



## Influence of the Technological Parameters of Jet Mixing Process on Chitosan/Alginate Nanoparticles

Sophia Milenkova, Ivan Bodurov and Maria Marudova

University of Plovdiv "Paisii Hilendarski", Faculty of Physics and Technology,  
24 Tsar Asen str., 4000 Plovdiv, Bulgaria

**Abstract.** In the field of new and non-conventional materials, polymers and polyelectrolytes have a particular place of interest. Considering the fact, that these compounds have a wide variety of features and that they can be used in numerous fields polyelectrolytes (PE) and their complexes (PECs) can be assumed as worthy alternates of many conventional materials. In this study, nano-sized PECs were successfully formed by using the jet-mixing technique. The turbidimetric method was used to evaluate the particle size. For this purpose the extinction-wave length dependence was determined. The refractive index of the colloid was also measured using laser refractometer. The dependences of the particles' size on the technological parameters of the mixer, as well as the influence of storage conditions and duration were found.

**Keywords:** jet mixing, polyelectrolyte complexes, nanoparticles, chitosan, alginate.

### 1. INTRODUCTION

In the past decades, nanotechnology and nanoscience have undergone rapid development. They include a wide range of scientific and industrial activities covering fundamental (physics, chemistry and biology) and applied (electronics, energy, material science) areas. One of the most significant achievements of nanotechnology and nanoscience is the production and application of nanosized particles. They can be manufactured from different materials in different shapes such as spheres, cylinders or tubes. Nanoparticles are used in electronics, energy, magnetism and optoelectronics, biomedicine, pharmacy, in the development of new materials, and in environmental monitoring (Liu, 2006).

One of the most common methods to create polymeric nanoparticles loaded with various hydrophobic compounds, including drugs, is the flash nanoprecipitation (Han et al, 2012). The method employs the principle of turbulent mixing and high supersaturation. The supersaturation promotes coprecipitation of the hydrophobic drug and the polymer to form nanoparticles. Mixing and precipitation occur within

milliseconds inside the small internal mixing chamber.

One of the known nanoparticle delivery devices based on flash nanoprecipitation is the (CIJ) mixer (Johnson and Prud'homme, 2003A). In this design, a syringe pump was used to drive two opposing liquid streams at high velocity into the mixing chamber. By this way, different types of nanoparticles have been successfully produced (Johnson and Prud'homme, 2003B; Johnson and Prud'homme, 2003C; Akbulut et al, 2009; Lince et al, 2009; Marchisio et al, 2006). The CIJ design was inspired by the simple T mixer that is commonly used to mix liquids or act as a chemical reactor (Hunter and Nash, 1935; Treybal, 1963).

To achieve a high efficiency of operation, the two streams entering the mixing chamber must move at the same speed. In practice, this requires approximately the same magnitude of the flows of the substances, which leads to limiting the highest possible precipitation rate. These reasons necessitate the development of other devices, one of which is the multi-inlet mixer (Liu et al, 2008; Gindy et al, 2008). In this design, the mixing chamber is connected to four inlets and the liquid streams meet at an angle as

opposed to the head-on impingement that is characteristic of the CIJ mixer. This type of device can be used for a wide range of solvents and materials. However, the construction is extremely complicated to manufacture, requires a considerable amount of cleaning time, as well as the need for costly numerically programmable pumps to control the flow of the inlet of the mixing chamber.

A new device for the production of polyelectrolyte nanocomplexes is proposed in the present work, which is a modification of the existing CIJ mixers and uses the principles of JET mixing. The effect of the technological parameters of jet mixing process on the particle size was investigated.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Middle molecular mass (Acros Organics), was used in the present research. Sodium alginate was bought from Sigma-Aldrich (Germany). All other used chemicals were with analytical grade.

### 2.2. Preparation of PEC Nanoparticles

Nano-sized PEC complexes were prepared by homemade JET mixer. The assembly consists of two electromechanical injectors located at an angle of 180° to each other, a mixing chamber and an outlet tube runner. In the experiments, a pressure pump, which provides constant pressure in the polyelectrolyte reservoir, was also used. The opening and closing of the injectors were controlled by software designed for this purpose. Thus, two oppositely directed aerosol jets form from the polyelectrolyte solutions, moving at high speed, were produced. The polyelectrolytes in the jets interact in the mixing chamber and form nano-sized PECs.

Chitosan and alginate complexes were prepared by mixing 0.1% solutions of the two polyelectrolytes in acetate buffer at pH 4.5 and ionic strength 100 mM. The solutions are JET mixing at room temperature under different pressures - 2, 3, 4, 5 and 6 bar. The shooting time and the pause time of the injectors were also varied - from 10 ms to 750 ms.

### 2.3. Particle Size Evaluation

Particle size was evaluated by the method, described in [Klein, 2002]. Briefly, light scattering theories describe the turbidity of a colloidal monodisperse system by the equation:

$$t = N_2 \pi r^2 K(\alpha, m) \quad (1)$$

Here,  $t$  is the turbidity,  $\text{cm}^{-1}$ ,  $N_2$  is the number of particles in  $\text{cm}^3$ ,  $r$  is the particle radius,  $K(\alpha, m)$  is the light scattering efficiency factor as a function of the relative size  $\alpha$  and the relative refractive index  $m$ :

$$\alpha = \frac{2\pi\mu_1 r}{\lambda} \quad (2)$$

$$m = \frac{\mu_2}{\mu_1} \quad (3)$$

$\mu_2$  is the refractive index of the dispersed phase,  $\mu_1$  is the refractive index of the dispersion medium, and  $\lambda$  is the wavelength in vacuum.

### 2.4 Stability of the Nanoparticles

The stability of the PEC nanoparticles was investigated, because of tendency of aggregation during the storage period. In order to survey the effect of storage conditions on the medium radius of the particles, three series of samples were prepared – “Reference”, which were stored at the pH at which the PECs were formed (pH=4.5), “pH 8”, for which the pH was changed from 4.5 to 8, and “20% ethanol”, when 20% of ethanol was added to the initial solution. The storage period was 15 days and the tests were taken on the first, third, seventh, tenth and fifteenth day after the preparation. All of the samples were stored at  $t = 4$  °C for the whole period of the survey.

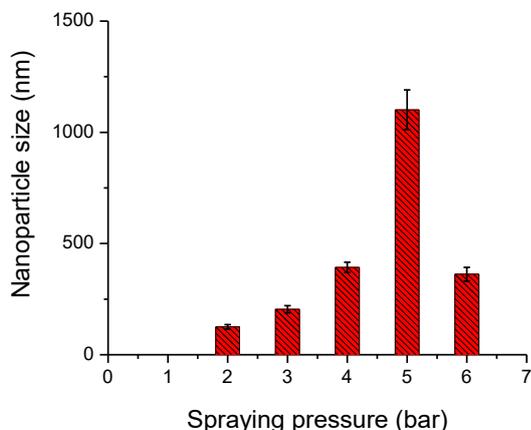
## 3. EXPERIMENTAL RESULTS AND DISCUSSION

### 3.1. Effect of Spraying Pressure on PECs Size

In the experimental setup used, the spraying pressure varied from 2 bar to 6 bar. The other setup of the experiments were as follows: shooting time - 100 ms, and pause time - 100 ms.



The dependence of the applied spraying pressure on the particle size is presented in Fig. 1.



**Fig. 1** Effect of the spraying pressure on the PEC nanoparticle size.

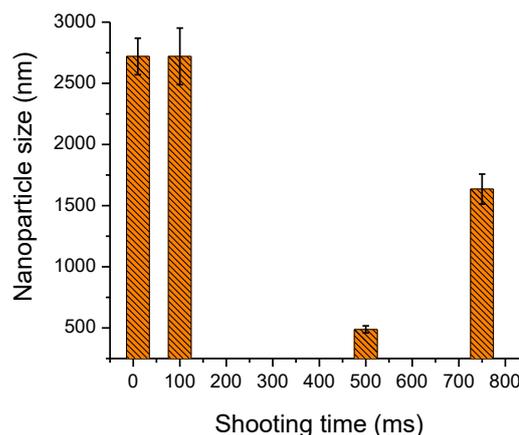
The smallest PEC sizes were obtained at spraying pressure 2 bar, which were 125 nm. It was found that the size of the resulting particles increased up to 1100 when the pressure increased to 5 bar. Upon further pressure increase up to 6 bar, a decrease in size was observed. This effect could be explained by the reduced mean free path of the PEC complexes when the pressure increases. As a result of more frequent collisions between them, at pressures up to 5 bar they aggregate. At a pressure of 6 bar, these aggregates are destroyed (Patwekar et al, 2016).

### 3. 2. Effect of Shooting Time on PECs Size

In order to determine the optimal shooting time of the jets, experiments were carried out at a pressure of  $p = 2$  bar, and a pause time between shots 500 ms. The shooting time was varied in the range of 10 ms to 750 ms and the PEC nanoparticle radius dependence of this time is shown in Fig. 2.

As the shooting time increases in the range from 10 ms to 500 ms, the particle size decreases. Further increase of the shooting time leads to a drastic increase of the particle size up to 1.7  $\mu\text{m}$ . The short shooting times do not have a significant impact on the size of the PEC since primary complexing is performed within 5 ms. Therefore, it can be considered that at all sho-

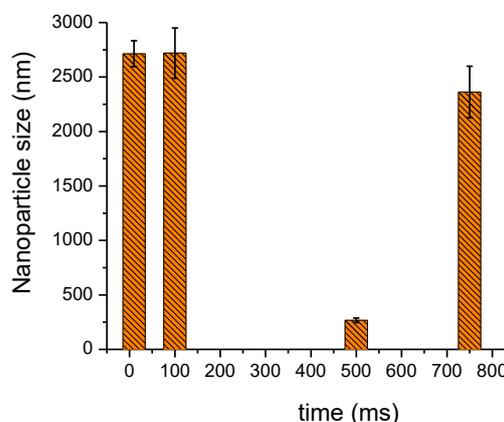
oting times used, PECs are formed in the time of collision between the two polyelectrolyte streams. Thus, the spraying time could have an impact on the formation of secondary complexes and their aggregation. At mixing times longer than 500 ms, there is again a rise in the mean radius of complexes due to aggregation (Ankerfors et al, 2010).



**Fig. 2** Effect of the shooting time on the PEC nanoparticle size.

### 3. 3. Effect of Pause Time on PECs Size

To determine the effect of pause time between two sprays in the range of 10 ms to 750 ms, all other process factors were retained unchanged:  $p = 2$  bar,  $\text{pH} = 4$ , ionic strength 100 mM, and spraying time equal to 500 ms. The dependence of the radius of the nanocomplexes on the pause duration is presented in the graph in Fig. 3.



**Fig. 3** Effect of the pause time on the PEC nanoparticle size.

At short breaks between sprays, at times shorter than 500 ms, complexes fail to leave the chamber before forming the complexes of the next spraying. Therefore, aggregation occurs between the particles of two consecutive sprays, resulting in an increase in the average size of the complexes. At a 500 ms pause there is enough time for the resulting complexes to drain through the pipeline before the next ones are formed. At times longer than this, an increase in complexes is observed.

### 3. 4. Investigation of PECs Stability during Storage

The stability of the complexes formed was studied for a 15 day storage period at 4 °C and different storage environment – Fig. 4.

It is noted that as the storage time increases, the average radius of the reference samples increases permanently. This process is due to nonreversible aggregation.

Changing pH of the acetate buffer from 4.5 to 8 significantly reduces the solubility of chitosan and reduces its reactivity. Therefore, a tendency for shrinkage is observed at the beginning of the storage period, where the particle radius decreases. After the initial contraction, the nanoparticle sizes were kept constant for 15 days.

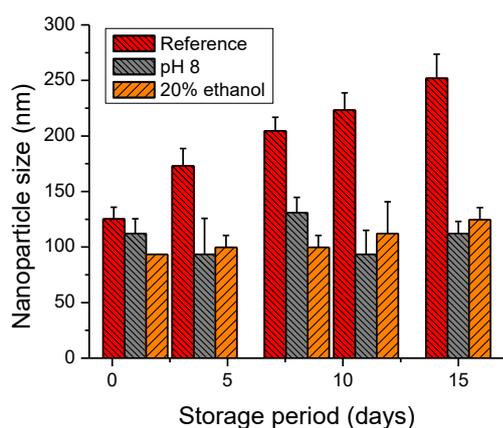


Fig. 4 PEC nanoparticle stability during storage.

Addition of ethanol into the acetate buffer again leads to reduced solubility of both the

electrolytes and shrinkage of the nanoparticles. They retain their size during the 15 day storage.

## 4. CONCLUSIONS

Based on the experiments and results obtained, the following conclusions can be drawn:

- The JET mixing method is suitable for the successful production of polyelectrolyte complexes with dimensions in the nanometric range.
- By controlling the technological parameters of the experimental setup, polyelectrolyte complexes of controllable sizes in the range of 80 nm to 2500 nm can be obtained.
- For the T-mixer system used, optimal shooting and pause times were found to be 500 ms and the optimal pressure was the lowest at which it was run -  $p = 2$  bar.
- Changing the pH of the solvent after the formation of the complexes or the addition of ethanol results in stabilization of the size of the complexes.

## ACKNOWLEDGEMENTS

The authors are thankful to Bulgarian Ministry of Education and Science - National Program for Young Scientists and Post-doctoral Students 2018/2020.

## REFERENCES

- Akbulut M., Ginart P., Gindy M. E., Theriault C., Chin K. H., Soboyejo W. and Prud'homme R. K., 2009. *Generic method of preparing multifunctional fluorescent nanoparticles using flash nanoprecipitation*, *Advanced Functional Materials*, 19, pp. 718-725.
- Ankerfors C., Ondaral S., Wågberg L., and Ödberg L., 2010. *Using jet mixing to prepare polyelectrolyte complexes: Complex properties and their interaction with silicon oxide surfaces*, *Journal of Colloid and Interface Science*, 351 (1), pp. 88-95.
- Gindy M. E., Panagiotopoulos A. Z. and Prud'homme R. K., 2008. *Composite block copolymer stabilized nanoparticle: Simulta-*



- neous encapsulation of organic actives and inorganic nanostructure, *Langmuir*, 24, pp. 83-90.
- Han J., Zhu Z., Qian H., Wohl A. R., Beaman C. J., Hoye T. R. and Macosko C. W., 2012. *A simple confined impingement jets mixer for flash nanoprecipitation*, *Journal of Pharmaceutical Sciences*, 101 (10), pp. 4018-4023.
- Hunter T. G. and Nash A. W., 1935. *Liquid-liquid extraction systems*, *Ind. Eng. Chem.*, 27, pp. 836-845.
- Johnson B. K. and Prud'homme R. K., (2003). *Mechanism for rapid self-assembly of block copolymer nanoparticles*, *Physical Review Letters*, 91, art. no. 118302.
- Johnson B. K. and Prud'homme R. K., 2003A. *Chemical processing and micromixing in confined impinging jets*, *AIChE Journal*, 49, pp. 2264-2282.
- Johnson B. K. and Prud'homme R. K., 2003B. *Flash nanoprecipitation of organic actives and block copolymers using a confined impinging jets mixer*, *Australian Journal of Chemistry*, 56, pp. 1021-1024.
- Klein V. I. and Ivanova N. A., 2002. *Spectro-turbidimetric Determination of the Size and Concentration of Terrestrial Particles in Potable Water*, *Russian Journal of Applied Chemistry* 75, No. 3, pp. 427-431.
- Liu W. T., 2006. *Nanoparticles and their biological and environmental applications*, *Journal of Bioscience and Bioengineering*, 102, pp. 1-7.
- Liu Y., Cheng C. Y., Liu Y., Prud'homme R.K. and Fox R.O., 2008. *Mixing in a multi-inlet vortex mixer (MIVM) for flash nanoprecipitation*, *Chem. Eng. Sci.*, 63, pp. 2829-2842.
- Johnson B. K. and Prud'homme R. K., 2003. *Mechanism for rapid self-assembly of block copolymer nanoparticles*, *Physical Review Letters*, 91 (11), art. no. 118302.
- Lince F., Marchisio D. L. and Barresi A. A., 2009. *Smart mixers and reactors for the production of pharmaceutical nanoparticles: Proof of concept*, *Chemical Engineering Research and Design*, 87, pp. 543-549.
- Liu Y., Cheng C. Y., Liu Y., Prud'homme R. K. and Fox R.O., 2008. *Mixing in a multi-inlet vortex mixer (MIVM) for flash nanoprecipitation*, *Chem. Eng. Sci.*, 63, pp. 2829-2842.
- Marchisio D. L., Rivautella L. and Barresi A. A., 2006. *Design and scale-up of chemical reactors for nanoparticle precipitation*, *AIChE Journal*, 52, pp. 1877-1887.
- Patwekar S. L., Potulwar A. P., Pedewad S. R., Gaikwad M. S., Khan S. A. and Suryawanshi A. B., 2016. *Review on polyelectrolyte complex as novel approach for drug delivery system*, *IJPPR*, 5, pp. 97-109.
- Treybal R. E., 1963. *Liquid extraction*, 2nd ed. New York: McGraw-Hill.