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Impact of physical parameters on particle size and reaction yield when using the ionic gelation method to obtain cationic polymeric chitosan–tripolyphosphate nanoparticles

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ABSTRACT

Ionic gelation is the most frequently used method to obtain chitosan–tripolyphosphate nanoparticles due to its simplicity and because it does not generate waste solvents in the samples prepared.

This paper presents a study of the physical factors involved in this method for obtaining nanoparticles in order to determine which of them significantly influences the particle size of polymeric nanoparticles made from low-molecular-weight chitosan, without any additional chemical treatment, with the aim of standardising and optimising the method conditions, in addition to establishing the reaction yield.

The results indicate that stirring speed during ionic gelation reaction is decisive for the size of the nanoparticles obtained. Furthermore, it thus follows that the stirring speed during ionic gelation significantly affects reaction yield, and therefore, by manipulating this parameter a greater proportion of nanoparticles of a given size range can be obtained.

1. Introduction

Due to the advantages it offers, the use of chitosan for obtaining cationic polymeric nanoparticles as non-viral vectors in drug delivery and gene transfer has been extensively studied (Bulmer et al., 2012; Fan et al., 2012; Gaspar et al., 2011; Ji et al., 2009; Tsai et al., 2011). However, it also presents various drawbacks. As a natural substance, the degree of purity varies widely since it is influenced by the extraction process, which determines the molecular weight (Wang et al., 2008). Low-molecular-weight (LMW) chitosan is the most suitable for use as a non-viral vector for gene delivery, especially with respect to transfection efficiency (Kong et al., 2012) and the degree of deacetylation, i.e., the positive charge density (Csaba et al., 2009), which determines its aqueous solubility.

Therefore, the variability present in the initial chitosan will undoubtedly determine the characteristics of the nanoparticles obtained.

These nanoparticles can achieve a size of up to 1000 nm and a positive zeta potential of between +20 mV and +60 mV (Calvo et al., 1997). Chitosan–tripolyphosphate nanoaparticles are intended for in vivo administration, so that no harmful residue exists that may jeopardise the safety of the organism to which they are delivered. The ionic gelation method meets this requirement, since the method does not require the addition of any organic solvents, thus avoiding the problem of elimination of residues prior to delivery into living organisms (Dash et al., 2011).

There is a considerable body of literature on the production of chitosan–tripolyphosphate using this method, with many variations concerning the concentrations and ratios of the initial components. However, there is little literature on the operational parameters for preparing chitosan–tripolyphosphate nanoparticles by ionic gelation as regards its effect on the particle size and surface charge of the final nanoparticles.

The ionic gelation method involves a complexation between the negative and positive charges of tripolyphosphate and chitosan, respectively, to determined pH values, behaving as a metastable thermodynamic system (López-León et al., 2005) which is highly sensitive to variations in ionic strength. For this reason, the time spent in the reaction, the stirring rate applied during the same, as well as the rate of addition of tripolyphosphate in the chitosan solution are factors which should be assessed in order to optimise the method for obtaining nanoparticles and evaluating production performance. It should be borne in mind that colloidal systems consisting of nanoparticles have a high tendency to aggregate. The mechanical energy associated with the reaction stirring speed may exceed the electrostatic repulsion energy between nanoparticle

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Table 1
The 2^3 experimental design in detail. Each of the three factors was studied at two levels. The letters rpm stand for revolutions per minute.

Run	Time of ionic gelation reaction (min)	Stirring speed during ionic gelation (rpm)	Time elapsed for TPP addition (min)
1	15	700	5
2	15	500	10
3	10	500	10
4	10	700	5
5	15	700	10
6	15	500	5
7	10	500	5
8	10	700	10

positive surface charges, thus eventually triggering aggregation phenomena. This represents a recurrent problem in the formation of single nanoparticles (Vauthier et al., 2008). Furthermore, if samples are subjected to lyophilisation in order to concentrate and preserve nanoparticle samples, the formation of aggregates can greatly hinder their redispersion, thus affecting particle size (Bozdag et al., 2005; Abdelwahed et al., 2006). At this point, the crucial role of the stirring speed should be emphasised, as being one of the factors mentioned above which is directly implicated in aggregation.

Regarding the characteristic size range of chitosan–tripolyphosphate nanoparticles, it is also of interest to analyse to what extent it is possible to obtain a higher percentage of a given particle size, depending on the mechanical parameters of the reaction. This information would contribute to defining a methodology for systematic and controlled production.

2. Materials and methods

2.1. Materials

LMW chitosan with a 75–85% degree of deacetylation came from Sigma–Aldrich (Spain). Sodium tripolyphosphate, glacial acetic acid and sodium acetate 3-hydrate were obtained from Panreac (Spain). Qualitative filters were supplied by Filterlab (Spain). Ultrapure water was obtained using the Milli-Q A10 system (Millipore Ibérica, Spain).

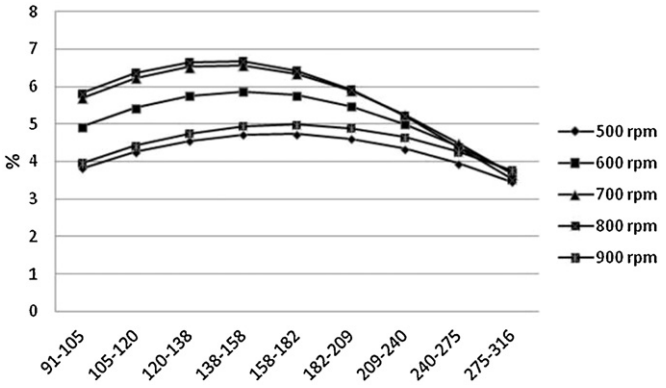

Fig. 1. Percentage of nanoparticles obtained in each measured interval.

2.2. Chitosan–tripolyphosphate nanoparticle production

Chitosan–tripolyphosphate nanoparticles were produced using a modified ionic gelation method (Calvo et al., 1997). Briefly, chitosan was dissolved at 0.2% (w/v) in acetic buffer solution at pH = 5 (Gan et al., 2005; Nasti et al., 2009). The solution was magnetically stirred for 3 h and then filtered to discard any chitosan not dissolved in buffer. Tripolyphosphate was dissolved in ultrapure water to obtain a 0.84 mg/ml concentration. Next, 12 ml of tripolyphosphate solution was added dropwise to 30 ml of chitosan solution (Vila et al., 2002). The final suspension was then filtered. The reaction was carried out under 8 different conditions to evaluate the effect of the physical parameters of the ionic gelation method on nanoparticle properties and the aggregation tendency. The factors studied were stirring speed, time of ionic gelation reaction and elapsed time for the addition of tripolyphosphate to chitosan solution. All these factors were tested at two different levels. The resulting samples were subjected to further analysis.

2.3. Experimental design to test effect of physical parameters on particle characteristics

A 2^k experimental design of three factors at two levels (2^3 design) was used to study the influence of several operational parameters of the ionic gelation method. The factors studied were: reaction time of ionic gelation, stirring speed during the reaction,

Table 2
For each run, surface weighted mean and *Z* potential values are shown.

Run	Time of ionic gelation reaction (min)	Stirring speed during ionic gelation (rpm)	Time elapsed for TPP addition (min)	Surfaced weighted mean *D*[3,2] (nm)	*Z* potential (mV)
1	15	700	5	127	40.3
2	15	500	10	296	39.8
3	10	500	10	278	39.7
4	10	700	5	156	40.3
5	15	700	10	131	40.3
6	15	500	5	227	39.9
7	10	500	5	208	39.8
8	10	700	10	113	39.3

Table 3
Results of nanoparticle size and zeta potential according to stirring speed applied during ionic gelation.

Rpm	Surface weighted mean (nm)				*Z* potential (mV)			
	Replicate			Mean value	Replicate			Mean value
500	214	207	203	208	41	38	41	39.8
600	207	151	196	185	41	40	42	41.0
700	190	193	121	156	40	41	40	40.3
800	206	159	132	166	40	43	41	41.2
900	231	223	197	217	40	43	42	41.8