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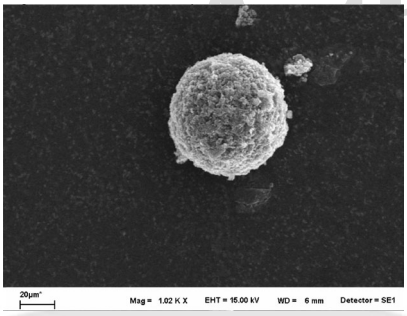
Review

The scheme shows an SEM micrograph of a poly(lactic acid) (PLA) microparticle and the available methods to prepare it. What we tried was not only to summarize the most recent available information on this topic but also to offer a kind of guide to any researcher in the field for the selection of a method to obtain nano/microparticles, by providing experimental details and references useful that we have not found in other reviews.



PLA Nano- and Microparticles for Drug Delivery: An Overview of the Obtension Methods

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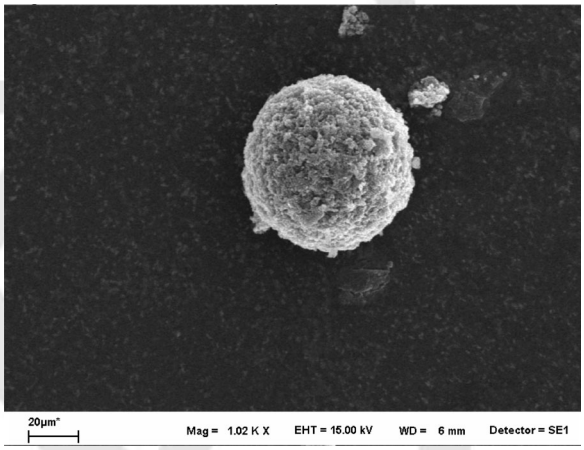
PLA Nano- and Microparticles for Drug Delivery: An Overview of the Obtension Methods

Q1 Authors, please consider Methods of Preparation instead of "Obtention Methods!

Verónica Lassalle,* María Luján Ferreira

The controlled release of medicaments remains the most convenient way of drug delivery. Therefore, a wide variety of reports can be found in the open literature dealing with drug delivery systems. In particular, the use of nano- and microparticles devices has received special attention during the past two decades. PLA and its copolymers with GA and/or PEG appear as the preferred substrates to fabricate these devices. The methods of fabrication of these particles will be reviewed in this article, describing in detail the experimental variables associated with each one with regard to the influence of them on the performance of the particles as drug carriers. An analysis of the relationship between the method of preparation and the kind of drug to encapsulate is also included. Furthermore, certain issues involved in the addition of other monomeric substrates than lactic acid to the particles formulation as well as novel devices, other than nano- and microparticles, will be discussed in the present work considering the published literature available.



1. Introduction

Controlled drug delivery (CDD) technology appears as one of the most prominent areas of human health care science. The carriers commonly used for the purpose of CDD are macromolecules and, being essentially multidisciplinary, involve more than one scientific approach. Poly(lactic acid) (PLA) is the favourite macromolecule since it is one of the most well-known bioabsorbable polymers, non-toxic and with good biodegradability, useful in several biomedical applications. The preparation of di- or triblock copolymers of PLA ■ **authors: ok?** ■ with different substrates, such as glycolic acid (GA), caprolactone (CL) and poly(ethylene glycol) (PEG) has received much attention recently. The synthesis of these materials is associated with medical applications because copolymerisation of lactides and glycolides with other substrates leads to biodegradable materials (polyesters) that can be degraded in shorter times compared to the corresponding homopolymers.[1] The common route of preparation is basically the chemical ring-opening copolymerisation and condensation.[2] How-

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Since 1999 to the date she acts as Assistant of the Professor in the Chemical Department of UNS.

M. L. Ferreira studied Chemistry at the Universidad Nacional del Sur (UNS), Bahía Blanca, Argentine from 1984 to 1988. She performed the PhD studies at the Pilot Plant of Chemical Engineering (PLAPIQUI), an institute dependent on the National Research Council of Argentina (CONICET) from 1989 to 1993. During this period she worked mainly in Ziegler-Natta Catalysts for olefin polymerization. These studies were directed by Dr. Daniel Damiani (PLAPIQUI, Argentine) On December 1993 she received her PhD in Chemistry from the UNS. On April 1994 she started her postdoctoral studies on the heterogeneization of metallocene catalysts for the alfa-olefin polymerization. From 2001 to the date she works in the field of homogeneous and heterogeneous enzymatic catalysis, with the focus on the application of lipases in the fields of oleochemistry and pharmaceutics.

Since 1999 to the date she acts as Professor in the Chemical Department of UNS.

Dr. Ferreira has 77 publications in international and national peer-review journals in her different fields of interest. She has received the Dr. Ranwell Caputto Prize (2003) and the Dr. Bernardo Houssay Prize (2004). She has participated in several projects with local industries, such as national and international Congress and Symposia.

ever, recently some few articles have appeared dealing with the enzymatic synthesis of polyesters.[3]

It is therefore possible to find several types of drugs carriers. Among them, liposomes and polymeric particles are the most important ones. The last systems are preferred from the point of view of stability and because they offer the possibility of modulation of the drug-release profile. Furthermore, these systems exhibit improved efficacy, reduced toxicity, enhanced patient compliance, and convenience in comparison to conventional dosage forms. On the other hand, a potential limitation is that they are too rapidly removed from the bloodstream, which may affect their performance in CDD and targeting. To solve this problem, some strategies have recently been implemented and basically consist of the addition of hydrophilic monomers during the fabrication of the particles.[4]

The application of PLA-based nano- and microparticles as the reservoir of different kinds of drugs to be released in a controlled way has been an object of study in the last years.[5] In spite of this, to the best of our knowledge, there is not enough information on the relationship between the properties of PLA particles and the methods used for their preparation. The goal of this review is to summarize the available information about the preparation of nano- and microparticles based on PLA polymers and copolymers to provide abundant and clear experimental data, which is not found in the open literature. Our contribution focuses on the resulting characteristics and performance of the obtained particles, depending on the applied preparative technique.

A better knowledge of the experimental parameters involved in the preparation process can be a valid tool to improve the drug-release efficiency and to the understanding of the mechanisms of the release system, especially considering the required particle size. In reference to this, a summary of the most common drugs, and the suitable methods to encapsulate them, is also included in this report.

2. PLA Nano- and Microparticles

One first classification of these particles can be made taking into account their size and preparation process. The term microparticle designates systems larger than 1 μm whereas nanoparticle is used to define submicron particles. The particle's size is a fundamental parameter related to the way of administration of the drug. For example, it is widely reported in the open literature that injectable microparticles from PLA based polymers and copolymers have been successfully employed to deliver a wide variety of medicaments, including cytotastics, anti-inflammatory agents, peptides, hormones, etc.[6] However, when the drug has to be directed to target tissues via systemic circulation or across the mucosal membrane (as well as in the cases of oral administration) particles of less than 500 nm are required.[4a,7] On the other hand, in many publications appears the term nano- and microparticle as synonym of nano- and microsphere and similar to nano- and microcapsule when they are quite different.

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Nano- and microcapsules are composed of a polymeric wall containing an inner core where the drug is entrapped; therefore the drug is completely inside the particle. Nano- and microspheres however consist of a solid polymeric matrix in which the drug can be dispersed; therefore it is distributed throughout the whole particle.

This review is restricted to the study of micro and nanospheres (also called nano- and microparticles or simply particles herein) of PLA- and some copolymers. According to the information provided by the literature, the available methods employed to obtain microspheres may also produce nanospheres by adjusting some experimental conditions as we are going to demonstrate in the following sections.

3. Preparation of Nano- and Microparticles of PLA and PLA-Copolymers

There are several techniques potentially useful for the preparation of biodegradable PLA-based polymeric particles. The selected method determines the characteristics of spheres, including the size, as it was discussed, as the most important property because it is strongly related to the administration mode. Another property influenced by the preparation process is the ability to interact with active principles contained in the drugs formulation.[8] As a consequence, a deep knowledge of the experimental parameters (solvents, temperature, kind of stabilizer, stirring rate, etc.) involved in each method is crucial as well as the effect that can produce the change of them on the characteristics of the resulting particles.

In the following paragraphs the most studied methods to obtain PLA-based nano- and microparticles will be presented with a focus on the factors affecting the final size, structure, morphology and performance of such particles as drug-controlled release systems.

3.1 Emulsion-Based Methods: Evaporation or Extraction of Solvent

These methods include the elimination of the solvents where the polymer is dissolved in. This elimination can be achieved by evaporation or by extraction. The formation of an emulsion is a necessary prerequisite. Aqueous and oily phases can be present, according to the nature of the continuum phase of the formed emulsion.

The polymer is contained in the organic phase and the emulsifier is present in the aqueous phase. The emulsified organic drops containing the polymer and the active principle form nano-and microspheres by the elimination of the organic solvent.[9]

3.1.1 Aqueous Phase

Simple Emulsions (o/w)

In this method the organic phase containing the polymer and the active principle emulsifies in an aqueous phase that contains a stabilizing agent. Then the evaporation of the organic solvent hardens the obtained nano- and microspheres.[10]

Double (Multiple) Emulsions (w/o/w)

This method constitutes a modification of the former one. Here the active principle to deliver dissolves in water (aqueous phase) and the polymer dissolves in an organic solvent (organic phase). The mixture of both solutions gives an emulsion (o/w). The resulting emulsion is then slowly added to an aqueous media that contains a stabilizer agent. The organic solvent is removed, leading to the formation of nano- and microspheres.

Gao et al. have used this technique to produce polymeric particles from a polymer synthesised by the coupling reaction of D,L-PLA and [6-(2-aminoethyl)amino-6-deoxy]-h-cyclodextrin ■ **Author, please check: β-cyclodextrin?** ■ (CDen) using *N,N*-dicyclohexylcarbodiimide as the catalyst. Basically, the procedure consisted in suspending CDen and PLA in a dichloromethane/acetone solvent mixture. Then, water was added under sonication, producing a w/o emulsion. After this, an aqueous solution of poly(vinyl alcohol) (PVA) was added under sonication originating a w/o/w emulsion. Finally, the organic solvent was removed by evaporation under reduced pressure and nanoparticles were recovered by ultracentrifugation. The obtained particles were successfully employed to load bovine serum albumin (BSA), a model protein.[11]

Freitas et al. prepared PLA microspheres through the emulsion solvent evaporation method. In this case microspheres were employed as a biodegradable polymeric carrier for the non-steroidal anti-inflammatory drug, nimesulide. According to the information provided by the authors this method showed an adequate encapsulation rate of water-insoluble compounds. As a consequence it was chosen to develop this research after a screening of others methods.[12] The experimental procedure involved the preparation of an aqueous solution containing different concentrations of PVA under heating and stirring. The organic phase including different amounts of PLA dissolved in chloroform was then slowly added to the aqueous solution containing PVA under stirring. The biphasic solution was kept under stirring, until all chloroform was evaporated. As a result, microspherical particles were produced. In order to verify the formation of PLA microspheres, the samples were analysed by optical microscopy. Zambaux and coworkers have also explored the preparation of PLA nanoparticles containing protein C, a

plasma inhibitor, through the double-emulsion method. They employed dichloromethane as organic solvent and PVA or human serum albumin (HSA) as a surfactant. They emphasized that the influence of some parameters associated with the method, such as sonication and the kind of organic solvent, was crucial for the performance of the particles.[14] In a typical experiment, an aqueous solution of the protein was emulsified in dichloromethane containing a certain amount of polymer. Then the solution of surfactant was added (PVA or HSA) and sonicated for a short period of time. Under these conditions a double emulsion was obtained. This double emulsion was then diluted in a solution of the surfactant, and the system was maintained under magnetic stirring. After this the solvent was evaporated and the nanoparticles were recovered by ultracentrifugation.

Recently, Faisant and coworkers have reported the preparation of 5-fluorouracil (5-FU)-loaded, poly[lactide-*co*-(glycolic acid)] (PLGA)-based microparticles, which can be used for the treatment of brain tumours. The authors focused their work on the search of the optimum experimental conditions such as temperature or pH and the effect of these parameters on the resulting drug release kinetics for *in vitro* drug release. The procedure detailed by these authors consisted mainly in the dispersion of the drug in dichloromethane under rapid stirring during a short period of time (commonly 4 min). Then the PLGA was added to the dispersion and stirred, allowing the complete polymer dissolution. To obtain the emulsion, an aqueous PVA solution was added to the system. The emulsion was maintained under stirring and then an extra addition of water was required in order to allow the microparticles hardening. Finally the resulting microparticles were separated by filtration.[15]

Although the extraction or evaporation of solvent or emulsion-based methods is very simple, there are several variables that have to be adjusted to optimise the properties of the obtained nano- and microparticles. Some of the important factors affecting the final particle's characteristics are detailed in the following paragraphs.

a) Solvents The PLA or PLGA solvent has to be immiscible with the emulsion solvent. Additionally, its boiling point has to be lower than the boiling point of the emulsion solvent in order to ensure a complete evaporation.

The polymer's solvents commonly employed for these purposes are ethyl acetate, which is rather non-toxic, and dichloromethane. Both solvents are easily removed and suitable to dissolve polymers. Other more toxic solvents are chloroform and acetonitrile. When the drug has also to be dissolved, a mixture of solvents is utilized, and the most widely used mixtures are dichloromethane/alcohol (ethanol, methanol and propylene glycol). Zambaux and coworkers have explored the influence of the organic solvent on the entrapment of C protein onto PLA nanoparticles. They have studied ethyl acetate, dichloromethane and acetone/dichloromethane, and found the mixture acetone/dichloromethane (1:1) as the most suitable solvent system.[14]

b) Surfactants Despite of the importance of PLA-based nano- and microparticles in biomedical applications, there is still a lack of information related to the influence of experimental conditions in the fabrication process on the final properties. Specifically, the size and shape of the resulting colloidal particles are strongly influenced by the presence of additives whose principal function is to stabilize the former nano- and microspheres but at the same time can induce changes in the properties of them. The function of these compounds is to form a protecting thin layer around the oil drops, polymer and drug, with the aim to reduce the coagulation and to stabilize the emulsion. The most commonly employed emulsifiers in this kind of system are hydrophilic polymeric colloids and anionic or cationic surfactants. PVA is the most widely used, however polyvinylpyrrolidone (PVP), alginates, gelatines, methyl cellulose or lecithin can also be employed.

Recent reports suggest that a fraction of the stabilizer always results linked to the particles despite of the washing and purification processes. The stabilizers not only can define some properties of nano- and microparticles but also can directly affect the performance of them in the controlled delivery of medicaments.[17] For example, the effect of the surfactant on the entrapment efficiency of C protein onto PLA was studied by Zambaux and coworkers. To do this, the activity of the protein as well as the efficiency of entrapment on the PLA particles was evaluated employing PVA and HSA as surfactants. The data demonstrated that HSA was more effective in entrapping the protein, but the rate of release was lower. It is important to point out that the parameters were analysed in terms of the anticoagulant activity of C protein.[14]

Freitas and coworkers have observed that the amount of surfactant may affect the nano- and microparticles properties. They added 1.3% and 8% of PVA to the aqueous solution and determined that the lower and higher concentrations of surfactant promoted the coagulation of the particles. Thus, the adequate amount of PVA proposed by these authors was 3%.[12]

c) Concentration of the PLA-Based Polymer, Stirring Rate and Amount of Aqueous PhaseThe amount of polymer dissolved in the organic phase plays an important role in the characteristics of nano- and microparticles. In spite of this, there are not enough published data about it. Freitas et al. have investigated the influence of this factor and covered a range of concentrations between 0.05% and

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0.15% (w/v) of PLA in the organic phase. They found that the particle's diameter increases with higher polymer concentrations, which is in agreement with other contributions.[10,11b] However there is a strong relationship between PLA amount, volume of aqueous phase and the rate of stirring. The combination of these three parameters would determine the particle's size. As a result, they found that 0.15% of PLA in organic solution; 50 ml of water and 11 000 rpm were the optimum conditions.[12]

d) Active PrincipleDue to the fact that this method involves an aqueous emulsion, its application is restricted to poorly water-soluble drugs. In case the drug is insoluble in the polymer's solvent, it can be pulverised or micronised to provide a homogeneous distribution of the drug between the microspheres and the emulsion.[18]

3.1.2 Oil Phase

Emulsion (o/o)

This is another modification of the emulsion o/w where the continuum phase is formed by an organic liquid such as a mineral oil. This technique is especially suitable to encapsulate hydrophilic active principles.[8]

This method was employed to fabricate microspheres used as a CDD system for the long-term inhibition of vascular endothelial growth factor (VEGA), anti-VEGA, RNA a tamer (EYE) and its mediated responses. In this particular case the procedure was the following: a few mg of the selected drug was suspended in a solution of PLGA in dichloromethane under strong stirring during 1 min. Then the coacervating agent, polydimethylsiloxane (PDMS), was added maintaining the system under stirring. In this way, phase separation of PLGA dissolved in dichloromethane, and formation of microspheres occurred. The coacervating mixture containing the microspheres was then poured by treatment with heptane under constant agitation, allowing the hardening of the microspheres, which were finally collected by filtration.

Emulsion Solid Oil Water (s/o/w)

This emulsion type is not so extensively utilized and it has emerged as an alternative to the double emulsion in order to avoid the water-organic solvent interface during the first emulsion.[8] This method is excellent in terms of the protein integrity because solid-state proteins are stable in an organic solvent. However, some micronisation treatment of the protein material is required in order to ensure an efficient protein entrapment.[19]

Morita et al. have employed this method to entrap a model protein (BSA). In particular, they investigated the effects of various amphiphilic polymers on the kinetics of protein release from reservoir-microspheres prepared by solid in oil in water emulsion technique (s/o/w).[4b]

The simple emulsion o/w has low efficiency to entrap hydrophilic drugs. Using the double (multiple) emulsion-method was an attempt to overcome this problem. However, this method requires large amounts of solvents and needs a stabilizer. The presence of residual stabilizers on the resultant nano- and microparticles is also a potential problem. The double emulsion requires many steps, rigid control of temperature and viscosity of the inner w/o emulsion, and it is difficult to encapsulate high concentrations of hydrophilic drugs. If the experimental parameters are not adequately adjusted, a wide size distribution will be obtained.

3.2 Nanoprecipitation

The method of nanoprecipitation was first described by Fessi et al. and is based on the interfacial deposition of polymers following displacement of a semi-polar solvent miscible with water from a lipophilic solution.[20] It constitutes an easy and reproducible technique that has been widely used in the preparation of PLA- and PLGA-based nanoparticles.[20,21] Some of the advantages associated with this method are: (1) large amounts of toxic solvents are the avoided and (2) submicron particle sizes with narrow size distribution are the obtained (3) without the use of external energy sources. In spite of this, the principal limitation is related to the drug solubility. Since nanoprecipitation was proven to be inappropriate for the entrapment of water-soluble molecules, most of the drug incorporation studies focused on poorly water-soluble and amphiphilic compounds highly soluble in water miscible organic solvents.[22]

In spite of this, Govender et al. have employed this method to prepare PLGA nanoparticles focusing the investigation on the delivery of procaine hydrochloride, a water-soluble drug.[23] The experimental procedure consists in the dissolution of PLGA copolymer and a specified quantity of the drug in acetonitrile. The organic phase was then added drop-wise into the aqueous phase under stirring until the complete evaporation of the organic solvent takes place. Afterwards the influence of some parameters associated to the microparticles formation, such as the pH of the aqueous phase, changes in the formulation of organic phase by addition for example of fatty acids, PLA oligomers, etc., as well as the chemical structure of the tested drug were evaluated in terms of the drug incorporation efficiency expressed as drug content (wt.-%).

These authors demonstrated that a better drug entrapment could be achieved increasing the aqueous phase pH from 5.8 to 9.3 as well as by replacing procaine hydrochloride with procaine dehydrate in the formulation. The

incorporation of charged moieties containing carboxylic groups also improved drug entrapment, in particular when lauric acid was added.

A similar study was developed by Barichelo et al. who explored the possibility to encapsulate water-soluble as well as amphiphilic drugs in PLA- and PLGA-based particles.[24] As it is well-known, nanoprecipitation is not the most suitable method to produce these particles, but it is also true that the available methods potentially useful for these purposes are scarce. As a consequence the goal of this research was to compare the performance of nanoparticles obtained by nanoprecipitation to encapsulate drugs that differ in their lipophilicity. The size of the resulting particles was determined by laser light scattering and it was in the range of 160–170 nm, with a relatively narrow particle size distribution. They also estimated the efficiency of loading and found, in agreement with other authors, that the highly hydrophilic drugs suffer from problems of low affinity to the polymer, leading to unsatisfactory loading efficiency. The obtained results revealed that more lipophilic drugs do not suffer from the problems of leakage of the drug to the external medium, leading to improved drug content in the nanoparticles. They have not defined any relationship between the particle size and the amount of loaded drug in the PLGA nanoparticles. On the other hand, they demonstrated that insulin exhibits great affinity for the lipophilic surfaces because it absorbs through a mechanism similar to self-aggregation. In addition to this they determined that the amount of encapsulated insulin depends on the pH of the buffer solution employed, and that the optimum one was near the insulin's isoelectric point. These observations are consistent with those obtained with albumin as protein.[25]

The use of this technique for the encapsulation of a poorly water soluble drug, the anti-inflammatory agent indomethacin (IND), suitable for magnetic drug targeting in PLA particles have recently been reported by Timko et al.[26] The principal aim of this work was to prepare PLA-magnetic nanospheres. The procedure was similar to the one described previously. In brief, PLA and the drug were dissolved in a mixture of acetone and chloroform (2:1) allowing the complete solubilisation of the drug. Then the organic phase was added drop-wise to the aqueous phase containing a dilute solution of a magnetic compound. The biphasic system was maintained under stirring until the complete evaporation of the organic solvent. At this time the formation of a turbid solution, where nanoparticles were dispersed, took place. The characteristic bands of PLA and the drug in the IR spectra of the obtained magnetite-PLA-IND nanospheres confirmed the successful drug entrapment. In addition, an optimum pH of 3 was found, where the drug entrapment efficiency showed a maximum.

The nanoprecipitation procedure was also applied by Chorny et al. to prepare PLA nanospheres loaded with a liphophilic compound (AG 1295). They also investigated the influence of some variables on the nanoparticles performance (carrier size, drug release rate and drug recovery yield).[22]

It is well known that a complex relationship exists between the experimental variables involved in the nanoprecipitation method and the size of the resulting nanoparticles. Many speculations appear in the open literature about the role of a non-solvent for the polymer on the particle size and shape, but to our knowledge, there is not enough precise information. One of the most recent works is the one carried out by Peltonen et al. that describes the formulation of PLA-based nanospheres by nanoprecipitation employing acetone, methanol and ethanol as the polymer non-solvent and chloroform as solvent.[27] The performance of each solvent in the fabrication of nanoparticles was evaluated in terms of particles's size and some of their physicochemical properties. The influence of the amount of non-solvent was also analyzed. In the case of acetone, a high aggregation tendency was evidenced. The smallest and almost spherical particles were originated with a low volume of the inner phase.

When methanol was used, the formed particles were large (approximately 1 100 nm) with a wide size distribution. The amount of aggregated polymer was markedly decreased. They also pointed out, in agreement with other authors, that the amount of inner solvent highly affected the size and the shape of the resulting particles. Furthermore, the polarity of the solvent seemed not to be the main factor that determines the size of nanospheres. On the contrary, the existence of intermolecular interactions between the solvent and the polymer chains could be the key to the amount of aggregated polymer. Increasing the volume of the inner phase, relative to the outer one, can result in an increase of the particle size. Another parameter to take into account is the viscosity of the selected non-solvent. The authors found that higher viscosity levels could avoid the aggregation of the polymer efficiently. Finally, the best non-solvent found was ethanol due to the fact that it leads to the maximum number of particles and the most spherical ones.

Chorny et al. suggested that the size of nanospheres is mainly dependent on the amount of polymer employed and the incorporation of the polymer non-solvent in the organic phase. They determined that higher polymer concentrations in organic solution lead to smaller nanoparticles. The same effect was observed by the addition of ethanol as the polymer's non-solvent, due to the reduced solubility of PLA in ethanol that causes an early precipitation of the polymer upon contact with the aqueous phase. According to this information, these authors found a method to obtain ultra small particles.[22]

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The main problem with the nanoprecipitation method is the frequent agglomeration of particles due to the lack of a stabilizer. This can be solved ensuring rapid and efficient stirring, by slow addition of the organic phase to the aqueous phase, and by the selection of an adequate solvent system. However, one difficulty is the need to remove of residual solvent from the final nano- and microparticles. In this case a possible solution can be spray-drying using supercritical fluids.

3.3 Salting Out

One alternative to the widely applied emulsion and nanoprecipitation procedures is the salting-out method. This method involves the use of a solution including the polymer and, eventually, the drug in a water-miscible solvent such as acetone or tetrahydrofuran (THF). The solution is emulsified under vigorous stirring in an aqueous gel containing the salting-out agent and, if required, a stabilizer. The addition of a high amount of water to the o/w emulsion allows the formation of nanospheres that can be purified and recovered by cross-flow filtration. The compounds commonly employed as salting-out agents are electrolytes such as magnesium chloride, sodium chloride or magnesium acetate and non-electrolytes such as sucrose. This method is especially suitable when high quantities of polymer and drug are required. The need of intensive purification of the resulted nanospheres as well as the incompatibility of most of the salts employed with the bioactive compounds are the principal limitations associated with this technique.[28]

3.4 Spray-Drying

Another way to obtain nano- and microspheres is the spray-drying method where the drug is solubilised or dispersed in an organic solution of the polymer that is then nebulised in a hot-air flow. The solvent is instantaneously evaporated and dried nano- and microparticles are finally recovered. This method seems to be more versatile, compared with the methods previously described, from the solubility parameters of polymer and drug point of view.[29] For example Bodmeier employed this technique to produce microspheres with a water-soluble drug (theophyline) as well as water insoluble one (progesterone). In the first case the drug was suspended while in the second case it was dissolved in polymer solution. In both cases the size of particles was less than 5 μm.[30] Bishara et al. have produced microparticles from D,L-PLA and octreotide (an octapeptide somatostatin analogue) employing spraying procedure. It basically consisted in mixing an acetonitrile solution of polymer and peptide with stirring. Then the solution was sprayed into liquid nitrogen containing isopropyl alcohol. After evaporation of the liquid nitrogen, the particles were recovered by ultracentrifugation. The size of the obtained particles ranged between 2.20 and 4 μm and the percentage of drug loading was between 52 and 99%, which demonstrated that this method was adequate to this purpose. Contrary to the conventional methods (solvent evaporation/emulsion and nanoprecipitation), the spray-drying involves a very rapid procedure potentially useful at the industrial scale that can be carried out under mild conditions.[31]

In spite of the mentioned advantages related to the spray-drying method, non-uniform particle sizes are obtained, which represents an important limitation principally with regard to the administration way of the nano- and microparticles.

As a consequence, novel preparative methods based on spray system have recently emerged. For example the case of the Prolease Technology, a method oriented to the encapsulation of proteins and peptides in order to ensure their stability. Basically this procedure consists in micronising a protein powder by spray freeze-drying, and then to suspend it in an organic polymer solution. After this, the suspension is atomized into a vessel containing liquid N_2 and frozen ethanol. The atomized droplets in the liquid N_2 are deposited on the surface of frozen ethanol. As the liquid N_2 evaporates, the frozen ethanol liquefies so that the frozen polymeric droplets will transfer into the ethanol where the polymer solvent is evaporated, yielding solid microspheres. As it was commented earlier, this novel methodology is specially suitable for the encapsulation of unstable proteins in contact with common polymer solvents such as dichloromethane or ethyl acetate.[32]

Another method included in the group of spray-derived techniques is the ultrasonic atomization. In this case the atomized drug/polymer dispersion was sprayed into a non-solvent where the polymer solvent was extracted, resulting in the microparticles formation.[33a] Similarly, the polymeric solution was also atomized by acoustical excitation in the work developed by Berckland and coworkers on the controlled release of rhodamine B from PLGA matrix. They designed a special device able to fabricate predefined particle size distributions via continuous variation of the process parameters. In brief, the PLGA solution was pumped though a small gauge or circular orifice while an ultrasonic transducer, controlled by a frequency generator, disrupted the stream into uniform droplets. Besides the control of the particle size, the atomization method offers the possibility of scale-up and to process polymer at ambient or reduced temperature. Furthermore, the authors established that extra benefits could be achieved by combining this methodology with some of the conventional techniques (e.g. double emulsion, nanoprecipitation).[33b]

With the aim to maintain protein and peptides stability, Yeo and coworkers proposed a novel system based on the production of reservoir-type microcapsules by causing the event to occur on the surface of an aqueous droplet generated by a dual microdispenser system (in the same way, ink-jet nozzles or ultrasonic atomizer could also be employed). Microparticles are formed though the collision of two droplets of the aqueous and the polymer solution. Following the collision the polymer droplet spread over the aqueous droplet and the mass transfer between the two liquids resulted in the formation of a polymer membrane on the surface of the aqueous droplet. The microparticles were collected in a water bath to complete the solidification of the polymer membrane. In this contribution the effect of the polymer's solvent on the formation of the particles was also evaluated, resulting in the selection of ethyl acetate as the most adequate one. The evaluation of the solvent was based on its physical characteristics as well as on the ability of the polymeric solution to spread on the aqueous droplet surface.

Finally, this report includes an interesting comparison between the conventional double emulsion technique and the method here proposed, where the advantages of the droplet generation method are highlighted. For example, the proposed method does not include emulsification that means that the contact between the aqueous and polymeric solution is restricted to the surface of the aqueous droplet. They also claimed that the stability of entrapped proteins could be preserved during prolonged drug release, since it has been pointed out that the accumulation of acidic components degradation could affect the integrity of some therapeutic agents in the most PLGA nano- and microparticle systems. In contrast, when interfacial methods are applied the polymer/drug interface is restricted to the polymer membrane covering the surface of the aqueous droplet. As a consequence the damage caused by the polymeric matrix to the drug is minimal.[32c] The feasibility of the proposed method was demonstrated by encapsulating lysozyme, a model protein.[33d]

One of the problems of spray-drying is that there may be very significant loss of product during the process due to the adhesion of the microparticles to the inside wall of the spray dryer apparatus and/or the agglomeration of the particles. A possible solution would be the double nozzle spraying drying technique that employs an anti-adherent.

The benefits associated with the use of supercritical fluids have determined the application of this kind of technology in several and different fields; thus, the production of nano- and microparticles is a clear example of this.[34a] The versatile operating conditions that are possible with supercritical fluids provide the flexibility in the control of the size of the particles that span from microns to nanometres. The possibility of fabricate polymeric particles in a solvent-free system is the most attractive advantage provided by these techniques, especially with regard to the application of nano- and microparticles in the biomedical field. Further advantages that this method offer, e.g., over conventional spray-dying, are the low critical temperatures for processing (34 °C) and the avoidance of oxygen exposure during atomisation, both parameters are particularly important to encapsulate drugs like antigens and proteins.[34b]

The use of PLA-based polymers to carry out these novel approaches have rapidly emerged. Cooper and coworkers[34c] and Kompella and Koushik[34d] have reviewed the use of the mentioned supercritical techniques in the encapsulation of various pharmaceutically active compounds on PLA, PGA and PLGA polymers. Sze and coworkers have encapsulated *p*-hydroxybenzoic acid and lysozyme onto PLA using supercritical CO_2. They evaluated several parameters associated to the method such as pressure, temperature, solution concentration, polymer solvent system, etc. Uniform PLA spheres of about 2 μm were obtained. The increase in the temperature aroused agglomeration and slight plasticization on the obtained particles. The effect of spraying rate was also demonstrated since a size reduction was noticed when the spray velocity increased. From the encapsulation efficiency point of view, lower values were reached compared with those obtained using conventional techniques. In spite of this, the authors highlighted valuables benefits offered by supercritical process such as small amounts of organic solvent are required, with the use of a non-toxic antisolvent combined with the rapid processing time and moderate temperatures.[34e]

3.5 Miscellaneous Methods

A novel in-situ PLGA microsphere formation process has been proposed by Jain and coworkers. It basically involves a stable dispersion of PLA/PLGA microglobules (also called premicrospheres) in a continuous phase consisting of an acceptable vehicle mixture. The resultant dispersion in contact with aqueous buffer or physiological fluids hardened and the microglobules turned into microparticles entrapping the drug.[7a,35a,35b] Excluding the use of toxic organic solvents and precluding the need for reconstitution of the PLGA microspheres before their administration are the most important benefits offered by this procedure. The same authors reported the success in the controlled release of cytochrome c by microspheres fabricated through this novel technique.[35c]

Other techniques such as hot melt microencapsulation[29c,36] and the polymerization of monomers by various methods including emulsion, suspension, and dispersion techniques are currently employed in order to obtain

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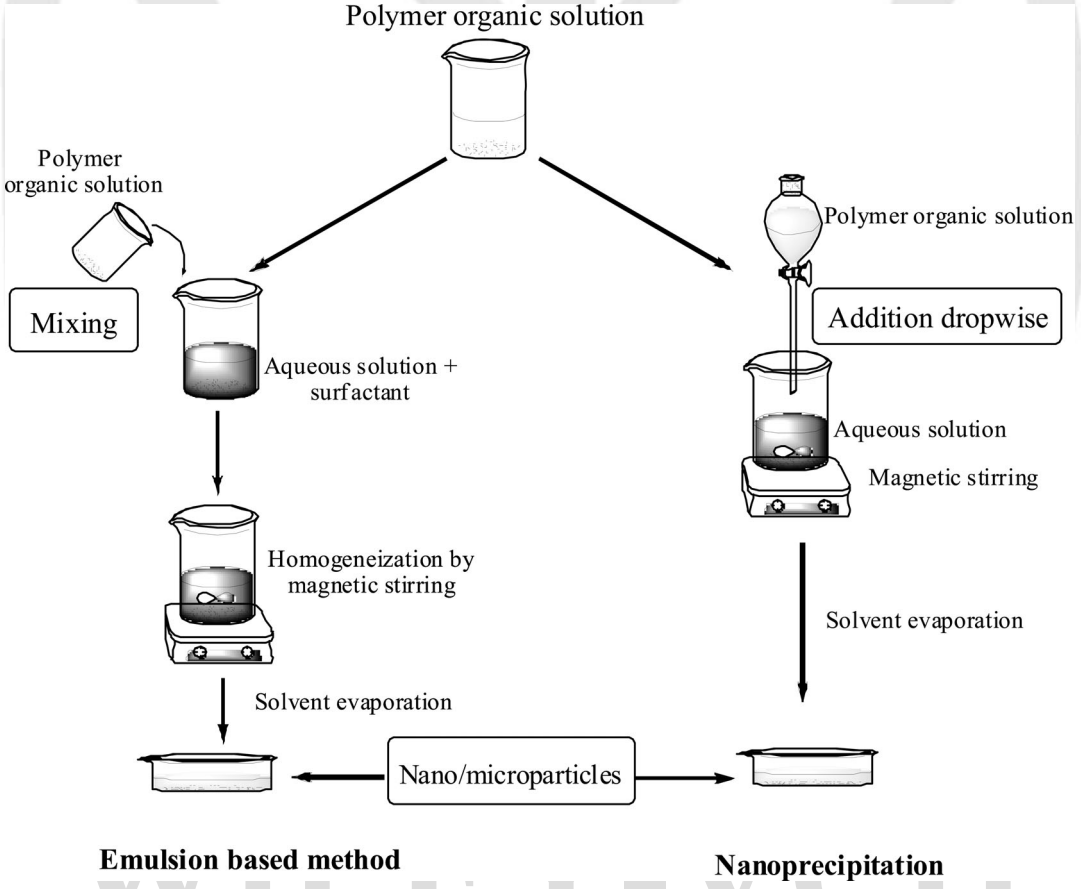
nano- and microparticles.[5a,37] However, to the best of our knowledge, there are no published articles leading with the use of PLA based polymers and copolymers, thus the mentioned processes are excluded of the scope of this review.

4. Comparison between Emulsion-Based Techniques and Nanoprecipitation

According to the bibliographic antecedents, emulsion-based and nanoprecipitation appear as the most widely used techniques in the fabrication of PLA and PLGA nano- and microparticles destined to the entrapment of drugs. The articles in the open literature do not show the differences between both techniques in a clear way. As a consequence, the selection of the most suitable method is not an easy task. In Figure 1 the experimental steps involved in each technique are illustrated. It is clear that both processes begin with an organic PLA/PLGA solution and the contact of it with an aqueous solution (polymer non-solvent) seems to mark the difference between both methods (see step 2 in Figure 1).

Although the rate and time of stirring, kind of solvent systems, the presence of surfactants, etc. may influence the morphology and size of nano- and microparticles, the nanoprecipitation technique leads to smaller size particles. To support this fact, preliminary results obtained in our laboratory are also included. We have prepared nano- and microparticles with PLA synthesized using lipase as biocatalyst. Both methods were tested:

- in nanoprecipitation acetone was used as polymer solvent and the organic solution was added in a controlled way (by means of a syringe) to the aqueous solution, under stirring. The evaporation of organic solvent was allowed while maintaining the stirring. After water evaporation (48 h later), solid nanoparticles were recovered and analysed by scanning electronic microscopy (SEM).
- in emulsion based preparation, dichloromethane was used as polymer solvent. The organic solution was mixed with an aqueous solution of chitin (CS) ■ **Authors: Please consider using CH instead of CS; or chitosan**



■ *Figure 1.* Comparison between the experimental steps involved in emulsion-based and nanoprecipitation methods.

instead of chitin! ■ (0.5%) and PVA (3%). The mixture was homogenised by stirring, promoting the organic solvent evaporation. The solid nanoparticles, recovered after water evaporation, were examined by SEM.

SEM micrographics of nanoparticles are shown in Figure 2. Figure 2a corresponds to the one obtained by nanoprecipitation. Besides the agglomeration of the particles it may be appreciated that they are quite uniform, spherical and that the sizes are lower than 200 nm. Figure 2b presents the nanoparticles obtained by emulsion method. In this case a matrix of CS-PVA coexists with nanospheres; as a consequence broader dispersion in the particle size is observed. In spite of this the average size seems to be in the order of 200 nm.

In a comparative way Table 1 summarizes some of the principal characteristics of both techniques. Evaluating these characteristics as well as the information commented earlier, the nanoprecipitation seems to be the most versatile method. However, the exposed points are referred to drugs encapsulation but nothing is revealed about the properties of the nanospheres by themselves, without the drug present at the preparation step.

The scarce number of reports on the issue available in the open literature also employs the efficiency of encapsulation as a comparative parameter to evaluate the performance of each method. For instance, Gao et al. have prepared a polymer by the coupling reaction of D,L-PLA and CDen using *N*,*N*-dicyclohexylcarbodiimide as the catalyst, as previously described. They also fabricated nanospheres of PLA-based polymers and a model protein (BSA) using the nanoprecipitation method and compared its performance with the method of double emulsion. As a conclusion of their work the authors ensured that both methods are useful to produce nanoparticles but they established that double emulsion leads to higher encapsulation efficiency than nanoprecipitation.[11a]

Bilati et al. have estimated the entrapment of three different model proteins [tetanus toxoid (TT), lysozyme (LY) and insulin (IN)] into PLA and PLGA using double-emulsion and nanoprecipitation methods. They investigated diverse experimental conditions such as different solvents, application of ultrasound, etc. The data provided by the authors suggest that the particles produced by double emulsion method exhibited the smallest size when containing TT. With respect to the values of efficiency of entrapment, entrapment yield and nanoparticle yield, all of them resulted noticeably lower than the same parameters evaluated from nanoparticles prepared by double emulsion. This tendency was evident for the three drugs studied.[18]

With these observations in mind, the choice of the method to produce nano and/or microparticles is strongly dependent on the identity of the drug that is going to be encapsulated. By adjusting some of the experimental variables associated to the preparative procedure of each method, it is possible to reach the desired properties of the generated particles. In general terms, hydrophobic water-insoluble drugs are more efficiently encapsulated by the simple emulsion method (w/o) or nanoprecipitation, while the double emulsion (w/o/w) method seems to be the most suitable to encapsulate hydrophilic water-soluble drugs.

Another point to consider is the way of administration of the particles that at the same time will be given by the therapeutic action of the drug.

Table 2 summarizes the most relevant drugs and the most suitable methods applied for their encapsulation. The suitability of the technique was estimated as a function of entrapment yield/efficiency or efficiency/yield of loading.

The data included in Table 2 show that the emulsion-based methods lead to bigger particles compared with nanoprecipitation. As a consequence, the former technique is appropriate to obtain particles whose main administration way is injection; while the particles produced by nanoprecipitation offer also the alternative of oral administration. As an illustrative example the cases of IN and lyzozyme are presented in Table 2. These drugs can be successfully encapsulated by emulsion and nanoprecipitation, thus the selection of the method will depend on the required administration way.[7b,49]

5. Incorporation of Different Monomeric Substrates on PLA-Based Nano- and Microparticles

In addition to PLA and PGA block copolymers employed as matrix in drug released systems, there are a vast variety of information in the open literature dealing with incorporation of novel monomeric substrates, such as CS, PEG, as well as several anhydrides and carboxylic and fatty acids onto the polymeric system to induce changes in some of their specific properties (size, surface reactivity, hydrophobic character, etc.).[50]

In this context, PEG is considered one of the most promising polymers, being employed in several commercial applications (Ocaspar® and Neulasta™).[51] The incorporation of PEG to PLA based copolymers contributes to modify the polymeric matrix by adding a hydrophilic part that can change the physicochemical properties of hydrophobic PLA/PLGA segments, obtaining particles that exhibit long circulation properties.[52]

Considering the available hydrophilic polymers, PEG has been found to be particularly effective, probably due to its chain flexibility, electrical neutrality and absence of functional groups which avoids undesired interactions with biological components *in vivo*, forming a protective coating on the particle surface.[53]

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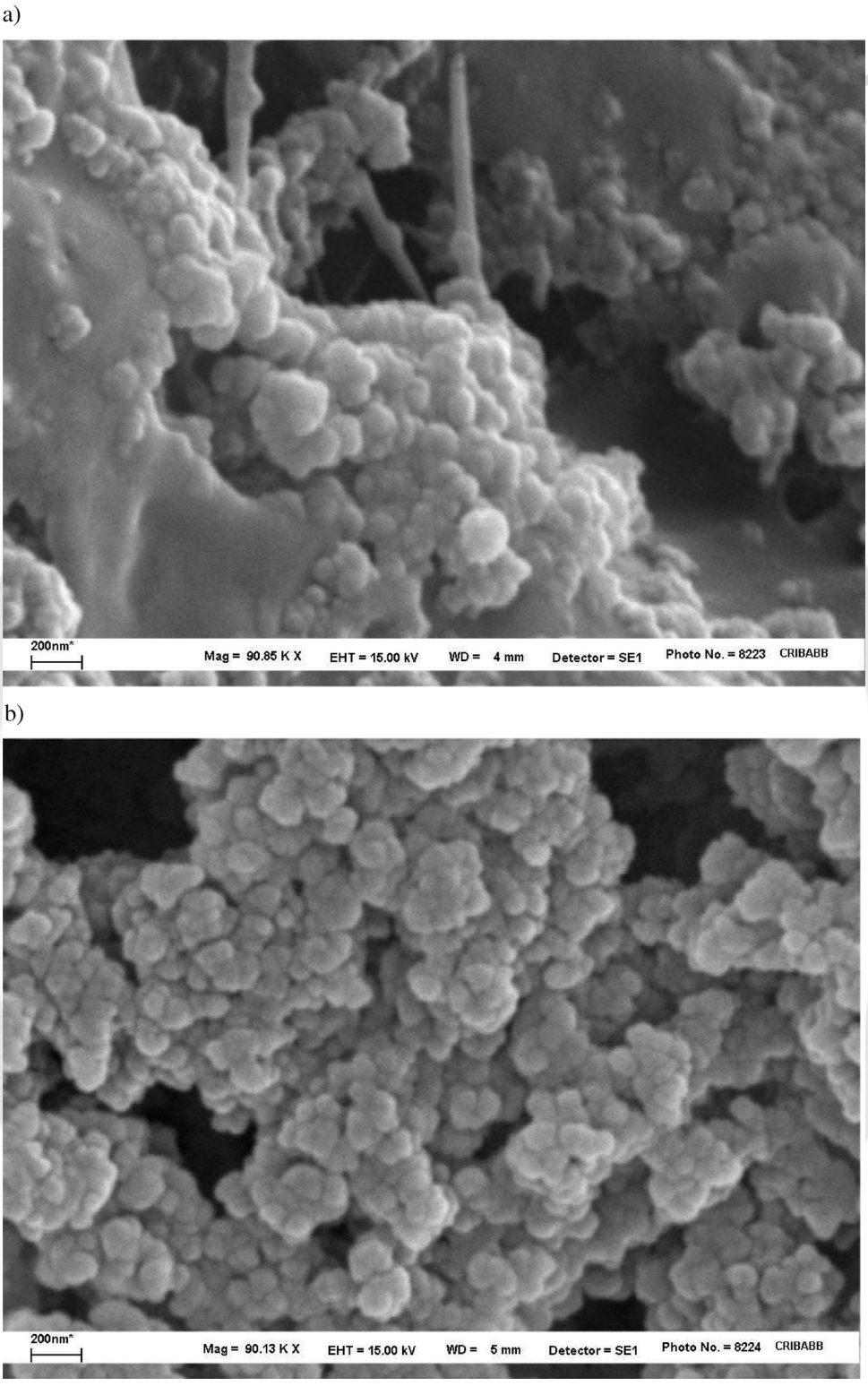

Figure 2. (a) SEM micrograph of PLA nanoparticles prepared by nanoprecipitation. (b) SEM micrograph of PLA nanoparticles prepared by emulsion-based technique.

Table 1. Comparison between the main characteristics of particles produced by emulsion or nanoprecipitation techniques.

Emulsion	Nanoprecipitation
w/o/w allows entrapment of hydrophilic proteins by means of it dissolution in water before the encapsulation process. Strongly dependent of pH	Allows principally hydrophobic compounds to be entrapped.
Protein structure can be altered by interfaces and agitation stress.	No degradation of proteins because interfaces and high shearing rates are avoided.
A wide range of particle size can be obtained by adjusting the conditions and rate of agitation.	Leads to agglomeration because it is not usual to incorporate stabilizers.
s/o/w is used as an alternative to solve some inconveniences associated to w/o/w method.	Requires mild shear forces and sub-micron particles are obtained. Not suitable for hydrophilic compounds because of considerable leakage of the drug in aqueous phase during the process.

The morphology, degradation and drug loading efficiency of nano- and microparticles containing PEG strongly depend on their chemical composition and structure. An example is the article published by Avgoustakis that highlights the important properties of PLA-PEG and PLGA / PEG nanoparticles with regards to their application in the drug delivery field.[4a] By the first time he described the available methods to produce nanoparticles and established the influence of the molecular weight of PEG on the size of the nanoparticle depending on the preparative method. Thus, when the nanoparticles were produced by emulsion-based techniques, he found that the size of the nanoparticles was independent of the PEG content when low $\overline{M}_w$ PEG was used. On the contrary, when relatively high $\overline{M}_w$ PEG (10 and 20 kDa) was employed, the particle's size initially increased and after reaching a maximum, decreased with increasing PEG content. As a difference when nanoprecipitation method was employed he found that the size of PLA/PLGA nanoparticles was similar to the size of nanoparticles prepared from PLA/PEG. Also the $\overline{M}_w$ of PEG did not have any effect on the size of the nanoparticles. With regard to this point, Petracchia et al. have presented a complete investigation evaluating the influence of the incorporation of PEG in PLA nano- and microparticles obtained from emulsion-based techniques.[54] Specifically they explored properties such as the particle's size, and the possibility to control the rate of the drug release by changing the proportion and $\overline{M}_w$ of the PEG added to the formulation of the particles. The analysis indicated that the addition of PEG leaded to a reduction in the particles size independently of the $\overline{M}_w$ of it. They employed lidocaine and predisolone as drugs and determined that the presence of the PEG reduced the rate of drug loading which can be attributed to an interaction between the drug and the PEG.

There are also several reports in the open literature that compare the performance of PLA/PEG nanoparticles with PLA ones in the controlled delivery of a variety of bioactive agents (conventional and antitumour drugs, proteins and genes) and all of them coincided in that the presence of PEG originated particles that can be stored for long periods as lyophilised powders, and that are longer time circulating which permits to reach the affected sites.[4a,54,55]

Another valid alternative to the use of the PEG, is the polysaccharide CS whose OH groups supply an hydrophilic character to nano- and microparticles.[56] CS has been recognized for its mucoadhesivity, biodegradability and ability to enhance the penetration of large molecules across mucosal surfaces.[57] These are other reasons to be an eligible alternative to the PEG. The addition of CS to the system is usually accompanied by the incorporation of lecithin to the organic phase, where the polymer is dissolved. The real objective of this procedure is to promote interactions between polymer and CS that will be facilitated by the ionic interaction between the negatively charged surfactant (lecithin) and the positively charged CS molecules.

Ravi Kumar et al. published the formulation of cationically modified PLGA nanoparticles with well-defined size and shape.[55b] For this purpose they added CS, PVA, and PVA-CS to the particles formulation and compared their performance in the binding of DNA. The preparation of nanospheres was achieved by a modified emulsion-evaporation method. The analysis of nanospheres by dynamic light scattering demonstrated a unimodal size distribution when PVA and PVA-CS mixture were aggregated. On the contrary, they could not find evidence of the formation of nanoparticles when CS alone was used. They concluded, in agreement with other authors, that the presence of PVA is necessary for the formation of the

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Table 2. Methods for the preparation of nano- and microparticles for various drugs employing PLA-based polymers as the matrix. The resultant particle sizes and loading efficiency are also included.

Drug	Preparation method	Size	Efficiency[a)] %	Ref.
BSA	Double emulsion	140–250 nm	44–71	[7a,11a]
Protein C	Double emulsion	175–316 nm	57–70	[14,38]
Nimesulide (anti-inflammatory)	Double emulsion	42.9 nm–2.1 μm	10–28	[12]
Tetanus toxoid	Double emulsion	353–1 153 nm	93–96	[18]
Triclosan (non-cationic antimicrobial agent)	Double emulsion	175–460 nm	63.8–89.3[b)]	[39]
Irinotecan hydrochloride (antitumor agent)	o/o emulsion	50 μm	80[b)]	[40]
25-Merphosphorothionate oligonucleotide	Double emulsion	450 nm	27[b)]	[40]
Lysozyme	Double emulsion	369–459 nm	84–88	[18]
Leuprolide acetate	o/o emulsion	microparticules		[41]
Gene	Double emulsion	207–231 nm	99	[42]
Insulin	Double emulsion	53 μm	75–80	[43]
		1 402–1 038 nm	73–87	[18]
		15–25 μm	95	[44]
		2–5 μm		[45]
Insulin	Nanoprecipitation	337 nm	94	[46]
		133–302 nm	13–22	[18]
		160–170 nm	41–61	[24]
Procain hydrochloride	Nanoprecipitation	159–207 nm	14–62	[23]
Indomethacin	Nanoprecipitation	168 nm	94	[24]
Lysozyme	Nanoprecipitation	137–351 nm	34–59	[18]
Cyclosporin A	Nanoprecipitation	169 nm	84	[24]
Ketoprofen	Nanoprecipitation	67 nm	46	[24]
Tyrphostin	Nanoprecipitation	65–143 nm	56–68	[22]
Steroids	o/w emulsion	90–250 nm	0.02–1.10[c)]	[47]
β-Lactoglobulin	Double emulsion	6.6–7.3 μm	16–62[b)d)]	[48]
Cloricromene/cloricromene acid	Nanoprecipitation	102–340 nm	2.6–3.9[e)]	[49]

[a)]Refers to the yield of loading and/or entrapment; [b)]Entrapment efficiency; [c)]wt.-% steroid/nanoparticle; [d)]Depending on the pH; [e)]Entrapment efficiency defined as mass of drug in nanoparticle/mass of drug used in formulation.

nanoparticles, but PVA alone could not give the electropositive charge required and CS alone is not enough to stabilize the particles.[58] As a consequence, the best choice seems to be a mixture of both in order to obtain not only cationic particles but also nanoparticles with uniform size and spherical shape.[59]

In reference to this topic, Vila et al. have compared the effect of addition of CS and PEG on PLA-based nanoparticles, evaluating its performance to load proteins and to deliver them in an active form.[46] The data collected by the authors indicated that the size of nanoparticles could change, from 190 to 300 nm as a function of the added substrate. Smallest particles were obtained employing PEG, while CS produced particles of near 500 nm. An analysis of the efficiency of the encapsulation of the model drugs was also included in this article. It was suggested that the highest value of the efficiency (≈90%) was obtained with CS, while a very low one (≈31%) was registered employing PEG.

According to the available bibliographic references it is clear that the most widely employed substrates to induce changes in the properties of PLA-based nano- and microparticles are PVA, PEG and CS. However, the techniques of preparation of nanoparticles are versatile because they admit the incorporation of other compounds that bring specific properties to the particles.

The surface modification of PLA-based nano- and microparticles is another strategy widely utilized in order to generate materials able to interact with polar substrates.[60a] Fahmy and coworkers prepared surface-modified PLGA nanoparticles by addition of fatty acids (palmitic acid) with the aim to provide a high density of functional group onto the particle's surface. They evaluated the performance of modified particles in the encapsulation of BSA and found that the surface treatment enhanced the protein encapsulation efficiency as well as the particle yield.[60b]

In this context, special attention deserves the incorporation of magnetic compounds (based on iron oxides) into PLA-based nano- and microparticles that lead to "magnetic nano- and microparticles" useful for the magnetic drug targeting. The aim of the drug targeting is to carry the desired amount of drug to the required target and release it at a controlled rate. Among the ways to control the targeting specificity, there is a possibility to use the magnetically-guided particles as drug carriers. By application of an external magnetic field, magnetic particles could be retained within a target organ for a given period of time limiting the spreading of the particles in the general circulation. For this purpose, the magnetic particles should be entrapped into a particulate biodegradable polymer matrix to improve the drug loading and the release profile. The published information with regard to this novel topic is very extensive; although the articles including PLA-based polymers are more limited.[61] The application of PLA-based magnetic nanoparticles as magnetic drug targeting system was reported by Timko and coworkers using Indomethacin as bioactive principle.[26] They prepared the magnetic particles by nanoprecipitation, and studied the effect of pH on the percentage of drug entrapment. They found an optimum pH value (pH = 3) where the drug entrapment was maximum (41%). Analyses about the size of the resulted composites were not included in this contribution.

The incorporation of Zn to nano- and microparticles formulation has been reported by Ishihara et al.[47] They found that Zn increased the encapsulation efficiency of bethamethasone phosphate in the nanoparticles by formation of a water-insoluble complex with the drug, favouring the formation of nanoparticles by interaction with a carboxyl group in the PLA-based molecule. They also pointed out that the presence of Zn delayed the degradation of the matrix polymer and enlarged the size of the resultant particles, which is suitable for intravenous administration and accumulation in inflammatory sites.

Table 3 includes the mentioned substrates (polymer, monomer, compounds) commonly used as modifiers, and the changes that they induce on the nano- and microparticle's properties/performance. The most adequate methods employed to the preparation of modified nano- and microparticles are also indicated.

6. Alternative PLA-Based Systems, Different from Nano- and Microparticles, Useful in CDD

In spite of the vast variety of information dealing with the use of PLA-based copolymers nano- and microparticles as drug delivery systems, there are several articles that describe other strategies able to promote this phenomenon from biodegradable polymers. One example is the procedure

Table 3. Summary of the most relevant substances (or additives) included to nano- and microparticles formulations. The effect of additives on the characteristics and performance of the resulting particles and the method commonly used to prepare them.

Added compound/s	Preparation method	Effect on nano- and microparticle characteristics and/or performance	Ref.
PEG	DE	Particles with long circulation properties Reduction of particle sizes	[4a,54,55]
CS	DE	Provide an active site (+ charge) to ionic interactions	[46,55b,56,58,59]
PVA	DE	Contributes to stabilization of nano- and microparticles	[17,55b]
Fatty acids (surface modification)	DE	Enhances specific interactions by providing a high density of surface functional groups	[60]
Iron oxides	DE, Polymerisation, Nanoprecipitation	Specific application for magnetic drug targeting	[26,61]
Zn	DE	Increment of particle sizes; Delays degradation of polymeric matrix	[47]

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proposed by Miyajima et al., who analyzed the release profiles of acidic and neutral drugs from PLA matrices.[62] They fabricated cylindrical matrices by the heat compression method. The PLA rods were prepared mixing PLA with the drug and dissolving them in dichloromethane. After evaporation of solvent, the mass was dried under vacuum. The dried powder was pressed moulding leading to transparent tubes. Acidic and neutral studied drugs were dissolved in the PLA rods. The principal aim of this work was to determine the release mechanism. On this way the authors found that the release profiles from PLA rods showed two stages: during the first one the rods remained as drug dissolved matrices and the drug diffused though hydrated homogeneous amorphous polymer matrices. And in a second stage the rods transformed to the drug dispersed matrices resulting from the precipitation of drugs in the rods. Then the drugs were able to diffuse through the water-filled micro pores of the polymer matrix. Similar devices were produced by other authors with different drugs such as BSA[52a] and amoxycillin.[63]

Micellar systems are another option that deserves attention in the drug carrier field. Agrawal et al.[51] and Jeong et al.[64] have synthesized PLGA-grafted poly(L-lysine) (PLL) (PLL-*graft*-PLGA) to demonstrate its micelle-forming property in an aqueous solution. The PLL-*graft*-PLGA micelles could be used to produce compact nanoparticulate complexes with plasmid DNA, which could efficiently protect the complexed DNA from enzymatic degradation by DNase I. The grafting of PLGA onto PLL backbone was promoted by reaction of carboxylic terminal groups of PLGA with primary amine groups present in PLL. PLGA-g-PLL provided an opportunity to form a self-assembled micellar structure in an aqueous phase. In this publication the graft copolymer was dissolved in dimethyl sulfoxide and subjected to dialysis to obtain micelles in aqueous medium.

7. Concluding Remarks

The utilization of nano- and microparticles based on PLA and related polymers and copolymers in the field of controlled release of medicaments became a prominent area of research due to its excellent biocompatibility and biodegradability.

The available techniques to produce such particles demonstrated to be very versatile since they are useful to entrap various classes of drugs. On this way, several drug profiles as well as noticeable enhancement in the production and performance of particles and in the loading efficiency can be achieved by adjusting some of the experimental variables involved in the preparation process.

The incorporation of a wide variety of compounds, principally PEG, CS, PVA, and iron oxides to PLA-based nano- and microparticles offers the possibility of enlarge the applicability of these materials leading to more versatile compounds, able to interact with specific medicaments in a specific environment.

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