

# Effects of Different Parameters on the Characteristics of Chitosan–Poly(acrylic acid) Nanoparticles Obtained by the Method of Coacervation

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Received 23 April 2008; accepted 23 July 2008

DOI 10.1002/app.29231

Published online 21 November 2008 in Wiley InterScience (www.interscience.wiley.com).

**ABSTRACT:** Chitosan–poly(acrylic acid) polyelectrolyte complex nanoparticles were prepared by coacervation under mild experimental conditions without the use of any organic solvents or surfactants. The influence of some experimental parameters such as the pH of the polyelectrolyte solutions, their concentrations, and the purification procedure on the particle dimensions and their size distribution was studied in detail. The physicochemical properties of the obtained complex were characterized with Fourier transform infrared spectroscopy, transmission electron microscopy, scanning electron microscopy, and dynamic light scattering. It was found that for solution concentrations below 0.1 wt %, it was possible to obtain suspensions of nanometer-sized particles. Furthermore, it was established that the pH values of the reactant solu-

tions had a great influence on both the particle size and the yield of the complex that was formed. The most convenient pH values for obtaining chitosan–poly(acrylic acid) particles with a nanometric size and optimum yield (near 90%) were found to be 4.5–5.5 for chitosan and 3.2 for poly(acrylic acid). Additionally, the effects of dialysis and ultrasonic treatment on the stability of complex suspensions, prepared under different experimental conditions, were clarified so that recommendations could be made to bring this system into practical use. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 111: 2362–2371, 2009

**Key words:** biodegradable; biological applications of polymers; particle size distribution; polyelectrolytes; polysaccharides

## INTRODUCTION

Polyelectrolyte complexes (PECs) are formed by the interaction between macromolecular polyacids and polybases or their salts and are stabilized by ionic bonds.<sup>1</sup> PECs are very interesting materials for different applications because their properties (swelling, permeability, and others) can be modified by external stimuli, such as the pH of the medium.

Particular interest is currently found in the preparation of PEC particles with biocompatible polyelectrolytes in aqueous media to be used for the transport of biological macromolecules and other therapeutic compounds throughout living organisms. In this connection, the advantages of nanoparticles over microparticles for many *in vivo* applications have been stressed. Because of their minute size, nanoparticles exhibit a number of distinct advantages over microparticles, including higher

intracellular uptake. In terms of intestinal uptake, apart from their particle size, the nature and charge properties of nanoparticles seem to influence their uptake by intestinal epithelia.<sup>2</sup> For this reason, the use of nanoparticles for the delivery of substances through intravenous, oral, and mucosal routes is currently receiving a great deal of attention.

A simple method of preparing nanoparticles involves the formation of PECs by complex coacervation, that is, by the mixing of solutions of oppositely charged polyelectrolytes under specific conditions. It can be seen in the literature that a lot of research has been carried out on PECs with chitosan (CHI) as the polycation and poly(acrylic acid) (PAA),<sup>3,4</sup> dextran sulfate,<sup>5</sup> alginate,<sup>6</sup> and tripolyphosphate<sup>7</sup> as polyanions for the preparation of PEC membranes, gels, capsules, and microparticles or nanoparticles.<sup>8–11</sup>

CHI is a natural polymer composed of units of glucosamine and *N*-acetylglucosamine. It is a biocompatible, biodegradable, and nontoxic polymer frequently proposed for applications in pharmaceutical and biomedical fields.

The formation of the complex between CHI and PAA has been widely studied, and a considerable amount of literature has been published on this

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Contract grant sponsor: Instituto de Salud Carlos III; contract grant number: PI050385.

theme.<sup>12</sup> Also, work has been carried out on the preparation of CHI-PAA PEC nanoparticles by coacervation;<sup>13,14</sup> the authors studied the effects of various parameters, such as the ratio of the initial polyelectrolyte concentrations, temperature, and incubation time, on the morphology of the resulting nanoparticles. However, the influence of factors such as the pH of the polyelectrolyte solutions, their concentrations, and the purification procedure on the particle dimensions of the resulting PEC is not yet clear. Therefore, in this work, a detailed study was carried out to assess the influence of these experimental parameters on the particle size and particle size distribution of the CHI-PAA PEC.

## EXPERIMENTAL

### Materials

CHI was purchased from Aldrich (Sigma-Aldrich, Madrid, Spain) and used as supplied. The degree of deacetylation, determined by potentiometric titration,<sup>15</sup> was 79.7%. The average molecular weight was  $1.13 \times 10^5$  Da, as determined by viscometry with an Ubbelohde viscometer.<sup>16</sup>

PAA (weight-average molecular weight =  $4.5 \times 10^5$  Da) was purchased from Aldrich and used as supplied. All other reagents were analytical-grade and were used without further purification.

### Preparation of the CHI-PAA nanoparticles

CHI-PAA particles were prepared by a dropping method through the mixing of positively charged CHI with negatively charged PAA. A certain volume (determined by the ratio of primary amino groups in CHI to carboxylic groups in PAA) of an aqueous solution of PAA of a determined concentration was added dropwise and with high-speed magnetic stirring (ca. 1300 rpm) into the corresponding volume of an aqueous solution of CHI [dissolved in a 1% (w/v) acetic acid solution]. The ratio of primary amino groups (in CHI) to carboxylic groups (in PAA) was fixed at 1.25. The resulting suspension was kept in a glass vessel. Some of these suspensions were put in dialysis membrane bags (Visking 20/32 dialysis tubing with a molecular exclusion size of 10,000 Da; Boehringer Ingelheim, Heiderberg, Germany) placed in distilled water, the pH value of which was adjusted with acetic acid to 4.5. This procedure continued with magnetic stirring for 24 or 48 h to remove both excess CHI and some of the low-molecular-weight inorganic substances used to adjust the pH of the reaction solutions.

pH measurements of the polymer solutions and resulting suspensions were carried out with a Metrohm (Herisau, Switzerland) 654-pH-meter at 25°C.

For the ultrasonic treatment of CHI-PAA suspensions, an ultrasonic bath (Transsonic T 460, Elma, Germany) was employed.

The yield of CHI-PAA complex formation was determined gravimetrically. The resulting particles were separated from the aqueous phase by ultracentrifugation (Optima L 100 XP, Beckman Coulter, Fullerton, CA, USA) at 41,000 rpm at 5°C for 40 min. The sediment particles were freeze-dried (-110°C and 24 h) and weighed. To calculate the yield of the complex, it was assumed that the composition of the particles in terms of the CHI/PAA ratio was 1 : 1.

### Characterization

The morphology of these particles in suspension was determined by transmission electron microscopy (TEM; H-7100 EM, Hitachi; Tokyo, Japan). The samples were placed on a nitrocellulose-covered copper grid at room temperature without staining.

The particle distribution profile and mean diameter of the CHI-PAA complex in suspension were determined by a light scattering (LS) method with an LS 32 particle size analyzer (Beckman Coulter). All LS measurements were performed in distilled water with pH 4.5 (adjusted with hydrochloric acid) at 25°C with an angle detection of 90°. Each sample was measured three times, and the reported values were the averages of these measurements.

Scanning electron microscopy (SEM) was used to ascertain the morphology of lyophilized CHI-PAA particles obtained by separation from the aqueous phase by ultracentrifugation (Optima L 100 XP ultracentrifuge, Beckman Coulter; 41,000 rpm at 5°C for 40 min) and freeze drying (-110°C, 20 h). SEM analysis was performed with an S-4700 field emission scanning electron microscope (Hitachi, Tokyo, Japan).

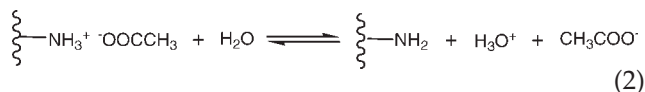
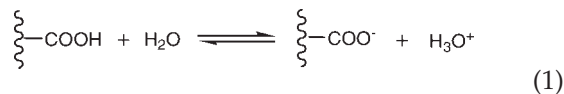
Fourier transform infrared (FTIR) studies were performed to confirm the presence of complex formation between CHI and PAA. FTIR spectra were measured with a PerkinElmer FTIR spectrophotometer (Madrid, Spain) provided with an attenuated total reflectance sampling attachment. Spectra were run at a resolution of  $4 \text{ cm}^{-1}$ , and the average of four scans was taken.

## RESULTS AND DISCUSSION

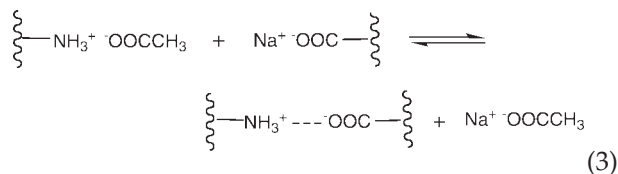
### Effect of the polymer concentration on the particle size distribution

The two polymers involved in the formation of the interpolymeric complex CHI-PAA are both weak polyelectrolytes, whose dissociation equilibria in

water can be represented by reaction (1) for PAA and reaction (2) for chitosan acetate:

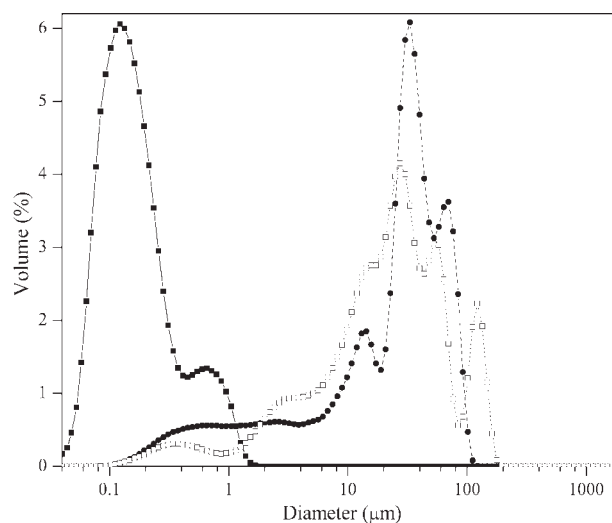


The complex formation between chitosan acetate and sodium polyacrylate can be expressed as follows:



The CHI-PAA complex is obtained spontaneously in the form of a suspension when the reactants are mixed at appropriate concentrations.<sup>13</sup> Thus, nanometric particles are obtained when the concentrations of both polyelectrolytes are fixed at 0.02<sup>13</sup> or 0.1 wt %.<sup>14</sup> The obtained nanometric particles are unstable and tend to aggregate. Chen et al.<sup>14</sup> concluded that the best results are obtained when the molar ratio of the polymers in the reaction mixture is  $n(\text{CHI}/\text{PAA}) = 1.25$  because excess CHI provides the particles with a net positive charge, thus reducing their tendency to aggregate and consequently increasing the stability of the CHI-PAA complex suspensions. The effect of the polymer concentration on the size of nanoparticles has previously been reported. For instance, for the CHI-poly(ethylene oxide) system, it has been reported that an increase in the CHI or poly(ethylene oxide) solution concentration increases the size of the CHI-poly(ethylene oxide) nanoparticles formed.<sup>17</sup> However, the upper limiting concentration of the polyelectrolyte solutions, which would lead to the formation of complex nanoparticles, has not been reported. This is very important from a practical point of view because the higher the allowed concentration is of the reactants, the smaller the volume is of the solutions to be handled to obtain a reasonable amount of nanometric particles.

With this in mind, experiments were carried out to obtain the complex by the dropping method (see the Experimental section) through the variation of the concentration of each reactant from 1 to 0.1 wt %. Specifically 1, 0.5, 0.2, 0.15, and 0.1 wt % solutions of each polyelectrolyte were tested at pH values around 3.5 and 3.2 for the CHI and PAA solutions, respectively. It was observed in these experiments that at concentrations of 1 and 0.5 wt

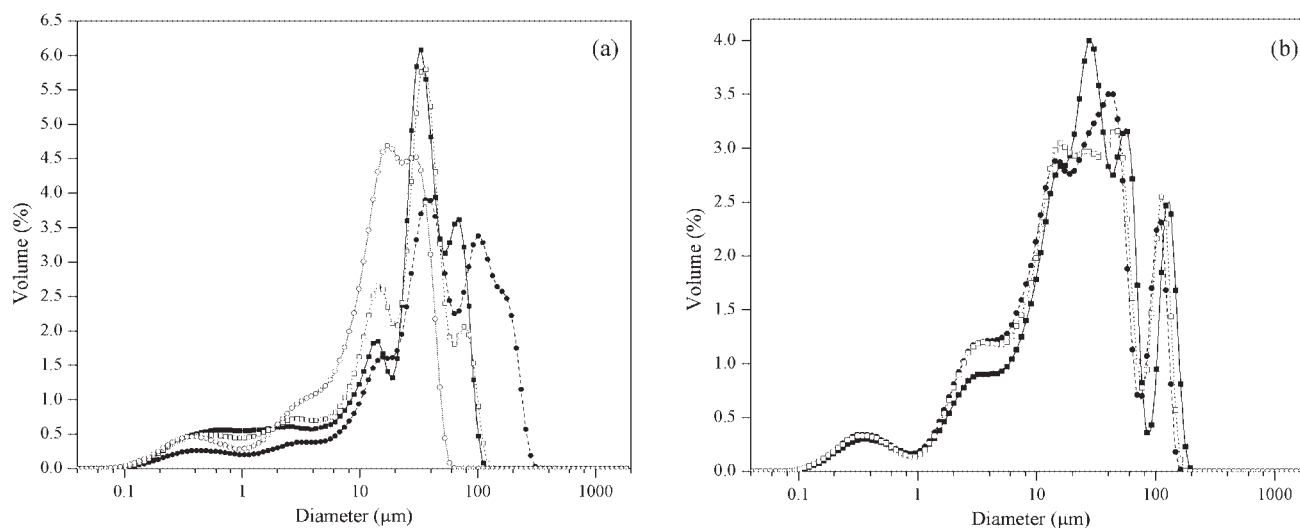


**Figure 1** Influence of the polymer concentration (CHI and PAA) on the particle size distribution: (■) 0.1, (●) 0.15, and (□) 0.2%.

%, the complex precipitated in the form of strands and clumps, rather than as a particle suspension. At concentrations of 0.2 and 0.15 wt %, a suspension and a precipitate were both formed. However, the experiment with 0.1 wt % solutions gave an apparently stable suspension without the formation of a precipitate or agglomerates. The particle size distributions of the supernatant solutions from the experiments carried out with the initial polyelectrolyte concentrations of 0.15 and 0.2 wt % and of the suspension obtained with the initial reactant concentrations of 0.1 wt % are shown in Figure 1. It can be seen that in the experiments with 0.15 and 0.2 wt % solutions, the particles in the supernatant showed a multimodal distribution, with approximately 90% of the particles having sizes between 1 and 100  $\mu\text{m}$ . However, with the 0.1 wt % solutions, the complex was obtained mainly (98%) in the form of nanometric particles with diameters ranging from 40 nm to 1  $\mu\text{m}$ .

Sonication has been used in several nanoparticle preparation procedures to promote the formation of smaller particles.<sup>18,19</sup> In our study, the CHI-PAA complex particles prepared at polyelectrolyte concentrations of 0.15 and 0.2 wt % were subjected to ultrasonic treatment to find out if in this way the particle sizes could be reduced to nanometric dimensions. To this end, successive 10-min treatments were applied to the complexes obtained at these concentrations. It has already been mentioned that the supernatant liquid of this experiment contained 90% of the particles between 1 and 100  $\mu\text{m}$  in size (see Fig. 1).

After the particles (0.15 wt %) were treated by ultrasound for 10 min, contrary to expectations, their diameters increased to 350  $\mu\text{m}$  [see Fig. 2(a)]. This apparently contradictory result may be attributed to the fact that during the ultrasonic treatment, some of



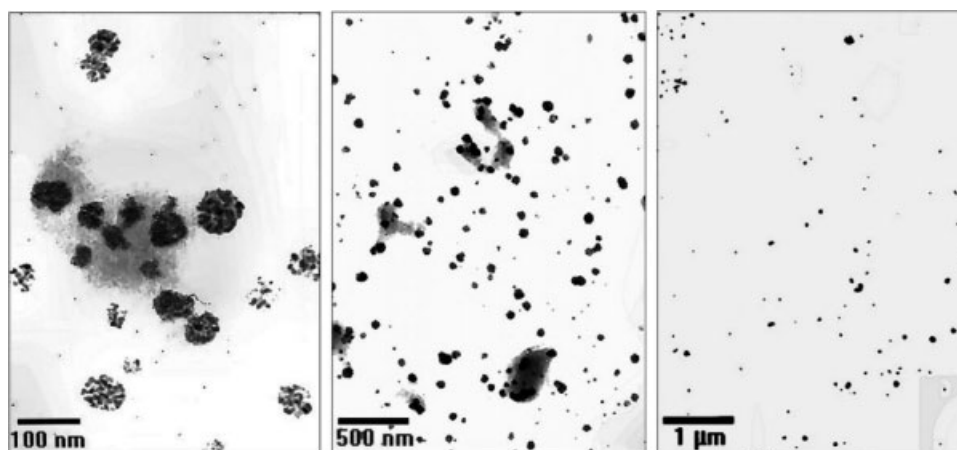
**Figure 2** Influence of ultrasonic treatment on particle distribution of CHI-PAA suspensions obtained initial at concentrations of (a) 0.15 and (b) 0.2%: (■) 0, (●) 10, (□) 20, and (○) 30 min.

the previously settled particles became dislodged and moved to form part of the supernatant suspension, thus increasing not only the proportion of micrometric particles but also their average size. To verify this hypothesis, this new suspension was treated ultrasonically for 10 min more, at which point it was clearly seen that the process of dislodging the particles increased the fraction of lesser size with the consequent disappearance of those particles whose diameters ranged from 100 to 350  $\mu\text{m}$ . When the ultrasonic treatment time was increased to 30 min, it was possible to further reduce the size of the micrometric particles down to a maximum value of approximately 60  $\mu\text{m}$ . This value remained virtually invariable even after a further 20-min treatment.

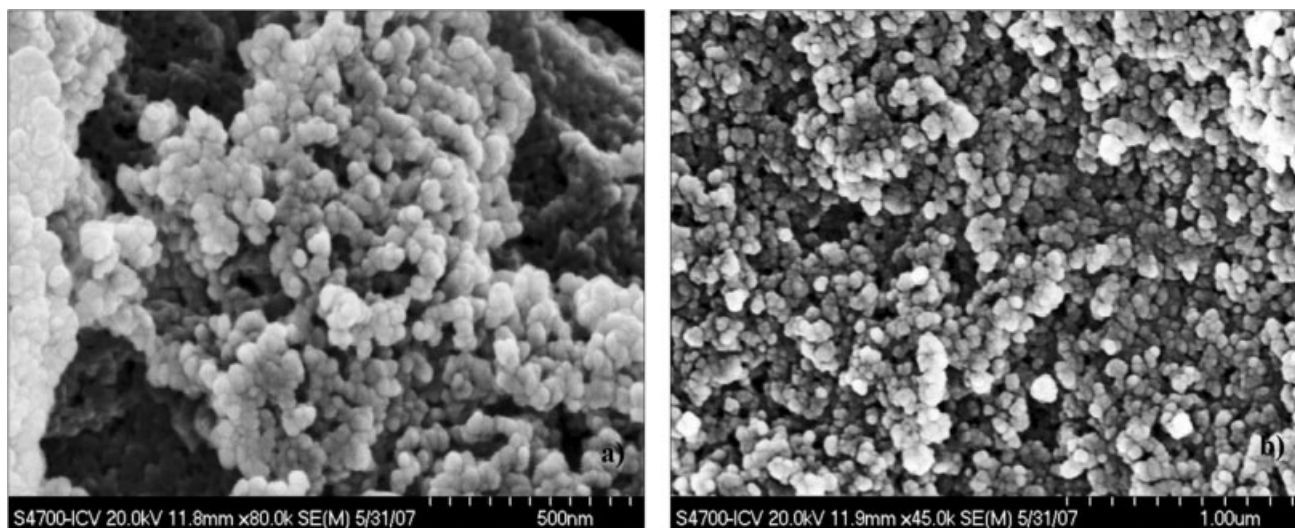
Ultrasound was also applied for the same periods of time to the supernatant liquid obtained from the initial 0.2 wt % solutions. This treatment was found

not to substantially modify the size distribution of the particles [Fig. 2(b)], and this indicates that sizes between 100 nm and 200  $\mu\text{m}$  (>85%) are not a product of aggregation of the particles but a result of the degree of initial dispersion of PAA in the CHI solution. When higher reactant concentrations were used, both solutions (CHI and PAA) became more viscous, and this restricted the dispersion of PAA in CHI and as a result caused the increase in the size of the resulting complex particle. Even excess CHI, which should provide a positive charge on the particle surface and contribute in this way to the stability of the particle formed, could not prevent clumping together or precipitation of the micrometer-sized particles (formed at concentrations of 0.15 wt % and higher).

Confirming this is the fact that as a result of the ultrasonic treatment, it was not possible to



**Figure 3** TEM microphotographs of CHI-PAA nanoparticle suspensions obtained from 0.1% solutions.



**Figure 4** SEM microphotographs of lyophilized CHI-PAA nanoparticles obtained from 0.1% solutions of CHI and PAA: (a) without dialysis of the suspension and (b) after dialysis (24 h) of the CHI-PAA suspension in an aqueous solution with pH 4.5.

significantly increase the fraction of particles of nanometric size [Fig. 2(a,b)]. This means that this procedure is of little use for obtaining CHI-PAA complex nanoparticles from polyelectrolyte solutions with concentrations equal to or greater than 0.15 wt %.

From these results, it is evident that only at polyelectrolyte concentrations below 0.1 wt % is it possible to obtain CHI-PAA nanometric particles.

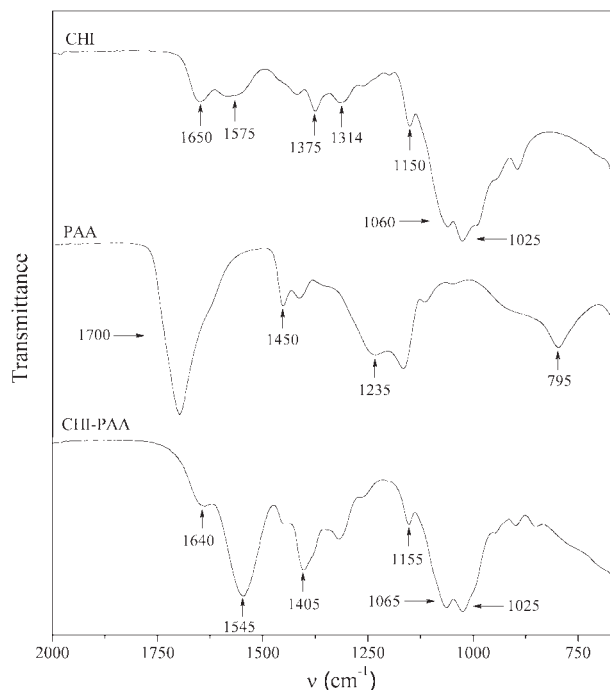
### Characterization

The morphology of particles in suspension obtained from 0.1 wt % solutions of CHI and PAA was determined by TEM. Figure 3 shows the TEM photographs of suspensions of CHI-PAA complexes at various magnifications, in which the homogeneous distribution of the spherically shaped nanometric particles can be observed.

The particles were separated from the aqueous phase by ultracentrifugation and freeze drying (see the Experimental section) and analyzed by SEM to ascertain their morphology. Figure 4 shows the SEM photographs of CHI-PAA lyophilized particles (1) obtained directly from the corresponding suspension and (2) obtained after dialysis of the suspension for 24 h in dialysis membrane bags placed in distilled water with the pH adjusted to 4.5 with acetic acid. The majority of the particles have an almost spherical shape with a quite uniform size distribution. This is in agreement with the size measurements obtained with the LS particle size analyzer. No substantial differences between the dialyzed and non-dialyzed particles can be observed.

### FTIR analysis

The IR spectra of CHI, PAA, and the PEC (CHI-PAA) are shown in Figure 5. The spectrum of CHI exhibits the distinctive absorption bands at 1650 (amide I), 1575 ( $\text{-NH}_2$  bending), and 1314  $\text{cm}^{-1}$  (amide III). The absorption bands at 1150 (antisymmetric stretching of the C—O—C bridge) and 1060 and 1025  $\text{cm}^{-1}$  (skeletal vibrations involving the C—O stretching) are characteristic of its saccharide



**Figure 5** FTIR spectra of CHI, PAA, and CHI-PAA nanoparticles obtained from 0.1% solutions of CHI and PAA.

TABLE I  
Dependence of CHI-PAA Complex Formation on pH

Experiment	pH		Resulting suspension	Mean diameter ( $\mu\text{m}$ )	<1- $\mu\text{m}$ -particle content (%)	Yield (%)
	CHI	PAA				
1	5.53	5.4	5.51	$44.33 \pm 0.03$	5.1	115.7
2	5.53	3.2	5.45	$0.49 \pm 0.01$	87.4	107.4
3	4.50	3.2	4.48	$0.477 \pm 0.008$	90.1	97.5
4	2.87	5.4	2.98	$0.138 \pm 0.003$	100	43.6
5	2.87	3.2	2.90	$0.149 \pm 0.005$	100	41.2

The starting concentration of both polyelectrolytes (CHI and PAA) was 0.1 wt %.

structure.<sup>20</sup> The IR spectrum of PAA exhibits the characteristic absorption band at  $1700\text{ cm}^{-1}$  due to the C=O stretching vibration of the carboxylic groups.

The absorption band at  $1700\text{ cm}^{-1}$  is absent in the CHI-PAA spectrum. Instead three new bands can be observed at 1640, 1545, and  $1405\text{ cm}^{-1}$ . The bands at 1553 (antisymmetric  $\text{COO}^-$  stretching) and  $1405\text{ cm}^{-1}$  (symmetric  $\text{COO}^-$  stretching) present in the spectrum of CHI-PAA, together with the small band at  $1640\text{ cm}^{-1}$  (attributed to the formation of  $\text{NH}_3^+$ ), are indicative of the formation of a complex between the amino groups of CHI and the carboxylic groups of PAA.<sup>21,22</sup>

### Influence of pH

It has been explained previously that the interpolyelectrolyte complex CHI-PAA is formed by the interaction between positively charged CHI and negatively charged PAA. The fact that CHI is a weak polybase and PAA is a weak polyacid suggests that their interaction must be strongly influenced by the degree of dissociation of both polymers in an aqueous solution, which, in turn, is dependent on the pH. In fact, it has been stated that the complex composition ( $Z$ ) can be expressed in terms of the dissociation degrees of the polyelectrolytes by the following relationship:

$$Z = \frac{[\text{Polycation}]}{[\text{Polyanion}]} = \frac{\alpha}{\alpha'}$$

where  $\alpha$  and  $\alpha'$  are the dissociation degrees of the polyanion and the polycation, respectively.<sup>1</sup>

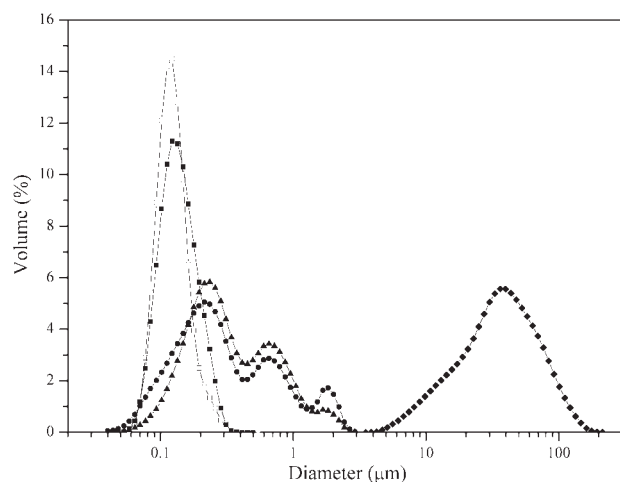
The  $\text{p}K_a$  values of CHI and PAA are 6.5 and 4.8, respectively,<sup>23</sup> so that under acidic conditions, when the pH is less than 4, most carboxylic groups of PAA are in the nondissociated form ( $-\text{COOH}$ ), whereas the majority of amine groups of CHI are protonated ( $-\text{NH}_3^+$ ). At pH values from 4.5 to 5.9, both polyelectrolytes (CHI and PAA) are partly ionized, and this should render the formation of the

complex by electrostatic interaction more favorable. When pH values increase to 6 or more, almost all of the amine groups in CHI are in the  $\text{NH}_2$  form, and CHI becomes insoluble. This circumstance led us to carry out the formation of the CHI-PAA complex under different pH values of the corresponding polyelectrolyte solutions (CHI and PAA) to assess the influence of this parameter on the particle size distribution and yield of the complex formed. For this study, a concentration of 0.1 wt % of both polymers was selected on the basis of the results of the dependence of the initial polymer concentration on nanoparticle formation.

The pH values of 0.1 wt % aqueous solutions of CHI [in 1% (w/v) acetic acid solution] and PAA (in distilled water) were 2.87 and 3.2, respectively. Because at this pH most of the carboxylic groups in PAA are in the nondissociated form ( $-\text{COOH}$ ), which makes their interaction with the  $\text{NH}_3^+$  groups of CHI less favorable, in this study, the pH of the two polymer solutions was increased by the addition of 1M sodium hydroxide to 5.4 (for PAA) and to 4.50 and 5.5 (for CHI). The last value is considered to be the upper limit to prevent phase separation due to the insolubility of CHI at higher pH values. The experimental conditions are displayed in Table I. The CHI-PAA complexes in each experiment were prepared at the same temperature and stirring speed by the dropping of PAA into a CHI solution.

In experiment 1, in which the pH of both reactant polyelectrolytes was close to 5.5, a white suspension together with some precipitate was obtained. In experiments 2 and 3, in which only the pH values of the CHI solutions were adjusted to 5.53 and 4.50, respectively, the formation of a stable, white suspension was observed. However, under the conditions of experiments 4 and 5, in which the pH values of the resulting suspensions were acidic, opalescent suspensions were obtained, indicating the formation of mainly nanometric particles.

The size distributions of the suspensions of the CHI-PAA complexes obtained in these experiments are shown in Figure 6, and their mean diameters values are collected in Table I. From these results, it



**Figure 6** Dependence of CHI-PAA complex formation on the pH: (◆) experiment 1, (●) experiment 2, (▲) experiment 3, (■) experiment 4, and (□) experiment 5.

is clear, therefore, that when both reactants were at a pH close to 5.5 (experiment 1), a suspension of mainly microparticles (94.9% between 1 and 200  $\mu\text{m}$  in size) was obtained. This could be due to the fact that with PAA at pH = 5.5, the increase in the charge density of PAA molecules produced an increase in the intramolecular and intermolecular electrostatic repulsive forces, which then caused an increase in the dimensions of the macromolecular coil of PAA and, consequently, in the mean size of the CHI-PAA particles. On the other hand, with the pH of the CHI solution increasing, the positive charge density of the molecule (resulting from the protonated amino groups) was diminished. This influenced the effectiveness of stabilization by electrostatic repulsion of the CHI-PAA complex particles, which were surrounded by a layer of CHI, which was in excess in the suspension.

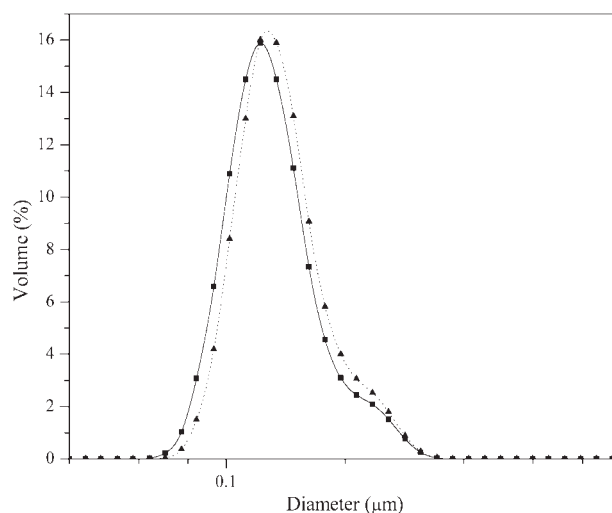
Under the conditions of experiments 2 and 3, a broad trimodal distribution of mainly nanometric particles ( $\sim 90\%$   $< 1 \mu\text{m}$ ) with maxima at 225, 656, and 1830 nm was found. Because the pH value of PAA solutions was 3.3, the carboxylic groups were almost fully protonated, causing a decrease in the volume of the macromolecular coil of PAA and contributing to a fine dispersion of PAA in CHI at first and then to the formation of a PEC membrane on the surface of the PAA core. Because of the excess CHI and reduction of the resultant pH (compared to experiment 1), CHI molecules on the particle surface maintained a net positive charge, which ensured their stability.

On the other hand, under the conditions of experiments 4 and 5, in which the pH of the reaction medium was near 3, all the particles of the complex were nanometric with a unimodal distribution at a

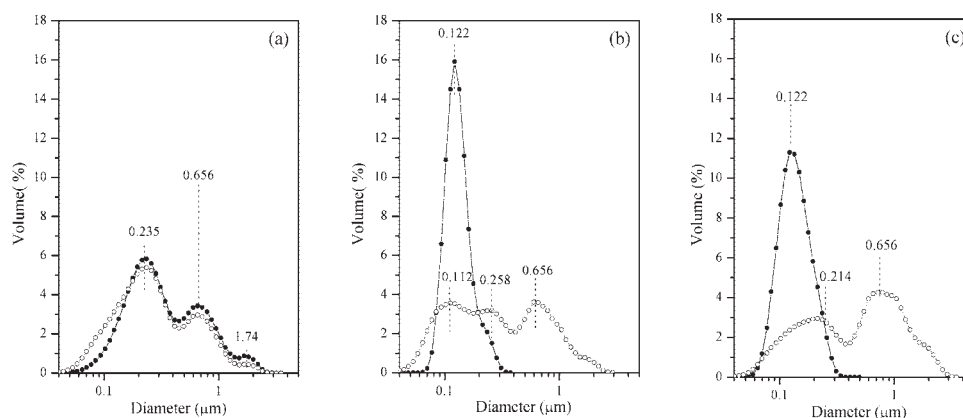
maximum of  $\sim 122 \text{ nm}$ . The formation of such nanometric particles under these conditions was due both to the good dispersion of PAA in the CHI solution and to the stabilizing power of the protonated CHI molecules at these pH values, which guaranteed the nanometric size and long-term stability of the suspension. In Figure 7, it can be seen that the particle size distribution of the suspension obtained in experiment 4 did not vary appreciably after the suspension was kept still for 1 month under atmospheric conditions (the temperature was between 20 and 23°C).

The results obtained in this study are in agreement with those reported by Hu et al.,<sup>13</sup> who observed an increase in the size of the nanoparticles of the CHI-PAA complex obtained by the polymerization of acrylic acid in the CHI matrix when these particles were incubated in an aqueous medium with a pH between 4 and 7. In fact, an increase in the particle size with the pH of the medium has been reported not only for the CHI-PAA system<sup>24</sup> but also for the preparation of CHI-tripolyphosphate<sup>25</sup> and CHI-glycyrrhetic acid<sup>26</sup> nanoparticles.

In experiments 1–5, the weight of the CHI-PAA particles was determined to assess the influence of pH on the yield. To this end, the resulting particles were separated from the aqueous phase by ultracentrifugation. The collected solid was freeze-dried and weighed. The yield was calculated as already described. The results are displayed in Table I, where it can be seen that the higher the pH was of the resulting suspension, the greater the yield was of particles. It can also be observed that the value of the resulting pH was basically determined by the pH of the CHI solution, which was in excess in the reaction mixture. For the same reason, in



**Figure 7** Stability of nanoparticle suspensions obtained from experiment 4 (CHI = 2.87 and PAA = 5.4): (■) the original sample and (▲) the sample measured after 1 month.



**Figure 8** Influence of dialysis on the particle size distribution of CHI-PAA complexes from (a) experiment 3, (b) experiment 4, and (c) experiment 5: (●) sample without dialysis and (○) dialyzed sample.

experiments 4 and 5, in which the pHs of PAA differed considerably but the final pHs of the two suspensions were practically the same ( $<3$ ), similar yields of about 40% were obtained. As pointed out previously, under these conditions, the carboxyl groups of PAA were mainly nondissociated (COOH), and so their interaction with the protonated amine groups of CHI did not favor the formation of the complex. As stated previously, at pH 5.5, the interactions between both polyelectrolytes were favored, and this was reflected in the yields of experiments 1 and 2, which were more than twice the yields obtained at pHs 2.90 and 2.98.

Yield values greater than 100% may be attributed to the presence of excess CHI surrounding the particles of the complex and to low-molecular-weight salts (specifically, sodium acetate resulting from the addition of sodium hydroxide to the CHI solution) trapped inside the particles. To confirm this supposition, the suspension obtained in experiment 3 was dialyzed for more than 48 h to remove the excess CHI (both that in suspension and that covering the particles) as well as any possible low-molecular-weight impurities that might have arisen from the adjustment of the pH of CHI with sodium hydroxide. It was found that the yield of the complex obtained from the dialyzed suspension was 14% less than that in experiment 3, that is,  $84 \pm 2\%$ . This supported the supposition that part of the lyophilized solid from the nondialyzed suspensions corresponded to excess CHI and low-molecular-weight salts.

It is clear from these experiments that a compromise must be reached regarding the conditions used to obtain the CHI-PAA particles in terms of their yield and size because, although the majority of the nanometric particles are obtained from polymer solutions of pH values higher than 3, the yield of the complex formed under such conditions is rather low. However, the results indicate that if the pH of the initial CHI solution is varied between 4.5 and 5.5 and

the initial pH of the PAA solution is not modified, then a yield of around 90% of particles of the CHI-PAA complex is obtained, and of these, more than 90% are nanometric in size (experiments 2 and 3).

#### Effect of dialysis on the particle size distribution

It has already been explained that the experimental conditions used to obtain the CHI-PAA complex as nanoparticles with a satisfactory yield required the reactant (CHI) to be in excess and sodium hydroxide to be added to modulate the pH values of the starting polymer solutions (CHI and PAA). For this reason, the resultant suspensions were usually purified to remove excess CHI and low-molecular-weight salts. Purification of nanoparticles is generally achieved by centrifugation on an appropriate bed. Then, the particles are washed and redispersed<sup>18,27</sup> or washed and freeze-dried.<sup>13</sup> Dialysis has also been used for the purification of particles,<sup>28–30</sup> however, its effects on the particle size distribution and the stability of the suspension have not been reported. The purification procedure employed in the current study was dialysis, and so the effect of this procedure on the particle size distribution of suspensions obtained at different pHs was investigated.

The dialysis medium used was distilled water adjusted to pH 4.5 with acetic acid (see the Experimental section) because, according to reports from Hu et al.,<sup>13</sup> at this pH the CHI-PAA complex remains more stable. The suspensions of the CHI-PAA complex obtained from experiments 3–5 were selected for this study, and the particle size distributions of these dialyzed suspensions were measured by the LS method. The results are shown in Figure 8 and Table II.

The effect of this procedure on the size distribution of the nanoparticles depended on the pH of the resulting suspensions. In experiment 3, for which



**TABLE II**  
**Influence of Dialysis on the Particle Size Distribution of CHI-PAA Complexes Obtained in Experiment 3 ( $\text{pH}_{\text{CHI}} = 4.45$ ,  $\text{pH}_{\text{PAA}} = 3.2$ ), Experiment 4 ( $\text{pH}_{\text{CHI}} = 2.87$ ,  $\text{pH}_{\text{PAA}} = 5.4$ ), and Experiment 5 ( $\text{pH}_{\text{CHI}} = 2.87$ ,  $\text{pH}_{\text{PAA}} = 3.2$ )**

Experiment	Dialysis treatment	Mean diameter ( $\mu\text{m}$ )	<1- $\mu\text{m}$ -particle content (%)
3	—	$0.477 \pm 0.008$	90.1
3	24 h	$0.40 \pm 0.01$	93.5
4	—	$0.138 \pm 0.003$	100
4	24 h	$0.39 \pm 0.06$	90.2
5	—	$0.149 \pm 0.005$	100
5	24 h	$0.64 \pm 0.03$	77.4

the pH was 4.48, no considerable difference in the particle size distribution after 24 h of dialysis was evident. A small increase in its nanometric fraction was noted together with a slight reduction in their sizes possibly due to the removal of a layer of CHI from the surface of the complex and the release of salts from the particles. The latter should have an effect on the size of the particles because of the decrease in the osmotic pressure resulting from the decrease in the salt content. However, in the dialyzed suspensions of experiments 4 and 5, a drastic change both in the distribution profile and in the particle size of the complex was observed. In both cases, a wide distribution of particle sizes ranging from 40 to 1200 nm was found (Fig. 8 and Table II) with a considerable increase in their average sizes. This could have resulted from an increase in the degree of swelling of PAA due to the increase in its ionization at pH 4.5 with the consequent strengthening of the intermolecular and intramolecular repulsive forces. On the other hand, the increase in the pH of the medium with the resultant decrease in the surface charge density of the particles may have led to the agglomeration of the nanometric particles, causing an increase in the particle size and broadening of the particle size distribution.

In this way, dialysis should cause a loss of stability of the CHI-PAA particles over a period of time. To verify this, a study was made of the particle size distribution in the dialyzed and nondialyzed suspensions obtained in experiment 3 after both were held without movement for 2 weeks at a temperature of 23°C. In both cases, a precipitate was formed, but more so in the case of the dialyzed suspension. Ultrasonic treatment was applied for 20 min to these systems, and it was observed that the precipitate that formed from the nondialyzed suspensions completely disintegrated, whereas in the case of the dialyzed system, the disintegration was only partial. The measurement of the size distribution showed that the particles from the nondialyzed suspension had the same size

distribution as a freshly prepared sample, whereas the dialyzed suspension, despite its ultrasonic treatment, showed only aggregates of particles of a size greater than 100  $\mu\text{m}$ . This confirmed that the removal of a layer of CHI on the surface of the complex and the decrease in the surface charge density produced by dialysis destabilized the suspension of the complex by a lack of repulsion between particles, and this caused aggregation and precipitation of bigger complex particles over a period of time.

These results lead to two conclusions of practical interest. First, if the requirement is to obtain nanometric CHI-PAA complex particles, then the recommendation is to use the dialyzed suspension within a relatively short period after its preparation to prevent its irreversible aggregation. Second, it is clear that after dialysis treatment, the particle distributions in experiments 3–5 did not differ widely, and, moreover, the distribution in the first experiment showed a greater fraction of nanometric particles (see Table II). Considering that the yield from experiment 3 was twice as much as that of experiments 4 and 5 (Table I), one may conclude that the conditions of the former are more favorable, in a practical sense, for obtaining nanometric particles of CHI-PAA when their purification by dialysis treatment is required.

## CONCLUSIONS

CHI-PAA nanoparticles were prepared by the method of coacervation under mild experimental conditions without the use of any organic solvents or surfactants. This form of preparation and also the fact that the complex is composed of hydrophilic, nontoxic, and biodegradable polymers (CHI and PAA) make the resulting system very suitable for biomedical applications.

The study of the influence of the starting concentrations of CHI and PAA solutions on the particle size distribution of the CHI-PAA complex revealed that below 0.1 wt %, it is possible to obtain nanometric particle suspensions. Furthermore, it has been established that the pH values of the reactant solutions have a great influence on both the particle size and the yield of the complex that is formed. The most convenient pH values for obtaining CHI-PAA particles with a nanometric size and an optimum yield (nearly 90%) are 4.5–5.5 for CHI and 3.2 for PAA. Additionally, the effects of dialysis and ultrasonic treatment on the stability of complex suspensions prepared under different experimental conditions have been clarified so that recommendations could be made to bring this system into practical use.

The authors thank the International Inter-University Agreement between Complutense University (Madrid, Spain) and

the University of Havana (Havana, Cuba) and especially Complutense University and the Santander Group, who through their financing of the Program for Distinguished Visitors to Complutense University enabled this research work to be carried out.

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