Iron-containing nanoparticles based on the 2-hydroxypropyl-β-cyclodextrin in aqueous solutions*

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The water-soluble hybrid nanoparticles based on high-substituted 2-hydroxypropyl-β-cyclodextrin were formed *in situ* during the reduction of iron(+2) salts by hypophosphite ion in alkaline medium. These nanoparticles are high sensitive to temperature and ultrasonication. It was established that the temperature and ultrasonication time of exposure increase leads to the successive dissociation of the nanoparticles.

Key words: 2-hydroxypropyl-β-cyclodextrin, hybrid nanoparticles, thermostability, ultrasonication.

Nonequivalent interactions in aqueous solutions of β-cyclodextrin and its derivatives are widely used for the synthesis of water-soluble substances based on magnetically ordered iron oxides.[1—5] Earlier[5] we showed that self-assembling of 2-hydroxypropyl-β-cyclodextrin (HPCD) in the presence of iron salts and reducing agent at ~20 °C brings to the formation of hybrid organic-inorganic nanoparticles (HN) with different composition, structure, physical and chemical properties which depend on molecular ratio of the reagents. An important characteristic of the obtained nanoparticles is their ability to bind acid-basic dyes. It should be noted that hydroxypropylcyclodextrins are widely used as supports for pharmaceuticals to solubilize water non-soluble compounds and dosage forms, decrease their toxicity, improve bioavailability and biocompatibility.[6—9]

When studying nanocomposite materials there is a particular interest in the relationships between their properties and external factors such as temperature, high-frequency alternating magnetic field, electromagnetic irradiation etc. Composites with magnetic nanoparticles are known to be sensitive to magnetic field exposure, that is applied in medicine for the targeted drug delivery.[10]

In this work the influence of the external parameters, in particular, temperature and ultrasonication, on the behavior of aqueous solutions of iron-containing nanoparticles and ability to bind phenolphthalein by two different HN based on HPCD and nonorganic compounds (salts, oxides).

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Experimental

Materials. 2-Hydroxypropyl-β-cyclodextrin with molar mass of M = 1540, that corresponds to the degree of substitution of 6.8 was used. Sodium hydroxide (Sigma, USA), phenolphthalein (Merck, Germany), sodium hypophosphite (Merck, Germany), Mohr´s Salt ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, Reachim, Russia) without additional purification were also used. Sodium tetraborate (Reachem, Russia) was recrystallized from water.

Synthesis and separation of iron-containing hybrid nanomaterials based on HPCD. *A. Synthesis of HN I.* 2 mmol of NaOH were added to an aqueous solution containing 0.05 mmol of HPCD and 0.5 mmol of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$. The mole ratio $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$: $NaH_2PO_2 \cdot H_2O$ was equal to 1 : 1 and Fe^{2+} : HPCD was 10 : 1. Then an aqueous solution of sodium hypophosphite (0.5 mmol) was added by drops to the reaction mixture under the intensive stir. The end of reaction was defined visually when sedimentation of brown colored iron hydroxide was observed. The high-molecular product formation was controlled by gel permeation chromatography (GPC). The adducts were purified from the low-molecular additives by flow dialysis against distilled water.

B. Synthesis of HN II. Synthesis, purification and separation of HN II was similar to those of HN I. The initial solution included 0.05 mmol of HPCD, 5 mmol of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ and 20 mmol of NaOH. The mole ratio $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$: $NaH_2PO_2 \cdot H_2O$ was equal to 1 : 1 and Fe^{2+} : HPCD was 100 : 1.

Spectrophotometric titration. Spectrometric measurements were carried out on a spectrophotometer Ultrospec-4050 (LKB, Switzelland) using a quarts cell with an optical path length of 1 sm. Phenolphthalein titrations were performed in 0.01 M solutions of sodium borate (pH 9.2) at λ = 553 nm.

Dynamic light scattering (DLS). The particle size was determined by a dynamic light scattering method on photometer ALV-5 (ALV, Germany), equipped with a 25 mWt He—Ne-laser (λ = 632.8 nm). The scattering angle was 90°. Autocorrelation

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functions of scattering light intensity were obtained by means of 280-channel Photocor-M correlometer. Before the measurements, the samples were purified by passing through the membrane Millipore filter (average pore size of 1.2 μm) two or tree times. The results were processed by Tikhonov normalization method. The radius of the equivalent hydrodynamic sphere was determined by the Stokes equation.

Ultrasonic exposure. Ultrasonic treatment was performed in an ultrasonic bath Saphir TTC-6580. The working frequency was 35 kHz, power was 100 Wt. The exposure of the aqueous solutions of HN I and HN II was in an immersion liquid (water) at 25 °C.

Results and Discussion

Hybrid nanoparticles I and II separated from the alkaline aqueous solution of high-substituted HPCD, iron(+2) salts and sodium hypophosphite $NaH_2PO_2 \cdot H_2O$, represent substances which form stable aqueous solution. According to the element analysis, compositions of HN I and II are essencially different, though they are products of the self-assembling process of the same monomers, HPCD molecules. We showed[5,11] that non-covalent bonding of HPCD occurs only in the presence of iron(+2) salts and hypophosphite ion as a reducing agent. In addition, it was sensitive to the reaction mixture composition. The highest yields of HN I and II were obtained at the molar ratio of the reagents HPCD : Fe^{2+} : $H_2PO_2^{1-}$ of 1 : 10 : 10 and 1 : 100 : 100 respectively. In the solution of HN II, that was obtained at 100-fold excess of iron in the initial mixture, the fraction of iron was 0.1 wt.%. The maximum iron concentration (4 wt.%) retained only in the supramolecular assembly HN I, and these nanoparticles exhibited noticeable magnetic properties. Besides, HN I sample contained a significant amount of inorganic salts (as sulfates or phosphates), which were captured from the aqueous solution during the synthesis and seemed to participate in stabilization of supramolecular assembly. HN I were found to be partly soluble in water, while solubility of HN II was considerably higher and close to that of the initial HPCD. Dynamic light scattering analysis showed that in the aqueous solutions of HN I with a higher content of iron the effective diameter was 400 nm, while that of HN II constituted 800 nm. By means of DLS method, particle size of HN I and II was established to be independed of its concentration in the solution.[5]

β-Cyclodextrin and its derivatives are known to form complexes with an acid-basic indicator, like phenolphthalein. Its composition used to be 1 : 1.[12,13] The complex formation substantially change the spectral characteristics of the initial solution of the dye. The colored aqueous-alcoholic solutions of phenolphthalein (in the borate buffer, pH 9.2) are decolorized in the presence of β-cyclodextrin and HPCD. The dependence of the optical density of phenolphthalein solution on the dye concentration in the absence of both HPCD and nanoparticles at 25 °C is shown in Figure 1 (curve *1*). Curve *4* corresponds to the titration of an aqueous solution of HPCD with an aqueous-alcoholic solution of phenolphthalein. The form of the curve definitely indicates on bonding of the dye by the cyclodexitrin molecules. At the same time the color of solution is observed only after all active cites of oligosaccharide in the solution being occupied. A different correlation is observed when the solutions of HN I and II were titrated with phenolphthalein. Figure 1 (curves *2* and *3*) shows that in this case a decrease in the light absorption by phenolphthalein was less evident in comparison with aqueous-alcoholic solutions of the dye in HPCD. Thus, in the solutions of HN I and II, a partial binding of the colorant occurs which is explained by the steric factor (screening of macrocycle cavities in the nanoparticle solution by the adjacent molecules of HPCD). The complexes including phenolphthalein appear to be formed just on the nanoparticle surface.

We found that after heating the solutions I and II up to 46 °C an amount of the bonded phenolphthalein considerably increased. The titration curves, obtained at this temperature (see Fig. 1, curves *5* and *6*), approached to the titration curve of free HPCD (curve *4*). Special experiments were performed to show the independence of optical density of phenolphthalein of its concentration in the range of temperature 25—46 °C. Thus, when heated, a decomposition of nanoparticles occurs due to the destruction of non-covalent hydrogen bonds formed in the self-assembling.

The influence of temperature on the ability of HN I and II to bond the dye is described by the titration curves of I and II with phenolphthalein at different temperatures (Figs 2 and 3). Present results indicate a gradual increase of the bonded phenolphthalein in both solutions I and II when the temperature increases. The bonding ability of HN I and II in respect to phenolphthalein was calculated

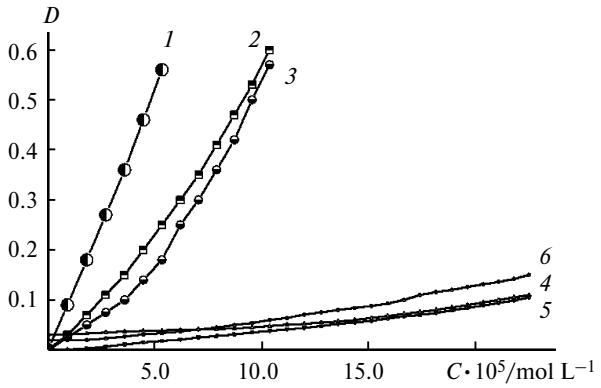

Fig. 1. Dependence of optical density of phenolphthalein (553 nm) on its concentration at the absence of nanoparticles (*1*); in the presence of HN I (*2*), HN II (*3*), HPCD (*4*) at 25 °C; in the presence of HN I (*5*), HN II (*6*) at 46 °C; C_{HPCD} = 1 wt.%, C_{HN}= 1 wt.%, borate buffer, pH 9.2.

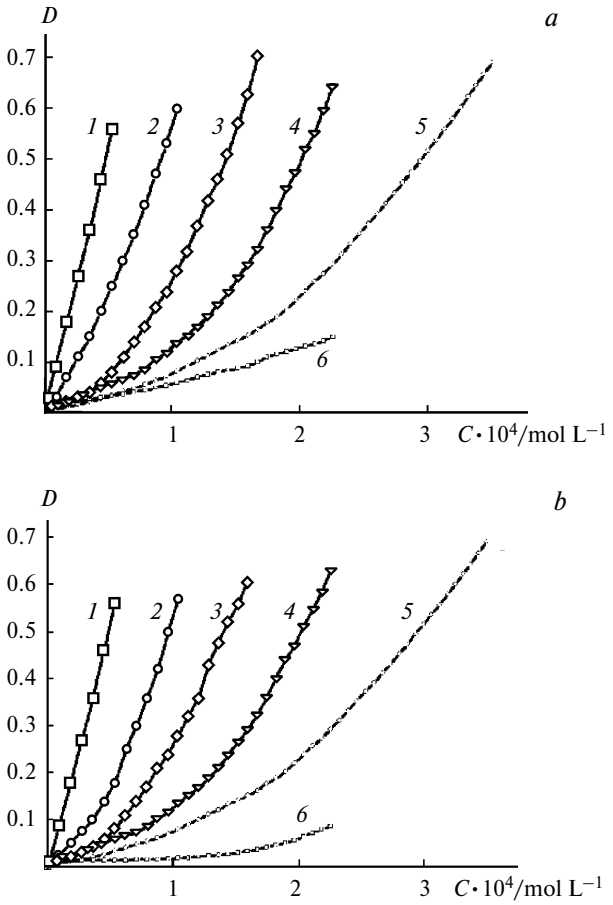

Fig. 2. Dependence of optical density of phenolphthalein (553 nm) on its concentration at the absence of nanoparticles (*1*) and in the presence of HN I (*a*) and HN II (*b*) at different temperature: 25 (*2*), 29 (*3*), 32 (*4*), 37 (*5*), and 46 °C (*6*); C_{HN} = 1 wt.%, borate buffer, pH 9.2.

according to the method which provided a quantative determination of fraction of the active cites (γ) in cyclodextrin solutions.[14] The correlation between values of γ for HN I and II and temperature is shown in Figure 3. The obtained data give evidence to the presence of free molecules of HPCD in the solutions I and II at the enhanced temperature, which means the subsequent decomposition of nanoparticles.

Thermostability of HN I and II in an aqueous solutions was studied using dynamic light scattering method. In both cases, temperature appeared to affect strongly the stability of nanoparticles. Hence, the result of the thermal treatment of the adducts is different. When heating the solution I from 25 to 35 °C the hydrodynamic radii of nanoparticles were almost invariable (Fig. 4). In this temperature range, the function of the particle-size distribution was characterized by the only mode with the maximum at 200—210 nm. The further heating to 37 °C resulted in a slight increase in the effective hydrodynamic radius until the value of 250 nm, while at

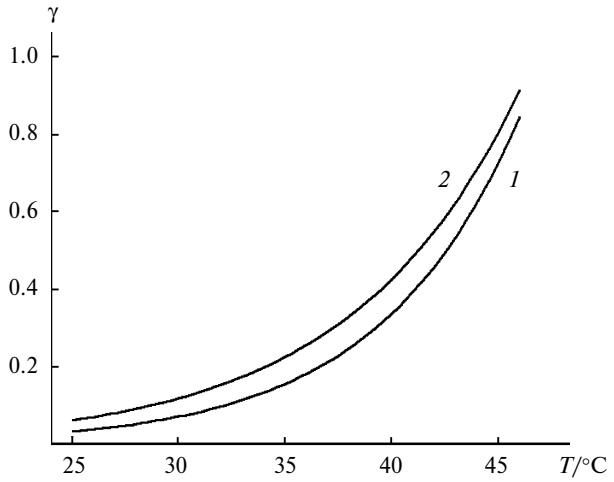

Fig. 3. Dependence of active sites fraction (γ) on temperature in the aqueous solutions of HN I (*1*) and II (*2*).

42 °C a total destruction of nanoparticles was observed (see Fig. 4).

When heating, the decomposition of nanoparticles was accompanied with the orange coloration and a sol of the hydrated iron(+3) oxide formation. Thus, the HN I destruction was proved to be irreversible at 42 °C. As a result, there were monomers of HPCD and a residue containing hydrated iron oxide left in the solution. The presence of non-associated HPCD molecules in the solution as a result of the thermal treatment of HN I was proved by phenolphthalein titration. The titration curve of the thermal treated solution I was equal to the curve which characterized free HPCD (see curves on Fig. 1).

When heating the solution II from 25 to 36 °C the effective hydrodynamic radii of nanoparticles tend to steady-state values of ~400 nm (Fig. 5). At 37 °C the recorded value decreased drastically until the value of

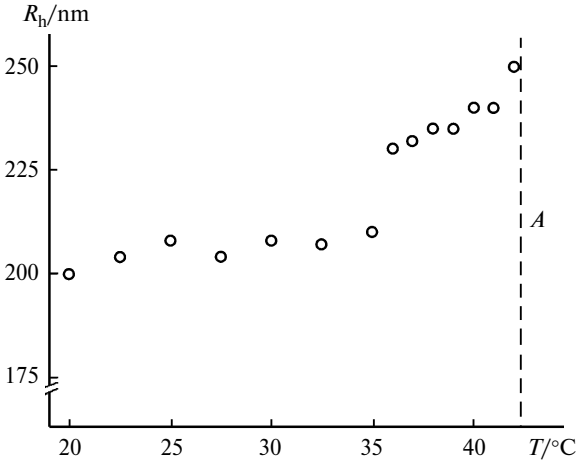

Fig. 4. Correlation between the radius of the effective hydrodynamic sphere (R_h) HN I and temperature (at heating). Concentration of HN I is 1 wt.%; *A* is complete destruction of the particles.

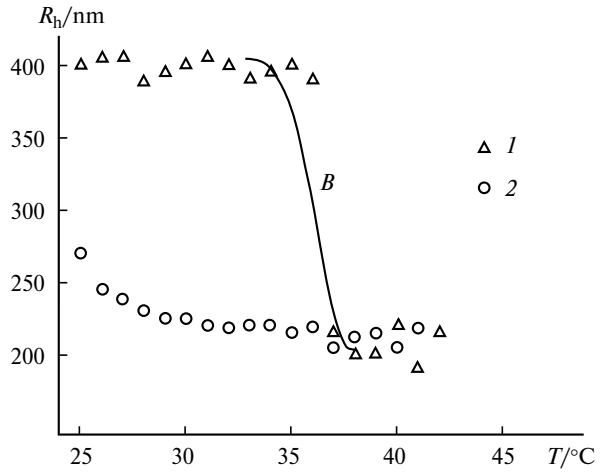

Fig. 5. Correlation between the radius of the effective hydrodynamic sphere of HN II and temperature; *1*, heating, *2*, cooling. Concentration of HN II is 1 wt.%; *B* is partial decomposition process.

200 nm. Unlike for HN I, the further heating to 46 °C did not lead to the decomposition of HN II and the size of the particles within the temperature interval of 37—46 °C did not change. A variation of the effective radii of HN II at cooling the solution up to the initial temperature is shown in Figure 5 (curve 2). Obviously, temperature reduction did not bring to the initial nanoparticle size, although a slight increase in the effective radius was noted (from 200 to 275 nm).

Thus, at enhanced temperature a subsequent decay of the nanoparticles HN I and II occurs as well as migration of HPCD molecules from their surface. When heating there are nanoparticles being in equilibrium with free HPCD molecules in both solutions I and II. HN I decomposed completely at 42 °C, while HN II were enough stable up to 46 °C.

A similar effect of decomposition of the nanoparticles based on HPCD was observed under the ultrasonic treatment. Figure 6 represents the relationship between the effective hydrodynamic radius of HN I and II and exposure time under the sonication in an aqueous solution at ~20 °C. Within two minutes of treatment the hydrodynamic radius for the adducts I and II decreased by a half. After the treatment followed by incubation at ~20 °C for 24 h the effective hydrodynamic radius of HN I did not change, while that of HN II showed an almost 1,5-fold increase compared with the initial value and reached the value of 334 nm. We suppose that after the sonication, fragmentated species of HN I included nanomagnetic domains capable to attract each other that resulted in the ensuing formation of new agglomerates. On the contrary, HN II decomposed irreversibly under the sonication. This result is likely to be attributed to the fact that an amount of iron in HN II was low and such species do not have pronounces magnetic properties.

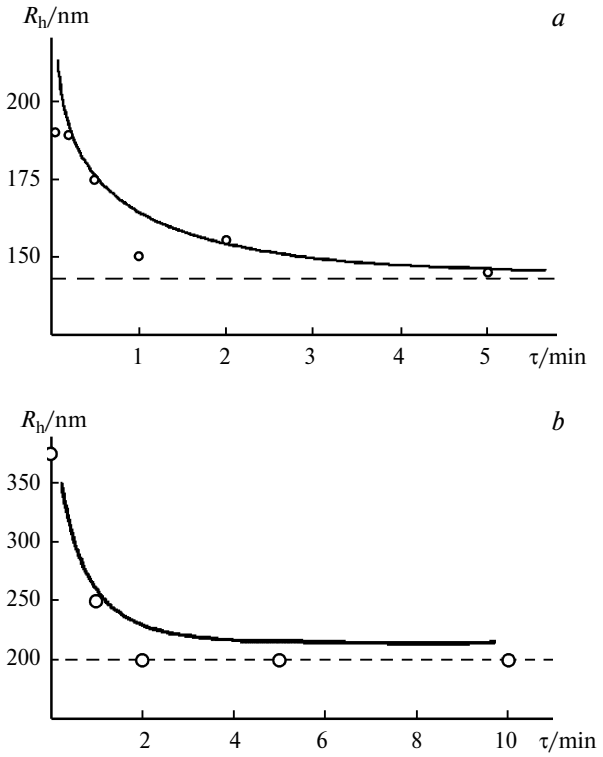

Fig. 6. Influence of ultrasonic treatment on the effective hydrodynamic radius of HN I (*a*) and II (*b*). Concentration of HN I and II is 1 wt.%; $T = 25$ °C; τ is exposure time.

The obtained results provide the evidence that the formation mechanism of NP I and II is different. Probably the formation of magnetically ordered HN I operates not only through intermolecular hydrogen bonds, but through the attraction of nanomagnetic domains to each other. The self-assemling of HN II is likely to be the result of intermolecular hydrogen bonding only.

Thus, HN based on HPCD, which are formed in an aqueous solution in the presence of iron(+2) salts and a reducing agent, undergo the partial or total decomposition at thermal or ultrasonic treatment. The sensibility of the nanoparticles to the internal factors can be used for the development of pharmaceuticals for the target drug delivery systems, which sequentially dissociate in the nidus of living organisms.

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