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A. SOBCZAK-KUPIEC[*], B. TYLISZCZAK[**], K. KRUPA-ŻUCZEK[*], D. MALINA[*], M. PIĄTKOWSKI[**], Z. WZOREK[*]

GOLD NANOPARTICLES AS A MODIFYING AGENT OF CERAMIC-POLYMER COMPOSITES

NANOCZĄSTECZKOWE ZŁOTO JAKO MODYFIKATOR KOMPOZYTÓW POLIMEROWO-CERAMICZNYCH

Much effort has been invested in the development of biomaterials for the repair or replacement of hard tissue. The synthesis of composites based on mineral and organic constituents is nowadays extremely important for the development of materials for biomedical applications. In this paper we report the preparation and characterization of ceramic-polymer composites doped with gold nanoparticles. Properties and applications in medicine and dentistry of colloidal gold nanoparticles depends upon their size and shape. The influence of the presence of the metallic nanoparticles on the degradation process was investigated by pH and conductivity analyses of water filtrates. The nanocomposites were characterized with the use of X-ray Diffaction (XRD) and Fourier Transformed Infrared Spectroscopy (FT-IR) methods.

The results of *in vitro* tests confirmed that it is possible to produce hydroxyapatite/polymer (HA/polymer) composites doped with gold nanoparticles (AuNPs) for medical applications. Tests proved that content of gold nanoparticles in composites had influence on degradation behaviour of HA/Polymer/AuNPs in water environment.

Keywords: gold nanoparticles, ceramic-polymer composites, biomaterials

Obecnie wiele starań jest wkładanych w badania dotyczące rozwoju biomateriałów do naprawy i wymiany tkanki kostnej. Otrzymywanie kompozytów opartych na składnikach mineralnych i organicznych jest niezwykle istotne dla rozwoju materiałów do zastosowań biomedycznych. Właściwości i zastosowanie nanocząstek złota w medycynie i stomatologii zależy przede wszystkim od ich wielkości i kształtu. W pracy opisano metodykę otrzymywania oraz charakterystykę kompozytów ceramiczno-polimerowych domieszkowanych nanocząstkami złota. Badano wpływ obecności nanocząstek metalicznych na proces degradacji w środowisku wodnym z wykorzystaniem analiz pH i przewodnictwa. Charakterystykę nanokompozytów wykonano z zastosowaniem dyfrakcji promieni rentgenowskich (XRD) i spektroskopii w podczerwieni z transformacją Fouriera (FT-IR).

Na podstawie badań *in vitro* stwierdzono, że możliwe jest otrzymanie kompozytów polimerowo-ceramicznych modyfikowanych nanocząstkami złota do zastosowań biomedycznych. Badania wykazały, że zawartość nanocząstek złota wpływa na degradację kompozytów HA/Polymer/AuNPs w środowisku wodnym.

1. Introduction

The wide range of possibilities as well as the increasing importance of nanomaterials in medicine has become apparent in today's world. Nanotechnology enables the use of technology that deals with nano-meter sized objects. It is expected that nanotechnology will be developed at several levels: materials, devices and systems. The nanomaterials' level is the most advanced nowadays, both in scientific knowledge and in commercial applications [1-3]. Due to their unique properties, metallic nanoparticles have gained special interest in the last years. One of the key point to use these nano-objects in chemistry, biology or physics is to control their incorporation in the host matrix and their impact on the material developement and final properties [4-5].

Extensive research has been undertaken to develop polymer/hydroxyapatite biomimetic composites as new bone substitute materials due to the presence of hydroxyapatite (HA) which is a bone inorganic component [6-8]. Calcium phosphates occupy an exceptional place among ceramic biomaterials. The calcium phosphates applied as biomaterials could be both synthetic and natural origin. The first group comprises implants made of synthetic hydroxyapatite (HAp), tricalcium phosphate (TCP) and bi-phase implant materials (BCP) containing hydroxyapatite, calcium orthophosphate and α or β TCP [6].

Hydroxyapatite/polymer composites were used in various biomedical fields such as dental material, bone substitute and hard tissue [9]. Hydroxyapatite/polymer composite becomes an important representative of bone substitute materials since they combine the osteoconductivity and bone bonding ability of HA with the resorbability and simplicity of the polymer matrix processing [10].

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[*] INSTITUTE OF INORGANIC CHEMISTRY AND TECHNOLOGY, CRACOW UNIVERSITY OF TECHNOLOGY, 24 WARSZAWSKA ST., 31-155 CRACOW, POLAND

[**] DEPARTMENT OF CHEMISTRY AND TECHNOLOGY OF POLYMERS, CRACOW UNIVERSITY OF TECHNOLOGY, 24 WARSZAWSKA ST., 31-155 CRACOW, POLAND

Silver, gold and other noble metals are important because of their antibacterial effects [11-12]. Gold has been used to treat juvenile arthritis and palindromic rheumatism, and was once used to treat tuberculosis [13].

Gold particles have been used for many decades as therapeutic agents for the treatment of human diseases such as rheumatoid arthritis [14-15]. AuNPs are particularly attractive for use in biological applications for several reasons. First, gold is a noble metal with inert chemical properties, resistant to corrosion and has low toxicity based on past clinical experience. Gold nanoparticles are also relatively easy to synthesize [16]. Second, the gold surface can be easily functionalized with biological molecules, such as antibodies and nucleic acids. There are two types of nanosized gold particles for biomedical use: gold colloids and gold nanoshells. Gold nanoshells are a unique class of nanoparticles because they have tunable plasmon resonance and, similar to quantum dots (QDs), can be used as in vivo imaging agents [17]. From described regards, in this work, we undertake the study concerning synthesis and characterization of HA/polymer composites doped with gold nanoparticles.

2. Experimental part

2.1. Experimental Part

FT-IR analyses were carried out with the use of Scimitar Series FTS 2000 spectrophotometer produced by the Digilab company within the basic infrared range of 400-4000 cm^{-1}. The sample of 0.0007 g was pressed with 0.2000±0.0011 g KBr into a pellet. The Deuterated Tri-Glycine Sulfate (DTGS) detector was used for mid-IR range measurements. There were 16 scans, and the resolution was 4 cm^{-1}.

The phase composition of the samples was analysed with the use of X-ray diffraction with Philips X'Pert diffractometer equipped with a graphite monochromator PW 1752/00, Cu Kα 1.54 nm, Ni filter (40 kV, 30 mA).

The multifunction device CX-742 produced by the Elmetron company was applied in the pH and conductivity analyses.

The weight and number average molecular weight of solution ingredients after degradation was measured by gel permeation chromatography GPC method at 35°C, relative to poly(ethylene glycol)/poly(ethylene oxide) standards on POLYSEP-GFC-P4000 column (Phenomenex) and a refractive index detector. The water with a flow rate of 0.5 ml/min was used as the eluent.

2.2. Materials

All chemicals were of reagent grade and used without further purification. Hydrogen tetrachloroaurate (III) trihydrate (0.21% solution), trisodium citrate, acrylic acid, ammonium persulfate and potassium hydroxide were purchased from POCh (Poland). Diacrylate ethylene glycol M = 525 Da as crosslinking agent was purchased from Sigma Aldrich, however the solution of sodium acrylic copolymer Dispex N40 came from Ciba Specialty. All the chemicals were used without further purification. Doubly distilled water was used for the preparation of all samples.

2.3. Gold nanoparticles AuNPs

The gold nanoparticles were prepared by sodium citrate reduction method [18] with modifications. The synthetic method developed for this experiment consistently produces a stable gold nanoparticles, provided that conditions are properly controlled [19].

2.4. Hydroxyapatite HA

Hydroxyapatite (HA) applied in our research was animal origin and was received in the calcining process of bone sludge in two stages. In the first stage the material was calcined at a temperature of 600°C in a chamber kiln with electric heating in air atmosphere within 180 minutes. In the next stage the unified material from the first stage was calcined at 950°C while kept at maximum temperature within 150 minutes in the chamber kiln [20].

2.5. Preparation of HA/PAA/AuNPs nanocomposites

The monomer used in polymerization process was acrylic acid (Mw = 72,06 g/mol). Preparation process of hydroxyapatite(HA)/ poly(acrylic) acid (PAA) composites modified with gold nanoparticles consist of following stages: neutralization of acrylic acid by 40% potassium hydroxide solution, preparation of HAp dispersion in Dispex N40 (dispersing agent), polymerization and finally crosslinking of the product. In connection with neutralization, which was exothermic reaction, the rapid increase of temperature was proceed. Then the mixture was cooled and before prepared HAp dispersion was added. Next, the proper amount (1-5%) of gold nanoparticles suspension (AuNPs) with concentration of 50 ppm, initiator and crosslinking agent were added. After homogenization the mixture was microwave irradiated for 3 minutes to enhance polymerization and crosslinking reactions.

3. Results and disscuusion

3.1. FT-IR analysis

Spectral analyses were made for all HA/PAA composites doped with gold nanoparticles. Figure 1 demonstrates the FT-IR spectra of obtained materials. On the basis of these investigations it was found that vibration characteristic for functional groups present in polymer matrix, were observed. The board absorption band at the range of 3500-3000 cm^{-1} corresponded to stretch vibrations of hydroxide group, the band broadening was a result of water molecules absorbed into the polymer matrix. In the range of 2970-2930 cm^{-1} the observed valence vibrations were from the CH_2 group, however bands in the range of 1150-350 cm^{-1} came from torsional vibrations of this functional group. The absorption band at 1640 cm^{-1} was corresponded to tensile vibrations of C=O group. The band in the range of 1580-1475 cm^{-1} was a result of deforming vibration of –NH and C-N groups. However bands from CH_2-CO group were visible in the range of 1435-1390 cm^{-1}. The bands in the range of 1100-1200 cm^{-1} were corresponded to valence vibrations of -C-N- group. No absorption bands from hydroxyapatite were observed. The lack of the most intensive band at 1200-1000 cm^{-1} was corresponded to asymmetric stretch vibrations of P-O bond, as well as absorption at 964 cm^{-1} was from symmetric vibrations of P-O bond. Vibrations of O-P-O bonds assigned to the absorption bands within wavenumbers range of 604-563 cm^{-1} were not noticed. In the spectra of obtained composites the low intensity bands at 1457 and 881cm^{-1} corresponded to stretching vibrations of CO_3^{2-} were not observed.

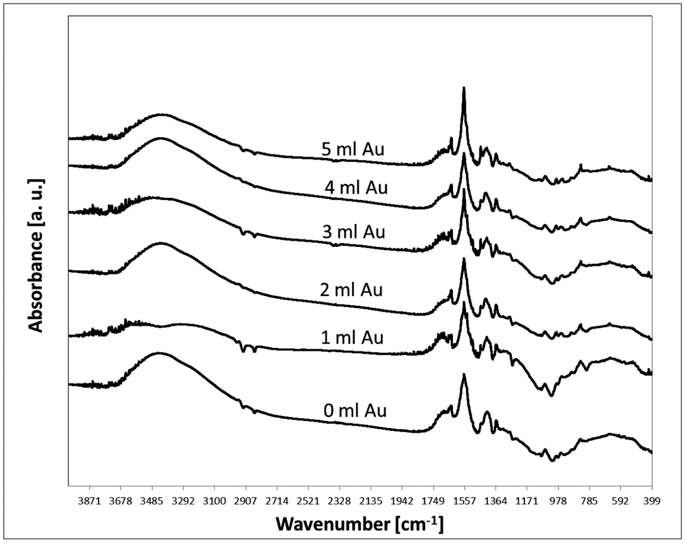

Fig. 1. FT-IR spectra of composites modified with AuNPs

3.2. XRD analysis

The XRD analyses of ceramic-polymer composites modified with gold nanopatricles were carried out. Figure 2 demonstrates the typical XRD pattern. The amorphous polymer matrix on the XRD pattern was observed as "background noises" and it covered peaks came from hydroxyapatite HA. The content of ceramic particles in composite materials amounted to 1% and it could be near the limit of phase determination by roentgenographic method.

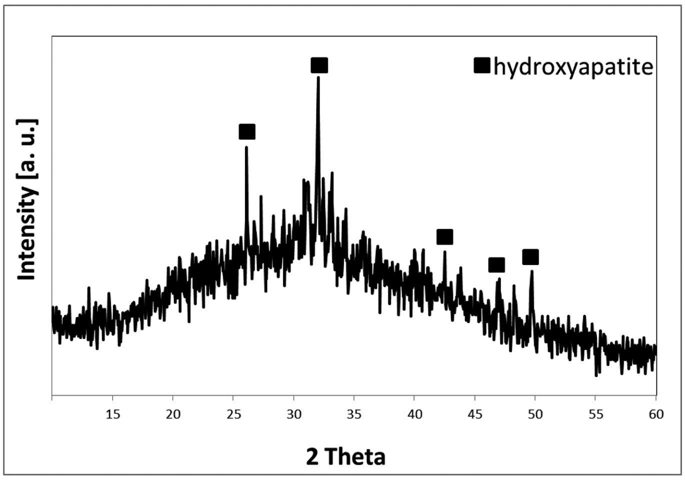

Fig. 2. Typical XRD pattern of ceramic-polymer composite with AuNPs (2% Au)

3.3. Degradation of composites in water environment

Composites with different amount of gold nanoparticles and weight amounting to 1.0 g were prepared with the use of microwave irradiation. Redistilled water was applied in order to evaluate the degradation process of composite samples. Chemical stability was evaluated on the basis of pH value and electrical conductivity of distilled water changes. Electrical conductivity and pH were measured on Elmetron CX device equipped with silver-silver chloride electrode and conductometer. The time of soaking was 14 days at 37°C.

Figure 3 demonstrates changes of pH of distilled water as a function of immersion time.

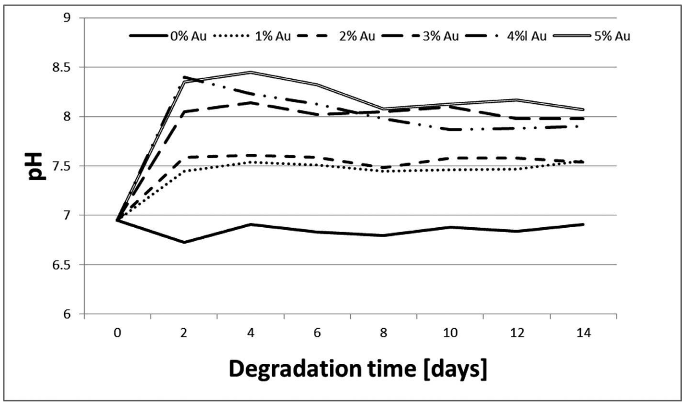

Fig. 3. The changes of pH value of distilled water

Figure 3 demonstrates changes of pH value of redistilled water as a function of degradation time of ceramic-polymer composited modified with gold nanoparticles. For composites without gold nanoparticles insignificant decrease of pH value of water filtrates during ten days of degradation was observed. However for all composites doped with gold nanoparticles the increase pH value was noticed.

The initial pH value of redistilled water at temperature 25°C was amounted to 6.95. In the case of HA/polymer/AuNPs composites the rapid growth of pH value in the first period of degradation was observed. The presence of gold nanoparticles could be influenced on releasing of calcium ions from composite material and it could cause the increase in pH value of water filtrates. The distinct effect of metallic gold nanoparticles content on the pH value of water filtrates was observed.

Figure 4 demonstrates changes of electric conductivity of distilled water as a function of immersion time of composite materials. The conductivity measurement confirmed that all composite samples were gradual degraded. The degradation rate was dependent on gold nanoparticles content. It was found that degradability of composite materials was diversified. The electric conductivity of fresh redistilled water applied in our research amounted to ~ 2 μS/ cm at 25°C. During the entire soaking period, for all composite samples electrical conductivity was increased because of gradual ions releasing in water environment. Increase in electrical conductivity was characterised by linearity and it was a result of progressive degradation process.

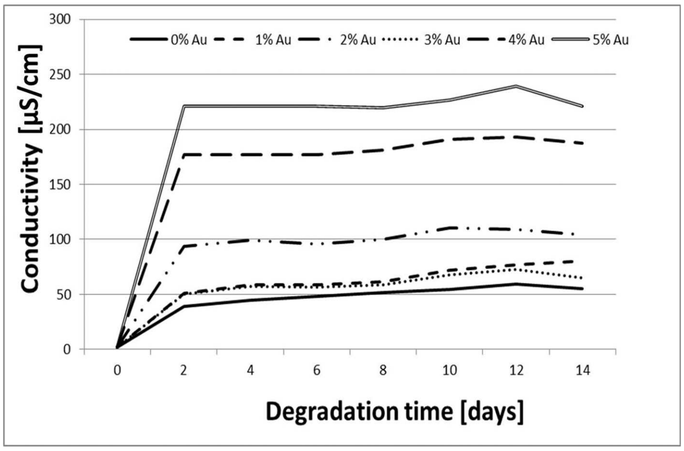

Fig. 4. Changes of electric conductivity of distilled water vs. degradation time

The largest propensity to degradation exhibited samples containing 4 and 5% of gold nanoparticles (AuNPs). Samples of composites doped with 0-3% of AgNPs were distinguished by the high chemical stability and at the end on soaking period electrical conductivity amounted below 100 μS/cm.

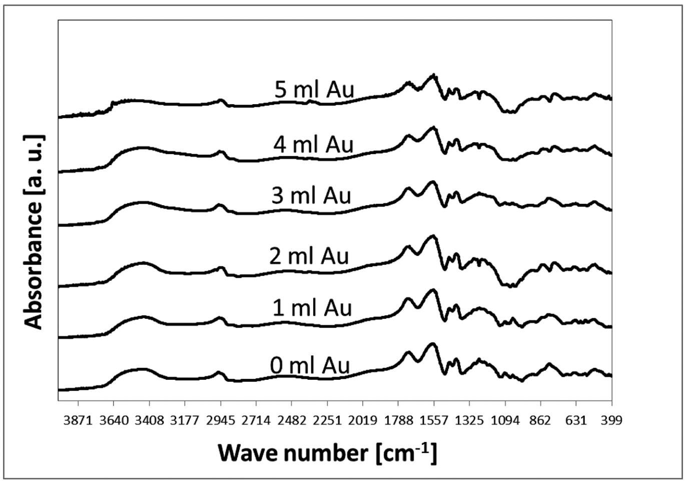

Fig. 5. FT-IR spectra of HA/polymer/AuNPs composites after degradation in water environment

The FT-IR analyses were realized for ceramic-polymer composites doped with AuNPs after immersion in redistilled water at 37°C (figure 5). The results indicated that 14-days degradation period caused insignificant decomposition of polymer matrix. The FT-IR spectra after degradation in water environment for all composite samples were of similar shape. Infrared investigations confirmed that content of gold nanoparticles had no considerable effect on degradation of polymer matrix.

The weight average molecular weight (Mw), number average molecular weight (Mn) and polidispersity index (PDI) of solution ingredients after degradation were measured by GPC method. In samples with water used as the degradation medium two polymer fractions were observed: high-molecular weight fraction with Mw 2 500 000 Da (HMWF) and low-molecular weight fraction 250 000 Da (LMWF), respectively. However their concentration was relatively low and no significant differences correlated with gold concentrations were observed.

TABLE 1
Results of gel permeation chromatography analyses (GPC) of water filtrates

Sample	Mw		Mn		PDI	
	HMWF	LMWF	HMWF	LMWF	HMWF	LMWF
0% Au	2 620 800	675 600	1 906 600	517 500	1.37	1.31
1% Au	2 268 000	118 800	1 659 100	97 200	1.37	1.22
2% Au	2 268 000	314 700	1 659 100	248 100	1.37	1.27
3% Au	3 110 900	72 000	2 248 500	60 000	1.38	1.20
4% Au	1 987 700	254 900	1 461 300	202 600	1.36	1.26
5% Au	2 487 600	158 700	1 813 400	128 400	1.37	1.24

4. Conclusions

In order to clinical risk assessment of implant application, analysis of its decomposition is requisite. Investigations concerning degradation process are necessary in the case when implant material is resorbable and this material remains in organism longer than 30 days. Degradation process of composite materials is connected with many factors including mass changes, releasing compounds and ions, cracking and components stratification. The results of *in vitro* tests confirmed that it is possible to produce hydroxyapatite/polymer composites doped with gold nanoparticles for medical applications. Tests proved that content of gold nanoparticles in composites had influence on degradation behaviour of HA/PAA/AuNPs. Analyses of electric conductivity of distilled water showed in all cases the increase in conductivity. Measurements confirmed that hydroxyapatite dense materials characterises different susceptibility to degradation. Research results indicated that the degree of degradation of ceramic-polymer composites with AuNPs content was closely connected with content of modifying agent. The increase of gold nanoparticles content caused decrease of chemical stability of composites, what was confirmed by pH and electric conductivity measurements.

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