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Article

Recrystallization of $CaCO_3$ submicron magnetic particles in biological media

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Abstract. ***Background and Objectives*:** The development of magnetic theranostics is associated with the determination of the behavior of magnetic carriers in biosimilar media. In this work, we analyze the formation of different crystalline phases from magnetic mineral submicron calcium carbonate particles during incubation under conditions of cell cultivation *in vitro* for 3 days. The study of mineralmagneticsubmicron particles recrystallization was analyzed by XRD and electron scanning microscopy. The shape of calcium carbonate particles begins to change from elliptical to spherical under cell culture cultivations. As the amount of magnetite nanoparticle particles in calcium carbonate increases, the recrystallization process is faster with fallout of calcite, vaterite and magnetite phases. ***Materials and Methods*:** Scanning electron microscopy, processing of results using a self-written Python code, XRDwere utilized in this study. Results: The study of the process of recrystallization of magnetic mineral particles shows has shown that increasing the content of magnetic carriers leads to accelerated recrystallization of particles with simultaneous precipitation of calcite, vaterite and magnetite phases. ***Conclusion*:** Magnetic mineral submicron calcium carbonate particles are promising targets for theranostics with the self-destruction property in biological environments.

Keywords: calcium carbonate, recrystallization, vaterite, magnetic nanoparticles, encapsulation

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Перекристаллизация субмикронных магнитных частиц $CaCO_3$ в биологических средах

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Аннотация. Развитие магнитной тераностики связано с определением поведения магнитных носителей в биосредах. В данной работе анализируется образование различных кристаллических фаз из магнитных минеральных субмикронных частиц карбоната кальция при инкубировании в условиях культивирования клеток *in vitro* в течение 3 суток. Исследование перекристаллизации минеральных магнитных субмикронных частиц проводили методами рентгеноструктурного анализа и электронной сканирующей микроскопии. При увеличении количества наночастиц магнетита в карбонате кальция процесс рекристаллизации протекает быстрее с выпадением фаз кальцита, ватерита и магнетита. Форма частиц карбоната кальция начинает изменяться от эллиптической к сферической. Магнитные минеральные субмикронные частицы карбоната кальция являются перспективными мишенями для тераностики, обладая свойством саморазрушения в биологических средах.

Ключевые слова: карбонат кальция, перекристаллизация, ватерит, магнитные наночастицы, капсулирование

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Introduction

Technologies for obtaining systems for theranostics of various classes of diseases are based on approaches that consist in encapsulating drugs to minimize side effects, as well as the formation of shells that would have functionalization (expanding the range of diagnostic approaches, increasing the time of circulation of the carrier in the body, active targeting of drug carriers, prolonged release of the drug, etc.) [1]. One of the basic systems for drug delivery is calcium carbonate nano- and microparticles in various polymorphic modifications, vaterite ($CaCO_3$) is most commonly used. $CaCO_3$ particles of submicron size can be obtained using various synthesis schemes. Using CO_2 barbotage, nanoscale calcite is obtained, whose shape and size can be varied by changing synthesis conditions such as temperature, synthesis time, etc. Slow carbonization yields spherical submicron calcite. The reverse emulsion method yields nanoscale carbonate with low polydispersity, and precipitation of Ca^{2+} and CO_3^{2-} solutions is the easiest and most commonly used method to produce carbonate in production [2].

The term “theranostics” was introduced, which means both diagnostic and therapeutic action of a single molecule [3]. Liposomes are most commonly used as drug carrier systems due to their ease of preparation, developed technological approaches for their preparation, but their main disadvantages are instability and low buffer capacity [4]. Calcium carbonate particles are biocompatible, biodegradable, more than half of the internal volume of the particles are occupied by pores, sensitivity to various buffers allows to achieve their self-destruction, wide possibilities in varying the shape and size of particles, which makes mineral microparticles of calcium carbonate promising drug delivery systems for various medical applications [5]. The most stable form of calcium carbonate crystal is calcite but it does not contain pores and the porous form is vaterite [6]. Two methods are often used to load the drug inside the synthesized capsule, passive – via adsorption (ADS) and active – via co-synthesis (COS) [7]. The adsorption capacity of catalase loaded by COS method is up to 6 times higher than that of catalase loaded using ADS [8].

In 2023 it was shown that the presence of a magnetic field causes the formation of small particles [9], mainly spherical vaterite or needle-shaped aragonite. The magnetic field of different frequencies changes

the morphology and adhesion of calcium carbonate deposits on the surface [10]. Shapes and sizes are very important parameters for tuning the magnetic properties of these particles. Therefore, recently there has been tremendous interest in fabrication of magnetic nanoparticles of various sizes and shapes [7]. Microcrystals doped with magnetic nanoparticles have been used as templates to fabricate hollow polyelectrolyte magnetic microcapsules and to manipulate them in space with a permanent magnet to enable their delivery and separation of materials using a magnetic field [11].

In this regard, the aim of this work was to study the process of recrystallization of magnetic mineral particles of calcium carbonate in solutions used for cell cultivation *in vitro*, during 3 days with a subsequent characterization of particle morphology by scanning electron microscopy (SEM) and formed crystalline phases by X-Ray diffraction analysis (XRD).

1. Materials and methods

1.1. Materials

Calcium chloride ($CaCl_2$), natrium carbonate (Na_2CO_3), iron (III) chloride ($FeCl_3$), iron (II) chloride ($FeCl_2$), sodium hydroxide (NaOH), citric acid ($C_6H_8O_7$) were purchased from Sigma-Aldrich (German), glycerol ($C_3H_8O_3$) was purchased from LenReactiv (Russia). Deionized (DI) water from Milli-Q was used to make all solutions.

1.2. Preparation magnetic carriers

Magnetite nanoparticles (MNPs) were obtained by chemical precipitation of iron (II) and (III) salts [12]. $CaCO_3$ particles were synthesized via precipitation reaction by mixing $CaCl_2$ and Na_2CO_3 solutions in glycerol under vigorous stirring. The obtained vaterite was washed three times in deionized water and afterwards dried by lyophilization method [13].

Doping of $CaCO_3$ with magnetic nanoparticles (1.7 mg/mL) was performed by the freezing method [14]. 10 mg of vaterite was incubated at –20°C in the presence of 2 ml of MNPs solution and then washed with deionized water. For samples with 3 loading steps, the above steps were repeated 2 more times.

1.3. Studying the process of degradation magnetic carriers

Samples with 1 and 3 loads of magnetic particles were topped up to 2 ml with Dulbecco's Modified Eagle Medium (DMEM, cell culture, content: 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate, ThermoFisher, USA). The control samples were immediately centrifuged at 6000 rpm for 1 min, the supernatant was removed, and the samples were frozen. The samples for recrystallization were placed in a thermoshaker at a constant temperature of 37°C with constant stirring at 500 rpm for 1 day. After 1 day, 2 samples were washed in the above described manner, the remaining samples were left on the thermoshaker for another 1 and 2 days respectively with the same washing method. All the obtained samples were lyophilically dried (Labconco, USA) for X-ray analysis (Fig. 1).

1.4. X-ray phase analysis

X-ray phase analysis of powder samples was carried out using an X-ray diffractometer DRON-8T, manufacturer's software (JSC IC Burevestnik, Russia), PDF-2 database version 2.2102 (International Center for Diffraction Data, USA, version 2021) and Crystallography Open Database (http://www.crystallography.net/). The powders were placed in placer on a low background oriented monocrystalline silicon substrate. X-ray diffractograms were recorded using CuK α-radiation, a parabolic Goebel mirror, and a Mythen 2R1D position-sensitive detector with 640 channels. The registration discreteness of the 2θ angle was 0.0144°. The focal beam position was fixed ($\theta F = 1°$), and the detector position θD was varied so that the range of angles $2\theta = \theta F + \theta D$ was from 10 to 100°. The step for the center channel of the detector was 0.2°, and the point exposure time was 6 s. Information on the quantitative phase composition was obtained by full-profile analysis of X-ray diffractograms using the Rietveld method.

1.5. SEM analysis

During recrystallization, the surface morphology of the magnetic calcium carbonate particles was characterized using a MIRA II LMU scanning electron microscope (SEM) (Tescan, Czech Republic), operating voltage was 30 kV under secondary electron mode. Analysis of SEM-images with the help of DemoVega software and a code written in Python programming language allowed them to construct a size distribution, and visualization of the obtained data was performed with the help of Origin software.

2. Results and discussion

Using the protocol described in Paragraph 1.2, samples of calcium carbonate particles were obtained with one and three cycles of freezing and

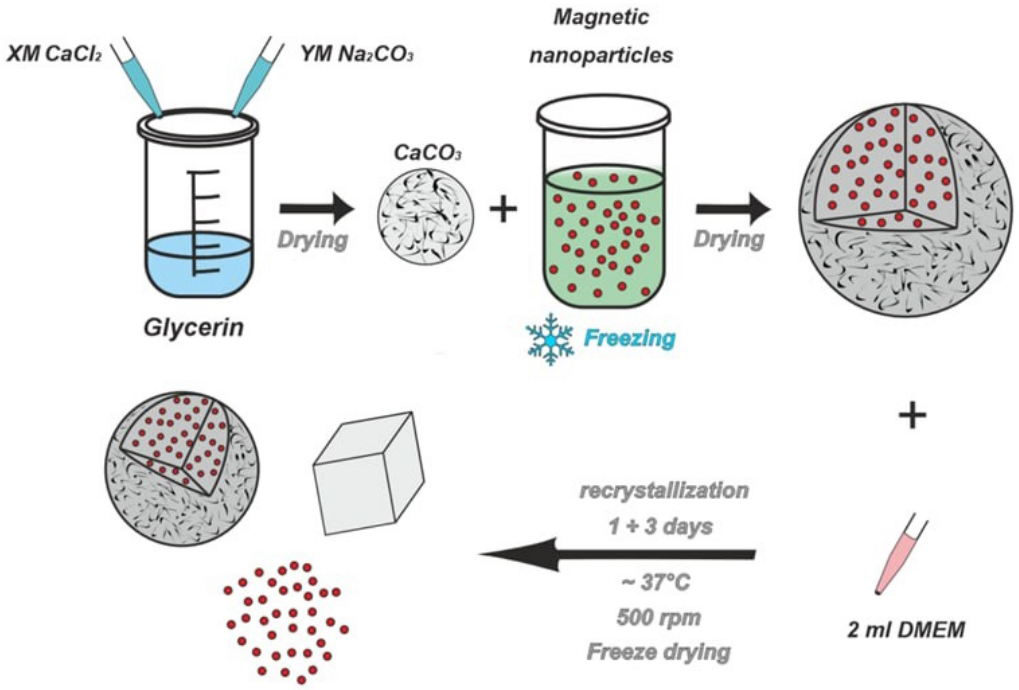

Fig. 1. Scheme for obtaining magnetic particles of calcium carbonate vaterite followed by their recrystallization with precipitation of calcite and magnetite phases (color online)

thawing of magnetite nanoparticles, which exposed to cell medium at +37°C under constant agitation with sampling every 24 hours for 3 days for analysis by SEM and XRD. Schematically, the steps of our experimental work shown in Fig. 1; the experimental results relating particle surface morphology, particle size distribution and XRD data shown in Fig. 2.

Analysis of XRD spectra (Fig. 2) shows that there is no recrystallization of vaterite with calcite and magnetite phase falling separately. It is impossible to visualize the change in diameter of particles with 3 freezing cycles over time during recrystallization due to the fact that clusters of particles are formed, where it is difficult to isolate individual particles for further analysis in contrast to particles with 1 cycle of magnetic nanoparticles introduction (Fig. 2). According to XRD data, the precipitation of calcite and magnetite phase occurs, which also indicates that the recrystallization process occurs under conditions close to the conditions of cell cultivation *in vitro*.

The diameter of particles with 1 cycle of freezing of magnetic nanoparticles when incubated in nutrient medium during 3 days, a coarse fraction of particles appears (Table), while the number of elliptical particles decreased. The means of major b and minor a axes of elliptical particles increases by the 3rd day, but the ratio (b/a) of the mean of major axis of elliptical particles to minor axis decreases (Table). Elliptical particles with magnetic nanoparticles are rounded when incubated in nutrient media over time.

In Ref. [15] the authors observed an intense diffraction peak around 29.4° (2θ), due to diffraction (104), and low-intensity peaks around 23.1, 31.4, 36.0, 39.4, 43.2, 47.1, 47,4, 48.5, 56.6, 57.5, 60.7 and 64.8° assigned to (012), (006), (110), (113), (202), (024), (018), (116), (211), (112), (122) and (220), which confirms the formation of $CaCO_3$ nanoparticles with the calcite polymorph. The resulting materials had a clearly defined crystalline structure and were in good agreement with the JCPDS standard sample (JCPDS card no: 86–0174) for similar calcite nanoparticles. In the study of the recrystallization process, the increase of magnetite phase from 0 to 3 days of observation increased, which is characterized by noise amplification of XRD spectra, as well as the observation of characteristic peaks at angles 35, 36, 47, 68° (2θ) (Fig. 2). For calcium carbonate samples with a single freeze-thaw cycle, no precipitation of the calcite phase was observed after 3 days of incubation under cell line culturing conditions, only the vaterite phase persisted (characteristic vaterite peaks at angles 21, 50, 55° (2θ), Fig. 2, *a*–*d*). When the amount of magnetite in the particles increases, the presence of calcite phase in the original sample and its complete disappearance by the third

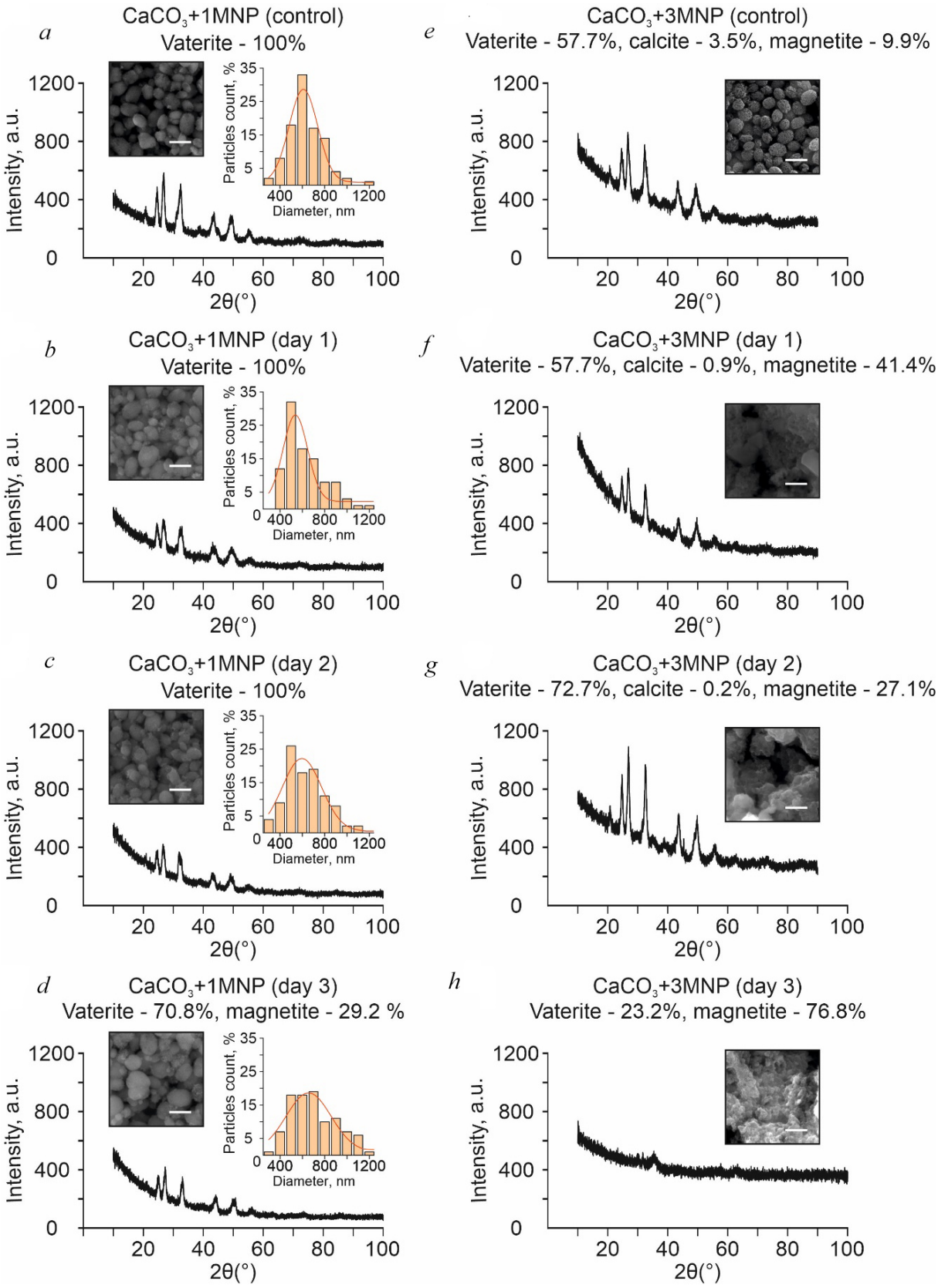

Fig. 2. Characterization of shape and size (SEM-images (*a–h*), XRD spectra (*a–h*), particle size distribution (*a–d*) with theoretical normal distribution curves (red lines in particle size distribution graph, *a–d*) constructed on the basis of experimental data using modeling) of calcium carbonate particles obtained at the ratio of $CaCl_2$ and Na_2CO_3 salt concentrations 1.00 : 1.00 with 1 (*a–d*) and 3 (*e–h*) freezing/thawing cycles in magnetite nanoparticle solutions with observation of particle recrystallization in cell media at +37°C for 3 days. Scale on SEM images – 1 μm

Table. Percentage ratio of spherical and elliptical (*a*, *b* – arithmetic mean values of lengths of major and minor axes of elliptical particles, *x*, *y* – arithmetic errors of values of lengths of major and minor axes of elliptical particles) particles in relation to the total number of particles in the group, diameter of particles in longitudinal and transverse parts of particles and calculation of their ratio to determine the approximation of the shape of particles to different groups

Parameters	Control	1st day recrystallization	2nd day recrystallization	3rd day recrystallization
Number of round particles, %	9.72	2.05	3.36	4
Number of elliptical particles, %	90.28	97.95	96.64	96
$\bar{b} \pm \bar{x}$, nm	682 ± 121	683 ± 153	678 ± 144	769 ± 183
$\bar{a} \pm \bar{y}$, nm	502 ± 77	534 ± 111	524 ± 107	606 ± 142
$\bar{b}/\bar{a}$	1.36	1.28	1.29	1.27

day of observation was observed (characteristic calcite peaks at angles 48, 57° (2θ), Fig. 2, *e–h*).

Conclusion

The variety of magnetic carriers for drug delivery is limited by the specificity of their behavior in tests simulating real application conditions. In our work it has been shown that magnetic mineral particles of calcium carbonate undergo changes in shape and size under conditions of cell cultivation. As the magnetic phase in the media increases, there is a more abrupt transition from elliptical porous to spherical porous particle shape, and then to non-porous cubic particle shape. The diameter of the particles increases at the same time. Thus, magnetic nanoparticles are the driver of the recrystallization process of mineral particles, which subsequently self-destruct. Along with many approaches to changing the shape and size of calcium carbonate particles, it is our approach that is the most reproducible, which gives it an advantage for use in further technological processes when scaling up production.

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