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Localization of Acoustic Energy in Gel Systems on Solid-Phase Inhomogeneities

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Abstract—The thermal effects of ultrasound on an agarose gel containing nanoparticles of iron(III) hydroxide and barium sulfate are comparatively studied. The agarose matrix is shown to interact differently with iron(III) hydroxide and barium sulfate. The relative change in ultrasound absorption due to modifier particles located in the gel is estimated. The highest thermal effect is observed for systems in which modifiers are located on separate elements of the matrix bulk. Production of "containers" with ultrasound-controlled drug release on the basis of thermosensitive gels containing solid-phase inclusions is discussed as an example of possible application of the effects described.

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The great interest in the problem of localization of acoustic energy stems from the use of ultrasound in sonodynamic cancer therapy. In this application, either very high intensity ultrasound ("burning" therapy) or low- and medium-intensity ultrasound in combination with sonosensitizers is used [1–3]. In the presence of sonosensitizers, the tumor is destroyed, which slows down its growth and sometimes leads to its complete regression. Often, sonosensitizers per se are not drugs, and some of them form intracellular solid-phase inclusions.

In the former case, localization of acoustic energy is achieved by high precision focusing, which is tomographically controlled; in the latter case, especially precise focusing is not required since healthy tissues remain unaffected during this treatment. However, in the second case, additional concentration of acoustic energy on vital parts of tumor cells would significantly improve the efficiency of ultrasound treatment. We suggested using artificial solid-phase inclusions as original sonosensitizers, which play the role of acoustic energy "concentrators." These solid inclusions can either be introduced into a biopolymer or synthesized in situ [4]. Solid-phase sonosensitizers stimulate thermal (and presumably cavitation) effects in adjacent parts of polymeric structures and thereby ensure localization of acoustic energy in a volume whose dimensions are determined by the size of inclusions and that can be significantly smaller than the ultrasound wavelength.

This work is aimed at estimating the thermal effects of ultrasound on modified hydrogel systems and their correlation with location of a solid modifier.

EXPERIMENTAL

The polymeric matrices of an agarose hydrogel, which mimics real biological systems in many respects, and a poly(*N*,*N*-diethylacrylamide) gel were studied.

An *agarose gel* was prepared by dissolving solid agarose (Difco, United States) in water on heating to 90°C in a concentration of 1.5 and 3.0 wt %. Then, the solution was slowly cooled to 20°C, which resulted in its gelation.

Barium sulfate, iron(III) hydroxide, and hydroxyapatite were used as modifiers. Solid iron hydroxide and barium sulfate were introduced as inclusions distributed over the gel volume by counterdiffusion of the reagents.

The agarose gel was modified by successive impregnation, first, with a 0.1 or 0.2 M solution of $FeCl_3$ and, then, with a 4 M aqueous ammonia or with a 0.04 or 0.08 M solution of $BaCl_2$ and then with a 1 M Na_2SO_4 solution. Impregnation with each of the solutions lasted for 48 h. The gel samples thus prepared contained 1 and 2 wt % iron(III) hydroxide and barium sulfate.

Electron microscopy. Gel samples with a volume of 1 mm^3 were placed in liquid propane for 30 s and then in acetone cooled to –95°C, which was gradually heated to room temperature. The resulting material was dried (Hitachi HSP-2) in a CO_2 atmosphere. The samples were coated with gold in an ion coater (EIKO IB-3). The resulting samples were studied under a Hitachi S-405 microscope at an accelerating voltage of 15 kV.

X-ray diffraction. X-ray diffraction patterns were recorded on a DRON-4 automated diffractometer (CuK_α radiation, $\lambda = 0.154178$ nm) in Bragg–Brentano geometry with a graphite monochromator in the diffracted beam. Measurements were taken in the point-

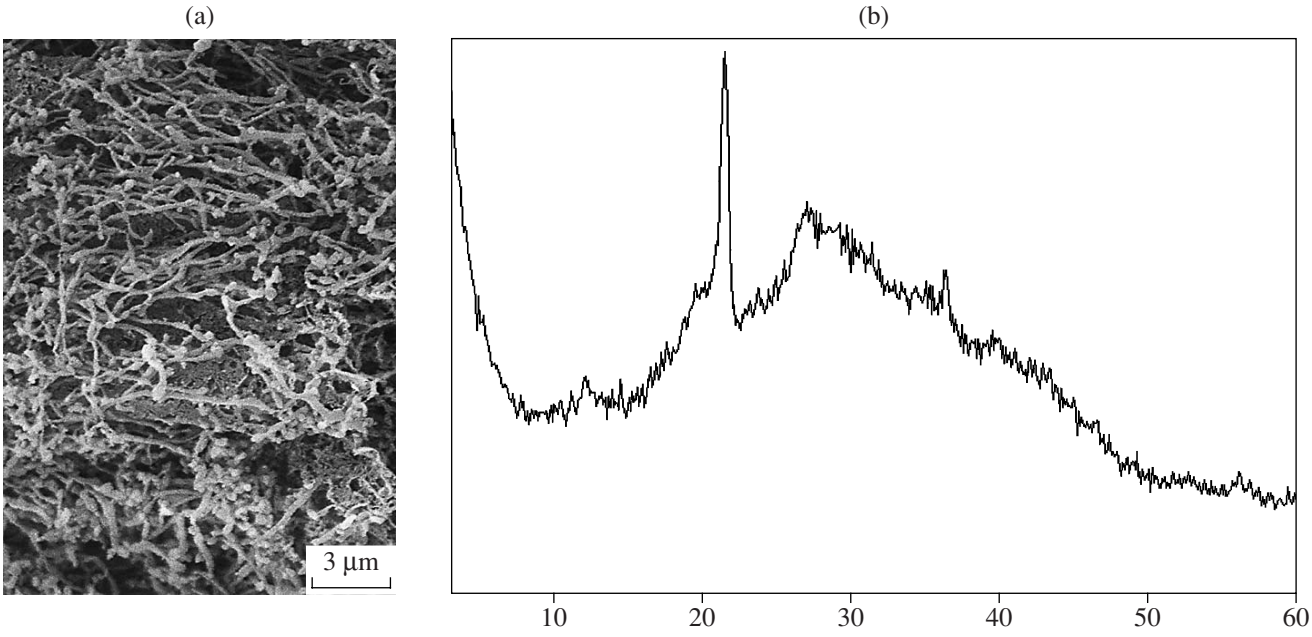

Fig. 1. (a) Electron microscopy image and (b) X-ray diffraction pattern of an agarose hydrogel modified with iron(III) hydroxide.

by-point scanning mode with the increment $\Delta 2\theta = 0.1°$. The counting time per point was 3–5 s, and the 2θ range was 3.0°–60.0°.

Thermal effects of the acoustic field on modified gel bodies were estimated by means of a thermocouple. An ultrasound emitter and a cylindrical sample (3 cm in diameter and 3 cm in height) were placed coaxially and immersed in a thermostated vessel with degassed water. Ultrasound was applied to the sample from one end and the thermocouple was introduced from the other end (along the symmetry axis of the system). The temperature was measured at the center of the sample. The distance between the emitter and the gel sample was 10 cm. To estimate the thermal effects of the acoustic field, ultrasound with a frequency of 2.64 MHz and an intensity of 2 W/cm^2 was used.

A poly(*N*,*N*-diethylacrylamide) (PDEAA) was obtained by copolymerization of *N*,*N*-diethylacrylamide and *N*,*N*'-methylenebis(acrylamide) in an aqueous solution as described in [5]. The lower critical solution temperature (LCST) of the PDEAA hydrogel samples was 31°C.

The PDEAA gel with inclusions located at the center was obtained as follows. A hydroxyapatite grain 2 mm in diameter was suspended at the center of a spherical mold. The mold was filled with the reaction mixture, which was polymerized at 18–20°C.

The resulting samples of PDEAA gel were kept in a saturated solution of ferroceron for 48 h, and, thus, containers containing the drug were obtained.

The kinetics of ferroceron release was studied on PDEAA gel samples. A gel sample was placed in a cell with a sound-transparent bottom filled with water. The content of the cell was pumped through the cell of a detector by means of a peristaltic pump. The cell was placed in a thermostat and was sonicated in the ultrasound field with a frequency of 2.64 MHz and an intensity of 1 W/cm^2. The kinetics of ferroceron release from the gel was determined from the change in its concentration in the external volume. The concentration was measured spectrophotometrically on a Specol-221 instrument in the flow mode ($\lambda = 495$ nm).

RESULTS AND DISCUSSION

Figures 1 and 2 show the microphotographs and X-ray diffraction patterns of samples of the agarose hydrogel modified with iron(III) hydroxide and barium sulfate. The photograph of the agarose gel modified with iron(III) hydroxide show the uniform distribution of the highly dispersed modifier over the matrix threads and thereby no sites of preferable localization of the solid phase are observed (Fig. 1a). X-ray diffraction (Fig. 1b) also points to the absence of iron(III) hydroxide crystallites of noticeable sizes.

A radically different situation is observed for barium sulfate. The microphotograph (Fig. 2a) shows the existence of a dispersed crystalline phase localized on separate elements of the host matrix, which is also supported by X-ray diffraction (Fig. 2b).

These data are evidence of the existence of at least two types of localization of the solid phase crystallizing in hydrogels; the probability of each type dominating is determined by the nature of the polymer matrix and the modifier. Analogous results were obtained for iron hydroxide and calcium salt of cobalt octa-4,5-carboxyphthalocyanine in the polyacrylamide gel matrix [6].

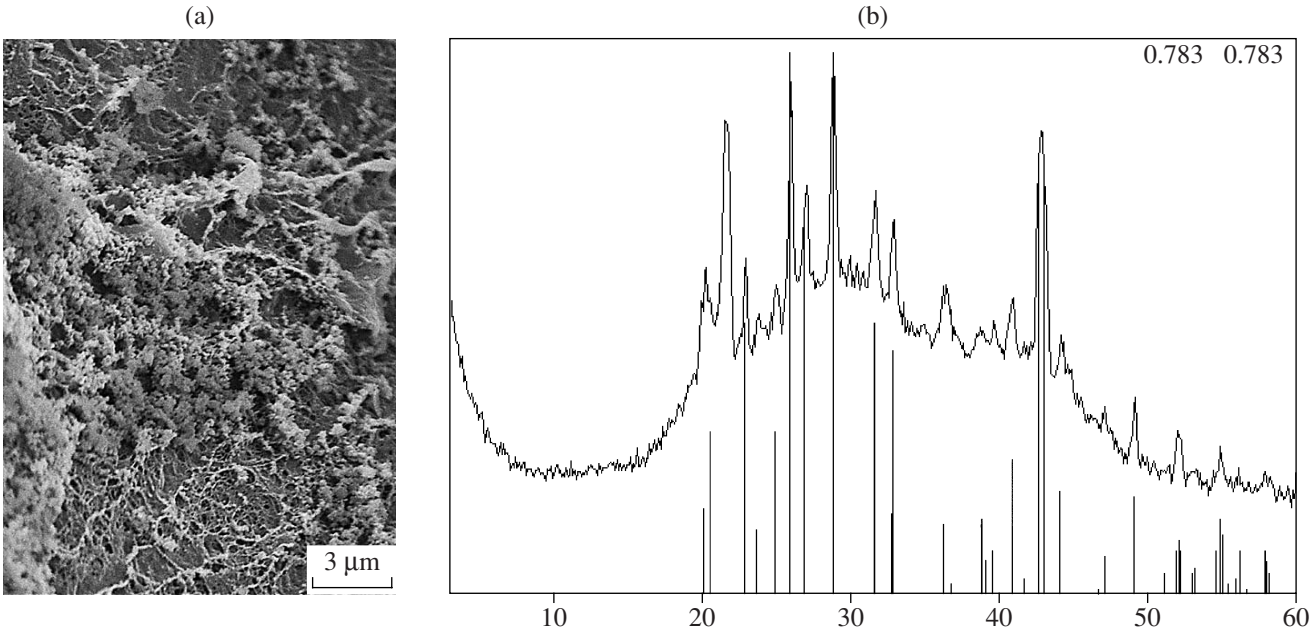

Fig. 2. (a) Electron microscopy image and (b) X-ray diffraction pattern of an agarose hydrogel modified barium sulfate.

The existence of two types of localization of a modifier allows us to assume that thermal effects of ultrasound on such systems can also be different.

Samples of modified hydrogel systems were used for studying thermal effects induced in them by ultrasound. Figure 3 shows typical temperature growth curves for utrasonicated agarose hydrogel samples modified with iron hydroxide and barium sulfate.

For comparison of the thermal effects of ultrasound in modified hydrogels, the temperature growth curves were approximated by the following function:

$$\Delta T = A[1 - \exp(-Bt)].$$

This equation can be derived on the basis of thermal balance conditions in the approximation of a uniform distribution of temperature over the sample bulk. The coefficient A is proportional to the absorbed power and inversely proportional to the heat transfer coefficient, whereas the coefficient B is directly proportional to the heat transfer coefficient. The physical meaning of the parameter A is that it is the maximal achievable (stationary) temperature under the given conditions of energy absorption and heat transfer. Then, the ratio of the stationary temperature for the modified gel (A_M) to the stationary temperature of the unmodified gel (A_0) will show the change in the acoustic power absorbed by the gel caused by the presence of the modifier, $K = A_M/A_0$. The table presents the stationary temperatures (A) and changes in the absorbed acoustic power (K) for the modified hydrogel systems.

As follows from the table, the thermal effects for the samples modified with barium sulfate and iron(III) hydroxide are significantly different. On average, the values of acoustic power absorption for the samples modified with barium sulfate exceed by a factor of 1.5–2.0 the values for iron(III) hydroxide. It is worth noting that, for the samples modified with iron(III) hydroxide, a decrease in ultrasound absorption is sometimes observed. This may be due to a change in the mechanical characteristics of the matrix network when it is incrusted with the modifier, which entails a change in the viscoelastic properties of the sample. A maximal increase in the absorbed power is achieved for a 3% agarose gel modified with 2% barium sulfate.

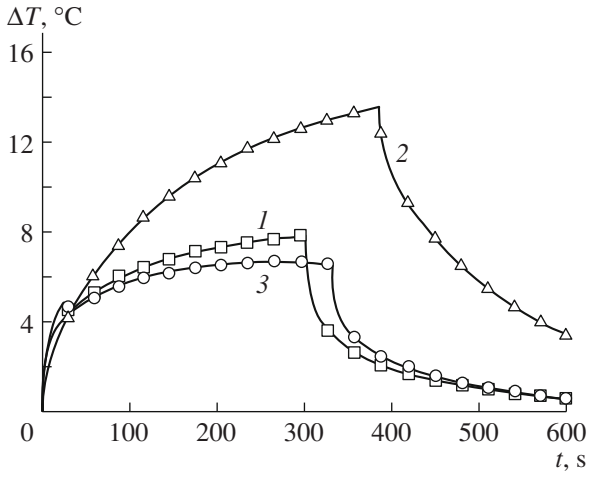

Fig. 3. Thermal effects induced by the acoustic field applied to an agarose hydrogel (*1*) without a modifier and (*2*, *3*) modified with (*2*) barium sulfate and (*3*) iron(III) hydroxide (2 wt %). Ultrasound characteristics: frequency, 2.64 MHz; intensity, 2 W/cm^2.

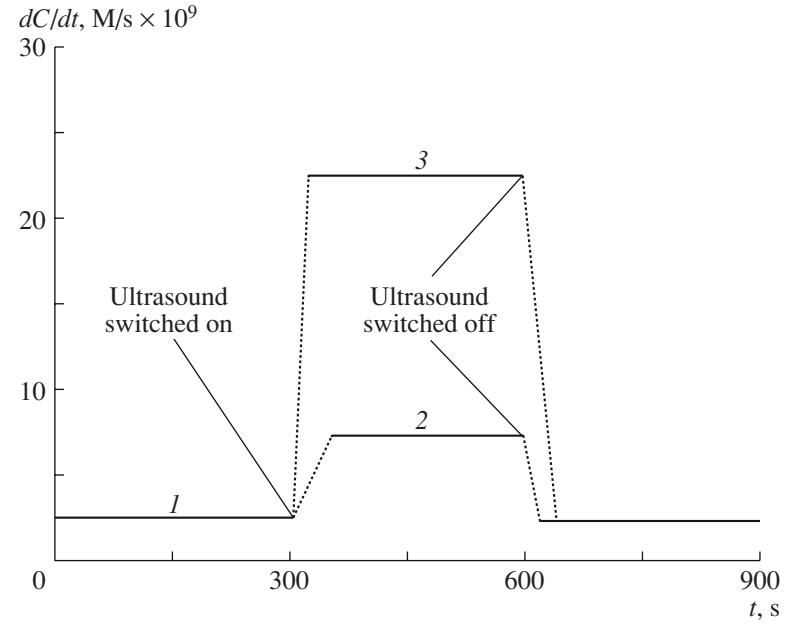

Fig. 4. Effect of ultrasound on the dynamics of ferroceron release from the modified and unmodified samples. Segment *1* corresponds to the background release of ferroceron in the absence of the ultrasound field. Segment *2* corresponds to ferroceron release from the unmodified gel stimulated by ultrasound. Segment *3* corresponds to ferroceron release from the gel with an HA grain at the center stimulated by ultrasound. Dashed lines correspond to the experimentally not determined ferroceron release rates. Ultrasound characteristics: frequency, 2.64 MHz; intensity, 1 W/cm^2.

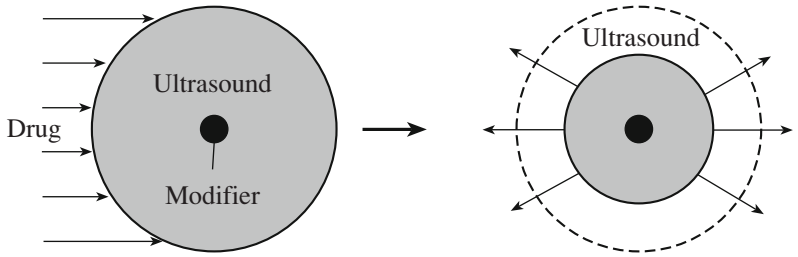

Fig. 5. Scheme of drug release induced by ultrasound from a thermosensitive hydrogel with the solid-phase modifier at the center.

In our opinion, the thermal effects induced by ultrasound in hydrogel systems make it possible to design materials with remote-controlled properties. Such materials can be based, for example, on a thermosensitive hydrogel capable of undergoing structural phase transition at some temperature. When an ultrasonic field is applied, the hydrogel is heated due to ultrasound absorption and, as the critical temperature is achieved, sharply changes its volume [5], which can be used for release the preloaded drug from the gel volume.

We studied the kinetics of ferroceron release from a hydrogel container induced by ultrasonic treatment. Figure 4 shows the plots of the ferroceron release rate versus time for the container without and with the ultrasound absorber. Region 1 in the plot corresponds to the background release of ferroceron in the absence of an ultrasound field, and regions 2 and 3 correspond to the release of ferroceron from the gel stimulated by ultrasound. These data show that ultrasound increases the release rate and that, for a sample with the modifier (HA), this rate is three times as large as for a sample without the modifier.

The experimental data related to drug transport allow us to assume that, for creation of similar materials, it is reasonable to use macroscopic inclusions with acoustic properties rather different from those of the hydrogel. This difference is responsible for strong ultrasound absorption and, hence, heat release. Location of the solid-phase inclusion at the center of a sample ensures that the gel density gradient due to compression on passing through the LCST is directed from the center to the periphery. Inasmuch as passing beyond the critical point is accompanied by the appearance of solution flows as a result of compression, the introduc-

tion of the ultrasound absorber into the center of the gel matrix makes it possible not only to decrease the ultrasound treatment duration but also to direct the substance flow from the gel center to its periphery (Fig. 5). In our opinion, most promising for directed transport is a container with a relatively large ultrasound absorber (10^{-3} m) at the center.

For other cases when the direction of solution flows in a container is unimportant (for example, for enzymatic reactors with ultrasonically controlled activity) while the change in its structure is important, a uniform distribution of a highly disperse modifier (10^{-6} m) is preferable.

Our findings show that the manifestation of acoustic thermal effects significantly depends on the character of the interaction and distribution of a modifier in the hydrogel matrix, which is determined by the chemical nature of the modifier and polymer matrix.

Thus, solid inclusions of different size can be treated as original concentrators of acoustic power.

The use of large inclusions into thermosensitive gel bodies makes it possible to create containers with ultrasonically controlled drug release. In solving the problem of directed transport, introducing different modifiers can change the degree of absorption of ultrasound by the container and, hence, its permeability for the drug.

Further development of this line of investigation implies solving the following problems: (1) elucidating characteristic features of phase formation in gel bodies as a function of the inclusion and matrix nature, (2) estimating changes in the viscoelastic properties of hydrogels caused by their modification, and (3) studying the mechanisms of emergence of thermal and cavitation effects of ultrasound on hydrogel systems modified with solid phases.

Calculated stationary temperatures (A) and the increase in the absorbed acoustic power (K)

Sample	Weight fraction, %			
	1.5		3.0	
	A, °C	K	A, °C	K
Unmodified	5.7	1.0	8.0	1.0
$BaSO_4$ 1%	8.8	1.5	11.3	1.4
$BaSO_4$ 2%	9.6	1.7	14.5	1.8
FeOOH 1%	6.0	1.0	7.8	1.0
FeOOH 2%	7.4	1.3	6.8	0.9

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