# ELABORATION AND CHARACTERIZATION OF POLYMERS/CERAMIC FILMS

Roxana G. POPESCU[1], Grațiela T. TIHAN[2], Nicoleta M. ŞULCĂ[3], Ioana DEMETRESCU[4]

*Scopul acestui studiu a fost acela de a dezvolta noi materiale polimerice realizate prin metoda "casting solution" din gel de colagen şi soluţii apoase de polietilenglicol amestecate cu hidroxiapatită, în diferite rapoarte gravimetrice. Filmele preparate au fost caracterizate prin spectroscopia în infraroşu, care arată prezența unor grupe funcționale în filmele polimerice. Analiza termogravimetrică a relevat faptul că filme polimerice pe bază de polimeri naturali şi sintetici sunt mai stabile termic decât matricea de polimer natural singură. Microscopia electronică de baleiaj utilizată pentru a analiza morfologia filmelor a arătat o distribuție a componentei anorganice în faza organică.*

*The goal of the present study was to develop new polymeric materials made from collagen gel and aqueous solutions of polyethylene glycol mixed with hydroxyapatite in different gravimetric ratio by casting solution method. The prepared films were characterized by infrared spectroscopy which shows the presence of functional groups in the polymeric films. Thermogravimetric analysis revealed that polymeric films based on natural and synthetic polymers are more thermally stable than pure natural polymer. Scanning Electron Microscopy used for examining the morphology of the films showed a uniform distribution of the inorganic component into the organic phase.*

**Keywords:** hydroxyapatite, polyethylene glycol, thermogravimetric analysis

## 1. Introduction

In recent years polymeric materials based synthetic and natural polymers have been investigated in various applications in tissue engineering to improve the biocompatibility [1-5]. Synthetic polymers such as polyethylene glycol (PEG) are widely used in biomaterial applications [6]. Polyethylene glycols are polymers obtained by oxyethylene polymerization. PEG is one of biocompatible materials which have received much attention due to the following properties: non-toxicity

[1] Assistent, Department of General Chemistry, University POLITEHNICA of Bucharest, Romania, e-mail: zgirianroxana@yahoo.com
[2] Assistent, Department of General Chemistry, University POLITEHNICA of Bucharest, Romania
[3] PhD Student, Department of Polymer Science and Technology, University POLITEHNICA of Bucharest, Romania
[4] Assistent, Department of General Chemistry, University POLITEHNICA of Bucharest, Romania,

and immunogenicity, hydrophilicity and high flexibility [7]. Moreover, PEG has a wide range of solubility and is non-biodegradable. Collagen (CG), one of the longest, fibrous structural proteins is a natural polymer extracted from animal or human sources and provides an excellent basis for biomaterials [8]. As a natural ceramic material, hydroxyapatite (HA) [9] represents a material with good mechanical properties and good bioactivity and osteoconductivity, being the main inorganic component of bones. HA/collagen composites have been extensively investigated [10, 11]. The properties of collagen based materials are usually modified by organic and inorganic additives [12]. Both collagen and hydroxyapatite exhibit excellent biocompatible properties [13, 14].

The present article describes the synthesis and characterization of polymers/ceramic films based on the polymer blend CG/PEG 4000 (in different gravimetric ratios) and hydroxyapatite, with potential applications in bone tissue engineering.

## 2. Experimental part

### 2.1. Materials

Collagen gel (3.2% dry substance) was obtained in the Collagen Department of the Romanian National Research & Development Institute for Textiles and Leather from bovine skin. Polyethylene glycol (average molecular weight 4000, PEG 4000), glycerin as lubrication agent, and aqueous solution of formaldehyde ($CH_2O$) as cross-linking agent were from Merck. HA powder ($Ca_5HO_{13}P_3$) was from Fluka.

### 2.2. Preparation method

Using the "casting solution method", at room temperature, polymeric films CG/PEG/HA have been prepared [15]. It involves the following steps: a) the collagen gel and glycerin are mixed at 30÷35$^{o}$C, and stirred for 5 minutes; b) the mixture is blended with an aqueous solution of PEG, for 10 minutes, until a homogeneous mixture is obtained, followed by the addition of the cross-linking agent at room temperature; c) the obtained composition is added to an aqueous suspension of hydroxyapatite, stirred for 30 minutes until a white gel is formed, which is cast onto Petri dishes, at 25$^{o}$C, in order to facilitate the cross-linking process (by water evaporation). The films are stored at 25$^{o}$C for approximately 30 days, when thin, white and uniform films are obtained. The polymeric films are detached and washed with distilled water to remove the traces of cross-linking agent that could influence cell growth and proliferation.

Until now, our research studies have shown that this method of obtaining polymeric films and their preparation for cell growth [16, 17] is a very good method, since osteoblast and fibroblast cells grow and proliferate with a normal phenotype. Different types of prepared polymeric films are presented in Table 1.

*Table 1*

**Polymeric films composition**

| Sample | Gravimetric ratios | |
|---|---|---|
| | PEG : CG | (CG + PEG) : HA |
| **A** | 1:1 | 1:2 |
| **B** | 1:2 | 1:2 |
| **C** | 1:3 | 1:2 |
| **D** | 2:1 | 1:2 |
| **E** | 3:1 | 1:2 |

### 2.3 Methods

The physico-chemical parameters of the samples were determined by Fourier Transform spectroscopy (ATR/FT-IR) using a Perkin Elmer FT-IR spectrophotometer, in the attenuated total reflection mode (ATR). The spectra are resulted from averages of 16 scans at 1cm$^{-1}$ resolution, between 600 and 4000cm$^{-1}$. For structural characterization the studies have been completed by using UV-Vis spectroscopic techniques on thin films with Perkin Elmer Lambda 950 UV-VIS spectrometer. The surface wettability was determined using KSV Instruments's CAM 100 by measuring the contact angle on the surface of the films. To evaluate thermal stability of the CG/PEG/HA films, thermal gravimetric analysis (TGA) was performed on a Q500 TA instrument. HITACHI S2600N Scanning Electron Microscope (SEM) was used for examining the morphology of films (GC/PEG/HA).

## 3. Results and discussion

Various ratios of CG/PEG/HA films were prepared and evaluated.

### 3.1. ATR/FT-IR study

The identification of functional groups in the HA powder, pure CG, pure PEG and polymeric films as well as the interfacial modification were performed by ATR/FT-IR analysis. The main FT-IR peak positions of various functional groups from spectra of the pure PEG [18], CG [19] and HA [20] samples are presented in Table 2.

*Table 2*

**FT-IR characteristics of the starting materials**

| ν, [cm$^{-1}$] | Bands | ν, [cm$^{-1}$] | Bands | ν, [cm$^{-1}$] | Bands |
|---|---|---|---|---|---|
| **PEG 4000** | | **CG** | | **HA** | |
| 3438 | $\nu_{OH\ free}$ and $\nu_{OH\ as}$ | 3308 | $\nu_{OH\ as}$ | 3570 | $\nu_{OH\ free}$ |
| 2891 | $\nu_{CH2\ sim}$ | 1635 | $\nu_{C=O}$ (AI) | 2786 | $\nu_{CH2\ sim}$ |
| 1467 | $\delta_{CH2}$ | 1555 | $\delta_{N-H}$ (AII) | 1408 | $CO_3^{2-}$ [21] |
| 1359 | $\delta_{CH3}$ | 1407 | N-H | 1087 and 1019 | $\nu_{P-O\ as}$ from $PO_4^{3-}$ |
| 1107 | C-O-C | 1245 | $\nu_{C-N}$ (AIII) | 962 | $\nu_{P-O\ sim}$ |
| | | 1084 | $\nu_{C-OH}$ | 632 | $\nu_{OH}$ |

IR spectra recorded on polymeric films based on PEG, CG and HA (Fig. 1) show the most representative bands:

- The peak 3308cm$^{-1}$ of CG is found in all ternary biopolymeric films, with small shifts. This band reveals the presence of hydrogen bonds formed between polyethylene glycol and collagen molecules, and between polymer molecules and hydroxyapatite. It is noted that when the amount of collagen increases (A, B, C films) the wavenumber decreases, showing a lower degree of association by hydrogen bonds. When the amount of PEG changes the band from CG (3308cm$^{-1}$) is not significantly influenced (A, D, E films);
- The spectra of polymeric films A, B and C show the absorption band corresponding to the stretching vibration of C=O ($\nu_{C=O}$) from amide I (at 1659cm$^{-1}$, 1646cm$^{-1}$, and 1646cm$^{-1}$ respectively). A shift of this band to lower wavenumbers (about ~ 13cm$^{-1}$) is observed when the CG content increases, due to the breaking of hydrogen bonds from CG which will lead to decreasing of the degree of association by hydrogen bonds. This results in a decrease in hydrogen bonding to hydroxyapatite, too. In the case of sample D and E, the amide I band appears at 1661cm$^{-1}$.
- The amide II band from CG is stable in all the samples, and is not influenced by the variation of CG or PEG content.
- The peak corresponding to N–H bond in pure CG appears at 1407cm$^{-1}$. This peak shifts to lower values of wavenumber (1403cm$^{-1}$, 1395cm$^{-1}$) in the polymeric films with higher CG content. In the films with increased PEG content this band shifts to higher wavenumbers (1411cm$^{-1}$). This is probably due to the linking of HA with CG and PEG;
- The absorption band at 1245cm$^{-1}$ which is present in CG, appears in all the films at 1241cm$^{-1}$ showing C–N–C bonds formed between polymer molecules;
- The peak at 1023cm$^{-1}$ in A, B and C films, corresponding to the stretching vibration of P-O ($\nu_{P\text{-}O\ as}$) from $PO_4^{-3}$ proves the hydroxyapatite presence and it is not affected by the increase of CG content. When the gravimetric ratio between polymers and HA is constant (1:2), but the gravimetric ratio between PEG and GC increases from 1:1 to 3:1, a shift to lower values of wavenumber is observed;
- The peak at 628cm$^{-1}$ corresponds to thestretching vibration of O–H ($\nu_{OH}$) and proves the HA presence too. The small shift to lower wavenumbers is due to the hydrogen bonds formed between polymers and HA.

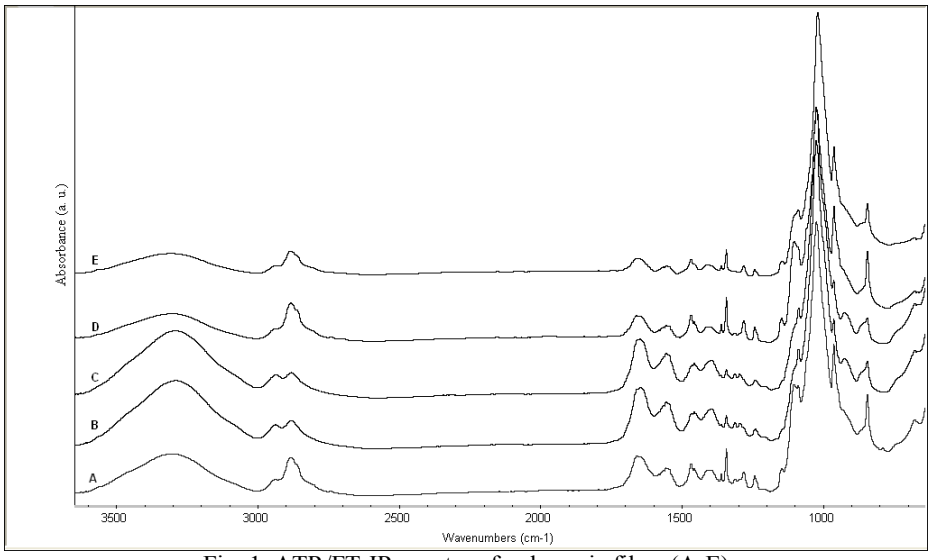



Fig. 1. ATR/FT-IR spectra of polymeric films (A-E)

Using the main data obtained from ATR/FT-IR spectra the ratio between the intensity of the bands $A_I/A_{II}$ and $A_{OH}/A_I$ calculated. They reflect the hydrolysis degree of the polymer films. The association degree between the two polymers and the association degree between the two polymers with hydroxyapatite were calculated using equation 1 [22], where $\nu_I$ and $\nu_{II}$ represents the wavenumber of the amide I and II, respectively.

$$\Delta\nu = \nu_I - \nu_{II} \quad (1)$$

The main characteristics obtained from ATR/FT-IR spectra processing are presented in Table 3.

*Table 3*

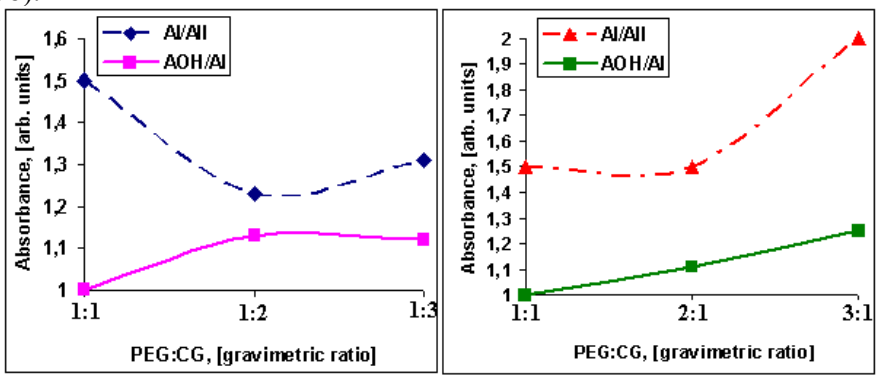
**Spectral characteristics of the polymeric films**

| Sample | $A_I/A_{II}$ | $A_{OH}/A_I$ | $\Delta\nu$ |
|---|---|---|---|
| **A** | 1,5 | 1 | 103 |
| **B** | 1,23 | 1,13 | 90 |
| **C** | 1,31 | 1,12 | 89 |
| **D** | 1,5 | 1,11 | 105 |
| **E** | 2 | 1,25 | 105 |

Analyzing the obtained results some aspects are evidenced:

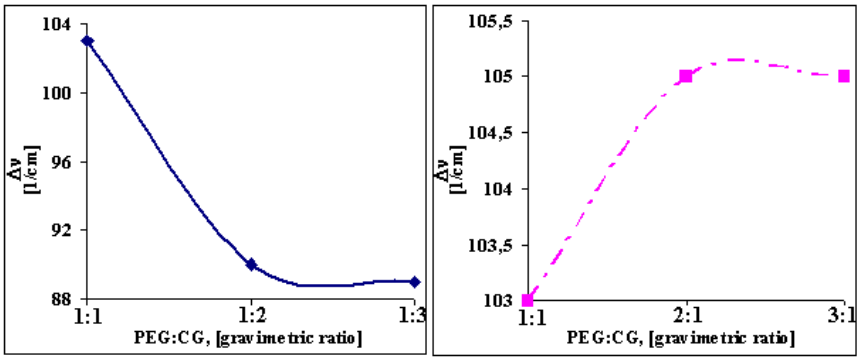
- The hydrolysis degree shown by $A_I/A_{II}$ and $A_{OH}/A_I$ ratios decreases with increasing CG content and therefore the association with HA is reduced (Figs. 2a and 3a);
- The hydrolysis degree presents a slight increase when PEG content increases. The association degree of the two polymers and their association degree

with the HA are facilitated by the increase in synthetic polymer content (Figs. 2b and 3b).




a) b)

Fig. 2. Hydrolysis degree versus polymer content (A- E films)




a) b)

Fig. 3. Degree of association versus polymer content (A- E films)

### 3.2. UV-Vis-NIR study

Both pure components and polymeric films samples were examined at room temperature, with UV-Vis-NIR spectroscopy technique. The characteristic bands of HA powder, pure CG and pure PEG are presented in Table 4.

*Table 4*

**Characteristic bands from UV-Vis-NIR spectra of the starting materials**

| λ, [nm] PEG 4000 | Band | λ, [nm] CG | Band | λ, [nm] HA | Band |
|---|---|---|---|---|---|
| 1226 | $\nu_{-CH2}$ | 218 | -CONH- | 231 | π →π* (P=O ) |
| 1438 | $\nu_{O-H\ free\ and\ asoc.}$ | 1194 | $CH_2$ | 267 | n→π* (P–O–) |
| 1741 | $\nu_{O-H\ free}$ | 1460 | OH asoc. inter/intramolecular | 1438 | $\nu_{O-H}$ from P–OH |
| 1937 | $\nu_{O-H}$ and $\delta_{N-H}$ | | | 1953 | $\delta_{O-H}$ |

In the case of polymeric films based on PEG, CG and HA all the spectra recorded in the UV-Vis-NIR domain (Fig. 4) show the characteristic bands of the pure compounds: electronic transition type $\pi \rightarrow \pi^*$ of P=O bond, absorption bands attributed to the amide unit -CONH- and to the $-CH_2-$ group, the stretching vibration of O–H bond from P–OH ($\nu_{O\text{-}H}$), bands corresponding to the stretching vibration of the O–H bond ($\nu_{O\text{-}H\ free}$), and the deformation vibration of the O–H bond ($\delta_{O\text{-}H}$).

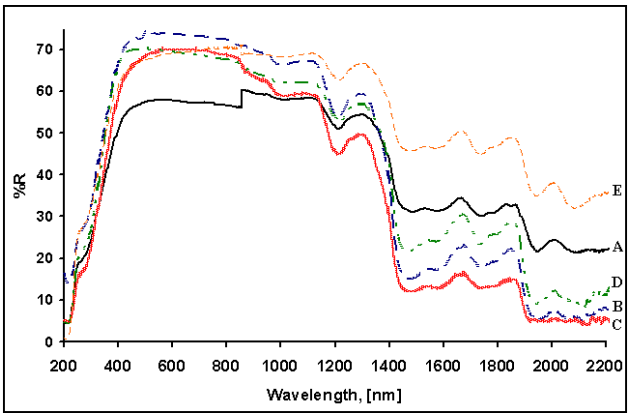



Fig. 4. UV-Vis-NIR spectra of the polymeric films with different PEG/GG gravimetric ratios

### 3.3 Contact angle measurements

The contact angle is one of the parameters used to characterize wettability of surfaces. By definition, contact angle values between 0 and 90° indicate a hydrophylic surface, and values higher than 90° indicate a hydrophobic character. The test was performed on polymer samples of 20mm x 20mm using a drop of distilled water (10µL), at room temperature. In all experiments contact angle between 33 and 40° with 0.1÷0.2 standard deviation were obtained. The contact angle values for all the polymeric films are presented in Fig. 5.

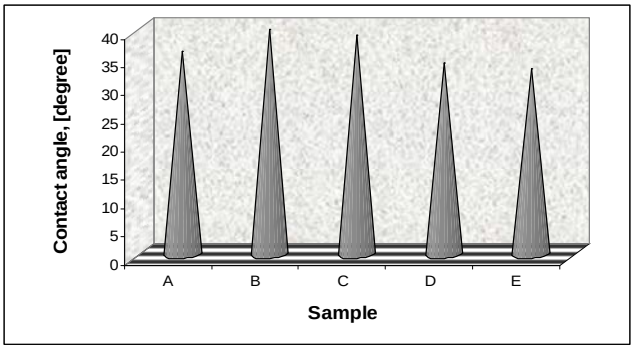



Fig. 5. Contact angle values of the polymeric films

The measurement requires the drop of water to stay a certain time on the surface. Distortions due to gravity effects are minimal when the droplet volume is $<10^{-4}$mL, and the drop can be assumed hemispherical.

First, the measurements have shown that the incorporation of a higher quantity of hydrophilic PEG [23] (A, D, E films) leads to the decrease of the contact angle values, and thus to the increase of hydrophilic character of the polymeric films. Also, the introduction of larger quantities of CG [24] (A, B, C films) leads to a slight decrease of the film hydrophylicity. Moreover, literature provides data on the correlation between contact angle, hydrophilic or hydrophobic character of the polymer and its biocompatibility. A hydrophilic character promotes cell adhesion and suggests that polymeric films could be used successfully in a wide variety of biomedical applications [25-27].

**3.4. TGA analysis**

TGA is an important method for analysis of polymeric composite materials with inorganic components, like HA. It is used for thermal stability determination and also to determine the weight loss of material as a function of heat, in controlled atmosphere. Samples in the range of 1.5–2mg were used.

To evaluate the thermal stability of the pure components, TGA studies were performed in the range 25 to 600$^{o}$C, at 10°C/min heating rate, under nitrogen. The results are presented in Fig. 6.

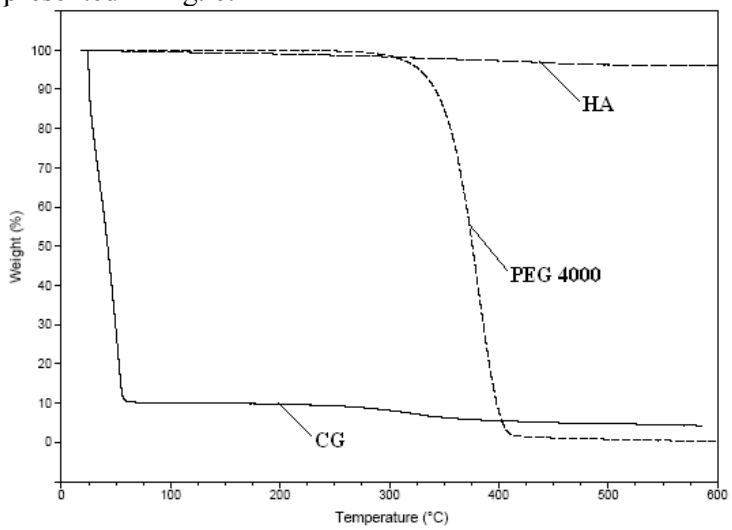



Fig. 6. TGA thermograms of PEG, CG, HA

From the thermograms of pure CG and PEG a steep decrease in weight can be observed when the temperature increases. The total weight loss was about 95.6% (CG) and 99.6% (PEG). Although PEG 4000 shows a higher loss in mass, it resists without deteriorating, up to approx. 350$^{o}$C. Comparatively, CG with

lower stability [25] is degraded (approx. 90%) at 50°C. The TGA curve of HA powder exhibits only a small weight loss, 4.3% [26].

The TGA data for the polymeric films have been recorded under the same conditions, and are presented in Table 5 and Fig. 7.

*Table 5*

**Principal parameters of the TGA plots for the films**

| Sample | $T_{onset\ 3\%}$ [$^0$C] | Weight loss at 600$^0$C [%] |
|---|---|---|
| **A** | 72 | 52 |
| **B** | 70 | 49 |
| **C** | 65 | 47 |
| **D** | 85 | 54 |
| **E** | 117 | 56 |

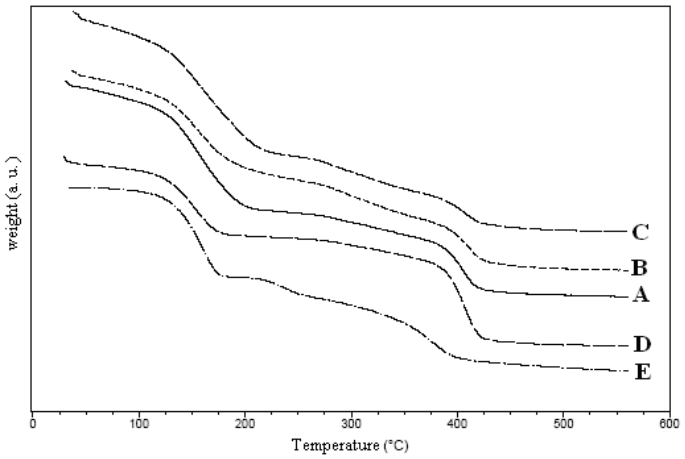



Fig. 7. TGA thermograms of the polymeric films

The thermostability of the films is determined by studying the temperature at which 3% of the sample is degraded. As expected samples with higher collagen content (samples B and C) are thermally less stable ($T_{onset}$ decreases). Increase in PEG 4000 content (samples D and E) leads to higher thermal stability ($T_{onset}$ increases).

The variation in weight loss triggers opposite effects. As CG content increases weight loss decreases and as PEG content increases weight loss increases. This is shown by TGA: at 600°C, CG presents a weight loss lower than PEG 4000.

HA, which is a component with the same concentration in all the samples does not interfere.

Thermogravimetric analysis revealed that composite polymers films, based on two polymers (one natural and one synthetic) are more thermally stable than the single components (CG or PEG, respectively).

### 3.5 Scanning Electron Microscopy (SEM)

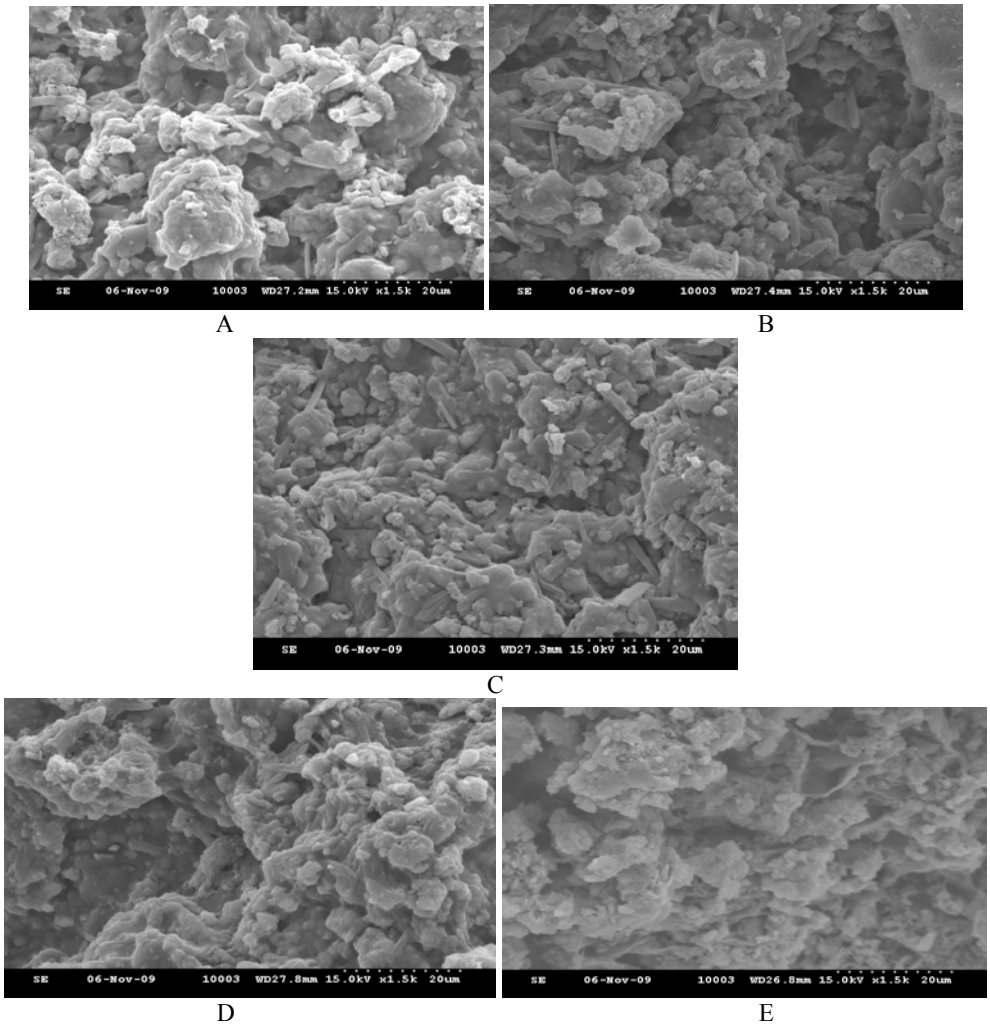



Fig. 8 SEM images of the samples A – E (x 1.5k)

SEM images of the samples are presented in Fig. 8. You can observe a uniform distribution and a good contact angle between the HA granules and collagen-polyethylene glycol complex. The HA is almost spherical and rod

shaped from the morphological point of view. The studied films, with different gravimetric ratios between CG and PEG, but with the same gravimetric ratio between (CG+PEG):HA present a similar morphology, the polymer matrix not having apparent influence.

## 4. Conclusions

We report the synthesis and characterization of new polymeric films based on a blend of two polymers with hydroxyapatite in different ratios. The main data obtained from FT-IR and UV-Vis-NIR spectra have shown that the hydrolysis degree slightly increases with the increase in synthetic polymer content. An increase in natural polymer content leads to a decrease in hydrolysis degree, too. Therefore, the degree of association with the ceramic material is improved by increasing the PEG content. The presence of the hydrogen bonds formed between PEG and CG molecules, and between these two polymers and hydroxyapatite is evidenced by ATR/FT-IR spectra. The contact angle values suggest that for polymeric films the increase in synthetic polymer content determines the increase of the hydrophilic character of the films, and the increase in natural polymer content determines the increase of the hydrophobic character. A low value for the contact angle indicates a hydrophilic material. TGA measurements provide valuable results that can be used to select films for different applications. SEM images show a uniform distribution of the inorganic phase into the collagen - polyethylene glycol complex.

## R E F E R E N C E S

[1] *A. Slovikova, L Vojtova, J. Jančař,* Chemical Papers, 2008, **62**, no. 4, pp. 417–422
[2] *R.G. Popescu, A. Morega, D. Iordachescu, I. Demetrescu*, Mat. Plast., 2009, **46**, no. 2, pp. 140 – 143
[3] *L. Liu, X. Xu, S. Guo, W. Han,* Carbohydrate Polymers, 2009, **75**, pp. 401–407
[4] *S. Sarkar, A. Chourasia, S. Maji, S. Sadhukhan, S. Kumar, B. Adhikari.* Bull. Mater. Sci., October 2006, **29**, no. 5, pp. 475–484
[5] *J.Y. Yang, D.Y. Lee, S.J. Park, Y. Byun*, Biomaterials 2004, **25**, pp. 3663
[6] *D.I. Zeugolis, R.G. Paul, G. Attenburrow*, J. Biomed. Mater. Res., Part B: Appl. Biomater, 2008, pp. 343 – 352
[7] *M. Rutnakornpituk, P. Ngamdee,* Polymer 2006, **47**, pp. 7909-7917
[8] *A. Huc*, J. Am. Leather Chem. Assoc., 1985, **80**, pp. 195-212
[9] *Y. Zhai, F.Z. Cui,* J. of Crystals growth, 2006, **291**, pp. 202-206
[10] *D. Lickorish, J. Ramshaw, J.A. Werkmeister, V. Glattauer, C.R. Howlett*, J. Biomed Mater Res. 2004; **68A,** pp. 19–27
[11] *M. Kikuchi, H.N. Matsumoto, T. Yamada, Y.K.K. Takakuda, J. Tanaka*, Biomaterials 2004, **25**, pp. 63–69
[12] *M. Olteanu, D. Achimescu, Z.Vuluga, V.Trandafir*, Rev. Roum. Chim. 2008, **53**, no. 2, pp. 157–63

[13] *M. Kikuchi, T. Ikoma, S. Itoh, H. N. Matsumoto, Y. Koyama, K. Takakuda, K. Shinomiya, J. Tanaka*, Composites Science and Technology, 2004, **64**, pp. 819–825
[14] *T. Taguchi, L Xu, H. Kobayashi, A. Tanigichi, K. Kataoka, J. Tanaka*, Biomaterials, 2005, **26**, pp. 1247
[15] *I. Ionescu Bujor, D. Iordăchescu, R. G. Popescu, H. Iovu, I. Demetrescu,* Filme biopolimerice pe bază de colagen, polietilen glicol şi hidroxiapatită (Biopolymeric films based on collagen, polyethylene glycol and hydroxyapatite), Brevet no. 122673, 2009, România
[16] *G. Tihan, I. Ionescu-Bujor, D. Iordăchescu, I. Kostrakievici*, Hydroxyapatite effect in enhancing cell growth on new ternary biopolymeric films, Buletinul Ştiinţific al U.P.B., Seria B, 2005, **67**, no. 3, pp. 27-32
[17] *G.T. Tihan, I. Ionescu Bujor, A. Meghea, I. Demetrescu*, Filme biopolimerice ternare (Thernary biopolymeric films), Brevet no. 122287, 2009, România
[18] *M.P. Patil, N.J. Gaikwad*, Acta Pharm., 2009, **59**, pp. 57–65
[19] *N.F. Mohd Nasir, M.G. Raha, N.A. Kadri, S.I. Sahidan, M.Rampado, A Azlan*, American Journal of Biochemistry and Biotechnology 2006, **2,** no. 5, pp. 175-179
[20] *X. Wang, Y. Lia, J. Wei, K. de Groot*, Biomaterials, 2002, **23,** pp. 4787–4791
[21] *J. Barralet, J.C.Knowles, S.Best, W. Bonfield*, J. of Materi. Science: Materials in Medicine, 2002, **13**, pp. 529-533
[22] *M.G. Albu, M.V. Ghica, M. Giurginca, V. Trandafir, L. Popa, C. Cotrut*, Rev. Chim. (Bucureşti), 2009, **60,** no. 7, pp. 666-672
[23] *S.J. Chang, C.C. Niu, C.F. Huang, S.M. Kuo,* J. of Med. and Biological Engineering, 2007, **27**, no. 1, pp. 41-46
[24] *Y., Tang, C. Cao, X. Ma, C. Chen, H. Zhu*, Biomed. Mater., 2006, **1,** pp. 242–246
[25] *K. E. Geckeler, R. Wacker, W. K. Aicher,* Naturwissenschaften, 2000, **87**, pp. 351-354
[26] *Y. Tamada, Y. Ikada*, J. of Biomed. Mater. Research, 1994, **28**, pp. 783-789
[27] *K. Geckeler, R. Wacker, F. Martini, A. Hack, W. Aicher*, Cell. Physiol. and Biochem., 2003, **13**, pp. 155-164
[28] *B. P. Chan and K. F. So,* J. Biomed Mater Res, 2005, **A 75**, pp. 689-701
[29] *X. Zhang, Y. Li*, G. Lv, Y. Zuo, Y. Mu*, Polym. Degrad. and Stab., 2006, **91**, pp. 1202-1207.