ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 6, Issue 7 , July 2019

On Obtaining Inter Polyelectrolyte Complexes of Chitosan Bombyx Mori with Collagen

KhudoiberdievSh.Sh., ErgashevK.Kh., Vokhidova N.R., RashidovaS.Sh.

100127, Institute of chemistry and physics of polymers of the Academy of sciences of the Republic of Uzbekistan, Tashkent

ABSTRACT: Interpol electrolyte complexes (IPEC) based on bombyx mori chitosan with collagen were obtained. IPEC has been studied by conduct metric, potentiometric, viscometric, and also by UV and IR spectroscopic, microscopic methods.

It has been established that at the mass ratio of chitosan collagen (R) R = 4.225, the functional groups of macromolecules —COOH and NH_2 will be mutually compensated. This is confirmed by conduct metric and UV spectroscopic methods. It was found that, under the chosen synthesis conditions, nanoparticles are formed in all ratios in the size range from 25 to 400 nm. The resulting complexes of chitosan with collagen are of interest in obtaining bactericidal films for veterinary medicine and medicine.

KEYWORDS*:* chitosan bombyx mori, collagen, interpol electrolyte complexes, nanoparticles

I. INTRODUCTION

In recent years, studies on the production of chitosan (Chitosan) interpol electrolyte complexes with polyanions and polyampholytes of natural and synthetic origin have been of interest [1–5].

Chitosan, a natural polycation, is obtained from renewable raw materials with a number of properties that are attractive in many fields of science, biotechnology and medicine. ChZ has unique physicochemical and biological properties, the environments of which can highlight its biocompatibility, biodegradability, antimicrobial action, etc. The attention of researchers is attracted by the ability of chitosan to form covalent and non-covalent complexes with other polyelectrolytes [6]. These complexes have specific properties that define new areas of their use.

The conformational, molecular weight characteristics, pH, concentration, molar ratio of polyelectrolytes, the degree of DE acetylation and other factors affect the complexation of chitosan complex, which requires a specific approach to the preparation of complexes based on it [6-7]. On this basis, the preparation of chitosan complexes with polyampholyte - collagen and the study of the process of their complexation is relevant in a fundamentally applied aspect. In this work, we studied the interaction of chitosan with collagen in aqueous solutions.

II. MATERIALS AND RESEARCH METHODS

To obtain polymeric complexes, Bombyx mori chitosan (ChZ) (molecular weight 15,000, degree of DE acetylation 85%), purified collagen, acetic acid of the chemically pure grade, and deionized water were used. Chitosan Bombyx mori was obtained according to the method [8], collagen was isolated from animal waste - the skin according to [9].

UV spectroscopic studies were carried out with a SPECORD 210 spectrophotometer in the range of 190–1000 nm. Accuracy of photometry UV with potassium dichromate in accordance with Ph.Eur. ± 0.01. The morphology of the films of interpol electrolyte complexes was studied using an AFM Agilent 5500 atomic-force microscope (USA) at 22 ° C. We used silicon cantilevers with a hardness of 9.5 N / m with a happiness of 145 kHz. The maximum scanning area for AFM in X, Y is 15x15 μm2, in Z – 1 μm.

The viscosity of polymer solutions was studied in Ubbelode's viscometer [10] with a solvent expiration time to = 91 s, at 298.15 ± 0.05 K in acetate buffer.

Conduct metric titration was carried out on a Mettler-Toledo AG instrument, Analytical CH-8603 Schwerzenbach, Switzerland, potentiometric titration was carried out on a pH meter 150 MI with a glass electrode as a measuring, at 20 ° C, Russia.

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 6, Issue 7 , July 2019

III.RESULTS AND DISCUSSIONS

Determination of the degree of chitosan DE acetylation by potentiometric titration

Weighed 0.13 g of the chitosan sample and dissolved in 50 ml of a 0.02 M HCI solution. Then the pH of the solution was measured in a pH meter of 100 M, which is equal to 1.98. The ChZ solution was titrated with a 0.1 M NaOH solution to pH = 6.3 (Fig. 1).

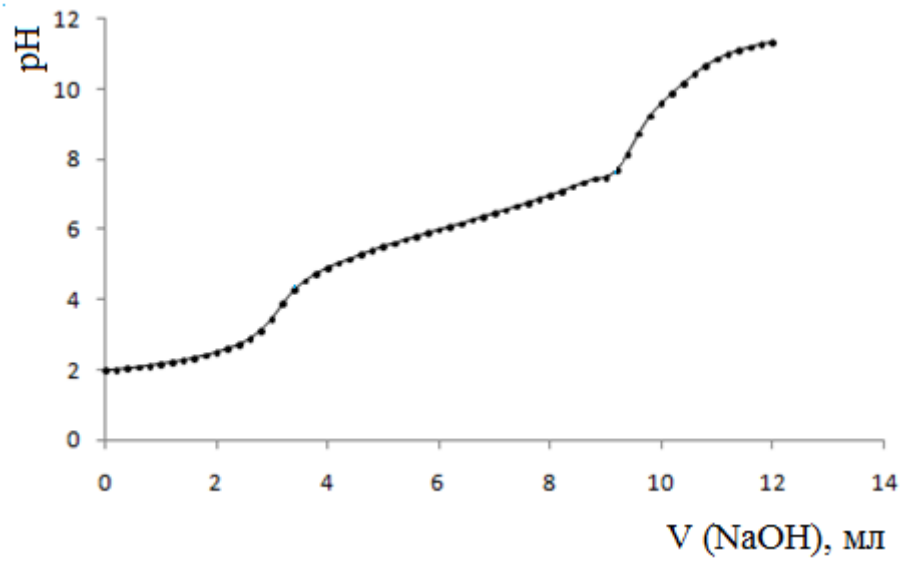


Fig. 1. Potentiometric titration of chitosan solution with 0.1 M sodium hydroxide solution

According to the following formula, the degree of DE acetylation of chitosan was calculated, which is equal to0,85 (85%)[11]:

$$DD = \frac{\Delta V \cdot C_{NaOH} \cdot 10^{-3} \cdot 16}{M \cdot 0{,}0994}$$

ΔV=6,8mlNaOH; M_{chz}=0,133 g

$$DD = \frac{6.8 \cdot 0{,}1 \cdot 10^{-3} \cdot 16}{0{,}13 \cdot 0{,}0994} = 0{,}85$$

Determination of the degree of chitosan DE acetylation by conduct metric titration

0.147 g of chitosan was weighed and dissolved in 20 ml of a 0.1 N solution of HCl, the initial pH of the solution was 1.56. Then, the ChZ solution is titrated with 0.1 M NaOH every 30 seconds and the amount of alkali consumed and the conductivity of the solution are fixed (Fig. 2).

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 6, Issue 7 , July 2019

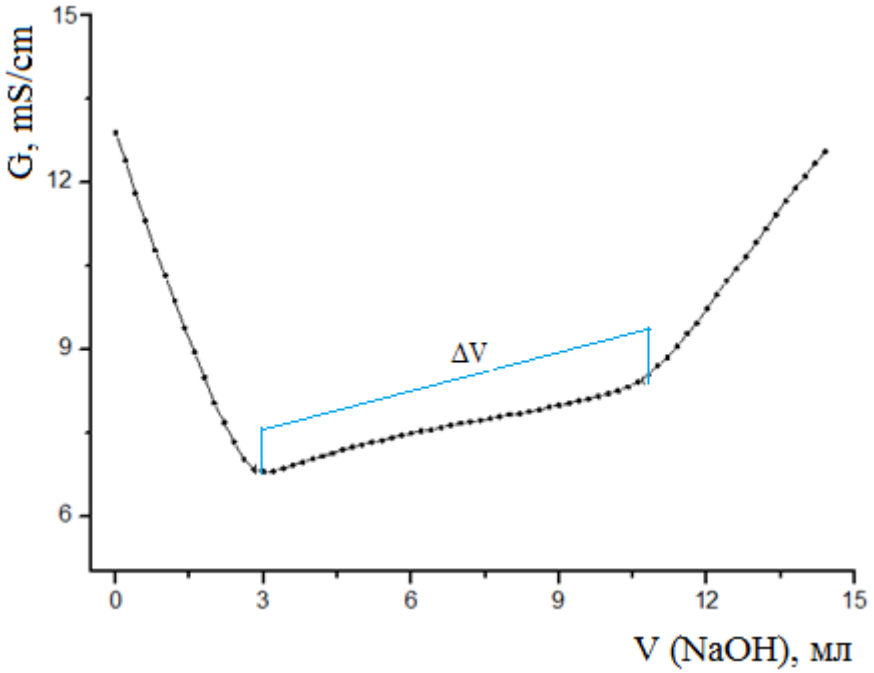


Fig. 2. Conduct metric titration of chitosan solution with 0.1 M sodium hydroxide solution

ATS is calculated by the formula, which is equal to 85%[12]:

$$\%DD = \frac{[base](V_2 - V_1) \times 161}{m}$$

ΔV=10.8-3=7.8 mlNaOH;

DD=0.1×7.8×161/147=0.85 (85%)

SSC-spectroscopic study of collagen

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 6, Issue 7 , July 2019

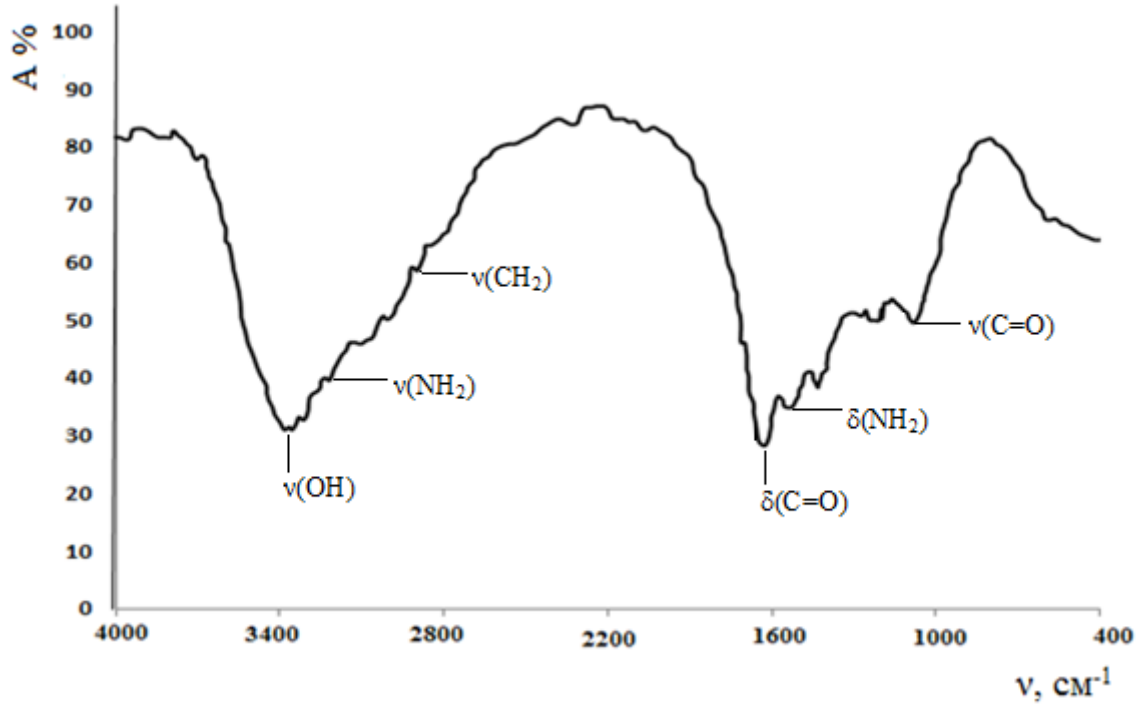


Fig. 3. IR spectrum of collagen macromolecules

According to the results of the IR spectrum of collagen, it can be seen that the collagen macromolecule has absorption bands in the range of 500-4000 $см^{-1}$. At 3200–3600 $см^{-1}$, the absorption bands of the NH_2, N – H, –OH groups are observed, and at 1100 $см^{-1}$there are absorption bands of –C – N = and C = O groups. In the range of 3800–3000 $см^{-1}$, a wide absorption band with a maximum at 3450 $см^{-1}$is observed, which is associated with stretching vibrations of the –OH and $–NH_2$ groups. Absorption bands at 2900 $см^{-1}$and 2960 $см^{-1}$indicate stretching vibrations of the –CH– (CH_2) groups.

In the structure of collagen, there are absorption bands of the amide 1 and amide II groups at 1500–1700 $см^{-1}$, more precisely, the absorption bands of the carbonyl group> C = O are observed at 1650 $см^{-1}$. The absorption bands at 1540 $см^{-1}$, 1460 $см^{-1}$, 1335 $см^{-1}$, 1240 $см^{-1}$are quite intense. The absorption bands at 1060 $см^{-1}$and 1115 $см^{-1}$are the doublet; at 1540 $см^{-1}$, the absorption bands from the deformation vibration —NH_2 from the Amide II groups are observed.

Determination of isoelectric point of 1% collagen solution

In order to obtain stable chitosan collagen complexes, the effect of pH on the behavior of collagen macromolecules was studied. Since collagen is a polyampholyte, its isoelectric point is determined. For this prepared 1% collagen solution with an initial pH of 4.95. Then, solutions of collagen with pH 2.0 were prepared by titration with 0.03 N solution of HCl; 2.5; 3.0; 3.5; 4.0; 4.5. And also prepared solutions of collagen with a pH of 5.5; 6.0; 7.0; 8.0; 9.0; 10 by titration with a 0.02 N solution of NaOH. After that, at 298 K, the viscosity of each solution was measured (Fig. 4).

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 6, Issue 7 , July 2019

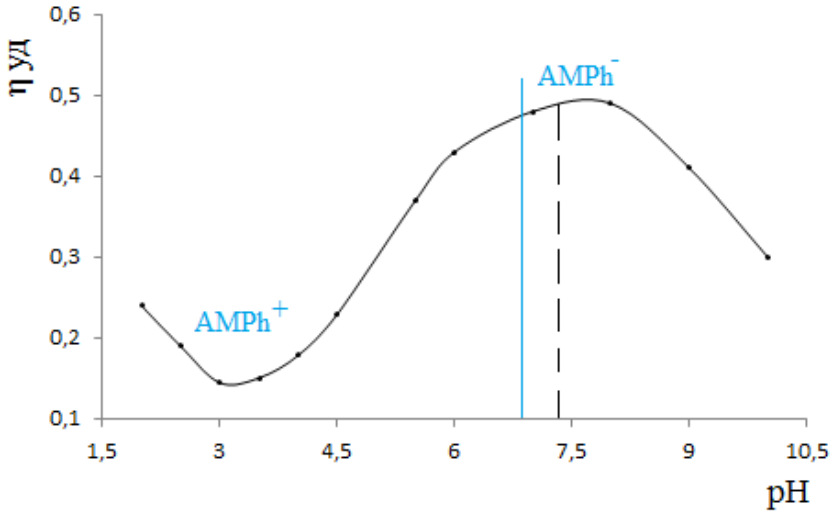


Fig. 4. The effect of pH on the specific viscosity of a 1% collagen solution

Based on the titration, it has been established that the structure of collagen, which is a polyampholyte, contains amino and carboxyl groups, i.e. at pH = 3.1, the amino groups are protonated and the interaction of chitosan with collagen occurs due to its carboxyl groups. On this basis, it is possible to carry out a directed synthesis of one or another functional group.

The study of the interaction of macromolecules chitosan Bombyx mori with collagen
Viscometric method

The interaction of chitosan macromolecules with collagen has been investigated by the viscometric method (Fig. 5). To do this, a 0.5% acetic acid solution of ChZ was prepared and it was titrated with a 0.2% aqueous solution of collagen and the solution expiration time was recorded. The measurement was carried out at pH-3.1, i.e. collagen amino groups are shielded; chitosan amino groups are supposed to be interconnected by –COOH groups.

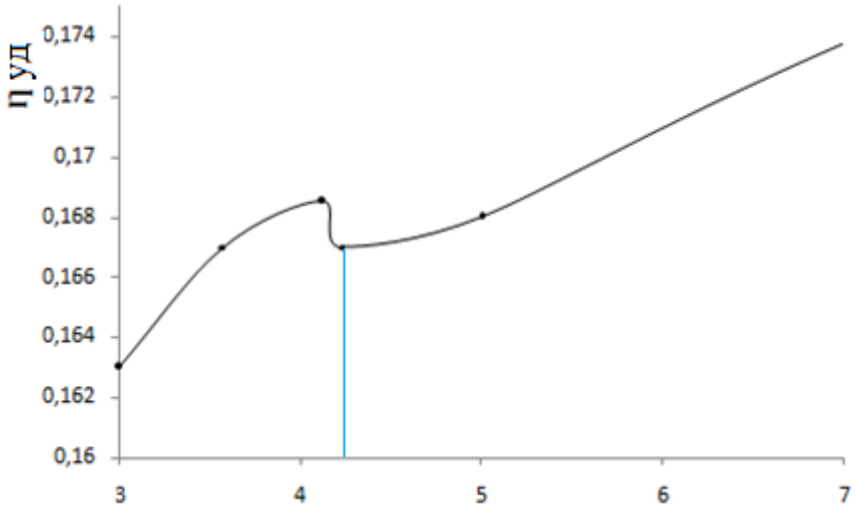


Fig. 5. The dependence of the viscosity of the solution on the mass ratio of ChZ. collagen (R)

The results indicate that no turbidity was observed during the measurements. It should be noted that during the titration a viscosity jump was detected at a ratio of R = 4.225. This is probably due to a decrease in the particle size of

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 6, Issue 7 , July 2019

IPEC due to the maximum interaction of the chromium amino groups with the carboxyl group of collagen, after which, with an increase in the content of collagen in the reaction mixture, the viscosity of the solution increases accordingly.

Conduct metric method

Prepared 12 ml of a 0.5% acetic-aqueous solution of X3 with a pH of 2.96. Also 0.2% aqueous solution of collagen with a pH of 6.2. The ChZ solution was titrated with 0.1 ml of collagen solution for 30 seconds. with fixing the conductivity of the solution (Fig. 6).

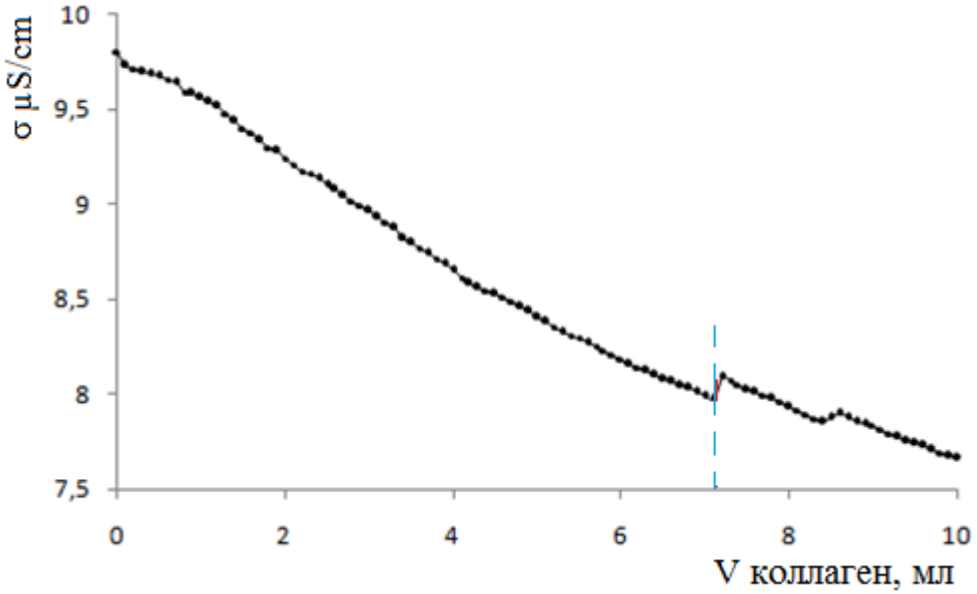
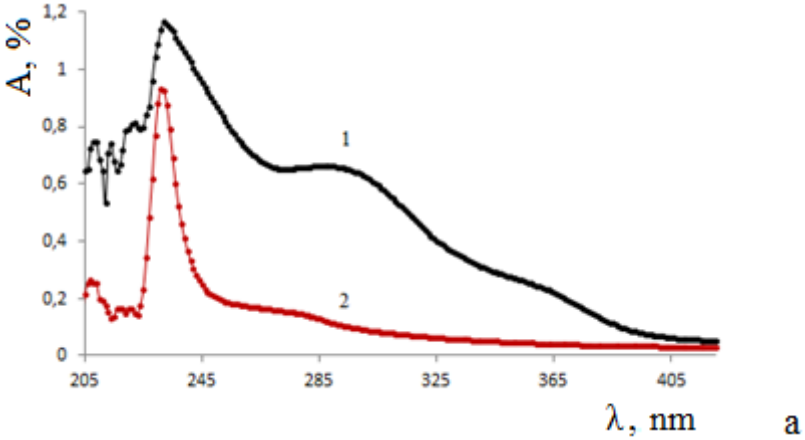


Fig. 6. Conduct metric titration of chitosan solution with collagen

The results show that after the mass ratio of chitosan: collagen R = 4.225, a jump is observed in the titration curve, the curve is identical to the curve of the original collagen. That is, in this ratio, the functional amino groups of chitosan are mutually compensated by the –COOH collagen groups, that a change in the line indicates the presence of unreacted collagen in the system.

UV spectroscopic studies of chitosan collagen and their complexes

UV spectroscopic studies of solutions of chitosan, collagen and their complexes were carried out at different ratios (Fig. 7a).



From the UV spectrum of the ChZ solution (Fig. 7a, 1 line) it can be seen that the absorption bands of acetamide and amino groups appear at λmax = 235 and 290 nm, respectively.

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 6, Issue 7 , July 2019

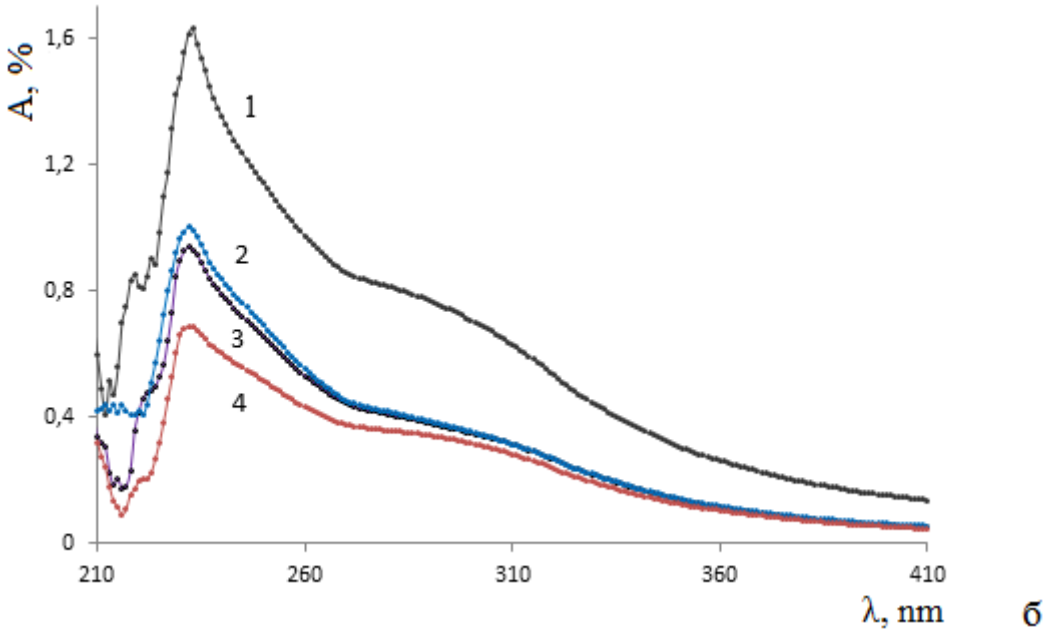


Fig. 7. UV spectra of chitosan (1) and collagen (2) (a) and interpolyelectrolyte complexes of chitosan with collagen (b): 1)R=4,225; 2) R=3,57; 3)R=4,1; 4) R=8,57

In the UV spectrum of collagen, absorption bands associated with amino and carboxyl groups in the range of 200–400 nm are observed (Fig. 7a, line 2), which, when producing complexes with chitosan, these absorption bands overlap.

In the spectra of chitosan collagen complexes (Fig. 7b), all absorption bands are observed that relate to both chitosan and collagen. It should be noted that only the intensity of the absorption bands of functional groups changes with a change in the ratio of chitosan: collagen. With a ratio of R = 4.225, a relatively high absorption intensity is observed, which confirms the results obtained above. Perhaps this is due to the high degree of binding of X3 with collagen.

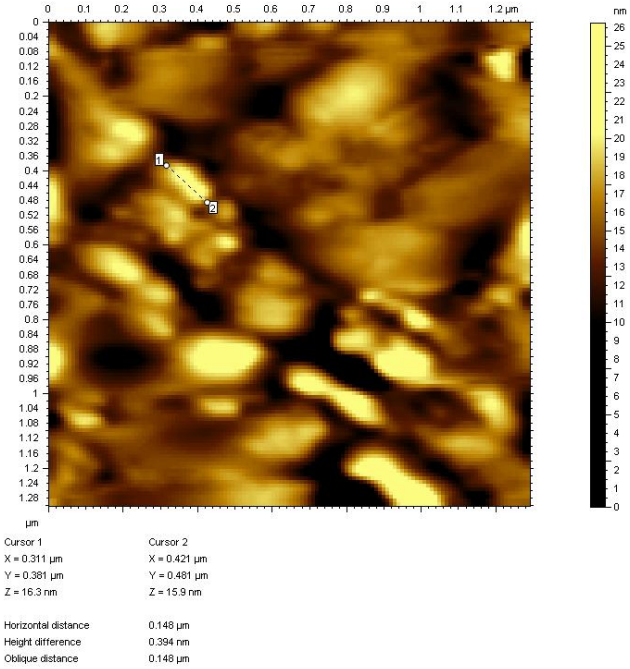
AFM studies of films of complexes with different proportions of chitosan collagen

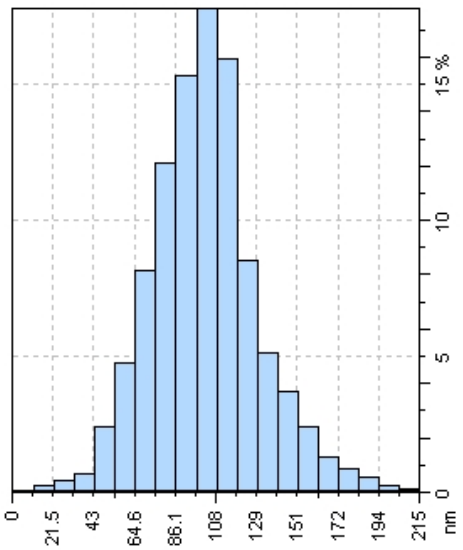
Films of chitosan-collagen complexes were obtained by dry molding using various ratios:R=3,57; R=4,1; R=4,225; R=8,57. Their morphology was studied by an atomic force microscope (Fig. 8)

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

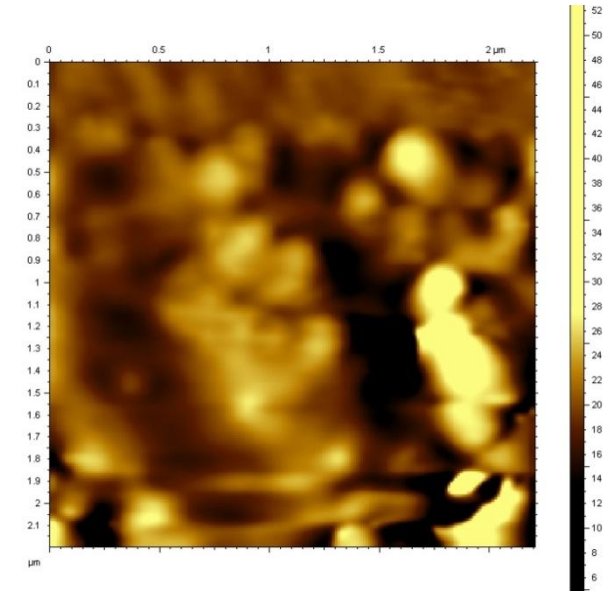
Vol. 6, Issue 7 , July 2019

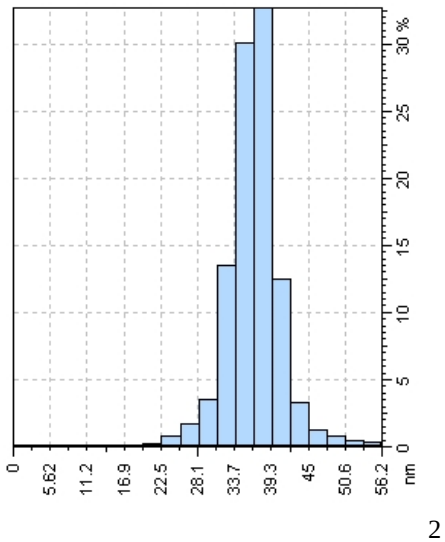




1

50-150 nm





2

25-45 nm

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 6, Issue 7 , July 2019

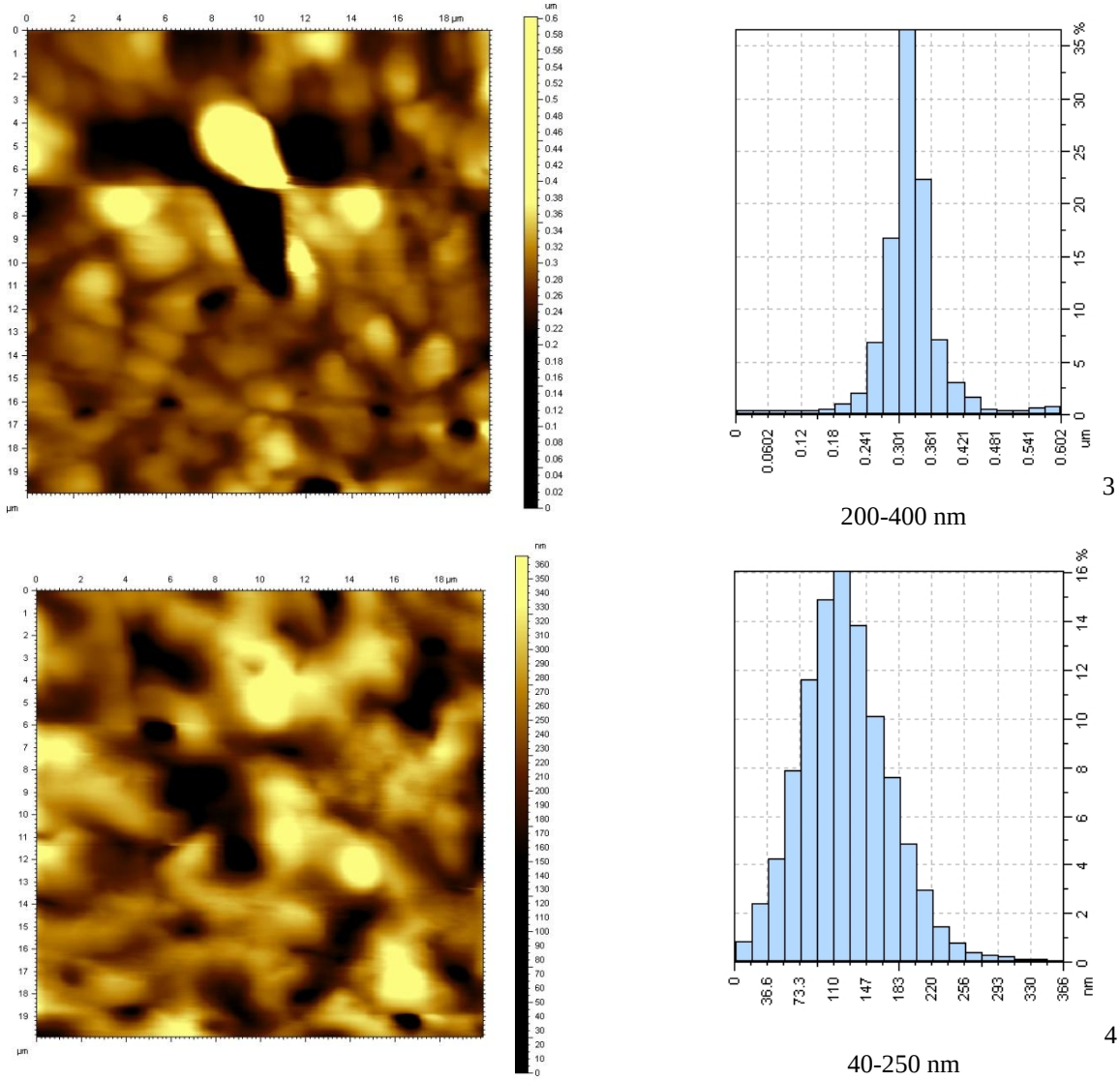


Fig. 8. AFM-images of samples of X-collagen and histograms of the distribution of particles on the polymer matrix:1) R=3,57 2) R=4,1 3) R=4,225 4)R=8,57

The results of microscopic studies indicate that chitosan-collagen complexes, regardless of the ratio, form nanoparticles in the chosen synthesis conditions. It should be noted that with an increase in the content of collagen, the particle size increases. These data are in good agreement with the literature data [13]. With R = 3.57; R = 4.1 and R = 8.57 nanoparticles are formed in a wide range of sizes from 25 to 250 nm. At R = 4.225, nanoparticles are formed in the range of 200–400 nm and the particles are relatively evenly distributed throughout the polymer matrix.

IV.CONCLUSION

Thereby, we have studied the process of the formation of interpolyelectrolyte complexes of chitosan and collagen at various ratios: R = 3.57–8.57. It was revealed that, under the chosen synthesis conditions, complexes are formed through the interaction of chromium amino groups with carboxyl groups of collagen at pH <7. The interaction of macromolecules is studied in solutions. It is established that with the mass ratio of chitosan collagen R = 4.225, the functional groups of macromolecules will be mutually compensated. This is confirmed by conductometric and UV spectroscopic methods. It was found that, under the chosen synthesis conditions, nanoparticles are formed in all ratios

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 6, Issue 7 , July 2019

in the size range from 25 to 400 nm. The resulting complexes of chitosan with collagen are of interest in obtaining bactericidal films for veterinary medicine and medicine.

REFERENCES

1. Kabanov A.V., Kabanov V.A. // High-altitude. Comm. A. 1994. T. 36. № 2. S. 198.
2. *VandeVordP.J., MatthewH.W.T., DeSilvaS.P., MaytonL., WuB.,WooleyP.H.*// J. Biomed. Mater. Res.2002. V. 59. № 3. P. 585.
3. *Onishi H., Mashida Y.* // Biomaterials. 1999. V. 20. №2. P. 175.
4. *Lui Q., Dunn E.T., Grandmaison E.W., Goosen M.F.* // Application of Chitin and Chitosan / Ed. by M.F.A Goosen. P.A Lancaster: Technomic Publ., 1997. P. 3.
5. *Dutta P.K., Tripathi S., Mehrotra G.K., Dutta J.* // Food Chem. 2009. V. 114. № 4. P. 1173.
6. Krayukhina MA, Samoilova N.A., Yamskov I.A. Polyelectrolyte chitosan complexes: formation, properties and application. Successes of chemistry 77 (9) 2008. p. 854-869
7. Volkova I.F., Gorshkova M.Y., Izumrudov V.A. Water-soluble non-stoichiometric polyelectrolyte complexes of chitosan and polystyrene sulfonate anion. High-Molecular Compounds, Series A, 2008, Vol. 50, No. 9, p. 1664-1655
8. RashidovaS.Sh., MilushevaR.Yu. Chitin and chitosan Bombyx mori. Tashkent: Fan, 2009.
9. Temirova.I., T.ZH. Kodirov "Charm and mine technology". Tashkent, Turon -, 2005. 255 b
10. Rafikov S.R. Viscometer to determine the viscosity of solutions of high-molecular compounds. // High-altitude. Comm. 1959. Ser. A.№10. (one). S. 1858-1860.
11. Wang Q.Z. et al. Protonation constants of chitosan with different molecular weight and degree of acetylation/ Carbohydrate Polymers 65 (2006) 194-201
12. Elson Santiago de Alvarenga. Characterization and Properties of Chitosan. Biotechnology of Biopolymers. pp.91-108. Doi: 10.5772/17020 Source: InTech.

13.Bo-yi Yu, Pei-Hsun Chou, Chang-an Chen, Yi-ming Sun And Shieh-Shiuh Kung. C3A Cell Behaviors on Micropatterned Chitosan–Collagen–Gelatin Membranes. Journal of biomaterials applications Volume 22 — November 2007. P.