
LETTERS
TO THE EDITOR

Synthesis of Magnetic Nanoparticles with Radiopaque Marker

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Abstract—A formulation superior in absorption compared to any biological tissue, including bone one, has been obtained by immobilization of radiopaque substance iodixanol on the surface of magnetic nanoparticles.

Keywords: radiopaque substances, magnetic nanoparticles, spacer, surface immobilization

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Radiopaque substances are widely used in cancer and heart disease diagnostics, for example, to visualize and differentiate benign and malignant tumor by the difference in vascular permeability of tumors [1]. Generally, iodine compounds are used for these purposes, yet their usage for visualization is complicated by fast systemic clearance from blood pool to extravascular space.

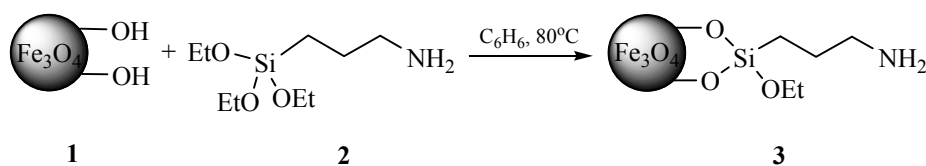
To prolongate the effect of iodixanol, the most popular radiopaque substance, conjugation or nano-encapsulation has been used. Nanogel which is a conjugate of hyaluronic acid with iodixanol has been applied in breast cancer chemotherapy and diagnostics [2]. Computer tomography has been studied for characterization of primary nodes of lung carcinoma in genetically modified mice with the use of radiopaque substance encapsulated in liposomes [1]. Such researches have been carried out using the liposomal iodide [3], in particular, liposomes loaded with iodixanol and doxorubicin [4]. Liposomal-iodide nanoparticles covered with polyethylene glycol, have been used for visualization of atheroma in animal models [5].

Synthesis of such systems based on liposomes and biocompatible acids is complicated, involving nano-encapsulation (which means preparation of double emulsions [6]), concentrating systems, repeated filtration, etc. On top of that, magnetic nanoparticles exhibiting inherent radiopaque effect may be used; this property can be enhanced by immobilization of radiopaque substance on their surface.

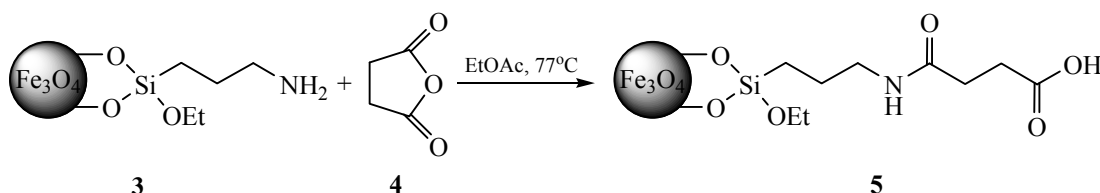
The surface modification of magnetic nanoparticles was performed with the aim of immobilization of radiopaque substance iodixanol {5,5'-[(2-hydroxy-1,3-propanediyl)-bis(acetylimino)]bis[*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide]} on their surface. The surface of magnetic nanoparticles **1** was modified via amination with 3-(triethoxysilyl) propane-1-amine **2** (Scheme 1) to immobilize the radiopaque substance, and then carboxylated spacer **5** was synthesized via the treatment of the aminated magnetic nanoparticles **3** with succinic anhydride **4** (Scheme 2).

Sorption and carbodiimide methods were used to immobilize radiopaque substance iodixanol **6**. Water-

Scheme 1.



Scheme 2.



soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) was used as a dehydrating agent. Tenfold molar excess of carbodiimide was used in carbodiimide synthesis. Covalent bound immobilized iodixanol **7** was obtained via the carbodiimide method (Scheme 3).

The coating thickness of 3-(triethoxysilyl)propane-1-amine could not be determined by electron microscopy. Both samples were nearly spherical nanoparticles. The aminated magnetic nanoparticles **3** did not agglomerate, since they were coated with aminosilane, unlike the precursors which clearly revealed the agglomerates in the microscopy images.

The total amount of amino groups in the aminated magnetic particles **3** was determined by back titration. The total amount of the amino groups did not provide the information on the sorption capacity of the modified surface of magnetic nanoparticles towards the active substance. Hence, the amount of accessible amino groups was additionally determined using the fluorescence dye (Indocyanine Green). The amount of accessible amino groups (0.048 mmol/g) was lower than their total amount in the sample (0.81 mmol/g) approximately by an order of magnitude. That typical situation has been previously studied in detail [7, 8].

The amount of carboxyl groups at the surface of magnetic nanoparticles **5** determined by back titration was found of 52 mmol/g, meaning that almost 65% of the total amount of amino groups at the surface of the particles **3** interacted with succinic anhydride, yielding the carboxylated spacer **5**. Since the length of spacer was increased, we expected the amount of the accessible functional groups available for immo-

bilization of iodixanol via carbodiimide method to be increased.

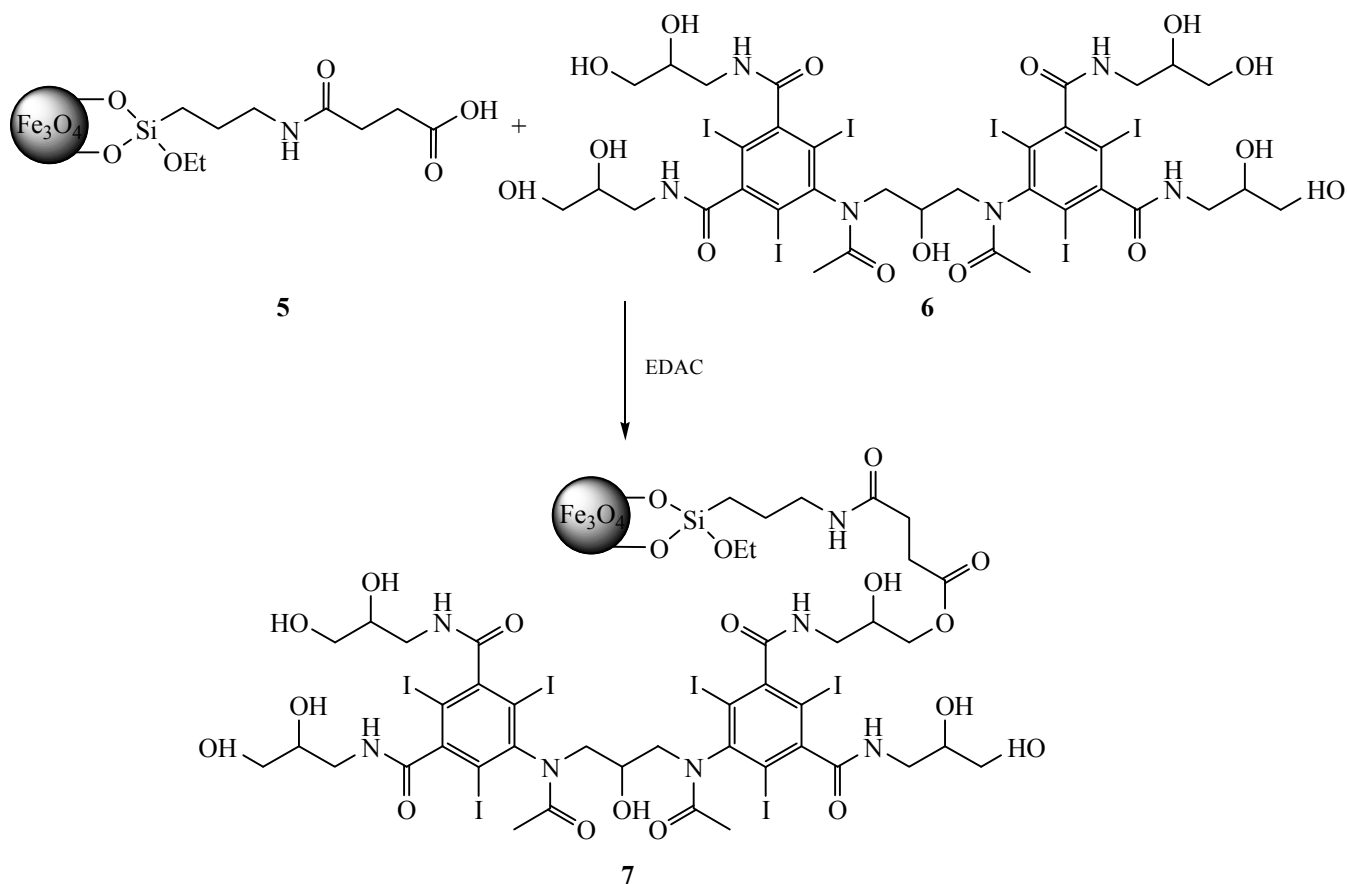
The amount of iodine in the samples with iodixanol immobilized via carbodiimide method was found to be 0.2% according to the results of X-ray fluorescence analysis. Besides, the signals corresponding to iron compounds, trace amounts of related impurities (manganese), silicon from 3-(triethoxysilyl)propane-1-amine, and sulfur from sulfate electrolytes used in the nanoparticles synthesis were detected. The content of iodine in the original formulation (solution for injections) was of 30 wt.%. However, since iodixanol was covalently bound with the surface in the obtained sample, the effect of contrasting was expected to be pronounced. Coordination-ionic mobilization of iodixanol did not occur, according to the X-ray fluorescence analysis data.

The samples of three concentrations were studied by X-ray image intensifier in comparison with biological tissues of rabbit. 3-mL specimens with mass concentration 20 mg/mL were tested.

The absorption of the obtained formulation was found much greater than that of any biological tissue (including the bone one). This fact can be used in the development of promising contrasting substances based on magnetic nanoparticles conjugated with radio-paque substances.

Microscopy images of the nanoparticles were obtained using a JEM-1400 STEM (JEOL, Japan) transmission electron microscope with cold cathode. The magnetic nanoparticles used in this study were synthesized as described elsewhere [9].

Scheme 3.



Amination of magnetic nanoparticles. A suspension of magnetic nanoparticles **1** was freeze-dried using a Vaco 2 system (ZirBus, Germany) at -50°C and pressure 3 Pa. Anhydrous benzene used for the synthesis was obtained via azeotropic mixture distillation followed by distillation over alkali. A mixture of 2 g of dried magnetic nanoparticles and 25 mL of 5% solution of 3-(triethoxysilyl)propane-1-amine **2** in anhydrous benzene was refluxed during 2 h at 80°C in a constant-temperature cell connected with a LT-105a liquid thermostat (LOIP, Russia). An excess of the reagent was removed via repeated washing with anhydrous chloroform using magnetic separation; finally, the products was washed with ethanol. During the modification and washing, the reaction mixture was vigorously stirred. The aminated magnetic nanoparticles **3** were freeze-dried.

Determination of the total amount of amino groups in the samples of aminated magnetic nanoparticles (3). 1 mL of 0.1 M hydrochloric acid was added to 50 mg of the aminated magnetic nanoparticles **3**. The

mixture was left for 15 min for neutralization with periodical shaking; then the suspension was centrifuged for 5 min at 3000 rpm. The supernatant was titrated with 0.1 M NaOH with methyl orange as indicator. The total amount of amino groups was calculated from the amount the alkali used for titration.

Determination of the amount of accessible amino groups. 1 mL of the solution of fluorescent dye Indocyanine Green (1 mg/mL) and 1 mL of water were added to a suspension of 50 mg of the aminated nanoparticles **3** in 2 mL of water. Sorption of the dye was performed in 15-mL polypropylene tubes during 2 h using an LS-220 shaker (LOIP, Russia) at 300 min^{-1} , then the solution was centrifuged for 5 min at 3000 rpm and washed with distilled water ($\times 5$) and centrifuged. In order to determine the amount of the adsorbed Indocyanine Green, 10 mL of 0.1 M solution of sodium hydroxide was added to the washed residue. The desorption was performed for 15 min, then the solution was centrifuged, and 1 mL of the supernatant was sampled. The amount of fluorescent dye was

analyzed spectrophotometrically, using a Unico 2802S spectrophotometer (Unico Sys, USA) at $\lambda = 700$ nm.

Synthesis of carboxylated spacer (5). A mixture of 1 g of aminated magnetic nanoparticles **3** and 10 mL of 5% solution of succinic anhydride **4** in anhydrous ethyl acetate was refluxed for 2 h. Upon the completion of the synthesis, the reaction product was repeatedly washed with ethyl acetate and ethanol, and freeze-dried. In order to determine the amount of carboxyl groups at the surface, 1 mL of 0.1 M solution of NaOH was added to 50 mg of nanoparticles **5**, then the mixture was stirred for 15 min and titrated with 0.1 M solution of hydrochloric acid (indicator—methyl orange) until a slight pink color of the solution.

Synthesis on immobilized iodixanol (7). A mixture of 1.5 g of lyophilized iodixanol in anhydrous DMF, 100 mg of magnetic nanoparticles **5**, and 0.5 mL of the carbodiimide were mixed in 15-mL polypropylene tube using an LS-220 shaker at 300 min^{-1} during 2 h. The obtained product was washed with distilled water ($\times 5$) using the magnetic separation and concentrated to 10 mg/mL. The amount of iodine in the obtained formulations was determined using a EDX 800 HS energy-dispersive X-ray fluorescence spectrometer (Shimadzu, Japan).

Assessment of capability of visualization of the nanoparticles with an immobilized substance was performed using a BV Endura X-ray image intensifier (Koninklijke Philips N.V., Finland) operating in Thorax regime at voltage of 48 kV.

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CONFLICT OF INTEREST

No conflict of interest was declared by authors.

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