REVIEW

FABRICATION OF NANOPARTICLES WITHIN POLYMERIC PORES FOR CONTROLLED RELEASE OF DRUG

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ABSTRACT

Nanotechnology, a multidisciplinary scientific undertaking, involves creation and utilization of materials, devices or systems on the nanometer scale. The field has enabled the development of an amazing variety of methods for fabricating nanoparticles in recent years. The technology is expected to create innovations and play a critical role in the field of biopharmaceuticals especially in controlled release of drug delivery. Sol-gel technique is one of the most widely used techniques to fabricate porous nanoparticles within the polymer. Such nanoparticles have also applications in vascular drug delivery and release, site-specific targeting (passive as well as active targeting), as well as transfusion medicine.

This article concentrates mainly on fabrication of porous nanoparticles, its characterisation and its use for controlled release of drug. It also encompasses the strategies that have been used to translate and fabricate a wide range of particulate carriers e.g., nanospheres, liposomes, micelles, oil-in-water emulsions, with prolonged circulation and/or target specificity. With regard to the targeting issues, attention is particularly focused on the importance of physiological barriers. we have also critically reviewed and assessed the fate and activity of biodegradable/bioerodable polymeric drug delivery vehicles because the uniformity in degradation of these polymers is questionable.

Keywords: Nanotechnology, controlled-release, nanofabrication, biopharmaceuticals, porous nanoparticles

INTRODUCTION

Technology is generally regarded as the utilisation or application of science to benefit society. Over the past few years a substantial amount of work has been done in the field of nanotechnology, and nanotechnology has been developed to such an extent that it becomes possible to fabricate, characterise and specially tailor the functional properties of nanoparticles for biomedical applications and diagnosis (John *et al*., 2002; Valter and Cinzia, 2004; Jae-Hyung and Lonnie, 2003; Meng Shi *et al*., 2003; Mu and Feng, 2001; Christopher and Maria, 2001; Ajay Kumar and Mona Gupta 2005). The field of nanotechnology seeks to exploit distinct technological advantages of reduced dimensional scales to sizes approaching individual molecules and their organised aggregates. The reason why nanophase and nanostructures are attracting so much attention is because of their potential applications in different areas of science especially in the field of biomedicine (Meng Shi *et al*., 2003; Zafar and Prasanna, 2004; Alexis and Santosh Kumar, 2004; Harjit and Johansen, 2005; Robert, 2005; Ajay Kumar and Mona Gupta, 2005; Serguei *et al*., 2002; Gorka *et al*., 2004). As we reduce the size of the particle the properties and behaviour of molecules shows deviation from bulk characteristics. Surface properties of nanoparticles are the key factors, which determine the *in vivo* fate of such a carrier. Nanoparticles are widely used in several areas of drug delivery and cosmetics (Zafar and Prasanna, 2004; Alexis and Santosh, 2004; Adam and Chris, 2001; Manja *et al*., 2000; Manja *et al*., 2001; Bhattacharjee *et al*., 2002). These particles are usually smaller than 100 nm, they are formed by nanocrystals or by drug-polymer complexes or by creating nanoscale shells that entrap drug molecules (Omathanu and Ramesh, 2001; Marie-Christine and Jean-Christophe, 1999; Breitenbach, 2002; Zafar and Prasanna, 2004; Manja *et al*., 2000; Pirjo and Manja, 2000).

Nanoparticles have unusual properties that can be exploited to improve drug delivery, because of their fine size, they are often taken up by cells where larger particles will be excluded or cleared from body. Recent strategies include the use of polymers to increase circulation time as well as the use of polymers in competition with binding groups to reduce non-specific attachments or uptakes (Sarah and Tejal, 2003).

Nanoparticles

Nanoparticles are submicron-sized polymeric colloidal particles with a therapeutic target of interest, encapsulated within their polymeric matrix or absorbed or conjugated onto the surface (Jayanth and Vinod, 2003; Gorka *et al*.,

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2004; Omathanu and Ramesh, 2001; Breitenbach, 2002; Manja *et al*., 2000).

As nanoparticles are intermediates between the molecular and solid states, they combine chemical accessibilities in solution with physical properties of bulk phase. They are thus ideal elements for the construction of nanostructured materials and devices with adjustable physical and chemical properties. Nano sized particles have physical and chemical properties of neither the atoms nor the bulk counterpart. Based on their unique mesoscopic physical, chemical, thermal and mechanical properties nanoparticles offer a high potential for several biomedical applications (Valter and Cinzia, 2004; Ajay and Mona, 2005; Michael *et al*., 2003; Bhattacharjee *et al*., 2002).

Significance of particle size

The submicron size of nanoparticles offers a number of distinct advantages over micro particles. Nanoparticles have in general relatively higher intracellular uptake compared to microparticles (Mu and Feng, 2001; Robert, 2005; Adam and Chris, 2001).

Polymers in drug delivery

Advances in the polymeric science have led to the development of several novel drug-delivery systems. A proper consideration of surface and bulk properties can aid in designing of polymers for various drug-delivery systems. Biodegradable polymers find widespread use in drug delivery, as they can be degraded to non-toxic monomers inside the body. Polymeric delivery systems are mainly intended to achieve either a temporal or spatial control of drug delivery (Omathanu and Ramesh, 2001; Samuel, 1995; Glen and Teruo, 1996; Dennis and Adi, 2002). Biocompatible and non toxic polymers such as poly (lactic-co-gucolic) (PLGA) have been employed for sustained release of drugs and macromolecular delivery and the development of single-shot vaccines that can immunise against multiple diseases (Gorka *et al*., 2004). As a result of their biodegradability these polymers form biologically compatible products that are removed from the body at a very slow rate, and thus do not affect normal cell function (Omathanu and Ramesh, 2001).

Polymeric nanospheres

It is apparent that a relatively successful approach for prolonging the circulation times of colloidal particles in the blood is to create a steric surface barrier of sufficient density. Because of the possible immunological cones-quences associated with some bacterial polysaccharides and the high cost of recombinant complement regulators, tremendous efforts have been directed to design synthetic polymers that can fulfill these criteria. It has long been known that stabilization of emulsion systems may be achieved at the interface by the addition of an emulsifying agent or a surfactant. In a pioneering experiment Geyer (1967) demonstrated that intravenously injected lipid emulsions prepared with high molecular weight members of POE/POP copolymer nonionic surfactants (poloxamers and poloxamines) as emulsifiers remained in the blood for relatively long periods. This behavior was initially thought to arise from the interference of surfactants with the lipoprotein-lipase activity (Hart and Payne, 1971). Later, it was suggested that high molecular weight POP/POE surfactants in some way prevent lipid particles from sticking to the blood vessel endothelium as well as inhibiting recognition by macrophages (Jeppsson and Rossner, 1975).

Poloxamers consist of a central POP block that is flanked on both sides by two hydrophilic chains of POE (fig. 2). A slightly different structure is exhibited by the poloxamines that are tetrafunctional block copolymers with four POE/POP blocks joined together by a central ethylene diamine bridge (fig. 2). Numerous investigators have now demonstrated that such copolymers adsorb onto the surface of oil-in-water emulsions or any hydrophobic nano-particulate systems [e.g., polystyrene, gold, PLGA, poly(phosphazene), poly(methyl methacrylate) and poly(butyl 2-cyanoacrylate)] via their hydrophobic POP center-block (Troster *et al*., 1990; Moghimi *et al*., 1993; Stolnik *et al*., 1994; Storm *et al*., 1995; Vandorpe *et al*., 1997; Monfardini and Veronese, 1998). This mode of adsorption leaves the hydrophilic POE side-arms in a mobile state as they extend outward from the particle surface and provide stability to the particle suspension by a repulsion effect through a steric mechanism of stabilization involving both enthalpic and entropic contributions (Moghimi *et al*., 1993). The strength of polymer adsorption and the resultant polymer conformation is dependent on the proportion and the size of both POP and POE segments as well as the physicochemical properties of the nanoparticle surface (Moghimi and Hunter, 2000). With the help of field-flow fractionation, electron spin resonance and conventional labeling techniques, detailed analytical characterization of the adsorption complexes formed between poloxamers and polystyrene beads of different sizes have been accomplished. These studies have also pointed toward the importance of nanoparticle surface curvature on polymer chain mobility and conformation (Li *et al*., 1994). For a given triblock polymer, it was found that both surface concentrations and adlayer thicknesses are strongly related to the particle size, such that smaller particles (below 100 nm) take up fewer polymer molecules per unit area than the larger ones. The reduced crowding around each POE chain results in thinner adlayers and higher chain mobilities. Therefore, the surface density decreases with decreasing particle size. For a particle of a given size, it is the size of the surfactant's hydrophobic center block (POP), rather than its flanking tails, that determines the surface concentration or density. Thus, triblocks of similar POP size showed comparable surface concentration, while the longer POE chains were associated with thicker adlayers as well as greater chain dynamics (Li *et al*., 1994). Therefore, by keeping the particle size constant, one can gain insight into

the effect of POE chain lengths on plasma protein adsorption and phagocytosis. Indeed, among the various copolymer members, poloxamine-908, poloxamine-1508, poloxamer-238, and poloxamer-407 (figure 1) have proved to be among the most effective copolymer nonionic surfactants for prolonging the circulation time of hydrophobic nanoparticles of 15 to 150 nm in mice and rats. For example, reported half-lives of poloxamine-908-coated nanospheres in mice and rats vary from a few hours to 1 to 2 days, depending on both the particle size and its initial surface hydrophobicity (Storm *et al*., 1995; Monfardini and Veronese, 1998; Moghimi and Hunter, 2000).

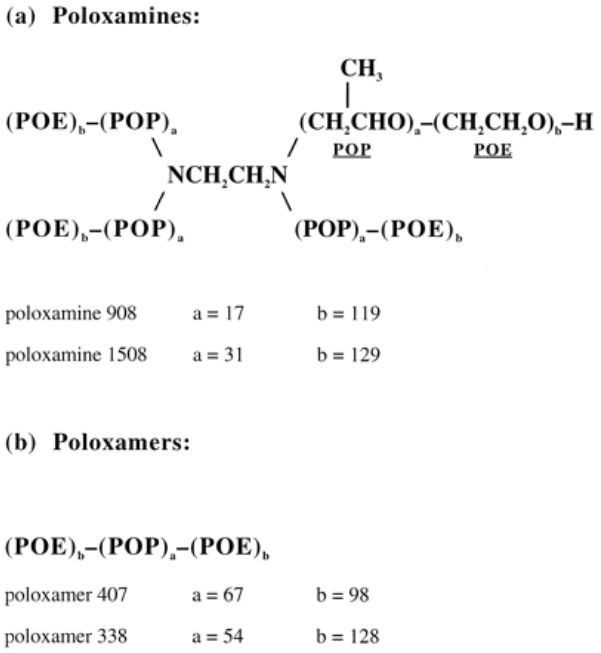

Fig. 1: The structure of selected poloxamines (a) and poloxamers (b).

Submicron sized PEG-ylated poly(isobutyl 2-cyanoacrylate) nanoparticles have also been produced by an emulsion/polymerization method (Peracchia *et al*., 1997c). Here, PEG was anchored to the nanoparticles through either only one end-group or through both end groups. The latter outcome surely limits the mobility of PEG chains and interaction with blood opsonins and, hence, particle retention time in the blood (Peracchia *et al*., 1997b). Efforts have also been made to form long-circulating nanospheres from diblock copolymers such as PLA-PEG (expressing a molecular weight of 20,000) in a one-step procedure, but again with rather disappointing results (Gref *et al*., 1994). Based on indium-111 labeling studies, the circulation half-life of PLA-PEG particles (90-150 nm) was less than 1 h in rats. Furthermore, the presented data indicated that at 90 min after injection, the circulatory pool of nanoparticles was less than 20% of the injected dose whereas the liver-associated activity was in the order of 30% of the initial dose (Gref *et al*., 1994). No explanation was provided for the missing 50% of the injected activity.

An interesting approach, however, has been the covalent attachment of semitelechelic poly[*N*-(2-hydroxylpropyl) methacrylamide]s of different molecular weights to nanospheres based on methyl methacrylate, maleic anhydride, and methacrylic acid (Kamei and Kopecek, 1995). One polymer preparation, with a weight average molecular weight of 18,800, was able to dramatically extend the circulation time of nanospheres (half-life of 12-15 h) in rats, whereas control preparations were cleared rapidly from the blood by the liver.

Micelles (self-assembly constructs)

Multiblock copolymers such as POE-poly(L-lysine), POE-poly(β-benzyl-L-aspartate, POE-poly(ε-caprolactone) and poly(acrylic acid)-poly(methyl methacrylate) as well as those that have been used in particle coatings (e.g., poloxamers, poloxamines, PEG-PLA, PEG-PLGA) also self-disperse in water to form spherical polymeric micelles with diameters in the size range of 15 to 80 nm (Yokoyama, 1992; Jones and Leroux, 1999). Some of these micellar structures have been suggested as promising long-circulating carriers of poorly water soluble and amphiphilic drugs, because of their small size and hydrophilic shell (Yokoyama *et al*., 1990, 1991; Yokoyama, 1992; Kwon *et al*., 1993, 1994; Hagan *et al*., 1996; Zhang *et al*., 1997a,b). Nonetheless, the effectiveness of such systems will depend on their critical micelle concentration. Upon intravenous injection, micelles are often diluted to less than their minimum micelle concentration and polymer molecules are known to behave in a dramatically different way. Furthermore, little information is known with regard to the stability of micellar systems in vasculature and their extent of interaction with blood and cellular components. Micellar stability may be enhanced by cross-linking procedures to produce a solid outer core (Thurmond *et al*., 1996; Bütün *et al*., 1999). However, such strategies may be rather premature for the design of a stable long-circulating micellar construct, as the reduced surface energy will probably result in rapid accumulation in liver macrophages.

Liposomes

For incorporation into the liposomal bilayer, numerous lipid derivatives of PEG have been made using lipids that, for example contain a primary amino group, an epoxy group or a diacylglycerol moiety (Blume and Cevc, 1990; Klibanov *et al*., 1990; Allen and Hansen, 1991; Allen *et al*., 1991a,b; Lasic *et al*., 1991; Mori *et al*., 1991; Maruyama *et al*., 1992; Parr *et al*., 1994; Kirpotin *et al*., 1996; Woodle, 1998). Alternatively, activated PEG can be anchored to reactive phospholipid groups of preformed liposomes (Senior *et al*., 1991). Another strategy has utilized the transfer of PEG-phospholipid conjugates from the micellar phase into the lipid bilayer of preformed vesicles (Uster *et al*., 1996). It was found by trial and error that PEG-grafted liposomes with extended circulation half-lives are in the size range of 70 to 200 nm and contain 3 to 7 mol% of methoxy-PEG-2000 grafted to DSPE or DPPE in addition to various

amounts of phospholipids and cholesterol (Klibanov *et al*., 1990; Allen *et al*., 1991b; Woodle and Lasic, 1992; Woodle, 1998). The circulation half-lives of such vesicles is 15 to 24 h in rodents, and as high as 45 h in humans. To date, these are the best engineered long-circulating particles. Only recently, the biophysical basis of these observations was realized. It was shown that mixtures of PEG-phospholipid conjugates and phospholipids exist in primarily three physically distinct states (Bedu-Addo *et al*., 1996a). These included a lamellar phase with components exhibiting some miscibility, a lamellar phase with components phase separated, and mixed micelles. The relative proportion of the three states in a given mixture was dependent on PEG chain length, acyl chain length and the degree of unsaturation of the PEG-phospholipid conjugate, and the acyl chain composition of the phospholipid. For example, beyond 7 mol% short-chain PEG-DPPE conjugates (PEG molecular weight in the region of 1000-3000) show a strong tendency to form mixed micelles with DPPC. Long-chain PEG-DPPC conjugates (PEG molecular weight of either 5000 or 12,000) above 8 mol% formed phase separated lamellae with the phospholipid due to PEG chain-chain interactions; the PEG chain-chain interactions can be reduced by using PEG-dioleoylphosphatidylcholine due to the presence of the kink in the acyl chain. Changes in phospholipid composition also alters the miscibility of PEG-PE with phospholipids. Increasing phosphatidylcholine acyl chain length increases fatty acid chain-chain interaction and so the van der Waals cohesive force. This results in more tightly packed phosphatidylcholine and PEG-PE molecules and a greater tendency toward PEG chain-chain entanglement and micelle formation. The extent of demixing of PEG-PE in bilayers, therefore, decreases in the order of C18:0 > C16:0 > C14:0. These observations explain why long-circulatory PEG-ylated liposomes usually contain 3 to 7 mol% short-chain PEG-PE (PEG molecular weight in the range of 1000-3000). Large chain PEG-PE (PEG with a molecular weight of either 5000 or 12,000) seem unsuitable for preparing certain formulations of long-circulating liposomes; phase separation generates domains less enriched with PEG-PE and could lead to poor steric protection of the liposome surface and subsequent destabilization by lipoproteins and opsonic attack (Bedu-Addo *et al*., 1996a).

Inclusion of a high concentration of cholesterol (above 30 mol%) within the liposomal bilayer can further improve surface protection by PEG (molecular weight of 1000-3000)-PE (5-7 mol%). This is due to an increase in bilayer cohesive strength and, hence, a reduction in the formation of phase separated lamellae (Bedu-Addo *et al*., 1996b). Because of its relatively inflexible structure cholesterol thus acts as a spacer keeping lipid chains apart and reducing PEG chain-chain interaction.

Apart from PEG lipid derivatives, other effective alternative materials for prolonging the circulation time of liposomes include phosphatidylpolyglycerols and phospholipid derivatives of amphiphilic poly(vinyl pyrrolidone), poly(vinyl alcohol), poly(2-methyl-2-oxazoline), poly(2-ethyl-2-oxazoline), poly(acryl amide), poly(acryloy morphine), and *N*-(2-hydroxylpropyl)methacrylamide (Torchilin *et al*., 1994, 1995; Maruyama *et al*., 1994; Woodle *et al*., 1994; Takeuchi *et al*., 1996; Zalipsky *et al*., 1996; Shtilman *et al*., 1999; Whiteman *et al*., 1999).

Oil-in-water emulsions

Apart from poloxamers and poloxamines, PEG-PE and Tweens have all been used as emulsifiers for the production of long-circulatory oil-in-water emulsions. Again, the blood retention time of such emulsions was shown to be related to PEG chain length and its surface density (Liu and Liu, 1995; Lundberg, 1997).

Consideration for selection of polymers

The selection and design of polymer is a challenging task because of their inherent diversity of structures and requires a thorough understanding of surface and bulk properties of polymer that can give the desired chemical, interfacial, mechanical and biological functions. The choice of polymer in addition to physicochemical properties is dependent on the need of for extensive biochemical characterisation and specific preclinical tests to prove its safety. Bulk properties that need to be considered for controlled delivery system includes chemical structure of polymer, chemical composition, molecular weight, adhesion, solubility based on the release mechanism and its site of action. A range of material has been employed to control the release of drug and other active ingredients. In order for a polymer to be successfully used for controlled release of drug the material must be chemically inert, free from impurities, have an appropriate physical structure and readily processable (Omathanu and Ramesh, 2001).

Polymers in nanoparticle

The fate of any polymer used will be determined by its method of synthesis, subsequent degree of purity and its behavior on administration into the individual biological system. Synthetic polymers exist as populations with a statistical distribution, i.e., they are polydisperse systems which vary in molecular weight. The degree of polydispersity will tend to be greatest if the product has been synthesized by free radical polymerization, which is nonselective with chain termination occurring, by combination or diproportionation. On the other hand, starburst polyamidoamine dendrimers are a relatively new class of spherical macromolecules, which have been reported as monodisperse (Kukowska-Latallo *et al*., 1996). Ironically, the future usefulness of these monodisperse systems in medicine remains to be revealed, since *in vivo* studies so far have indicated their propensity to localize in the liver and spleen (Malik *et al*., 2000).

There have been some preliminary studies on the effects of specific molecular weight fractions present within a polydisperse system to discern individual biological

activities *in vivo* on both short-term and long-term administration (Emanuele *et al*., 1999; Toth *et al*., 1997). Variation in polymer molecular weight fractions has been linked to tissue toxicity (Toth *et al*., 1997).

For example, Flocor (CytRx, GA, USA), a well defined fraction of poloxamer-188 (molecular weight 8964, polydispersity 1.0280) only reduced nephrotoxicity by 68% in a recent clinical trial (Emanuele *et al*., 1999) when compared with the native poloxamer-188 (RheothRx) following intravenous administration. This suggests that the nephrotoxic constituent(s), which are below the renal threshold, had not been removed or that the polymer is inherently toxic in its own right or a joint effect of the two.

Limited studies with polydispersed radiolabeled POP/POE copolymers have indicated that the primary route of polymer excretion is renal and the minor route is biliary (Wang and Stern, 1975; Willcox *et al*., 1978; Rodgers *et al*., 1984). In addition, radiolabeled copolymers have been found in all organs, particularly in the liver, lung and skeletal muscles, 24 h after intravenous administration into the dog (Willcox *et al*., 1978). Long-term effects of accumulated polymers in these organs still remain to be evaluated.

Interspecies and intraspecies responses to polymeric systems

It should also be emphasized that different species may respond differently to the administered polymeric materials. For example an early study with polyelectrolyte poly(ethylene sulfonate) clearly demonstrated a broad spectrum of antitumor activity in mice following subcutaneous administration (Regelson and Holland, 1958). However, the antitumor activity of this polymer was found to be reduced in humans and it was also too toxic for clinical use (Regelson and Holland, 1958; Breslow, 1976). Variation in interspecies activity has also been observed with pyran copolymer. This material was able to induce interferon production in mice. A synergistic effect between pyran copolymer and the foot-and mouth-disease vaccine in mice (Campbell and Richmond, 1973) was observed. A 1.2 mg dose of pyran copolymer plus the vaccine protected 80% of the mice, with no antiviral activity observed for the copolymer alone. This activity was not carried over to cattle and pigs where dosages were too high and toxic side-effects were observed (Sellers *et al*., 1972; McVicar *et al*., 1973). Similar to the above observations, variation in complement activation in serum of healthy individuals by PEG-bearing liposomes has also been observed. These observations seem relevant to nanoparticle production with polymers and suggest caution in extrapolating animal model responses to humans. One may even experience variations among different individuals.

Controlled drug delivery systems

Conventional dosage forms such as oral delivery and injections are predominant routes for drug delivery. However these types of dosage are not easily able to control the rate or drug delivery or the target area of the drug and are often associated with an intermediate or rapid drug release. Consequently the initial concentration of the drug in the body peaks above the level of toxicity and then gradually diminishes over time to an ineffective level. The duration of therapeutic efficacy then become dependent on the frequency of administration, and half life of the drug and high dosage of non targeted drugs are often administered to achieve an effective blood concentration (Sarah and Tejal, 2003).

Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a pre-designed manner. The release of active agent may be constant over a long period of time, it may be cyclic over long period, or the environment or other external events may trigger it. In any case the purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under and overdosing. Other advantages of using controlled release drug delivery are; maintenance of drug levels within desired range, need of fewer administration, optimal use of drug in question and patient compliance (Omathanu and Ramesh, 2001; Glen and Teruo, 1996).

Where there are some advantages of controlled release of drugs, there are certain disadvantages, e.g., possible toxicity, non-compatibility of the material used, undesired by-products obtained by degradation of material and the higher cost of controlled release system as compared to traditional pharmaceutical formulations (Dennis and Adi Eisenberg, 2002).

An immense amount of interest has been increasingly placed on controlled release of drug delivery systems to maintain the therapeutic efficacy of these drugs. There are a number of mechanisms that can provide such controlled release of drugs, including transdermal patches, implants, bio-adhesive systems, and micro encapsulation (Sarah and Tejal, 2003). Controlled release of drugs using degradable polymers is well known and research is being carried out in designing newer class of materials including whose release rate can be changed in vivo (Omathanu and Panchagnula, 2001).

Fabrication of nanoparticles

Different methods can be used to prepare nanoparticle systems depending upon particle size requirement, thermal and chemical stability of the active agent, reproducibility of the release of kinetic profiles, stability of the final product, and residual toxicity associated with the final product (Meng Shi *et al*., 2003; Sarah and Tejal, 2003; Cory and Daniel, 2004; Sunil and Nadagouda, 2005; Xianghui and Zhenhua, 2004; Bhattacharjee *et al*., 2002).

Sol-gel technique

Sol gel technique is a versatile synthetic tool for construction of inorganic or organically modified inorganic networks and can be adopted to suit the requirements of nanofabrication by means of controlled reaction in presence of suitable additives, tem-plating compounds, seed particles or compartmented reaction environment (Valter and Cinzia, 2004). Advantages to this technique are low temperature and mild reaction conditions at all stages, which can satisfy the most demanding requirements for biomedical applications (Valter and Cinzia, 2004; Manja *et al*., 2000; Janet and Pablo, 2002).

Sol-gel technique involves formation of a colloidal suspension, which is obtained by controlled acid or base catalysed hydrolysis and partial condensation of metal alkoxide precursor in alcoholic or other non-hydrolytic organic solvents then condensation or dispersion of colloidal particles leads to viscous gel depending on the composition, pH and reaction conditions. The internal network structure depends on respective rates of hydrolysis and condensation, and therefore on steric hindrance, inductive effect of alkyl substituents and number of alkoxy groups on the silicon atoms, as well as catalysis and solvent (Valter and Cinzia, 2004; Manja *et al*., 2000; Janet and Pablo, 2002).

Acid catalysed hydrolysis at low H_2O/Si ratio yields poorly branched polymer sols, while highly condensed ones are produced under basic catalysis at high H_2O/ Si ratio. Thin films or hard nanospheres with controllable thickness or size may be prepared by such sol-gel technique. If the final gel or particle is nanostructured hybrid, a last thermal treatment can be employed to remove the organic fraction and to produce a porous material or hollow sphere (Valter and Cinzia, 2004; Xianghui and Zhenhua, 2004; Pirjo and Manja, 2000; Janet and Pablo, 2002).

The interfacial properties play a key role not only because of their importance for nanostructure formation during hybrid synthesis, but also in relation to final application of the hybrid material.

While a lot of metal alkoxide and alkoxoy derivatives can be used to build sol-gel networks, the high hydrolytic instability of most of the compounds in aqueous media limits the application exclusively to silicon alkoxide (Valter and Cinzia, 2004; Janet and Pablo, 2002).

Among the various possibilities, high dilution, control of pH and addition of surfactants can prevent or effectively slow down gelation, allow better control on the degree of hydrolysis and condensation of organosilane precursors and provide additional ordering through compartmentalisation (Valter and Cinzia, 2004). On the other hand water can be an ideal reaction medium for structure build-up at nanometric scale (Valter and Cinzia, 2004; Janet and Pablo, 2002).

Fabrication of nanoparticles within polymeric pores

Tetraethyl orthosilicate (TEOS), and poly acrylic acid (PAA) are used as a source of SiO_2 and inert polymer respectively. Different PAA contents and gelation temperatures are used in order to obtain samples with different nanostructure. The synthesis is carried out with an excess of water with respect to stiochiometric relationship. Nitric acid is used to catalyse the reaction at a pH of 0.45, these reaction conditions give rise to hydrolysis and polycondensation of silica and sterification of PAA occurs. The H_2O/TEOS ratio is kept 9.5 for all samples and PAA and TEOS monomeric molar ratio varies from 0.07 to 0.25. Strong stirring is applied until homogeneous, transparent solutions are obtained. The solutions are then heated to a temperature range of 40 ^{0}C to 95 ^{0}C in sealed vassals in order to avoid evaporation. Gelation and ageing of samples is carried out at the above temperature range and different nanostructures are obtained (figure 2) (Janet and Pablo, 2002).

Organic phase is eliminated by soaking gel samples in a solution of 1:1 water and ethanol. Finally the samples are heated at 400^{0}C, 700^{0}C and 1000^{0}C at a rate of 1^{0}C per minute. Then samples are cooled at a rate of 10^{0}C per minute (Janet and Pablo, 2002).

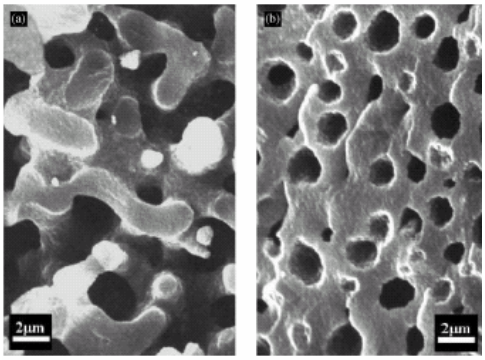

Fig. 2

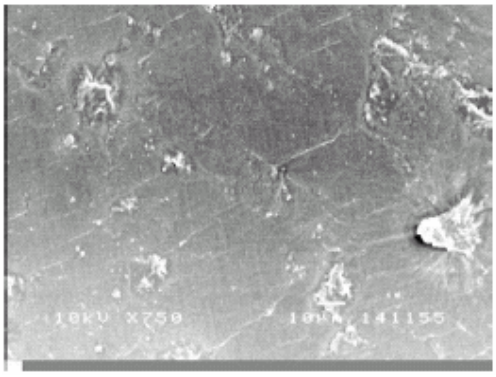

Fig. 3

Characterization of samples

Scanning electron microscopy (SEM) (figure 3), N2 adsorption/desorption porosimetry and BET surface are employed for nanostructural characterisation of samples. The gels are then classified according to differences in porosity, which are detected by SEM. The surface state of samples and deposition of appetites are monitored by means of FTIR reflectance spectroscopy and SEM (figure 2) (Meng Shi *et al*., 2003; Alexis and Santosh, 2004; Mu and Feng, 2001; Gang and Si-Shen Feng, 2002; Xianghui and Zhenhua, 2004; Janet and Pablo, 2002; Sang and Joo, 2004).

Particle size measurement

The diameter of nanoparticles are measured by using a Nikon polarising microscope and analysed by the computer software (Jae-Hyung and Lonnie, 2003; Meng Shi *et al*., 2003; Janet and Pablo, 2002).

Drug loading into nanoparticles

Drug loading in nanoparticulate system can be done by two methods, incorporation and incubation, which differs in a way that earlier is employed during the preparation of particles and later after the formation of particles.

In these systems, the drug is embedded into the matrix or adsorbed onto the surface. Efficiency of loading is largely dependent upon method of preparation and physicochemical properties of drug. Maximum drug loading can be achieved by incorporation but it may get affected by process parameters, such as method of preparation or presence of additives etc. (Alexis and Santosh, 2004; Harjit and Johansen, 2005; Gorka *et al*., 2004; Sunil and Nadagouda, 2005; Janet and Pablo, 2002).

Controlled-release mechanisms

There are three primary mechanisms by which active agents can be released from a delivery system: diffusion, degradation, and swelling followed by diffusion. Any or all of these mechanisms may occur in a given release system. Diffusion occurs when a drug or other active agent passes through the polymer that forms the controlled-release device. The diffusion can occur on a macroscopic scale—as through pores in the polymer matrix—or on a molecular level, by passing between polymer chains. Examples of diffusion-release systems are shown in figures 4 and 5.

In figure 3, a polymer and active agent have been mixed to form a homogeneous system, also referred to as a matrix system. Diffusion occurs when the drug passes from the polymer matrix into the external environment. As the release continues, its rate normally decreases with this type of system, since the active agent has a progressively longer distance to travel and therefore requires a longer diffusion time to release.

For the reservoir systems shown in Figures 5a and 5b, the drug delivery rate can remain constant. In this design, a reservoir—whether solid drug, dilute solution, or highly concentrated drug solution within a polymer matrix—is surrounded by a film or membrane of a rate-controlling material. The only structure effectively limiting the release of the drug is the polymer layer surrounding the reservoir. Since this polymer coating is essentially uniform and of a nonchanging thickness, the diffusion rate of the active agent can be kept fairly stable throughout the lifetime of the delivery system. The system shown in Figure 4a is representative of an implantable or oral reservoir delivery system, whereas the system shown in Figure 4b illustrates a transdermal drug delivery system, in which only one side of the device will actually be delivering the drug (Alexis and Santosh Kumar, 2004; Ajay and Mona, 2005; Manja *et al*., 2000; Janet and Pablo, 2002).

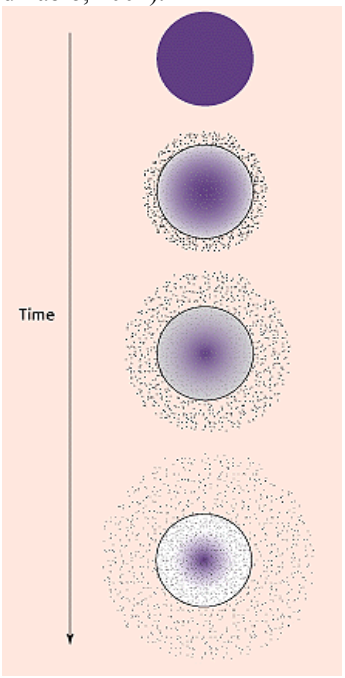

Fig. 4: Drug delivery from a typical matrix drug delivery system.

Biodegradable systems

All of the above-described systems are based on polymers that do not change their chemical structure beyond what occurs during swelling. However, a great deal of attention and research effort is being concentrated on biodegradable polymers. These materials degrade within the body because of natural biological processes, eliminating the need to remove a drug delivery system after release of the active agent has been completed. Most biodegradable polymers are designed to degrade because of hydrolysis of the polymer chains into biologically acceptable, and progressively smaller, compounds. In some cases, the polymers will eventually break down, enters the Kreb's cycle. Degradation may take place through bulk hydrolysis, in which the polymer degrades in a uniform manner throughout the matrix. For some degradable polymers, most notably the polyanhydrides and polyorthoesters, the degradation occurs

only at the surface of the polymer, resulting in a release rate that is proportional to the surface area of the drug delivery system (Meng Shi *et al*., 2003).

The situation regarding the fate and activity of biodegradable/bioerodable polymeric drug delivery vehicles for parenteral use is complicated, because at present there is no study available, which demonstrates these polymers will degrade in a uniform manner *in vivo*. Clearly the *in vivo* environment will vary depending on the site of parenteral administration, composition of tissue fluids and disease state which further complicates the uniformity of the degradation process. PLGA polymers have been used widely as biomaterials for medical applications over the last 30 years and are regarded as "biocompatible" and "nontoxic". This has been due to the wide variety of materials achievable by varying the molar ratios of the lactic acid and glycolic acid moieties. For example, high molecular weight crystalline PLGA has been used effectively as surgical sutures and bone fixation nails and screws (Daniels *et al*., 1990; Pulapura and Kohn, 1992). Conversely low molecular weight amorphous PLGA has been researched widely for controlled drug delivery applications (Asano *et al*., 1990; Wang and Wu, 1997). However, there is little information available regarding the rate of degradation as well as toxicological problems associated with PLGA and related biodegradable particulate drug delivery systems following parenteral administration. A 2-fold increase in the rate of degradation of PLA has been observed in plasma compared with buffer or water at 37°C (Mason *et al*., 1981). The specific plasma constituents responsible were not identified. The large surface area of the colloid, which is gradually presented to opsonic and other immunoregulatory proteins, may potentiate these effects. This surface area will be markedly increased once the degradation process starts. It will be essential to control the rate of degradation in the *in vivo* environment to ensure even dosage delivery assuming that the polymer and its by products are completely inert *in vivo*. Faster in vivo rates of degradation have been attributed, in part, to the foreign body response (Tokiwa and Suzuki, 1977; Williams and Mort, 1977; Ali *et al*., 1994). Investigation into the phagocytosis of both whole (≤ 38 μm) and predegraded PLA particles following intraperitoneal administration into mice has shown that predegradation of PLA particles induced cell damage (e.g., swollen mitochondria, widened endoplasmic reticulum, disappearance of membrane ruffling) on day 2 of the experiment with evidence of necrotic cells and cell debris on days 3 and 4. This clearly indicates that degradation products of the so called "bioinert" PLA can be cytotoxic to immune cells (Lam *et al*., 1993). Therefore, there is a clear need for a wide range of *in vivo* studies on biodegradable polymers to determine the nature and fate of the degradation products. The physicochemical nature of the particle (size, shape and porosity will also affect the degradation rate (Matlaga *et al*., 1976; Grizzi *et al*., 1995). The autocatalytic nature of some polymers will further accelerate degradation rates and this may be potentiated when the polymers are isolated or have restricted transport away from a region. For example, hydrolysis of the esteratic link of PLA will result in the formation of more acid groups and, hence, increased availability of protons to feed the esteratic hydrolysis. PLG polymers with the acid group capped (as an ester) have been demonstrated to have reduced rates of degradation compared with the free acid and that the length and type of capped moiety can be used to control the rate of degradation (Tracy *et al*., 1999). Perhaps the ideal drug delivery application for biodegradable polymer microparticulate systems is for the delivery of biologically active polymers. If controlled rates and site of degradation can be achieved it is an ideal opportunity for a sustained release "biologically active polymer-polymer therapeutic system".

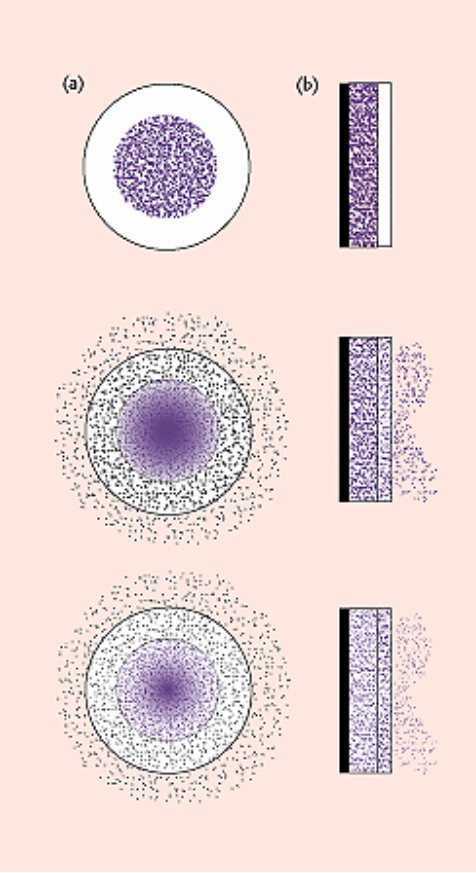

Fig. 5: Drug delivery from typical reservoir devices: (a) implantable or oral systems, and (b) transdermal systems.

The precise role of polymer stereochemistry on enzyme degradation and immunological response following parenteral administration has not yet been determined. Stereochemical preference for copolymer bonds has been demonstrated (Li *et al*., 2000), degradation of stereocopolymers (L-, DL- and meso-lactides) by proteinase K that showed preferential degradation of bonds with the stereochemical configuration of L-L, L-D and D-L bonds as opposed to D-D bonds. However enzymatic attack in the case of the meso form may have been facilitated by

increased rates of water uptake. A comparison of the effect of poly-L- and D-lactides on inflammatory response was found to be reduced in the case of polymers containing the D-isomer (Gogolewski *et al*., 1993). Whether different stereochemistry can modulate biological responses is still subject to speculation.

CONCLUSION

The ability to deliver pharmacologically active drugs in a controlled manner into targeted tissues provides therapeutic treatments that are efficient and exhibit good patient compliance. Recent advances in the field have provided novel drugs with improved pharmacokinetics and biopharmaceutical properties, which have gained the attention of the scientific community and motivated and inspired many biotechnological and pharmaceutical companies. Nanoporous polymeric systems have great utility in controlled release and targeting studies of almost all class of bioactive molecules. Recently, porous nanoparticles within polymeric matrix are also extensively explored in gene delivery. However, studies toward optimization of process parameters and scale up from the laboratory to pilot plant and then, to production level are yet to be undertaken. Majority of studies carried out so far are only in *in vitro* conditions. More *in vivo* studies need to be carried out (Sunil and Nadagouda, 2005). Future studies should also explore the relationship between in vitro and/or in vivo complement activation by such copolymers and clinical signs and symptoms of hypersensitivity to poloxamer/poloxamine based nanoparticles.

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