., Björn, H., 1959. Passage of tissue fluid into human gingival pockets. Acta Odont. Scand. 17, 11–21.

Brill, N., Krasse, B., 1958. The passage of tissue fluid into the clinically healthy gingival pocket. Acta Odont. Scand. 16, 233–245.

Calvo, P., Vila-Jato, J.L., Alonso, M.J., 1996. Comparative in vitro evaluation of several colloidal systems, nanoparticles, nanocapsules, and nanoemulsions, as ocular drug carriers. J. Pharm. Sci. 85, 530–536.

Carrillo, J., Castillo, M., Hernández, H., Zermeño, J., 2000. Estudio epidemiológico de las enfermedades periodontales en pacientes que acuden a la facultad de estomatología de la UASLP. Revista ADM LVII, 205–213.

Colombo, A.P., Briancon, S., Lieto, J., Fessi, H., 2001. Project, design and use of a pilot plant for nanocapsule production. Drug Dev. Ind. Pharm. 27, 1063–1072.

Doelker, E., 1985. Propiétés des polymères et principes de préparation des systèmes médicamenteux polymériques. In: Buri, P., Puisieux, F., Doelker, E., Benoît, J.P. (Eds.), Formes Pharmaceutiques Nouvelles, aspects technologique, biopharmaceutique et medical. Technique et Documentation, Paris, pp. 34–35.

Drisko, C.H., 1996. Non-surgical pocket therapy, pharmacotherapeutics. Ann. Periodontol. 1, 491–566.

Esposito, E., Cortesi, R., Cervellati, F., Menegatti, E., Nastruzzi, C., 1997. Biodegradable microparticles for sustained delivery of tetracycline to the periodontal pocket, formulatory and drug release studies. J. Microencapsulat. 14, 175–187.

Fine, D.H., Pechersky, J.L., McKibben, D.H., 1969. The penetration of human gingival sulcular tissue by carbon particles. Arch. Oral Biol. 14, 1117–1119.

Fitzgerald, J.F., Corrigan, O.I., 1996. Investigation of the mechanisms governing the release of levamisole from poly-lactide-co-glycolide delivery systems. J. Control. Rel. 42, 125–132.

Friedman, M., Steinberg, D., 1990. Sustained-release delivery systems for the treatment of dental diseases. Pharm. Res. 7, 313–317.

Fuentes, A., Olvera, B., Piñón, E., López, M., Ganem, A., Quintanar, D., 2001. Evaluación de recubrimiento de película a partir de soluciones acuosas de copolímeros acrílicos originalmente acuoinsolubles con surfactantes. In: XXXIV Congreso Nacional de Ciencias Farmacéuticas, Manzanillo, México, 21–25 October; Asociación Farmacéutica Mexicana, AC, pp. 026, 83–84.

Furuichi, Y., Ramberg, P., Krok, L., Lindhe, J., 1997. Short term effects of triclosan on healing following subgingival scaling. J. Clin Periodontol. 24, 777–782.

Ganem-Quintanar, A., 1997. Étude de la perméabilité de la muqueuse orale: evaluation de différentes approaches pour augmenter le passage des principes actifs, Ph.D. thesis. Université de Genève/Université Claude Bernard, Lyon, Genève.

García-Godoy, F., De Vizio, W., Volpe, A.R., Ferlauto, R.J., Miller, J.M., 1990. Effect of a triclosan/copolymer/fluoride dentifrice on plaque formation and gingivitis, a 7-month clinical study. Am. J. Dent. 3, S15–S26.

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

Jain, R.A., 2000. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 21, 2475–2490.

Kreuter, J., 1994. Nanoparticles. In: Kreuter, J. (Ed.), Colloidal Drug Delivery Systems. Marcel Dekker, New York, pp. 219–220.

Lawter, J.R., Lanzilotti, M., Brizzolara, N., Fransson, C., Christersson, L.A., Johanson, O., 1990. Sustained drug delivery to the periodontal pocket. In: Proceedings of the 17th International Symposium on Controlled Release of Bioactive Materials, Controlled Release Society Inc., Nevada, USA, 22–25 July, pp. 230–231, D344.

Levy, M.Y., Benita, S., 1990. Drug release from submicronized o/w emulsion, a new in vitro kinetic evaluation model. Int. J. Pharm. 66, 29–37.

Markland, P., Yang, V., 2002. Biodegradable polymers as drug carriers. In: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. Marcel Dekker, New York, pp. 143–144.

Medlicott, N.J., Rathbone, M.J., Tucker, I.G., Holborow, D.W., 1994. Delivery systems for the administration of drugs to the periodontal pocket. Adv. Drug. Deliv. Rev. 13, 181–203.

Mu, L., Feng, S.S., 2002. Vitamin E TPGS used as emulsifier in the solvent evaporation/extraction technique for fabrication of polymeric nanospheres for controlled release of paclitaxel (Taxol®). J. Control. Rel. 80, 129–144.

Niwa, T., Takeuchi, T., Hino, T., Kunou, N., Kawashima, Y., 1993. Preparations of biodegradable nanospheres of water-soluble and

insoluble drugs with D,L-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. J. Control. Rel. 25, 89–98.

NOM-062-ZOO-, 1999. Especificaciones técnicas para la producción uso y cuidado de los animales de laboratorio, 2001. Norma Oficial Mexicana. Diario Oficial de la Federación, Segunda Seccion, vol. 575, pp. 20–72.

Okada, H., Doken, Y., Ogawa, Y., Toguchi, H., 1994. Preparation of 3-month depot injectable microspheres of leuprorelin acetate using biodegradable polymers. Pharmacol. Res. 11, 1143–1147.

Piñón-Segundo, E., 2000. Diseño de un sistema terapéutico nanoparticulado destinado al tratamiento de la gingivitis y la periodontitis, Tesis Licenciatura. Facultad de Estudios Superiores Cuautitlán. Universidad Nacional Autónoma de México, México.

Piñón-Segundo, E., Jacques, Y., Ganem-Quintanar, A., Quintanar-Guerrero, D., 2000. Optimization of a process to obtain nanoparticles of triclosan for periodontal treatment. In: Proceedings of the 27th International Symposium on Controlled Release of Bioactive Materials, Controlled Release Society Inc., París, France, 7–13 July, p. 8133.

Piñón, E., Chávez, F., Ayala, H.A., Fuentes A., Ganem, A., Quintanar, D., 2001. Desarrollo de un sistema nanoparticulado biodegradable destinado al tratamiento de la gingivitis y la periodontitis. In: XXXIV Congreso Nacional de Ciencias Farmacéuticas, vol. 023. Asociación Farmacéutica Mexicana, AC, Manzanillo, México, 21–25 October, pp. 81–82.

Piñón-Segundo, E., 2003. Desarrollo tecnológico y caracterización de partículas submicrónicas poliméricas como vectores terapéuticos para el tratamiento de enfermedades periodontales, Tesis Maestría, Programa de Maestría y Doctorado en Ciencias Químicas. Universidad Nacional Autónoma de México, México.

Quintanar-Guerrero, D., Fessi, H., Allémann, E., Doelker, E., 1996. Influence of stabilizing agents and preparative variables on the formation of poly(D,L-lactic acid) nanoparticles by an emulsification–diffusion technique. Int. J. Pharm. 143, 133–141.

Quintanar-Guerrero, D., Ganem-Quintanar, A., Allémann, E., Fessi, H., Doelker, E., 1998a. Influence of the stabilizer coating on the purification and freeze drying of poly(D,L-lactid acid) nanoparticles prepared by an emulsion-diffusion technique. J. Microencapsulat. 15, 107–119.

Quintanar-Guerrero, D., Allémann, E., Fessi, H., Doelker, E., 1998b. Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. Drug Dev. Ind. Pharm. 24, 1113–1117.

Radwan, M.A., 1995. in vitro evaluation of polyisobutylcyanoacrylate nanoparticles as a controlled drug carrier for theophylline. Drug Dev. Ind. Pharm. 21, 2371–2375.

Ramberg, P., Furuichi, Y., Volpe, A.R., Nabi, N., Gaffar, A., Lindhe, J., 1995. The effect of triclosan on developing gimgivitis. J. Clin. Periodontol. 22, 442–448.

Ratcliff, P.A., 1966. Permeability of healthy gingival ephitelium by microscopicalii observable particles. J. Periodontol. 37, 291–293.

Rosling, B., Dahlén, G., Volpe, A.R., Furuichi, Y., Ramberg, P., Lindhe, J., 1997a. Effect of triclosan on the subgingival microbiota of periodontitis-susceptible subjects. J. Clin. Periodontol. 24, 881–887.

Rosling, B., Wannfors, B., Volpe, A.R., Furuichi, Y., Ramberg, P., Lindhe, J., 1997b. The use of a triclosan/copolymer dentrifice may retard the progression of periodontitis. J. Clin. Periodontol. 24, 873–880.

Schwach-Abdellaoui, K., Vivien-Castioni, N., Gurny, R., 2000. Local delivery of antimicrobial agents for the treatment of periodontal diseases. Eur. J. Pharm. Biopharm. 50, 83–99.

Soppimath, K.S., Aminabhavi, T.M., Kulkarni, A.R., Rudzinski, W.E., 2001. Biodegradable polymeric nanoparticles as drug delivery devices. J. Control Rel. 70, 1–20.

Tolo, K.J., 1971. A study of permebility of gingival pocket epithelium to albumin in guinea pigs and norwegian pigs. Arch Oral Biol. 16, 881–888.

Waaler, S.M., Rölla, G., Skjörland, K., Ögaard, B., 1993. Effects of oral rinsing with triclosan and sodium lauryl sulphate on dental dental plaque formation, a pilot study. Scan. J. Dent. Res. 101, 192–195.

Washington, C., 1990. Drug release from microdisperse systems, a critical review. Int. J. Pharm. 58, 1–12.