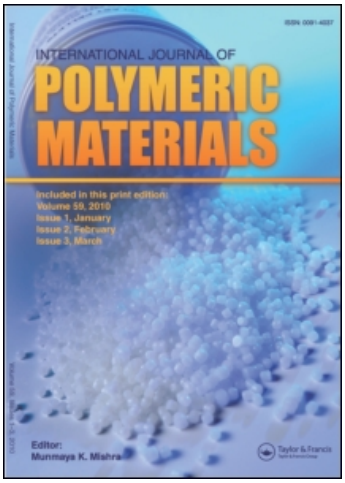


International Journal of Polymeric Materials
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713647664

Biodegradable Polymeric Microparticles in Biomedical Applications

K. Z. Gumargalieva[a]; Daniel Horak[b]; G. E. Zaikov[a]
[a] Institute of Chemical Physics Academy of Sciences of Russia ul, Moscow [b] Institute of Macromolecular Chemistry Academy of Sciences of the Czech. Republic, Prague, Czech Republic

To cite this Article Gumargalieva, K. Z. , Horak, Daniel and Zaikov, G. E.(1998) 'Biodegradable Polymeric Microparticles in Biomedical Applications', International Journal of Polymeric Materials, 42: 1, 83 — 117
To link to this Article: DOI: 10.1080/00914039808041062
URL: http://dx.doi.org/10.1080/00914039808041062

Intern. J. Polymeric Mater., 1998, Vol. 42, pp. 83–117
Reprints available directly from the publisher
Photocopying permitted by license only

Biodegradable Polymeric Microparticles in Biomedical Applications

K. Z. GUMARGALIEVA[a], DANIEL HORAK[b] and G. E. ZAIKOV[a,*]

[a] *Institute of Chemical Physics Academy of Sciences of Russia ul. Kosygina, 4, 117334, Moscow;*
[b] *Institute of Macromolecular Chemistry Academy of Sciences of the Czech. Republic. 16206, Prague, 6 Czech Republic*

(Received 4 November 1997)

Factors affecting the drug release from biodegradable microparticles are reviewed. It is shown that spherical microparticles ranging in diameters from below 1 μm to over 100 μm along with the nanoparticles with the size less than 1000 nm are particularly effective. The role of molecular weight, molecular weight distribution and co-polymer ratio and distribution is also discussed. Important drug parameters are the solubility of the drug in biological fluids and possible polymer particle-drug interactions. The role of microporosity may be essential in delivery of high molecular weight substances.

Keywords: Drug release; polymeric microparticles; biodegradation; particle size; clinical applications

INTRODUCTION

A wide range of polymeric materials are nowadays available for biomedical applications. The number of materials that are approved for human use, however, is small. They are recently receiving much attention mainly as a means of controlled drug delivery over a prolonged period of time targeting drugs to specific sites in, for example, cancer chemotherapy [1] and intracellular parasitic diseases [2]. If drug delivery devices are resistant to biodegradation, upon repeated treatments they accumulate in the liver of the patient which

* Corresponding author.

could eventually result in adverse effects. As a consequence, many hydrophilic addition polymers have been banned from medicinal use. Therefore, the use of biodegradable polymers is required to serve as drug carriers in these systems [3]. Degradable systems have the advantage of obviating the need to surgically remove the drug-depleted device which can always be the source of infection [4]. Potentially, degradable matrix systems also have a number of other advantages such as simplicity of design and predictability of release if the release is controlled solely by matrix degradation [5].

For many medical applications it is desirable to have a biodegradable polymer in the form of spherical microparticles [6] in diameters ranging from below 1 μm to over 100 μm and nanoparticles [7] with a size less than 1000 nm. The spherical sample geometry offers distinct advantages relative to the more conventional film and fiber geometries, since microspheres not only can control delivery of the incorporated agent, but also protect the agent, such as proteins and peptides, from proteolytic degradation. Moreover, their application is easier than that of other forms: they can be subcutaneously, intravenously and intramuscularly injected. Nanoparticles can have a matrix-type structure or can be nanocapsules with a reservoir-like structure, consisting of a solid shell and an inner liquid core. Various other particulate systems have been described, including emulsions [8, 9], phospholipid vesicles [10, 11], microcapsules [12, 13] and microspheres prepared from a variety of materials, such as polysaccharides [14–16], alkylcyanoacrylates [17, 18], lactic acid and related polymers [19, 20] and proteins [21–23].

The drug release from biodegradable microparticles is governed by various properties of the polymer, drug and carrier system [24]. Polymer dependent factors include the molecular weight and molecular weight distribution, the co-polymer ratio and distribution, and the crystallinity. Effective ways to increase the drug release from microspheres are to increase the drug loading or to decrease molecular weight of polymer or to prefer amorphous to crystalline polymer. Important drug dependent parameters are the solubility of the drug in dissolution or biological fluids, the molecular weight and possible polymer-drug interactions. These can be influenced through pH changes. Carrier system dependent factors comprise the type of microparticle (microphere *vs.* microcapsule), the drug loading, the

physical state of the drug in the polymer matrix (dissolved *vs.* dispersed), the drug loading, the physical state of the drug in the polymer matrix (dissolved *vs.* dispersed), the particle size and particle size distribution, and the porosity and internal structure of the microparticles [25]. An increase in matrix porosity enhances the drug release because of the easier accessibility of the drug by dissolution fluids. Porous microspheres are also essential to deliver high molecular weight substances which cannot diffuse out of a nonporous matrix and to deliver substances which have high affinity for polymer and are not released unless the matrix is eroding.

BIODEGRADATION

Polymeric implant materials designed to safely degrade in the body are usually referred to as degradable, biodegradable, resorbable or absorbable polymers [26]. Some general review articles on biodegradation of polymers [27] and biodegradable polymers for medical applications have been written [28, 29]. For clinical applications, it is often important to distinguish clearly between "biodegradable polymers" and "bioabsorbable polymers" [30]. Biodegradable polymers are polymers which are decomposed in the living body but whose degradation products remain in tissue long-term. On the other hand, bioabsorbable polymers can be defined as polymers which degrade after implantation into nontoxic products which are then eliminated from the body or metabolized therein [30]. Polymers that are decomposed by enzyme-specific reactions are called "enzymatically degradable polymer" while polymers that are decomposed by contact with water or serum are "nonenzymatically degradable polymers" [31]. Technically, only enzymatically degradable polymers should be considered as truely "biodegradable". However, in this review, nonenzymatically degradable polymers will also be included. In nonenzymatically degradable polymers, a relatively fast degradation rate is found for synthetic polymers consisting of easily hydrolyzable chemical bonds, such as amides, enamines, esters, orthoesters, acetals, urea, urethane, glycosides and related groups and carbonate linkages [30].

A few critical requirements for the design of biodegradable/bioerodible polymers must be considered. First, they should hydrolyze

at an appropriate, well-defined, rate to suit a specific application [26]. Second, their degradation products must be nontoxic and preferably should be normal constituents of a human biochemical metabolic pathway. Besides these two factors, the other important quality is biological compatibility [32]. Finally, the mechanical and chemical properties of the polymer must be able to satisfy the requirements of the device, *e.g.*, stiffness, permeability, elasticity or blood compatibility, depending on the nature of the device [26]. If the degradation occurs at the surface of the particles and approaches the center, surface erosion is achieved. In contrast, bulk erosion is characterized by degradation that occurs simultaneously through the particle [33]. Five important factors control the type of erosion taking place: 1) bond stability, 2) hydrophobicity, 3) crystallinity, 4) dissolution of the degradation products and 5) porosity. In any degradation process the first step is the penetration of water into the polymer, followed by the hydrolysis of the chemical bond and dissolution of the degradation products. If water penetration is slower than hydrolysis and dissolution, surface erosion will take place. However, if water penetration is faster than the dissolution rate, bulk erosion will dominate. Ideal polymers for surface erosion would have a hydrophobic backbone but very labile linkages connecting monomers [33]. Since the degradation rate is proportional to the surface are of micropheres, the degradation rate increases with decreasing microsphere size (increasing surface area) [33]. Porous materials degrade at higher rate than the nonporous ones [33]. Degradation also depends on drug loading: microspheres with higher drug loading degrade faster than those with low drug loading [33]. Crystalline regions will erode more slowly than amorphous regions [33].

Natural polymers such as polypeptides, polysaccharides, polynucleotides and bacterial polyesters are generally degraded in biological systems by hydrolysis followed by oxidation. Most of these biopolymers are enzymatically biodegradable [30], except for highly crystalline celluloses and "hard" proteins like keratin and silk fibroin. On the other hand, most non-enzymatically degradable polymers are synthetic polymers, including aliphatic polyesters and polycarbonates prepared from fatty acids and diols. These polymers are hydrolyzed into low molecular weight oligomers and then to monomers by water or serum. For this reason, degradation rates for nonenzymatically

degradable polymers are almost the same *in vivo* compared with *in vitro* [30]. From the standpoint of the degradation mechanism, biodegradable polymers may be divided into three general classes: 1. polymers whose main chain bounds are hydrolyzed to produce oligomers and monomers; 2. polymers which are converted to soluble polymers by hydrolysis of side groups; and 3. cross-linked polymers which are solubilized by a dissociation of their cross-links. In the latter two cases, the molecular weight of the resulting soluble polymers should be low enough so that they can be excreted by kidney dialysis [34].

ENZYMATICALLY DEGRADABLE BIOPOLYMER MICROPARTICLES

All naturally occuring polymers such as starch, cellulose, proteins, nucleic acids and lignin are biodegradable, since they have been existing on earth for a length of time sufficient for enzymes and microorganisms to evolve that can degrade or utilize these materials as food. Unfortunately, all of these materials which evolved through on aqueous medium contain very polar groups, mostly either peptide (— CONH — or ester (— COO — in their main chain and therefore decompose upon heating before they melt [35]. It might seem that natural polymers would be well suited for use as biomedical materials because of their structural similarity to components in host tissues. However, there are some significant disadvantages to natural polymers. These include: 1. strong physiological activities and the potential for rejection, 2. a difficulty in evaluating degradation rates *in vivo* because of differences in enzyme concentrations in different parts of living tissues; and 3. the mechanical strength of natural polymers is generally insufficient. For these reasons, their application as biomedical materials has been limited to only a few specific areas.

Polysaccharides

The polysaccharides [30] occupy a central position among the natural polymers and are ubiquitous throughout nature. Modified cellulose,

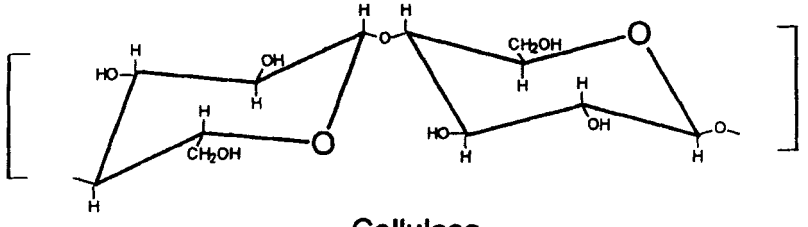
starch, various dextrans, alginic acid, hyaluronic acid, chitin and chitosan are some polysaccharides that may serve as potential biomaterials.

Cellulose and its Acetate

Cellulose (β-1,4-polyanhydroglucose) (Fig. 1) is the major naturally occurring storage and structural polymer in the vegetable kingdom [30]. Although native cellulose is poorly digestible in living tissue, it can be made biodegradable through modifications that disturb its higher ordered structure. One of such modifications include the reaction of acetic anhydride with cotton linters of wood pulp, resulting in cellulose acetate [36]. Cellulose esters can also be produced from recycled paper and sugar cane [37]. Commercially available cellulose acetate has a degree of substitution between 1.7 and 3.0. Cellulose acetate is biodegradable [38], if its degree of substitution is less than 2.5. Rates of biodegradation decrease as the degree of substitution increases.

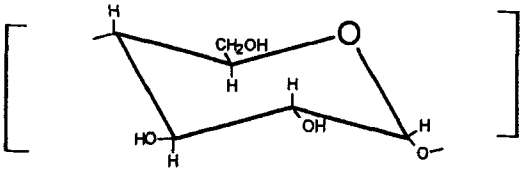
Starch

Starch [30] (Fig. 2) consists of glucopyranose residues in an α-*D*-(1,4)-linkage that yield *D*-glucose upon hydrolysis. Starch is produced in plants and contains the polysaccharides amylose [α-(1,4)-linked glucose] and amylopectin [α-(1,4)-linked glucose main chains with α-(1,6)-linked branches of α-(1,4)-linked glucose] [36]. Corn is the primary source of starch, although potato, wheat and rice starch also have markets in Europe, the Orient and the USA [39]. Starch is a



Cellulose

FIGURE 1



Starch

FIGURE 2

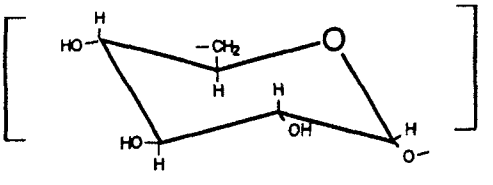
potentially useful polymer for thermoplastic biodegradable materials because of its low cost, availability and production from annually renewable resources. The degradation rate and extent to which the starch gel could be degraded by α-amylase are dependent on the degree of modification of starch chains during the crosslinking reaction [40].

Dextran

Dextrans [30] consist of α-*D*-glucose units joined predominantly by 1–6 glycosidic bonds (Fig. 3). They are cleaved by dextranases which are not present in blood, but are present in the liver, spleen, intestinal mucosa and colon [41].

Alginic and Hyaluronic Acid

Sodium alginate is a linear polymer of β-(1–4)-*D*-mannuronic acid and α-(1–4)-*L*-glucuronic acid residues [30] (Fig. 4). Aliginic acid



Dextran

FIGURE 3

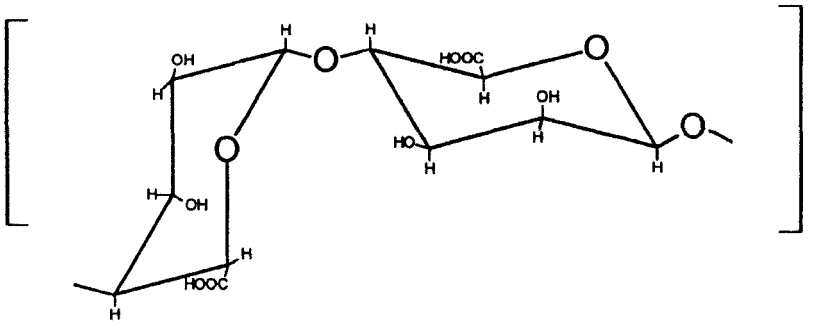


FIGURE 4

forms water-soluble salts with monovalent cations, low molecular weight amines, and quaternary ammonium compounds. Upon introduction of polyvalent cations such as Ca^{2+}, Be^{2+}, Cu^{2+}, Co^{2+}, Al^{3+} and Fe^{3+} it becomes water-insoluble and forms gels. The degree of cross-linking is dependent on various factors such as pH, ionic strength, type of counterion, and the functional charge density of these polymers [42]. The polymeric anionic character of aliginate renders the polymer suitable for enteric coating of oral delivery systems [43]. Similarly like alginates, also gellan which is of microbial origin, is capable of gelation in the presence of mono- and divalent ions [44]. Gellan beads show a slightly slower release than obtained with aliginate beads [44].

Hyaluronic acid is a naturally occurring nonsulfated glycosaminoglycan consisting of a linear sequence of *D*-glucuronic acid and *N*-acetyl-*D*-glucosamine (Fig. 5). It is present in connective tissue, in the synovial fluid of articular joints, and in the vitreous humor of the eye. In dilute solutions under physiological conditions, hyaluronate molecules exist as random coils with their own solvent shells [30].

Chitin and Chitosan

Chitosan is deacetylated chitin and a linear polymer of 2-amino-2-deoxy-β-*D*-glucan (Fig. 6). It is abundant in nature as a major hexose of the crustacean skeleton, insects, etc. It contains primary and

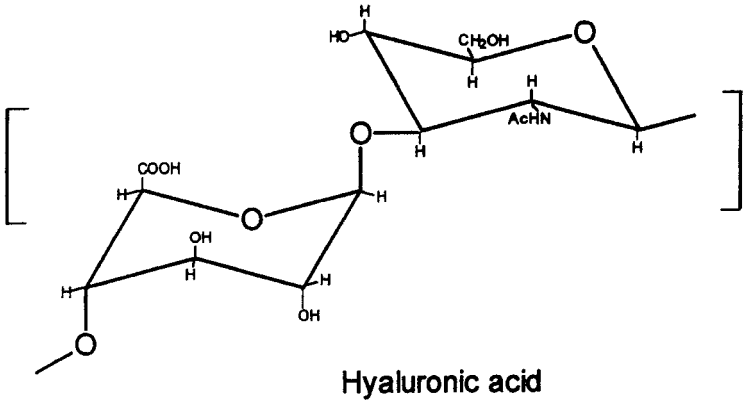


FIGURE 5

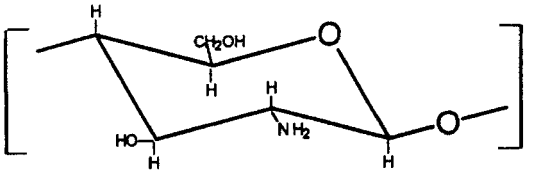


FIGURE 6

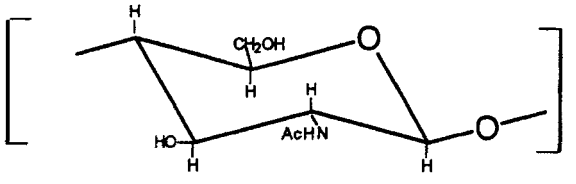


FIGURE 7

secondary alcoholic groups and its amine group renders the polymer soluble in dilute organic acids. Chitin (Fig. 7) can be degraded by chitinase. Its immunogenicity is exceptionally low in spite of the presence of nitrogen atoms. The polymeric cationic character along

with its potentially reactive functional groups has given it unique properties for utilization in controlled release systems [45]. Chitosan has ability of gelling by controlling inter and intramolecular interaction of its positively charged amino groups with the tripolyphosphate counterion [46].

Proteins

Albumin and Heparin

Serum albumin is a blood plasma protein that is soluble in water half saturated with a salt such as ammonium sulfate. Albumin (molecular weight 68 000) has a sigle free sulfhydryl (-SH) group, which on oxidation forms a disulfide bond with the sulfhydryl group of another serum albumin molecule thus forming a dimer. The resulting albumin-cross-linked networks are susceptible to proteolytic degradation. Degradation of albumin microspheres depends on concentration of glutaraldehyde used in preparation of protein microspheres. Iodine labelling, drug loading and scaling up batch sizes has no effect [47]. Heparin is a well-known anticoagulant and widely used for the prevention and treatment of thrombotic diseases [48].

Collagen and Gelatin

Collagen is the major component of all mammalian tissues, including skin, bone, cartilage, tendons, and ligaments [30]. Different types of collagenous material are known, from collagen type I to X. Among them, the most abundant and widely investigated material is collagen type I. It contains high content of glycine and the imino acids proline and hydroxyproline, hydroxylysine and small amount of aromatic and sulfur containing amino acids.

Gelatin [30] is a variant of collagen, that is, a denaturated collagen. Since a heat denaturation method cannot be applied to the microsphere preparation of gelatin, microspheres or hydrogels of gelatin require stabilization by chemical cross-linking.

Other Poly(Amino Acid)s

The polymers synthesized from α-amino acids present a unique opportunity and distinct advantage over other polymeric systems because of the wide choice of amino acids with different side-group functionalities [49]. These side groups can be manipulated to afford tailor-made systems for specific applications. However, the antigenicity of polypeptides containing three or more amino acids excludes their use in biomedical applications [50]. Due to these difficulties, only a few synthetic polypeptides have been investigated as implant materials [51]. Their greatest potential lies in the area of drug-delivery systems, where research has concentrated mainly on homopolymers and copolymers derived from *L*-glutamic acid (*L*-Glu) and *L*-aspartic acid (*L*-Asp). This is mainly because of the side-chain carboxyl groups, which enable attachment of other functional groups including drug molecules (polyagents). Other systems are based on a sequential polydepsipeptide containing a tripeptide sequence *L*-alanyl-*L*-alanyl-*g*-ethyl *L*-glutamyl and an α-hydroxy acid *L*-lactic acid, such as poly[Ala-Ala-Glu(OEt)-Lac]. In $[Glu(OMe)_m(Sar)_n$ and $[Lys(z)]_m$ polysarcosine segment in polypeptide $(Sar)_n$ synthesized by *N*-carboxyyanhydride method is soluble either in water or in organic solvents which enables the preparation of microcapsules by a solvent evaporation technique [52]. The membrane permeability of these polypeptide microcapsules is pH-sensitive [53].

Polyesters

Poly(β-hydroxyalkanoates)

Poly(β-hydroxyalkanoates), PHA, such as poly(β-hydroxyvalerate), PHV, and their copolymers, are naturally occuring bacterial polyesters synthesized by microbial fermentation [36]. They accumulate as energy storage reserves within the cells of many bacteria and fungi under growth-limiting conditions [54] in the same way that animals use fat. For example, *Alcaligenes eutrophus NCIB 11599* can accumulate 80% of total cell dry weight as PHA [55], and the species is used in the commercialized production of poly(hydroxybutyrate-*co*-valerate). Polyhydroxybutyrate, PHB, random copolymers of butyrate and valerate, PHBV, long-side-chain PHAs [56], novel functionalized side

chains [57], and copolymers containing 4-hydroxybutyrate, 4-HB, [58] or 3-hydroxybutyrate, 3-HB, [59] are biosynthesized depending on the carbon source, the organism and the growth conditions [60]. Biosynthesis of PHB with inexpensive carbon sources such as methanol or hydrogen gas and carbon dioxide [61], and by halophilic microorganisms, *Haloferax mediterranei*, with glucose [62] or starch [63] as the carbon source, have also been demonstrated. The biodegradability of PHB, PHBV (with various valeric acid contents), longer side chains and 4-PHB has been well documented [64]. In addition, some of the esterase enzymes involved in the initial hydrolytic depolymerizations step have been identified and characterized [65]. However, the degradation rates *in vivo* appear to be extremely slow.

Poly(caprolactone)

Poly(ε-caprolactone), PCL, is an aliphatic polyester synthesized by the ring-opening polymerization of ε-caprolactone with a catalyst like stannous octanoate in the presence of an initiator that contains an active hydrogen atom [36]. Molecular weight vary from 2000 to 80000 Da [66]. PCL can be degraded by microorganisms (enzymes, lipases) and also by a hydrolytic mechanism under physiological conditions [67]. Also, low-molecular-weight fragments of PCL are reportedly taken up by microphages and degraded intracellularly [68]. As the molecular weight of PCL increases the rates of biodegradation decrease [69]. PCL degrades at a much slower pace than PLA and can therefore be used for the design of long-term, implantable drug delivery systems that remain active for more than one year. Interest in slowly degrading polymers has, however, decreased.

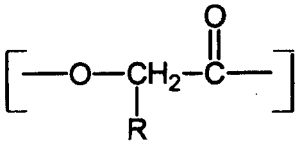
NONENZYMATICALLY DEGRADABLE SYNTHETIC MICROPARTICLES

By contrast to naturally occurring polymers, synthetic polymers have not, in general, been in existence long enough to have enzymes or microorganisms evolve that can utilize them as food. Degradable

synthetic polymers offer several advantages over enzymatically degradable natural polymers. Generally, the nonenzymatically degradable synthetic polymers are less immunogenic and more biocompatible than many natural polymers. Synthetic polymers can be prepared reproducibly under carefully controlled conditions and can be made available, if needed, in almost unlimited quantities. The mechanical properties of some synthetic polymer materials are maintained even in harsh biological environments, and are easily modified by chemical and physical modifications. Thus, degradable synthetic polymers have been considered to be more useful as biomedical materials than the natural polymers.

Poly(α-hydroxy esters)

Poly(α-hydroxy esters) such as poly(glycolic acid), PGA, or poly(lactic acid), PLA, are crystalline polymers with relatively high melting point. They are obtained by ring-opening polymerization of cyclic glycolides and lactides or the direct condensation of lactic and glycolic acids and depending on the method of synthesis, the molecular weight can be varied. Poly(glycolic acid) often called poly(glycolide) (Fig. 8), is the simplest linear, aliphatic polyester [30]. Poly(lactic acid) also called poly(lactide), is more hydrophobic than PGA, due to the presence of an extra methyl group. It has been studied since the 1960s as degradable polymer [70]. Since lactic acid is a chiral molecule, it exists in two stereoisomeric forms (enantiomers) which give rise to four morphologically distinct polymers. They degrade more slowly than a *DL* mixture [71]. The *L*-form is highly crystalline and the melt



Polyglycolide (R = H), polylactide ($R = CH_3$)

FIGURE 8

transition temperature decreases with lower molecular weight [72]. *D,L*-Lactide is the racemic polymer significantly more amorphous, with a correspondingly lower melting point. The release rate of drugs molecularly dispersed in poly(lactide) microspheres is governed by glass temperature of the polymer matrix [73]. Rates of hydrolysis and thus the drug release rate increase in polymers with lower glass temperature, *i.e.* as molecular weight decrease. Variations in the molecular weight of polymer, however, usually alter not only glass temperature but also the particle size and surface area of prepared microspheres. These factors also affect the drug release rate of the microspheres. Adjusting the glass temperature of microspheres without altering other physical properties may be achieved by γ-irradiation of the particles after preparation [74]. Introduction of poly(ethylene glycol) within poly(*DL*-lactide) increases the degradation rate [75]. The role of enzymatic versus chemical hydrolysis is, to date, not clear [76]. Certain hydrolytic enzymes, such as pronase, proteinase K and bromelain, accelerate the rate of PLA degradation, other enzymes such as liver esterase do not [77]. Nevertheless it is supposed that in aqueous environments, such as body fluids, PGA and PLA degrade by simple hydrolysis of the ester backbone. The polymer breaks down to lactic or glycolic acid, a normal product of carbohydrate metabolism. Furthermore, the degradation products are ultimately metabolized to carbon dioxide and water or are excreted via the kidneys [30]. Poly(glycolic acid) exhibits a more rapid rate of bioabsorption than poly(lactic acid). Poly(lactic acid-*co*-glycolid acid) (PLGA) materials can be prepared so that their degradation rates range between PLA and PGA extremes. Especially low molecular weight PLGA microspheres are known to be able to incorporate high peptide load [78].

If formulated as nanoparticles, the major problem is their rapid clearance from the bloodstream, which is non-specific and characteristic for all colloidal drug carriers. Therefore surfactants containing poly(ethyleneoxide) have been explored to alter surface properties such as surface charge and hydrophobicity of poly(lactide) nanoparticles. *In vivo* studies with rats confirmed that the circulation half-life of these particles was significantly higher than the half-life of the degradable part alone [79].

Poly(*ortho* esters)

Poly(*ortho* esters), POE, are a family of synthetic degradable hydrophobic polymers that can undergo an erosion process confined to the polymer-water interface under certain conditions. The properties of POEs can ne controlled to a large extent by the choice of the diols used in the synthesis. The *ortho*-ester linkage is far more stable in base than in acid. Upon hydrolysis, these POEs release acidic by-products that autocatalyze the degradation process, resulting in degradation rates that increase with time.

Polyanhydrides

Polyanhydrides, such as poly(sebacic anhydride), SA, hydrolyze more quickly (*in vivo* after 72 days) than poly(lactic acid) [80]. By varying the ratio of the aliphatic to the aromatic monomers during the copolymerization of sebacic acid and 1,3-bis-(carboxyphenoxypropane), CPP, the type of erosion taking place could be controlled. Polymers with high SA content erode more quickly than those with a high CPP content, causing increased rates of protein release. It is theoretically possible to deliver drugs for periods ranging from hours to years just by changing the ratio of SA to CPP in the polymer [81]. The hydrophobeicity and crystallinity of the polyanhydrides are two of the most important factors controlling the type of erosion [82]. Polymers made of aromatic monomers degrade much more shortly than polymers of aliphatic monomers. Poly(adipic anhydride) prepared by ring-opening polymerization of oxepan-2,7-dione initiated with triethyl amine [82] displays surface erosion which completely releases entrapped drug after 7 h at 37°C. Characteristic for the surface-eroding polyanhydrides is the approximately constant release rate [82]. On the contrary, bulk-eroding polyesters, such as poly(lactic-*co*-glycolic acid), reveal an *S*-shaped release profile at low loadings.

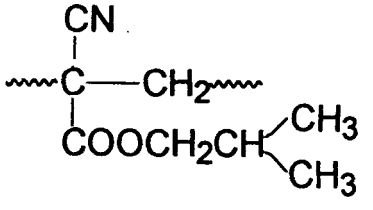
Poly(alkylcyanoacrylates)

Previously, polymers based up on alkylcyanoacrylates received considerable attention, however, the interest in these systems has

declined because of concerns over toxicity. Formaldehyde is produced during degradation of the polymers *in vivo*. Poly(isobutyl 2-cyanoacrylate) (Fig. 9) degrades in two enzyme-free media at pH 7 and pH 12 in the presence of rat-liver microsomes [83]. The formaldehyde-producing degradation route is less efficient, and the ester hydrolysis is catalyzed by enzymes. The degradation of poly(ethylcyanoacrylate), PECA, microspheres which are produced by dispersion polymerization occures mainly by surface erosion [84]. Higher degradation rates are observed with the PECA microspheres produced with higher poly(ethylene oxide-*co*-propylene oxide) stabilizer and lower hydrochloric acid concentrations.

Polyphosphazenes

Polyphosphazenes with hydrolytically labile substituents also have a potential for biodegradable applications. With proper choice of substituents, polymers can be prepared which can degrade into harmless products [30]. Certain side-group structures impart hydrolytic sensitivity to polyphosphazenes, examples being those with amino acid ester or imidazolyl side groups [85]. For example, the ethyl glycinato derivative [36] hydrolyzes slowly in aqueous media to yield ethanol, glycine, phosphate, and ammonia. Polyphosphazenes are suitable for a production of microparticles that range in size from 5 to 200 μm or small 100–200 nm.



Poly(isobutyl-2-cyanoacrylate)

FIGURE 9

PREPARATION METHODS

A great number of techniques are available for the preparation of biodegradable microspheres. They include solvent evaporation after aqueous microemulsification, organic phase separation, often referred as coacervation, dispersion polymerization, spray-drying, hot melt encapsulation [33], supercritical fluid technology [86], etc. The last two techniques are potentially suitable for the encapsulation of peptide and protein drugs. Hot melt encapsulation has, however, the disadvantage that the drug must be exposed to higher temperature. The most popular methods for the preparation of biodegradable microparticles are therefore solvent evaporation and phase separation techniques since they can readily be performed in the laboratory without specialized equipment. The choice of one particular method is primarily determined by the solubility characteristics of the drug and of the polymer. If the core material is water insoluble, either of the methods can be considered. For water-soluble core materials, only the organic phase separation procedure is suitable [87]. Organic phase separation method results in the formation of microcapsules (core-shell structure) *vs.* microspheres (matrix structure) formed by the solvent evaporation method. The disadvantage of both these manufacturing processes stems from the fact that commonly used sovents for polymers are harmful compounds like halogenated hydrocarbons, cyclic ethers, nitrides or amides.

Organic Phase Separation Method

In the organic phase separation method, the wall-forming polymer is precipitated around a dispersed drug phase. This can be induced by the addition of nonsolvents to the polymer, addition of a second polymer incompatible with the wall-forming polymer, and change in temperature [88]. As an example of a nonsolvent (coacervating agent) added to a polymer solution containing an active compound, silicon oil can be named [89]. The dispersion is then transferred into a hardening agent, where solid microspheres are formed.

Temperature can be used as another means to induce phase separation. Phase separation induced by temperature change is used for the preparation of gelatin microspheres crosslinked with glutar-

aldehyde [90] or uncrosslinked [8]. It takes advantage of the fact that gelatin is soluble in a hot aqueous solution but insoluble on cooling to room temperature. The extent of crosslinking is an effective parameter in the control of the size of the microspheres. When the amount of crosslinker is increased, the size of the microspheres decreases and this occurrence may be explained by increased crosslinking density resulting in the formation of densely packed structure like helical formation causing a shrinkage of the particles. Increasing crosslinking results in slower rate of water evaporation and leads to the formation of microspheres with a smooth surface. Another insolubilization process may involve spraying of warm aqueous gelatin solution through a cappilary into cold water containing glutaraldehyde forming a Schiff base [91]. Narrow particle size distribution results. The particle size is about 1 mm and smaller, depending on the concentration of the crosslinker, the conditions of spraying and the temperature of the medium [90]. Drug-loaded gelatin microspheres can also be prepared by ultrasonically emulsifying an aqueous drug/gelatin solution in excess sesame seed oil [92]. In these systems environmentally undesirable solvents such as diethyl ether or chloroform are required to remove the oil phase of the emulsion and the resulting microspheres often contain residual surfactants used as emulsifiers.

Solvent Evaporation Method

In the solvent extraction/evaporation method, an aqueous solution of the drug (*e.g.*, peptide, aclarubicin [93], adriamycin [94]) is dispersed or emulsified into an organic polymer solution (typically polyanhydride [95], poly(lactide-*co*-glycolide), poly(lactide-*co*-glycolide) and poly(-ethylene glycol), poly(*d,l*-lactide) [93], or poly(*D*, *L*-lactide-*co*-ethylene glycol) [75] in dichloromethane), to form a primary water in organic emulsion (w/o), which is then emulsified by sonication into an external aqueous or oil (*e.g.*, linseed or peanut oil) continuous phase [96] to form a w/o/w or w/o/o emulsion. The organic solvent diffuses from the emulsion droplets into the external aqueous (or oil) phase and evaporates. Inner oil phase gives solid dispersion. Resulting microspheres are usually collected by centrifugation, washed with water and lyophilised [97]. Advantage of w/o/o double emulsion technique, when compared with more conventional protein encapsulation using water-

in oil-in water (w/o/w) methods, include much higher protein entrapment, a marked reduction of burst effect and more uniform and controllable release characteristics [98]. Typical size is 75–150 μm or even in tens of micrometers. A reduction in particle size of polylactide and poly(lactide-*co*-glycolide) microspheres to a submicron size range can be achieved by introduction of water-miscible organic solvent to the organic phase of the emulsification solvent evaporation protocol. This modification was named "spontaneous emulsification solvent evaporation method" [99]. Further reduction in the particle size to sub-200 nm range can be reached applying a preparation method based on precipitation of the polymer from its solution by the addition of miscible nonsolvent [100].

In most cases, a surfactant is required to stabilise the dispersed phase. Poly(vinyl alcohol), poly(vinylpyrrolidone), or methyl cellulose are the most frequently used polymeric surfactants [101], however, bovin serum albumin emulsifies poly(α-hydroxy acids) as well [102]. Mean particle size of the microspheres is inversely related to the stirring speed and the emulsifier (surfactant) counterion. Sorbitan monooleate (Span 80) as an emulsifier and *n*-hexanol as a cosurfactant are used in preparation of porous chitosan beads with typical diameter 70 or even 250 μm in toluene continuous phase. Hydroxypropyl chitosan hollow microbeads are manufactured from w/o (toulene) emulsion stabilized with sorbitan monooleate by crosslinking hydroxyl groups with epichlorohydrin under alkaline condition [103]. Using an oil-in-oil solvent removal technique, poly(adipic anhydride) microspheres with an average size 40 μm are prepared by adding methylene chloride solution of polymer to sesame oil containing Span 80 under vigorous stirring [82]. Using the same technique [104] poly(glycolic-*co*-lactic acid) is dissolved in dimethyl formamide containing cisplatin and the solution was added dropwise to liquid paraffin containing Span 80 resulting in 5 to 40 μm microspheres. Solvent extraction/evaporation method involves intrinsic variables, such as solvent-polymer interaction parameters, molecular weight of polymer, and extrinsic variables, such as dispersed phase/continuous phase ratio, temperature, dispersed phase composition. These variables control the particle size. Higher viscosities of the organic phase caused by higher molecular weights or by increasing solvent/polymer ratio result in larger particles [75].

Dispersion Polymerization

The term of dispersion polymerization usually covers the polymerization of apolar monomers by using the dispersion media in which all ingredients, including monomer, are completely soluble initially. The nucleation occurs when the polymer chains reach to a certain molecular weight at which they become insoluble in the dispersion medium. Thermal initiation is usually selected and macromolecular stabilizers are used to prevent the aggregation of microspheres in the medium. Under optimum conditions in the system with fast particle nucleation in the initial period of the polymerization, followed with a continuous size increase of growing particles, and accompanied with an efficient stabilization, monosize microspheres in the micron-size range can be obtained [105]. In the dispersion polymerization of ethylcyanoacrylate the polymerization occurs via an anionic mechanism involving initiation by nucleophillic attack on the β-carbon of ethylcyanoacrylate [106]. This polymerization is performed by using a stabilizer system containing a poly(ethylene oxide-*co*-propylene oxide) and dextran in the aqueous hydrochloric solution as a dispersion medium. Phosphoric acid is then used as a catalyst. The average size in the range 0.5 – 2.5 μm and the size distribution (monodispersity) of poly(ethylcyanoacrylate) microspheres does not change significantly with the phosphoric acid concentration and molecular weight and concentration of dextran. The microsphere size significantly decreases with increasing poly(ethyleneoxide-*co*-propyleneoxide) and hydrochloric acid concentration [84].

Poly(*D*,*L*-lactide), poly(*L*,*L*-lactide) and poly(ε-caprolactone) particles smaller than 5 μm can be prepared in heptane-1,4-dioxane mixed solvent by the dispersion ring-opening polymerization initiated with tin(II) 2-ethylhexanoate in the presence of poly(dodecylacrylate)-*g*-poly(ε-caprolactone) stabilizer [107].

Other Techniques

Various other techniques for preparation of biodegradable particles are described. For example, microspheres with size of 150 μm are prepared from 2-vinyl-4,4-dimethyl-2-oxazolin-5-one derivatized starch through interfacial crosslinking with dipropyleneglycol diacry-

late by a water-in-oil polymerization using Pluronic F68 emulsifier [108]. An interesting technique to prepare thermo-sensitive poly(γ-benzyl *L*-glutamate-*b*-*N*-isopropylacrylamide) core/shell nanoparticles 70–360 nm in size consists in dialysing the block copolymer dissolved in *N*, *N*-dimethylformamide [109]. Nanoparticle size depends on the nature of the solvent, temperature and dialysis speed. Such particles release higher content of drug (indomethacin) at 28°C than 34° and if the content of hydrophobic glutamate blocks is lower.

Thermal denaturation at an elevated temperature (95–170°C) or chemical cross-linking in vegetable oil or organic solvent emulsions are two basic methods for the production of albumin microspheres. In the latter case, an aqueous protein is prepared by dissolving a lyophilised quantity of human serum albumin in a phosphate buffer, saline and drug (*e.g.*, doxorubicin or daunorubicin). Solution of human serum albumin is added to an oil phase consisting of petroleum ether and cotton seed oil containing Span 80 and the mixture homogenised. The droplets are stabilized by cross-linking effect of varying amount of glutaraldehyde. The diameter of albumin microspheres prepared by these methods ranges from 0.2 μm to 100 μm.

Thermal condensation of oligoamino acids synthesized by oligomerization of amino acid *N*-carboxyanhydrides, such as *L*-aspartic and *L*-glutamic acids, *L*-phenylalanine and *L*-tyrosine under benzylamine [110] or *L*-tyrosine methyl ester [111] intiation produces proteinoids. The driving force for microspheres formation might be related to a combination of hydrogen-bonding, hydrophobic interaction, and charge transfer interaction. The capability of an amino acid oligomer to form microspheres is strongly affected by its composition, which is selected to balance the hydrophilicity and hydrophobicity. The stability of microspheres the diameter of which varies from 0.5 to 20 μm, has been found to be pH dependent. They appear at low pH's (<4) and spontaneously disassemble under neutral pH conditions.

BIOMEDICAL APPLICATIONS

Drug Delivery

Particulate drug delivery systems, often also termed as micro (or nano)sphere-based controlled release systems, are utilised in various

areas of modern medicine as a means of improving therapeutic efficiency of bioactive materials such as proteins and peptides, antigens and other drugs [112]. Much attention has focused especially on poly(lactic acid), poly(glycolic acid) and poly(*D*,*L*-lactide-*co*-glycolide) particles [113] in which a branched peptide immunogen (200 M), representing a portion of the principal neutralizing determinant of HIV-1, indomethacin [114], progesterone [115], aclarubicin HCl, somatostatin acetate [116], thyrotropin releasing hormone [117], a somatostatin antagonist [118], several LH-RH analogues [119], porcine somatotropin [120], interleukin-2 [121], norgesterol, bone morphogenic protein [122] have been encapsulated. Alginate microspheres have also been used to encapsulate various vaccines, antigens, microorganisms and cells [30, 123]. Ideally, the controlled release device enables the agent to be delivered over prolonged time periods. Its advantages include the use of lower amounts of drug and hence, reduction in costs and toxic side effects, and improved patient compilance. Recent advances in delivery systems are focusing on drug targeting. Drug targeting is especially useful for cancer treatment whereby the cancer drug is selectively delivered into the malignant tumor cells. Decreasing the particle polymer molecular weight is believed to result in a faster drug release rate due to the faster degradation of the polymer matrix [124]. Once encapsulated, the active agent can exist in several different physical states: crystalline, molecularly dispersed in the polymer matrix, or existing as a metastable dispersion with the carrier matrix. This can also greatly influence the drug release rate and storage stability of the microspheres [125]. The level of drug loading in the microspheres significantly affects the initial burst phase. To prevent the loss of encapsulated materials and to reduce the burst effect, the microcapsules can be coated with another polymer that forms a membrane at the bead surface. This membrane acts as a rate controlling barrier to drug release and thereby prolongs release of drug when compared to uncoated microspheres. The most well-known and promising system is the encapsulation of alginate beads with poly(*L*-lysine) [126] or with a polyelectrolyte complex of sodium alginate, an anionic polysaccharide, and a derivative of chitosan, a cationic polysaccharide [127]. The release rate of drug from such microcapsules may depend mainly on two factors. One is the thickness and the other is the compactness of the

membrane that forms the outer shell of the microcapsule. This can be controlled by the pH where the capsules are formed and the kind of groups in the chitosan [127].

Particulate drug delivery systems are gaining attention for use in oral, systemic and parenteral drug delivery routes [128].

Oral Drug Administration

Oral drug administration is the most popular, convenient, and traditionally preferred mode of delivery of therapeutic agents. Conventionally, therapeutic drug levels are achieved by taking multiple and regularly spaced daily doses of medicine [129]. The need to remember to take the pills or tablets frequently is obviated by using colloidal particulate carriers such as microspheres as an oral delivery system. They are ideal in providing a constant therapeutic and nontoxic level of the drug. Many small drug molecules are chemically and structurally stable and remain unchanged by oral delivery. However, macromolecular drugs such as heparin, insulin and human growth hormone are very sensitive to the components of the gastrointestinal tract. Specific digestive processes, especially hydrolysis, can efficiently transform these agents into molecular fragments that are pharmacologically ineffective since their activity is directly dependent on specific molecular structures [110]. One primary approach to protect the incorporated drug in the system as well as the system itself from degradation in the gastric juice is to coat (encapsulate) the drug system with biocompatible, nontoxic polymers, such as alginate, that are insoluble at acidic pH of the stomach but dissolve in the alkaline pH of the intestine and release the drug. On the other hand, different pH sensitivity is obtained at chitosan coats on gelatin microspheres containing the anticancer drug methotrexate [129]. Chitosan, which is soluble at acidic pH but insoluble at alkaline pH, prolonged the release of methotrexate in intestinal fluid. It is possible to control release rate of methotrexate by selecting appropriate concentrations of the coating polymers or by varying the size of the gelatin microspheres [129]. Chitosan/gelatin hybrid polymer network microspheres loaded with cimetidine are suggested for treatment of gastric ulceration [130].

In some cases, oral administration of protein and polar macromolecular drugs results in extremely low to no bioavailability due to their poor membrane permeability from the gastrointestinal tract. Therefore proteinoid microspheres are being developed aimed at delivering therapeutics such as polysaccharides and proteins, *e.g.*, insulin and heparin, which are being presently administered *via* injection. To encapsulate a drug, the proteinoid material is dissolved in an aqueous solution at pH 7. This solution is added to a therapeutically desirable drug solution at low pH and vigorously shaken. Under these acidic conditions [131], the proteinoids form stable microspheres with encapsulated drugs ranging in size from 5 to 10 μm. The size is usually dependent on the proteinoid used and the agitation, *i.e.*, smaller spheres are formed by ultrasonic agitation (0.5 μm) compared to hand agitation (10–15 μm). In this manner, the proteinoid microspheres are able to protect a therapeutic agent, such as standard and low-molecular weight heparin, heparinoids, calcitonin, human growth hormone, vaccines, monoclonal antibody IgG2a, polar compounds such as sodium cromolyn and desferoxamine, from gastric acids and enzymes in the stomach and then release it into the intestinal adsorption area [48]. By using proteinoid microsphere oral delivery system, high plasma levels and higher bioavailability are obtainable [48]. In essence, proteinoids have a dual function; in the microsphere phase, proteinoids have the ability to protect drug cargoes from acids and enzymes; and as soluble materials, the proteinoids facilitate the absorption of a therapeutic agent from the intestine into the blood stream [48].

Other oral biodegradable carriers include poly(lactide) and poly(-lactide-co-glycolide) particles and poly(alkylcyanoacrylate) nanoparticles with physically entrapped or adsorbed drugs. Oral immunization via targeting to Peyers patches by antigen encapsulated in poly(lactide-co-glycolide) microparticles has been studied and its many advantages compared to parenteral administration of vaccines have been shown [132]. Target diseases include influenza, polio, measles, pertunis, cholera, tetanus and so on. Nanoparticles of ~100 nm diameter containing covalently bound peptide prepared by copolymerization of lutenizing hormone releasing hormone-vinylacetate with *n*-butylcyanoacrylate are also described [133].

Parenteral Drug Administration

Micro- and nanoparticles, such as those based on chitosan, human albumin or poly(caprolactone-β-(*d,l*) lactide), are a promising vehicle for parenteral delivery of the protein and polar macromolecular drugs, genes, antigens and vaccines [48], which are currently administered via injection into the muscle to provide enhanced immune response. To avoid difficulty during injection, particles with high degree of sphericity and monodispersity and with much smaller size than those administered orally are required. By gellation of chitosan in the presence of poly(ethylene oxide) and poly(ethylene oxide-*b*-propylene oxide) pH-sensitive nanoparticles are formed, the size of which increases with the increasing concentration and molecular weight of both stabilizers [46]. Temperature-sensitive nanoparticles for parenteral administration are developed from poly(γ-benzyl *L*-glutamate-*b*-*N*-isopropylacrylamide) [109]. The uptake of micron-size monosized polymeric particles by white blood cells and macrophages is higher than that of polymeric particles in the submicron size range [134]. Injectable poly(lactide) microspheres containing antimetabolic agent adriamycin are suggested as a therapeutic agent for glaucoma filtering surgery [94]. Poly(glycolic-*co*-*L*-lactic acid) particles containing cisplatin are promising to enhance the antitumor effect of the drug and to reduce its systemic toxicity [104]. They enable to increase drug dose to the level 5-times higher than its LD_{50} value. A sustained release of cisplatin can be achieved over 20 days without a large initial burst. About 70% of the incorporated drug is released within the initial 7 days and the microspheres are completely digested after about 8 months. Cisplatin loaded poly(lactic-*co*-glycolic acids) microspheres ranging from 180 to 250 μm were suggested for direct injection into brain tumors [124]. Release of cisplatin, approximately 80% of which took place within over 30 days, is a combination of diffusion and erosion processes.

Intraarterial Applications

Popular drug carrier used in intraarterial applications are albumin microspheres with entrapped adriamycin. In order to overcome problems connected with low adriamycin payloads and with a large

burst effect usually observed (80–90% of the drug is released within 30 min. in any aqueous medium), highly negatively charged albumin-heparin conjugate microspheres having thus ion-exchange properties are developed [135]. Pendent amino groups of the lysine residues of the albumin are used to crosslink the material in order to obtain stable microspheres. The anionic groups of the heparin (such as sulfamate groups and carboxylic acid groups) allow ionic interaction with the adriamycin, thus enabling high payloads (up to 34%) and ion-controlled drug release. Other biodegradable microspheres, which behave as an ion-exchanger and which can be loaded with adriamycin up to a payload of 60%, are based on sulfamated polylysine stabilized using glutaraldehyde or oxidized dextran as a crosslinking agent [136].

Another microspherical particles of about 58 μm diameter are produced from polydepsipeptide (poly(Ala-Ala-Glu(OEt)-Lac)) containing β-estradiol. If implanted into the animal, the effective pharmacological level of the drug is kept during the first 12-week period. This is followed by an incomplete supplying of drug until the implant is perfectly degraded in the 25th week from the start of implantation [137].

Chemoembolization

Classical cryostatic agents widely used for the treatment of cancer have, in general, a narrow therapeutic window. Due to the high systemic levels of the drug needed to achieve sufficiently high drug levels at the tumor site, severe side effects such as cardiomyopathy can occur [138]. A reduction of the side effects can be achieved if the drug is targeted to the site of action using microspheres which is known as chemoembolization [139]. Chemoembolization refers to transcatheteral administration of drug-loaded particles of the requisite size (15–60 μm), to cause tumor infarction by occlusion or to induce transient ischemia within the tumor vasculature, whereupon the entrapped drug (*e.g.*, 5-fluorouracyl, mitomycin) can be released locally [140]. The great advantage of this procedure consists in that toxicity of the microsphere-formulated drug decreases as compared with the free drug, thus improving the therapeutic index [141]. An essential aspect of chemoembolisation as a targeting technique is the biodegradation of the system [83]. Therefore microspheres and microcapsules from

ethylcellulose [13], starch [14], casein [22], human serum albumin [142, 143], poly(*D*,*L*-lactide) [19, 144], hyaluronic acid [145] (sympo Berlin 1992) and poly(isobutyl 2-cyanoacrylate) [83] have been utilised for targeting as (chemo)embolic agents in the treatment of vascular and tumoral lesions. Particles greater than 7 μm in diameter become embolised in the first capillary bed encountered after intravenous administration [6]. Then larger and larger particles follow, obturating blood vessels from periphery to proximal region of the tumor.

Other Applications

Other applications include the use of the particles in designing supports for cell growth and the design of cyanoacrylic nanoparticles as a lysosometric and biodegradable drug carrier [146]. Cyanoacrylic nanoparticles have been also used in applications such as ocular drug carriers to enhance the corneal penetration of polar drugs and as carriers with monoclonal antibodies for targeting cytotoxic agents to tumor cells [147]. Likewise polyanhydride microspheres with incorporated timololol maleate seem to be promising in ophthalmology [82]. Strarch particles are suggested as a model of the human oocyte during its transport through the oviduct (Fallopian tube). These biocompatible oocyte surrogates could thus provide the information about functional disorders of tubal transport, unavailable yet by classical diagnostic methods, in female patients who were indicated for tubal patency examination because of long-term unexplained infertility [40].

References

[1] Gupta, P. K. (1990). Drug targeting in cancer chemotherapy: A clinical perspective, *J. Pharm. Sci.*, **79**, 1–14.

[2] Laakso, T., Stjarnkvist, P. and Sjoholm, I. (1987). Biodegradable microspheres VI: Lysosomal release of covalently bound antiparasitic drugs from starch microparticles, *J. Pharm. Sci.*, **76**, 134–140.

[3] Visscher, G. E., Robison, R. L., Maulding, H. V., Fong, J. W., Pearson, J. E. and Argentieri, G. J. (1985). Biodegradation of and tissue reaction to 50:50 poly(*DL*-lactide-*co*-glycolide) microcapsules, *J. Biomed. Mater. Res.*, **19**, 349–365.

[4] Langer, R. S. and Peppas, N. A. (1981). Present and future applications of biomaterials in controlled drug delivery systems, *Biomaterials*, **2**, 201–214.

[5] Langer, R. and Peppas, N. A. (1983). Chemical and physical structure of polymers as carriers for controlled release of bioactive agents: A review, *Rev. Macromol. Chem. Phys.*, **C23**, 61–126.

[6] Illum, L. and Davis, S. S. (1982). The targeting of drugs parenterally by use of microspheres, *J. Parenter. Sci. Technol.*, **36**, 242–247.
[7] Gurny, R., Peppas, N. A., Harrington, D. D. and Banker, G. S. (1981). Development of biodegradable and injectable latices for controlled release of potent drugs, *Drug Dev. Ind. Pharm.*, **7**, 1–25.
[8] Hashida, M., Muranishi, S., Sezaki, H., Tanigawa, N., Satomura, K. and Hikasa, Y. (1979). Increased lymphatic delivery of bleomycin by microsphere in oil emulsion and its effect on lymph node metastasis, *Int. J. Pharm.*, **2**, 245–256.
[9] Lidgate, D. M., Fu, R. C., Byars, N. E., Foster, L. C. and Fleitman, J. S. (1989). Formulation of vaccine adjuvant muramylpeptides. 3. Processing optimisation, characterisation and bioactivity of an emulsion vehicle, *Pharm. Res.*, **6**, 748–752.
[10] Roerding, F. H., Daemen, T., Baker-Woundenburg, I. A. J. M., Storm, G., Crommelin, D. J. A. and Scherphof, G. L. (1987). Drug Delivery Systems: Fundamentals and Techniques, Ellis Horwood, Chichester, pp. 66–80.
[11] Florence, A. T. and Baillie, A. J. (1989). Non-ionic surfactant vesicles-alternatives to liposomes in drug delivery, in: *Novel Drug Delivery and its Therapeutic Application* (Prescott, L. F. and Nimmo, W. S., Eds.), John Wiley, New York, pp. 281–296.
[12] Kato, T., Nemoto, R., Mori, H., Takahashi, M. and Harada, M. (1981). Arterial Chemoembolization with mitomycin C microcapsules in the treatment of primary or secondary carcinoma of the kidney, liver, bone and intrapelvic organs, Cancer, **48**, 674–680.
[13] Kerr, D. J., Goldberg, J., McArdle, C. S. and Whateley, T. (1990). Intrahepatic arterial administration of mitomycin C microcapsules, *Br. J. Cancer*, **62**, 527–533.
[14] Tuma, R. F. (1984). The use of degradable starch microspheres for transient occlusion of blood flow and for drug targeting to selected tissues, in: *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects* (Davis, S. S., Illum, L., McVie, J. G. and Tomlinson, E., Eds.), Elsevier, Amsterdam, pp. 189–203.
[15] Artursson, P., Arro, E., Edman, P., Ericsson, J. L. E. and Sjoholm, I. (1987). Biodegradable microspheres. V. Stimulation of macrophages with microparticles made of various polysaccharides, *J. Pharm. Sci.*, **76**, 127–133.
[16] Laakso, T., Andersson, J., Artursson, P., Edman, P. and Sjoholm, I. (1986). Acrylic microspheres *in vivo*. X. Elimination of circulating cells by active targeting using specific monoclonal antibodies bound to microparticles, *Life Sci.*, **38**, 183–190.
[17] Kreuter, J. and Hartmann, H. R. (1983). Comparative study on the cytostatic effects and the tissue distribution of 5-fluorouracil in a free form and bound to polybutylcyanoacrylate nanoparticles in sarcoma 180-bearing mice, *Oncology*, **40**, 363–366.
[18] Wasser, P. G., Muller, U., Kreuter, J., Berger, S., Munz, K., Kaiser, E. and Pfluger, B. (1987). Localization of colloidal particles (liposomes, hexylcyanoacrylate nanoparticles and albumin nanoparticles) by histology and autoradiography in mice, *Int. J. Pharm.*, **39**, 312–327.
[19] Flandroy, P., Grandfils, C., Collignon, J., Thibaut, A., Nihard, N., Barbette, S., Jérome, R. and Teyssie, P. (1990). (*D,L*)-polylactide microspheres as embolic agent. A preliminary study, *Neuroradiology*, **32**, 311–315.
[20] Verrijk, R., Smoders, I. J. H., Bosnie, N. and Begg, A. C. (1992). Reduction of systemic exposure and toxicity of cisplatin by encapsulation in polylactide-*co*-glycolide, *Cancer Res.*, **52**, 6653–6656.
[21] Miyazaki, S., Hashiguchi, N., Yokouchi, C., Takada, M. and Hou, W.-M. (1986). Antitumour effect of fibrinogen microspheres containing doxorubicin on Ehrlich ascites carcinoma, *J. Pharm. Pharmacol.*, **38**, 618–620.

[22] Chen, Y., Willmott, N., Anderson, J. and Florence, A. T. (1987). Comparison of albumin and casein microspheres as a carrier for doxorubicin, *J. Pharm. Pharmacol.*, **39**, 978–985.
[23] Chen, Y., Willmott, N., Anderson, J. and Florence, A. T. (1988). Haemoglobin, transferrin and albumin/polyaspartic acid microspheres as carriers for the cytotoxic drug adriamycin. I. Ultrastructural appearance and drug content, *J. Control. Release*, **8**, 93–101.
[24] Kissel, T., Brich, Z., Bantle, S., Lancranjan, I., Nimmerfall, F. and Vit, P. (1991). Parenteral depot-systems on the basis of biodegradable polyesters, *J. Control. Release*, **16**, 27–42.
[25] Washington, C. (1990). Drug release from monodisperse systems: A critical review, *Int. J. Pharm.*, **58**, 1–12.
[26] Ratner, B. D. (1989). Biomedical applications of synthetic polymers, in: *Comprehensive Polymer Science*, **7** (Allen, G., Bevington, J. C., Aggarwal S. L., Eds.), Pergamon Press , Oxford, pp. 201–247.
[27] Zaikov, G. E. (1985). Quantitative aspects of polymer degradation in the living body, *J. Macromol. Sci. Rev. Macrmol. Chem. Phys.*, **C25**, 551–597.
[28] Barrows, T. H. (1986). *Clin. Mater.*, **1**, 233.
[29] Vert, M., Christel, P., Chabot, F. and Lerry, J. (1984). In: *Macromolecular Biomaterials* (Hastings, G. W. and Duckeyne P., Eds.), CRC Press, *Boca Raton*, p. 119.
[30] Hayashi, T. (1994). Biodegradable polymers for biomedical uses, *Prog. Polym. Sci.*, **19**, 663–702.
[31] Ikada, Y. (1989). Kobunshi Shinsozai Binran (Japanese), Maruzen, Tokyo, p. 322,
[32] Hubbell, J. A. (1994). Chemical modification of polymer surfaces to improve biocompatibility, *Trends Polym. Sci.*, **2**, 20–25.
[33] Mathiowitz, E. and Langer, R. (1992). Polyanhydride microspheres as drug delivery systems, in: *Microcapsules and Nanoparticles in Medicine and Pharmacy* (Dunbrow, J., Ed.), CRC Press, Boca Raton, pp. 99–123.
[34] Kimura, Y. (1993). In: *Biomedical Applications of Polymeric Materials* (Tsurutu, T., Ed.), CRC Press, Boca Raton, pp. 163–189.
[35] Bailey, W. J. (1989). Ring-opening polymerization, in: *Comprehensive Polymer Science*, **3** (Allen, G., Bevington, J. C., Eastmond, G. C., Ledwith, A., Russo, S. and Sigwalt, P. Eds.), Pergamon press, Oxford, pp. 283–320.
[36] Mayer, J. M. and Kaplan, D. L. (1994). Biodegradable materials: Balancing degradability and performance, *Trends Polym. Sci.*, **2**, 227–235.
[37] Elion, G. R. (1993). US Patent 5 244 945.
[38] Buchanan, C. M., Gradner, R. M. and Komarek, R. J. (1993). Aerobic biodegradation of cellulose acetate, *J. Appl. Polym. Sci.*, **47**, 1709–1719.
[39] Wurzburg, O. B. (1986). Modified starches: Properties and uses, CRC Press, Boca Raton.
[40] Rypáček, F., Matějková, S. and Uher, J. (1995). Biodegradable hydrogel microspheres for studies of oocyte transport in human salpinx, *36th Microsymposium High-Swelling Gels*, Prague, SL1.
[41] Ammon, R. (1963). The occurrence of dextranase in human tissues, *Enzymologia*, **25**, 245–251.
[42] Pfister, G., Bahadir, M. and Korte, F. (1986). Release Characteristics of herbicides from Ca alginate gel formulations, *J. Control. Release*, **3**, 229–333.
[43] Edelman, E. R., Mathiowitz, F., Langer, R. and Klagsbrun, M. (1991). Controlled and modulated release of basic fibroblast growth factor, *Biomaterials*, **12**, 619–626.
[44] Santucci, E., Alhaique, F., Carafa, M., Coviello, T., Murtas, E. and Riccieri, F. M. (1996). Gellan for the formulation of sustained delivery beads, *J. Control. Release*, **42**, 157–164.

[45] Chandy, T. and Sharma, C. P. (1991). Bidegradable chitosan matrix for the controlled release of steroids, Biomater., *Artif. Cells Immobilization Biotechnol.*, **19**, 745–760.
[46] Calvo, P., Remuñán-López, C., Vila-Jato, J. L. and Alonso, M. J. (1997). Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers, *J. Appl. Polym. Sci.*, **63**, 125–132.
[47] Magee, G. A., Halbert, G. W. and Vilmott, N. (1995). Effect of process variables on the *in vitro* degradation of protein microspheres, *J. Control. Release*, **37**, 11–19.
[48] Ottenbrite, R. M., Zhao, R. and Milstein, S. (1996). A new oral microsphere drug delivery system, *Macromol. Symp.*, **101**, 379–387.
[49] Gonsalves, K. E. and Mungara, P. (1996). Synthesis and properties of degradable polyamides and related polymers, *Trends Polym. Sci.*, **4**, 25–33.
[50] Anderson, J. M., Spilizewski, K. L. and Hiltner, A. (1985). In: *Biocompatibility of Tissue Analogs*, **1** (Williams, D. F., Ed.), CRC Press, Boca Raton, p. 67.
[51] Lescure, F., Gurny, R., Doelker, E., Pelaprat, M. L., Bichon, D. and Anderson, J. M. (1989). Acute histopathological response to a new biodegradable polypeptidic polymer for implantable drug delivery system, *J. Biomed. Master. Res.*, **23**, 1299–1313.
[52] Kidchob, T., Kimura, S. and Imanishi, Y. (1996). Preparation, structure and release profile of polypeptide microcapsules, *J. Control. Release*, **40**, 285–291.
[53] Kidchob, T., Kimura, S. and Imanishi, Y. (1997). pH-Responsive release from polypeptide microcapsules, *J. Appl. Polym. Sci.*, **63**, 453–458.
[54] Steinbuechel, A. (1992). Biodegradable plastics, *Curr. Opin. Biotechnol.*, **3**, 291–297.
[55] Holmes, P. A., Collins, S. H. and Wright, L. F. (1984). US Patent 4 477 654.
[56] Gross, R. A., DeMello, C., Lenz, R. W., Brandl, H. and Fuller, R. C. (1989). Bisynthesis and characterization of poly(β-hydroxyalkanoate)s produced by pseudomonas oleovorans, *Macromolecules*, **22**, 1106–1115.
[57] Hazer, B., Lenz, R. W. and Fuller, R. C. (1994). Biosynthesis of nethyl-branched poly(β-hydroxyalkanoate)s produced by pseudomonas olevorans, *Macromolecules*, **27**, 45–49.
[58] Nakamura, S., Doi, Y. and Scandola, M. (1992). Microbial synthesis and characterization of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate), *Macromolecules*, **25**, 4237–4241.
[59] Nakamura, S., Kunioka, M. and Doi, Y. (1991). Biosynthesis and characterization of bacterial poly(3-hydroxybutyrate-*co*-3-hydroxypropionate), *Macromol. Rep.*, **A28**, 15–24.
[60] Page, W. J. and Knosp, O. (1989). Hyperproduction of poly-β-hydroxybutyrate during exponential growth of azobacter vinelandii UWD, *Appl. Environ. Microbiol.*, **55**, 1334–1339.
[61] Collins, S. H. (1987). Choice of substrate in polyhydroxybutyrate synthesis, *Spec. Publ. Soc. Gen. Microbiol.*, **21**, 161–168.
[62] Anton, J., Meseguer, I. and Rodriguez-Valera, F. (1988). Production of an extracellular polysaccharide by Haloferax mediterranei, *Appl. Environ. Microbiol.*, **54**, 2381–2386.
[63] Allen, A. L. and Ball, D. H. (1992). Continuous cultivation of haloferax mediterranei for the production of polyhydroxyalkanoates, *Proc. Am. Chem. Soc. Div. Polym. Mater. Sci. Eng.*, **67**, 132–133.
[64] Doi, Y., Kanesawa, Y., Kunioka, M. and Saito, T. (1990). Biodegradation of microbial copolyesters: Poly(3-hydroxybutyrate-*co*-hydroxyvalerate) and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate), *Macromolecules*, **23**, 26–31.
[65] Schimmer, A., Jendrossek, D. and Schlegel, H. G. (1993). *Appl. Environ. Microbiol.*, **59**, 1222.

[66] Benedict, C. V., Cameron, J. A. and Huang, S. J. (1983). Polycaprolactone degradation by mixed and pure cultures of bacteria and yeast, *J. Appl. Polym. Sci.*, **28**, 335–342.
[67] Pitt, C. G., Chasalow, F. I., Hibionada, Y. M., Klimas, D. M. and Schindler, A. (1981). Aliphatic polyesters. I. The degradation of poly(ε-caprolactone). *In vivo*, *J. Appl. Polym. Sci.*, **26**, 3779–3787.
[68] Woodward, S. C., Brewer, P. S., Moatamed, F., Schindler, A. and Pitt, C. G. (1985). The intracellular degradation of poly(ε-caprolactone), *J. Biomed. Mater. Res.*, **19**, 437–444.
[69] Fields, R. D., Rodriguez, F. and Finn, R. K. (1974). Microbial degradation of polyesters: Polycaprolactone degraded by P. pullulans, *J. Appl. Polym. Sci.*, **18**, 3571–3579.
[70] Kulkarni, R. K., Pani, K. C., Neuman, C. and Leonard, F. (1966). Poly(latic acid) for surgical implants, *Arch. Surg.* (Chicago), **93**, 839–854.
[71] Kulkarni, R. K., Moore, E. G., Heggyeli, A. F. and Leonard, F. (1971). Biodegradable poly(lactic acid). Polymers, *J. Biomed. Mater. Res.*, **5**, 169–181.
[72] Jamshidi, K., Hyon, S.-H. and Ikada, Y. (1988). Thermal characterization of polylactides, *Polymer*, **29**, 2229–2234.
[73] Aso, Y., Yoshioka, S., Li Wan Po, A. and Terao, T. (1994). Effect of temperature on mechanism of drug release and matrix degradation of poly(*D,L*-lactide) microspheres, *J. Control. Release*, **31**, 33–39.
[74] Yoshioka, S., Aso, Y. and Kojima, S. (1995). Drug release poly(*dl*-lactide) microspheres controlled by γ-irradiation, *J. Control. Release*, **37**, 263–267.
[75] Celikkaya, E., Denbas, E. B. and Piskin, E. (1996). Poly(*DL*-lactide)/poly(ethylene glycol) copolymer particles. I. Preparation and characterization, *J. Appl. Polym. Sci.*, **61**, 1439–1446.
[76] Vert, M., Li, S. M., Spenlehauer, G. and Guerin, P. (1992). Bioresorbability and biocompatibility of aliphatic polyesters, *J. Mater. Sci. Mater. Med.*, **3**, 432–446.
[77] Williams, D. F. (1981). *Eng. Med.*, **10**, 5.
[78] Mehta, R. C., Thanoo, B. C. and DeLuca, P. P. (1996). Peptide containing microspheres from low molecular weight and hydrophilic poly(*d,l*-lactide-*co*-glycolide), *J. Control. Release*, **41**, 249–257.
[79] Bazile, D., Prud'Homme, C., Bassoullet, M.-T., Marland, M., Splenlehauer, G., Veillard, M. and Stealth, M. (1994). PEG-PLA nanoparticles avoid uptake by the mononuclear phagocytes system, *J. Pharm. Sci.*, **84**, 493–498.
[80] Pekarek, K. J., Dyrud, M. J., Ferrer, K., Jong, Y. S. and Mathiowitz, E. (1996). *In vitro* and *in vivo* degradation of double-walled polymer microspheres, *J. Control Release*, **40**, 169–178.
[81] Leong, K. W., Brott, B. C. and Langer, R. (1985). Bioerodible polyanhydrides as drug-carrier matrices. I. Characterization, degradation and release characteristics, *J. Biomed. Mater. Res.*, **19**, 941–955.
[82] Albertsson, A.-Ch., Carlfors, J. and Sturesson, C. (1996). Preparation and characterization of poly(adipic anhydride). microspheres for ocular drug delivery, *J. Apply. Polym. Sci.*, **62**, 695–705.
[83] Huang, S. J. (1985). Biodegradable Polymers, in *Encycl. Polym. Sci. and Eng.*, **2**, Mark, H. F., Bikales, N. M., Overberger, Ch. G. and Menges, G., Eds., John Wiley, New York, pp. 220–243.
[84] Tuncel, A., Cicek, H., Hayaran, M. and Piskin, E. (1995). Monosize poly(ethylcyanoacrylate) microspheres: Preparation and degradation properties, *J. Biomed. Mater. Res.*, **29**, 721–728.
[85] Alkock, R.Polyphosphazenes, in *Encyclopedia of Polymer Science and Engineering* (Mark, H. F., Bikales, N. M., Overberger, Ch. G. and Menges, G. Eds.), **13**, John Wiely, New York 1988, pp. 31–41.
[86] Bleich, J., Müller, B. W. and Waßmus, W. (1993). Aeorosol solvent extraction system: A new microparticle production technique, *Int. J. Pharm.*, **97**, 111–117.

[87] Alex, R. and Bodmeier, R. (1990). Encapsulation of water-soluble drugs by modified solvent evaporation method. I. Effect of process and formulation variables on drug entrapment, *J. Microencapsulation*, **7**, 347–355.
[88] Ruiz, J. M. and Benoit, J. P. (1991). *In vitro* peptide release from poly (*D,L*-latic acid-*co*-glycolic acid copolymer). 50/50 microspheres, *J. Control. Release*, **16**, 177–186.
[89] Thomasin, C., Johansen, P., Alder, R., Bemsel, R., Hottinger, G., Altorfer, H., Wright, A. D., Wehrli, G., Merkle, H. P. and Gander, B. (1996). A contribution to overcoming the problem of residual solvents in biodegradable microspheres prepared by coacervation, *Eur. J. Pharm. Biopharm.*, **42**, 16–24.
[90] Akin, H. and Hasirci, N. (1995). Preparation and characterization of crosslinked gelatin microspheres, *J. Appl. Polym. Sci.*, **58**, 95–100.
[91] Chatterji, P. R. (1989). Gelatin with hydrophilic/hydrophobic grafts and glutaraldehyde crosslinks, *J. Appl. Polym. Sci.*, **37**, 2203–2212.
[92] Yoshioka, T., Hashida, M., Muranishi, S. and Sezaki, H. (1980). *Chem. Pharmacol. Bull*, **28**, 1009.
[93] Wada, R., Hyon, S.-H., Ikada, Y., Nakao, Y. Yoshikawa, H. and Muranishi, S. (1988). Lactic acid oligomer microspheres containing and anticancer agent for selective lymphatic delivery I. *In vitro* studies, *J. Bioactive Compat. Polym.*, **3**, 126–136.
[94] Hyon, S.-H. (1996). Ophthalmic drug delivery systems using biodegradable polymers, *Polym. Preprints*, **37**(2), 125–126.
[95] Chiba, M., Hanes, J. and Langer, R. (1997). Controlled protein delivery from biodegradable tyrosine-containing poly(anhydride-*co*-imide) microspheres, *Biomaterials*, **18**, 893–901.
[96] Wang, H. T., Schmitt, E., Flanagan, D. R. and Linhardt, R. J. (1991). Influence of formulation methods on the *in vitro* controlled release of protein from poly(ester) microspheres, *J. Control Release*, **17**, 23–32.
[97] Wada, R., Hyon, S.-H. and Ikada, Y. (1995). Kinetics of diffusion-mediated drug release enhanced by matrix degradation, *J. Control Release*, **37**, 151–160.
[98] Yek, M. K., Jenkins, P. G., Davis, S. S. and Coombes, A. G. A. (1995). Improving the delivery capacity of microparticle system using (resorbable) blends of poly(*DL*-lactide-*co*-glycolide) and poly(ethylene glycol), *J. Control Release*, **37**, 1–9.
[99] Niwa, T., Takeuchi, H., Hino, T., Kunou, N. and Kawashima, Y. (1994). *In vitro* drug release behaviour of *D,L*-lactide/glycolide copolymer (PLGA). nanospheres with naferelin acetate prepared by a novel spontaneous emulsification solvent evaporation method, *J. Pharm. Sci.*, **83**, 727–732.
[100] Fawaz, F., Bonini, F., Guyot, M., Lagueny, A. M., Fessi, H. and Devissaguet, J. P. (1996). Disposition and protective effect against irritation after intravenous and rectal administration of indomethacin loaded nanocapsules to rabbits, *Int. J. Pharm.*, **133**, 107–115.
[101] Fong, J. W. (1981). French Patent No. FRA 81 11694.
[102] Boury, F., Marchais, H., Benoit, J. P. and Proust, J. E. (1997). Surface characterization of poly(α-hydroxy acid). microspheres prepared by a solvent evaporation/extraction process, *Biomaterials*, **18**, 125–136.
[103] Ha, B.-J., Lee, O.-S. and Lee, Y.-S. (1996). Novel hollow microbeads based on hydroxypropyl chitosan by w/o emulsion cross-linking method, *Macromo. Reports*, **A33**, 297–308.
[104] Itoi, K., Tabata, C.-Y., Ike, O., Shimizu, Y., Kuwabara, M., Kyo, M., Hyon, S.-H. and Ikada, Y. (1996). *In vivo* suppressive effect of copoly(glycolic/*L*-lactic acid) microspheres containing CDDP on murine tumor cells, *J. Control Release*, **42**, 175–184.
[105] Shen, S., Sudol, E. D. and El-Aasser, M. S. (1993). Control of particle size in dispersion polymerization of methyl methacrylate, *J. Polym. Sci.*, Part A, **31**, 1393–1402.

[106] Douglas, S. J., Illum, L., Davis, S. S. and Kreuter, J. (1984). Particle size and size distribution of poly(butyl-2-cyanoacrylate) nanoparticles, *J. Colloid Interface Sci.*, **101**, (1984). 149–158.

[107] Sosnowski, S., Gadzinowski, M. and Slomkowski, S. (1996). Poly(*L,L*-lactide) microspheres by ring-opening polymerization, *Macromolecules*, **29**, 4556–4564.

[108] Duarte, M. G., Brunnel, D., Gil, M. H. and Schacht, E. (1997). Microcapsules prepared from starch derivatives, *J. Mater Sci. Mater Med.*, **8**, (1997). 321–323.

[109] Cho, Ch.-S., Cheon, J.-B., Jeong, Y.-I., Kim, I.-S., Kim, S.-H. and Akaike, T. (1997). Novel core-shell type thermo-sensitive nanoparticles composed by poly(γ-benzyl *L*-glutamate) as the core and poly(*N*-isopropylacrylamide) as the shell, *Macromol. Rapid Commun.*, **18**, 361–369.

[110] Yang, J.-Z., Antoun, S. and Ottenbrite, R. M. (1996). Amino acid oligomer microspheres as drug delivery systems. I. Synthesis of oligo(amino acids) via NCAs and their microsphere formation, *J. Bioactive Compat. Polym.*, **11**, 219–235.

[111] Yang, J.-Z., Wang, M. and Ottenbrite, R. M. (1996). Amino acid oligomer microspheres as drug delivery systems. II. Oligomerization of NCAs initiated with *L*-tyrosine methyl ester, *J. Bioactive Compact. Polym.*, **11**, 236–247.

[112] Pitt, C. G. (1990). The controlled parenteral delivery of polyeptides and proteins, *Int. J. Pharm.*, **59**, 173–196.

[113] Jeffery, H., Davis, S. S. and OHagan, D. T. (1993). The preparation and characterization of poly(lactide-*co*-glycolide). microparticles II: The entrapment of a model protein using a (water-in-oil)-in-water emulsion solvent evaporation techniques, *Pharm. Res.*, **10**, 362–368.

[114] Richey, T. and Harris, F. W. (1995). Preparation of characterization of indomethacin-loaded microspheres, *Polym. Preprints*, **36**(2), 255–256.

[115] Carrio, A., Schwach, G., Coudane, J. and Vert, M. (1995). Preparation and degradation of surfactant-free PLAGA microspheres, *J. Control. Release*, **37**, 113–121.

[116] Herrmann, J. and Bodmeier, R. (1995). The effect of particle microstructure on the somatostatin release from poly(lactide). microspheres prepared by a w/o/w solvent evaporation method, *J. Control. Release*, **36**, 63–71.

[117] Heya, T., Okada, H., Tanigawara, Y., Ogawa, Y. and Toguchi, H. (1991). Effects of counteranion of TRH and loading amount on control of TRH release from copoly(*dl*-lactic/glycolic acid) microspheres prepared by an in-water drying method, *Int. J. Pharm.*, **69**, 69–75.

[118] Mason-Garcia, M., Vaccarella, M., Horvath, J., Redding, T. W., Groot, K., Orsolini, P. and Schally, A. V. (1988). Radioimmunoassay for octapeptide analogs of somatostatin Measurement of serum level after administration of long-acting microcapsule formulations, *Proc. Natl. Acad. Sci.*, USA 85, 5688–5692.

[119] Ruis, J. M. and Benoit, J. P. (1991). *In vivo* peptide release from poly(*DL*-latic acid-*co*-glycolic acid). copolymer 50/50 microspheres, *J. Control Release*, **16**, 177–186.

[120] Wyse, J. W., Takahashi, Y. and DeLuca, P. P. (1989). Instability of porcine somatotropin in polyglycolic acid microspheres, *Proc. Int. Symp. Control Rel. Bioact. Mater.*, **16**, 334–360.

[121] Hora, M. S., Rana, R. K., Taforo, T. A., Nunbeerg, J. H., Tice, R. R., Gilley, R. M. and Hudson, M. E. (1989). Development of controlled release microspheres formulation of interleukin-2, *Pro. Int. Symp. Control. Rel. Bioact. Mater.*, **16**, 509–510.

[122] Lee, S. C., Shea, M., Battle, M. A., Kozitza, K., Ron, E., Turek, T., Schaub, R. G. and Hayes, W. C. (1994). Healing of large segmental defects in rat femurus is aided by RhBMP-2 in PLGA matrix, *J. Biomed. Mater. Res.*, **28**, 1149–1156.

[123] Bowersock, T. L., HogenEsch, H., Suckow, M., Porter, R. E., Jackson, R., Park, H. and Park, K. (1996). Oral vaccination with alginate microsphere systems, *J. Control. Release*, **39**, 209–220.

[124] García-Contreras, L., Abu-Izza, K. and Lu, D. R. (1997). Biodegradable cisplatin microspheres for direct brain injection. Preparation and characterization, *Pharm. Develop. Technol.*, **2**, 53–65.

[125] Benoit, J. P., Courteille, F. and Thies, C. (1986). A physiochemical study of the morphology of progesterone-loaded poly(*D*,*L*-lactice). microspheres, *Int. J. Pharm.*, **29**, 95–102.

[126] De Vos, P., De Haan, B. and Van Schilfgaarde, R. (1997). Effect of the alginate composition on the biocompatibility of alginate-polylysine microcapsules, *Biomaterials*, **18**, 273–278.

[127] Lee, K. Y., Park, W. H. and Ha, W. S. (1997). Polyelectrolyte complexes of sodium alginate with chitosan or its derivatives for microcapsules, *J. Appl. Polym. Sci.*, **63**, 425–432.

[128] Donbrow, M. (1992). in *Microcapsules and Nanoparticles in Medicine and Pharmacy* (Donbrow, M., Ed.), CRC Press, Boca Raton, p.1–14.

[129] Narayani, R. and Rao, K. P. (1995). pH-Responsive gelatin microspheres for oral delivery of anticancer drug methotrexate, *J. Appl. Polym. Sci.*, **58**, 1761–1769.

[130] Yao, K. D., Xu, M. X., Yin, Y. J., Zhao, J. Y. and Chen., X. L. (1996). pH-Sensitive chitosan/gelatin hybrid polymer network microspheres for delivery of cimetidine, *Polymer Int.*, **39**, 333–337.

[131] Santiago, N., Milstein, S., Rivera, T., Garcia, E., Zaidi, T., Hong, H. and Buck, D. (1993). Ozal immunization in rats with microspheren encapsulating influenza virus, *Pharm. Res.*, **10**, 1243–1247.

[132] Davis, S. S., Illum, L. and Stolnik, S. (1996). Polymers in drug delivery (1996). *Curr. Opin. Colloid Interface Sci.*, **1**, 660–666.

[133] Hillery, A. M., Toth, I., Shaw, A. J. and Florence, A. T. (1996). Co-polymerised peptide particles (CPP). I: Synthesis, characterisation and *in vitro* studies on a novel oral nanoparticulate delivery system, *J. Control. Release*, **41**, 271–281.

[134] Tabata, Y. and Ikada, Y. (1988). Macrophage phagocytosis of biodegradable microspheres composed of *L*-lactic acid/glycolic acid homo and copolymers, *J. Biomed. Mater. Res.*, **22**, 837–858.

[135] Cremers, H. F. M., Lam, K., Wolf, R. F. E., Blaauw, E. H., Schakenraad, J. M., Verrijk, R., Kwon, G. S., Bae, Y. H., Kim, S. W. and Feijen, J. (1994). Degradation and intrahepatic compatibility of albumin-heparin conjugate microspheres, *Biomaterials*, **15**, 577–585.

[136] Cremers, H. F. M., Lens, J. P., Seymour, L. and Feijen, J. (1995). Biodegradable ion-exchange microspheres based on modified polylysines, *J. Control. Release*, **36**, 167–179.

[137] Yoshida, M., Asano, M., Kumakura, M., Katazaki, R., Mashimo, T., Yuasa, H., Imai, K and Yamanaka, H. (1990). A new biodegradable polydepsipeptide microsphere for application in drug delivery systems, *Colloid Polym. Sci.*, **268**, 726–730.

[138] Doroshow, J. H. (1991). Doxorubicin-induced cardiac toxicity, *New Engl. J. Med.*, **324**, 843–845

[139] Davis, S. S. and Illum, L. (1988). Polymeric microspheres as drug carriers, *Biomaterials*, **9**, 111–115.

[140] Noteborn, H. P. J. M. and McVie, J. G. (1989). Chemoembolization in regional chemotherapy, in: *Drug Delivery in Cancer Treatment*, ESO Monographs (Domellöf, L., Ed.), Springer Verlag, Berlin-New York, pp. 55–78.

[141] Codde, J. P., Burton, M., Kelleher, D. K., Archer, S. G. and Gray, B. N. (1990). Reduced toxicity of adriamycin by incorporation into ion exchange microspheres: A therapeutic study using a rat liver tumor model, *Anticancer Res.*, **10**, 1715–1718.

[142] Goldberg, J. A.,Willmot, N. S., Anderson, J. H., Mc Currach, G., Bessent, R. G., McKillop, J. H. and McArdle, C. S. (1991). The biodegradation of albumin

microspheres used for regional chemotherapy in patients with colorectal liver methastatses, *Nucl. Med. Commun.*, **12**, 57–63.

[143] McArdle, C. S., Lewi, H., Hansell, D., Kerr, D. J., McKillop, J. H. and Willmott, N. S. (1988). Cytotoxic-loaded albumin microspheres: A novel approach regional chemotherapy, *Br. J. Surg.*, **75**, 132–134.

[144] Grandfils, C., Flandroy, P. and Jérome, R. (1996). Control of the biodegradation rate of poly(*D*,*L*-lactide) microparticles intended as chemoembolization materials, *J. Control. Release*, **38**, 109–122.

[145] Lozsen, N. E., Pollak, S. T., Hezs, K., Leshchinez, E. A. and Balaezs, E. A. (1992). Use of Hylan gel composition for embolization of cezebzal vessel in a rabbits, *4th World Biomaterial Symp.*, Berlin, p. 576.

[146] Couvreur, P., Kante, B., Roland, M., Guiot, P., Bauduin, P. and Speiser, P., (1979). Polyalkylcyanoacrylate nanocapsules as potential lysosomotropic carriers: Preparation, morphological and sorptive properties, *J. Pharm. Pharmacol.*, **31**, 331–332.

[147] Manil, L., Trupel, L. R. and Couvreur, P. (1986). Isobutylcyanoacrylate nanoparticles as a solid phase for an efficient immunoradiometric assay, *Biomaterials*, **7**, 212–216.