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Polysaccharides Based Nanoparticles as Protein Oral Delivery System

M.V. Konovalova[1,*], D.S. Khramova[2], A.V. Ilyina[1], D.V. Kurek[1]

[1] *Centre "Bioengineering", Russian Academy of Sciences, 7/1, Prospect 60-Letia Oktyabrya, 117312 Moscow, Russia*
[2] *Institute of Physiology, Komi Science Centre, Urals Branch of the Russian Academy of Sciences, 50, Pervomajskaya Str., 167982 Syktyvkar, Republic of Komi, Russia*

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Ovalbumin loaded chitosan and pectin based composite nanoparticles were obtained. The mean size, morphology and zeta potential of the nanoparticles were determined by dynamic light scattering (DLS) and atomic force microscopy (AFM). Effect oral administration of obtained nanoparticles on the immune response was studied.

Keywords: Composite nanoparticles, Pectin, Chitosan, Ionotropic gelation, Oral administration.

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1. INTRODUCTION

Polysaccharides are widespread biopolymers, which can be used as drug delivery systems [1]. Recently, a lot of attention is given to chitosan, pectin and their derivatives through their high biocompatibility, biodegradability, low toxicity and high affinity to macromolecules and cells. Currently, oral delivery is still the preferred and common route of drug administration. However, the main disadvantages of this method are instability of drugs under the action of different environmental conditions, such as low pH in the stomach, protease affection in small intestine, and microflora digestion in colon, particularly relates to drugs based on bioactive substances, peptides, proteins, nucleic acids, as well as their limited absorption in the intestine due weak adhesion. Solution of the problem of this route of administration is the creation of new drug delivery systems. Composite, nano-sized drug delivery systems are able to overcome a variety of biological barriers in the organism (intestine - blood channel, blood channel - tissue, blood-brain barrier), reduce the side effects (toxicity and allergenicity), prolong of a drug action in the organism [2]. In this study, foundation of creation of composite particles for oral administration is the complexation between oppositely charged polysaccharides and proteins.

2. MATERIALS AND METHODS

2.1 Materials

For nanoparticles preparation crab shell chitosan with molecular weight 200 kDa and deacetylation degree 86% (ZAO "Bioprogress", Moscow region, Russia), lowmethoxyl willow herb pectin with 14% degree of esterification (Vyatka State University, Kirov region, Russia) and ovalbumin (AppliChem, Germany) were used.

2.2 Preparation of Nanoparticles

Chitosan was dissolved in 0.25% acetic acid solution. The pH of chitosan solutions was initially adjusted with 0.25% NaOH to 5.2. Pectin and ovalbumin solutions were prepared in deionized water. Chitosan/Ovalbumin-nanoparticles (Chi/OVA-NPs) were obtained in a one-step procedure based on the ionotropic gelation with sodium tripolyphosphate (TPP) with following cover nanoparticles with pectin and 0.5 mM $CaCl_2$.

PEG-2000 was added to 0.4% solution of N-acetyl-chitosan to final concentration of 5 mg/ml. After that 0.1% ovalbumin solution was added to N-acetyl-chitosan solution at vigorous stirring at 30 rpm. Then 0.1% TPP (pH 7.0) was added to this solution. The formed dispersion was additionally stirred at 30 rpm during 20 min at 22°C. Chi/OVA-NPs were separated by centrifugation (14000 g, 20 min) and then resuspended in deionized water.

Nanoparticles (3.2 mg/ml) was injected under gentle stirring into a beaker containing pectin solution (1.25 mg/ml) and stirred for 30 min. After that, Chitosan/Ovalbumin/Pectin-nanoparticles (Chi/OVA/Pec-NPs) were separated by centrifugation (14000 g, 20 min) and then resuspended in 0.5 ml $CaCl_2$. Then Chi/OVA/Pec-NPs were separated by centrifugation (14000 g, 10 min) and freeze-dried.

2.3 Size and Zeta Potential Measurements

The mean particle size was measured by AFM using NTEGRA Prima microscope (NT-MDT, Russia) in a semi-contact mode with cantilevers NSG01 and zeta potential were determined by DLS using 90 Plus Particle Size Analyzer (Brookhaven instruments corporation, USA) at a scattering angle 900 and 661 nm wavelength.

2.4 Loading Efficiency

The loading efficiency (*LE*) value was calculated according to the following equation:

$$LE = [(W_0 - W)/W_0] \cdot 100\%,$$

where W_0 is the total amount of OVA used to prepare nanoparticles, W is the amount present in the supernatant after centrifugation at 14000 rpm for 20 min.

*mariya.v.konovalova@gmail.com

Samples were analyzed for OVA content using Bradford method.

2.5 In Vitro Release Studies

The release of OVA from Chi/OVA/Pec-NPs were carried out in model gastric and intestinal juices which were prepared according to procedure described in the article [3]. The Chi/OVA/Pec-NPs were incubated in a solution model gastric juice for 2 hours, after that Chi/OVA/Pec-NPs were separated by centrifugation (14000 g, 20 min) and then nanoparticles were resuspended in model gastric juice and were incubated during the 24 hours (t = 37°C). Samples were analyzed for OVA content using Bradford method.

2.6 Oral Immunization

Immunization was carried out according to the procedure described in the article [4]. Antibody titers to OVA were assessed using an enzyme-linked immunosorbent assay (ELISA).

2.7 Statistical Analysis

Results are presented as mean ± standard deviation (SD). The significance of differences between means was evaluated using Mann-Whitney test.

3. RESULTS AND DISCUSSION

Composite particles were formed by ionotropic gelation method. At first complex between oppositely charged chitosan and ovalbumin was obtained, then complex was cross-linked with TPP. The yield of particles ranged from 14 to 16%. Maximum loading efficiency of OVA (60%) was obtained at a ratio OVA:Chi equal 1:3.6 (Table 1).

Table 1 – Influence of the ratio of ovalbumin and chitosan on the loading efficiency of protein

№	Ratio of ovalbumin and chitosan	Concentration, mg/ml			Loading efficiency, %
		Chi	OVA	TPP	
1	1:5,3	2,10	0,40	0,08	23
2	1:4,0	1,42	0,35	0,12	49
3	1:3,8	1,60	0,42	0,09	48
4	1:3,6	1,80	0,50	0,10	60
5	1:3,2	1,80	0,55	0,14	46

Further, this ratio of polymers was used for the preparation of the particles. The Chi/OVA-NPs were coated by pectin and treated with $CaCl_2$ solution for additional crosslinking. Amount of pectin was selected empirically (Fig. 1) to obtain a stable, nano-sized particles. The graph shows that the pectin concentration of 1.25 mg/ml is sufficient for the formation of stable particles.

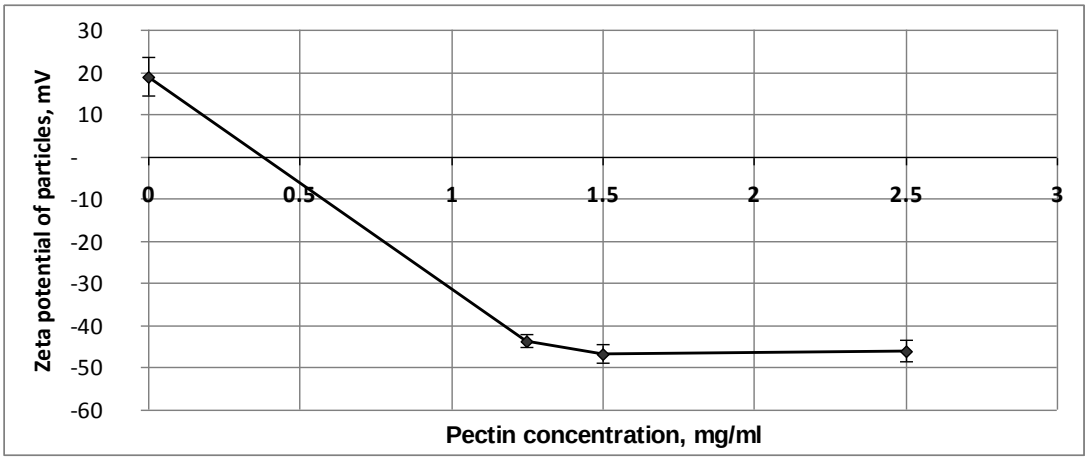

Fig. 1 – The influence of the pectin concentration on zeta potential of composite particles

According to the data AFM (Fig. 2) and the DLS particles are not covered by pectin have a size of 50 ± 10 nm and zeta potential of 19 ± 4.6 mV. After covering the pectin particle size slightly increased to 60 ± 10 nm, and the zeta potential was equal to -44 ± 1.5 mV.

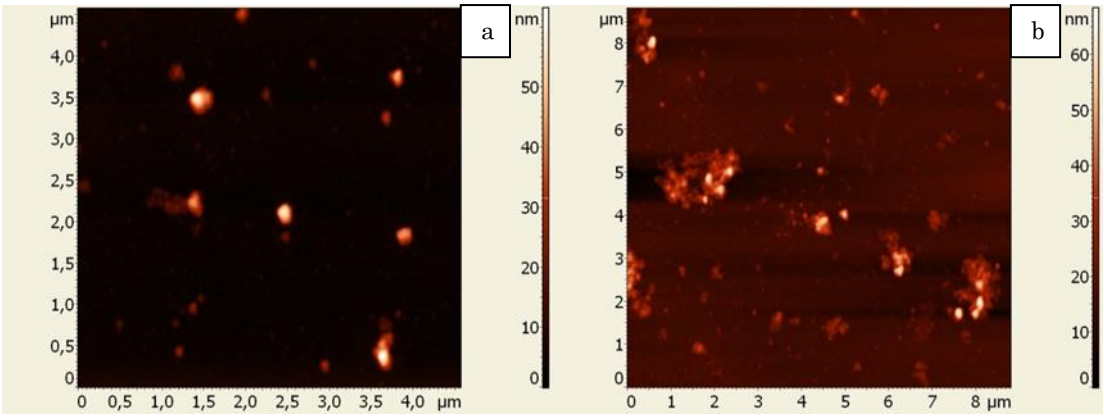

Fig. 2 – Morphology and particle size measured by AFM of Chi/OVA-NPs (a), Chi/OVA/Pec-NPs (b)

Investigation of the stability of the composite particles in model gastric and intestinal juices (*in vitro)* showed that ovalbumin completely released from the particles in the gastric environment in 2 hours.

The effect of the composite nanoparticles on the immune response was studied. In the experiment, female A/HeJ mice (20 – 25 g) were immunized three times with the antigen administered orally. Repeated oral immunizations were carried out on day 7 and 14 after the primary immunization. On day 21 blood samples were taken from animals. Fig. 3 shows ELISA results. Found that the composite particles reduced immune response comparing to control and have an immunosuppressive action.

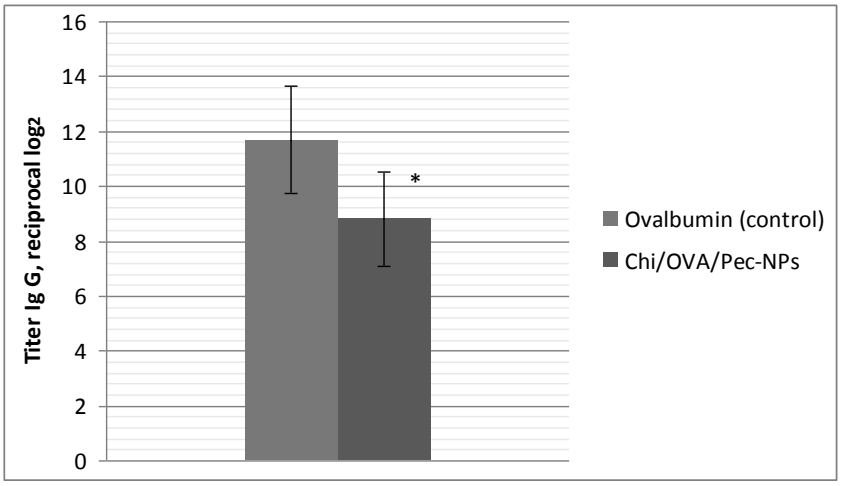

Fig. 3 – Effect of OVA loaded composite nanoparticles on immunoglobulin G (IgG) production. Data are presented as mean ± SD (n = 9). *P < 0.05 comparing to control

4. CONCLUSIONS

Thus, developed a method for producing chitosan and pectin based ovalbumin loaded composite nanoparticles release the protein in the gastric environment and possess immunosuppressive action. In further studies is planned to create nanoparticles having immunoenhancing action by using pectin, which has ability to be resistant to proteases and amylases, which are active in the gastrointestinal tract.

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