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**SYNTHESIS AND CHARACTERIZATION
OF MAGNETIC NANOPARTICLES $\text{Fe}_3\text{O}_4@\text{SiO}_2$
DECORATED WITH AMINO GROUPS**

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The oxidation of ferrous sulfate in aqueous medium was used to prepare hydrophilic nanoparticles of Fe_3O_4 with the hydrodynamic size of 114 ± 59 nm. By means of sol-gel method, the nanoparticles were coated with a layer of amorphous SiO_2 , which provided the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles with the size of 188 ± 36 nm. The reaction of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ with 3-aminopropyltriethoxysilane resulted in attaching of amino groups to the surface of the particles. During the reaction lasting 1 hour, the original size of particles (190 ± 40 nm) remained unchanged, and the total nitrogen content was 2.21 %, and the nitrogen content corresponding to reactive amino groups was 1.22 %. A prolongation of the reaction time up to

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3 hours resulted in production of aggregates and increase in the total nitrogen content up to 3.75 %. However, the content of reactive nitrogen increased only negligibly (1.30 %). The spherical shape of the $Fe_3O_4@SiO_2-NH_2$ particles produced and their hydrodynamic size are suitable for applications to catalysis as well as for biomedical applications.

Introduction

In the recent past, the number of published papers have been increasing with the focus on possible bio-medical applications of magnetic micro- and nanoparticles, particularly in the field of magnetic resonance imaging (MRI) [1,2], magnetic separation, and targeted application of medical drugs [3-6]. The targeted action of a medical drug in this case is based upon the principle of local application of external magnetic field [3-6]. These systems tend to be used for targeted transport of the corresponding active substance to the attacked tissue, which mainly concerns cytostatics, e.g., doxorubicin [6,7]. For this reason, effort was made to develop superparamagnetic iron oxide nanoparticles (SPIONs) [2-9] for use as new drug delivery vehicles. These SPIONs represent small, synthetically prepared particles of $\gamma-Fe_2O_3$ (maghemite) or Fe_3O_4 (magnetite) with the average size between 10 nm and 200 nm. The magnetic particles can be coated on the surface with natural or synthetic biocompatible polymers, such as dextran [10,11] or poly(ethylene glycol) [12-15]. Another possibility consists in coating of these magnetic nanoparticles with a layer of Si(IV)-oxide ($Fe_3O_4@SiO_2$) [2-9]. The layer of SiO_2 ensures high chemical inertness over practically whole pH scale, and at the same time, it enables the surface of such particles to covalently bind organic molecules. For instance, with the help of 3-aminopropyltriethoxysilane (APTES), the surface of these particles can be decorated relatively easily with primary aliphatic amino groups [16-18]. From the standpoint of medical applications, the key role is played by the particles size, which determines the half-time of their circulation in the patient's body [6]. Particles smaller than 10 nm are rapidly excreted, mainly through kidneys [1,2]. Particles greater than 200 nm are concentrated in spleen and then excreted from the organism by action of phagocyte cells [6]. In both cases of the particles of such size (i.e. < 10 nm and > 200 nm), their concentration in blood plasma is rapidly decreased. Therefore, the particles of the size range from 10 nm to 200 nm represent the optimum from the standpoint of longer-time circulation [2-6]. On the other hand, the particles smaller than 2 nm usually are not suitable for medical applications, because after their diffusion through cell membranes they damage the intra-cellular organelles, and thus they can potentially be toxic [19]. Besides their utterly medical applications, magnetic nanoparticles are also used in biochemical and biological branches: the surface of such micro- and nanoparticles is used to attach enzymes, which are afterwards

easily separated from the reaction medium [20]. The possibility of rapid and simple separation of catalyst (whether an enzyme or a classic catalyst) makes it possible to considerably accelerate, and/or automate, as the case may be, the given processes. The catalyst removed by magnetic separation can be reused, which in its consequences is environmentally friendly [20]. In the recent past, there appeared reports [21,22] describing applications of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles as the carriers of the catalysts that had been applied earlier in homogeneous systems [20]. However, in enantioselective catalysis, this area has not yet been sufficiently developed for technological applications. The published results show that this type of catalysts acting in a semi-homogeneous system can be very promising even on industrial scale [20]. The aim of this present paper was to use a thorough literature search for finding and verifying a laboratory viable method of the preparation of magnetic nanoparticles of magnetite coated with a nanolayer of SiO_2 decorated with amino groups. The purpose of the work was to prepare such nanoparticles that will have suitable properties as carriers of organic molecules for the targeted transport of medical drugs or as enantioselective catalysts.

Theory

Preparation Methods of Magnetite (Fe_3O_4) Nanoparticles

Literature [1-18] describes a number of methods for preparation of magnetic nanoparticles of magnetite (Fe_3O_4). The most common method consists in coprecipitation of ferrous and ferric ions in aqueous alkaline medium [23]. The shape and size of obtained particles depend on the reaction conditions, i.e. the type of the salts used, temperature, reaction medium (pH, ionic strength), and the speed of introduction of the starting solution of the mixture of both the salts. The key role is played by the addition of substances which can be adsorbed on the surface of the particles formed. In the presence of these substances, the particles formed exhibit a relatively narrow range of size distribution [23]. The particles produced are stabilized on their surface by this additive, which prevents their aggregation and sedimentation. The additives used include chelate-forming compounds, such as citric acid or oleic acid. Another alternative is the addition of natural or synthetic polymers, such as dextran [10,11] or poly(ethylene glycol) [12-14]. Beside the coprecipitation method, the hydrophilic Fe_3O_4 nanoparticles can also be prepared by hydrolysis and partial oxidation of ferrous sulfate in a solution of sodium nitrate in the presence of sodium citrate and sodium hydroxide [24] (Fig. 1).

The above method [24] on gram scale can be used to prepare the hydrophilic Fe_3O_4 nanoparticles with the size from about 20 nm to 40 nm; these can particularly be used in medical applications. Other methods of preparation of magnetic Fe_3O_4 nanoparticles in aqueous medium include, e.g., the Massart me-

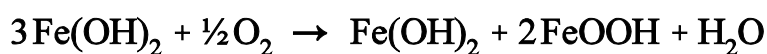
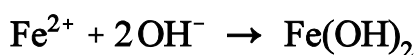


Fig. 1 The principle of preparation of magnetic nanoparticles of Fe_3O_4 by oxidation method

thod [25], in which the magnetic nanoparticles are stabilized by addition of perchloric acid or tetramethylammonium hydroxide. The preparation of particles with a narrow size distribution range makes use of microemulsion technique [10]: a stable dispersion of organic phase in water produces micelles which surround the magnetic nanoparticles. The morphology of nanoparticles produced in this way is usually governed by the structure and size of the micelles [10]. In non-aqueous media, the hydrophobic Fe_3O_4 nanoparticles are produced most frequently by thermal decomposition of precursors which contain ferric ions [26-28]. For example, the decomposition of ferric triacetylacetonate [$\text{Fe}(\text{acac})_3$] in diphenyl ether in the presence of 1,2-hexanediol, oleic acid and oleylamine at the temperature of 265 °C gave cubic monodispersion Fe_3O_4 nanoparticles with the size of 4 nm [26]. The decomposition of $\text{Fe}(\text{acac})_3$ in boiling pyrrolidine-2-one gave Fe_3O_4 nanoparticles of average size 5 ± 1.2 nm, which were stabilized on their surface with pyrrolidine-2-one [27]. Thermal decomposition of ferric triacetylacetonate in tri(ethylene glycol) [28] gave the nanoparticles of average size about 10 nm, or in dibenzyl ether in the presence of oleylamine their size was about 9 nm [29]. Also similarly the thermal decomposition of ferric complex of cupferron (FeCup_3) gave Fe_3O_4 nanoparticles [29]. Other methods are based on decomposition of iron pentacarbonyl $\text{Fe}(\text{CO})_5$ [30]. The reduction/hydrolysis of $\text{Fe}(\text{acac})_3$ with sodium borohydride (NaBH_4), which was described recently [31], appears to be a suitable method, too. This method was realized under much milder conditions (methanol, 78 °C) as compared with the described pyrolyses [26-28]. Both hydrophilic and hydrophobic Fe_3O_4 nanoparticles of 5-8 nm size were prepared in this way [31]. Industrial processes use the thermal decompositions of the above-mentioned precursors with action of ultrasound [32] or laser pyrolysis technique [33]. A number of types of magnetic micro- and nanoparticles of Fe_3O_4 are industrially available, the largest producers in the world being the firms Magnetics (USA), Miltemyi Biotec (Germany) and Iontosorb (The Czech Republic) [34].

Methods of Preparation of Fe₃O₄@SiO₂ Nanoparticles

A number of applications [1-4], however, necessitate magnetic nanoparticles whose surface is coated with relatively inert layer of SiO₂, which means preparation of nanoparticles with Fe₃O₄ nucleus and SiO₂ covering. In literature [1-7], such nanoparticles are called “core–shell” and are denoted as Fe₃O₄/SiO₂ or Fe₃O₄–SiO₂. Most often and preferably, these particles are denoted as Fe₃O₄@SiO₂ [35]. The most common way of silanization is the so-called sol-gel method, i.e., controlled base-catalyzed hydrolysis of tetraethyl orthosilicate (TEOS) proceeding at the surface of magnetite particles in aqueous ethanol in the presence of ammonia solution or sodium hydroxide solution (Stöber’s process) [36] (Fig. 2).

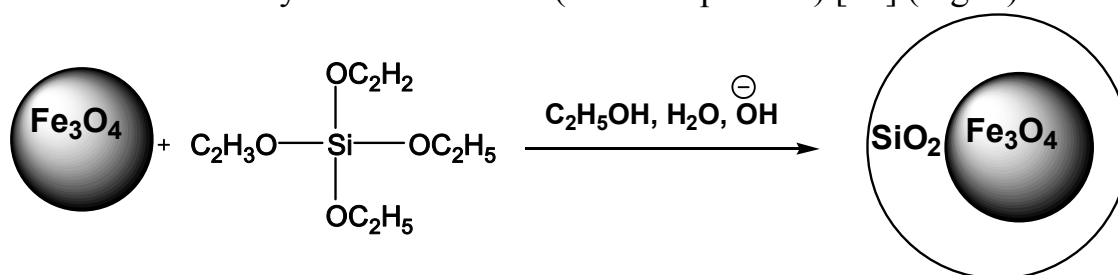


Fig. 2 Sol-gel method of preparation of magnetic nanoparticles Fe₃O₄@SiO₂: base-catalyzed hydrolysis of TEOS (Stöber’s process)

The process of coating of magnetite particles with silicon dioxide presented in Fig. 2 is seemingly very simple. However, if we want to prepare well-defined particles of Fe₃O₄@SiO₂, i.e., particles of particular diameter, monodisperse as far as possible, with a given size of silicon dioxide layer of particular porosity, the coating process has to be optimized [37,38]. Like in the case of preparation of the Fe₃O₄ particles themselves, the coating process is also controlled by many reaction parameters, particularly the reactor and its stirrer geometry, stirring rate, temperature, reaction medium, and the amount of TEOS and rate of its introduction. Fundamental role is played by the overall concentration of Fe₃O₄ particles and, last but not least, addition of surfactants or polymers preventing the aggregation of particles. With regard to the high specific mass of magnetite nanoparticles, their aggregation with formation of clusters proceeds very easily [39]. This results in production of relatively large polydisperse particles of Fe₃O₄@SiO₂, which are not suitable for medical applications [39]. A number of published reports [37-40] suggest such coating conditions which lead to well-defined and, as far as possible, uniform nanoparticles of Fe₃O₄@SiO₂. For instance, one of the methods [40] consists in a high dilution process, where the recommended concentration of starting nanoparticles should not exceed 12 mg l⁻¹. This method, however, is not suitable for preparation of a larger, practically applicable, amount of Fe₃O₄@SiO₂ particles. The temperature during the

hydrolysis of TEOS proceeding at the surface of Fe_3O_4 in the system ethanol/water/ammonia should not exceed 25 °C; otherwise the undesirable aggregates are formed [39]. However, the hydrolysis at the temperature of 25 °C proceeds very slowly and takes from 12 to 48 hours, depending on the ratios of ethanol/water/ammonia. In order to achieve the highest possible uniformity of the produced $\text{Fe}_3\text{O}_4@\text{SiO}_2$ particles, it is possible to make use of the microemulsion technique [41]. In this case, the hydrolysis of TEOS proceeds in a microemulsion of / Fe_3O_4 -water/surfactant/organic phase/. Water covers the nanolayer of hydrophilic Fe_3O_4 particle, and subsequent hydrolysis produces amorphous SiO_2 on its surface [41]. The size of this nanolayer is controlled by reaction conditions, particularly by the presence of non-ionic surfactants, such as Triton X-100 [polyoxyethylene(10)isooctylphenyl ether, 4-(C_8H_{17}) $\text{C}_6\text{H}_4(\text{OCH}_2\text{CH}_2)_n\text{OH}$, $n \sim 10$], and Igepal CO-520 [polyoxyethylene(5)nonylphenyl ether, 4-(C_9H_{19}) $\text{C}_6\text{H}_4(\text{OCH}_2\text{CH}_2)_n\text{OH}$, $n \sim 5$]. In these cases, the organic phase is *n*-heptane or cyclohexane [41]. Another method [37] of preparation of well-defined uniform nanoparticles of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ is based on the application of intensive ultrasound 20 kHz (sonochemical approach); the produced monodisperse nanoparticles of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ had the size of 4-8 nm, and the thickness of SiO_2 nanolayer was from 1 to 3.5 nm, depending on the reaction conditions [37].

Methods of Preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ Nanoparticles

For a number of applications in the field of chemistry, biochemistry and medicine it is necessary to bind organic molecules by covalent bonds to the surface of nanoparticles of Fe_3O_4 or $\text{Fe}_3\text{O}_4@\text{SiO}_2$. One of the methods achieving that aim consists in attaching of these molecules by means of primary amino groups which already have been bound to the surface of the particles [16-18]. Organic molecules (ligands) can be attached to such amine-decorated surface ($\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$) by means of relatively stable bonds (amide, carbamate) or hydrolysis-sensitive labile bonds (imine). Several different reagents can be used for attaching amino groups to the surface of nanoparticles [18], e.g., (3-aminopropyl)triethoxysilane (APTES), (3-aminopropyl)trimethoxysilane (APTMS), (3-aminopropyl)dimethylethoxysilane (APDS) or *N*-(3-trimethoxysilylpropyl)trimethoxysilane (APTED) (Fig. 3).

Previous papers [16-18] described tests and optimization of experimental conditions for introduction of amino groups onto the surface of nanoparticles Fe_3O_4 or $\text{Fe}_3\text{O}_4@\text{SiO}_2$. The most common method is the reaction with APTES in organic solvent with addition of water: acid- or base-catalyzed polymerization and hydrolysis proceed on the surface of the particles. However, the reaction needs refluxing for 48-72 hours; if microwave radiation is applied, the reaction time can be reduced down to the order of min [18]. The use of different reagents (APTMS, APDS, APTED) leads to results which differ in the content of attached amino

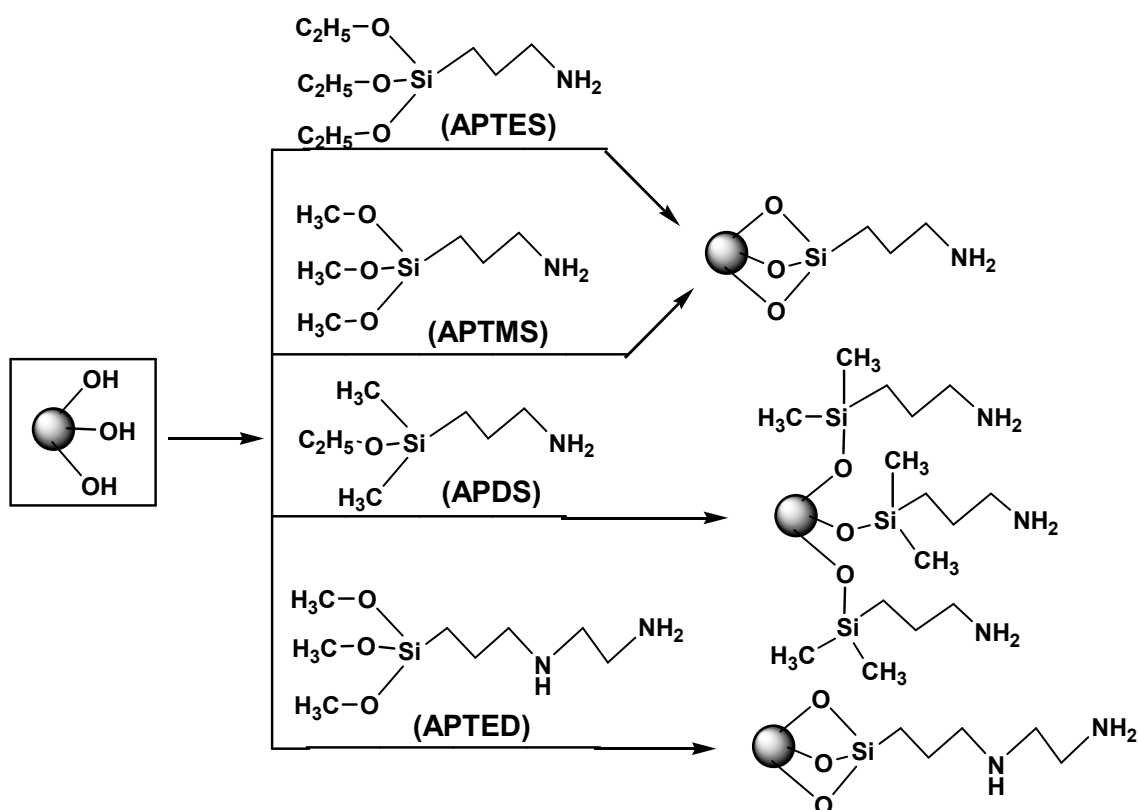


Fig. 3 Principle of decoration of surface of magnetic particles Fe_3O_4 or $\text{Fe}_3\text{O}_4@\text{SiO}_2$ with primary amino groups [18]

groups from 73 to 128 mmol g^{-1} . The best results were obtained with the reagent APTES in toluene with traces of water and with acid catalysis using 4-toluenesulfonic acid, and application of microwave radiation of 800W for a period of 5 min. Other authors pointed out [42] that the application of microwaves leads to a change in the arrangement of the molecules bound to the surface and to formation of cyclic structures. The reaction can also be accelerated by the use of Lewis acids; however, with the use of tin tetrachloride the reaction had explosive character [18]. Another paper [16] describes the effects of solvent, temperature (18, 50, and 70 °C), time (1-24 hours), applied amount of APTES (0.2 and 2 %) and the effect of ultrasound upon the nitrogen content and the morphology of prepared $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$. In the series: water, ethanol, tetrahydrofuran, the obtained results of bound nitrogen were 0.14 %, 0.31 % and 1.86 %, respectively (determined by means of microanalysis). The evaluation of these experiments revealed [16] that the content of reactive amino groups is lower than that corresponding to the nitrogen content determined by microanalysis. The difference was explained by formation of multi-layer arrangement on the surface of nanoparticles. A part of amino groups (“sequestered amino groups”) remains hidden inside the polymeric layer and, therefore, these amino groups are not available for chemical reactions. A simple spectrophotometric method was developed and tested for determination of reactive amino groups [16]. The method

is based on the reaction of reactive amino groups with 4-nitrobenzaldehyde giving an imine bond (Schiff's bases). After removal of excess 4-nitrobenzaldehyde, the imine bond is hydrolyzed. The concentration of 4-nitrobenzaldehyde released by the hydrolysis is then determined spectrophotometrically at the wavelength of 283 nm. The nitrogen content determined in this way corresponds to the reactive amino groups at the surface (Fig.4).

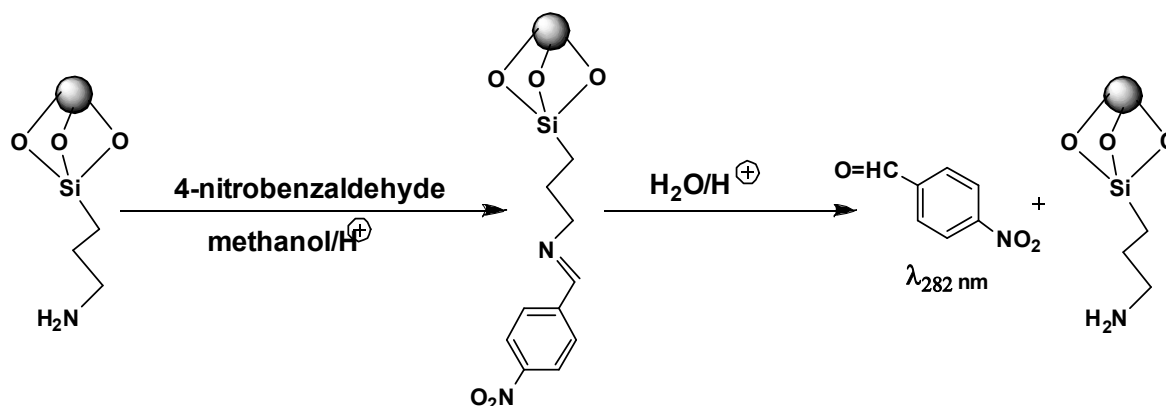


Fig. 4 Principle of spectrophotometric determination of reactive amino groups of magnetic particles $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ [16]

Materials and Methods

Microanalysis

The microanalyses were performed on an apparatus of FISON'S Instruments, EA 1108 CHN.

Spectrophotometric Measurements

The measurements were performed on a UV-vis spectrophotometer Hewlett-Packard 8453 diode-array, in a 1-cm cuvette at the temperature of 25 °C in methanol solution.

Dynamic Light Scattering (DLS)

The average particle size and size distribution of the prepared particles were determined by DLS using a Zetasizer Nano ZS (Malvern Instruments, UK). The measurements were performed at the temperature of 25 °C with a scattering angle of 173°, using disposable sizing cuvettes (80 mg l⁻¹).

Powder X-Ray Diffraction

The powder X-ray diffraction data (Cu K α , $\lambda = 1.5418 \text{ \AA}$) were collected on a D8 Advance diffractometer (Bruker AXS, Germany) with Bragg-Brentano θ - θ goniometer (radius 217.5 mm) equipped with a secondary beam curved graphite monochromator and Na(Tl)I scintillation detector. The generator was operated at 40 kV and 30 mA. The scan was performed at room temperature from 2° to 70° (2θ) in 0.02° steps with a counting time of 10 s per step.

The Scanning Electron Microscopy (SEM)

The SEM of prepared particles was carried out on a Jeol 5600LV apparatus with detector of secondary electrons (SEI). In order to increase its conductivity, the sample was coated with Au layer (0.2 nm) with the help of dust sprinkling equipment Balzers.

Spectrophotometric Determination of Reactive Amino Groups [16]

A precise amount of about 1 mg nanoparticles Fe $_3$ O $_4$ @SiO $_2$ -NH $_2$ in 1 ml Eppendorf vessel was mixed with 1 ml anhydrous acetic acid solution (0.8%) in methanol (coupling solution). After mixing, the particles were separated by action of external magnet and then again mixed with the coupling solution; the procedure was repeated four times. The washed particles were treated with 1 ml solution of 4-nitrobenzaldehyde (7 mg in 10 ml coupling solution). After 3 hour stirring at room temperature, the particles were separated and again washed with coupling solution four times. The hydrolysis was carried out by addition of 1 ml hydrolytic solution (75 ml water, 75 ml methanol, and 0.2 ml glacial acetic acid). After one hour of treatment, the magnetic particles were separated, and the remaining clear solution was measured spectrophotometrically at the wavelength of 283 nm. By means of calibration curve, the absorbance value was used to determine the concentration of released 4-nitrobenzaldehyde, which was converted into the nitrogen content corresponding to reactive amino groups.

Preparation of Fe $_3$ O $_4$ Nanoparticles [24]

A boiling solution of 2.78 g (10 mmol) ferrous sulfate heptahydrate in 5 ml water was added at once into boiling solution of 1.4 g (35 mmol) sodium hydroxide, 1.05 g (5 mmol) citric acid, and 85 g (1 mol) sodium nitrate in 95 ml water, whereupon the mixture was refluxed 1 hour. After cooling, the Fe $_3$ O $_4$ particles were separated

with magnet and subsequently washed with 3×100 ml distilled water and 2×100 ml methanol and dried in a vacuum drying oven at the temperature of 40°C . The yield was 700 mg of nanoparticles.

Preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ Nanoparticles

A 500 ml three-necked round-bottom flask equipped with a stirrer and placed in an ultrasonic bath was charged with 80 mg Fe_3O_4 nanoparticles in 20 ml distilled water. After 30 min dispersing by action of ultrasound (450 W), the mixture was treated with 4 ml $2\text{NH}_3 \cdot \text{H}_2\text{O}$ (25%) solution, 12 ml water, and 200 ml absolute ethanol denaturized with petrol. After 10 min, the stirred (300 min^{-1}) reaction mixture placed in ultrasonic bath was treated with a solution of 140 mg (0.672 mmol) TEOS in 10 ml absolute ethanol, which was added drop by drop during 10 min. After 24 hour stirring at the temperature of 40°C , the particles $\text{Fe}_3\text{O}_4@\text{SiO}_2$ were separated by means of a magnet and subsequently washed with 3×50 ml distilled water and 2×50 ml methanol and dried in a vacuum drying oven at the temperature of 40°C . The yield was 120 mg $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles.

Preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ Nanoparticles

A mixture of 100 mg $\text{Fe}_3\text{O}_4@\text{SiO}_2$, 50 ml water, and 100 ml 3-aminopropylethoxysilane (0.452 mmol) in 200 ml tetrahydrofuran placed in a round-bottom flask was submitted to action of ultrasound (450 W) at the temperature of 40°C for different time periods (1 hour and 3 hours). The particles of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ were separated by means of a magnet and subsequently washed with 3×50 ml distilled water and 2×50 ml methanol and dried in a vacuum drying oven at the temperature of 40°C . In the nanoparticles prepared (about 100 mg), the total nitrogen content was determined by microanalysis, and the content of reactive amino groups was determined spectrophotometrically.

Results and Discussion

First of all, we verified the preparation of hydrophilic nanoparticles of Fe_3O_4 by the method of partial hydrolytic oxidation of ferrous sulfate [24]. The reaction was performed by pouring ferrous sulfate solution into sodium nitrate solution containing sodium citrate and sodium hydroxide. The obtained nanoparticles of Fe_3O_4 were separated by means of a permanent magnet, washed and dried in vacuum. In this way, we prepared about 700 mg Fe_3O_4 from 2.78 g (10 mmol) ferrous sulfate heptahydrate (yield 91 %). Figure 5(a) presents the powder X-ray

diffraction diagram showing the diffraction peaks 18.3, 30.1, 35.5, 43.1, 53.5, 57 and 62.6° (2θ). In accordance with the results published earlier [24], in this way we prepared clusters of cubic spinel nanocrystals of Fe₃O₄ with average size of crystals in cluster equal to 13 nm. This size was calculated according to Sherrer's formula [37].

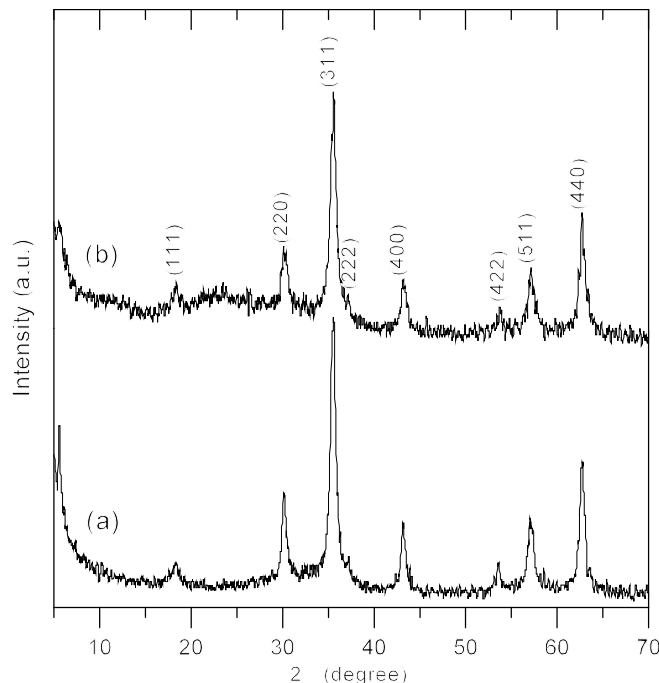


Fig. 5 Powder X-ray diffraction diagram of (a) the Fe₃O₄ nanoparticles prepared by the method of partial hydrolytic oxidation of ferrous sulfate and (b) Fe₃O₄@SiO₂ nanoparticles prepared by sol-gel method

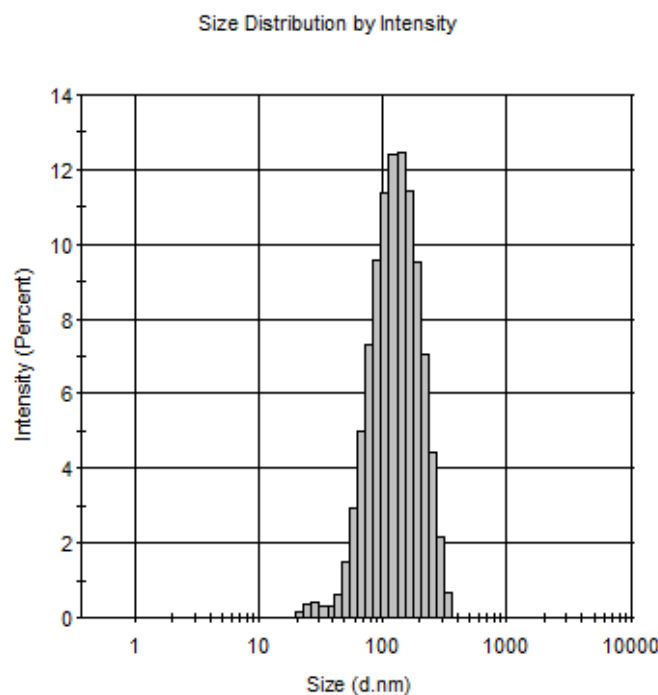


Fig. 6 Diagram of distribution of hydrodynamic size of Fe₃O₄ nanoparticles prepared by method of partial hydrolytic oxidation of ferrous sulfate

Furthermore, the hydrodynamic size of the prepared Fe_3O_4 nanoparticles was determined by means of dynamic light scattering (DLS).

The diagram in Fig. 6 shows that the hydrodynamic size of particles was 114 ± 59 nm. These hydrophilic nanoparticles of Fe_3O_4 were then coated with a layer of SiO_2 by means of sol-gel method, i.e., controlled hydrolysis of TEOS proceeding on the surface of the Fe_3O_4 particles in aqueous ethanol in the presence of ammonia solution (Stöber's process) [36]. The preparation of $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ nanoparticles having the narrowest possible distribution curve required ultrasonic treatment throughout the reaction time (24 hours). The action of ultrasound restricted the formation of conglomerates [42,43]. The hydrodynamic size of prepared particles $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ was determined by means of DLS and was equal to 188 ± 36 nm (Fig. 7).

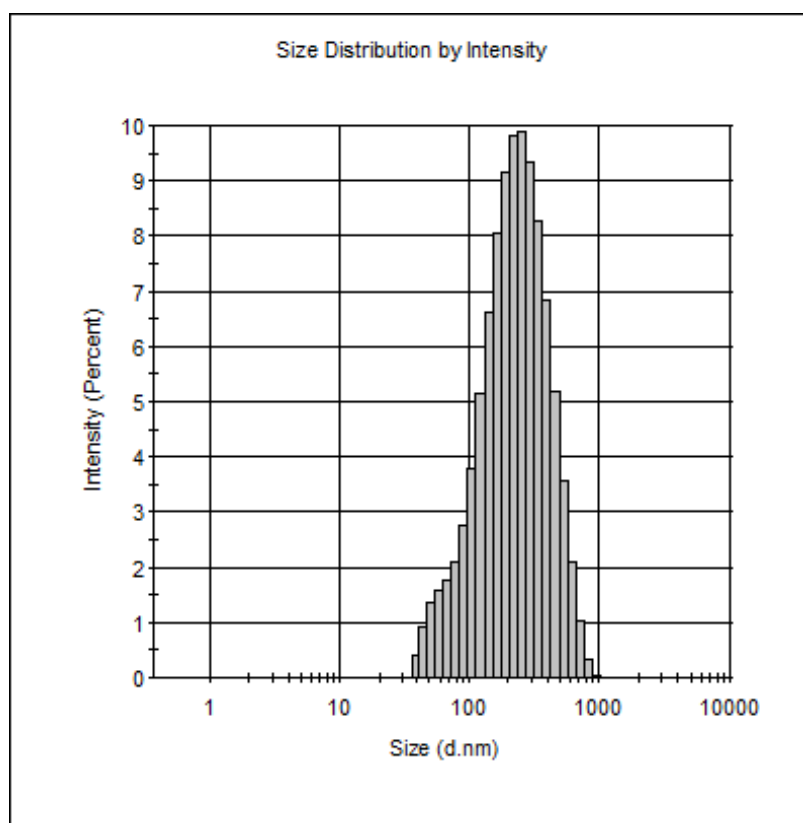


Fig. 7 Diagram of distribution of hydrodynamic size of nanoparticles $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ prepared by sol-gel method

Moreover, the morphology of prepared nanoparticles was studied by means of scanning electron microscopy (SEM) (Fig. 8.).

From Fig. 8 it is obvious that the prepared nanoparticles have spherical shape. During drying of the solution of nanoparticles on microscopic slide, there occurs distinct formation of agglomerates. Next, the prepared nanoparticles of $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ were submitted to reaction with APTES in the presence of water in

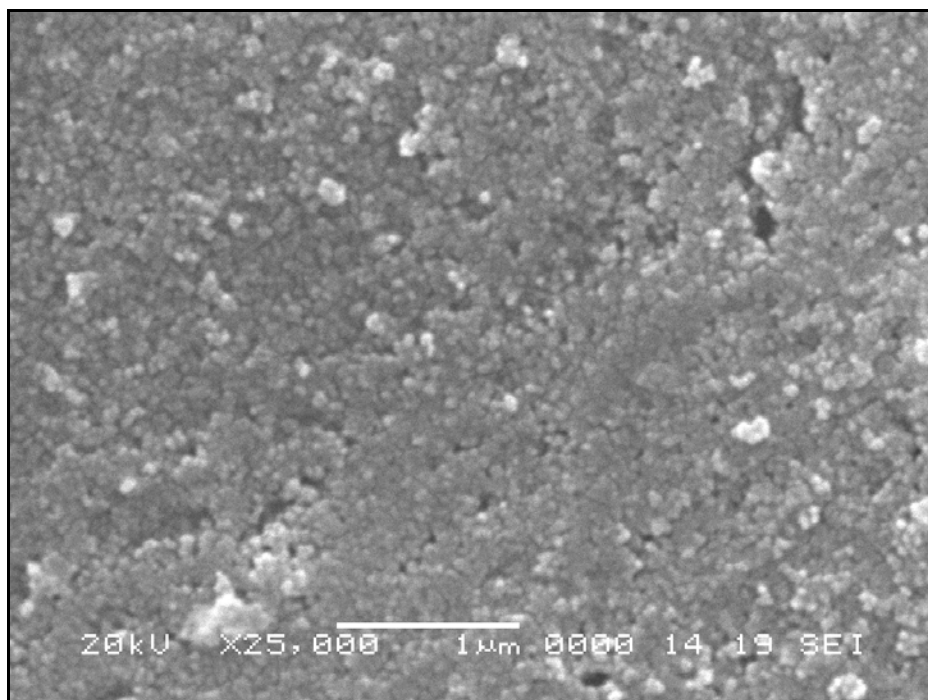


Fig. 8 SEM of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles prepared

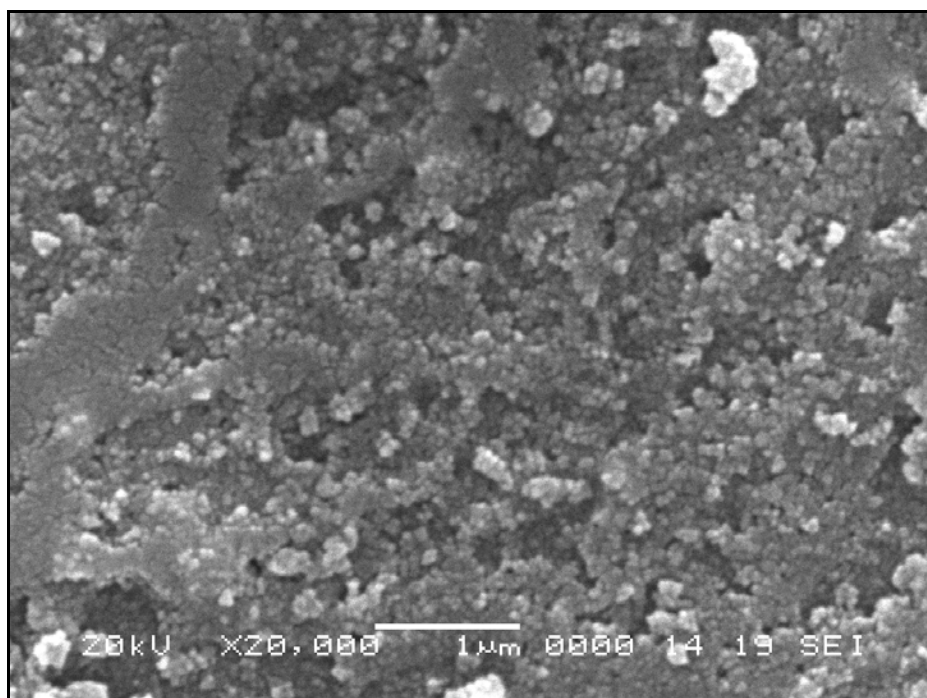


Fig. 9 SEM of particles $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ (after 1-hour reaction)

tetrahydrofuran at the temperature of 40 °C and under the action of ultrasound (450 W). The overall nitrogen content was determined by microanalysis, and the

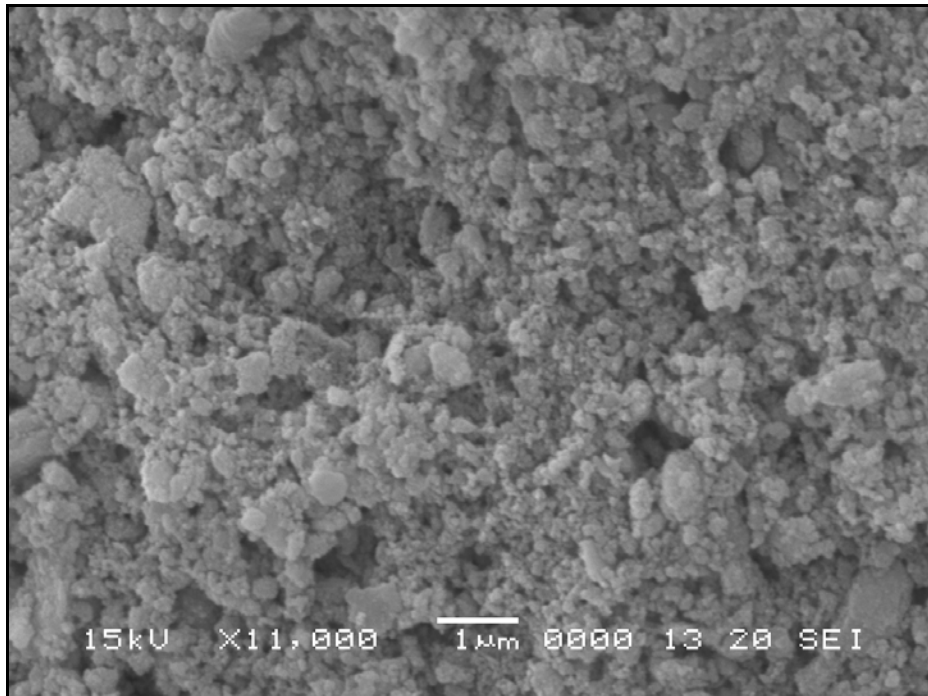


Fig. 10 SEM of particles $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ (after 3-hour reaction)

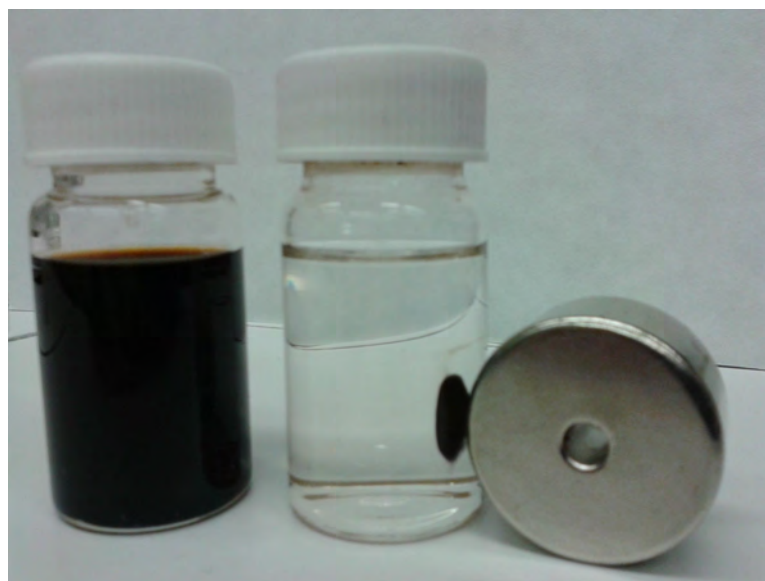


Fig. 11 Separation of nanoparticles $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ by application of external magnetic field

content of reactive amino groups was determined spectrophotometrically [16].

After 1 hour of reaction, the total nitrogen content determined by microanalysis was 2.21 %, and the nitrogen content corresponding to reactive amino groups determined spectrophotometrically was 1.22 %. After 1 hour of reaction, the hydrodynamic diameter of the particles remained practically unchanged (190 ± 40 nm), and the morphology of prepared $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ particles was comparable

with that of the starting $\text{Fe}_3\text{O}_4@\text{SiO}_2$ particles (Fig. 9). Prolongation of the reaction time to three hours increased the total nitrogen content to 3.74 %. The nitrogen content corresponding to reactive amino groups increased only negligibly, namely to 1.30 %. After this longer reaction time, however, aggregates with the size of microparticles were formed (Fig. 10.).

Figure 11 shows the possibility of separation of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ particles from aqueous medium by means of external magnet. Beside the simple separation, the application of magnetic field can also be used for targeted therapy in the cases where a medical drug is bound by means of a labile bond to the surface of nanoparticles [44]. The spherical shape and hydrodynamic distribution of sizes of the prepared particles of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ are suitable for medical applications.

Conclusion

This present paper gives an up-to-date survey of literature concerning the laboratory-applicable methods of preparation and characterization of magnetic particles of magnetite coated with a nanolayer of SiO_2 with amino groups attached to the surface. The preparation methods were tested with regard to the application in the fields of both medicine and catalysis. The oxidation method of preparation of Fe_3O_4 was verified experimentally. The Fe_3O_4 nanoparticles prepared had the size equal to 114 ± 59 nm. Also verified was the sol-gel method of preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ by controlled hydrolysis of TEOS, and the particles prepared by this method had the size equal to 188 ± 36 nm. The reaction of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ with 3-aminopropyltriethoxysilane was used for introduction of amino groups on the surface. The prepared particles of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ had the hydrodynamic size equal to 190 ± 40 nm. The optimum reaction time of amination is 1 hour; a longer reaction time increases the total nitrogen content, but the content of reactive nitrogen is increased only negligibly; on top of that, aggregates of microparticle sizes are formed. The prepared nanoparticles of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ were used subsequently for preparation of a pH-sensitive targeted conjugate of isoniazid [44].

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