ISSN1027-5495. Functional Materials, 31, No.4 (2024), p. 527-537
doi:http://dx.doi.org/10.15407/fm31.04.527

Biogenic hydroxyapatite-based composites modified by magnetite and chitosan: long-term bioresorption and adsorption activity

A. Synytsia[1], O. Sych[1,2], O. Bykov[1], O. Olifan[1], T. Babutina[1], O. Bydulina[1], O. Khomenko[1]

[1]Frantsevich Institute for Problems of Materials Science, NAS of Ukraine,
3 O. Pritsaka Str., Kyiv 03142, Ukraine,
[2]Institute of High Pressure Physics, Polish Academy of Sciences,
Sokołowska Str., 29/37, 01-142 Warsaw, Poland

Received July14, 2024

The work is devoted to the investigation of the long-term resorption of BHA/magnetite/chitosan composites with a magnetite content of 1, 5, 25, and 50 wt.% in physiological solution for different periods of time (2, 10, 16, and 31 days) and their adsorption activity toward methylene blue. It was shown that the content of magnetite significantly affects the rate of resorption of materials, in particular at the initial stages; the highest rate of resorption is observed during the first 2 days. In the next 8-10 days, a sharp decrease in the rate of resorption of all composites is observed, followed by stabilization on the 15th day of the *in vitro* experiment. At the same time, the most significant weight loss of test samples occurs when added more than 5 wt. % of magnetite. The dynamics of the dissolution process is also confirmed by the presence of Ca, P and Fe in physiological solution, a change in the pH of the saline, a decrease in the size and smoothing of composite particles, and an increase in the specific surface area. The adsorption activity for methylene blue increases with increasing amounts of chitosan and magnetite in the composites up to 168 mg/g for 25% magnetite (2.5% chitosan) compared to 108 mg/g for pure BHA. The obtained results confirmed the controlled resorption and high adsorption properties of BHA/magnetite/chitosan composites, which provides prospects for their medical application.

Keywords: hydroxyapatite, magnetite, chitosan, composite, biomaterial, resorption, adsorption activity

Композити на основі біогенного гідроксиапатиту, модифіковані магнетитом та хітозаном: дослідження довготривалої біорезорбції та адсорбційної активності.
А. Синиця, О. Сич, О. Биков, О. Оліфан, Т. Бабутіна, О. Будиліна, О. Хоменко

Робота присвячена дослідженню довготривалої резорбції композитів БГА/магнетит/хітозан із вмістом магнетиту 1, 5, 25 та 50 мас.% у фізіологічному розчині протягом різних проміжків часу (2, 10, 16 та 31 добу) та їх адсорбційної активності щодо метиленового синього. Показано, що вміст магнетиту значно впливає на швидкість резорбції матеріалів, зокрема на початкових етапах, де найвища швидкість резорбції спостерігається протягом перших 2 діб. В наступні 8-10 діб спостерігається різке зменшення швидкості резорбції всіх композитів з подальшою стабілізацією на 15 добу експерименту *in vitro*. При цьому, найбільш суттєва втрата ваги дослідних зразків відбувається при введенні більш ніж 5 мас.% магнетиту. Динаміка протікання процесу розчинення також підтверджується наявністю Ca, P та Fe у фізіологічному розчині, зміною pH розчину, зменшенням розміру та згладжуванням частинок композитів та збільшенням питомої поверхні. Адсорбційна активність для метиленового синього зростає зі збільшенням кількості хітозану та магнетиту в композитах до 168 мг/г для 25% магнетиту (2,5% хітозану) порівняно з 108 мг/г для чистого БГА. Отримані результати підтверджують контрольовану резорбцію та високі адсорбційні властивості композитів БГА/магнетит/хітозан, що відкриває перспективи для їх медичного застосування.

1. Introduction

The modern intensive development of materials science inspires researchers to continuously search for new ways of using natural and synthetic components to create effective and environmentally friendly biomaterials. One of such promising directions is the development of biocomposites based on synthetic and biogenic hydroxyapatite (BHA). Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is the main mineral component of bones and teeth, which is generally responsible for the hardness and strength of bone tissue. Due to its natural properties, hydroxyapatite is widely used in medicine in the form of implants for full or partial bone replacement, as a biocompatible and bioactive coating of metal implants that degrade too quickly in the body [1-4]. In addition, due to sufficiently large adsorption properties, hydroxyapatite is used for targeted delivery of cells or for separation of proteins, viruses, etc. [5, 6].

To improve the natural properties of hydroxyapatite, its modification with various organic and inorganic additives is widely used [7, 8]. Thus, BHA-based composites modified by magnetite and chitosan are considered promising [9, 10]. The addition of magnetite significantly increases osseointegration, mechanical and resorption properties, compared to pure hydroxyapatite [11-13]. Moreover, the high magnetic characteristics of magnetite allow the use of BHA-based composites as magnetically sensitive materials for magnetotherapy, hyperthermia, or drug delivery [14-16]. Chitosan, as a natural collagen analog and a biopolymer with unique physicochemical properties, improves bioactivity, biocompatibility, biomineralization, and adsorption properties of composites and coatings based on them [17-19].

In our previous work [12], we investigated the resorption of composites based on BHA modified with magnetite and chitosan, kept in a model solution for 2 days. It was established that an increase in the magnetite content leads to an increase in the composites resorption. Moreover, the use of magnetite obtained by chemical precipitation in amount of 5-50% allows achieving a resorption rate of 2.5-5.3 wt.% per day, which is 3.5-7.5 times higher than "pure" biogenic hydroxyapatite and 1.2-2 times higher than composites with magnetite obtained by decomposition in nitrogen media.

However, it would also be advisable to study the resorption of composites over a long period of time, which more realistically reproduces conditions in a living organism. It will also allow obtaining accurate information about dissolution dynamics, interaction with the model solution, and evaluating the stability of the obtained biomaterials. In addition, it is relevant to study the adsorption properties of BHA/magnetite/chitosan composites, as this will allow us to assess the prospects of using the created materials as carriers loaded with drugs for the regeneration or treatment of damaged bone tissue.

Therefore, the purpose of this work was to study the long-term resorption of BHA/magnetite/chitosan composites during 31 days, as well as to evaluate their adsorption activity towards methylene blue, which was chosen as a model solution.

2. Materials and methods

Composite materials based on biogenic hydroxyapatite modified by magnetite (1, 5, 25 and 50 wt. %) and chitosan (10 wt.% of magnetite) were used as the research materials [9]. Magnetite used in composites was obtained by the chemical precipitation of iron chlorides (chem.) and by the thermolysis of iron oxalates (therm.) [20].

The resorption properties of composites were studied *in vitro* according to the technology described in the previous work [12]. The rate of resorption (solubility) of the materials was determined as the specific mass loss during the experimental period – after 2, 10, 16 and 31 days of exposure to physiological solution.

The content of chemical elements (Ca, P and Fe) in physiological solution after interaction with composite materials during 31 days (after 2, 10, 16 and 31 days of the experiment) was monitored by the photocolorimetric method using the FEK-56M device. The pH of the physiological solution was also monitored during resorption of the materials using a laboratory pH meter ADWA AD1030 (ATC, Hungary).

Phase composition, specific surface area (SSA), and microstructure were studied for all composites before and after 2, 10, 16, and 31 days of exposure to physiological solution. X-ray diffraction analysis of the composite powders was performed using a DRON-3 diffractometer. The microstructure was studied by scanning electron microscopy (SEM) using a Tescan Mira 3 LMU microscope (Tescan, Czech Republic). The AMIS software (automatic microstructure analyzer) [21] was used to evaluate the particle shape using the Saltykov method [22].

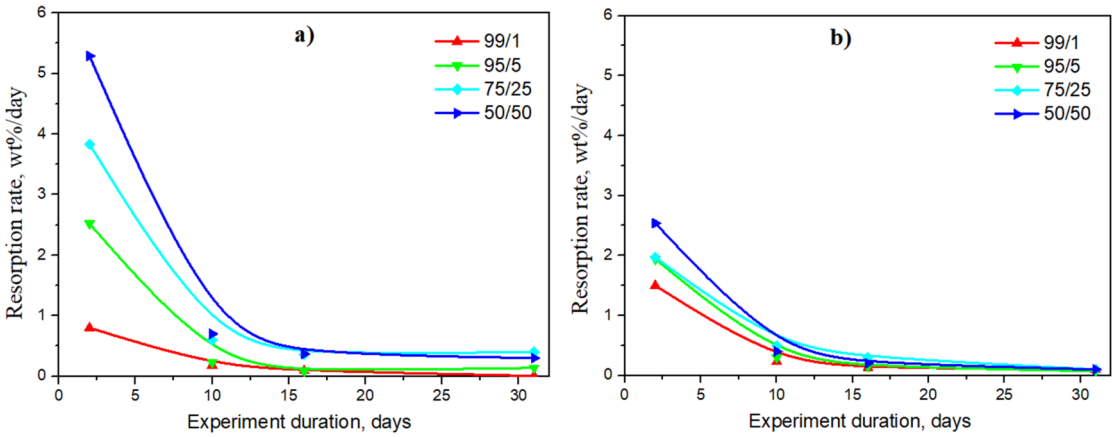

Fig. 1 – Composite resorption rate over 31 days: a) – BHA/magnetite(chem.)/chitosan composites; b) – BHA/magnetite(therm.)/chitosan composite

The SSA was evaluated by the BET isotherm analysis using a Gemini 2360 instrument (Micromeritics, USA) according to ISO 9277:2010. The skeleton density was measured using a helium pycnometer (AccuPyc II 1340, Micromeritics) according to ISO 12154:2014. Before the measurements of density and SSA, the materials were dried at 50 °C for 5 h in vacuum (VacPrep 061 Sample Degas System by Micromeritics). The particle size of the composites was estimated from SEM images and by calculation based on the SSA and density results [12].

The adsorption properties were studied on powder samples of composites according to the method described in [23]. Composite samples were saturated with an aqueous solution of methylene blue with a concentration of 1500 mg/ml for 45 minutes. The optical density was determined by the photocolorimetric method using the FEK-56M. The residual mass concentration of methylene blue in the solution was determined on the basis of the obtained optical density values and the compiled calibration graph.

Adsorption activity was determined by formula:

$$X = \frac{(C_1 - C_2 \cdot K) \cdot V}{m},$$

where C_1 is the mass concentration of the initial solution of methylene blue, mg/l; C_2 is the mass concentration of methylene blue solution after contact with samples, mg/l; K is the dilution factor of the solution taken for analysis (K = 10); V is the volume of the indicator solution taken for the investigation, ml; m is the mass of the sample, g.

3. Results and discussion

The results of the long-term investigation of the resorption BHA/magnetite/chitosan composites are presented in Fig. 1. Comparison of the resorption rates of composites with different composition allows us to conclude that the magnetite content significantly affects the resorption of materials.

It has been shown that in the long-term experiment, the most intense resorption process occurs during the first 2 days. However, as we showed earlier [12], the rate of the resorption process is significantly affected by the type and amount of magnetite in the composites. Thus, the resorption rate of the composite containing 1% magnetite (chem.) is 0.8 wt.% per day and is comparable to the resorption rate of pure BHA (0.8 wt.% per day), while the introduction of 1% magnetite (therm.) increases resorption by 17% compared to pure BHA. Increasing the amount of magnetite (chem.) to 5% leads to a 3-fold increase in the rate of resorption (2.52 wt.% per day), and the introduction of 50% – by 6 times (5.29 wt.% per day) (Fig. 1(a)). At the same time, the resorption rate of the composite with 50% magnetite (therm.) content is almost 2 times lower – 2.54 wt.% per day (Fig. 1(b)). In the next 8-10 days, a sharp decrease in the resorption rate of all composites with subsequent stabilization was observed. It was established that after the 15 days of the experiment, the resorption rate became constant without significant changes, regardless of the type and amount of magnetite introduced into the composites.

The nature of the resorption process is also confirmed by the results of the elemental analysis of filtrates of physiological solutions

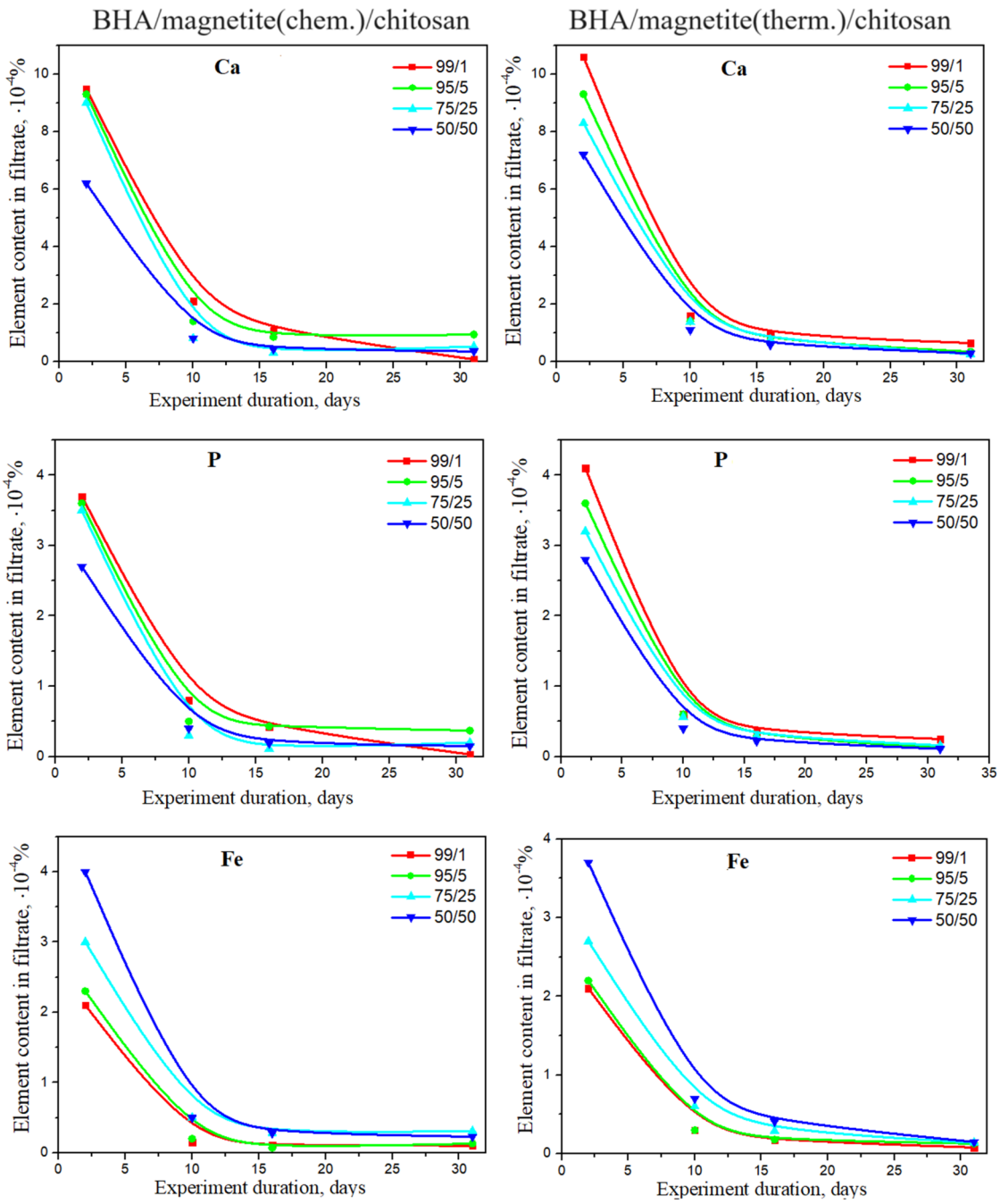

Fig. 2 – Changes in the content of calcium, phosphorus and iron in physiological solution after interaction with composites

after each of the stages of *in vitro* experiments (Fig. 2). The analysis of the specific content of chemical elements in the filtrates of the solution during the long-term experiment shows a gradual decrease in the dynamics of the release of Ca^{2+}, PO_4^{3-} and Fe^{2+} ions, which is directly related to the slowdown in the process of material resorption (Fig. 1).

Also, the release of iron ions during the long-term experiment established that the most intense saturation of the solution with Fe^{2+} ions occurs during the first 2 days. However, the assessment of the specific content of iron ions indicates a slowdown in the process of iron release after the 10 days of the experiment; the actual content did not exceed $4.5 \cdot 10^{-4}$% and $4.0 \cdot 10^{-4}$ % for the composite with the maximum magnetite (chem.) and magnetite (therm.) content, respectively. At the same time, the specific release of iron ions into the model solution is practically

Table 1 - Crystal structure parameters of BHA/magnetite/chitosan composites before and after interaction with physiological solution

BHA/magnetite ratio in composites	Size of the crystallites (Å)										Crystal cell volume V (Å^3)				
	Da					Dc									
	starting [12]	after saline				starting [12]	after saline				starting [12]	after saline			
		2 days [12]	10 days	16 days	31 days		2 days [12]	10 days	16 days	31 days		2 days [12]	10 days	16 days	31 days
Standard HA 09-432	9.418	—	—	—	—	6.884	—	—	—	—	528.80	—	—	—	—
BHA [23]	9.411	—	—	—	—	6.878	—	—	—	—	527.54	—	—	—	—
magnetite – chemical precipitation (5 min)															
99/1	9.451	9.453	9.424	9.446	9.444	6.895	6.885	6.883	6.879	6.880	533.3	532.8	529.4	531.6	531.4
95/5	9.407	9.436	9.436	9.426	9.437	6.909	6.886	6.885	6.882	6.885	529.5	531.0	530.9	529.6	531.7
75/25	9.406	9.450	9.450	9.436	9.447	6.857	6.892	6.886	6.883	6.885	525.4	533.0	532.1	530.7	531.9
50/50	9.405	9.461	9.450	9.436	9.443	6.874	6.893	6.880	6.894	6.896	526.5	534.3	532.3	531.6	532.7
magnetite – thermal decomposition (N_2)															
99/1	9.454	9.427	9.444	9.442	9.444	6.889	6.885	6.879	6.8913	6.886	533.2	529.9	531.3	532.1	531.9
95/5	9.393	9.450	9.428	9.431	9.433	6.860	6.892	6.877	6.8743	6.877	524.2	533.0	529.4	529.5	531.1
75/25	9.394	9.470	9.423	9.435	9.437	6.851	6.884	6.883	6.8922	6.892	523.6	534.6	529.4	531.4	532.3
50/50	9.410	9.457	9.419	9.426	9.426	6.866	6.886	6.889	6.8843	6.889	526.5	533.3	529.2	529.7	531.9

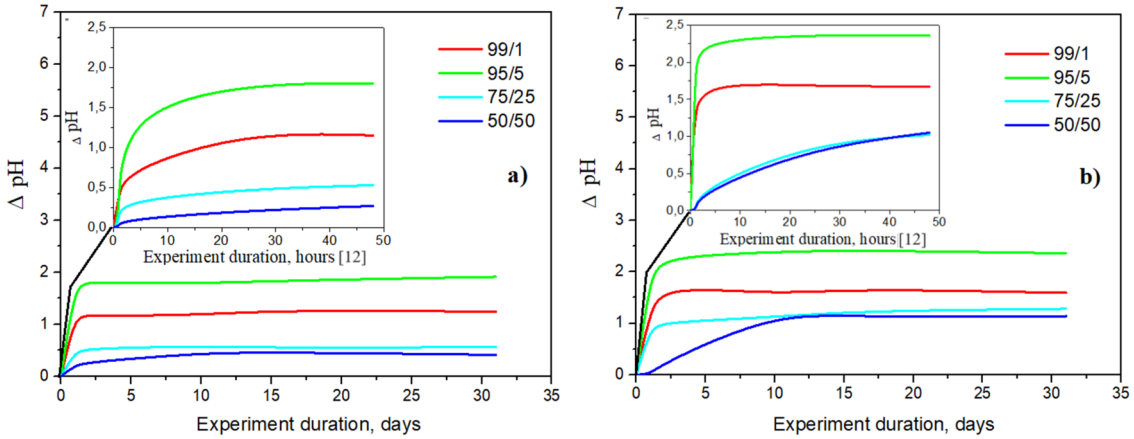

Fig. 3 – Change in the physiological solution pH in the presence of composites for 31 days
a) – BHA/magnetite(chem.)/chitosan composites; b) – BHA/magnetite(therm.)/chitosan composites

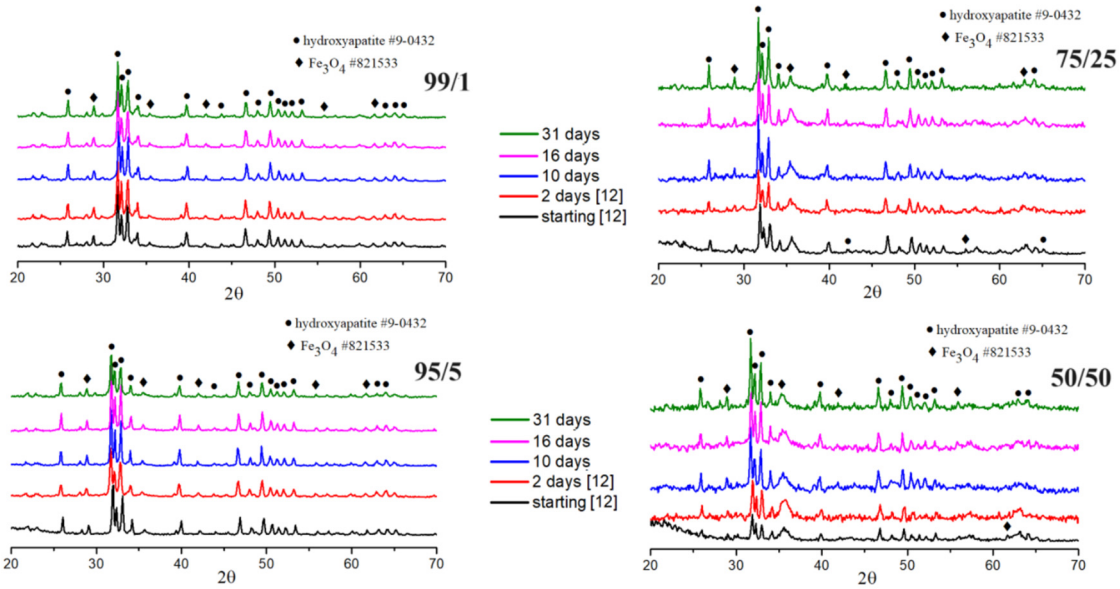

Fig. 4 – XRD patterns for BHA/magnetite (chem.)/chitosan composites before and after interaction with physiological solution for different periods of time

the same for all samples (~0.1 - 0.15·10^{-4}%). The obtained data confirm the previously obtained results on the absence of cytotoxicity of the composites [12].

The interaction of saline with BHA/magnetite/chitosan composites is also confirmed by the change in pH of the solution over time (Fig. 3). As can be seen, the most intense change in pH occurred during the first 2 days of the experiment with subsequent stabilization. This is due to the active dissolution of composites, as well as the release of calcium, phosphorus and iron elements into the model solution. For composites with 1-5% magnetite, regardless of the type, a rapid increase in the pH during the first 24 h of the experiment is characteristic, while for composites with 25-50% magnetite, the pH increases gradually, without sharp changes. This is in complete agreement with the results of chemical analysis of the filtrates presented in Fig. 2; in addition, the release of Ca^{2+} and PO_4^{3-} ions, which are present in the BHA crystal lattice, affects the resorption rate during the first 2 days.

The phase composition was controlled before and after interaction with the model solution (Figs. 4 and 5). XRD analysis showed that, as in the case of samples after 2-day exposure in physiological solution [12], the phase composition of BHA/magnetite/chitosan composites does not change after a 31-day *in vitro* experiment. The presence and superimposition of the characteristic reflection peaks of the starting materials is observed: hydroxyapatite (at 2θ angles of 32-34°, 40°, 46°, 50°), magnetite (at 2θ angles of 35°, 57° and 63°), as well as

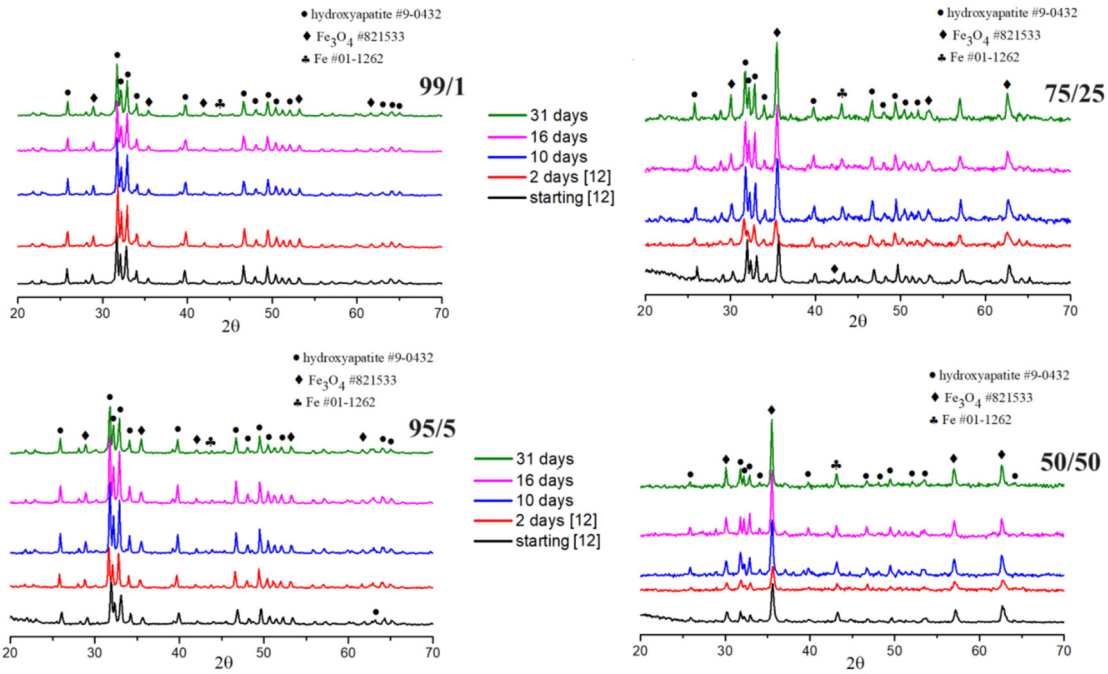

Fig. 5 – XRD patterns for BHA/magnetite (therm.)/chitosan composites before and after interaction with physiological solution for different periods of time

Table 2 – Skeleton density of BHA, magnetite, chitosan and composites

BHA/magnetite ratio in composites	Skeleton density, ± 0.02 g/cm^3				
	starting [12]	after saline			
		2 days [12]	10 days	16 days	31 days
BHA [23]	3.09	-	-		
Chitosan [24]	1.49	-	-		
Magnetite (precipitation) [20]	4.22	-	-	-	-
Magnetite (decomposition) [20]	4.74	-	-	-	-
magnetite - chemical precipitation (5 min)					
99/1	3.07	3.01	2.90	2.91	2.92
95/5	3.10	3.09	3.05	3.04	3.06
75/25	3.08	3.17	3.16	3.15	3.16
50/50	3.08	3.38	3.18	3.14	3.17
magnetite – thermal decomposition					
99/1	3.04	3.03	2.99	3.00	3.02
95/5	3.13	3.11	3.11	3.12	3.08
75/25	3.05	3.18	3.13	3.11	3.14
50/50	3.33	3.43	3.45	3.46	3.47

impurities of iron (at 2θ angles ~44-46°, Fig. 5), which is typical for a composite modified with magnetite (therm.). In addition, the dependence remains the same: the higher the magnetite content, the more intense its reflection peaks on the diffraction patterns. The resorption of the composites is confirmed not only by the change in the intensity of the characteristic peaks in XRD patterns, but also by the lattice parameters (Table 1). The higher the magnetite content, the higher the change of crystal lattice parameters after contact of composites with physiological solution.

Moreover, the effect of saline on lattice parameters of BHA/magnetite composites was also confirmed based on the skeleton density (Table 2). Increasing the magnetite content up to 25-50% leads to an increase in density after contact with a saline solution, whereas with a magnetite content of 1-5% a decrease in density

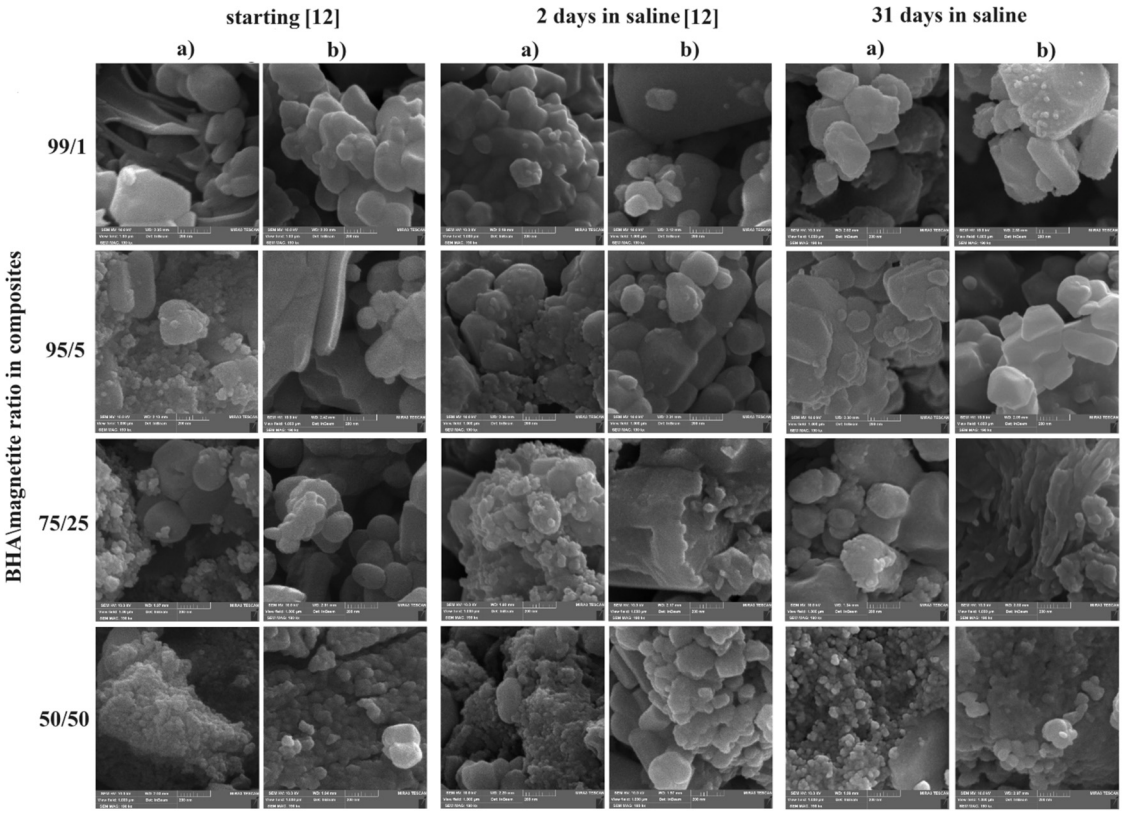

Fig. 6 – Microstructure of composites before and after 2 and 31 days of interaction with physiological solution: a) – BHA/magnetite(chem.)/chitosan composites b) – BHA/magnetite(therm.)/chitosan composites

Table 3 – Particle size of BHA/magnetite/chitosan composites calculated based on SSA data

BHA/magnetite ratio in composites	Particle size, nm				
	starting [12]	after saline			
		2 days [12]	10 days	16 days	31 days
magnetite – chemical precipitation (5 min)					
99/1	376	273	234	206	187
95/5	194	167	127	109	92
75/25	58	44	45	46	46
50/50	42	27	33	34	33
magnetite – thermal decomposition					
99/1	411	381	244	214	142
95/5	304	364	272	262	211
75/25	150	110	119	122	117
50/50	106	86	102	104	94

is observed. Changes after interaction with the model solution can also be evaluated by changes in the morphology and size of the particles studied using SEM (Fig. 6). Changes in the morphology of particles after 2 days of exposure to physiological solution were described in detail in our previous work [12]. Analysis of the results after a long-term experiment confirmed a decrease in the minimum particle size of the composites, as well as a smoothing of the material particles, compared to the original powder composites; this was also confirmed by calculating the particle sizes of BHA/magnetite/chitosan composites based on SSA data (Table 3). At the same time, elongated hexagonal structures of hydroxyapatite and small rounded particles of magnetite can be distinguished even after 31 days of exposure to physiological so-

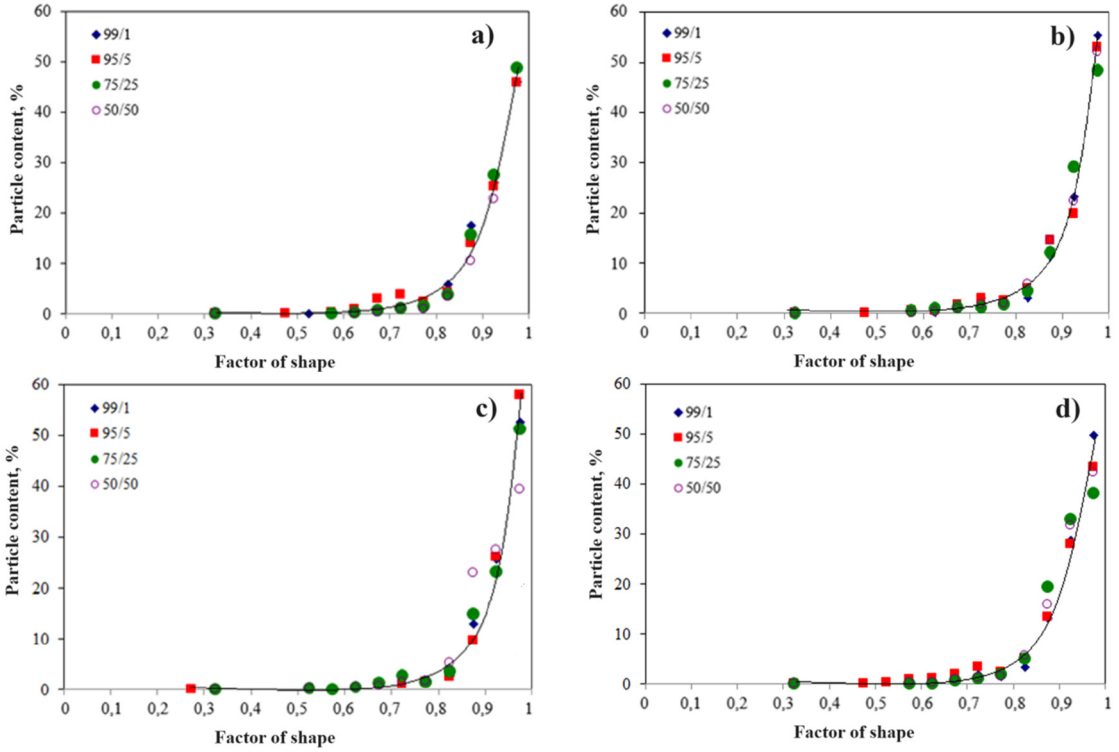

Fig. 7 – Shape factor of composites before (a, b) [25] and after 31 days (c, d) of exposure to physiological solution: a, c) – BHA/magnetite(chem.)/chitosan composites b, d) – BHA/magnetite(therm.)/chitosan composites

lution that was also confirmed by evaluation of the shape factor (Fig. 7). It was found that 92-96 % of composites particles after interaction with saline have the shape factor greater than 0.8, which indicates the formation of regular-shaped powders. The increase in the number of spherical particles compared to the «starting» composites (89-95 % of which had a shape factor greater than 0.8) confirms the change in the morphology of materials after interaction with saline.

The adsorption activity of materials is one of the important parameters that determine their effectiveness in medical use. One of the main factors influencing the adsorption activity of composites is the SSA. The greater the specific surface of the material, the more active centers it has for interaction with biological agents or drugs. In addition, it was shown that the interaction of the experimental composites with physiological solution leads to an increase in the SSA, as well as a decrease in the skeleton density, which also confirms the resorption process (Table 4). Thus, after 2 days of exposure to the model solution, the specific surface area of composites containing magnetite (chem.) increases by an average of 17%, and after 31 days – by 34%. For composites containing magnetite

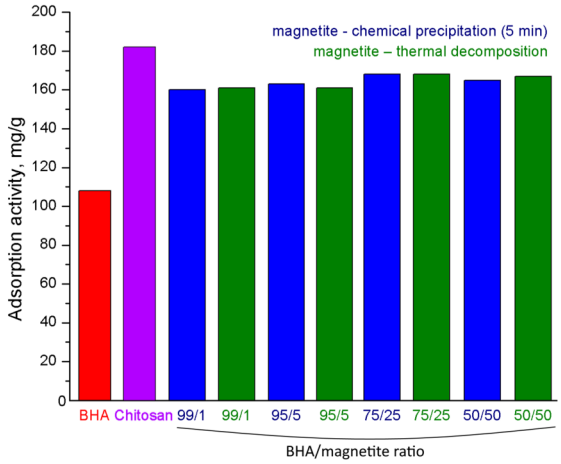

Fig. 8 – Adsorption activity of starting BHA, chitosan and BHA/magnetite/chitosan composites

(therm.), these values are slightly lower – 10% and 14%, respectively. These data are fully consistent with the previously described rate of resorption of materials (Fig. 1).

As can be seen from the presented results (Fig. 8), the adsorption activity of the composites is significantly higher than for pure BHA. It is also shown that despite the small specific surface area, chitosan has more than 1.5 times higher adsorption activity compared to BHA. This is due to its chemical structure, since free

Table 4 – Specific surface area of BHA, magnetite, chitosan and composites

BHA/magnetite ratio in composites	SSA, ± 2 m^2/g				
	starting	after saline			
		2 days [12]	10 days	16 days	31 days
BHA [23]	10.8	-	-		
Chitosan [24]	0.8	-	-		
Magnetite (precipitation) [20]	141	-	-	-	-
Magnetite (decomposition) [20]	25	-	-	-	-
magnetite – chemical precipitation (5 min)					
99/1	5	7	9	10	11
95/5	10	12	16	18	21
75/25	34	41	42	41	41
50/50	47	52	58	56	57
magnetite – thermal decomposition					
99/1	5	5	8	9	14
95/5	6	7	7	7	9
75/25	11	15	16	16	16
50/50	13	17	17	17	18

amino groups are able to actively interact with molecules of biological substances. Therefore, it is natural that the adsorption activity of composites increases with respect to methylene blue with an increase in the amount of, first of all, chitosan and magnetite. At the same time, the highest index of adsorption activity is observed for the composite with 25% magnetite content (168 mg/g), regardless of the synthesis method. This is probably due to the optimal interaction between the components of the composites and the creation of a large number of active adsorption centers on the surface of the material.

4. Conclusions

Based on the investigation of resorption and interaction of BHA/magnetite/chitosan composites with physiological solution in thermostatic conditions, it was established that despite the amount of magnetite, all materials are resorbed *in vitro*. It is shown that the content of magnetite in BHA/magnetite/chitosan composites significantly affects the rate of material resorption. Composites with 50% magnetite demonstrated the highest resorption rate (5.29 wt.% per day for magnetite (chem.) and 2.54 wt.% per day for magnetite (therm.) during the first 2 days. However, despite the difference in the rate of resorption during the first 12 days, the obtained data indicate that the amount of magnetite in the composite does not affect the long-term (more than 31 days) dissolution of the materials, which slows down significantly after 15 days. This is confirmed by the results of the elemental analysis of the filtrates: a gradual decrease in the yield of Ca, P and Fe is observed, as well as a change in the pH of the physiological solution when interacting with the composites. The course of the resorption process is also confirmed by the change in size and smoothing of the surface of the particles, as well as an increase in the specific surface area of the composites after exposure to physiological solution. The investigation of adsorption towards to methylene blue showed a significant effect of chitosan on the adsorption properties of the composites. At the same time, the highest adsorption activity –168 mg/g was determined for composites with 25% magnetite, regardless of the synthesis method, compared to pure BHA (108 mg/g).

Thus, it can be concluded that the use of BHA/magnetite/chitosan biocomposites is highly promising. The amount of magnetite and chitosan in the composite will allow regulation of the resorption rate and adsorption activity, which is an important factor for various medical applications. For example, composites with a high content of magnetite (25-50%) provide rapid resorption in the initial stages, which can be useful for rapid filling of bone defects or

drug delivery, due to a high index of adsorption activity. At the same time, the stabilization of the resorption rate after 15 days indicates the possibility of long-term use of such materials without the risk of rapid destruction.

References

1. E. Fiume, G. Magnaterra, A. Rahdar, et al., *Ceram,* **4**, 542, (2021). https://doi.org/10.3390/ceramics4040039
2. W. Dai, S. Li, H. Jia, et al., *J. Mater. Sci. Technol,* **295**, 207, (2024). https://doi.org/10.1016/j.jmst.2024.04.032
3. S.R. Paital, N.B. Dahotre, *Mat. Sci. Eng. R,* **1**, 66, (2009). https://doi.org/10.1016/j.mser.2009.05.001
4. M. Rahman, Y. Li, C. Wen, *J. Magnes. Alloy,* **8**, 929, (2020). https://doi.org/10.1016/j.jma.2020.05.003
5. J.S. Son, M. Appleford, J.L. Ong, J.C. Wenke, et al., *J. Controlled Release,* **153**, 133 (2011). https://doi.org/10.1016/j.jconrel.2011.03.010
6. R. Li, K. Chen, G. Li, et al., *J. Mol. Struct,* **34**, 1120, (2016). https://doi.org/10.1016/j.molstruc.2016.05.017
7. I. Ielo, G. Calabrese, G. De Luca, S. Conoci, *Int. J. Mol. Sci,* **23**, 9721, (2022). https://doi.org/10.3390/ijms23179721
8. H. Shi, Z. Zhou, W. Li, et. al., *Cryst,* , **11**, 149, (2021). https://doi.org/10.3390/cryst11020149
9. A. Synytsia, O. Sych, T. Babutina, et al., *Funct. Mater,* **29**, 299, (2022). https://doi.org/10.15407/fm29.04.506
10. T. Varadavenkatesan, R. Vinayagam, S. Pai, et al., *Prog. Org. Coat,* **151**, 106056, (2021). https://doi.org/10.1016/j.porgcoat.2020.106056
11. H.Y. Xu, N. Gu, *Front. Mater. Sci*, **8,** 20, (2014). https://doi.org/10.1007/s11706-014-0232-1
12. A. Synytsia, P. Zaremba, S. Zahorodnia, et al., *Funct. Mater,* **29,** 506, (2022). https://doi.org/10.15407/fm29.04.506
13. F. Heidari, M. Razavi, M.E. Bahrololoom, et al., *Mater. Sci. Eng. C,* **65**, 338, (2016). https://doi.org/10.1016/j.msec.2016.04.039
14. M. Kamitakahara, N. Ohtoshi, M. Kawashita, et al., *J Mater Sci: Mater Med,* **27**, (2016). https://doi.org/10.1007/s10856-016-5704-7
15. S. Mondal, P. Manivasagan, S. Bharathiraja, et al., *Nanomater,* **7**, 426, (2017). https://doi.org/10.3390/nano7120426
16. D.A.C. Ferreira-Ermita, F.L. Valente, E.C. Carlo-Reis, et al., *Results in Materials*, **5**, 100063, (2020). https://doi.org/10.1016/j.rinma.2020.100063
17. M.A. Nazeer, E. Yilgör, I. Yilgör, *Carbohydr. Polym,* **175**, ,38, (2017). https://doi.org/10.1016/j.carbpol.2017.07.054
18. M.K. Hassan, S.A.A. Abdelrehim, T.A. Elkhoosly, et al, *Thin Solid Films*, **798**, 140378, (2024). https://doi.org/10.1016/j.tsf.2024.140378
19. D.-E. Radulescu, I.A. Neacsu, A.-M. Grumezescu, E. Andronescu, *Polym,* **14**, 899, (2022). https://doi.org/10.3390/polym14050899
20. A. Synytsia, O. Sych, A. Iatsenko, et al., *Appl Nanosci,* **12**, 929, (2022). https://doi.org/10.1007/s13204-021-01797-5
21. A.I. Khomenko, E.V. Khomenko, P*owder Metall Met Ceram,* **46**, 100, (2007). https://doi.org/10.1007/s11106-007-0016-6
22. S.A. Saltikov The determination of the size distribution of particles in an opaque material from a measurement of the size distribution of their sections. Elias, H. (eds) Stereology. Springer, Berlin (1967).
23. O. Sych, O. Otychenko, N. Ulianchych, et al., *AdvNanoBioM&D*, **2**, 287, (2018).
24. O. Sych, N. Pinchuk, T. Tomila, et al., *AdvNanoBioM&D,* **4**, 617, (2020).
25. A. Synytsia, O. Sych, V. Zenkov, et al., *Surface,* **15**, 97, (2023). https://doi.org/10.15407/Surface.2023.15.097 [in Ukrainian]