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TEM use for the study of chitosan microspheres and nanospheres obtained from its salts with several acids

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Abstract. Aminopolysaccharide-containing dispersed systems were obtained by coacervation based on a water–oil emulsion of a chitosan solution in glycolic or tartaric acid (a salt water-soluble form of the polymer) followed by neutralization of the salt form of the polymer with triethanoamine. Morphology was studied and the size of dispersed chitosan particles was estimated by the methods of transmission electron and polarization microscopy. The spherical shape of the solid-phase constituent of the dispersed system was established, whose size varied from 50 nm to 115 μm. The influence of the acid nature on the morphological structure, size and fineness of the formed chitosan-containing particles is shown. The disperse system formed using glycolic acid was unstable. The use of tartaric acid was found to contribute to the stabilization of a highly dispersed system. The effect of the component ratio of the dispersed system on the morphology and size of spherical chitosan-containing structures was studied.

Keywords: transmission electron microscopy, polarizing microscopy, chitosan, microspheres, nanospheres, chitosan tartrate, chitosan glycolate

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Применение ПЭМ для изучения микро- и наносфер хитозана, полученных из его солей с разными кислотами

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Аннотация. Методом коацервации на основе водно-масленной эмульсии раствора хитозана в гликолевой или винной кислоте (солевая водорастворимая форма полимера) с последующей нейтрализацией солевой формы полимера триэтаноамином получены полисахаридсодержащие дисперсные системы. Методами просвечивающей электронной и поляризационной микроскопии изучены морфология и определен размер дисперсных частиц хитозана. Установлена сферическая форма твердофазной составляющей дисперсной системы, размер которой варьируется от 50 нм до 115 мкм. Показано влияние природы кислоты на морфологическую структуру, размер и дисперсность сформированных хитозансодержащих частиц. Дисперсная система, сформованная с использованием гликолевой кислоты нестабильна. Обнаружено, что применение винной кислоты способствует стабилизации высокодисперсной системы. Исследовано влияние соотношения компонентов дисперсной системы на морфологию и размер сферических хитозансодержащих структур.

Ключевые слова: просвечивающая электронная микроскопия, поляризационная микроскопия, хитозан, микросферы, наносферы, тартрат хитозана, гликолят хитозана

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Introduction

Currently, much attention is paid to obtaining materials with efficient targeted delivery of biologically active substances on polymer matrices. In particular, the use of a matrix based on a chitosan-containing material may help stabilize a drug substance, prevent its biodegradation, and increase its biological activity [1–4]. The formation of dispersed chitosan particles is often based on the formation of a complex colloidal system, resulting in a wide range of sizes (microspheres and nanospheres) [5–9]. The multistage nature of the process of their obtaining is also undesirable [10, 11]. In this regard, it is necessary to control both the size of the resulting spheres and the structure of their walls, which transmission electron microscopy (TEM) can be successfully applied for. It is also noteworthy that, despite active research, chitosan-containing materials are still obtained on the basis of aqueous acetic solutions of chitosan [12–14]. The use of biologically active acids in combination with chitosan would make it possible to expect a synergistic effect and expand the application scope of spherical structures of microsizes and nanosizes [15, 16].

In this work, the effect of the acid nature on the formation of microspheres and nanospheres obtained in the course of the interfacial reaction of the polymer-analogous chitosan salt → base transformation was studied by the TEM method.

Materials and Methods

We used chitosan with an average viscosity molecular weight of 700 kDa, a deacetylation degree of 80 mol.% produced by CJSC Bioprogress (RF), 1.5% aqueous solution of glycolic acid (Sigma-Aldrich, USA), 6% aqueous solution of tartaric acid (KhimReaktiv SIAB, China), 50% aqueous solution of triethanolamine (CJSC Baza No. 1 Khimreakivov, RF), vegetable oil (MEZ Yug Rusi Ltd., RF).

The objects of our study were microspheres and nanospheres obtained from chitosan solutions in glycolic and tartaric acids. Polymer solutions with a concentration of 3 wt % were prepared by dissolving an air-dry sample of the polymer in the calculated volume of an aqueous acid solution with stirring on a magnetic stirrer at 22 ± 2°C for ~ 5–7 h. The polymer in solution was in the salt form (chitosan glycolate or tartrate). The conversion of chitosan into its basic form was carried out by treatment with an aqueous TEA solution. Particles were obtained by coacervation in two stages. First, an emulsion was prepared by mixing vegetable oil with a chitosan solution in glycolic or tartaric acid under stirring (1,000 rpm) and keeping the emulsion on a stirrer for 3 min. At the second stage, a neutralizing agent (TEA) was added and the system was kept for an hour on a stirrer until dispersion was formed. The mass ratio of all three components was varied.

Before microscopy, sample preparation was carried out by centrifugation (12,000 rpm) of the resulting dispersed system for 5 min, followed by removal of the upper oil part and dispersion with water. The procedure was repeated 5 times. Next, the aqueous dispersion was applied onto an aluminum substrate with a formvar coating and dried under vacuum on a K450X Carbon Coater for 5 min. The size of solid-phase nanoobjects was estimated by TEM using a MIRA\\LMU microscope at an accelerating voltage of 30 kV and a conducting current of 400 pA with a dark and bright field detector (TED/TEB). The size of solid-phase microobjects was assessed on a

LaboPol-2 polarizing microscope (RF) with crossed nicols and ×10 magnification. The photos were taken with a DMC 300, 3 MPx USB camera (China). Microsphere size calculations were performed from digital photos using image analysis software.

Gravimetric measurements were carried out on an Ohaus Discovery analytical balance (USA), weighing accuracy ± 0.01 mg.

Results and Discussion

The study of our disperse system in a polarizing microscope at several magnifications made it possible to observe clear solid-phase structures of micron size and spherical shape with a thin dark shell (Fig. 1). There was some difference in the morphology of the spherical particles formed from chitosan solutions in glycolic and tartaric acids. E.g., the use of tartaric acid made it possible to obtain particles with an almost ideal spherical shape. The use of glycolic acid led to some distortion of the spherical shape of the microparticles and their aggregation. It turned out that the acid nature affects not only the morphology, but also the dispersion degree of the resulting systems. For example, the size of the solid-phase spherical structures varied within ~ 50 nm – –115 μm and ~ 2.7 – 33 μm for chitosan tartrate and chitosan glycolate, respectively.

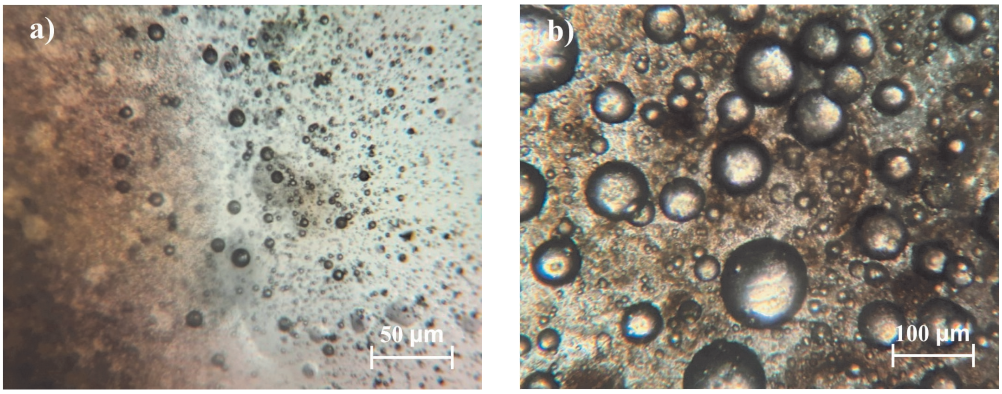

Fig. 1. Optical photos of spherical structures obtained from a chitosan solution in glycolic (*a*) acid and tartaric (*b*) acid

The TEM method made it possible to establish that the obtained dispersions also contain nanometer-sized particles (Fig. 2). Nanoparticles based on chitosan tartrate, like microparticles obtained from a similar polymer solution, predominantly had a spherical shape. The size of the spherical chitosan structures depended on the volume ratio of the aqueous (chitosan solution and TEA solution) and oil media. Such sizes are comparable with currently known analogues obtained using chitosan acetate and NaOH (~ 0.5–10 μm) [7].

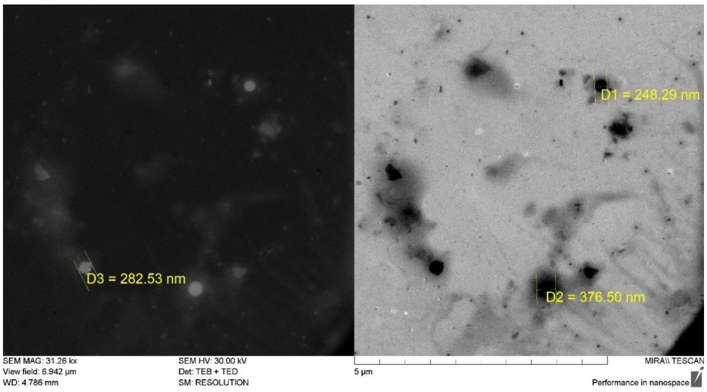

Fig. 2. TEM photo of the spherical structures obtained from a chitosan solution in tartaric acid

At the next stage, the kinetic stability of our resulting disperse systems was assessed. The objects were kept at room temperature (22 ± 2°C) for 30 days. During the experiment, it was found that dispersions of chitosan-containing microparticles and nanoparticles with a variable mass ratio of the components (oil: aqueous solution of the salt form of chitosan: TEA) were unstable and delaminated into few layers, namely: oil layers without and with some content of a small fraction of dispersed particles, and a layer of solid white precipitate. The dispersed systems of chitosan glycolate particles obtained at component ratios of 1:2:2 and 2:1:1 underwent phase separation within 30 min, while those obtained at component ratios of 1:1:1, 1:1:2, 1:2:1 and 2:1:2 did within 24 h. The dispersed system of chitosan tartrate particles was kinetically more stable. No phase separation was observed within a month for the 1:2:1 oil:chitosan tartrate:TEA system.

Conclusion

Thus, it was found that the nature of the acid-solvent, other things being equal, affects the size and structure of the resulting microspheres and nanospheres. Spherical particles obtained from a chitosan solution in tartaric acid had a wider range of sizes, in contrast to those obtained from a chitosan solution in glycolic acid. Using the PM and TEM methods, a relationship was found between the size of the obtained chitosan microspheres and nanospheres and the ratio of aqueous (chitosan solution and TEA solution) and oil media, which

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