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The manufacturing techniques of various drug loaded biodegradable poly(lactide-*co*-glycolide) (PLGA) devices

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Abstract

A considerable research has been conducted on drug delivery by biodegradable polymeric devices, following the entry of bioresorbable surgical sutures in the market about two decades ago. Amongst the different classes of biodegradable polymers, the thermoplastic aliphatic poly(esters) like poly(lactide) (PLA), poly(glycolide) (PGA), and especially the copolymer of lactide and glycolide, poly(lactide-co-glycolide) (PLGA) have generated immense interest due to their favorable properties such as good biocompatibility, biodegradability, and mechanical strength. Also, they are easy to formulate into different devices for carrying a variety of drug classes such as vaccines, peptides, proteins, and micromolecules. Also, they have been approved by the Food and Drug Administration (FDA) for drug delivery. This review discusses the various traditional and novel techniques (such as in situ microencapsulation) of preparing various drug loaded PLGA devices, with emphasis on preparing microparticles. Also, certain issues about other related biodegradable polyesters are discussed.

Keywords: Biodegradable; Poly(lactide-*co*-glycolide) (PLGA); Bioresorbable

1. Introduction and historical perspectives of drug delivery by PLGA devices

In order to avoid the inconvenient surgical insertion of large implants, injectable biodegradable and biocompatible polymeric particles (microspheres, microcapsules, nanocapsules, nanospheres) could be employed for controlled-release dosage forms [1]. Microparticles of size less than 250 μm, ideally less than 125 μm are suitable for this purpose [2]. Biodegradable polymers are natural or synthetic in origin and are degraded in vivo, either enzymatically or non-enzymatically or both to produce biocompatible, toxicologically safe by-products which are further eliminated by the normal metabolic pathways [3]. Drugs formulated in polymeric devices are released either by diffusion through the polymer barrier, or by erosion of the polymer material, or by a combination of both diffusion and erosion mechanisms [4]. The polymers selected for the parenteral administration must meet several requirements like biocompatibility, drug compatibility, suitable biodegradation kinetics and mechanical properties, and ease of processing [4,5].

A wide variety of natural and synthetic biodegradable polymers have been investigated for drug targeting or prolonged drug release. However, only a few of them are actually biocompatible. Natural biodegradable polymers like bovine serum albumin (BSA), human serum albumin (HSA), collagen, gelatin, and hemoglobin have been studied for drug delivery [1]. The use of these natural polymers is limited due to their higher costs and questionable purity [1].

Since the last two decades, synthetic biodegradable polymers have been increasingly used to deliver drugs, since they are free from most of the problems associated with the natural polymers [1–8]. Poly(amides), poly(amino acids), poly(alkyl-α-cyano acrylates), poly(esters), poly(orthoesters), poly(urethanes), and poly(acrylamides) have been used to prepare various drug loaded devices [1–7]. Amongst them, the thermoplastic aliphatic poly(esters) like PLA, PGA, and especially PLGA have generated tremendous interest due to their excellent biocompatibility and biodegradability [1–17].

The discovery and the synthetic work on low molecular weight oligomeric forms of lactide and/or glycolide polymers was first carried out several decades ago [3,5]. The methods to synthesize high molecular weights of these polymers were first reported by Lowe [3]. During the late 1960s and early 1970s a number of groups had

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published pioneering work on the utility of these polymers to make sutures/fibers [2,3,5,12]. These fibers had several advantages like good mechanical properties, low immunogenicity and toxicity, excellent biocompatibility, and predictable biodegradation kinetics [2,3,5,12]. The wide acceptance of the lactide/glycolide polymers as suture materials, made them an attractive candidate for biomedical applications like ligament reconstruction, tracheal replacement, ventral herniorrhaphy, surgical dressings, vascular grafts, nerve, dental, and fracture repairs [3,5,9].

Many researchers have investigated and documented the biodegradation, biocompatibility, and tissue reaction of PLA and PLGA [5,14]. The first work on parenteral controlled release of drugs using PLA was reported by Boswell, Yolles, Sinclair, Wise, and Beck [3,5]. Since then an ocean of literature on drug delivery using PLA, and especially PLGA has been published. Various polymeric devices like microspheres, microcapsules, nanoparticles, pellets, implants, and films have been fabricated using these polymers for the delivery of a variety of drug classes. Also they are easy to formulate into drug carrying devices for various applications, such as, orthopaedic drug delivery; they have also been approved by the FDA for drug delivery use [1–17].

2. Physico-chemical and biological properties of PLGA

The understanding of the physical, chemical, and biological properties of the polymer is helpful, before formulating a controlled drug delivery device. The various properties of the polymer and the encapsulated drug directly influence other factors like the selection of the microencapsulation process, drug release from the polymer device, etc. [1].

The polymer PLA can exist in an optically active stereoregular form (L-PLA) and in an optically inactive racemic form (D,L-PLA) [1,5,9]. L-PLA is found to be semicrystalline in nature due to high regularity of its polymer chain while D,L-PLA is an amorphous polymer because of irregularities in its polymer chain structure [3,9]. Hence the use of D,L-PLA is preferred over L-PLA as it enables more homogeneous dispersion of the drug in the polymer matrix [9,13]. PGA is highly crystalline because it lacks the methyl side groups of the PLA [3,9]. Lactic acid is more hydrophobic than glycolic acid and hence lactide-rich PLGA copolymers are less hydrophilic, absorb less water, and subsequently degrade more slowly [1,3,13].

The physical properties such as the molecular weight and the polydispersity index affect the mechanical strength of the polymer and its ability to be formulated as a drug delivery device [3,5,12]. Also these properties may control the polymer biodegradation rate and hydrolysis [3,12]. The commercially available PLGA polymers are usually characterized in terms of intrinsic viscosity, which is directly related to their molecular weights [3].

The mechanical strength, swelling behavior, capacity to undergo hydrolysis, and subsequently the biodegradation rate are directly influenced by the crystallinity of the PLGA polymer [3]. The resultant crystallinity of the PLGA copolymer is dependent on the type and the molar ratio of the individual monomer components (lactide and glycolide) in the copolymer chain [1]. PLGA polymers containing 50 : 50 ratio of lactic and glycolic acids are hydrolyzed much faster than those containing higher proportion of either of the two monomers [5,12]. PLGAs prepared from L-PLA and PGA are crystalline copolymers while those from D,L-PLA and PGA are amorphous in nature [3,5]. Gilding and Reed have pointed out that PLGAs containing less than 70% glycolide are amorphous in nature [18]. The degree of crystallinity and the melting point of the polymers are directly related to the molecular weight of the polymer [3,5].

The T_g (glass transition temperature) of the PLGA copolymers are above the physiological temperature of 37°C and hence they are glassy in nature [3,5]. Thus, they have a fairly rigid chain structure which gives them significant mechanical strength to be formulated as drug delivery devices [3,5]. Jamshidi et al. have reported that T_g of PLGAs decrease with decrease of lactide content in the copolymer composition and with decrease in their molecular weight [19].

The PLGA polymers should have considerable mechanical strength, since the drug delivery devices formulated using them are subjected to significant physical stress [3,5]. Different factors like the molecular weight, copolymer composition (lactide/glycolide ratio), crystallinity, and geometric regularity of individual chains significantly affect the mechanical strength of the polymer [1,3,5].

Both, in vitro and in vivo the PLGA copolymer undergoes degradation in an aqueous environment (hydrolytic degradation or biodegradation) through cleavage of its backbone ester linkages [1–3,5,12,13]. The polymer chains undergo bulk degradation and the degradation occurs at uniform rate throughout the PLGA matrix [3,13]. Thies and Bissery have reported that the PLGA biodegradation occurs through random hydrolytic chain scissions of the swollen polymer [20]. The carboxylic end groups present in the PLGA chains increase in number during the biodegradation process as the individual polymer chains are cleaved; these are known to catalyze the biodegradation process [3,5]. The biodegradation rate of the PLGA copolymers are dependent on the molar ratio of the lactic and glycolic acids in the polymer chain, molecular weight of the polymer, the degree of crystallinity, and the T_g of the polymer [3,5,13]. A three-phase mechanism for the PLGA biodegradation has been

proposed [21]:

1. Random chain scission process. The molecular weight of the polymer decreases significantly, but no appreciable weight loss and no soluble monomer products formed.
2. In the middle phase a decrease in molecular weight accompanied by rapid loss of mass and soluble oligomeric and monomer products are formed.
3. Soluble monomer products formed from soluble oligomeric fragments. This phase is that of complete polymer solubilization.

The role of enzymes in any PLGA biodegradation is unclear [3,5]. Most of the literature indicates that the PLGA biodegradation does not involve any enzymatic activity and is purely through hydrolysis [3]. However, some investigators have suggested enzymatic role in PLGA breakdown based upon the difference in the in vitro and in vivo degradation rates [5].

The PLGA polymer biodegrades into lactic and glycolic acids [1–3,5,12,13]. Lactic acid enters the tricarboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbon dioxide and water [1–3,5,9]. In a study conducted using ^{14}C-labeled PLA implant, it was concluded that lactic acid is eliminated through respiration as carbon dioxide [22]. Glycolic acid is either excreted unchanged in the kidney or it enters the tricarboxylic acid cycle and eventually eliminated as carbon dioxide and water [3].

3. Manufacturing techniques of various biodegradable PLGA devices

3.1. Microparticles

Although, a number of microencapsulation techniques have been developed and reported to date, the choice of the technique depends on the nature of the polymer, the drug, the intended use, and the duration of the therapy [1,2,4,5,10]. The microencapsulation method employed must include the following requirements [1,2,23]:

(i) The stability and biological activity of the drug should not be adversely affected during the encapsulation process or in the final microsphere product.
(ii) The yield of the microspheres having the required size range (upto 250 μm, ideally <125 μm) and the drug encapsulation efficiency should be high.
(iii) The microsphere quality and the drug release profile should be reproducible within specified limits.
(iv) The microspheres should be produced as a free flowing powder and should not exhibit aggregation or adherence.

3.1.1. Solvent evaporation and solvent extraction process

3.1.1.1. Single emulsion process. This process involves oil-in-water (o/w) emulsification. The polymer is first dissolved in a water immiscible, volatile organic solvent (dichloromethane (DCM) most commonly used). The drug is then added to the polymer solution to produce a solution or dispersion of the drug particles (particle size of the drug added to be <20 μm) [4]. This polymer–solvent–drug solution/dispersion is then emulsified (with appropriate stirring and temperature conditions) in a larger volume of water in presence of an emulsifier (like poly(vinyl alcohol) (PVA)) to yield an o/w emulsion. The emulsion is then subjected to solvent removal by either evaporation or extraction process to harden the oil droplets [10]. In the former case the emulsion is maintained at reduced pressure or at atmospheric pressure and the stir rate reduced to enable the volatile solvent to evaporate [4,10]. In the latter case the emulsion is transferred to a large quantity of water (with or without surfactant) or other quench medium, into which the solvent associated with the oil droplets is diffused out [4,10]. The solid microspheres so obtained are then washed and collected by filtration, sieving, or centrifugation [4]. These are then dried under appropriate conditions or are lyophilized to give the final free flowing injectable microsphere product.

It should be noted that for the solvent evaporation process in a way is similar to the extraction method, in the sense that the solvent must first diffuse out into the external aqueous dispersion medium before it could be removed from the system by evaporation [4,10]. The rate of solvent removal by the extraction method depends on the temperature of quench water or other medium, ratio of emulsion volume to quench water/medium volume and the solubility characteristics of the polymer, the solvent, and the dispersion medium. The rate of solvent removal by evaporation method strongly influences the characteristics of the final microspheres and it depends on the temperature, pressure, and the solubility parameters of the polymer, the solvent, and the dispersion medium [10]. Very rapid solvent evaporation may cause local explosion inside the droplets and lead to formation of porous structures on the microsphere surface [10]. The solvent removal by extraction method is more faster (generally <30 min) than the evaporation process and hence the microspheres made by the former method are more porous in comparison to those made from the latter method under similar conditions [10].

One of the disadvantages of the o/w emulsification method is poor encapsulation efficiencies of moderately water-soluble and water-soluble drugs [1,4,10]. The drug would diffuse out or partition from the dispersed oil phase into the aqueous continuous phase and microcrystalline fragments of the hydrophilic drugs get deposited on the microsphere surface and dispersed in the PLGA matrix [24,25]. This would result in poor trapping of the

hydrophilic drug such as salicylic acid and initial rapid release of the drug (burst effect) [1]. The o/w emulsification process is therefore widely used to encapsulate lipid-soluble drugs like steroids [1].

In order to increase the encapsulation of the water-soluble drugs, an oil-in-oil (o/o) emulsification method was developed [1,10,26]. A water-miscible organic solvent like acetonitrile is employed to solubilise the drug in which PLGA or PLA are also soluble. This solution is then dispersed into an oil such as light mineral oil in presence of an oil soluble surfactant like Span to yield the o/o emulsion. Microspheres are finally obtained by evaporation or extraction of the organic solvent from the dispersed oil droplets and the oil is washed off by solvents like *n*-hexane. This process is also sometimes referred as water-in-oil (w/o) emulsification method [1].

The formation of the microspheres is affected by a number of factors. The main variables that influence the microencapsulation process and the final microsphere product are: (a) the nature and solubility of the drug being encapsulated; (b) the polymer concentration, composition, and molecular weight; (c) the drug/polymer ratio; (d) the organic solvent used; (e) the concentration and nature of the emulsifier used; (f) the temperature and stirring/agitation speed of the emulsification process; and (g) the viscosities and volume ratio of the dispersed and continuous phases [1,4,5,10].

3.1.1.2. Double (multiple) emulsion process. This is a water-in-oil-in-water (w/o/w) method and is best suited to encapsulated water-soluble drugs like peptides, proteins, and vaccines, unlike the o/w method which is ideal for water-insoluble drugs like steroids [1,4,5]. A buffered or plain aqueous solution of the drug (sometimes containing a viscosity building and/or stabilizing protein like gelatin) is added to an organic phase consisting of PLGA and/or PLA solution in DCM with vigorous stirring to form the first microfine w/o emulsion. This emulsion is added gently with stirring into a large-volume water containing an emulsifier like PVA to form the w/o/w emulsion. The emulsion is then subjected to solvent removal by either evaporation or extraction process. In the former case the emulsion is maintained at reduced pressure or at atmospheric pressure and stirred to enable DCM to evaporate. In the latter case the emulsion is transferred to a large quantity of water (with or without surfactant) with stirring, into which DCM is diffused out. The solid microspheres so obtained are then washed and collected by filtration, sieving, or centrifugation. These are then dried under appropriate conditions or are lyophilized to give the final free flowing microsphere product.

Use of solvents such as ethyl acetate and hydrophilic stabilizers like Pluronic F68, PEG 4600, BSA, HSA or sodium glutamate for protein/peptide drugs have been reported [27]. Singh et al. used a blend of PVA and PVP in the outer aqueous phase to make PLA/PLGA microspheres [28]. Cohen et al. have used an outer aqueous PVA phase saturated with DCM to prepare PLGA microspheres [29]. Alpar et al. have reported preparation of PLA microspheres in which the inner aqueous phase contained MC besides PVA or PVP [30,31]. They found that particles containing PVP were more hydrophobic, exhibited higher drug loading and encapsulation efficiency, and showed decreased burst effect as compared to those containing PVA [30,31]. The addition of a stabilizing polymer (BSA), reduced the net encapsulation efficiency of the protein drug [30].

A number of hydrophilic drugs like the peptide leuprolide acetate, a luteinizing hormone-releasing hormone (LH-RH) agonist [32–37], vaccines [21,27–29,31,38–72], proteins/peptides [30,73–86], and conventional molecules [87–99] have been successfully encapsulated by this method. Various formulation and process variables significantly affect the final microsphere product and the drug release from them.

3.1.2. Phase separation (coacervation)

This process consists of decreasing the solubility of the encapsulating polymer by addition of a third component to the polymer solution in an organic solution [1,4,5]. At a particular point, the process yields two liquid phases (phase separation): the polymer containing coacervate phase and the supernatant phase depleted in polymer. The drug which is dispersed/dissolved in the polymer solution is coated by the coacervate. Thus, the coacervation process includes the following three steps: (i) phase separation of the coating polymer solution, (ii) adsorption of the coacervate around the drug particles, and (iii) solidification of the microspheres [100].

The polymer is first dissolved in an organic solution. The water-soluble drugs like peptides and proteins are dissolved in water and dispersed in the polymer solution (w/o emulsion). Hydrophobic drugs like steroids are either solubilized or dispersed in the polymer solution. An organic nonsolvent is then added to the polymer–drug–solvent system with stirring which gradually extracts the polymer solvent. As a result the polymer is subjected to phase separation and it forms very soft coacervate droplets (size controlled by stirring) which entrap the drug. This system is then transferred to a large quantity of another organic nonsolvent to harden the microdroplets and form the final microspheres which are collected by washing, sieving, filtration, or centrifugation, and are finally dried [4,100].

This process, unlike the o/w emulsification method is suitable to encapsulate both water-soluble as well as water-insoluble drugs, since its a non-aqueous method. However, the coacervation process is mainly used to encapsulate water-soluble drugs like peptides, proteins, and vaccines. The addition rate of first nonsolvent should be such that the polymer solvent is extracted slowly, so

that the polymer has sufficient time to deposit and coat evenly on the drug particle surface during the coacervation process [4]. The concentration of the polymer used is important as well, since too higher concentrations would result in rapid phase separation and nonuniform coating of the polymer on the drug particles. Due to absence of any emulsion stabilizer in the coacervation process, agglomeration is a frequent problem in this method [4]. The coacervate droplets are extremely sticky and adhere to each other before the complete phase separation or the hardening stages of this method. Adjusting the stirring rate, temperature, or the addition of an additive is known to rectify this problem [4].

Compared to the solvent evaporation/extraction process, the requirement of solvents for the polymer are less stringent since the solvent need not be immiscible with water and the boiling point can be higher than that of water [4]. DCM, acetonitrile, ethyl acetate, and toluene have been used in this process [100–111]. The nonsolvents affect both the phase separation and the hardening stages of the coacervation process. The nonsolvents should not dissolve the polymer or the drug and should be miscible with the polymer solvent [100–108]. The second nonsolvent should be relatively volatile and should easily remove the first viscous nonsolvent by washing. Some of the oils used as the first nonsolvent are silicone oil, vegetable oils, light liquid paraffin, low molecular weight liquid polybutadiene, and low molecular weight liquid methacrylic polymers [4,100–111]. Examples of the second nonsolvent include aliphatic hydrocarbons like hexane, heptane, and petroleum ether [4,100–111].

In the phase separation method the phase equilibrium is never reached and hence the system is constantly out of equilibrium [4]. Therefore, the formulation and process variables significantly affect the kinetics of the entire process and ultimately the characteristics of the final microspheres. In a classic article, Nihant et al. have investigated the effect of several process factors on the coacervation process [102]. With increase in the aqueous phase/organic phase volume ratio from 0.02 to 0.12% w/w the 'stability window' (an area in the phase diagram where the dispersed aqueous phase is efficiently coated by the coacervate) was unmodified and only got slightly narrower [102]. An SEM picture revealed that the morphology of the particles changed from a spherical shape for 0.02 ratio to a deformed one at higher ratio of 0.12. Above water contents of 0.12, the microspheres became brittle and spontaneously released the encapsulated drug solution during filtration [102]. With decrease in the stirring rate from 800 to 400 rpm for the aqueous drug dispersion in PLGA/DCM solution, the particle size increased from 40.0 to 51.5 μm and for 300 rpm no microparticles were formed [102]. Similarly, with decrease in the stirring rate from 200 to 130 rpm for the phase separation by adding silicone oil, the particle size increased from 40.0 to 58.0 μm and for 100 rpm no microparticles were formed. For the addition rate (of silicone oil) of 18 ml/min, microparticles of the size 40.0 μm were formed and their size decreased to 39.1 μm when the addition rate was decreased to 5.7 ml/min [102]. However, with further decrease in the addition rate to 0.65 ml/min, the particle size increased to 53.1 μm and aggregates were formed and in certain cases no microparticles were formed. The authors concluded that microencapsulation by coacervation is a complex process that depends on the interplay of several kinetic parameters [102]. In another paper, the same group has reported the effects of weight, volume, composition, and viscosity of the coacervate and supernatant phases on the size distribution, surface morphology, and internal porosity of the final microparticles [110].

Various researchers have also reported microencapsulation by coacervation [101–109,111]. Vidmar et al. induced phase separation of a drug-PLA-DCM suspension by addition of *n*-heptane to give particles in the range of 50–500 μm and in another study they used chloroform instead of DCM to dissolve the polymer [1]. Nakano et al. used an ethyl acetate solution of PLA/carboxymethyl ethyl cellulose blend and suspended the drug particles in it prior to inducing phase separation by adding ethyl ether to finally give smooth microspheres having mean size of 16.4 μm [1]. Fong et al. carried out microencapsulation at low temperature, where the drug was suspended in PLA/toluene solution at $-65^{\circ}C$ and phase separation was induced by dropwise addition of isopropanol with constant stirring to yield microspheres in the range of 25–50 μm [112]. Mandal et al. added the suspension of water-soluble diltiazem or metoprolol in PLGA/DCM solution to a silicone oil : DCM solution (1 : 6 ratio) with stirring and the coacervates obtained were hardened by petroleum ether to yield microspheres with high encapsulation efficiencies [101]. In an article, Ruiz et al. have concluded that the polymer properties such as hydrophobicity or chain length, viscosity of the silicone oil used, the concentration of the polymer, and the polymer solvent/silicone oil ratio greatly affected the overall coacervation process and thereby the characteristics of the final microsphere product [113]. Leelarasamee et al. have reported preparation of PLA microcapsules by solvent partitioning to achieve phase separation [107]. A solution of hydrocortisone and PLA in DCM was slowly injected into a mineral oil stream with a constant injection rate and needle size. As DCM partitioned into the mineral oil phase, the polymer precipitated and encapsulated the drug. The microcapsules were finally washed with hexane and they had a size of 250 μm with 90% yield [107].

3.1.3. Spray drying

As discussed in the previous sections, injectable biodegradable PLA and PLGA microparticles have been

successfully prepared by double-emulsion and phase-separation methods. The coacervation method tends to produce particles which are agglomerated, there is difficulty in mass production, the method requires large quantities of organic solvent, and it is difficult to remove residual solvents from the final microsphere product [114]. The double-emulsion method on the other hand requires many steps, rigid control of the temperature and viscosity of the inner w/o emulsion, and is difficult to encapsulate higher concentration of hydrophilic drugs [1,114]. Contrary to these methods, the spray drying method is very rapid, convenient, easy to scale-up, involves mild conditions, and is less dependent on the solubility parameter of the drug and the polymer [1,114,115].

A solution of PLGA, hexafluro-2-propanol, benzene, and the drug was sprayed to produce microcapsules of less than 125 μm [116]. Bodmeier and Chen prepared microspheres by spray drying where a water-soluble drug (theophylline) was suspended or a water-insoluble drug (progesterone) was dissolved in a PLA/DCM solution and then spray dried to produce particles of less than 5 μm [117]. Due to incompatibility of the hydrophilic drug and PLA, needle-shaped crystals grew on the microsphere surface, while the progesterone–PLA solution gave smooth particles. The nature of the solvent used, temperature of the solvent evaporation, and presence of PLA microspheres during the spray-drying process affected the polymorphic form of progesterone. A major problem encountered with this technique was the formation of fibers due to insufficient force available to break up the polymer solution. An efficient dispersion of the filament into polymer droplets was dependent on the type of polymer and the viscosity of the spray solution. Other groups have also reported successful preparation of PLGA and PLA particles using the spray-drying technique [115,118–122].

A solution of the polymer, DCM, and the drug piroxicam was spray-dried to yield microspheres that were hollow (no solid core) [115]. D,L-PLA microparticles were more spherical and smooth than those made from D,L-PLGA. The microspheres were in the size range of 1–15 μm, with an high drug encapsulation efficiency of 99.0% [115]. Men et al. have shown that PLGA microparticles prepared using spray-drying technique produced particles in the size range of 1 to 15 μm and drug loading of 4.4–6.6 μg/mg microspheres, while PLA microspheres prepared using coacervation technique yielded particles having diameter in the range of 20–90 μm with relatively low drug loading of 3.5 μg/mg microspheres [121]. In order to protect an hepatitis vaccine from the harmful effects of the solvent, a mixture of the antigen powder and an hydrophilic polymer, Hydroxypropyl cellulose (HPC) was first spray-dried to produce core microparticles [122]. These were then suspended in PLGA/ethyl acetate solution and spray dried to yield double-walled microparticles in the size range of 4–22 μm. The first coating layer of HPC protected the antigen from solvent during the second encapsulation process with PLGA [122].

There may be a significant loss of the product during spray-drying, due to adhesion of the microparticles to the inside wall of the spray-drier apparatus, and can also produce agglomeration of the microparticles [114]. In order to rectify these problems, a novel double-nozzle spray-drying technique was developed which involved use of mannitol as an anti-adherent [114]. A solution or a dispersion (w/o emulsion) of the drug in PLGA solution was sprayed from one nozzle and from another nozzle an aqueous mannitol solution was sprayed simultaneously and the process completed to give the final microspheres. The surface of the spray-dried microspheres were coated with mannitol and the extent of agglomeration was decreased [114]. Also this method produced microspheres with higher yield and encapsulation ratio as compared to those prepared from the double-emulsion method [114].

A novel low-temperature spraying method for preparing PLA and PLGA microspheres has been reported by Khan et al. [123] and the group at Alkermes Inc. (ProLease® technology) [124,125]. First the protein powder and optional excipients were suspended in the PLA/PLGA solution in acetone, ethyl acetate, or DCM. This suspension was then sprayed into a vessel containing liquid nitrogen overlaying a frozen extraction solvent like ethanol. The liquid nitrogen is subjected to evaporation causing the polymer solvent from the frozen droplets to be extracted then by liquid ethanol. Microspheres were then filtered and the residual solvents evaporated by filtration. The microspheres were 50–60 μm in size with drug encapsulation efficiency more than 95% [123–125].

3.2. Nanoparticles

As described in the previous sections, injectable microparticles from PLA and PLGA have been successfully prepared to deliver drugs like peptides, proteins, and vaccines over a period of days, weeks, or even months at a constant rate depending upon the degradation behavior of the polymer employed [126]. However due to their large size, it was impossible to direct the drug to target tissues via systemic circulation or across the mucosal membrane [11]. Following oral administration, particles less than 500 nm can cross the M cells in the Payer's patch and the mesentery on the surface of the gastrointestinal mucosa, delivering the drug to the systemic circulation [11].

Nanoparticles (nanospheres and nanocapsules) could be prepared by the same methods as those described for microparticles, except that manufacturing parameters are adjusted to obtain nanometer-size droplets [10].

This is obtained by using a relatively small dispersed phase : dispersion medium ratio and a substantially higher stirring speed [10]. Song et al. have prepared PLGA nanoparticles by o/w emulsification/solvent evaporation technique to produce nanoparticles having size of 150 nm, drug loading of 15.5% w/w, encapsulation efficiency of 62% and yield of 85% [127]. Many formulation/process variables affecting microsphere production also influence nanoparticle production, in more or less the similar way. Dawson and Halbert prepared nanoparticles by the o/w emulsification technique and used response surface methodology to determine the effect of some variables on the size distribution of PLGA nanoparticles [128]. The authors found that the homogenization pressure and PLGA concentration have a linear and thus predictable effect on both size and polydispersity of the particles. Tween 80 concentration and the DCM (organic phase) concentration had a greater effect on the diameter and polydispersity [128].

A novel method of preparing PLA and PLGA nanoparticles was described by Müller et al. [129]. Magnetite (used for magnetic resonance imaging) was first dispersed in ethanol by sonication. This dispersion was then incorporated in the polyester polymers and the temperature slowly increased with stirring to remove ethanol [129]. Heating and stirring was continued to melt PLA/PLGA polymer to facilitate homogeneous dispersion of magnetite in them. After solidification of the magnetite-containing polymer, they were grounded [129]. PLA/PLGA nanoparticles were then obtained by high pressure homogenization of the magnetite-containing polymer particles dispersed in aqueous poloxamer 188 solution [129]. Leroux et al. prepared PLA nanoparticles by a reversible salting-out process using a cross-flow filtration technique which involved the use of magnesium salts [130]. A 90% drug entrapment was achieved by this method.

Production of nanospheres for indomethacin and 5-fluorouracil (water-insoluble and water-soluble drugs, respectively) and for nafarelin acetate (NA), a LH-RH analog, by a novel spontaneous emulsification-solvent diffusion method was reported by Niwa et al. [131,132]. The drug and PLGA were first dissolved in acetone-DCM mixture and then emulsified in an aqueous PVA solution using a high-speed homogenizer (o/w emulsification). The rapid diffusion of acetone in the aqueous phase resulted in faster deposition of polymeric film on the droplet and yielded nanospheres less than 500 nm [131,132]. The same group also reported nanospheres production for NA by the emulsion-phase separation method in an oil system [133]. An aqueous solution of NA was emulsified in acetone-DCM mixture containing dissolved PLGA using a homogenizer at 15 000 rpm. To this was added a mixture of Triester oil (caprylate and caprate triglyceride) and HGCR (hexaglycerine condensed ricinoleate), which resulted in phase separation of PLGA. PLGA coacervates precipitated around the aqueous emulsion droplets and were hardened by evaporation of the solvent to yield nanospheres in the range of 500–800 nm [133]. Murakami et al. have reported preparation of PLGA latex by a method based on double coacervation of PVA and PLGA [134]. PLGA was first dissolved in a mixture of acetone and DCM, ethanol, or methanol. This PLGA solution was then dispersed in aqueous PVA solution with stirring [134]. The latex dispersion was then freeze-dried to obtain powder latex having size of approximately 300 nm. The authors concluded that the coacervated (adsorbed) PVA molecules prevented the aggregation of PLGA nanoparticles due to its steric hindrance [134].

After an intravenous injection, the nanoparticles are taken up by cells of the mononuclear phagocyte system mainly the Kupffer cells of the liver and the spleen macrophages and are essentially lost [135,136]. This is considered as the major hurdle to target delivery of drugs to other organ/tissue sites within the body [135,136]. Several groups have tried to address this problem. Gref et al. prepared polyethylene glycol (PEG)-coated nanospheres by employing a amphiphilic diblock copolymer of PLGA (75 : 25, lactide : glycolide) and PEG (molecular weight 5000–20 000) [135]. The nanospheres were prepared by a single-step o/w solvent evaporation technique, where the PEG fraction migrated to the surface of the nanospheres forming a protective cover. After 5 min of the injection of coated or uncoated nanospheres, 15% of coated nan ospheres were found in the liver and 60% of them in the blood with a significantly improved circulating time [135]. In comparison, 40% of uncoated nanospheres were found in liver and only 15% of them in the blood. Also 4 h post-injection, 30% of coated nanospheres were still circulating in the blood while the plane nanospheres had completely disappeared from the circulation. Blood circulation time for the nanoparticles increased as the molecular weight of the PEG increased from 5000 to 20 000 due to increased thickness of the protective PEG layer which prevented their opsonization [135]. Stolnik et al. [136] and Dunn et al. [137] reported preparation of PLGA nanospheres by o/w-emulsification/solvent evaporation and further coating them by the diblock copolymer PLA : PEG (ratio of 2 : 5 and 3 : 4). The coated particles had increased hydrophilicity and decreased surface charge (as determined by measuring their surface zeta potentials) and were sterically stabilized particles. These particles exhibited reduced protein adsorption and liver uptake and increased blood circulation time as compared to uncoated PLGA nanospheres [136,137]. Leroux et al. have also reported similar improved site specific drug delivery of nanoparticles after coating them with PEG [130]. Other groups have also reported successful preparation of PLGA nanoparticles [138–141].

3.3. Implants and miscellaneous devices

Preparation of drug-loaded implants from PLA or PLGA have been reported by many groups [142–146]. Kunou et al. have designed nail-like ganciclovir incorporated D,L-PLGA implant (length-5 mm, diameter–1 mm) for intraocular drug delivery to treat cytomegalovirus retinitis [147]. Wang et al. have reported preparation (by compression) of 5-fluorouracil D,L-PLGA subconjunctival coated and uncoated implants/matrices (5 mg, diameter—2.5 mm, thickness—1.2 mm) using drug : PLGA ratio of 9 : 1, 8 : 2, and 7 : 3 [148]. Lin et al. and others have investigated the performance of various antibiotics loaded PLA and PLGA implants, beads and cylinders [149–151].

Lemmouchi have reported in vitro and in vivo performance of drug-loaded rods of various polyesters prepared by the melt extrusion process [152]. Mandal et al. prepared PLGA tablets by compressing physical mixture of zidovudine (AZT) and PLGA (50 : 50) and AZT loaded PLGA microcapsules [153]. Other groups have also reported preparation of PLA/PLGA compressed tablets with some involving use of heat [154–156].

In an article, Schmitt et al. have reported preparation of amaranth incorporated pellets (diameter—3.7 mm, thickness—3.1 mm) from D,L-PLGA which was purchased from three different sources: DuPont, Birmingham Polymers, and Henley Chemicals [157]. The authors prepared the pellets by melt-pressing spray-dried PLGA with a 4 mm standard concave punch and die set [157]. Tracy et al. prepared drug-free PLGA pellets by a similar method but used a Carver Laboratory Press instead [158].

Schade et al. have described preparation of aqueous colloidal D,L-PLGA dispersion by a spontaneous emulsification-solvent diffusion technique followed by drying of these dispersions to form biodegradable latex films [159]. In order to study the effect of hydrophilic excipient on the drug release from the hydrophobic PLGA (50 : 50) film, Song et al. prepared double-layer films (150 μm thickness), where the drug releasing layer consisted of drug/hydrophilic additive/PLGA (10 : 10 : 80 ratio) and a protecting layer consisting of PLGA only [160]. A combined solvent casting and melt-compression method was utilized to prepare these films [160]. In an effort to study drug-induced D,L-PLA hydrolytic degradation, Li et al. prepared caffeine incorporated PLA circular plates (1.5 mm thick) and films (0.3 mm thin) [161]. Other researchers have also described drug delivery through PLGA films [162,163].

Preparation of low-density PLGA (85 : 15) foams having high interstitial void volume was reported by Hsu et al. [164,165]. First PLGA was dissolved in glacial acetic acid at different concentrations. The solutions were then frozen and lyophilized and the solvent recovered in a dry ice/acetone cooled trap to give foams which had leaflet or platelet structures. Lyophilization helped to achieve control of the specific gravity and interstitial void volume of the foam. The drug isoniazid (INH) was then impregnated into the foams, by immersing a weighed quantity of foam into an aqueous INH solution of known concentration and finally lyophilizing them to remove water. The foams were then subjected to matrices preparation by different methods using high-pressure extrusion to prolong in vitro release of INH and to understand the mechanism of its release [164,165]. Lee et al. have described preparation of biodegradable drug incorporated L-PLA porous membranes (10 mg, 1 cm × 1 cm) for periodontal therapy by an in-air drying phase inversion technique which involved use of solvents like DCM and ethyl acetate [166]. Whang and Healy have described preparation of BSA incorporated D,L-PLGA scaffolds having different pore sizes and very high porosities by an emulsion freeze-drying method [167]. Pore size was controlled by varying polymer inherent viscosity and/or volume fraction of the dispersed aqueous phase and/or polymer concentration (w/v%) [167]. Ovalbumin-loaded D,L-PLA granules were prepared by first emulsification (o/w emulsion) of the vaccine followed by lyophilization of the emulsion and then compression-molding the powder into rods [168]. These were then grounded by pestle and mortar into granules which were then sized by using appropriate sieves (20–100 μm) [168,169].

3.4. In situ formed devices

The traditional methods of preparing PLGA microparticles suffer from drawbacks such as: (i) the microspheres need to be reconstituted (suspended) in an aqueous media, before they could be injected in the body, (ii) the hazards and environmental concern associated with the use of organic solvents like methylene chloride for the solubilization of PLGA polymer, and (iii) residual organic solvents remaining in the final microsphere product. Although PLGA implants have been fabricated to deliver a variety of drug classes, they have not received much commercial success, primarily due to difficulty in administration; they require minor surgical incision or special type of pellet injector (trocar), thereby causing inconvenience to the patients.

To improve patient acceptance, a novel implant system has been developed which is intramuscularly or subcutaneously administered as a liquid and subsequently solidifies in situ [170,171]. First PLA or PLGA is dissolved by heating in a water-miscible, biocompatible solvent (this may also act as a plasticizer for the polymer). The polymer solution is then cooled under ambient condition and the drug is dispersed into it by homogenization or alternatively, the drug is dissolved in a solvent (like propylene glycol) which is miscible with the polymer solvent and water. This polymer–solvent–drug system has a viscous consistency but is sufficiently syringeable to

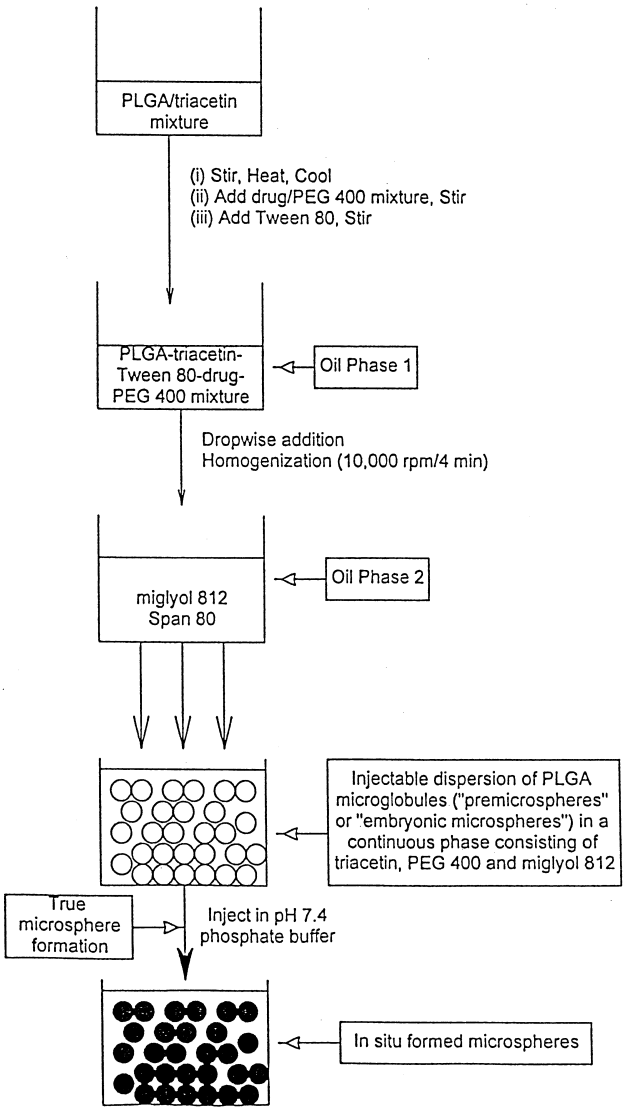

Fig. 1. Schematic representation of the novel in situ PLGA microencapsulation process.

be enabled to be injected intramuscularly or subcutaneously by conventional syringe and needle. When injected, it comes into contact with water from aqueous buffer (in vitro condition) or physiological fluid (in vivo condition) and as a result the polymer precipitates and forms a gel matrix (solidifies) entrapping the drug (in situ/in vitro or in situ/in vivo implant formation). The polymer solvent dissipates and diffuses out of the system and water diffuses into the polymer matrix. Due to water-insoluble nature of the polymer, it precipitates/coagulates to form a solid implant in situ, from which the drug is released in a controlled fashion.

A number of researchers have extensively reported drug delivery using this method [170–187]. They employed the combination of a host of biocompatible solvents and biodegradable polyesters besides PLA and PLGA to deliver a variety of therapeutic drug classes [170–187]. Although this implant system precludes the need for any surgery for its administration, it has a number of disadvantages: (i) the safety of solvents like NMP used to formulate these systems is questionable and not well documented, (ii) the injection of these liquid implant systems and their subsequent solidification produce non-uniform matrix implants having variable consistency and geometry, and (iii) due to formation of matrix implants having inconsistent texture, shape and size, the drug release from them is variable and unpredictable [188].

Jain et al. have described a novel method for in situ preparation of injectable biodegradable PLGA microspheres which did not involve the use of any unacceptable organic solvents [188–190]. The delivery system is a dispersion of PLGA microglobules ('premicrospheres' or 'embryonic microspheres') in an acceptable vehicle mixture (continuous phase) and whose integrity is maintained by the use of appropriate stabilizers (Fig. 1). A solution of PLGA, triacetin, a model protein (cytochrome *c*), PEG 400, and Tween 80 (Oil Phase 1) is added dropwise with continuous homogenization to Miglyol 812-Span 80 solution (Oil Phase 2), thereby inducing phase separation (coacervation) of PLGA and forming PLGA microglobules (containing cytochrome *c*) dispersed in the continuous phase (Fig. 1). This novel drug delivery system (NDDS) is a dispersion and has a viscous consistency, but is sufficiently syringeable. When injected, it comes in contact with water from aqueous buffer or physiological fluid and as a result, the microglobules harden to form solid matrix type microparticles entrapping cytochrome *c* (in situ formed microspheres) (Fig. 1). Cytochrome *c* was then released from these microspheres in a controlled fashion [188–190].

The above novel microencapsulation process overcomes some of the disadvantages associated with other methods by (1) excluding the use of unacceptable organic solvents like DCM or NMP and using acceptable vehicle mixture instead to prepare biodegradable PLGA microspheres, (2) forming drug containing PLGA microglobules ('premicrospheres' or 'embryonic microspheres') which could be considered as precursors to the final microsphere product; these on coming into contact with water harden to form discreet PLGA microspheres which subsequently exhibit non-variable, predictable, and controlled drug release profile, and (3) precluding the need for reconstitution of the PLGA microspheres before their administration [188,189]. Thus, this novel microencapsulation method could be viewed as a modified coacervation process, wherein cytochrome *c* containing microglobules are converted to microspheres in situ.

Jain et al. have also described a modified form of the above in situ microencapsulation process [188,191]. This method can be terminated at various stages to yield different syringeable mixtures (Fig. 2). These on injection,

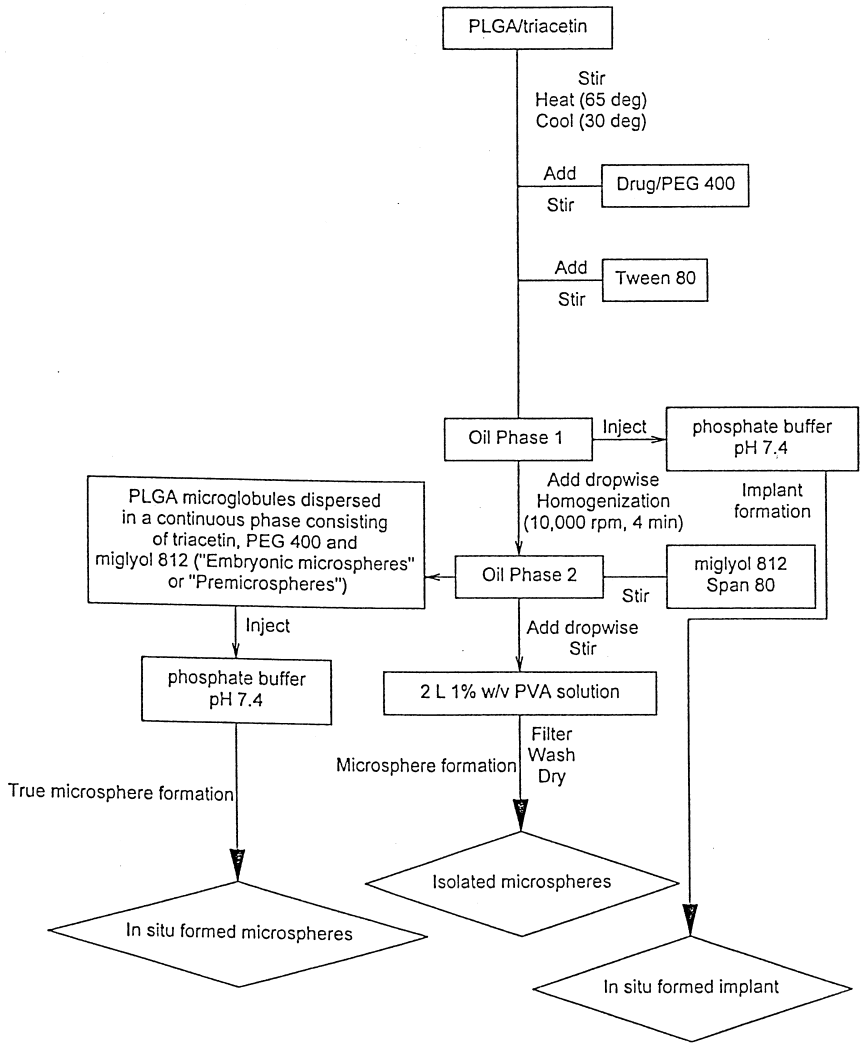

Fig. 2. Schematic representation of the novel modified microencapsulation process to produce various injectable biogradable PLGA devices.

came into contact with water and formed solid matrix type implant or microspheres (in situ formed implant or microspheres respectively) entrapping cytochrome *c* (Fig. 2). The method can also be made to produce injectable, isolated microspheres. (Fig. 2). The protein, cytochrome *c*, was encapsulated by these three devices and was released in a controlled fashion [188,191].

4. Conclusion

Administration of drugs using biodegradable PLGA polymers has generated immense interest due to its excellent biocompatibility and biodegradability. Also, they are easy to formulate into drug carrying devices and have been approved by the FDA for drug delivery use. The various biodegradable PLGA devices fabricated from different techniques are versatile in terms of the various classes of drugs encapsulated, the different time period of their release, and the diverse routes of their delivery. PLGA microparticles, in particular, are important drug delivery systems where various drug release profiles can be achieved by adjusting the PLGA composition, molecular weight, drug loading, microparticle size, porosity, and other factors. The newer techniques for fabricating PLGA devices, such as the in situ formed microspheres, is evidence of continued efforts by researchers throughout the world to optimize the drug delivery through PLGA polymer. Some drugs formulated into PLGA microparticles and other devices have already been introduced into the market and many more are undergoing clinical trials.

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