

Potential for Scaled-up Manufacture of Chitosan Nanoparticles Using the Spinning Disc Processor

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ABSTRACT

Spinning disc processing (SDP) is an innovative and robust continuous flow technology that has great potential for the scaled up production of nanoparticles. This study examined the feasibility of producing chitosan nanoparticles by SDP technology. Analysis by dynamic light scattering suggested that chitosan nanoparticles produced by SDP (19.6 ± 2.8 nm diameter, $n = 4$) were similar in size compared to those produced by the conventional ionotropic gelation method (30.6 ± 12.5 nm, $n = 3$), while laser Doppler anemometry detected significant differences ($p < 0.05$) in the zeta-potential of the nanoparticles (SDP: 53.3 ± 4.3 mV, $n = 3$; conventional: 38.7 ± 6.1 mV, $n = 3$). The SDP-manufactured chitosan nanoparticles were spherical and discrete when viewed under the transmission electron microscope. In summary, SDP technology can be a reliable method for the commercial production of chitosan nanoparticles of a consistent quality

Keywords: chitosan nanoparticles, spinning disc processing

1 INTRODUCTION

Chitosan has been widely investigated for biomedical applications because of its biocompatibility, biodegradability, and mucoadhesive properties [1]. This polymer has been employed in drug and gene delivery [2], wound dressings [3] and tissue engineering [4]. In recent years, nanoparticles of chitosan have also been synthesized to impart increased reactivity and biological activity, particularly in the fields of drug and gene delivery [5, 6]. Currently, chitosan nanoparticles are manufactured by many laboratories using batch processing methods, some of which utilize toxic chemicals and harsh processing conditions [7], are limited in production capacity and present the operator with little control over the characteristics of the nanoparticles. To realize the commercial potential of chitosan nanoparticles, a large scale production method must be available that will yield a product of acceptable quality to the regulatory authorities. The production method must also be cost effective, flexible and readily validated.

The aim of this study was to investigate the potential of the spinning disc processing (SDP) technology for scaled up production of chitosan nanoparticles. SDP uses a small equipment for continuous flow processing. Reactants are introduced on a rotating disc where they are mixed under controlled conditions to produce nanoparticles of requisite size (Figure 1). It is simple to operate, and is readily adapted to bulk manufacturing without necessarily increasing the size of the processor. The manufacturing process may be controlled by optimizing the feed rates of reactants, along with the temperature and speed of mixing on the spinning disc. The SDP has been shown to be a reliable and efficient method for fabricating nanoparticles under 10 nm, with remarkable control over the size, shape and agglomeration of the nanoparticles [8-10]. The continuous flow technology further eliminates batch-to-batch variations in the nanoparticle production.

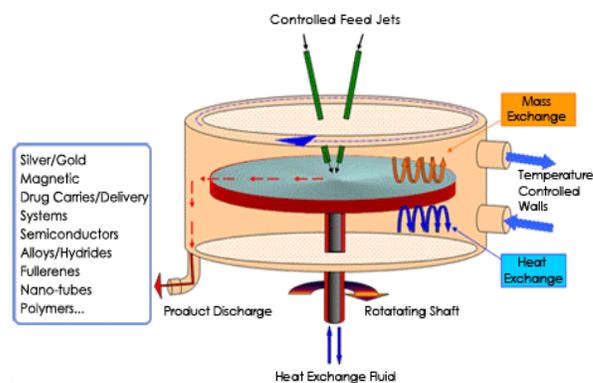


Figure 1: Schematic Diagram of the Spinning Disc Processor [10]

A wide variety of inorganic nanoparticles, including: metal coated carbon nanotubes [11], mesoporous silica capsules [12] and zinc oxide nanoparticles [13], has been successfully produced using the SDP. There is however, considerably less experience in using the SDP to manufacture organic nanoparticles. To assess the SDP method for scaled up production, chitosan nanoparticles produced using the SDP were compared to those synthesized using conventional batch processing methods based on ionotropic gelation of chitosan with the

tripolyphosphate anion. Comparable concentrations of chitosan and counterions were used. However, whereas the conventional method employed a beaker to cause bulk mixing of the reactants under magnetic stirring (1000 rpm), the SDP introduced the reactants at controlled flow rates onto a disc, which was rotated at specified speeds (1000-3000 rpm) that forced the reactants to flow outwards as very thin films. Intimate mixing of the reactants was effected on the disc and the effluent was collected for analysis.

2 MATERIALS AND METHODOLOGY

2.1 Materials

All reagents used were at least of analytical grade. Chitosan (medium molecular weight) and sodium tripolyphosphate (TPP) were purchased from Sigma Aldrich Australia.

2.2 Production by Spinning Disc Processing

Chitosan nanoparticles were manufactured by mixing sodium tripolyphosphate (TPP, 0.10 %w/v in MilliQ water, 50 mL, feed rate: 1.1 mL/s) and chitosan (0.25 %w/v in aqueous acetic acid, 50 mL, feed rate: 1.5 mL/s) on the spinning disc of the SDP. A spinning disc processor (SDP, Triton SDR Type P100, Protensive, UK) attached with a 10-cm stainless steel grooved disc was used for this study. The effects of acetic acid concentration (0.1 to 1 M) for dissolving the chitosan, along with the rotating speed of the spinning disc (1000 to 3000 rpm), on the characteristics of the manufactured nanoparticles were evaluated.

2.3 Production by conventional method

Chitosan in solution (0.25 %w/v in acetic acid, 8.2 mL) were condensed into nanoparticles through the addition of sodium tripolyphosphate (0.10 %w/v in MilliQ water, 6 mL) under magnetic stirring at 1000 rpm at room temperature. The effects of acetic acid concentration (0.1 to 1 M) on nanoparticle characteristics were evaluated.

2.4 Physical Characterisation

Particle size was measured by dynamic light scattering and the zeta potential of the nanoparticles was determined by laser Doppler anemometry (Zetasizer Nano ZS, Malvern Instruments, Version 4, Worcestershire, UK). The morphology of the nanoparticles was examined under a transmission electron microscope (TEM, JOEL 2100, Japan).

2.5 Statistical Analysis

Data summary were expressed as mean \pm standard deviation (S.D.). Data on particle sizes were analyzed by

One-Way ANOVA with Post-Hoc Tukey's Test (SPSS Version 11, Lead Technologies, Inc.). A p -value ≤ 0.05 was considered to be significant.

3 RESULTS AND DISCUSSION

The most widely applied batch processing method for manufacturing chitosan nanoparticles is through the condensation of the polymer by ionic interaction with tripolyphosphate anions [14]. This principle was also applied for the manufacture of chitosan nanoparticles by SDP technology.

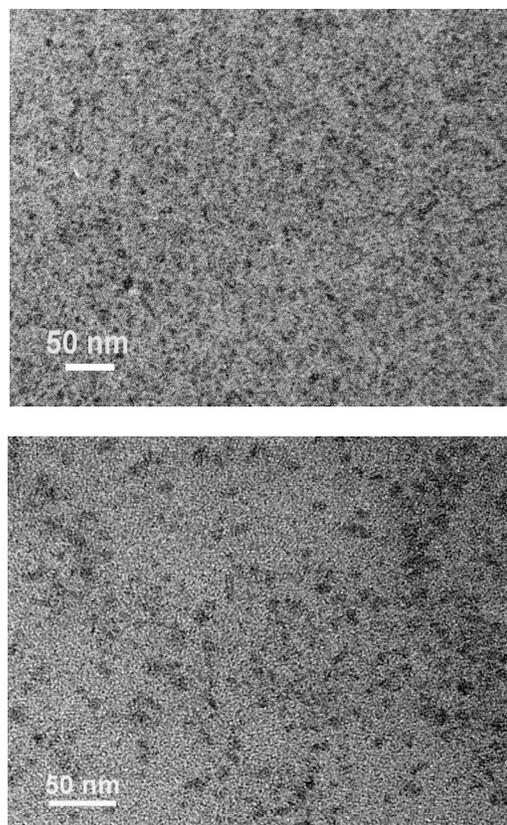


Figure 2: Transmission electron micrograph of chitosan nanoparticles on Formvar[®] coated gold grids. Instrument was operated at 120.0 kV, spot 1, alpha 3. (A) Particles prepared by SDP technology. (B) Particles prepared by conventional ionotropic gelation method using the same chitosan and TPP solutions as in the SDP method.

Using 0.25% w/v of chitosan in 1M acetic acid, and 0.10% w/v of TPP in water as feed solutions, chitosan nanoparticles less than 50 nanometers were successfully manufactured by SDP. TEM micrographs (Figure 2A) showed uniformly sized, discrete and spherical particles of about 20 nm in diameter were produced. Corresponding chitosan nanoparticles produced by the conventional method exhibited a much wider size variation (Figure 2B).

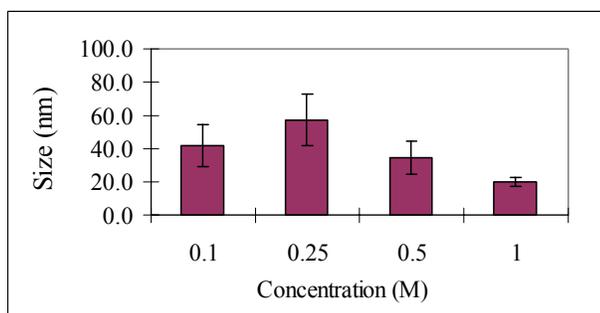


Figure 3. Effects of acetic acid concentration on chitosan nanoparticle size (mean \pm S.D., $n = 4$). Nanoparticles were manufactured by SDP. Significant differences ($p < 0.05$) were found amongst the mean sizes of nanoparticles produced using 0.25, 0.5 and 1 M. However, no statistical difference was found between nanoparticles produced using 0.1 and 1 M of acetic acid ($p > 0.05$).

The TEM results were supported by dynamic light scattering analysis data. Nanoparticles prepared using the SDP showed much smaller size deviation from the mean (19.6 ± 2.8 nm, $n = 4$) than those manufactured by the conventional method (30.6 ± 12.5 nm, $n = 3$). This capacity to yield nanoparticles of narrow size range is a known feature of the SDP [8], and has been attributed to the intense mixing achievable on the spinning disc. In contrast, nanoparticles manufactured by the conventional method had diameters that ranged from 16.8 to 41.3nm. There was therefore a significant size overlap with the SDP nanoparticles, resulting in a lack of significant difference ($p > 0.05$) in mean size between the two lots of nanoparticles.

To evaluate the effects of pH, different concentrations of acetic acids were used to prepare the nanoparticles at pH 2.7 to 3.5 (Figure 3). Nanoparticles prepared on the SDP using 0.25, 0.5 and 1 M of acetic acid had significantly different mean sizes, but lowering the acetic acid concentration to 0.1 M did not produce nanoparticles of significantly different mean size from those prepared using 1 M acetic acid. Comparable data were obtained for chitosan nanoparticles produced by the conventional method using different concentrations of acetic acid ($p > 0.05$, Table 1), however the zeta-potential of nanoparticles produced at pH 2.7 (38.7 ± 6.1 mV, $n = 3$) were significantly lower than the SDP-manufactured nanoparticles (53.3 ± 4.3 mV, $n = 3$, $p < 0.05$). The data are in agreement with published studies. In one study [15], an enlargement of particle size with rising pH was noted between the pH range of 3.2 to 12.2. This was attributed to an increase in particle aggregation due to the reduction in the protonation of the amino groups in chitosan molecules. Studies have also shown that the zeta potential of chitosan nanoparticles typically varied as a function of manufacture pH [16, 17], and could well vary between 30.8 to 68.9 mV within a batch [18].

Table 1. Comparison of nanoparticles manufactured by SDP and conventional methods using different concentrations of acetic acid (HAc).

SDP	Size (nm)	ZP (mV)
0.1M HAc	41.6 ± 12.8	43.1 ± 3.6
0.5 M HAc	34.6 ± 10.1	42.4 ± 2.8
1M HAc	19.6 ± 2.8	$53.3 \pm 4.3^*$
Conventional		
0.1M HAc	25.0 ± 7.9	48.7 ± 4.8
0.5 M HAc	28.8 ± 9.0	38.7 ± 5.3
1M HAc	30.6 ± 12.5	$38.7 \pm 6.1^*$

The size and zeta potential (ZP) is summarized (mean \pm S.D., $n \geq 3$). *Significant differences ($p < 0.05$) were detected between the zeta-potential values of nanoparticles produced by both methods using 1M acetic acid.

The mixing speed achievable on the SDP could range from 300 to 3000 rpm. Figure 4 depicts the effects of increasing the speed of the rotating disc on the mean size and size distribution of chitosan nanoparticles. No significant differences were found in the mean sizes of nanoparticles manufactured at 1000, 2000 and 3000 rpm, although higher rotating speeds resulted in an increase in the size range of the nanoparticles produced. The wider particle size distribution might be associated with the lower stability of the rotating disc operating at higher speeds.

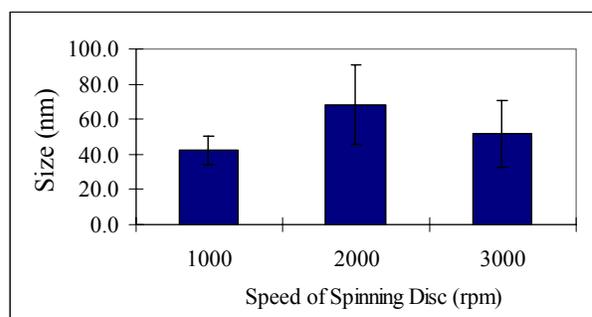


Figure 4. Size distribution of chitosan nanoparticles manufactured by SDP operating under different spinning disc speeds (mean size \pm S.D., $n = 4$). No statistical difference was observed in the mean size of nanoparticles formed between 1000 and 3000 rpm ($p > 0.05$)

4 CONCLUSIONS

In summary, the data demonstrate the validity of manufacturing chitosan nanoparticles through SDP technology. Discrete, spherical chitosan nanoparticles could be successfully manufactured by SDP technology using chitosan and TPP solutions typically employed in batch processing methodologies based on the ionotropic gelation principle. The nanoparticles had comparable mean size and

zeta potential as corresponding nanoparticles manufactured by the conventional method, although there was evidence the SDP method was superior in producing nanoparticles of a narrower size range. Nanoparticle size was not significantly affected by the spinning disc rotating speed, suggesting that it could be a robust method for manufacturing chitosan nanoparticles.

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