NEW CELLULOSE-BASED MATERIALS AS TRANSDERMAL TRANSFER SYSTEMS FOR BIOACTIVE SUBSTANCES

NARCIS ANGHEL, SORIN LAZĂR, BIANCA-IULIA CIUBOTARU, LILIANA VEREȘTIUC* and IULIANA SPIRIDON

"Petru Poni" Institute of Macromolecular Chemistry, 41A, Gr. Ghica-Voda Alley, 700487, Iasi, Romania
**Faculty of Medical Bioengineering, "Grigore T. Popa" University of Medicine and Pharmacy, 9-13 Kogalniceanu Str., 700454, Iasi, Romania*
✉ *Corresponding author: Narcis Anghel, anghel.narcis@icmpp.ro*

Dedicated to the 70th anniversary of the Department of Pulp and Paper, "Cristofor Simionescu" Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi

The purpose of this study was to obtain and characterize some cellulose/collagen biocomposites comprising different antioxidant and anti-inflammatory substances. The structure of the obtained materials was investigated using scanning electron microscopy (SEM) and attenuated total reflection infrared spectroscopy (FTIR). Additionally, the mechanical and biological characteristics of the materials were evaluated in order to identify some potential applications. The properties of the studied materials suggest that they may potentially be applied as skin care products in the field of cosmetics.

Keywords: cellulose, collagen, hesperidin, quercetin

INTRODUCTION

The growing interest in biomaterials based on cellulose is due to numerous possibilities to enhance the functionalities and quality of the current generation of materials, as well as to their nontoxic character and low cost, as compared to other materials. The use of various natural substances, such as polysaccharides[1,2] and proteins, with different medical applications, is well documented in the literature. Natural compounds, such as flavonoids, provide numerous opportunities for their exploitation, due to their antimicrobial, antibacterial and anti-inflammatory activity,[3,4] in the context in which consumers are more interested in their health nowadays.

Collagen is recognized for its biocompatibility and biodegradability, as well as for good permeability. Due to the repetitive array of receptor–recognition motifs present in its structure, collagen could improve the adhesion and differentiation of cells.[5] The most abundant collagen in the human body, type I collagen has a fibrillar morphology.[6] That is why, combining collagen and cellulose, it is possible to obtain materials with improved properties.

Cellulose is the most abundant polysaccharide in the world. It consists of linear glucose rings connected to each other through β (1→4) glycosidic bonds. Some parameters, such as shape, size, and crystallinity, vary as a function of cellulose sources and the delignification process applied. Its amorphous region is easily accessible, while the crystalline region is harder to penetrate by various reactants.[7] Nanoengineering of this polymer could allow the controlled delivery of various bioactive materials, including chemotherapeutic agents, anti-inflammatory drugs and antimicrobial compounds.[8] There are some studies dedicated to the development of some biomaterials based on bacterial[9,10] or cellulose derivatives[11] and collagen. In this study, we have used microcrystalline cellulose as matrix.

As we have mentioned above, the inclusion of natural compounds with antimicrobial and anti-inflammatory activity could improve the final properties of the developed materials.

This study seeks to develop new cellulose/collagen formulations comprising different fillers and to investigate their mechanical and morphological properties. We have also characterized the bioadhesive properties of all the formulations in order to determine their potential for cosmetic or dermatological applications.

EXPERIMENTAL

Materials

Cellulose (CEL) from Sigma Aldrich (~20 micrometers) was used as matrix. Collagen hydrolysate, a polypeptide made by further hydrolysis of denatured collagen, with a molecular weight of 96 kDa, hesperidin, quercetin, acetyl salicylic acid, and ascorbic acid were purchased from Sigma-Aldrich and used without further purification.

Biocomposites manufacture

The base matrix for these biocomposites was made by mixing cellulose with collagen. Each component had its role. Thus, cellulose functions as a reinforcing material that confers the mechanical strength of the support. Collagen was chosen to ensure bioadhesiveness.[12]

The materials were obtained in the form of foil by the casting method, using the ratio of cellulose: collagen: bioactive principle of 4: 1: 0.05. The base matrix components were solubilized in DMAc/LiCl[13] and then the bioactive compound was added at room temperature. The composites were dried in a vacuum oven at 40 °C in order to remove the solvent, washed with distilled water and then air dried at room temperature.

The bioactive materials were thus chosen to belong to the categories of anti-inflammatories (acetyl salicylic acid, *e.g.* aspirin)[14] and antioxidants (ascorbic acid, hesperidin and quercetin).[15-18]

The samples were coded as follows: CEL – cellulose; CCO – cellulose/collagen; ASP = CCO + acetyl salicylic acid; ASC = CCO + ascorbic acid; HES = CCO + hesperidin and QER = CCO + quercetin.

Methods

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR) was used to evaluate the interactions between the materials components.

64 scans of all the samples were acquired using a Bruker Vertex 70 (Billerica, MA, USA) ATR-FTIR spectrometer, equipped with an ATR device (ZnSe crystal), with a 45 angle of incidence. The scanning was recorded in the range from 4000 cm^{-1} to 600 cm^{-1} and a spectral resolution of 2 cm^{-1}.

Compression test

The samples were placed between the compression plates of a computer-controlled Shimadzu testing machine, with a 500 N load cell. An initial compressive contact of 0.1 N was applied. The data were registered at a stroke of 1 mm/min. The test was performed at 22 °C and the compressive modulus was calculated from the slope of stress–strain curves between 0 and 10% strain.

Scanning electron microscopy (SEM)

The surface of all the specimens was analyzed by SEM (FEI QUANTA 200ESEM instrument), with an integrated EDX system, GENESIS XM2i EDAX with an SUTW detector. The samples were analyzed with a low-vacuum secondary electron detector at an accelerating voltage of 25.0 kV, at room temperature and 0.050 Torr internal pressure. The experiment was performed in triplicate and the magnification is indicated on the figure.

Bioadhesivity test

An TA.XTplus® analyzer from Stable Micro Systems was used to evaluate the adhesion force and work of adhesion. Before the tests, the samples were cut to a standard size to allow dropping on the support (a mobile cylinder component of the equipment). Bladder tissue and cellulose membrane with a surface of 4 cm^2 were placed in the sample device. During the recordings, the cylindrical holder with the pieces of samples was lowered with 1 mm/s. The contact force and the contact time were established at 1 gF and 30 s, respectively. The test was performed several times for each sample.

RESULTS AND DISCUSSION

FTIR analysis

The effects of different fillers within the cellulose/collagen hydrogels were investigated in terms of mechanical, morphological and biological properties.

FTIR measurements were performed in order to evaluate the crystallinity and hydrogen bond intensities for the obtained materials (Fig. 1). The total crystallinity index (TCI) was calculated as the ratio between the intensity of the band at 1376 cm^{-1} and that of the -CH_2- peak (2902 cm^{-1}) chosen as reference, while the hydrogen bond intensity (HBI) – as the ratio between the absorbance at 3400 cm^{-1} and that at 1362 cm^{-1}, according to Colom and Carrillo.[19]

As can be seen from Table 1, the presence of collagen in the polymeric matrix causes a decrease of the total crystallinity by about 40%, compared to that of cellulose, together with a significant increase, on average of 80%, of the intensity of hydrogen bonds, which is otherwise

expected. As hesperidin and quercetin are amorphous substances, they decrease the total crystallinity, but establish new interactions through hydrogen bonds with the other components of the blends, which is reflected in the mechanical properties and bioadhesive characteristics of the new materials, as will be seen later.

The presented data show a close correlation between the chemical structure of the bioactive principles and the intensity of the hydrogen bonds, hesperidin being best retained by the polymeric material.

Morphology analysis

Further insights into the loaded cellulose-collagen materials were provided by SEM investigation.

The micrographs achieved with the scanning electron microscope (SEM) show that the obtained biomaterials have a relatively homogeneous structure (Fig. 2).

In the case of hesperidin (hesperitin-7-rutinoside), a high degree of organization of the biomaterial can be observed (Fig. 2d), due to the interaction of the remaining rutinoside with the polymeric matrix. It appears that hesperidin forms clusters with parallel orientation, which are repeated at regular intervals. By comparison, the presence of salicylic acid and quercetin decreases the degree of structural organization and causes the appearance of pores with the corresponding consequence, *i.e.* the decrease of the intensity of the hydrogen bonds between the components of the biocomposite.

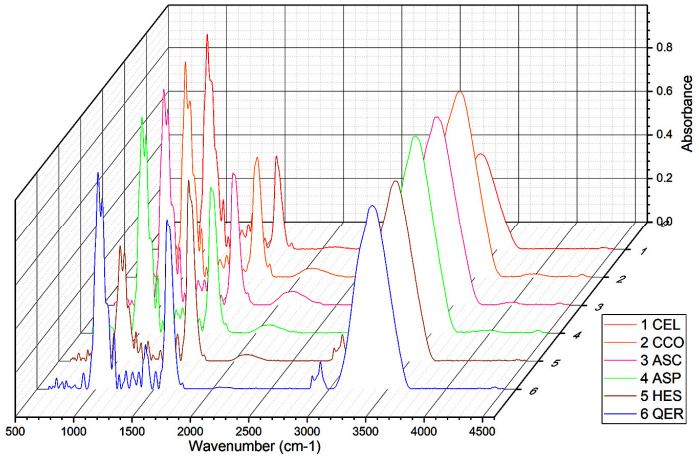

Figure 1: FTIR spectra of the studied biomaterials

Table 1
Total crystallinity index (TCI) and hydrogen bond intensity for the studied materials

Sample	Total crystallinity index (TCI) A1376/A2902	Hydrogen bond intensity (HBI) A3336/A1336
CEL	0.841	5.069
CCO	0.495	10.713
MAT	0.500	8.367
ASC	0.665	7.664
ASP	0.928	10.671
HES	0.402	30.667
QER	0.624	10.948

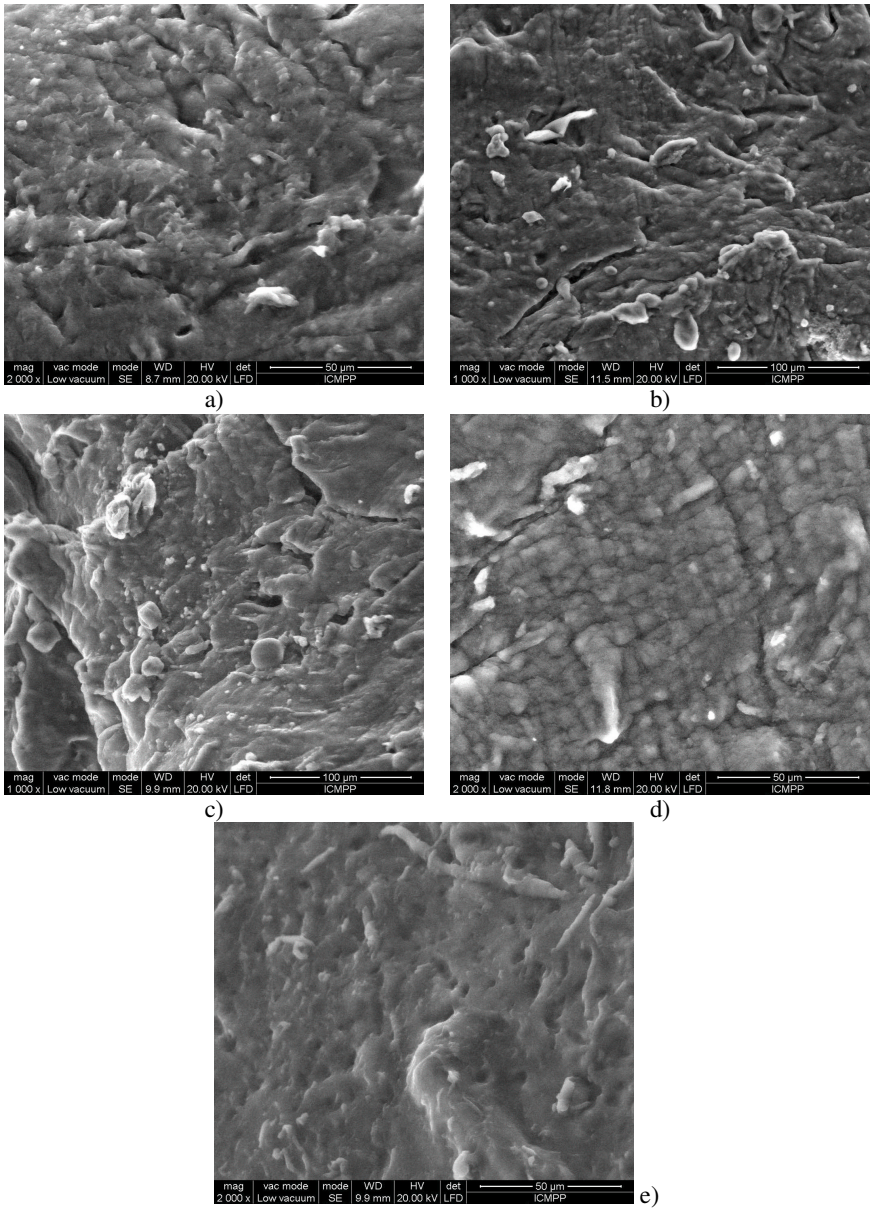

Figure 2: SEM micrographs for the tested materials; a) CCO; b) ASC; c) ASP; d) HES; e) QER

Mechanical properties

The results shown in Figure 3 indicate that the incorporation of different fillers into the cellulose/collagen polymer matrix resulted in a slow increase in compression strength, as compared to that of the matrix. The highest value of the compression modulus was observed for the cellulose (E_c = 2.79 kPa), whereas the quercetin-loaded material had the lowest stiffness (E_c = 1.97 kPa). An interesting behavior was remarked for the material comprising hesperidin. Its presence in the biocomposite caused a decrease in the elasticity of the material, which correlates very well with the value of the hydrogen bond intensity between the components (Table 1) and the degree of structural organization (Fig. 2d).

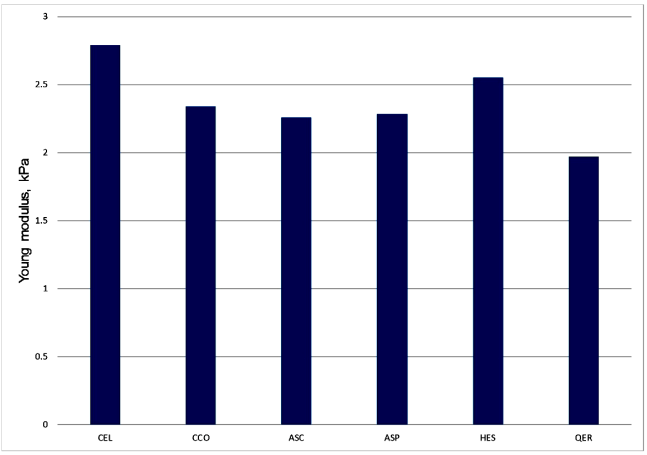

Figure 3: Mechanical properties of the composite biomaterials

Table 2
Adhesion force and work of adhesion for the obtained biomaterials

Sample	Adhesion force, (gF × 0.00980665), N	Work of adhesion, (N×s)
CEL	6.5	0.0059
CCO	15.0	0.0309
ASC	6.7	0.0066
ASP	5.9	0.0057
HES	8.5	0.0115
QER	5.2	0.0057

Bioadhesive properties

The term bioadhesion is used to describe the attachment of a drug carrier system/formulation to an epithelial tissue due to the action of interfacial forces.[20] The data (Table 2) suggest that the fillers interfere with the ability of the materials to adhere to the substrate. According to Table 2, the cellulose-collagen film presented the highest bioadhesive strength. The experimental data evidenced that the addition of collagen to the cellulosic matrix increased the bioadhesive force with 130.77%. This adhesion improvement can be due to the increase of the hydrogen bond intensities between the materials and the membrane, as evidenced by the FTIR spectra (Table 1). The filler addition has induced a decrement of the bioadhesion force, which decreased for all the formulations (except that comprising hesperidin) to values similar or lower than that of cellulose. The greater force of bioadhesion and the mechanical adhesion work of the matrix containing hesperidin are due to the homogeneous structure, without pores, of the biomaterial.

According to the parameters calculated from the FTIR spectra, the TCI values are lower while the HBI values are higher, compared to those of the cellulose matrix. This means that more hydroxyl groups are available to interact by inter- and/or intramolecular hydrogen bonding. Thus, the presence of the filler encourages the formation of more hydrogen bonds and the polymer mobility is reduced. Rheological studies will clarify these aspects.

The work of adhesion values are in good agreement with the findings for the bioadhesive forces.

CONCLUSION

This paper describes the preparation and properties of novel cellulose collagen biomaterials comprising different bioactive compounds. It has been found that the incorporation of different fillers into the cellulose collagen polymer matrix resulted in a slow decrease in tensile strength, as compared to that of the matrix. The bioadhesive properties of all the formulations were evaluated as a function of their composition and it was found that the presence of hesperidin resulted in the best bioadhesion properties, while that of quercetin had the opposite effect.

The final properties of the studied materials suggest that they may potentially be applied as skin care products in the field of cosmetics.

The present study is groundwork for future research, the structure–property relationships developed for these formulations will be further analyzed by rheological tests.

REFERENCES

[1] S. Rençber, C. N. Cheaburu-Yilmaz, F. Aydin Köse, S. Yaprak Karavana and O. Yilmaz, *Cellulose Chem. Technol.*, **53**, 655 (2019), https://doi.org/10.35812/CelluloseChemTechnol.2019.53.64

[2] M. D. Turliuc, A. I. Cucu, A. Cărăuleanu and C. F. Costea, *Cellulose Chem. Technol.*, **52**, 505 (2018), http://www.cellulosechemtechnol.ro/pdf/CCT7-8(2018)/p.505-513.pdf

[3] I. Spiridon, *Cellulose Chem. Technol.*, **52**, 543 (2018), http://www.cellulosechemtechnol.ro/pdf/CCT7-8(2018)/p.543-550.pdf

[4] A. B. Ciubara, R. C. Tudor, L. Nechita, O. Tita, A. Ciubara *et al.*, *Rev. Chim.*, **69**, 1247 (2018), http://www.revistadechimie.ro/article_eng.asp?ID=6300

[5] B. J. Huang, J. C. Hu and K. A. Athanasiou, *Biomaterials*, **98**, 1 (2016), https://doi.org/10.1016/j.biomaterials.2016.04.018

[6] M. Dobrota, S. Vlad, L. Gradinaru, M. Butnaru and G. Pircalabioru, *Cellulose Chem. Technol.*, **53**, 211 (2019), https://doi.org/10.35812/CelluloseChemTechnol.2019.53.21

[7] R. J. Moon, A. Martini, J. Nairn, J. Simonsen and J. Youngblood, *Chem. Soc. Rev.*, **40**, 3941 (2011)

[8] A. Sheikhi, J. Hayashi, J. Eichenbaum, M. Gutin, N. Kuntjoro *et al.*, *J. Control. Release*, **294**, 53 (2019), https://doi.org/10.1016/j.jconrel.2018.11.024

[9] S. B. Lin, C. P. Hsu, L. C. Chen and H. H. Chen, *Food Hydrocoll.*, **23**, 2195 (2009), https://doi.org/10.1016/j.foodhyd.2009.05.011

[10] C. Wiegand, P. Elsner, U. C. Hipler and D. Klemm, *Cellulose*, **13**, 689 (2006), https://doi.org/10.1007/s10570-006-9073-0

[11] J. Kozlowska, N. Stachowiak and A. Sionkowska, *Polym. Test.*, **69**, 350 (2018), https://doi.org/10.1016/j.polymertesting.2018.05.042

[12] Y. Liu, S. C. Ng, J. Yu and W. B. Tsai, *Colloid. Surfaces B: Biointerfaces*, **174**, 316 (2019), https://doi.org/10.1016/j.colsurfb.2018.10.077

[13] S. Bano and Y. S. Negi, *Carbohyd. Polym.*, **157**, 1041 (2017), https://doi.org/10.1016/j.carbpol.2016.10.069

[14] A. V. Sorokin, Z. H. Yang, B. L. Vaisman, S. Thacker, Z. X. Yu *et al.*, *J. Nutr. Biochem.*, **35**, 58 (2016), https://doi.org/10.1016/j.jnutbio.2016.05.012

[15] E. A. González and M. A. Nazareno, *LWT - Food Sci. Technol.*, **44**, 558 (2011), https://doi.org/10.1016/j.lwt.2010.09.017

[16] O. Sagdic, I. Ozturk, G. Ozkan, H. Yetim, L. Ekici *et al.*, *Food Chem.*, **126**, 1749 (2011), https://doi.org/10.1016/j.foodchem.2010.12.075

[17] T. Barzegar, M. Fateh and F. Razavi, *Sci. Hortic.*, **241**, 293 (2018), https://doi.org/10.1016/j.scienta.2018.07.011

[18] M. Sikora and M. Świeca, *Food Chem.*, **239**, 1160 (2018), https://doi.org/10.1016/j.scienta.2018.02.038

[19] X. Colom and F. Carrillo, *Eur. Polym. J.*, **38**, 2225 (2002), https://doi.org/10.1016/S0014-3057(02)00132-5

[20] R. Shaikh, T. R. Raj Singh, M. J. Garland, A. D. Woolfson and R. F. Donnelly, *J. Pharm. Bioallied Sci.*, **3**, 89 (2011), https://doi.org/10.2147/IJN.S75474