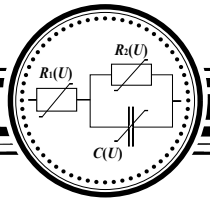
Innovations in applied physics



Article DOI: 10.18500/0869-6632-003184

Changes of the structure and permeability of lipid membranes caused by nanoparticles and pulsed electromagnetic effects

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Received 3.04.2025, accepted 25.06.2025, available online 3.07.2025, published 30.09.2025

Abstract. *Purpose.* The work is devoted to the development of effective and safe biocompatible means and methods of encapsulation, targeted delivery and controlled release of drugs in aqueous environments, including living systems. *Methods.* For encapsulation of medicinal compounds in colloidal carriers, originally created nanostructured biomimetic lipid membrane vesicles were used — nanocomposite liposomes, the membranes of which are functionalized with magnetite and gold nanoparticles. To solve the problem of safe controlled release of an encapsulated substance into aqueous media, an approach has been developed based on the use of powerful ultrashort electrical pulses with a duration of less than 10 ns, providing a non-thermal effect of selective controlled electroporation of nanocomposite lipid membranes containing conductive nanoparticles. *Results.* The effect of controlled selective change in permeability and decapsulation of nanocomposite liposomes was registered by fluorimetry methods in experiments with the anticancer antibiotic doxorubicin and the fluorescent dye carboxyfluorescein, which were loaded into liposomal carriers as model molecular compounds. Encapsulated substances were released from nanocomposite liposomes after exposure to ultrashort electrical pulses with an efficiency of up to 98%, while no significant changes in the structural and functional state of natural and pure lipid membranes were recorded. The data on changes in membrane permeability correlated well with the results on structural changes in nanocomposite liposomes recorded by transmission electron microscopy and atomic force microscopy. *Conclusion.* A theoretical model of non-thermal interaction of nanostructured liposomal capsules with ultrashort electrical pulses has been developed, within the framework of which an expression has been obtained for the critical value of the electric field strength that determines the threshold for the occurrence of the electroporation effect in a conducting aqueous medium. The key role of electrically conductive nanoparticles in increasing the sensitivity of the structure and conductivity of nanocomposite liposomes to external ultrashort electric sunlight is shown. The theoretically described mechanism of change in the structure and conductivity of lipid membranes containing electrically conductive nanoparticles explains the selective controlled nature of ultrashort pulse action on nanocomposite liposomal containers.

Keywords: lipid membranes, liposomes, magnetite nanoparticles, gold nanoparticles, nanocomposite vesicles, controlled drug delivery, electrical pulses, controlled electroporation.

Acknowledgements. The work was carried out within the framework of the state assignment (FFWZ-2025-0013).

For citation: Gulyaev YuV, Cherepenin VA, Taranov IV, Vdovin VA, Yaroslavov AA, Kravtsov ID, Grigoryan IV, Koksharov YuA, Khomutov GB. Changes of the structure and permeability of lipid membranes caused by nanoparticles and pulsed electromagnetic effects. Izvestiya VUZ. Applied Nonlinear Dynamics. 2025;33(5):709–730. DOI: 10.18500/0869-6632-003184

Introduction

The study of the structure and permeability of lipid biomembranes is of fundamental importance in biophysical studies of life processes, since the structural state of biomembranes is directly related to the functional state of the corresponding organelles and the cell as a whole. Studies of changes in the structure and permeability of biological and biomimetic lipid membranes caused by natural and artificial factors are currently an important area of fundamental science and applied research. An important interdisciplinary area of such research with high potential for biomedical applications is the development of new effective drug therapy methods aimed at solving the problem of controlled targeted delivery of drugs and genes directly to target areas and cells of the body (spatiotemporal coupling and control of drug delivery).

Currently, methods and technologies are being developed for the targeted delivery of drugs and genes directly to target areas of the body using colloidal carrier particles of various natures. The purpose of these methods is to encapsulate drugs, localize drug carriers in target areas, and control the release of encapsulated drugs from the carrier as a result of various effects [1–3]. Promising systems for such targeted controlled drug delivery should ensure that drugs are delivered to the right place at the right time and in the right quantity, that is, to ensure the spatiotemporal coherence of the action of drugs. An important criterion in the creation of such systems, determining the prospects for their actual clinical application in practice, is the biocompatibility, non-toxicity and safety of the materials used and external control influences. To do this, it is necessary to effectively solve a number of interrelated problems. The first problem is related to the development of optimal biocompatible colloidal drug encapsulation systems that enable targeted capsule delivery to target areas of the body. An equally important problem is the development of methods for the effective controlled release of encapsulated substances from capsules into the target area of the body.

Currently, colloidal drug carriers of various types are being developed, including micelles, polymer particles and complexes, vesicles, porous silicon particles, etc. [4–9]. Self-organizing lipid biomimetic colloidal vesicles, liposomes, have long been used as a model system in biophysical studies of lipid membranes and are currently one of the few systems used in real biomedical and cosmetic practice [10–13]. The liposomal lipid bilayer membrane is similar in composition and structure to biological membranes, which determines the biocompatibility of liposomes. The size of liposomes can vary widely and corresponds to the size of biogenic vesicles, in particular microvesicles and exosomes. Since 2022, 14 types of liposomal products have been allowed and used in clinical practice [14]. A further step in the development of biomimetic liposomal drug carriers may be their functionalization with nanoparticles capable of providing liposome sensitivity to external controlling physical influences.

A promising direction for creating controlled colloidal systems for targeted delivery of genes and drugs is the inclusion of magnetite nanoparticles in the structure of drug carriers and the management of spatial localization of colloidal magnetic drug carriers for their targeted delivery in the body using an external magnetic field. Usually, single-domain superparamagnetic and ferromagnetic particles of magnetic materials are used for these purposes [15–17].

Recently, magnetic nanoparticles of iron oxides (mainly magnetite Fe_3O_4) have been widely used in biomedicine due to their low toxicity, relatively high saturation magnetization, stable structure and magnetic characteristics [18–25]. Various colloidal magnetic systems for controlled drug delivery are currently being developed, including highly promising nanocomposite magnetic liposomes containing superparamagnetic iron oxide nanoparticles [26–28].

Another promising nanomaterial actively used for biomedical applications are gold nanoparticles [29, 30]. Gold nanoparticles can be used in biosensors [31, 32] or for sensitive to external stimuli (for example, photosensitive) drug delivery systems [33]. For some medical purposes, methods such as magnetothermal therapy may exhibit some side effects, such as heat shock, so non-thermal physical stimuli such as ultrasound [34, 35] or an external electric field [34], can be used as fairly safe methods to change the structure and permeability of drug carriers, causing the release of the encapsulated substance. An external electric field applied to lipid membranes can cause the formation of pores in the membranes, which leads to the effect of electroporation [36]. Electroporation can be reversible or irreversible. Irreversible electroporation can be used in non-thermal therapy of some tumor tissues [37] or can potentially cause destruction of nanocomposite lipid vesicles, which leads to effective and rapid drug release.

The methods used to release encapsulated substances from colloidal carriers must be safe and at

the same time ensure the effectiveness of drug release and selectivity of action, primarily on drug carriers. In this work, using a number of independent experimental methods and theoretical estimates, selective activation and a controlled increase in the permeability of nanocomposite lipid membranes compared with control liposomes have been demonstrated. Effective selective release of encapsulated compounds from nanocomposite lipid vesicles as a result of external pulsed electrical action was found. At the same time, the ultrashort electrical action used did not lead to significant changes in the structural and functional state of natural and pure lipid membranes. The selectivity of the external control action only on the drug carriers is fundamentally important for the safe control of the delivery and release of drugs in the target areas of the body.

1. Experimental part

1.1. Synthesis of nanocomposite liposomal capsules. For selective activation of nanocomposite liposomal capsules (NLC) used for targeted drug delivery, NLC with increased sensitivity to an external electric field have been synthesized. They are based on single-layer liposomes synthesized from amphiphilic compounds phosphatidylcholine (PC) — 80% and stearoyl-spermine (SS) — 20% with a characteristic size of about 200 nm. The outer and inner surfaces of the liposomal membrane are connected with conductive magnetite nanoparticles with a shape close to spherical and a characteristic size of 6 nm. An adapted classical Massart method was used to obtain an aqueous suspension of cationic ligand-free magnetite nanoparticles Fe_3O_4 with an average size in the range of 4...6 nm [38] (Fig. 1, *a*).

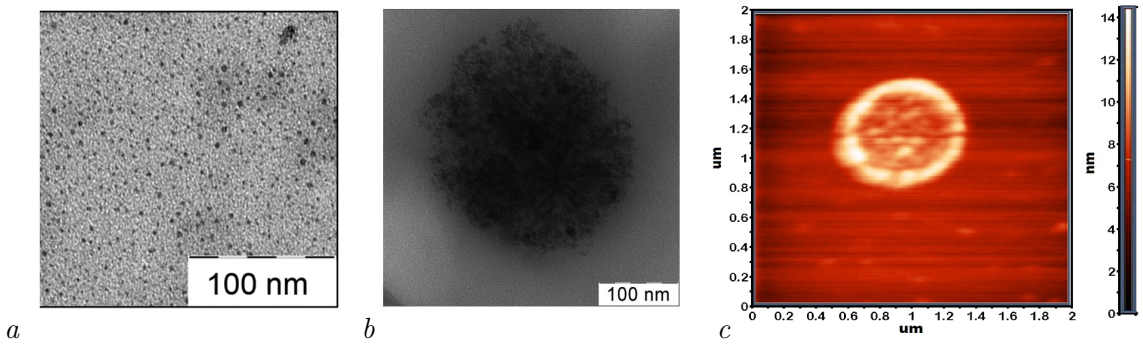


Fig. 1. TEM images of magnetite nanoparticles (*a*), NLC before exposure to USEP (*b*) and AFM image of undamaged NLC before exposure to USEP (*c*)

To prepare NLC, the method [39] was used, which differs from the previously used method [40] in that the pre-synthesized colloidal magnetite nanoparticles were initially introduced into the initial buffer solution of PC and SS, which was then exposed to ultrasound. As a result of this method, colloidal magnetite nanoparticles bound to SS amino groups on both the outer and inner surfaces of the liposomal membrane, which ensured the localization of magnetite nanoparticles on both surfaces of the membrane [41]. The structure of synthesized NLC containing magnetite nanoparticles on both surfaces of the liposomal membrane was studied by transmission electron microscopy (TEM) (Fig. 1, *b*) and atomic force microscopy (AFM) (Fig. 1, *c*) [28, 39, 40]. The size of the new nanocomposite liposomal capsules averaged 150...200 nm. The study of the effect of UCEP on NLC was conducted in conditions close to real biological conditions. NLC were found in aqueous electrolyte solutions, including saline (150 mM NaCl). The dye carboxyfluorescein was loaded into the internal volume of the NLC at a concentration of self-extinguishing fluorescence [41]. The effects of decapsulation and changes in the permeability of liposomal membranes were recorded by changing the fluorescence parameters of carboxyfluorescein upon its release from the internal volume of liposomal capsules into the external environment.

1.2. Experimental stand for the effect of ultrashort electrical pulses on NLC. The study of the effect of ultrashort electric fields (USEP) on aqueous suspensions of NLC was carried out on a specially created experimental setup. The scheme of the experimental setup used is shown in Fig. 2, *a*.

A high-voltage pulse of variable amplitude was generated by a FID Technology source and supplied

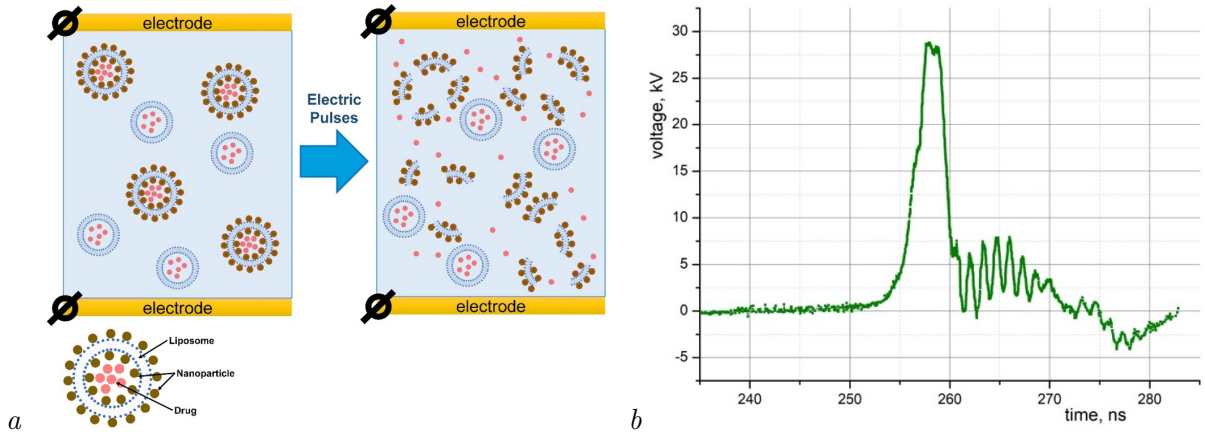


Fig. 2. Schematic diagram illustrating the effect of selective decapsulation of drug carriers based on NLC caused by external USEP (*a*) and a characteristic oscillogram of electrical voltage pulses applied to the cuvette with NLC (*b*) (color online)

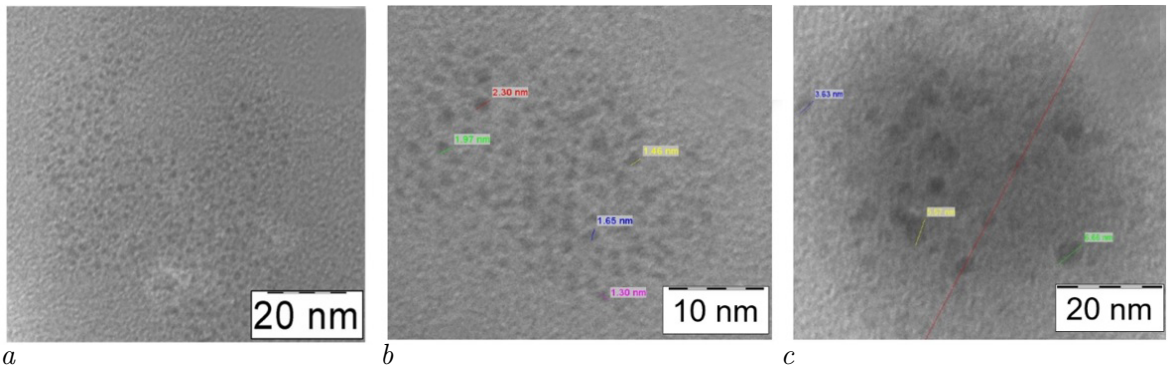
via a coaxial cable to the electrode system. A 3-meter-long high-voltage coaxial cable ensured stable operation of the generator when pulses were reflected from the load. Plane-parallel electrodes with an adjustable gap made of gold-coated copper were placed in a cuvette with an aqueous suspension of nanocomposite vesicles. The shape of the voltage pulse on the electrodes was monitored by a high-voltage, high-frequency probe, an Aktakom ASA-6039, and recorded by a high-speed Infinium MSO 9404 oscilloscope with a bandwidth of 4 GHz. The system of plane-parallel electrodes located in the cuvette with the test sample made it possible to change the interelectrode gap in the range from 1 to 5 mm, which additionally made it possible to adjust the electric field strength in the affected area. A typical waveform of a unipolar electrical pulse on electrodes with the test sample is shown in Fig. 2, *b*. This installation provides the generation of unipolar ultrashort electrical pulses in an aqueous suspension of nanocomposite vesicles. The shapes of the generated unipolar ultrashort pulses were measured directly during the pulse action on the studied samples. The pulse duration on the electrodes when exposed to aqueous suspensions of nanocomposite vesicles at half height was about 5 ns, the front duration was 2 ns, the peak voltage ranged from 1 to 30 kV, and the pulse repetition rate was about 0.1 Hz (Fig. 2, *b*).

1.3. Preparation and examination of liposomes containing hydrophobic gold nanoparticles. The synthesis of hydrophobic gold nanoparticles was carried out by an original method, by reducing gold ions from goldophenylphosphine chloride $Au(P(C_6H_5)_3)Cl$ with sodium borohydride in the presence of stabilizing water-insoluble amphiphilic ligands (stearic acid, octadecylthiol). 5.2 mg of ligand and 5 mg of $Au(P(C_6H_5)_3)Cl$ was dissolved in 15 ml of chloroform. Next, a two-phase system was formed, which is an aqueous medium, an aqueous solution of $NaBH_4$ (4 mg of $NaBH_4$ in 2 ml of water), and a phase solution of a complex of goldophenylphosphine chloride and ligand in chloroform (2.6 mg in 15 ml of chloroform). In this system, gold ions were reduced by sodium borohydride to form gold metal nanoparticles in chloroform and stabilized by ligand and phenylphosphine molecules.

In Fig. 3 the characteristic TEM images of hydrophobic gold nanoparticles obtained with a different ratio of precursor and ligand are shown.

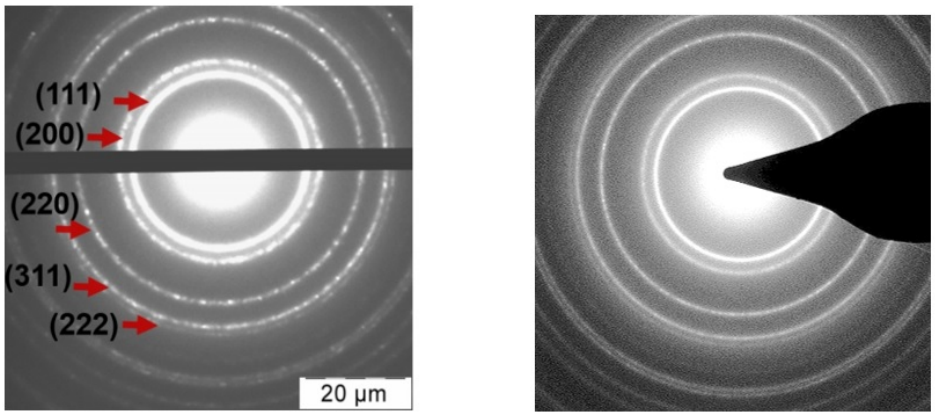
Electron diffraction patterns (Fig. 4) of synthesized hydrophobic gold nanoparticles and a reference sample were obtained by transmission electron microscopy.

A comparison of electronic diffraction patterns shows a coincidence of diffraction reflections, which confirms that the nanoparticles synthesized and used in this work are metallic gold nanoparticles. The synthesis of aqueous salts of control liposomes was carried out using standard techniques adapted for the purposes of this work [28]. Fluka phosphatidylcholine, a natural lipid that is the main component of biological membranes, was used as the base component of liposomal membranes. Small monolamellar liposomes from the natural electroneutral lipid phosphatidylcholine were obtained by a standard method using ultrasound exposure (voicing). The hydrodynamic diameter of the obtained liposomes, determined by the method of dynamic light scattering, was 50...300 nm.



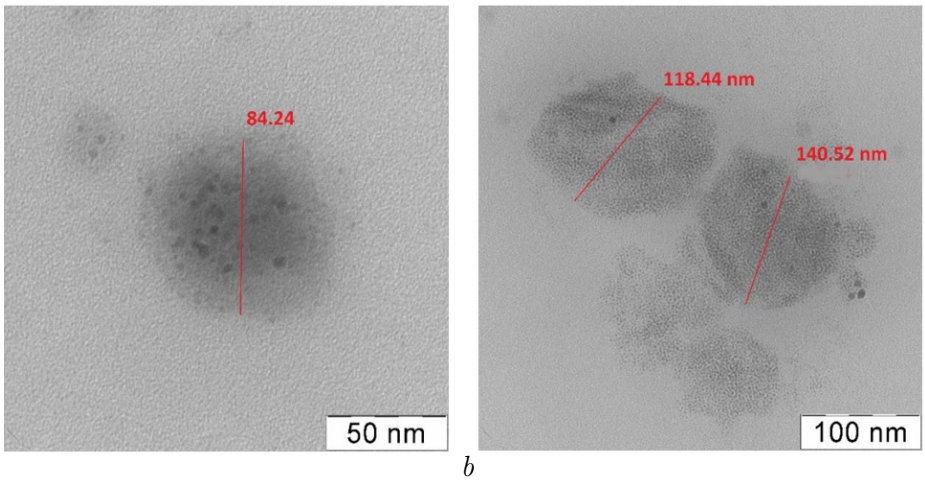
a *b* *c*

Fig. 3. TEM images of hydrophobized gold nanoparticles synthesized with different precursor-to-ligand ratios. *a* — Maximum amount of ligand, the ligand-to-precursor ratio is 5.2 mg ligand and 5 mg precursor, *b* — the ratio is 2.6 mg ligand and 5 mg precursor, *c* — Minimum amount of ligand, the ratio is 1.3 mg ligand and 5 mg precursor



a *b*

Fig. 4. Electron diffraction patterns of a sample of synthesized hydrophobized gold nanoparticles (left) and a sample of standard colloidal gold nanoparticles (right)



a *b*

Fig. 5. Representative electron microscopic images of nanocomposite liposomes with hydrophobized metallic gold nanoparticles. Images were obtained by transmission electron microscopy

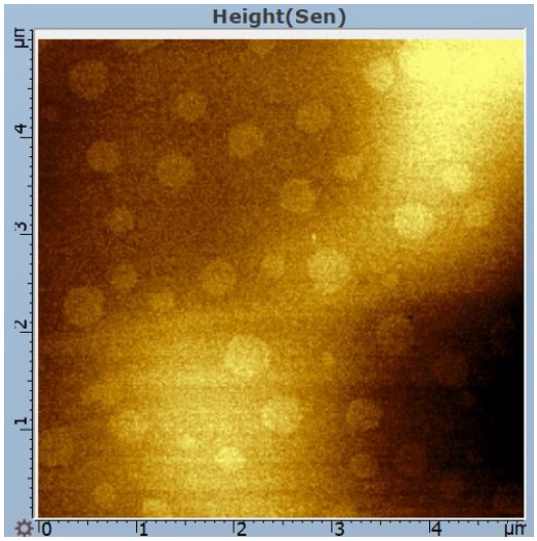


Fig. 6. Representative topographic images of nanocomposite liposomes containing hydrophobized gold nanoparticles. Images obtained by AFM

Synthesis of new stable nanocomposite liposomes with a diameter of 50 mm...300 nm with hydrophobic gold nanoparticles localized in the inner hydrophobic region of the lipid membrane was produced by adding previously synthesized low molecular weight gold nanoparticles to the lipid mass at the initial stage of liposome formation. In Fig. 5 characteristic electron microscopic images of nanocomposite liposomes with hydrophobic metallic gold nanoparticles are presented. From Fig. 5 it can be seen that the hydrophobic gold nanoparticles are fairly uniformly distributed inside the liposomal membrane, with liposomes having a quasi-spherical shape and dimensions of 100...200 nm.

From Fig. 5 it can be seen that the hydrophobic gold nanoparticles are fairly uniformly distributed inside the liposomal membrane, and liposomes having a quasi-spherical shape and dimensions of 100...300 nm. Characteristic images of nanocomposite liposomes with hydrophobic gold nanoparticles obtained by the AFM method are shown in Fig. 6.

1.4. Decapsulation of NLC. The effects of decapsulation caused by the exposure of USEP on NLC containing carboxyfluorescein in the internal volume were investigated in the framework of the following experimental scheme. A conductive suspension of liposomal capsules is located between flat electrodes with gaps of $L = 1$ and 2 mm. Ultrashort voltage pulses $U_0 = 110$ kV with a half-height duration of $\tau \cong 5$ ns are applied to flat metal electrodes. During the action of an electric pulse in an aqueous medium (away from liposomal containers), an electric field with a voltage of $E_W = U_0/L$. The release of the carboxyfluorescein dye, initially encapsulated in the intra-liposomal space, into the external environment was recorded by changing the fluorescence intensity of a suspension of liposomal capsules using a Hitachi F 3000 spectrofluorimeter.

2. Exposure and discussion

2.1. Impact of USEP on NLC. The experimental data obtained on the change in fluorescence intensity as a result of exposure to USEP on NLC are presented in the Table.

An increase in the fluorescence intensity indicates the release of carboxyfluorescein into the external environment as a result of exposure to USEP. The data obtained show that the effect of disruption of the integrity of liposomal membranes (decapsulation) is significantly higher when exposed to USEP on NLC containing conductive nanoparticles, compared with a similar effect on membrane vesicles not bound to magnetite nanoparticles. The results obtained demonstrate the key role of conductive nanoparticles in increasing the sensitivity of nanostructured liposomal capsules to external ultrashort electrical action. Also, the destruction of NLC caused by exposure to USEP was independently confirmed by atomic force

Table. Change in the fluorescence intensity of a suspension of NLCs containing encapsulated carboxyfluorescein in an aqueous solution of NaCl (0.15 M) under the action of ultrashort electrical pulses of 5 ns duration

Sample	Electric field, kV/cm	Fluorescence intensity
Nanocomposite liposomes + carboxyfluorescein at 0.15 M solution of NaCl	3 ± 0.5	59.4 ± 0.05
Nanocomposite liposomes + carboxyfluorescein at 0.15 M solution of NaCl	10 ± 0.5	81.8 ± 0.05
Liposomes without nanoparticles Fe_3O_4 + carboxyfluorescein at 0.15 M solution of NaCl	3 ± 0.5	14.6 ± 0.05
Liposomes without nanoparticles Fe_3O_4 + carboxyfluorescein at 0.15 M solution of NaCl	10 ± 0.5	17.8 ± 0.05

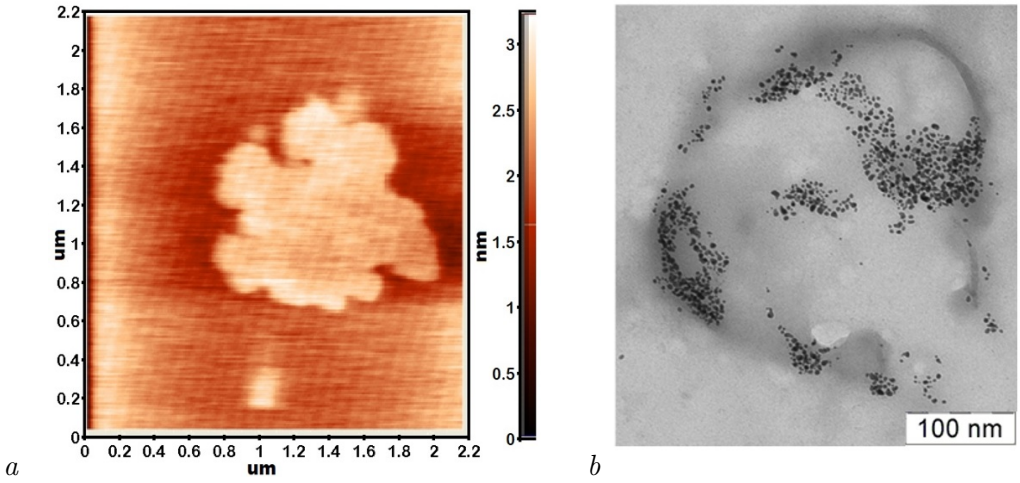


Fig. 7. AFM image (a) and TEM image of NLC destroyed by the action of USEP (b)

microscopy (AFM) (Fig. 7, a) and transmission electron microscopy (TEM) (Fig. 7, b). A comparison of the fluorescence intensity caused by exposure to USEP with a field strength of 10 kV/cm on NLC and the fluorescence intensity of NLC completely destroyed by chemical methods allowed us to conclude that the encapsulated substance was released from nanocomposite liposomes after exposure to ultrashort electrical pulses with an efficiency of up to 98%.

2.2. Model of ultrashort electrical action on NLC. Understanding the mechanisms by which USEP can change the structure and permeability of NLC containing conductive nanoparticles on the outer and inner surfaces of the membrane can be helped by considering the following task. For the values of the parameters of the problem under consideration, the condition of quasi-stationarity of the electromagnetic field $c \cdot \tau \gg l$ (c is the speed of light) [42]. Since the duration of the electrical pulse τ satisfies the conditions $\tau \gg \{\sigma_{\text{in}}^{-1}, \sigma_{\text{out}}^{-1}, \}$, where $\sigma_{\text{in}}^{-1}, \sigma_{\text{out}}^{-1}$ are the specific electrical conductivities of water-salt solutions outside and inside the capsules, then the inner and outer regions of the capsule can be considered a conductor, and the liposomal membrane can be considered a dielectric with permittivity $\varepsilon_L = 2.7$. The electric potential $\Phi(\vec{r})$ and the electric current density $j(\vec{r})$ satisfy Laplace equation, Ohm law in differential form, and the continuity equation

$$\Delta\Phi = 0; \quad \vec{j} = \sigma\vec{E}; \quad \partial\rho/\partial t + div\vec{j} = 0, \tag{1}$$

where σ is the specific conductivity of the medium, $\vec{E}$ is the electric field strength, ρ is the charge density. When exposed to USEP, the shape of the liposomal container can change while maintaining a constant volume. As suggested in [40], the shape of the liposome changes from initially spherical to an elongated

ellipsoid of rotation with the largest semi-axis parallel to the external field $\vec{E}_W$.

By choosing an ellipsoidal coordinate system ξ, ζ, ϕ centered in the center of the liposome and with the largest semi-axis parallel to $\vec{E}_W$, we get that the system in question consists of 3 regions. The region «0» is the inner part of an elongated ellipsoid of rotation: $-b^2 \leqslant \xi \leqslant -\xi_0$, $-a^2 \leqslant \zeta \leqslant -b^2$, $0 \leqslant \phi \leqslant 2\pi$, $a \geqslant b = c$ are the main semi-axes of the elongated ellipsoid of rotation, it is conductive. The region «1» is an ellipsoidal layer with a dielectric constant ε_L : $-\xi_0 \leqslant \xi \leqslant 0$, $-a^2 \leqslant \zeta \leqslant -b^2$, $0 \leqslant \phi \leqslant 2\pi$. The area «2» represents the outer part of the ellipsoid: $\xi_0 \leqslant \xi$, $a^2 \leqslant \zeta \leqslant -b^2$, $0 \leqslant \phi \leqslant 2\pi$ and is conductive.

The solution of problem (1) for the electric potential $\Phi(\xi, \zeta)$ with boundary conditions can be found in the following form:

$$\Phi(\xi, \zeta) = \Phi_1(\xi, \zeta) + \Phi_2(\xi, \zeta), \tag{2}$$

where

$$\Phi_1(\xi, \zeta) = \begin{cases} -\dfrac{n(1+s(\Delta))E_W x(1-\frac{R(\xi)}{n})}{(1-n)s(\Delta)}, & \xi \geqslant 0, \\ 0, & \xi \leqslant 0, \end{cases}$$

$$\Phi_2(\xi, \zeta) = \begin{cases} \dfrac{n+s(\Delta)E_W x(1-\frac{R(\xi)}{n+s(\Delta)}}{(1-n)s(\Delta)}, & \xi \geqslant -\Delta, \\ 0, & \xi \leqslant -\Delta, \end{cases}$$

$R(\xi) = \frac{ab^2}{2}\int_{\xi}^{\infty} \frac{d\xi\prime}{(\xi\prime+a^2)^{3/2}(\xi\prime+b^2)}$, $s(\Delta) = \frac{1-e^2}{e^2}(\frac{1}{2e}\operatorname{arcsh}\frac{2\delta e}{\delta^2-e^2} - \frac{1}{2e}\operatorname{arcsh}\frac{2e}{1-e^2} - \frac{1}{\delta}+1)$, $n = \frac{1-e^2}{e^2}\times(\frac{1}{2e}\operatorname{arcsh}\frac{2e}{1-e^2} - 1)$ is the coefficient of depolarization of liposomes, $\Delta = a^2 - (a-d)^2$ is the liposomal membrane thickness parameter, d is the membrane thickness, $x = \pm\sqrt{\frac{(\xi^2+a^2)(\zeta^2+a^2)}{a^2-b^2}}$ is the Cartesian coordinate along the semimajor axis of the ellipsoid, $\delta = \sqrt{1-\Delta/a^2}$, $e = \sqrt{1-b^2/a^2}$ is the eccentricity.

The electric field strength inside the membrane, defined as

$$E_{\text{in}} = -\frac{1}{h_\xi(\xi=0)}\frac{\partial\Phi_2}{\partial\xi}(\xi=0),$$

where $h_\xi(\xi=0) = \frac{2ab}{\sqrt{-\xi}}$ is the Lame coefficient, taking into account (2), it takes the following form:

$$E_{\text{in}} = \frac{b}{a}\frac{x}{\sqrt{-\xi}}\frac{s(\Delta)+1}{s(\Delta)(1-n)}E_W, \tag{3}$$

and in the case of a weakly elongated ellipsoid shape ($e \to 0$), it coincides with the well-known formula Schwan [43]:

$$E_{\text{in}} = \frac{3}{2}\frac{R}{d}E_B\cos\theta, \tag{4}$$

where R is the radius of a sphere whose volume is equal to the volume of a liposome; θ is a spherical coordinate. The intramembrane field strength reaches its highest value in the polar region ($\zeta = -b^2$)

$$E_{\text{in}} = \frac{(s(\Delta)+1)}{s(\Delta)}\frac{1}{1-n}E_W. \tag{5}$$

There is a surface charge with a surface density of on both surfaces of the liposomal membrane [41]:

$$\Sigma(\zeta) = \frac{(b\varepsilon_W)}{4\pi\cdot a\cdot e}\sqrt{\frac{\zeta+a^2}{-\zeta}}\frac{s(\Delta)+1}{s(\Delta)(1-n)}E_W. \tag{6}$$

This leads to the appearance of opposite charges on spherical conductive nanoparticles located in the polar region of the ellipsoid on opposite surfaces of the liposomal membrane.

$$Q = \frac{s(\Delta)+1)}{s(\Delta)(1-n)}\varepsilon_W\cdot r^2\cdot E_W, \tag{7}$$

where r is the radius of the nanoparticle. At a sufficiently high interaction energy of two nanoparticles located on opposite surfaces of the liposomal membrane $U_E = Q^2/\varepsilon_L D$, where D is the distance between

the centers of the nanoparticles, possibly destruction of the liposomal membrane. Thus, the condition for the destruction of the liposomal membrane is the following:

$$U_E = \pi r^2 \alpha, \tag{8}$$

$\pi r^2\alpha$ is the surface energy of the liposomal membrane per nanoparticle, α is the coefficient of surface tension of the liposomal membrane. From the condition (8), taking into account (5), (7), it is easy to obtain an expression for the critical value of the electric field

$$E_W^c = \frac{\sqrt{\pi\alpha D\varepsilon_L}}{r\varepsilon_W} \frac{1 - \frac{1-e^2}{e^2}\frac{1}{2e}\operatorname{arcsh}\frac{2e}{1-e^2} - 1}{(1 + \frac{e^2}{1-e^2}(\frac{1}{2e}\operatorname{arcsh}\frac{2\delta e}{\delta^2-e^2} - \frac{1}{2e}\operatorname{arcsh}\frac{2e}{1-e^2} - \frac{1}{\delta} + 1)^{-1}}, \tag{9}$$

causing destruction of the liposome membrane due to the presence of two nanoparticles located on opposite sides of the membrane. In the case of $\varepsilon_L = 2.7$, $\varepsilon_W = 80$, $\alpha \cong 1$ mN/m [42, 45], $r = 3$ nm, $d = 3$ nm, $D/r = 3$ the critical field value is

$$E_W^c = 0.7 \quad \text{kV/sm}. \tag{10}$$

The magnitude of the critical value of the electric field (10) turns out to be less than the field used in experiments on the effect of ultrashort electric pulses on conducting aqueous suspensions of NLC, that is, the obtained estimate (10) agrees well with the observed experimental results. At the same time, the field (9), which destroys NLC, is insufficient to destroy cell membranes with a characteristic size of 1 μm . It would result, according to the formula Schwan [42], to the emergence of a transmembrane potential of $\Phi_{TMP}^c \cong 100$ mV, insufficient for irreversible electroporation. Thus, the combination of the effects of USEP and the use of NLC containing conductive nanoparticles on both surfaces of the liposomal membrane creates a real basis for a selective method of controlled drug release in a given area of a living organism. Remote targeting selectivity is very important for applications involving controlled drug delivery in vivo, as it avoids damage to surrounding cells by ensuring that the structure and permeability of only nanocomposite liposomal capsules are altered. It should also be noted that the found critical value of the electric field (10) caused by the interaction of nanoparticles located on opposite surfaces of the liposomal membrane is less than the previously found [40] critical value of the field for liposomal containers containing nanoparticles only on the outer liposomal surface. This result indicates a greater sensitivity to external pulsed electrical action of nanostructured liposomal containers containing conductive nanoparticles on both the outer and inner surfaces of the lipid membrane. Similar effects were observed when exposed to USEP nanocomposite liposomes functionalized with gold nanoparticles [47].

2.3. Registration of changes in the properties of liposomes containing hydrophobic gold nanoparticles by electron paramagnetic resonance. The change in the properties of the liposome membrane when gold nanoparticles are embedded in them was investigated by the method of electron paramagnetic resonance (EPR). EPR spectroscopy is a useful tool for studying the plasticity of biological membranes, as it can provide information about the local properties of membrane lipid molecules by including special label molecules (probes) containing a stable free radical (that is, a molecule containing at least one unpaired electron). Nitroxide-containing spin tags are among the most widely used probes of this type. In them, the free radical is located in the tail hydrophobic part of the molecule, which is embedded inside the membrane. The chemical structure of the n-DOXYL-stearic acid spin tags used in the work ($n = 5, 12, 16$) is such that as the value of n increases, the stable radical is located at an increasing distance from the hydrophilic head of the probe molecule.

Liposomes containing gold nanoparticles in the shell and not containing nanoparticles (control) were dispersed in water to which nitroxyl spin tags were added. EPR spectra were recorded at room temperature on an X-band EPR spectrometer Varian E-4. To test the effect of temperature, several spectra were recorded at elevated temperatures (up to 60°C) temperatures.

Fig. 8 shows typical EPR spectra of a sample with liposomes containing gold nanoparticles and a control sample of the same (prepared by the same method) liposomes, but without nanoparticles. The specific electronic structure of the radical fragment of the nitroxyl radical, which contains an unpaired electron localized on the nitrogen nucleus, determines the characteristic appearance of the EPR spectrum of the nitroxyl radical, which has parameters that can be used to analyze the rotation correlation time of the spin label.

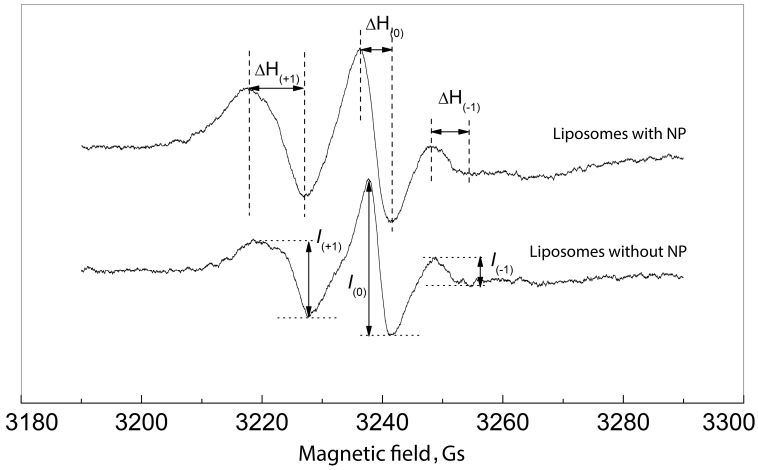


Fig. 8. EPR spectra of liposomes containing gold nanoparticles and liposomes without nanoparticles (control), recorded at room temperature

In Fig. 8 the parameters of the EPR spectra are noted, which are usually used to analyze the nature of the spin label movement in the liposome membrane. In general, the parameters of liposome spectra with and without nanoparticles are close to each other.

It is important to note that heating samples with liposomes (both with and without nanoparticles) to 60°C did not lead to a noticeable change in the parameters of the EPR spectra shown in Fig. 8. In this temperature range, liposomes, regardless of the presence of nanoparticles in the membrane, are quite stable. Therefore, to destroy the liposome shell, it is necessary to use non-thermal methods of exposure, for example, USEP.

Conclusion

The paper presents an original approach to the development of effective and safe biocompatible agents and methods for the encapsulation and controlled release of drugs into aqueous media based on the encapsulation of medicinal compounds into colloidal carriers, which are nanostructured lipid membrane vesicles (nanocomposite liposomes), the membranes of which are functionalized by magnetite and/or gold nanoparticles. The safe controlled release of the encapsulated drug from nanocomposite vesicles was realized by a method based on the use of powerful ultrashort electrical pulses with a duration of less than 10 ns, providing a non-thermal effect of selective controlled electroporation of nanocomposite lipid membranes containing conductive nanoparticles polarized in an externally applied electric field. A theoretical model of the non-thermal interaction of nanostructured liposomal capsules with ultrashort external electrical pulses has been developed, in which an expression for the critical value of the applied electric field strength is obtained, determining the threshold for the occurrence of the effect of electroporation and decapsulation in a conductive aqueous medium. The key role of electrically conductive nanoparticles in increasing the sensitivity of the structure and conductivity of nanocomposite liposomes to external ultrashort electrical action is shown.

The described mechanism of changing the structure and conductivity of lipid membranes containing electrically conductive nanoparticles explains the selective controlled nature of the effect of ultrashort pulses on nanocomposite liposomal containers. Further detailed theoretical description of the behavior of nanocomposite liposomes in external electric fields requires consideration of a number of nanoscale factors, such as local interactions of molecular dipoles with polarized nanoparticles in the presence of an external electric field, elastic properties of lipid membranes, liposome shape changes under the influence of an external field, etc. Fluorimetry methods have shown that ultrashort electrical pulses cause the release of doxorubicin and the fluorescent dye carboxyfluorescein loaded into liposomal carriers as model molecular compounds from nanocomposite liposomes. The encapsulated payload was released from

nanocomposite liposomes after exposure to USEP with an efficiency of up to 98%. The data on changes in membrane permeability correlate well with the results on structural changes in nanocomposite liposomes, independently recorded by transmission electron microscopy and atomic force microscopy. It has been shown that electrical impulses lead to significant destruction of nanocomposite liposomal membranes. The experimental data obtained demonstrate, and the theoretical estimates describe, the mechanism of selective activation and a controlled increase in the permeability of nanocomposite lipid membranes compared with control liposomes. It has also been shown that the used method of NLC activation, which leads to effective selective release of encapsulated compounds, does not significantly alter the structural and functional state of natural and pure lipid membranes. The selectivity of external control action only on drug carriers is fundamentally important and necessary for the safe management of drug delivery and release in target areas of the body. Consequently, the results obtained open up opportunities for the development of a technological platform for promising spatiotemporally guided drug therapy based on biocompatible biomimetic colloidal lipid membrane drug carriers functionalized with nanoparticles and effective safe selective control action of ultrashort electrical impulses on such drug carriers.

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