Modified Smart Collagen Biomaterials for Pharmacy and Adhesive Applications

by
Ján Matyašovský,[1] Ján Sedliačik,[2*] Igor Novák,[3] Peter Šimon,[4] Peter Jurkovič,[1] Peter Duchovič[1] and Angela Kleinová[3]
[1]*VIPO, a.s., Partizánske,*
Gen. Svobodu 1069/4, 958 01 Partizánske, Slovakia
[2]*Faculty of Wood Sciences and Technology, Technical University in Zvolen,*
Masaryka 24, 960 53 Zvolen, Slovakia
[3]*Polymer Institute, Slovak Academy of Sciences,*
Dúbravská cesta 9, 845 41 Bratislava 45, Slovakia
[4]*Faculty of Chemical and Food Technology, Slovak Technical University,*
Radlinského 9, 812 37 Bratislava, Slovakia

Abstract

Collagen has widespread use for preparation of cell cultures, dermal cosmetics, food and medicines, while achieving biocompatibility with the biological environment. Unmodified native collagen is relatively difficult to process for intended applications. This work presents potential application possibilities of modified collagen biopolymer for pharmacy and adhesive applications.

Firstly, the application possibilities of collagen in the system of controlled drug release were verified. Dissolution profile of the matrix Ambroxol hydrochloride and Venlafaxine confirmed potential of crosslinked and plasticized collagen as a pharmaceutical agent for the solid medicinal product. This product was prepared by direct compression with controlled drug release within the 8-11 hours with almost zero-order kinetics.

Secondly, low temperature plasma was applied to achieve the biocompatibility and to modify the film surface of the collagen type I. Collagen film was treated in two plasma environments; low temperature plasma N_2/H_2 was used for grafting the amino groups and CO_2 plasma ensured grafting the carboxyl groups. The surface functional groups of collagen were applied in further reactions (e.g., antimicrobial pretreatment with strong biocide properties). Functionalized collagen film provided stronger adhesion and compatibility. Prepared non-formaldehyde collagen thermoplastic adhesive was tested for the technical applications. The results have shown that the collagen adhesive bond gained high strength, flexibility and required strong gluing, e.g. books or wood veneered materials.

Introduction

In recent years, the environmental processing of secondary raw materials from various industrial productions to obtain products with high-added value has gained very high attention. Fibril proteins of skin, mainly collagen and keratin are perspective biopolymers for biomedicine, pharmacy, cosmetics and other technical applications (e.g. bonding). Food and leather industry are among the biggest polluters of the environment. Biopolymer waste from manufacturing of meat and skins is not recovered complexly, and remains as the environmental load. Other wastes such as chromium shavings, limed fleshings, animal fat, wool, hair, feathers, keratin hydrolysate from skins processing contain a mixture of various substances of the chemical and biological origin. These wastes can be on one hand inert and biodegradable, and on the other hand possibly hazardous. They contribute to global environmental pollution, and therefore, the main attention focuses on their further processing and use. The last couple of years are characterized by the rapid development of advanced technologies aimed specifically on new applications, quality parameters, and pricing of new biologically active natural substances.[1-5]

Almost all secondary raw materials described above contain collagen protein because this biopolymer is widespread throughout the animal kingdom. Collagen protein is one of the technically most important proteins. Collagen is the main component of connective tissues where affects their proper function-ability and determines mechanical properties. This is due to its specific structure and a high degree of the internal molecule organization.[6]

*Corresponding author e-mail: sedliacik@tuzvo.sk
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Collagen represents 25-30% of all proteins in the body and represents the main organic component of skin, bone and cartilage. Moreover, it is an important part of blood vessel walls, basement membrane and corneas. It also has the support and protection functions and belongs to the key life protein processes in a healthy organism mainly as a component of the extracellular matrix proteins.[7-8]

Collagen with other proteins has the amphoteric polyelectrolyte nature that causes ionic reactions to occur depending on the pH of the environment. This means that some side chain groups are ionized in the alkaline and some in the acid pH. The collagen molecule charge changes with pH. It has a positive charge in strong acids and a negative charge in strong alkalis. The isoelectric point of the native collagen occurs at pH 7 and under slight chemical affect could be changed within the range of 4.5-8.0. Most of the physical and chemical properties show the extreme values in this range.[9]

Collagen is one of the transitional colloidal system – gel from the physical and chemical point of view. Its most important feature is the swelling ability. After immersion in water, the collagen fiber partially swells (exothermic process). This process is accompanied by a change of the fiber volume, length and flexibility. Some water in the swollen collagen can be mechanically removed, the other water type is moisturizing that is colloidally bounded and removable only by drying.[10]

Numerous articles have been published about applications of collagen as a drug carrier, but only a few collagen-based systems are currently available or get into clinical testing. Therefore, there is a continuing effort to improve collagen products and find new processing ways and use. Advantages of the collagen application as a drug carrier are following:

- good biocompatibility and well characterized, low antigenic properties,
- degradation to physiologically well tolerated compounds,
- individual preparation steps take place in the aqueous environment,
- improved cell penetration and wound healing.

However, the collagen application has also some disadvantages:

- the high cost of pure collagen type 1 preparation,
- the composition variability of the insulated collagen (crosslinking density, fiber size, the trace contamination, etc.),
- hydrophilicity, which results in the higher swelling and more rapid drug release compared to synthetic polymers,
- variability in the rate of the enzymatic degradation compared to hydrolytic decomposition,
- complicated manipulation.

Benefits of collagen applications will lead to the intensive future research and new product development in tissue engineering. The interest in collagen medical applications in the formulation of dosage drug forms has been widely investigated.[11-14]

In order to apply collagen in pharmacy or as an industrial adhesive, it needs to be modified by, for example, crosslinking or plasticizing. The main disadvantage of the chemical agents used in the collagen crosslinking for pharmaceutical or cosmetics applications is a potential toxic effect of residues. Therefore, the research focuses on the alternative physical methods such as dry heat, UV and gamma-radiation. The increase of denaturation temperature and resistance against degradation by collagenases is achieved by collagen exposure to dry heat and UV-radiation with the wavelength of 254 nm. However, partial denaturation is expected during this process. When using the heat, reduction of the actual water content to the minimum before preparations is very important because even a small amount of moisture can cause collapse of the original structure and lead to proteolysis.[15] Little dehydration induces the formation of amides and esterification. Dry heat sterilization causes partial denaturation and formation of crosslinked bonds. Although, tensile strength can be achieved by dry heat, degradation in vivo can be substantially changed. A combination of degradation and crosslinking enables non-specific enzymes to attack and solubilize the crosslinked fragments. The sensitivity of collagen to trypsin was increased at heat treatment, while degradation by pepsin and lysosomal enzymes was reduced.[16]

Crosslinking under UV – radiation is initiated by free radicals, which are forming on aromatic residues of amino acids. They can create only a limited crosslink density due to the low content of tyrosine and phenylalanine in collagen.[17] Therefore, the exposure doses are short and the maximum value of cross-linking is rapidly reached. The mechanical strength is also increased under UV – radiation.

Collagen is also possible to use as a modifier of polyvinyl alcohol intended for thermoplastic processing to blow extruded films. Proteins such as wheat gluten, corn zein, soy protein, myofibrillar proteins, and whey proteins have been successfully formed into films using thermoplastic processes such as compression molding and extrusion. Thermoplastic processing can result in a highly efficient manufacturing method with commercial potential for large-scale production of edible films due to the low

moisture levels, high temperatures, and short times used.[18-20] Another way of modification of the properties and processability of collagen is plasticizing and compounding with suitable types of polymers.[2] The possibility of usage of the biodegradable polymer film of ethylene-vinyl acetate (EVAc) and modified protein as a bio-based and biodegradable hot-melt adhesive has also been studied.[21] Thermo-oxidative stability of different materials and biopolymers was tested by differential scanning calorimetry (DSC). The method is based on determination of the end of induction period, or the beginning of the main oxidation process.[22-23] Films of EVAc and collagen copolymers have been modified with plasma, and the influence of low-temperature atmospheric discharge plasma on the change of surface properties has been studied. The chemical changes of collagen films modified by plasma were analyzed using Fourier Transform Infra Red – Attenuated Total Reflection (FTIR-ATR) spectroscopy.[24]

The aim of the experiments is focused on verification of the possibility of collagen application not only as the potential binder (adhesive) of tablets, but also as an original pharmaceutic excipient based on physically crosslinked collagen for the purposes of the formulation, manufacturing and other usage of the solid dosage forms with the controlled drug release. Matrix tablets containing drug, Ambroxol hydrochloride and Venlafaxine are to be prepared by direct pressing. The technical applications are focused on the preparation of non-formaldehyde collagen thermoplastic adhesive with required quality for gluing of books or wood veneered materials.

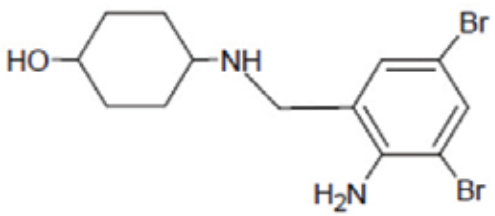
Experimental

Experimental investigation was carried out using the published data about collagen as the perspective biomaterial for drugs (in various dosage forms) and transport systems related to solid medicament forms. Used measurement methods were performed according to European Pharmacopoeia.[25]

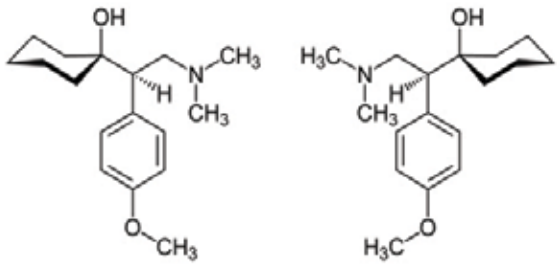
Material and Methods

Used Drug and Excipients:

Ambroxol hydrochloride – Antitussive; white or yellowish crystalline powder, soluble in methanol and water, practically insoluble in methylene chloride, pH = 4.5-6.0, heavy metals max. 20 ppm.



Venlafaxine hydrochloride – antidepressant, racemic mixture, easily soluble in water and methanol, soluble in absolute ethanol, low solubility, practically insoluble in acetone, molar mass 277.402 g/mol, melting point 215-217°C.



Collagen BS – prepared from porcine skins, crosslinked, plasticized, the particle distribution of 45 Mesh - max. 0.355 mm prior the crosslinking and plasticization, pH 5.1, Bloom 220 at the concentration of 6.67% and temperature of 10°C – subsequently crosslinked and plasticized.

Collagen HS – prepared from bovine hides, heat-treated, plasticized, particle size of 45 Mesh - max. 0.355 mm prior the crosslinking and plasticization, pH 5.5, Bloom 176 at the concentration of 6.67% and temperature of 10°C – subsequently crosslinked and plasticized.

Collagen K 12 – characterized as a collagen heat-treated powder. The sample is a mixture of particles less than 200 Mesh - 0.074 mm and the particles distribution of 60 Mesh - max. 0.25 mm. Collagen K 12 is light brown powder, plasticized, compressible, with the ability to produce matrix tablets.

Ethylene Vinyl Acetate – Evatane® 1080 VN 5 Ethylene Vinyl Acetate

Methocel K 100 McR – cellulose ethers, water-soluble polymers derived from cellulose, viscosity 75 000-140 000 cP, hydroxypropoxyl (HP) substitution 9.5-11.5%, methoxyl (MeO) substitution 22.0-24.0%, particle size through 100 mesh – 0.125 mm min 90%.

Measurement Methods:

Tablet Pressing

Tablets were pressed on universal press (VEB Elmo-Thurm), under the pressure of 1000 – 1500 kPa, with tablet diameter of 12 mm.

Mechanical Testing of Tablets

The tablet resistance test to fracture determines the force required to break the tablet under defined conditions. The used device (Schleuniger 2E Hardness Tester) was calibrated according to European Pharmacopoeia.

The test of friability of uncoated tablets was carried out on the tablet friability tester (Erweka TA) under defined conditions. The permitted weight loss of the tablet after exposure on friability test is 1%.

The test of mass uniformity was carried out on the laboratory weight (Mettler AE 163).

Disintegration of Tablets

This test investigates if tablets are disintegrated within the determined time, in liquid medium and in determined experimental conditions. Tablets are placed in a basket below the level of water at the temperature of 37 ± 2°C and the basket is rotated at 30 rpm. The end of the test is when no residue of the tablet remains in a basket. If the tablet does not disintegrate within 15 minutes, it is marked as non-compliant.

Dissolution of Tablets

The test quantifies the release rate of active substances from tablets and it was done under the following conditions:

- dissolution medium: purified water,
- media volume: 900 ml,
- media temperature: 37°C ± 0.5°C,
- basket rotation: 100 per min.

Drug concentration was determined spectrophotometrically (Philips PU 8620 UV/VIS/NIR) at a wavelength of 246 nm, and the flow cell of 10 mm.

Surface and Adhesion Properties of EVAc Films with Collagen

The contact angles were determined by measurements of five test liquids with different polarity. Eight replicated measurements were used to test the contact angle for each testing liquid. The test liquids drops (V = 5 μl) were placed on the surface of the film samples with a micropipette (Biohit, Finland). The contact angle measurements were performed using a professional device equipped with a web camera (Advex, Czech Republic) and the appropriate software.

The shear strength of adhesion joints was tested on dynamometer (Instron 4301, USA) using overlapped sheets of aluminum with 20×10 mm dimensions of overlapping.

Plasma Modification

The surface of the film samples was treated under air, N_2/H_2 and CO_2 as the processing gases. The atmospheric Diffusive Coplanar Surface Barrier Discharge (DCSBD) plasma was applied in a laboratory-scale plasma system operated at a reduced pressure of 80 Pa. The system consists of two circular brass electrodes with dimensions of 240 mm in diameter and 10 mm in thickness. They were placed in a parallel orientation, between which DCSBD plasma was induced.

ATR-FTIR Spectroscopy Measurements

ATR-FTIR measurements were performed with an FTIR ™NICOLET spectrometer (Thermo Scientific, USA) using a single bounce ATR accessory equipped with a Ge crystal. For each measurement, the spectral resolution was 2 cm^{-1} and 64 scans were performed.

Plywood Preparation

Rotary-cut veneer sheets of birch wood (*Betula verrucosa* Ehrh.) free from defects were used for the experiments with the following dimensions: 300×300 mm with 1.5 mm thickness and the moisture content of approximately 6%. Three-layer experimental plywood panels were laboratory-prepared using prepared thermoplastic films. The film was placed between veneers, then laid up and hot-pressed in a laboratory press using the pressing temperature of 130°C, pressure of 1.8 MPa, and time of 5 min. The shear strength of plywood samples was tested according to EN 314-1 and 314-2.[26-27] After pressing, plywood was cooled under the steel plate for 1 hour, ready panels were conditioned for 7 days and strength of samples (Figure 1) was tested after immersion in water for 24 hours.

Marking of Prepared Films:

- **No. 77** – (80% EVAc copolymer + 20% modified collagen) – thickness 0.10 mm
- **No. 84** – (70% EVAc copolymer + 30% modified collagen) – thickness 0.13 mm
- **No. 98** – (50% EVAc copolymer + 50% modified collagen) – thickness 0.09 mm

Results and Discussion

1. Applications of Modified Collagen in Pharmacy

The aim was to prepare and verify the functionality of pharmaceutical excipients not only as the filler of solid medicament forms – uncoated tablets, but also as the binder and mainly as an additive of the matrix of tablets with the controlled drug release.

Figure 1. Test piece according to EN 314-1.

Non-crosslinked Collagen Hydrolysate as the Tablet Binder

Non-crosslinked "Collagen BS" hydrolysate was tested as an adhesive binder. The tablets with collagen concentration of 5%, 10%, 11%, 15%, and 22% were pressed from prepared granules. The effect of collagen concentration on basic parameters was evaluated, i.e. disintegration of tablets, friability of uncoated tablets – mechanical resistance to abrasion, and resistance of tablets to fracture – mechanical compression strength. Disintegration of tablets meet the requirements of European Pharmacopoeia – tablets were disintegrated within 15 minutes, the disintegration time (from 56.6 s to 680.3 s) was extended with an increase of concentration of collagen in the tablet, see Figure 2.

The resistance of tablets to breakage (radial strength of tablets in pressure) was satisfactory over collagen concentration of 11% in the granulate (from 72.5 N at 11% concentration to 138.6 N at 22% concentration), see Figure 3.

Mechanical resistance of uncoated tablets to abrasion (friability) – weight loss from 7.76% to 0.34% decreased with increasing concentrations of collagen, see Figure 4.

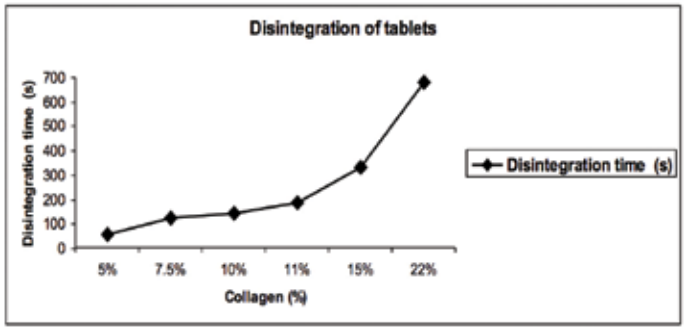


Figure 2. Influence of collagen concentration on the disintegration of tablets.

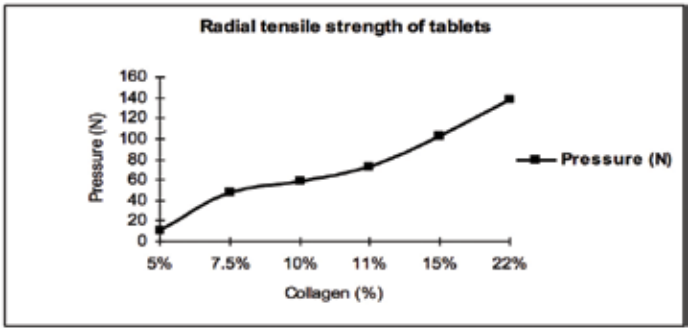


Figure 3. Influence of the collagen concentration on the tablet fracture resistance.

Applications of the non-crosslinked collagen hydrolysate confirmed the following:

- The tablet disintegration process meets the requirements of European Pharmacopoeia. Tablets were disintegrated within 15 min, which corresponds to requirements for uncoated tablets. The disintegration time was extended with the increasing dry matter content of collagen in the tablet.

- Resistance of tablets against fracture (radial tensile strength of tablets) was satisfactory for the samples prepared from granules with the collagen content higher than 11%. The same is valid also for friability of uncoated tablets.

- A strong binding effect of collagen was confirmed on the physical and technological parameters of prepared granules. The optimal fraction with regard to the tablet preparation process occurred at about 0.5 mm.

Non-crosslinked collagen with concentrations of 11%, 15% and 22% was confirmed as an effective adhesive. Granules and tablets complied with the parameters required by Pharmacopoeia.

Based on the obtained results, Zentiva Hlohovec pharmaceutical company (Slovakia) prepared tablets "Gastrogel" in the amount of 5 kg where the non-cross-linked collagen hydrolysate with concentration of 20% was applied as a binder of granules. After final treatment, the granulate obtained required properties for the tabletation process, Table I.

The molded sample tablets performed the comparable quality parameters as reference samples from the regular production.

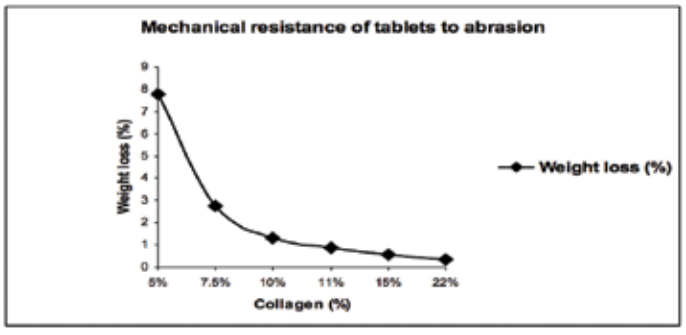


Figure 4. Influence of the collagen concentration on the mechanical strength of uncoated tablets.

Stability of tablets in an aqueous medium was investigated as the concentration of dissolved collagen in a standard solution of 0.1 mol/dm^3 hydrochloric acid and evaluated with a biuret agent. The sensitivity of the proposed dissolution methodology of collagen is from concentration of 0.5 g/dm^3. To determine the effect of molecular weight on the dissolution time of non-crosslinked collagen, the following samples were tested:

- No. 1 Collagen HS (bovine, the particle size of 0.355 mm - 45 Mesh, 176 Bloom),
- No. 2 Collagen BS (porcine, the particle size of 0.355 mm - 45 Mesh, 220 Bloom).

Measurements have shown (Figure 5) that the solubility of collagen samples is independent on the origin of the raw material and the molecular weight. The samples were dissolved within 2 hours, and the dissolubility process was almost linear. To enhance the stability, collagen was modified by dry heat in laboratory conditions. Prior to the experiment, there was important to reduce the actual water content to a minimum. Based on the obtained results, temperature of 150°C was chosen for crosslinking of samples no. 1 and no. 2 for 120 min exposure time.

Measurements have shown that the crosslinked samples no. 1 and 2:

- reduced dissolution of collagen compared with the non-crosslinked samples,
- the influence of collagen origin was not observed,
- the influence of molecular weight (measured as Bloom) was not observed.

Table I
Physical parameters of tablets.

Physical parameters of tablets	
diameter	12 mm
weight	0.6004 g
height	3.43 mm
resistance against fracture	227 N
abrasion resistance	after 4 min 0.06% after 10 min 0.08%
disintegration	2 min 30 s

Tests of Controlled Release of Ambroxol from the Tablet Matrix

The analytical method was developed to determine the controlled dissolution of Ambroxol HCl and the wavelength was determined to measure the concentrations of Ambroxol in UV spectra at 246 nm.

Ambroxol HCl drug controlled release was tested from tablets made with non-crosslinked collagen. The tablets did not exhibit any controlled drug dissolution and they disintegrated within 2 hours in each used solution, as shown in Figure 6.

In the next step, tablet samples were prepared using crosslinked collagen to achieve the controlled drug release from the tablet

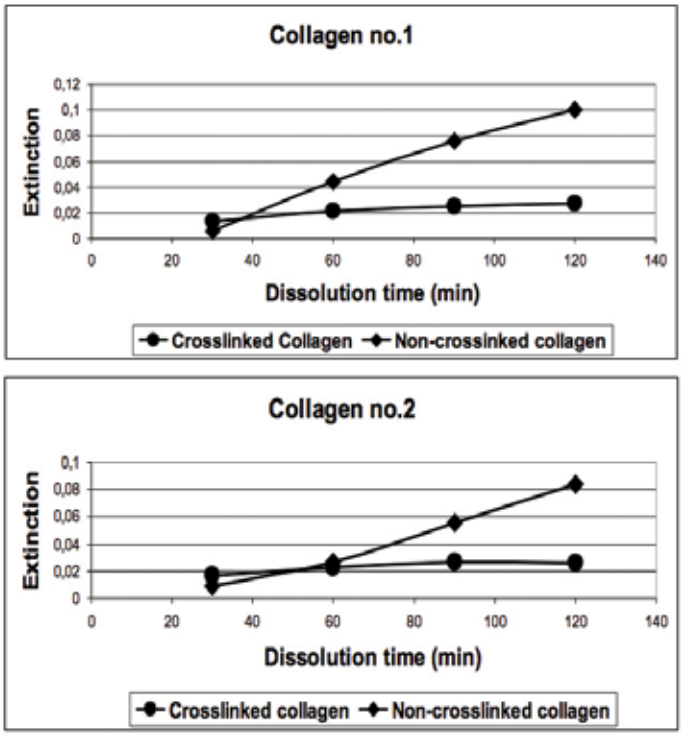


Figure 5. Determination of the solubility changes of non-crosslinked and heat-treated collagen samples.

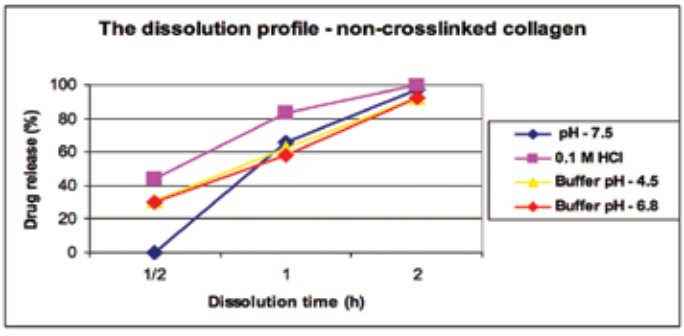


Figure 6. Dissolution time of Ambroxol HCl from tablets based on non-crosslinked collagen.

matrix. Time dissolution of Ambroxol from tablets with additionally treated collagen by heat can be seen in Figure 7.

The dissolution time of Ambroxol HCl from tablets based on non-crosslinked and crosslinked collagen was measured, and the following was found:

- tablet samples based on non-crosslinked collagen ensured the drug release within the time range of max. 120 min. The release was not linear, but steep with limit approaching to 100% of the released drug,
- the time of release was extended for tablet samples after collagen crosslinking, within 8 hours 84% of the drug was released in the environment of 0.1 mol/dm^3 HCl, and only 68% in a solution of pH 6.8. This means a possibility of controlled release of this modification up to 12 hours.

Tests of crosslinking of collagen tablets have shown that the physical methods of crosslinking can be effective. Physically crosslinked collagens for the excipients applications as for the controlled drug release must be modified by plasticization because their binding effect decreased significantly. For the tablet preparation, new collagen samples were prepared and the ability to form tablet matrix by dry compression was assessed.

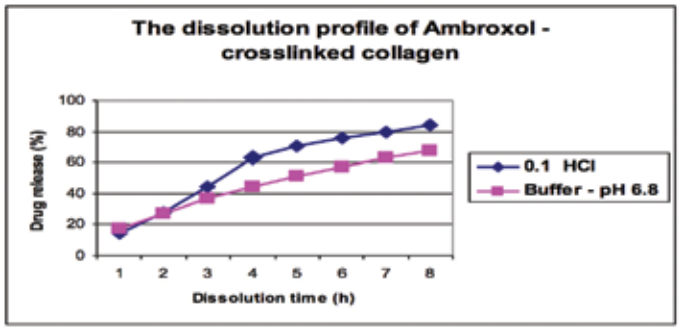


Figure 7. Dissolution time of Ambroxol from tablets with additionally heat-treated collagen,

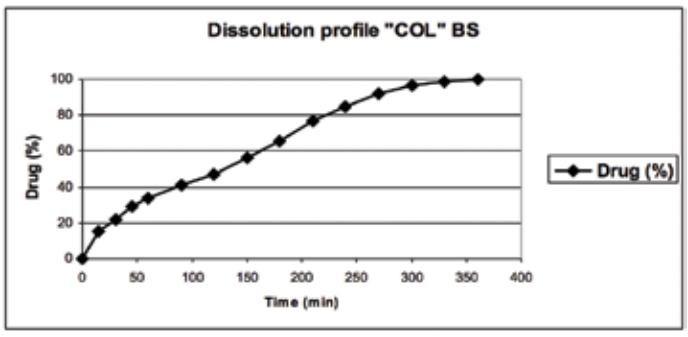


Figure 8. Dissolution profile of Ambroxol drug from the tablets prepared by direct compression of heat-treated and plasticized collagen "COL" BS.

Measurements of the time dissolution of drug Ambroxol from the tablets prepared of different types of collagen are in Figures 8, 9 and 10.

Based on the achieved results and evaluation of physical parameters, it can be concluded that the tablet skeleton with samples of modified collagen has satisfactory physical parameters.

Mechanical and physical parameters of tablets **Collagen HS:**

- Test "*fracture toughness*" – 61.8 N,
- Test "*friability of uncoated tablet*" – 2.4476%.

Mechanical and physical parameters of tablets **Collagen BS**:

- Test "*fracture toughness*" – 70.6 N,
- Test "*friability of uncoated tablets*" – 1.908%.

Mechanical and physical parameters of tablets **Collagen K12**:

- Test "*fracture toughness*" – 71.2N.
- Test "*friability of uncoated tablets*" – 1.373%.

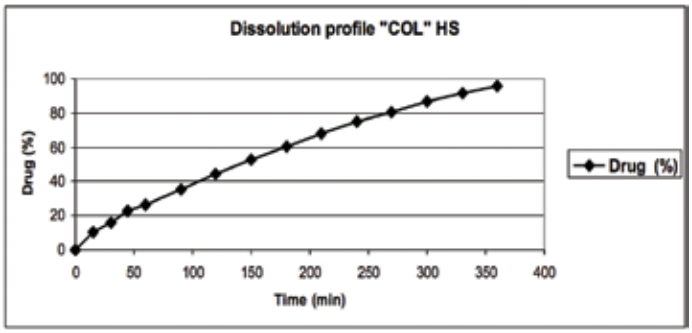


Figure 9. Dissolution profile of Ambroxol drug from the tablets prepared by direct compression of heat-treated and plasticized collagen "COL" HS.

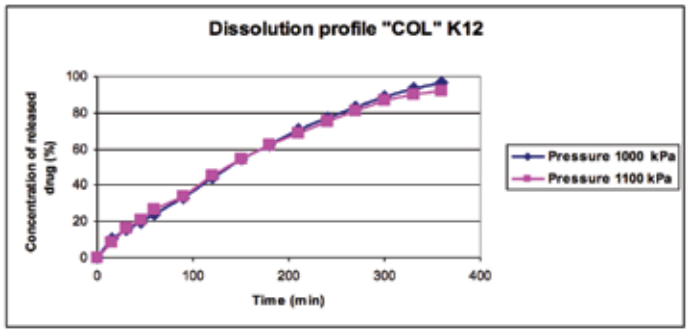


Figure 10. Dissolution profile of Ambroxol drug from the tablets prepared by direct compression at 1000 and 1100 kPa of heat-treated and plasticized collagen "COL" K 12.

The results of dissolution of heat-treated and plasticized collagens have confirmed:

- crosslinked collagen samples (COL HS, COL BS and COL K12) are suitable for application of the controlled drug release,
- crosslinked collagen samples (COL HS, COL BS and COL K12) have a comparable dissolution time of drug Ambroxol HCl from tablets,
- the assumption of the drug dissolution time extension with a pressure increase was not confirmed.

To verify the drug dissolution time extension, collagen mixtures "COL" (BS, HS and K12) with Ambroxol drug were modified with supplementary additives with 10% of weight.

- COL HS + additive 1 based on Ca^{2+},
- COL HS + additive 2 based on Mg^{2+},
- COL BS + additive 1 based on Ca^{2+},
- COL BS + additive 2 based on Mg^{2+},
- COL K 12 + additive 1 based on Ca^{2+},
- COL K 12 + additive 2 based on Mg^{2+},
- COL HS + additive 3 with a buffering effect,
- COL BS + additive 3 with a buffering effect,
- COL K 12 + additive 3 with a buffering effect.

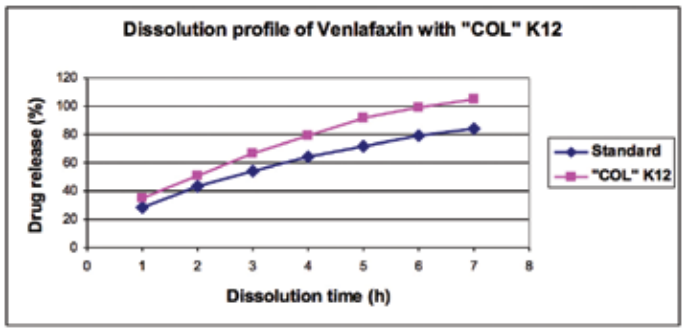


Figure 11. Dissolution profiles of the drug Venlafaxin.

The dissolution results have revealed:

- dissolution profile of the drug release from tablets can be changed by additives,
- to extend the dissolution up to 18 h, addition of 10% of alkali additive based on Ca^{2+} into the matrix of modified collagens "COL" 12, HS and BS is needed,
- similarly, the dissolution profile is improved by Mg^{2+} based additives,
- the dissolution time of the controlled drug release is shorter with additive 3 with a buffering effect.

Skeleton of tablets from heat-treated and plasticized collagen "COL" K12 were prepared to test the dissolution profile of Venlafaxin HCl. These skeletons were compared with a standard, which is based on modified cellulose Methocel K 100 McR. Dissolution profiles can be seen in Figure 11.

Tests confirmed that the modified collagen "COL" K12 provides a controlled release of Venlafaxin up to 6 hours.

Table II

Contact angles of water on the surface of unmodified and plasma modified sample films with collagen.

Sample No.	Type of film	unmodified	DCSBD plasma N_2/H_2	Plasma CO_2
		contact angle (°)		
1	collagen film + keratin	97.4 ± 4.6	80.2 ± 2.6	64.2 ± 1.6
2	collagen film	88.2 ± 3.5	76.2 ± 2.1	58.1 ± 2.2
84	EVAc film + 30% collagen	94.0 ± 2.3	64.4 ± 1.9	56.1 ± 2.0
98	EVAc film + 50% collagen	87.8 ± 2.1	61.1 ± 1.7	52.2 ± 2.3

2. Surface and Adhesion Properties of the Film Samples with Collagen

Surface and Adhesion Properties of Collagen Based Thermoplastic Hot-melt Films

In the further research of collagen application, thermoplastic hot-melt films based on collagen biopolymer were prepared. The experiments were concentrated on the surface and adhesion properties of the samples EVAc films with collagen, and on the adhesive bond strength under shear load. Films no. 84 and 98 were modified by plasma, and the impact of discharge atmospheric low-temperature plasma on the surface properties modification was studied.

The selection and amount of additives changed the parameters of thermoplastic films, e.g. elongation of the film, hydrophilicity, hydrophobicity, viscosity, melting and solidification points. The static contact angles of re-distilled water were measured at different points on the film surface with collagen and they are presented in Table II.

Sample 2 – collagen film is more hydrophilic than the sample 1 according to the water contact angle. A similar difference was shown for samples 84 and 98. The sample 98 that contained 50% of collagen was more hydrophilic, and its hydrophilicity value from the wetting perspective was similar as hydrophilicity of collagen.

Comparison of the values of hydrophilicity and polarity of plasma-treated samples in various gases has shown that the most effective treatment was obtained using a CO_2 process gas. Using this treatment the highest wetting of the sample film surface was obtained.

The shear strength of the adhesive bonds was tested for samples 84 and 98 with aluminum adhesive joints. Adhesively bonded aluminum-collagen samples were prepared at the temperature of 120°C, and aluminum sheets were fixed by overlapping in a hydraulic press at a pressure of 50 $N.cm^{-2}$ and results are presented in Table III.

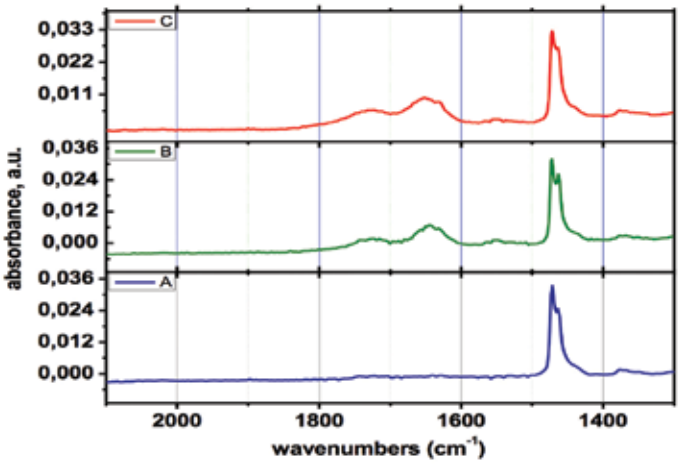


Figure 12. FTIR spectra of EVAc film no. 84.

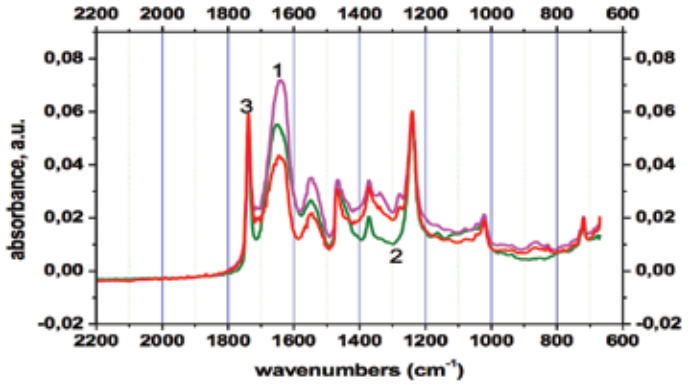


Figure 13. FTIR spectra of EVAc film no. 98.

Table III

Results of the shear strength of adhesive bonds with aluminum.

Sample No.	Type of film	Unmodified	DCSBD plasma N_2/H_2	Plasma CO_2
		The adhesive bond strength ($N.cm^{-2}$)		
84	EVAc film + 30% collagen	0.8 ± 0.1	2.2 ± 0.1	3.5 ± 0.2
98	EVAc film + 50% collagen	0.0	0.0	0.0

Sample 84 – the adhesive bond strength increased after plasma modification
Sample 98 – the adhesive joint failed to measure

FTIR-ATR spectra of thermoplastic hot-melt film sample no. 84 based on collagen modified by plasma in various atmospheres are shown in Figure 12 where:

- curve A is unmodified sample no. 84,
- curve B is sample modified with DCSBD plasma in a N_2/H_2 process gas,
- curve C is sample modified with DCSBD plasma in a CO_2 process gas .

FTIR spectra of the sample no. 84 are typical spectra of polyethylene. The content of acetate units in copolymer is most likely under the detection limit and therefore, it is not registered in the spectra. After plasma affection (in air vs. O_2), the formation of new absorption bands can be seen in the area between 1800 and 1600 cm^{-1}. They belong to the oxidation products (carbonyl, carboxyl, hydroxyl, peroxy groups, and potential degradation products containing the double bond – the band with max at approx. 1640 cm^{-1}, which can also be assigned to the OH groups).

FTIR-ATR spectra of thermoplastic hot-melt film sample no. 98 based on collagen modified by plasma in various atmospheres are shown in Figure 13 where:

- curve 1 – unmodified sample no. 98,
- curve 2 – sample modified with DCSBD plasma in a N_2/H_2process gas,
- curve 3 – sample modified with DCSBD plasma in a CO_2process gas.

The bands typical for the acetate group (1736 cm^{-1}) are present in the spectra of the sample 98, as well as the band amino-group that belongs to collagen (1640 cm^{-1}). Plasma treatment of samples in air vs in oxygen leads to changes in the area of the carbonyl and amino groups (the change of shape of absorption bands comparing to the changes of the intensity ratio of the functional groups (acetate and amino). Under the action of plasma in the oxygen, the volume of the amino-groups has decreased, resulting in a decrease of the absorption band intensity. Similarly, in the area under 1400 cm^{-1} the formation of new absorption bands was observed, most likely caused by oxidation of the material.

Table IV
Shear strength of plywood and statistic evaluation.

Plywood samples after the dry test

Sample No.	**Avg x (MPa)**	Std dev s (MPa)	Coeff of var v_k (%)	Value min (MPa)	Value max (MPa)	Number of samples (n)
77	**2.88**	0.20	7.1	2.66	3.08	15
84	**2.27**	0.59	25.9	1.63	2.73	15
98	**1.77**	0.11	5.98	1.64	1.86	15

Plywood samples after immersion in water 24 h

Sample No.	**Avg x (MPa)**	Std dev s (MPa)	Coeff of var v_k (%)	Value min (MPa)	Value max (MPa)	Number of samples n
77	**1.64**	0.22	13.1	1.26	1.95	15
84	**1.47**	0.13	9.1	1.19	1.68	15
98	**0.35**	0.04	10.8	0.25	0.39	15

Note. European standard EN 314-2 requires the value of shear strength to be 1.0 MPa.

Determination of the Bond Quality of Plywood Boards with Collagen Films

The shear strength test is commonly used as a fundamental indicator of the adhesive performance in plywood. Investigated non-formaldehyde collagen thermoplastic adhesive film samples based on collagen have different effects on the shear strength of plywood, as stated in Table IV. Performance of plywood samples from veneer bonded with samples no. 77 and 84 were corresponding to the requirements, but the strength of the sample no. 98 was low and do not meet the requirements of EN standard.

Conclusions

There is a large interest in the application of biopolymers especially from the perspective of the novel dosage forms with the controlled drug release. These medicament formulations represent significant added values from the perspective of comfort and benefits to patients. Replacement of the currently used chemically modified excipients (cellulose- and starch-based) by proteins such as collagen and keratin helps to reduce burden on the human body. Moreover, these two proteins are sources of amino acids essential for life. This feature significantly increases potential for the application of biopolymers in the pharmacy.

Non-crosslinked collagen hydrolysates with concentrations of 5%, 10%, 11%, 15% and 22%, were tested as an adhesive binder. Tablets were pressed from the prepared granules. The impact of collagen concentration on the basic parameters (disintegrating of the tablet, friability of uncoated tablets, mechanical resistance to abrasion of tablets, tablets resistance to fracture, mechanical strength in compression) was evaluated. Non-crosslinked collagens with concentrations of 11%, 15% and 22% were shown to work well as binders, and granules and tablets performed parameters in accordance with the requirements of European Pharmacopoeia. Tablets prepared from non-crosslinked collagen hydrolysate did not show extended release of Ambroxol. The release of drug was not linear and tablets disintegrated within 2 hours.

Prepared samples of thermally crosslinked collagen tablets extended the time of controlled drug release for more than 8 hours. To use the physically crosslinked collagens as excipients for the drug controlled release, they must be modified by plasticization because the binding effect decreased significantly. The controlled dissolution time was achieved for Ambroxol during 6-10 hours. The dissolution profile of drug release from tablets can be changed by additives. It can be extended up to 18 hours by addition of 10% of alkaline additive based on Ca^{2+} and Mg^{2+} into a matrix of modified collagens "COL" K12, HS and BS or it can be shortened with buffering effect additives. Tests confirmed that modified collagen "COL" K12 provides controlled release of the drug Venlafaxin up to 6 hours.

These tablets meet the requirements of the European Pharmacopoeia. This confirmed the potential of crosslinked collagen to be used as a pharmaceutical excipient for solid medicaments with the controlled drug release.

The surface properties of films with collagen were measured by the static contact angles. Comparison of the values of hydrophilicity and polarity of plasma-treated samples at various process gases showed that the most effective treatment was achieved by using CO_2 as a process gas. The surface of samples was strongly polarized and hydrophilized, and the best wettability of the surface by water was reached.

Investigations of the adhesion properties and quality of plywood bonding with collagen thermoplastic film confirmed that standard requirements for the adhesive bond shear strength are fulfilled for the samples no. 77 (film with 20% of modified collagen) and no. 84 (film with 30% of modified collagen). In the case of the sample no. 98 (film sheet with 50% of modified collagen), the required shear strength of the adhesive bond was not met.

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