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Stevanović, M., 2017. Chapter 11 - Polymeric micro- and nanoparticles for controlled and targeted drug delivery, in: Andronescu, E., Grumezescu, A.M. (Eds.), Nanostructures for Drug Delivery, Micro and Nano Technologies. Elsevier, pp. 355–378. https://doi.org/10.1016/B978-0-323-46143-6.00011-7

Polymeric micro and nanoparticles for controlled and targeted drug delivery

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Nanotechnology, among others, has great potential in the field of medicine and pharmacy because nanoobjects have comparable dimensions to biological entities. Polymer-based particles play an integral role as vehicles in the controlled delivery of different forms and types of active substances, such as anticancer drugs, antihypertensive and immunomodulatory agents, medical imaging contrast media, hormones, vitamins and different macromolecules, such as nucleic acids (deoxyribonucleic acid, ribonucleic acid), proteins, antibodies, etc. The release of the active agent may be constant over a long period, it may be cyclic over a long period, or it may be triggered by the environment or other external events. The purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under- and overdosing. Other benefits of using controlled-delivery systems can include the maintenance of drug levels within a desired range, the need for fewer administrations, optimal use of the drug in question, and increased patient compliance. This review article reports on obtaining polymeric micro and nanoparticles with special emphasis on obtaining polyester particles, incorporation of different active substances within polymer matrix, degradation and release process of active substances from the polymeric particles, physiochemical and biological properties of such obtained systems, as well as about their application as drug delivery systems.

Keywords: nanotechnology, drug delivery, polymers, encapsulation, carriers, microparticles, nanoparticles

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1. Introduction

Controlled drug delivery is a very multidisciplinary field and compared with conventional delivery systems offers numerous advantages such as improved efficacy, better stability of the incorporated (encapsulated or immobilized) substances against (e.g. enzymatic) degradation, reduced toxicity, an easy administration etc. (Mu et al. 2003., Ratnam et al. 2006., Langer 2001., Feng et al. 2004., Kumar et al. 2004). Polymers are often use as carriers for the active substances. Polymers in which active substances can be successfully encapsulated are usually polyesters, polysaccharides, etc. Controlled delivery of active substances requires that the encapsulated or immobilized material retain its performances such as biological activity or the activity of acceptable degradation products (Stevanović et al.2009, Sahoo et al. 2007; Sumer et al. 2008; Besenbache et al. 2007; Moghimi et al. 2005, Wong et al. 2007). Methods for the encapsulation/immobilization of the active substances is commonly performed not only to retain and/or to slowly release them, but to provide a more stable environment for the encapsulated/immobilized species (Ishida et al. 2003) In this way, treatments and procedures that would not otherwise be possible are now in use.

Particles, whether nano or micro, occupy unique position in controlled release of active substances because of their different and specific properties from the bulk material (Mainardes et al. 2005, Stevanović 2011b, Stevanović 2012a). Possibility to control particle size, surface properties and release of active substances in order to achieve the site-specific action of the substances at the optimal rate and dose regimen is one of the major challenges in designing micro and nanoparticles as a delivery system (Ito et al. 2007). Micro and nanoparticles can be used to deliver a wide variety of active substances such as hydrophilic or hydrophobic drugs, vitamins, minerals, different nutrients, etc. The ways to administrate them in the body are different (Nafee et al. 2007; Kozubek 2001; Lian et al. 2001; Medina et al. 2004; D'Emanuele et al. 2004; Liu et al. 1999; Stevanović et al. 2007; Yoo et al. 2006).

One often hears of a vitamins, minerals or other nutrients deficit in human body, which are crucial for normal physiologic functioning of the human body. For example, system for the controlled delivery of vitamins can bring to the more balanced and efficient concentration of vitamins throughout the extended period of time (Stevanović et al. 2009, Stevanović et al 2014). These systems can be prepared from a variety of materials such as polysaccharides, liposomes, synthetic polymers, etc. (Nafee et al. 2007; Kozubek et al. 2001; Lian et al. 2001., Medina et al. 2004, D'Emanuele et al. 2004., Liu et al. 1999., Stevanović et al. 2007., Yoo et al. 2006). Active substances can be delivered in systems that would

otherwise be unacceptable or aggressive to them (Vasir et al. 2005; Greene et al. 2007). For instance, water-soluble substances can be delivered in non-aqueous systems such as ointments. The nature and matrix of the carriers can be chosen from a wide variety of materials (usually selected from a list of materials "generally recognized as safe" (GRAS) for applications in pharmaceutical or food industry (Rados et al. 2004) to meet the needs of the application as well as regulatory demands, e.g., biodegradability. Such particles i.e. carriers can be multifunctional and serve for the delivery of the different substances in the same time. Actually, mutually chemically incompatible actives can be formulated together. The additional way to exploit this technology is to encapsulate one substances that can facilitate, or stabilize, a second (Vandervoort et al. 2002; Stevanović et al. 2007). By isolating some substances, formulation and processing issues are minimized; undesirable properties can be masked, etc.

The purpose of this chapter is to point out some important issues related to various methods, which are employed to encapsulate active substances within polymeric carriers, such as thermal phase separation, melt dispersion, solvent evaporation, spray drying, homogenization of water and organic phases, etc. This review also gives a comparison of the characteristics of polymeric micro- and nanoparticles prepared by different methods. Encapsulation efficiency, release rate, size distribution of particles with encapsulated active substances, are some of the parameters which are used for the evaluation of the encapsulation system characteristics.

2. Polymeric micro and nanoparticles for a controlled delivery of active substances

Polymers have been extensively studied as carriers of active substances in the pharmacy, food industry, etc. (Legrand et al., 1999). For controlled drug delivery polymers which are biodegradable and bioerodible represent the most important type of materials. Polymers in which vitamins and other active substances can be successfully encapsulated are polyesters, polysaccharides, etc. Polyesters are synthetic polymers that have ester linkages. They are used as fibers, films, in composites and elastomers, etc. Polyesters like polylactides, polyglycolides, poly(lactide-co-glycolides), poly (ε-caprolactone) are approved by the World Health Organization (WHO) and Food and Drug Administration (FDA) as substances that can be used in medicine and pharmacy. They are biodegradable polymers with numerous advantages, such as excellent processing characteristics, biocompatibility and biodegradation at rates that can be tailored for the intended application. In degradation covalent bond cleavage by chemical reactions while erosion occurs by the dissolution of chain fragments in noncrosslinked systems

without chemical alterations to the molecular structure (Liecht et al. 2010). For dissolution to occur, the polymer must absorb the surrounding solution and must interact with solution via charge interactions or hydrogen bonding mechanisms. Degradation as well as erosion can occur from the surface or from the bulk of the material (Stevanović et al. 2011a). When degradation starts from surface, the polymeric material is rapidly eliminated from the surface of the particle, but the polymer volume fraction stay quite unchanged. Inversely, when the degradation starts from the bulk, not enough change occurs in the size of the polymeric particle up to it is nearly completely degraded, but the fraction of polymer residual in the carrier decreases during time. The most dominant process is defined by the rates of solution penetration within the polymeric particle, diffusion of the degradation products, and degradation or dissolution of the polymer structure. Polyesters micro and nanoparticles are used for the controlled delivery of several classes of medicaments such as anticancer agents, growth factors, antibiotics, antimicrobial agents etc. In the literature different reviews can be found dealing with this topic, such as the nanoparticle formation mechanisms (Moinard-Checot et al., 2006), the classification of particulated systems (Letchford and Burt, 2007), methods for the preparation of carriers (Vauthier and Bouchemal, 2008) etc. It is commonly assumed that a systems described as a microsphere is comprised of a fairly homogeneous mixture of polymer and active agent, whereas microcapsules have at least one discrete domain of active agent and sometimes more (Singh et al 2010). Some of the variants on microparticle structures are given in **Figure 1**.

Please insert Figure 1 about here

3. Formulation of micro- and nano- polymer particles

Based on the selected material, techniques for obtaining polymeric particles for delivery of active substances can be commonly divided into several groups and they are dispersion of preformed polymers, polymerization of monomers, and ionic gelation or coacervation of hydrophilic polymers. In the literature, other methods such as supercritical fluid technology, particle replication in non-wetting templates have also been described (Gratton et al. 2007; Mishima et al. 2008; Kang et al. 2008). For example, for the preparation of the particles from the polyester polymer poly(lactide-co-glycolide) (PLGA) many approaches are proposed. The emulsification-evaporation method (Prabha et al. 2004; Sahoo et al. 2005; Song et al. 2006; Astete et al. 2007), emulsification-solvent diffusion method (Zhang

et al. 2006; Lee et al. 2005), nanoprecipitation (Bilati et al. 2005; Govender et al. 1999) spray-drying (Rivera et al. 2004; Atuah et al. 2003; Takashima et al. 2007) are all widely used in making PLGA micro and nanoparticles of various sizes.

These methods have a comparable primary steps, where an aqueous medicament solution is emulsified in an organic solution in order to form a water/oil dispersion. The medicament can be also dispersed as a powder (solid particles) in an organic polymeric solution, or co-dissolved in a solvent together with the polymer. Afterward the solution or dispersion is treated according to the one of the above-mentioned techniques.

In solvent extraction or evaporation procedure, the polymer is dissolved in an organic solvent such as dichloromethane, acetone, acetonitrile, chloroform or ethyl acetate. These solvents are also used as a solvent for the hydrophobic drugs. The mixture which contains polymer and drug is then emulsified in a water solution containing a surfactant, stabilizing or emulsifying agent to produce an oil-in-water emulsion. When a stable emulsion is formed, the organic solvent is evaporated either by decreasing the pressure or by continuous stirring. Different parameters have influence on the particle size and morphology such as the type and concentration of stabilizer, speed of the homogenization and the polymer concentration (Kwon et al. 2001). For the purposes of obtaining small particle size, high-speed homogenization or centrifugation can be employed (Zambaux et al. 1998; Stevanović et al. 2007a, b, c, d). Stevanović et al. have shown that by varying parameters like aging time (after the non-solvent is added), time of centrifugation, it is possible to influence morphology, size, agglomeration and uniformity of poly (lactide-co-glycolide) particles (Stevanović et al. 2007d). For example, poly(lactide-co-glycolide) particles produced by physicochemical solvent/non-solvent method with the shortest aging time with non-solvent, the longest time and highest velocity of the centrifugal processing has the smallest particles and the highest uniformity (Stevanović et al. 2007c).

Solvent diffusion technique is an adapted version of solvent evaporation process (Niwa et al. 1993). In this procedure, a water-miscible solvent is used together with the water-immiscible organic solvent as an oil phase. Thanks to a spontaneous diffusion of solvents, an interfacial turbulence arises among the two phases, leading to the creation of small particles. Solvent evaporation as well as solvent diffusion methods can be employed for both, hydrophobic or hydrophilic drugs. In the case of hydrophilic drugs, a multiple water/oil/water emulsion needs to be formed with the drug dissolved in the internal aqueous phase (Stevanović et al. 2009a).

The coacervation method is a technique consists of three parts which are under a continuous agitation: initial part in which a solution must be made with three immiscible phases: the core material (i.e. active substance), the coating material (i.e. polymeric material) and a solvent; second part in which the liquid coating is placed around the core material, which is achieved by mixing the coating phase with the solvent phase (in which the active substances reside); and third part, the coating is rigidized thermally or by desolvation (Thomasin et al. 1998a; Thomasin, et al. 1998b).

Recently, Guan and coworkers (Guan et al. 2015) have described coaxial electrospray method to produce multilayer PLGA micro- and nano-particles with well controlled shape and size. ALA-PDT is a technology used in the treatment for skin and other cancers. In their work, ALA-loaded PLGA nanoparticles with high drug loading efficiency were produced via coaxial electrospray and used for the PDT treatment in HSC-3 cells.

Spray-drying gives an attractive and relatively simple alternative to the aforementioned processes. In this case, the solution containing antigen or water/oil emulsion is atomized in a flow of drying air at a elevated temperature. The organic solvent is quickly evaporated leaving behind solid micro or nano-particles that are separated from the drying air and collected in a deposition chamber (Gander et al. 1996; Tamber et al. 2005).

Park et al. described methods based on filling micro-molds with polymeric particles, in contrast to polymer melts, to prepare microstructures composed of multiple materials, with complex geometries, and produced using mild processing conditions (Park et al. 2007). Polymer particles with sizes of about 1 to 30 μm have been made from poly (lactide), poly (glycolide) and poly(lactide-co-glycolide) by spray drying and emulsion methods either with or without incorporated drug. These polymeric particles have been filled into micro-molds at room temperature and melted or attached together to form microstructures according to different protocols (Park et al. 2007).

Emulsion method produced poly (lactide-co-glycolide) spheres of 100-250μm (Choi et al. 2005), 45μm (Martinez-Sancho et al. 2004) and 30μm (Daugherty et al. 1997) in diameter. Changing in parameters of the emulsion procedure led to obtaining spherical particles with smaller diameters up to 10 μm (Jeong et al. 2003).

Usually, the emulsion process is most appropriate for water insoluble medicaments such as steroids, while the double emulsion process is the most often used to incorporate water soluble medicaments such as peptides (Makadia and Siegel, 2011).

Poly (lactide-co-glycolide) spherical microparticles with encapsulated drug paclitaxel have been prepared by spray drying method. These particles were with size range of 1-8μm (Liu et al. 2007). Xu and coworkers (Xu et al 2011) have described a micro-emulsion method to encapsulate Nile Red dye (and LY294002 in carboxylic acid group terminated PLGA. The average size of the microspheres was of about 1μm.

Further modifications of the process with additional evaporation produced spherical particles with diameters in submicron scale. The first submicron spherical particles obtained were 570-970 nm (Feng et al. 2004) and 244-260 nm (Murakami et al. 2000) in diameter. PLGA particles obtained by physicochemical solvent/non-solvent method were in the size range of 110-170 nm (Stevanović et al. 2007b Stevanović et al. 2009b).

PLGA nanoparticles can also be synthesized by the nanoprecipititation method. In the study of Bilati et al. (Bilati et al. 2005) have shown that the mean particle size was closely dependent on the type of non-solvent selected. When alcohols were used, the final mean size increased in the sequence: methanol<ethanol<propanol. The nanoparticles obtained ranged from about 85 to 560nm in size (Bilati et al. 2005).

Recently, Alshamsan (Alshamsan 2014) has shown that nanoprecipitation is more efficient than emulsion solvent evaporation method to encapsulate cucurbitacin I in PLGA nanoparticles. It is likely that cucurbitacin I escapes with the organic solvent after the emulsification step to the aqueous phase leading to ineffective entrapment in the polymeric matrix. Avoiding emulsification seems efficient in increasing cucurbitacin I disposition in the instantly-precipitating NPs. Therefore, nanoprecipitation method increases cucurbitacin I entrapment in PLGA NPs and possibly other water-insoluble polar drugs.

With spray-drying applied for the preparation of cationic PLGA nanospheres as gene delivery vectors, in order to minimize aggregation and loss of gene transfection efficiency, the mean particle diameter was 100–250nm (Takashima et al. 2007).

Jin et al. examined PLGA nanoparticles with encapsulated paclitaxel, etanidazole or paclitaxel+etanidazole prepared by o/w and w/o/w emulsification-solvent evaporation method (Jin et al. 2007). The prepared nanoparticles were spherical with reaching between 80 and 150nm in size. The drug encapsulation efficiency was higher for paclitaxel and lower for etanidazole. With the emulsion evaporation method using sodium dodecyl sulfate as a surfactant, the size of the obtained particles ranged from 40 to 70 nm (Astete et al. 2007).

3. The most widely used incorporation techniques

In pharmaceutical, medical and food industry encapsulation as well as immobilization techniques are widely used (Wieland-Berghausen, et al. 2002; Ravi Kumar et al. 2000, Stupar et al. 2014). Encapsulation protects components that are sensitive to the environment or for providing time-released delivery of active substances (**Figure 2**). Certain ingredients are easy to encapsulate using conventional techniques. Others, such as sensitive water-soluble components like vitamin C, are very difficult to encapsulate so that controlled protection and release is provided. **Figure 2** represents micropraticle with an encapsulated active substance.

Please insert Figure 2 about here

The choice of a particular method of encapsulation is mainly determined by drug solubility and molecular stability considerations. Many studies have been done on vitamin C (ascorbic acid) with variables optimized to determine the most stable way of encapsulating this vitamin. The commonly utilized techniques for encapsulation of the active substances such as vitamins, minerals or other nutrients within micro or nanoparticle are thermal phase separation (coacervation), spray chilling and cooling, melt dispersion, solvent evaporation, fluidized bed coating, spray drying, homogenization of water and organic phases, rotational suspension separation, extrusion and inclusion complexation, etc. Some of them will be described here.

3.1 Thermal phase separation

By use of coacervation (thermal phase separation), the active substance is coated with a polymer shell leading to the formation of microcapsules (Wieland-Berghausen, et al. 2002). Ethyl cellulose is the most widely used polymer in the coacervation process (Miller et al. 1964; Yoshiyuki et al. 1983; Benita et al 1982; Yalabik-Kas, H. S. 1983; Uddin et al. 2001). Several factors such as homogenization speed, cooling rate, concentration of phase-separation inducing agent and viscosity/molecular weight of ethyl cellulose show an effect on the encapsulation efficiency and the release behavior of the encapsulated drug. Uddin et. al. have been encapsulated vitamin using this technique (Uddin et al. 2001). Ethyl cellulose has been used as the wall forming material. Molecular weight of the ethyl cellulose was varied and it was determined that molecular weight of ethyl cellulose and the addition of polyisobutylene significantly

influenced the aggregation and release rate of microcapsules which contains ascorbic acid. It was determined that microencapsulation product size decreased as the molecular weight of ethyl cellulose increased.

Bachtsi and coworkers (Bachtsi et al 1996) have described obtaining of the poly(vinyl alcohol) (PVA) microcapsules which were prepared by the coacervation method. This is followed by the chemical cross-linking of the coacervated PVA membrane with glutaraldehyde. They have examined the influence of several parameters such as size of the particles, the degree of cross-linking of the coacervated PVA membrane, influence of the amount of the desolvating agent, the concentration of the surfactant used in the synthesis. It has been also studied the ionic strength of the release medium on the release rate of the oil from the microcapsules into a model surfactant solution. It was found that the oil release rate exhibited a first-order kinetic model. The permeability coefficient of the santosol oil for the PVA coacervated/cross-linked membrane was shown to vary from 10-4 to 10-6 cm/s and was strongly dependent both on the environmental conditions of the release medium and the physical and morphological characteristics of the microcapsules.

In the work of Nihant and coauthors (Nihant et al. 1995) phase separation of poly (lactide-co-glycolide) solutions in dichloromethane (CH2Cl2) has been described. The phase separation of PLGA was induced by the addition of a silicone oil to promote protein microencapsulation. The process is very fast and that is the reason why the system is out of equilibrium. In this paper the influence of the main processing parameters on the encapsulation process has been examined and has underlined that kinetics of the main encapsulation steps has a great effect on the appearances of the final microspheres.

A phase separation is a method which is also used for the encapsulation of the medicament chlorpromazine hydrochloride. Chlorpromazine hydrochloride is a highly water-soluble drug. In this procedure which is described in the work of Lin et al. (Lin et al. 1986) ethylene-vinyl acetate was used as agent to induce coacervation. The influence of ethylene-vinyl acetate concentration on the drug release properties of chlorpromazine hydrochloride microcapsules and tableted microcapsules was examined. Microcapsules were obtained by the deposition of ethyl cellulose round solid chlorpromazine hydrochloride particles and by using 0–6% ethylene-vinyl acetate as a coacervation-inducing agent. It was shown that the size of the particles depends on the concentration of coacervation agent and decrease with concentration increase. The concentration of coacervation agent also has influence on the

surface topography of the microcapsules, atmospheric stability, powder flowability, the release rate from untabletted or tabletted microcapsules.

Simovic and coworkers have prepared porous silica–lipid hybrid microcapsule (SLH) for delivery of drug indomethacin (Simovic et al. 2010). SLH is oral delivery system for indomethacin fabricated from Pickering emulsion templates. Indomethacin forms an electrostatic complex with cationic lipid present in the oil phase. The silica–lipid hybrid microcapsule have been prepared either by spray drying (range of about 1 to 5μm) or phase coacervation (range of about 20 to 50μm) showed a specific internal porous structure with pores in the range of 20-100nm. Experiments of dissolution under sink conditions as well as in the presence of electrolytes shown a decreased extent of dissolution; which is confirmation of the lipophilic nature the drug–lipid complex as well as its location in the oil phase. In vivo experiments exhibited complete medicament absorption and statistically higher fasted state bioavailability than the water suspensions and oil/water submicron emulsions of indomethacin. It is hypothesized that the silica–lipid hybrid microcapsule improve oral absorption via complete solubilisation of drug–lipid electrostatic complexes during enzymatic lipolysis in the gastrointestinal tract.

Poulain et al have used coacervation method for preparing biocompatible polymeric microspheres with sizes of about 0.5–5 μm as carriers for drugs for the treatment of thrombosis (Poulain et al. 2003). In the case of the drugs used for the treatment of thrombosis, the pharmacological activity of serine protease inhibitors, is often linked to the presence of amidine functions. In this paper, inulin and inulin acetate with or without 1,12-dodecanedicarboxylic acid, were selected for the preparation of the microspheres. (E,E)-bis(amidinobenzylidene)cycloheptanone [(E,E)-BABCH] is used as model drug. The influence of different parameters such as drug mass, velocity and time of stirring during synthesis have been examined. It was shown that encapsulation efficiency reached 65% irrespective to the nature of the polymer, when it is used a stirring time of 30 min, a high stirring speed and a centrifugation during 15 min. The release of the model drug was studied in vitro and exhibited three characteristic phases which are: the initial phase i.e. a rapid ‘drug burst’. (in the first 5 min, 58–62% of the drug were delivered), a slow diffusion phase which lasted 33 h and an increasing rate until complete drug release was observed for 2.5 days.

Mao and coworkers have examined obtaining of chitosan-DNA nanoparticles by complex coacervation (Mao et al. 2001). They have studied influence of different parameters during synthesis such as concentrations of ingredients, temperature of the solutions, pH and molecular weights of chitosan and

DNA. The particle size was optimized to be from 100 to 250 nm when the ratio between amino and phosphate group was adjusted to be from 3 to 8 while the concentration of the chitosan was 100 μg/ml. The particles were with the narrow size distribution. These particles have had a positive zeta potential of about 12-18mV. By the electrophoretic mobility method it has been shown that chitosan-DNA nanoparticles protect the encapsulated plasmid DNA from the degradation. It has been also shown, the transfection efficiency of as prepared nanoparticles depends on the cell-type. The presence of fetal bovine serum in the concentration of 10% did not interfere with the transfection ability. The authors have developed three different schemes to conjugate transferrin or KNOB protein to the nanoparticle surface. When the transferrin was conjugated to the particles a maximum of four-fold increase in their transfection efficiency in HEK293 cells and HeLa cells were obtained. In the case of KNOB conjugation to the nanoparticles, gene expression level in HeLa cells has been improved by 130-fold. Conjugation of polyethylene glycol on the nanoparticles provide lyophilization without aggregation. Also, bioactivity of the particles was preserved for at least 1 month in storage. The clearance of the PEGylated nanoparticles in mice after intravenous administration was slower than in the case of unmodified nanoparticles at 15 min, and with higher depositions in kidney and liver. However, no difference was observed at the 1-h time point.

Stott et al have used complex coacervation technique for the coacervation of cationic antidepressants and counter-ions of anionic bile salts (Sctott et al. 1996). Complex coacervation represent the technique of the separation of an aqueous mixture of oppositely charged ions into a dense coacervate oil phase and a dilute equilibrium phase (Sctott et al. 1996). Stott et al have investigated coacervation of cationic tricyclic antidepressants and counter-ions of anionic bile salts sodium cholate or sodium deoxycholate, and the surfactant sodium lauryl sulfate. As cationic tricyclic antidepressants they have used amitriptyline, imipramine and doxepin. The samples have been characterized by different techniques such as microscopy, particle size analysis chromatography, titration and thermogravimetric analysis. For the purpose of the enhancing transdermal flux of charged species, the authors have selected two systems: amitriptyline with sodium deoxycholate, which separates into two distinct phases, and amitriptyline with sodium lauryl sulfate which remains as a sol. Amitriptyline with sodium deoxycholate produced an 18-fold increase and amitriptyline with sodium lauryl sulfate 22-fold in comparison with aqueous solution. The experiments were conducted using human epidermal membrane with an aqueous receptor and the flux from a 0.025 M aqueous solution which is above the critical micelle concentration was 3.0 ± 0.54 μg/cm2/h. The flux from an amitriptyline - sodium deoxycholate

coacervate donor was 6.6 ± 0.71 μg/cm2/h, which is a 2.2-fold increase. The amitriptyline with sodium lauryl sulfate, on the other hand, reduced the flux in comparison with the aqueous solution. Based on the results from the study it was concluded that the increased lipophilicity of the coacervate oil phase can increase the transdermal flux of charged species.

Complex coacervate method is also used in the study of Jin et al. (Jin et al. 2008.) for the obtaining of the injectable and thermo-reversible gels. These gels have been formed in aqueous solution with two oppositely charged biomacromolecules. High molecular weight gelatin type A has been used as positively charged biomacromolecule while chondroitin 6-sulfate has been used as negatively charged. A negative, thermo-sensitive polysaccharide, methylcellulose containing a salting-out salt, ammonium sulfate has been co-formulated with previously mentioned compounds. By using complex coacervation and a thermo-reversible gel confirmed synergistic effects on the complex formation the release rates of model proteins and in situ gel depot formation. Gels showed sustained release patterns of the model protein over twenty-five days with slight initial bursts. Innovative in situ gel carrier systems exhibited advantages of complex coacervation and temperature responsiveness for efficient protein drug delivery since it was achieved high protein loading, sustained protein release, simplicity of administration, an aqueous environment without toxic organic solvents, and a simple preparation method.

Matrigel™ hydrogels and poly(lactide-co.glycolide) scaffolds have been examined as a carriers of angiogenic factors (des Rieux et al 2011.) To achieve tissue regeneration one of the most important requirements is a good vascularization. The delivery of the angiogenic factors, such as VEGF, has been widely examined for creating a vascular network within the tissue. des Rieux et al. have examined if the incorporation of VEGF within nanoparticles could enhance angiogenesis in vivo as compared to free VEGF. By complex formation with dextran sulfate and coacervation by chitosan, nanoparticles with encapsulated VEGF were synthesized. After two weeks, the release of the VEGF which have been incorporated within hydrogels has reduced from 30% to 1% while release of the VEGF which have been incorporated within PLGA scaffolds has increased from 20% to 30% compared with free VEGF. In vivo experiments showed VEGF encapsulation improved angiogenesis in both type of 3D matrices. In the case of Matrigel™hydrogels more endothelial and red blood cells were observed as well as in the case of PLGA scaffolds. Encapsulation of VEGF in Matrigel™ hydrogels as well as in PLGA scaffolds as a carriers enhanced VEGF efficiency. Encapsulation of VEGF within hydrogels as well as scaffolds is a promising approach for tissue regeneration engineering.

There are studies which deals with the association of bioactive molecules to casein, the major milk protein, and investigate the evidence of their efficacy in adapting the release and/or enhancing, the bioavailability of the associated compounds (Elzoghby et al. 2011.). Casein has a lot of interesting properties that make it a good candidate for traditional and innovative drug delivery systems. In the literature, the capacity of casein to change drug dissolution from compacts has been reported. The high tensile strength of casein films, exploit its use as a film-coating for tablets. For the preparation of innovative casein-based systems, i.e. hydrogels for the controlled release of bioactives, as crosslinkers very often were used a naturally occurring genipin and a natural tissue enzyme, transglutaminase. Since casein possess emulsifying and bubble-forming properties it have been used in order to develop casein floating beads. This beads have been used to increase the residence time of drugs in the stomach. Also, microparticles of casein have been prepared by emulsification method and by crosslinking with glutaraldehyde, enzymatic crosslinking by transglutaminase, coacervation and electrostatic complexation. Methods used for the preparation of different casein nano-systems for the delivery of active substances are also enzymatic crosslinking, graft copolymerization, heat-gelation and polyelectrolyte ionic complexation. It can be concluded that casein-based formulations are promising materials for controlled drug delivery.

The coacervation method has been used for the preparation of chitosan nanoparticles with encapsulated small interfering RNA (siRNA) (Lee et al. 2009.). siRNA has been widely examined as a potential therapeutic for treatment of different diseases. Fast degradation and low intracellular association, however, limited the usage of siRNA, in vitro and in vivo. Chitosan nanoparticles with encapsulated small interfering RNA have been synthesized in the presence of polyguluronate. Polyguluronate was isolated from alginate. The samples have been characterized by different methods to evaluate their properties such as size, surface charge, morphology, and interaction with siRNA. It was found that the mean diameter of the particles depends on the ratio of chitosan and siRNA. The size of the particles was 110-430nm. Nanoparticles exhibited low cytotoxicity and were suitable for delivery of siRNA to HEK 293FT and HeLa cells. The chitosan nanoparticles were considered as a good candidate for siRNA delivery.

In the work of Carrasquillo and coworkers, coacervation method was used for the preparation of poly(d,l lactide-co-glycolide) microspheres with encapsulated bovine serum albumin as a model protein (Carrasquillo et al. 2001.). Bovine serum albumin in powder form has been obtained by spray-freeze drying and after that suspended in methylene chloride containing poly(d,l lactide-co-glycolide).

Thereafter coacervation has been performed by adding silicon oil and microsphere hardening in heptane. The structure of the bovine serum albumin has been characterized by Fourier-transform infrared spectroscopy. Structural perturbations in bovine serum albumin were reduced during the spray-freeze drying step by using the excipient trehalose. The encapsulation of bovine serum albumin within poly (d,l lactide-co-glycolide) microspheres was performed without significant structural perturbations of the protein. The encapsulated bovine serum albumin had a similar monomer content and the same secondary structure as non-encapsulated. There was a small initial burs release and a prolonged and continuous sustained release phase of the protein from the polymeric matrix.

A key factor in determining the bioavailability of intranasally administered drugs is often rapid mucociliary clearance. The usage of mucoadhesive micro- and nanoparticles provide a potential strategy for improving retention of drugs within the nasal cavity, and thus improve the resulting pharmacokinetic profile. The study of Lim et al. describes the comparison of a number of novel, potentially mucoadhesive microspheres, prepared by solvent evaporation, composed of hyaluronic acid, chitosan glutamate and a combination of the two with microcapsules of hyaluronic acid and gelatin prepared by complex coacervation (Lim et al. 2000.). The particles had a mean diameter of about 20µm (hyaluronic acid), 28 µm (hyaluronic acid/ chitosan glutamate) and 30µm (chitosan glutamate). The encapsulation of a model drug, gentamicin sulphate was 46.90±0.53% (hyaluronic acid), 28.04±1.21 (hyaluronic acid/ chitosan glutamate) and 13.32±1.04 (chitosan glutamate). The release of the drug was characterized and it was shown that the release of gentamicin from hyaluronic acid and hyaluronic acid/chitosan glutamate was 50% longer than in the case of chitosan glutamate and was best modelled as a release from a carrier. The mucociliary transport rate of the particles across an isolated frog palate has been used to determine the degree of mucoadhesion of each system. The rank order of mucoadhesion for the microspheres and the microparticles was hyaluronic acid = hyaluronic acid/chitosan glutamate> chitosan glutamate > hyaluronic acid /gelatin> chitosan glutamate ins. The encapsulation of gentamicin did not affect the mucoadhesive properties. The combination of hyaluronic acid with chitosan may afford additional advantages in combining the mucoadhesive potential of hyaluronic acid with the penetration enhancing effect of chitosan.

3.2 Melt dispersion

In the melt dispersion technique, the drug-containing molten wax phase is emulsified into a heated, emulsifier-containing external phase. Depending on the solubility of the drug, the external phase can be

either aqueous (for water-insoluble drugs) or nonaqueous (for water-soluble drugs). On cooling the emulsion, the liquid droplets congeal and suspension of the wax microparticles is formed. The microparticles are then separated, mostly by filtration or centrifugation, sometimes washed to remove free drug crystals and surfactants, dried and sized. Carnauba wax was used in the melt dispersion method for encapsulation of ascorbic acid (Uddin et al. 2001).

In the study of Mathiowitz et al. a “hot melt” microencapsulation has been described as a method for obtaining polyanhydride microspheres with encapsulated dyes (acid-orange),p-nitroaniline or insulin (Mathiowitz et al. 1987.) Poly[bis(p-itcarboxy phenoxy)propane anhydride] copolymerized with sebacic acid has been used as a model polymer. The degradation of the microspheres depends on the amount of the hydrophilic drug which are used in the synthesis. Insulin-incorporated microspheres implanted into diabetic rats resulted in normoglycemia for a period of 3 to 4 days.

3.3 Solvent evaporation

The emulsification-evaporation method (Prabha et al. 2004; Sahoo et al 2005; Song et al. 2006; Astete et al. 2007), spontaneous emulsification-solvent diffusion method (Zhang et al. 2006, Lee et al. 2005), nanoprecipitation method (Bilati et al. 2005; Govender et al. 1999), solvent evaporation and spray-drying (Rivera et al. 2004; Atuah et al 2003; Takashima et al. 2007), are all widely used in preparing micro and nanoparticles of various sizes. Each of these methods employs a similar first step, where an aqueous drug solution is emulsified in an organic polymer solution to form a water-in-oil dispersion (w/o) (O'Donnell et al 1997). If appropriate, the ingredient may also be dispersed as a solid substance in an organic polymer solution, or dissolved in a common solvent together with the polymer. In solvent evaporation technique, the drug substance is dispersed homogeneously in the polymer. The particle size is found to be influenced by the type and concentration of stabilizer, homogenizer speed and the polymer concentration (Kwon et al. 2001). However, this technique leads to low encapsulation efficiencies for water-soluble drugs (Wieland-Berghausen et al. 2002; Bodmeier et al. 1994).

The solvent evaporation method has been used to explore influences of changing the temperature during the experiments, core-to-wall ratio and the influence of the presence of the plasticizers (triethyl citrate) on the release rate of the microencapsulated ascorbic acid (Uddin et al. 2001). Results exhibited that the presence of plasticizer reduced the release rate. The ratios of the core to wall which have been used were 1:1 and 3:1. This parameter had no significant effect on the release rate. Also, the two temperatures used in the experiments of 28°C and 55° C, exhibited insignificant influence on the release

rate. In the experiments of spray drying, four different polymer-coating formulations, whether singly or as a mixture, have been used. Those were gel, starch, ethyl-cellulose and β-cyclodextrin. The loss of ascorbic acid during spray drying was 20%. Different coating materials resulted in particles-capsules with sizes in the range from 90 μm to 280μm. The ratio of encapsulated ascorbic acid was less than 50%. This indicates and means that less than 50% of ascorbic acid was actually microencapsulated.

Moderately uniform-sized biodegradable poly (lactide) microcapsules have been successfully produced by combining a shirasu porous glass membrane emulsification method and multiple emulsion-solvent evaporation method (Liu et al 2005.). An aqueous phase containing lysozyme was used as the internal water phase. Poly (lactide) and arlacel 83 were dissolved in a mixture solvent of dichloromethane and toluene which was used as the oil phase. These two solutions were emulsified by a homogenizer to form a water/oil primary emulsion. These primary emulsion was filtrated through the uniform pores of a shirasu porous glass membrane into the external water phase by the pressure of nitrogen gas to form the uniform water/oil/water droplets. Afterward, the solid polymer microparticles were obtained by evaporating the solvent. It is essential to avoid the phase separation of primary emulsion during the membrane emulsification. It was shown that when the density alteration of the internal water phase and oil phase was reduced to nearly zero and arlacel 83 was employed as the oil emulsifier, the phase separation was not observed within 24 h. The drug encapsulation efficiency was found to be dependent on poly (lactide) molecular weight, additive type and its concentration in the internal water phase, the emulsifier type and concentration in the oil phase, the NaCl concentration and the pH value in the external water phase. In comparison with the stirring method, it was found that the size was more uniform and the drug encapsulation efficiency was much higher when the microcapsules were prepared by shirasu porous glass membrane emulsification method and the highest drug encapsulation efficiency of 92.20% was obtained.

The emulsification is the major and first step of the emulsification solvent evaporation (Rosca et al. 2004.). However, the second step which is the solvent transport out from the emulsion droplets has great influence on the particle morphology as well as microparticles encapsulation and release behavior. Rosca and coauthors have studied the mechanism of the solvent elimination from the emulsion droplets and its influence on the particle morphology, encapsulation and release behavior. Very often, the solvent is highly volatile that makes the solvent elimination process very fast thus difficult to observe. The initial emulsion was monitored by optical microscope under controlled solvent evaporation conditions. In this study, the results from the optical microscopic observations validated with laser

diffractometry analysis showed that in single emulsion formulations, spherical microparticles are formed by accelerated solvent elimination due to the combined effects of high solvent volatility and polymer precipitation. The solvent expulsion accompanied by significant shrinkage generates on the microparticle surface a thin layer of nanoparticles confirmed by electron microscopy and laser diffractometry. During the intense solvent removal, the encapsulated substance is drained, affecting the loading efficiency. Therefore, it will concentrate near the microparticle surface contributing to the initial burst release. In the procedure such as in the case of double emulsion formulations, particles with different morphologies are generated due to the presence of the aqueous-phase droplets inside the emulsion droplet. During the solvent elimination, these microdroplets normally coalesce under the pressure of the precipitating polymer. Depending mostly on the polymer concentration and emulsification energies, the final microparticles will be a mixture of honeycomb, capsule or plain structure. During the shrinkage due to the incompressibility of the inner droplets, the precipitating polymer wall around them may break forming holes through which the encapsulated substance is partly expulsed. Through these holes, the encapsulated substance is further partitioning with the external aqueous phase during solvent evaporation and contributes to the initial burst release during the application (Rosca et al. 2004.).

3.4 Spray drying

Spray-drying is a well-known technique that generates solid formulations from solution or suspension and has special applications in the pharmaceutical and food industry. Consisting of a single step process that involves atomisation of the liquid feed, which is injected into a drying chamber containing hot air or nitrogen. The droplets instantly dry into solid particles that are subsequently collected in the drying chamber. The two factors that can influence the properties of the output material are the spray-dried particle formulation factors and the spray-drying parameters (Seville et al. 2007). Spray-drying was used as preparation method of vitamin C/Eudragit® microspheres (Esposito et al. 2008). Vitamin C/Eudragit® microspheres obtained by this method showed potential for delivery of vitamin C by oral rout (Esposito et al. 2008). Spray-drying was, also, used by Finotelli and co-workers for obtaining microcapsules with different content of ascorbic acid for application in the food industry as fortification (Finotelli et al. 2005).

Jensen et al. have examined the influence of different parameters on the poly(D,L-lactide-co-glycolide) nanoparticles with encapsulated small interfering RNA which have been synthesized by spray drying

technique (Jensen et al. 2010.). Poly(D,L-lactide-co-glycolide) nanoparticles with encapsulated small interfering RNA have been convert into nanocomposite microparticles intended for inhalation. The spray drying procedure has been optimized by a statistical design of experiment and by characterizing powder appearances after systematic variation of the formulation parameters. The parameters which have been varied were concentration, carbohydrate excipient and the ratio of nanoparticles to excipient. Trehalose, lactose and mannitol were used as carbohydrate excipients. The parameters were varied to observe there influence on moisture content, size of the particles and their morphology and powder yield. The recognized optimum conditions were applied for spray drying of small interfering–encapsulated nanocomposite microparticles. The result of this condition was a system with a low water content (0.78% w/w) and with optimal size for inhalation. When mannitol was used as carbohydrate excipient a significantly lower moisture content has been obtained than in the case when trehalose or lactose were used. Also, larger amounts of nanoparticles lead to changes in the surface morphology of the spray-dried particles. The integrity and biological activity of the small interfering RNA were preserved during the spray drying process. Jensen et al. have concluded that spray drying is a very good method for synthesis of nanocomposite microparticles comprising small interfering RNA within poly(D,L-lactide-co-glycolide) nanoparticles for potential use in inhalation therapy.

3.5 Spray chilling and spray cooling

Spray chilling and spray cooling are similar methods to spray drying in that core material is dispersed in a liquified coating or wall material and atomized but there is generally no water to be evaporated. The core and wall mixture are atomized into either cool of chilled air which causes the wall to solidify around the core (Risch 2009). In spray chilling, the coating is typically a fractionated or hydrogenated oil. In spray cooling, the wall is typically oil, although other materials can be used. These methods, actually differ only in the melting point of the wall material used and are most often used to encapsulate solid materials such as vitamins, minerals or acidulants.

3.6 Homogenization of water and organic phases

Spherical nanoparticles of the poly (D,L-lactide-co-glycolide) (PLGA) were produced using physicochemical method with solvent/non-solvent systems (Stevanović et al. 2007a; Stevanović et al 2007b; Stevanović et al. 2007c). The encapsulation of the ascorbic acid, folic acid, silver nanoparticles (AgNp) or AgNp together with ascorbic acid in the PLGA polymer matrix have bee successfully

performed by homogenization of water and organic phases (Stevanović et al.2007a,b; Stevanović et al. 2008a, b; Stevanović et al. 2009c; Stevanović et al 2012b; Stevanović et al. 2013; Stevanović et al 2014)). Typically, the PLGA commercial granules have been dissolved in acetone and after that the aqueous solution of the ascorbic acid has been added in PLGA solution in acetone while continuously been homogenized. Concentration of ascorbic acid in water was varied in order to obtain particles with different ratio of PLGA and ascorbic acid (PLGA/ascorbic acid 85/15%wt, PLGA/ascorbic acid 70/30%wt, PLGA/ ascorbic acid 50/50%wt and PLGA/ ascorbic acid 30/70%wt) (Stevanović et al. 2007a; Stevanović et al 2007b). The introduction of 15% of ascorbic acid does not influence the size, while further increase of ascorbic acid concentration increases the size of PLGA particles. After the precipitation with alcohol methanol or ethanol, the particles are stabilized due to the zeta potential created by stabilizer. This was followed by centrifugation, decantation and drying of the particles. Polyvinyl pyrrolidone (povidone, PVP) or polyvinyl alcohol (PVA) were used as a stabilizer of the particles.

Stevanović et al. described obtaining of the poly (lactide-co-glycolide) particles with encapsulated silver nanoparticles stabilized by polyglutamic acid (PLGA/AgNpPGA) together ascorbic acid (PLGA/AgNpPGA/ascorbic-acid particles). PLGA/AgNpPGA/ascorbic-acid particles have been synthesized by a physicochemical method with solvent/non-solvent systems which are spherical, have the mean diameter of 775 nm and a narrow size distribution with a polydispersity index of 0.158. The encapsulation efficiency of AgNpPGA/ascorbic-acid within PLGA was determined to be greater than 90%. The entire amount of encapsulated ascorbic acid was released in 68 days, and the entire amount of AgNpPGAs was released in 87 days of degradation (Stevanović et al. 2014). Schematic representation of degradation and release of the PLGA/AgNpPGA/ascorbic-acid particles is given in Figure 3.

Please insert Figure 3 about here

Desai *et. al.* reports the encapsulation of vitamin C within sodium alginate beads by an alternative approach (Desai et al 2005). The alternative encapsulation procedure mostly includes immobilization of vitamin C in hydrated zinc oxide layers and subsequently encapsulation of as prepared particles in sodium alginate beads. Vitamin C has been immobilized in hydrated zinc oxide layers by a coprecipitation method. Fourier transform infrared spectroscopy (FTIR) exhibited that the vitamin C has been found to be stable during and after its immobilization.

Hydrated zinc oxide as an inorganic matrix for immobilization of vitamin C was, also, used by Yang and coworkers (Yang et al. 2003). Encapsulation of vitamin C within a biocompatible layered inorganic material was achiewed by coprecipitation reaction, in which the layered inorganic lattice and its intercalate of vitamin C are simultaneously formed. The nanometer sized powders of vitamin C intercalate thus prepared was again encapsulated with silica nanosol to form a nanoporous shell structure. This ternary nanohybrid of vitamin C layered inorganic core-SiO2 shell exhibited an enhanced storage stability and a sustained releasing of vitamin C. Furthermore, the encapsulation of vitamin C with inorganic mineral was very helpful in delivering vitamin C molecules into skin through stratum corneum, facilitating transdermal penetration of vitamin C in topical application.

3.7 Extrusion

The extrusion is a relatively new process compared to spray drying and it often used in food industry (Risch 2009). This process begins by forming a low moisture (5-10%) carbohydrate melt. An emulsifier is added to the melt and then the material for the encapsulation is added with vigorous agitation. This molten emulsion is forced through a die into a cold isopropanol bath. The melt solidifies into an amorphous structure which is broken into small rod shaped pieces by mechanical agitation. The pieces are recovered by centrifugation, dried under a vacuum, mixed with a free flowing agent and stored.

By hot melt extrusion a thermodynamically stable dispersions of amorphous quinine, a model drug, within an amorphous polymeric platform were produced (Jones et al 2014.). Characterization of the pre-extrudates and extrudates was performed using differential scanning calorimetry, X-ray diffraction and Raman spectroscopy. Water uptake by the raw materials was determined using dynamic vapour sorption technique. Additionally, the presence or absence of crystalline drug following storage at 25 °C/60% relative humidity and 40 °C/75% relative humidity in a sealed glass jar, and at 40 °C/75% relative humidity in an open glass jar for 3 months was determined using powder X-ray diffraction. Amorphous quinine was generated in situ during extrusion from both quinine base (5%, 10%, 20% w/w drug loading) and from quinine hydrochloride (5%, 10% w/w drug loading) and remained thermodynamically stable as a solid-in-solid dispersion within the polymeric extrudates. When processed with polymer, quinine hydrochloride (20% w/w) was converted to amorphous quinine hydrochloride. Whilst stable for up to three months when stored under sealed conditions, this amorphous form was unstable, resulting in recrystallization of the hydrochloride salt following storage for 1 month at 40 °C/75% relative humidity

in an open glass jar. The behavior of the amorphous quinine hydrochloride (20% w/w) extrudate was related, at least in part, to the lower stability and the hygroscopic properties of this amorphous form.

Carriers for the sustained release of ibuprofen have been produced by hot-melt extrusion method (Brabander et al. 2003.). Ibuprofen was used as the model drug and ethyl cellulose as sustained release agent. Since release of ibuprofen has been slow from ibuprofen/ethyl cellulose matrices at ratio 60:40 w/w, respectively, Brabander and coworkers modified the procedure in such a way that excipient hydroxypropyl methylcellulose and xanthan gum has been added to the formulation. This affected on the ibuprofen release. Initially burst release has been observed for the preparations containing hydroxypropyl methylcellulose and a nearly time independent release was seen for the xanthan gum based mini-matrices. The ibuprofen release from the mini-matrices has been generally diffusion controlled. Also, swelling of the matrices played an imperative role to achieve complete drug release. The preparations have been stable during one year storage at 60% relative humidity and room temperature. At an elevated humidity and temperature the ibuprofen release increased.

This method, hot-melt extrusion was also used in the work of Follonier and coworkers for the preparation of the sustained release pellets (Follonier et al. 1995.). As a model drug in this study diltiazem hydrochloride has been used. The obtained preparations, pellets, can be packed into hard gelatin capsules. With this method it was achieved in vitro release at the rates which are slow enough to rich the therapeutic plasma levels for the drug with a once or twice daily administration. The release profile was dependent on polymer to drug ratio and this was examined by double exponential decay equation to distinguish among surface release and diffusion release phases. In order to achieve optimal release of the drug, different parameters such as type of the polymer, addition of additives for the purpose of forming pores, addition of hydrophilic polymers, etc were studied. The probability of reaching a nearly zero order release without any coating has been investigated with poly (ethylene-co-vinyl acetate). Poly (ethylene-co-vinyl acetate) is inert polymer. Assuming that this could be reached by including polymers with pH-dependent behavior, the influence of cationic and enteric additives has been evaluated. At the end, swelling agents have been included into the pellets, which have been able to reduce the burst release.

Fule et al. have examined influence of different surfactants on the formulations of solid dispersions and a model antiulcer drug lafutidine prepared by hot melt extrusion (Fule et al. 2014.). PEG 400, Lutrol F127, Lutrol F68 have been used as surfactants. Synthesized amorphous glassy solid dispersion have been found to be thermodynamically and physicochemically stable. Vice versa, traces of crystalline

antiulcer drug lafutidine not observed in the extrudates according to differential scanning calorimetry, scanning electron microscopy, X-ray diffraction and Raman spectroscopy. Based on this methods it was revealed drug- polymer molecular miscibility and surface interaction at micro level. By 1H–COSY NMR spectroscopy method it was confirmed miscibility and interaction among antiulcer drug lafutidine solid dispersions and solubilizing agent (amphiphilic Soluplus®), with chemical shift drifting and line broadening. Also, computational modelling (MD simulation) showed intermolecular interaction between molecules. Dissolution rate and solubility of antiulcer drug lafutidine was enhanced remarkably in developed solid dispersions systems. Also, it was shown that ratio between polymer and surfactants is a very important and has significant role in dissolution rate enhancement of antiulcer drug lafutidine solid dispersions. The developed system has promising potential for oral delivery and might be an effective approach for improving the therapeutic potential of antiulcer drug lafutidine.

4. Characteristics of polymeric micro- and nanoparticles with encapsulated vitamin as an active substance and their comparison

Encapsulation efficiency, release rate, size distribution of particles, are some of the parameters which are used for evaluating encapsulation system characteristics.

Uddin *et al.* evaluated the influence of process perimeters on ascorbic acid properties (Uddin et al. 2001). Different methods for the encapsulation of ascorbic acid have been chosen. Uddin and coauthors selected spray drying technique, thermal phase separation, melt dispersion method as well as solvent evaporation. It is well known that ascorbic acid is highly oxidative substance and this, for example, can cause a difficulties in food systems. During the processing steps, ascorbic acid can change color from white to yellow which have an effect on food colors. Also, ascorbic acid can react with other constituents and come to undesirable variations in the color and perception of the food. The results confirmed that with the encapsulation of ascorbic acid it will be prevented its color change, delay its core release rate, and also cover its acid taste (Uddin et al. 2001). Regarding the thermal phase separation method it was shown, molecular weight of ethyl cellulose and the addition of polyisobutylene significantly affected the agglomeration and release rate of microparticles. There is a noteworthy change in release ratios with various molecular weights of ethyl cellulose. The initial release rate of ascorbic acid was lower when the ethyl cellulose with higher molecular weight was used. The

molecular rate was not a parameter for complete release of ascorbic acid from the microcapsules since high or low molecular weight gave a release of ~1.0 in 20 minutes.

In the second method which was melt dispersion method, spherical capsules were prepared with carnauba wax (Uddin et al. 2001). The ascorbic acid release rate has been slower in this case than in the case when the capsules were prepared with ethyl cellulose. This shows that carnauba wax keeps the ascorbic acid more stable. In the solvent evaporation method, a higher molecular weight of ethyl cellulose and the addition of plasticizer have been found to be significant for encapsulation. During the spray drying process, loss of ascorbic acid during microencapsulation has been found to be least. Uddin *et al* showed that the loss of ascorbic acid which is encapsulated by spray drying has been 20% (Uddin et al. 2001). It was also shown that starch and β-cyclodextrin have influence on the degradation of the capsules with encapsulated ascorbic acid.

Trindade and Grosso examined the stability of ascorbic acid encapsulated in granules made of rice starch and also in granules made of gum arabic (Trindade et al. 2000). Capsules prepared from starch have been larger than those prepared from gum arabic. Microcapsules prepared from starch have been with sizes of about and less than 57 μm and with a mean size of 20.5 μm while 90% of gum arabic capsules had sizes less than 27 μm with mean size of 8.0 μm.

Using the simplistic method, spherical and uniform particles powder has been obtained from the commercial granules of poly(DL-lactide-co-glycolide), where the mean particles size are in the range from 110 to 170nm. The various concentrations of the vitamin C have been encapsulated within PLGA particles. The results of the determination of the particle yield for various PLGA/ascorbic acid ratios were similar for each of the samples and in all cases greater than 50% (Stevanović et al. 2007a; Stevanović et al 2007b). The loading efficiency was determined to be greater than 90% in all ratios of PLGA/ascorbic acid particles (Stevanović et al. 2007a; Stevanović et al 2007b). The degradation of the PLGA without and with ascorbic acid has been followed as well as morphological changes which occurred during the degradation (Stevanović et al. 2007a; Stevanović et al. 2008). The degradation have been tracked for eight weeks and it has been determined that PLGA completely degrades within this period fully releasing all encapsulated ascorbic acid (Figure 8.). In the first 24 days, the samples degrade slower while latter the pace of the degradation increases. In the first 24 days of the degradation, for all samples, less than the 10% of the encapsulated ascorbic acid have been released (Stevanović et al. 2007a; Stevanović et al 2007b). At the beginning the particles maintain the initial shape, but after 24 days the particles start being agglomerated, creating the porous film, where the porosity increases until the complete degradation of the samples. By the end of the experiment the nanoparticles have fully

degraded and there were no more traces of them in the solution. PLGA degrades via backbone hydrolysis (bulk erosion) and the degradation products are the monomers, lactic acid and glycolic acid. It could be expected that the faster degradation of the lower molar mass fraction, present in copolymer, increases the local acidity, thereby, accelerating the hydrolysis of higher molar mass species. In another words, when acid accumulation creates a local pH drop, catalytic degradation of the polymer itself occurs. The different ionized forms of the ascorbic acid have different redox properties, so that the redox-chemistry of the ascorbic acid is highly pH dependent (Bors et al 1997; Rozario et al. 2005; Buettner 1988). Ascorbic acid decomposes into biologically inactive compounds by auto-oxidation only at alkaline pH (Kim et al. 1996). In the solution with low pH, decomposition of the ascorbic acid can happen for example under the influence of the enzymes (enzymatic oxidation) (Kim et al. 1996).

The biological behaviour of PLGA nanospheres without and with encapsulated ascorbic acid is discussed in terms of *in vitro* toxicity in human hepatoma cells and *in vivo* biodistribution in rat after intravenous injection (Stevanovic et al. 2009). Neither PLGA nanospheres nor PLGA/ascorbic acid 85/15% nanoparticles significantly affected the viability of the HepG2 cells (Stevanovic et al. 2009). PLGA nanospheres with encapsulated ascorbic acid exhibit prolonged blood circulation accompanied by time dependent reduction in lung, liver and spleen, and addition in kidney, stomach and intestine (Stevanovic et al. 2009).

Vitamin C/Eudragit® microspheres obtained by spray-drying method have been incapable to provide slower release of the drug with respect to the free form of ascorbic acid. However, these microparticles have a morphology and size distribution that make these particles promising for the delivery of ascorbic acid in the treatment of colorectal cancer (Esposito et al. 2002).

The procedure of obtaining microparticles with encapsulated ascorbic acid by spray drying was described by Finotelli and co-workers (Finotelli et al. 2005). The morphology of the Capsul/vitamin C particles was observed by a scanning electron microscopy, whose analysis showed a tendency of agglomeration. Particle size analysis showed a multi-modal particle size distribution, but with a main mode in intermediate diameters range (4–8 μm). The particle yield was 52%. Ascorbic acid stability was studied for particles stored, at both, room temperature and at 45°C showing 100% of retention at the beginning. Microcapsules containing 20% of ascorbic acid recovered by a mixture presented 7% of ascorbic acid reduction in samples for up to 60 days stored at 28°C temperature.

Spray drying technique was also used by Desai and co-workers for encapsulation of ascorbic acid in tripolyphosphate cross-linked chitosan microparticles (Desai et al. 2005a; Desai et al. 2005b; Desai et al. 2006a; Desai et al. 2006b) The particles were with spherical shapes and a mean size of about 6 μm to-

9.0. The ascorbic acid encapsulation efficiency was 58%. Encapsulation efficiency has been decreased as the amount of tripolyphosphate solution has been increased. Also the quantity of crosslinking influenced the release rate and the size of the particles.

5. Conclusion

This chapter reports on polymers for a controlled delivery of active substances. It focuses on most widely used encapsulation techniques for the encapsulation of active substances within polymeric carriers such as thermal phase separation, melt dispersion, solvent evaporation, spray drying, homogenization of water and organic phases, etc. This chapter also gives a comparison of the characteristics of polymeric micro- and nanoparticles prepared by different methods. Presently there is great interest for the polymer-carrier systems in the field of medicine, pharmacy and food industry based on the need for a system with a controlled and balanced release of active substances. It is foreseen that applications of different methods in micro- and nanoparticle research for this purposes will expand in the near future.

Acknowledgements

M.S. acknowledges the support from the Ministry of Education, Science and Technological Development of the Republic of Serbia

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