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Microscopic and spectral study of the release kinetics of betamethasone dipropionate from vaterite carriers in aqueous media

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The development of novel delivery systems for water-insoluble glucocorticosteroids (GCSs) enabling their transportation to the inflammatory site in skin is essential for improvement of their therapeutic efficiency. In this context, the design of different carriers for GCS encapsulation, which allows enhancement of the local drug concentration and, thus, reduction of the overall dose and side effects for this GCS, is of particular interest. Here, we propose to use porous metastable submicron vaterite particles, since such carriers possess a high sorption capacity and ability to release the encapsulated drug during their degradation. We demonstrated the possibility of their efficient loading with betamethasone dipropionate (BD) glucocorticosteroid. A comprehensive assessment of the BD encapsulation was performed using a combination of various spectroscopic methods and electron microscopy. In addition, we optimized the methodology for studying the BD release kinetics in model aqueous media *in vitro*. Namely, the introduction of a nonionic surfactant as a solubiliser to the aqueous suspension of the BD-loaded carriers provided an increase in accuracy for spectroscopic determination of the drug amount released at different time intervals. The data obtained in the study of drug release kinetics using the proposed method demonstrated a good correlation with the results of carriers' morphology monitoring by means of scanning electron microscopy and energy dispersive X-ray spectroscopy.

Keywords: Raman spectroscopy, UV spectrophotometry, scanning electron microscopy, energy dispersive X-ray spectroscopy, glucocorticosteroids, encapsulation, vaterite, targeted drug delivery.

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Introduction

Glucocorticosteroids (GCSs) are important agents for treatment of a wide range of inflammatory diseases, which includes dermatoses of various nature [1–3]. However, non-rational use of GCSs (i.e., long-term or uncontrolled use, dose escalation, and unfounded change of the used steroid class) has many side effects, which may include exacerbation of chronic diseases and development of secondary infections [4]. In this regard, the development of approaches ensuring effective use of existing GCS drugs is an important problem. An increase in their local concentration and the likelihood of accumulation in the skin and its appendages may promote the improvement of therapeutic efficacy of topical glucocorticoids.

The use of various targeted delivery systems is considered to be a promising way to increase the local bioavailability of GCSs [5]. In recent decades, an approach based on the immobilization of GCSs into various nanosized and submiron carriers (liposomes; niosomes; ethosomes; micelles; nanoemulsions; polymer, composite, or inorganic particles; etc) has become widespread [6,7]. Topical application of such a carriers facilitates the drug transportation through the skin. Moreover, it enables the control of the payload release profile.

In the context of GCS immobilization, the use of porous submicron calcium carbonate ($CaCO_3$) particles in the form of vaterite, which are easy to synthesize and have a low cost, is of great interest [8]. The high porosity of vaterite particles allows for efficient loading of a wide range of biologically active substances within their volume [8–10]. The feasibility of using vaterite carriers for drug encapsulation is also attributed to their biocompatibility [11–13] and biodegradability [14,15]. In addition, it has been demonstrated numerous times the payload release from such carriers is driven by their degradation, both *in vitro* [16–18] and *in vivo* [14,19– 21].

In the current research, the possibility of betamethasone diproportionate (BD)immobilization into vaterite carriers was studied.. The structural formula of BD is presented in Fig. 1, *a*. This drug is a GCS of the first class of activity (drugs of the highest potency) and is capable of providing a rapid therapeutic effect in the treatment of non-infectious inflammatory dermatoses [22]. The efficiency

of BD immobilization into vaterite particles was evaluated by UV spectrophotometry, high-performance liquid chromatography (HPLC), and Raman spectroscopy (RS). The process of BD release from the carriers in model aqueous media was studied using combination of UV and energy-dispersive X-ray spectroscopy (EDXS) with scanning electron microscopy (SEM). In order to improve the reliability of the BD concentration estimation, the effect of a polar solvent and a nonionic surfactant (nSA) on the solubility of the drug in an aqueous carrier suspension was also assessed.

Materials and methods

Materials

Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), sodium carbonate (Na_2CO_3), ethylene glycol (EG), betamethasone dipropionate (BD), polysorbate 20 (Tween 20) (all the above reagents were produced by Sigma-Aldrich, United States), ethanol 96% and hydrochloric acid (HCl) (EkoKhimAnalit, Russia), high-purity acetonitrile (Cryochrom, Russia), and Dulbecco's phosphate-buffered saline (DPBS) without calcium and magnesium (Gibco, United States) were used in the study. All chemical reagents were subjected to additional purification. Deionized water obtained from the Milli-Q Purification System, Millipore, Merck, USA was used in all the experiments. The ethanol concentration was 96% unless otherwise stated.

Synthesis of the BD-loaded vaterite carriers

At the first stage, the unloaded vaterite particles were synthesized in a mixture of water and EG [17]. To this end, equal volumes of 1-M $CaCl_2$ and Na_2CO_3 solutions were first mixed with EG in a ratio of 1:5 and then mixed with each other under constant stirring using an IKA RO 10 (IKA, Germany) magnetic stirrer operating at a rate of 700 rpm for 2.5 h at room temperature. The solid phase ($CaCO_3$ particles) was separated from the residual solution using an Eppendorf 5810 R (Eppendorf, Germany) centrifuge at 5000 rpm within 10 min. In order to remove residual EG and inorganic ions, the sediment was rinsed twice with deionized water and ones with ethanol 70%. The obtained particles were freeze-dried at a temperature of -50°C for 2 h using a FreeZone system (Labconco, United States).

Further, the BD drug was immobilized into the synthesized carriers using a crystallization-induced adsorption method [23]. To do this, 40 mg of vaterite particles were mixed with 0.5 mL of a solution of BD (2 mg/mL) in ethanol, and the obtained mixture was diluted with water to 2 mL. The ethanol concentration in the resulting mixture was 24%. The obtained suspension of vaterite particles was vigorously agitated using a V-3 vortex (ELMI, Latvia)vigorously agitated using a V-3 vortex (ELMI, Latvia) and kept in a freezing chamber at -20°C under the slow constant mixing (TetraQuant, Russia). Following complete freezing of this suspension, the samples were thawed at room temperature. The solid phase ($CaCO_3$+BD particles) was then separated using an Eppendorf 5430 (Eppendorf, Germany) centrifuge. The supernatant was removed for spectrophotometric determination of the BD concentration, the BD solution was again added to the particle sediment, and the entire freezing/thawing/supernatant sampling cycle was repeated. Following that, the containers were again freeze-dried for 8 h.

The morphology of the obtained $CaCO_3$ and $CaCO_3$+BD containers was studied by SEM using a MIRA II LMU (Tescan, Czech Republic) setup at an operating voltage of 20 kV. The obtained SEM images were processed in ImageJ software to calculate the average size of the carriers. A minimum of 200 measurements per one sample image was performed.

Spectral study of the BD loading efficiency

The BD content in the carriers was assessed with the use of UV spectrophotometry and HPLC with a photometric detector. Spectrophotometric studies were performed following two distinct protocols. The first one involved estimating the amount of BD loaded into vaterite particles as a difference between its known initial concentration and the determined residual concentration of BD in the supernatant. The optical density of solutions was measured in a standard quartz cell ($l = 1$ cm) with a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan); the calibration curve was obtained in a 1:4 ethanol-water mixture (24% ethanol). The maximum of the absorption spectrum of BD in 24% ethanol was at a wavelength of 239 nm (Fig. 2, *a*). This and subsequent calibration curves were plotted without subtraction of the baseline from the optical density spectra of BD solutions.

The second protocol was meant for estimating the efficiency of BD release from the carriers after their dissolution by adding hydrochloric acid. In this case, the optical density of solutions was measured with a CLARIOstar Plus (BMG Labtech, Germany) multi-mode plate analyzer. To release all of the immobilized GCS, $CaCO_3$+BD particles were dissolved according to the following reaction:

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 \uparrow + H_2O.$$

For this purpose, 0.2 M HCl was added dropwise to a weighed portion of the carriers and mixed thoroughly with them until the $CaCO_3$ phase was dissolved completely. Following that, ethanol was added, and the mixture was stirred again until the released BD was dissolved completely. The 0.2 M HCl/ethanol ratio in the resulting mixture was 1:4. The prepared ethanol solution contained BD and Ca^{2+} and Cl^- ions. A calibration curve was plotted in this case by dissolving a weighed portion of unloaded vaterite particles in a similar way and then adding a known amount of BD powder. The maximum of the absorption spectrum of BD in this mixture was at a wavelength of 242 nm.

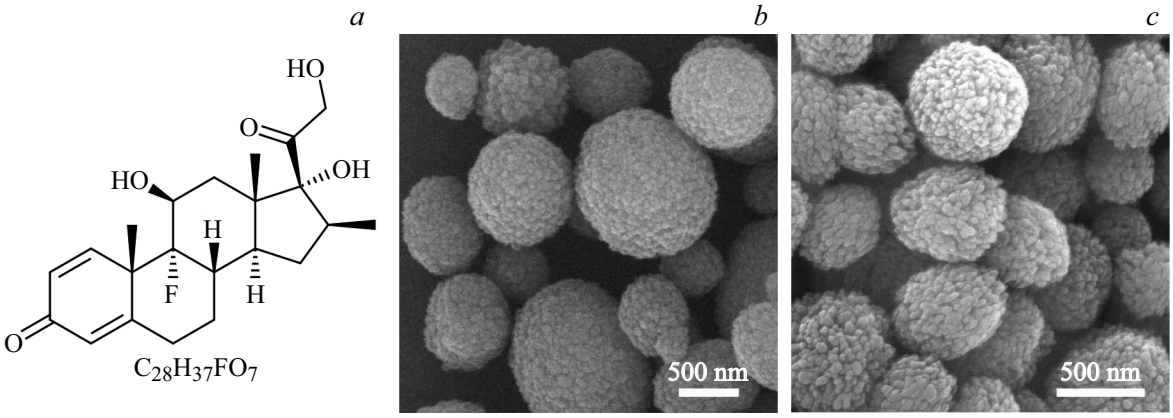


Figure 1. Structural formula of BD (*a*). SEM images of unloaded $CaCO_3$ containers (*b*) and containers loaded with BD (*c*).

HPLC studies of supernatants collected for analysis during the synthesis of $CaCO_3$+BD carriers were performed using an LC-20 Prominence (Shimadzu, Japan) chromatograph. A Spherisorb ODS2 C18 column (80 Å, 5 μm, 4.6 × 250 mm) was used in measurements; acetonitrile (mobile phase A) and deionized water (mobile phase B) in a ratio of 52/48 (A/B) served as mobile phases. Detection was performed at a wavelength of 254 nm. The characteristic chromatographic peak of BD was observed at a retention time of 12 min 13 s (Fig. 3, *a*).

The efficiency of the carrier loading with BD (loading capacity, %LC) was calculated as follows:

$$\%LC = \frac{m_{\text{BD in}(CaCO_3+\text{BD})}}{m_{(CaCO_3+\text{BD})}} \times 100, \qquad (1)$$

where $m_{(CaCO_3+\text{BD})}$ is the mass of the carriers and $m_{\text{BD in}(CaCO_3+\text{BD})}$ is the mass of BD in these carriers.

Raman spectroscopy studies were also carried out to verify that BD was immobilized into the vaterite particles. A Renishaw InVia spectrometer (Renishaw, UK) with a laser excitation wavelength of 532 nm and an 50× /0.5 n.a. objective was used for measurements. Raman spectra of dry weighed portions of $CaCO_3$, and $CaCO_3$+BD and BD powders were measured separately. Raman spectra were recorded at several points of each sample at a laser power of 2.5 mW; the recording time was 10 s. The peak position error was less than 0.1 cm^{-1} (the system was calibrated prior to measurements after laser thermal stabilization with the use of a reference silicon sample).

To quantify the BD content in the studied samples via Raman spectroscopy, a calibration curve was plotted by measuring the spectra of a BD solution in ethanol within the 0.0625−1.0000 mg/mL BD concentration range. Prior to measurements, a droplet of the studied solution of 20 μL was distributed uniformly over the surface of a quartz substrate and left to dry. The study was carried out at a laser power of 12.5 mW; the recording time was 30 s. The chemical composition of objects was identified in a 7 × 7 array of points with a step of 20 μm. A total of 49 individual Raman spectra were obtained for each sample and used to calculate the signal averaged over a sample. The calibration curve was plotted based on the Raman signal intensity at the peak with a wavenumber of 1660 cm^{-1} that corresponds to BD. Similar measurements were performed for supernatants collected during the synthesis of $CaCO_3$+BD carriers, which were pre-concentrated by a factor of 10. This was done by lyophilizing 0.5 mL of the examined supernatant and resuspending the required weighed amount of BD in 50 μL of ethanol.

Kinetics of BD release from the carriers in water

At the first stage, the process of BD release from the obtained carriers was studied in deionized water. Water (1 mL) was added to weighed portions of $CaCO_3$+BD particles (4 mg), the samples were then introduced into a Digital Shaking Drybath shaker (Termo Scientific, United States) and incubated at room temperature under continuous shaking for 96 h. Three separate tubes with particle samples were prepared for each time point (5 min, 24, 48, 72, and 96 h of incubation).

The change of state of the carriers over time was assessed based on morphological investigation using SEM data by sampling 1 μL of suspensions for analysis at each specific time point. BD release profiles were studied by UV spectrophotometry. In order to dissolve the crystals of BD released by a certain time point, 1 mL of a polar proton-donor solvent (ethanol) was added to the suspension of $CaCO_3$+BD carriers and mixed thoroughly prior to measuring the absorption spectra [24]. The samples were then centrifuged for 2 min at 5000 rpm, and supernatants were collected for spectrophotometric analysis. A weighed amount of BD powder was dissolved in a 1:1 ethanol-water mixture to plot the corresponding calibration curve.

Kinetics of BD release from the carriers in an ethanol-water mixture

At the next stage, the release profile of BD from the carriers was studied in an ethanol-water mixture (1:1). A

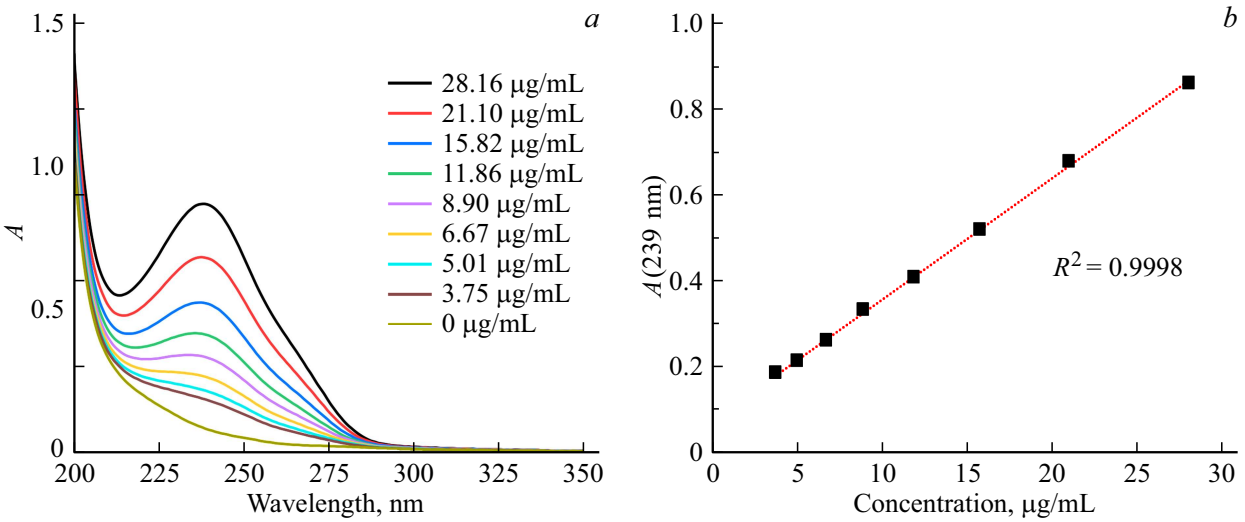


Figure 2. Absorption spectra of BD solutions of various concentrations in 24% ethanol (*a*) and calibration curve for spectrophotometric determination of BD in containers (*b*).

weighed portion (20 mg) of $CaCO_3$+BD particles was introduced into an ethanol-water mixture (20 mL in volume) and incubated for 72 h at room temperature under continuous stirring with a shaker. Samples (2 mL) of the studied suspension were collected at different time points (5 min, 24, 48, and 72 h of incubation). Following centrifugation of these samples, the sediment was analyzed by SEM, while supernatants were analyzed by UV spectrophotometry.

Kinetics of BD release from the carriers in a DBPS-Tween 20 mixture

At the final stage, the kinetics of BD release from the carriers in DPBS with the addition of an nSA (Tween 20) was studied. A dialysis bag with a pore diameter of 14 kDa containing a weighed amount (40 mg) of $CaCO_3$+BD particles and 5 mL of a DPBS-Tween 20 mixture (0.1%) was used for incubation. The sample was incubated for 144 h in 40 mL of the DPBS-Tween 20 mixture under continuous stirring with a magnetic stirrer at 37°C. The sample aliquots were taken at different time points (5 and 30 min and 1, 6, 24, and 144 h) and their optical density was assessed. Following incubation, the suspension of containers was studied by SEM and EDX using an Inca Energy 350 spectrometer (Oxford Instruments, UK).

Results and discussion

Immobilization of BD into the vaterite carriers

Vaterite carriers were synthesized by crystallization from solution by mixing the salts of calcium chloride and sodium carbonate [25]. It is known that an amorphous $CaCO_3$ precipitate forming in the process of such a synthesis as a result of rapid mixing of the reaction solution transforms into ordered spherulites due to colloidal aggregation. Varying the process conditions, one may obtain spherulites of various sizes ranging from 0.4 to 50 μm [26,27]. We aimed at producing submicron particles, since this size was shown optimal in terms of accumulation in skin appendages [14]. The synthesis was carried out in a viscous medium of water-ethylene glycol mixture [26,28], which enables the formation of porous spherical $CaCO_3$ particles with an average size of $0.9 \pm 0.4\,\mu$m (Fig. 1, *b*).

The obtained particles were loaded with a water-insoluble BD drug via crystallization-induced adsorption. This method involves the incorporation of organic molecules into vaterite particles in an aqueous medium with controlled freezing of their suspension. The active substance is then loaded into the pores of vaterite under the influence of a moving crystallization front of the solution [23]. This method allows the active substance to be loaded from a water-alcohol mixture when the suspension has frozen completely. In the present study, BD was immobilized into the vaterite particles from 24% ethanol. The SEM image of the obtained carriers is shown in Fig. 1, *c*.

According to the UV spectrophotometry data, the mass of BD in 1 mg of the obtained carrier powder was $49 \pm 6\,\mu$g (%$LC = 4.9$ % w/w). This exceeds significantly the concentration of the active substance in conventional pharmaceutical products (creams, emulsions, and ointments; e.g., Beloderm and Akriderm), which contain 0.05% BD, and opens up new opportunities for enhancing the efficacy of this GCS. It is important to note that the loading capacity estimated in different ways (i.e., by analyzing the supernatant during synthesis and by the carrier dissolution) matched perfectly; therefore, these two methods may be used for characterization as equivalent.

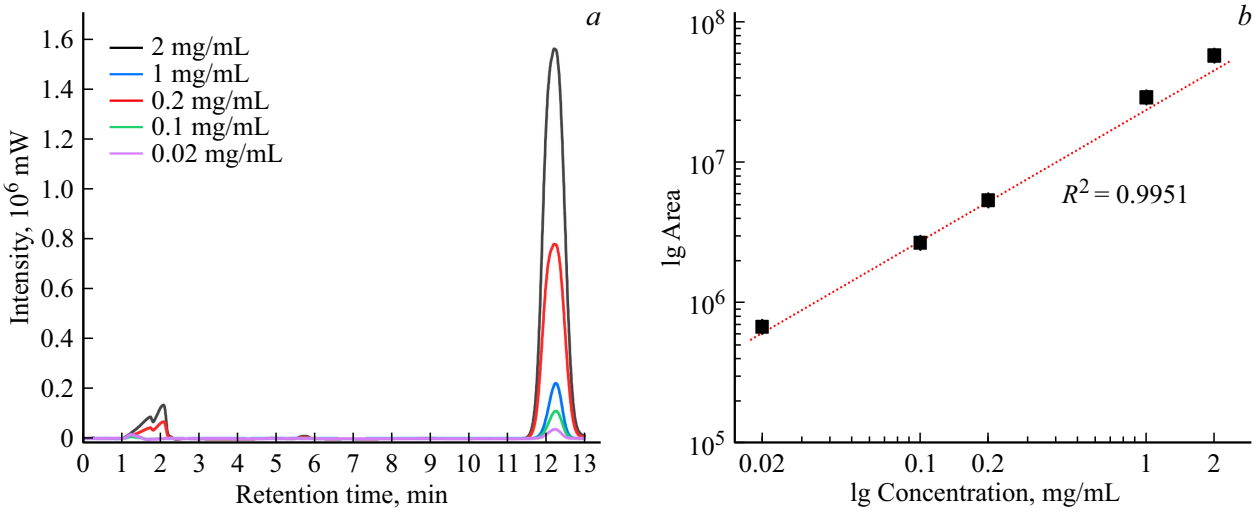


Figure 3. Chromatograms of BD solutions of various concentrations (*a*) and calibration curve plotted based on them (*b*). The BD retention time was 12 min 13 s.

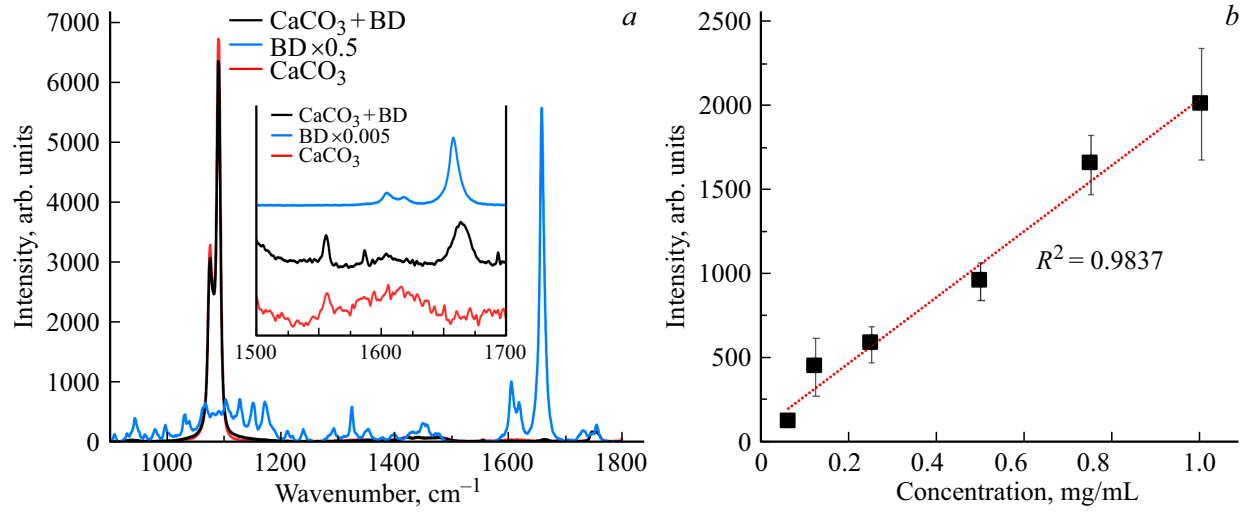


Figure 4. Raman spectra of the BD-loaded vaterite carriers (black curve, $CaCO_3$+BD) in comparison with unloaded vaterite particles (red curve, $CaCO_3$) and BD powder (blue curve, BD) (*a*). Calibration curve for the determination of BD in ethanol by Raman spectroscopy at the peak with a wave number of 1660 cm^{-1} (*b*). Data points correspond to „mean ± standard deviation" values calculated based on 49 independent measurements at each point.

The absorption spectra of the BD solutions of various concentrations in 24% ethanol and the calibration curve for its spectrophotometric determination are presented in Fig. 2.

HPLC data revealed that 1 mg of the obtained carriers contain 48 ± 3 μg of BD, which is within the error of spectrophotometric determination of the BD concentration. The chromatograms of BD solutions of various concentrations and the calibration curve are shown in Fig. 3.

Raman spectra of the obtained containers (Fig. 4, *a*) were also studied to verify the fact of inclusion of BD into the vaterite matrix. Vaterite (1075 and 1089 cm^{-1}) [29] and glucocorticoid (1660 cm^{-1} in the inset of Fig. 4, *a*) [30,31] peaks in the measured spectra of $CaCO_3$+BD containers indicated that the drug was present in the vaterite matrix.

Raman spectroscopy was also used to quantify the BD content in solutions. To this end, a calibration curve was plotted using the average intensities of the Raman signal at the peak with a wave number of 1660 cm^{-1} for BD solutions within the 0.0625–1.0000 mg/mL concentration range (Fig. 4, *b*). When the obtained points were approximated with a linear function, coefficient of determination R^2 was 0.984; i.e., Raman spectroscopy is generally suitable for the determination of BD, but the large spread of Raman signal intensity values at each point is indicative of a slightly worse reproducibility of the results (compared to the spectrophotometric method).

The use of Raman spectroscopy to examine one of the supernatants collected during the synthesis of $CaCO_3$+BD

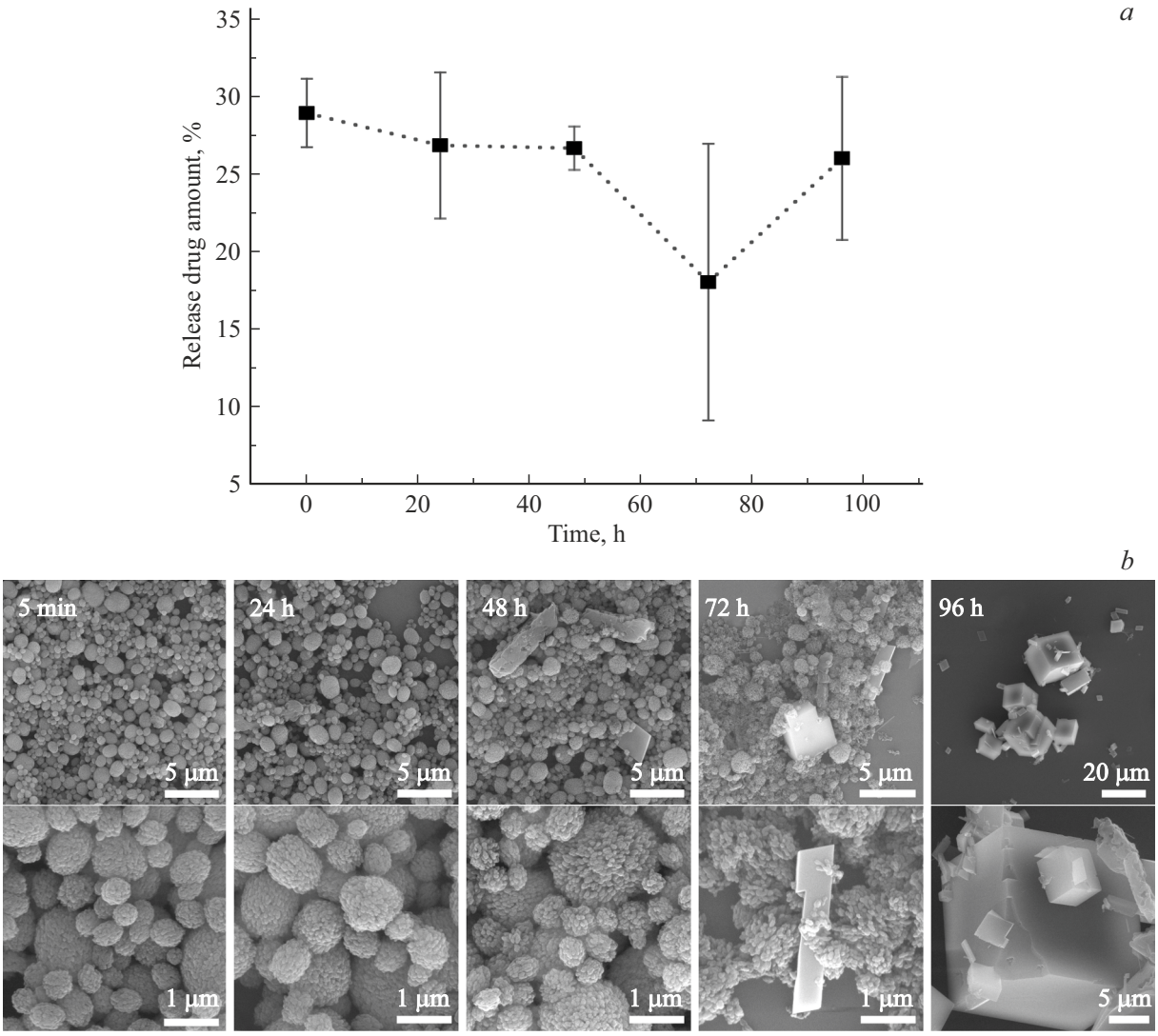


Figure 5. Kinetics of BD release from the carriers in deionized water (*a*). Typical SEM images of the $CaCO_3$+BD carriers obtained at different time points upon their incubation in deionized water: 5 min, 24, 48, 72, and 96 h (*b*).

carriers and concentrated by a factor of 10 prior to measurements allowed us to establish that the concentration of BD in the concentrate was 0.22 ± 0.03 mg/mL; i.e., the concentration in the original solution was 0.022 mg/mL. This agrees in general with the results of spectrophotometric analysis (0.018 mg/mL), but confirms that the error of determination by Raman spectroscopy is higher (the mean values differ by 22%) due to the lower sensitivity of this method. Based on these results, we chose the spectrophotometric method for further study of the kinetics of drug release from the $CaCO_3$+BD carriers in aqueous media.

Study of the BD release from the carriers in aqueous media

The immobilized drug gets released from porous $CaCO_3$ carriers and undergoes a the dissolution in the aqueous phase as a result of its desorption and degradation of the carriers [10]. Thus, the release profile represents the interaction of these two processes and depends on the dispersion medium [17]. Specifically, if the solvent cannot convert efficiently the immobilized substance into a molecularly dispersed state, the desorption process is slow. In contrast, this process is intensified if the medium is a suitable solvent that penetrates into the vaterite matrix and dissolves the drug contained within, ensuring its rapid diffusion in the medium. In addition, it is known that vaterite, being a metastable form of calcium carbonate, recrystallizes into thermodynamically stable calcite in an aqueous medium [32,33]. The speed of this process also affects the rate of release of the immobilized substance. The released drug may either pass into the solvent or precipitate out (if its solubility in a given medium is limited) [10].

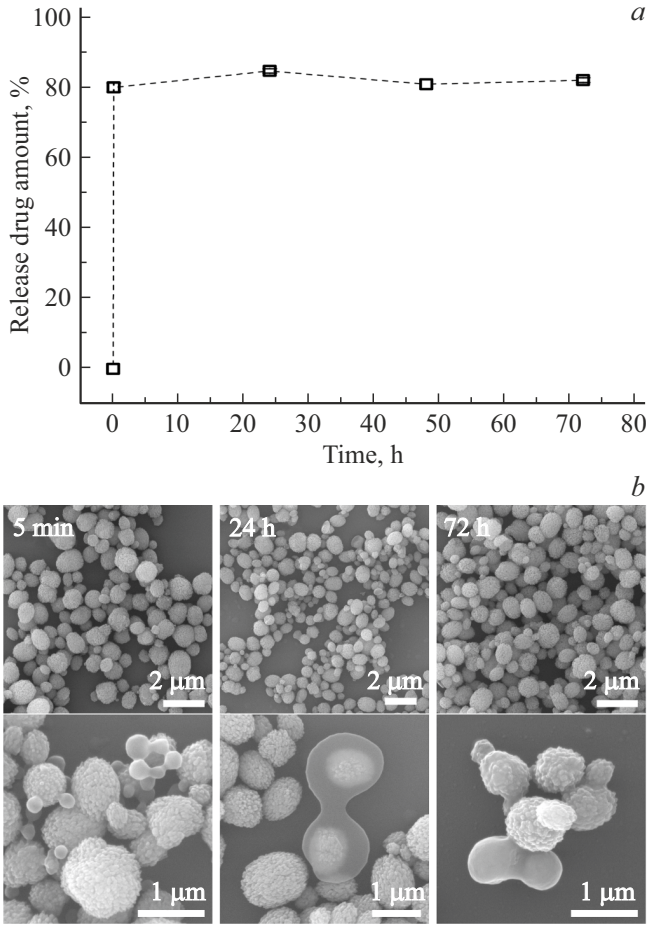


Figure 6. Kinetics of BD release from the carriers in 1:1 ethanol-water mixture (*a*). Typical SEM images of the $CaCO_3$+BD carriers obtained at different time points upon their incubation in a 1:1 ethanol-water mixture: 5 min, 24, and 72 h (*b*).

Modeling the drug behavior under physiological conditions, which includes the examination of the release kinetics from the carriers in aqueous media simulating biological fluids, is an important stage of the study of targeted drug delivery systems. In the present research, the kinetics of BD release from the vaterite carriers in deionized water was studied first. The drug release profile is shown in Fig. 5, *a*. Figure 5, *b* presents the typical SEM images of the $CaCO_3$+BD carriers obtained at different time upon their incubation in deionized water.

Betamethasone dipropionate is almost insoluble in water and, when released from the vaterite carriers, it forms a crystalline precipitate, distorting the data on its concentration in the studied suspensions. This is confirmed by the curve of BD release kinetics in water (Fig. 5, *a*), which features a fairly large spread of values at each specific time point. The results of SEM investigation provided an opportunity to trace structural and morphological changes that occur in the process of their incubation in water (Fig. 5, *b*). It was found that the degradation of $CaCO_3$+BD particles in an aqueous medium proceeded for 96 h. Within this period, the vaterite phase was substituted completely by the calcite phase, and porous spherical containers recrystallized into smooth cubic crystals. It is important that the majority of vaterite particles retained their original morphology 24 h after the onset of incubation; the presence of only of individual calcite crystals was noted. After 48 h of incubation, BD crystals of various shapes (prismatic, rhombohedral, and columnar) formed, and the size and number of such crystals grew with time. It should be noted that after 72 h of incubation, the majority of the carriers became amorphous, which was accompanied by the release of immobilized BD. Calcite particles then formed from amorphous calcium carbonate. SEM images reveal that the formation of large calcite crystals was accompanied by the capture of smaller particles of solid BD. A combination of the mentioned phenomena occurring over time is what induces the large spread of values and the negative slope of the kinetic curve in Fig. 5, *a*.

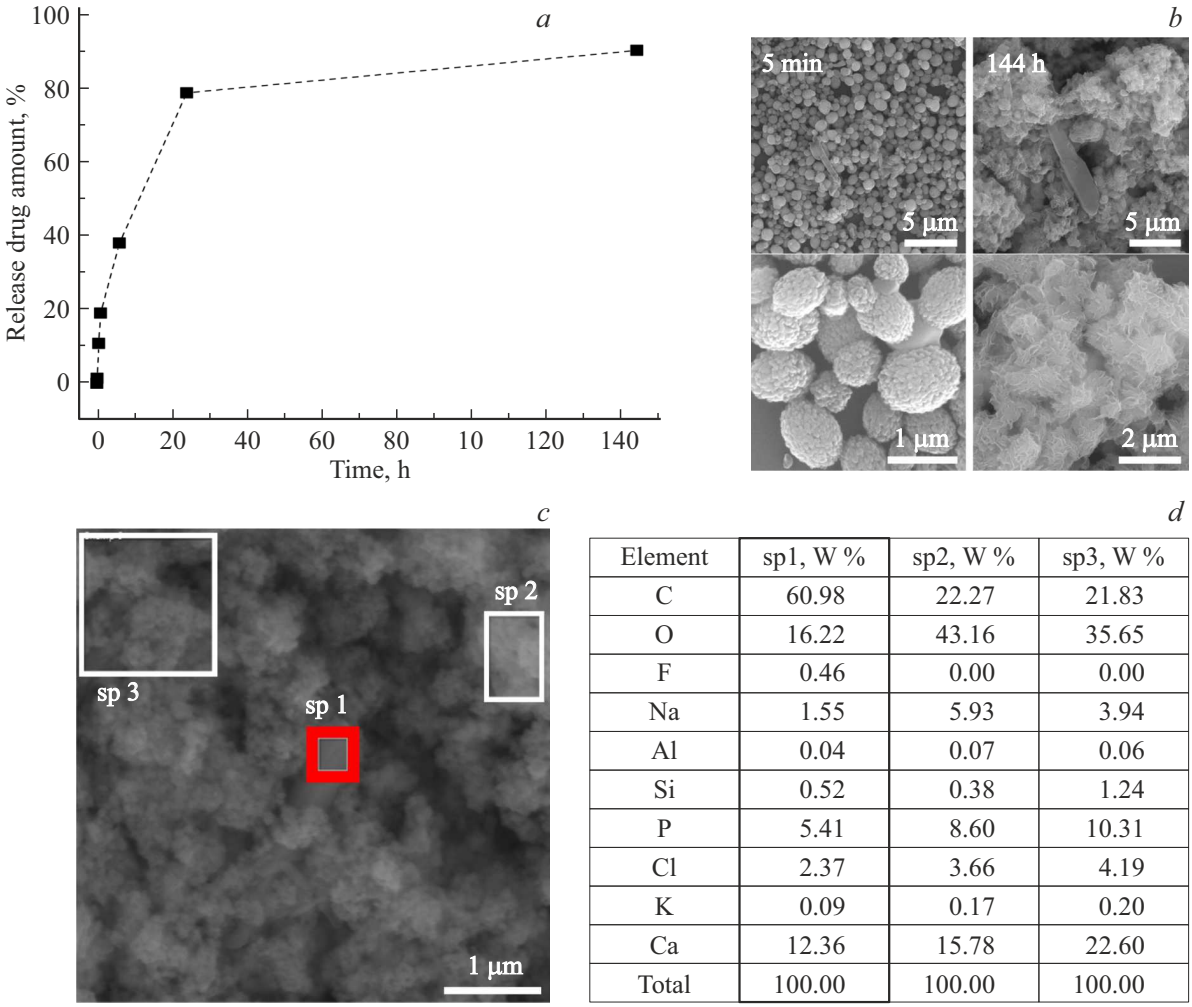
At the next stage, the release profile of BD from the $CaCO_3$+BD carriers was studied in a 1:1 ethanol-water mixture. This dispersion medium was chosen due to the need to prevent the formation of GCS crystals during its release and simulate the dynamics of this process in an „open system" [34]. The obtained results are presented in Fig. 6.

Figure 6, *a* shows clearly that the $CaCO_3$+BD carriers released up to 80% of the drug contained in them within 5 min of incubation in an ethanol-water mixture. This rapid („burst") release of BD is attributable to its higher solubility in ethanol, which penetrates into the pores of vaterite. With further incubation of the carriers in this solvent, the amount of the released drug did not increase, since all the available immobilized substance was already extracted from the solid phase in the first 5 min, and degradation of the matrix, which could contribute to the release of the remaining 20% of BD, was not observed (Fig. 6, *b*). The carriers retained their original morphology throughout the entire observation period, which agrees well with literature data that are indicative of a high stability of vaterite in ethanol [17].

Thus, the study of the release kinetics for the immobilized BD in a water-ethanol mixture was invalid. These experiments were more relevant to extraction of the drug contained in the $CaCO_3$+BD carriers than to modeling of their behavior in an open system.

At the final stage, the process of BD release was studied in an aqueous buffer solution (DPBS) with the addition of a nonionic surfactant solubiliser (Tween 20). It is known that this nSA enhances the solubility of BD in the phosphate buffer, thus providing an opportunity to model an „open system" for the studied carriers [35]. The obtained results are presented in Fig. 7.

Within the first 6 h of incubation of the $CaCO_3$+BD carriers in the DPBS-Tween 20 mixture, the drug release proceeded at a high rate (Fig. 7, *a*). A total of 38% of the immobilized BD amount was released from the carriers within this time interval. Further, the slope of the kinetic curve decreased, but the process of active release continued. In 24 h, the mass of released BD doubled; i.e.,



Element	sp1, W %	sp2, W %	sp3, W %
C	60.98	22.27	21.83
O	16.22	43.16	35.65
F	0.46	0.00	0.00
Na	1.55	5.93	3.94
Al	0.04	0.07	0.06
Si	0.52	0.38	1.24
P	5.41	8.60	10.31
Cl	2.37	3.66	4.19
K	0.09	0.17	0.20
Ca	12.36	15.78	22.60
Total	100.00	100.00	100.00

Figure 7. Kinetics of BD release from the carriers in a DPBS-Tween 20 mixture (*a*). Typical SEM images of the $CaCO_3$+BD the carriers obtained after 5 min and 144 h of their incubation in the DPBS-Tween 20 mixture (*b*). EDX data obtained after 144 h of the carrier incubation in the DPBS-Tween 20 mixture (*c*): the studied regions are marked with rectangles in the SEM image; the elemental composition of the sample (W%) corresponding to these regions is presented in the table (*d*).

77% of the drug was released from the carriers within 24 h. The kinetic curve then became flatter and reached saturation. By the end of the carriers incubation in the DPBS-Tween 20 solution (in 144 h), 90% of the drug was released from the carriers. According to the SEM data (Fig. 7, *b*), the remaining 10% precipitated out and formed columnar crystals. The results of EDX analysis of the elemental composition of these crystals confirmed that they were related to BD, since fluorine (Fig. 1, *a*) was found in the spectra recorded at the corresponding sections of the sample (sp 1 in Figs. 7, *c, d*).

In addition, the SEM image obtained after 144 h of incubation of $CaCO_3$+BD particles in DPBS revealed the formation of hydroxyapatite crystals in the sample (spherical particles with a spike surface in Fig. 7, *b*), which agrees well with literature data indicating the transformation of vaterite particles into hydroxyapatite ones upon incubation in phosphate-buffered saline [36]. The EDX analysis of formed structures confirmed the presence of calcium, phosphorus, and oxygen atoms, which are characteristic of hydroxyapatite, in the samples (sp 2 and sp 3 in Fig. 7, *c*). Presumably, hydroxyapatite particles formed according to the following exchange reaction [37]:

$$10CaCO_3 + 6Na_2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6Na_2CO_3 + 4H_2CO_3.$$

Thus, vaterite carriers loaded with BD were found to remain relatively stable in the DPBS-Tween 20 mixture for an hour. Further, the process of the immobilized drug release intensified, and 77% of loaded BD was released into solution within 24 h. Therefore, despite the high porosity of vaterite particles, the process of drug release from them was not „burst." The profile of BD release from the $CaCO_3$+BD carriers may be classified as a „sustained release."

Conclusion

The feasibility of loading submicron vaterite particles with water-insoluble GCSs was demonstrated for BD drug. The loading efficiency was evaluated spectroscopically. It was found that the BD content in the resulting carriers was 4.9 % w/w, which is significantly higher than the concentration of the active substance in conventional forms (0.05 % w/w). Protocols for spectrophotometric assessment of the BD concentration in solution, which were developed in the course of research, provide fine agreement with independent HPLC investigation however are much simpler than chromatographic measurements. A comprehensive study of therelease process for water-insoluble BD from the carriers in model aqueous media was carried out with the use of UV and energy-dispersive X-ray spectroscopy combined with scanning electron microscopy. It was demonstrated that the data obtained by different methods are correlated. A comparison of a polar solvent and a nonionic surfactant, which were added to the carriers suspension,, revealed that the use of a surfactant is efficient, since it allows one to increase the solubility of BD in aqueous media and model the behavior of vaterite particles loaded with the drug in an open system. Based on the results of examination of the BD release kinetics in phosphate buffer, the obtained $CaCO_3$+BD carriers were classified as drug delivery systems with a sustained release profile. This makes them a promising alternative to existing pharmaceutical forms.

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Conflict of interest

The authors declare that they have no conflict of interest.

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