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Experimental evaluation of structure formation in solutions of biopolymer mixtures and prediction of the properties for biomedical materials obtained

A. S. Shurshina[†], V. V. Chernova, M. Yu. Lazdina, A. S. Titlova, E. I. Kulish

[†]anzhela_murzagil@mail.ru

Bashkir State University, Ufa, 450076, Russia

The experimental evaluation of structure formation in solutions of individual polymers, as well as in their mixtures with other polymers in order to predict the properties of materials obtained on their basis was the aim of this work. We used chitosan, sodium salt of chitosan succinyl, sodium salt of carboxymethyl cellulose, and polyvinyl alcohol. Distilled water, 1% acetic acid, and buffer systems based on acetic acid and sodium acetate with pH = 3.80, 4.25, and 4.75 were used as solvents. It was found that the type of aggregates formed in polymer mixtures is determined by the nature of the solvent and the concentration of the polymers in the initial solution. A correlation has been shown between the nature of aggregates formed in solutions and some physicochemical properties of film materials obtained from solutions, namely, the degree of enzymatic hydrolysis, as well as the rate and degree of water vapor sorption by film materials. Thus, the studies carried out show that by varying the nature of the solvent, the concentration and nature of the polymers in the initial solutions, it is possible to control the structure formation in mixed polymer solutions, and, therefore, the properties of the resulting composites.

Keywords: polymers, sorption, enzymatic hydrolysis, structure formation.

1. Introduction

Today in medicine, traditional textile dressings are used to protect and heal wounds: gauze, bandages, etc. [1]. Unfortunately, they do not provide adequate protection for the skin, for example, during healing of burn wounds. In this case, the dressings soaked with exudate become vapor and gas tight, which can cause accelerated multiplication of microorganisms on the wound surface. This, accordingly, requires replacement of the dressings, which injures the forming epithelium and prolongs the recovery of skin tissues, and can also provoke the formation of scars [2]. In this regard, the problem arises of finding new protective materials that would be devoid of these disadvantages and at the same time would have a number of advantages, such as good moisture absorption capacity, vapor and gas permeability, biocompatibility with body tissues, bacteriostaticity, biodegradability, etc. [2]. A promising basis for the creation of such protective film coatings are polymers, for example, chitosan (ChTS) and its derivatives [3,4]. ChTS-based materials meet almost all the requirements for protective materials. Moreover, one of its main advantages is the ability to biodegrade under the action of enzymes secreted by the wound surface. The biodegradability makes it potentially possible to create such film coatings that could be applied to the wound once without requiring replacement, and gradually decomposed (lysed) on the wound as new tissue grew. At the same time, the ability for enzymatic degradation, as shown by experiments, has become in some way a problem for the protective film material based on ChTS, since it degrades too quickly on the wound and is completely lysed in two to three days. In this regard, ChTS, used to create a protective film material, needs modification, the purpose of which is to increase the service life of the protective material on the wound by reducing the rate of enzymatic degradation. Colloidal particles [5], low molecular weight electrolytes [6,7], alcohols [8], as well as other polymers [9] can act as modifiers of chitosan. For example, mixing solutions of two polymers with each other can result in an increase in aggregation processes [10], which is understandable, given the low affinity of different polymers to each other. However, the nature of the forming supramolecular structures in the case of solutions of polymer mixtures is not unambiguous. This fact is primarily determined by the type of possible intermolecular interactions between polymers — the preference for polymer1-polymer1 (homoaggregates) or polymer1-polymer2 (heteroaggregates) contacts. In polymer mixtures, there is a high probability of the formation of supramolecular structures which are homoaggregates of both polymers and are characterized by an increased packing density and a slower rate of chemical transformations [11,12].

It is clear that the type of aggregates formed, along with the general characteristics of the conformational-supramolecular state of polymers in solution, is one of the determining factors that has a significant effect on physicochemical properties of the material formed from the solution [13–15]. This is possible due to the fact that the film formation process at room temperature occurs rather quickly, which results in the nonequilibrium state of the polymer.

In this case, the relaxation processes are so slowed down that the polymer molecules do not come to equilibrium not only during the evaporation of the solvent, but also during the subsequent stay of the film at room temperature. The structure, and hence the properties of polymers in such a film, are determined by the state of the polymer molecules in the initial solution [16–18]. Consequently, by varying the structure formation of polymers in solution, it becomes possible to regulate the physicochemical properties of the material formed from the solution.

In this regard, the purpose of this work was to experimentally evaluate the structure formation in solutions of individual polymers of ChTS and its water-soluble derivative — sodium salt of succinyl chitosan (SChTS), as well as in their mixtures with other polymers, in order to predict the degree of enzymatic degradation and other properties of materials obtained on their basis.

2. Experimental

2.1. Materials

The following polysaccharides were used as objects for the study:

- chitosan (ChTS) (TS 9289-067-00472124-03) with the degree of deacetylation 82%, M_{sd}=113 kDa and intrinsic viscosity 7.8 g/dl produced by ZAO“Bioprogress” (Shchelkovo, Russia);
- sodium salt of chitosan succinyl (SChTS) with $M_{sd}=67$ kDa (TS 9284-027-11734126-08) produced by ZAO “Bioprogress” (Shchelkovo, Russia). The degree of substitution of samples of SChTS for amino groups was 75%. The degree of deacetylation of the initial ChTS samples, from which the SChTS were obtained, is 82%.
- sodium salt of carboxymethylcellulose (CMC) with $M_w=260$ kDa. The degree of substitution of CMC is 80%. CMC brand Blanose CMC 7 MFPH is manufactured by Ashland Inc. (France);
- polyvinyl alcohol (PVA) grade 11/2 and $M=35$ kDa produced by OOO Reakhim.

2.2. Solution preparation

1% acetic acid (AA) and buffer solutions with pH = 3.80 (BS-1), pH = 4.25 (BS-2) and pH = 4.75 (BS-3) were used as solvents for ChTS and PVA. Also, in the case of PVA, SChTS, and CMC, distilled water was used as a solvent. Polymer solutions were prepared by stirring on a magnetic stirrer for a day at room temperature.

2.3. Film preparation

The films from individual polymers or their mixtures were obtained by pouring 1% solutions onto the glass surface in a Petri dish. The thickness of the films was monitored with a thickness gauge with an accuracy of 0.01 mm.

2.4. Solution characterizations

Intrinsic viscosity $[\eta]_0$ was determined using an Ubbelohde viscometer at a temperature of $T=(25\pm1)$°C using the Baranov approach [19]. To determine the indicator of aggregation of polymers in solution δ, the method described in detail in [20] was used. Any deviation of the indicator δ from the value $\delta=1.00$ indicates that the system is structured.

2.5. Water vapour uptake

Experiments on water sorption by films were carried out at 100% humidity under thermostated conditions ($T=25$°C). The relative amount of water m_t absorbed by the film sample at the time t was determined by the gravimetric method by keeping the film samples in a desiccator in water vapour for a certain time t and calculated by the formula:

$$m_t=(\Delta m_t)/m_0,$$

where m_0 is the initial mass of the polymer in the film, Δm_t is the difference between the mass of the film at time t and the initial mass of the polymer in the film.

The experimental accuracy with 95% confidence interval and 5 replicates does not exceed 3%.

The diffusion coefficient was determined according to [21] by processing the sorption data.

2.6. Enzymatic hydrolysis of film samples

To carry out an experiment simulating the process of enzymatic hydrolysis of chitosan on a wound surface, film samples were placed on a substrate moistened with the solution of an enzyme preparation in water and kept for a certain time at a constant temperature (36 ± 0.1°C). The enzyme used was hyaluronidase produced under the Liraza trademark (Microgen CJSC, Moscow, Russia). The principal possibility of the enzymatic destruction of ChTS under the influence of nonspecific enzymes modeling the enzymatic composition of the wound surface was established earlier [22]. The volume of the enzyme preparation solution (0.1 ml) was selected so that the entire surface of the film was covered with the solution. The activity of the enzyme preparation was 0.64 c.u. Exposure of ChTS on a support soaked in the solution of an enzyme preparation was accompanied by a decrease in the intrinsic viscosity, and, therefore, in the molecular weight of the polymer, due to the process of enzymatic hydrolysis of the polymer. For all the films, the dependence of the change in viscosity on the time of exposure in contact with the enzyme complex was of the same type. The most significant drop in intrinsic viscosity occurred in the initial period within 10 minutes. Further enzymatic exposure of the film samples had an insignificant effect on the degree of drop in intrinsic viscosity.

After exposure of the film to the solution of the enzyme preparation, the process of enzymatic hydrolysis was stopped by boiling for 30 min in bain marie. Next, the film was dissolved in 1% acetic acid to determine the value of the intrinsic viscosity of the polymer [η]. The difference $\Delta\eta=[\eta]_{t-}[\eta]_0$, where t=10 minutes can serve as a measure of the depth of the enzymatic hydrolysis.

3. Results and discussion

3.1. *Effects of the nature of the solvents*

The study of the hydrodynamic properties of macromolecules in solutions allows one to obtain important information about the state of the polymer: the size of an individual coil (according to the values of the intrinsic viscosity), the thermodynamic quality of the solvent (according to the value of the Huggins constant), as well as the degree of intermolecular interaction (according to the value exponent δ depending on viscosity on concentration $\eta \sim C^{\delta}$).

Any deviation of the indicator δ from the value $\delta = 1.00$ evidences that this system is aggregated, and macromolecules in it interact with each other.

As can be seen from the data presented in Table 1, the solution of ChTS in acetic acid, as well as solutions of SChTS and CMC in water in the region of high dilutions, are solutions of individual molecules. Perhaps, this is due to the presence of a charge on these macromolecules, which leads to polyelectrolyte swelling and repulsion of macromolecules from each other in solution, which, in turn, suppresses their tendency for aggregation.

PVA solutions in all studied solvents are aggregated systems of interacting macromolecules. ChTS solutions in buffer solutions are also aggregated. This fact is understandable if we take into account that in buffer solutions the effect of polyelectrolyte swelling is suppressed and the mutual repulsion of like-charged molecules in solution (as in the case in the solution of acetic acid) is no longer observed.

There is another possible reason for the intensification of the aggregation processes of chitosan during the transition from solutions in acetic acid to solutions in acetate buffers. This is a deterioration in the quality of the solvent, as evidenced by both an increase in the value of the Huggins constant with an increase in the pH of the solvent, and a decrease in the values of the intrinsic viscosity. Replacement of the solvent in the case of PVA does not affect either the characteristics of the macromolecular coil or the value of the aggregation degree of the solution, since the quality of the solvent (the value of the Huggins constant) practically does not change.

When mixing equally concentrated (0.1 g/dl) polymer solutions with each other, one can expect both the additive behavior of mixed solutions and deviations from additivity due to the rearrangement of the polymer structure. It was shown that the values of the degree of aggregation are higher than the additive values for system ChTS-PVA in work [20]. Similar patterns were observed for all studied systems (SChTS-CMC-water, SChTS-PVA-water, ChTS-PVA-AA, ChTS-PVA-BS-1, ChTS-PVA-BS-2, ChTS-PVA-BS-3) that indicates an increase in intermolecular interactions of macromolecules in mixtures. At the same time, with the deterioration of the thermodynamic quality of the solvent, i.e. when going from acetic acid to a more alkaline buffer, higher deviations from additive values are observed.

Similar to the work [20] in which the system ChTS-PVA was studied, polymer mixtures are characterized by increased (compared to additive) values of the Huggins constants, which indicates a deterioration in the quality of the solvent.

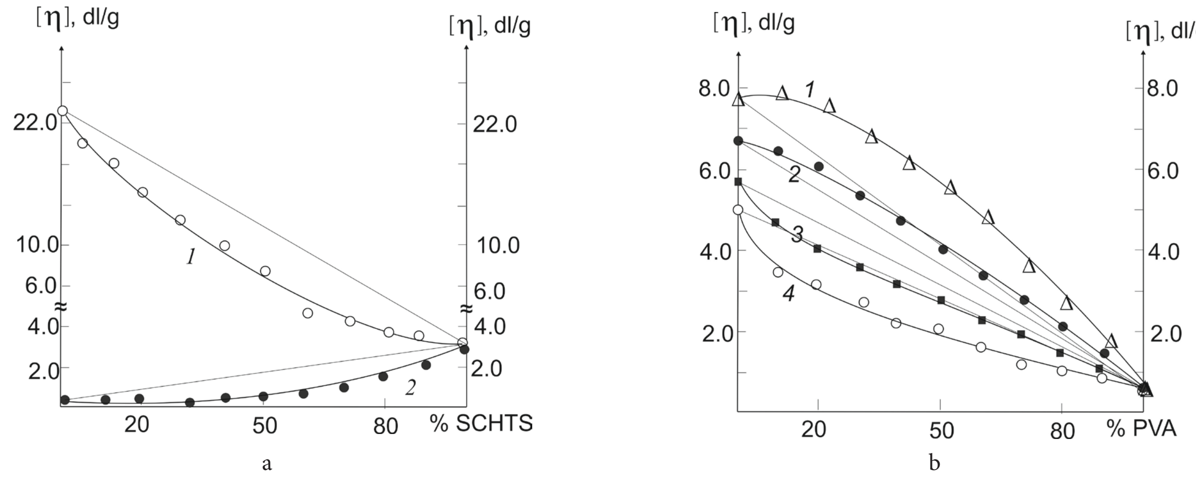
Analysis of the dependence of the intrinsic viscosity on the ratio of polymers in the mixture indicates that the type of aggregates formed is different for different systems (Fig. 1).

As can be seen from the data in Fig. 1a for the SCHTS-PVA-water and SCHTS-CMC-water systems, the intrinsic viscosity values deviate to the lower side from the additive values. At the same time, for mixtures of ChTS-PVA,

Table 1. Characteristics of the studied polymers obtained from viscometric data.

Polymer	Solvent	δ	K_x	[η], dl/g
ChTS	AA	1.00	0.48	7.80
	BS-1*	1.25	0.54	6.80
	BS-2*	1.28	0.60	5.70
	BS-3*	1.35	0.62	5.00
SChTS	water	1.00	0.47	3.36
CMC	water	1.00	0.48	22.8
PVA	water	1.10	0.81	0.50
	AA	1.10	0.80	0.50
	BS-1*	1.10	0.80	0.50
	BS-2*	1.10	0.80	0.50
	BS-3*	1.10	0.81	0.49

*[20]



a

b

Fig. 1. Dependence of the intrinsic viscosity for the systems: 1 — SChTS-CMC-water, 2 — SChTS-PVA-water (a); 1 — ChTS-PVA-AA, 2 — ChTS-PVA-BS-1, 3 — ChTS-PVA-BS-2 and 4 — ChTS-PVA-BS-3 (b) on the ratio of polymers in the mixture.

deviations of viscosity from additive values to the lower side only take place for solutions of these polymers in BS-2 and BS-3. When BS-1 and AA are used as solvents, the values of the characteristic viscosity deviate from the additive values upward. It can be assumed that the formation of homoaggregates in a mixture of polymers is accompanied by a deviation of the intrinsic viscosity to a smaller side, and of heteroaggregates to a larger one from the additive values.

Thus, the experimental studies of the viscosity of solutions of polymer mixtures made it possible to determine the type of formed aggregates which are largely determined by the nature and thermodynamic quality of the selected solvent, as well as the concentration of the initial polymer solutions. But what is really important is that on the basis of these experimental data, it is possible to determine some of the properties of materials obtained from solutions.

3.2. Effect of the nature of the solvents and the polymer on the process of water vapour sorption

For example, due to the fact that homoaggregates are characterized by an increased (in comparison with heteroaggregates) packing density, it is clear why films obtained from solutions of polymer mixtures in which homoaggregates are formed (SChTS-CMC-water, SChTS-PVA-water), are characterized by a lower degree of sorption of water vapour in comparison with materials obtained from solutions of polymer mixtures, in which heteroaggregates ChTS-PVA-AA are formed (Fig. 2a). Similar regularities take place for the values of the diffusion coefficients of water vapour by polymer films (Fig. 2b).

3.3. Enzymatic hydrolysis of film samples

Moreover, for film materials obtained from solution, there is a clear correlation between the data on the sorption capacity of film materials and the degree of their enzymatic hydrolysis (Table 2).

Analysis of the data given in Table 2 allows us to conclude the following. Firstly, attention is drawn to the fact that the worse the quality of the solvent used in the preparation of the film, in relation to ChTS (replacement of acetic acid with buffer solutions), the lower the degree of enzymatic hydrolysis of CTS in the film material. Secondly, in the case when heteroaggregates are formed in the solution of polymer mixtures (ChTS-PVA film obtained from a solution in AA), the degree of enzymatic hydrolysis of ChTS is higher, and when homoaggregates (ChTS-PVA films obtained from solutions in BS) are less, rather than for an individual ChTS film. Thirdly, as the quality of the solvent deteriorates in relation to chitosan, the degree of its enzymatic decomposition in the film mixtures of ChTS-PVA decreases. Consequently, by varying the type of aggregates obtained, it becomes possible to regulate the service life of the material on the wound.

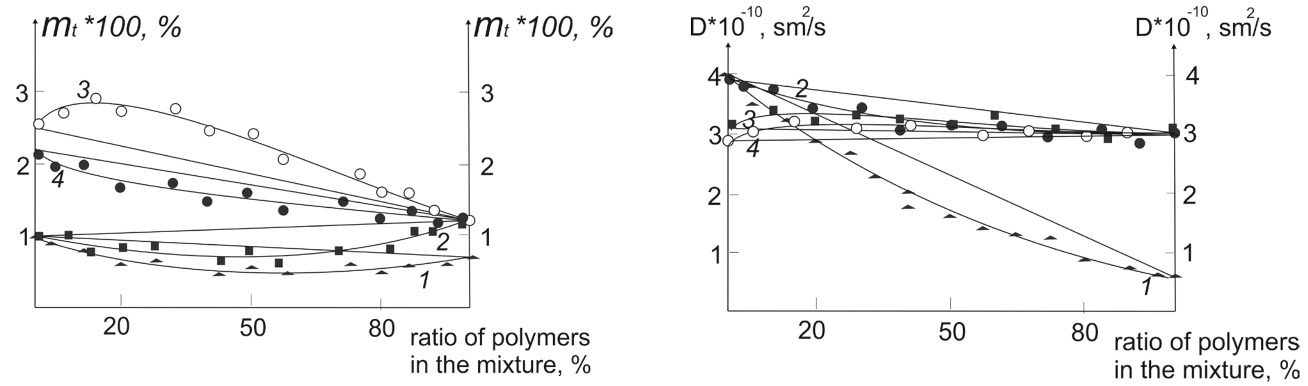
Table 2. Degree of enzymatic decomposition of ChTS in film polymeric materials ChTS-PVA.

Used solvent	The ratio of polymers in a mixture of ChTS: PVA	Δh, g/dl
AA	100:0	0.42
	80:20	0.51
	60:40	0.48
BS-1	100:0	0.39
	80:20	0.37
	60:40	0.35
BS-2	100:0	0.34
	80:20	0.32
	60:40	0.29
BS-3	100:0	0.31
	80:20	0.25
	60:40	0.23

Thus, the studies carried out show that by varying the nature of the solvent, the concentration and nature of the polymers in the initial solutions, it is possible to control the structure formation in mixed polymer solutions, and, therefore, the properties of the resulting composites.

4. Conclusions

1. The most important physicochemical parameters characterizing the polymer-polymer system in solution — the characteristic viscosity [η], the Huggins constant K_x and the exponent δ, characterizing the course of aggregation processes — have been determined by the method of viscometry for the SChTS-PVA-water, CMC-PVA-water and



a b

Fig. 2. Dependence of the amount of water mt absorbed in 24 hours (a) and the diffusion coefficient on the ratio of polymers in the mixture (b) for film samples SChTS-CMC-water (1), SChTS-PVA-water (2), ChTS-PVA-AA (3) and ChTS-PVA-BS-2 (4).

chitosan-PVA systems in a number of solvents. It was shown that the deterioration of the thermodynamic quality of the solvent used in the preparation of the film is accompanied by a decrease in the degree of enzymatic hydrolysis of chitosan in the film material, i. e. leads to an increase in the service life of the material on the wound surface.

2. It has been found that for all the studied polymer mixtures, the formation of aggregates from macromolecules takes place even in the region of high dilutions. In this case, the type of aggregates formed (homo- or hetero-) is determined by the nature of the solvent used and the concentration of polymers in the initial solution.

3. A correlation has been established between the nature of aggregates formed in solutions and some physicochemical properties of film materials obtained from solutions. It is shown that in the case when heteroaggregates are formed in a solution of polymer mixtures, the degree of enzymatic hydrolysis of chitosan, as well as the rate and degree of water vapor sorption by film materials, is higher than for films made from an individual polymer. In the case when homoaggregates are formed in solutions of polymer mixtures, the degree of enzymatic hydrolysis, the rate and degree of water vapor sorption by film materials, on the contrary, are lower.

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