Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México

Influence of three different colloidal systems on the oxytetracycline-lecithin behavior

A. Faustino-Vega, M. A. Alvarez-Polo, B. Gasca, M. J. Bernad-Bernad

Received January 20, 2009, accepted February 21, 2009

Prof. Dr. Maria Josefa Bernad-Bernad, Departamento de Farmacia, Universidad Nacional Autónoma de México, Av. Universidad 3000 F, 04510 México DF
bernadf@gmail.com

Pharmazie 64: 505–509 (2009) *doi: 10.1691/ph.2009.9027*

Oxytetracycline (OTC) is a wide spectrum antibiotic, but it is known to be degradated when it is stored under adverse conditions. It is classified as short-acting based on serum half-lives of 6–8 h. The stabilizing effect of colloidal carrier systems and their ability to sustain drug release for OTC was investigated using solid dispersion, liposomes and solid lipid nanoparticles with lecithin. Analysis of chemical stability showed that OTC is more resistant in colloid systems than in the free form. The characterization results show clear differences between the prepared systems. Release studies show that a sustained release is achieved when the OTC is formulated in these carriers.

1. Introduction

Oxytetrarycline (OTC) is one of the most widely used antimicrobial substances both in human therapy and for veterinary purposes. It is used in therapeutics, as a prophylactic and as a growth promoter in husbandry, cattle, swine, and poultry. Several scientists have examined the photolysis (Pouliquen et al. 2007), hydrolysis (Doi and Stoskopf 2000), sorption and other pharmaceutical processes including OTC (Gratacós-Cubarsí, et al. 2007). Some of the degradation products of oxytetracycline are active antimicrobials and they can give a dark color to the solution (Sanderson et al. 2005). However, it is important to maintain the initial color, since the color change is associated with degradation and, consequently, the drug is unsuitable as a commercial product.

Several studies have been carried out to ensure the stability of OTC through formulation. For example, the stability of OTC was increased by using technological strategies like complexation with hydroxypropyl-β-cyclodextrin (Moreno-Cerezo et al. 2001). Also, reverse micelles were found to increase the OTC half-life from 329 h to 2402 h (Sah 2006), and the incidence and levels of OTC degradation products were reduced in the presence of Mg^{2+} and PEG 400 (Tongaree et al. 2000).

OTC is classified as short-acting based on serum half-lives of 6–8 h. As such, the administration and dosing intervals are often very costly in terms of labour. Colloidal carriers show great potential as multipurpose delivery systems, especially in targeting, controlling release, and increasing drug stability (Lasic 1998; Leuner and Dressman 2000; Muhammad 2000; Müller et al. 2000; Papageorgiou et al. 2006; Podio et al. 2000; Shigeo Yamamura 1 1996). Lecithin-based systems are becoming increasingly popular as carriers for drugs because of their ability to include the drug and their low toxicity (Deol and Khuller 1997; Lasic 1993). Examples of such lipid-based systems include colloidal solid dispersion (SD), solid lipid nanoparticles (SLN) and liposomes (Andresen et al. 2005; Ann Clark et al. 2001; Arnardottir et al. 1996; Zhong et al. 2005).

Previous studies have demonstrated that OTC stability and OTC delivery can be controlled by pharmaceutical technology. However, studies that describe the effect of lecithin-based carriers on the stability and release of the drug have not been published to date.

In the present paper, a preformulation study leading to the development of new OTC colloidal formulations with phosphatidylcholine (PC) has been carried out.

2. Investigations, results and discussion

2.1. Storage stability studies

A 6-month storage stability study was conducted on all three lipid-based formulations in comparison to freshly prepared samples (control). Visual observation showed a white color for the four samples at time zero (free OTC, SD, SLN and liposomes), while dark-colored solutions were observed for the free OTC solutions after six months. The other three samples maintained their visual appearance (Fig. 1).

The UV-Vis spectra show a new band at around 360 nm after 6 months for free OTC, while the spectra remain unchanged for the other three samples (Fig. 2). The increase in stability could be attributed to the inclusion of OTC in colloidal systems, which protects them from degradation factors such as light.

From these observations it is not possible to indicate a ranking order correlation in terms of stability. Interestingly, all formulations at the end of the 6-month storage period demonstrated good stability.

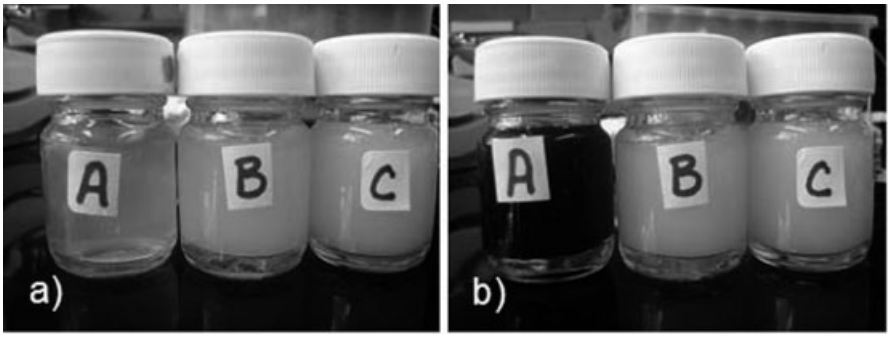

Fig. 1:
Physical appearance of OTC samples: a) Time 0 and b) After 6 months (A = OTC dissolution, B = OTC in SLN and C = OTC in liposomes)

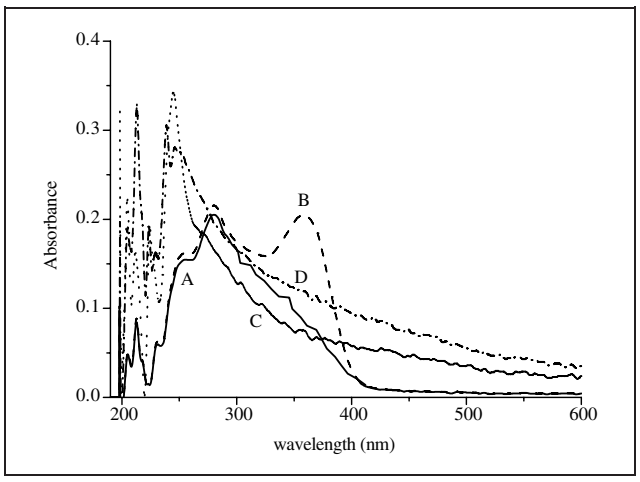

Fig. 2: UV-Vis spectra. A and B are free OTC, C and D are liposomes at time zero and 6 months, respectively

2.2. Morphology

An SEM study was carried out to investigate the morphology of the colloids. It can be seen that the liposomes and SLN had spherical or ellipsoidal shapes (Fig. 3). Drug crystals were not visible in these systems, although they were observed in SD. Furthermore, in SD, particles with defined forms were not observed.

2.3. Size and particle distribution

In parenteral formulations, particle size is one of the most important physicochemical characteristics. The reason for this is that large particles are clinically unacceptable due to the formation of emboli. The colloid type and the process employed allow the formulation to influence the size distribution of particles. Moreover, it is known that the half-lives of nanosystems are dependent on the size of the carriers (Senior et al. 1985). In consideration of these facts, the particle size is one of the most important factors in the development of dispersion systems.

The particle size measurements on colloid systems (Table 1) revealed that the technique used to obtain the nanosystems led to different particle sizes. The size and polydispersity indices (PDI) of the resulting particles were small with SLN and increased for liposomes and SD, respectively.

For SLN, the presence of Tween 20 decreases the surface tension of the aqueous medium, thus favoring a surface area increase of the OTC-PC mixture in the emulsification process, decreasing the drop size and, therefore, generating particles of reduced size (7.57 nm). The PDI was 0.2, which shows size homogeneity. In the case of liposomes the particle size was larger (214 nm), probably due to the formation of complex structures. Liposomes form lipidic bilayers that include a volume of the aqueous medium in which they were prepared. Besides, it has been reported (Zhong et al. 2005) that the thin layer hydration technique generates uni-, oligo- and multilamelars liposomes, and this is consistent with the obtained PDI of 0.5. In the case of SD, the particle size and PDI are the largest of the three preparations. This situation is attributed mainly to the preparation process, in which a surfactant was not used and energy was not applied to diminish the size, thus giving thick colloidal dispersions and multimodal distribution (319 nm, PDI 0.642).

Any product to be administered by a parenteral route has to be sterilized. It is well known that free radicals are formed during γ-sterilization in all samples due to the high energy of the γ-rays. These radicals can recombine without modification of the sample or undergo secondary reactions, which might lead to chemical modifications of the sample. Also, Schwarz et al. (1995) investigated the impact of different sterilization techniques (steam sterilization at 121 °C for 15 min and 110 °C for 15 min) and the results indicate that particle aggregation might occur as a result of the treatment. Therefore, filtration is a good technique to apply to colloid nanosystems. Our results show that SLN can be sterilized by this method.

2.4. Zeta potential

The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersions. In gen-

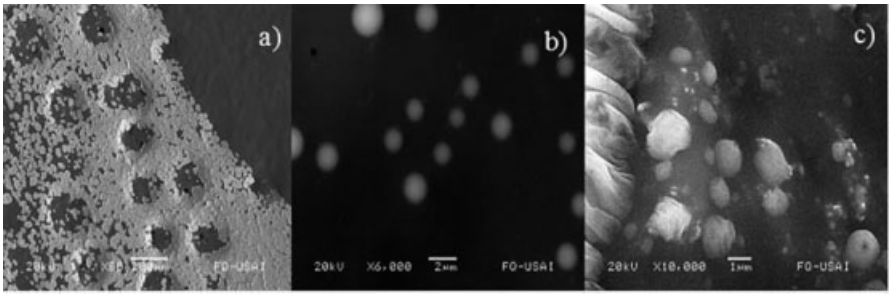

Fig. 3:
Scanning electron micrographies (SEM) of colloidal dispersions. a) Solid dispersion (SD), b) solid lipid nanoparticles (SLN), c) liposomes

Table: Characterization parameters of different colloidal carriers with OTC

	Size (nm)	PDI	ζ (mV)	Entrapment efficacy
SLN	7.57	0.249	−3.02	79%
Liposomes	214	0.54	−23.8	45%
Solid dipersion	319	0.642	−11.3	81%

eral, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. However, this rule cannot be strictly applied to systems that contain steric stabilizers, because the adsorption of a steric stabilizer will decrease the zeta potential due to the shift in the shear plane of the particle.

The prepared systems present zeta potential values that increase in the order SLN > SD > liposomes, as can be seen from Table 1. Liposomes show the most negative zeta potential value (−23.8 mV) (cf. −68 mV for empty vesicles), and this is the nearest to the limit of stability (−30 mV). This finding is probably because several OTC molecules remain adsorbed onto the vesicle surface and, as a result, the most negative zeta potential is neutralized for the drug. It is clear that PC, a zwitterion at pH = 7.5, exposes the negative groups towards the outer surface. Other research shows the effect of the drug on the liposome zeta potential (Fatouros and Antimisiaris 2002). In the case of SD, the zeta potential is −11.30 mV and this value indicates the decrease of net negative charge at the particle surface in comparison with that found for liposomes. Lecithin is present in the surface but it is not organized, rather it is adsorped on the OTC crystal surface in a random way. Finally, for SLN the zeta potential is −3.02 mV, which makes it the most unstable preparation of the colloids studied here. This zeta potential value is a result of neutralization of the negative charges of the PC by the incorporation of phosphates during the SLN formation; this leads to a decrease in the charges at the particle surface to the point where they are almost completely eliminated.

2.5. Drug entrapment efficacy

The prerequisite to obtain a sufficient loading capacity is a sufficiently high solubility of the drug in the lipid. The chemical nature of the lipid is also important because lipids that form highly crystalline particles with a perfect lattice (e.g. monoacid triglycerides) lead to drug expulsion (Westesen et al. 1997). More complex lipids that are mixtures of mono-, di- and triglycerides and also contain fatty acids of different chain lengths form less perfect crystals with numerous imperfections offering space to accommodate the drugs (Mehnert et al. 1997).

Although the lipid and the lipid concentration are the same in the three systems studied, the entrapped percentage varies from one system to another. There are reports that describe how the entrapment efficacy increases with the nanosystem size and it is also reported that smaller liposomes have a better ability to encapsulate (Ferdous et al. 1998).

The results in Table 1 show that liposomes are the particles that encapsulate the smallest quantity of OTC followed by SLN and SD. The low entrapped percentage in liposomes is probably due to the fact that this system is like a core-shell model with a drug enriched shell, with the OTC hardly included in the inner cavity.

The percentage of OTC entrapment in SLN and SD is high, probably because the manufacturing techniques involve the incorporation of the drug from the beginning together with the lecithin and CH_2Cl_2. Taking into account the great affinity of OTC for organic media, it is clear that the drug does not diffuse towards aqueous media, thus increasing the encapsulation within these colloids.

2.6. In vitro drug release

As shown in Fig. 4, release of pure OTC was poor but was higher than for the OTC formulations, with about 51.46% released after 48 h. These results are understandable because the drug is dissolved in the donor and it only has to diffuse through the membrane to arrive at the receptor. The poor solubility of OTC (3.083×10^{-4} M) in aqueous phosphate buffer solution can be attributed to its hydrophobic nature, poor wettability, and probably particle agglomeration during the release runs. The release rate decreased significantly when OTC was formulated in lecithin carriers. SD showed the greatest decrease with the unformulated drug, followed by liposomes and SLN. Overall, the entrapment of the drug into colloids creates a better microenvironment for the OTC and, as a result, its release from the formulation is slower than the release of the pure drug. In this case, the inclusion within carriers decreased the release rate by around 5, 3.5 and 2.5 times, respectively, when compared to the control formulation.

The released quantities of OTC are in good agreement with the colloid size – the higher the surface area the quicker the release. SLNs also have a surfactant in their formulation that favors the release of OTC. In addition, a burst effect is observed for the three formulations. This initial fast release of the drug from the colloidal systems can be explained by the presence of free OTC and drug desorption from the outer surface of the nanosystems. The burst can be exploited to deliver an initial dose when desired.

In conclusion, we successfully incorporated the lipophilic OTC into different colloidal systems. From visual observations and UV-Vis spectroscopy it is apparent that the three nanosystems increase the drug stability, compared with pure OTC, to at least 6 months. The characterization studies show the effect of the manufacturing technique on the type of system formed. It is not possible to state definitively which is the best colloid system to formulate OTC. However, these results suggest that SLNs are appro-

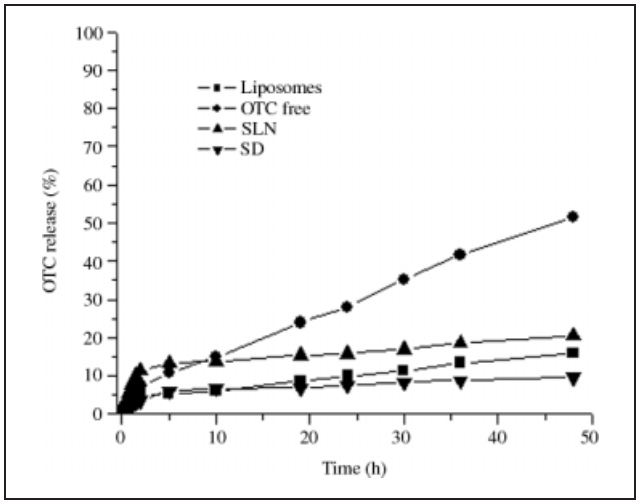

Fig. 4: In vitro OTC cumulative release: ● OTC free, ■ Liposomes, ▲ SLN, ▼ SD

priate to be considered in formulations that require sterilization, that liposomes present the most appropriate zeta potential values to ensure physical stability. Although the SD have relatively large sizes and zeta potential values that indicate unstable systems, they represent a simple system to stabilize and sustain release of the OTC with lecithin.

The three systems described here can be stabilized by adding electrolytes to the dispersion medium. For future studies, the incorporation of stability enhancers like electrolytes or stabilizing polymers (like polyvinyl alcohol) should provide valuable information on the long-term storage physical stability of the colloidal formulations.

3. Experimental

3.1. Materials

All chemicals were A.R. grade and were used without further purification. OTC was kindly donated by Moléculas Finas de Mexico S.A. de C.V. L-α-phosphatidylcholine, Type X-E minimum 60% TLC (Lecithin), NaH_2PO_4 and Tween 20 were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). CH_2Cl_2 and acetone were purchased from Baker. NaOH was supplied by Mallinckrodt (Paris, KY, USA).

All samples were prepared in buffered solutions, which were made with filtered (0.02 μm) water.

3.2. Preparation of OTC/PC systems

3.2.1. Solid dispersion (SD)

OTC and lecithin SD were prepared by the solvent evaporation method (Chiou and Riegelman 1971) using CH_2Cl_2, with accurately weighed amounts of OTC and PC dissolved in minimum quantities of CH_2Cl_2. The solvent was evaporated under reduced pressure. Colloidal particles were produced by dispersion of the solid dispersion system composed of the lipid-based formulation containing the drug and carrier into aqueous buffer solution (0.1 M, pH = 7.5). The resulting suspension was sonicated for 30 min and stored until further use. The final drug/lecithin ratio was 1 : 1 (mol : mol).

3.2.2. Solid lipid nanoparticles (SLN)

The SLNs were produced by a solvent emulsification/evaporation technique (Mehnert and Mader 2001). OTC and lecithin were dissolved in CH_2Cl_2 and then emulsified in an aqueous buffer solution (0.1 M, pH = 7.5) of Tween 20 (5% w/v). The emulsion was obtained by agitation using a homogenizer (CAT X-120, PolyScience, Niles, IL) for 2 minutes at 14 000 rpm. The CH_2Cl_2 was then removed from the emulsion by evaporation under vacuum with constant agitation for 3 hours. The final drug/lecithin ratio was 1 : 1 (mol : mol).

3.2.3. Liposomes

Liposomes with or without the drug were prepared by the thin film hydration method (Bangham et al. 1965; Zaru et al. 2007). In brief, the PC (83 mg) and the drug (50 mg) were dissolved in CH_2Cl_2 and the final lipid solution was placed in a round-bottomed flask, which was subsequently connected to a rotary evaporator (Laborota 4000 Heindolph) under reduced pressure at 35 °C in order to evaporate the organic solvent. This resulted in the formation of a thin film of lipid including OTC. The thin film was hydrated with aqueous buffer solution (pH = 7.5 and 0.1 M). The resulting liposome dispersions were placed in a bath-type sonicator for 1 h. The final drug/lecithin ratio was 1 : 1 (mol : mol).

3.3. Storage stability studies

All OTC formulations were stored in closed glass vials for a period of 6 months at a temperature of 4 °C. Visual observation and UV-Vis spectrophotometry were conducted at time zero and at 6-month intervals to assess changes in drug characteristics.

3.4. Characterization of the colloidal systems

3.4.1. Morphology

Scanning electron microscopy (SEM) was employed to evaluate the surface morphology of colloids using an SEM JEOL JSM 5900 LV microscope operating at 20 kV. Liquid samples were deposited on vitreous carbon conductive double-sided tape and dried at room temperature and a 1% w/v phosphotungstic acid aqueous solution was added as a staining agent.

3.4.2. Particle size and zeta potential

The median diameter and polydispersity index (PI) of samples were assessed by photon correlation spectroscopy (PCS, dynamic light scattering, DLS) using a Nanosizer ZS ZEN3600 (Malvern Instruments Ltd., Malvern, UK) at 25 °C. Each sample was analyzed five times. The cumulative distribution of the particle size was obtained at the same time with intensity-weighting, volume-weighting and number-weighting while the value of the PI showed the width of the distribution. The particle size analysis data were evaluated using the volume-weighting pattern and these were of median diameter.

The zeta potential was determined using a Nanosizer ZS ZEN3600 (Malvern Instruments Ltd., Malvern, UK) by electrophoretic mobility. Values reported are the mean value of five results. The electrophoretic mobility measurements were converted to zeta potential using the Smoluchowski equation.

3.4.3. Drug entrapment efficiency

The drug-entrapment percentage was determined by ultracentrifugation (Optima TL1000 Ultracentrifuge, Beckman). In each case a 3 mL sample was placed in a polycarbonate centrifuge tube and ultracentrifuged at 40000 rpm for 15 min at 4 °C. The supernatant was separated and analyzed by UV-Vis spectroscopy (Ocean Optics S2000) at 358 nm. The value obtained is considered as free OTC and the difference with the total drug concentration is the drug entrapment percentage. The drug entrapment efficiency (Eq. 1) was expressed as a percentage of the drug difference between the initial amount of OTC and the free amount in the supernatant relative to the total amount used for the colloid preparation.

$$\mathrm{EE}(\%) = \left(\frac{Q_t - Q_f}{Q_t}\right) \times 100 \qquad (1)$$

where EE is the entrapment efficiency, Q_t the theoretical amount of OTC that was added, and Q_f is the amount of drug detected only in the supernatant.

3.4.4. In vitro drug release from colloidal dispersions

Drug release from formulations containing different OTC colloid systems was measured through a regenerated cellulose membrane (MW 12,000–14,000, SpectraPor) in a horizontal Franz-type diffusion cell. The formulation (3 mL) was placed on the membrane surface in the donor compartment while the receptor contained 5 mL of pH 7.5 phosphate buffer. Both donor and receptor solutions were stirred at 300 rpm and kept at 37 °C. Samples were collected and then the withdrawn solution was replaced with fresh buffer to maintain sink conditions up to 48 h. Samples were immediately analyzed by UV-Vis spectrophotometry.

Acknowledgements: This work was suported by CONACYT, Mexico (ref. 177587 and ref. 178022). We thank I.Q Iván Puente Lee for technical support during the SEM experiments.

References

Andresen TL, Jensen SS, Jorgensen K (2005) Advanced strategies in liposomal cancer therapy: Problems and prospects of active and tumor specific drug release. Progr Lipid Res 44: 68–97.

Ann Clark M, Blair H, Liang L, Brey RN, Brayden D, Hirst BH (2001) Targeting polymerised liposome vaccine carriers to intestinal M cells. Vaccine 20: 208–217.

Arnardottir HB, Sveinsson SJ, Kristmundsdottir T (1996) The release of clindamycin phosphate from a suspension of different types of liposomes and selected topical dosages forms. Int J Pharm 134: 71–77.

Bangham AD, Standish MM, Watkins JC (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 13: 238–252.

Chiou WL, Riegelman S (1971) Pharmaceutical applications of solid dispersion systems. J Pharm Sci 60: 1281–1302.

Deol P, Khuller GK (1997) Lung specific stealth liposomes: stability, biodistribution and toxicity of liposomal antitubercular drugs in mice. Biochim Biophys Acta – General Subjects 1334: 161–172.

Doi AM, Stoskopf MK (2000) The kinetics of oxytetracycline degradation in deionized water under varying temperature, pH, light, substrate, and organic matter. J Aquatic Animal Health 12: 246–253

Fatouros DG, Antimisiaris SG (2002) Effect of amphiphilic drugs on the stability and zeta-potential of their liposome formulations: A study with prednisolone, diazepam, and griseofulvin. J Colloid Interface Sci 251: 271–277.

Ferdous AJ, Stembridge NY, Singh M (1998) Role of monensin PLGA polymer nanoparticles and liposomes as potentiator of ricin A immunotoxins in vitro. J Control Release 50: 71–78.

Gratacós-Cubarsí M, Fernandez-Garcí AA, Picouet P, Valero-Pamplona A, García-Regueiro J-A, Castellari M (2007) Formation of tetracycline degradation products in chicken and pig meat under different thermal processing conditions. J Agricult Food Chem 55: 4610–4616.

Lasic DD (1993) Liposomes: From Physics to Applications. Elsevier, Amsterdam, pp. 63–107.
Lasic DD (1998) Novel applications of liposomes. Trends Biotechnol 16: 307–321.
Leuner C, Dressman J (2000) Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm 50: 47–60.
Mehnert W, Mader K (2001) Solid lipid nanoparticles – production, characterization and applications. Adv Drug Deliv Rev 47: 165–196.
Mehnert W, zur Mühlen A, Dingler A, Müller RH (1997) Solid lipid nanoparticles – a novel carrier system for cosmetics and pharmaceutics / 2nd communication: drug incorporation, drug liberation, sterilisation. Pharm Ind 59: 511–514.
Moreno-Cerezo JM, Córdoba-Díaz M, Córdoba-Díaz D, Córdoba-Borrego M (2001) A stability study of tetracycline and tetracycline cyclodextrins in tablets using a new HPLC method. J Pharm Biomed Anal 26: 417–426.
Muhammad JH (2000) Pharmaceutical Solid Dispersion Technology. CRC Press, Washington D.C., USA.
Müller RH, Mäder K, Gohla S (2000) Solid lipid nanoparticles (SLN) for controlled delivery – a review of the state of the art. Eur J Pharm Biopharm 50: 161–177.
Papageorgiou GZ, Bikiaris D, Karavas E, Politis S, Docoslis A, Park Y, Stergiou A, Georgarakis E (2006) Effect of physical state and particle size distribution on dissolution enhancement of nimodipine/PEG solid dispersions prepared by melt mixing and solvent evaporation. AAPS J 8: E623–E631.
Podio V, Zara GP, Carazzone M, Cavalli R, Gasco MR (2000) Biodistribution of stealth and non-stealth solid lipid nanospheres after intravenous administration to rats. J Pharm Pharmacol 52: 1057–1063.
Pouliquen H, Delepee R, Larhantec-Verdier M, Morvan ML, Le Bris H (2007) Comparative hydrolysis and photolysis of four antibacterial agents (oxytetracycline oxolinic acid, flumequine and florfenicol) in deionised water, freshwater and seawater under abiotic conditions. Aquaculture 262: 23–28.
Sah H (2006) Degradation patterns of tetracycline antibiotics in reverse micelles and water. Biomed Chromatogr 20: 1142–1149.
Sanderson H, Ingerslev F, Brain RA, Halling-Sørensen B, Bestari JK, Wilson CJ, Johnson DJ, Solomon KR (2005) Dissipation of oxytetracycline, chlortetracycline, tetracycline and doxycycline using HPLC-UV and LC/MS/MS under aquatic semi-field microcosm conditions. Chemosphere 60: 619–629.
Schwarz C, Freitas C, Mehnert W, Müller RH (1995) Sterilization and physical stability of drug-free and etomidate-loaded solid lipid nanoparticles. Proc Intern Symp Control Rel Bioct Mater 22: 766–767.
Senior J, Crawley JCW, Gregoriadis G (1985) Tissue distribution of liposomes exhibiting long half-lives in the circulation after intravenous injection. Biochim Biophys Acta 839: 1–8.
Shigeo Yamamura 1 JAR (1996) Characterization and dissolution behavior of nifedipine and phosphatidylcholine binary systems. Int J Pharm 130: 65–73.
Tongaree S, Goldberg AM, Flanagan DR, Poust RI (2000) The effects of pH and PEG 400-water cosolvents on oxytetracycline-magnesium complex formation and stability. Pharm Devel Technol 5: 189–199.
Westesen K, Bunjes H, Koch MHJ (1997) Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J Control Release 48: 223–236.
Zaru M, Mourtas S, Klepetsanis P, Fadda AM, Antimisiaris SG (2007) Liposomes for drug delivery to the lungs by nebulization. Eur J Pharm Biopharm 67: 655–666.
Zhong H, Deng Y, Wang X, Yang B (2005) Multivesicular liposome formulation for the sustained delivery of breviscapine. Int J Pharm 301: 15–24.