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Research Article

A Novel Aerosol Method for the Production of Hydrogel Particles

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A novel method of generating hydrogel particles for various applications including drug delivery purposes was developed. This method is based on the production of hydrogel particles from sprayed polymeric nano/microdroplets obtained by a nebulization process that is immediately followed by gelation in a crosslinking fluid. In this study, particle synthesis parameters such as type of nebulizer, type of crosslinker, air pressure, and polymer concentration were investigated for their impact on the mean particle size, swelling behavior, and morphology of the developed particles. Spherical alginate-based hydrogel particles with a mean particle size in the range from 842 to 886 nm were obtained. Using statistical analysis of the factorial design of experiment it was found that the main factors influencing the size and swelling values of the particles are the alginate concentration and the air pressure. Thus, it was demonstrated that the method described in the current study is promising for the generation of hydrogel particles and it constitutes a relatively simple and low-cost system.

1. Introduction

Polymeric nanoparticles have been widely used as carriers in drug delivery systems because they offer several advantages over larger particles including a larger surface area, increased dissolution rate, enhanced bioavailability of insoluble drugs, mass transfer from the particles into the surrounding medium, and ability to cross the cellular membranes [1, 2]. Thus, nanoparticles have become a new formulation option, specifically in the aerosol delivery field, where the aerosol droplet size is crucial to the efficacy of inhalation therapy [3].

Technologies such as supercritical fluid extraction (SCF), electrospray, high-pressure homogenization, solvent displacement, and spray drying have been commonly used to produce nano- and microparticles [1, 3, 4]. Supercritical fluid extraction (SCF) is a method that depends on the coprecipitation of a drug and polymer together using mainly carbon dioxide as a supercritical fluid (nonsolvent), to extract the solvent from the drug emulsion or solution. However, when highly viscous solutions are used, unsteady particle size, particle agglomeration, and incomplete encapsulation can occur which makes the supercritical fluid extraction a very material-specific method [4–6]. Electrospray is a method that depends on dispersion of liquids into small charged droplets when an electrostatic field is applied and it was first described by Lord Rayleigh. The electrospray setup comprises a nozzle connected to a high-voltage supply and to the liquid or solution to be atomized. Once the high electrical field is applied, the electrostatic forces created disperse a liquid stream into highly fine charged droplets. Experiments using electrospray method have demonstrated that particles as small as 250 nm can be produced. However, a wide droplet size distribution can be produced as a consequence of fission of droplets. Also, this method is restricted to high-voltage operation, rising safety and reliability issues in the consumer use [7–9]. High-pressure homogenization is another method that has been used to produce nanosuspensions. Here, an aqueous solution containing the drug is passed through a narrow homogenization gap using very high velocities [10, 11]. The size reduction of the particles occurs due to

induced cavitation forces within the fluid. The main disadvantage of this method is the temperature-induced drug degradation [12]. Solvent displacement is considered a simple method for preparation of nanodispersions or nanospheres. This method involves the mixing of an aqueous phase in presence of an emulsifier with an organic solution containing the dissolved active compound and the polymer [13]. The rapid diffusion of the organic solvent in the aqueous medium leads to an instantaneous precipitation of nanoparticles. Even though solvent displacement offers a rapid obtaining of nanoparticles, the use of suitable water-miscible solvents to produce spontaneous emulsification may make it difficult. Another limitation of this method is that it is used mainly for lipophilic drugs because of the miscibility of solvent with the aqueous medium [14]. Spray drying is a process that force fluid through a nozzle generating a mist, that dried produces a fine powder, and it has become in one of the widely used techniques for the production of micro- and nanoparticles. Spray drying variations such as spray freeze-drying into liquid and air nebulization spray drying have been reported as well [1]. The principal disadvantages of the spray drying technique are the formation of aggregates hardly disintegrated by shaking caused for the stress during the drying process and thermal destabilization [15].

Even though these techniques are industrially relevant and relatively easy to scale up [1, 6], problems such as instability, degradation, contamination by the solvent, and a broad particle size distribution have been associated with these processes. Therefore, particle engineering using appropriate systems that preserve the integrity of particles and offer optimal physicochemical properties for drug delivery has been required. Hence, new nanoparticles production methods have emerged for pharmaceutical applications [7, 16]. Lately, a microfluidic method has been used to direct the assembly of liposomes-poly (*N*-isopropylacrylamide) hybrid nanoparticles. As a result, this method has produced narrowly dispersed lipid-hydrogel hybrid nanoparticles for controlled release applications [17]. In addition, it has been found that this technique is able to provide highly monodisperse spherical polymeric microparticles. Nevertheless, the production of shape-controlled nonspherical microparticles is still limited. Modifications of this method such as surface acoustic wave microfluidic atomization (SAW) have been used to generate aerosols with a narrow size distribution within a size range that is optimal for inhalation therapy [18].

In addition to all these processing techniques, alternative polymeric nanoparticles production methods that involve polyelectrolyte complex formation, solvent evaporation single/double emulsion methods, emulsion polymerization, and ionotropic gelation techniques have been reported [1]. For example, in the polyelectrolyte complex formation technique, a polycation and a polyanion are dissolved and mixed to produce nanoparticles. The formation of polyelectrolyte complexes involves the ionic interactions of the ionogenic groups of the oppositely charged polyelectrolytes and generally the reaction takes place in an aqueous solution. However, precipitation and aggregation can occur when both oppositely charged groups approach to unity and low-molecular weight electrolytes are used [19, 20]. In the case of the solvent evaporation emulsion method, a polymer is dissolved in an organic solvent, followed by emulsification in an aqueous phase. Here, the polymeric solution is dispersed into nanodroplets using high-energy homogenization and a dispersing agent in a nonsolvent medium. Subsequently, the polymer precipitates as nanospheres. During a second step, the organic solvent is evaporated leading to formation of solid nanoparticles [21]. This method has been designed and used particularly for the incorporation of hydrophobic entities into biodegradable micro- and nanoparticles and it is difficult to scale up because of the high-energy requirements [14]. In the emulsion polymerization method, a monomer is added to an aqueous surfactant-containing solution, followed by in situ polymerization of the dispersed phase generating nanoparticles. Because of the requirement of surfactants, monomers, toxic solvents, and initiator, the use of this method has been reduced [1, 14]. Another method that has been widely used to develop hydrogel nanoparticles is ionotropic gelation, in which polysaccharides are dissolved in an aqueous phase and added to solutions containing counterions. The gelation process is caused by the formation of inter- and intramolecular crosslinks within a charged polymer chain, mediated by the presence of oppositely charged ions. Moreover, it has been described that the quality of those hydrogel nanoparticles can be further improved by the polyelectrolyte complexation technique [20, 22, 23].

Even when a diversity of methods have been designed for hydrogel nanoparticle production, the need for systems that efficiently manufacture particles in a simple way with suitable size and polymeric systems that ensure the production of nanoparticle with optimal properties without using toxic solvents is still required.

This study investigates a novel method of generating hydrogel microparticles using biocompatible polymers, nontoxic solvents, and the potential for continuous processing. The synthesis method includes nebulization of polymeric solutions into a gelation fluid followed by separation and drying. For this new particle generation method, we investigated the effects of various processing parameters such as polymer concentration, air pressure, type of crosslinking agent, and type of nebulizer on the size, swelling, and appearance of the resulting polymeric microparticles.

2. Materials and Methods

2.1. Materials. Chitosan (Cs) (medium MW, % N-deacetylation; about 76.4%, as determined by elemental analysis), monomethoxy-poly(ethylene glycol) (m-PEG, MW 5000 Da), succinic anhydride and 1-hydroxybenzotrizole (HOBt), sodium alginate (low viscosity, 250 cps for 2% w/v aqueous solution at 25°C), and 4-Dimethylaminopyridine (DMAP) were purchased from Aldrich (St. Louis, MO). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCL) was purchased from Fluka Chemical Corp (Milwaukee, WI). Phthalic anhydride, triethyl amine, hydrazine monohydrate, dioxane, and dimethylformamide (DMF) were obtained from Sigma-Aldrich (St. Louis, MO, SIAL). Calcium chloride anhydrous was purchased from

EMD Chemicals Inc. (Darmstadt, Germany). Ethanol of analytic grade and CCl_4 were used as received.

2.1.1. Preparation of PEG-g-Chitosan Copolymer. The synthesis of the copolymer of m-PEG grafted onto chitosan (Cs) was carried out by using a modified method previously reported by our group [24]. Briefly, Cs (10 g) was reacted with phthalic anhydride (44.8 g, 5 mol equivalent to pyranose ring) in DMF (200 mL) at 130°C under nitrogen atmosphere for 8 hours to obtain N-phthaloyl chitosan (NPHCs), which was collected after precipitation in cold water, washed with metanol, and dried under vacuum.

The m-PEG macromer was modified to obtain m-PEG-COOH by reacting with succinic anhydride. In brief, an amount of m-PEG (100 g, 20 mmol) was reacted with succinic anhydride (2.4 g, 24 mmol), DMAP (2.44 g, 20 mmol), and triethylamine (2.02 g, 20 mmol) and dissolved in dry dioxane (350 mL). Then, the mixture was stirred at room temperature under nitrogen atmosphere for 48 h and the dioxane was then evaporated under vacuum. The final mixture was taken up in CCl_4, filtered, and precipitated in diethyl ether to obtain m-PEG-COOH.

To graft m-PEG-COOH onto NPHCs, m-PEG-COOH (37.9 g) was stirred with NPHCs (5.0 g, 0.4 mol equivalent to m-PEG-COOH) in DMF containing HOBt (3.4 g, 3 mol equivalent to m-PEG-COOH) until obtaining a clear solution. EDC·HCl (4.25 g, 3 mol equivalent to m-PEG-COOH) was added and stirred at room temperature overnight. The mixture was dialyzed in distilled water and washed thoroughly with ethanol to remove impurities.

To synthesize the PEG-g-Cs copolymer, a deprotection of the masked NH_2 groups of PEG-g-NPHCs was carried out by using hydrazine monohydrate. Briefly, PEG-g-NPHCs (4.0 g) was heated to 100°C and stirred in 15 mL of DMF under nitrogen atmosphere. Then, hydrazine monohydrate (20 mL) was added and the mixture was stirred for 2 h. The final mixture was then dialyzed against a mixture of water and ethanol (1 : 1) and dried at 40°C under vacuum.

N-Phthaloyl Chitosan (NPHCs). EA $(C_8H_{13}NO_5)_{0.2363}$ $(C_6H_{11}NO_4)_{0.016}$ $(C_{14}H_{13}NO_6)_{0.747}$, Anal. Calculated (%): C, 55.74; H, 4.84; and N, 5.23, found (%): C, 60.31; H, 4.83; and N, 4.92. FT-IR (ν_{max}, cm^{-1}), 3281 (OH stretching and NH bending), 2961 (C–H stretching), 1775 and 1698 (C=O anhydride), 1395 (C=C, phthaloyl), 1058 (C–O, pyranose), and 732 (aromatic ring).

m-PEG-COOH (yield 98%). EA ($C_{23}1H_{460}O_{117}$), Anal. Calculated (%): C, 54.35 and H, 9.02, found (%): C, 56.8 and H, 9.19. FT-IR (ν_{max}, cm^{-1}) 3496 (OH stretching), 2882 (C−H stretching), 1733 (C=O of carboxylic group), and 1102 (C−O−C stretching).

PEG-g-NPHCs (5.47 g). EA, found (%): C, 56.16; H, 4.96; and N, 5.15. FT-IR (ν_{max}, cm^{-1}) 3423 (OH stretching and NH bending), 2879 (C−H stretching), 1736 (C=O ester and anhydride), 1703 (C=O anhydride), 1096 (C−O−C stretching), and 723 (aromatic ring of phthaloyl).

PEG-g-Cs. EA, found (%): C, 40.46; H, 4.71; and N, 14.44. FT-IR (ν_{max}, cm^{-1}) 3312 (OH stretching, NH bending, and intermolecular H-bonding), 2879 (C−H stretching), 1708 (C=O ester), and 1096 (C−O−C stretching).

2.2. Methods

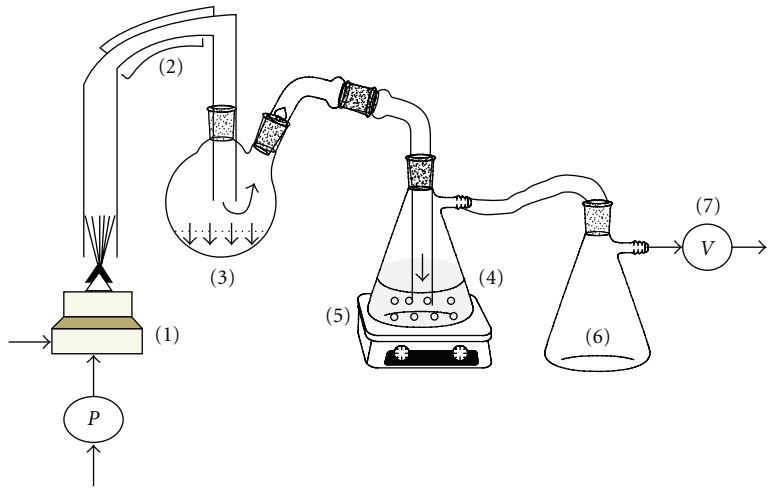
2.2.1. Spray Gelation-Based System and Preparation of the Hydrogel Particles. The hydrogel particles were developed via air-jet nebulization of small sprayed droplets of alginate solutions followed by either ionotropic gelation in aqueous Ca^{2+} solution or both ionotropic and polyelectrolyte complexation in (1 : 1) aqueous solution of PEG-g-Cs/$CaCl_2$ as crosslinkers. Briefly, an (0.1% w/v) aqueous solution of the synthesized copolymer, PEG-g-Cs was prepared using a few mLs of 0.06 M acetic acid and then made up to the predetermined volume with distilled water. Also, aqueous solutions of $CaCl_2$ (0.2 M) and sodium alginate (0.5 and 1% w/v) were prepared. Alginate solutions of different concentrations were added to different air-jet nebulizers (Pari LC Plus and Aerotech II) and aerosolized by using compressed air delivered at controlled pressures of 20 and 40 psi (Table 1). The generated sprayed droplets were then collected into the crosslinking solutions containing either $CaCl_2$ (0.2 M) or a (1 : 1) mixture of PEG-g-Cs/$CaCl_2$ under continuous mild stirring throughout a vacuum system leading to gelation of the aerosol droplets. The obtained swollen hydrogel particles were transferred to scintillation vials and freeze-dried. The resulting hydrogel powder was then washed with water to remove any residual crosslinker and then refreeze-dried. The yield (%) of the resulting dry hydrogel particles powders was calculated and then the powders were stored at room temperature in a desiccator until further investigation.

A schematic illustration of the complete spray gelation-based system developed in this study is shown in Scheme 1. The system consists of a regulated compressed air source directly connected to a nebulizer that delivers the polymeric solutions as sprayed droplets to a feed zone comprising tubing carrying droplets. This tubing carrying droplets was assembled to an aerodynamic aerosol classifier connected to a secondary vacuum system responsible of transporting the sprayed droplets to the crosslinker solutions (collecting fluids). An alternate empty glass collector was directly assembled to the primary vacuum system to recover the hydrogel microparticles dragged from the secondary vacuum system.

2.2.2. Characterization of PEG-g-Cs Graft Copolymer. The elemental analysis for all products involved in the synthesis of PEG-g-Cs graft copolymer was carried out by using a Costech ECS4010 Elemental Analyzer coupled to a Thermo-Finnigan Delta Plus Isotope Ratio Mass Spectrometer. FT-IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer. Differential Scanning Calorimetry (DSC) was used to analyze all synthesized polymers using a DSC 2920 (Modulated DSC, TA Instruments, Woodland, CA). 10 to 12 mg were weighted into aluminum pans and sealed. All measurements were performed in a range from −40 to 400°C at a heating rate of 10°C/min.

Table 1: Parameters and compositions of the hydrogel particles developed by the new spray gelation-based method.

Nebulizer	Sample code	Air pressure (psi)	Crosslinker	% Alginate (w/v)	GSD
Pari LC Plus	PN1	20	$CaCl_2$	0.5	1.2
	PN2	20	PEG-g-Cs/$CaCl_2$	0.5	1.1
	PN3	40	$CaCl_2$	0.5	1.3
	PN4	40	PEG-g-Cs/$CaCl_2$	0.5	1.1
	PN5	20	$CaCl_2$	1	1.1
	PN6	20	PEG-g-Cs/$CaCl_2$	1	1.3
	PN7	40	$CaCl_2$	1	1.3
	PN8	40	PEG-g-Cs/$CaCl_2$	1	1.2
Aerotech II	AN1	20	$CaCl_2$	0.5	1.3
	AN2	20	PEG-g-Cs/$CaCl_2$	0.5	1.2
	AN3	40	$CaCl_2$	0.5	1.1
	AN4	40	PEG-g-Cs/$CaCl_2$	0.5	1.1
	AN5	20	$CaCl_2$	1	1.1
	AN6	20	PEG-g-Cs/$CaCl_2$	1	1.4
	AN7	40	$CaCl_2$	1	1.2
	AN8	40	PEG-g-Cs/$CaCl_2$	1	2.2



(1) Nebulizer
(2) Drying system
(3) Aerodynamic aerosol classifier(s)
(4) Collecting fluid
(5) Collected nanoparticles
(6) Particle trapper
(7) Vacuum

Scheme 1: General scheme of the composition of the new spray gelation-based system used for the production of hydrogel particles.

2.2.3. Characterization of Hydrogel Particles

Particle Size Measurements. The average size of the prepared hydrogel particles suspended in ethanol was determined by dynamic light scattering, DLS (Malvern nanosizer, Malvern Instruments Ltd., Worcestershire, UK). The particle size was also estimated using microscopy techniques including light microscopy (Leica DMI6000B scope) with Leica application suite advanced fluorescence 2.2.0 build 4765 software. Particle sizes for each hydrogel formulation were recorded to be statistically analyzed to obtain the mean particle size, the standard deviation, and the geometric standard deviation (GSD).

Dynamic Swelling Study. The swelling behavior of the hydrogel particles in water was studied by determining the increase in the mean particle diameter after 2, 5, 10, 60, and 120 minutes. The measurements of the swollen hydrogel particles were recorded to estimate the swollen mean size.

Scanning Electron Microscopy (SEM). Scanning electron microscopy analysis was carried out to study the surface morphology of the developed particles by using a Zeiss Supra 40 VP scanning electron microscope. The dried particles were mounted on aluminum studs and coated with a 50/50 mixture of Au/Pd and scanned using an accelerating voltage of 10 kV.

2.2.4. Optimization of the Hydrogel Particles by a 2^k Factorial Design. A 2^k factorial design was used to determine the effect of formulation's variables such as type of nebulizer, type of crosslinker, polymeric concentration and the compressed air pressure on the hydrogel nanoparticle size, and swelling for further optimization and investigations. In addition, statistical significance was determined using ANOVA ($P < .05$) for each response variable (size and swelling). Statgraphics Plus version 5.0 software was used.

3. Results and Discussion

In the present study, a novel spray gelation-based method was developed for the production of hydrogel particles by using sprayed microdroplets from nebulizers. In addition, the effects of different processing parameters on the characteristics of the developed hydrogel particles such as size and morphology were assessed. The setup of this novel method is very simple and easy and did not require large amounts of material. It just involved the preparation of the polymeric solution to be sprayed and the crosslinking agent.

3.1. Synthesis of PEG-g-Cs Copolymer. The PEG-g-Cs copolymer synthesized through a modified method reported previously by our group was successfully obtained [12]. A 98% yield of m-PEG-COOH was obtained and characterized by FT-IR and elemental analysis (EA). In the same way, the synthesis of NPHCs was confirmed by FT-IR through the appearance of the bands at 1395 and 732 cm^{-1}, which correspond to the aromatic C=C and the aromatic ring of the phthaloyl group, respectively. The grafting of m-PEG-COOH onto NPHCs was carried out in DMF (grafting %; 9.34). Afterwards, the PEG-g-Cs copolymer was obtained by deprotection of NH_2 groups of PEG-g-NPHCs copolymer using $N_2H_4 \cdot H_2O$. The FT-IR and EA were also used to confirm the synthesis of the PEG-g-Cs copolymer. In the DSC of Cs, (data were reported elsewhere) [12], endothermic and exothermic peaks were shown at 90°C and 312°C, respectively. The former peak is attributed to the loss of moisture and the exothermic peak observed is mainly due to the decomposition of Cs. In the case of PEG-g-Cs, an endothermic peak at 55°C corresponding to the melting of the grafted PEG was observed. In addition, an endothermic peak was shown at 119°C as a consequence of the loss of water. Moreover, two exothermic events appeared at 261 and 310°C and were related to the crystallization and decomposition of the graft copolymer, respectively [12].

3.2. Preparation of Hydrogel Particles. Different concentrations of alginate solutions were used to prepare hydrogel particles through a spray gelation process for drug delivery applications. As shown in Table 1, several hydrogel particle formulations were obtained based on the combination of parameter such as nebulizers, crosslinkers, and applied air pressure. In addition, the geometric standard deviation (GSD) of the different formulations was listed.

Since nebulizers have been therapeutically used to convert liquids into aerosols and it has been proven that they can produce fine droplets (~1 μm) [1], we proposed that nebulizers may be appropriate for delivering the alginate solutions, as example for other polymer solutions, into small droplets under the principles of jet nebulization as a new alternative method for production of hydrogel particles. Alginate solutions of different concentrations were added to the nebulizer and the driving force for the atomization of the alginate droplets was generated by the high-velocity air that passes through a small nozzle within the device and at the same time draws fluid to be nebulized via the venturi effect [25]. Then, after the sprayed anionic polyelectrolyte alginate solutions were passed directly into an aqueous solution containing divalent cations such as Ca^{2+} or polycation such as PEG-g-Cs copolymer. Following this rapid reaction, insoluble network structures were formed and gelation was induced because of the diffusion of cations into the alginate droplets or by the formation of polyelectrolyte complexes, forming three-dimensional lattice of ionically crosslinked polymer resulting in the formation of hydrogel particles, which were later dried by freeze-drying. After the final powder was collected and weighed, the spray gelation yield was calculated resulting in a range between 30% and 90%.

Aerotech II and Pari LC Plus nebulizers were chosen in this study because of the several literature reports [26–28], in addition to our prestudy screening experiments using different types of nebulizers. Also, Aerotech II and Pari LC Plus were found to have greater aerosol output efficiencies, produce droplets with small sizes (<5 μm), and show higher efficiency in the aerosolization of viscous formulations. Also, it has been reported [25] that the main determinants of a droplet size produced by a nebulizer include the characteristics of the solutions such as viscosity and the velocity of the gas. Thus, the influence of polymer concentration, air pressure, and crosslinker type on droplet sizes was studied in these investigations.

3.3. Particle Size Analysis. Figure 1 shows the influence of the formulation's parameters on the mean size of the hydrogel particles. The mean size of the particles developed using the Pari LC Plus and Aerotech II nebulizers was found to be 842 and 886 nm, respectively, with a standard deviation of 7%–12%. The particle size of the obtained hydrogels was also confirmed by DLS technique where, according to the data, Pari LC Plus and Aerotech II showed very similar sizes (around 858 nm and 889 nm, resp.).

To verify the existence of significant differences between both sets of particle size data upon using different nebulizers (Pari LC Plus or Aerotech II), statistical analysis was carried out. The obtained results showed minor statistical differences ($P = .0445$) (Table 2). These minor statistical differences can be attributed to practical differences. As shown in Figure 1(a), Pari LC Plus nebulizer generated hydrogel particles with hardly comparable sizes (PN1 = PN5, PN4 > PN8, PN6 > PN2) of particles with different alginate concentrations, even when it seemed that 0.5% produced smaller particle size. The data also showed that the air pressure is a determinant factor on the droplet size because upon applying higher pressure (40 psi), all formulations

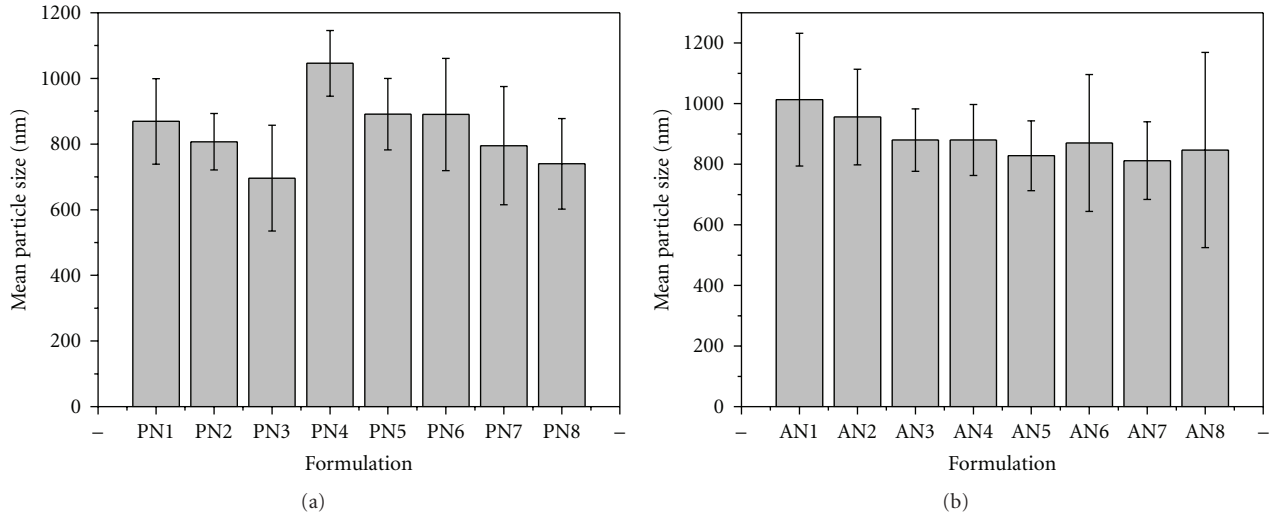


Figure 1: Mean particle size (diameter, d, nm) of the dried hydrogel particles produced by spray gelation-based method using the (a) Pari LC Plus and (b) Aerotech II nebulizers.

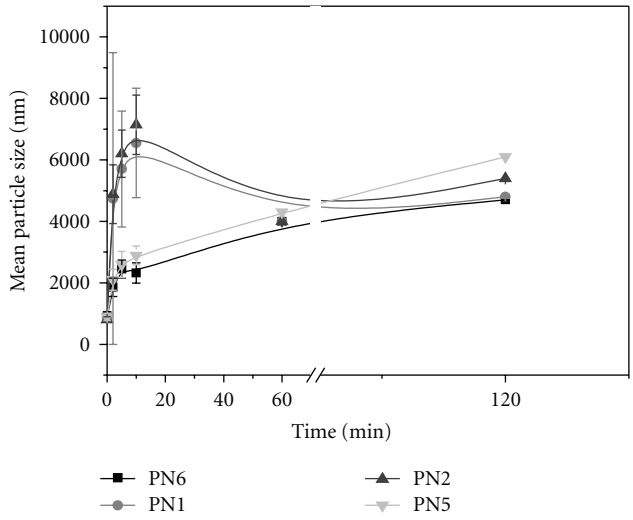


Figure 2: Swelling behavior of hydrogel particles based on the concentration of alginate solutions (PN1 and PN2, 0.5% and PN5 and PN6, 1%) after 2, 5, 10, 60, and 120 minutes.

(PN3, PN7, and PN8) showed the smallest particles size, except for PN4. The influence of the type of crosslinker on the particle size was not clearly seen.

On the other hand, in comparison with Pari LC Plus, Aerotech II formulations showed very slightly larger particle sizes and as mentioned before, we attributed this increase to practical differences (Figure 1(b)). Nevertheless, they presented more uniform sizes. This effect may be related to the presence of a baffle that covers the aerosol stream region preventing the formation of larger particles due to the centered pressure applied during atomization into a constant direction. In the case of Aerotech II formulations, relatively smaller hydrogel particles were produced when 1% alginate solutions were used, particularly this effect could be seen in AN7 and AN5 formulations. This was probably because a higher concentration of polymer shows a larger capacity of being crosslinked leading to the formation of smaller size. In addition, it appears that the type of the crosslinkers used in this study has no significant effect onto the size of the developed particles (see Table 2).

3.4. Swelling Equilibrium Study. The swelling profiles of the hydrogel particles in water are shown in Figure 2. The effect of alginate concentrations and type of crosslinker on the swelling behavior of the formulations developed using Pari LC Plus (PN1, PN2, PN5, and PN6) was investigated. The swelling extents were estimated by determination of the increase in the mean diameter (nm) of hydrogel particles after 2, 5, 10, 60, and 120 minutes by microscopy. As seen in Figure 2, the formulations containing 0.5% alginate (PN1 and PN2) showed a faster initial swelling after 2 min in comparison with the formulations of 1% alginate, increasing their size more than four times. For example, the size of PN1 and PN2 increased from 869 and 807 nm when dried to 4,647 and 4,884 nm after 2 min, respectively. A continuous increase in the mean diameter was observed after 10 min, where the hydrogel particles reached values of 6,551 and 7,144 nm, respectively. From the figure, it can be noted that increasing the alginate concentration to 1% has reduced the swelling values of the hydrogel particles. These data are consistent with the crosslinking extent, where as the alginate concentration increases, the interaction of alginate with the divalent cations is enhanced, leading to the formation of a smaller mesh size in the meshwork that limits the access of water. In this way, PN5 and PN6 formulations showed smaller sizes when swollen. In the effect of type of crosslinker onto swelling, it was observed that, with keeping the alginate concentration constant at 0.5%, both $CaCl_2$ and the PEG-g-Cs/$CaCl_2$ mixture showed a similar effect on the swelling of the hydrogel particles. The same phenomenon was observed in the 1% alginate formulations. These data were confirmed

Table 2: Summary of the analysis of variance for mean particle size.

Factor	Sum of squares	DF	Mean square	F-ratio	*P-value
Nebulizer	23232.0	1	23232.0	4.34	.0445
Crosslinker	11907.0	1	11907.0	2.23	.1446
Alginate	42126.8	1	42126.8	7.88	.0081
Air Pressure	34347.0	1	34347.0	6.42	.0159

*$P < .05$, DF: Degree of Freedom.

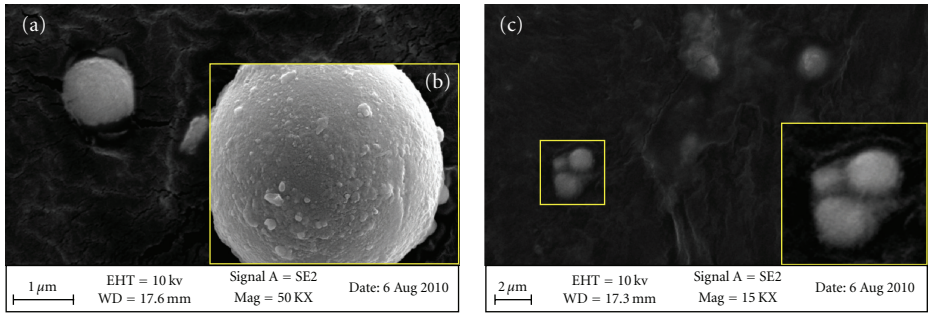


Figure 3: Scanning electron micrographs of alginate particles produced using the Aerotech II nebulizer using spray gelation: (a, b) AN7 and (c) AN8.

by the P-value obtained by the analysis of variance, which showed a nonsignificant difference between both crosslinkers (Table 2). After 60 min, a continuous increment in the swelling behavior of formulations containing 1% alginate was also seen. However, from the figure, it can be noted also that some of the hydrogel formulations (with 0.5% alginate such as PN1 and PN2) showed a swelling decrease at equilibrium. Generally, the fast initial swelling of hydrogel formulations is attributed to their hydrophilic nature (osmotic driving forces). This is followed by occurrence of a resistance due to the cohesive forces exerted by the crosslinked polymer chains. These cohesive forces tend to resist further hydrogel expansion and encourage some of swelling fluid to be expelled which explains the swelling decrease stage [29]. The extent of these two opposing forces (osmotic and cohesive) depends mainly on the crosslinking magnitude of hydrogels, and generally, the equilibrium is reached when a balance is achieved between these two forces.

3.5. Scanning Electronic Microscopy. It is well known that the final particle morphology (hollow and solid particles) and the size of the sprayed or aerosol particles are dependent on the solution characteristics, precursor concentration, viscosity, droplet size, evaporation rate, and operating parameters such aerosol generator, flow rate, and drying technique [30, 31]. Hence, in this study we investigated the effect of different concentrations, two aerosol generators (nebulizers), and different air pressures on the appearance of the hydrogel particles. Figure 3 shows some scanning electron micrographs of the morphology of hydrogel particles generated by the spray gelation method. The developed hydrogel particles presented, in general, spherical shapes with highly rough surfaces, especially in Pari LC Plus formulations, where 1% alginate solutions and 40 psi air pressure were applied (Figure 3(b)). Furthermore, Aerotech II formulations also presented spherical shapes under the same conditions (Figure 3(c)). It seems that the concentration of both polymer and crosslinker used played an important role in the formation of spherical, solid, and well-crosslinked particles; however, the type of nebulizer did not significantly change the morphology of the particles. This may be attributed to the fact that both nebulizers operated under the same mechanism. Further investigation of the drying mechanism along with some other parameters has to be carried out to determine their effects on the hydrogel morphology.

3.6. Analysis of the Hydrogel Particles Synthesis by a 2^k Factorial Design. In this study, a discontinuous 2^k factorial design was carried out to determine the effects of nebulizer, crosslinker, air pressure, and alginate concentration and their interaction on the obtained hydrogel formulations. Considering that the combination of those factors would modify the properties of the particles, variations of each factor in two levels were analyzed. The two levels of each factor were coded with a low and high level to be analyzed by response surface methodology and Pareto charts. In this design, the particle size and equilibrium swelling values were used as response variables. Since type of nebulizer and crosslinker are not numeric values, -1 and 1 levels were assigned for each nebulizer and crosslinker.

The analysis of variance for particle size obtained by Statgraphics 5.0 shows the most influential factors in the size and swelling behavior of the hydrogel particles at the 95% confidence level. Then, all those factors and/or interactions with a $P < .05$ value were considered the most relevant factor for either particle size or swelling. Thus, the obtained results showed that four effects are influencing the particle size. Those effects and their interactions can be clearly

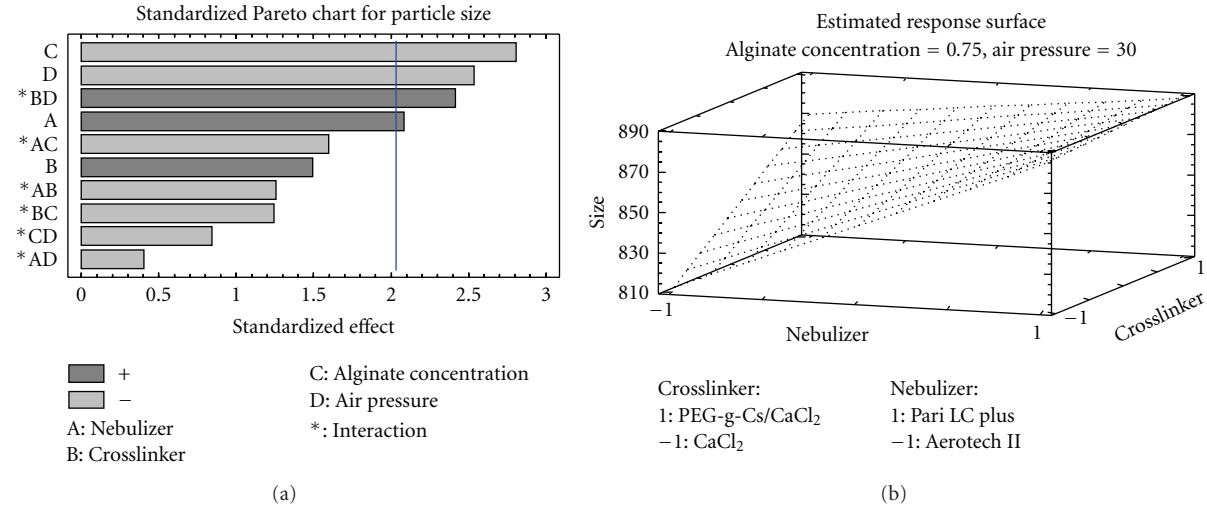


Figure 4: Statistical estimation of the effect of formulation's parameters and their interactions on the particle size of hydrogel particles represented by (a) Pareto chart and (b) response surface methodology.

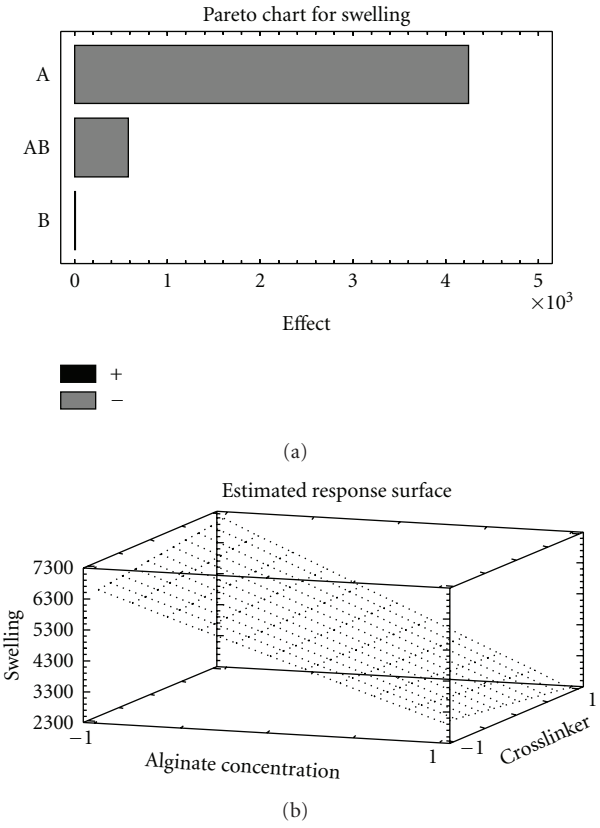


Figure 5: (a) Pareto chart and (b) response surface methodology graph of the effect of alginate concentration and crosslinker on the swelling behavior of hydrogel particles.

seen in the standardized Pareto chart (Figure 4(a)), where it is showed that alginate concentration, air pressure, the interaction between crosslinker and air pressure, and the type of nebulizer are the main factors that modify the size of the hydrogel particles. The order in which they appear corresponds to their importance in affecting the particle size. From the figure, it seems that the alginate concentration is the principal determinant of the size of particles, followed by the air pressure. Therefore, modification of any of these two factors would have a more relevant effect on the particle size than the other factors. We also used surface response methodology to illustrate the way in which particle size would respond to variations in the parameter used during the production of particles (Figure 4(b)). The results showed that when the alginate concentration and air pressure are maintained in their intermediate values, by using Pari LC Plus nebulizer, smaller particle sizes can be obtained. The effect of the type of crosslinker is considered not significantly different. This effect can be clearly observed by the pronounced slope that gives rises to the minimum peak on the presented surface. Therefore, the data shown here are consistent with the particle size data found experimentally, where Pari LC produced relatively smaller particle sizes than Aerotech II.

In the case of the swelling behavior, the crosslinker and alginate concentration were the factors modified in this study for Pari LC Plus formulations and the results in Pareto chart (Figure 5(a)) showed that alginate concentration is a fundamental factor in the processing of the particles and any modification in its value would lead to an increase or reduction in the swelling values. According to the surface response methodology graph, particles with 0.5% alginate would reach a larger size when swollen than the formulations with 1% alginate (Figure 5(b)). These data are very consistent with our experimental results, as seen in Figure 2. Also, the interaction between both factors plays an important function, showing dependence on the alginate concentration used. However, the type of the crosslinker by itself does not have an effect on the swelling behavior of particles (Figure 5).

4. Conclusion

It has been demonstrated that the spray gelation-based system described in this study can be used as an alternative method for the preparation of hydrogel particles with well-defined shapes and sizes around 840 nm. Moreover, the obtained results indicated that the size of the hydrogel particles can be optimized by changing the polymer concentration and the applied air pressure. In addition, the developed method offers many advantages over other methods such relative simplicity, low cost, and ease of collecting fluid and it does not involve the use of toxic solvents. All these advantages make this new technique valuable for the preparation of hydrogel particles for various applications particularly for drug delivery purposes. However, as a novel method, additional effort has to be done for the investigation and optimization of the particle size and aerosolization.

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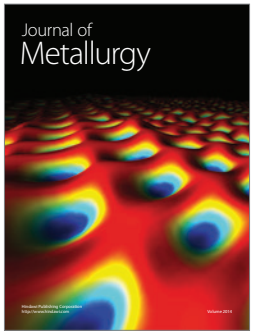

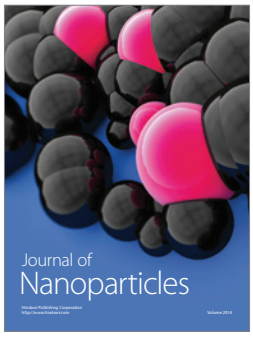

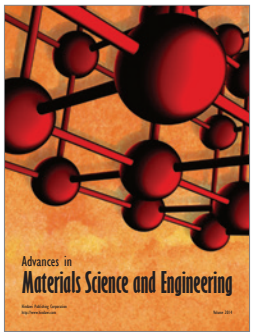

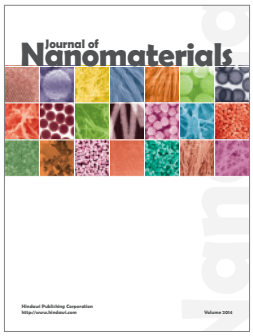

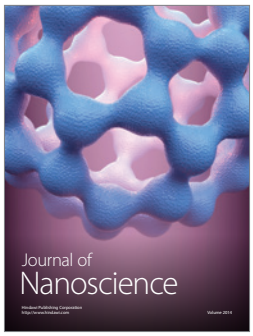

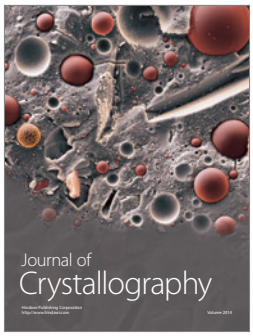

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