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Influence of collagen modifications on qualitative parameters of thermoplastic adhesive mixtures and its microbiological stability

JÁN MATYAŠOVSKÝ[1)], JÁN SEDLIAČIK[2)], IGOR NOVÁK[3)], PETER DUCHOVIČ[1)], PETER JURKOVIČ[1)]
[1]VIPO a.s., Gen. Svobodu1069/4, 958 01 Partizánske, Slovakia
[2]Faculty of Wood Sciences and Technology, Technical University in Zvolen, T.G. Masaryka 24, 960 01 Zvolen, Slovakia
[3]Polymer Institute, Slovak Academy of Sciences, Dúbravská cesta 9, 845 41 Bratislava, Slovakia

Abstract: *Influence of collagen modifications on qualitative parameters of thermoplastic adhesive mixtures and its microbiological stability.*This work presents the possibility to use the modified biopolymer collagen for preparation of ecologic, biologically degradable thermoplastic adhesives. Collagen prepared from secondary raw materials of the leather and food industry was applied as a starting material for the preparation of thermoplastic, formaldehyde-free adhesives intended for use in woodworking, furniture and paper industries. Glued joint obtained high strength and flexibility after application of modification plasticisation agents based on collagen. Modifications of collagen glue with keratin biopolymer increased its resistance to water and the strength of the glued joint. Prepared samples of hot-melt adhesive had higher bonding strengths than standard commercial adhesives. The highest tensile strengths were achieved by applying of undiluted adhesive with the application of 2.5% keratin hydrolysate into hot-melt adhesive. As collagen is a natural polymer easy biodegradable in the aquatic environment, the research has focused on the possibility of its microbiological stabilization with aqueous solutions of ionic and colloidal silver. The highest microbiological activity was observed in a sample of ionic silver sulphate solution with a concentration of 2000 ppm Ag^+. Its 1% concentration was applied for antibacterial thermoplastic stabilization of formaldehyde-free collagen glue.

Keywords: hot-melt adhesive, plasticizer, collagen, keratin, viscosity, bacterial stability, colloid silver

INTRODUCTION

Possibilities of effective processing and applications of leather tanned and non-tanned waste for different products are described in (Pünterer 1995, Buljan et al., Matyašovský et al. 2011, Matyašovský 2008). Controlled enzymatic hydrolysis has the advantage in lower consumption of energy, especially when using commonly available commercial proteases of microbial origin (Kolomazník et al. 1999, Kolomazník et al. 2000, Sun and Zhong 2000). The advantage of this procedure can be the control of medium mole weight of hydrolysate by selection of reaction time of enzymatic hydrolysis.

Collagen is the main component of connective tissues and also belongs among technically most important fibrous proteins. Characteristic property of collagen molecule is the strength and three dimensional spiral structure created with α-chains, which rotate to regular right-handed scroll. Molecules are created mostly of glycine (26-28 %) and proline (over 15 %). Native collagen without modification is relatively difficult to process for proposed application. Collagen, similarly as other proteins, has character of amphoteric polyelectrolyte, what causes, that its ion reactions run in the dependence on pH value. Isoelectric point of native collagen is at value of pH 7.5 and it can be changed by mild affect with chemicals in the interval pH 4.5 – 8.0. Most of physical and chemical parameters of collagen have in this interval extreme values, stability is changing, reactivity, ability of hydration etc. (Blažej et al. 1978, Blažej et al. 1984). This property of collagen was used at modification of adhesives for woodworking industry and production of modified

thermoplastic collagen adhesives. Mutual combination of individual components of the mixture and their concentrations enable to modify physical and mechanical properties of adhesives and their biologic degradation (He 2016).

Chemical crosslinking of collagen can be combined with casting steps as spinning, moulding or additive manufacturing techniques. The effects of the processing steps on the final materials properties are discussed especially with regard to the thermal and the physical properties (Meyer 2019). However, collagen is also suffering from the poor physical and chemical properties (mechanical strength, thermo-stability, resistance to enzyme and so on). Therefore, the modification of collagen in preparation process is necessary (Liu et al. 2019). Collagen prepared from secondary raw materials of the leather and food industry was applied as a starting material for the preparation of thermoplastic, formaldehyde-free adhesives intended for use in woodworking, furniture and paper industries.

The aim of our work is the proposal of technology of adhesives preparation and investigation of the influence of obtained modified collagen on parameters of adhesive dispersions and gluing. For increasing of microbial stability, modification with colloid silver was laboratory tested, with regards to the significant antibacterial properties with the aim to prolong the lifetime of protein adhesives.

MATERIALS

Chemicals and raw materials for modification of adhesive mixtures:

- collagen and keratin biopolymers,
- acids, alkalis, proteases,
- urea, glycerine, Ag_2SO_4,
- paper glued materials with different thickness,
- calcium sulphate, titanium dioxide.

METODS

Labelling of the tested samples:

Sample No. 1 collagen adhesive reference – 40% technical gelatin, water 50%, hydrolysis 90 minutes, plasticization by 15% glycerin, the melting temperature collagen thermoplastic dispersion 55 - 60°C and reverse solidification temperature of the adhesive 30 °C, dry content of matter 55.2%.

Sample No. 2 collagen white adhesive - 40% technical gelatin, water 50%, hydrolysis 90 minutes, plasticization by 15% glycerin, modification by 5% titanium dioxide, the melting temperature collagen thermoplastic dispersion 55–60°C and reverse solidification temperature of the adhesive 35°C, dry content of matter 60.2%.

Sample No. 3 – collagen white adhesive + inorganic filler - 40% technical gelatin, water 50%, hydrolysis 90 minutes, plasticization by 15% glycerin, modification by 5% titanium dioxide + 5% calcium sulphate, the melting temperature collagen thermoplastic dispersion 55 – 60°C and reverse solidification temperature of the adhesive 35- 38°C, dry content of matter 65.5%.

Keratin was prepared using sheep wool ‘Merino’ of the following composition: nitrogen 12.15 %, ash 2.53 %, sulphur 2.21 %, fat 7.16 %. The wool was separated, washed, defatted and dried at room temperature. Keratin hydrolysates was prepared by acid hydrolysis. 900 mL distilled water and 100 mL of concentrated HCl were added into 100 g of sheep wool. The hydrolysis proceeded at the boiling point in closed glass vessel for 5 hours. Prepared hydrolysate was filtered, neutralized and concentrated to 40% dry of matter.

RESULTS

The solution was focused on the plasticization of collagen by application of trihydric alcohols, amines, modification by the biopolymer of keratin etc. in order to achieve the desired adhesive parameters, e.g. film ductility, hydrophilicity, hydrophobicity, viscosity, melting and solidification points (Liu et al. 2019). The technology of collagen hydrolysate preparation was made in a duplicator with heating to the temperature max. 60 °C, by stirring and with the possibility to add chemicals in liquid and solid form. The following chemicals were used to prepare collagen hydrolysate: water, collagen, HCl, and lyotropic agent. The device was supplemented by dosing of modified collagen dispersion into PE containers in which the dispersion cools and forms a solid gel, while preventing the thermoplastic collagen adhesive from drying out and microbiological contamination of the hot-melt adhesive.

The formulation of model collagen thermoplastic dispersion and quality parameters of collagen adhesive at 60 °C after plasticization:

pH: 6.5 ± 0.5,
viscosity: 2100-2200 mPa.s,
dry content matter, refraction value: 55-60 %,
open time: short,
surface stickiness of adhesive: immediate,
storage ability: min. 6 month at laboratory temperature.

The melting and reverse solidification temperature of the adhesive (30-60) °C depends on the collagen concentration and the modifying additives, pH, time and temperature of hydrolysis.

Table 1. Qualitative parameters of collagen after plasticisation.

The change of viscosity of developed collagen adhesives with temperature					
Sample No. 1		Sample No. 2 white adhesive		Sample No. 3 white adhesive + inorganic filler	
Temperature (°C)	Viscosity (mPa.s)	Temperature (°C)	Viscosity (mPa.s)	Temperature (°C)	Viscosity (mPa.s)
50	3125	50	8013	50	12550
55	2513	55	5613	55	8663
60	2113	60	4500	60	6825
65	1825	65	3713	65	5575

Since the water and moisture resistance of the bonded joint is an important parameter of the quality of the adhesive dispersion, the experiments were focused on testing of the surface properties of collagen foils and the collagen foils with the keratin. Static wetting contact angles were measured at different locations on the film surface samples. As the collagen foil, according to the contact angles of water, is more hydrophilic than the collagen foil with keratin, the solution was also focused on the possibility of modifying the collagen glue using the keratin biopolymers in order to increase the bond strength.

Description and composition of tested hot melt adhesives and tensile strength according to EN 3376:

1. DAVIS BL 150 – standard thermoplastic collagen adhesive, dry content matter 55.3%,
2. sample No. 1 – hot-melt collagen adhesive – prepared in VIPO, dry content matter 55.2%,
3. sample No. 2 – hot-melt collagen adhesive + 2.5% keratin hydrolysate, dry matter 54.8%,
4. sample No. 3 – hot-melt collagen adhesive + 5% keratin hydrolysate, dry matter 54.4%.

Table 2. Parameters of collagen adhesives in comparison with standard DAVIS BL 150 according to EN 3376.

Thermoplastic adhesive	MPa concentrated	MPa diluted 1:1	MPa diluted 1:1 1:2	MPa diluted 1:1 1:4
DAVIS BL 150 – standard	1.33	0.77	0.18	0.14
Sample No. 1	1.58	1.25	0.32	0.21
Sample No. 2	2.40	0.92	0.29	0.18
Sample No. 3	2.25	0.82	0.25	0.15

According to obtained results, adhesives prepared in VIPO reached higher tensile strength as standard commercial adhesive DAVIS. Highest tensile strength was reached with the application – sample No. 2 hot-melt collagen adhesive + 2,5% keratin hydrolysate, dry content matter 54.8%. Thermoplastic adhesives based on collagen have the value of pH in interval (6-7) and temperature of application must not exceed 70 °C, because adhesive will degrade.

As the collagen is a natural polymer that is easy biodegradable in the aquatic environment, research has focused on the possibility of its microbiological stabilization with aqueous solutions of ionic and colloidal silver. Silver sulphate Ag_2SO_4 p.a. was selected for the preparation of Ag^+ ionic solution and colloidal silver.

For preparation of Ag nano-particles with controlled size, two-stage reduction was applied, where combination of smaller (approx. 10-20 nm) and bigger particles with dimensions up to hundred nm is created. Colour shade of colloidal silver depends on wavelength of the spectra and from the size and shape of nano-particles and surrounding, in which are dispersed. For example, small nano-particles of silver in water are yellow, as they have absorbency peak at wave length approx. 400 nm. Stabilized silver sulphate solution – the ionic form of silver was tested as a reference standard for particles.

Figure 1. Images of water dispersions of silver particles prepared by reduction of Ag^+ in ammonia. From left: dispersions – ion silver; nano-particles of silver with the size approx. 25 to 50 nm.

Research was also aimed on preparation and application of colloid silver and ion silver with the aim to prolong lifetime of collagen adhesives. Scanning electron microscope (SEM) - JEOL JIB-4000 was used for control measurements of the size and aggregation of silver particles. Procedure: polymer foil was powdered by fine layer of Au (with the thickness of 4 nm), and then, thin layer of colloid Ag was spread, and water evaporated at laboratory temperature.

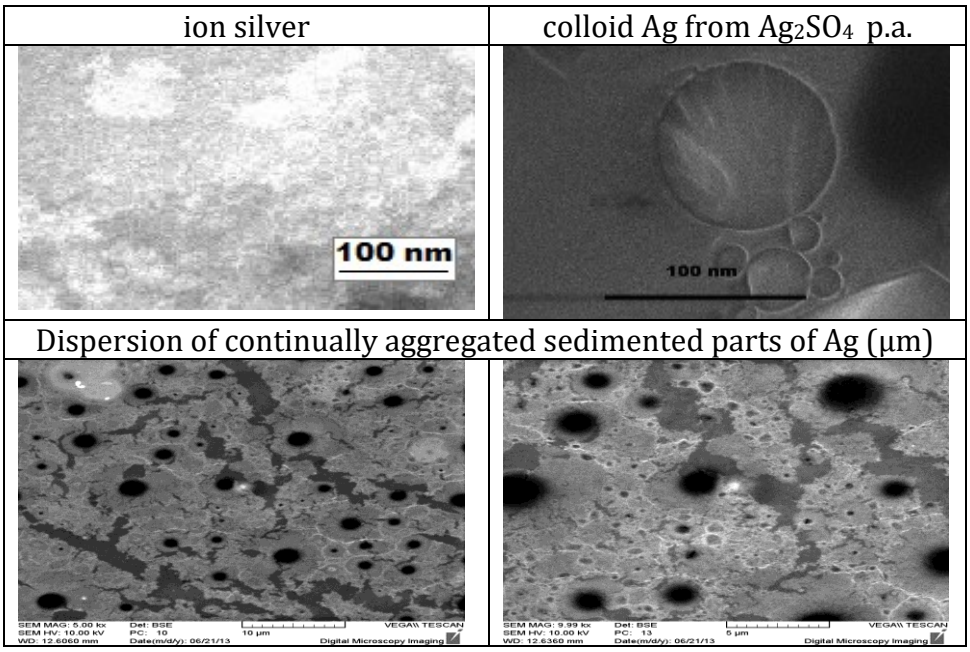

Figure 2. SEM images illustrating of ion and nano-particles of silver.

The testing of the effect of concentration of ionic and colloidal silver on microbiological activity was performed according to the relevant EN standards on samples given in Tab 3.

Table 3. Testing the effect of the size of Ag particles on microbiological activity.

Tested strain	Minimal inhibition concentration in μg/ml		
	Ag > 100 nm	Ag (25-100) nm	Ion Ag+
Pseudomonas aeruginosa, 168/79	13.50	4.75	0.98
Staphylococcus aureus, 43/60	6.75	6.75	1.91
Candida albicans, NCTC 49/64	27.00	12.96	3.86
Aspergillus niger	–	16.75	5.25

Results of microbiologic activity of water solutions of Ag^+ salts and Ag colloids depend mainly on the size of particles and on the type of bacteria strain. The highest microbiologic activity performed the sample of ionic solution of silver sulphate with the concentration of 2000 ppm Ag/litre. Its 1% concentration was applied for the antibacterial thermoplastic stabilization of formaldehyde-free collagen adhesive. Testing the influence of the concentration of ionic silver on microbiologic activity was performed according to appropriate EN standards on following samples:

1. bactericidal effect on gram-negative bacteria: *Pseudomonas aeruginosa* was confirmed at 0.25 % concentration and time of 5 min,
2. bactericidal effect on gram-positive bacteria: *Staphylococcus aureus* was confirmed at 0.5 % concentration and time of 24 h,
3. fungicidal effect on vegetative cells: *Candida albicans* was confirmed at 0.5% concentration and time of 24 h,
4. fungicidal effect for *Aspergillus niger* was confirmed at 1 % concentration and time of 24 hours,
5. sporicidal effect for *Bacillus subtilis* was confirmed at 80 % concentration after 24 h.

Collagen modified with colloid silver

Morphology of collagen samples with colloid silver was investigated by dual scanning microscope SEM FEI Quanta 3D (The Institute of Electrical Engineering of Slovak Academy of Sciences). Procedure: metallisation of underlay foil was realised by powdering of Au in thin layer with the thickness of 3 nm.

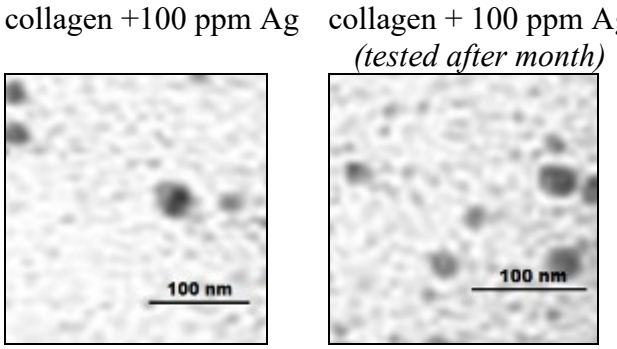

Figure 3. SEM images of colloid silver particles and collagen hydrolysate – images illustrate long-term stability of nanoparticles of silver with collagen at the value of pH=5.7.

In laboratory conditions, there were prepared 2 samples of collagen hydrolysates, one without modification with colloid silver and another with its modification with the aim to compare IR spectra of these samples. IR spectra of samples of collagen and collagen modified with colloid Ag were comparable even after one month, accordingly to the research of Nogueira et al. (2019).

CONCLUSIONS

Experiments were aimed to testing the influence of modification of collagen colloids on properties of thermoplastic collagen adhesive. Laboratory tests confirmed biopolymers of animal origin (e.g. technical leather glue, gelatine etc.) as suitable raw materials for preparation of thermoplastic collagen adhesives. Evaluation of raw material from leather industry from renewable sources was investigated with the aim to apply them after modification for gluing of book sheets. Tested modifications of collagen enable to prepare thermoplastic collagen adhesive with required parameters. For increasing of the lifetime of adhesive, application of colloid silver was laboratory tested. Results obtained in VIPO Partizánske and in industrial conditions of printing company confirmed, that collagen adhesive prepared in VIPO is possible to use for technical application in woodworking, paper or similar industries. The glued joint with collagen obtained high strength and flexibility by application of plasticizing agents. Obtained experimental results confirmed, that modified collagen gel is possible to use for preparation of ecologic thermoplastic adhesives with comparable parameters of competitive commercial adhesives.

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Streszczenie: *Wpływ modyfikacji kolagenu na parametry jakościowe mieszanin klejów termoplastycznych i ich stabilność mikrobiologiczną*. W pracy przedstawiono możliwości zastosowania modyfikowanego kolagenu biopolimerowego do wytwarzania ekologicznych, biodegradowalnych klejów termoplastycznych. Jako materiał wyjściowy do wytwarzania bezformaldehydowych klejów termoplastycznych przeznaczonych do stosowania w przemyśle drzewnym, meblarskim i papierniczym zastosowano kolagen wytworzony z surowców wtórnych przemysłu skórzanego i spożywczego. Zastosowanie modyfikatorów na bazie kolagenu pozwoliło uzyskać wysoką wytrzymałość i elastyczność połączeń klejowych. Modyfikacje kleju kolagenowego przy zastosowaniu biopolimeru keratynowego zwiększyły jego odporność na wodę i wytrzymałość złącza klejowgo. Przygotowane próbki kleju

topliwego charakteryzowały się większą wytrzymałością spoin klejowych niż standardowe kleje handlowe. Najwyższe wytrzymałości na rozciąganie uzyskano przez zastosowanie nierozcieńczonego kleju topliwego z dodatkiem 2,5% hydrolizatu keratyny. W związku z tym, że kolagen jest naturalnym, łatwo biodegradowalnym polimerem w środowisku wodnym, badania skupiły się na możliwości jego stabilizacji mikrobiologicznej za pomocą wodnych roztworów srebra jonowego i koloidalnego. Najwyższą aktywność mikrobiologiczną zaobserwowano w próbce roztworu jonowego siarczanu srebra o stężeniu 2000 ppm Ag+. 1% roztwór tego związku zastosowano do antybakteryjnej stabilizacji kleju kolagenowego.

Corresponding author:

Prof. Ing. Ján Sedliačik, PhD.
Faculty of Wood Sciences and Technology
Technical University in Zvolen
T.G. Masaryka 24
960 01 Zvolen
Slovakia
e-mail: jan.sedliacik@tuzvo.sk

ORCID ID:

Sedliačik Ján 0000-0003-0014-594X