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SPECTRAL CHARACTERIZATION OF SOME COLLAGEN BASED COMPOSITE FOR DENTAL APPLICATION

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In this paper we present the spectral results obtained for collagen based materials with anti-inflammatory substance. The results obtained confirm the presence of the active substance and of the cross-linking agent and, on the other hand, they allow to predict the materials behavior in different conditions. Thus, this study could be useful in choosing the optimal composition for such kind of materials.

Keywords: collagen, niflumic acid, ibuprofen, FT-IR analysis, UV-VIS analysis

1. Introduction

Infrared spectra are important tool in determining material characteristics. Thus, hydrolysis degree, triple helix integrity, cross-linking degree and material degradation could be estimated based on IR spectra analysis [1-3].

Correlating the data of IR spectra with UV-VIS-NIR spectra results important information could be obtained with respect to the material behavior.

The aim of this study was to establish the influence of the active substance and cross-linking agent on properties of collagen sponges taking into consideration the final application of these dental materials.

2. Experimental part

2.1. Materials

The collagen (CG) used for the composite material synthesis was obtained from bovine derma at the Research Institute for Leather and Footwear from National Research and Development Institute for Leather and Textile according to the procedure described in reference [4]. The experiments were performed with collagen type I with fibrilar structure. The collagen gel has a concentration of

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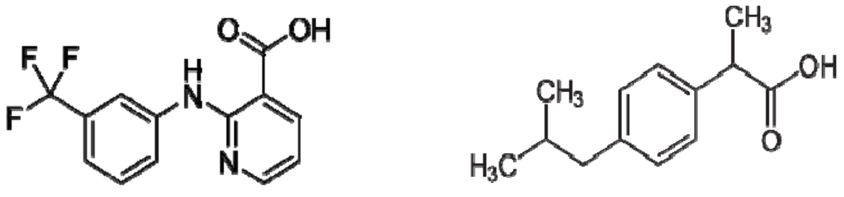
2.11 %, pH = 2.5 and it was free of fat or ash. Glutaraldehyde (GA) was purchased from Merck (Germany). Sodium hydroxide and phosphate buffer solution (PBS), pH, 7.4 were of analytical grade. The active substances (SA) used were: niflumic acid (NA) (Fig. 1a) from ICN Biomedicals Inc. (USA), Lot No. 80845, Cat. No. 155832; and ibuprofen (IB) (Fig. 1b) produced by Hubei Granules – Biocause Pharmaceutical Co Ltd, China, lot C100 – 1112284M.



a) b)

Fig. 1 Chemical structures of the active substances used: a) niflumic acid, b) ibuprofen

2.2. Preparation of collagen based composites

The solutions pH was adjusted to 7.4 using NaOH 1M solution and mechanical stirring. The antiinflamatory active substance at a concentration of 1 g/L was embedded into collagen gel. Both, collagen gels with antiinflamatory substance and without (as reference) were cross-linked with GA. The GA concentrations with respect to the dry substance were 0.25, 0.5, 0.75 and 1 %. All the samples had a concentration of 4 % drug with respect to the dry substance (0.1 % with respect to collagen gel). The homogeneous gells were lyophilised in order to obtain 3D collagen systems (collagen sponges) [5]. Tabel 1 presents the composition of the collagen sponges with active substance.

Table 1

Composition of collagen sponges with active substance

Sample	CG s.u.		pure AS		GA	
	[g]	[%]	[g]	[%]	[g]	[%]
CG + AS	0.2	90.91	0.02	9.09	0	0
CG + AS + 0.25% GA	0.2	90.70	0.2	9.07	0.0005	0.23
CG + AS + 0.5% GA	0.2	90.50	0.2	9.05	0.001	0.45
CG + AS + 0.75% GA	0.2	90.30	0.2	9.03	0.0015	0.67
CG + AS + 1% GA	0.2	90.10	0.2	9.00	0.002	0.90

where: CG – collagen gel; AS – active antiinflamatory substance; GA – glutaraldehyde

As references, similar samples have been prepared without active anti-inflammatory substance. The composition of these samples is presented in table 2.

Table 2

Composition of collagen sponges without active substance

Sample	CG s.u.		GA	
	[g]	[%]	[g]	[%]
CG	0.2	100	0	0
CG + 0.25% GA	0.2	99.75	0.0005	0.25
CG + 0.5% GA	0.2	99.50	0.0010	0.50
CG + 0.75% GA	0.2	99.25	0.0015	0.75
CG + 1% GA	0.2	99	0.0020	1

2.3 Methods

IR absorption spectra were recorded using a Perkin Elmer Spectrum 100 FT-IR spectrometer equiped with ATR and diamond devices. The IR spectra were recorded for the domain 4000 - 600 cm^{-1}, and each spectrum is the result of 4 scans. Suplimentary information can be obtained from the spectra as following:

☞ The ratio of the band intensities A_{OH}/A_I ($A_{\sim 3300}/A_{\sim 1630\ cm^{-1}}$) and A_I/A_{II} ($A_{\sim 1630}/A_{\sim 1550\ cm^{-1}}$) could be correlated with the ***hydrolysis degree*** [6];

☞ The ratio of the band intensities A_{III}/A_{COO^-} ($A_{\sim 1240}/A_{\sim 1450\ cm^{-1}}$) offer information about the ***triple helix integrity*** [7];

☞ The ratio of the band intensities A_I / A_{OH} ($A_{\sim 1630}/A_{\sim 3300\ cm^{-1}}$) is associated with the ***cross-linking degree***. For high values of this ratio one can affirm that the cross-linking degree is high [8].

☞ The difference $\Delta \nu = \nu_I - \nu_{II}$, put in evidence the presence of the ***distortion process***. ν_I and ν_{II} represents the wavenumbers of amide I and II respectively. It is considered that for $\Delta \upsilon < 100$ a distortion process does not happen [9].

UV – VIS – NIR absorption spectra were recorded in the wavelength range 200 – 2000 nm using Jasco V670 Spectrometer equipped with integrated sphere.

3. Results and discussion

3.1. FT-IR spectra of collagen based composites

Table 3 presents the results obtained for the IR spectra and interpretation for raw materials [10, 11].

Figs. 2 – 4 present the main absorption bands recorded for collagen based sponges. There are bands corresponding to raw materials as well as to new bonds formed between the compounds.

It can be noticed that ν_{NHas}+ν_{OHas} band from 3294 cm^{-1} in collagen sponge is shifted to higher wavenumbers in the collagen sponge containing GA, and GA with active substance, respectively (Fig. 2). This band highlights the hydrogen bonds formed between collagen molecules and active substance. In the case of collagen based composites with IB content, the association degree by hydrogen bonds increases when GA content is lower, while for collagen based composites with NA the GA content does not significantly influence the shift of this band.

Table 3

ATR/FT-IR spectral data for raw materials

NA		**IB**		**CG gel**	
ν, [cm^{-1}]	**Band**	**ν, [cm^{-1}]**	**Band**	**ν, [cm^{-1}]**	**Band**
3012	ν_{O-H}	2955	ν_{CH2}	3308	$\nu_{NHas} + \nu_{OHas}$
1662	$\nu_{C=O}$	2924	ν_{CH2}	1635	$\nu_{C=O};\nu_{C-N};\delta_{C-C-N}$
1581	δ_{N-H}	1701	C=O	1555	δ_{N-H}; ν_{C-N}
1531	$\nu_{C=C}$ aromatic	1365	ν_{c-c-o}; δ_{C-H}; δ_{CO-H}	1339	ν_{C-N}; C – C
1326	$\delta_{O-H\ acid}$	1230	CO–H in-plane bending (H-bonded)	1245	ν_{C-N}
1175	ν_{C-F}	1168	Φ CH in-plane bending; C^3–C^{24} stretching	1084	ν_{C-OH}
1146	ν_{C-F}	1073	CH2 twisting; C^{26}H3 rocking; C^{19}H3 rocking; C^{14}–H^{20} bend		
1104	$\nu_{C-OH\ acid}$	780	Φ, CH_3, C=O		
1066	$\nu_{C-OH\ acid}$				
973	$\delta_{C-H\ aromatic\ in\ plane}$				
770	$\delta_{C-H\ aromatic\ trisubstituted\ 1,2,3\ out\ of\ plane}$				
696	$\delta_{C-H\ aromatic\ disubstituted\ 1,3\ out\ of\ plane}$				

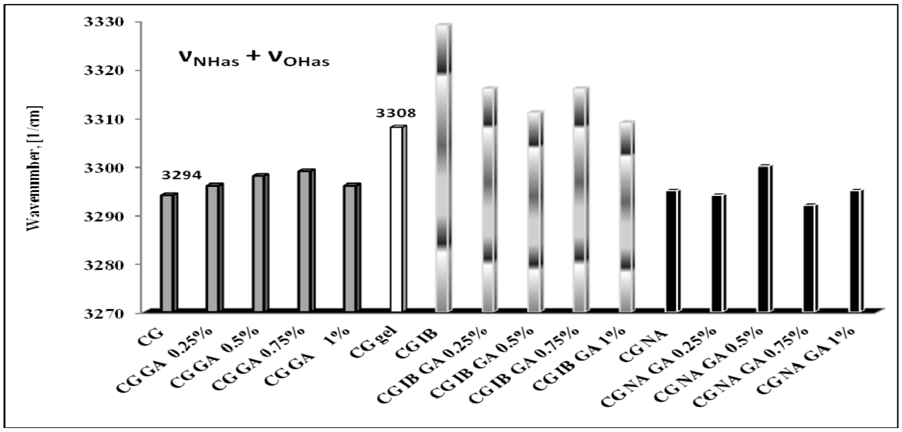


Fig. 2 Shift of $\nu_{NHas} + \nu_{OHas}$ band for collagen based composites

By analyzing the spectral data recorded for CG gel (table 3) and for collagen sponges with/without IB and different contents of GA, the ν_{NHas}+ν_{OHas} band registered at 3308 cm^{-1} is slightly shifted to smaller wavenumbers in the absence of IB, while the presence of IB induces a shift to higher wavenumbers. This behavior indicates an increase in the association degree by hydrogen bonds. For collagen sponges with/without NA, the association degree by hydrogen bonds decreases as evidenced by $\nu_{NHas}+\nu_{Ohas}$ band shifting to smaller wavenumbers.

The absorption band characteristic to stretching vibration of C=O ($\nu_{c=o}$) from amide I presented at 1630 cm^{-1} in the collagen sponge without GA, IB and NA as well as the absorption bands of the deformation vibration of the N–H bond ($\delta_{N\text{-}H}$) and the bond stretching vibration C–N ($\nu_{C\text{-}N}$) from the amide II presented at 1549 cm^{-1} in collagen sponges can be found in the collagen based sponges with active substance slightly shifted towards higher wavenumber, suggesting an association through hydrogen bonds formed between collagen molecules and the active substance (Figs. 3 and 4). It can be noticed an increasing of the association degree with the decrease of the GA content as the strength of the δ_{NH} increased.

The increasing of the association degree through hydrogen bonds due to the active substance is confirmed in the case of samples with NA by the amide II band which is slightly shifted from 1555 cm^{-1} to 1559 cm^{-1}(Fig. 4).

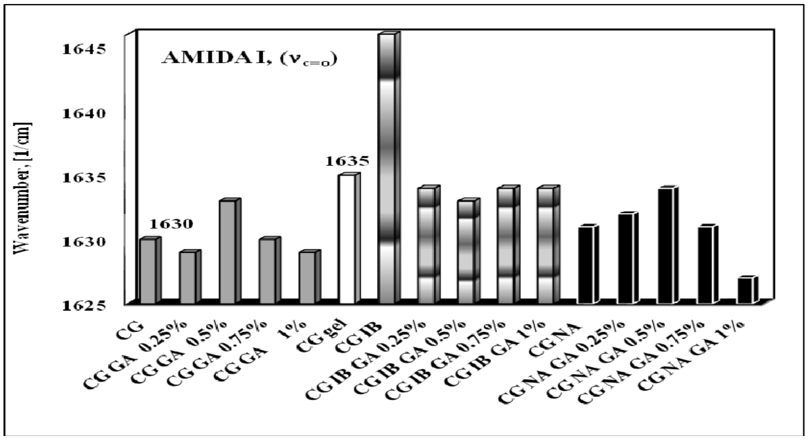


Fig. 3 Band shift of amide I for collagen based composites

The amide III band due to the contribution of the bonds C – C and stretching vibrations of the bonds C – N ($\nu_{C\text{-}N}$) are in the region 1340 ÷1331 cm^{-1} for composite materials with active substance (Fig. 5).

The peak at 1237 cm^{-1} is at the same wavenumber in all the samples and is due to the C – N – C bond presented in all polymeric molecules (Fig. 5).

The characteristic band of the C – OH bond from hydroxyproline units, due to the stretching vibrations of the bond C – OH ($\nu_{C\text{-}OH}$) is presented for all collagen sponges at 1081 cm^{-1}.

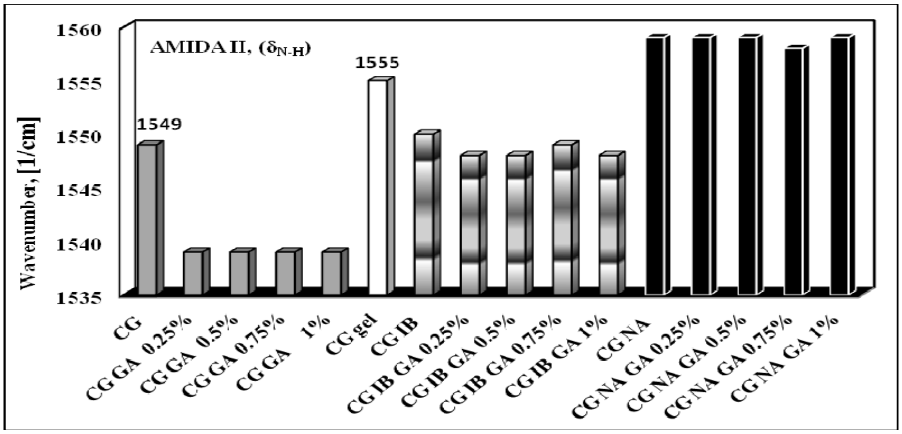


Fig. 4 Band shift of amide II for collagen based composites

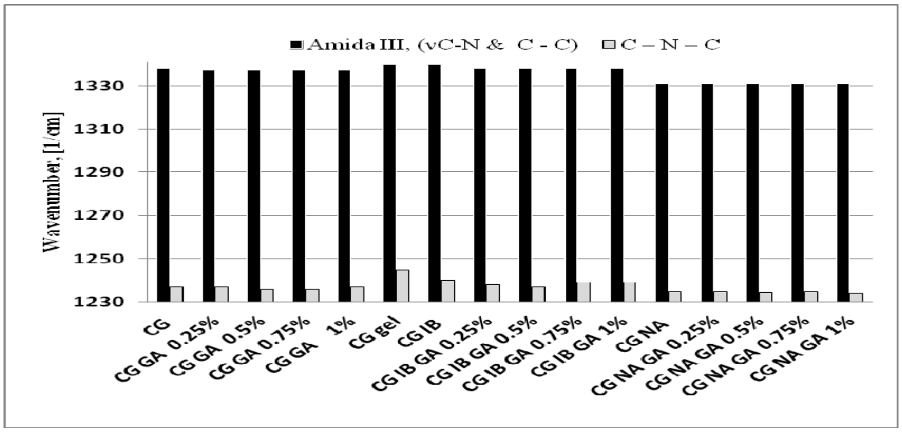


Fig. 5 Band shift of amide III and C – N – C bond for collagen based composites

The characteristic bands of the IB registered at 1365 cm^{-1} and 1168 cm^{-1} are observed at the same wavenumbers for all the samples with IB (~1365 cm^{-1}; ~ 1165 cm^{-1}). The twisting CH_2 band, rock bands $C^{26}H_3$ and $C^{19}H_3$, and C^{14}–H^{20}, which for IB are presented at 1073 cm^{-1}, could be seen at 1059 ÷ 1057 cm^{-1} in the collagen sponges. At the same time, the absorption band assigned to Φ, CH_3, C=O from 780 cm^{-1} for IB is registered at 787 cm^{-1} for the samples with this active substance.

Concerning the sponges with NA the maximum absorption band corresponding to $\delta_{N\text{-}H}$ recorded at 1581 cm^{-1} for collagen is present at approximately 1590 cm^{-1}, showing once again that NA increases the association degree through hydrogen bonds.

The samples with NA show at around 1520 cm^{-1} the stretching vibration of the C = C bond ($\nu_{C=C\ aromatic}$), at 1118 cm^{-1} and 1068 cm^{-1} the stretching vibration of C – OH bond ($\nu_{C-OH\ acid}$) and in the domain 1068 ÷ 697 cm^{-1} the specific aromatic compounds intense bands.

Table 4

Spectral data of collagen based sponges with/without IB/NA and different content of GA

Sample	**A_{OH}/A_I**	**A_{III}/A_{COO^-}**	**A_I/A_{OH}**	**$\Delta\upsilon$**
CG	0.4111	1.0017	2.4326	84
CG GA 0.25%	0.3869	0.9892	2.5845	90
CG GA 0.5%	0.3909	0.9554	2.558	94
CG GA 0.75%	0.3741	0.9370	2.6733	91
CG GA 1%	0.3779	0.9373	2.6465	90
CG IB	0.4492	0.7938	2.2261	96
CG IB GA 0.25%	0.4416	0.8495	2.2645	86
CG IB GA 0.5%	0.4399	0.8876	2.2734	85
CG IB GA 0.75%	0.4334	0.8385	2.3075	85
CG IB GA 1%	0.4047	0.8466	2.471	86
CG NA	0.4304	0.7282	2.3235	72
CG NA GA 0.25%	0.4111	0.7039	2.4328	73
CG NA GA 0.5%	0.4611	0.6842	2.169	75
CG NA GA 0.75%	0.4317	0.6741	2.3164	73
CG NA GA 1%	0.4586	0.656	2.1804	68

The ATR/FT-IR spectra data presented in table 4 suggest the following aspects:

- ✓ ***The hydrolysis degree*** calculated as the ratio A_{OH}/A_I increases when NA or IB is present. Nevertheless, in the cases of IB samples, increasing the GA content lead to a decrease of this degree, while for samples with NA increasing the cross-linking agent amount favours an increase of the hydrolysis degree.
- ✓ The comparative study of the ratios A_{III}/A_{COO^-} for the samples with SA but without cross-linking agent suggests that the ***triple helix structure integrity* is kept**. Adding GA determine some changes in helicoidally structure, as expected, these changes being more important in the case of collagen sponges with NA.
- ✓ From the ratio A_I/A_{OH} values it can be affirmed that the cross-linking degree is high and slightly increases with the increasing of the GA content. Some exceptions present the samples with NA where apparently the GA amount does not influence the cross-linking degree.
- ✓ As $\Delta\upsilon$ values are not higher than 100 one may suggest that there is no any distortion process.

3.2. UV-VIS-NIR spectra of collagen based composites

Fig. 5 presents the UV-VIS-NIR recorded for raw collagen in gel form.

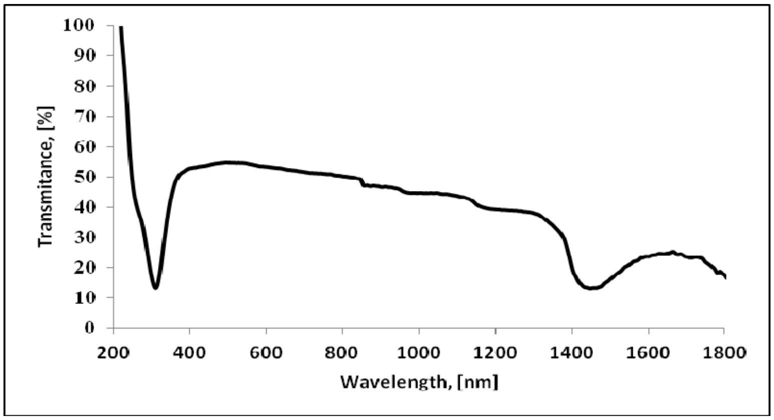


Fig. 6 UV-VIS-NIR spectrum for CG gel

An absorption band can be seen at 309.5 nm corresponding to the electronic transition n→π* of the -CO-NH- bond from the polypeptidic structure. In NIR region could be seen at 1448 nm the OH groups inter and intramolecular associated by hydrogen bonds ($\nu_{OH\ associated}$).

The UV-VIS-NIR spectra of collagen based sponges with and without active substance (IB, NA) and different contents of GA are showed in figs. 7 – 9, while table 5 presents the assignments of the main bands.

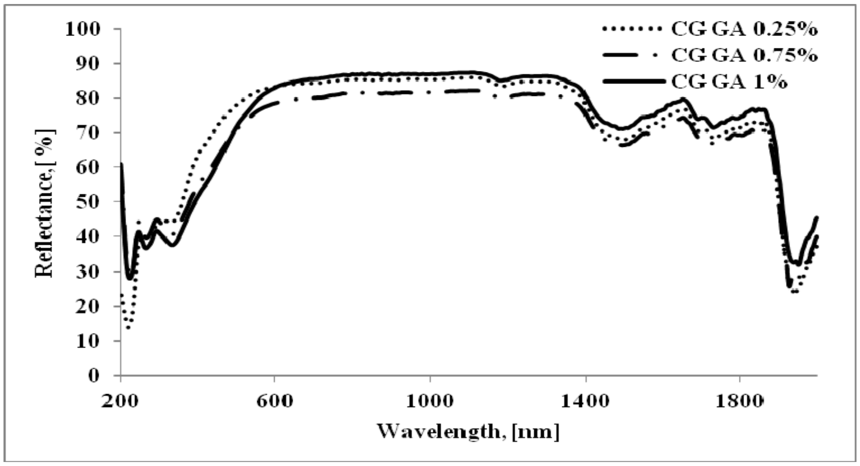


Fig. 7 UV-VIS-NIR spectra of CG – GA sponges

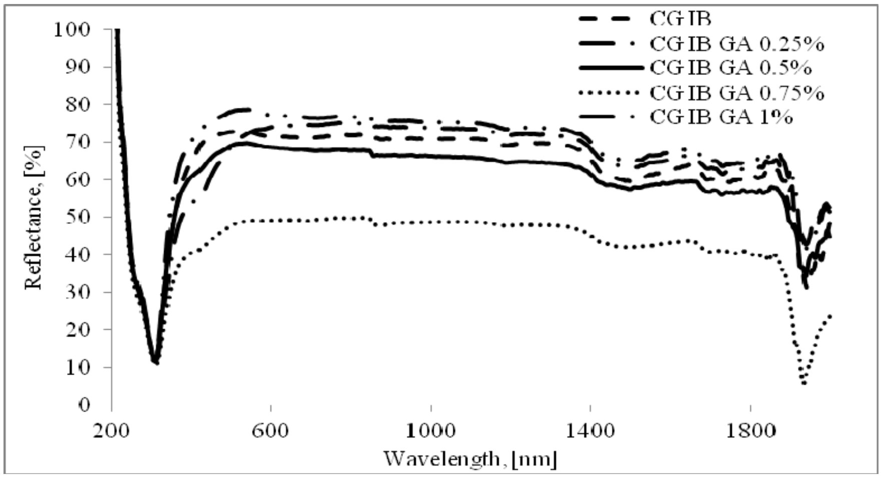


Fig. 8 UV-VIS-NIR spectra of CG – IB – GA sponges

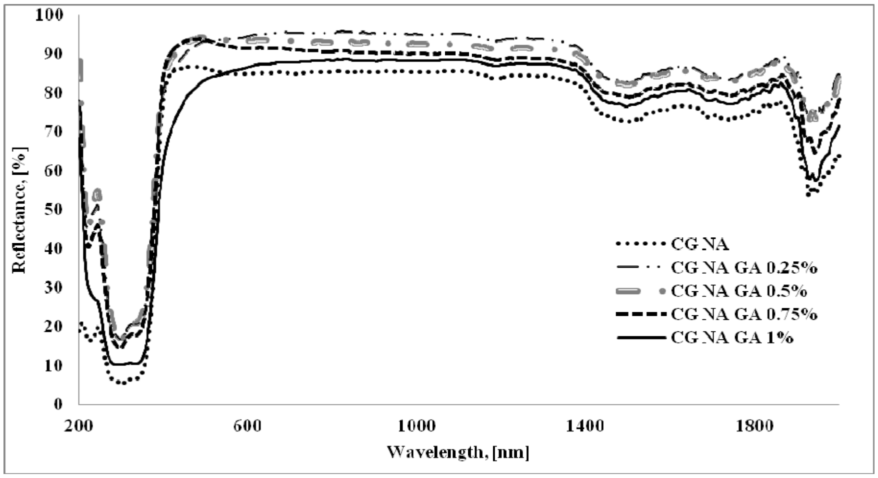


Fig. 9 UV-VIS-NIR spectra of CG – NA – GA sponges

In summary one may affirm that:

- In UV region there is a relatively large band at 317 ± 17 nm corresponding to the n→π* electronic transition of the -CO-NH- bond from the amidic structure;
- In NIR domain, between 1182 – 1193 nm could be seen the CH_2 band. At 1499 ± 4 nm there is the characteristic band for the valence vibration of the O – H bond. The presence of this band suggests that OH groups are associated by intermolecular hydrogen bonds. At ~ 1950 nm there is a relatively intense absorption band corresponding to deformation vibration of O – H bond ($\delta_{O\text{-}H}$).

Table 5

Assignements of the UV-VIS-NIR bands for the collagen based sponges

Sample	λ, nm	Band	λ, nm	Band	λ, nm	Band	λ, nm	Band
CG GA 0.25%	332	-CO-NH-	1186	ν_{CH2}	1502	$\nu_{OH\ ass}$	1942	$\delta_{O\text{-}H}$
CG GA 0.75%	333		1183		1498		1947	
CG GA 1%	334		1182		1488		1952	
CG IB	308		1191		1502		1942	
CG IB GA 0.25%	307		1188		1500		1940	
CG IB GA 0.5%	308		1188		1498		1939	
CG IB GA 0.75%	310		1190		1502		1947	
CG IB GA 1%	309		1190		1495		1939	
CG NA	304		1193		1498		1944	
CG NA GA 0.25%	299		1183		1499		1949	
CG NA GA 0.5%	299		1193		1499		1944	
CG NA GA 0.75%	299		1192		1503		1942	
CG NA GA 1%	306		1185		1498		1944	

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3.3 Correlation of FT-IR and UV-VIS-NIR results

The association degree by hydrogen bonds between collagen molecules and between collagen and the active substance could be revealed by using eq. 1. The results obtained for the studied materials are presented in table 6.

Table 6

FT-IR and UV-VIS-NIR spectra data for collagen based sponges

CG sponges			CG IB sponges			CG NA sponges		
Sample	FT-IR	NIR	Sample	FT-IR	NIR	Sample	FT-IR	NIR
	A_{OH}/A_I	$\Delta\lambda_{OH}$		A_{OH}/A_I	$\Delta\lambda_{OH}$		A_{OH}/A_I	$\Delta\lambda_{OH}$
CG	0.41		CG IB	0.45	53.5	CG NA	0.43	50
CG GA 0.25%	0.39	53.5	CG IB GA 0.25%	0.44	52	CG NA GA 0,25%	0.41	51
CG GA 0.5%	0.39		CG IB GA 0.5%	0.44	50	CG NA GA 0,5%	0.46	50.5
CG GA 0.75%	0.37	49.5	CG IB GA 0.75%	0.43	53.5	CG NA GA 0,75%	0.43	55
CG GA 1%	0.38	40	CG IB GA 1%	0.40	46.5	CG NA GA 1%	0.46	49.5

$$A_{OH}/A_I = f(\Delta\lambda_{OH}) \tag{1}$$

where, $\Delta\lambda_{OH} = \lambda_{OH_1} - \lambda_{OH_2}$,

λ_{OH_1} represents the wavelength corresponding to OH group recorded in NIR domain for raw collagen;

λ_{OH_2} represents the wavelength corresponding to OH group recorded in NIR domain for collagen based composite materials with and without active substance and different amount of cross-linking agent.

Graphical evolution corresponding to eq. 1 for the studied materials is presented in Fig. 10. The shift of the maximum wavelength to the lower frequencies as well as the high values of the ratio A_{OH}/A_I suggests that the association degree is increasing by adding the active substance due to the hydrogen bonds.

At the same time it can be observed that the most powerful association by intermolecular hydrogen bridges, in the case of collagen based composite materials with IB is favored by a low content of GA (0 – 0.25%), while for collagen based sponges with NA increasing the cross-linking agent content determines an increase of the association degree by hydrogen bonds.

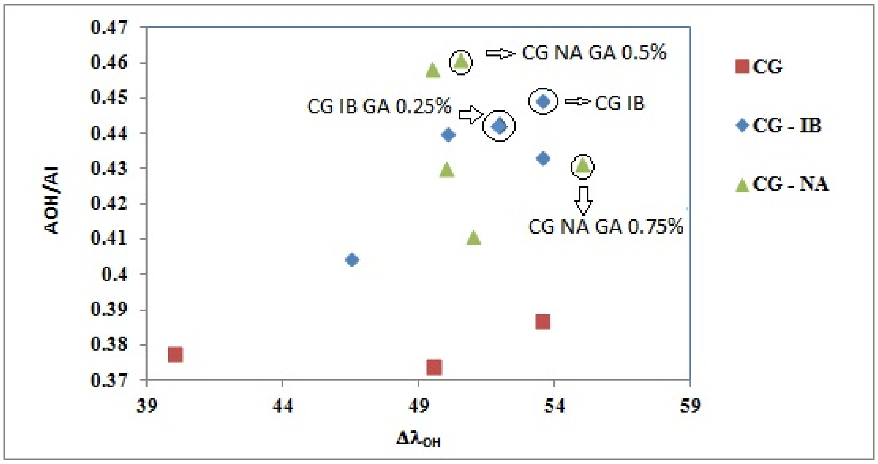

Fig. 10 A_{OH}/A_I (IR) versus $\Delta\lambda_{OH}$ (NIR)

4. Conclusion

In this paper spectral results obtained for collagen based sponges for dental applications were presented. It was proved that the cross-linking agent as well as the active substance presence has influence on the materials characteristics.

Taking into consideration the results obtained one can predict the behavior of these materials in different conditions. Considering other tests for these kinds of materials, water up-take and enzymatic degradation are important. It is expected that adding active substance will increase the water absorbed content while materials with cross-linking agent will have a higher resistance to the enzymatic degradation.

Having in mind the final application of these materials the optimum composition will be chosen by corroborating with results obtained from other complementary analyses. Nevertheless it is expected that the optimum amount of cross-linking agent is 0.5 for collagen based materials with anti-inflammatory substance.

REFERENCES

[1]. *I.B. Ionescu, D. Iordăchescu, Roxana Gabriela Popescu, I. Demetrescu, H. Iovu*, "Compoziție de film biopolimeric pe bază de gel de colagen şi procedeu de obținere a acesteia", Brevet de invenție Nr. 122673, Oficiul de stat pentru invenții şi mărci. BOPI nr. 11/2009

[2]. *G.T. Tihan, I.B. Ionescu, I. Demetrescu, A. Meghea*, "Filme biopolimerice ternare", Brevet de invenție, Nr. 122287, Oficiul de stat pentru invenții şi mărci, 2009

[3]. *C.R. Barbaresso, I. Rău, R.G. Zgarian, A. Meghea, M.V. Ghica*, "Niflumic acid-collagen delivery systems used as anti-inflammatory drugs and analgesics in dentistry", Comptes rendus - Chimie, vol. 17, no. 1, 2014, pp. 12-17

[4]. *M.G. Albu*, "Collagen gels and matrices for biomedical applications", Lambert Academic Publishing, Saarbrücken, Germany, 2011

[5]. *A. Lungu, I. Titorencu, M.G. Albu, N.M. Florea, E. Vasile, H. Iovu, V. Jinga, M. Simionescu*, Digest Journal of Nanomaterials and Biostructures, vol. 6, no. 4, 2011, pp. 1897

[6]. *M.G. Albu, M.V. Ghica, M. Giurginca, V. Trandafir, L. Popa, C. Cotrut*, Rev. Chim. (Bucharest), vol. 60, no. 7, 2009, pp. 666 – 672

[7]. *M.E. Andrews, J. Murali, C. Muralidharan, W. Madhulata, R. Jayakumar*, Colloid Polym Sci, vol. 281, 2003, pp. 766 – 770

[8]. *Y. Garcia, N. Hemantkumar, R. Collighan, M. Griffin, J.C. Rodriguez-Cabello, A. Pandit*, Tissue Eng Part A, vol. 15, no. 4, 2009, pp. 887-899

[9]. *G. David, B.C. Simionescu, S. Maier, C. Balhui*, Digest Journal of Nanomaterials and Biostructures, vol. 6, no. 4, 2011, pp. 1575-1585

[10]. *M.L. Vueba, M.E. Pina, L.A.E. Batista de Carvalho*, Journal of Pharmaceutical Sciences, vol. 97, no. 2, 2008, pp. 845–859

[11]. *N.F. Mohd Nasir, M.G. Raha, N.A. Kadri, S.I. Sahidan, M.Rampado and A Azlan*, American Journal of Biochemistry and Biotechnology, vol. 2, no. 5, 2006, pp. 175-179.