10.2478/v10063-010-0004-4

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXV, 4 SECTIO AA 2010

Surface modification of bioceramics by PEG grafting♣

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Surface modification of Hydroxyapatite (HAp) and β-Tricalcium phosphate (β-TCP) powders was performed using hexamethylene diisocyanate (HMDI) as a coupling agent. Polyethylene glycol (Mw = 2000) was grafted to the surface of these ceramic materials. Different methods were used to characterize modified surfaces. Fourier transform infrared (FT-IR) and Attenuated Total Reflection Fourier Transform Infrared (FTIR/ATR) technique analyses confirmed the modification reaction on HAp and β-TCP surfaces. BET isotherm analysis showed the changes in textural properties of materials after modification. Elemental analysis was performed to confirm a presence of selected elements from modifier and coupling agent. Examination of dispersion stability of materials in different solvents show better stability for samples of β-TCP than HAp.

1. INTRODUCTION

Ceramic biomaterials are very interesting materials used for bone substitutes in many fields like orthopaedic, dental and plastic surgeries mainly due to their biocompatibility. Hydroksyapatite and tricalcium phosphate are the most popular among, so-called calcium phosphate bioceramics [1-3].

Compounds like hydroxyapatite (HAp) and related tricalcium phosphate (β-TCP) have received considerable attention in the field of biomaterials, chromatography and biomineralization. Interest in hydroxyapatite as a bioma-

♣This article is dedicated to Professor Tadeusz Borowiecki on the occasion of his 65th birthday

terial developed due to its bioactivity, biocompatibility, osteoinductivity, osteoconductivity and its spontaneous interfacial osteointegration when implanted. It has chemical formula $Ca_{10}(PO_4)_6(OH)_2$ and Ca/P ratio of 1.67. Hydroxyapatite in the particulate form can be produces by using a variety of methods. The characteristics of HAp powders have significant effects on the subsequent products with Hap being in the form of dense or porous structure, in coatings or in composite. There is several wet, dry and hydrothermal method of HAp production leading to the manufacturing of dense or porous manufacturing Hydroxyapatite is soluble in acidic solution, insoluble in alkaline solution and slightly soluble in distilled water. The solubility of HAp changes in the presence of amino acids. Dissolution rate depends on the shape, porosity, crystal size and crystallinity of HAp implants. The use of hydroyapaptite implants is attributed mainly to HAp good biocompatibility and bioactivity [1-5]. β-Tricalcium phosphate is one of polymorph of tricalcium phosphate having formula $Ca_3(PO_4)_2$. β-TCP has similar properties to HAp but its main attribute is resorbability connected with biocompatibility, high solubility and bioactivity. Therefore it is extensively used in orthopedic and maxillo-facial surgery [2,3,6-8]. It has been accepted and used as a material for bone repair in the form of ceramic blocks, granules and cements [2,9,10]. Unfortunately, weak mechanical properties and the lack of resorbability in case of HAp limit their use in modern medicine as individual biomaterials [2,3,6,11,12]. These disadvantages bring about the necessity of surface modification of those biomaterials.

The character of biomaterial surface is essential for the interactions between materials and livings systems and for fabrication of biomaterials and biomedical devices. Polyethylene glycol (PEG) surface modification was used for a wide range of biomedical microdevices for the improvement of material biocompatibility and device efficacy.

There are several methods of surface modification. Generally they are divided into biological, chemical and physicochemical methods. All of possibly methods of surface modification are presented in the Figure 1 [13]. I have used the method named grafting on the surface in presented work.

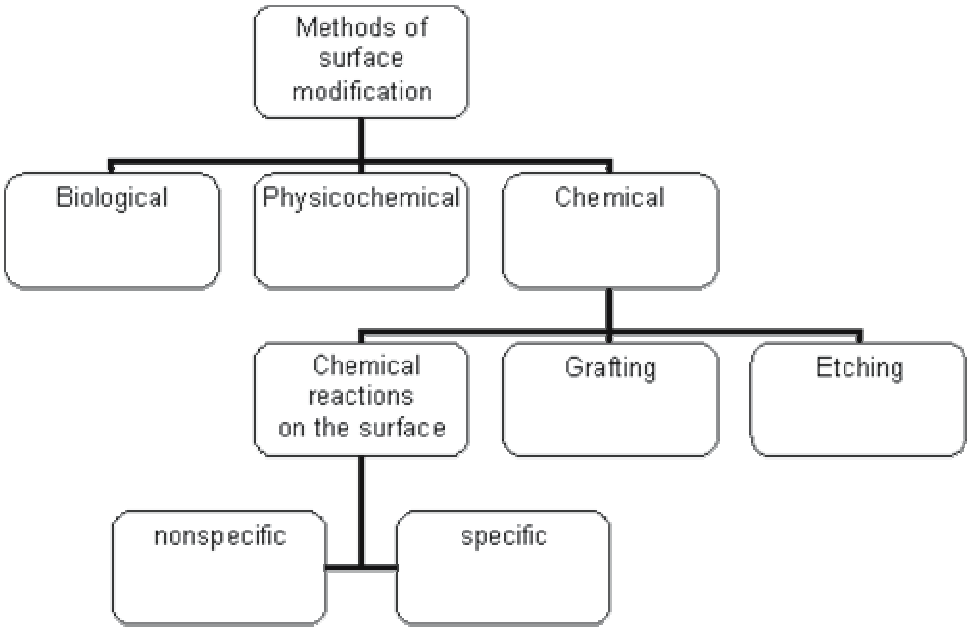

Fig. 1. Methods of surface modification of biomaterials.

The main aim of this study was to modify HAp and β-TCP by use of polyoxyethylene glycol as a modifier and hexamethylene diisocyanate as a coupling agent. PEG is a biodegradable material with high biocompatibility. It possess a variety of properties pertinent to biomedical and biotechnical applications [14,15]. PEG is biocompatible with biological material, weakly immunogenic and therefore approved by FDA (Food and Drug Administration) for internal consumption. Therefore, we have selected PEG modification [16, 17] as a tool to improve biological properties of HAp and β-TCP samples.

The following methods were used to assess the modification: elemental analysis, Fourier Transform Infrared spectroscopy (FTIR), Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR), textural properties analysis, Zeta potential measurements and analysis of dispersion behavior of biomaterials in different solvents.

2. MATERIALS AND METHODS

Hydroxyapatite (HAp) powder with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ and polyethylene glycol (PEG, Mw=2000) were purchased from Fluka. β-Tri-calcium phosphate, hexamethylene diisocyanate (HMDI) and N,N-dimethy-lformamide (anhydrous, DMF) were obtained from Sigma-Aldrich.

2.1. Surface grafting reaction

Modification process consisted of 3 steps. At the first, after drying at 120^{o}C for 48 h, 4g of biomaterial powders were charge to round-bottom three-necked flask together with 75 ml dry DMF, 3 ml HMDI and 0.06 ml dibutytindilaurate as a catalyst. The suspension was stirred with a mechanical stirrer and bubbled with nitrogen. The temperature was increased to 60^{o}C and kept for 4h under the protection of nitrogen. In the second step, 20g PEG (Mw=2000) were added to the suspension together with 20 ml DMF and stirred for 8 h. At the end, the powders were separated by vacuum filtration and further washed by DMF and ethanol 3 times. After that, the powders were dried at 50^{o}C for 24 h.

2.2. Characterization of surface-grafted biomaterials

Fourier Transform Infrared (FT-IR) spectroscopy was used to determine the functional groups grafted to biomaterial. The spectra were recorded on a Vertex 70 (Brucker) in 400–4000 cm^{-1} region by using KBr tablets.

The Fourier Transform Infrared Attenuated Total Reflection (FTIR/ATR) technique possesses an ability to depict processes occurred on the surface of biomaterial. These spectra were recorded on Equinox 55 (Brucker) by using a Zn/Se crystal.

The specific surface area, pore volume and pore size were determinated by Brunauer-Emmett-Teller (BET) nitrogen gas adsorption using ASAP 2020 (Micrometric Instruments Co.) surface area and pore size analyzer.

The zeta potential (ζ) was measured using a Zetasizer Nano ZS (Malvern Instruments) analyzer.

Elemental Analysis was performed on a EuroScience Elementar Analyzer (Euro EA).

Modified biomaterials were also evaluated by determination of the dispersion stability. The dispersion process consisted of three steps:

I. Preparation of samples (weighting and extensive stirring the biomaterial sample with a solvent)
II. Dosage of dispersion samples (simultaneous dosing using the syringe piston to a series cuvettes)
III. Measurement of dispersion stability (scanning and graph drawing) by using Sediment Analyzer Program [18,19].

3. RESULTS AND DISCUSSION

3.1. Study about the reactivity of hydroxyl group of ceramic biomaterials

During the modification process following reactions occurred: at first, the hydroxyl group of HAp reacts with coupling agent (isocyanate). The grafting of PEG chains to the semi-product product was achieved in the second step of the

modification procedure. The structure of the product of this process in given in Figure 2.

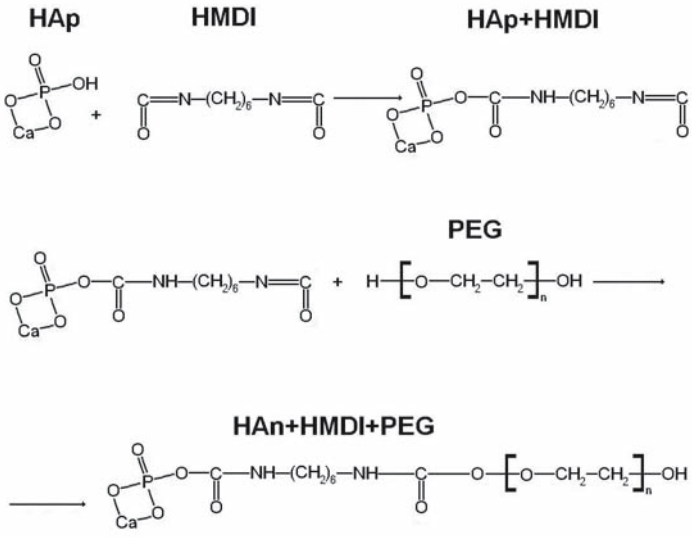

Fig. 2. Proposed reaction scheme of modification of HAp by the use of PEG.

The reaction process is more complicated in the case of TCP, because, this material does not contain hydroxyl groups. Therefore, the coupling agent is connected to TCP by phosphoric group. The second step of the reaction is similar as in the case of HAp (Figure 3).

At the level of 3300 cm^{-1} the signal from the stretching vibrations of amino groups (N-H) was found. Furthermore, near to 2800 and 1500 cm^{-1} the signals for stretching and bending, respectively, vibrations characteristic to methylene groups (CH_2) were found. Moreover, the bands for the carbonyl (C=O) and amide groups (C(O)-NH) were found at the level of 1600cm^{-1}, and the C-O-C ether band is visible at approximately 1200 cm^{-1}. All of mentioned groups were also identified by means of ATR technique (Figure 5).

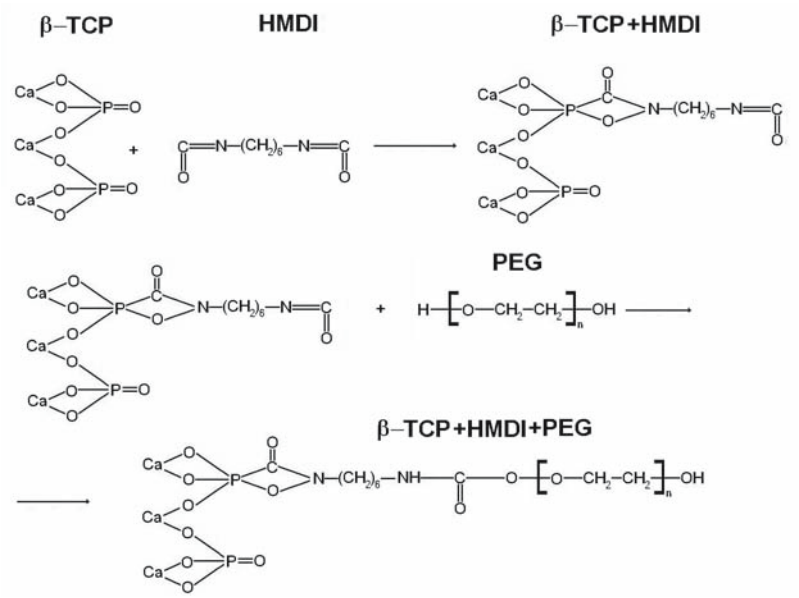

Fig. 3. Proposed reaction scheme of modification process of β-TCP by the use of PEG.

3.2. FT-IR and FTIR/ATR spectra study of PEG grafting

FR-IR spectra of unmodified and PEG-grafted HAp are presented in Figure 4.

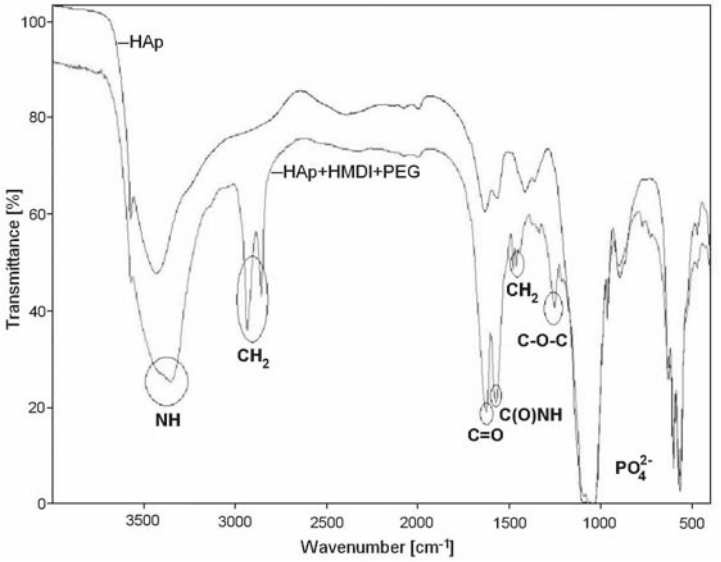

Fig. 4. FT-IR spectra of unmodified and PEG-grafted hydroxyapatite.

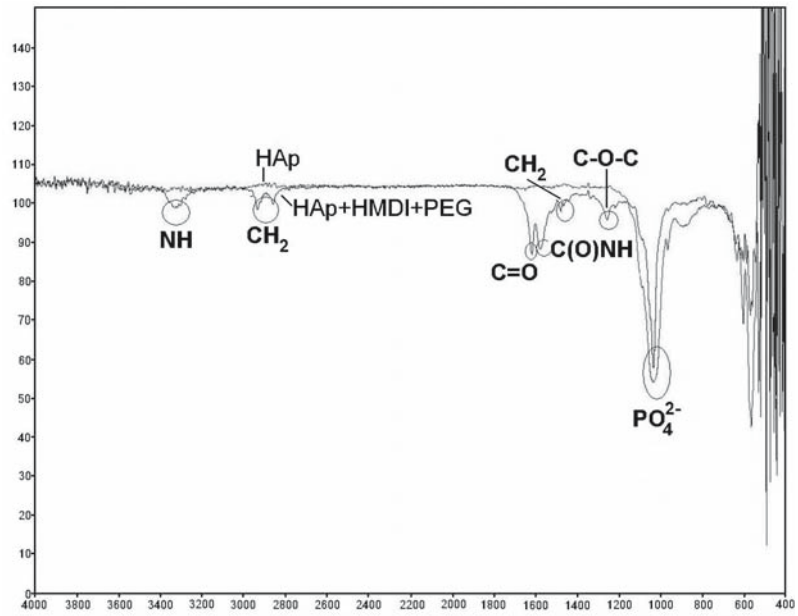

Fig. 5. FTIR/ATR spectra of HAp before and after PEG-grafting.

In the case of β-TCP, results similar to HAp were achieved. The same groups as in the case of HAp are present on the FTIR spectrum of modified β-TCP (Figure 6).

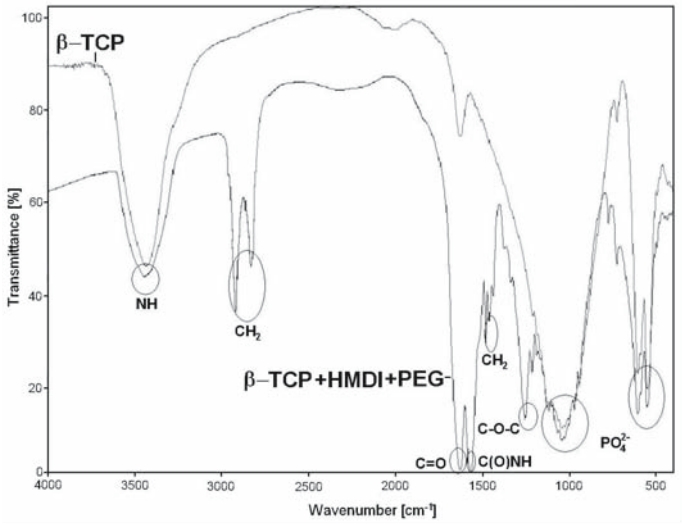

Fig. 6. FT-IR spectra of unmodified and PEG-grafted β-tricalcium phosphate.

There is lack of phosphoric group band in ATR spectrum of β-TCP (Figure 7). This could be explained by the fact, that phosphoric group reacts with HMDI coupling agent.

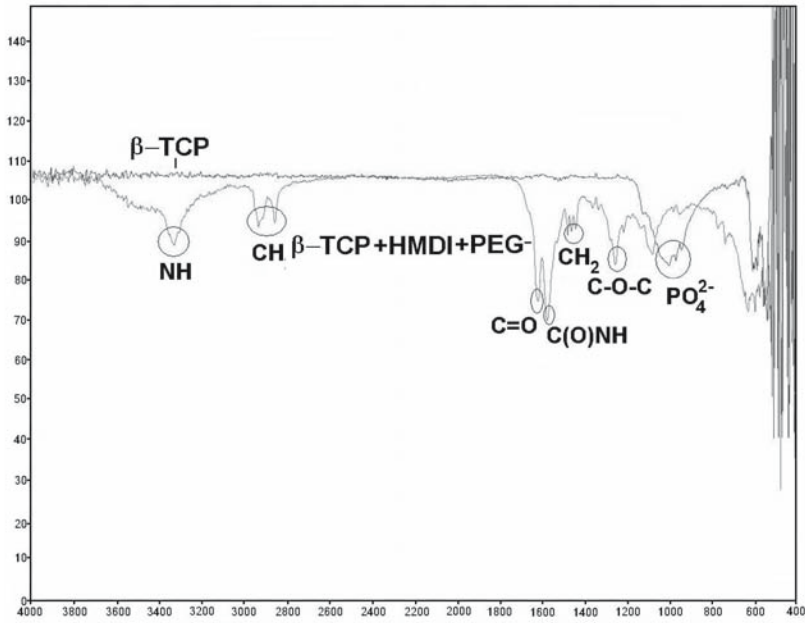

Fig. 7. FTIR/ATR spectra of β-TCP before and after PEG grafting on the surface.

All functional groups with responding wavenumber values are presented in the Table 1.

Tab. 1. All of functional groups grafted on the surface of HAp and β-TCP.

Functional group		Wavenumber [cm^{-1}]
name	formula	
ether	C-O-C	1255
amide	C(O)NH	1575
carbonyl	C=O	1625
methylene	CH_2	2857
		2934
amino	NH	3337

3.3. Textural properties

Surface area, pore volume and pore size of HAp and β-TCP before and after modification were determined by means of BET isotherms. The comparison of results showed that modification process did not change significantly the hydroksyapatite surface layer. However, there were some changes in pore size and volume. On the other hand, in case of β-TCP there were significant increase in surface area and pore volume with concomitant decrease in pore size.

Tab. 2. Textural properties of HAp and β-TCP before and after surface modification.

	BET Surface area [m^2/g]	Pore volume [cm^3/g]	Pore size [nm]
	Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$		
unmodified	30.12	0.22	27.99
PEG-modified	23.69	0.12	22.20
	β-Tricalcium phosphate $Ca_3(PO_4)_2$		
unmodified	1.17	0.005	43.47
PEG-modified	9.34	0.05	27.45

3.4. Analysis of zeta potential

The analysis of zeta potential showed that modification improved stability of HAp. However, it is still not satisfactory. Moreover, chemical stability of β-TCP before and after modification was appropriate. Generally, we can say that materials possess the chemical stability under value of -30mV but in our case the most important region is between 7 and 8 pH because we have to had a neutral pH like in human body (Figure 8).

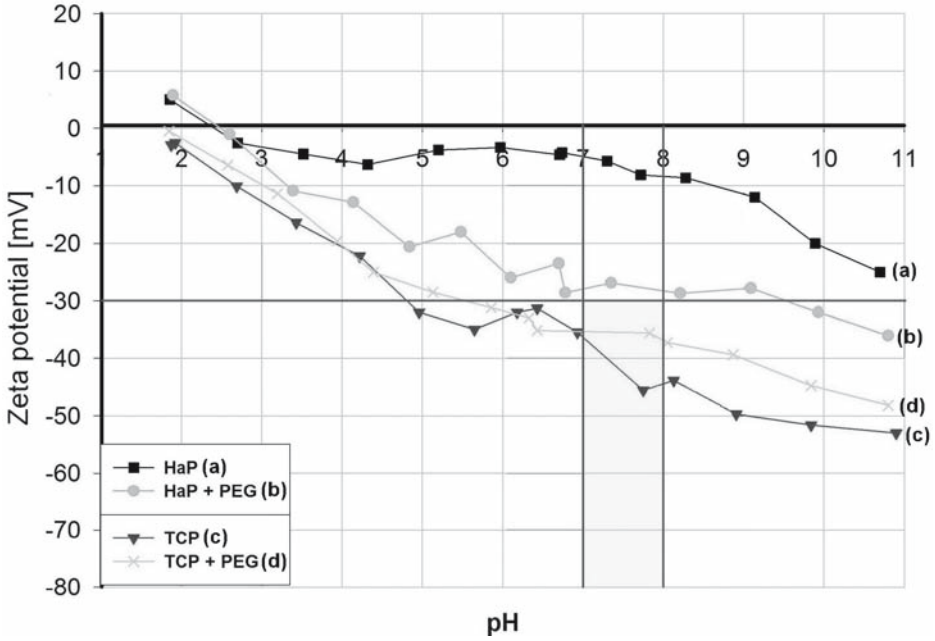

Fig. 8. Zeta potential of biomaterials.

3.5. Elemental analysis

The elemental analysis is the basic method used to evaluate the content of nitrogen. carbon and hydrogen within the substance. By means this method, we have found, that proportion of these elements is strictly related to the amount of modifier and coupling agent and it is showed in the Table 3.

Tab. 3 Results of elemental analysis of PEG-modified materials.

	%N	%C	%H
HAp+HMDI+PEG	2.95	12.64	2.63
TCP+HMDI+PEG	7.79	23.50	4.26

3.6. Dispersion of biomaterials

The dispersion of HAp and β-TCP modified by PEG were evaluated by the use of different media. Least stable dispersion was obtain in water while the most stable one were observed in SBF as a medium (Figure 9). The dispersion graphs of biomaterials in 15% SBF before and after modification are presented on the Figure 10. As it is shown the modification changed dispersion properties of biomaterials and dispersion of β-TCP (even before modification) is more table that in the case of HAp.

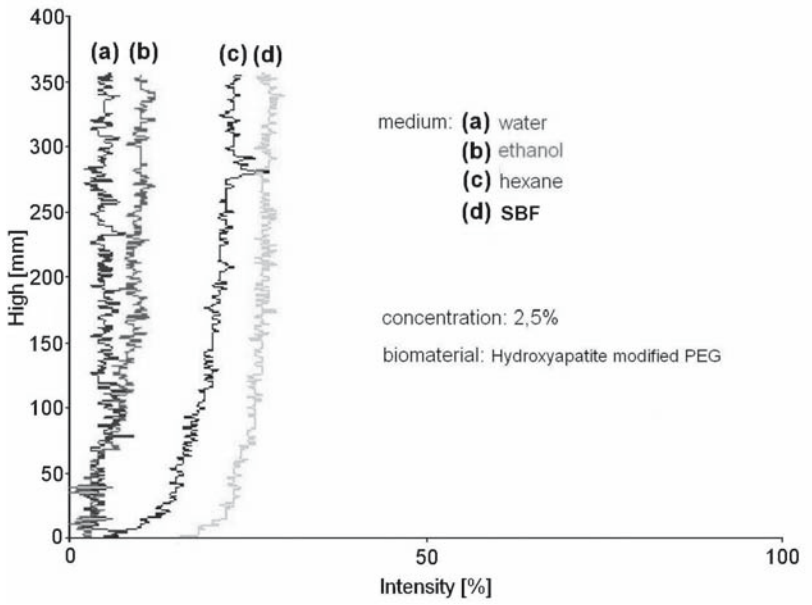

Fig. 9. Graph of dispersion of unmodified and PEG-grafted HAp and β-TCP in water ethanol hexane and simulated body fluid (SBF). The more intense signal (right shifted) indicates the more stable dispersion.

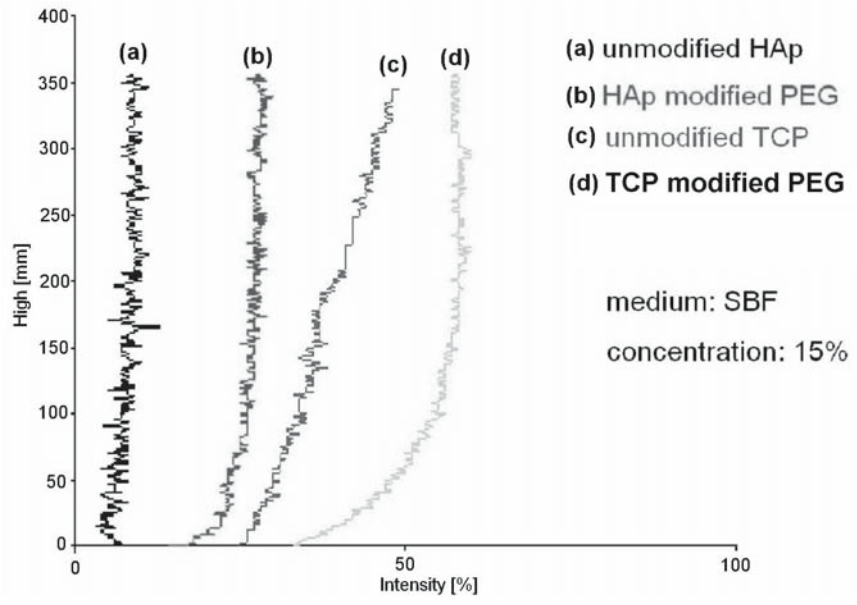

Fig. 10. Graph of dispersion of materials in 15% SBF (simulated body fluid).

4. CONCLUSIONS

Surface modification of HAp and β-TCP was successfully performed by grafting of biodegradable polymer (polyethylene glycol) molecules with using of HMDI as a coupling agent. New functional groups derived from PEG and HMDI were found in FTIR spectra. All of mentioned groups were also identified by means FTIR/ATR technique. Elemental analysis confirmed that content of elements like carbon, nitrogen and hydrogen is strictly related to the amount of modifier and coupling agent. BET isotherm revealed changes in textural properties after modification. Analysis of zeta potential showed that β-TCP samples, both before and after modification were chemically stable and that modification improved chemical stability of hydroxyapatite. Differences in dispersion of modified and unmodified materials were observed. The most stable dispersion were obtained in SBF medium. β-TCP particles were better dispersed in the examined media than HAp ones.

Acknowledgement. This paper was supported by DS. 32/045/10 PUT grant was is gratefully acknowledged.

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CURRICULA VITAE

Professor Adam Voelkel was born in 1952 in Leszno. He studied chemistry at the Poznań University of Technology, graduated in 1976. He received his Ph.D. in 1981, D.Sc. in 1990 (at UMCS Lublin) and professorship in 1997. He was vice-dean of the Faculty of Chemical Technology in 1993–1999 and dean in 1999–2005. Member of Committee of Chemistry of Polish Academy of Sciences. Author of 190 published papers, including the chapters in the books, reviews. His research interest is focused on application of chromatographic techniques in the determination of surface and bulk properties of the variety of materials. The inverse gas chromatography was used in the procedure enabling the estimation of solubility parameter and its increments corresponding to different types of intermolecular interactions for chemical compounds of different nature, consistence and activity including surfactants, extractants, nanomaterials, natural products. Physicochemical characteristics of the examined materials were further used to predict their activity in technological systems. Recently, his investigations are focused on the physicochemical characterization of nanomaterials and prediction of their behaviour in dispersed systems. Chromatographic techniques like GC, GC-MS, HPLC, HPLC-MS with different sample preparation systems, e.g. HS-SPME, along with methods of surface analysis were applied for the examinations of the stability of dental materials. GC, GC-MS, HPLC, HPLC-MS were used for examination of the degradation processes of phenolic compounds. His responsibility includes lectures on the physicochemistry and technology of surface active agents, chromatographic techniques, application of chromatographic methods, leading the research group – division of organic chemistry, supervising the research group of several students, Ph.D.students. He was advisor of 11 completed doctoral thesis.

Katarzyna Adamska was graduated at Wroclaw University of Technology at Faculty of Chemistry in 1999. In 2002 she started Ph.D. studies at Poznan Univeristy of Technology at Institute of Chemical Technology and Engineering. In 2007 she recived her Ph.D. Her research interest is focused on application of inverse gas chromatography for characterization different materials. The work she has been doing up to present is related to estimation of bulk and surface properties e.g. raw materials, products, excipients used in pharmacy. She is an author of 6 papers, co-author of plenary lectures and posters.

Magdalena Woźniak was born in 1985 in Poznan. She was graduated at Poznan University of Technology at Faculty of Chemical Technology in 2009. Her Master's thesis was entitled "The characteristic of surface layer of biomaterials". After graduation, she started Ph.D. studies at Poznan University of Technology at Institute of Chemical Technology and Engineering. Her field of research is related to biomaterials, especially their modification and characteristic of their surface layer. She actively takes part in different conferences and congresses in the field of chemical technology.