Study of the supramolecularly ordered layered structure of chitosan gel films

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Abstract. Structural and morphological features of chitosan gel films with a radially periodic structure, obtained by neutralizing the salt form of the polymer with sodium hydroxide or triethanolamine, were visualized by scanning electron microscopy. The formation of such supramolecularly ordered layered structures was found to obey diffusion kinetics and the regularities of Liesegang periodic precipitation. The revealed dependence of the morphostructure of our chitosan gel films on the neutralizing reagent used is due to differences in the diffusion rate of inorganic and organic substance, as well as some spatio-temporal features of the mass transfer process.

1. Introduction

Studies of the physicochemical regularities of mass transfer during the formation of supramolecularly ordered structures will make it possible to control the process of layered ordering and obtain multilayer materials (capsules, plates, and films) with desired properties. An urgent solution to the problem of the short duration of drug substance in the biological environment, the uncontrolled release of the biologically active compound in the human body is the use of systems with targeted drug delivery to the zone of planned localization [1]. The targeted prolonged release of a drug from the polymer system makes it possible to effectively carry out therapy, simultaneously eliminating any negative effect of the drug upon the gastrointestinal tract and other side effects. Multilayer chitosan-containing gel films promising for targeted drug delivery were obtained in our work [2]. The method for their preparation is based on the interfacial reaction of polymer-analogous chitosan salt → base transformation [2]. Strong alkali (NaOH) was used as a neutralizing reagent. To increase the variety of layered structures and, at the same time, increase the environmental friendliness of the process of their preparation, it is advisable to utilize an organic alkaline reagent, e.g., triethanolamine. In this work, we performed a comparative analysis of the morphostructure of chitosan gel films obtained with an organic and inorganic base.

2. Materials

We used chitosan with a viscosity-average molecular weight of 700 kDa, a deacetylation degree of 80 mol% produced by Bioprosess Ltd. (RF) and a 1.5% aqueous solution of glycolic acid (Sigma-Aldrich, USA). Based on these reagents, a polymer solution with a concentration within 1.5–4 wt% was prepared, wherein chitosan was in the salt form (chitosan glycolate). Chitosan was converted into
its basic form with a 5% aqueous solution of NaOH (Khimreakiv Corp., RF) or 50% aqueous solution of triethanolamine ("Baza No. 1 Khimreakiv" Corp., RF).

3. Experimental methods
The objects of our study were gel films obtained from a chitosan solution in glycolic acid by converting the polymer into its basic form. The neutralizing agent effect on the surface and internal morphostructure of the samples was evaluated by scanning electron microscopy on a MIRA LMU microscope at a voltage of 15 kV and a conducting current of 400 pA. To explore the surface structure, the samples were prepared as follows: a glass mold with removable sides was filled with a chitosan solution, a solution of NaOH or triethanolamine was applied to the center of its surface until the salt groups were completely neutralized. The gel film sample was removed from the mold, washed with distilled water until neutral pH, and dried in air. To obtain a sample for exploring its internal structure, a chitosan solution in the mold was poured with an excess of a neutralizing agent, after the formation of the basic chitosan form, the sample was immersed in ethyl alcohol to remove water, then removed from the mold, cut into two parts with a medical scalpel, and dried in air. Before microscopic studies, a 5 nm thick layer of gold was deposited on the samples using a K450XCarbonCoater.

4. Results
During our experiment, it was found that the obtained chitosan-containing gel films were formed in the form of supramolecularly ordered radial periodic structures with bulky circular zones (Figure 1 and 2). An increase in the initial polymer concentration increased the number of circular zones, but insignificantly affected the width of rings with a common serial number.

![Figure 1. SEM photos of the surface of a gel film obtained from a 4 wt% chitosan solution in glycolic acid after NaOH neutralization: (a) – general view; (b) – central concentric zones.](image-url)

Earlier, the surface structure of a chitosan gel film was examined [2], in the preparation of which sodium hydroxide was used as a neutralizing agent. Three regions differing in morphostructure were identified, namely: the primary interface of an almost circular shape with spherulite formations, central concentric zones and peripheral edge rings, whose structural features and orientational ordering depend on the polymer concentration (Figure 1 a). Presumably, the formation of spherulite structures depends on the "ring" growth of crystallites, induced by the nuclei of a new phase formed upon contact of chitosan glycolate with NaOH, which is characteristic of the process of aggregation of spherulites during crystallization in high-viscosity polymer systems. The topographic morphology of the central circular zones of the gel film is represented by an array of circularly symmetric micro-rings (Figure 1 b). The width of these micro-rings varies in the range of ~2–6 μm. The circular shape of the
mutual orientation of the supramolecular formations is also preserved at the periphery, however, some compaction of the material structure is observed.

When using an organic neutralizing agent (triethanolamine), three regions differing in morphostructure were also observed (Figure 2 a). However, there were a number of significant differences. First, the primary gel-film interface is represented by supramolecular formations as scale-like particles arranged in ribbon-like structures (Figure 2 b). Possibly, this orientation is formed as a result of the presence in the sample of a complex salt of tris (2-hydroxyethyl) ammonium glycolate, which has a pseudotricyclic structure [3]. The width of micro-rings of circular symmetry in the central zone differs insignificantly and varies in the range of 2–7 μm, and it is 400–700 nm at the periphery (Figure 2 c, d). Some differences in the supramolecular ordering of the central and edge ring regions of the periodic structure may be due to some kinetic features of mass transfer [4], which is currently being investigated.

![Figure 2. SEM photos of the surface of a gel film obtained from a 4 wt% chitosan solution in glycolic acid after TEA neutralization: (a) – general view; (b) – primary interface; (c) – central rings; (d) – peripheral structure.](image)

In addition, the morphology of the internal structure on a perpendicular section of the sample was studied. It was found that the formation of circular zones occurred not only on the surface, but throughout the entire volume of the sample (Figure 3).
Figure 3. SEM photo of a perpendicular section of a gel film obtained from a 4 wt% chitosan solution in glycolic acid after NaOH neutralization.

The results obtained for the formation of spatially separated concentric rings in the structure of our chitosan gel-film show common features with the main regularities of the Liesegang phenomenon [5–8]. To confirm this assumption, the spatiotemporal evolution of the formation of the chitosan solid phase in the gel film in the course of the studied reaction of polymer-analogous transformation was analyzed by the method from Ref. [2]. It was proved that for both neutralizing reagents the formation of a chitosan base layer obeyed diffusion kinetics and the regularities of Liesegang periodic sedimentation.

Thus, as a result of using the method of scanning electron microscopy, features of the structure of gel films of the chitosan base obtained using an organic and inorganic alkaline reagent were visualized. It was found that the supramolecular ordering of the samples depended on the neutralizing reagent nature. This makes it possible to obtain gel films with a predetermined structure. The use of triethanolamine makes it possible to prepare multilayer structures with a large number of periodic formations.

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