ISSN 1027-5495. Functional Materials, 29, No.2 (2022), p. 299-304.
doi:https://doi.org/10.15407/fm29.02.299

Biogenic hydroxyapatite-based composites modified by magnetite and chitosan: synthesis, phase composition and structure

A.O.Synytsia, O.E.Sych, T.E.Babutina, T.V.Tomila, O.I.Bykov

Frantsevich Institute for Problems of Materials Science, National Academy of Sciences of Ukraine, 3 Krzhyzhanovsky Str., 03142 Kyiv, Ukraine

Received January 11, 2022

In the present work, a method of synthesis of biogenic hydroxyapatite-based composites modified by magnetite (1, 5, 25, 50 % by weight) and chitosan was developed. The composition and structure were studied by X-ray diffraction analysis (XRD), infrared (IR) spectroscopy and scanning electron microscopy (SEM). According to X-ray phase analysis, the particle size varies from 43.6 nm to 53.8 nm for composites containing magnetite obtained by chemical precipitation and from 43.6 nm to 50.1 nm for composites containing magnetite obtained by thermal decomposition in a nitrogen environment. The investigation of morphology showed that composite materials, regardless of the ratio of BHA and magnetite and its type (method of production) are characterized by significant agglomeration of rounded particles.

Keywords: biogenic hydroxyapatite, magnetite, chitosan, nanopowders, biocomposites.

Композити на основі біогенного гідроксиапатиту, модифіковані магнетитом та хітозаном: синтез, фазовий склад та структура. *А.О.Синиця, О.Є.Сич, Т.Є.Бабутіна, Т.В.Томила, О.І.Биков*

В даній роботі розроблено метод синтезу біогенних композитів на основі гідроксиапатиту, модифікованих магнетитом (1, 5, 25, 50 % мас. складу) та хітозаном. Склад іструктуру досліджували методами рентгеноструктурного аналізу (РФА), інфрачервоної (ІЧ) спектроскопії та скануючої електронної мікроскопії (СЕМ). За даними рентгенофазового аналізу, розмір частинок коливається від 43,6 нм до 53,8 нм для композитів, що містять магнетит, отриманих хімічним осадженням, і від 43,6 нм до 50,1 нм для композитів, що містять магнетит, отриманих термічним розкладом в середовищі азоту. Дослідження морфології показало, що композиційні матеріали, незалежно від співвідношення БГА та магнетиту та його типу (способу отримання) характеризуються значною агломерацією округлих частинок.

1. Introduction

Due to its biological compatibility, unique bioactivity, structural and chemical similarity to human bone tissue, hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is widely used in modern medicine and materials science as an analogue of the inorganic component of bone material. This is one of the most stable modifications of calcium orthophosphate in the human body.

Biogenic hydroxyapatite (BHA) is obtained from the bones of cattle, fish bones, eggshells, algae, corals, mollusk shells, and others [1]. It is generally used because the crystal structure of natural HA is too complex to be accurately mimicked by synthetic crystalline apatites, which can lead to poor adhn and low mechanical strength, as well as reduced resorption capacity.

At the same time, the urgent task of modern biomaterials is the development of multifunctional magnetically sensitive composite materials. Magnetite (Fe_3O_4) nanoparticles are currently the most widely

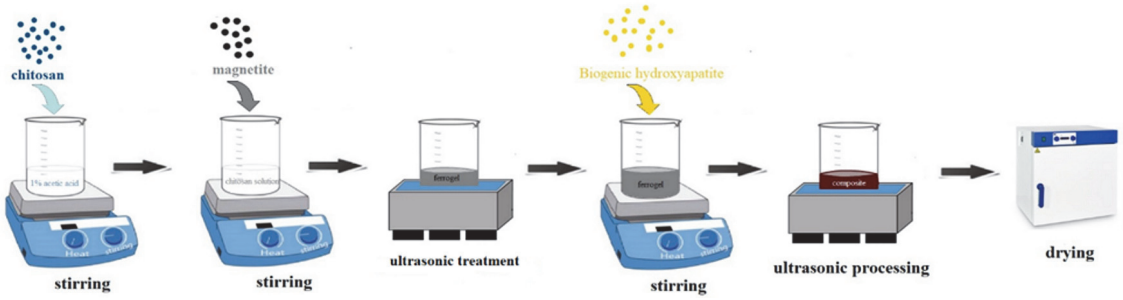

Fig. 1. Specific surface area of composites with magnetite obtained by chemical precipitation and magnetite obtained by thermal decomposition.

used particles of iron oxides for biomedical purposes [2]. The biomedical use of magnetic nanoparticles can be considered depending on whether they are inside the body (*in vivo*) or outside it (*in vitro*). *In vivo* use can be further classified into diagnostic (MRI) and therapeutic (hyperthermia and drug delivery) applications. The two main factors to consider are the size and modification of the surface. The nanoparticles have a huge surface area that can be modified with a large number of functional groups for crosslinking with tumor-targeted ligands, for diagnostic imaging or delivery of a therapeutic agent [3].

The use of the polymer component can improve the biocompatibility of materials, their surface and mechanical properties. Chitin is the second most common biopolymer after cellulose. Chitosan is made from chitin, which is a natural polysaccharide found in crabs, shrimp, lobsters, corals, jellyfish, butterflies, sundews and mushrooms [4]. Chitosan has specific properties that make it a promising biomaterial for use in tissue engineering. This polymer is demonstrated as excellent biocompatibility, almost non-toxic to humans and animals, has high bioactivity, biodegradability, selective permeability, polyelectrolyte action, antimicrobial activity, ability to form gel and film, chelate, reactivity and absorption [5].

Special attention is paid to development of hydroxyapatite-based composite materials due to their wide range of medical uses. A sufficient number of studies have been published on the study of hydroxyapatite/magnetite composites and their use as carriers in drug delivery, treatment of cancerous tumors with hyperthermia, and orthopedics [6–9]. However, in our opinion, insufficient attention has been paid to the BHA-based composite modified with magnetic nanoparticles and the polymer component.

That is why the aim of this work is the synthesis of a biogenic hydroxyapatite-based composite modified by magnetite and chitosan, the investigation of its phase composition and structure for possible further use as a biomaterial.

2. Experimental

As a starting material, we used biogenic hydroxyapatite obtained by annealing the bones of cattle at 800°C in air for 4 hours [10]. Chitosan powder ($(C_6H_{11}NO_4)_n$, M = 500.000, Wako Pure Chemical Industries Ltd.) was selected as the organic polymer.

Magnetite powder was obtained in two ways: by the method of chemical precipitation of iron chlorides and by the method of thermal decomposition from iron oxalates [11].

The first precipitation process was performed from aqueous solutions of $FeCl_2{\cdot}4H_2O$ and $FeCl_3{\cdot}6H_2O$ with a molar ratio of Fe^{2+}:Fe^{3+} 1:2. A 25 % solution of NH_4OH (ammonia) was slowly added dropwise to the mixture to obtain smaller nanoparticles. The mixture was stirred on a magnetic stirrer for 5 min at 80°C. The resulting black precipitate was washed with distilled water and ethanol.

The second method of magnetite synthesis is the thermal decomposition of FeC_2O_4 (iron oxalate) in a Mars furnace at 470°C for 2 h in nitrogen medium.

BHA/magnetite/chitosan composites were obtained according to the scheme shown in Fig. 1. First, a homogeneous 0.1 % solution of chitosan in a 1 % acetic acid solution was prepared. Magnetite, previously obtained by precipitation or decomposition, was added to the chitosan solution, mixed thoroughly, and sonicated for 3 minutes. To the formed ferrogel was added BHA powder in the amount of 99, 95, 75 and 50 wt.%, with ultrasonic treatment for 5 minutes and dried at 50°C for 18 hours.

Specifc surface area (SSA) was measured by gas (nitrogen) adsorption-BET method.

Chemical composition was controlled by X-ray fuorescence EXPERT 4L analyzer (INAM, Ukraine).

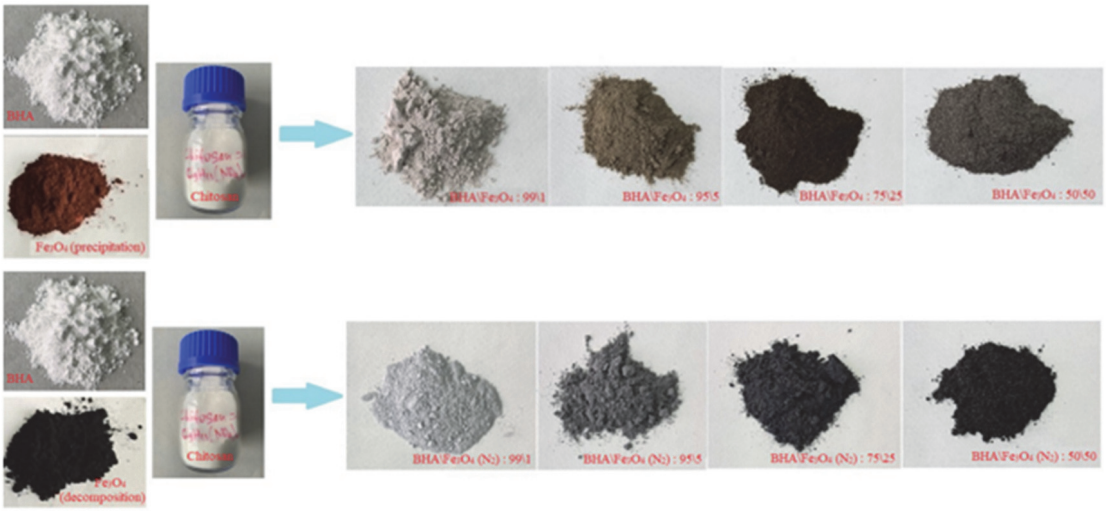

Fig. 2. General view of powder composites with different mass ratios of BHA and magnetite, obtained by different methods.

The phase composition of synthesized composites was studied by X-ray diffraction analysis using a DRON-3 diffractometer (Bourevestnik, Russia) in Cu–Kα radiation with $\lambda = 1.54178$Å^3 at room temperature in the angle range 2θ 10–80°. The obtained diffraction patterns were processed using the JCPDS (International Center for Diffraction Data) and PDF (Powder Diffraction Files) files.

Infrared (IR) spectra were recorded by an Fourier spectrometer FSM-1202 (Infraspectrum, Russia) in the frequency range 4000–400 cm^{-1} using KBr pellets technology.

The microstructure of composite powders was observed by scanning electron microscopy (SEM) using a Tescan Mira 3 LMU microscope (Tescan, Czech Republic).

3. Results and discussions

The appearance of obtained powder composites are presented in Fig. 2.

The values of the specific surface area of composites are schematically presented in Fig. 3. The increase in the specific surface area of powder composites is associated with an increase in Fe_3O_4 content, because the synthesized magnetite, according to studies [11], is nanoscale, with a higher specific surface area in powders obtained by chemical precipitation from iron chloride for 5 min. These data fully confirm the study of the specific surface area of the hydroxyapatite-based composites.

The XRD patterns for raw materials and the obtained composites are shown in Fig. 4 and Fig. 5. As can be seen from the diffraction patterns, a multiphase material was formed in which the main characteristic

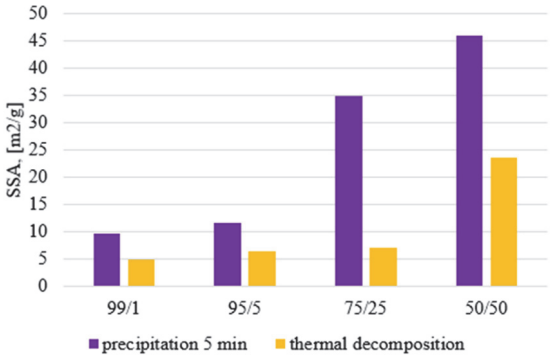

Fig. 3. Specific surface area of composites for composites, which include: magnetite obtained by chemical precipitation and magnetite obtained by thermal decomposition.

lines of hydroxyapatite (JCPDS No. 9-0432) well overlap with the diffraction peaks of the synthesized materials. In addition, the characteristic peaks of magnetite (JCPDS No. 821533) are also presented at values of angle 2θ 35.5°, 57.2° and 62.9°, which increase in proportion to the amount of Fe_3O_4 in the material. The diffraction patterns do not show a clear characteristic peak of chitosan (2θ 20.1°), but the X-ray spectra of the composites, compared to pure biogenic hydroxyapatite, are somewhat amorphous, which may indicate the presence of chitosan phase.

Also, according to X-ray phase analysis, the particle size varies from 43.6 nm to 53.8 nm for composites containing magnetite obtained by chemical precipitation and from 43.6 nm to 50.1 nm for composites containing magnetite obtained by thermal decomposition in a nitrogen media.

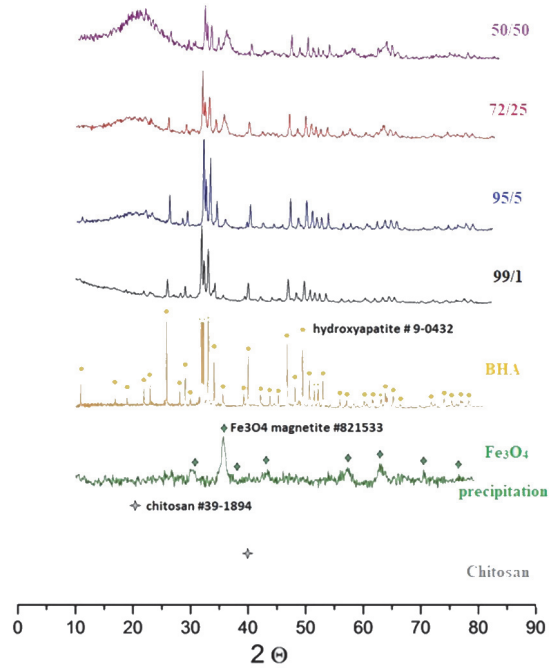

Fig. 4. XRD patterns for composites with different mass ratios of BHA and magnetite obtained by chemical precipitation.

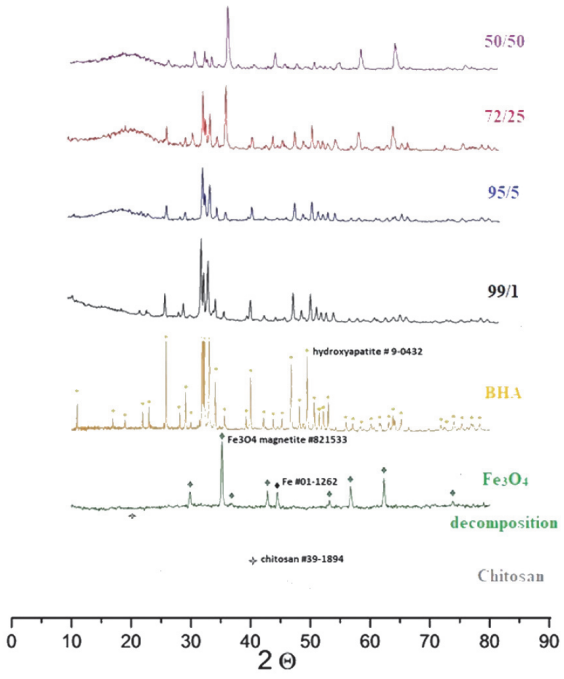

Fig 5.XRD patterns for composites with different mass ratios of BHA and magnetite obtained by thermal decomposition.

IR data are presented in Fig. 6 and Fig. 7. As can be seen, there is an overlap of infrared absorption bands of BGA, magnetite and chitosan. The IR spectrum is typical

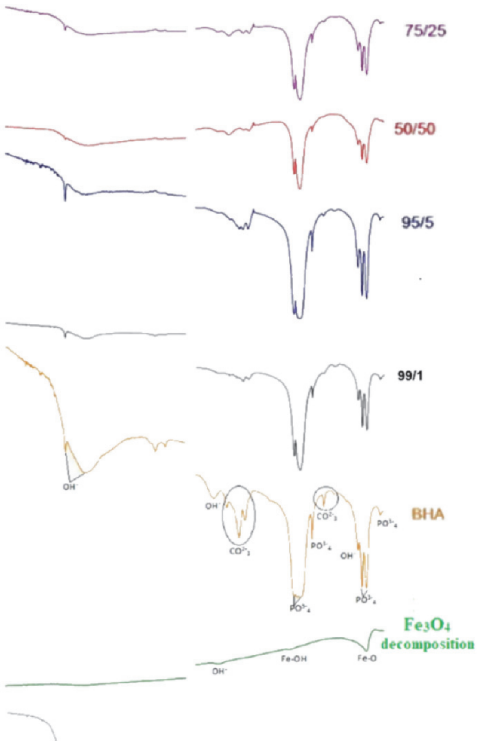

Fig. 6. IR for composites, which include magnetite obtained by chemical precipitation.

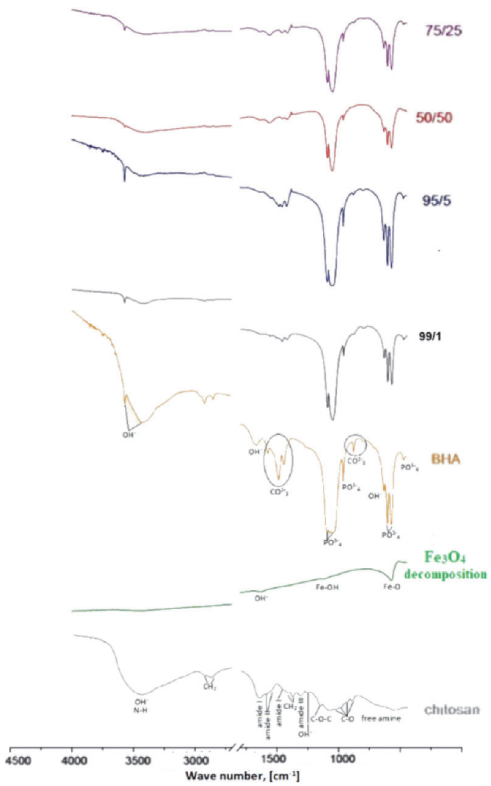

Fig. 7. IR for composites that include magnetite obtained by thermal decomposition.

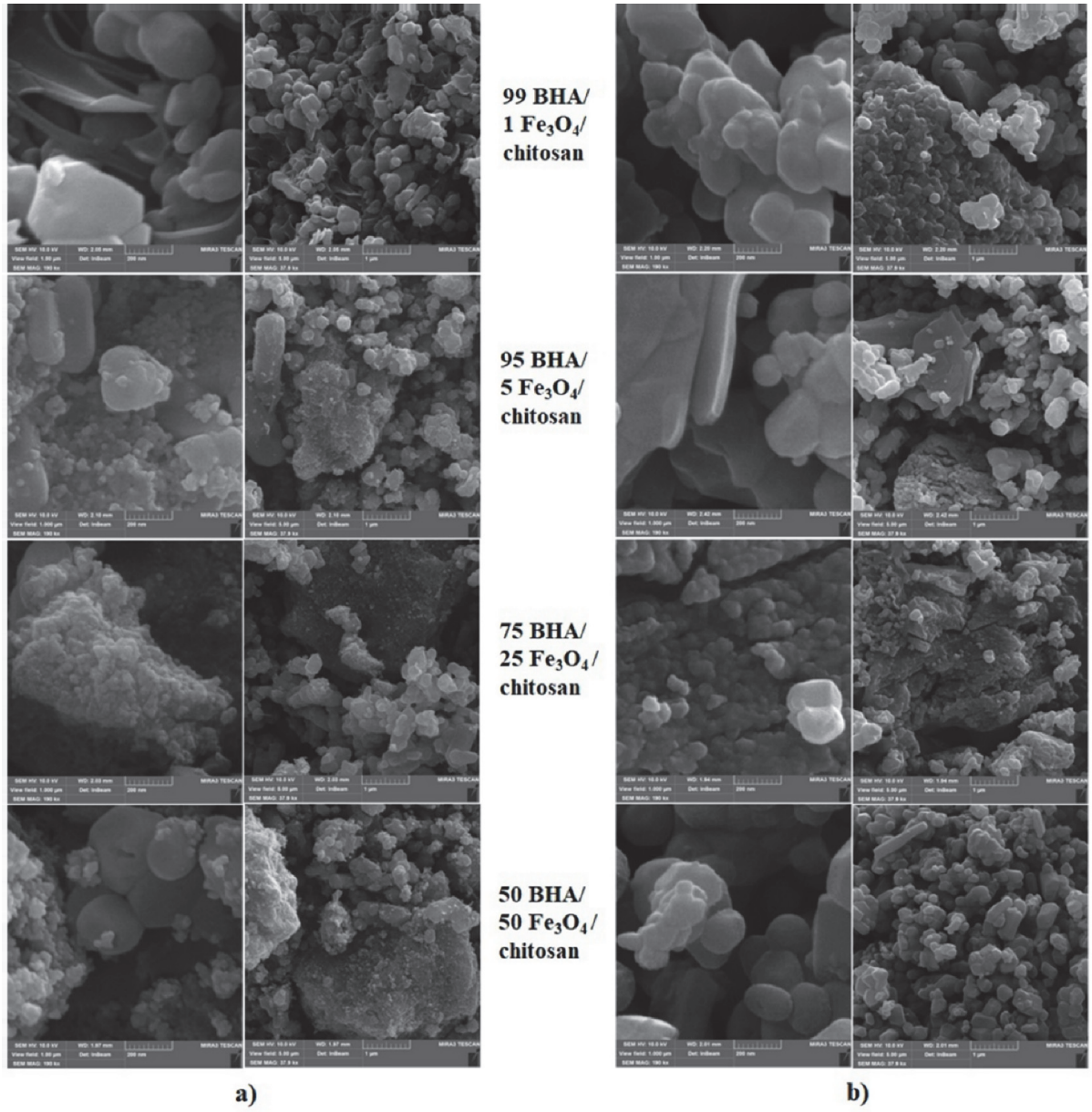

Fig. 8. Microstructure of the obtained composite materials, which include: a) magnetite obtained by chemical precipitation; b) magnetite obtained by thermal decomposition.

for hydroxyapatite-based composites. Due to their high intensity, the spectra of BHA with characteristic absorption bands of functional groups PO_4^{3-}, OH^- and CO_3^2 overlap the absorption bands of Fe–O functional groups, deformation oscillations of Fe–OH groups, which are characteristic of magnetite, as well as methylene (CH_2) and amide bands. I (C = O), amide II (NH deformation NH_2), amide III (CN), deformation oscillations CH_3, asymmetric oscillations COC and CO bonds, vibrational oscillations of hydroxyl groups, inherent in the structure of chitosan, which also leads to their broadening and reducing the intensity.

The control of the chemical composition on the iron content in the obtained composites was also carried out. It was found that in samples 99 BHA/1 Fe_3O_4/Chitosan and 95 BHA/5 Fe_3O_4/Chitosan the percentage of iron content from the total weight of the composite does not exceed 2.28 %. It should be noted that compared to some other nanoparticles, magnetite particles show relatively low toxicity to cell cultures [12] and the allowable amount of iron is about 10 g/kg [13] or about 3 % by

weight. Therefore, the above samples of composites are promising for further study and use in biomedicine.

The morpholo8gy of the obtained powder composites in the BHA/magnetite/chitosan system is presented in Fig. 8. For composite materials, regardless of the ratio of BHA and magnetite and its type (method of production) is characterized by significant agglomeration of rounded particles. In addition, hexagonal prisms can be seen in the structure — particles of this shape are characteristic of BHA, which crystallizes in hexagonal syngony.

4. Conclusions

Composite powders of biogenic hydroxyapatite/magnetite/chitosan based on magnetite obtained by chemical precipitation for 5 minutes, as well as thermal decomposition in a nitrogen media with different ratio of components (wt.% BHA/magnetite: 99/1, 95/5, 75/25 and 50/50 with a chitosan content of 0.1 %) were obtained.

According to the results of X-ray diffraction and IR spectroscopy, it was found that the composites consist of phases of hydroxyapatite, magnetite and chitosan. According to the control of chemical composition, it was found that in samples 99 BHA/1 Fe_3O_4/Chitosan and 95 BHA/5 Fe_3O_4/Chitosan, the percentage of iron content from the total weight of the composite does not exceed 2.28 %, which is acceptable for biomaterials.

The composites are nanoscale and characterized by the formation of agglomerates of complex shape, the specific surface area depends on the type and ratio of components and is 4.8–23.6 and 9.6–46.0 m^2/g for composites based on magnetite obtained by thermal decomposition and chemical precipitation, respectively. Sufficiently high specific surface of the obtained materials will allow functionalizing the surface of materials for further use.

References

1. M.Sadat-Shojai, M.T.Khorasani, E.Dinpanah-Khoshdargi, A.Jamshidi, *Acta Biomater.*, **9**, 7591 (2013). https://doi.org/10.1016/j.actbio.2013.04.012
2. M.R.Ghazanfari, M.Kashefi, S.F.Shams, M.R.Jaafari, *Biomedical Applications, Biochem.Res.*, 1 (2016). https://doi.org/10.1155/2016/7840161
3. H.Fatima, K.S.Kim, *Korean J.Chem.Eng.*, **34**, 589 (2017). https://doi.org/10.1007/s11814-016-0349-2
4. F.Shahidi, R. ????????, *Adv.Food Nutr.Res.*, **49**, 93 (2005). https://doi.org/10.1016/S1043-4526(05)49003-8
5. J.Synowiecki, N.A.Al-Khatteb, *Crit.Rev.Food Sci.Nut.*, **43**, 145 (2003). https://doi.org/10.1080/10408690390826473
6. A.Tampieri, T.D'Alessandro, M.Sandri, *Acta Biomater.*, **8**, 843 (2012). https://doi.org/10.1016/j.actbio.2011.09.032
7. T.Iwasaki, *Adv.Topics*, 175 (2017). http://dx.doi.org/10.57725/54344
8. S.Sprio, S.Panseri, A.Adamiano, *Frontiers in Nanosci.Nanotech.*, **3**, 1 (2017). https://doi.org/10.15761/ FNN.1000145
9. J.He, H.Hu, X.Zeng, *Reg.Biomater.*, **4**, 97 (2017). https://doi.org/10.1093/rb/rbw039
10. O.Sych, N.Pinchuk, A.Parkhomey et al., *Funct.Mater.*, **14**, 430 (2007).
11. A.Synytsia, O.Sych, A.Iatsenko et al., *Appl. Nanosci.* (2021). https://doi.org/10.1007/s13204-021-01797-5
12. S.M.Hussain, K.L.Hess, J.M.Gearhart et al., *Toxicol in Vitro*, **19**, 975 (2007). https://doi.org/10.1016/j.tiv.2005.06.034
13. S.T.Shah, W.A.Yehye, O.Saad, *Nanomater. (Basel)*, **7**, 306 (2017).