Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 325 (2006) 172–179
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

PLA/PLGA nanoparticles for sustained release of docetaxel

T. Musumeci [a], C.A. Ventura [b], I. Giannone [a], B. Ruozi [c], L. Montenegro [a], R. Pignatello [a], G. Puglisi [a,*]

[a] *Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Catania, Viale A. Doria, 6-I-95125 Catania, Italy*
[b] *Pharmaco-Chemical Department, Faculty of Pharmacy, University of Messina, Viale Annunziata, I-98168 Messina, Italy*
[c] *Department of Pharmaceutical Sciences, University of Modena and Reggio Emilia, Via Campisi 183, 41100 Modena, Italy*

Received 30 March 2006; received in revised form 9 June 2006; accepted 17 June 2006
Available online 23 June 2006

Abstract

This study investigates the potentiality of nanosphere colloidal suspensions as sustained release systems for intravenous administration of docetaxel (DTX). Nanospheres were prepared by solvent displacement method using polylactic acids (PLA) at different molecular weight and polylactic-co-glycolic (PLGA) as biodegradable matrices. The systems were characterized by light scattering analysis for their mean size, size distribution and zeta potential and by scanning electron microscopy (SEM) for surface morphology. The average diameters of the nanoparticles ranged from 100 to 200 nm. Negative zeta potential values were observed for all systems, particularly the nanospheres produced with the lowest molecular weight PLA showed a zeta potential value of −28 mV. Differential scanning calorimetry analysis (DSC) suggested that DTX was molecularly dispersed in the polymeric matrices. A biphasic release of DTX was observed for all colloidal suspensions, after a burst effect in which about 50% (w/w) of the loaded drug was released a sustained release profile for about 10 days was observed. To evaluate the influence of the polymeric carrier on the interaction of DTX with biological membranes, we performed an *in vitro* study using lipid vesicles made of dipalmitoylphosphatidylcholine (DPPC) as a biomembrane model. DSC was used as a simple and not invasive technique of analysis. DTX produced a depression of DPPC pretransition peak, no variation of the main phase transition temperature and a significative increase of ΔH value, showing a superficial penetration of the drug into DPPC bilayer. Kinetic experiments demonstrated that the release process of DTX form nanospheres is affected by the molecular weight of the employed polymers.

Keywords: Docetaxel; Nanoparticles; PLA/PLGA; *In vitro* release; Biomembrane models; DSC

1. Introduction

Docetaxel (Taxotere®) (DTX, Fig. 1) is a second-generation taxane derived from the needles of the European yew tree, *Taxus baccata* (Herbst and Khuri, 2003). The synthesis of DTX starts from 10-deacetylbaccarin III, a non-cytotoxic constituent of European yew tree needles (Aapro and Bruno, 1995).

DTX acts by disrupting the microtubular network that is essential for mitotic and interphase cellular functions. It promotes the assembly of tubulin into stable microtubules and inhibits their disassembly, causing inhibition of cell division and eventual cell death. DTX shows 1.9-fold higher than paclitaxel affinity for microtubule. The clearance of DTX depends on the cytochrome P450 (CYP) 3A isoforms, notably CYP3A4 and CYP3A5, and the membrane transporter P-glycoprotein (ABCB1) (Aapro, 1996).

DTX shows a very low water solubility, then presently the only available formulation for clinical use consists of a solution (40 mg/ml) in a vehicle containing high concentration of Tween 80®. This vehicle has been associated with several hypersensitivity reactions and has shown incompatibility with common PVC intravenous administration sets (Gelderblom et al., 2001). It interferes with the normal binding of DTX to serum proteins in a concentration dependent-manner and can modulate the pharmacokinetics of DTX *in vivo* (Baker et al., 2004).

In order to eliminate the Tween 80®-based vehicle and in the attempt to increase the drug solubility, alternative dosage forms have been suggested, including liposomes (Immordino et al., 2003; Alexopoulos et al., 2004) and cyclodextrins (Grosse et al., 1998).

* Corresponding author. Tel.: +39 095222215; fax: +39 0957384211.
E-mail address: puglisig@unict.it (G. Puglisi).

0378-5173/$ – see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2006.06.023

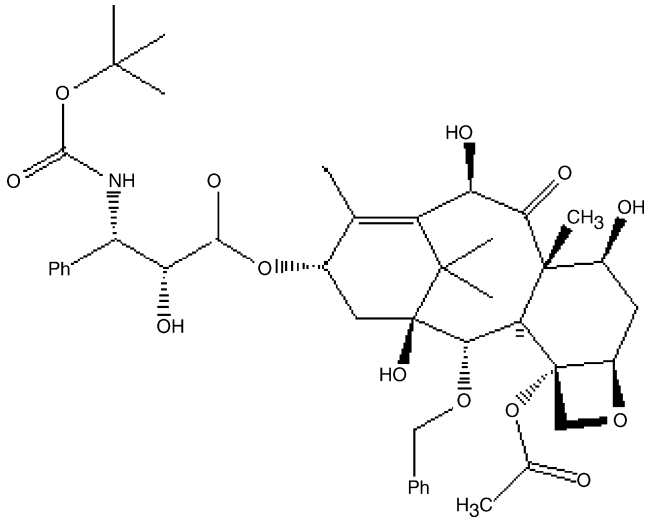

Fig. 1. Chemical structure of docetaxel.

Polymeric nanoparticles show some advantages with respect to other drug delivery systems, such as more stability during storage (Müller et al., 2001). Moreover, these colloidal systems, after intravenous administration, may extravasate solid tumours and into inflamed or infected sites, where the capillary endothelium is defective (Barratt, 2000; Brigger et al., 2002), thus passively targeting drug-loaded nanoparticles to the tumour site. Polymeric nanoparticles could reduce the multi-drug resistance that characterizes many anticancers drugs, including DTX, by a mechanism of internalization of the drug (Davda and Labhasetwar, 2002), reducing its efflux from cells mediated by the P-glycoprotein (Brigger et al., 2002; Mu and Feng, 2003a,b).

In this work, we developed polymeric drug delivery systems for intravenous administration of DTX, able to improve the drug solubility. Polymeric nanoparticles were prepared by a "solvent deposition method" (Giannavola et al., 2003) using as matrix poly(DL-lactic acid) (PLA) and poly(DL-lactic-co-glycolic) acid (PLGA) at different molecular weights. The systems were characterized for surface morphology, size distribution and surface chemistry. Technological studies were performed to evaluate the drug encapsulation efficiency and *in vitro* release. Moreover, by using DSC as a simple, powerful and precise method, we investigated the influence of DTX on DPPC biomembrane model assembly and the drug–membrane interaction as a function of DTX molar fraction. The influence of the polymeric carrier on the kinetic interaction of DTX-loaded nanoparticles with DPPC liposomes was also investigated.

2. Materials and methods

2.1. Materials

Polylactic acid (PLA) RES 203 (M_w 16,000), RES 206 (M_w 109,000), RES 207 (M_w 209,000) and polylactic-co-glycolic acid (RG502H, 50:50) were purchased from Boheringer Ingheleim (Germany). Polyoxyethylene sorbitan monoleate (Tween 80®) and docetaxel (DTX) was purchased from Sigma–Aldrich S.r.l. (Milan, Italy). Dipalmitoyl-*rac*-glycero-3-phosphatidylcholine (DPPC) was purchased from Genzyme Pharmaceuticals (Liestal, Switzerland). Hydroxypropyl-β-cyclodextrin (HP-β-Cyd) with 0.6 degree average substitution, was kindly provided by Roquette Italia (Cassano Spinola, Italy). All the other chemicals and solvents were of analytical reagent grade. Deionized double-distilled water was used throughout the study.

2.2. Preparation of the DTX-loaded nanospheres

PLA and PLGA nanospheres were prepared following a described solvent deposition method (Perracchia et al., 1998; Giannavola et al., 2003). DTX (0.5% and 1%, in weight drug/polymers) and polymer (75 mg) were dissolved in acetone (20 ml). The organic phase was poured into 40 ml of a water/ethanol mixture (1:1 v/v), containing 0.5% (w/v) of Tween 80®, under magnetic stirring, thus forming a milky colloidal suspension. The organic solvent was then evaporated off under vacuum by a rotavapor. The PLA or PLGA colloidal formulations were purified from untrapped DTX and unabsorbed surfactant by centrifugation (15,000 × *g*) for 1 h at 5 °C using a Beckman (Fullerton, CA) model J2-21 centrifuge equipped with a Beckman JA-20.01 fixed-angle rotor. The obtained surnatants were collected and the pellets resuspended in water (50 ml), then centrifuged again under the same conditions. This operation was repeated three times. After final washing nanospheres were resuspended in 5 ml of water solution containing 5% (w/v) HP-β-Cyd as lyoprotectant agent (Musumeci et al., in press) and freeze-dried (Edwards Modulyo 4 K).

2.3. Size and zeta potential measurements of nanoparticles

PLA and PLGA nanospheres mean size was determined by photon correlation spectroscopy (PCS) (Zetamaster, Malvern Instruments Ltd., Worcs, England). The experiments were carried out using a 4.5-mW laser diode operating at 670 nm as light source. Size measurements were carried out at a scattering angle of 90°. To obtain the mean diameter and polydispersity index of colloidal suspensions, a third-order cumulant fitting correlation function was performed by a Malvern PCS submicron particle analyzer. The real and imaginary refractive indexes were set at 1.59 and 0.0, respectively. The following parameters were used for experiments: medium refractive index 1.330, medium viscosity 1.0 mPa s and a dielectric constant of 80.4.

Each freeze-dried sample (100 μg) was suitably diluted with filtered water (10 ml) to avoid multiscattering phenomena and placed in a quartz cuvette. The size analysis of each sample consisted of 30 measurements, and the result was expressed as mean size ± S.D.

Zeta potential distribution was measured with the Zetamaster particle electrophoresis analyzer setup equipped with a 5-mW HeNe laser (633 nm). Measurements were performed on the same samples prepared for size analysis (100 μl) diluted with 10 ml of filtered water. Zeta limits ranged from −120 to 120 V. Strobing parameters were set as follows: strobe delay −1.00, on time 200.00 ms, off time 1.00 ms. A Smoluchowsky constant

F (K_a) of 1.5 was used to achieve zeta potential values from electrophoretic mobility.

2.4. Differential scanning calorimetry (DSC)

DSC scans of empty and DTX-loaded nonanoparticles were performed on a Mettler DSC 12E equipped with a Haake thermocryostate model D8-G. A Mettler TA89E and FP89 system software was used for the data acquisition. Indium was used to calibrate the instrument. The samples were scanned at a speed of 5 °C/min in 30–250 °C temperature range.

2.5. Surface morphology

Scanning electron microscopy (SEM) was performed to evaluate the surface morphology of nanoparticles using a SEM Philips model 500. Nanoparticle samples were dried for 24 h before the analysis. A small amount of nanoparticles was stuck on a double-sided tape attached on a metallic sample stand, then coated, under argon atmosphere, with a thin layer of gold, using a POLARON E5100 SEM coating unit.

2.6. DTX encapsulation efficiency

Lyophilized nanospheres (50 mg) were dissolved in 1 ml of acetonitrile and the drug amount was determined by HPLC analysis. The encapsulation efficiency was determined as the mass ratio of entrapped DTX in nanoparticles to the theoretical amount of drug used in their preparation. The entrapment of DTX PLA and PLGA nanospheres was expressed as loading capacity.

2.7. In vitro DTX release

Five hundred milligrams aliquots of freeze-dried DTX-loaded nanoparticles were poured in screw-capped tubes and suspended in 5 ml of isotonic pH 7.4 phosphate buffer solution (PBS). The tube was placed under magnetic stirring in a water bath maintained at 37 ± 0.5 °C. At fixed time intervals the tubes were taken out from the water bath and centrifuged at 12,000 rpm for 1 h. The pellets were resuspended in 5 ml of fresh PBS and placed back into the water bath to continue release measurement (Mu and Feng, 2003b). The collected supernatants were extracted three times with 5 ml of dichloromethane. The extraction solvent was evaporated and DTX residue was solubilized in 500 μl of acetonitrile. The obtained solution was analyzed by HPLC to determine the drug concentration. The experiments were carried out in triplicate.

The extraction efficiency (91.9 ± 0.4%) was evaluated in triplicate using PBS solutions (5 ml) containing different known concentrations (1.0–0.7 μg/ml) of pure DTX. All solutions were submitted to the same extraction procedure above described.

2.8. HPLC analysis

HPLC analysis was performed at room temperature using a 1050 Hewlett-Packard apparatus (Hewlett-Packard, Milano, Italy) on a 5 μm Hypersil ODS cartridge (125 mm × 4 mm i.d.) (Hewlett-Packard) equipped with a 5 μm Hypersil 100 RP-18 guard cartridge (4 mm × 4 mm i.d.) (Hewlett-Packard) and eluted isocratically with acetonitrile/water (65/35 v/v). The flow rate was fixed at 1 ml/min and detection was obtained by UV detection at 230 nm. The linear regression coefficient determined in the range 0.7–37.9 μg/ml was 0.9999 (n = 6). The method sensitivity was 0.01 μg/ml (signal to noise ratio, 3:1).

2.9. Liposome preparation

DPPC multilamellar liposomes were prepared in the presence and absence of free drug, at a temperature above the gel–liquid crystalline phase transition. Chloroform stock solutions of lipid and drug were mixed in order to obtain the chosen molar factions of the drug. The solvents were removed under nitrogen in a rotoevaporator and the resulting film was kept for 4 h at 40 °C under high vacuum (Buchi T-50) to remove the residual solvents.

DPPC vesicles were prepared by adding 400 μl of phosphate buffer solution (pH 7.4) to the film, then alternatively vortexed (3 min) and warmed in a water bath at 45 °C for 5 min. This procedure was repeated two times. The biomembrane suspension was left at room temperature for 3 h to anneal the bilayer structures and reach a partition equilibrium of the drug within the vesicles. Afterwards, aliquots of 40 μl (1 mg of the lipid) were transferred in 40 μl DSC aluminium pans and submitted to DSC analysis. All samples were submitted to four heating/cooling cycles in the temperature range 20–60 °C at a scanning rate of 1 °C/min. Data from the first scan were always discarded to avoid mixing artifacts. Due to periodic recalibration, a control sample consisting of a drug-free DPPC biomembrane suspension accompanied each set of experiments. The endothermal peak coming from the second scan of the control sample was used as a reference template. All samples, after calorimetric scans, were extracted from the pans and aliquots were used to determine the amount of the phospholipids by a phosphorus assay (Bartlett, 1959).

2.10. Kinetic experiments

DTX-loaded PLA R207 and PLA R203 nanospheres were added to the DPPC liposomes in amounts to obtain a 0.06 molar fraction of the drug with respect to the lipid (10 mg). Unloaded PLA R207 and PLA R203 nanospheres were added to the liposomes (10 mg in lipids) in the same amount in which the polymer is present in DTX-loaded nanospheres. Forty microlitres of each sample were placed in 40 μl DSC aluminium pans and incubated in a Haake thermocryostate model D8-G at a temperature (37 °C) lower than the polymer glass transition temperature, found at 43 and 40.5 °C for PLA R207 and PLA R203, respectively. The samples were analyzed immediately after preparation and after different incubation times (12, 24, 36 and 48 h).

Each sample was submitted to the following procedure: (i) a first scan (from 10 to 37 °C) to detect drug release bringing the sample to 37 °C; (ii) 60 min at 37 °C, to detect drug release after a long incubation time at a temperature simulating '*in vivo*' temperature; (iii) a subsequent scan from 10 to 37 °C, after the

60 min incubation at 37 °C followed by cooling down the sample to 10 °C.

3. Results and discussion

3.1. Nanosphere characterization

DTX-loaded PLA and PLGA nanospheres were obtained by a solvent displacement method and freeze-dried. HP-β-Cyd was used as lyoprotectant agent because our previous studies (Musumeci et al., in press) demonstrated its ability to protect nanosphere suspensions to physical aggregation due to dehydratation, during the freeze-drying process. HP-β-Cyd is able to maintain unchanged the mean size of nanospheres and their technological properties. Moreover, due to its high tolerability and biocompatibility this cyclic oligosaccharide does not compromise any *in vivo* administration way.

SEM was used to investigate the morphology of nanoparticles. As showed in Fig. 2 both PLGA RG502H and PLA R203 nanospheres displayed a spherical shape with a smooth surface and no aggregation was observed. No difference was observed in the morphological properties of nanoparticles due to presence of the drug. Similar pictures were obtained for the other systems (not shown).

DTX-loaded nanoparticles were characterized to evaluate the effect of the different polymers on mean particle size, size distribution and surface charge.

Using polylactides as matrices for the particles, different characteristics can be obtained by modifying the formulation parameters such as polymer molecular weight and monomer stereochemistry. The presence of an anionic surfactant is important to reduce the dynamic interfacial tension and to stabilize the nanosuspension. The surfactant is adsorbed on the nanosphere surface, increasing the steric repulsion between particles. In this study, Tween 80® was used at 0.5% (w/v) concentration, because as reported by Giannavola et al. (2003), this concentration being sufficient to obtain small PLA and PLGA nanoparticles and permits to remove the excess of surfactant by centrifugation and washing.

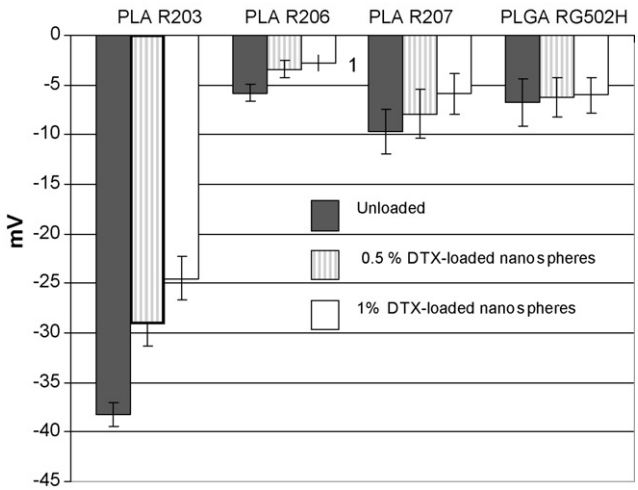

Fig. 3. Zeta potential of unloaded and DTX-loaded nanospheres (0.5% and 1%, w/w). Data are the average of 30 measurements ± S.D.

Zeta potential values are showed in Fig. 3. In Table 1 the particle size and polydispersity index of unloaded and different DTX-loaded nanoparticle formulations are reported.

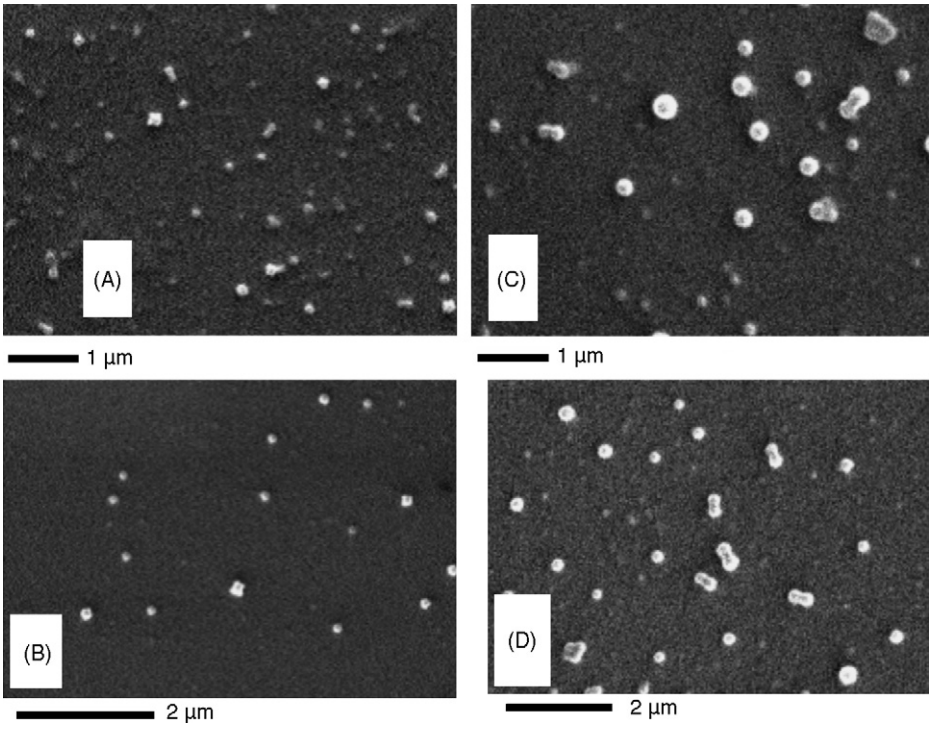

Fig. 2. Scanning electron micrographs of unloaded PLGA RG502H nanospheres (A); unloaded PLA R203 nanospheres (B); DTX-loaded PLGA RG502H nanospheres (0.5%, w/w) (C); DTX-loaded PLA R203 nanospheres (0.5%, w/w) (D).

Table 1
The effect of the different type of polymers on the particle size, polydispersity index and encapsulation efficiency of DTX-loaded nanoparticles

Type of polymers	M_w[a]	DTX (%, w/w)	Particle size (nm)	P.I.[b]	E.E. (%)
Polylactic acid					
PLA R203	16000	0.0	100.7 ± 2.9	0.058 ± 0.007	–
PLA R203	16000	0.5	129.1 ± 1.3	0.052 ± 0.025	16.13 ± 1.3
PLA R203	16000	1	157.2 ± 1.2	0.210 ± 0.034	22.12 ± 2.1
PLA R206	109000	0.0	118.5 ± 1.2	0.097 ± 0.012	–
PLA R206	109000	0.5	122.5 ± 1.9	0.144 ± 0.003	14.21 ± 0.8
PLA R206	109000	1	128.4 ± 3.4	0.298 ± 0.072	19.08 ± 1.6
PLA R207	209000	0.0	137.5 ± 1.9	0.136 ± 0.080	–
PLA R207	209000	0.5	142.0 ± 4.8	0.261 ± 0.040	10.52 ± 2.0
PLA R207	209000	1	179.4 ± 1.7	0.403 ± 0.030	16.91 ± 1.3
Polylactic-co-glycolic acid					
PLGA RG502H		0.0	98.7 ± 1.7	0.095 ± 0.023	–
PLGA RG502H		0.5	157.2 ± 2.9	0.172 ± 0.008	16.82 ± 2.6
PLGA RG502H		1	172.0 ± 4.9	0.470 ± 0.070	23.09 ± 1.3

Data are shown as mean ± S.D. obtained from three formulations.
[a] M_W, mean molecular weight of PLA and PLGA polymers used for nanosphere preparations.
[b] P.I., polydispersity index.

All formulations showed a mean diameter in the range 100–200 nm, suitable to obtain an effective intracellular uptake of nanoparticles. A decrease of unloaded PLA nanoparticle size increasing polymer molecular weight was observed. This trend can be the result of the enhancement of lipophilicity of the polymer with increasing molecular weight. The consequent reduced water solubility of the polymer, probably caused, during the preparation procedure, a faster precipitation of the polymer, with formation of a greater number of nanospheres and a limited size increase. All unloaded formulations showed low polydispersity index, evidencing the formation of very homogeneous systems.

The addition of the drug in the formulations probably produces an expansion of the polymeric matrix, increasing particle size, particularly in the presence of the highest drug amount. In all cases, the size was in an accepted range for parenteral use.

Zeta potential of nanoparticles was negative due to the presence of terminal carboxylic groups in the polymers. Müller (1991) demonstrated that a high potential value, of about −25 mV, ensures a high-energy barrier that stabilizes the nanosuspensions. The PLA R203 particles seemed to possess the ideal surface charge, ranging between −38 and −24 mV for unloaded and DTX-loaded nanoparticles, respectively. Probably, the less negative value of the other systems was due to the higher molecular weight of polymers with respect to PLA R203. In fact, regardless the molecular weight of PLA, only two carboxylic groups are present for each polymer molecule, an increase in the molecular weight of the polymer then reduces the percentage of carboxylic groups present in the nanospheres (Fig. 3). Moreover, a higher concentration of surfactant could be adsorbed on the particle surfaces with the formation of a denser surfactant film on the nanosphere surface, thus eliciting a reduced electrophoretic mobility.

The presence of DTX in PLA nanospheres always reduced the negative zeta potential value; probably, there was a masking effect of the superficial carboxylic groups by the drug adsorbed on nanosphere surface. No influence was exerted by DTX on the zeta potential value of PLGA nanospheres.

DSC studies were performed to investigate the physical state of the drug in the nanoparticles, because this aspect could influence the *in vitro* and *in vivo* release of the drug from the systems. Different combinations of drug/polymer may coexist in the polymeric carriers, such as: (i) amorphous drug in either an amorphous or a crystalline polymer and (ii) crystalline drug in either an amorphous or a crystalline polymer. Moreover, a drug may be present either as a solid solution or solid dispersion in an amorphous or crystalline polymer. Fig. 4 shows the DSC thermograms of pure DTX, unloaded PLA R203 nanoparticles, DTX-unloaded PLA R203 nanoparticles physical mixture and

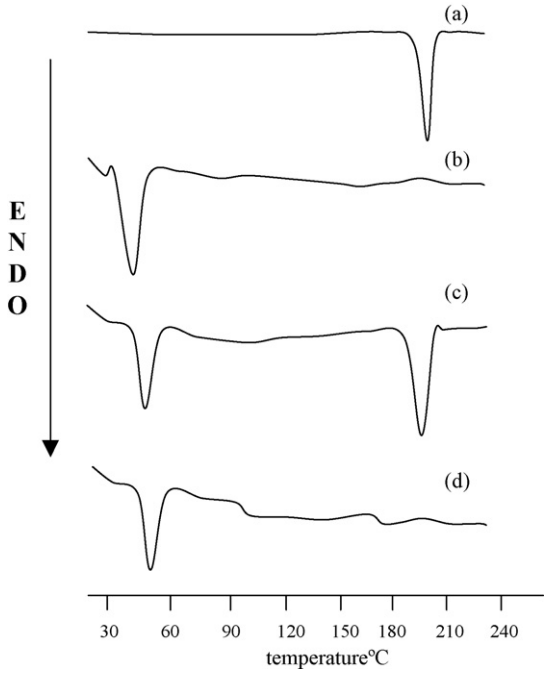

Fig. 4. DSC curves of DTX (a), unloaded PLA R203 nanoparticles (b), DTX-unloaded PLA R203 nanoparticles physical mixture (0.5%, w/w) (c), DTX-loaded PLA R203 nanoparticles (0.5%, w/w) (d).

DTX-loaded PLA R203 nanoparticles. Pure DTX showed a single sharp endothermic melting peak at 188.9 °C (Fig. 4a), that was slightly shifted to a lower temperature (186 °C) (Fig. 4c) in the thermogram of the physical mixture. DTX melting peak totally disappeared in the calorimetric curve of loaded nanoparticles, evidencing the absence of crystalline drug in the nanoparticles samples, at least at the particle surface level. It might be hypothesized that the polymer inhibited the crystallization of DTX during nanoparticles formation. Therefore, it could be concluded that DTX in the nanoparticles was in an amorphous or disordered crystalline phase of a molecular dispersion or a solid solution state in the polymer matrix after the production (Dubernet, 1995). The polymer glass transition temperature was not influenced by the preparation procedure. Similar results were obtained for the other systems (data not reported).

3.2. Entrapment efficiency and in vitro drug release from nanospheres

The entrapment efficiency of DTX in the nanospheres prepared by the solvent displacement method ranged from about 10–16% and from 17% to 23% for nanoparticles prepared using 0.5% and 1% of DTX, respectively (Table 1). This low entrapment efficiency was probably due to the high affinity of the drug for the organic solvents used during the nanosphere preparation that caused a diffusion of DTX away from the polymer matrix (Layre et al., 2005). PLA R203 and PLGA RG502H nanospheres showed the better entrapment efficiency. Probably, a more higher surface adsorption of the drug was observed for these polymers with respect to PLA R206 and PLA R207, as demonstrated by the *in vitro* release where a higher entrapment efficiency was associated to a higher burst effect (Fig. 5). In fact, all nanosphere formulations produced a biphasic DTX release profile with an initial burst effect in which DTX release ranged between 40% and 68% within the first sampling time (24 h). This fast release is related to DTX adsorbed on the nanoparticle surface (Magenheim et al., 1993) and/or to the release of the drug encapsulated near to nanosphere surface. After this phase, a constant slow DTX release until 70% and 95% of the loaded drug amount was observed within 10 days, showing a typical sustained and prolonged drug release that depends on drug diffusion and matrix erosion mechanisms (Holland and Tighe, 1992).

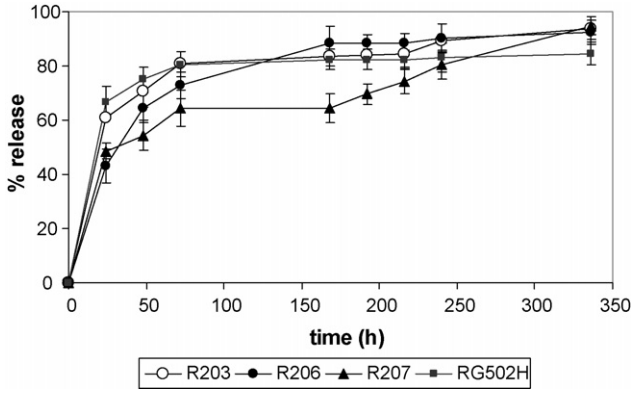

Fig. 5. DTX release from PLA at different molecular weights and PLGA nanoparticles prepared with 0.5% (w/w) theoretical amount of the drug. Release experiments were carried out in isotonic PBS (pH 7.4), at 37 ± 0.5 °C, immediately after sample preparation. Each point represents the mean value of three different experiments ± S.D.

3.3. Interaction with biomembrane models

The drug–cell membrane interaction could have a decisive influence on partitioning, orientation, and conformation of the drug as well as on the functioning of cell membranes. An *in vitro* drug–biomembrane interaction study may provide important and useful information for understanding of the drug pharmacology as well as optimizing the nanosphere formulation.

Pure lecithins, such as DPPC, have well-defined thermotropic properties (Estep et al., 1978; Jain, 1988a) and hence any change in these properties can be easily related to the kind of drug–membrane interaction and to the localization in the ordered structure of the bilayer (Jain, 1988b) of a foreign comound. To evaluate the thermotropic properties of lecithin-based vesicles, a DSC analysis was carried out.

The DSC thermograms obtained for pure DPPC liposomes or in the presence of DTX are showed in Fig. 6.

Two endothermal transitions were observed for pure DPPC liposomes the sharp acyl chain melting transition ($P_\beta \rightarrow L_\alpha$) at 41 °C is associated to the passage from a highly ordered gel state, in which phospholipid acyl chains are in *all-trans* conformation, to a less packed and more fluid liquid crystal phase due to an increased *trans–gauche* isomerization of acyl chains. A small broad pretransition, so-called $L_\beta \rightarrow P_\beta$, was observed at 36 °C, due to a conformational change and/or packing order rearrangement at the level of phospho-diester groups and glycerol backbone of DPPC.

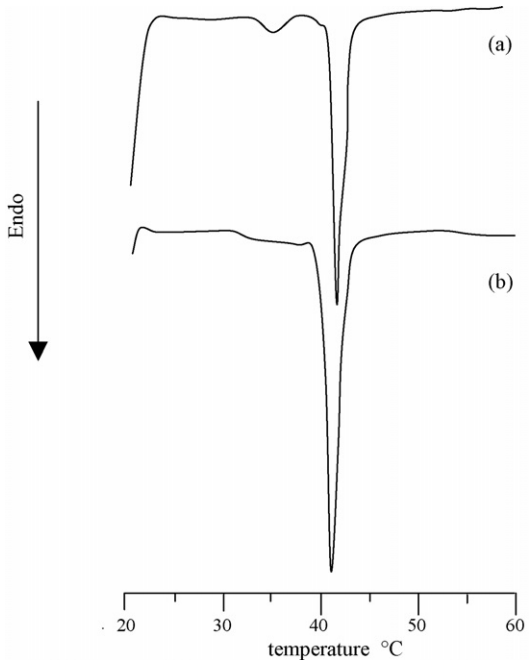

Fig. 6. DSC scans in the heating mode of colloidal suspensions of multilamellar DPPC vesicles prepared in the absence (curve a) and in the presence of 0.06 molar fraction of DTX (curve b).

Table 2
Thermotropic parameters of mesophase transition from gel to liquid crystalline phase of DPPC multilamellar vesicle dispersions at different molar fractions of DTX

DTX molar fraction	Transition temperature (K)	ΔH (kJ mol^{-1})	$\Delta T_{1/2}$ (K)
0	314.3	−37.41	1.9
0.01	314.2	−38.87	2.1
0.02	314.2	−40.44	2.2
0.03	314.2	−42.87	2.6
0.04	310.1	−43.10	2.7
0.06	314.1	−45.25	2.8

The incorporation of DTX in DPPC liposomes produces at all the considered molar ratios the disappearance of the pretransition peak. Because of this pretransition is highly sensitive to the presence of foreign molecules in the polar region of the phospholipids, the loss of pretransition cannot be ascribed to a specific molecular change. No influence on the main phase transition temperature of DPPC bilayer was exerted by DTX (Table 2) and a little broadening of this peak was observed (see $\Delta T_{1/2}$ values in Table 2). However, a significative increase of ΔH value associated to the main phase transition was observed with increasing the DTX molar ratio. The light broadening of the main transition peak evidences a limited loss of co-operativity between phospholipids alkyl chains, due to the superficial penetration of the drug within the bilayers. The increase of ΔH value is due to an increase of van der Waals reticular energy of the hydrophobic chains due to the lower electrostatic repulsion forces among choline groups produced by a drug able to electrostatically interact with DPPC polar heads. Thus, it is conceivable that the bulky DTX is not able to deeply penetrate into the bilayer, limiting its interaction with the polar headgroup layer of the phospholipids. Similar results were obtained by Zhao and Feng (2004) for paclitaxel and are in agreement with the biological evidences about an active transport of taxanes within viable cells (Smith et al., 2005).

In Fig. 7 the release kinetics of DTX from nanospheres to DPPC liposomes are shown. The 0.06 molar fraction of DTX dispersed in PLA R207 and PLA R203 nanospheres was chosen to follow the release.

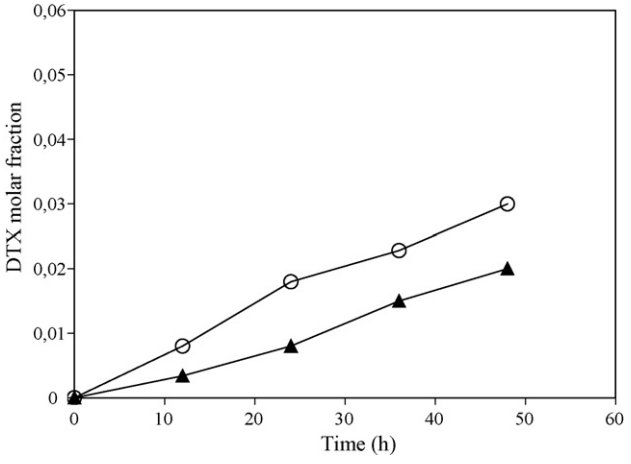

Fig. 7. Molar fraction of DTX released from PLA R207 (▲) and PLA R203 (◯) to void liposomes after incubating at 37 °C for 12, 24, 36 and 48 h.

A limited amount of DTX was transferred from the PLA R207 nanospheres to liposomes, and after 48 h the DTX amount interacting with DPPC biomembranes corresponded to about a 0.02 molar fraction. These findings showed that the drug was not able to diffuse into the external medium through the polymer matrix in a rigid glassy state. Therefore, the higher drug release observed in the *in vitro* assay was probably due also to the degradation of the polymer structure. Calorimetric studies performed using unloaded PLA R207 nanospheres demonstrated no presence of free lactic acid due to the polymer degradation. In fact, we did not observed any shift of DPPC transition temperature nor broadening of the calorimetric peak (data not shown). Different results were obtained in the presence of DTX-loaded PLA R203 nanospheres. In this case the more rapid interaction of DTX with DPPC liposomes (DTX release corresponding to a 0.03 molar fraction after 2 days of incubation) was related both to the highest amount of DTX adsorbed on nanosphere surface, thus rapidly released (see the high burst effect in Fig. 5) and also to the fast degradation of this polymer with respect to PLA R207, due to its low molecular weight.

In conclusion, the obtained results demonstrated that biodegradable polymeric colloidal systems made up of PLA or PLGA can entrap DTX providing a sustained drug release. Nanospheres with suitable size for parenteral administration were always obtained. The interaction studies with a biomembrane model suggested that diffusion is not the only kinetic process involved in DTX release, but a complete erosion of polymer matrix and longer times are needed to obtain complete drug release.

Acknowledgment

This work was financially supported by Italian MIUR.

References

Aapro, M., Bruno, R., 1995. Early clinical studies with docetaxel. Eur. J. Cancer 31A, S7–S10.
Aapro, M., 1996. The scientific rationale for developing taxoids. Anticancer Drugs 7, 33–36.
Alexopoulos, A., Karamouzis, M.V., Stavrinides, H., Ardavanis, A., Kandilis, K., Stavrakakis, J., Georganta, C., Rigatos, G., 2004. Phase II study of pegylated liposomal doxorubicin (caelyx) and docetaxel as first-line treatment in metastatic breast cancer. Ann. Oncol. 15, 891–895.
Baker, S.D., Zhao, M., He, P., Carducci, M.A., Verweij, J., Sparreboom, A., 2004. Simultaneous analysis of docetaxel and the formulation vehicle polysorbate 80 in human plasma by liquid chromatography/tandem mass spectrometry. Anal. Biochem. 324, 276–284.
Barratt, G.M., 2000. Therapeutic applications of colloidal drug carriers. Pharm. Sci. Technol. Today 3, 163–171.
Bartlett, G.R., 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234, 466–468.
Brigger, I., Dubernet, C., Couvreur, P., 2002. Nanoparticles in cancer therapy and diagnosis. Adv. Drug Deliv. Rev. 54, 631–651.
Davda, J., Labhasetwar, V., 2002. Characterization of nanoparticle uptake by endothelial cells. Int. J. Pharm. 233, 51–59.
Dubernet, C., 1995. Thermoanalysis of microspheres. Thermochim. Acta 248, 259–269.

Estep, T.N., Mountcastle, D.B., Biltonen, R.L., Thompson, T.E., 1978. Thermal behaviour of synthetic sphingomyelin–cholesterol dispersion. Biochemistry 18, 2112–2117.

Gelderblom, H., Verweij, J., Nooter, K., Sparreboom, A., 2001. Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. Eur. J. Cancer 37, 1590–1598.

Giannavola, C., Bucolo, C., Maltese, A., Paolino, D., Vandelli, M.A., Puglisi, G., Lee, V.H., Fresta, M., 2003. Influence of preparation conditions on acyclovir-loaded poly-D,L-lactic acid nanospheres and effect of PEG coating on ocular drug bioavailability. Pharm. Res. 20, 584–590.

Grosse, P.Y., Bressolle, F., Pinguet, F., 1998. *In vitro* modulation of doxorubicin and docetaxel antitumoral activity by methyl-β-cyclodextrin. Eur. J. Cancer 34, 168–174.

Herbst, R.S., Khuri, R., 2003. Mode of action of docetaxel a basis for combination with novel anticancer agents. Cancer Treat. Rev. 29, 407–415.

Holland, S.J., Tighe, B.J., 1992. Biodegradable polymers. In: Garderton, D., Jones, T. (Eds.), Advances in Pharmaceutical Science, vol. 6. New York, pp. 101–164.

Immordino, M.L., Brusa, P., Arpicco, S., Stella, B., Dosio, F., Cattel, L., 2003. Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing docetaxel. J. Contr. Release 91, 417–429.

Jain, M.K., 1988a. Properties of bilayer. In: Jain, M.K. (Ed.), Introduction to Biological Membranes. John Wiley & Sons Inc., New York, pp. 86–121.

Jain, M.K., 1988b. Order and dynamics in bilayers and solute in bilayers. In: Jain, M.K. (Ed.), Introduction to Biological Membranes. John Wiley & Sons Inc., New York, pp. 122–146.

Layre, A.M., Gref, R., Richard, J., Requier, D., Chacun, H., Appel, M., Domb, A.J., Couvreur, P., 2005. Nanoencapsulation of a crystalline drug. Int. J. Pharm. 298, 323–327.

Magenheim, B., Levy, M.Y., Benita, S., 1993. A new *in vitro* technique for evaluation of drug release profile from colloidal carriers-ultrafiltration technique at low pressure. Int. J. Pharm. 94, 115–123.

Mu, L., Feng, S., 2003a. PLGA/TPGS nanoparticles for controlled release of paclitaxel: effects of the emulsifier and drug loading ratio. Pharm. Res. 20, 1864–1872.

Mu, L., Feng, S.S., 2003b. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol®): PLGA nanoparticles containing vitamin E TPGS. J. Contr. Release 86, 33–48.

Müller, R.H., 1991. Charge determinations. In: Müller, R.H. (Ed.), Colloidal Carriers for Controlled Drug Delivery and Targeting Modification, Characterization and In Vivo. CRC Press, Boca Raton, FL, pp. 57–97.

Müller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. Adv. Drug Deliv. Rev. 47, 3–19.

Musumeci, T., Vicari, L., Ventura, C., Gulisano, M., Pignatello, R., Puglisi, G., in press. Lyoprotected nanospheres formulations for paclitaxel controlled delivery. J. Nanosci. Nanotechnol.

Perracchia, M.T., Vauthier, C., Desmaele, D., Gulik, A., Dedieu, J.C., Demoy, M., D'Angelo, J., Couvreur, P., 1998. Pegylated nanoparticles from a novel methoxypolyethylene glycol cyanoacrylate–hexadecyl cyanoacrylate amphiphilic copolymer. Pharm. Res. 15, 550–556.

Smith, N.F., Acharya, M.R., Desai, N., Figg, W.D., Sparreboom, A., 2005. Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. Cancer Biol. Ther. 4.

Zhao, L., Feng, S., 2004. Effects of lipid chain length on molecular interactions between paclitaxel and phospholipid within model biomembrane. J. Colloid Int. Sci. 274, 55–68.