EXPERIMENTAL CONTRIBUTIONS IN THE SYNTHESIS OF PLGA NANOPARTICLES WITH EXCELLENT PROPERTIES FOR DRUG DELIVERY: INVESTIGATION OF KEY PARAMETERS

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Biodegradable polymers are materials used in multiple applications including biomedicine, nanomedicine and medical engineering. Especially, PLGA (DL-lactide-co-glycolide) nanoparticles are studied as formulations for drug delivery and controlled release. The aim of this study was to investigate the influence of key parameters in synthesis process, optimized them in order to obtain nanoparticles with optimum diameter and polydispersity. The nanoparticles (NPs) were obtained by emulsion-solvent evaporation method. The mean diameter and polydispersity of synthesized nanoparticles where studied by DLS, the morphological characteristics by SEM and AFM. The results show that concentration and molecular mass of surfactant, type of organic phase and method of homogenization exhibit a significant influence on formulations.

Keywords: biodegradable polymeric nanoparticle, biomedical application, emulsion-solvent evaporation method

1. Introduction

In the last years, nanotechnology has emerged as versatile instrument in the pharmaceutical and biomedical fields, exhibiting a significant impact towards the development of approaches for the diagnosis and treatment of a variety of diseases. In this order, nanoscale structures (<1000 nm) have been used as delivery devices for a wide range of therapeutic compounds, starting from small molecule drugs to diagnostic imaging agents [1] [2]. These substances are usually enclosed inside formulations and here their release from the devices is controlled by the nanocarrier matrix composition [3]. The drug delivery device protects the therapeutic substance encapsulated inside against degradation in biological environment, improves the therapeutic properties, controls the release rate and

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decreases the toxicity of the active agent by reducing the administration frequency [4].

Thus, polymeric nanoparticles are the most distinguished carriers due to their higher structural integrity, stability and capability to control the release profile of the drug. In addition, they are easily synthesized and can be modified and functionalized, becoming versatile systems for a variety of biomedical applications.

Among these formulations, biodegradable polymeric nanoparticles stand out, exhibiting multiple advantages opposite to other carriers because they show excellent biocompatibility, better control release profile and higher versatility. One of the most important properties of these polymers is the ability to degrade into components that can be metabolized by the body [5]. Polymeric nanoparticles can be synthesized from natural or synthetic polymers. Among the natural polymers are chitosan and gelatin, synthetic polymers utilized in this field are PLGA, poly-lactic acid (PLA), poli-ε-caprolactone (PCL) [6].

One of the most important biodegradable synthetic polymers used in preparation of various drug delivery formulations is PLGA. Its remarkable biocompatibility and biodegradability derive from the resulting monomers in hydrolysis process [7] which can be metabolized by the body through the Krebs cycle. This is the main factor which determines a lower systemic toxicity of the drug delivery formulations based on PLGA [8]. The degradation rates of PLGA can vary from weeks to months depending on its physical properties [9].

Based on the desired administration route, the size and surface properties of the nanocarriers should be optimized. For example, if the nanoparticles are synthesized for intravenous administration, they should be smaller than 500 nm (the diameter of the smallest blood capillaries is around 4μm), and ensure a good circulation into the bloodstream without forming any aggregates or embolisms. [2], [10]. Also, the risk for larger nanoparticles to be entrapped and eliminated from the body by the liver, spleen and bone marrow mononuclear phagocytic systems (MPS) exists [11]. Consequently, depending on the desired delivery route and properties of the final formulation, multiple protocols to obtain nanoparticles were created [2]. The second step after the protocol is selected consists of setting the parameters in order to obtain the best characteristics for the drug delivery formulation.

The aim of this work was to design the formulation with the best diameter and polydispersity for biomedical applications. This was gained by emulsion-solvent evaporation method, studying and modifying some parameters, exhibiting important functions in the synthesis process and on final characteristics of nanocarriers.

2. Experimental

2.1. Materials:

(PLGA) poly (lactic-co-glycolic) acid 50:50, acid terminated, with Mw. 40000-75000 Da, polyvinyl alcohol (PVA) with Mw 30000-70000 Da, 87-90% hydrolyzed, (PVA) with Mw 80000-124000 Da, 87-89% hydrolyzed, (PVA) with Mw 31000-50000 Da, 87-90% hydrolyzed, (PVA) with Mw 88000 Da, 88% hydrolyzed, dichloromethane (DCM) >99,9%, all being purchased from Sigma Aldrich (USA) and ultrapure water. All chemicals were used without further purification.

2.2. Methods:

2.2.1. Synthesis of PLGA nanoparticles

As illustrated in Fig.1, PLGA nanoparticles (NPs) were prepared by standard emulsion-solvent evaporation method [12]. Specific amount of PLGA was dissolved in 4 mL DCM, resulting organic phase. This phase was added dropwise to 20 mL 2% aqueous solution of PVA with 10mL/h dropping speed, under ultrasonication in an ice bath. The obtained mixture was homogenized by sonication for 15 min on ice (20 kHz, 220 V, CVX 130, Produced by Sonics& Materials INC, USA). Afterwards, solvent evaporation was achieved by magnetic stirring at 1000 rpm for 3 hours at 35°C. The NPs were recovered by centrifugation at 9000 rpm for 15 min, washed three times with distilled water at 35°C-38°C, to remove all residual surfactant. The obtained NPs were either used freshly, lyophilized (D-37520, Osterode am Harz, Germany) or dried in oven for further experiments.

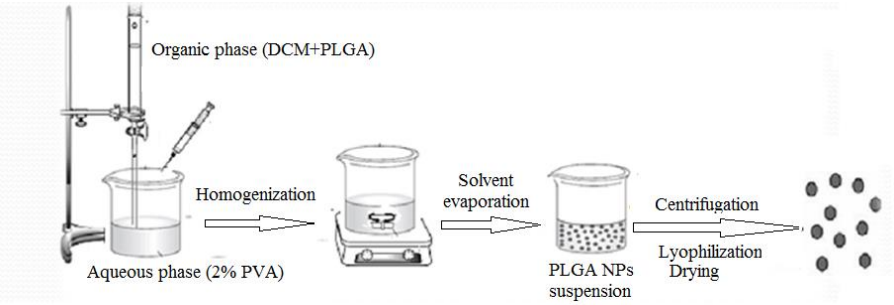

Fig. 1. The synthesis of PLGA NPs by solvent-evaporation method

2.2.2. Size distribution, zeta potential and morphology

Hydrodynamic diameter and zeta potential values were measured by Dynamic Light Scattering using Malvern Zeta Sizer ZEN 3600 (Worcestershire, UK, DLS). PLGA NPs fresh samples were suspended in distilled water and 12 successive cycles were run at 25°C.

The Laser Doppler Electrophoresis Technique was used to determine the zeta potential (PZ) of the NPs. All the measurements were done in triplicate to ensure the reproducibility and the data were presented in mean ± SD.

The morphology of the PLGA NPs was studied by Scanning Electron Microscopy (QUANTA INSPECT F, SEM) and Atomic Force Microscopy (Agilent 5500, AFM). For SEM analysis, the samples dried or lyophilized were coated with a gold layer. Examinations were performed at 30 kV. For AFM study, a drop of the NPs suspension was placed on a lamella. After drying, the sample was analyzed through contact method.

3. Results and discussion

3.1. Size distribution and zeta potential analyses

The DLS analysis was employed to determine the hydrodynamic diameter and zeta potential.

NPs size was as much as 106.2 ± 1.42 nm in diameter with polydispersity index (I) of 0.21±0.016. Fig. 2 indicates a monomodal curve, suggesting a narrow size distribution without the formation of aggregates. The zeta potential was generally moderately negative (-17.6 mV). This negative value may be influenced by the –COOH end groups of polymer. In theory, positive or negative values of zeta potential determines the stability of the suspensions [13]. The obtained values indicate that suspension of NPs is medium stabile.

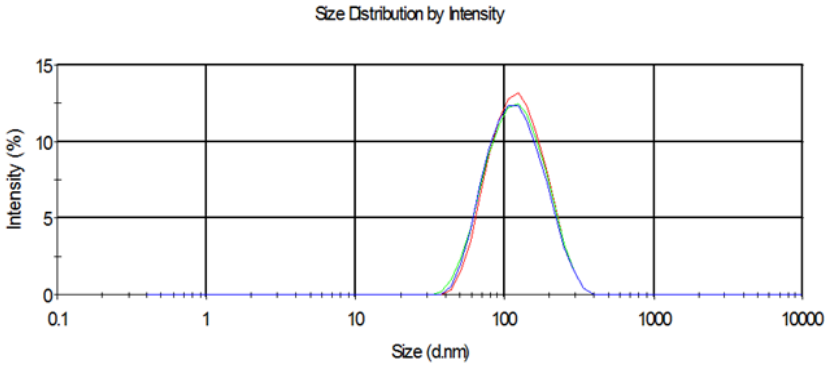

Fig. 2. Hydrodynamic diameter of PLGA NPs from dynamic light scattering measurement

3.2. Optimization of the formulation parameters

The efficacy of a preparation method is usually judged by the particle size and distribution of the obtained carriers [14].

In this context, using emulsion-solvent evaporation method, various parameters having an essential role in final characteristics of synthetized carriers were evaluated in order to reach optimal preparation conditions for an ideal device for drug delivery. The following parameters were taken into account: surfactant

content in the formulation, PLGA concentration in the organic phase, aqueous to organic phase volume ratio, type of organic solvent and sonication power. The influence of each parameter was independently determined.

3.2.1. Influence of surfactant concentration

To obtain NPs with better characteristics by emulsion solvent-evaporation method, the stabilization of globules from primary emulsion is a decisive factor. The surfactant exhibits a crucial role in the emulsification and stabilization process protecting the droplets against coalescence [15]. Thus, the influence of different concentrations of PVA in the external aqueous phase was studied. The size and I of synthesized nanoparticles with 1.0%, 1.5%, 2.0% and 5% PVA are studied (Fig. 3).

The data indicate that with increasing the concentration of PVA the size of NPs decreases. The smaller size particles were synthesized when PVA concentration was 2.0%, producing nanoparticles having size 108.2 nm ±0.75 and I 0.215±0.008. If the PVA concentration increased to 5%, the dimension of NPs also increased. This is due to an increase in the PVA concentration leading to the formation of an aqueous phase with higher density and to a decrease in the net share stress in the emulsification process, which results in an enhancement of the mean diameter of the NPs [16].

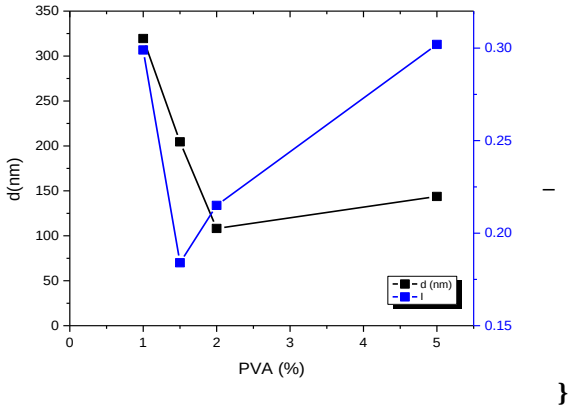

Fig. 3. The size and I index of PLGA NPs at different concentrations of APV

3.2.2. Influence of the molecular weight and the hydrolysis degree of PVA

Thus, PVA with different molecular weight and hydrolysis degree was employed (Tab. 1).

Table 1

The influence of different type of PVA on the mean diameter of PLGA NPs

Mw PVA (KDa)	Hydr. degree of PVA (%)	d (nm)	I
80-124	87-89	416.9 ± 0.42	0.420 ± 0.090
30-70	87-90	111.4 ± 2.25	0.218 ± 0.012
31-50	87-90	172.9 ± 15.59	0.219 ± 0.012
88	88	259.4 ± 7.51	0.221 ± 0.031

The results show that the molecular weight of PVA exhibits a directly influence on the final characteristics of NPs. It can be seen that PVA with higher molecular weight forms large size NPs. Probably it is due to the large chains of stabilizer that remain trapped on the NPs surface and thus lead to the formation of largest drug delivery formulations. Also, surfactant with low molecular weight (PVA with Mw= 31-50 KDa) is not able to stabilize the primary emulsion, resulting in formation of NPs with larger mean diameter and I.

Another parameter which can influence the hydrodynamic characteristics of synthesized PLGA NPs is the hydrolysis degree of PVA. The hydrolysis degree influences the removal of the residual surfactant on the NPs surface. PVA partially hydrolyzed is removed easier from the surface of the NPs than the fully hydrolyzed type, forming smaller NPs with lower I value. The higher hydrolysis degree of PVA involves a higher number of –OH groups in their structure, forming an orderly arrangement, in which water penetrates harder and polymer solubilization occurs in a difficult manner. Consequently, the surfactant remove is tough, forming aggregates on the surfaces of the NPs, leading to an increasing of mean dimension and I value.

PVA with Mw 30-70KDa and 87-90% hydrolyzed was the best surfactant, forming the NPs with optimum hydrodynamic characteristics, lower dimension and polydispersity.

3.2.3. The ratio between internal and external phase

Table 2

The influence of phase ratio on mean diameter of NPs

Phase ratio	d (nm)	I
4/20	110.9 ± 0.85	0.236 ± 0.019
2/20	197.2 ± 1.66	0.308 ± 0.042

The fraction between the organic and the aqueous phase of the emulsion is one of the most important parameter which influences the stability, dispersity and size of the formed globules [15]. The organic phase was varied between 2 mL and 4 mL, aqueous phase remained constant, and its influence on mean diameter and size distribution of NPs was examined (Tab. 2). As the volume of internal phase increases, the mean diameter of NPs decreases. This result is due to a better dispersion of polymer in organic phase. Also, a large amount of organic solvent prevents the coalescence of droplets in the aqueous phase diffusion. The samples were formed in the presence of 2% PVA. Similar results were reported in literature, the lower ratio of organic-aqueous phase produces NPs of smaller size [17].

3.2.4. Nature of the solvent

To obtain drug delivery systems with optimal size through solvent evaporation method, the main criterion for selecting organic solvent is polymer solubilization and if it is possible immiscibility of organic solvent with aqueous phase [17]. Also, it was examined the possibility to use a miscible solvent- acetone mixture. This possibility would enable a simpler purification process of synthesized formulations.

Small particles with a size of up to 106 nm were obtained using water-immiscible solvent (Tab.3). These results were attributed to higher interfacial tension between aqueous and organic phase. The surfactant remains at the organic-aqueous interface during the diffusion process exhibiting an adequate protective effect to stabilize the formed droplets.

Table 3

The influence of solvent type on the mean diameter of PLGA NPs

Solvent	d (nm)	I	PZ (mV)
DCM	106.2 ± 1.42	0.210 ± 0.016	- 17.6

Acetone	135.9 ± 1.05	0.199 ± 0.021	- 4.13

The stability of obtained NPs was assessed by zeta potential analysis. Significant repulsive forces appear between the NPs as a result of extreme zeta potential values (positive or negative) which, on the one hand prevent particle aggregation and, on the other, ease the redispersion process [18]. NPs obtained using DCM exhibit a higher value of zeta potential, and are more stable than NPs synthetized using water miscible solvent.

3.2.5. Polymer concentration

The polymer concentration into organic phase is another important parameter to consider when forming polymeric NPs. Several concentrations of polymer into organic phase were employed (Fig. 4).

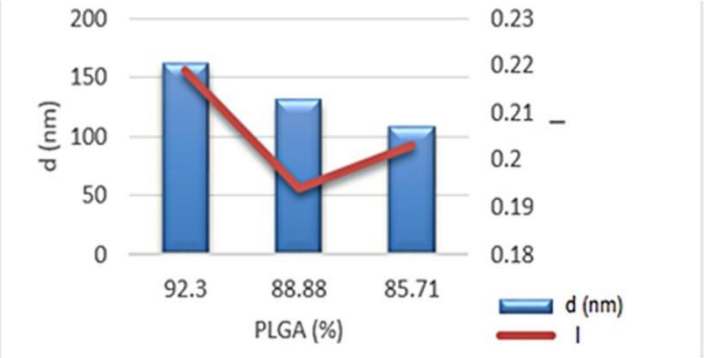

Fig. 4. The influence of polymer concentration on the mean diameter of the PLGA NPs

It is easily to observe that an increase of PLGA concentration leads to an increase of the NPs diameter [17], [2], [16]. The size of NPs increases from 108.2 nm to 162 nm if the PLGA concentration into organic phase was increased from 85.7% to 92.3%. A higher PLGA concentration into the organic phase makes the solution more viscous; increasing the viscosity of the internal phase makes the formation of primary emulsion more difficult, resulting in the larger size of NPs.

3.2.6. Method of homogenization

The homogenization process was performed by two methods: sonication and magnetic stirred (Tab.4.)

It can be concluded that homogenization by sonication is more efficient yielding NPs with better characteristics than NPs homogenized by magnetically stirred. It is due to a better dispersion of droplets into aqueous medium, forming NPs with smaller size and distribution. NPs formed by sonication have mean diameter 10 times lower than mean diameter of NPs obtained by magnetically stirred.

Table 4

The size and polydispersity index of PLGA NPs homogenized by different methods

The method of homogenization	d (nm)	I
Sonication (70%)	110.9 ± 0.85	0.236 ± 0.019
Magnetic stirred (1000 rpm)	1016 ± 543.8	0.758 ± 0.221

3.2.7. The power of sonication

Among the technical parameters, the power of sonication, also exhibit an important effect on the globules size. Thus, there were examined three amplitudes to determine the optimum power for homogenization the organic phase, and obtain NPs with better diameter and I (Tab 5).

*Tab**le** 5*

The influence of the power of sonication on the mean diameter of polymeric NPs

Power of sonication	d (nm)	I
40%	197.8 ± 6.92	0.353 ± 0.006
70%	103.7 ± 0.43	0.22 ± 0.011
90%	142.9 ± 9.10	0.35 ± 0.061

A lower power of sonication forms NPs with larger dimension. Sonication at higher values leads to raise of temperature of primary emulsion, resulting premature evaporation of organic solvent and coalescence of droplets [16]. In this context, the mean diameter and I of final formulations increases considerable. The optimum power of sonication to homogenize the droplets was found to be 70%.

3.2.8. The dropping speed

The influence of dropping speed on the mean diameter of PLGA NPs was determined by synthesized NPs at four values of dropping speed of organic phase into aqueous (Fig. 5). As the dropping speed decreases, the mean diameter of NPs decreases. Probably at higher dropping speed the organic phase is not very good dispersed into aqueous phase, resulting NPs with larger dimension that NPs prepared at lower speed. At this dropping speed, the polymer phase is better dispersed into external phase, forming NPs with lower size and optimum characteristics for drug delivery.

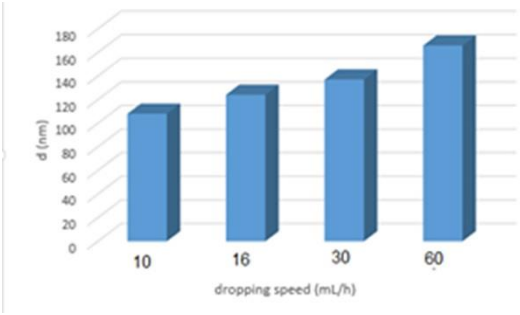

Fig. 5. The influence of dropping speed on the characteristics of biodegradable NPs

3.3. Morphology of nanoparticles

Under SEM and AFM observations (Fig. 6 and 7), the NPs exhibit a fine spherical shape without any cracks. The mean diameter was approximately 87 nm, these results are in concordance with the diameter value achieved by DLS analysis.

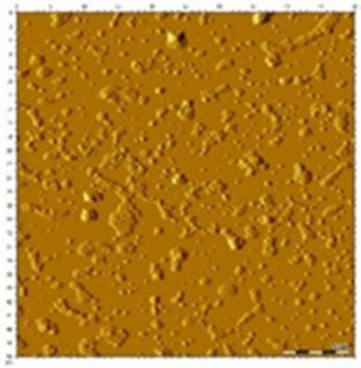

Fig. 6. AFM image of PLGA NPs dried at 37°C

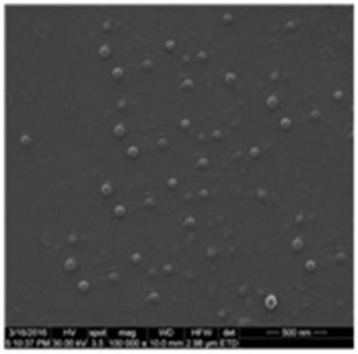

Fig. 7. SEM image of PLGA NPs dried at 37°C

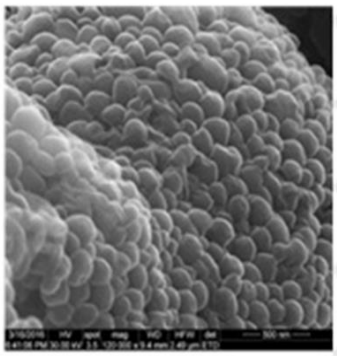

Fig. 8. SEM image of lyophilized PLGA NPs

The morphological characteristics of dried NPs were determined by SEM analysis, showing regular spherical shape and smooth surface. The SEM image reveals low surface molecular aggregations. Similar morphological results were

observed when the sample was analyzed by AFM technique. In the AFM image, the NPs show some aggregates, this being probably caused by the residual surfactant remained on the surface of the NPs. In Fig. 8, SEM image shows lyophilized PLGA NPs. There are spherical shapes with some deformations and porous surface due to lyophilization process. The essential purpose of the lyphilization process is to improve the NPs stability. Through this procedure, the colloidal suspensions are transformed into solid form which prevents the aggregation and degradation of the polymer NPs [19]. Also, through freeze-drying process NPs are formed with porous structures, which is important in the mechanism of drug release from the drug delivery devices.

4. Conclusions

The most commonly employed method to synthesized polymeric NPs for drug delivery is solvent-evaporation. The obtained NPs are spherical with or without porosity, depending on the method of drying. Parameters like concentration of stabilizer and polymer, ratio between external and internal phase, type of organic solvent and method of homogenization proved to be decisive factors for the synthesis of PLGA NPs with optimum characteristics for drug delivery systems.

The smaller size of formulations with higher stability was obtained using the organic phase immiscible with aqueous phase, intermediate concentration of surfactant, lower polymer concentration into organic phase and lower dropping speed with homogenization method by sonication.

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