DEVELOPMENT AND CHARACTERIZATION OF COLLAGEN – CARBOXYMETHYLCELLULOSE MATERIALS FOR LENSES

ALEXANDRA-CRISTINA BURDUŞEL[1], IZABELA STANCU[1], ION BOGDAN MIHAI[1] ŞTEFANIA MARIN[2], CIPRIAN CHELARU[2], ANDRADA SERAFIM[1], DIANA DRĂGUŞIN[1], MĂDĂLINA GEORGIANA ALBU KAYA[2], GHEORGHE COARĂ[2]

[1]*University Politehnica Bucharest, Faculty of Medical Engineering, 1-7 Gheorghe Polizu St., Bucharest, Romania*

[2]*INCDTP - Division Leather and Footwear Research Institute, 93 Ion Minulescu St., 031215, Bucharest, Romania, albu_mada@yahoo.com (corresponding author)*

Cataract is one of the most common causes of blindness for people over 40 years old. Hydrogels are three – dimensional structures with increased properties which can be successful used in medical application such prosthesis or intraocular soft contact lenses. Sodium carboxymethylcellulose (CMC - Na) is the cheaper option currently available on the market with properties like biocompatibility similar with pHEMA. The aim of this study was to develop polymeric crystalline materials based on collagen (COL) and carboxymethylcellulose cross-linked with EDC/NHS for cataracts or other eye-disease. Type I fibrillar collagen gel with various ratios of CMC-Na hydrogels were lyophilized and tests tested by optic and scanning electronic microscopy, FT-IR spectroscopy and water absorption. The obtained gels were crosslinked with EDC/NHS and lyophilized in order to obtain spongious forms. They are porous structures with pore sizes between 25-81 µm. The samples with CMCNa presented more uniform and dense matrices and the crosslinked ones are more resistant, being more proper as material for lenses for be used in cataract.

Keywords: collagen, carboxymethylcellulose, EDC/NHS.

INTRODUCTION

Cataract is the most common cause of vision loss in people over age 40 and is the principal cause of blindness in the world. In fact, there are more cases of cataracts worldwide than there are of glaucoma or other eyes diseases combined, according to Prevent Blindness America (PBA) (Gretchyn *et al*., 2016).

Poly(hydroxyethyl methacrylate), pHEMA, hydrogels are highly biocompatible and transparent materials, with a high thermal stability, resistance to acid and alkaline hydrolysis and tuneable mechanical properties. These properties make them particularly useful as a basis of biomedical devices, such as catheters, intrauterine inserts, prosthesis or intraocular and soft contact lenses (pHEMA being the main material for lenses).

Collagen is the most widely used tissue-derived natural macromolecule, and it exhibits attractive properties including good biodegradability, weak antigenicity, excellent biocompatibility and unique fibril-forming properties (Engel *et al*., 2005; Lee *et al*., 2001; Madhan *et al*., 2002).

Carboxymethylcellulose (CMC) is one of the major low-cost, commercially available derivatives of cellulose used in industrial applications (Heinze and Koschella, 2005). An interesting potential application is the preparation of hydrogels with superabsorbent properties (Chang and Zhang, 2011). CMC has several advantageous properties for gel synthesis, such as good water solubility and the presence of reactive hydroxyl and carboxymethyl groups. The non-toxic nature and biocompatibility of such gels is advantageous for biomedical applications (Caló and Khutoryanskiy, 2015).

1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide Hydrochloride / N-Hydroxysuccinimide (EDC/NHS) is a crosslinking agent complex for collagen and it offers

transparent lens and is non-toxic, while glutaraldehyde is yellowish and toxic in a certain amount.

The aim of this study was to develop polymeric crystalline lens made of collagen and carboxymethylcellulose cross-linked with EDC/NHS for people who suffer from cataracts or other eye-disease.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel (Col) having a concentration of 2,85% (w/v) was extracted from calf hide using technology currently available at the Research-Development Textile Leather National Institute Division Leather and Footwear Research Institute – Collagen Department (Albu *et al*., 2011). EDC/NHS was purchased from Sigma-Aldrich (China), Sodium Carboxymethylcellulose (CMC-Na) from Fluka and collagenase from Sigma-Aldrich, China. The other reagents as HCl or NaOH were of analytical grade.

Preparation of Collagen Sponges

The concentration of each collagen gel was adjusted at 1% and 5.5 pH using 1M sodium hydroxide. 2% carboxymethylcellulose was added to half of collagen gel (w/v), then the collagen gels were cross-linked with 2:1 and 1:1 ratios of EDC/NHS as Table 1 presents.

Table 1. Composition and name of collagen gels

Code of gels	Col, %	CMC-Na, g	EDC/NHS
AX 3.1	1	-	1:1
AX 3.2	1	-	2:1
AX 3.3	1	8	1:1
AX 3.4	1	8	2:1

The collagen-CMC-Na gels were cast in polystyrene Petri dishes of 3 cm diameter at 20°C. The collagen gels were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) and spongious forms were obtained.

Methods

FTIR-ATR Analysis

FT-IR spectral measurements were recorded by spectrophotometer Jasco FT/IR-4200. All the spectra were recorded at the following parameters: spectral range 4000-600 cm^{-1}, resolution 4 cm^{-1} with 30 acquisitions per each sample.

Water Absorption

In order to determine the water absorption, the collagen gels were first immersed in water. At scheduled time intervals, the samples were withdrawn and weighed. The water absorption was calculated using the following equation:

% Water up-take = (Wt –Wd)/Wd (g/g) (1),

where Wt denotes the weight of the swollen samples at immersion time t, and Wd denotes the weight of the dry samples. All the samples were studied in triplicate.

Optical Microscopy Study

All images were captured with a Leica Stereomicroscope model S8AP0, 20-160x magnification capacity.

Scanning Electron Microscopy

The scanning electron microscopic (SEM) images of sponge samples were registered using Quanta 200 FEI.

RESULTS AND DISCUSSION

The collagen gels with compositions according to Table 1, based on collagen and CMC-Na, cross-linked with EDC/NHS were freeze-dried and spongious matrices with the appearance and codification presented in Figure 1(a-d) were obtained.

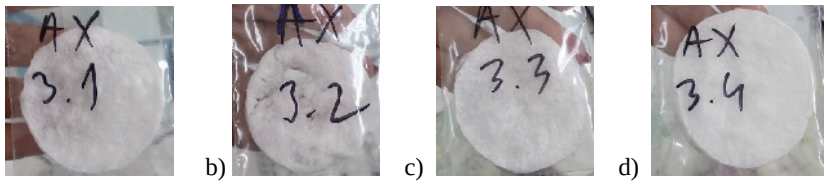


Figure 1. Collagen spongious forms:
a) Coll-R(1:1) b) Coll-R(2:1) c) Coll-R(1:1)-CMC-Na d) Coll-R(2:1)-CMC-Na

The samples from Table 1 were analyzed by FT-IR spectroscopy, water absorption, optical and scanning electron microscopy.

From the FT-IR spectra (Figure 2a, b) the typical bands from collagen can be observed: amide A, B, I, II and III (Albu, 2011) at 3291 cm^{-1}, 3070 cm^{-1}, 1631 cm^{-1}, 1549 cm^{-1} and 1237 cm^{-1}, respectively.

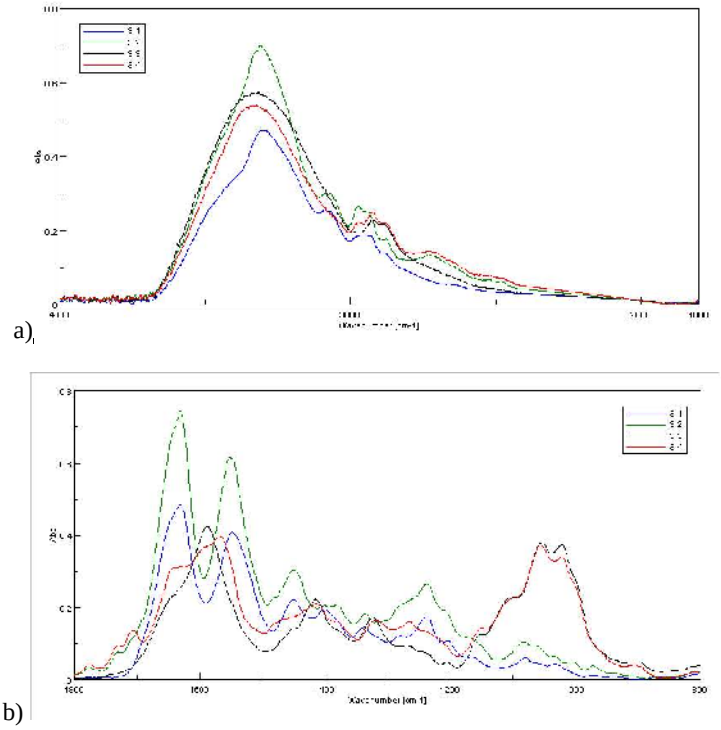


Figure 2. FT-IR spectra of matrices: a) 4000-1800 cm^{-1} and b) 1800 – 800 cm^{-1}

The cross-linking agent, EDC/NHS modify the collagen structure as it can be observed from amide A which shifted from 3292 cm^{-1} to 3306 cm^{-1}, amide II from 1549 cm^{-1} to 1553 cm^{-1} and also the appearance of new peak at 1730 cm^{-1}. The CMCNa presents characteristic peaks at 1595 cm^{-1} (stretching vibration of carboxylate group), 3402 cm^{-1} (O–H stretching vibration), 2912 cm^{-1} (C–H stretching vibration), 1421 cm^{-1} (–CH2 scissoring vibration), 1322 cm^{-1} (–OH bending vibration), 1060 cm^{-1}, (>CH–O–CH2 stretching vibrations), respectively (Liu *et al.*, 2016). The structure of Coll-CMCNa presented many changes compared with control one (Coll – AX 3.1.) such as: there is no amide I and amide A shift from 3291 cm^{-1} to 3316 cm^{-1}, amide II from 1549 cm^{-1} shifted to 1588 cm^{-1} because of carboxylate group from CMCNa and specific peaks of CMCNa appeared at 1322 cm^{-1} and 1052 cm^{-1}. The crosslinking from sample A.X. 3.4 are highlighted by shifted of amide A (from 3292 to 3323 cm^{-1}) and the peak from 1705 cm^{-1}.

The water up-take for all the studied samples is presented during 1, 2, 4 and 24 hours in Figure 3.

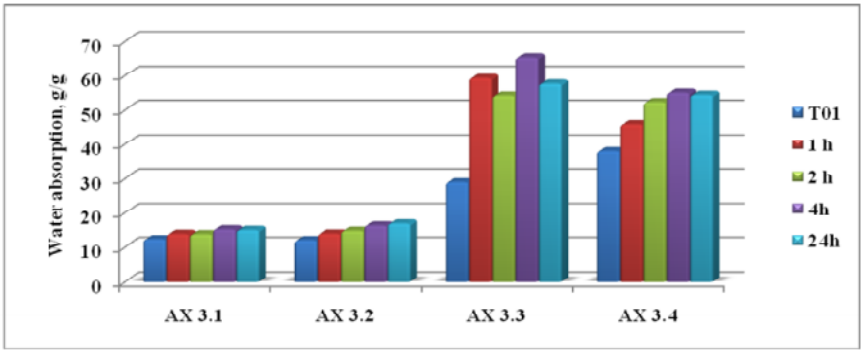
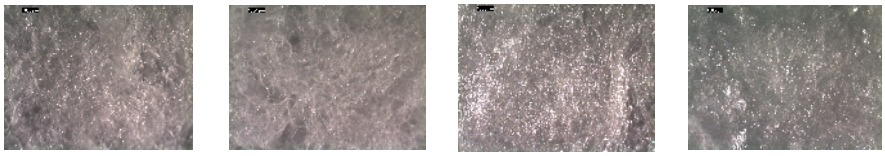


Figure 3. Water up-take during 24 hours for spongious forms

It can be observed that samples AX 3.1 and AX 3.2 have the capacity of water absorption under 20 g/g, compared to AX 3.3 and AX 3.4 that have a water absorption capacity over 50 g/g. Therefore samples with CMC-NA are about 2.5 times more hydrophilic then the others.

Figure 4 presents optical microscopy images for obtained matrices.



AX 3.1 AX 3.2 AX 3.3 AX 3.4

Figure 4. Optical microscopy (20x)

It can be observed a denser structure for samples AX 3.1 and AX 3.2 which explain why the lower amount of water was absorbed. The samples with CMC – Na (AX 3.3 and AX 3.4) present interconnected pores and a higher homogeneity.

Figure 5 presents the scanning electron microscopy for AX 3.2 sample.

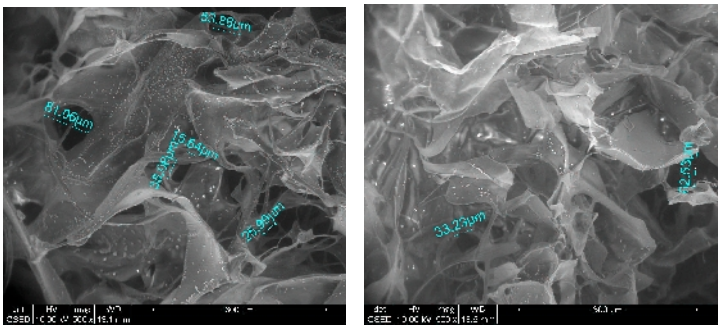


Figure 5. SEM images for AX 3.2

It presents a denser structure with interconnected pores of about 25 – 81 µm.

CONCLUSIONS

Type I fibrillar collagen and sodium carboxymethylcellulose were chosen as polymer for lens materials because of their transparency and biocompatibility. The obtained gels were crosslinked with EDC/NHS and lyophilized in order to obtain spongious forms. They are porous structures with pore sizes between 25-81 μm. The samples with CMCNa presented more uniform and dense matrices and the crosslinked ones are more resistant, being more proper as material for lenses for be used in cataract.

Acknowledgements

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