Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India

Development, physical characterization, micromeritics and *in vitro* release kinetics of letrozole loaded biodegradable nanoparticles

N. Mondal, T. K. Pal, S. K. Ghosal

Received November 19, 2007, accepted December 3, 2007

Prof. (Dr.) S. K. Ghosal, Division of Pharmaceutics, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700 032 India
profskghosal_ju@yahoo.co.in

Pharmazie 63: 361–365 (2008) *doi: 10.1691/ph.2008.7365*

The present investigation was aimed at preparing and characterizing biodegradable nanoparticles of letrozole with poly (D,L-lactide-co-glycolide) monomer ratio 50 : 50. The nanoparticles were prepared by direct precipitation technique. Different formulations were prepared by changing polymer-drug ratio at two different levels of phase volume ratio. The prepared nanoparticles were evaluated for the recovery, drug entrapment efficiency, micromeritic properties and particle size distribution profile, surface morphology and *in vitro* release kinetics. The results show that the nanoparticles recovery and drug entrapment efficiency vary from 37 to 79% and 12 to 27% respectively. Span value and mean diameter (MD) of different formulations were found to vary from 0.937 to 2.462 and 146 nm to 267 nm, respectively. Field Emission Scanning Electron Microscopy (FESEM) revealed the particles to be spherical with smooth surfaces. Release kinetics fitted the Higuchi model and ensured the capability of sustaining the drug release from the nanoparticles.

1. Introduction

Recently increasing attention has been paid on formulating therapeutic agents in biodegradable and biocompatible nanocomposites like liposomes, nanocapsules, nanospheres, micellar systems and conjugates (Brigger et al. 2002; Drummond et al. 1999; Kim et al. 2001; Onishi et al. 2003; Yoo et al. 2001; Zhang et al. 2004). Since these systems are submicron in size, they can be used to provide nonspecific-targeted delivery of drugs, to improve bioavailability, to sustain drug release, to make the delivery system applicable parenterally and to improve the stability of the drug against enzymatic reactions (Brannon-Peppas 1995; Labhasetwar 2003; Moghimi et al. 2001).

PLGA is composed of lactic acids and glycolic acids linked together by ester bonds. It is a biocompatible and biodegradable compound less toxic than other polymers (Anderson and Shive 1997) and has been extensively used for controlled drug delivery systems (Hans and Lowman 2002; Jain 2000; Park 1995; Sahoo et al. 2002; Soppimath et al. 2001; Uhrich et al. 1999; Vert et al. 1998). The polymer degradation takes place with formation of free carboxylic end groups.

Nanoparticles from biodegradable polymers are commonly prepared by solvent evaporation, monomer polymerization, nano precipitation and salting out procedures (Quintanar-Guerrero et al. 1998). The precipitation method developed by Fessi et al. (1999) was an easy and reproducible technique to prepare nanoparticles.

Letrozole is a potent, specific, non-steroidal, third generation aromatase inhibitor, used therapeutically to treat hormone-sensitive breast cancer in postmenopausal women (Budavari 2001). An uninterrupted supply of an anti-tumor agent is vital for patient's health. A long-term drug delivery system would improve patient's compliance and ensure a continuous supply of drug. Thus letrozole was selected as a model drug to be encapsulated into PLGA nanoparticles and also to understand the effect of variation in polymer-drug ratio at two different levels of phase volume ratio (1 : 5 and 2 : 5).

Letrozole loaded nanoparticles were prepared using the precipitation technique. Poloxamer 188 was used as stabilizer. Poloxamers are used in a variety of oral, parenteral and topical formulations and are generally regarded as non-toxic and non-irritant materials (Rowe 2003). The different formulations of nanoparticles were characterized with regard to nanoparticles recovery, drug entrapment efficiency, size, shape, micromeritics and *in vitro* drug release kinetics.

2. Investigations, results and discussion

The nanoparticles were formed by direct precipitation. The internal phase (acetone) is completely miscible with the external phase, thus the method allows the formation of nanoparticles without prior emulsification (Mathiowitz 1999). In this case, polymer precipitation takes place when the organic solution diffuses rapidly into the aqueous phase and is successively evaporated. The use of poloxamer keeps the precipitated particles suspended and discrete.

Formulations and nanoparticles recovery as well as drug entrapment efficiencies are shown in Fig. 1. It was observed that nanoparticles recovery was increased up to the

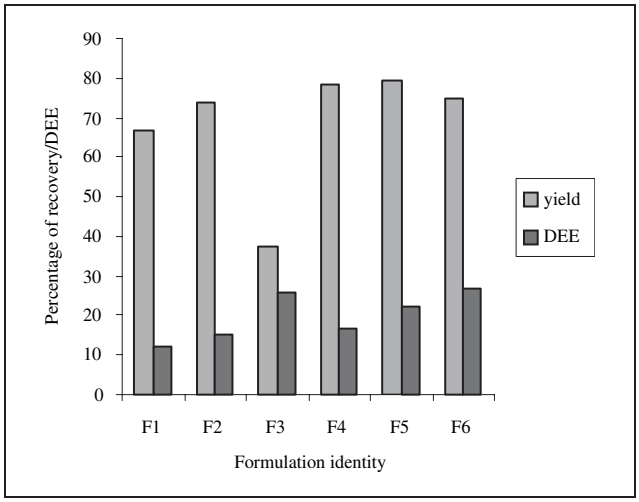


Fig. 1: Nanoparticles recovery and drug entrapment efficiency of different batches

polymer drug ratio 10 : 1 in case of two different levels of phase volume ratio. But yield was decreased when the polymer-drug ratio was increased to 20 : 1. This might be because of agglomeration of polymeric particles due to increased viscosity of the internal phase since a higher amount of polymer is present at a higher polymer-drug ratio. Hence at a certain polymer-drug ratio nanoparticles recovery may be highest and when the amount of polymer was increased beyond the specific ratio, agglomeration of polymer may take place and eventually nanoparticles recovery is decreased. Increase in polymer-drug ratio resulted in increase in drug entrapment efficiency. A lower fraction of drug resulted in more efficient drug entrapment.

Particle size distribution pattern is shown in Fig. 2 and different micromeritic properties are shown in Table 1. Particle size distribution profile is a very important parameter for nanoparticulate drug delivery systems, specifically for parenteral application. For drug delivery, most of the sites are accessible through either microcirculation by blood capillaries or pores present at various surfaces and membranes. For any moiety to remain in the vasculature,

Table 1: Micromeritic properties of nanoparticles

Formula	PDR*	PVR**	D_{10}	D_{50}	D_{90}	MD*** (nm)	Span
F1	5 : 1	1 : 5	74	142	300	161	1.595
F2	10 : 1	1 : 5	65	129	263	169	1.530
F3	20 : 1	1 : 5	79	184	532	267	2.462
F4	5 : 1	2 : 5	80	130	201	146	0.937
F5	10 : 1	2 : 5	77	124	195	140	0.955
F6	20 : 1	2 : 5	65	128	256	167	1.494

*PDR: polymer drug ratio; **PVR: phase volume ratio; ***MD: Mean diameter; D_{10}: 10% of the particles is smaller than this diameter; D_{50}: 50% of the particles is smaller than this diameter; D_{90}: 90% of the particles is smaller than this diameter.

it needs to have its own dimension narrower than the cross-sectional diameter of the narrowest capillary, which is about 2000 nm (Gupta and Kompella 2006). Actually for efficient transport, the arithmetic mean diameter of nanoparticles should be smaller than 300 nm. However, other micromeritic properties are also to be considered. Span which is calculated as $(D_{90} - D_{10})/D_{50}$ (where D_{10}: 10% of the particles is smaller than this diameter; D_{50}: 50% of the particles is smaller than this diameter; D_{90}: 90% of the particles is smaller than this diameter) is an useful parameter in determining the size distribution pattern of particles (Sarkar et al. 2005). A large span value may be due to one or more of the following reasons (a) very high D_{90} and very small D_{10} (b) moderate D_{90} and D_{10} but very small D_{50}. On the other hand a small span value may result from moderate D_{90}, D_{50} and comparatively smaller D_{10}. A very high span value with a very high D_{90} is undesirable since that indicates the presence of a considerably higher percentage of larger particles. Ideally a small span value with smaller D_{10} and moderate D_{90} and D_{50} is more suitable for such purposes (Sarkar et al. 2005).

According to Table 1, the mean diameter of nanoparticles prepared with a solvent-non-solvent ratio 1 : 5 varied from 161 nm to 267 nm, when polymer drug ratio was increased from 5 : 1 to 20 : 1. This can be explained by the fact that a greater amount of polymer results in a more viscous dispersed phase, making the dispersion between two phases difficult and originating larger particles.

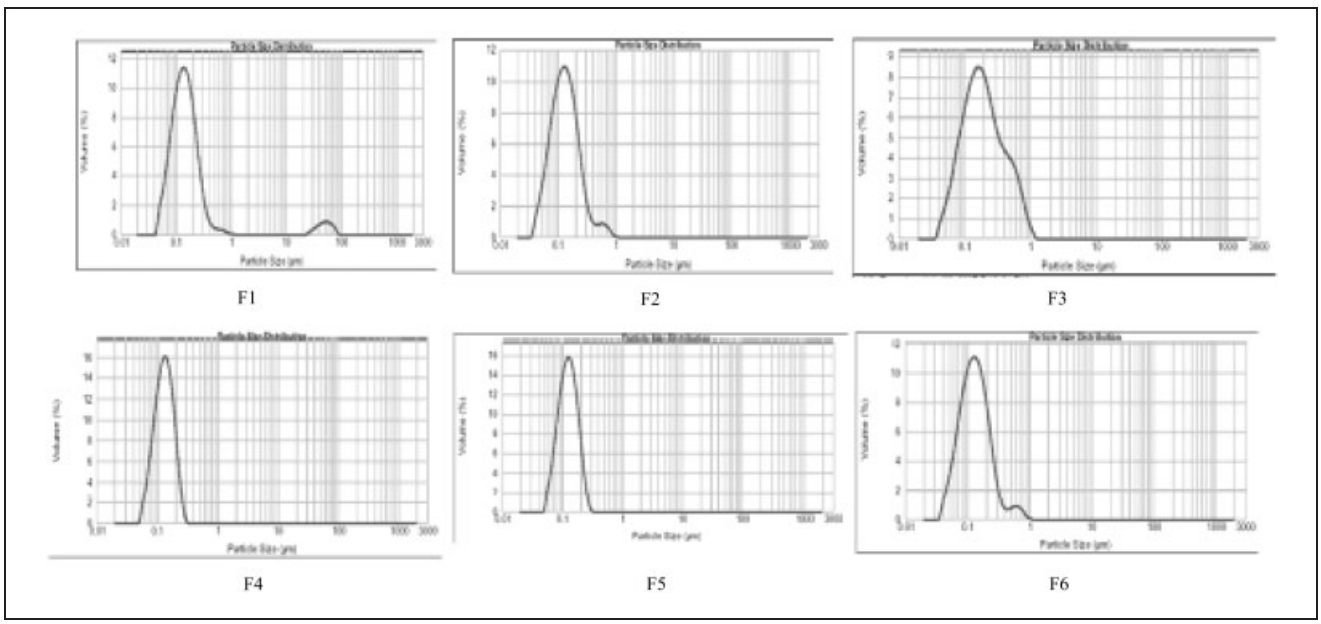
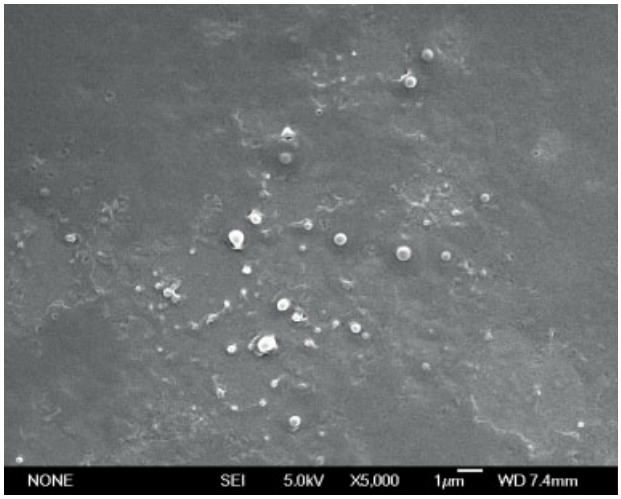
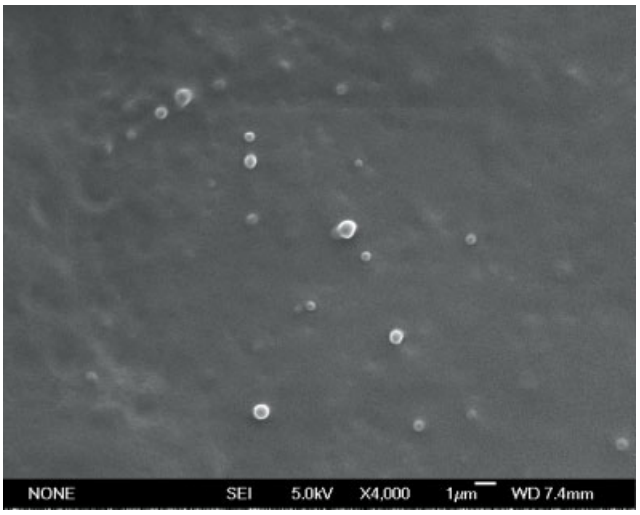


Fig. 2: Particle size distribution pattern of different formulations



(a)



(b)

Fig. 3: Scanning electron micrographs

An increase in mean particle diameter is also observed when the polymer drug ratio is increased when the phase volume ratio was kept 2 : 5 but mean diameter of all three formulations in second set (F4, F5, F6) are less compared

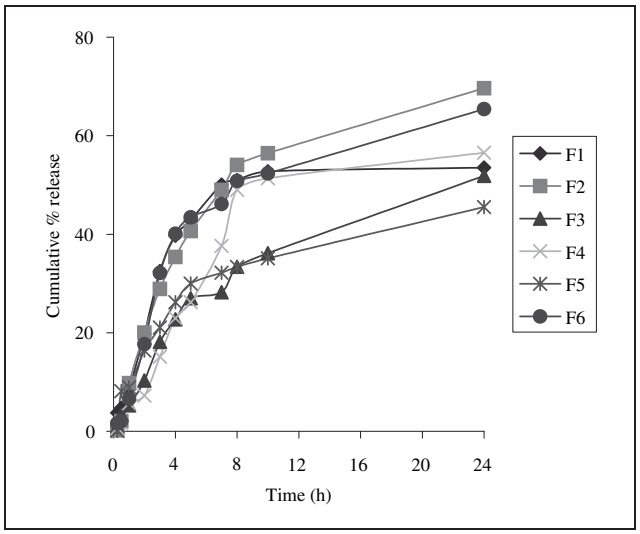


Fig. 4: Drug release profile from nanoparticles of different batches

Table 2: Correlation coefficients of different mathematical models and release rate constant (k) for letrozole from nanoparticles

Formula	Correlation coefficients			
	Zero order	First order	Higuchi model	k* (mg $h^{-0.5}$)
F1	0.517	0.564	0.962	13.873
F2	0.714	0.846	0.922	17.594
F3	0.830	0.907	0.973	12.293
F4	0.716	0.767	0.886	15.478
F5	0.705	0.781	0.914	10.363
F6	0.661	0.788	0.881	16.444

*k denotes release rate constant according to Higuchi model.

to first set of formulations (F1, F2, F3). Enhancement of phase volume ratio from 1 : 5 to 2 : 5 resulted in increase in volume of the organic phase and consequently a decrease in viscosity of the disperse phase and finally resulted in a small MD and narrow distribution profile of nanoparticles.

Scanning electron micrographs of PLGA nanoparticles are shown in Fig. 3. The surface structure of nanoparticles prepared by the solvent displacement technique was found to be spherical and smooth in nature.

When only spherical particles are present the release can be more controlled. The homogeneity in particle size indicates the stability and eventually security in case of parenteral administration.

The drug release study from PLGA nanoparticles was performed in phosphate buffer pH 7.4 using a Kesary-chein diffusion cell apparatus with dialysis membrane. The release profiles of all six formulations are shown in Fig. 4. In different formulations about 45–69% of drug was released over the period of 10 h followed by a sustained release up to more than 24 h. The sustained release may be due to diffusion of drug from the matrix of PLGA nanoparticles to release medium. From Fig. 4 it was observed that as polymer concentration increased release rate was decreased.

The results of kinetic evaluation are presented in Table 2 based on different models i.e., zero order, first order and Higuchi. From the tabulated results it is clear that the release kinetics of all formulations followed the Higuchi model since correlation coefficients of the Higuchi model were higher than that of the zero order or the first order models. The results indicated that the various drug polymer ratios did not interfere with release kinetics. Since release kinetics fitted the Higuchi model, the nanoparticles may be capable of sustaining the release of letrozole successfully.

From the study the following conclusions can be drawn: The direct precipitation method allowed the preparation of spherical drug loaded particles of PLGA. Higher proportion of internal phase resulted in smaller particles with narrower size distribution profile. Narrow particle size distribution profile along with a small average particle size in all formulations may make the nanoparticulate suspension to be applied parenterally. Extensive analysis of release kinetics revealed that the release data fitted the Higuchi model ensuring the sustained release of drug from nanoparticles.

3. Experimental

3.1. Materials

Poly (D,L-lactide-co-glycolide) with a monomer ratio of 50 : 50 (Resomer 503 H) with inherent viscosity 0.32–0.44 dl/g was purchased from Boeh-

ringer Ingelheim, Germany, poloxamer 188 (Lutrol 68) was generously supplied by Ranbaxy Research laboratory. Gurgaon, India. Letrozole was a kind gift from Sun Pharma Advanced Research Centre (SPARC, Vadodara, India), potassium dihydrogen orthophosphate, sodium hydroxide, acetone were procured from Mecrk, India. Milli-Q water was used through out the study. Other excipients and solvents were of analytical grade and were used without further purification.

3.2. Preparation of letrozole loaded nanoparticles

The preparation of nanoparticles was based on the solvent displacement process. The required amount of polymer was dissolved in acetone. The organic phase was added to the aqueous phase containing 0.5% w/v poloxamer 188 at a constant flow rate (0.3 ml/min) under mechanical stirring at 2000 rpm. Acetone was removed at room temperature with constant stirring at 2000 rpm for 5 h. Finally the nanoparticles were isolated by centrifugation ant $10\,000 \times g$ at 4 °C for 30 min and washed with water and dried under vacuum.

3.3. Quantitative analysis of letrozole by HPLC

HPLC analysis were performed with a Jasco HPLC system (Jasco, Japan) equipped with a Jasco – PU-980 pump and a Jasco – UV-975 UV detector (set at the wavelength of 240 nm) and Clarity Lite software. For analysis a reversed phase FinePak C_8 column (4.6 mm × 250 mm, Jasco, Japan) was eluted with water, acetonitrile and methanol (50 : 30 : 20 v/v/v) in isocratic mode. The flow rate of 1 ml/min was maintained and the column effluent was monitored continuously at 240 nm. Quantitation of the drug was carried out by measuring the peak areas in relation to those of standard chromatograms under identical conditions. A calibration curve was prepared by plotting the concentration and peak area through X-axis and Y-axis respectively. The method showed the linearity over 1–50 μg/ml. The retention time was 9.8 min. Under these conditions the limit of detection and limit of quantitation were found to be 0.207 μg/mL and 0.627 μg/mL, respectively.

3.4. Size measurement

Nanoparticle mean diameter and Span value as an index of polydispersity were determined by laser diffractometry using a Mastersizer 2000 (Malvern laser diffraction particle size analyzer, Malvern Instruments, U.K.). The Mastersizer comprises of Helium-Neon laser as a light source. This is then focused by a Fourier lens to a detector, which consists of a large number of photosensitive elements radiating outward from the center. The intensity of the scattered light is measured. The volumetric particle size distribution is calculated by using an optical model and mathematical deconvolution procedure.

For particle size analysis, the samples were dispersed in Milli-Q water. Measurements were carried out at 30 °C using a Helium-Neon laser at an angle of 90°.

3.5. Determination of nanoparticles recovery

Initially formed nanoparticulate suspension was centrifuged at 25,000 rpm at 4 °C for 1 h. The supernatant liquid was removed and solid portion is washed twice with fresh water and again centrifuged for 10 min under the same conditions. This step was repeated twice. Solid portion was isolated and dried under vacuum. Weight of the dried powder was determined. The percentage of nanoparticles recovery was calculated by the following equation:

Nanoparticles recovery (%) = (weight of recovered particles/weight of drug and polymer used) × 100

3.6. Determination drug loading

A known weight of prepared nanoparticles was taken in 100 ml volumetric flask. 40 ml of methanol was added and shaken on a rotary flask shaker for 2 h. The volume was made up to 100 ml and filtered through membrane filter with porosity of 0.22 μm to obtain a completely clear solution. Samples were then assayed spectrophotometrically by a previously reported method (Mondal et al. 2007) at 238 nm using a double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to a computer loaded with spectra manager software. UV Probe was employed with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. The percentage drug loading was then calculated.

3.7. In vitro drug release:

For determination of the *in vitro* release of letrozole, the Keshary-Chein diffusion cell apparatus was used (Leo et al. 2004). A cellulose dialysis membrane (cutoff 12000, HIMEDIA, Mumbai, India) was adapted to the terminal portion of the donor compartment. Phosphate buffer of pH 7.4 was taken in the receptor compartment of the cell. The amount of nanosuspension, sufficient for establishing sink conditions was measured and put over the membrane in the donor compartment. The donor compartment was fitted to the receptor compartment in such a way that the dialysis membrane was just in contact with the release media. The study was carried out for 24 h with continuous stirring at 37 °C with the help of a thermostatic water bath. At different time intervals, aliquots of 1 ml were withdrawn and immediately replaced with the identical volume of fresh medium. The aliquots were filtered and concentration of letrozole in the release medium were assessed by the proposed HPLC method. The release test data were obtained on average of five parallel studies. The amount of letrozole obtained from the drug release studies was calculated from a linear regression equation. For evaluation of release kinetics, the obtained release data were fitted into zero order, first order and Higuchi equation. Selection of the best model was based on comparison of the relevant correlation coefficients.

3.8. Particle morphology

The morphology of nanoparticles was observed by Field Emission Scanning electron microscopy (FE-SEM) (JEOL 67007, JAPAN). A drop of nanoparticulate suspension was placed on a metallic surface. After drying under vacuum, the sample was coated with a gold layer. Observations were performed at 5 KV.

Acknowledgements: One of the authors, N Mondal is grateful to UGC, Govt. of India [F.10–17/2004(SA-I)] for financial assistance and to East India Pharmaceutical Works Ltd (Kolkata, India) to carry out particle size analysis. Authors are also grateful to Sun Pharmaceuticals Advanced Research Centre (Vadodara, India) for sending letrozole as gift sample and Ranbaxy Research Laboratory (Gurgaon, India) for sending poloxamer 188 as gift sample.

References

Anderson JM, Shive MS (1997) Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Del Rev 28: 5–24.

Brannon-Peppas L (1995) Recent advances on the use of biodegradable microparticles and nanoparticles in controlles drug delivery. Int J Pharm 116: 1–9.

Brigger I, Dubernet C, Couvreur P (2002) Nanoparticles in cancer therapy and diagnosis. Adv Drug Del Rev 54: 631–651.

Budavari S (ed.) (2001) The Merck Index, An Encyclopedia of Chemicals, drugs and biologicals, Merck and Co., 13th Edn, White house Station, NJ, 974.

Chew NYK, Chan HK (2002) Effect of powder polydispersity on aerosol generation. J Pharm Pharm Sci 5: 162–168.

Drummond DC, Meyer O, Hoong K, Kirpotin DB, Papahadjopoulos D (1999) Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. Pharm Rev 51: 691–744.

Fessi H, Puisieux F, Devissaguer JP, Ammoury N, Benita S (1989) Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm R1–R4.

Gupta RB, Kompella UB (eds.) (2006) Nanoparticle Technology for Drug Delivery, Taylor & Francis, 1st Edn, New York, 119.

Hans ML, Lowman AM (2002) Biodegradable nanoparticles for drug delivery and targeting. Curr Opin Solid State Mat Sci 6: 319–327.

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

Kim SY, Lee YM (2001) Taxol-loaded block copolymer nanospheres composed of methoxy poly(ethylene glycol) and poly(caprolactone) as novel anticancer drug carriers. Biomaterials 22: 1697–1704.

Labhasetwar V (2003) Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv Drug Del Rev 55: 329–347.

Leo E, Brina B, Forni F, Vandelli MA, (2004) *In vitro* evaluation of PLA nanoparticles containing a lipophilic drug in water-soluble or insoluble form. Int J Pharm 278; 133–141.

Mathiowitz, E (eds.) (1999) Encyclopedia of Controlled drug Delivery, John Wiley & Sons, Inc, 1st Edn, New York, 650.

Moghimi SM, Hunter AC, JC (2001) Murray Long-circulating and target specific nanoparticles: theory and practice Pharmacol Rev 53: 283–318.

Mondal N, Pal TK, Ghosal SK (2007) Development and validation of spectrophotometric method for estimation letrozole in bulk and pharmaceutical formulation. Pharmazie 62: 597–598.

Onishi H, Machida Y, Machida Y (2003) Antitumor properties of irinotecan-containing nanoparticles prepared using poly (DL-lactic acid) and poly (ethylene glycol)-block-poly(propylene glycol-block-poly (ethylene glycol). Biol Pharm Bull 26: 116–119.

Park TG (1995) Degradation of poly(lactide-co-glycolide acid) microspheres: effect of copolymer composition. Biomaterials 16: 1123–1130.

Quintanar-Guerrero D, Allemann E, Fessi H, Doelker E (1998) Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. Drug Devel Ind Pharm 24: 1113–1128.

Rowe RC (ed.) (2003) Handbook of Pharmaceutical Excipients, Pharmaceutical Press, 4th Edn, 1 Lambeth High Street, London SE 1 7JN,UK, 447.

Sahoo SK, Panyam J, Prabha S, LAbhasetwar V (2002) Residual polyvinyl alcohol associated with poly(D,L-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake. J Control Release 82: 105–114.

Sarkar A, Rano R, Mishra KK, Sinha IN (2005) Particle size distribution profile of some Indian fly ash-a comparative study to assess their possible uses. Fuel Proc Technol 86: 1221–1238.

Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE (2001) Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release 70: 1–20.

Uhrich KE, Cannizzaro SM, Langer RS, Shakeshelf KM (1999) Polymeric systems for controlled drug release. Chem Rev 99: 3181–3198.

Vert M, Schwach G, Engel R, Coudane J (1998) Something new in the field of PLA/GA bioresorbable polymers. J Control Rel 53: 85–92.

Yoo HS, Park TG (2001) Biodegradable polymeric micelles composed of doxorubicin conjugated PLGA-PEG block copolymer. J Control Release 70: 63–70.

Zhang L, Hu Y, Jiang X, Yang C, Lu W, Yang YH (2004) Camptothecin derivative-loaded poly (caprolactone-co-lactide)-b-PEG-b-poly(caprolactone-co-lactide) nanoparticles and their distribution in mice. J Control Release 96: 135–148.