THE INFLUENCE OF THE TYPE SOLVENT ON THE STRUCTURE OF CHITOSAN BLENDS WITH HYALURONIC ACID

Katarzyna Lewandowska*, Alina Sionkowska, Sylwia Grabska

Nicolaus Copernicus University in Toruń,
Faculty of Chemistry, Department of Chemistry of Biomaterials and Cosmetics,
7 Gagarin Street, 87-100 Toruń Poland
e-mail: reol@chem.umk.pl

Abstract

The influence of the type solvent on the structure of chitosan, hyaluronic acid and their blend films was investigated. Aqueous acetic acid, hydrochloric acid, sodium chloride and aqueous acetic acid/NaCl were used as solvents for chitosan, hyaluronic acid and Ch/HA solution blends. Ch, HA and their blend films were prepared by casting technique. The homogeneity and morphology of chitosan blends were ascertained from the tapping-mode atomic force microscopy (AFM) and scanning electron microscopy (SEM). The changes of topography images are considered by determining the root mean square (RMS, Rq) deviation in the image data. The surface roughness of chitosan, hyaluronic acid and Ch/HA blended films was altered by mixing. The structure of chitosan blends with hyaluronic acid depends on the blend composition and on the solvent used for preparing the blend.

Key words: *chitosan, hyaluronic acid, polymer blends, surface properties, microscopy.*

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1. Introduction

In this paper, the blends composed of chitosan with hyaluronic acid at different component ratio have been prepared as material designed for different applications e.g. in cosmetic industry or biomedical applications. Nowadays, there is a tendency toward a greater use of natural polymers that are obtained from renewable resources. It is known, chitosan and hyaluronic acid can be considered as good candidates for the preparation of new biopolymer blends because of their potential attractive properties such as biocompatibility, biodegradability, solubility in an aqueous solution, and non-toxicity for humans [1-5].
The purpose of this study was to evaluate the physico–chemical properties of chitosan/hyaluronic acid blends. The properties of chitosan (Ch), hyaluronic acid (HA) and their blends have been investigated by the tapping-mode atomic force microscopy (AFM) and scanning electron microscopy (SEM). Atomic force microscopy (AFM) and scanning electron microscopy (SEM) are useful methods for the study of structure and homogeneity of polymer blends [6-9].

2. Materials and Methods

2.1 Materials

Hyaluronic acid (HA) is a commercial polymer from Aldrich Company with a viscosity average molecular weight of 1.8x106. Chitosan (Ch) sample has a degree of deacetylation of 78% with a viscosity average molecular weight of 0.59 x106. The polymeric samples were solubilized separately in the solvent. The polymer concentration in the solution was kept constant at 1wt. %. Chitosan was solublized in aqueous 0.1mol/dm3 CH3COOH/0.2 mol/dm3 NaCl. For the hyaluronic acid, we used different solvents such as 0.3 mol/dm3 NaCl, 0.1mol/dm3 CH3COOH/0.2 mol/dm3 NaCl and 0.1 mol/dm3 HCl. Ch/HA blends were prepared from mixed polymer solutions. The composition of Ch/HA was 80/20, 50/50 and 20/80. The films were prepared by casting one drop of approximately 20-30 µl on the glass surface of 1 cm2. The films were not removable, because they were thin and fragile.

2.2 Methods

Topographic imaging was performed in air using a multimode scanning probe microscope with a Nanoscope IIIa controller (Digital Instruments Santa Barbara, CA) operating in the tapping mode, at room temperature. Surface images, using the scan widths ranging from 1µm to 10 µm, with a scan rate of 1.97 Hz were acquired at fixed resolution (512x512 data points). The roughness parameter such as the root mean square (Rq) was calculated for scanned area (1µm x 1µm) using Nanoscope software.
The morphology of the samples was studied using Scanning Electron Microscopy (SEM) Quanta 3D FEG.

3. Results and Discussion

The surface properties of Ch/HA blend films were observed using AFM microscopy. Examples of AFM images for chitosan, hyaluronic acid and Ch/HA blends are shown in Figures 1 and 2. The corresponding roughness values are presented in Table 1.

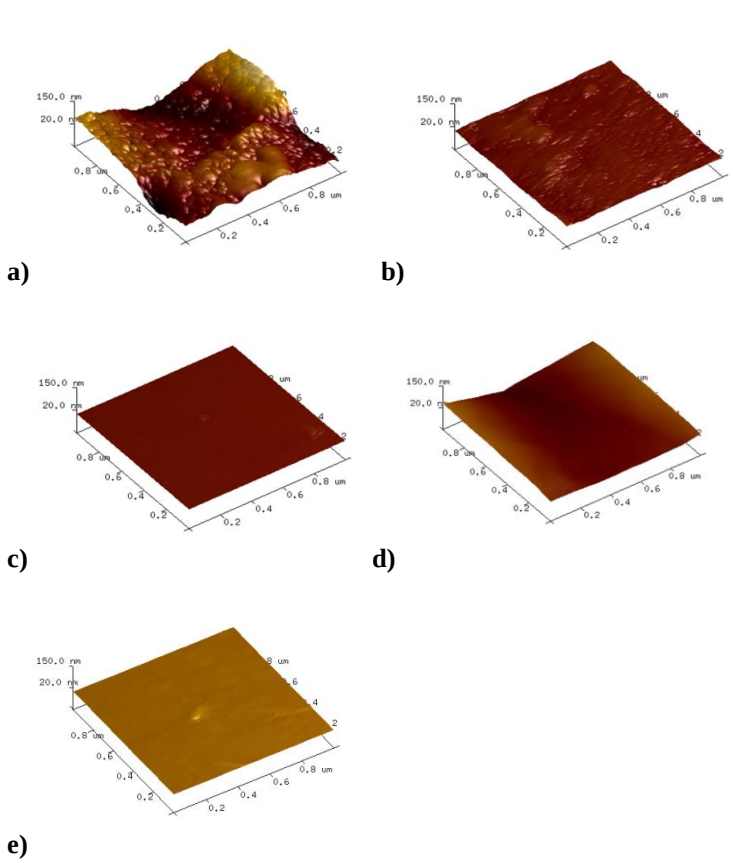

Figure 1. AFM images of the surface of films: a) Ch, b) HA – films cast from 0.3 mol/dm3 NaCl c) HA – films cast from 0.1 mol/dm3 HCl, d) HA – films cast from 0.1mol/dm3 CH3COOH/0.2 mol/dm3 NaCl, e) 0.3 mol/dm3 NaCl (solvent film).

Table 1. The roughness parameters (Rq) for films of different compositions

Sample	Rq (nm)
NaCl	1.0
Ch	29.8
HAa	4.1
HAb	0.6
HAc	11.2
80/20a	33.2
20/80a	19.0
80/20b	8.1
20/80b	21.2

a HA dissolved in 0.3 mol·dm-3 NaCl; **b** HA dissolved in 0.1 mol·dm-3 HCl;
c HA dissolved in 0.1 mol·dm-3 CH3COOH/ 0.2 mol·dm-3 NaCl,

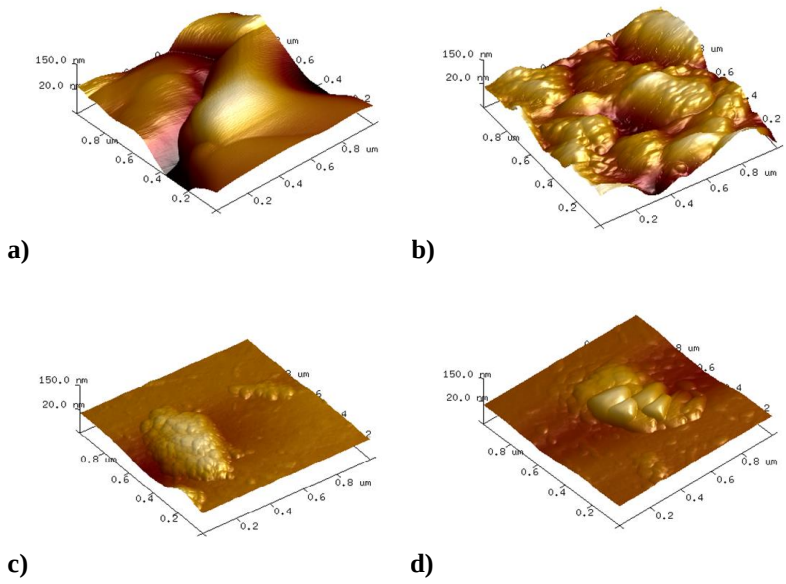

Figure 2. AFM images of the surface of films made of Ch/HA: a) 80/20 (HA – dissolved in 0.3 mol/dm3 NaCl), b), 20/80 (HA – was dissolved in 0.3 mol/dm3 NaCl), c) 80/20 (HA –dissolved in 0.1 mol/dm3 HCl), d) 20:80 (HA – dissolved in 0.1 mol/dm3 HCl),

As it can be seen in Figure 1e, the surface of solvent film is rather flat and does not contain any symptom of surface roughness. The surface morphology of chitosan film exhibits a well-defined island structure which can result from the crystallinity of the sample. In the case of HA films, the surface morphology depends on the ionic strength of the solution and the solvent used for preparing the films (Figures 1b-d). The HA film has a relatively flat and smooth surface when the film was casted from 0.1 mol·dm-3 HCl solution. The roughness parameter of HA film is the lowest among the investigated polymer samples (Table 1). For the HA films in different solvents, the observed changes in morphology are related to the polyelectrolyte properties. In the 0.1 mol·dm-3 HCl solution, the ionisation of the carboxylic acid groups of the HA is suppressed due to the low pH of the solvent medium. In this solution, HA is not a polyelectrolyte. These reasons could be responsible for the extremely low roughness of the film produced from this system. These conclusions were confirmed by FTIR results as is shown in our previous paper [10].

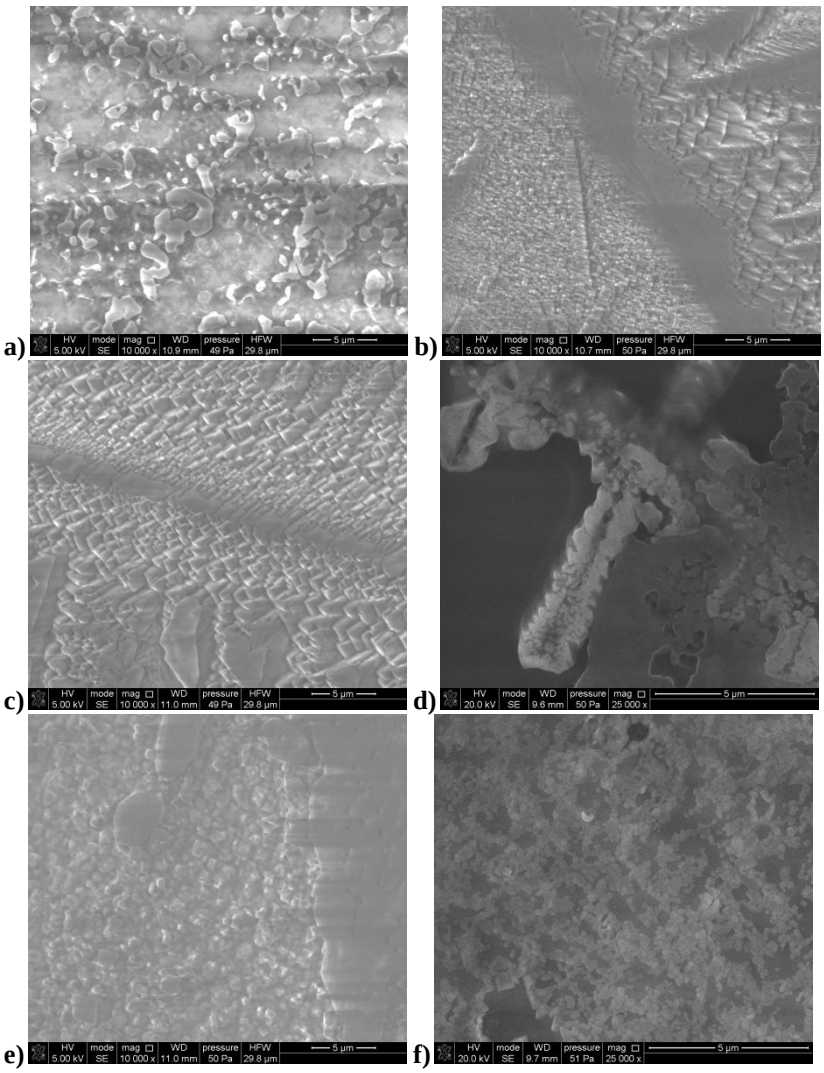

Figure 3. SEM microphotograph of the surface of chitosan, HA and their blend films.
a) Ch, **b)** HA – films cast from 0.3 mol/dm3 NaCl c) HA – films cast from 0.1 mol/dm3 HCl, **d)** HA – films cast from 0.1mol/dm3 CH3COOH/0.2 mol/dm3 NaCl **e)** 80/20 (HA – dissolved in 0.3 mol/dm3 NaCl), **f)** 80/20 (HA – dissolved in 0.1 mol/dm3 HCl).

In the case of Ch/HA blends, the surface morphology of blend depends on its composition and the solvent used for preparing the films (Figures 2a-d). AFM images of all the chitosan blends have also revealed that the chitosan is concentrated on the surface of the blends. The roughness of the Ch/HA blends decreases with the decreasing chitosan content for the blend films in 0.1 mol·dm-3 HCl solution (Table 1). This may indicate the improvement of the homogeneity of these blends when the HA was dissolved in 0.1 mol·dm-3 HCl solution. The attractive force and/or electrostatic interactions between components in the blends may lead to a decrease in the size of the microdomains, as shown in Figure 2. It is well known, chitosan and hyaluronic acid are all polyelectrolytes, in which the properties are strongly associated with the electrostatic interactions that determine the shape of the

macromolecule [5]. In the aqueous solution of a polyelectrolyte, the macromolecules are stretched due to the electrostatic repulsive forces between the charges on the functional groups. The addition of salt to the solution induces an increase in the solution ionic strength and screens the electrostatic charges. Then, the macromolecule conformation reduces to the statistical coil conformation. In the case of the 0.1 mol×dm-3 HCl solution, HA is a non-ionic polymer, whereas ionic groups of chitosan are screened (solvent: 0.1mol/dm3 CH3COOH/0.2 mol/dm3 NaCl). Thus, in these blends, mainly interactions predominate by hydrogen bond.

Figure 3 shows SEM microphotographs of the top surfaces of unmodified polymer films and of their blends when HA was dissolved in 0.3 mol/dm3 NaCl and 0.1 mol/dm3 HCl, respectively. It could be observed that the SEM microphotographs of all investigated samples show ordered structures with oval-shaped particles. The smallest nanodomains (below 1μm) are observed with the Ch/HA blend film when the HA was dissolved in 0.1 mol·dm-3 HCl solution. The observed changes in the SEM data are in agreement with the AFM results.

4. Conclusions

At the presented paper the structure of chitosan blends with hyaluronic acid in different solvents was studied. The surface morphologies of chitosan, hyaluronic acid and Ch/HA blended films were altered by mixing. For the chitosan and hyaluronic acid films the AFM and SEM images showed a crystalline structure. In the case of Ch/HA blends, the crystalline structure is visible both in AFM and SEM micrographs suggesting that the mixing of polymers does not disturb the crystallization process. For the Ch/HA blends when the HA was dissolved in 0.1 mol·dm-3 HCl solution, the images show smaller nanodomains on the surface in comparison to the unmodified polymers and other blends.

5. Acknowledgement

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6. References

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