

COMPOZITE COLAGEN/COCHILIE DE MOLUSCĂ POTENȚIALE BIOMATERIALE PENTRU SUBSTITUȚIA OȘOASĂ MOLLUSC SHELL/COLLAGEN COMPOSITE AS POTENTIAL BIOMATERIAL FOR BONE SUBSTITUTES

MARIA FICAI^{1*}, ECĂTERINA ANDRONESCU, GEORGETA VOICU, DENISA FICAI,
MĂDĂLINA GEORGIANA ALBU², ANTON FICAI¹

¹ Universitatea POLITEHNICA București, str. Gheorghe Polizu nr. 1, sector 1, București, România

² Institutul Național de Cercetare Dezvoltare Textile și Pielărie (INCDTP), str. Ion Minulescu nr. 93, sector 3, București, România

În această lucrare ne-am concentrat atenția asupra caracterizării cochiliei unei specii de corbicula și sinteza și caracterizarea unor materiale compozite de tip colagen/cochilie. Cochilia cât și materialul compozit au fost caracterizate prin XRD, SEM, FTIR și ATD-TG. Se poate concluziona că cochilia de moluscă conține peste 98% CaCO₃ și mai puțin de 2% fază organică. Materialul compozit s-a obținut prin amestecarea cochiliei de moluscă fin măcinată cu colagen în raport masic 4:1. Imaginile SEM înregistrate pe materialul compozit denotă o bună omogenitate a materialului, nanofibrele de colagen acționând drept lianți ai particulelor de carbonat.

In this work our attention was focused on the characterization of a corbicula species shell and the synthesis and characterization of collagen/mollusc shell composite materials. The mollusc shell and the composite material were characterized by XRD, SEM, FTIR and DTA-TG. It can be concluded that mollusc shell contain more than 98% of CaCO₃ and less than 2% organic phase. The composite material was obtained by mixing fine milled mollusc shell and collagen in 4:1 weight ratio. The recorded SEM images of the obtained composite material exhibit a very good homogeneity; the collagen nanofibrils acting as glue between carbonate particles.

Keywords: mollusc shell, calcium carbonate, collagen, composite, bone graft

1. Introduction

Synthetic bone grafts are very important because of the limited amounts of natural bone grafts. The natural bone grafts materials include autografts (achieved from the patient), allografts (achieved from other fellow of that species) and xenografts (achieved from other species). The use of these graft materials are firstly reported by Gallie and Toronto in 1914 [1]. Since then, a lot of synthetic materials were tested as bone substitutes: metals and alloys [2, 3]; coated metals and alloys (especially, with ceramic materials) [4-6]; polymeric [7]; ceramic [8-10], composite materials [11-15].

Between the natural materials extensively studied were: bovine bone [16-18], coral [19], deer

antler [20]. The roles of bone [21, 22] and mollusc shell are very similar (Table 1).

The remarkable properties of bones are not only due to the collagen and hydroxyapatite but also due to the other components such as carbonate, citrate, etc.

During the time, many carbonate based materials were obtained [23]. Many of these materials were obtained in order to use them as biomaterials.

Mollusc shell is a remarkable composite material composed from calcium carbonate as main inorganic phase and less than 2 % of organic phase acting as true "glue" for the inorganic phase.

The collagen is also a remarkable biomaterial and starting from these premises we obtain

Table 1

Comparative roles of bone and mollusc shell / Comparație între rolurile oaselor și a cochiliei de moluscă

Bone / Os	Mollusc shell / Scoică
- protective role for many organs / rol protector pentru mai multe organe	- protective roles for entire mollusc / rol protector pentru întreaga moluscă
- act as a mineral reservoir of Ca ²⁺ and PO ₄ ³⁻ , especially / acționează ca un rezervor mineral în special de Ca ²⁺ și PO ₄ ³⁻ ,	- act as a mineral reservoir for Ca ²⁺ and CO ₃ ²⁻ / acționează ca un rezervor mineral pentru Ca ²⁺ și CO ₃ ²⁻
- provide the mechanical support for animal and human body / asigură ajutor mecanic pentru corpul animalelor și omului	

* Autor corespondent/Corresponding author,

Tel.: +40 21 402 39 60, e-mail: ficaimaria@yahoo.com

collagen/mollusc shell composite material with potential biomedical use.

2. Experimental

Type I collagen (M.W. 300.000) gel was obtained by extraction from bovine's derma hides. The extraction process allows extracting the collagen molecules with triple helix native conformation.

For this work, mollusc shells from *corbicula* species were used (Figure 1). The shells were plenty rinsed with water and ethylic alcohol, milled in a planetary ball mill for 30 minutes at 150 RPM and dried by freeze drying.

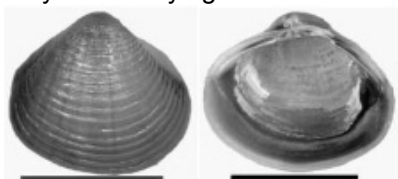


Fig. 1 - *Corbicula* species shell; bar corresponding to 1 cm
Cochilie de moluscă caracteristică speciei de corbicula, scala 1cm.

The collagen/mollusc shell composite material was obtained by milling of 25 g collagen gel 2% with 2 g mollusc shell. For good homogenisation the milling process was set for 30 minutes at a rate of 150 RPM. After milling, the material was dried in a controlled atmosphere at 30 ± 2 °C.

The obtained material was investigated by X-ray diffraction, IR spectroscopy, scanning electron microscopy and thermal analysis.

X-ray diffraction analysis was performed using a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu K α radiation from a Cu X-ray tube (run at 15mA and 30 kV) was used. The samples were scanned in the Bragg angle, 2θ range of 10 – 80.

SEM analyses were performed on a HITACHI S2600N electron microscope with EDAX, at 15 keV, in primary electrons fascicle, on samples covered with a thin silver layer.

The IR spectra were recorded on a Shimadzu 8400 FTIR Spectrometer on 500 – 4000 cm^{-1} range, using a resolution of 2 cm^{-1} , for mollusc shell and also for collagen/mollusc shell composite material.

The differential thermal analysis (DTA) coupled with thermo gravimetric analysis (TGA) was performed with a Shimadzu DTG-TA-50H, at a scan rate of 10 °C/min, in air.

3. Results and discussion

The X-ray diffraction pattern (Figure 2) confirms calcium carbonate presence as the main mineral phase of mollusc shell. Between the different crystallographic forms of calcium carbonates the mollusc shell contains calcite and aragonite as main mineral phases. Calcite: aragonite weight ratio, determined by semi-quantitative XRD was 9:1. The calcite: aragonite ratio was estimated as follows: 1 g of mollusc shell was analysed by XRD and the ratio between the intensities of peaks recorded at 29.57 and 47.64 was calculated (I_1^0); in the second step, 1 g of calcite is added to 1g of mollusc shell and the ratio between the intensities of the same peaks was

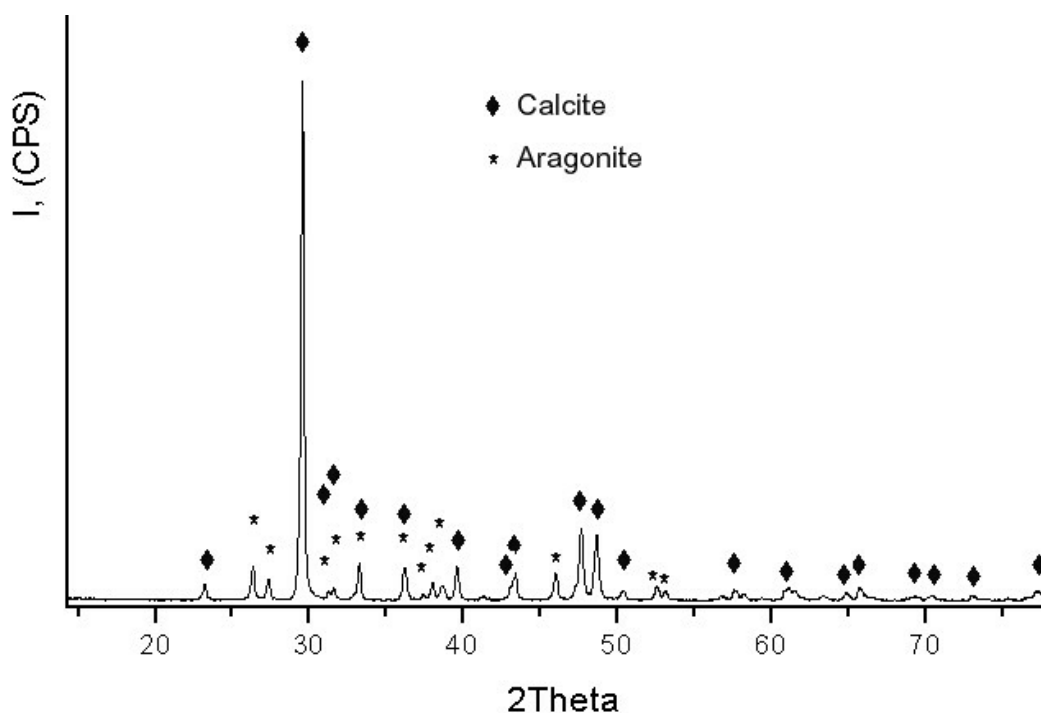


Fig. 2 - XRD pattern of collagen - mollusc shell composite material / *Difractograma de raze X a materialului compozit de tip colagen/cochilie.*

calculated (I_2^0). The weight ratio between calcite and aragonite is estimated by Eq. 1.

$$\frac{x}{y} = \frac{I_2^0 - I_1^0}{2 \cdot I_1^0} \dots \dots \dots \text{(Eq. 1)}$$

No other mineral, such as vaterite - CaCO_3 [24] and dolomite - $\text{CaMg}(\text{CO}_3)_2$ [25], gypsum - $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ [25] or basanite $2\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ [25] which usually occur in molluscs shells can be identified by XRD.

The XRD pattern (Figure 2) was also recorded for collagen/mollusc shell composite material which confirms that the adding of collagen gel doesn't modify the composition of carbonate mineral phase.

Infrared spectroscopy (Figure 3) was used for both mollusc shell and collagen/mollusc shell characterisation. In the IR spectra of both materials the carbonate peaks can be easily identified, the most intense being at 2522, 1789, 1473 (vs), 860 and 706 cm^{-1} .

The bands of organic phase present in 1200 – 1700 cm^{-1} range are overlapped with the main peak of calcium carbonate. Because of the low concentration of organic phase from the mollusc shell and the moderate molar absorptivity coefficient these peaks can be identified only by deconvolution. In collagen/mollusc shell composite material, the collagen peaks can be easily identified. The main organic peaks are: 1630 cm^{-1} corresponding to C=O, 1530 cm^{-1} corresponding to NH deformation, peaks which can be assigned to collagen.

The infrared spectrum of collagen/mollusc shell composite exhibit an increase of collagen peaks height because of the increase of organic matter content.

The scanning electron microscopy (Figure 4) was used in order to study the morphology of collagen/mollusc shell composite material and also the interaction which occurs between mineral mollusc shell particles and collagen fibrils and fibers.

The recorded scanning electron images of collagen/mollusc shell composite material exhibits a stratified structure due to the geometrical shape of lamellar mollusc shell and due to the strong interaction between the two phases.

The collagen molecules from the collagen gel interact with calcium carbonate lamellae and cover many of them. The collagen covered calcium carbonate lamellae are joined by many fibrils and fibres.

At high magnification (Figure 4d) molecular size collagen can be observed. It is important to mention that the obtained composite is very stable in the electron beam being possible to magnify up to 100.000 fold with minimal burn.

Energy dispersive X-Ray analysis (Figure 5) was recorded in order to establish the main elemental composition of the material. The EDS analysis was recorded on the collagen/mollusc shell composite materials, at low magnification (500 X). The elemental composition of the composite is: Ca, P and O.

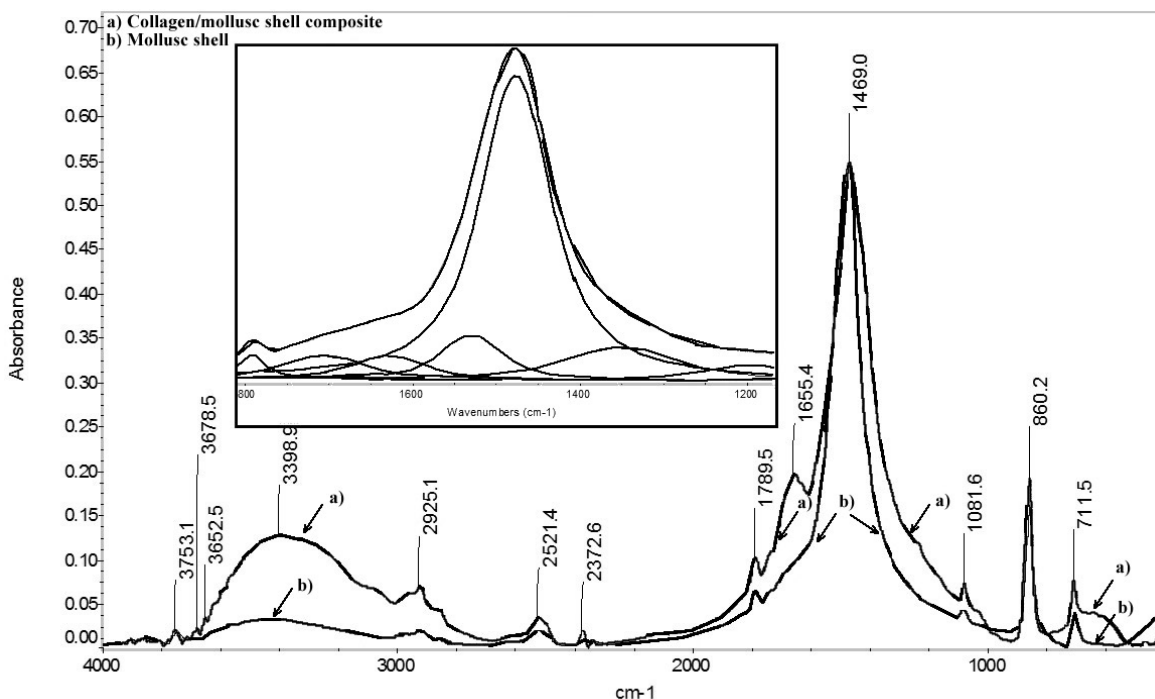


Fig. 3 - Infrared spectra of a) mollusc shell; b) collagen/mollusc shell composite materials; detail corresponding to deconvolution of composite material peaks between 1200 – 1800 cm^{-1} / *Spectrele IR ale a) scoicii de moluscă, b) materialului compozit de tip collagen/scoica de moluscă; detaliu corespunzător domeniului 1200 – 1800 cm^{-1} pentru materialul compozit.*

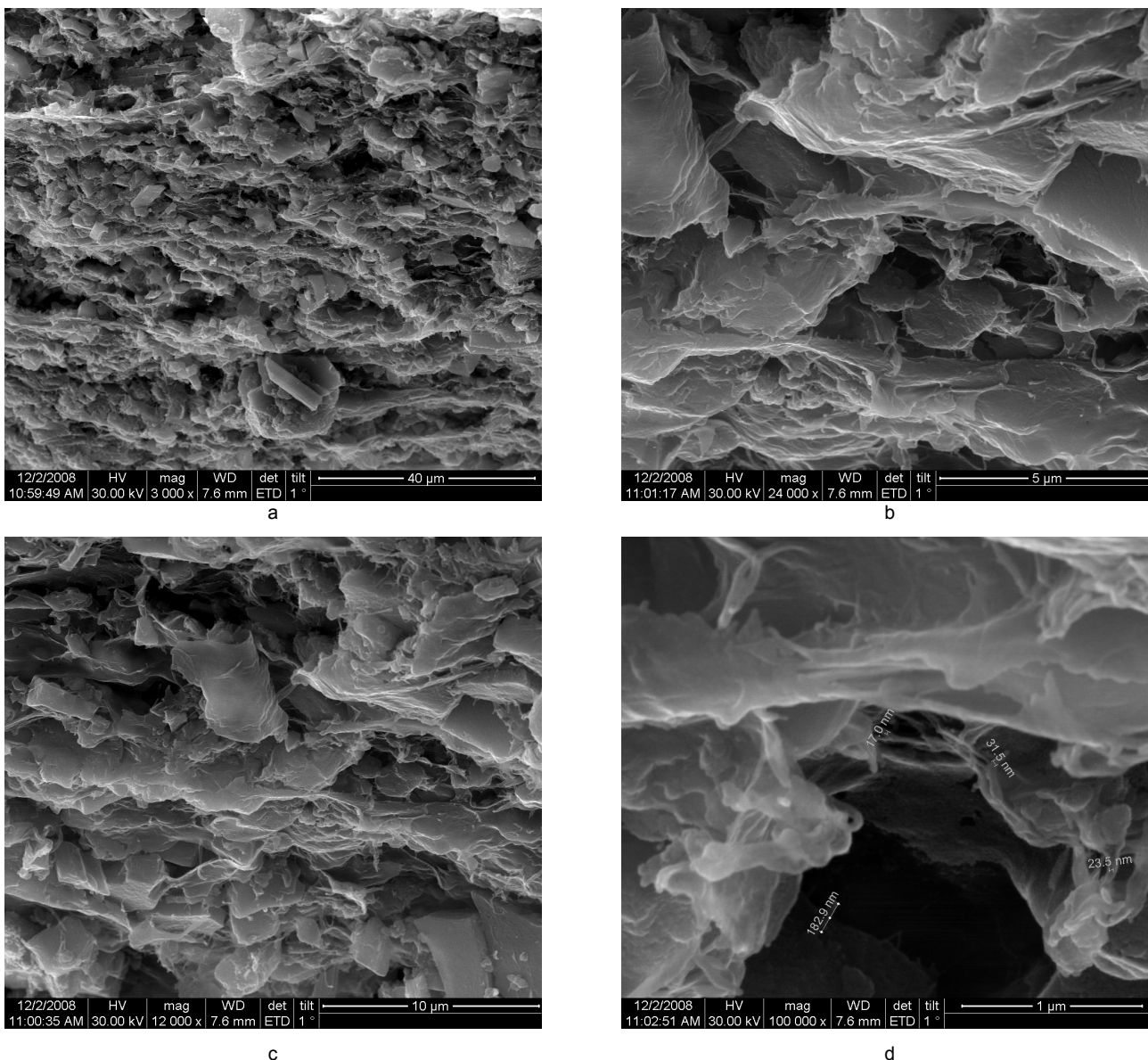


Fig. 4 – SEM images of collagen/mollusc shell composite material at different magnifications: a) 3.000, b) 12.000, c) 24.000 and d) 100.000x / Imagini SEM caracteristice materialului compozit collagen/cochilie de molușcă la diverse mărimi: a) 3.000, b) 12.000, c) 24.000 și d) 100.000x.

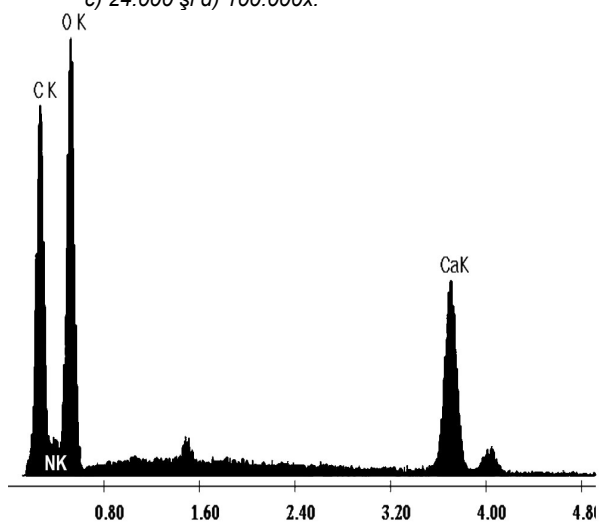


Fig. 5 - EDS spectrum of collagen-mollusc shell composite. Spectrul EDS al materialului compozit collagen-cochilie de molușcă.

In order to characterize the composition and the thermal stability of the mollusc shell and the obtained collagen/mollusc shell composite material, the both materials were analysed by DTA-TG (Figure 6). The effects recorded in DTA for mollusc shell and collagen/mollusc shell are similar but because of their different composition, the intensities of peaks are different. The endothermic effect associated with free water evaporation can be identified at ~72 °C; the exothermic effects associated with the organic phase decomposition appears in the 250-550 °C region while the endothermic effect corresponding to calcium carbonate decomposition appear at 736 °C.

Based on TG curves recorded for mollusc shell and for collagen/mollusc shell composite, the composition can be estimated. Subsequently, the mollusc shell composition is:

1.83 % organic matter and 98.17% CaCO₃

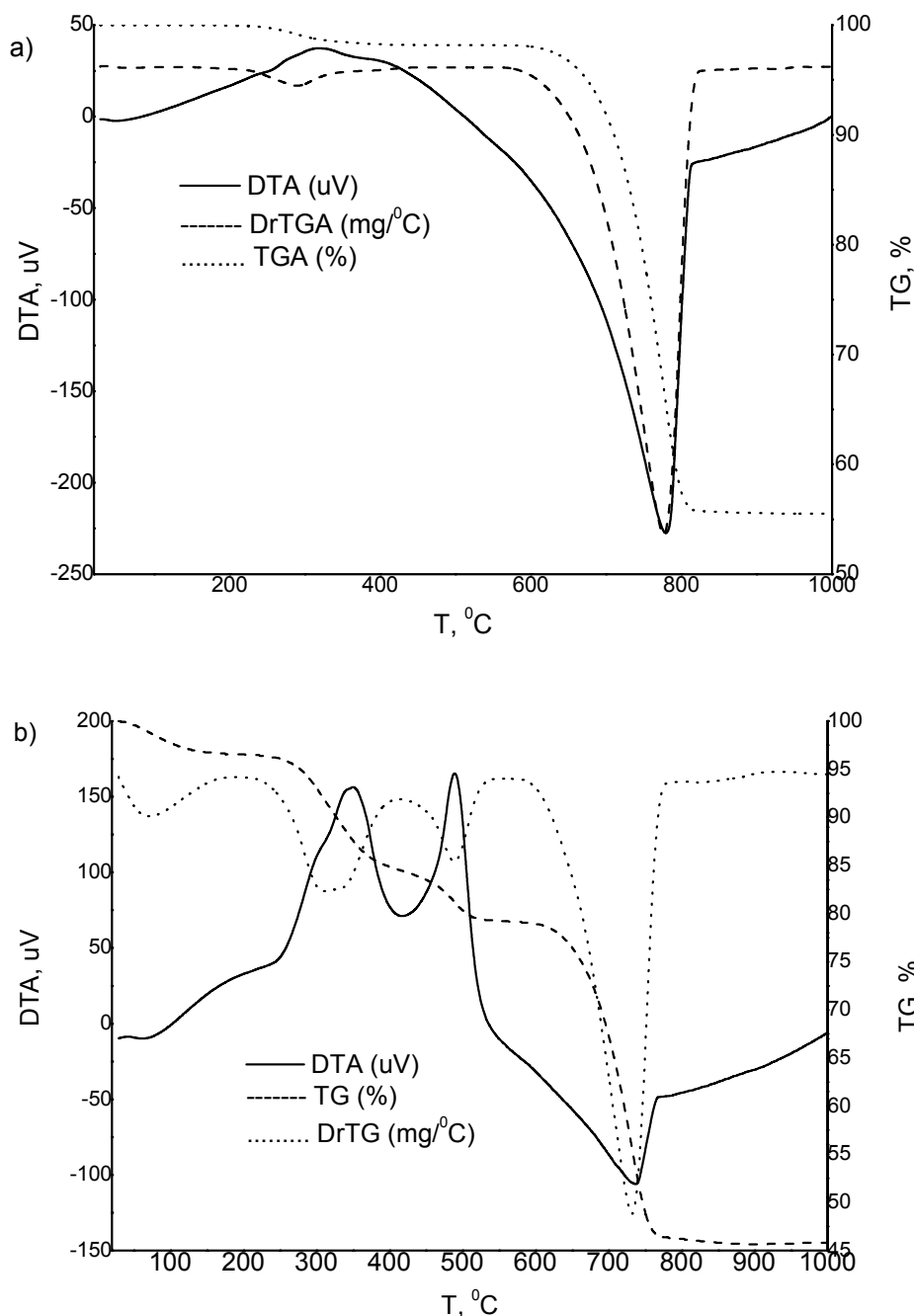


Fig. 6 - DTA – TG analyses of a) mollusc shell and b) collagen/mollusc shell composite material / *Analizele ATD-TG ale a) cochiliei de moluscă și b) ale materialului compozit de tip collagen/cochilie de moluscă.*

while the collagen/mollusc shell composition is 3.43 % water, 17.42 % collagen and 79.15 % CaCO_3 .

Also, is important to note that the CaCO_3 decomposition shifts to lower temperature in collagen/mollusc shell composite probably, due to the carbonate layers destructuration.

4. Conclusions

Based on the presented data some conclusion can be laid.

The mollusc shell was characterized by XRD, FTIR and DTA-TG. XRD and FTIR analyses collagen gel. The obtained composite material

were used in order to make the qualitative attributions: calcium carbonate – mainly composed by calcite and aragonite while the DTA-TG analyses permit quantitative attributions. Based on these analyses, the composition of the mollusc shell is: organic matter ~ 1.83% and calcium carbonate ~98,17%. Based on the intensities of the main XRD peaks it can conclude that mollusc shell contains calcite and aragonite; the calcite: aragonite masic ratio being 9:1.

The collagen/mollusc shell composite material was obtained by mixing finely powdered mollusc shell and the corresponding amount of

contain 3.43 % water, 17.42 % collagen and 79.15 % CaCO₃.

SEM images confirm a good compatibility of collagen and calcium carbonate from mollusc shell; the calcium carbonate particles being enclosed by collagen network.

Mollusc shell is compositional very similar with the coral which is widely used for bone grafting. Based on this, we expect that this collagen/mollusc shell composite material to be potential material for bone grafting.

Acknowledgements

This work was supported by Romanian National Authority for Scientific Research. Authors recognise financial support from the European Social Fund through POSDRU/89/1.5/S/54785 project: "Postdoctoral Program for Advanced Research in the field of nanomaterials"

REFERENCES

1. W.E. Gallie and M.B. Toronto, The history of a bone graft; The Journal of Bone and Joint Surgery 1914, **s2-12**, 201.
2. T.K. Monsees, K. Barth, S. Tippelt, K. Heidel, A. Gorbunov, W. Pompe, et al., Effects of different titanium alloys and nanosize surface patterning on adhesion, differentiation, and orientation of osteoblast-like cells; Cells Tissues Organs 2005, **180**(2), 81.
3. A.A. Minea and I.G. Sandu, Correlation between aluminium alloys plasticity and heat treating technology; Materiale Plastice 2007, **44**(4), 370.
4. Y.L. Cai, C.Y. Liang, S.L. Zhu, Z.D. Cui and X.J. Yang, Formation of bonelike apatite-collagen composite coating on the surface of NiTi shape memory alloy; Scripta Materialia 2006, **54**(1), 89.
5. C.Y. Liang, H.S. Wang, J.J. Yang, Y. Yang and X.J. Yang, Surface modification of cp-Ti using femtosecond laser micromachining and the deposition of Ca/P layer; Materials Letters 2008, **62**(23), 3783.
6. M.A. Lopez-Heredia, J. Sohler, C. Gaillard, S. Quillard, M. Dorget and P. Layrolle, Rapid prototyped porous titanium coated with calcium phosphate as a scaffold for bone tissue engineering; Biomaterials 2008, **29**(17), 2608.
7. G.R. Castro, B. Panilaitis, E. Bora and D.L. Kaplan, Controlled release biopolymers for enhancing the immune response; Molecular Pharmaceutics 2007, **4**(1), 33.
8. O. Suzuki, H. Imaizumi, S. Kamakura and T. Katagiri, Bone regeneration by synthetic octacalcium phosphate and its role in biological mineralization; Current Medicinal Chemistry 2008, **15**(3), 305.
9. H.N. Liu, H. Yazici, C. Ergun, T.J. Webster and H. Bermek, An in vitro evaluation of the Ca/P ratio for the cytocompatibility of nano-to-micron particulate calcium phosphates for bone regeneration; Acta Biomaterialia 2008, **4**(5), 1472.
10. A. Melinescu, M. Preda, L.E. Sima, S.M. Petrescu and I. Teoreanu, In vitro testing of hydroxyapatite bioceramics; Romanian Journal of Materials 2008, **38**(3), 233.
11. A. Ficai, E. Andronescu, V. Trandafir, C. Ghitulica and G. Voicu, Collagen/hydroxyapatite composite obtained by electric field orientation; Materials Letters 2010, **64**(4), 541.
12. H.L. Luo, G.Y. Xiong, Y. Huang, F. He, W. Wang and Y.Z. Wan, Preparation and characterization of a novel COL/BC composite for potential tissue engineering scaffolds; Materials Chemistry and Physics 2008, **110**(2-3), 193.
13. C.H. Yao, J.S. Sun, F.H. Lin, C.J. Liao and C.W. Huang; Biological effects and cytotoxicity of tricalcium phosphate and formaldehyde cross-linked gelatin composite; Materials Chemistry and Physics 1996, **45**(1), 6.
14. C. Târdei, L. Moldovan and O. Crăciunescu, Hybrid bioresorbable composites with high bioactivity properties; - Romanian Journal of Materials 2010, **40**(1), 41.
15. M.L. Popescu, R.M. Piticescu, A. Meghea and V. Trandafir, The influence of the collagen concentration on the grain size of hydroxyapatite collagen nanopowders; UPB Sci Bull, Series B 2008, **70**(4).
16. S.M. Bowman, J. Zeind, L.J. Gibson, W.C. Hayes and T.A. McMahon, The tensile behavior of demineralized bovine cortical bone; Journal of Biomechanics 1996, **29**(11), 1497.
17. V.B. Rosen, L.W. Hobbs and M. Spector, The ultrastructure of anorganic bovine bone and selected synthetic hydroxyapatites used as bone graft substitute materials; Biomaterials 2002, **23**, 921.
18. N. Sasaki, A. Tagami, T. Goto, M. Taniguchi, M.O. Nakata and K. Hikichi, Atomic force microscopic studies on the structure of bovine femoral cortical bone at the collagen fibril-mineral level; Journal of materials science: Materials in medicine 2002, **13**, 333.
19. J.B. Park and J.D. Bronzino, editors. Biomaterials, Principles and Applications. Boca Raton, London, New York, Washington: CRC Press, 2003.
20. C. Hellmich, J.F. Barthelemy and L. Dormieux, Mineral-collagen interactions in elasticity of bone ultrastructure - a continuum micromechanics approach; European Journal of Mechanics A/Solids 2004, **23**(5), 783.
21. S. Bandyopadhyay-Ghosh, Bone as a Collagen-hydroxyapatite Composite and its Repair; Trends Biomater Artif Organs 2008, **22**(2), 112.
22. M.-M. Giraud-Guille, E. Belamie and G. Mosser, Organic and mineral networks in carapaces, bones and biomimetic materials; C R Palevol 2004, **3**, 503.
23. T. Materna, H.J. Rolf, J. Napp, J. Schulz, M. Gelinsky and H. Schliephake, In vitro characterization of three-dimensional scaffolds seeded with human bone marrow stromal cells for tissue engineered growth of bone: mission impossible? A methodological approach; Clinical Oral Implants Research 2008, **19**(4), 379.
24. D.E. Jacob, A.L. Soldati, R. Wirth, J. Huth, U. Wehrmeister and W. Hofmeister, Nanostructure, composition and mechanisms of bivalve shell growth; Geochimica et Cosmochimica Acta 2008, **72**, 5401.
25. D. Medakovic, R. Slapnik, S. Popovic and B. Grzeta, Mineralogy of shells from two freshwater snails *Belgrandiella fontinalis* and *B. kuesteri*; Comparative Biochemistry and Physiology Part A 2003, **134**, 121.
