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THE EFFECT OF CONFORMATIONAL TRANSITION OF GELATIN-POLYSACCHARIDE POLYELECTROLYTE COMPLEX ON ITS FUNCTIONAL PROPERTIES

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ABSTRACT

The blends of gelatin and shear-thinning hydrocolloids (guar gum, kappa-carrageenan and xanthan gum) were examined to determine the effect of the conformational change on the functional properties of the solutions. The polyelectrolyte complexes of 0.5% gelatin/0.5% polysaccharide in 70 mM KCl or 70 mM NaCl were investigated by the laboratory rheometer and conductivity meter in the temperature range 25 – 45 °C. The rheological data were fitted by the power-law and Herschel-Bulkley model to obtain the flow parameters. The functional properties of the samples were substantially affected by the conformational change of the polysaccharide, as well as by the type of the hydrocolloid and salt solution. There was an evident change of viscosity and conductivity of the solutions upon heating, corresponding to the helix-coil transition of the polysaccharide at temperature about 35 °C. The type of the salt solvent had an effect on the gelation properties of the samples. Gelatin/kappa-carrageenan blend in NaCl provided a gel of high consistency at ambient temperature (20 – 25 °C), whereas the blend in KCl did not gel in the studied temperature range. The potential stability of the blends was determined by zeta-potential analysis. The low values of ζ-potential indicate that the gelatin/polysaccharide blends are electrically unstable systems which tend to coagulate. The mixtures of gelatin/polysaccharide electrostatic complexes may have a great potential in many food applications.

Keywords: biopolyelectrolyte complex; conformational transition; flow parameter; gelatin-polysaccharide blend; hydrocolloid

INTRODUCTION

The biopolyelectrolyte complexes (PECs) in aqueous and salt solutions, formed by oppositely charged polysaccharides and proteins, were largely investigated in the last years. Particularly, the formation and physicochemical properties of these complexes were intensively studied **(Derkach et al., 2015; Viebke, Piculell and Nilsson, 1994)**. Other research in the field of the polyelectrolyte complexes might be of great value in extending our knowledge of the protein/polysaccharide electrostatic complexation. The effect of the addition of hydrocolloids employed in these complexes and the hydrocolloid gels on the properties of food products can have a significant importance for manufacturing of food products **(Bojňanská, Šmitalová and Vollmannová, 2016; Kumbár et al., 2017)**. Notably, the investigation of electrostatic interaction between gelatin and polysaccharides seems to be beneficial for new practical applications of PECs in the food industry. Gelatin, which is broadly used in food production, is limited by its rheological properties, thermal stability, etc. The compatibility of gelatin with different ionic polysaccharides, such as guar gum, xanthan gum and kappa-carrageenan, is provided by the formation of PECs. It is possible to obtain new modifications of rheological properties of gelatin-based products using gelatin-polysaccharide combinations **(Derkach et al., 2015)**.

One of the most common hydrocolloids in the polyelectrolyte complexes is carrageenan, an anionic polysaccharide, which plays a role of a basis in the electrostatic complexation. The κ-carrageenan molecule is negatively charged in aqueous solution due to one negatively charged sulphonic acid group per carrabiose unit. In the presence of a positively charged natural polyelectrolyte, κ-carrageenan self-assembles with the complementary polyelectrolyte, such as gelatin. The complexation with gelatin is provided by the electrostatic interaction between positively charged groups in gelatin and negatively charged sulfate groups in κ-carrageenan leading to conformational changes of gelatin macromolecules **(Derkach et al., 2015; Voron’ko et al., 2016)**.

Gelatin, which is widely employed in the PECs, is protein (polypeptide) extracted by denaturation of collagen from mammalian or fish tissues **(Fang et al., 2006)**. In solution at moderate temperature above 40 °C, gelatin is present in the form of flexible, disordered coils which associate into triple helices, similar to native collagen. In other words, the polypeptide chains of gelatin have a random coil conformation at higher temperatures; this conformation is slowly transformed into single helix by lowering temperature. By sufficient concentration, gelatin bonds with two other molecules to form a triple helix upon cooling. The triple helix is an insoluble semi-rigid rod partly consisting of collagen due to unfinished renaturation. Above the gelation temperature T_g (ca. 40 °C), gelatin behaves as a viscous liquid (*sol state*), under T_g as a thermoreversible weak gel (*gel state*). However, the gelatin exhibits the increase in the gel strength upon cooling to below 30 °C. At temperature above 35 °C, gelatin melts due to the dissociation of triple helices **(Pelc et al., 2014; Sarbon, Badii and Howell, 2015)**.

The electrostatic interactions are the main forces in the formation and stabilization of PECs, notably polysaccharide-protein complexes, although the hydrogen bonds and hydrophobic interactions are also important. As stated by **Voron'ko et al. (2016)**, at high temperatures, when biopolymer macromolecules in solution assume the random coil conformation, hydrophobic interactions contribute to a large extent to complex stabilization. On the other hand, below and at the temperature of gelatin coil to helix conformational transition, the electrostatic interactions and hydrogen bonds play a decisive role in complex formulation.

In the field of food industry, the formation of an electrostatic complex between a polysaccharide and a protein, such as gelatin, plays an important role in many applications. The complexation is utilized for stabilizing of emulsions, design of low-calorie and low-starch food products, encapsulation of flavours, etc. The protein/polysaccharide complexes can be also used as new emulsifying agents providing unique emulsification properties **(Cao et al., 2016; Li et al., 2012)**. Moreover, the specific hydrophilic and polydisperse character of gelatin provides the electrostatic complexes a great nutritional value in the production of dietary food. The polyelectrolyte complexes of gelatin/polysaccharide can have health benefits for an average customer as a substitute of high-sugar content food products. In other words, the complexes can prevent some diseases such as diabetes resulting from a high consumption of sugars in an unhealthy diet **(Li and Nie, 2016)**.

The goal of this research is to study the rheological and other functional properties of the gelatin/polysaccharide mixtures dependent on the temperature, salt solvent and hydrocolloid employed in the mixture. The effect of the conformational transition of the biopolyelectrolyte complex on the functional properties will be examined.

MATERIAL AND METHODOLOGY

Materials

The powder samples of polysaccharides, guar gum (No. G4129), kappa-carrageenan (No. 22048, viscosity of 0.3% solution at 25 °C reported as 5 – 25 mPa.s) and xanthan gum (No. G1253, viscosity of 1% solution reported as 800 – 1200 mPa.s^{-1}) were delivered by Sigma-Aldrich Co. (St. Louis, USA). Gelatin, potassium chloride (KCl) and sodium chloride (NaCl) were obtained by the Czech company IPL – Ing. Petr Lukeš (Uherský Brod, Czech Republic). All chemicals were of analytical grade.

The samples of hydrocolloids were stored in a dry laboratory place at room temperature (ca 25 °C) and relative humidity about 40% (vol.).

Samples preparation

The solution of 1.0% gelatin was prepared by dissolving the sample in 70 mM KCl or 70 mM NaCl. The gelatin was swelled in a minor amount of the solvent. Afterwards, the solution was heated at 45 °C to dissolve completely. Then the solution was cooled under ambient temperature.

The polysaccharide solutions of 1.0% w/w concentration were prepared by dissolving the samples in 70 mM KCl or 70 mM NaCl and mixing by a magnetic stirrer. Since the powder samples were only partially soluble in cold solvent, the solutions were treated at room temperature (ca 25 °C) for a long time and heated at 80 – 89 °C for a short time to make easy the total dispersion. 0.5% w/w polysaccharide solutions were prepared in the same way as the reference samples.

The salt mixtures of gelatin/polysaccharide were prepared by blending the equal amounts of the stock solutions of 1.0% gelatin and 1.0% polysaccharide to achieve the final concentration of 0.5% w/w. The dispersions of gelatin/polysaccharide were stirred at 45 °C for 10 min. The solutions were kept at room temperature for 72 h to release air bubbles. Subsequently, the samples were stored in a refrigerator at temperature about 4 °C. Before the analyses, the samples were gently and carefully mixed and shaken to ensure the homogeneous consistency of the solutions.

Methods

Viscosimetric analysis

The viscosimetric analysis of the samples was performed using universal laboratory rheometer HAAKE RheoStress 1 (Thermo Scientific, USA) with automatic software. The model type of cylinder-cylinder geometry was used. The thermal control was ensured by thermostat HAAKE AC 200 (Thermo Scientific, USA).

Prior to testing, the samples were thermally equilibrated in the water bath. The viscosimetric measurements were performed at specific temperatures: 25 °C, 30 °C, 35 °C, 40 °C and 45 °C. The temperature was kept constant with an accuracy of ±0.5 °C using the heating circulator. The viscosity was measured in the shear rate between 2 and 200 s^{-1}. The shear rate was rising and decreasing, the duration of one cycle was 180 s.

The measurement was performed three times for each sample. The experimental data were statistically analysed and fitted by suitable rheological models, power-law (Ostwald-de Waele) model and Herschel-Bulkley model. The flow parameters were evaluated using the software SigmaPlot 2002 Version 8.0 (SPSS Inc., USA). The differences between the values of consistency coefficient (k), flow behaviour index (n) and yield stress (τ_0) were considered at significant level p <0.0001.

Arrhenius model

The effect of temperature on the viscosity of the blends was evaluated using the Arrhenius equation. The values of determination coefficient (R^2) were stated at specified shear rates (20 s^{-1} and 100 s^{-1}) for all solutions.

The form of Arrhenius model is shown in Eq. (1):

$$\eta = A\exp\left(\frac{E_a}{RT}\right) \tag{1}$$

where: η is the dynamic viscosity (Pa.s), A is the pre-exponential factor (-), E_a is the activation energy (J.mol^{-1}), R is the molar gas constant (8.3144598 J.$K^{-1}mol^{-1}$) and T is the thermodynamic temperature (K) **(Marcotte et al., 2001)**.

Conductivity measurement

The conductivity of the blends was determined by conductometer Mettler-Toledo (USA) in the temperature range 25 – 45 °C. The temperature of the samples was ensured by the water bath Julabo MA-4 (Germany). Each sample was measured three times at specific temperatures: 25 °C, 27 °C, 29 °C, etc. The conductivity of 0.5% polysaccharide solutions as reference materials was also determined.

The conductivity of each sample was calculated as an average value of three measurements. The data were evaluated by plotting the graphs of conductivity C (mS.cm^{-1}) versus temperature (°C).

Zeta potential analysis

The zeta potential of 0.5% gelatin/polysaccharide blends was measured by Zeta Potential Analyzer, Zeta Plus (Brookhaven Instruments Corporation, USA). The appropriate amount of the sample (1.6 ml) was poured into the squared polystyrene cuvette. The temperature of the samples was equilibrated at 25 °C by automatic software. Prior to testing, the pH-values of the solutions were measured by pH meter Five Easy Plus FEP 20 (Mettler-Toledo, Switzerland); the obtained results were entered as parameters for the following analysis. The values of zeta potential of the solutions, as well as the effective diameter of the particles were analysed by the software. The data were compared for the samples to evaluate the potential stability of the blends.

The zeta potential (ζ-potential) measurement consists in determining the electrostatic potential at the surface of shear which extends out from particle surface of the examined colloidal system. Generally, the electrostatic potential (y) is a function of distance from the particle surface and can be expressed by Eq. (2):

$$y = y_0 e^{-kx} \tag{2}$$

where y_0 is the potential at the surface and k is a parameter that measures the decrease of the potential with distance. Similar to y, the ζ-potential decreases in an exponential fashion. The parameter k^{-1}, called the double layer thickness, plays an important role in determining the distance from the particle surface; k^{-1} is a function of temperature, dielectric constant of the liquid and ionic strength due to free ions (typically by added salt like KCl) in the bulk of the fluid **(Anonym, 2000)**.

RESULTS AND DISCUSSION

Viscosimetric analysis

The flow parameters obtained by fitting the rheological models, Ostwald-de Waele and Herschel-Bulkley models, to the data are summarized in **Chyba! Nenašiel sa žiaden zdroj odkazov.** to **Chyba! Nenašiel sa žiaden zdroj odkazov.**. The Herschel-Bulkley parameters were evaluated without the extrapolation of the yield stress values. The Herschel-Bulkley model was the most suitable model for all samples in terms of determination coefficient (R^2). The high values of R^2 (0.99) obtained by fitting the Herschel-Bulkley model indicate a good fit of this model for the hydrocolloid blends.

The blends of gelatin/polysaccharide were dissolved in 70 mM KCl or 70 mM NaCl to eliminate contributions to the viscosity from intramolecular electrostatic repulsions which are typical for the polymer samples in distilled water **(Kupská et al., 2014)**. The blends were prepared in two different salt solvents to follow the effects of helix-coil transition induced by external stimulus, i.e., by the change of temperature and type of the solvent.

Table 1 Flow parameters of 0.5% w/w gelatin/polysaccharide blends in 0.07M KCl (for upward curves).

		Herschel-Bulkley model				**Ostwald-de Waele model**		
Sample	***t* (°C)**	**τ_0 (Pa)**	**k (Pa.s^n)**	***n***	**R^2**	**k (Pa.s^n)**	***n***	**R^2**
Guar gum/gelatin	25	-7.008	6.830	0.22	0.9990	1.956	0.38	0.9905
	30	-0.9089	0.8898	0.45	0.9998	0.5246	0.53	0.9982
	35	-0.7405	0.5905	0.51	0.9998	0.3434	0.59	0.9980
	40	-0.6377	0.4812	0.53	0.9997	0.2826	0.61	0.9980
	45	-0.5032	0.3679	0.56	0.9998	0.2265	0.64	0.9984
Kappa-carrageenan/ gelatin	25	0.1580	0.0139	0.83	0.9962	0.0438	0.64	0.9895
	30	0.1794	0.0015	1.18	0.9675	0.0201	0.72	0.9425
	35	0.2516	0.0404	0.55	0.9198	-	-	0.9085
	40	0.1044	0.0030	1.07	0.9906	0.0120	0.83	0.9824
	45	0.3453	0.0038	0.89	0.6532	-	-	0.5522
Xanthan gum/gelatin	25	3.282	0.8333	0.36	0.9998	3.264	0.187	0.9931
	30	2.10	1.489	0.27	0.9985	3.177	0.180	0.9961
	35	1.179	1.989	0.23	0.9985	2.953	0.186	0.9978
	40	2.057	1.292	0.28	0.9993	2.919	0.185	0.9963
	45	1.647	1.077	0.32	0.9988	2.281	0.224	0.9959

Table 2 Flow parameters of 0.5% w/w gelatin/polysaccharide blends in 0.07M NaCl (for upward curves).

		Herschel-Bulkley model				**Ostwald-de Waele model**		
Sample	t **(°C)**	τ_0 **(Pa)**	k **(Pa.s^n)**	n	R^2	k **(Pa.s^n)**	n	R^2
Guar gum/gelatin	25	-2.823	2.493	0.31	0.9987	0.9213	0.45	0.9927
	30	-0.4414	0.3243	0.58	0.9998	0.2066	0.65	0.9986
	35	-0.2869	0.1849	0.66	0.9998	0.1253	0.72	0.9989
	40	-0.2373	0.1557	0.67	0.9999	0.1086	0.73	0.9991
	45	-0.1773	0.1166	0.70	0.9999	0.0848	0.76	0.9993
Kappa-carrageenan/ gelatin	25	4.80	0.7628	0.56	0.9983	3.093	0.35	0.9867
	30	9.877	0.2149	0.76	0.9977	5.478	0.25	0.9427
	35	3.009	1.083	0.48	0.9992	2.674	0.34	0.9946
	40	2.084	1.044	0.39	0.9889	2.386	0.28	0.9953
	45	0.208	0.0727	0.68	0.9998	0.126	0.59	0.9982
Xanthan gum/gelatin	25	3.30	0.6827	0.38	0.9997	3.112	0.184	0.9913
	30	2.288	0.8347	0.36	0.9985	2.428	0.220	0.9976
	35	1.911	0.940	0.34	0.9987	2.332	0.219	0.9944
	40	1.394	1.184	0.30	0.9990	2.221	0.222	0.9970
	45	0.973	1.375	0.28	0.9992	2.106	0.227	0.9983

Table 3 Flow parameters of 0.5% w/w polysaccharide solutions in 0.07M KCl (for upward curves).

		Herschel-Bulkley model				**Ostwald-de Waele model**		
Sample	t **(°C)**	τ_0 **(Pa)**	k **(Pa.s^n)**	n	R^2	k **(Pa.s^n)**	n	R^2
Guar gum	25	-1.321	1.183	0.418	0.9997	0.6125	0.520	0.9971
	30	-1.340	1.214	0.407	0.9998	0.6182	0.511	0.9970
	35	-1.322	1.186	0.408	0.9998	0.6006	0.513	0.9970
	40	-1.268	1.116	0.412	0.9997	0.5614	0.518	0.9969
	45	-1.179	1.015	0.424	0.9997	0.5175	0.529	0.9970
Kappa-carrageenan	25	6.039	0.3985	0.60	0.9608	3.286	0.29	0.9521
	30	7.20	0.0370	0.98	0.9559	2.890	0.29	0.9125
	35	10.53	0.0063	1.24	0.8550	5.970	0.17	0.7617
	40	7.584	0.0189	1.05	0.8714	3.652	0.22	0.8074
	45	6.255	0.0269	0.96	0.8979	3.032	0.23	0.8520
Xanthan gum	25	2.014	0.903	0.35	0.9995	2.350	0.223	0.9948
	30	1.792	0.977	0.34	0.9998	2.248	0.227	0.9972
	35	1.580	1.083	0.32	0.9998	2.223	0.225	0.9979
	40	1.311	1.197	0.30	0.9998	2.152	0.228	0.9986
	45	1.139	1.299	0.29	0.9998	2.139	0.227	0.9989

Note: t, temperature; τ_0, yield stress; k, consistency coefficient; n, flow behaviour index; R^2, coefficient of determination. The hyphen means that no relevant value was possible to determine by the fitting of the model. The number of replicates, 3.

There was a clearly visible substantial change of the rheological behaviour of the blends reflecting a conformational transition of the helix to coil at temperature about 35 °C. The change corresponds to the conformational ordering of the polysaccharide in the gelatin/polysaccharide complex. The gelatin/ polysaccharide electrostatic complexation is enhanced due to the conformational transition of the polysaccharide from double helix to random coil upon heating. At higher temperatures (above 40 °C), gelatin in the complexes is present in the form of disordered coils. On the other hand, gelatin/polysaccharide complexes dissociate during cooling by the conformational ordering of the polysaccharide (coil to double helix transition) as reported by **Cao et al. (2016)** for gelatin/κ-carrageenan complexation.

The conformational transition temperature of the polysaccharide increases with the addition of salts such as KCl or NaCl. This is due to the reduction of electrostatic complex coacervation of gelatin/polysaccharide mixture with increasing salt concentration; the coacervation is entirely screened after exceeding a certain limit of the salt. For example, the electrostatic complex coacervation of 0.75% gelatin/κ-carrageenan mixture is completely screened when KCl concentration is more than 100 mM **(Cao et al., 2016)**. The samples in the present study showed different behaviour of the polysaccharide solutions and gelatin/polysaccharide blends in 70 mM KCl or 70 mM NaCl. The polysaccharide conformational transition affected the rheological properties of the blends; the flow parameters of pure polysaccharide solutions in 70 mM KCl were evidently different from the parameters of the blends as can be seen in **Chyba! Nenašiel sa žiaden zdroj odkazov.** to **Chyba! Nenašiel sa žiaden zdroj odkazov.**.

As shown in the tables, temperature had a considerable effect on the flow behaviour of the studied solutions. The consistency coefficient (k) of the blends of guar gum/gelatin and κ-carrageenan/gelatin predominantly

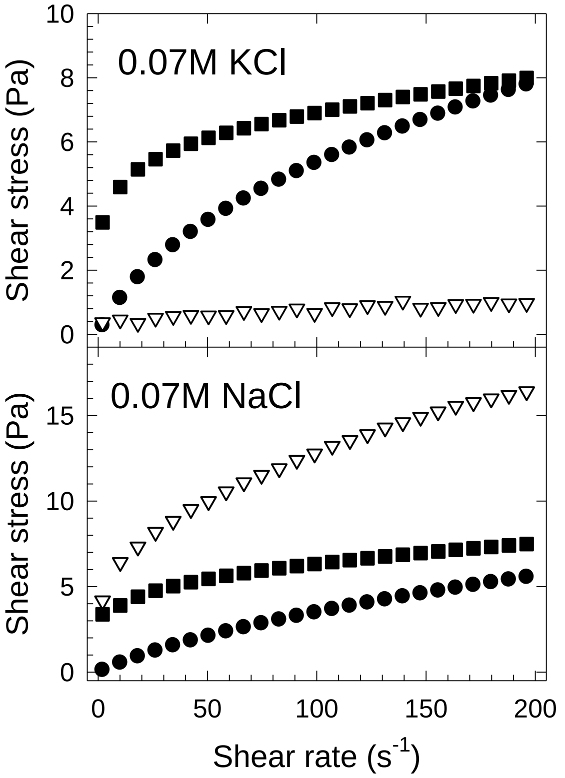

Figure 1 Flow curves of 0.5% w/w gelatin/polysaccharide blends in 0.07M KCl and 0.07M NaCl at 35 °C (upward shear rate): full circle – guar gum/gelatin, empty triangle – κ-carrageenan/gelatin, full square – xanthan gum/gelatin.

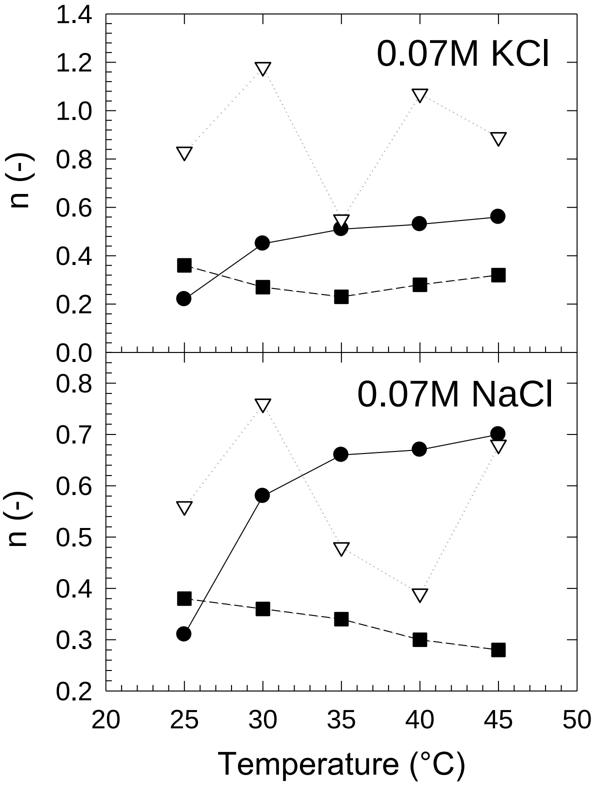

Figure 2 Temperature dependence of flow behaviour index (n) (Herschel-Bulkley model) of 0.5% w/w gelatin/polysaccharide blends in 0.07M KCl and 0.07M NaCl: full circle – guar gum/gelatin, empty triangle – κ-carrageenan/gelatin, full square – xanthan gum/gelatin.

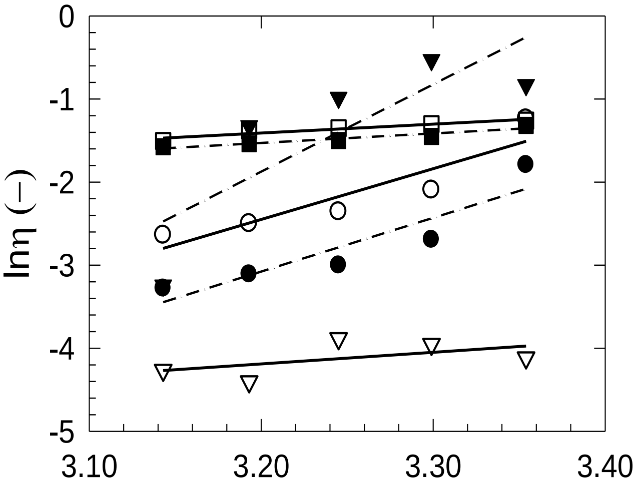

Figure 3 Arrhenius plots of 0.5% w/w gelatin/polysaccharide blends in 0.07M KCl (empty symbols) and in 0.07M NaCl (full symbols) (viscosities read at shear rate 20 s^{-1}): circle – guar gum/gelatin, triangle – κ-carrageenan/gelatin, square – xanthan gum/gelatin. Linear regression curves: full line – in 0.07M KCl, dash-dot line – in 0.07M NaCl.

decreased with the temperature, whereas the flow behaviour index (*n*) increased with the temperature. The pure polysaccharide solutions of guar gum and κ-carrageenan showed the same trend. However, the xanthan gum/gelatin blends and pure xanthan solution exhibited an opposite behaviour of *n* versus temperature, as compared to other samples. For xanthan gum/gelatin blend in NaCl and pure xanthan solution in KCl, the flow behaviour index predominantly decreases, whereas the consistency coefficient increases within the studied temperature range (25 – 45 °C). For xanthan gum/gelatin in KCl, there is an evident step change of flow behaviour index at 30 – 35 °C. This change probably corresponds to the conformational transition of gelatin macromolecules, which undergo a helix to coil transition at temperature above 30 °C **(Derkach et al., 2015)**; the change at temperature around

35 °C could demonstrate the conformational ordering of xanthan gum, i.e., the transition from single coils to double helices.

Overall, xanthan gum provides more viscous blends at higher temperatures, in contrast to guar gum and κ-carrageenan blends which tend to more Newtonian behaviour at elevated temperatures. The blends of xanthan gum also exhibit a significant yield stress (τ_0) within the whole temperature range. Although the values of τ_0 were predominantly decreasing with the temperature, the yield stress was noticeable even at 45 °C, the highest temperature under study (1.647 Pa for the blend in KCl, and 0.973 Pa for the blend in NaCl, respectively, compared to 1.139 Pa for the pure xanthan solution). The viscosity stability of xanthan blends at elevated temperatures (relatively high at low-shear conditions) and the non-negligible values of yield stress result from the weak intermolecular associations of xanthan structure, typical even at very low hydrocolloid concentration. This rheological behaviour of xanthan gum is a consequence of the progressive alignment of the rigid xanthan molecules with the shearing force **(Marcotte et al., 2001)**.

For kappa-carrageenan and gelatin blends, the solutions in NaCl exhibit substantially larger values of yield stress and consistency coefficient, and comparatively lower values of flow behaviour index, than the samples in KCl. This fact can be related to the presence of the potassium or sodium cations which effect the gelation properties of the blends. It means that the flow parameters of the κ-carrageenan/gelatin blends, including yield stress values, are notably affected by the type of salt solvent. K-carrageenan/gelatin blends in NaCl provided a gel of high consistency at temperature about 25 °C, whereas the blend in KCl did not undergo a sol to gel transition within the studied temperature range. Therefore, the differences in the flow parameters between the blends in KCl and NaCl were substantive, particularly between the values of *k* and τ_0. This behaviour proves that κ-carrageenan can be largely used as co-gelators of gelatin-based solutions via interaction of charged gelatin with the polysaccharide macroions leading to formation of PECs. As stated by **Derkach et al. (2015)** the addition of κ-carrageenan to aqueous gelatin solutions affects the gelation kinetics of the blends, as well as the thermal stability of the system;

Table 4 Arrhenius parameters at 20 s^{-1} and 100 s^{-1} (upward shear rate) of 0.5% w/w gelatin/polysaccharide blends in 0.07M KCl and 0.07M NaCl.

Sample	Arrhenius equation (20 s^{-1})				Arrhenius equation (100 s^{-1})		
	Solvent	η_0 (mPa.s)	E_A (kJ.mol^{-1})	R^2	η_0 (mPa.s)	E_A (kJ.mol^{-1})	R^2
Guar gum/gelatin	KCl	2.64x10^{-7}	50.94	0.8438	1.03x10^{-4}	34.02	0.8234
	NaCl	4.52x10^{-8}	53.90	0.8398	2.45x10^{-5}	36.62	0.8682
Kappa-carrageenan/ gelatin	KCl	0.1728	11.63	0.2940	n.c.	n.c.	n.c.
	NaCl	3.576x10^{-13}	87.53	0.6613	1.494x10^{-11}	75.30	0.7083
Xanthan gum/gelatin	KCl	7.873	8.926	0.9113	4.995	6.727	0.9747
	NaCl	5.498	9.545	0.9153	4.163	6.992	0.96

Note: η_0, pre-exponential factor; E_A, activation energy; R^2, coefficient of determination; n.c., not computed value. The number of replicates, 3.

Table 5 Conductivity of 0.5% w/w gelatin/polysaccharide blends in the temperature range 31 – 41 °C.

t (°C)	Guar gum/gelatin		K-carrageenan/ gelatin		Xanthan gum/gelatin	
	C (mS.cm-1)					
	KCl	**NaCl**	**KCl**	**NaCl**	**KCl**	**NaCl**
31	11.37	9.43	11.35	9.59	10.45	8.94
33	11.40	9.53	11.65	9.98	10.55	8.94
35	11.42	9.67	11.73	10.09	10.75	8.94
37	11.54	9.80	11.88	10.61	11.31	9.19
39	11.75	10.10	12.09	10.69	11.34	9.48
41	12.15	10.20	12.22	10.88	11.41	9.51

Note: *t*, temperature; *C*, conductivity. The number of replicates, 3.

Table 6 Data of zeta potential analysis of 0.5% w/w gelatin/polysaccharide blends in 0.07 M KCl and 0.07 M NaCl.

Sample	Solvent	*ζ-potential* (mV)	*D* (nm)	*pH* (-)
Guar gum/gelatin	KCl	-0.99 ±0.36	7922 ±1280	4.83
	NaCl	1.00 ±0.20	3226 ±919	5.00
Kappa-carrageenan/ gelatin	KCl	2.37 ±0.84	6385 ±478	6.61
	NaCl	-7.21 ±0.95	14463 ±734	6.44
Xanthan gum/gelatin	KCl	-4.93 ±1.02	54020 ±9863	4.59
	NaCl	-5.01 ±1.72	28891 ±6874	4.63

Note: *ζ-potential*, zeta potential; *d*, effective diameter; *pH*, pH-value; ±values indicate the standard deviation. The number of replicates, 10.

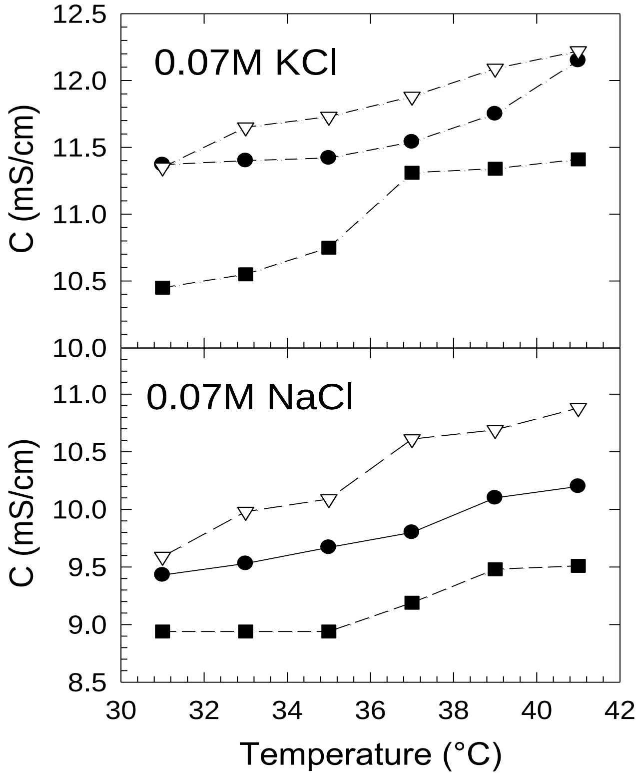

Figure 1 Temperature dependence of conductivity (C) of 0.5% w/w gelatin/polysaccharide blends in the temperature range 31 – 41 °C: full circle – guar gum/gelatin, empty triangle – κ-carrageenan/gelatin, full square – xanthan gum/gelatin.

moreover, the gelatin/κ-carrageenan ratio has a substantial influence on the flow parameters of the blends. In other words, according to the type of the solvent and concentration of the components, the blends of gelatin/κ-carrageenan tend to form a gel. The blends of gelatin with guar gum or xanthan gum in the present study did not undergo a sol to gel transition between 20 – 45 °C.

In the case of guar gum, the rheological behaviour of the gelatin/guar gum blends and pure guar gum solution was considerably different. The flow parameters of 0.5% guar gum in KCl varied in a relatively narrow range whereas the parameters of the gelatin/guar gum blends both in KCl and NaCl exhibit an evident change with the temperature. This fact can probably be attributed to the synergistic effect of gelatin on the flow properties of gelatin/guar gum blends. Besides, the values of *k* and *n* are comparatively different for the blends in KCl and NaCl, respectively. The presence of chaotropic cations (K^+) and kosmotropic cations (Na^+) leads to the reduction of electrostatic complex coacervation of polysaccharide/gelatin system **(Cao et al., 2016)**. The mechanism of action of these ions is opposite: the chaotropic electrolytes (KCl) suppress the electrostatic repulsion effect on the polymer chains; moreover, the chaotropic ions can enhance the dissolution of colloidal aggregates which are ordinarily present in polysaccharide solutions under ambient conditions **(Ma and Pawlik, 2007; Wang et al., 2015)**. On the other hand, the kosmotropic electrolytes (NaCl) promote interactions between water molecules and thus stabilize intramolecular interactions in protein macromolecules such as gelatin **(Moelbert et al., 2004)**. These specific properties of the salt solvents affect the flow behaviour of guar gum/gelatin blends. The effect of Na^+ ions on the gelatin macromolecules seems to tend the guar gum/gelatin blends to more Newtonian fashion at higher temperatures, as compared to the effect of K^+ ions in the same system showing more pseudoplastic behaviour (higher values of *k*, and lower values of *n*). The flow curves of gelatin/polysaccharide blends both in KCl and NaCl read at 35 °C are shown in **Chyba! Nenašiel sa žiaden zdroj odkazov.**. The temperature dependence of flow behaviour index of the blends is presented in **Chyba! Nenašiel sa žiaden zdroj odkazov.**.

Arrhenius model

The temperature dependency of the blends' viscosity was investigated by the Arrhenius model. The activation energy (E_A), which was calculated according to the model, represents an energy barrier of the reorientation of water molecules in the biopolymer system **(Baranowska et al., 2008)**. It means, that the activation energy reflects the sensitivity of the molecular structure to the temperature and its change at a specified shear rate **(Wang et al., 2015)**. The Arrhenius parameters in the present study are stated in **Chyba! Nenašiel sa žiaden zdroj odkazov.**. As can be seen from the table, relatively high values of E_A were determined for the blends of guar gum/gelatin and for κ-carrageenan/gelatin blend in NaCl; these blends are relatively high temperature-dependent samples indicating a lower resistance of their structure at elevated temperatures. On the other hand, the lowest values of E_A were calculated for the mixtures of xanthan gum and gelatin which were less affected by temperature. This fact proves that xanthan gum/gelatin blends have the ability to retain their gel network and viscosity at higher temperatures which is in compliance with the temperature dependency of apparent viscosity observed for xanthan gum solutions **(Marcotte et al., 2001; Marcotte et al., 2001)**.

A relatively high difference between the values of activation energy was determined for kappa-carrageenan/gelatin blends in KCl and NaCl, respectively. However, the Arrhenius model seems to be not suitable for κ-carrageenan/gelatin blends because of low values of coefficient of determination (R^2). Therefore, the Arrhenius model was not applied in some cases. In all cases, the activation energy of the samples in 70 mM NaCl was higher than for the samples in KCl; it means that the blends in NaCl need higher amount of activation energy which is needed to promote the viscous flow than the samples in KCl. In the same way, the activation energy read at 20 s^{-1} was higher than E_A read at 100 s^{-1} for all samples. This is in accordance with the fact that the structure of a hydrocolloid and its change with the shearing plays an important role in the temperature dependency of the solutions. As stated by **(Marcotte et al., 2001)** the decrease in activation energy of the hydrocolloid solutions is, in varying degree, associated with an increase of the applied shear rate, depending on the type of hydrocolloid. The observations indicate that the energy necessary for the reorientation of water molecules in the gelatin/polysaccharide blends is obviously influenced by the type of hydrocolloid and salt solvent, as well as by the applied shear rate.

The Arrhenius plots of gelatin/polysaccharide blends read at shear rate of 20 s^{-1} are shown in **Chyba! Nenašiel sa žiaden zdroj odkazov.**.

Conductivity measurement

The effect of conformational transition on conductivity of gelatin/polysaccharide blends depending on temperature was examined. As stated by **Cao et al. (2016)**, pure gelatin solution contributes only negligibly to the conductivity of gelatin/polysaccharide blends. For that reason, the difference in conductivity between pure polysaccharide solution and gelatin/polysaccharide mixture reflects the difference between the polysaccharide in free and complex states. The average values of conductivity *C* (mS.cm^{-1}), determined for the gelatin/polysaccharide blends, are summarized in Table 5 (the data of conductivity for pure polysaccharide solutions used as reference materials are not stated).

As can be seen from the graph (**Figure 1**) there is an evident change of conductivity at temperature about 35 °C, both for blends in KCl and NaCl, which can be attributed to the helix-coil transition of the polysaccharide. This conformational change entails a steep rise of conductivity above 35 °C. As in the case of rheological behaviour, the change in the slope of conductivity can be considered as confirmation of the effect of polysaccharide conformational ordering on the functional properties of gelatin/polysaccharide complexes.

Zeta potential analysis

The potential stability of the gelatin/polysaccharide blends was evaluated based on the results of zeta potential (ζ-potential) analysis. The experimental data of ζ-potential, pH-values of the solutions and effective diameter of the particles are summarized in **Chyba! Nenašiel sa žiaden zdroj odkazov.**.

The zeta potential is a potential difference between the fluid volume and the thin layer of the counter ions tied to the particle surface. It represents the potential at the plane where slip with respect to bulk solution occurs. In other words, ζ-potential is an electrokinetic potential at the interface between compact and diffusive part of electric double layer. The electric charge of the double layer affects the stability of a colloidal system; ζ-potential is a function of interface order and its sign is opposite to the charge of the ions of outer layer in the electric double layer.

The zeta potential value is dependent on the nature of the surface, its charge (related to pH), the electrolyte concentration in the solution, and the nature of the electrolyte and solvent.

The most important factor for ζ-potential determination is the pH-value. The zero value of ζ-potential indicates the least stable state of the colloidal system at a defined pH, i.e., an isoelectric point of the system **(Anonym, 2000; Delgado et al., 2007)**.

Zeta potential analysis was used to characterize the gelatin/polysaccharide blends. More specifically, the analysis determined the potential difference among the blend medium and the layer of fluid related to the dispersed particles. The colloidal particles (when dispersed in an aqueous medium) prone to interact with other close particles to form aggregates or clusters by attractive forces or remain dispersed via electrostatic repulsion. The balance between attractive and repulsive forces depends on surface charge characteristic which is determined by material and solution type; for example, an electrostatically stabilized colloid is possible to coagulate by adding enough electrolyte such as KCl. Thus, the zeta potential provided information about the magnitude of the electrostatic repulsive forces of the examined colloid systems **(Gerzhova et al., 2016; Wu et al., 2015)**.

In the present study, the low values of ζ-potential determined for all samples (less than ±10 mV) indicate the instability of gelatin/polysaccharide blends, which tend to coagulate. It means that the electrostatic repulsion between similarly charged nearby particles in the solutions is weak and the blends do not resist aggregation. It suggests that the particles of the blends are not small enough, and the attractive forces between them exceed the electrostatic repulsion.

The highest ζ-potential (in absolute value) was measured for κ-carrageenan/gelatin in NaCl (7.21 mV) with corresponding effective diameter of the particles 14463 nm. On the other hand, the lowest values of ζ-potential were determined for guar gum/gelatin blends both in KCl and NaCl (around ±1.0 mV) which represent the least electrically stabilized blends of all samples.

CONCLUSION

The results of the present study demonstrate that the conformational transition of gelatin/polysaccharide polyelectrolyte complex has a substantial influence on the functional properties of gelatin/polysaccharide blends. The noticeable change of flow parameters and conductivity of the solutions upon heating, determined about 35 °C, corresponds to the helix-coil transition of the polysaccharide. The flow behaviour and gelation properties of the blends were also affected by the type of hydrocolloids and salt solution; gelatin/kappa-carrageenan blend in NaCl showed a gel of high consistency at ambient temperature (ca. 25 °C), while the same blend in KCl did not gel even at refrigerator temperatures. The potential stability of the blends was investigated by zeta-potential analysis. On the basis of relatively low values of ζ-potential, the gelatin/polysaccharide blends can be characterized as electrically unstable systems which tend to coagulate.

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