

LABILE COLLAGEN MATRIX: TRANSFORMATION OF HIERARCHICAL STRUCTURE AT NANO- AND MICRO-LEVELS INFLUENCED BY CHEMICAL TREATMENT

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ABSTRACT

Porous structure both of natural skin and collagen matrix obtained by chemical treatment of the skin with alkaline and acidic solutions, tannage with a Cr^{3+} -containing solution, vegetable retannage and modification with bentonite was researched using scanning and transmission electron microscopy as well as standard contact porometry. Each stripe of porogrammes has been related to elements of multilevel structure. The pores were recognized using octane as a working liquid, the results obtained by this manner were used to determine loosening-compaction and ordering-disordering at each level of the structure, which includes nanosized macromolecules, microfibrils, fibrils and primary fibres of micron size. The measurements performed in aqueous media allowed us to determine hydrophilic pores and estimate their functions, secondary fibres have been also found by this manner. As for initial skin, porometric measurements diagnosed also non-collagen hydrophobic and hydrophilic inclusions, which form their own structure between microfibrils, fibrils, primary and secondary fibres of the matrix. The structure due to these inclusions is similar to that for collagen macromolecules. Microfibrils and fibrils have been found to form both ordered and disordered structures. Contribution of porosity of each organization level into the total porosity has been estimated, changes of collagen structure caused by chemical treatment and modification of inorganic ion-exchanger have been analyzed. Recommendation dealt to obtaining of materials for sorption and membrane separation have been given.

Key words: collagen, porosity, macromolecules, fibrils, fibres, standard contact porometry, bentonite.

INTRODUCTION

Collagen is a group of naturally occurring proteins found, in nature, exclusively in animals, especially in the flesh and connective tissues of mammals [1]. It is the main component of connective tissue, and is the most

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abundant protein in mammals, making up about 25% to 35% of the whole-body protein content. Collagen, in the form of elongated fibrils, is mostly found in fibrous tissues such as tendon, ligament and skin, and is also abundant in cornea, cartilage, bone, blood vessels, the gut, and intervertebral disc. Membranes based on natural [2] or artificial [3] collagen are also considered as prospective materials for sorption [2] and membrane [3] separation processes. These membranes are rather attractive from economical point of view.

Functional properties of the membranes are determined by not only chemical composition of the surface, but also their porous structure [4]. Porosity of natural collagen depends undoubtedly on treatment conditions. The aim of this work was to elucidate a change of porous structure influenced by both chemical treatment and modification with inorganic ion-exchanger. These researches are necessary to develop membranes for separation processes. The method of standard contact porometry was used to determine both hydrophilic and hydrophobic pores within a wide interval of 3×10^{-10} - 3×10^{-4} m [5]. Earlier the method of analysis of porometry data using geometrical model has been proposed to make correlation between each stripe of the porogramme and structure element [6]. This approach is useful because collagen is characterized by multilevel structure: its treatment causes a change of functional properties influenced by transformation of porous structure.

EXPERIMENTAL

The samples taken from cattle skin skirt were preserved in a saturated NaCl solution followed by soaking (sample I) [7]. Sample I was treated serially with solutions containing: (i) $\text{Ca}(\text{OH})_2$ and Na_2S , (ii) $(\text{NH}_4)_2\text{SO}_4$, (iii) pancreatine, (iv) H_2SO_4 , CH_3COOH and NaCl (the aim of the last procedure was to nap fibres). Non-collagen inclusions of proteins, carbohydrates and animal fat were removed and collagen matrix was obtained by this manner (sample II). Sample II was tanned with a $\text{Cr}(\text{OH})\text{SO}_4$ solution (sample III) and further retanned with tannides and modified with bentonite (sample IV). Tannage provides bridge formation between aminogroups of adjacent polypeptide chains, retannage causes bonding of carboxyle groups through tannin. Cherkassy bentonite (Ca-form) was activated with a $\text{Na}_4\text{P}_2\text{O}_7$ solution [8], this activation method causes no change of micron size of particles (*Fig. 1*).

Porometric measurements were performed at 0.1 MPa using octane or water as working liquids. The samples were previously vacuumized at 353 K. The scin and collagen matrix as well as bentonite particles were also investigated using a JEM-1230 transmission electron microscope and a Jeol-6700 scanning electron microscope.

RESULTS AND DISCUSSION

First let us consider differential porogrammes ($\frac{dV}{d(\log r)}$ - $\log r$, where V is

the volume, r is the radius) obtained using octane, which does not provide high swelling degree of the samples. The porogramme for sample II demonstrates maximum attributed to macromolecules ($\log r=0.5$ (nm)), intensity of the stripe is lower comparing with sample I (Fig. 2). As for initial skin, narrow peak for pores formed by macromolecules was found indicating their ordered structure. Nanosized macromolecules are visible in TEM image (Fig. 3). Macromolecules form microfibrils, their maximum is not visible for sample I, but it appears in the case of sample II ($\log r=1.4-1.6$ (nm)). A board between peaks for pores formed with fibrils and primary fibres ($\log r \geq 1.8$ (nm)) is rather diluted.

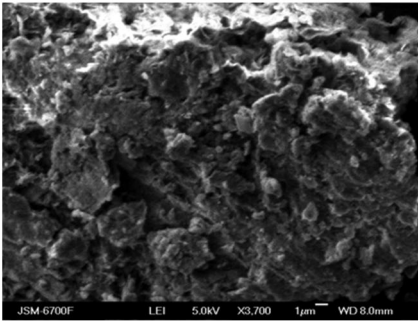


Fig. 1 – SEM image of bentonite particles

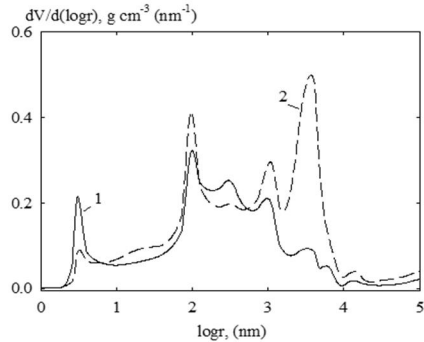


Fig. 2 – Differential pore volume distribution for samples I (1) and II (2). Octane was used as a working liquid

Higher intensity of macromolecule stripe for the initial sample is probably caused by non-collagen inclusions, which form their own porous structure. Their porous structure is evidently similar to that for macromolecules. Pores between microfibrils, fibrils and primary fibres are filled with the inclusions, after removal of which the peak intensity increases for sample II comparing with sample I.

Since no shift of the maxima at $\log r > 2.5$ (nm) towards higher r values is observed for sample II comparing with sample I, non-collagen inclusions cork pores at all the hierarchical levels, which are higher than microfibrils. After removal of these corks (sample II), the peak at $\log r = 2$ (nm) related to fibrils becomes narrower and more intensive indicating ordering of the structure at this level. Moreover the peak due to microfibrils ($\log r = 1-2$ (nm)) appears: these structure elements become more ordered.

Non-collagen inclusions are placed also between macromolecules:

intensity of the stripe at $\log r=0.5$ (nm) of differential surface distribution ($\frac{dS}{d \log r} - \log r$) increases (Fig. 4).

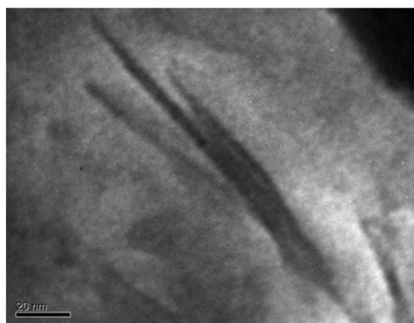


Fig. 3 – TEM image of sample 2

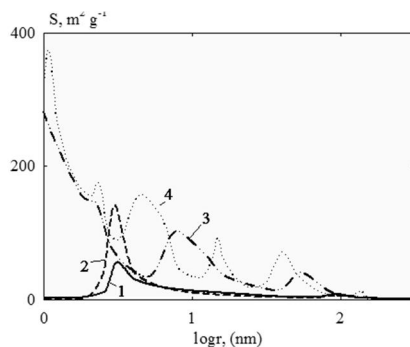


Fig. 4 – Differential pore surface distribution for samples I (1,3) and 2 (2, 4). Octane (1, 2) and water (3, 4) were used as working liquids

After swelling of samples I and II in water, higher porosity was found comparing to that for octane (Fig. 5). This is due to functional groups of polypeptide chains, which are able to co-ordinate water. Maxima related to secondary fibres ($\log r \geq 5$ (nm)) becomes visible. If octane is used, pores between these structure elements are outside a sensitivity limit of the porometry method, though secondary fibres are visible in SEM image (Fig. 6). Moreover additional maximum at $\log r \geq 0$ (nm) appears in the case of sample II. This stripe is also visible in the $\frac{dS}{d \log r} - \log r$ plot both for samples I and II. Thus it is

possible to say about zones of ordered and disordered macromolecules. These additional peaks are due to hydrophilic inclusions between macromolecules of sample I, namely soluble proteins, mucopolysaccharides, albumin etc.. Their function is nutritious. Removal of these inclusions causes a decrease of swelling due to weakening of surface hydrophilicity.

Reversal of intensity of the maxima attributed to macromolecules for initial and chemically treated skin, which was observed in octane and water media, shows hydrophobicity of non-collagen inclusions between microfibrils, fibrils and fibres. These inclusions are fats and lipids, their functions are not only nutritious, but also regulatory: they control water inflow towards lowest elements of hierarchical structure. The hydrophobic inclusions also cork pores resulting in stretching of the fibres. Chemical treatments leads to removal of these inclusions and ordering of porous structure. However more intensive peaks caused by secondary fibres (sample II show) hydrophilic inclusions like

proteoglycans, which are associated with collagen fibres and provide stability of structure on this organization level. Perhaps the spaces between microfibrils, fibrils and fibres are filled with both hydrophilic and hydrophobic inclusions, they act in opposite direction during swelling.

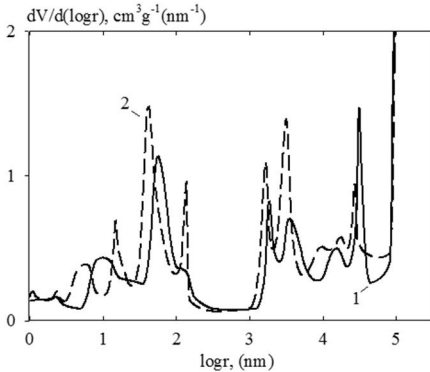


Fig. 5 – Differential pore volume distribution for sample I (1) and II (2). Water was used as a working liquid

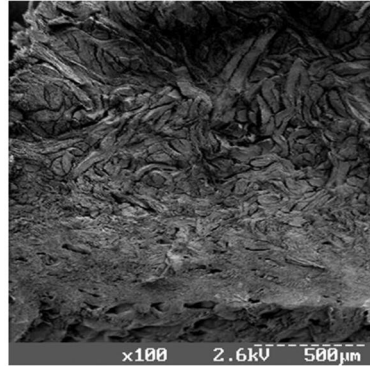


Fig. 6 – SEM image of sample II

When sample I swells in water, splitting of the maximum due to microfibrils is visible: a smoothed peak is related to disordered microfibrils ($\log r=0.8$ (nm)), the sharp one is attributed to regular microfibrils ($\log r=1.2$ (nm)). Pores due to regular ($\log r \approx 1.6-1.8$ (nm)) and irregular ($\log r \approx 2.1$ (nm)) fibrils are also observed: these elements are also visible in CEM image (Fig. 7).

The results of porometric measurements allow us to make conclusions regarding to functions of different structure elements of skin. Regular pores formed by macromolecules, microfibrils and fibrils are responsible for water transport. Irregular pores due to microfibrils and fibrils control water inflow. Hydrophobic inclusions plays the same role, moreover they are against filling with water of pores between primary and secondary fibres, which are responsible for gas and heat exchange. Irregular pores caused by macromolecules as well as hydrophilic inclusions between them provide water retention.

Tanning leads to disordering of porous structure of macromolecules (smoothing and decrease of maximum intensity at $\log r=0.5$ (nm)) (Fig. 8). Sharper maximum at $\log r=1.3$ (nm) for sample III comparing with that for sample II indicates more ordered structure in the level of microfibrils. The same regularity has been found for fibrils. At the same time irregular fibrils and primary fibres are more disordered comparing with those for sample III. This is evidently due to removal of proteoglycans, thus the pores between irregular

fibrils, primary and secondary fibres are filled both with hydrophilic and hydrophobic inclusions, which acted in opposite directions during swelling in water and octane media. The disordering effect is also visible for macromolecules from porogrammes for samples II and III. This effect is caused by cross-linkage of adjacent polypeptide chains and, as a result, thinning of these structure elements. The cross-linkage is due to Cr(III) complex formation with aminogroups. Retannage (sample IV), which provide formation of additional bridges between polypeptide chains caused slight disordering of microfibrils comparing with sample III. During this process tannins are bonded with carboxyl groups.

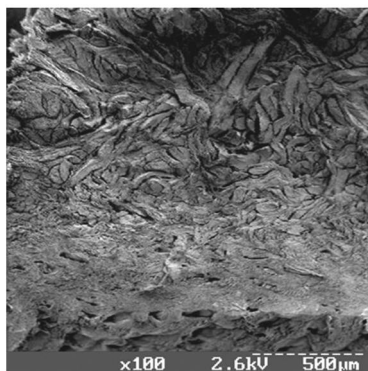


Fig. 7 – SEM image for ordered and disordered fibrils

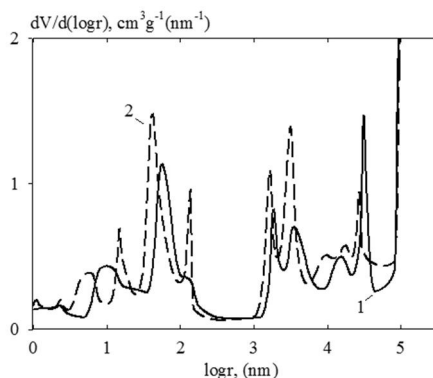


Fig. 8 – Differential distribution of pore volume for sample III (1) and 4 (2). Octane was used as a working liquid

Ordering at the level of regular fibrils (increase of intensity and narrowing of corresponding maximum for sample III comparing with sample II) is probably caused by removal of hydrophilic corks of protein-carbohydrate inclusions. Influence of these inclusions on porous structure of regular fibrils and lower elements is probably inconsiderable. However hydrophilic inclusions bond irregular fibrils between each other. This is also valid for fibres: the stripes attributed to these structure elements become wider, their intensity decreases after removal of protein-carbohydrate corks.

Deposition of bentonite plate-like particles of micron size occurs only in macropores formed with primary (*Fig. 9*) and evidently secondary fibres. Inorganic ion-exchanger corrugate these pores. This is confirmed with a slight shift of corresponding maxima towards lower r values for sample IV comparing with sample III. The particles evidently act as a press squeezing the fibres. As a result, porous structure of fibrils becomes more ordered – intensity of the maximum at $\log r=2$ (nm) increases.

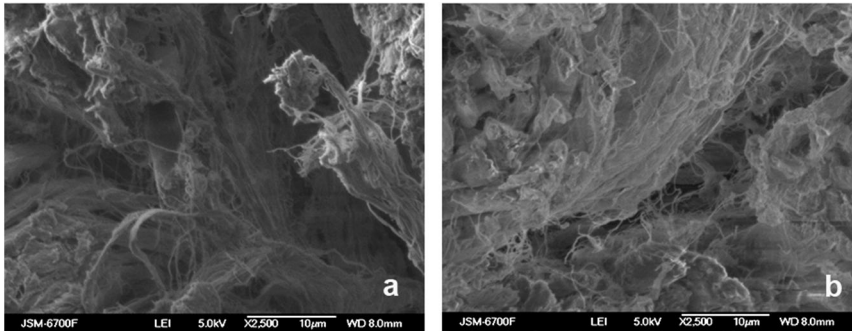


Fig. 9 – SEM image of primary fibres free from inorganic filler (a) and covered with bentonite particles (b)

The porogrammes were calculated for each stripe within the intervals, where no overlapping of peaks is observed. Increase of pore volume were found from integral porogrammes within pre-determined intervals of radius. Then a contribution of each level into total volume of solid phase was calculated as $\frac{\varepsilon(1-\varepsilon_0)}{\varepsilon_0}$, where ε is the total porosity, ε_0 is the porosity due to

structure element. The contribution of solid into total volume of fibres for each organization level is shown in *Fig. 10* (the calculations have been done for the porogrammes obtained using octane). This parameter reflects loosening or compaction of collagen structure.

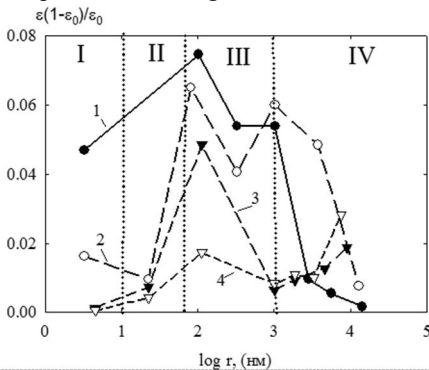


Fig. 10 – Contribution of solid phase into total volume of fibres on each organization level for samples I (1), II (2), III (3), IV (4). Octane was used as a working liquid. Levels: macromolecules (I), microfibrils (II), fibrils (III), primary fibres (IV)

It is seen that chemical treatment, tannage and retannage leads to loosening of structure on the levels of macromolecules, microfibrils and fibrils. In the case of sample II this is caused by removal of hydrophilic inclusions, which are not associated with collagen, and also hydrophobic non-collagen constituents. In the case of samples III and IV the loosening is evidently caused by thinning of macromolecules, microfibrils and fibrils due to cross-linkage of polypeptide chains. Regarding to the level of primary fibres, sample II shows more compacted structure

probably due to removal of non-collagen corks. Moreover compaction of primary fibres can be caused by ordering of fibrils, which join bundles of regular fibrils.

Removal of hydrophilic protein-carbohydrate inclusions associated with collagen during tannage also leads to compaction of primary fibres. However irregular fibrils become more disordered, as a result, a shift of corresponding maximum towards primary fibres ($\log r \approx 3$ (nm)) is observed. Thus irregular fibrils can be identified only in water media.

CONCLUSIONS

Porometric measurements in a wide diapason of pore radii followed programme calculation allows us to estimate a change in collagen structure caused by chemical treatment and modification of inorganic ion-exchanger. The matrix based on natural collagen is attractive to create sorption organic-inorganic materials: synthesis conditions must exclude formation of large aggregates in pores formed with secondary fibres since large particles cannot provide high sorption rate. In this case modifying agent has to corrugate pores. Fragmentation of this material under special conditions allows one to obtain nanoparticles [9], which could be deposited not only on the primary fibres, but also on the surface of structure elements of lower organization level. At the same time exclusion of large pores is necessary for membrane separation. This can be reached by means of corking of pores with aggregates of inorganic nanoparticles [6]. In this case a use of sol-gel method is recommended for matrix modification.

REFERENCES

- [1] Collagen: Structure and Mechanics (Eds. by P. Fratzl), Springer, 2008.
- [2] Xuepin Liao, Hwei Ma, Ru Wang, et al. Sci., 2004, Vol. 243, No 1-2, P, 235-241.
- [3] Y.Chen, R.W.Crawford, A.Oloyede, Med Sci Monit.,2007, Vol.13, No4, P.101-105.
- [4] N. P. Berezina, N. A. Kononenko, O. A. Dyomina, N.P. Gnusin, Adv. Colloid. Interface Sci., 2008, Vol. 139, No 1-2, P. 3–28.
- [5] Yu.M. Volfkovich, V.E. Sosenkin, V.S. Bagotzky, J. Power Sources, 2010, Vol. 195, No 17, P. 5429--5441.
- [6] Yu.M. Volfkovich, Yu.S. Dzyazko, V.E. Sosenkin, N.F. Nikolshaya, Ukrainian Chemistry Journal (Ukrainskii Chimicheskii Jurnal), 2010, Vol. 76, No 12, P. 80-86.
- [7] F. O'Flaerty, W.T. Roddy, R.M. Lollar, The Chemistry and Technology of Leather: Evaluation of Leather, Krieger, 1977.
- [8] O.R. Mokrousova, A.G. Danilkovich, Scientific proceedings of Riga Technical University. Material science and applied chemistry, 2006, Series 1, Part 14, P. 83–91.
- [9] Z. Darvishi, A. Morsali, Colloids Surfaces A: Physicochem. Eng. Aspects, 2011, Vol. 377, No 1-3, P. 15-19.