Poly(lactide-co-glycolide)-based Micro and Nanoparticles for the Controlled Drug Delivery of Vitamins

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Abstract: Controlled drug delivery systems and polymeric carriers have undergone significant development in recent years. Polymers like polylactides (PLA), polyglycolides (PGA), poly(lactide-co-glycolides) (PLGA), are approved by the World Health Organization (WHO) and Food and Drug Administration (FDA) as materials that can be used in medicine and pharmacy. Owing to their biodegradable nature, polymer materials, such as copolymer poly(DL-lactide-co-glycolide), are widely used in various medical applications; controlled release of delivering drugs, carriers in the tissue engineering, fixation of bone fractures, chirurgical strings, etc. Polymeric particles are used for the controlled delivery of several types of medicaments, including anticancer agents, antihypertensive agents, immunomodulatory drugs, hormones, vitamins and macromolecules, such as nucleic acid, proteins, peptides, antibodies, etc. Preparation of poly(lactide-co-glycolide) submicron spheres poses serious challenges. The present review attempts to address some important issues related to micro/nanoparticle-based delivery systems comprising poly(lactide-co-glycolide), with a special reference to PLGA for the controlled delivery of vitamins. A range of topics is discussed, including formulation aspects of micro- and nanoparticles, the effects of particle size and size distribution, most commonly used incorporation techniques, surface modification with stabilizers, surface functionalization, and factors affecting degradation and drug release rate.

Keywords: poly(lactide-co-glycolide), micro- and nanoparticles, drug delivery, drug release, vitamins delivery.

1. INTRODUCTION

Nanotechnology has become a rapidly growing field with potential applications ranging from electronics to cosmetics [1-7]. Creating nanomaterials such as nanoparticles, nanorods, nanowires, nanotubes and thin films is the key component for a successful development of nanotechnology owing to their extraordinary physical and chemical properties resulting from the nanosize effect [7-9]. Fueled by flourishing development in preparation of nanomaterials, a number of applications in the biomedical field have been proposed, and some of them, such as DNA sensors, controlled drug delivery, tumor therapy etc., are coming close to successful development [9].

Conventional drug delivery implies periodic dosing of a compound, which results in drug levels oscillations around a desired steady state level, and between the side effect level and the minimum therapeutic level, within the ideal therapeutic window [10]. The most part of the drug content tends to be released rapidly after the administration, which may cause a rapid increase of the drug concentration in the body. Concentration oscillations of the administered drug may cause alternating periods of ineffectiveness and toxicity [7]. Controlled drug delivery strategies have made a dramatic impact on medicine. Controlled drug release can be achieved by a combination of carrier materials and active agents [11]. Carrier matrices are usually formed from biocompatible materials such as solid lipid nanoparticles [12-14], inorganic materials [15, 16] or spheres fabricated from biodegradable polymers [17, 18]. In general, controlled-release polymer systems deliver drugs in the optimum dosage for long periods. Apart from the maintenance of optimum therapeutic drug concentration in blood or in a cell, the advantages of controlled delivery systems include predictable and reproducible release for extended periods of time, enhancement of activity duration for short half-life drugs, reduction of side effects, frequent dosing and waste of drug, optimized therapy, and better patient compliance [19].

Polymer micro- and nanospheres can be employed to deliver medication in a rate-controlled and sometimes targeted manner. Biodegradable polymers can be natural polymers, modified natural because they are always biodegradable [19]. Drug delivery systems prepared through the combination of biodegradable and biocompatible materials make a major focus area in the engineering of medical devices [10]. Micro- and nanospheres fabricated from a biodegradable polymer for drug delivery systems have become increasingly important owing to the fact that such systems enable controlled drug release at desired sites [10].

While advantages of controlled drug delivery can be significant, potential disadvantages cannot be ignored: possible toxicity or non-biocompatibility of the materials used, undesirable by-products of degradation, the fact that surgery may be required to implant or remove the system, the possibility of patient discomfort caused by the delivery device, and the higher cost of controlled-release systems compared to traditional pharmaceutical formulations [18].

A number of polymers have been investigated for formulating biodegradable nanoparticles, such as polylactide (PLA), polycaprolactone (PCL) and poly(lactide-co-glycolide) (PLGA). These are biocompatible and biodegradable polymers which have recently been the subject of extensive investigation [19-21]. However, due to copolymer crystallization, low biodegradation rate or poor flexibility, the application of polymer nanoparticles is limited. For example, in case of homopolymer poly(L-lactide), due to its crystalline and low biodegradation rate, drug release from relevant drug delivery devices is mainly controlled by drug diffusion similar to that in non-degradable drug carriers [22]. Biodegradation of polymers involves cleavage of hydrolytically or enzymatically sensitive bonds in a polymer, leading to polymer erosion. Depending on the mode of degradation, polymeric biomaterials can be further classified into hydrolytically degradable polymers and enzymatically degradable polymers. The most part of naturally occurring polymers undergo enzymatic degradation [19]. Biodegradation of hydrolysable polymers proceeds in a diffuse manner, with amorphous regions degrading prior to the complete split of crystalline and cross-linked regions [20]. Factors affecting biodegradation of polymers might be: chemical structure, chemical composition, distribution of repeat units in multimers, presence of ionic groups, presence of unexpected units or chain defects, configuration structure, molecular weight, molecular weight distribution, morphology (amorphous/semicrystalline, microstructures, residual stresses), presence of low-molecular-weight compounds, processing conditions, annealing, sterilization process, storage history, shape, site of implantation, adsorbed and absorbed compounds (water, lipids, ions, etc.), physicochemical factors (ion exchange, ionic strength,
pH), physical factors (shape and size changes, variations of diffusion coefficients, mechanical stresses, stress-and-solvent-induced cracking, etc.), mechanism of hydrolysis (enzymes versus water) [19, 22].

The objective of this review is to highlight the current status of poly(lactide-co-glycolide) as a drug delivery vehicle. This review covers synthesis, the effect of particle size and size distribution, commonly used incorporation techniques, surface modification by stabilizers, surface functionalization, and factors affecting degradation and drug release rate.

2. POLY(LACTIDE-CO GLYCOLIDE)

Structure, properties and applications of nanoparticles are strongly affected by the properties of the polymer used in their formulation. For each application and drug, one must evaluate the properties of the system (drug and particle) and determine the optimal formulation for a given drug delivery application. Polymers based on poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(caprolactone) (PCL), and their copolymers have been extensively employed as systems for drug delivery [23-29]. PLGA (Fig. (1)) and PLA have been approved by the FDA for numerous clinical applications, such as sutures, bone plates, abdominal mesh, and extended-release pharmaceuticals [30-35].

![Chemical structure of PLGA polymer. The "m" component represents lactic acid and "n" component represents glycolic acid.](image)

Biomedical uses of PLA have been reported since the 1960s [31]. Tissue response to such biodegradable materials is characterized by minimal localized inflammation and foreign body reaction that lessen with time. No toxic effects have been associated with the use of such polymers, biodegraded via a random, non-enzymatic process into homopolymers of lactic acid and glycolic acid, known products of cellular intermediary metabolism [36-38]. PLGA degrades through hydrolysis of its ester linkages in the presence of water. It has been shown that the time required for the degradation of PLGA is related to the ratio of monomers used in its production: the higher the content of glycolide units, the lower the time required for degradation. An exception to this rule is copolymer with 50:50 ratio of D- and L-lactic acid monomers, which undergoes faster degradation (about two months) in both in vitro and in vivo conditions [39-41]. Miller et al. have shown that PLGA 50:50 is the fastest degrading composition, with the degradation rate being decreased when either lactide or glycolide content of the copolymer was increased [42].

PLGA can be synthetized by a polycondensation reaction, or via ring-opening polymerization of cyclic diesters (Fig. (2)) [43-46]. Ring-opening polymerization is currently the preferred method for the synthesis for PLGA and PLA due to shorter reaction times and higher monomer conversion rates [44-46].

![Ring-opening polymerization of glycolide to polyglycolide.](image)

Using the properties of polyglycolide and poly(l-lactide) as a starting point, it is possible to copolymerize these two monomers to extend the range of homopolymer properties [47-49]. Glycolide monomer is synthesized through dimerization of glycolic acid. PGA is highly crystalline with a high melting point and a glass-transition temperature of 35—40°C [50, 51]. Lactide is a cyclic dimer of lactic acid, which has two optically isomeric forms, d and l [48]. L-lactide is the naturally occurring isomer, whereas dl-lactide is the synthetic blend of dl-lactide and l-lactide. The homopolymer of l-lactide (PLLA) is a semicrystalline polymer, while poly(dl-lactide) (DLPLA) is an amorphous polymer due to irregularities in its polymer chain structure [52, 53]. The greatest part of PLA types used in biological applications exist in the racemes D, L form (DLPLA) and are amorphous polymers. DLPLA and PLGA have glass transition temperatures above body temperature [44].

The selection of reactants and the synthesis conditions determine the physiochemical properties of the resulting polymers, such as hydrophilicity, mechanical strength, glass transition and crystallinity [54-56]. The parameters that can be used to describe the final polymers include weight or number-average molecular weight, polydispersity, the ratio of lactide and glycolic acid monomers, the ratio of D- and L-lactic acid monomers, the end-group functionality, the segment length of monomeric repeat units, etc [57-60].

3. FORMULATION ASPECTS OF MICRO AND NANO POLYMERIC PARTICLES

Depending on the nature and matrix of the selected material, methods for obtaining polymer particles can be generally divided into three groups: dispersion of preformed polymers, polymerization of monomers, and ionic gelation or coagervation of hydrophilic polymers. However, other methods such as supercritical fluid technology and particle replication in non-wetting templates (PRINT) have also been described in the literature [61-63]. Many approaches are proposed for the preparation of PLGA particles. The emulsification-evaporation method [64-67], spontaneous emulsification-solvent diffusion method (SESD) [20, 68], nanoprecipitation method [69, 70] and spray-drying [71-73] are all widely used in preparing PLGA particles 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). If appropriate, the drug may also be dispersed as a solid powder in an organic polymer solution, or codissolved in a common solvent with the polymer. The solution or dispersion is then processed according to one of the aforementioned methods. During the nanoparticle formation using emulsification-evaporation and SESD approaches, toxic organic solvents such as CH₂Cl₂ and CHCl₃ are usually employed [68]. To meet the requirement for the clinical use, residual solvents should be completely removed from PLGA particles [74].

In solvent extraction or evaporation method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as a solvent for the hydrophobic drug. The mixed polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form an oil-in-water (o/w) emulsion. After the formation of a stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. The particle size is found to be influenced by the type and concentration of stabilizer, homogenizer speed and the polymer concentration [75]. In order to obtain small particle size, high-speed homogenization or centrifugation may be employed [76, 77]. By changing parameters like aging time (after the non-solvent is added), or time and velocity of centrifugal processing, it is possible to influence morphology, size and uniformity of PLGA particles [77]. For example, PLGA powder obtained by physicochemical solvent/non-solvent method with the shortest aging time with non-solvent, the longest time and highest velocity of
and melted or bonded together to form microstructures according to the processing method. Microparticles were filled into micromolds at room temperature with or without encapsulating model drug compounds. These polymers and microparticles of 1 to 30 μm in size were made from PLA, PGA and PLGA using spray drying and emulsion techniques either homogenous precipitation was applied for the preparation of microcomposites, with particles 150–200 nm in size, and nanocomposite, with particles 40±5 nm in size [91].

Spontaneous emulsification or the solvent diffusion method is a modified version of solvent evaporation method [78]. In this method, a water-miscible solvent is used along with a small amount of the water-immiscible organic solvent as an oil phase. Due to a spontaneous diffusion of solvents, an interfacial turbulence arises between the two phases, leading to the formation of small particles. As the concentration of water-miscible solvent increases, a decrease in the size of particle can be observed. Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drugs, multiple w/o/w (water-in-water) emulsion needs to be formed with the drug dissolved in the internal aqueous phase.

In the coacervation technique the coating precipitates onto a droplet of the drug [79]. Coacervation consists of three stages taking place under constant agitation: first, a solution must be formed with three immiscible phases: the core material (active ingredient), the coating material and a solvent; second, the liquid coating is deposited around the core material, which is accomplished by mixing the coating phase with the solvent phase (in which the active ingredients reside); and third, the coating is rigidized thermally or by desolvation [79, 80]. Spray-drying offers an attractive and relatively simple alternative to the previous methods. Here, the antigen solution or W/O emulsion is atomised in a flow of drying air at a slightly elevated temperature. The organic solvent is rapidly vaporized leaving behind solid micro and nanoparticles that are separated from the drying air in a cyclone and collected in a deposition chamber [81, 82].

Park et al. developed methods based on filling micromolds with polymer microparticles, as opposed to polymer melts, to produce microstructures composed of multiple materials, having complex geometries, and made using mild processing conditions [83]. Polymer microparticles of 1 to 30 μm in size were made from PLA, PGA and PLGA using spray drying and emulsion techniques either with or without encapsulating model drug compounds. These polymer microparticles were filled into micromolds at room temperature and melted or bonded together to form microstructures according to different protocols [83].

Emulsion process produced PLGA spheres of 100-250 μm [84], 45 μm [85], 30 μm [86] in diameter. Modification of the emulsion process led towards obtaining spheres with smaller diameters up to 10 μm [87]. PLGA microspheres with encapsulated paclitaxel were prepared using spray drying technique. The particles were in the size range of 1-8 μm, suitable for intraperitoneal and intrapleural lymphatic targeting delivery [88]. 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 [89] and 244-260 nm [90] in diameter. PLGA particles obtained by physicochemical solvent/non-solvent method were in the size range of 110-170 nm [23]. Calcium phosphate was incorporated into the PLGA polymer matrix by emulsion procedure using solvent–non-solvent system [91]. The use of calcium phosphates (CP) and CP-based composite biomaterials in medical treatment is currently an interesting field of research aimed at developing different biomaterials for the reconstruction of human tissue [91-94]. Two kinds of composites were prepared: microcomposite, with particles 150–200 nm in size, and nanocomposite, with particles 40±5 nm in size [91].

Sonochemistry is a widely used method for obtaining nanostructured composite materials due to resulting chemical effects, like surface influence of radicals, and physical effects, like intensive dispersion, homogenisation and emulsification [92]. Sonochemical homogenous precipitation was applied for the preparation of PLGA-hydroxyapatite (PLGA/HAp) particles [95]. The morphology and spatial arrangement of obtained particles processed at low temperature during the synthesis process were determined. The most regular morphology was obtained for PLGA/HAp composite with 90:10 weight percent ratio fabricated at lower (8°C) bulk temperature. These spherical particles were in the range between 30 and 300 nm in size and they had highly regular spatial arrangement [95].

PLGA nanoparticles can also be synthesized by the nonprecipitation method as described by Bilati et al. [69]. It was 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 560 nm in size [69]. With spray-drying applied for the preparation of cationic PLGA nanoparticles as gene delivery vectors, in order to minimize aggregation and loss of gene transfection efficiency, the mean particle diameter was 100–250 nm [73].

Jin et al. examined PLGA nanoparticles with encapsulated paclitaxel, etanidazole or paclitaxel+etanidazole prepared by o/w and

Fig. (3). SEM images of PLGA particles with different aging time in the presence of non-solvent, and with different time and velocity of the centrifugal processing: a) 10 min and 15 min on 1500 rpm (bar 5 μm) b) 30 min and 30 min on 3000 rpm (bar 1 μm) c) 5 min and 60 min on 4000 rpm (bar 1 μm) d) 5 min and 60 min on 4000 rpm (bar 0.5 μm) e) 5 min and 120 min on 6000 rpm (bar 0.5 μm).
w/o/w emulsification-solvent evaporation method [96]. The prepared nanoparticles were spherical with size between 80 and 150 nm. 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 [67].

4. THE EFFECT OF PARTICLE SIZE AND SIZE DISTRIBUTION

PLGA particles allow the encapsulation of medicaments within the polymer matrix, and the crucial requirements for the controlled and balanced release of the medicament in the body are their ideal spherical shape and narrow size distribution [97]. The size and shape of particles play the key role in their adhesion and interaction with the cell. Drug release dynamics (rate and concentration) depend on morphology, particle porosity, etc. Chemical structure, molecular weight, composition, as well as the synthesis conditions, are parameters which influence the final morphology of the polymer [97, 98]. The direct relation between these parameters and morphology is insufficiently examined, thus making it a topic of many research studies.

A possible mechanism enabling the particles to pass through gastrointestinal (and other physiological) barriers could be: (1) paracellular passage—particles “kneading” between intestinal epithelial cells due to their extremely small size (<50 nm); (2) endocytotic uptake—particles absorbed by intestinal enterocytes through endocytosis (particles size<500 nm); and (3) lymphatic uptake—particles adsorbed by M cells of the Peyer’s patches (particle size <5 μm) [99].

Jani et al. [100, 101] observed that particles with mean diameters of 50 and 100 nm showed a higher uptake in the rat intestine than larger particles. The uptake of nanoparticles was followed by their appearance in the circulatory system and distribution to different tissues. After the administration of equal doses, 33% of the 50 nm and 26% of the 100 nm nanoparticles were detected in the intestinal mucosa and gut-associated lymphoid tissues (GALT). In the case of 500 nm nanoparticles, only 10% were localized in intestinal tissues. Particles bigger than 1 μm in diameter yielded fairly low uptake and were exclusively localized in Peyer’s patches. Although particles>3 μm were found occasionally in the follicle-associated epithelia (FAE), the passage to associated lymphoid tissues could not be observed.

Nanoparticles offer a number of advantages over microparticles. For example, nanoscale particles can travel through the blood stream without sedimentation or blockage of the microvasculature. Small nanoparticles can circulate through the body and penetrate tissues like tumors. In addition, nanoparticles can be taken up by cells through natural means, such as endocytosis. Nanoparticles have already been used to deliver drugs to target sites for cancer therapeutics [102] or deliver imaging agents for cancer diagnostics [103]. These vehicles can be engineered to recognize biophysical characteristics that are unique to the target cells, minimizing thus drug loss and toxicity associated with the delivery to non-desired tissues.

Since the smallest capillaries in the body are 5–6 μm in diameter, particles distributed into the bloodstream must be much smaller than 5 μm and they must not form aggregates. An advantageous feature of particles smaller than 220 nm is that they could be easily sterilized by filtration, since the sizes of bacteria and viruses are larger [98].

It has been reported that the size of PLGA particles ranged from 100 to 500 nm, the standard deviation being up to 30% or more. The size of PLGA particles has been traditionally measured using photon correlation spectroscopy (PCS, also called dynamic light scattering). However, PCS measurement may not be as precise as measurements using transmission electronic microscopy (TEM). The latter reflect the exact particle size, whereas PCS measures merely the hydrodynamic diameter of PLGA particles. For example, Astete et al. reported 38±0.2 nm and 67±0.2 nm PLGA particles from PCS, but large distribution (15–40%) was observed in TEM images [67]. Thirumala et al. reported 20±0.2 nm and 157±0.9 nm PLGA particles using PCS, but their standard deviation was broad (50–70%) in TEM images [70]. Therefore, the preparation of uniformly sized PLGA particles is still a challenge.

However, the use of nanosized particles for inhalation treatment has certain disadvantages. Their mass median aerodynamic diameter (MMAD) is not suitable for inhalation since their size is too small. It is expected that a large fraction of the inhaled dose will be exhaled, i.e. that the dose deposited in the lungs will be very small. It is reported that particles having 2–3 μm in diameter have the most effective properties in terms of the deposition in a deep lung site [104, 105]. In order to use nanoparticles for an inhalation treatment, nanocomposite particles with 2–3 μm in diameter were prepared as a blend of nanoparticles and additives. The nanocomposite particles are designed to decompose into primary nanoparticles after reaching the deep lung site [106].

5. COMMONLY USED INCORPORATION TECHNIQUES

A drug can be dissolved, adsorbed, entrapped, encapsulated or covalently attached to the surface of the particles and, depending on the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained [53, 106, 107]. Nanocapsules are vesicular systems in which a drug is confined to a cavity surrounded by a polymer membrane, while nanospheres are matrix systems in which a drug is physically and uniformly dispersed [107]. PLGA particles are used for the controlled delivery of several classes of medicaments like anticancer agents, antihypertensive agents, vitamins, immunomodulatory drugs, hormones and macromolecules like nucleic acid, proteins, peptides, antibodies, etc. Ideally, “the successful” system for a controlled delivery of medicaments should have high encapsulation efficiency, i.e., it should incorporate a substantial amount of the medicament.

In literature, two methods for incorporating medicaments into PLGA are described. Drugs may be incorporated either simultaneously with the formation of nanoparticles (incorporation method), or through an absorption of the drug after the formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption/absorption technique). Drug loading and entrapment efficiency largely depend on the solid-state drug solubility in a matrix material or polymer (solid dissolution or dispersion), which is related to the polymer composition, molecular weight, drug–polymer interaction and the presence of end-functional groups (ester or carboxyl) [70, 108, 109]. However, for nanoparticles prepared by a double emulsion process, the solid-state solubility of the drug in the polymer was not found to affect drug loading. Drug loading and encapsulation in the nanoparticles appeared to be governed by the partition coefficient of the drug between the organic phase and the external aqueous phase employed in nanoparticle preparation [110].

The way in which a drug is distributed in a medium may also influence its release profile [111].

For the multi-reservoir type microspheres composed of poly(dl-lactide-co-glycolide) and poly(dl-lactide), the influence of the drug-holding layer and the non-drug-holding layer on drug release profiles was studied by Matsumoto et al. [112]. Microspheres with the blend of PLGA and PLA were prepared by the W/O type emulsion-solvent evaporation technique; cisplatin was used as a model drug. The results of the study indicate that drug release from multi-reservoir type microspheres involves the following process: (a) rapid release of the drug near the surface of microspheres, (b) for-
mation of microspheres in the non-drug-holding layer by hydration and erosion, (c) degradation of the drug-holding layer, and (d) diffusion of the drug through microspheres [112].

6. SURFACE MODIFICATION BY STABILIZERS

The aggregation of PLGA particles during the process of particle formation is a notable problem regardless of the preparation method. In order to prevent the aggregation of PLGA particles, polymer stabilizers are often used. Furthermore, the size and shape of the particles can also be influenced by the stabilizer used. Stabilizers or surfactants are amphiphilic molecules that possess both hydrophilic and hydrophobic parts. The hydrophilic moiety is called the head and the hydrophobic part the tail (or tails) [113]. The hydrophobic part may consist of a single chain or may have up to four chains [113]. The head can be a charged or uncharged polar group. Depending on the nature of the head groups, stabilizers are classified as anionic, cationic, non-ionic and zwitterionic (amphoteric) [113, 114]. The type of the drug dissolved and the conditions of the target site will determine the type of surfactant used to carry the medicine.

The most commonly used stabilizers of polymer particles include polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), Tween 80, Floucon 127 (poloxamer 407), Flucon 68 (poloxamer 188), didodecyldimethyl ammonium bromide (DMAB), carbopol (prop-2-enonic acid), etc. [20, 53, 65, 68-70,115-122]. These stabilizers are deposited on the surface of PLGA particles and can affect the zeta potential, particle size and particle surface properties. PVA and PVP create negatively charged PLGA particles [122]. They induce specific zeta potential, which is the electrical potential that exists across the interface of all solids and liquids. The value of zeta potential is a very important characteristic of the particle and it has a significant influence on its stability. With the creation of the specific zeta potential, PVA (or PVP) reduces the agglomeration because the particles of the same charge are not attracted to each other [122, 123]. Also, the coating of particles with appropriate bioadhesive materials, such as polyvinyl alcohol, poly(ethylene glycol) (PEG), vitamin E TPGS, etc., can greatly improve their adhesion and absorption into the intestinal cells as well as the ability to escape from the multi-drug resistance pump proteins [124-127]. Protection offered by surfactants is primarily a function of their surface activity. Unlike proteins, which reduce antigen loss by decreasing the adhesion of particles to the surface, surfactants provide additional protection against irreversible aggregation of partially denatured antigens [82, 128].

According to literature data, polyvinyl alcohol (PVA) is a widely used stabilizer for the production of PLGA nanoparticles [122]. The influence of the concentration of PVA and the polymers tested on particle size and zeta potential value was evaluated before and after freeze-drying of the prepared particles by Vandervoort et al. [129]. Leaving PVA out of the formulation increased the size of the particles by over 1 μm [129]. The morphology (size and shape) and the uniformity of the PLGA particles can be modelled under different type of stabilizers. PLGA powder obtained in the experiment in which PVP was used as a stabilizer consists of highly uniform spherical particles with a low level of agglomeration and the particle size ranging between 110 to 170 nm, which means that they are smaller than those of PLGA spheres obtained in the experiment with PVA as a stabilizer (150 to 230nm) [122]. Zeta potential values were usually slightly negative; the most extreme zeta potential values were measured when poloxamer and carbopol were employed. The use of gelatin type A made it possible to achieve positive values [129].

It has been demonstrated that the type and concentration of stabilizer, homogenizer speed and polymer concentration determine the size of PLGA nanoparticles. Kwon et al. have shown that the application of didodecyl dimethyl ammonium bromide (DMAB) as stabilizer yields estrogen containing nanoparticles smaller than 100 nm [130].

However, although polymer stabilizers may prevent aggregation of nanoparticles, they are difficult to remove even through washing. Furthermore, most polymer stabilizers do not have functional groups for further modification, which significantly limits their biomedical application. So far, only a few studies demonstrated successful biomolecule conjugation using stabilizers and this process usually requires extended experimental time, e.g., 24 h for the reaction [131].

Also, the use of surfactants should be limited to the minimum level in order to avoid possible toxic and hypersensitivity reactions [132].

7. SURFACE FUNCTIONALIZATION

Great efforts have recently been made to make biomaterials more biocompatible. Generally, our living system recognizes biomaterials as foreign bodies through the surface contact. Therefore, a biomaterial that has a surface quite different (different surface properties such as crystallinity, topography, texture, defects, etc.) from that of the living structures may be very poor in interfacial biocompatibility [133]. Thus, the rationale for the surface modification of biomaterials is straightforward: retain the key physical properties while modifying only the outermost surface to influence biointeraction [134, 135]. Non-specific adsorption of plasma proteins on PLGA micro- and nanospheres is a great limitation of drug targeting. Also, a serious handicap in drug targeting is the rapid uptake of intravenously injected particulate drug carriers by the cells of the reticuloendothelial system (RES), comprising mainly the Kupffer cells of the liver and the macrophages of the spleen and bone marrow [136]. The circulation time of PLGA microparticles in a bloodstream in vivo is determined by the physicochemical characteristics of the particles, especially their size, surface charge and surface affinity [137]. It has long been known that the removal of particles by the spleen increases with increasing particle size [138, 139]. The adsorption of plasma proteins, on the other hand, is regarded as the key factor in explaining the organ distribution of microspheres.

In order to control the targeted drug delivery of intravenously delivered nanoparticles, nanoparticle interactions with other cells, such as macrophages must be controlled. Various approaches have been developed to control these interactions, ranging from changing the size of the particle to changing nanoparticle surface properties [140, 141]. In order to eliminate nonspecific protein adhesion and decrease uptake by macrophages, nanoparticles can be functionalized using protein replant materials, such as poly(ethylene glycol) (PEG) [140] and polysaccharides [103, 141]. Nonadhesive surface coatings increase the circulation time of the nanoparticles [140] and reduce toxic effects associated with non-targeted delivery [142, 143].

Because of the inert nature of most commercial polymers, they must undergo surface functionalization prior to attachment of a bioactive compound [144]. The second step is therefore to optimize surface functionalization techniques in order to introduce the desired type and quantity of reactive functional groups. The major methods of immobilizing a bioactive compound to a polymeric surface are adsorption via electrostatic interactions, ligand–receptor pairing and covalent attachment. Non-covalent adsorption is sometimes desirable, as in certain drug delivery applications.

Polymeric nanocarriers such as poly(DL-lactide-co-glycolide) have shown promising pharmacokinetics both at the whole-body and cellular levels (passive targeting) [2,145-147]. The active drug targeting is usually achieved by the chemical attachment onto a targeting component, that strongly interacts with the receptor(s) displayed on the target tissue, leading to the preferential accumulation of the drug in the targeted organ, tissue, or cells [2].
Bioactive compounds for surface functionalization of PLGA can be enzymes, peptides, polysaccharides, phospholipids analog, poly(ethylene glycol) (PEG), etc [124]. Cheng et al. [148] developed ~250nm nanoparticles-aptamer (NP-Apt) biocongeguates using poly(D,L-lactide)--block--poly(ethylene glycol) (PLA–b–PEG) copolymer and the A10 RNA Apt [149] that can bind the extracellular domain of the prostate specific membrane antigen (PSMA), and demonstrated their capability for active binding and uptake by the targeted cancer cells in vitro [150]. They also developed ~180 nm docetaxel-encapsulated nanoparticles-Apt bioconjugates using poly(D,L-lactide--co--glycolide)--block--poly(ethylene glycol) (PLGA–b–PEG) copolymer that showed remarkable antitumor efficacy in vitro after a single intratumoral administration to subcutaneous xenograft mouse models of prostate cancer.

In the active drug targeting, folic acid is often used as a ligand to encourage intracellular uptake of drugs [145,151-153]. Folates (the anion form) are low molecular weight vitamins required by eukaryotic cells, and their conjugates have the ability to deliver a variety of drugs or imaging agents to pathological cells without causing harm to normal tissues [2]. Folate targeting is an interesting approach for cancer therapy because it offers several advantages over the use of monoclonal antibodies [154]. More importantly, elevated levels of folate receptors are expressed on epithelial tumors of various organs such as colon, lung, prostate, ovaries, mammary glands, and brain [155]. Folate is known to be non-immunogenic, and folate-conjugated drugs and/or nanoparticles are rapidly internalized via receptor-mediated endocytosis [145, 156, 157].

Wang et al. have reported silk fibroin coating on PLGA and alginate microspheres [158]. Silk coating on the PLGA microsphere surface was heterogeneous with an average thickness of about 1 μm, where as it was homogenous with a thickness of about 10 μm on the alginate microsphere surface. Silk fibroin coatings not only stabilized microspheres against degradation but also sustained protein drug release from the microspheres by providing an effective diffusion barrier. Protein drug loading was not changed by silk coating in either case.

PLGA nanoparticles, modified with both alendronate and poly-ethylene glycol (PEG), were prepared by the dialysis method without additional surfactant in order to evaluate the potency of the bone-targeted drug delivery as described by Choi et al [159]. Alendronate, a targeting moiety that has a strong affinity for bone, was conjugated to PLGA polymer via carbodiimide chemistry. The surface-modified PLGA nanoparticles with various ratios of alendronate and mPEG densities on their surface had a strong and specific ability to adsorb onto hydroxyapatite.

Various approaches have been proposed to functionalize the surface of biodegradable PLGA microparticles and nanoparticles [160-165]. The negatively charged surface of PLGA microparticles has been functionalized by electrostatic binding of cationic surfactants, such as cetyltrimethylammonium bromide (CTAB) [166]. An alternative to the use of cationic surfactants is electrostatic coating with polycationic polymers, such as chitosan, poly(llysine) or poly(ethyleneimine) (PEI) [167-169].

8. FACTORS AFFECTING DEGRADATION AND DRUG RELEASE RATE

Poly(lactide-co-glycolide) (PLGA) is a highly biocompatible, mechanically processable polymer that degrades while yielding water soluble, non-toxic products of a normal metabolism [23, 111, 170-172]. The term "degradation" designates the process of polymer chain cleavage, which leads to the loss of molecular weight. Degradation induces subsequent erosion of the material, which is defined as the weight loss of the material brought about by the polymer chain cleavage [173]. For degradable polymers, two different erosion mechanisms have been proposed: homogeneous or bulk erosion, and heterogeneous or surface erosion [174, 175]. However, for most polymers, erosion has features of both mechanisms.

Although PLGA is insoluble in water, it is hydrolytically unstable and is degraded by hydrolysis of its ester bonds [176]. Through this hydrolytic attack, random chain scission occurs, causing it to degrade into lactic and glycolic acids [59]. Since PGA is more susceptible to hydrolysis than PLA, by changing the ratio of these two components, PLGA polymers can be synthesized with various degradation rates. Degradation first occurs in amorphous micro/nanospheres regions and is followed by a slower degradation in crystalline regions. This suggests that crystallinity in polymer chains can affect the degradation [111].

Various studies have revealed that in vitro and in vivo PLGA degradation are the result of several processes occurring simultaneously. These include water uptake, swelling, ester hydrolysis, diffusion of oligomers and degradation products, and local pH drop [177-182]. PLGA monomers, lactic acid and glycolic acid are non-toxic and can be removed from the body by normal metabolic pathways [180]. However, the biocompatibility of degraded oligomers and particles remains questionable. For example, oligomers and polymer particles can elicit inflammatory responses, sometimes causing tissue necrosis. This has been documented in several long-term studies [183, 184].

PLGA degrades via backbone hydrolysis (bulk erosion) and the degradation products include monomers, lactic acid and glycolic acid. It could be expected that the faster degradation of the lower molar mass fraction, present in the copolymer, increases the local acidity, accelerating thus the hydrolysis of higher molar mass species. In other words, when acid accumulation creates a local pH drop, catalytic degradation of the polymer itself occurs [23].

However, until now the degradation process has not been completely elucidated. From a general point of view, two phenomena are discussed. Firstly, degradation causes an increase in the number of carboxylic end groups, which are known to autoactivate ester hydrolysis [174, 185]. In the second stage, with increasing the degradation time, the amount of oligomer within the polymer matrix increases and soluble oligomers can escape from the whole mass of the polymer device. In larger specimens, only soluble oligomers located close to the surface can diffuse from the matrix before they are totally degraded, whereas oligomers located at more inward positions within the matrix remain entrapped and increase the acidity within it. The encapsulated oligomers increase the concentration of ester and carboxyl bonds, which results in an increased degradation rate and autocatalysis in comparison to the outer part of the specimen. These diffusion reaction phenomena [186] lead to a differentiation between the surface and the centre in larger specimens [187-189].

Apart from specimen size [187] and copolymer ratio, it has been shown that the degradation rate of PLGA is also affected by a number of other factors, such as the type of encapsulated medicament, initial pH, porosity, etc. [23, 190].

8.1. Effect of the type of encapsulated medicament

As for the design of biodegradable polymeric drug carriers, one must take into consideration the effect of the drug on the polymer degradation and drug release rate [191, 192]. This is especially relevant in the case of drug carriers with high drug loadings.

The physico-chemical properties of the incorporated drug(s) might significantly affect the resulting release patterns and degradation of the polymer matrix, especially at high initial drug loadings. For example, high content of freely water-soluble drugs can facilitate water penetration and lead to the creation of highly porous polymer networks upon drug leaching. In contrast, lipophilic drugs can hinder water diffusion into the system, slowing down polymer
degradation. In the case of significant amounts of acidic and basic active agents, additional effects on the PLGA degradation kinetics can be expected, because ester hydrolysis is catalyzed by acids and bases [193, 194].

Frank et al. have studied the effect of the chemical nature of the drug on matrix degradation and drug release behavior of degradable polymers, using lidocaine as a model drug in base and salt forms [194]. It is shown in this study that the drug in the base form has a substantial effect on the release characteristics, through an accelerating effect on matrix degradation. Siegal et al. investigated the process of degradation and drug release from 50:50 PLGA pellets containing 20% (weight) drug, for several common drugs (thiothixene, haloperidol, hydrochlorothiazide, corticosterone, ibuprofen, and aspirin) [195]. They found that the mechanism of pellet degradation and the parameters of the drug release rate vary as a function of the drug type. The presence of the drug may change the degradation mechanism from bulk erosion (control) to surface degradation (haloperidol), as well as affect the rate of pellet degradation [195]. The drug release profile, as defined by the time required for 100% release and the steady-state rate, also varies significantly. The drug release profile for the four drugs seems to follow the classical diffusion/reaction kinetics. However, efforts to correlate the release rate parameters to the drug chemistry (as defined by the density of OH groups) or hydrophilicity (as given by solubility in water) did not yield a strong relationship. Thus, Siegal et al. have concluded that drug incorporation affects the rate of polymer degradation and release rate significantly, but further studies are needed to determine the relationship between the drug properties and the release rate [195].

8.2. pH Controlled Release

pH of the release medium was found to be of great importance for the resulting release patterns [200]. Drug release from PLGA microspheres can range from days to months and, therefore, accelerated in vitro drug release testing methods are often used for manufacturing batch release [201].

Numerous advantages and drawbacks of PLGA and PLGA-based delivery systems for delivering macromolecular drugs have been mentioned in the literature [202]. However, PLGA has a negative effect on the protein stability during preparation and storage, primarily due to the acid-catalyzed nature of its degradation. Its hydrolysis leads to accumulation of acidic monomers, lactic and glycolic acids within the drug delivery device, thereby resulting in a significant reduction of pH of the microenvironment (Fig. (4)) [23] and denaturation of the encapsulated proteins [202]. Poor control of
the pH in PLGA delivery systems has been implicated as one of the most significant drawbacks [203].

Many pH modifiers, mostly basic salts, have been included in PLGA formulations in attempt to stabilize the pH, but these techniques may not prevent degradation reactions that are both acid and base labile, such as deamidation [204-206]. The inclusion of salts with the purpose of modifying pH has also proved to be problematic due to their poor solubility in a great part of organic solvents used to dissolve PLGA [206]. The addition of basic salts has been linked to an increased water uptake in PLGA matrices [206], which may promote hydrolytic peptide and protein degradation reactions. Buffers also have the potential to neutralize the acidic monomers produced by the PLGA degradation without producing a basic pH.

8.3. Control of Sphere Porosity

Another important factor influencing the degradation process of poly(DL-lactide-co-glycolide) particles is its porosity. Klose et al. reported how porosity and size of the particles affect the drug release mechanisms from PLGA-based microparticles [207]. Porous PLGA particles obtained through the water-in-oil-in-water solvent extraction/evaporation method with a medicament incorporated were suspended into a phosphate buffer solution pH 7.4 in order to monitor the degradation of PLGA and the release of the medicament [207]. In contrast to non-porous microparticles of identical composition, the relative drug release rate was found to decrease with increasing the drug delivery system size [207]. In contrast to non-porous microparticles of identical composition, the relative drug release rate was found to decrease with increasing the drug delivery system size [207]. In contrast to non-porous microparticles of identical composition, the relative drug release rate was found to decrease with increasing the drug delivery system size [207]. In contrast to non-porous microparticles of identical composition, the relative drug release rate was found to decrease with increasing the drug delivery system size [207]. In contrast to non-porous microparticles of identical composition, the relative drug release rate was found to decrease with increasing the drug delivery system size [207]. In contrast to non-porous microparticles of identical composition, the relative drug release rate was found to decrease with increasing the drug delivery system size [207]. In contrast to non-porous microparticles of identical composition, the relative drug release rate was found to decrease with increasing the drug delivery system size [207].

The size exclusion chromatography (SEC), differential scanning calorimetry (DSC) and gravimetric analysis measurements have shown that the polymer degradation rate increases with increasing particle size, demonstrating that autocatalytic effects play a significant role even in small and highly porous PLGA particles [207]. However, this effect is considerably less apparent in comparison with non-porous devices. It is important to note that this is compensated; the diffusion effect becomes more pronounced with increasing the device size. The presence of pores does not only increase the mobility of the involved species (drug molecules, acids and bases), but fundamentally alters the underlying drug release mechanisms. Generally, the release of medicament during the degradation process will be faster in case of porous particles. A factor related to the sphere porosity is also, the initial burst effect, which corresponds to the rapid initial release of drug and is normally followed by the relatively controlled linear release.

Sodium chloride, silicone oil, paraffin or pluronic f127 are often used as porogens [208-214]. For example, Kim et al. described a synthesis of porous PLGA microspheres using the emulsion method in which pluronic f127 was used as porogen [215]. Human growth hormone (rhGH) was encapsulated in porous PLGA microspheres. The protein-loaded porous microspheres were readily transformed to non-porous microspheres through a treatment with water-miscible solvents under non-aqueous and vapor conditions [215]. The resulting non-porous microspheres exhibited sustained release profiles over an extended period of time [215].

9. PLGA FOR CONTROLLED DELIVERY OF VITAMINS

Poly(lactide-co-glycolide) is a copolymer material that can also be used in creating systems for controlled delivery of vitamins. One often hears of a vitamin deficit in human body, and vitamins are crucial for its normal metabolic activity. System for the controlled delivery PLGA/vitamin can brings to the more balanced and efficient concentration of the vitamin throughout the extended period of time.

For example, ascorbic acid is a water soluble vitamin that cannot be synthesised and stored in the body. It has a variety of biological, pharmaceutical and dermatological functions, but it is very
unstable in air, light, heat, moisture, presence of metal ions, oxygen, and base, and it easily decomposes into biologically inactive compounds [216]. Ascorbic acid introduced in the body in a greater portion gets isolated from the body. However, the encapsulated ascorbic acid within the polymeric matrix should have a significantly higher efficiency [23, 97]. In order to overcome chemical instability of ascorbic acid, a considerable amount of research has been staged towards its encapsulation or immobilization [23, 97]. Stevanović et al. prepared PLGA particles by physicochemical solvent/non-solvent chemical methods and centrifugal processing. The encapsulation of ascorbic acid in the polymer matrix was performed through a homogenisation of water and organic phases. The mean size of nanoparticles containing PLGA/ascorbic acid in the ratio 85/15 %, was between 130 and 200 nm (Fig. (5)) [97]. The degradation of PLGA with and without ascorbic acid in vitro within physiological solution has been tracked for eight weeks and it has been determined that PLGA completely degrades within this period, releasing the full amount of the encapsulated ascorbic acid [23].

Preparation of poly(DL-lactide-co-glycolide) microspheres through the solvent evaporation method for the controlled delivery of vitamin A palmitate (RAP) is described by Martinez-Sancho et al. [217]. Various quantities of vitamin A (10-80mg) had been incorporated into the microspheres, and then their release was tracked. The release of vitamin A from the microspheres lasted for 49 days. The mean size of the microspheres was 21.79μm [217]. Recently, Ribeiro et al. described the solvent displacement method for the formation of β-carotene-loaded nanodispersions containing PLA and PLGA. β-carotene is a pigment converted into retinol in the body and also possesses provitamin A activity. Due to its antioxidant activity, β-carotene may play an important role in preventing degenerative diseases. Nanoparticles containing β-carotene were produced by interfacial deposition of the polymer, due to the displacement of acetone from the dispersed phase. Gelatin or Tween® 20 was used as a stabilizing hydrocolloid in the continuous phase. The solvent displacement method shows some advantages, such as low energy input, high entrapment efficiency, and high reproducibility [218].

It is well known that vitamin K₃ acts as a coagulant in the liver. PLGA is modified with 2-imino-2-methoxyethyl (IME)-thiogalactosides (Gal-PLGA) for the controlled delivery of vitamin K₃ in vivo [219]. Vitamin K₃ together with Gal-PLGA was showing coagulation activities during the entire measuring period after the intravenous application, while free K₃ was showing activities up to four hours after the administration.

Feng et al. concluded that vitamin E TPGS (d-α-tocopheryl polyethylene glycol 1000 succinate) has great advantages for the manufacturing of polymeric PLGA nanoparticles for the controlled release of paclitaxel and other anti-cancer drugs. They propose a novel formulation for the fabrication of PLGA particles containing vitamin E TPGS to replace the current method of clinical administration and, with further modification, to provide an innovative solution for oral chemotherapy. They have found that vitamin E TPGS could be a novel surfactant as well as a matrix material when blended with other biodegradable polymers. A drug encapsulation efficiency as high as 100% can be achieved and the release kinetics can be controlled [220, 221]. The size of PLGA particles emulsified with vitamin E ranges from 300 to 800nm [222].

Vitamin B₁₂ is a water soluble vitamin that can also be incorporated in the PLGA matrix. Fine particles of vitamin B₁₂ (0.2g) with 3μm in size and copolymer PLGA of molecular weight 10,000 with lactide/glycolide ratio 50/50 (1.8g) were dissolved in methylene chloride [223]. This was followed by a sonification in order to obtain homogeneous dispersion, which is used in the latter as an oil phase. Polyvinyl alcohol was used as the particle stabilizer.

Folic acid (folate-the anion form, vitamin B₉) is a very important vitamin, usually insufficiently introduced into the body. The particles of PLGA can be used for the controlled delivery of folic acid [224]. The obtaining of PLGA particles for the controlled delivery of aspirin and folic acid using the emulsion method (o/w or w/o/w) has been described by Kanthamneni et al. Different concentrations of folic acid and aspirin (20, 40 or 60%) were added into a polymeric solution. The efficiency of the encapsulation was between 83 and 91% wt.

Stevanović et al. successfully encapsulated folic acid into PLGA particles in various concentrations by physicochemical solvent/non-solvent method, thereby producing particles with different morphological characteristics (Fig. (6)) [145]. The particles of PLGA/folic acid with a lower content of folic acid had a higher

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uniformity, lower levels of agglomeration, smaller size. The nanoparticles of PLGA/folic acid 95/5% were spherical in shape and their mean size ranged between 140 and 240nm. The percentage yields for various PLGA/folic acid ratios were similar, and in all cases greater than 50%, whereas the loading efficiency was greater than 75%.

For the folic acid release from degrading PLGA, a number profile has been observed (Fig. (7)) [145]. In the first phase, there is a burst effect, caused by the release of the drug adsorbed to the outer particle surface. Initially, in the first day of the degradation, 17% of folic acid is released. The second phase is marked by a relatively slow release due to the diffusion of the drug out of the matrix (from the first until the 12th day). The third phase is a phase of an increased drug release, caused by (an extensive) polymer degradation, resulting in an increased permeability of the drug in the polymer matrix. More than 82% of the encapsulated folic acid was released before the end of the experiment.

The folic acid has been extensively investigated for its possible usage as a ligand for targeted delivery of particles with anticancer drugs with the purpose of reducing the drug’s non-specific action on healthy cells, but also with the aim to enhance the introduction of the drugs into the targeted cells. Such line of research results in numerous studies describing the conjugation of PLGA particles with folic acid [145, 151, 156, 225].

**CONCLUDING REMARKS**

This review outlines the research and developmental activities related to the application of PLGA and PLGA-based nanoparticles as drug delivery vehicles. The extensive interest in drugs encapsulated into PLGA particles brought forth the need to prepare such particles in larger quantities, thereby meeting the highest quality standards, all in order to make them suitable for clinical trials and commercialisation. The controlled release of medications from PLGA micro and nanospheres is achievable by manipulating the physical and chemical properties of the polymer, as well as those of the particles. Besides other medicaments, vitamins can also be encapsulated into PLGA particles. Owing to their undisputable importance, they were given a special consideration in this review.

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