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Novel electrohydrodynamic preparation of porous chitosan particles for drug delivery

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Abstract Uniform spherical chitosan particles of size <10 μm in diameter are important in drug delivery applications due to their excellent biocompability and biodegradability. A high concentration of chitosan in the particles can help to control the release of drugs and methods for processing high viscosity chitosan solutions are therefore required. In principle, any type of polymer, whether hydrophobic or hydrophilic, can be electrosprayed to obtain monodisperse particles of diameter <10 μm. In practice, however, electrospraying of biopolymers having viscosities of >100 mPa s results in particles >10 μm diameter. In this study, by reducing surface tension of a high viscosity chitosan suspension, it was found that smaller diameter particles could be prepared. Chitosan solutions were electrosprayed in the stable cone-jet mode to systematically study the relationship between particle diameter, viscosity and surface tension. Increasing viscosity resulted in larger diameter particles with a broad size distribution, but decreasing surface tension had the opposite effect. Results show that a chitosan solution having a viscosity of ~80 mPa s can be used to prepare chitosan particles of diameter ~2.5 μm which on drying reduced to particles of 500 nm.

1 Introduction

Hydrophilic molecules such as protein-based drugs need to be delivered using a systemic route since transdermal, oral, ocular or nasal delivery can result in low bioavailability. Biodegradable polymer-based microparticles provide a useful means of delivering proteins with controlled release as they minimise exposure of proteins to enzymes prior to reaching the target [1]. Microparticles made of commonly used biodegradable polymers such as poly glycolic acid or poly (D,L-lactic-co-glycolic acid) for protein-based drug delivery, can only be prepared in conjunction with the evaporation or extraction of organic solvents in oil-water dispersions due to its lipophilicity. This limits the loading of hydrophilic drugs due to leakage into the surrounding aqueous environment during preparation. Naturally occurring, strongly hydrophilic polymers, based on starch, polysaccharides and protein are better candidates for protein-based drug delivery as they are able to prevent this type of leakage [2].

Chitosan is derived from deacetylation of chitin which improves its solubility in an acidic aqueous environment. Chitin which has undergone more than 50% of deacetylation is classified as Chitosan [α (1–4) 2-amino 2-deoxy β-d-glucan] [3]; a cationic polymer which is suitable for drug delivery applications mainly due to its biocompatibility [4] and mucal adhesion [5, 6]. Moreover, the cations in a chitosan solution also make complex ions with negatively charged protein or DNA to prolong release of protein [7].

Until now, many methods based on double emulsions [8], spray drying [9], ion-induced gelation and solvent evaporation [10] have been used to prepare chitosan spheres with diameters from 250 nm to 100 μm. Each of these methods has their own limitations. The double emulsion method is sensitive to several properties such as hydrophilicity, biocompatibility, surface tension and viscosity of the polymer, crosslinker and the drugs to be encapsulated. This restricts the choice of materials available to prepare the particles. A crosslinker like

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glutaraldehyde is not only an antigenic agent but also allows protein to covalently attach to chitosan [11]. Ionic-induced gelation can result in nano-particles only in low concentration suspensions of chitosan [12] while the spray drying method requires the dispersion of a protein-based drug in an organic solvent resulting in denaturing of the protein. It is desirable to prepare chitosan particles of <10 µm diameter as these can pass through an intravenous capillary of 10 µm and also enhance surface area controlled diffusion using any type of polymer, crosslinker and drug.

Electrohydrodynamic processing can be controlled by varying the surface tension and viscosity of the medium [13]. The electric force is required to overcome surface tension forces to generate a jet and monodisperse droplets. Droplet diameter is influenced greatly by viscosity [14]. Chitosan solutions of different concentration and therefore viscosities can give control over drug release as higher viscosity liquid will transform into denser particles giving slow drug release [15]. This work explores the effect of the surface tension of a chitosan solution upon droplet and hence particle size using electrospraying as the parameter for the processing route for generating droplets/particles. A range of chitosan solutions with different viscosities are considered.

2 Experimental details

2.1 Preparation and characterisation of the solutions

Deionised water (99.3 ml) was mixed with 0.7 ml of acetic acid (glacial 100% analytical reagent grade) in a beaker using a magnetic stirrer. Subsequently chitosan powder (Harvard Biotech, MA, USA, molecular weight 731 g/mol) was added to this mixture gradually in order to prevent agglomeration. The amount of chitosan added to the mixture was varied from 0.5 g, 1 g and 2 g resulting in concentrations of 5 mg/ml, 10 mg/ml and 20 mg/ml. This process was performed at ambient temperature (22°C). Once the suspension had become clear in appearance, indicating the total dissolution of the chitosan, it was transferred to a sample bottle and stored in a refrigerator. In order to change the surface tension of each chitosan solution by 10%, different amounts of acetic acid was added, the quantity required was decided through a process of trial and error.

Viscosities and surface tensions of the chitosan solutions were measured at ambient temperature. The viscosity of the solutions were measured using a 'Viscoeasy' (Camlab Ltd., Cambridge, U.K.) rotational viscometer. The surface tension was measured with a Kruss Tensiometer (Model-K9, Kruss GmbH, Germany) using the plate method. Furthermore, in order to ensure the readings were correct, the plate was heated beforehand to 70°C and subsequently allowed to cool to the ambient temperature, to eliminate any remaining residue. Before use, the tensiometer was calibrated using distilled water and the viscometer was calibrated using silicone oil of known viscosity.

2.2 Particle preparation

Firstly, chitosan solutions were sonicated at power 30 W for 12 min to generate bubbles. The resulting bubble suspension was drawn into a 10 ml syringe and perfused through a steel capillary of 1.5 mm internal diameter using a Harvard syringe pump (PHD-4400) and subjected to electrohydrodynamic atomisation (Fig. 1).The diameter of the capillary was chosen to retain all bubbles in the suspension while it was flowing through. Chitosan solutions having different viscosities were subjected to electrohydrodynamic atomisation at a flow rate of 100 µl/min. The voltage used in the experiments solely depended on the concentration of chitosan in the solution. 9, 14 and 21 kV were used for solutions containing 5 mg/ml, 10 mg/ml and 20 mg/ml concentration of chitosan, respectively. The combination of flow rate and applied voltage resulted in the stable cone-jet mode in each case except in the case of the highest concentration suspension, which was difficult to electrospray.

Ion induced gelation was used as the primary mechanism of preparing the chitosan particles. 2.8 g of NaOH was dissolved in 80 ml of distilled water to make a basic solution which could instantly gel the droplets of electrosprayed chitosan. This solution was kept in a glass beaker and continuously stirred using a magnetic stirrer while the electrosprayed chitosan droplets were being collected in NaOH solution to prevent coalescence. The chitosan droplets collected in this way attained a spherical shape once left for an hour in the NaOH solution due to high

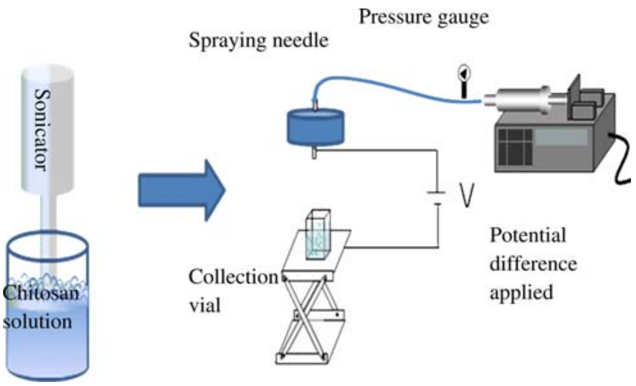

Fig. 1 Chitosan particle preparation procedure and electrospraying set-up

interfacial energy between the hydrophobic chitosan and the surrounding water.

2.3 Microscopy

Approximately 0.1 ml of the chitosan sphere suspension was extracted using a 1 ml syringe and placed on a glass slide to observe at least 300 particles after gelation under a Nikon Eclipse ME 600 microscope fitted with a CCD camera JVC KY-F55B. Micrographs of the particles were captured by the program AcQuis from Synoptics (version 4.0.1.3). These were used to measure their diameter and data acquired was transferred to Microsoft Excel(R) 2007 using Image Pro Express by Media Cybernetic Inc, version 5.0.1.26. All data was analysed and plotted using Excel(R) 2007.

Particles prepared by electrospraying were left for 4 h in the NaOH solution. To separate the particles, NaOH solution was removed from the top of the container. Distilled water was added to the separated particles to wash them further and was subsequently drained away from the top of the container after particles settled down at the bottom. This process was repeated six times before preparing samples for scanning electron microscopy.

A 1 ml of sample was carefully placed on an aluminium stub drop by drop. Subsequently the sample was dried at ambient temperature in a closed container. To reveal the inner particle structure, particles were sonicated in water at 8 W for 30 s using a sonicator (Misonix XL 2000, NY, USA). Subsequently, 1 ml of sample from this sonicated suspension was dried on an aluminium stub for electron microscopy. Scanning electron micrographs were captured at 10 kV and 20 kV accelerating voltages, respectively, to study both the coarse and fine details of the particles obtained.

3 Results and discussion

The addition of 0.5 g, 1 g and 2 g of chitosan and 0.7 ml of acetic acid to make up a total of 100 ml of solution results in a variation of viscosity from 40 mPa s to 825 mPa s. Similarly, the variation of the acetic acid concentration from 0.7 ml to 10 ml significantly decreases the surface tension without appreciable change in viscosity (Fig. 2). By adding an extra 7 ml of acetic acid to the 20 mg/ml chitosan suspension, viscosity decreased only by 3%, while surface tension drops by 24%. This shows that this chitosan solution is best suited to see the combined effect of surface tension and viscosity on chitosan particle generation. Increase in electrical conductivity due to addition of acetic acid and chitosan was not taken in to account.

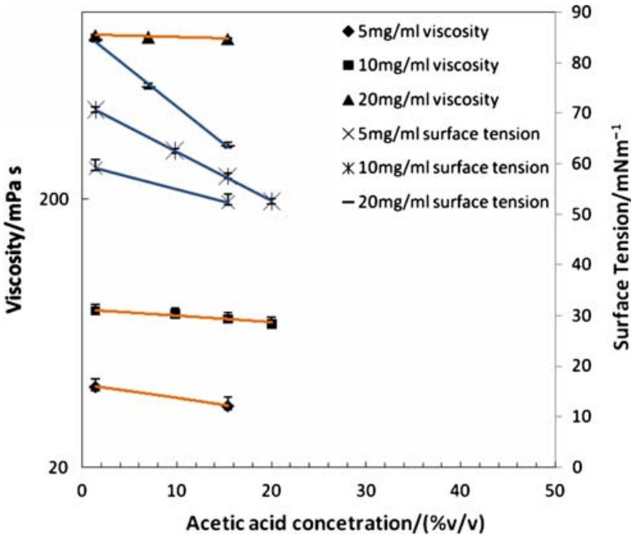

Fig. 2 Viscosity and surface tension of chitosan solutions prepared as a function of the acetic acid added

3.1 Effect of chitosan concentration

Since ionisation-mediated gelation of chitosan was employed to obtain the particles, the particles were left for 3–4 h in the NaOH solution before separation. This ensured proper gelation of particles. The molecular weight of the particles obtained from 5 mg/ml chitosan suspension was extremely low and due to the higher content of water in the 5 mg/ml chitosan suspension, the droplets spread out in the collecting medium (NaOH solution) as a result of very low interfacial tension. Hence, it was found that the higher viscosity chitosan solutions (in range of 10 mg/ml concentration) were needed to enable the preparation of

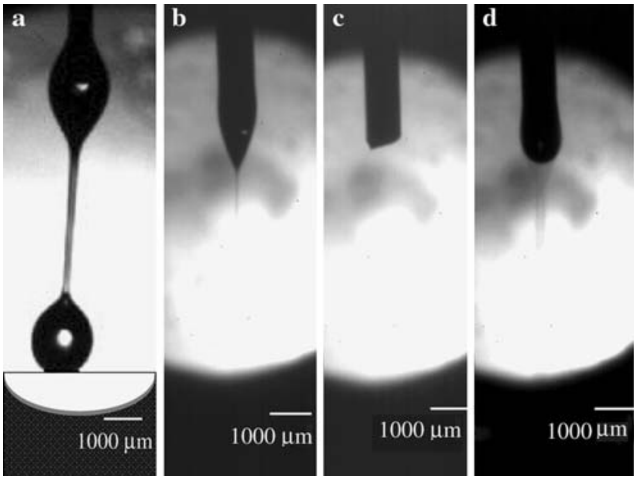

Fig. 3 High speed camera images (300 frames s^{-1}) showing different electrohydrodynamic modes of atomization of chitosan solution. **a** large droplet; **b** stable cone-jet mode; **c** multi-spindle mode; **d** unstable cone-jet mode

particles of diameter <10 µm. The 20 mg/ml suspension produced particles of diameter >10 µm.

3.2 Effect of viscosity

The 20 mg/ml suspension was difficult to spray with intermittent release of large diameter droplets being observed due to the extension of the viscous thread under the influence of electrical stress and relaxation (Fig. 3a). The possible near-monodisperse particles were obtained after electrospraying at a 100 µl/m flow rate in the stable cone-jet mode. Different modes of atomisation observed in electrohydrodynamic spraying are described in detail elsewhere [16]. Spraying in stable cone-jet mode (Fig. 3b) generates near monodisperse droplets because it is associated with the least amount of jet instability. However, as viscosity increases, the applied potential difference also needs to be increased from 9 kV to 21 kV to sustain the stable cone-jet mode. This shows that in order to overcome the viscous force, a dominant tangential electrical force is required [17].

It is characteristic of a polymer to act as an elastic material while under tension. In the simplest model, it can be described as a series of masses connected by springs.

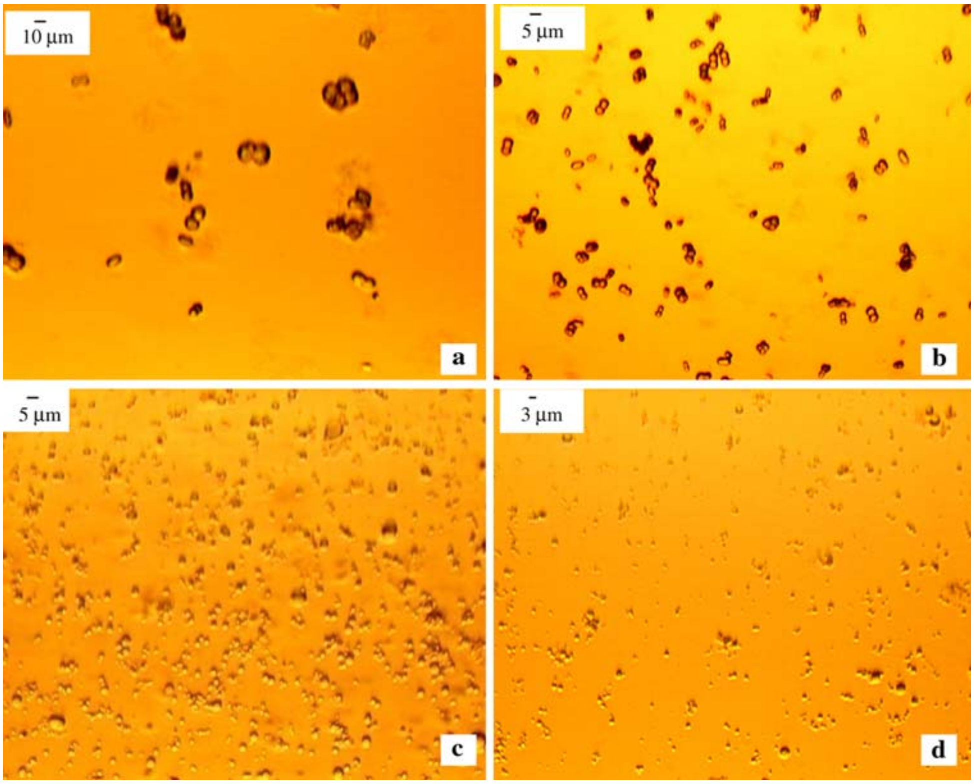

Fig. 4 Optical micrographs showing chitosan particle diameter and shape as collected in sodium hydroxide. **a** 10 mg/ml (concentration) 0.7 ml (acetic acid); **b** 10 mg/ml 4.9 ml; **c** 10 mg/ml 7.7 ml; **d** 10 mg/ml 10 ml

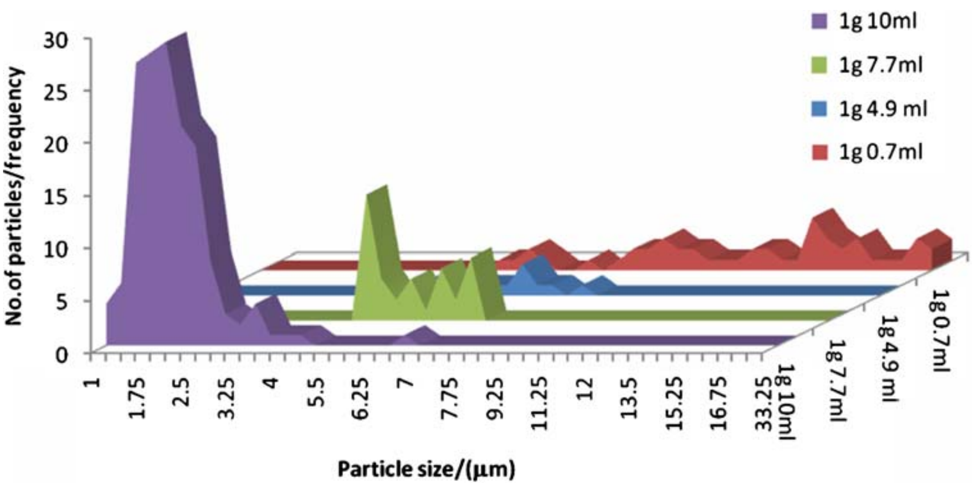

Fig. 5 Chitosan particle size distributions obtained from solutions of different surface tension but having nearly same viscosity. In the case of the 1 g 10 ml composition the few "rogue" particles at ~7 µm were not considered in polydispersivity calculations reported in the text

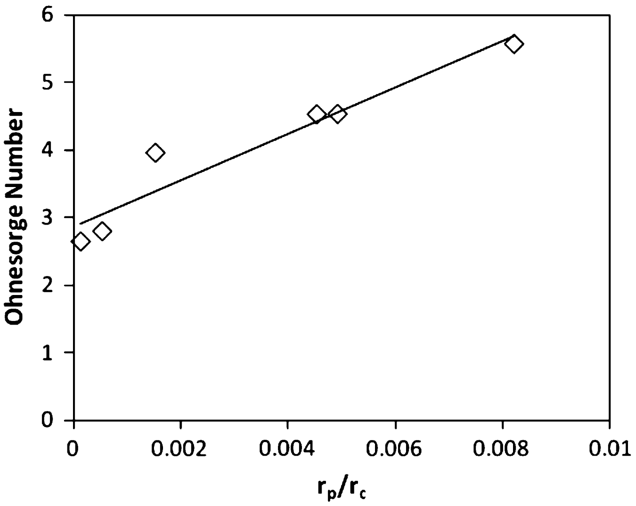

Fig. 6 Relationship between Ohnesorge number and normalized particle radius before drying

During electrospraying, the electric force stretches threads of liquid away from the capillary until it breaks but when polymer relaxation takes longer than electrohydrodynamic deformation, the polymer jet is deformed significantly and does not have time to relax [18]. In the deformed 'cone-jet' condition, the viscous liquid was forced out due to the extension of capillary threads, and eventually breaks up in to small droplets [19] (Fig. 3b). In the case of a shear thinning polymer suspension, the jet breaks in to droplets. Conversely, with an increase in polymer viscosity, the local shear viscosity does not decrease sufficiently resulting in large droplets formed under the action of the hydrodynamic forces alone (Fig. 3a). Figure 3c shows that there is not enough liquid available to affect the electrohydrodynamic force on the polymer solution droplet and this results in an irregular "flicker" of droplets (multi-spindle mode) [20]. Electrospraying of the 800 mPa s chitosan solution gives rise to the unstable cone-jet mode because the viscous stress and electrostatic stress are approximately in equilibrium (Fig. 3d). The stable cone-jet mode is key to the preparation of monodisperse <10 μm diameter particles. The viscosity of the 10 mg/ml solution was found to be ideally suited for obtaining particles of diameter <10 μm with a narrow size distribution.

3.3 Effect of surface tension

The 10 mg/ml solutions having different surface tension values were electrosprayed. The solution having a viscosity of 77 mPa s and surface tension of 71 mN/m gave particles ranging from 7 μm to 33 μm in diameter but, as surface tension was reduced, the mean diameter and its standard deviation also started to fall. With a reduction in surface tension of 12%, the mean particle diameter decreased from 12 μm to 3 μm. Optical micrographs of particles collected in NaOH solution are shown in Fig. 4. The agglomeration of the chitosan particles prepared was not investigated, however, it must be noted that chitosan particles tend to aggregate at physiological pH and temperature and in order

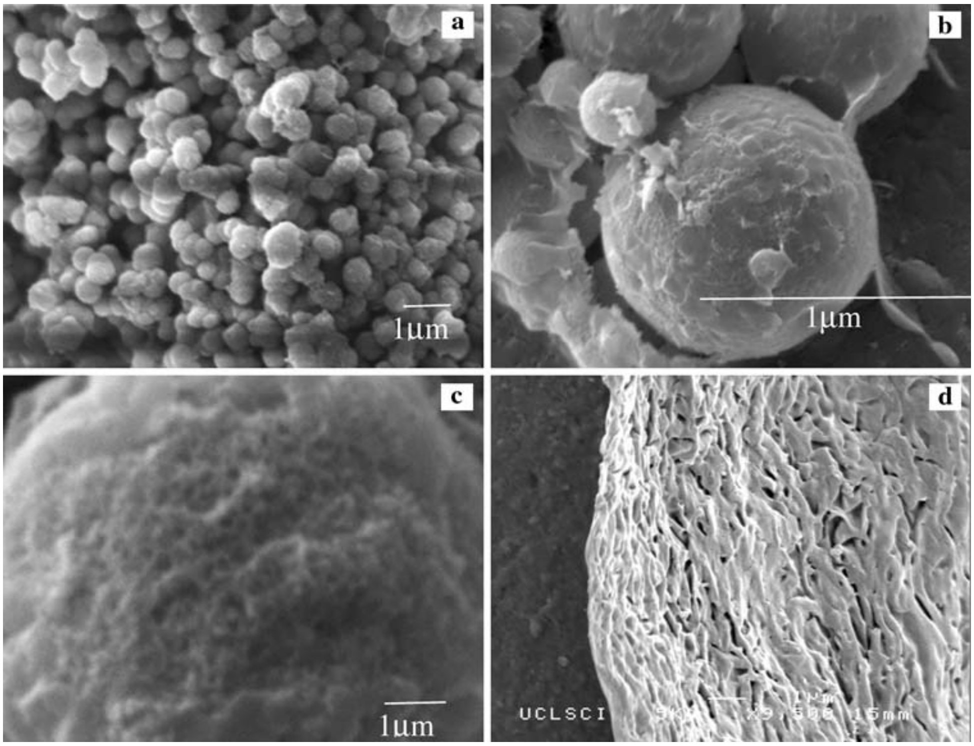

Fig. 7 Scanning electron micrographs of chitosan particles. **a** collection of particles; **b** single particle; **c** surface of particle; **d** cross section of particle

to evaluate their behaviour in vivo further investigation is necessary. The diameter measurements from the micrographs were further analysed and as shown in Fig. 5, surface tension was found not only to reduce mean particle diameter but also the width of the size distribution. Thus the polydispersivity index was reduced from 51% (1 g in 0.7 ml composition) to 10% (1 g in 10 ml composition). This may have been due to the lower surface tension gradient around the droplet. It was found that the diameter of the particles could only be reduced with the stable cone-jet mode, which also improved the monodispersity of the particles [21].

The consideration of surface tension and viscosity separately cannot determine the effect on the diameter and the size distribution of the chitosan particles prepared but the interaction between these two properties provides a better indication. The results show that r_p (mean radius of particles) is directly proportion to μ (viscosity) but inversely proportional to surface tension (γ). The combined effect of viscosity and surface tension can be described by the Ohnesorge number [22],

$$O_h = \frac{\mu}{\sqrt{\rho \gamma L_o}} \tag{1}$$

where ρ is density and L_0 is the characteristic length of the jet. To find the relationship between particle radius and the Ohnesorge number, the particle radius r_p is normalized with respect to r_c, the capillary radius, and plotted against Ohnesorge number as shown in Fig. 6. This was found to give the linear relationship

$$O_h = 344.8 \frac{r_p}{r_c} + 2.8 \tag{2}$$

The droplet diameter generated by electrospraying has previously been shown to be directly proportional to the Ohnesorge number and this work shows that diameter of the relics (particles) obeys a similar relationship [22].

As shown in Fig. 7a and b, after drying at ambient temperature and pressure, the diameter of smallest particles obtained is found to be ~500 nm. These particles are not only spherical but also monodisperse with a porous outer membrane and a sponge like internal structure (Fig. 7c, d). However, some of the particles seemed to agglomerate after drying on an aluminium stub, which could have been prevented by lyphilisation of particles after dispersion in liquid.

4 Conclusions

A pre-bubbled chitosan solution was successfully electrosprayed in the stable cone-jet mode to prepare particles down to 500 nm, which had a sponge like internal structure. The control of surface tension at a high viscosity (~80 mPa s) was found crucial to controlling the size distribution of the chitosan particles.

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