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The Effect of Graphene Oxide on the Properties and Release of Drugs from Apatite-Polymer Composites

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The effect of graphene oxide (GO) on the structural and mechanical properties of Hydroxyapatite-Alginate (HA-Alg) based composite, as well as the ability to release the Chlorhexidine (CHX) and Diclofenac Sodium (DS) from it are studied. HA-Alg beads were synthesized with different GO content (0.0004 % and 0.004 %). The formation under MW irradiation of calcium-deficient HA (Ca/P = 1.65) with small carbonate content is confirmed by XRD and XRF techniques. Alg acts as a dispersant and provides the uniform distribution of GO particles within the Alg matrix after sonification. GO nanoparticles in combination with cross-linked Alg macromolecules by calcium ions contribute to the enhancement of mechanical properties of the obtained beads. Distribution of GO particles in the Alg matrix enhances composites Young's modulus from 0.79 GPa in the HA-Alg sample to 1.33 GPa in the HA-Alg-GO sample. Calculated intra- and intermolecular interaction energies in the HA-Alg-GO complex confirm that the total stabilization energy consists of solvophobic interactions, van der Waals stacking energy, and H-bonds. CHX release is influenced by GO content and is primarily driven by matrix erosion. GO prolongs the release of the CHX for 48 h in a neutral medium. The release of the DS in a neutral medium is affected by GO content. In an acidic environment, DS release is controlled mainly by diffusion forces, which are slowed down by the clustering of DS through the formation of H-bonds and hydrophobic interactions between GO and DS.

Keywords: Alginate, Graphene oxide, Hydroxyapatite, Structural and mechanical properties, Structural modeling, Drug release.

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1. INTRODUCTION

The development of nanoscale drug carriers based on the latest biopolymer-inorganic composites has been the focus of many current studies. Similar structures correspond, to a certain extent, to the second level of the hierarchical bone structure of nine differentiated structural levels for lamellar bones. The specific nano-organization in these biopolymer-nonorganic structures is like this: type I collagen fibrils are reinforced in some way by Hydroxyapatite (HA; more precisely, calcium deficient HA – CDHA) nanocrystals. This fundamental principle causes a growing interest of medical material scientists to create the synthetic hybrid materials with mechanical properties similar to natural composites.

HA ($Ca_{10}(PO_4)_6(OH)_2$) is a bioceramic material used for clinical bone care and implantation. Being similar in structure, chemical composition and bioactivity to the native apatite, synthetic HA promotes the adhesion and proliferation of osteoblast cells that build new bone. One of the approaches to improve the insufficient mechanical properties of HA for high load-bearing is the hybridization of organic and inorganic constituents in one composite. The synergistic effect of inorganic particles, biopolymers and metal ions results in the development of new composites with enhanced mechanical properties and elasticity, capacity of drug encapsulation and sustained drug release [1, 2].

Alginate (Alg) as a natural biocompatible and biodegradable polymer is widely used for medical, pharmaceutical and food applications. Alg based composites are applied in bone and cartilage tissue engineering, as wound dressings, for cell cultivation in drug delivery systems [3]. It is a linear unbranched anionic polysaccharide obtained from brown seaweed and containing varying proportions of beta-D-mannuronate (M) and alpha-L-guluronate (G) residues. It is known the ability of sodium Alg to undergo gel formation by ionic interaction in an aqueous medium with transition metal cations. Ionically cross-linked Alg has been developed as a matrix for prolonged and controlled drug release [4].

Among the advanced nanomaterials, graphene-based materials received increasing attention in biomedicine because of their excellent mechanical strength, good biocompatibility and low toxicity. Graphene oxide (GO) has excellent hydrophilicity and stability in physiological environment compared to graphene and graphdiyne and is largely used for biomedical application, as reinforcement materials in tissue-engineering [5], for drug delivery [6, 7], cell and tissue imaging. The structure of the monatomic GO layer promotes adsorption and securing drug molecules to the GO surface that increases the efficiency of drug loading [8]. Compared with pristine graphene, GO demonstrates the higher stabilization behavior in aqueous media and may be used as nanoscale reinforcement fillers in biocomposites [9]. GO contains both hydrophobic and hydrophilic regions that give it the amphiphilic properties [5]. Bound on the

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surface of GO hydroxyl, epoxide, and carbonyl reactive groups can interact with polymers, biomolecules, DNA, proteins, quantum dots, giving them capabilities for various biological and medical applications [10]. Controlled drug release systems responding to pH, light, and magnetic fields are also created on graphene-based nanomaterials [11]. Although new evidences suggest that graphene-based materials are promising in the biomedical field, there are concerns about potential adverse effects on humans and the environment [12]. The results of the evaluation of the toxicity of graphene-containing materials remain controversial, indicating the need for new research and data before applying these new materials in clinical medicine [5].

The requirements for biomaterials such as bioactivity, bioresorbability, porosity, ability to induce bone formation and vascularization most meet by materials in the form of 3D scaffolds or beads.

In this work, we combined in one composite the characteristic features of GO, HA, Alg and investigated their influence on the properties of the new hybrid material. The ability of GO to maintain structural stability under the influence of external factors, amphiphilicity and ability to act as a drug carrier – there are some circumstances that initiated our efforts to incorporate GO into the hybrid nanocomposite materials. The following was also taken into account when performing this work. It is known, bone tissue, unlike other mineralized tissues, is constantly undergoing remodeling. In particular, osteoclasts in contact with bone under influence of acid phosphatase secrete lactic acid, which dissolves bioapatite due to the local acidic environment. Therefore, there is a question of studying the specificity of drug release under these conditions. Based on the above, the main purpose of this work was to investigate the effect of GO on the ability of the composite to prolonged release of different in structure drugs, namely hydrophilic Chlorhexidine (CHX) and hydrophobic Diclofenac Sodium (DS) in neutral and acidic media. It was also taken into account the influence of the mechanical and structural HA-Alg-GO composite properties.

2. EXPERIMENTAL SECTION

2.1 Chemicals

Calcium nitrate tetrahydrate $Ca(NO_3)_2{\cdot}4H_2O$, calcium chloride $CaCl_2$, diammonium dihydrophosphate ($(NH_4)_2HPO_4$), ammonium hydroxide NH_4OH were purchased from Sinopharm Chemical Reagent Co., Ltd. GO powder (Sigma-Aldrich, USA), sodium Alg of low viscosity (E407, Shanghai Chemical Company Ltd, China), commercially available pharmaceutical 0.05 % CHX bigluconate and DS were used. All reagents were analytical grade, commercially available and used as received. A Milli-Q system with the resistance of 18.2 MΩ cm was used to purify the water which was eventually used for preparing solutions and washing steps.

2.2 Composites Preparation

The technological scheme of composite beads formation is shown in Fig. 1 and includes the following steps.

GO powder was dispersed in the 2 % sodium Alg solution at GO concentrations of 15 and 150 μg/ml. The Alg-GO colloidal suspensions were sonicated for 10 min (40 kHz) and then stirred overnight at 37 °C in the dark.

HA hydrogel with moisture content of about 85 % was obtained according to the previously described method [4]. Briefly, 0.3 M $(NH_4)_2HPO_4$ solution was added dropwise to 0.5 M $Ca(NO_3)_2{\cdot}4H_2O$. After adjusting pH value to 10.5 by the addition of ammonia solution, the mother suspension transferred to the consumer microwave (MW) oven Samsung M1712NR for its irradiation for 3 min at a power of 600 W. After cooling, washing, and centrifugation (6 min, 8000 rpm) HA hydrogel was obtained.

Drugs containing HA-Alg-GO beads were prepared using $CaCl_2$ cross-linking agent by ionotropic-gelation method according to the early described technology [13]. Briefly, HA hydrogel was gradually added to Alg-GO colloidal suspensions with weight ratio 1:1 and treated with ultrasound (100 W) for 3 min. The resulting dispersions were dropped into 100 ml of 0.25 M $CaCl_2$, in which droplets were retained for 24 h to produce spherical beads. The formed beads were then collected by filtration, washed and dried at 37 °C for 24 h. The final GO content in the obtained HA-Alg-GO beads was 0.0004 % and 0.004 % (in terms of GO powder weight to the HA powder weight).

Pharmaceutical CHX (0.05 % solution) and DS (2.5 mg/ml) were used as model drugs. CHX is active against gram-positive and gram-negative bacteria. DS is a nonsteroidal anti-inflammatory drug used to treat pain and inflammatory diseases. The impregnation of the drugs was made by soaking of the 0.2 g of lyophilized beads (HA-Alg, HA-Alg-GO15 and HA-Alg-GO150) into 2 ml of CHX or DS solutions for 2 h followed by drying at 37 °C. Subsequently, the samples were called as HA-Alg-GO15 and HA-Alg-GO150, respectively. GO-free HA-Alg sample was used as the control.

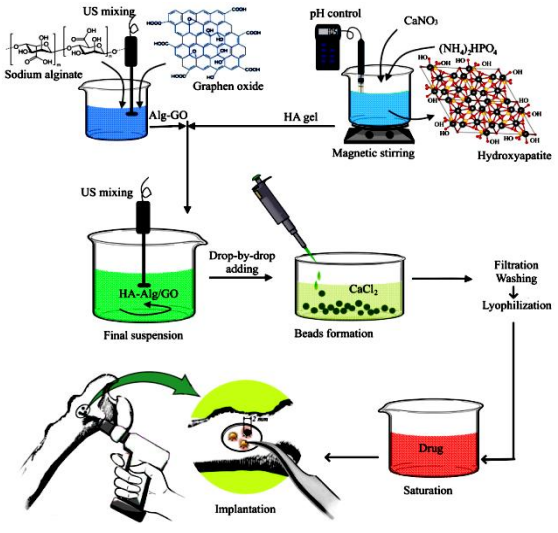

Fig. 1 – The scheme of drugs loading in a HA-Alg-GO composite

2.3 Methods

The characterization of the synthesized samples was performed by a transmission electron microscope (TEM, SELMI, Ukraine) at accelerating voltage of 90 kV. The dispersed sample was captured on a holey carbon film (1020 nm) of a Cu TEM grid prior to analysis.

The GO particle size distribution in water was determined by dynamic light scattering (DLS) on a Zetasizer Nano-ZS90 (Malvern, Worcestershire, UK) at room temperature. The instrument was equipped with a He-Ne laser (5 mW) operating at 633 nm. Results were analyzed under the Smoluchowski approximation. The autocorrelation function of the scattered light intensity was analyzed by the Malvern Zetasizer software.

X-ray diffraction (XRD) structural studies were performed using the Shimadzu XRD-6000 diffractometer with Cu-Kα radiation. The data were collected over 2θ range of 5.0-60.0° with a step of 0.02° and counting time of 2 s. Average crystallite sizes along [002] direction were estimated from the corresponding peak broadening by the Scherrer equation [14]. Calculation of the hexagonal lattice parameters a and c was performed according to the corresponding formulas using the Miller indexes. The Ca/P ratio was determined using the X-ray fluorescence spectrometer ElvaX Light SDD (XRF analysis). The voltage of the Rh anode X-ray tube was 12 kV. Calcium and phosphorous concentrations were estimated by the regression analysis method for HA samples only.

2.3.1 Structural Modeling

The structure of the triple complex HA-Alg-GO was built using HyperChem 8.0 software and energy minimized using the molecular mechanics method MM+. The spatial structure of the elementary cell of HA was reported [15] from X-ray analysis of the synthetic H6L sample and was taken by us from the American Mineralogist Crystal Structure Database in the form of AMC-file (code 0002297). This cell was further used to construct a flat crystal structure comprising four 2 × 2 cells, which well match the shape and dimensions of the corresponding rhomboid GO molecule by using the VESTA program (version 3.4.4). The GO structure [16] was built using HyperChem 8.0 software and energy minimized using Gaussian09W on DFT (B3LYP) level of theory with 6-31G** basis set. The structure of Alg was built using HyperChem 8.0 software (the Sugar Builder module) by alternating hyaluronic (HYL) and mannuronic (MAN) units linked by α-1.4 glycosidic bonds. The length of the oligosugar was set in a way to cover the perimeter of the interface between HA and GO and comprised 17 units: $(HYL\text{-}MAN)_8$-HYL. The hydrogen atoms of polysaccharide carboxylic groups were substituted by sodium atoms.

2.3.2 Swelling Degree Study

The swelling degree was quantified by measuring the changes in sample weight before and after immersion of the lyophilized beads into phosphate buffered saline (PBS) [17] at pH = 7.2 and pH = 4.0. The swelling degree (Sw) was calculated using the following equation:

$$\mathrm{Sw} = (Wt - W_0)/W_0 \cdot 100\ \%,$$

where W_0 is the initial sample weight and Wt is the final weight of the swollen sample.

2.3.3 Compressive Strength

To measure the strength under the uniaxial compression, control HA-Alg and two GO containing samples were molded into tablets with 5 mm diameter and 2.4-2.6 mm thickness by cold pressing using a hydraulic press (at ~ 100 MPa). Mechanical properties of the samples under load were investigated using an original test machine.

2.3.4 HPLC Determination of Drug Release

The experimental samples loaded with CHX and DS were placed into 6 ml of PBS at different pH (7.2 and 4.0) and incubated at 37 °C under continuous shaking of 80 rpm. The release of drugs was monitored hourly for the first day and then daily. Aliquots of 600 μl of PBS were taken from each test tube and an equal volume of fresh medium was then added to maintain a constant initial volume in the test tubes. High-Performance Liquid Chromatography (HPLC; Agilent Technologies 1200, detector with UV-Vis Abs, detection at $\lambda = 280$ nm, column C18 (Zorbax SB-C18 4.6 × 150 mm, 5 μm)) was applied. To determine CHX release the next mobile phase was applied: 68 % of 0.05 M potassium hydrogen phosphate buffer with 0.2 % triethylamine (pH = 3.0 at 21 °C); 32 % of acetonitrile. The isostatic treatment, elution rate of 2 ml/min, the temperature of the analytical column 40 °C, was used. The injection volume was 20 μl and detection was at 210 nm. The next mobile phase was used to determine DS release: 0.05 orthophosporic acid, pH 2.0 and acetonitrile as 35 and 65 %, respectively, at flow rate of 2.0 ml/min and the run time was 3 min. Degassing was achieved via sonification for 45 min. The injection volume was 20 μl and detection was at 254 nm.

2.3.5 Statistical Analysis

Drug release and swelling measurements were performed in triplicate. The range of the obtained experimental values is represented by error bars. Student t-test at a 95 % confidence interval were used to analyze statistical differences between obtained data groups.

3. RESULTS AND DISCUSSION

3.1 TEM and DLS Studies

TEM images and electron diffraction (ED) patterns of the synthesized composites (Fig. 2, A-C) indicate the formation of needle-like HA crystallites and their agglomerates of 100 nm and more. ED patterns confirmed the presence of a single HA phase. An increase in the content of the GO reduces the degree of crystallinity of the composite, as evidenced by the weakening of the diffraction ring [002] on the ED spectra. DLS measurement was performed for the monitoring of GO particle distribution in an aqueous solution. Degree of GO particle aggregation may affect their bioactivity [18]. Because of high specific surface area, GO tends to form irreversible agglomerates due to van der Waals interactions. GO water solution at room temperature by DLS data (Fig. 2D) is a polydisperse system with the Z-average particle size of about 1 μm at concentration of 0.15 mg/ml. Numerous oxygen functionalities in the basal planes and the borders of GO can generate the interfacial bonding with hydroxyl groups of Alg allowing homogeneous distribution of GO in the Alg matrix. TEM confirms that the dispersion of GO in the Alg solution under sonification leads to the distribution of GO with a nanoparticle size of 10-20 nm (Fig. 2C).

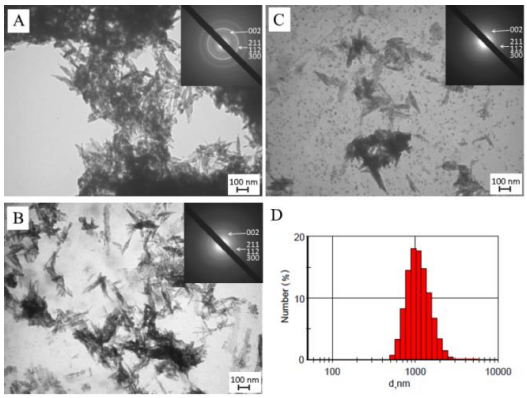

Fig. 2 – TEM images and ED spectra of experimental samples: A) HA-Alg; B) HA-Alg-GO15; C) HA-Alg-GO150; D) DLS (hydrodynamic size) data of GO (0.15 mg/ml) distribution in water

3.2 XRD Study

XRD analysis shows that under the influence of MW HA (JCPDS 01-086-0740) was synthesized. According to XRF data, the Ca/P atomic ratio in the obtained HA is 1.65. The crystalline lattice parameters calculated in planes (002), (211) for the obtained HA (a = 0.9385 nm; c = 0.6882 nm) also differ from the stoichiometric ones (a = 0.9421 nm; c = 0.6881 nm). The average crystallite size for diffraction peaks (002) is of about 30 nm. Those facts mean the formation of calcium deficient HA with a carbonate small content [19].

3.3 Structural Modeling of HA-Alg-GO System

The possibility of complexation between the studied compounds HA, Alg and GO in an aqueous mixture was testified by means of molecular mechanics. Arrangement of molecules is mainly stabilized by solvophobic/van der Waals interactions between GO and HA surfaces, and is additionally stabilized by the hydrogen bonds between the OH-groups of GO and oxygen atoms of HA (≈ 4 bonds). A few H-bonds were also noted between the OH-groups of Alg and OH-groups of GO (≈ 3 bonds), and oxygen atoms of HA (≈ 3 bonds). Within the framework of the MM+ method, we calculated the energies of intermolecular interactions in the triple HA-Alg-GO complex, viz. E_{total} = 6172.35 kcal/mol, E_{GO} = 3715.50 kcal/mol, E_{HA} = 1303.33 kcal/mol and E_{Alg} = 1440.94 kcal/mol. These calculations allowed to estimate the contribution of intermolecular interactions into the total energy of complex formation: $\Delta E = E_{total} - E_{GO} - E_{HA} - E_{Alg} \approx$ ≈ – 287 kcal/mol. This value should be increased by the magnitude of complex stabilization from solvophobic interactions. The latter could be estimated from solvent accessible surface areas (A) of each molecule in separate and in complex, viz. A_{total} = 4061.39 Å^2, A_{GO} = 1044.62 Å^2, A_{HA} = 2430.58 Å^2 and A_{Alg} = 3272.94 Å^2. Taking the microscopic surface tension, γ = 0.05 kcal/mol·Å^2, for water one can finally estimate the solvophobic contribution as $\Delta G_{hyd} = \gamma \cdot (A_{total} - A_{GO} - A_{HA} - A_{Alg}) \approx -$ 134 kcal/mol. It is seen that solvophobic interactions give one third of the total stabilization energy, whereas the rest is given by van der Waals stacking energy and H-bonds.

The above calculations helped to represent the structural pattern of the experimental HA-Alg-GO composite (see Fig. 3).

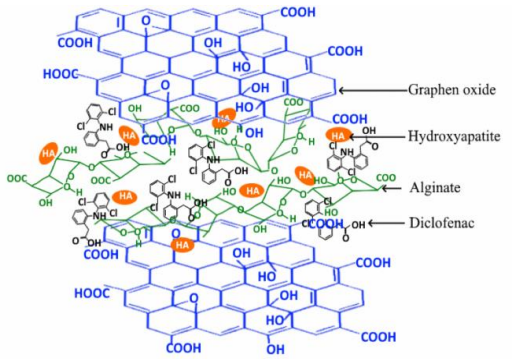

Fig. 3 – The calculated structural pattern of the HA-Alg-GO triple complex

3.4 Compressive Strength Study

The result of the compressive strength investigation showed that the addition of 0.0004 % and 0.004 % GO to the composites of HA and ionotropically cross-linked by Ca^{2+} ions Alg matrix, increased the Young's modulus to 0.84 and 1.33 GPa, respectively, compared to the GO-free sample, for which this value was 0.79 GPa. The uniform distribution of GO particles in the structure of the Alg matrix promotes stress distribution, thereby noticed enhancing the mechanical properties of the material, which makes it promising for use in the form of beads for hard bone tissue cure.

3.5 Swelling Degree Study

The swelling degree was determined to evaluate the ability of the experimental samples to maintain beads shape stability and their water loading capacity at different pH values (Fig. 4). It was proved, that addition of 0.0004 % and 0.004 % of GO in relation to HA, leads to an increase in the beads shape stability due to more stronger intermolecular interactions at both pH. It was noticed that GO reduces the composites swelling degree for about 10 % and 15 % in neutral and acidic media, respectively. It should be noted a decrease in the swelling degree of all samples, including GO-free sample, in an acidic medium compared to neutral one due to shrinkage of Alg matrix.

3.6 HPLC Study

The HPLC method was used to determine and compare the dynamics of CHX and DS release from experimental samples in PBS. It should be noted that the studied composites could potentially be used to treat bone tissue that is in the body in the process of continuous remodeling. Osteoclast cells are firmly adhered to the bone surface and dissolve the bone due to the formation of an acidic medium for dissolving calcium phosphate (pH 4 to 4.5) and the release of collagenase [20]. Osteoblast cells are constantly building a new bone and both processes are in equilibrium. Therefore, given the possibility of the existence of local zones with different acidity, the dynamics of drug release with different chemical structures and properties at pH 4.0 and 7.2 was investigated.

The dynamics of drug release is presented in Fig. 5 and Fig. 6. The quantity of released drugs was plotted against the incubation time. First of all, the behavior of

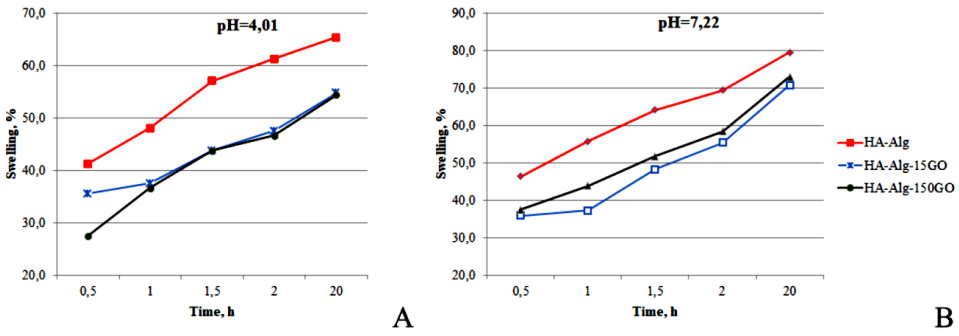

Fig. 4 – Dynamics of swelling of experimental samples during 20 h in PBS under acidic (A) and neutral (B) conditions

composite materials in the PBS common to both studies (with CHX and DS) should be noted. Experiments have visually proved that the addition of GO contributes to the beads shape stability and brings down the erosion rate of the matrix. The content of HA in beads also reduces the mobility of the Alg matrix, which may decrease the release of the drug in neutral medium. In acidic medium, on the one hand, there is a partial dissolution of HA, which promotes the diffusion of the drug, and on the other hand – shrinkage of Alg matrix reduces the diffusion. These circumstances affect the degree of release of both investigated drugs into PBS.

CHX molecules most likely do not form intermolecular bonds by a donor-acceptor mechanism with components of the composites in acidic and neutral environments and therefore their release is dominated by matrix erosion manner. In the first stage (up to 4 h), the release of surface adsorbed CHX molecules occurs. At both pH values, the CHX release from control GO-free HA-Alg samples is more intensive compared to GO-containing samples because of faster erosion of Alg matrix caused by ion exchange between Ca^{2+} ions of ionotropically cross-linked Alg matrix and Na^{+} ions of PBS (Fig. 5A, B).

Addition of GO to the composite contributes to the beads stability and limits the erosion rate, which in turn leads to a decrease in the release of CHX in PBS. Full release (about 95 %) of CHX at pH = 7.2 from the sample HA-Alg occurs after 72 h. At the same time, the GO content in the HA-Alg-GO15 and HA-Alg-GO150 samples slightly reduces the volume of full release (about 90 %), but prolongs the full CHX release time to 120 h. This is obviously due to the additional hydrogen bonds between the hydrophilic functional groups GO and Alg which slows the drug release from the composite structure.

In acidic medium (Fig. 5C), CHX release delay is more noticeable from GO-containing samples, apparently due to the increase in the number of bonds between OH, COOH and epoxide groups in the basal plane of GO and Alg functional groups. This fact reduces the CHX full release time to 48 h and its efficiency from GO-containing samples (60-80 %) compared to the control GO-free sample. In this case, the degree of CHX release decreases in proportion to the increase in GO content. The control GO-free sample in acidic medium shows 100 % CHX release for 120 h. Thus, CHX release from GO-containing samples is influenced by GO content and primary driven by matrix erosion.

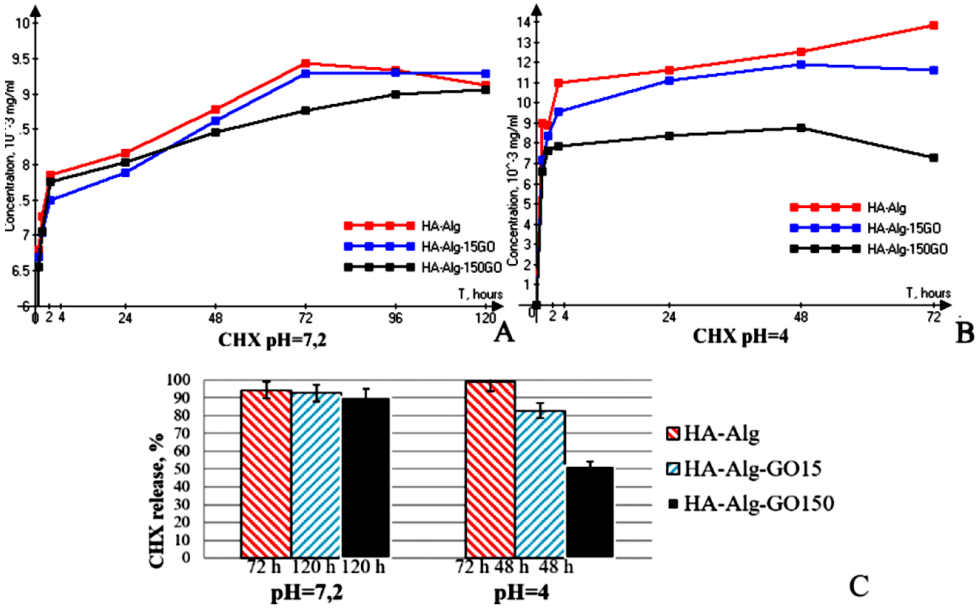

Fig. 5 – Dynamics of CHX release (mg/ml) from experimental composites into PBS medium (A, B); first stage and full CHX release in % (C) at different pH values

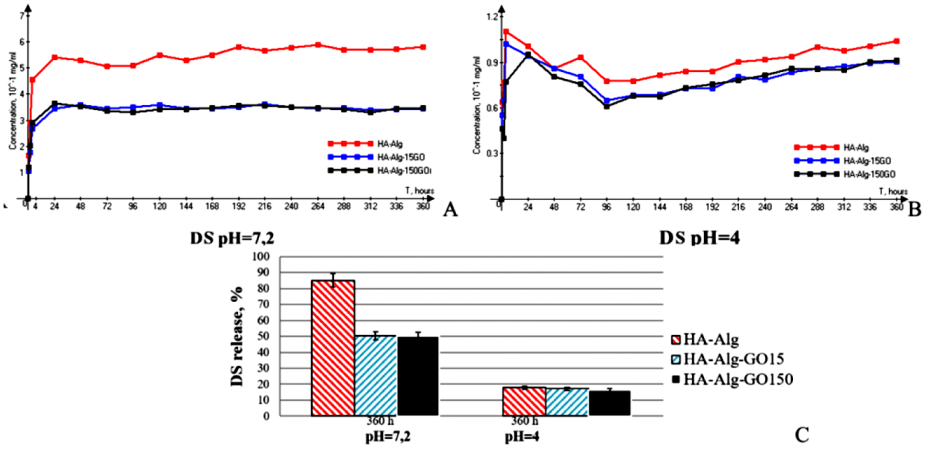

Fig. 6 – Dynamics of DS release (mg/ml) from experimental composites into PBS medium (A, B) and the percentage of DS release from GO-containing samples relative to the control GO-free one (C) at different pH values

Another situation arises with the release of DS. Thus, during the first 24 h in neutral medium (with pH = 7.2), about 70 % of DS was released from the GO-free control HA-Alg sample and drug concentration in PBS was 0.546 mg/ml (Fig. 6 A, C). The full DS release after 360 h was about 90 %.

At the same time, the release of DS from GO-containing samples as a percentage of total release from the control sample during the first day was about 40 %, and the concentration of DS in PBS was approximately 0.35 mg/ml. The reason for such a significant decrease in the DS release may be both a decrease in the ability of the GO-containing composite to absorb DS during saturation and the binding between functionalities of GO and DS, which prevent the diffusion of the drug from composites. The results obtained indicate that the release of DS from GO-containing samples in neutral medium is affected by the GO introduced into their composition. In acidic medium at pH = 4, a full DS release after 360 h was about 18 % for the GO-free sample and for GO-containing samples, DS release slightly decreased in proportion to the increase in GO content and was 15-14 % (Fig. 6 B, C). The significant decrease in total release of DS in an acidic environment compared to neutral one can be explained by the significant amount of hydrogen bonds between functional COOH, OH-groups of Alg and GO, on the one hand, and, on the other hand, by the formation of clusters of DS molecules with the participation of protons H^+, which solution PBS at pH=4 contains 10^3 times more than the neutral solution (pH = 7.2) (Fig. 7).

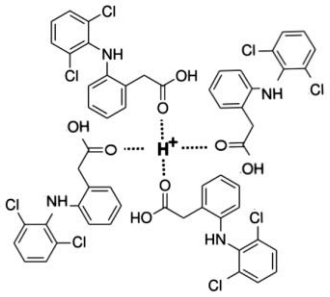

Fig. 7 – Scheme of the DS clusters formation in an acidic environment

In addition, GO is an amphiphilic substance because it contains not only hydrophilic regions along the edges but also hydrophobic ones in the basis plane. In acidic environments, GO hydrophobicity increases. Therefore, additional hydrophobic interactions between GO and DS are possible. Thus, after the first release stage of surface adsorbed DS, which lasts for 24 h, the second release stage begins from 24 to 96 h, where DS concentration in PBS slightly decreases. This means that the beads maintain their integrity during this period and the drug release does not occur. Indeed, increased beads shape stability has been experimentally proven in acidic medium at pH = 4, although partial dissolution of HA is possible under these conditions and may increase matrix mobility and promote further DS diffusion. During the period from 96 to 360 h, the concentration of released DS in an acidic environment gradually increased by 5 % for HA-Alg sample and by 10 % for GO-containing samples (Fig. 6B). Thus, DS release in an acidic environment is controlled mainly by diffusion forces, which are slowed down by the clustering of DS and hydrophobic interactions between GO and DS.

4. CONCLUSIONS

GO loaded HA-Alg beads with different content (0.0004 % and 0.004 %) were synthesized. The formation under MW irradiation of calcium deficient HA (Ca/P = 1.65) with small carbonate content is confirmed by XRD and XRF techniques. Alg acts as a dispersant and provides the uniform distribution of GO particles within Alg matrix after sonification. GO nanoparticles in combination with cross-linked by Ca^{2+} ions Alg macromolecules contribute to the enhancement of mechanical properties of the obtained beads. HA-Alg composite containing 0.004 % GO has a much higher Young's modulus (1.33 GPa) in comparison with GO-free HA-Alg (0.79 GPa). Calculation of the intra- and intermolecular interaction energies in the triple complex, containing HA, Alg and GO, confirmed that solvophobic interactions give one third of the total stabilization energy, whereas the rest is given by van der Waals stacking energy and H-bonds. HPLC demonstrates prolonged release of hydrophilic CHX and hydrophobic DS in acidic and neutral media. GO content in the compo-

site contributes to the beads shape stability and reduces the erosion rate of the matrix. CHX release from GO-containing samples is influenced by GO content and primary driven by matrix erosion. GO prolongs CHX release for 48 h in neutral medium and reduces release time and its efficiency in acidic medium. The release of DS from GO-containing samples in a neutral medium is affected by the GO introduced into their composition, while in an acidic environment DS release is controlled mainly by diffusion forces, which are slowed down by the clustering of DS and hydrophobic interactions between GO and DS.

Thus, the results obtained indicate that the drug loaded HA-Alg-GO composite can be used for the effective treatment of bone tissue diseases.

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Вплив оксиду графену на властивості та вивільнення лікарських засобів з апатит-полімерних композитів

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Вивчено вплив оксиду графену (GO) на структурно-механічні властивості композиту на основі гідроксиапатиту та альгінату (HA-Alg), а також здатність до пролонгованого вивільнення з нього хлоргексидину (CHX) та диклофенаку натрію (DS). Гранульований композит HA-Alg був синтезований з різним вмістом GO (0,0004 % та 0,004 %). Утворення при мікрохвильовому опроміненні кальцій-дефіцитного HA (Ca/P = 1,65) з малим вмістом карбонату підтверджується методами XRD та XRF. Alg діє як диспергатор і забезпечує рівномірний розподіл частинок GO в альгінатній матриці після ультразвукової обробки. Наночастинки GO, поєднанні із зшитими іонами кальцію макромолекулами альгінату, сприяють посиленню механічних властивостей отриманих гранул. Розподіл частинок GO в альгінатній матриці підвищує модуль Юнга від 0,79 ГПа в зразку HA-Alg до 1,33 ГПа в зразку HA-Alg-GO. Обчислені енергії внутрішньо- та міжмолекулярних взаємодій комплексу HA-Alg-GO підтверджують, що загальна енергія стабілізації складається з сольвофобних взаємодій, енергії ван дер-Ваальса та Н-зв'язків. GO впливає на вивільнення CHX, яке, в основному, відбувається за рахунок матричної ерозії. Вміст GO подовжує вивільнення CHX на 48 год у нейтральному середовищі. Динаміка вивільнення DS в нейтральному середовищі також контролюється вмістом GO, а в кислому середовищі – в основному дифузійними силами, які сповільнюються кластеризацією DS через утворення Н-зв'язків та гідрофобних взаємодій між GO та DS.

Ключові слова: Альгінат, Графен оксид, Гідроксиапатит, Структурні та механічні властивості, Структурне моделювання, Вивільнення лікарських засобів.