Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

The Characterization of Bioartificial Polymer Films Based on Collagen Filled with Oligoelements

G. Zgirian [a], I. Bujor [a], M. Giurginca [a], I. Rau [a], H. Iovu [a] & I. Demetrescu [a]

[a] University "Politehnica" Bucharest -Faculty of Industrial Chemistry, Bucharest, Romania

Version of record first published: 18 Oct 2010

To cite this article: G. Zgirian, I. Bujor, M. Giurginca, I. Rau, H. Iovu & I. Demetrescu (2004): The Characterization of Bioartificial Polymer Films Based on Collagen Filled with Oligoelements, Molecular Crystals and Liquid Crystals, 418:1, 291-298

To link to this article: http://dx.doi.org/10.1080/15421400490479460

Mol. Cryst. Liq. Cryst., Vol. 418, pp. 291/[1019]–298/[1026], 2004

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400490479460

THE CHARACTERIZATION OF BIOARTIFICIAL POLYMER FILMS BASED ON COLLAGEN FILLED WITH OLIGOELEMENTS

G. Zgirian, I. Ionescu Bujor, M. Giurginca, I. Rau, H. Iovu, and I. Demetrescu
University "Politehnica" Bucharest – Faculty of Industrial Chemistry, Bucharest, 781261, Romania

The purpose of this study is production and characterization of biofilms that possess mechanical properties of synthetic polymers and biocompatibility of the natural ones, being suitable for cell grows.

Two types of collagen hydrolysates the acid (HA) and the neutral forms were used as natural macromolecule and poly (vinyl alcohol – PVA) as a synthetic matrix, in order to obtain biofilms. As characterization methods FTIR Spectrometry, Atomic Force Microscopy (AFM), Differential scanning calorimetric measurements and Atomic Absorption Spectroscopy were used.

The correlation of the collagen content, the structure and properties of the bioartificial polymers is discussed.

Keywords: AFM; biofilms; collagen hydrolysates; DSC; oligoelements

INTRODUCTION

The biopolymers for biomedical applications should possess a combination of biocompatibility, physical-chemical and mechanical properties. Collagen is a protein which consists of three polypeptide chains wrapped around each other in a triple helix structure and is the major component of bone and connective tissue.

The range of potential applications have been targeted for collagen polymeric biofilms, artificial skin, included drug delivery, dialysis membranes, support for cell growth, etc. [1–3]. A technique to obtain biofilms whose properties depend on and may be monitored and controlled through compositional modification is polymer blending. As osteoblaste growth on films

Address correspondence to I. Demetrescu, University "Politehnica" Bucharest, Faculty of Industrial Chemistry, Bucharest, 781261, Romania. E-mail: i_demetrescu@chim.upb.ro

is a function of film characteristics this technique seems to be suitable. The present work is an approach of enhancing biocompatibility and mechanical properties of polymer blends based on collagen and poly(vinyl alcohol – PVA) adding various ions as calcium, magnesium and phosphate ions.

EXPERIMENTAL PART

Samples

The collagen hydrolysates denoted were HO_2, HO_8, and HA_4 where 2, 4 and 8 are hydrolysis times (hours), and the viscosity average molecular weight of the collagen hydrolysates mainly depends on the hydrolysis time, being 11,000–80,700 u.a. [3].

Films based on collagen filled with oligoelements were obtained using a "casting solution method" and final cross-linking step was achieved by dehydrothermal treatment (new patent) [4].

This study is devoted to various types of biofilms as following:

- A with PVA and collagen hydrolysates (HO_2) in different volumetric ratios, where collagen hydrolysates do not contain Ca^{2+} and Mg^{2+} ions.
- B with PVA and collagen hydrolysates (HO_8) in different volumetric ratios, where collagen hydrolysates do not contain Ca^{2+} and Mg^{2+} ions.
- C, D with PVA, collagen hydrolysates (HA_4) and Ca^{2+}, Mg^{2+} ions.
- E, F with PVA, collagen hydrolysates (HA_4) and Ca^{2+}, Mg^{2+}, PO_4 ions.

The amount of magnesium, calcium and phosphate in biofilms [4] was between 0.6–5%.

Technique

- The average viscosimetric molecular weight (M_V) was determined using Mark-Khun-Houwink equation: $[\eta] = K * M^{\alpha}$, were η is the intrinsec viscosity [dL/g], K and α are coefficients with specific values for different polymer – solvent system. It is to point out that the viscosimetric average molecular weight was almost simillar with gel chromatography average molecular weight [3].
- Infrared analysis was registered using a FTIR – Schimadzu spectrometer. Spectra were collected from the films and from KBr pellets.
- Surface analysis was performed with an Atomic Force Microscope. Images were acquired in standard contact mode. For quantitative analysis purposes maximum area images were selected and processed with AFM IMAGES ANALYSIS, a Windows application with Single Document Interface used in surface topography characterization. The program provide

2D and 3D visualization of AFM data. This data set can then be visualized and analyzed in various ways. We can extract **3D** profiles along polylines and calculate roughness and we can even evaluate the so-called fractal dimension of the specimen.

- Calorimetric measurements were carried out with a DSC CAHN 550 equipment. Argone was used as inert gas and the heating rate was $10^{\circ}C\ min^{-1}$.
- Atomic Absorption Spectroscopy, using an AAS 6 Vario apparatus with flame ionisation for cation content determination.

RESULTS AND DISCUSSION

Infrared Analysis

In the IR spectra of pure collagen hydrolysates, and also in case of collagen hydrolysate/PVA mixtures the well-known triple helical structure of collagen have been disappeared. The collagen hydrolysate spectrum presents the principal bands for the amide groups at about $1645\,cm^{-1}$ – corresponding to stretching vibration of C=O from amide I, and at $1556\,cm^{-1}$ corresponding to deformation vibration of N−H and stretching vibration of C−N from amide II respectively. Also the characteristic signal for NH groups is noticed at $1338\,cm^{-1}$. The isolated OH group from hydroxyproline unit gives a signal at $1082\,cm^{-1}$.

The differences between the spectra with various collagen concentration consist in the ratio between the intensity of some bands, first of all an increase of $3400\,cm^{-1}$ absorbtion band related to the OH of hydrogen bonding.

Differential Scanning Calorimetry

The samples were analyzed in sealed and nonsealed conditions in order to put in evidence water influence taking into account that both polymers used as blends components are highly water sensitive and non sealed system allows water evaporation.

Blends of PVA and collagen show a glass transition temperature around 35°C. According to literature data [5] thermal behavior of PVA/collagen mixtures indicates a remarkable enthalpy relaxation effect associated with the glass transition which appears as an endotherm peak close to 40°C independent of the PVA/collagen ratio. The main feature in DSC curve of such mixture is the endotherm denaturation at $T_d = 117^{\circ}C$.

In the second scan of PVA/collagen blends [5] the endotherm denaturation has disappeared indicating that all specific collagen structure colapsed.

TABLE 1 Effect of Hydrolysis Type and Time on Thermal Behavior of Pure Collagen Hydrolysates

Type sample	T_{2m} (°C)	Enthalpy (ΔH) (J/g)	Average viscozimetric molecular weight
Pure Collagen HO_2	81.51	525	80,700
Pure Collagen HO_8	81.38	231	16,250
Pure Collagen HA_4	80.73	677	11,000

T_{2m} = Second peak temperature.

Our samples being prepared with collagen hydrolysates do not present endotherm denaturation and the first glass transition phenomenon around 40°C (T_g) is a relaxation, and very small in all the cases.

The DSC curves of collagen hydrolysates/PVA blends permit a thermal characterization summarized in Tables 1, 2 and 3.

According to Table 1, T_{2m} is almost the same and independent on the type of sample and time of hydrolysis. The molecular weight also does not affect significantly the T_{2m} value. For neutral hydrolisates HO, an increase in hydrolysis time involves a decrease in enthalpy.

The synthetic components of the blends investigated is a partially crystalline highly hydrophilic polymer whose thermal properties is strongly influenced by adsorbed water. The distribution and interaction of water with protein in collagen have been the subject of numerous studies [6,7]. Water evaporation is the first process which occurs during sample heating. The DSC data on films without oligoelements show a second step around 200°C.

In the case of samples with calcium and magnesium, a small decrease is observed in ΔH_2 value and a significant increase for ΔH_3. The T_{2m} peak could be attributed to PVA melting taking into account that when PVA is subjected to DSC measurements using non sealed samples a melting endothermic process at 227°C is observed, associated with the crystalline polymer fraction. It is to point out that melting temperature of PVA is close to the glass transition temperature of pure collagen. A small variation in the melting temperature can be attributed to the alteration of the crystalline

TABLE 2 Oligoelements Effect on Thermal Parameters of Polymer Blends

Sample type	T_{2m} (°C)	ΔH_2 (J/g)	T_{3m} (°C)	ΔH_3 (J/g)
(A) HO_2/PVA	230	57	292	244
(B) HO_8/PVA	220	66	287	193
(D) HA_4/PVA	228	48	323	970

TABLE 3 Phosphate Effect on Thermal Parameters of Collagen Hydrolysates/PVA Blends

Sample type	T_m (°C)	ΔH (J/g)
HA_4 with calcium and magnesium		
(C) HA_4/PVA	231	56
(D) HA_4/PVA	228	48
HA_4 with calcium, magnesium and phosphate		
(E) HA_4/PVA	230	51
(F) HA_4/PVA	229	52

character of the PVA in the mixture. The T_{3m} peak seems to be more important in the oligoelement presence when the temperature and corresponding ΔH value are much greater.

It is to point out that in Table 3, samples D and F contains a double amount of collagen than C and E. Taking into account that all samples have Ca and Mg, the difference between sample behavior is due to phosphate and collagen changes. At the same collagen content, but lower content of phosphate ion induces a quite small decrease in T_m value and an increase in enthalpy. In fact phosphate ions do not influence thermal behavior.

Absence of important shift in temperature glass transition value upon mixing indicates that in collagen hydrolyzates/PVA blends each component contributes almost independently to the thermal properties of the blends, providing evidence that it is impossible to apply in this case the usual criterion for miscibility. In the literature [8] a study on thermal behavior of some mixtures of collagen hydrolysates with vinylic polymers present DSC data which show compatibility for these mixture at low temperature. By heating the mixture over the glass transition temperature this compatibility disappears.

It is known from thermodynamics that the miscibility is determined by the Gibbs free energy of mixing ΔG_m which is described by the relation:

$$\Delta G_m = \Delta H_m - T\,\Delta S_m \tag{1}$$

where T is absolute temperature and ΔS_m, ΔH_m are the entropic and enthalpic changes on mixing. The enthalpy of mixing for polymers is often small and positive.

The entropy for binary polymer mixture [2] can be written as:

$$\Delta S_m = -R\left(\frac{V}{V_R}\right)\left[\frac{\phi_1}{m_1}\ln\phi_1 + \frac{\phi_2}{m_2}\ln\phi_2\right] \tag{2}$$

In Eq. (2) R is gas constant, V is a total molar volume, V_R is a volume of the reference segment, ϕ_I is the volume fraction, and m_I is the degree of

polimerization. According to Eq. (2), the mixing entropy is varying with 1/degree of polymerization at larger molecular weight, while the entropy of mixing became smaller and can not compensate for endothermic enthalpies of mixing, in order to obtain a negative ΔG_m as should be the value in the case of two compatible components. A method of enhancing miscibility by building interaction as hydrogen bonding, such as those present in collagen hydrolysates – PVA blends is appropriate, but the interactions are rather small and the effect does not seems to be very important.

Surface Morphology

Regarding Atomic Force Microscopy data it is to point out that collagen content of hydrolysates used in biofilm preparation and various ions are important factors in changing surface morphology.

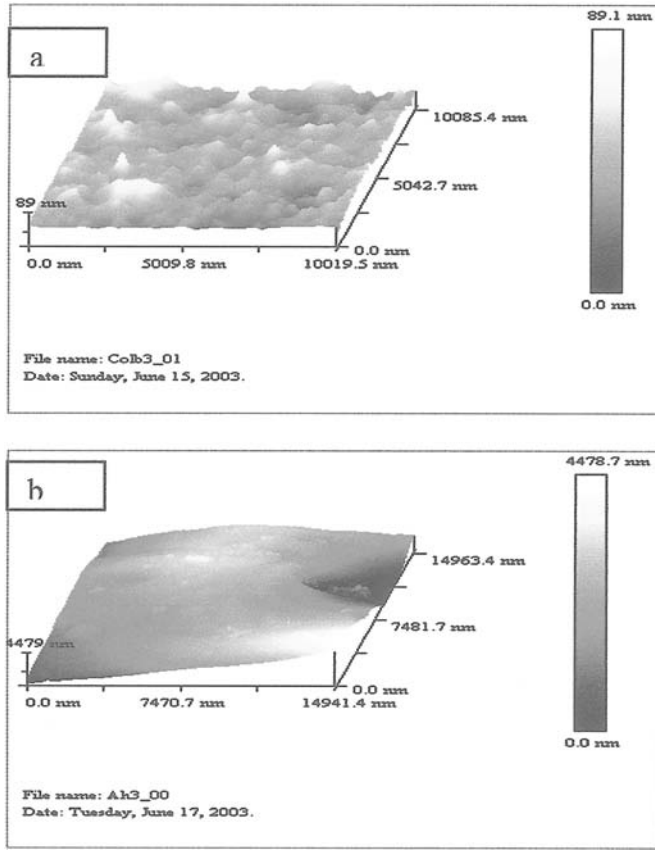

FIGURE 1 AFM images of biopolymer films sample (a) without calcium and magnesium ions sample (b) with calcium and magnesium ions.

TABLE 4 AFM Data

Samples	Content	Root-mean-square roughness (nm)	Fractal dimensions
Biopolymer film based on HA and PVA	with calcium, and magnesium ions	794.62	2.15
	with calcium, magnesium, and phosphate ions	712.90	2.14
Biopolymer film based on HO and PVA	without calcium, magnesium, and phosphate ions		
	0.3 g HO_8	30.38	2.27
	0.6 g HO_8	10.26	–

One of the main objectives of the present work is designing new biofilms structures based on acid and neutral collagen hydrolysates as support for cell. Osteoblaste growth on films is a function of biofilms characteristics, especially roughness. The roughness increase with depression and flakes in the surface is related to cell attachment. The roughness change is significant as could be seen in Figure 1 and Table 4.

The aspect of sample (a) and (b) in Figure 1 is an argument for the ion role in increasing roughness value. A concrete example is presented in Table 4 where the effects of various factors involved in changing biofilms roughness are evaluated.

Usually fractal dimensions are considered as part of space dimensions and a value between 2 and 3 is supposed to correspond to the capacity of a surface to partly fill a volume, without achieving it completely, out of having the whole value 3 which is needed. Our values of fractal dimensions are close to each other even the roughness is very different.

According to Table 4 phosphate ion and increase of collagen content induce a decrease in roughness value. As roughness value is direct related to adhesion and this is a factor of cell growth the AFM data proved to be a valuable criterion in biofilms selection.

In vitro observation of cell attachments reveals that all biofilms supported some cell attachments [4], PVA films presented a smooth surface, but even in this case osteoblast proliferation was observed.

CONCLUSION

1. Using various collagen hydrolysates with and without oligoelements and phosphate ion a series of biofilms with medical application were obtained. The average molecular weight of the collagen hydrolysates mainly depend on the hydrolysis time.

2. The absence of important T_g shifts with composition demonstrates that the blend components are almost immiscible.
3. The molecular average weight, the cross-linking, solubility, and oligoelement content of bioartificial film collagen/PVA, are in a direct relationship with roughness and cell adhesion.
4. Phosphate ion and increase of collagen content induce a decrease in roughness value both of them being related to adhesion and cell growth.

As roughness value is direct related to adhesion and this is a factor of cell growth, the AFM data proved to be a valuable criterion in biofilm selection.

REFERENCES

[1] Scotchford, C. A., Cascone, M. G., Downes, S., & Giusti, P. (1998). *Biomaterials*, *19*, 1–11.
[2] Giusti, P., Lazzeri, L., De Petris, S., Palla, M., & Cascone, M. G. (1994). *Biomaterials*, *15*(15), 1223–33.
[3] Bujor Ionescu, I., Trandafir, V., Demetrescu, I., Iovu, H., & Zgirian, G. (2003). *European Cell & Materials*, Vol. 5(Suppl 1), pp. 10–11.
[4] patent (N^0. A/00662/2003).
[5] Sarti, B. & Scandola, M. (1995). *Biomaterials*, *16*, 785–792.
[6] Pineri, M. H., Escoubes, M., & Roche, G. (1978). *Biopolymers*, *17*, 2799.
[7] Sasaki, N., Shiva, S., Yagihara, S., & Hikichi, K. (1983). *Biopolymers*, *22*, 2539.
[8] Vilcu, R., Olteanu, M., & Mandru, I. (1983). Proceeding 29th Symposium on Macromolecules IUPAC, Bucharest, pp. 494–497.