ABSTRACT
The rheological properties of hydrogels based on PEC of gelatin with marine polysaccharides such as chitosan have been studied. The rheological characteristics were measured in the mode of shear deformation. The interaction of gelatin and polysaccharide has been studied by the methods of UV and FTIR spectroscopy.

INTRODUCTION
A cationic polysaccharide chitosan is widely used as a "matrix" for the formation of (bio) polyelectrolyte complex (PEC) due to the attractiveness of its properties such as biocompatibility, biodegradability, low toxicity and relatively low manufacturing cost, due to the rich natural sources1.

Macromolecules of chitosan have a high density of positive charge in the acidic environment due to ionization of the free amino groups. Therefore, self-assembly of (bio)PEC occurs in acidic solutions of chitosan in the presence of the negatively charged polyelectrolyte2.

Gelatin is denatured collagen and has been widely used as a fundamental material for microspheres, sealants, tissue adhesives and carriers for controlled delivery systems. Gelatin has also been widely used in combination with other polymers for encapsulation3.

The aim of this work was to study the rheological properties of hydrogels based on chitosan-gelatin (bio)PEC formed in the aqueous phase, where pH is less than the isoelectric point of gelatin.

EXPERIMENTAL
Alkaline gelatin Type B from bovine skin 225 Bloom was used (from Sigma-Aldrich, USA). The gelatin sample contains the mass fractions as follows: 75% protein, 10.2% moisture, 1.6% ash (dry residue). Isoelectric point (pl) is 4.7 (determined by viscometric and turbidimetric methods). The viscosity-average molecular mass M, of gelatin was determined to be 96 kDa.

Chitosan from shrimp shells was used (from Sigma-Aldrich, Iceland). The degree of deacetylation was 86%; M, = 260 kDa.

Acetic acid (0.1 M) was used as the aqueous phase for preparing the systems under study (hydrosols and chitosan-gelatin hydrogels). pH values of the systems were in the range of 3.2 to 3.9 (< pl of gelatin).

The turbidimetric measurements of the hydrosols were carried out at 23 °C using a spectrometer T70 UV/visible (PG Instruments, UK).

Fourier transform infrared (FTIR) spectra of the sample under study were registered with IR-Fourier spectrometer Shimadzu IR Tracer-100 in the middle range of IR radiation (800–3600 cm⁻¹). FT-IR spectra were measured using dried samples of gelatin gels and gels formed by chitosan-gelatin PEC.

The rheological properties of hydrogels were measured in the mode of shear deformation.
deformation using rheometer Physica MCR302 (Anton Paar, Austria) at 14 °C. A cone-plate measuring cell (cell diameter of 50 mm, the angle between the cone and the plate 1°) was used.

RESULTS AND DISCUSSION

The stoichiometric (bio)polyelectrolyte complexes of gelatin with chitosan are formed in the range of the chitosan/gelatin w/w ratios about 0.80 in aqueous phase at pH values 3.2 – 3.9.

Interaction between gelatin and chitosan macromolecules is confirmed by the changes in the FTIR spectra. Fig. 1 presents parts of the FTIR spectra for samples of native gelatin as well as for chitosan-gelatin mixtures at different chitosan-gelatin w/w ratios Z.

Attribution of lines in the FTIR spectrum of gelatin (Fig. 1, spectrum 1) to the vibration of corresponding functional groups is based on data of the publications 7-8.

In the native gelatin spectrum, the main lines are wide band with peak at 3400 cm⁻¹ (vibration of NH-groups), characteristic absorption at 1654 cm⁻¹ (Amide I, stretching vibration of the CO and CN groups), 1538 cm⁻¹ (Amide II, vibration of NH and CN groups) and 1238 cm⁻¹ (Amide III, stretching vibration of NH and CN groups). Bands at 1165 cm⁻¹ are obliged to stretching vibration of carboxyl (–COOH) groups of Glu and Asp in gelatin 8-9.

Similar effects were observed in the study of blend films of gelatin added with sodium alginate4, κ-carrageenan6 and chitosan9.

Similar result was obtained in paper9 where authors studied the films from fish gelatin, chitosan and their mixtures. It is emphasized that fish chitosan-gelatin films revealed the formation not only of hydrogen bonds within and between polymer chains, but also of electrostatic interactions between –COO groups of gelatin and –NH₃⁺ groups of chitosan.

The rheological characteristics of hydrogels formed by stoichiometric (bio)PEC chitosan-gelatin (Z ≤ 0.80) were measured by dynamic mechanical analysis to obtain the amplitude and frequency dependencies of the components of the complex modulus G*. This measurement scheme seems appropriate, since the investigated gels are viscoelastic matters.

Fig. 2 shows the amplitude dependence of the storage G' and loss modulus G'' for gels where the concentration of gelatin is 1.0 % and different concentration of chitosan.

![Figure 2](image-url)
These samples reveal linearity of the mechanical properties until amplitude of strain $\gamma$ of about $10 - 30\%$. Thus, it is believed that these gels at low strains (in linear viscoelastic region) show solid state behaviour. The figure also shows that for all concentrations of chitosan values of storage modulus are much higher than values of loss modulus $G' > G''$. The measurements were performed in the linear viscoelastic region at the deformation amplitude $\gamma = 1\%$.

Fig. 3 shows the frequency dependence of the storage $G'$ and the loss modulus $G''$ for the chitosan-gelatin complex gels. The constancy of $G'$ over a wide frequency range (up to a frequency $\omega$ of $10\; s^{-1}$), and the lowest values of $G''$ indicate solid state behaviour of gels, which were investigated at small strains.

Module $G''$ increases with the angular frequency of up to $200\; s^{-1}$ and decreases with further increase of frequency. Increasing the concentration of chitosan (and hence chitosan-gelatin w/w ratios $Z$) causes increase in both $G'$, and $G''$ in the entire considered frequency range. In the $0.1-0.8\%$ concentration range of chitosan ($Z = 0.1-0.8$) under conditions with high concentrations of stoichiometric PEC, a decrease in rheological characteristics is observed (Fig. 4). This effect can be explained by the increase in the role of electrostatic repulsion between complexes carrying positive charges at pH values below pI of gelatin. Then the strengthening effect of chitosan (creating additional sites in the spatial network) is suppressed by the electrostatic repulsion of complexes.

CONCLUSION

The stoichiometric (bio)polyelectrolyte complexes of gelatin with chitosan are formed in the range of the chitosan/gelatin w/w ratios about $0.80$ in aqueous phase at pH values $3.2 - 3.9$. Chitosan-gelatin (bio)PEC appears due to electrostatic interaction between positively charged amino-groups in chitosan and negatively charged amino-acid Glu and Asp residues in gelatin and also due to hydrogen bonds.

The stoichiometric chitosan-gelatin complexes can be considered as new structure formers which show viscoelastic behavior in the linear range. Increasing the chitosan-gelatin w/w ratios causes the growth of hydrogels viscoelastic characteristics.
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REFERENCES


