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Review

# Poly-ϵ-caprolactone microspheres and nanospheres: an overview

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**Abstract**

Poly-ε-caprolactone (PCL) is a biodegradable, biocompatible and semicrystalline polymer having a very low glass transition temperature. Due to its slow degradation, PCL is ideally suitable for long-term delivery extending over a period of more than one year. This has led to its application in the preparation of different delivery systems in the form of microspheres, nanospheres and implants. Various categories of drugs have been encapsulated in PCL for targeted drug delivery and for controlled drug release. Microspheres of PCL either alone or of PCL copolymers have been prepared to obtain the drug release characteristics. This article reviews the advancements made in PCL-based microspheres and nanospheres with special reference to the method of preparation of these and their suitability in developing effective delivery systems.


*Keywords:* Poly-ε-caprolactone; Microsphere; Implant

## 1. Introduction

Technological advancements have brought many new innovative drug delivery systems into commercial circulation. Among these biodegradable microparticulate systems are interesting for controlled drug release and drug targeting. The most active area of contemporary research using synthetic biodegradable polymers such as polyester polymers focuses on controlled drug delivery of pharmaceuticals. Based on their biodegradability and biocompatibility, homo- and co-polymers derived from poly(lactic acid) (PLA) and poly glycolic acid (PGA) are being extensively utilized to prepare controlled release carriers for drugs and proteins. These aliphatic polyester polymers degrade by bulk hydrolysis of the ester bonds. Their therapeutic values have been enormously increased in the form of microparticles and nanoparticles, which have shown clear advantages for parenteral and oral drug delivery. Most of the research work has been directed at poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid) (PLGA) copolymers. The success of these for pharmaceutical applications has further led to the evaluation of aliphatic polyesters such as poly-ε-caprolactone (PCL).

It was in 1930s that the ring-opening polymerization of PCL was studied (Van Natta et al., 1934). The biodegradable property of this synthetic polymer was first identified in 1973. PCL is suitable for controlled drug delivery due to its high permeability to many

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drugs and non-toxicity (Murthy, 1997). This article reviews research on biodegradable microspheres and nanospheres prepared using PCL.

## 2. Physicochemical properties of PCL

There are various mechanisms which affect the polymerization of PCL and these are anionic, cationic, co-ordination and radical. Each method is affected by molecular weight ($M_n$), molecular weight distribution, end group composition and chemical structure of the copolymers (Murthy, 1997). PCL is a semi-crystalline polymer having glass transition temperature of −60 °C and melting point ranging between 59 and 64 °C, depending upon its crystalline nature of PCL. The number average molecular weight of PCL samples may vary from 10,000 to 42,500 and it is graded according to the molecular weight ($M_n$). PCL is soluble in chloroform, dichloromethane, carbon tetrachloride, benzene, toluene, cyclohexanone and 2-nitropropane at room temperature. It has a low solubility in acetone, 2-butanone, ethyl acetate, dimethylformamide and acetonitrile and is insoluble in alcohol, petroleum ether and diethyl ether. PCL can be blended with other polymers to improve stress crack resistance, dyeability and adhesion. Polycaprolactone is used in combination with polymers such as cellulose propionate, cellulose acetate butyrate, polylactic acid and polylactic acid-co-glycolic acid for manipulating the rate of drug release from microcapsules (Chang et al., 1986). Polymer blends have been categorized with three types compatible, exhibiting only a single $T_g$; mechanically compatible, exhibiting the $T_g$ values of each component but with superior mechanical properties and incompatible, exhibiting the enhanced properties of phase-separated materials (Koleske, 1978). Compatibility of these blends depends on the ratios employed and is generally used to control the permeability of the delivery systems.

Copolymers (block and random) of PCL can be formed using many monomers, e.g. ethyleneoxide, polyvinylchloride, chloroprene, polyethylene glycol, polystyrene, diisocyanates(urethanes), tetrahydrofuran (THF), diglycolide, dilactide, δ-valerlactone, substituted caprolactones, 4-vinyl anisole, styrene, methyl methacrylate and vinyl acetate.

## 3. Biodegradation

Degradation of PCL in comparison to polyglycolic acid and other polymers is slow making it suitable for long-term delivery extending over a period of more than one year. Biodegradation of this polymer can be enhanced by copolymers like polylactic acid and polyglycolic acid (Koleske, 1978).

Degradation of PCL is a bulk process that can be divided into two phases:

(1) $M_n$ loss up to 5000 due to chain scission.
(2) Onset of weight loss.

Degradation is autocatalyzed (Pitt, 1990). The kinetic patterns of PCL degradation are consistent with an autocatalytic process, whereby the liberated carboxylic acid end groups catalyze the hydrolysis, i.e. the cleavage of additional ester groups (Eq. (1)).

$$\text{–COOR} \cdots \text{HOOC–} + \text{H}_2\text{O} \rightarrow 2\text{COOH} + \text{ROH} \quad (1)$$

$$\text{COOH} = k(\text{COOH})_0 \text{ (ester)} \quad (2)$$

$$\eta = \eta_0 \exp(-akt) \quad (3)$$

$$M_n = M_n^0 \exp(-kt) \quad (4)$$

Assuming the concentration of ester groups is constant during the initial stages of hydrolysis, integration of Eq. (2) leads to Eqs. (3) or (4), which are related to the Mark–Houwink relationship (Eq. (5))

$$\eta = KM_n^{\text{a}} \quad (5)$$

This equation expresses the observed exponential decline in the intrinsic viscosity ($\eta$) and number average molecular weight ($M_n$). No weight loss is observed during the initial phase of the biodegradation process, which covers a molecular weight ($M_n$) range of 200,000 to 5000. The second phase of polymer degradation is characterized by a decrease in the rate of chain scission and the onset of weight loss. Weight loss has been attributed to an increased probability that chain scission of a low molecular weight polymer will produce a fragment small enough to diffuse out of the polymer bulk and the break up of the polymer mass to produce smaller particles with an increased probability of phagocytosis. The decrease in the rate of chain scission is associated with an increase in crystallinity, since cleavage takes place in the amorphous region of the polymer.

The in vivo degradation process was studied by implanting low molecular weight poly(ε-caprolactone-$^{14}$C) in rats and measuring the radioactivity in urine, feces, expired air and the residual activity at the implant site. It was observed that absorption was complete in 60 days, although only 60% of the radioactivity was accounted for and only 9 ± 4% of the original radioactivity remained after 120 days. ε-Hydroxycaproic acid, derived from complete hydrolysis of the polymer, and tritiated water were the only metabolites detected. The mechanism of bioabsorption was studied by electron microscopic examination of the tissue at the implant site. This revealed the presence of intracellular polymer particles and demonstrated the role of phagocytosis in the final stage of polymer degradation. Biodegradation of microspheres based on polycaprolactone and its copolymers had been studied (Chen et al., 2000).

## 4. PCL microspheres

Biodegradable polymers have been the major focus of attempts to develop improved delivery systems for pharmaceutical research. The commonly studied biodegradable polymers for controlled drug delivery are the aliphatic polyesters; poly(lactide), poly(glycolide), PCL and their copolymers (Thombre and Cardinal, 1990).

The advantages of PCL include its high permeability to small drug molecules, their failure to generate an acidic environment during degradation as compared to polylactides and glycolides, an exceptional ability to form blends with other polymers and degradation of PCL homopolymer being slow as compared to PLGA and polyglycolic acid-co-lactic acid making it more suitable for long term delivery systems extending to a period of more than one year (Koleske, 1978).

### *4.1. Techniques of microsphere preparation*

PCL microspheres can be prepared by several different methods as described below:

#### *4.1.1. o/w emulsion solvent extraction/evaporation method*

PCL microspheres can be prepared by an emulsion solvent extraction/evaporation technique. Several drugs have been encapsulated by this method including nifedipine and propranolol hydrochloride. In the solvent evaporation method, the required amount of polymer and drug are dissolved in an organic phase (e.g. methylene chloride) which is emulsified under stirring with polyvinyl alcohol (PVA) (0.25% w/w) solution to form an o/w emulsion. Stirring is continued for 3 h at about 500 rpm, to evaporate the organic phase. The microspheres so formed are filtered and dried (Perez et al., 2000).

PCL microcapsules were prepared by Barbato et al. (2001) using a simple emulsion solvent evaporation technique with a slight modification. PCL was dissolved in 10 ml of dichloromethane and emulsified into 100 ml of 4% w/v aqueous PVA solution with the help of a high speed homogenizer at 10,000 rpm. This emulsion was then magnetically stirred at 500 rpm followed by addition of 100 ml of distilled water. This increased the diffusion of organic solvent into the external aqueous phase to promote microsphere hardening. After completion of solvent evaporation the microspheres were collected by centrifugation, washed and dried.

#### *4.1.2. w/o/w emulsion solvent evaporation technique*

Various drugs such as bovine serum albumin (BSA) (Benoit et al., 1999), nifedipine and propranol HCl (Perez et al., 2000) have been encapsulated with PCL using the w/o/w emulsion method. Aqueous solutions of drug are emulsified with PCL in dichloromethane. The resulting w/o emulsion is then again emulsified with water containing PVA as an emulsifier. Stirring for 5 min at 1200 rpm leads the formation of a w/o/w emulsion. The stirring was continued to evaporate the organic solvent leading to formation of microspheres. After decantation the microparticles were filtered, washed and dried in an oven for 24 h at 50 °C (Perez et al., 2000).

BSA loaded PCL microspheres were also prepared by modified w/o/w emulsion technique (Sah et al., 1995). Aqueous solution of BSA was emulsified with organic solvent (methylene chloride) containing PCL, by homogenization. Shear rate was set at a particular rpm (11–23 rpm) to prepare w/o emulsion with different shear forces. Double emulsion, i.e. w/o/w was then prepared by pouring the primary w/o emulsion into 4% aqueous PVA solution. This w/o/w emulsion was kept at constant stirring for 30 min. Additional water was

slowly added to the emulsion over a period of 30 min. Microcapsules were collected by filtration and dried under vacuum. The existing methods of encapsulation of proteins and peptides like emulsification-solvent evaporation techniques, use an aqueous medium for processing, due to their hydrophilicity, proteins and peptides are likely to preferentially partition out into the aqueous processing medium leading to low entrapment efficiency. Considering this a novel method of microencapsulation of proteins and peptides was developed which leads to a high entrapment efficiency, i.e. (water in oil) in oil emulsion technique in which oil was used as processing medium in the expectation that hydrophilic proteins would find it unfavorable to diffuse out of the microspheres before they harden (Badri et al., 1999).

#### *4.1.3. Spray drying technique*

The spray drying technique was used for the preparation of PCL microspheres of ketoprofen by Giunchedi et al. (1994). The organic solution of the drug and two polymers, cellulose acetate butyrate and PCL was made in a mixture of dichloromethane and chloroform (1:1). The prepared solution was sprayed through a nozzle in a spray-drier under different experimental conditions. The spray flow rate was kept constant around 6 ml/min. Solid microspheres were collected into final bottom vessel of the spray-drier, and then harvested and kept under vacuum for 48 h.

#### *4.1.4. Solution-enhanced dispersion method*

Some techniques used for microencapsulation suffer from drawbacks such as the use of organic solvents, low encapsulation efficiencies or stability problems. To overcome these problems a new solution enhanced dispersion method (SED) was used for preparation of polymeric microparticles using supercritical fluid (Bodmeier et al., 1995). Two techniques were used to minimize the use of organic solvents during the preparation of microparticles. These techniques are based on the use of supercritical fluid like carbon dioxide. Technique in which microparticles were prepared by spraying a solution of polymer in mixture of carbon dioxide and organic solvent into air is termed as rapid expansion of supercritical solutions (RESS) (Phillips and Stella, 1993). In another method, organic polymer solution was atomized into a vessel containing compressed carbon dioxide. Microparticles were formed after precipitation of the polymer, caused by extraction of organic solvent into carbon dioxide and by carbon dioxide diffusion into droplets. This process is termed as aerosol solvent extraction system (ASES) (Bleich et al., 1993). This is more advantageous and promising technique for preparation of microparticles as the properties of carbon dioxide may be adjusted over a continuum throughout the gaseous, supercritical and liquid states by varying temperature and pressure. In this method, the amounts of toxic solvents such as dichloromethane is reduced. Ghaderi et al. (1999) also studied the solution-enhanced dispersion by supercritical fluids (SEDS), for the production of polymeric microparticles. PCL was one of the polymer used for preparation of microspheres by SEDS. Solutions of the polymers in organic solvents were dispersed and sprayed with supercritical $CO_2$. Extraction of the organic solvents resulted in the formation of solid microparticles. The amounts of highly toxic solvents such as dichloromethane were reduced in the process.

Microspheres formed with PCL were having diameters of 30–210 μm and showed a strong tendency to form films at high pressure. Above study led to the conclusion that SEDS process is a promising method for production of microparticles from biodegradable polymers without the use of toxic solvents.

#### *4.1.5. Hot melt technique*

In this technique, the polymers with low melting point were fabricated into microspheres by hot melt technique. The molten polymer was dispersed in a suitable dispersion medium and slowly cooled to form the microspheres. This method was particularly successful in case of microspheres, which were susceptible to hydrolysis in presence of moisture (Mathiowitz and Langer, 1987).

## 5. PCL microspheres as delivery system

Out of various drug delivery systems biodegradable microspheres based delivery systems occupy a hot seat. Various categories of drugs have been encapsulated in PCL microspheres for their effective delivery. Microspheres can be prepared either by PCL alone, or by using copolymers with PCL or PCL blends in

order to obtain the desired release characteristics. The fabrication of PCL microspheres from blends of PCL and polyethylene glycols has also been studied (Lin et al., 1998).

### 5.1. Microspheres based on PCL or PCL blends

Many drugs have been encapsulated making use of PCL alone. These include antigens, antihypertensive drugs, taxol, etc. Microspheres based drug delivery systems using PCL or PCL blends have shown to be useful in varied conditions which have been discussed below:

#### 5.1.1. Antigen

Jameela et al. (1996) showed that PCL has good permeability to proteins and unlike PLA and PGA, PCL degrades very slowly and does not generate an acidic environment which can adversely affect the antigenicity of the vaccine and hence, it can be used as a vaccine carrier. Considering this, PCL microspheres were prepared containing 3% BSA by a double emulsion technique. Adult wistar rats were used for immunogenicity studies. In vitro release studies showed a initial burst of 10–12% of the protein and in six months about 60.5% of the protein was released. There was no change in the pH of the buffer incubated with PCL microspheres over a six-month period, which indicated that protein antigenicity was not affected by encapsulation in PCL. Jameela et al. (1997) prepared microspheres by another technique, i.e. melt encapsulation technique. In vitro studies showed that the release rate of the protein from microspheres prepared by melt encapsulation technique was slower as compared to release from microspheres prepared by solvent evaporation technique. In vivo studies carried out in rat showed that a single injection of BSA-loaded PCL microspheres generated an immune response comparable in magnitude and time kinetics to that of conventional three-injection schedule.

In another study PCL microparticles loaded with BSA were prepared by w/o/w solvent evaporation technique (Youan et al., 1999a). In vitro dissolution studies of BSA microparticles were carried out in presence or absence of sodium dodecyl sulphate (SDS). The release pattern of BSA from microparticles was found to be significantly faster in medium containing SDS. Burst effect from the microspheres was found to be slow and was followed by a continuous release for 28 days.

PCL based microparticulate system intended for protein delivery was designed for oral immunization (Youan et al., 1999b). Microparticles were prepared by solvent evaporation method using BSA as a model antigen. Muramyl dipeptide (MDP) was added as a adjuvant. When MDP was entrapped alone, the entrapment efficiency (MEE) was $16.96 \pm 0.67\%$ μg/100 mg. But when BSA was coencapsulated with MDP, there was no significant effect on BSA entrapment efficiency (BEE). In this case, MEE ($48.58 \pm 1.42\%$) μg/100 mg was significantly higher compared with MDP alone. It was postulated that the intrinsic emulsifier effect of the coentrapped BSA contributed partly to stabilize the MDP containing emulsion during the process of preparation. Furthermore, the manufacturing process did not seem to alter the structural integrity of MDP in the microparticles. The latter were resistant to simulated gastric fluid and were smooth and spherical with an average diameter of less than 10 μm, hence were suitable for oral immunization. Release profile of BSA from PCL particles with mean diameter of $10.94 \pm 0.75$ μm containing $1.62 \pm 0.09\%$ BSA eluted into 0.1 M phosphate buffer solution (PBS) pH 7.4 without or after preincubation in simulated gastric fluid were studied for 72 h. It was concluded that BSA release was unchanged in both cases. These observations provided evidence that PCL microparticles were resistant to simulated gastric fluid and hence could afford some protection of adjuvant and/ or the antigen from proteolytic destruction in the stomach, allowing the entrapped drugs to pass intact into the intestine for presentation to the gut-associated lymphoid system. Moreover, the release profile of the protein in the two media was consistent with burst effect of less than 30% followed by a slow continuous release.

Microspheres based on poly-epsilon-caprolactone and its blends with PLGA were developed as a vaccine delivery system against brucellosis (Murillo et al., 2002). The antigenic extract in hot saline from Brucella ovis was microencapsulated by the spray-drying technique. Results indicated that formulation containing no PCL showed the highest encapsulation efficiency. Release rates were characterized by a high burst effect after 1 h of incubation, followed by a slow and continuous release. pH of the medium dropped

from 7.4 to 3.5 during release for the formulation based on PLGA while the presence of poly-epsilon-caprolactone attenuated the pH drop. Microspheres prepared with poly-epsilon-caprolactone showed the higher uptake by J744-macrophages and cell respiratory burst. All these characteristics suggested that the microsphere based antigenic formulation containing the higher ratio of poly-epsilon-caprolactone is susceptible to be used in animal vaccination studies.

#### 5.1.2. Antihypertensive drugs

Solvent evaporation method using o/w or w/o/w emulsion have been used for microencapsulation of lipophilic (nifedipine) and hydrophilic (propranolol HCl) drugs. The encapsulation efficiency for nifedipine was 91 and 83% respectively for o/w and w/o/w emulsions. Whereas, it was 37 and 57% for propranolol HCl, respectively. In vitro drug release studies revealed that varying the manufacturing process of microspheres varied the drug release characteristics of both the type of drugs. A controlled release profile was obtained for microparticles prepared by o/w method. While propranolol HCl microparticles prepared by w/o/w method showed a burst drug release (Perez et al., 2000).

#### 5.1.3. Taxol

Taxol microspheres with PCL were prepared by solvent evaporation method and tested for angiogenesis. In vitro studies have shown that 25% of loaded drug was released in 6 weeks from microspheres containing 5% taxol (Dordunoo et al., 1995). It was demonstrated that taxol released from the microspheres induced vascular regression and inhibited angiogenesis.

#### 5.1.4. Gentamicin

Systemically administered antibiotics for arthritis and persistent lameness are not effective for local drug therapy in eliminating infection. Controlled release techniques offer a novel approach for antibiotic delivery by targeting drug to the site of infection. The in vitro release kinetics of gentamicin from 50/50 drug loaded PLGA and PCL microspheres prepared by double emulsion technique was determined in synovial fluid The studies showed that drug release from PCL microparticles was variable but significantly higher than that from PLGA. This may be due to faster degradation and higher drug permeability of PCL as compared to PLGA (Sondhof et al., 1998).

#### 5.1.5. Ketoprofen

Ketoprofen microspheres were prepared using PCL, PCL and hydroxypropylmethylcellulose phthalate (HPMCP50) blend and hydroxypropylmethylcellulose phthalate (HPMCP50) alone (Guzman et al., 1996). Mean particle size of the microspheres of PCL or HPMCP50 was found to be $10.7 + 2.0\,\mu m$ and $10.9 + 1.8\,\mu m$, respectively. Blend of PCL and HPMCP50 increased the particle size of the microspheres to $30\,\mu m$. In vitro drug release studies showed that release rate of ketoprofen from PCL microspheres was rapid probably due to porosity of PCL while the release rate of ketoprofen from HPMCP50 was found to be pH dependent.

Ketoprofen is eliminated rapidly from blood after dosing due to its low plasma elimination half-life (1–3 h). In order to achieve and maintain therapeutic plasma level it must be administered at least twice daily. To avoid this, microspheres of ketoprofen using PCL and cellulose acetate butyrate were prepared using spray-drying method. Morphology, particle size distribution and in vitro release profiles from the microspheres were affected by polymeric composition. Temperature of the spray drying process did not affect the above properties (Giunchedi et al., 1994).

#### 5.1.6. Colchicine

Injectable microspheres of colchicine (antiproliferative agent) were developed for maintaining high tissue level of drug at site of vascular injury by entrapping colchicine in PEG coated biodegradable microspheres composed of PLA/PCL blends. Microspheres colchicines individually with PLA and PCL were also prepared for comparative studies. In vitro studies were carried out to evaluate sustained subdermal delivery of the antiproliferative drug. These studies showed that colchicine release was affected by particle size, loading and PLA/PCL composition. The amount of drug release was much higher from PLA microspheres as compared to PCL microspheres. These PEG coated PLA/PCL microspheres may have potential for targeting antiproliferative agents for prolonged period depending upon the PLA/PCL composition (Das et al., 2000).

*5.1.7. Chlorpromazine*

Chlorpromazine microspheres were prepared using PCL and polylactide system. In vitro studies have shown that microspheres of chlorpromazine prepared by using combination of PCL and polylactide showed drug release in biphasic manner consisting of an initial fast release phase followed by slow release phase (Chang et al., 1986).

It was further found that the mixture of PCL and cellulose acetate butyrate could control the drug release characteristics and size of microspheres containing chlorpromazine, prepared by solvent evaporation method (Chang et al., 1987a). Microspheres could be easily prepared using a combination of PCL with CAB, because other polymers like polymethylmethacrylate, polycarbonate form sticky mass with PCL, which prevents the recovery of microspheres. In vitro studies have shown that the drug release pattern changes with a change in polymer ratios.

In another study chlorpromazine–HCL and progesterone were encapsulated in PCL microspheres prepared by an emulsion-solvent evaporation technique. The effect of PVA addition in the continuous phase on the dissolution properties of the microspheres was studied. Both types of microspheres showed faster dissolution rates than those of the pure drugs. The achievement of a molecular or colloidal dispersion of drugs in the polymer matrix and the high permeability of the polymer to both the drugs and the water are suggested as possible reasons for the fast drug release (Chang et al., 1987b).

*5.1.8. Cyclosporine*

PCL microspheres containing cyclosporine were prepared by the solvent evaporation method. It was found that the stirring speed and the organic phase volume were the only parameters significantly affecting the microspheres size. It was observed that microsphere size decreases either by increasing the internal phase volume or the stirring rate. Cyclosporin microspheres of size 2.5 μm resulted in a high entrapment percentage (98 ± 0.66%), with the drug dissolved or molecularly dispersed within the dense polymeric matrix of microspheres. After 12 months of storage at 8 °C or at room temperature, PCL microspheres remained physically stable, although crystallinity of the polymer increased by 35% upon storage at both the temperatures. Freeze drying studies showed that microspheres could be successfully lyophilized in the absence of cryoprotectants without significant changes in the drug entrapment. This formulation offered the possibility of cyclosporin administration through different routes (Aberturas et al., 2002).

*5.1.9. Cisplatin*

Local delivery of agents capable of modulating vascular responses, have the potential to prevent restenosis. An antiproliferative agent, cisplatin, was entrapped in a series of surface coated biodegradable microspheres composed of poly(lactic acid) poly(caprolactone) blends with a mean diameter of 2–10 pm. The microspheres were surface coated with polyethylene glycol (PEG), chitosan (chit), or alginate (alg). A solution of cisplatin and a 50:50 blend of PLA–PCL dissolved in acetone–dichloromethane mixture was poured into an aqueous solution of PEG (or polyvinyl alcohol or Chit or Alg) with stirring using a high speed homogenizer, for the formation of microspheres. Drug release from the microspheres was much higher initially (20–30%), which was followed by a constant slow release extending over a 30-day period. Drug release depended on the amount of drug entrapped, on the presence of extra cisplatin in the dispersing phase and the polymer coatings (Chandy et al., 2002).

*5.1.10. E- and P-selectin*

In a variety of disease settings the expression of the endothelial selectins E- and P appears to be increased. This feature makes these molecules attractive targets around which to design directed drug-delivery schemes. One possible approach for achieving such delivery is to use polymeric biodegradable microspheres bearing a humanized monoclonal antibody (MAb) for E- and P-selectin, MAb HuEP5C7.g2. Based on this Dickerson et al. (2001) prepared microspheres from the biodegradable polymer, PCL, using a single emulsion process and PVA as a stabilizer. On incubation of the PCL microspheres with HuEP5C7.g2 the adhesion of the resulting HuEP5C7.g2 microspheres to E- and P-selectin under in vitro flow conditions was investigated and it was seen that the HuEP5C7.g2 PCL microspheres exhibited specific adhesion to Chinese hamster ovary cells stably expressing P-selectin (CHO-P) and 4-h IL-1β-activated human umbilical vein endothelial

cells (HUVEC). Whereas, HuEP5C7.g2 PCL microspheres exhibited little adhesion to parental CHO cells or unactivated HUVEC. Based on this it was postulated that the limited attachment efficiency was due to a low level of HuEP5C7.g2 adsorbed to the PCL microspheres. Although the attachment was limited, a significant percentage of the HuEP5C7.g2 PCL microspheres were able to remain adherent at relatively high shear (8 dyn/cm$^2$) which led to the conclusion that HuEP5C7.g2 PCL microspheres exhibited selective limited adhesion to cellular substrate expressing E- and P-selectin.

*5.1.11. Ribozymes*

Ribozymes are catalytic RNA that bind and cleave specific regions of target RNA. However, ribozymes are rapidly cleared from plasma so effective treatment of proliferative diseases rely on the repeated administration of these agents to maintain therapeutic ribozyme concentrations. Jackson et al. (2002) encapsulated ribozymes in injectable polymeric paste and microsphere formulations to allow for the controlled release of these agents over extended period of time. Ribozymes was incorporated in the PCL pastes. The release rate of ribozymes from PCL pastes was effectively controlled by altering the loading concentration of ribozymes in the paste.

*5.1.12. Bovine serum albumin*

PCL microspheres containing bovine serum albumin (BSA) were investigated in order to understand the relationship amongst morphology, drug distribution and in vitro release profiles and to develop controlled release devices for marine fishes in tropical area. It was seen that the presence of PVA in the internal water phase enhanced the stabilization of inner water droplets against coalescence. This resulted in a more uniform drug distribution and a slower BSA release. A low oil-phase volume yielded microspheres with a porous matrix and defective skin surface, which as a result gave a high initial BSA burst as well as a fast release profile. Microspheres fabricated from a low polymer concentration had less defective skin surface, but with a less tortuous inner matrix which also resulted in a more rapid BSA release. A higher BSA loading gave a larger concentration gradient between the emulsion droplet and the continuous water phase as well as between the microspheres and the in vitro medium. The former resulted in a lower encapsulation efficiency, whereas the latter yield a faster initial burst and a more rapid release profile (Yang et al., 2001). In another study spherical microspheres consisting of polymer blends 80:20 PEAD/PCL II and 40:40:20 PEAD/P(HB-HV)/PCL II containing a range of BSA loadings were prepared using a single emulsion technique with solvent evaporation (Atkins, 1997). It was found that 80:20 PEAD/PCL II microspheres had smooth surfaces while 40:40:20 PEAD/P(HB-HV)/PCL II microspheres consisted of a mixture of smooth surfaced, microporous and macroporous microsphere fractions. Irrespective of fabrication polymer, microspheres were produced in high yield (>75%) and BSA incorporation had no significant effect on microsphere size distribution which ranged from 0.6 to 5 μm and from 2.1 to 50 μm for 80:20 PEAD/PCL II and 40:40:20 PEAD/P(HB-HV)/PCL II microspheres, respectively. The reason for low encapsulation efficiencies (<14.5%) was loss of BSA by partitioning into the aqueous phase. In another study Huatan et al. (1995) studied the effect of inclusion of poloxamer 181 a triblock copolymer of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) into matrix of entrapped BSA in a novel ternary blend, comprising of high and low molecular weight PCL and it was observed that it retarded the rate of crystallization of the PCL, thereby enhancing particulate sphericity and regularity. On studying the effect of variables such as protein to polymer ratio, internal phase volume and emulsifier concentration in both the internal and external aqueous phases, on the properties of the microspheres it was observed that a mean particle size ranging from 10 to 42 μm was achieved by altering the internal phase volume of the primary emulsion, whilst a high protein entrapment (11% w/w) was possible with a protein to polymer ratio of 1:4. Native-PAGE analysis of the entrapped protein indicated a maintenance of bulk structural integrity.

Lin and Yu (2001) designed a study based on a 2(3) factorial experiment to compare the BSA loaded poly(epsilon-caprolactone) microparticles prepared by the hot-melt technique. The effect of the particle size of protein, protein/polymer ratio, and hydrophilic PEG on the surface morphology, particle size, yield of PCL microparticles, encapsulation efficiency of BSA, and in vitro release properties were studied.

It was seen that none of three variables affected the yield of microparticles prepared from eight formulations. However, the particle size of BSA significantly affected the size and the burst release as well as the cumulative release of protein in these microparticles. The initial loading of BSA in terms of BSA/PCL ratio and the amount of PEG blended with PCL significantly affected all of the properties, except the yield.

Lin et al. (2001) designed another study based on 2(4) factorial experiment to investigate the characteristics of bovine serum albumin (BSA) loaded PCL microparticles. The influences of polyvinyl pyrrolidone (PVP) concentration, BSA/PCL ratio, w/o/o/o ratio, and PEG/PCL ratio on the surface morphology, particle size, as well as the yield of microparticles, encapsulation efficiency of BSA, and in vitro release properties were evaluated. Surface topography revealed highly porous morphology in all microspheres irrespective of the formulations. Results revealed that the volume ratio of the multi-phases significantly affected the encapsulation efficiency of BSA in PCL microparticles, and the initial amount of BSA encapsulated by PCL in terms of BSA/PCL ratio significantly affected the amount of BSA released at the end of 14 days.

The particle morphology and in vitro release of BSA from porous and non-porous PCL-F127 blended microparticles were evaluated (Lin and Huang, 2001a). The BSA loaded PCL microparticles were prepared by the w/o/o/o emulsion-solvent evaporation method using two types of homogenizer, a Polytron homogenizer and a probe ultrasonicator and the effects of solvent evaporation rate on the crystallinity and the performance of the microparticles were studied. The microparticles prepared with a Polytron homogenizer were quite porous in structure, which created channels for protein to continuously diffuse out, and resulted in sustained- and controlled-release characteristics. In addition, the initial burst release of protein from the microparticles was also reduced. An influence of evaporation rate on the size of resulting microparticles was observed whereas it did not change the crystallinity of the final microparticles. Based on above studies it was concluded that by carefully controlling these variables microparticles with desirable release performance can be fabricated.

Lin and Huang (2001b) also studied the influence of pluronics on BSA-loaded poly(epsilon-caprolactone) microparticles prepared by the w/o/o/o solvent evaporation technique with an ultrasonicator. Surface topography revealed spherical shape of microparticles with a rough surface due to crystallization of PCL in the microparticles. The pluronics efficiently prevented microparticles from aggregation, and the size of microparticles prepared was significantly reduced. It was seen that incorporation of pluronic F127 significantly increased the encapsulation efficiency and decreased the burst release of BSA from PCL microparticles along with increase in the hydrophilicity of the matrix, which further retained protein in blended microparticles.

#### *5.1.13. Ethyl salicylate*

A method suitable for transfer of poly(ε-caprolactone) microspheres (synthesized by pseudoanionic dispersion polymerization of ε-caprolactone in heptane-1,4-dioxane mixed solvent) from heptane to water was developed (Gadzinowski et al., 2000). The method consisted of treating the microspheres with KOH-ethanol in the presence of surfactants nonionic (Triton X-405), anionic (SDS), and zwitterionic (ammonium sulfobetaine-2 (ASB)). In the pH values ranging from 3 to 11, suspensions for poly(ε-caprolactone) microspheres in water were stable for all three surfactants. Surface charge density determined by electrophoretic mobility varied for poly(ε-caprolactone) microspheres from $2.6 \times 10^{-7}$ to $8.9 \times 10^{-7}$ mol/m$^{-2}$, for particles stabilized with Triton X-405 and ASB, respectively. Ethyl salicylate was loaded in poly(ε-caprolactone) microspheres transferred into water and loading up to 38% (w/w) was obtained.

#### *5.1.14. Nerve growth factor (NGF)*

Nerve growth factor (NGF) may enhance axonal regeneration following injury to the central nervous system (CNS), such as after spinal cord injury. So biodegradable polymeric microspheres were prepared from PLGA 50/50, PLGA 85/15, PCL and a blend of PCL/PLGA 50/50 (1:1, w/w), where the latter was used to further tailor the degradation rate. Protein loading in the microspheres was varied, with highest amount of protein being encapsulated in PCL and minimum in PLGA 50/50. A two-phase release profile was observed for all polymers where the first phase resulted from release of surface proteins and the second phase resulted predominantly from polymer degradation.

The amount and bioactivity of released NGF was followed over a 91-day period using a NGF-ELISA and PC12 cells, respectively. NGF was found to be bioactive for 91 days (Cao and Shoichet, 1999).

*5.1.15. Indomethacin*

Indomethacin–polycaprolactone microspheres were prepared by a melt-dispersion technique without the use of organic solvents. The microspheres were prepared by cutting the indomethacin–PCL films into small pieces and dispersing into hot water containing 0.25% w/v polyvinyl alcohol (at 80 °C). The temperature was above the melting temperature of PCL causing the polymer to melt which on homogenization resulted in formation of spherical particles. Solid, free flowing microspheres without drug crystals were obtained after cooling. Due to the absence of free drug crystals, the drug release from PCL microspheres prepared by the melt method was slower when compared to microspheres prepared by the solvent evaporation method. The problem of residual solvents could be avoided with this melt-dispersion technique (Bodmeier and Chen, 1989).

*5.1.16. Nitrofurantoin*

Nitrofurantoin, an antibacterial agent, was encapsulated in PCL microspheres using the solvent evaporation process. Rod like crystals have been observed on surface on increasing the drug content. Nitrofurantoin formed crystalline domains dispersed in the polymer matrix. Size distribution and the drug loading of the microparticles can modulate the in vitro release rates of nitrofurantoin from the microspheres and tabletting them produced a much slower release rate. It was observed that release profile obeyed the Higuchi equation (Dubernet et al., 1987).

*5.1.17. Insulin*

Poly-epsilon-caprolactone microsphere based parenteral depot system for insulin was developed to maintain constant plasma drug concentrations over prolonged period of time for the effective control of blood sugar levels (Shenoy et al., 2003). This study revealed that biodegradable depot-forming poly-epsilon-caprolactone microspheres controlled the release of drug and plasma sugar levels more efficiently than plain insulin injection upon subcutaneous injection. These formulations, with their reduced frequency of administration and better control over drug disposition, may provide an economic benefit to the user compared with products currently available for diabetes control.

*5.1.18. 3,4-diaminopyridine (3,4-DAP)*

Poly-epsilon-caprolactone microparticles containing 3,4-diaminopyridine (3,4-DAP) and Eudragit RS microspheres containing 3,4-DAP prepared by different methods for the treatment of multiple sclerosis and Lambert-Eaton myasthenia syndrome were compared (Gibaud et al., 2002). Microparticles prepared with poly-epsilon-caprolactone showed lower drug incorporation along with lesser particle size as compared to eudragit RS microspheres. Scanning electron microscopy (SEM) of poly-epsilon-caprolactone microparticles did not show any crystal but marks of dissolved crystals were observed on the polymeric surface whereas eudragit RS microspheres showed many crystals on the surface and inside the microparticles.

*5.1.19. Heparin*

Heparin loaded microparticles were prepared by a double emulsification and evaporation process with pure or blends of biodegradable (poly-epsilon-caprolactone and poly(D,L-lactic-co-glycolic acid)) and of positively-charged non-biodegradable (Eudragit RS and RL) polymers. The effect of polymers and some excipients (gelatin A and B, NaCl) on the particle size, the morphology, the heparin encapsulation as well as the in vitro drug release was investigated (Jiao et al., 2002). Results revealed that microparticles prepared with Eudragit RS and RL exhibited higher drug entrapment efficiency but lower drug release within 24 h as compared to those prepared with PCL and PLAGA. The use of blends of two polymers in the organic phase was found to modify the drug entrapment as well as the heparin release kinetics compared with microparticles prepared with a single polymer.

*5.1.20. Injectable bone substitute (IBS)*

Poly(epsilon-caprolactone) microparticles (80–200 μm) manufactured by a solvent evaporation–extraction process were introduced into the IBS in a 5–50% (V/V) range. Injectability was evaluated by texture analysis. Results revealed that with less than 45% of particles, the material had rheological properties similar to those of the reference IBS, whereas injectability

decreased markedly with more than 45% of particles. A preliminary in vitro release study showed that this type of triphasic IBS could be efficient for drug delivery systems with osteoconduction properties (Iooss et al., 2001).

### 5.2. Microspheres based on PCL co-polymers

The co-polymerization of PCL with PLA and PGA enhances the biodegradation of PCL. Many drugs have been encapsulated using PCL with other copolymers.

#### 5.2.1. Steroids

By oral administration, steroids undergo first pass metabolism. To avoid this, microspheres of steroids were prepared to ensure continuous delivery of drug and to enhance the bioavailability. Microspheres of β-estradiol and progesterone were prepared by solvent evaporation method using co-polymers of L,L-lactide or D,L-lactide and caprolactone (Pitt et al., 1981). The best uniform release rate of β-estradiol was obtained using PCL with the copolymer containing 83–93% of D,L-lactide (Buntner et al., 1996). The effect of copolymer chain microstructure on progesterone and estradiol release from microspheres was studied (Buntner et al., 1998) and it was seen that initial release rate of progesterone from microspheres containing copolymer of L,L-lactide was higher than that observed in case of microspheres containing D,L-lactide based copolymer. A nearly constant release rate was found when using the copolymer containing 93% of D,L-lactide. Also in the case of β-estradiol, a more uniform release rate was obtained when D,L-lactide was used with PCL. On carrying out the in vivo studies of estradiol in male wistar rats it was observed that during the first 70 days the changes in the release rate were small and the release of estradiol was nearly at a constant rate. In this period about 72% of estradiol contained in the microspheres was released The rate of estradiol release during the in vivo study was slower than the in vitro release and was uniform for longer period of time. Thus, the results demonstrated that copolymers of D,L-lactide and PCL are interesting material for controlled release of progesterone and estradiol and the microspheres containing 20% β-estradiol may be utilized in the prophylactics of a menopausal osteoporosis development.

#### 5.2.2. 5-FU

5-FU microspheres using triblock copolymer of PCL and ethylene oxide were prepared by hot melt technique (Martini et al., 2000). In vitro release rate studies have shown a non linear release kinetics of drug associated with a pronounced burst release. In another study 5-FU microspheres prepared from copolymer of ε-caprolactone and L-lactide showed a fast initial release followed by a slow release, which stops at a limiting value depending on the copolymer composition. These polymers can be used as delayed drug delivery systems (Guerra et al., 2001).

#### 5.2.3. Insulin

The poly(ε-block D,L-lactide) microparticles (MP) prepared by solvent evaporation method were evaluated as a drug carrier for insulin. The in vitro release behaviour of insulin from PCL-microparticles was rapid initially and then slowed down exponentially. In vivo studies were carried out in diabetic rat to evaluate the hypoglycemic effects after subcutaneous administration of the microparticles. The blood glucose level in rat serum after administration of insulin containing PCL microparticles was effectively lowered (Limin et al., 2000).

#### 5.2.4. Bioactive compounds (diclofenac, nicardipine, dicumarol)

Microspheres using PCL and poly(ether ester amide) (PEEA) were prepared by emulsion solvent evaporation technique using diclofenac, nicardipine and dicumarol as model drugs. Comparative in vitro drug release studies showed that the release of diclofenac from all the prepared microspheres was very rapid (100% within 2 h) whereas nicardipine release was slower and biphasic. In case of nicardipine, the release was characterized by an initial rapid release in the first 8 h approximating a zero order release, followed by a slower phase lasting about 30 days. Release from PEEA microspheres was higher than from PCL microspheres. This may be due to low crystallinity of PEEA as compared to PCL, which further led to a facilitated access of water molecule through the polymeric matrix. The release of dicumarol from PEEA microspheres was very slow in comparison to PCL (Barbato et al., 2001).

*5.2.5. L-Methadone*

Microspheres containing 13–16% L-methadone were prepared from PCL–LA (lactic acid) using the solvent evaporation method. The rate of release from PCL-LA microspheres into an unbuffered aqueous reservoir was zero order and much slower as compared to when a pH 7.4 buffered reservoir was used. It was found that the release of L-methadone from microspheres of PCL–LA (75–85 mol% L-lactic acid) was complete within 48 h. It was shown that Fickian diffusion was responsible for the observed kinetics. The permeability and drug release from the microspheres can be controlled by both blending and changing the copolymer composition (Cha and Pitt, 1988).

*5.2.6. FITC-dextran*

Release rate of FITC-dextran from a novel biodegradable polymer, 2,2-bis(2-oxazoline) linked poly (epsilon-caprolactone) and poly(epsilon-caprolactone) alone was compared (Tarvainen et al., 2002). Results revealed that FITC-dextran release was notably faster from PCL-O microparticles than from those made of PCL. FITC-dextran release was a combination of diffusion and polymer degradation and thus, the faster degradation of PCL-O enhanced the release of FITC-dextran. Based on the above results it was concluded that the effects of the oxamide groups on drug release profiles were dependent on the drug release mechanisms.

The effect of pancreatin on FITC-dextran release from PCL and PCL-O microparticles, prepared by w/o/w double emulsion technique, was also studied (Tarvainen et al., 2003). It was seen that pancreatin increased FITC-dextran release from both PCL and PCL-O microparticles considerably. Results revealed that pancreatin present in intestinal fluid may substantially affect drug release from PCL based preparations.

## 6. PCL nanospheres

Nanospheres are colloidal drug delivery systems, which act as transport carrier compartments for drugs or other active molecules, with size ranging between 10 and 1000 nm. Drug particles may be encapsulated, dispersed or absorbed in the nanospheres. They are also called nanoparticles or nanocapsules depending upon whether the drug is in a polymeric matrix or encapsulated in the shell.

Nanospheres can be used for selective targeting via reticuloendothelial system to liver and to cells that are active phagocytically (Speiser, 1979). The size of nanospheres allows them to be administered intravenously via injection unlike many other colloidal systems which occlude both needles and capillaries, used as diagnostic agents. Injectable nanoparticulate carrier, have good applicability for specific drug delivery and medical imaging. But they cannot generally be used due to their elimination with in seconds after intravenous injection by the reticuloendothelial system. To overcome this limitation, monodisperse biodegradable nanospheres were developed from amphiphilic copolymers. These nanospheres exhibited increased blood circulation time and reduced drug accumulation in liver of mice (Gref et al., 1994). The efficacy of these colloidal particles as drug carriers is closely related to their interaction with proteins and enzymes in different body fluids. The interaction phenomenon between lysozyme, a positively charged enzyme that is highly concentrated in mucosa and two different drug carriers: nanocapsules made of an oily core coated by the polymer PCL and nanoparticles made solely of PCL was analyzed. Results showed that the interaction of lysozyme with these colloidal drug carriers is highly affected by their surface charge (Calvo et al., 1997). The influence of the PEG corona thickness and density, as well as the influence of the nature of the core (PLA, PLGA or PCL), on the competitive plasma protein adsorption, zeta potential and the particle uptake by polymorphonuclear (PMN) cells was studied (Gref et al., 2000). The conditions to stabilize PLGA and the PCL nanoparticles by freeze drying with several cryoprotective agents were identified (Saez et al., 2000). Studies indicated the necessity of adding sucrose, glucose, trehalose or gelatin to preserve the properties of nanoparticles regardless of the freezing procedure. Freeze drying of itraconazole-loaded nanosphere suspensions was also studied (de Chasteigner et al., 1996).

### 6.1. Techniques of nanosphere preparation

Different methods have been reported in literature for the preparation of drug entrapped nanoparticles including, emulsion polymerization method in continuous aqueous phase (Kreuter, 1991), emulsion

polymerization method in continuous organic phase (Birrenbach and Speiser, 1976), interfacial polymerization (Rollot et al., 1986), interfacial disposition (Fessi et al., 1989; Ferranti et al., 1999), solvent evaporation, desolvation of macromolecules (Marty et al., 1978), dialysis method, etc. (Kim et al., 2000). The general methods of preparation of PCL nanospheres are discussed below:

*6.1.1. Interfacial polymer disposition method*

Interfacial polymer disposition is a procedure of preparing nanospheres of biodegradable polymer following displacement of a semi-polar solvent, miscible with water from the lipophilic solution. In this method, first the polymer is dissolved in an organic solvent, usually acetone. Similarly the mixture of phospholipid is prepared in acetone by increasing temperature to near the boiling point. Then drug dissolved in benzyl benzoate is added to acetonic solution. The resulting organic solution is poured under stirring to water containing poloxamer. Immediately turning of aqueous phase into milky solution gives indication of nanocapsules formation. Acetone is removed under reduced pressure. The colloidal suspension thus formed is concentrated to desired volume by removal of water (Fessi et al., 1989). Spray-dried polymeric nanocapsules (NC) and nanospheres (NS) were prepared from poly(epsilon-caprolactone) suspensions containing diclofenac (DIC) using interfacial deposition of the polymer (Muller et al., 2001).

*6.1.2. Dialysis method*

Indomethacin loaded nanospheres of PCL were prepared by dialysis method (Kim et al., 2000). The polymer was dissolved in organic solvent (dimethylformamide). Drug was added to the above solution under stirring at room temperature. After removing the organic solvent dialysis was done for 24 h using cellulose membrane bag. The miceller solution was collected from the bag, sonicated and centrifuged to remove aggregated particles and unloaded drug. Lyophilization was done for two days to obtain nanospheres.

*6.1.3. Emulsion polymerization method*

The earliest nanoparticles prepared by the polymerization of a monomer were those proposed by Birrenbach and Speiser in the 1970s. In emulsion polymerization method droplets of water insoluble monomers are emulsified in an external aqueous and acidic phase that contains a stabilizer (Couvreur et al., 1982). The monomers polymerize relatively fast by an anionic polymerization mechanism, the polymerization rate being dependent on the pH of the medium (Donnelly et al., 1977). Thus, if the pH is neutral, the monomer polymerizes extremely fast, leading to the formation of aggregates. However, at acidic pH, between pH 2 and 4, the reaction is slowed, yielding nanospheres with a narrow-size distribution (frequently 200 nm). The system is maintained under magnetic agitation while the polymerization reaction takes place. Finally the colloidal suspension is neutralized and lyophilized following the incorporation of glucose as a cryoprotectant. Water soluble drugs may be associated with nanospheres either by dissolving the drug in the aqueous polymerization medium or by incubating blank nanospheres in an aqueous solution of the drug. High speed mixing or sonication is very critical step in emulsification of drug or monomer solution into external phase as it determines the size distribution of the nanoparticles. In order to achieve narrow particle size distribution ultrasonication or high speed homogenization is required. So these parameters are to be carefully monitored.

## 7. PCL nanospheres as delivery system

PCL nanospheres of numerous drugs have been investigated by various researchers. A brief account of these studies are being discussed below:

*7.1. Nanospheres based on PCL or PCL blends*

Nanospheres can be prepared either by PCL alone, or by using copolymers with PCL or PCL blends. Le Roy Boehm et al. (2000) investigated the ability of nanospheres (NS) to improve the biodelivery of new active ingredients (AI) to plants. Their aim was to obtain stable poly(epsilon-caprolactone) NS (PeC-NS) with the smallest size and the largest amount of encapsulated AI, using a nanoprecipitation method and they found that the highest encapsulation was obtained with Montanox 80 as surfactant.

A glimpse of studies with other drugs is given below:

*7.1.1. Indomethacin*

Calvo et al. (1996a) investigated the ability of different drug carriers to improve the ocular bioavailability of drugs in the rabbit eye. They prepared suspensions of submicron systems (nanoparticles, nanocapsules and microparticles) made of PCL and a submicron emulsion. Results indicated that the three submicron systems, i.e. nanoparticles, nanocapsules and emulsion, increased the indomethacin concentration by more than three-fold in the cornea, aqueous humour and iris-ciliary body at 0.5 and 1 h post-instillation. Furthermore, the ocular bioavailability of indomethacin was 300% after instillation of the submicron systems in comparison with the value obtained for a commercial solution. In contrast, the microparticles hardly increased the ocular bioavailability of indomethacin. On the other hand, the favourable ocular penetration of indomethacin when encapsulated in the colloidal carriers, rather than in the microparticles, led to the assumption that the colloidal nature of these carriers was the main factor responsible for the increased ocular bioavailability. PCL nanoparticles and nanocapsules as well as submicron emulsions were shown to be novel corneal drug carriers, thus representing a useful approach for increasing the ocular bioavailability of drugs. In another study by Calvo et al. (1996b) the comparative in vitro evaluation of these three different colloidal carriers, namely, nanoparticles and nanocapsules, was designed and their capacity for increasing the corneal penetration of drugs was investigated. The three systems differed in their inner structure and composition, but they had a similar size (200–250 nm) and a negative superficial charge (−16 to −42 mV). Indomethacin, which was used as a model drug, was dispersed at a molecular level within the colloidal systems, no chemical interaction between the polymer and the drug being detected. Release of the encapsulated indomethacin occurred very rapidly upon high dilution in a buffered medium and was independent of the composition of the system. The in vitro corneal penetration of the encapsulated indomethacin was more than three-fold than that of the commercial eye drops. This increased penetration was similar for the three formulations investigated, which therefore excluded the influence of the inner structure or chemical composition of the colloidal systems on the corneal penetration of indomethacin. Thus, it was stated that the main factor responsible for the favorable corneal transport of indomethacin was the colloidal nature of these carriers rather than their inner structure or composition.

*7.1.2. Cartelol*

Nanoparticles and nanocapsules of cartelol were prepared using PCL to increase the ocular absorption of drug. In vivo studies were performed on rabbits. Chronological variations in intraocular pressure were measured. The results were compared with aqueous cartelol eye drops. It was found that cartelol nanocapsules and nanoparticles produced more pronounced effects on intraocular pressure than plain cartelol eye drops. Nanocapsules were found to be better than nanoparticle due to entrapment of drug in oily core of the carrier. Thus, colloidal suspension prepared using PCL seem to be good carriers for ocular drug delivery (Marchal-Heussler et al., 1993).

*7.1.3. Flurbiprofen (FB)*

Lacoulonche et al. (1999) prepared PCL nanospheres loaded with flurbiprofen and evaluated them for stability, physicochemical characteristics of drug delivery system and drug release behavior. Results showed that stability of polymeric system was affected by temperature and initial pH value while drug release increased rapidly on dilution. In another study by Gamisans et al. (1999) flurbiprofen loaded-poly-epsilon-caprolactone nanospheres were prepared by solvent displacement method. Characterization by thermal methods, infrared spectroscopy and X-ray diffraction analysis revealed the dispersion state of the drug inside the nanospheres. This information predicted the stability of the particles and the drug release behaviour. The study had indicated the presence of a molecular dispersed system within the nanospheres. In another study, PCL nanospheres for ocular delivery were prepared to improve and prolong the corneal penetration of drugs specifically taken up by corneal epithelial cells without damaging the cell membrane (Gamisans et al., 2000). The main purpose of the investigation was to evaluate flurbiprofen (FB) release from PCL nanospheres. Studies on release pattern of FB from PCL nanospheres showed a characteristic biphasic pattern with an initial fast release phase much more marked for free drug, followed by a second much slower release phase. The fast initial release could be due to drug diffusion from near the

surface and second slow phase could be produced by diffusion of drug from the interior of nanospheres. Studies showed that the release rate of FB from the polymeric system was greater than that of free drug.

*7.1.4. Primidone*

Primidone loaded PCL nanocapsules were prepared by interfacial deposition technique. These were evaluated for the release pattern of the drug and encapsulation efficiency. The release profile of drug obtained from nanocapsules was compared with that from an oily control solution. It was observed that 100% of drug was released within 8 h from an oily control solution whereas the release from nanocapsules was found to be slower. On the other hand, encapsulation efficiency of nanocapsules was obtained upto 74% (Ferranti et al., 1999).

*7.1.5. Aceclofenac*

Aceclofenac nanocapsules were prepared by interfacial precipitation of PCL at o/w interface. The effect of various factors such as pH, entrapment efficiency, p$K_a$ of drug and polymer concentration on physicochemical properties of nanocapsules was studied. The studies showed that entrapment in PCL nanocapsules improved the bioavailability of drug from the ocular delivery system and strengthened the application of PCL nanospheres for the ophthalmic applications (Alonso et al., 2000).

*7.1.6. Diclofenac*

Spray-dried polymeric nanocapsules (NC) and nanospheres (NS) were prepared from poly(ε-caprolactone) suspensions containing diclofenac (DIC) using interfacial deposition of the polymer. Spray-dried powders were prepared by addition of 3% (w/v) Aerosil 200 into suspensions of NC or NS. These mixtures were fed into a spray-dryer. NC and NS suspensions had acceptable diameter, 340 and 247 nm, respectively. The yields of NC and NS spray-dried powders were 80 and 75% and the recovery of the DIC from the NC and NS was 99 and 93%, respectively. For spray-dried NC formulations, the SEM analyses of powders showed spherical microparticles of silicon dioxide, covered by nanoparticles (300 nm), while for spray-dried NS formulations the microparticles presented a rugged surface at the same magnification (Muller et al., 2001). Guterres et al. (2001) studied the effect of spray dried diclofenac loaded nanocapsules and nanospheres on gastrointestinal system following their oral administration.

*7.1.7. Amphotericin B*

Polycaprolactone nanospheres of Amphotericin B were prepared by solvent displacement process. When Amphotericin B and PCL were dissolved in a solvent mixture consisting of acetone and a cosolvent, a reproducible and monodisperse size distribution centred on 220 nm was obtained. It was seen that nanoparticles modified the aggregation state of Amphotericin B due to weak interaction between the drug and the polymer. FTIR showed the absence of drug incorporation into the core of these carriers and also that no chemical interaction between the drug and the polymer had occurred (Espuelas et al., 1997).

Espuelas et al. (1998) studied the effect of poloxamer on the adsorption of amphotericin B onto polycaprolactone nanospheres. In another study by Espuelas et al. (2002), in vitro antileishmanial activity of amphotericin B loaded in poly(epsilon-caprolactone) nanospheres coated with variable amounts of a non ionic surfactant poloxamer 188 was studied against AmB-susceptible (WT) and AmB-resistant (AmB(r)) strains of Leishmania donovani amastigotes in thioglycolate-elicited peritoneal macrophages. Based on the study which revealed that the activity was not influenced by the concentration of poloxamer 188, it was suggested that association of AmB with nanospheres may improve the capability of the drug to interact with ergosterol.

*7.1.8. Cyclosporine*

Marketed formulations of cyclosporine (cyclosporin A, CyA) show acute hemodynamic changes that result in high nephrotoxicity so encapsulation of these in nanoparticles (NPs) was investigated. It was proposed that use of nanoparticles as potential drug carriers would avoid the therapeutic risks of conventional formulations. Two different mechanisms for obtaining polymeric NPs loaded with CyA were studied with regard to their preparation and physicochemical characterization. Isobutyl-2-cyanoacrylate monomer (IBCA) was polymerized, whereas poly caprolactone (PCL, a preformed polymer) was precipitated; both reactions took place in an aqueous medium containing Pluronic F-68 (polyoxypropylene polyoxyethy-

lene block copolymer) as a surface active agent. The encapsulation efficiencies were 78.49 ± 5.87% and 84.85 ± 5.02%, respectively, and they remained stable over a wide range of drug concentrations. The polymeric NP had average sizes of 81 ± 25 nm and 95 ± 25 nm for poly-IBCA and PCL, respectively. In vitro activity of the drug and the carrier was tested by inhibition of lymphocyte proliferation induced by Concanavalin A. Drug-loaded PCL NPs and free CyA inhibited lymphocyte proliferation by 91.40 and 86.19%, respectively. However, drug-free NPs also exhibited statistically significant ($P < 0.05$) immunosuppressive activity (Guzman et al., 1993). Nanocapsules consisting of an oily core (Migliol 840) (MG) surrounded by a poly-epsilon-caprolactone (PECL) coat were prepared by solvent displacement technique and were evaluated as potential vehicles for the topical ocular administration of cyclosporin A (CyA). Results indicated that nanocapsules had a mean size in the range of 210–270 nm, a negative zeta potential (between −55 and −60 mV) and a maximum loading capacity of 50% (CyA/PECL ratio). These highly loaded nanocapsules displayed a thick spongeous polymer coating around the oily nanodroplets. The corneal levels of CyA were up to five times higher for the encapsulated CyA than for the oily solution of CyA. In addition, drug levels obtained from nanoparticles remained significantly higher than those obtained from the control group (oily solution) for a period of 3 days. Based on this it was concluded that the CyA-loaded nanocapsules are interesting vehicles for the improvement of the ocular penetration of CyA (Calvo et al., 1996c).

Molpeceres et al. (1999) described the application of the limited sampling strategies to demonstrate the advantages of using CyA incorporated in polymeric nanoparticles (CyA-NP) as compared to two reference sandimmune formulations which consisted of an emulsion of an oily solution in milk (SIM-EM) and a microemulsion (SIM-Neoral). The use of these limited sampling models manifested the coincidence between CyA-NP and SIM-Neoral as well as the advantages of both formulations over SIM-EM with regard to CyA monitoring. In another study Molpeceres et al. (2000) incorporated Cyclosporine (CyA) into polycaprolactone nanoparticles (PCL-NP) in order to increase its oral bioavailability and to control drug distribution, thereby potentially reducing its toxicity. The nephrotoxicity and immunosuppressive ability of cyclosporine (CyA) incorporated into polycaprolactone nanoparticles (CyA-NP) was assessed in vitro and in vivo and compared to the effects caused by free drug (Sandimmun) (Varela et al., 2001). Based on the data it was concluded that NP improved the oral bioavailability of CyA and its uptake by lymphocytes. However, specific immunosuppression and adverse effects were not simultaneously increased.

#### *7.1.9. Digitoxin*

Uptake of free digitoxin and digitoxin associated with PCL nanoparticles by rat glomerular mesangial cells was investigated (Guzman et al., 2000). No significant differences of drug uptake were observed between free DGT or DGT-NP at 30 min; however, the uptake of DGT, when it was associated to the PCL nanoparticles, increased by approximately two-fold at 60 min, whereas no significant changes were observed for free drug. The pharmacologic activity of the drug as evaluated by measuring the planar cell surface area (PCSA) revealed that free drug, or DGT-NP did not produce significant variations on the PCSA as compared with control cells after a 30-min incubation. Nonetheless, DGT-NP reduced the PCSA to 82.51 ± 8.42% of control values after 60 min of incubation.

#### *7.1.10. Atovaquone*

Atovaquone-loaded nanocapsules were prepared by interfacial deposition technique and the effect of using different polymers like poly-epsilon-caprolactone (PECL), poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLAGA) was evaluated on in vitro characteristics (Cauchetier et al., 2003). Results showed no release of atovaquone from PECL nanoparticles over 4 months as compared to 25.9% with PLA nanoparticles at four months and 18.9% with PLAGA nanoparticles from the third month.

#### *7.1.11. Tamoxifen*

Biodegradable poly(epsilon-caprolactone) nanoparticles for tumor-targeted delivery of tamoxifen were developed (Chawla and Amiji, 2002). Results revealed that a large fraction of the administered nanoparticle dose was taken up by MCF-7 cells through non-specific endocytosis. The nanoparticles were found in the perinuclear region after 1 h. Above study led to the conclusion that nanoparticle formulations

of selective ER modulators, like tamoxifen, can provide increased therapeutic benefit by delivering the drug in the vicinity of the ER.

*7.1.12. Metipranolol*

Losa et al. (1992) developed a formulation consisting of a colloidal suspension of polyepsiloncaprolactone nanocapsules with an oily core (Migliol 840) in which metipranolol was dissolved. On administering this formulation to rabbits, a reduction of intraocular pressure similar to that seen with commercial eye drops was observed. But, the evaluation of the cardiovascular side effects clearly showed lower conjunctival absorption of the encapsulated drug compared with the commercial drops. The direct (bradycardia) and the indirect evaluation showed that blockage of beta-adrenoreceptors was reduced greatly by the topical administration of the new formulation. Several formulations of metipranolol loaded polyisobutylcyanoacrylate and polyepsiloncaprolactone nanocapsules were developed to investigate the potential of polymeric nanocapsules for ocular delivery (Losa et al., 1993). Formulations differed in their polymer forming the coating and in the type and volume of the oil encapsulated. Analysis of particle-size distribution, electrophoretic mobility, and loading efficiency of the nanocapsules revealed that the type of oil is the most important factor influencing these properties. Results showed that the release profiles were not affected by the polymeric coating, which suggested that drug release from these systems is governed mainly by the partition of the drug between the oily core and the aqueous release medium. Though the polymer coat was not able to control the release of the drug, but it stabilizes the emulsion. Finally, the suitability of these formulations for ophthalmic administration was investigated. A drastic decrease in the drug's systemic side effects was observed although the pharmacologic response was not affected by the encapsulated metipranolol compared with the commercial eye drops.

*7.1.13. Isradipine*

Isradipine, a antihypertensive agent, was encapsulated by the nanoprecipitation method using various polymers like poly(epsilon-caprolactone), poly(D,L-lactide) and poly(D,L-lactide-co-glycolide). PCL nanoparticles were having size larger than nanoparticles prepared with the other polymers. PCL nanoparticles were in the semi-crystalline state as compared to PLA and PLAGA nanoparticles. Results revealed that these nanospheres can act as good candidate delivery system for oral administration, to reduce the initial hypotensive peak and to prolong the antihypertensive effect of the drug (Leroueil-Le Verger et al., 1998).

*7.1.14. Bovine serum albumin (BSA)*

Lamprecht et al. (2000) studied the influences of process parameters on nanoparticle preparation of BSA prepared by a double emulsion pressure homogenization technique. Two biodegradable polymers, poly(D,L-lactic-co-glycolic acid) 50/50 (PLGA) and PCL were used in experiments. Results revealed that on increasing the protein concentration in the inner aqueous phase BSA encapsulation efficiency decreased while the particle size was not influenced significantly. All release profiles were characterized by a initial burst effect, and a higher release rate with PLGA NP compared with PCL NP after 4 weeks.

*7.2. Nanospheres based on PCL co-polymers*

*7.2.1. Indomethacin*

Indomethacin (IMC) nanospheres were prepared by dialysis method using block copolymer Pluronic/PCL (PEO–PPO–PEO block copolymers/PCL). Drug release behaviour of nanospheres and their loading efficiency were studied. In vitro studies showed that the unloaded free indomethacin showed faster dissolution as compared to loaded IMC. The release behaviour of indomethacin from nanospheres was temperature dependent. They demonstrated that cytotoxicity of indomethacin-loaded Pluronic/PCL nanospheres was reduced when compared with the unloaded free indomethacin. Therefore these block copolymeric nanospheres could be useful as a drug carrier for targeted drug delivery (Kim et al., 2000).

Drug-loaded polymeric nanospheres consisting of the methoxy poly(ethylene glycol) (MePEG) and PCL which showed a narrow size distribution and average diameter of less than 200 nm were prepared (Kim et al., 2001). Nanospheres having a relatively high drug-loading efficiency of about 42% were obtained when the feed weight ratio of indomethacin (IMC) to polymer was kept 1:1. IMC pharmacokinetics in the IMC-loaded MePEG/PCL nanospheres (DMEP70)

using the rats as animal model was studied and IMC concentration in plasma was analysed with HPLC after i.v. bolus administered at a dose of 10 mg/kg in free IMC (control) and IMC-loaded MePEG/PCL nanosphere (DMEP70) groups via tail vein. Pharmacokinetics parameters (mean ± S.D.) such as the mean residence time (MRT, h), the steady-state volume of distribution ($V_{dss}$, l), the terminal half-time ($t_{1/2}$, h) and the plasma clearance (CL, l/h) of IMC in each groups (control versus DMEP70) were also determined. From these results, it was concluded that MEP70 has a significant potential for sustained release and the enhancement of circulation time of loaded drug by prolonging terminal half-life, increasing MRT and $V_{dss}$ of IMC. Therefore, the MePEG/PCL block copolymeric nanosphere system were being considered as promising biodegradable and biocompatible drug carrier vehicles for parenteral use and may be useful as sustained release injectable delivery systems for hydrophobic drugs.

#### *7.2.2. Taxol*

Methoxy poly(ethylene glycol) (MePEG) and PCL amphiphilic block copolymeric nanospheres of taxol were prepared as novel anticancer drug carriers. MePEG and PCL nanospheres prepared by dialysis procedure had core shell structure consisting of hydrophilic outer shell consisting of MePEG and hydrophobic inner core of PCL. Thus, high drug loading efficiency and suspending properties of nanospheres in water seemed to be useful for delivery of taxol. (Kim and Lee, 2001).

#### *7.2.3. Bovine serum albumin (BSA)*

BSA-loaded nanocapsules were prepared by means of a modified w/o/w double emulsion technology (Lu et al., 1999). A mixture of glycerin and water was used instead of the traditional stabilizer system in the preparation of poly(L-lactide) (PLLA) nanocapsules. Formation of the nanocapsules was assisted by the high viscosity of the mixture and the hydroxyl group of the glycerin. On comparing different polymers of poly(L-lactide) and polycaprolactone–poly(ethylene oxide) block copolymer (PCE), it was found that the entrapment efficiency of the BSA was strongly dependent on the hydrophilicity of the polymer. Results revealed that by using relatively hydrophilic PCE polymer as the entrapping material a lower entrapment efficiency of BSA along with smaller size of nanocapsules was obtained.

#### *7.2.4. Clonazepam*

Ryu et al. (2000) investigated the release of Clonazepam from core-shell type nanoparticles of poly (epsilon-caprolactone)/poly(ethylene glycol)/poly(epsilon-caprolactone) triblock copolymers. Core-shell type nanoparticles of poly(epsilon-caprolactone)/poly (ethylene glycol)/poly(epsilon-caprolactone) (CEC) block copolymer were prepared by a dialysis technique. Results revealed that clonazepam (CNZ) release kinetics were dominantly governed by diffusion mechanism.

## 8. Biocompatibility

Toxicity is a factor that must be considered before selection of material to be used in pharmaceutical formulations. Determination of degradation rate of polymer and local tissue clearance is important to predict the concentration present in the tissue and the resultant response. The inflammatory response of copolymer PCL and PLA after implantation in male wistar rats was studied (Pitt, 1990). These studies have shown that inflammation was moderate after 2 weeks of implantation and was greatest around the implanted area. The implantation of polymer devices that release neuroactive drugs in controlled manner is gaining interest. The tissue reaction of the implantable microspheres of PCL prepared by solvent evaporation method was studied by implanting them in brain of wistar rat (Menci et al., 1994). Results have shown that no necrosis was observed which accounts for biocompatibility of microspheres with the brain tissue. Inflammatory reactions in bones were less pronounced than in muscles. A pronounced inflammatory reaction in muscle might be due to a better vascularization of muscle tissue and a greater amount of implanted material. Inflammation may be due to high local concentration of degradation product and the transport potential of polymer (Nakamura et al., 1992; Ekholm et al., 1999). The injection of microspheres in body results in activation of neutrophiles and causes localized inflammation. The rapid activation of neutrophils by PCL microspheres was confirmed by measurement of superoxide anion gen-

eration. Activation of neutrophiles was measured by chemiluminescence. Neutrophiles activation released chemotactic factors leading to influx of massive number of neutrophiles into the affected site and causing inflammation. Phagocytosis of drug loaded polymeric microspheres by WBCs shows the main clearance mechanism by which foreign material eliminate from the body (Jackson et al., 2000; Tang et al., 1993). To prevent the phagocytosis of microspheres modification of microspheres surface can be done by steric stabilization (Jackson et al., 2000). Flow cytometry was used to study the effect of poly(epsilon-caprolactone) microspheres on apoptosis and cell cycle of fibroblast. Results revealed that poly(epsilon-caprolactone) microspheres purified in different ways showed different cytocompatibility with well-purified microspheres having good cytocompatibility (Luo et al., 2003).

## 9. Conclusion

PCL is biodegradable, biocompatible and water insoluble polymer suitable for controlled drug delivery due to a high permeability to many drugs and at the same time being free from toxicity. It has the ability to form compatible blends with other polymers. Biodegradation of PCL is very slow in comparison to other polymers, so it is most suitable for long-term delivery extending over a period of more than one year. Microspheres of certain drugs have exhibited a faster dissolution rate than pure drug due to an increase in surface porosity of microspheres. This property of polymer matrix requires further investigation. Release rate of drug from PCL depends on type of formulation, method of film preparation, PCL content, size and percent of drug loaded in the microcapsules. Due to a higher permeability of PCL it is blended with other polymers to improve stress, crack resistance, dyeability and control over release rate of drugs. Within the last decades, PCL polymers have been major area of concern to develop controlled delivery systems especially for peptides and proteins. Despite considerable research efforts and impressive progress made in recent years, the question of feasibility of injectable PCL microspheres as protein/peptide or vaccine delivery system remains open to debate. Microencapsulation techniques have been developed to allow incorporation of sensitive proteins into PCL polymers under mild conditions. The future of success of PCL microsphere or nanosphere formulation will primarily depend on commitment of pharmaceutical and biotechnology industries to development of this technology.

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