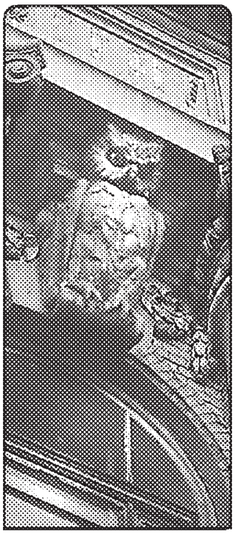
БИОФИЗИКА И МЕДИЦИНСКАЯ ФИЗИКА



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Article

Effect of electric field pulses on the suspension of microcontainers based on organic polymer and magnetite nanoparticles

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Abstract. ***Background and Objectives:*** Here, non-thermal effects induced in the suspension of hollow alginate silver microcontainers after application of short electric field pulses (about 1 ms) of high intensity (about 1 kV/cm) were studied as a prospective tool for remote activation of microcontainers. Alterations in microcontaner's shells were studied as a function of their composition. Magnetic nanoparticles immobilized within microcontainer's shells were found to enhance effects that occurred after application of electric field pulses. Alterations found in microcontaner's shells can be further employed for remote activation of microcontainers and release of encapsulated cargo. ***Results:*** The obtained results are the basis for further research of multifunctional microcontainers based on an organic alginate matrix and inorganic metal nanoparticles of silver and magnetite as drug carriers, the permeability, and structure of which can be switched using a non-thermal pulsed electrical action. ***Conclusion:*** The proposed microcontainers can be employed as carriers in new effective systems for encapsulation, targeted delivery, and controlled release of various substances in aqueous media responsive towards electric and magnetic fields which are promising in a wide range of biomedical tasks and other applications.

Keywords: hydrogel, microspheres, magnetite, silver nanoparticles, electric field

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Влияние импульсов электрического поля на суспензию микроконтейнеров, состоящих из органического полимера и магнитных наночастиц

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Аннотация. В данной работе были изучены нетепловые эффекты, индуцированные в суспензии полых альгинатных серебряных микроконтейнеров после приложения коротких импульсов электрического поля (около 1 мс) высокой интенсивности (около 1 кВ/см) как перспективный инструмент для удаленной активации микроконтейнеров. Изучены изменения в оболочках микроконтейнеров в зависимости от их состава. Было обнаружено, что магнитные наночастицы, иммобилизованные в оболочках микроконтейнера, усиливают эффекты, возникающие после приложения импульсов электрического поля. Изменения, обнаруженные в оболочках микроконтейнеров, могут в дальнейшем использоваться для дистанционного вскрытия микроконтейнеров и выпуска инкапсулированного вещества. Полученные результаты являются основой для дальнейших исследований многофункциональных микроконтейнеров на основе органической альгинатной матрицы и неорганических металлических наночастиц серебра и магнетита в качестве носителей лекарственных средств, проницаемость и структура которых могут изменяться с помощью нетеплового импульсного электрического воздействия. Предлагаемые микроконтейнеры могут быть использованы в качестве носителей в новых эффективных системах инкапсуляции, адресной доставки и контролируемого высвобождения различных веществ в водных средах с высокой чувствительностью к электрическим и магнитным полям, что является многообещающим подходом для широкого круга биомедицинских задач и других применений.

Ключевые слова: гидрогель, микросферы, магнетит, наночастицы серебра, электрическое поле

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Introduction

Currently, a key task promising progress in medicine, biotechnology, and cosmetology is the creation of effective systems for encapsulation and targeted delivery of biologically active substances into the body, enabling controlled release of the delivered compounds in a specific site and at the right time.

A wide variety of methods have demonstrated high efficiency of microcarriers-assisted delivery of drugs into the body [1, 2]. A promising approach to perform controlled localization of microcontainers loaded with biologically active substances and their targeted delivery to the body is modification of microcontainers by magnetic nanoparticles, which opens up the possibility of remote control by an external magnetic field [3, 4]. Thus, the functionalization of microcontainers with magnetic nanoparticles (MNPs) is a highly desirable task for promising drug delivery systems based on microcarriers. While magnetic field-assisted delivery of microcontainers to the specific site of the body was widely employed, electric fields have not been paid great attention. Probably the reason is the high absorbance of electric fields by tissues and similar difficulties that obstruct the application of electric fields [5]. For these reasons, electric fields have not been widely investigated as a stimulus for the remote opening of microcontainers. However, at the same time, electric fields offer a variety of advantages in terms of remote control over the release of bioactive substances within the body. A range of works employs electric stimuli in implantable devices which are limited in the number of applications [6–8]. A few works have demonstrated liposomes modified by magnetic and conductive nanoparticles with high responsiveness towards electric fields of high intensity [9, 10]. However, liposomes are not stable carriers with limited applicability. Few works have demonstrated high responsiveness of microcapsules loaded with conductive media, however, proposed approaches allow the fabrication of huge capsules with the inert response towards electric fields [11]. Ohmic heating induced by high electric fields (9.5 kV/cm) also was found to be effective in terms of control over properties of the microcapsules, but this system seems to be too hard for biomedical applications [12]. In this regard, the development of novel compositions for microcarriers combining high encapsulation ability with responsiveness towards electric fields is a promising task.

Here, we develop hollow microcontainers based on hydrogels which provide a prospect for encapsulation of different substances both low molecular weight and large macromolecules. Moreover, hydrogel-based microcontainers offer a possibility for modification of the shells in a number of ways.

1. Experimental section

1.1. Materials

Alginic acid sodium salt (SA) from brown algae, citric acid ($C_6H_8O_7$, 99.8%), and all inorganic salts were purchased from Sigma. Ultrapure water (resistivity >18.2 MΩ·cm) was used for all experiments.

1.2. Characterization techniques

The morphology of the microparticles and microcontainers characterization was performed by electron microscopy. Scanning electron microscopy (SEM) was performed by MIRA II LMU (Tescan, Czech) microscope at an operating voltage of 30 kV, in secondary and backscattering electron modes. Transmission (TEM) and scanning/transmission (STEM) electron microscopy imaging was performed by Titan 80-300 TEM/STEM (FEI, USA) electron microscope, equipped with a Schottky field emission gun, spherical aberration corrector (Cs probe corrector), and energy dispersive X-ray spectroscopy system (EDXS; EDAX, USA).

An electroporator (MicroPulser, Bio-Rad, USA) was used to study the effect of electric fields on the suspensions of the developed microcontainers by exposing the aqueous suspension of microcontainers to short (duration 1 ms) electrical impulses of high intensity. In this regard, 80 μL of the suspensions of microcontainers were placed in a plastic polypropylene cuvette with electrodes. The gap between the electrodes in the cell was 0.1 cm, and their length was 2 cm. The intensity of the electric pulses was 1 kV/cm. A series of 5 short pulses with a duration of 1 ms was applied, the time interval between pulses was 1 s.

1.3. Microcontainers synthesis

The spherical calcium carbonate ($CaCO_3$) microparticles were synthesized according to the protocol reported by Volodkin et al. [13] as follows: 1 mL of Na_2CO_3 (0.33 M) and 1 mL of $CaCl_2$ (0.33 M) were rapidly mixed in a glass vessel and stirred at 500 rpm for 1 min. The calcium carbonate microparticles were precipitated using centrifugation (2000 rpm, 2 min) and subsequently washed with pure ethanol. This procedure was repeated three times.

1.4. Preparation of samples of two types:

a) hollow silver alginate microcontainers

Hollow silver hydrogel microcontainers were prepared according to the previously published protocols [3, 14]. Briefly, 1 mL of 5 mg/mL SA was added to 20 mg of freshly prepared $CaCO_3$ microparticles and the dispersion was left agitated for 15 min in a shaker. Sodium alginate-coated $CaCO_3$ microparticles were then washed three times with deionized water. Next, 1 mL of 0.75 M silver nitrate solution was introduced to initiate cross-linking of sodium alginate. Then, 0.7 mL of 0.1 M ascorbic acid solution was slowly added to the microparticles both for $CaCO_3$ cores dissolution and silver nanoparticles synthesis. After that, the resulting microcontainers were collected by centrifugation and thoroughly washed with deionized water. The hollow silver alginate hydrogel microcontainers were stored in water.

b) hollow silver alginate microcontainers with MNPs immobilized within the shells

The synthesis of $CaCO_3$ cores coated with sodium alginate was performed according to the method *a* (hollow silver alginate microcontainers). Prior to the adding of ascorbic acid synthesis of hollow alginate microcontainers with magnetite nanoparticles immobilized in the shell was performed via absorption of MNPs on the $AgNO_3$ layer [10]. The mixture was agitated for 10 min and washed with deionized water. Then, 0.7 mL of 0.1 M ascorbic acid solution was added slowly to the microparticles both for $CaCO_3$ cores dissolution and silver nanoparticles synthesis. Finally, microspheres were washed with water and kept in it.

2. Results and discussions

Electrically induced activation of microcontainers offers a range of advantages including independence of the system and remote control. In this regard, a variety of parameters should be estimated to reach this possibility. As liquids are rich with ions that screen electric fields, it is of high importance to understand the potential difference on the surface of microcontainers. Corresponding calculations based on experimental data should be provided.

In this work, microcontainers of various compositions were prepared: a) hollow silver alginate microcontainers (Fig. 1, *A*) and b) hollow silver alginate microcontainers with MNPs (Fig. 1, *B–D*) immobilized within the shells (an average diameter of microcontaners is 4.6 ± 0.3 μm as determined from TEM/STEM images (Fig. 1)). Studying the morphology of the obtained samples was performed via the ultrasonic treatment of the microcontainers for 1 min at the frequency of 35 kHz with a power of 0.64 W/cm^2 to loosen shells followed by characterization by STEM (Fig. 1, *C, D*).

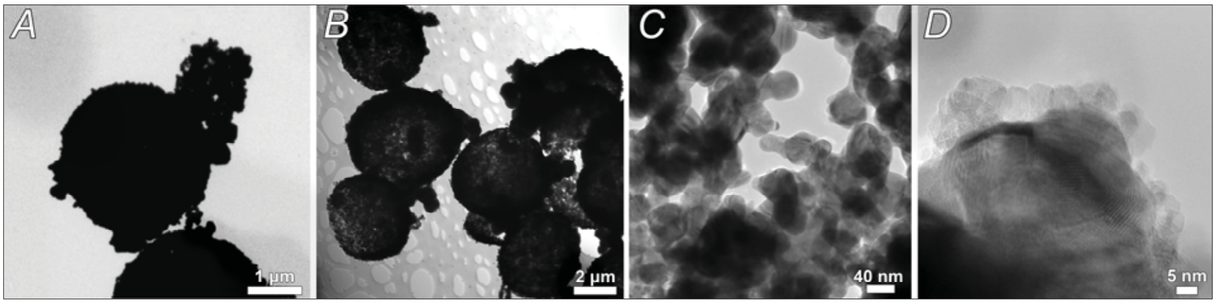

Fig. 1. TEM/STEM images of hollow silver alginate microcontainers: *A* – TEM image of the silver alginate microcontainers; *B* – STEM image of the silver microcontainers after ultrasonic exposure; *C* – STEM image of silver nanoparticles formed within the microcontainer's shell; *D* – TEM image of silver and magnetite nanoparticles within the microcontainer's shell

Heating should be carefully considered as an accompanying process during the activation of microcontainers which is able to induce undesirable toxic effects. The activation of microcontainers is supposed to be a non-thermal process caused rather by mechanical stress induced by the potential difference on the polar parts of each microcontainer as it was reported elsewhere [10]. However, electrodes are isolated from the suspension by a plastic layer and thus the breakdown of the system can not be reached at the voltages applied to the electrodes (according to the specification of the electroporator and the cuvette), that excludes joule heating of the system by electric currents.

The amount of magnetite nanoparticles immobilized within microcapsule shells was evaluated by the colorimetric titration method according to the previously published protocol [3]. MNPs content per sample was evaluated as 0.9 ± 0.1 mg, which is consistent with previously published results [3]. As previously described, the ζ-potential of microspheres was negative after all the layers were adsorbed, which proves chemical bonding between the components of the layers rather than electrostatic attraction [3].

As previously shown [10], the application of an electric field to a suspension of nanocomposite spherical particles induces polarization of the solution near the polar regions of the spheres, which, in turn, leads to the polarization of inorganic nanoparticles immobilized in the shell of the spheres. Dipole-dipole interaction of nanoparticles in the shells able to cause the mechanical stress and destruction of the shells. Using the expressions obtained in [10], we can estimate the minimum potential difference required for the destruction of hybrid alginate microcontainers.

The effect of a pulsed electric field on hybrid microcontainers containing magnetite nanoparticles with a characteristic size d_{np}= 6 nm in the shells was carried out as follows. A suspension of microcontainers was placed in a cuvette equipped with electrodes which were separated from water by a thin layer of plastic. The dielectric constant of the plastic can be neglected within the framework of the problem under consideration. As described above, a potential difference U_0 = 100 V was applied to the electrodes as a pulse with a duration of the order of 1 ms. Parameters of silver and magnetite nanoparticles employed for synthesis of microcapsules were widely studied over past few decades and can be found elsewhere, for example [15–17].

At the same time, the potential difference appeared on the spherical shell of the microcontainer $U_\kappa = U_0/\varepsilon_\kappa (d_w/\varepsilon_w + d_\kappa/\varepsilon_\kappa)^{-1} \cong \varepsilon_w/\varepsilon_\kappa\, d_\kappa/d_w\, U_0 = 2{,}9$ mV, where d_κ = 220 nm is the thickness of the alginate shell, ε_κ = 3 is the relative dielectric constant of the alginate shell, d_w = 2 mm is the thickness of the water layer, ε_w = 80 is the relative dielectric constant of water.

The voltage applied to the electrodes led to the appearance of the electric field, which can be considered spatially uniform over distances of the order of the microcontainer size. The external electric field led to polarization of the microcontainer (Fig. 2).

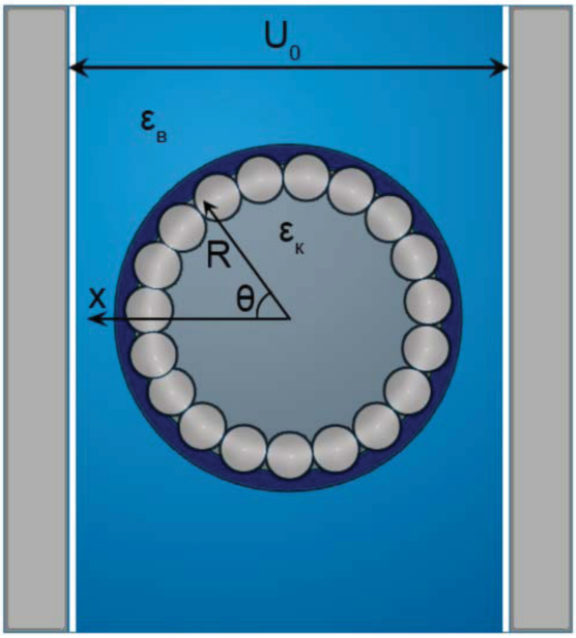

Fig. 2. Scheme of a hybrid alginate microcontainer with magnetite NP immobilized within the shell in the external electric field

The induced electric potential near the microcontainer can be found as a solution to the following electrostatic problem:

$$\begin{cases} \Delta\varphi = 0, \\ \varphi = 0, \quad r = R, \\ \varphi \to xE_w, x \to \pm\infty, \end{cases} \qquad (1)$$

where
$E_w = U_0/\varepsilon_w \ (d_w/\varepsilon_w + d_к/\varepsilon_к)^{-1} \cong \varepsilon_w/\varepsilon_к \ U_0/d_w$, R is the radius of the microcontainer.

The solution to the problem (1) is the following:

$$\begin{cases} \varphi = E_w x \left(1 - \frac{R^3}{r^3}\right), & r \ge R, \\ \varphi = 0, & r \le R, \end{cases} \qquad (2)$$

taking into account these equations, we find the electric field strength near the polar region ($\theta \cong 0, \pi$) of the microcontainer $\widetilde{E_к} = -\nabla\varphi = 3 \ \varepsilon_w/\varepsilon_к \ U_к/d_к$, $r \to R, \ \theta \to 0, \pi$.

The electric field $\widetilde{E_к}$, in turn, polarizes magnetite nanoparticles localized in the shell of an alginate microcontainer. Solving the problem for the electric field near a polarized nanoparticle similar to (1), the following equations can be obtained:

$$\begin{cases} \varphi = 3x \frac{\varepsilon_w}{\varepsilon_к} \frac{U_к}{d_к} \left(1 - \frac{a^3}{r^3}\right), & r \ge a, \\ \varphi = 0, & r \le a, \end{cases} \qquad (3)$$

where a is the radius of the nanoparticle. Potential (3) is the sum of the potential of the constant field $3x \ \varepsilon_в/\varepsilon_к \ U_к/d_к$ and the dipole potential $\varphi_\mu = \frac{1}{4\pi\varepsilon_0\varepsilon_к} \frac{(\vec{\mu}\vec{r})}{r^3}$ with the induced dipole moment $|\vec{\mu}| = 12\pi\varepsilon_0\varepsilon_в a^3 \frac{U_к}{d_к}$.

The induced dipole moments of nanoparticles localized in the polar regions of the microcontainer's shell are parallel to each other and directed in the direction of the external electric field. Therefore, repulsion between polarized nanoparticles occurs in the polar regions of the microcontainers as a result of their dipole-dipole interaction. The energy ϵ_μ of such a dipole-dipole interaction of polarized nanoparticles takes the form:

$$\epsilon_\mu = \frac{9\pi}{2} \varepsilon_0 \frac{\varepsilon_w^2}{\varepsilon_к} \frac{d_{np}^6}{l^3} \frac{U_к^2}{d_к^2}, \qquad (4)$$

where ε_0 is the electric constant, $d_{np} = 2a$ is the nanoparticle diameter, l is the distance between the nanoparticles.

The minimum potential difference $U_к^{min}$ induced at the microcontainer shell which leads to the destruction of the microcontainer can be found from the condition $\epsilon_\mu = kT$ as follows:

$$U_к^{min} = \frac{1}{\varepsilon_w} \frac{d_к}{d_{np}} \left(\frac{l}{d_{np}}\right)^{\frac{3}{2}} \sqrt{\frac{2kT\varepsilon_к}{9\pi\varepsilon_0 d_{np}}} = 2 \ mV. \qquad (5)$$

Here, the energy of the dipole-dipole repulsion of nanoparticles in the microcapsule shell was (at $T = 300$ K):

$$\epsilon_\mu \cong 2{,}2 \ 10^{-18} J = 534 \ kT, \qquad (6)$$

which led to the complete destruction of the microcontainers.

Experimentally, the alternations were studied using the electroporator system. We study the possibility of remote activation of hollow silver alginate microcontainers (with and without magnetite) by exposure to short electromagnetic pulses of high intensity (Fig. 3). Application of the electric pulses of the intensity described in the experimental section led to formation of alternations in the shells of microcontainers. SEM imaging showed the presence of the capsule's fragments which indicate effective activation of microcapsules (Fig. 3, *B*). It should be noted that the used parameters of the electric field did not induce significant alternations of the shells of original silver alginate microcapsules, while the presence of magnetite nanoparticles led to a higher sensitivity of the microcontainers to the electric field. The character of the alterations in microcontainers

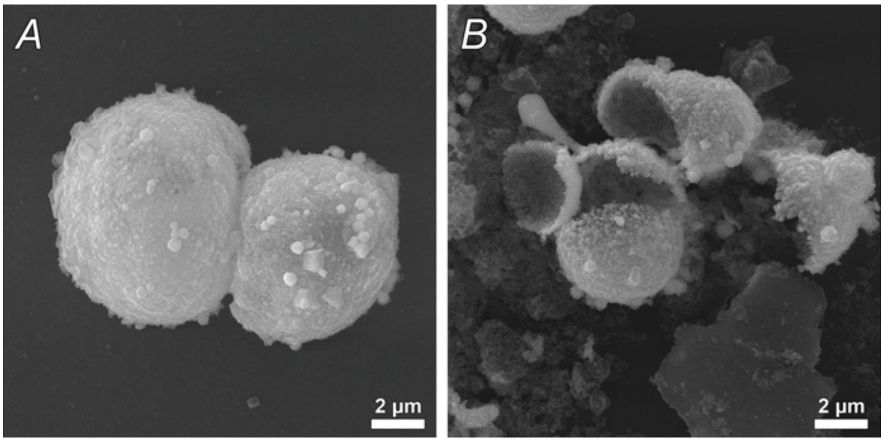

Fig. 3. SEM images of hollow alginate silver microcontainers before and after exposure to electric impulses: *A* – silver alginate microcontainers free of magnetite after exposure to electric impulses; *B* – alginate microcontainers with magnetite nanoparticles immobilized within the shells after exposure to electric impulses

shells together with the morphology to be similar before and after electric field application indicate a mechanical nature of the process.

Conclusions

In this study, the effects of short electric field pulses on the suspension of hollow silver alginate microcontainers with magnetite nanoparticles immobilized within the shells were studied as a prospective tool for remote activation of microcontainers. Non-thermal alternations of microcontainer's shells were shown to occur as a result of exposure to electric field pulses. Theoretical analysis allowed to conclude dipole-dipole interaction between nanoparticles to be the main reason of the alternations revealed after exposure to an electric field which were observed experimentally. In this way the effect of the electric field pulses was found to depend on the microcontainer's composition. In particular, the presence of magnetic nanoparticles within the shells significantly enhances alterations after exposure of microcontainers to electric field pulses. The proposed microcontainers can be employed as carriers in new effective systems for encapsulation, targeted delivery, and controlled release of various substances in aqueous media responsive towards electric and magnetic fields which are promising in a wide range of biomedical tasks and other applications.

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