Russian Chemical Reviews **79** (3) 219 – 229 (2010) © 2010 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2010v079n03ABEH004068

Synthesis of magnetic polymeric microspheres

A Yu Gervald, I A Gritskova, N I Prokopov

Contents

I.	Introduction	219
II.	Magnetic materials and magnetic nanoparticles	219
III.	Synthesis of magnetic nanoparticles	220
IV.	Synthesis and stabilization of magnetic fluids	221
V.	Synthesis of magnetic polymeric microspheres	221

Abstract. The key types of magnetic polymeric microspheres are considered. Methods of synthesis of different types of magnetic nanoparticles and of preparation of stable magnetic fluids on their basis are outlined. The overview of the methods for the manufacture of magnetic polymeric microspheres is presented. The effect of the synthesis conditions on the characteristics of magnetic polymeric microspheres such as the diameter and the particle size distribution and the content of magnetic material is discussed by particular examples. The application fields of magnetic polymeric microspheres are briefly surveyed. The bibliography includes 117 references.

I. Introduction

Magnetic polymeric microspheres represent a kind of magnetically controlled disperse systems widely used at present in solving different problems of biology, biotechnology, pharmaceutics and medicine as the carriers of biologically active compounds.[1] Magnetic microspheres were employed in magnetic separation of bio-molecules,[2] sell sorting,[3] drug delivery,[4] magnetic resonance imaging,[5,6] therapy and diagnostics,[7] extraction of malignant cells from the organism and hyperthermic treatment of neoplastic cells aimed at their inactivation[8] and also for antibody detection in immunologic responses or in agglutination diagnostic tests.[9]

Magnetic polymeric microspheres can also be considered as polymeric matrices with incorporated superfine magnetized particles.[10–16] The main idea underlying the synthesis of such polymeric microspheres was to combine the mechanical, optical and electrical properties of organic materials and inorganic nanoparticles in a single whole. The properties of magnetic polymeric microspheres are determined first of all by their sizes and the magnetic material bulk distribution. The possible morphological types of such microspheres are discussed in Section V.

The polymeric shell can consist of different kinds of polymers, *e.g.*, polystyrene (PS), cross-linked polyacrylamide, polyacrylates, polyacrolein, poly(vinyl alcohol) (PVA), poly(methyl methacrylate), polyamines, polyimines, poly(ethylene glycol), dextran, *etc.*[11,12] The functional groups introduced on the surface of polymeric microspheres to impart the affinity for one or another biological object can also be of different nature, namely:[14] carboxyl, amine, chloromethyl, amide, hydrazide, aldehyde, thiol, hydroxyl, epoxide, *etc.*[15,16]

For magnetic polymeric microspheres to be used in a particular field of biotechnology, the size distribution and the amount of the magnetic constituent are important. Thus the rate of extraction of magnetic polymeric microspheres from different media under the action of a magnetic field depended on the iron content and their size.[17] It is considered that the successful use of magnetic microspheres requires the content of magnetic material to be no less than 12 mass %.[18]

It should be emphasized that the currently accessible commercial magnetic dispersions characterized by the wide diversity as regards the particle size and the magnetic material content, are optimized and destined mainly for the processes based on magnetic separation.[10]

II. Magnetic materials and magnetic nanoparticles

Many important properties of a material depend substantially on the particle size and change upon transition from macroobjects to nanoobjects. This to the full extent applies to magnetic properties. Macroscopic samples of magnetically ordered materials below the ordering temperature were found to have the domain structure with magnetic spins oriented in the coordinated manner in each domain.[19] Upon application of the magnetic field, the magnetic domains tend to be oriented along the field direction and the sample as a whole becomes magnetized.

A Yu Gervald, I A Gritskova, N I Prokopov M V Lomonosov Moscow State Academy of Fine Chemical Technology, prosp. Vernadskogo 86, 119571 Moscow, Russian Federation. Fax (7-495) 434 87 11, tel. (7-495) 936 88 40, e-mail: gervald@bk.ru (A Yu Gervald), tel. (7-495) 936 82 52 (I A Gritskova), tel. (7-495) 936 82 04, e-mail: prokopov@mitht.ru (N I Prokopov)

Received 16 July 2009
Uspekhi Khimii **79** (3) 249 – 260 (2010); translated by T Ya Safonova

As a rule, ferromagnetics exhibit high positive magnetic permeability and are characterized by hysteresis (Fig. 1) and remnant magnetization, the latter strongly depending on the external effects and sample prehistory.[20] Above the Curie temperature, ferromagnetics become paramagnetics. Ferromagnetics include three d-metals (Fe, Co, Ni), six f-metals (Gd, Tb, Dy, Ho, Er, Tm), several alloys and compounds of the mentioned metals with each other and their compounds with other elements, certain Cr and Mn alloys and compounds with non-ferromagnetic elements, *etc*.

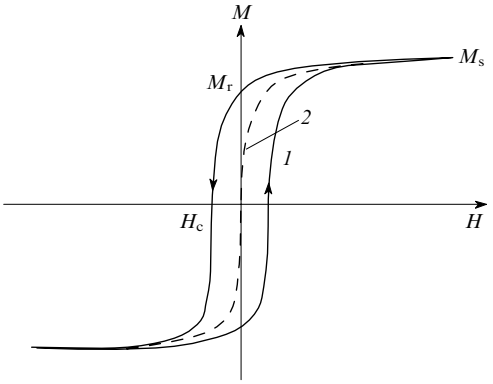


Figure 1. Magnetization curves of a ferromagnet (*1*) and a superparamagnet (*2*).
M_s is saturation magnetization, H_c is coercive force, M_r is remnant magnetization.

Ferrimagnetics are also magnetized in the external magnetic field, exhibit remnant magnetization, and pass to the paramagnetic state above the Curie point. However, the existence of several different sublattices of magnetic atoms (ions) leads to a more complex temperature dependence of magnetization as compared with ferromagnetics. A large class of ferrimagnetics is formed by ferrites, *i.e*., complex oxides with the general composition $MO \cdot Fe_2O_3$, where M is a bivalent metal (Fe, Ni, Co, Mn, *etc*.).[21]

As the particle size in the magnetic material decreases below a certain critical value, the domain structure is no more energetically favourable and the particles become single-domain. A system of such single-domain nanoparticles acquires new magnetic properties, *i.e*., passes to the superparamagnetic state.[22,23] Below, the critical diameters (d_{crit}) are shown for certain materials at room temperature.

Material	d_{crit}/nm	Material	d_{crit}/nm
Cobalt	70	Nickel	55
Iron	14	Magnetite	128

Superparamagnetic particles behave like the paramagnetic ones but have substantially higher magnetic moments. Superparamagnetic materials exhibit no remnant magnetization, the coercive force is zero and their magnetization dependence on the external magnetic field lacks the hysteresis loop (see Fig. 1).[24] For single-domain particles localized in a solid, only the magnetic moment vector changes its direction. In magnetic fluids, particles themselves can rotate like the molecules in paramagnetic gases.

Yet another distinguishing feature of superparamagnetics is the presence of heat fluctuations of the magnetic moment (analogous to fluctuation for common paramagnets).

To synthesize single-domain particles, it is necessary to control the particle size and prevent their aggregation.[21] The magnetic behaviour of aggregated particles considerably differs from that of individual particles; hence, aggregation results in the uncontrolled and irreproducible behaviour of magnetic materials.

III. Synthesis of magnetic nanoparticles

The choice of material for the production of magnetic nanoparticles is determined by quite a number of factors. For the further practical application, a material should be easily accessible and exhibit sufficiently high Curie temperature. Nanoparticles of magnetic materials are highly susceptible to aggregation; hence, any method of their synthesis should envisage the particle stabilization. The most popular nanoparticles are those of ferromagnetic d-metals and their compounds, first of all, magnetite (Fe_3O_4).

Iron nanoparticles can be manufactured by different methods. One of them is the reduction of iron salts by complex hydrides in surfactant-stabilized microemulsions. For example, nanoparticles 2–15 nm in diameter were synthesized[25] by the reduction of $FeCl_3$ with sodium borohydride in the presence of dodecyltrimethylammonium chloride. This method was also used for the synthesis of iron nanoparticles with the diameter of no less than 100 nm by reduction of $FeCl_2$ with $NaBH_4$ in the presence of sodium bis(2-ethylhexyl) sulfosuccinate (AOT).[26]

The efficient methods of synthesis of magnetic nanoparticles include thermolysis of a mixture of bivalent metal M (M = Co, Ni, Mn, Fe) and iron(III) oleates in octadec-1-ene at 300 °C. As the result, cubic nanoparticles of the corresponding ferrites MFe_2O_4 measuring from 9 to 24 nm and exhibiting high-degree monodispersion were formed.[27] A serious advantage of this method is the possibility of producing nanoparticles in amounts of up to 40 g in a single experiment.[28]

Several methods for the preparation of magnetic cobalt nanoparticles were described of which the following methods were used most widely: reduction of $CoCl_2$ in microemulsions stabilized with ionogenic surfactants,[29,30] reduction of $CoCl_2$ in micelles of amphiphilic block copolymers[31] and reduction of cobalt halides in a polystyrene matrix.[32] In another study,[33] the organometallic precursor $Co(\eta^3\text{-}C_8H_{13})(\eta^4\text{-}C_8H_{12})$ (C_8H_{13} is cyclooctenyl, C_8H_{12} is cycloocta-1,5-diene) was reduced with hydrogen in tetrahydrofuran containing polyvinylpyrrolidone giving rise to cobalt. Superparamagnetic nanoparticles of cobalt ferrite ($CoFe_2O_4$) were synthesized in the reaction of $FeCl_2$ with cobalt acetate.[34]

Magnetic nanoparticles were also manufactured by thermal decomposition of organometallic compounds,[35] dicobalt octacarbonyl serving as the starting material. Quick heating to 110 °C produced cobalt atoms, which was followed by their quick aggregation into clusters and spherical nanoparticles. Cobalt nanoparticles possessed a narrow size distribution and the diameter in the range of 2–30 nm depending on such parameters of the decomposition process as dicobalt octacarbonyl concentration and temperature.

Cobalt nanoparticles were synthesized by thermal decomposition of $Co_2(CO)_8$ in micelles of a styrene–4-vinylpyridine block co-polymer, which served as nanoreactors.[31] The decomposition was carried out at 110 °C for 1 h in toluene under argon atmosphere. Transmission electron microscopy has shown that the thus synthesized particles were spherical with the average diameter of 10 nm.

Iron nanoparticles were synthesized[36] by thermal decomposition of $Fe(CO)_5$ in argon atmosphere at 150 °C in the presence of block co-polymers of styrene with butadiene, phenyl vinyl ketoxime, 4-vinylpyridine, *etc*. By varying the polymer : solvent and iron : polymer ratios, nanoparticles with the diameter in the range of 2–20 nm were obtained; moreover, it was found that only systems with the particle size < 10 nm exhibited superparamagnetic properties.

The preparation of magnetic metal nanoparticles also involved sonication as a method of decomposition of organometallic precursors. This method was successfully used in the manufacture of cobalt nanoparticles from $Co(NO)(CO)_3$, nickel nanoparticles from $Ni(CO)_4$, iron–nickel alloy nanoparticles from a mixture of $Fe(CO)_5$ and $Ni(CO)_4$ and $CoFe_2O_4$ nanoparticles from solutions of $Fe(CO)_5$ and $Co(NO)(CO)_3$.[37]

Particles of ferromagnetic 3d-metals (iron, nickel and cobalt) are characterized by high magnetic moments; however, their drawbacks are electric conductivity and the susceptibility to surface oxidation upon contact with atmospheric oxygen,[22] which results in the partial loss of magnetic properties. From this viewpoint, preference should be given to nanoparticles of iron oxides, for example, magnetite, which has the cubic spinel structure and is a ferromagnetic at temperatures below 858 K.[38] The main advantage of magnetite is its resistance to oxidation, which ensures the stability of its magnetic characteristics in time.

There are several methods for the synthesis of magnetite particles of which one of the first methods dates back to 1852. In this synthesis, aqueous solutions of a mixture of Fe^{III} and Fe^{II} salts in the molar ratio 1 : 2 were treated with a base. This reaction is known as the method of chemical co-deposition. In 1938, Elmore reported a method of production of stabilized magnetite colloids.

In his method, an aqueous solution of $FeCl_2 \cdot 4\,H_2O$ and $FeCl_3 \cdot 6\,H_2O$ was treated with a solution of NaOH with stirring. The precipitate formed was washed with water and 0.01 M hydrochloric acid and then boiled in a soap solution to stabilize the colloidal particles. Methods proposed later involved sonication of an aqueous solution of iron(II) acetate and reduction of goethite [α-FeO(OH)] from a glycol–water solution at elevated temperatures and pressures followed by controlled crystallization.[39]

IV. Synthesis and stabilization of magnetic fluids

One of the methods for stabilization of magnetic nanoparticles is the preparation of magnetic fluids. Magnetic fluids or ferrofluids are surfactant-stabilized suspensions of colloidal magnetic nanoparticles in water or another liquid medium. The magnetic phase is most often represented by iron oxides of which magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) and haematite (α-Fe_2O_3) are most common (the latter is an antiferromagnet but in a certain temperature range exhibits weak ferromagnetism).[40] In the presence of a magnetic field, ferrofluids behave as single-phase systems, *i.e*., the system was not separated into liquid-carrier and magnetic nanoparticles.[41] Due to their unique structure and magnetic properties, the scope of application of magnetic fluids is very wide.[42]

A method for the manufacture of magnetic fluids was developed and later refined by Rosenzweig (see, *e.g*., Ref. 43). After grinding, the magnetic particles are dispersed in an organic dispersion medium containing oleic acid. Oleic acid is adsorbed on the particle surface thus providing the steric stabilization factor. This method solved the problem of synthesizing homogeneous and stable magnetic fluids in different organic solvents.

A process of dispersing magnetic particles in toluene was described.[44]

In the synthesis of magnetic particles, in contrast to the known methods, iron(II) sulfate was first oxidized with nitrite and then precipitated upon addition of concentrated ammonia. The synthesized magnetic particles were dispersed in an aqueous solution containing a fatty acid salt (*e.g*., oleate) as the surfactant; the addition of hydrochloric acid to the system promoted destabilization and aggregation of nanoparticles. Next, water was removed and nanoparticles with a surfactant layer on their surface were dispersed in toluene.

Other methods of the preparation of magnetic fluids are known. For example, barium ferrite nanoparticles were obtained[45] by mixing the following two inverse microemulsions (water in oil): one contained $Fe(NO_3)_3$ and $Ba(NO_3)_2$ and the other contained $(NH_4)_2CO_3$. Homogenization of the system led to the deposition of $BaFe_{12}O_{19}$ nanoparticles which were stabilized in the organic medium by a surfactant (a mixture of n-butanol and cetyltrimethylammonium bromide) present in the latter medium.

Yet another original procedure consisted of electroextraction of metals from aqueous solutions of the corresponding salts to afford cathodic deposits; the latter were immediately dispersed in organic media containing dissolved stabilizers.[46] This method was used for the preparation of polymer-stabilized suspensions of iron, cobalt, nickel in mineral and vegetable oils. The particle sizes were typically 70–150 nm, the particle concentrations did not exceed 0.1 mass %. Particles measuring 30–80 nm could be obtained by special selection of the process parameters.

Another no less original process was described[47] aimed at the preparation of fine particles of Fe, Co or Ni in a rotating cylindrical evaporation chamber. The metals were evaporated *in vacuo* onto the surface of a film of a liquid carrier (naphthalene) containing the pre-set concentration of a polymer. The polymer adsorption on the metal nanoparticles favoured their stabilization in the organic medium; the synthesized nanoparticles were agglomerated by heating in a flow of argon and isolated from the organic medium by centrifuging. Finally, the nanoparticles were redispersed in toluene containing the polymer by sonication. This method allowed manufacture of superparamagnetic particles measuring ~20 nm.

V. Synthesis of magnetic polymeric microspheres

Recently, the attention of scientists has become more and more attracted to the synthesis of magnetic polymeric microspheres. The first polymeric microspheres with a narrow size distribution were prepared by Dow Chemical in 1950, while the first composite microspheres were obtained upon acrylamide polymerization in the presence of magnetic iron oxide nanoparticles only in 1975. The

particle size distribution of the hydrophilic magnetic polymeric suspension prepared was rather broad, which made it unsuitable for the application in medicine and biotechnology.

The past decades were marked by the appearance of a large number of publications devoted to the methods of synthesis of magnetic polymeric microspheres for biotechnology and medicine. Thus magnetic polymeric microspheres were obtained *in situ* by the deposition of a polymer that served as the stabilizer on the surface of magnetic polymeric nanoparticles. The role of the stabilizer was played by water-soluble carboxymethyldextran,[48] polyethyleneimine,[49] poly(vinyl alcohol),[50] poly(ethylene glycol),[51] sodium polyoxyalkylene diphosphonates[52] and starch derivatives.[53]

An alternative method for the manufacture of magnetic polymeric microspheres is separate preparation of magnetic nanoparticles and polymeric microspheres followed by their mixing. Polymeric microspheres could be synthesized by various methods of emulsion, suspension or precipitation heterophase polymerization.[54, 55]

Heterophase polymerization of monomers can be carried out in the presence of magnetic nanoparticles with a surfactant adsorbed on their surface. For example, such magnetic nanoparticles were added to a mixture of styrene and glycidyl methacrylate after which the polymerization was carried out. The resulting magnetic polymeric microspheres had the diameter of 100 – 200 nm.[55] Magnetite-containing microspheres measuring 150 – 200 nm were synthesized by seed precipitation polymerization of methacrylic acid and hydroxyethyl methacrylate in the medium of ethyl acetate.[56] Magnetite particles with a narrow size distribution encapsulated into a co-polymer of methacrylic acid and hydroxyethyl methacrylate were synthesized[57] by the microemulsion seed co-polymerization; however, the content of magnetic material did not exceed 3.3 mass %.

Magnetic polymeric suspensions were prepared[58] by dispersing magnetite in an organic dispersion medium consisting of vinylaromatic monomers and a poorly soluble initiator, emulsifying this mixture in water and its polymerization. Polymeric microspheres with the magnetite content in the range from 0.5 mass % to 35 mass % were produced by this method. However, particle size distribution in suspensions was broad (from 30 to 5000 nm). A similar method was used[59] for the manufacture of magnetic composite microspheres of a hydrophobic cross-linked vinylaromatic polymer, which however afforded polymeric suspensions containing both microspheres with incorporated magnetic nanoparticles and 'empty' microspheres (containing no magnetic nanoparticles). The encapsulation of magnetic nanoparticles into hydrophilic polymers was also carried out[60, 61] by inverse miniemulsion polymerization.

Thus, magnetic polymeric microspheres can be divided into the following three types with respect to their morphology (Fig. 2):

— core – shell structures with a magnetic core inside a microsphere (Fig. 2 *a*);

— structures in which magnetic nanoparticles are uniformly distributed throughout the polymeric matrix (Fig. 2 *b*);

— structures representing a polymeric core with a surface layer of magnetic nanoparticles (Fig. 2 *c*).

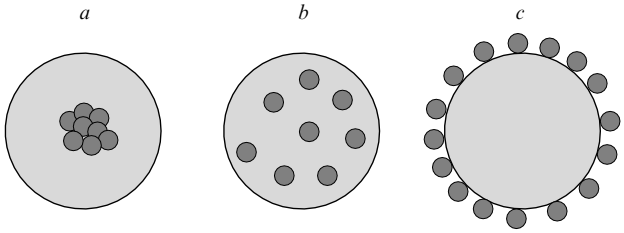


Figure 2. Morphology types of magnetic polymer microspheres (see text).

The different methods of preparation of magnetic polymeric microspheres can be classified into the following three groups:

— combination of magnetic nanoparticles and a polymer prepared separately;

— preparation of magnetic nanoparticles inside polymeric microspheres or in the presence of pre-formed polymers;

— polymerization of monomers in the presence of magnetic nanoparticles.

In the following sections, these methods are considered in more detail.

1. Combination of magnetic nanoparticles and a polymer prepared separately

As follows from the very title, this method of fabrication of magnetic polymeric microspheres makes use of preliminarily prepared magnetic nanoparticles. Depending on the nature and the size of nanoparticles and also on the polymer nature, synthesis of composite microspheres can be carried out by two different methods (Fig. 3), namely, by introducing magnetic nanoparticles into seed polymeric microspheres or by applying polymeric surfactants on the surface of an inorganic magnetic material. Evidently, the former method requires that the polymeric microspheres

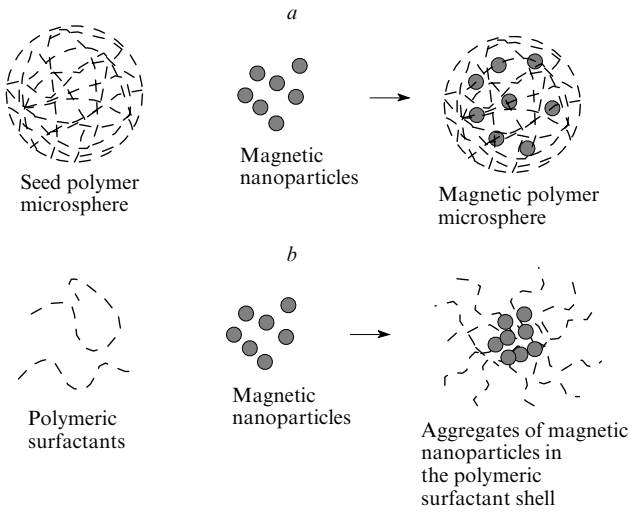


Figure 3. Synthesis of composite microspheres by incorporation of magnetic nanoparticles into preliminarily prepared polymer microspheres (*a*) and encapsulation of aggregated magnetic nanoparticles into polymeric surfactant (*b*).

have sufficiently large pores and the magnetic nanoparticles be sufficiently small.

The first attempts to synthesize polymeric microspheres for medicinal purposes were carried out with biocompatible and biodegradable polymers such as albumin, poly(vinyl alcohol), *etc*. The method of preparation [62,63] of such particles from albumin involved sonication of an inverse emulsion of an aqueous solution containing albumin, a surfactant and iron oxide nanoparticles in natural oil, which was followed by its heating to 110–165 °C or the addition of a cross-linking agent for fixing magnetic nanoparticles in the polymer. The magnetic microspheres contained 20 mass %–50 mass % Fe_3O_4 and had the average diameter of 1 μm.

This method also allowed manufacture of magnetic polymeric microspheres with smaller diameters (200–500 nm). This was achieved by varying the amounts of a solvent and a component as well as the intensity of sonication.[63] However, the size distribution of such microspheres was broad (coefficient of variation 20%) and the magnetite content was only 5 mass %–9 mass %.

In the preparation of magnetic polymeric microspheres from poly(vinyl alcohol), the emulsion was prepared from oil and an aqueous suspension of a surfactant and magnetic nanoparticles by mixing them in the presence of polyoxyethylated non-ionic surfactants and glutaraldehyde as the cross-linking agent.[64] The synthesized polymeric microspheres were characterized by a narrow size distribution, the diameter of an order of magnitude of several tens of micrometres and the content of magnetic material ~5 mass %.

Yet another method of preparation of magnetic polymeric microspheres consisted in mechanical trapping of magnetic particles by the polymer.[65] Magnetic polymeric microspheres were manufactured by mixing a solution of polystyrene (with the molecular mass ~50 000) in a volatile solvent (dichloromethane) to which iron oxide nanoparticles with the diameter of 200 nm were added with an aqueous solution of surfactant [poly(vinyl alcohol)], mechanical stirring was continued until the volatile solvent evaporated. Solid particles were washed with water and lyophilized. The resulting microspheres were several hundred micrometres in diameter and contained up to 50% Fe_3O_4 of the total mass. However, they were characterized by a broad size distribution and irregular shape.

A method was proposed that made in possible to introduce the magnetic material into pre-formed polymeric matrices.[66] The magnetic microspheres may consist of Sepharose gel (cross-linked agarose beads, Pharmacia, Sweden) with immobilized affinity ligands containing magnetic nanoparticles incorporated by adsorption on, or precipitation into, the beads; they were used for affinity chromatography. Their affinity properties were the same as those of non-magnetic polymeric particles. However, due to the weak fixation of magnetic nanoparticles inside the beads, washing with any buffer solution could result in desorption and liberation of ferrites, which is the major drawback of their use.

A method of preparation of magnetic polymeric microspheres that consisted of polymer adsorption on the surface of a magnetic material was studied for magnetic nanoparticles of two diameters [~10 nm (see Ref. 18) and 200–400 nm], which led to the formation of a thin polymeric layer on the surface of magnetic nanoparticles as the result of physical [67] or chemical [18,68] adsorption of the polymer. The chemical adsorption was caused by the interaction between functional groups of polymers (CO_2H, PO_3H_2, NH_2) and functional groups of magnetic nanoparticles introduced upon activation. Usually, hydrophilic polymers were employed, for example, poly(acrylic acid),[68] poly(methyl methacrylate),[67] poly(propylene glycol)[18] or polysiloxane derivatives. It deserves mentioning that a thin layer of a polymer adsorbed on inorganic magnetic nanoparticles did not change their supermagnetic properties.

2. Preparation of magnetic nanoparticles in polymeric microspheres

Composite particles can be obtained from functionalized polymeric microspheres pre-fabricated by heterophase polymerization. They are dispersed in solutions of salts of such metals as iron (a mixture of $FeCl_2$ and $FeCl_3$), cobalt or nickel followed by precipitation of the magnetic material incorporated.

Ugelstad *et al*.[69] pioneered the studies in the field of magnetic polymer microspheres. The method included four stages (illustrated in Fig. 4).

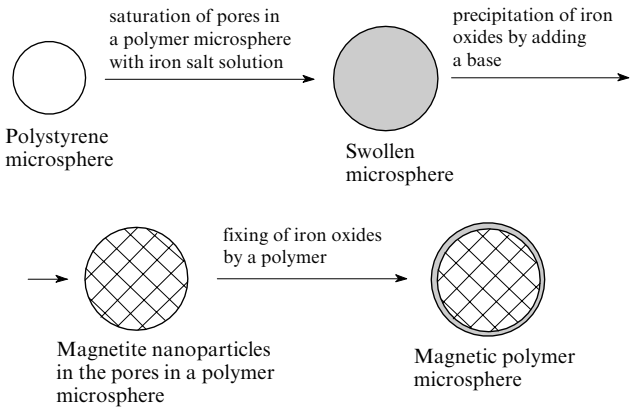


Figure 4. Scheme of the fabrication of magnetic particles developed by Ugelstad *et al*.

1. Synthesis of porous polymer microspheres (used later as seed particles) with the large diameter (~2–4 μm) and a narrow size distribution.

2. Diffusion of metal salts into pores of dispersed particles.

3. Precipitation of metal oxides in pores upon reaction with ammonia or sodium hydroxide.

4. Fixation of iron oxides in pores of the microspheres by a polymer formed in the seed polymerization.

Porous polymer microspheres to be later impregnated with solutions of Fe^{II} and Fe^{III} salts were prepared by seed polymerization of vinyl monomer in the presence of monodisperse seed particles measuring ~800–1000 nm. As a result of swelling, the volume of the microspheres increased 50–1000-fold. The two-stage swelling process was carried out in the presence of low-molecular-mass pore-forming solvents with low water-solubility that diffuse inside the particles. For example, such solvents can be represented by hexadecane or dioctyl peroxide, the latter also serving as the polymerization initiator. In the second stage of swelling, diffusion of a vinyl monomer into the activated latex seed particles occurred.

The swelling process was determined by the thermodynamic equilibrium between the following three phases: seed polymeric microspheres, monomer droplets and the continuous phase (water). The system equilibrium was characterized by the equality of the Gibbs energies of three phases where the Gibbs energy of each phase was related by their volume fractions, the surface tension and the size of seed microspheres. If porous particles were to be prepared, a volatile solvent introduced in the swelling stage was evaporated to leave pores in microspheres.[70–72]

The described swelling procedure was used for synthesizing porous monodisperse poly(styrene-*co*-divinylbenzene) particles measuring several micrometres.[73] Then, polymeric microspheres were chemically modified (by introducing NO_2 groups), dried, redispersed in an aqueous solution of Fe^{II} and Fe^{III} salts and treated with a base. The salt ratio $Fe^{II}:Fe^{III}$ varied in the range from 1 to 2 depending on the type of iron oxide (γ-Fe_2O_3 or Fe_3O_4) to be synthesized.

The synthesized polymer microspheres had the regular shape and the irregular porous surface[73,74] containing iron oxide nanoparticles. To encapsulate iron oxide into the polymer, in the last stage, co-polymerization of styrene with divinylbenzene was carried out. Such magnetic polymeric microspheres had the size up to 5 μm and contained up to 10 mass % of magnetic material.

For the covalent binding with functional groups of biomolecules, certain functional groups (amine, hydroxyl, carboxyl, thiol or aldehyde) should be present on the surface of magnetic polymer microspheres. These groups were obtained by seed polymerization of functional monomers on seed polymeric particles.[75,76]

Magnetic polyacrylamide microspheres cross-linked with *N*,*N*′-methylenebisacrylamide were manufactured[77] by dispersing polymer microspheres in a solution of iron salts in the presence of dextran followed by the precipitation upon addition of ammonia. Dextran served a dual role. On the one hand, it enhanced the compatibility of a polymer with biomolecules and, on the other hand, prevented aggregation of magnetic nanoparticles in the dispersion medium. Next, glycidyl methacrylate was added to the suspension, which adsorbed on the surface of the particles. This enhanced their stability and provided the presence of epoxide groups on the microsphere surface. The manufactured magnetic polymer microspheres had the inner diameter of $\sim$0.4 μm and contained 20 mass % – 25 mass % of magnetic iron oxides, which corresponded to a half of the initial amount of iron oxides introduced[70] during the synthesis.

The preparation of hydrophobic polymeric microspheres containing cobalt nanoparticles was described.[78] First, polystyrene suspensions with the average particle diameter of 0.35 μm were obtained. In the second stage, polymer microspheres were impregnated with styrene, divinylbenzene and dicobalt octacarbonyl in toluene. Then, seed co-polymerization was initiated as a result of which polymeric microspheres with the average diameter of 1.35 μm were formed. Thermolysis of cobalt carbonyl was carried out at 120 °C. The synthesized magnetic polymer microspheres exhibited superparamagnetic properties. However, carbon monoxide formed throughout the reaction led to deformation of the particle surface.[78]

The reduction of metal salts (Fe^{II}, Ni^{II}, Co^{II}) on the polymer particle surface is a method of manufacture of composite microspheres. Such polymer microspheres (measuring up to 300 nm) were used as conducting polymeric materials or as catalytic systems. The emulsion co-polymerization of styrene and a functional co-monomer, *e.g.*, acrylonitrile, acrylamide or *N*-vinylimidazole, was carried out.[79] The amount of immobilized metal depended on its nature, the initial concentration of the metal salts and the type of the polymer used. For iron, this amount was merely several percent, whereas for cobalt it reached 50% of the overall mass of a composite particle.

3. Monomer polymerization in the presence of magnetic nanoparticles

This approach to the fabrication of magnetic polymer microspheres employs different methods of heterophase polymerization in the presence of magnetic materials.

a. Emulsion and miniemulsion polymerization

Emulsion polymerizaton of monomers in the presence of inorganic seed particles is the most popular process for the preparation of composite microspheres.[80]

In this case, the inorganic material should meet the following requirement.

1. The surface of the material should be hydrophobic, which is achieved by adsorption of a surfactant[81,82] or chemical modification.[83] Reactive groups capable of interacting with the polymer functional groups or a substance that favours the hydrophobic interaction with the polymer surface should be present on the surface. The modifiers can be represented, for example, by silanes, titanates, dihydric acids, *etc*.

2. Inorganic particles should retain their colloidal stability. Nanoparticles are stabilized by adsorbing surfactants commonly used in emulsion polymerization.[84,85]

Encapsulation of inorganic nanoparticles into a polymer requires that the surfactant concentration was below the critical micelle concentration (CMC) to prevent the micelle formation in the original system and exclude the formation of polymer microspheres containing no inorganic material. Moreover, for polymerization, it was necessary that the initiator and the monomer be water-insoluble to rule out the formation of oligomeric water-soluble surfactants.[86]

Magnetic nanoparticles such as ferrites were often prepared in an aqueous phase and stabilized by either a single surfactant or a mixture of surfactants. In this case, polylayers could be formed on the particle surface where the first layer consisted of surfactant chemically bound to the iron oxide surface and the second was built of surfactant physically adsorbed on this surface (oleic acid and oleate) (Fig. 5).[86] The surface became negatively charged; the emulsion polymerization could be initiated by potassium persulfate (PP).

The effect of the ferrite : styrene mass ratio and the concentration of the initiator (PP), monomer, fluorescent dyes and surfactant on the encapsulation of iron oxide nanoparticles in the polymer was studied.[86] The diameter of polymeric particles was 50 – 150 nm depending on the surfactant concentration, the content of magnetite nanoparticles was in the range of 12 mass % – 14 mass %. During the polymerization, the system remained stable and the final amount of coagulum was merely several percent.

Magnetic polymer particles with the diameter exceeding 1 μm were prepared[62,87] in several stages by emulsion polymerization of acrylamide in inverse emulsions. They were used in the PP-induced seed polymerization of styrene, methyl methacrylate or vinyl acetate. The polymer obtained

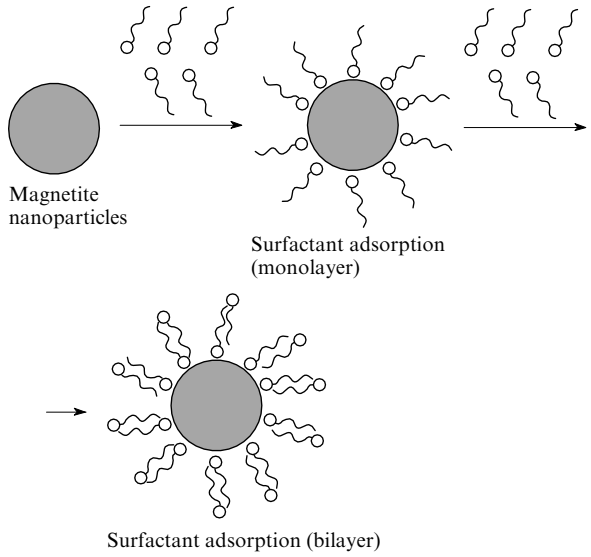


Figure 5. Stabilization of magnetite nanoparticles by surfactant monolayer and bilayer.

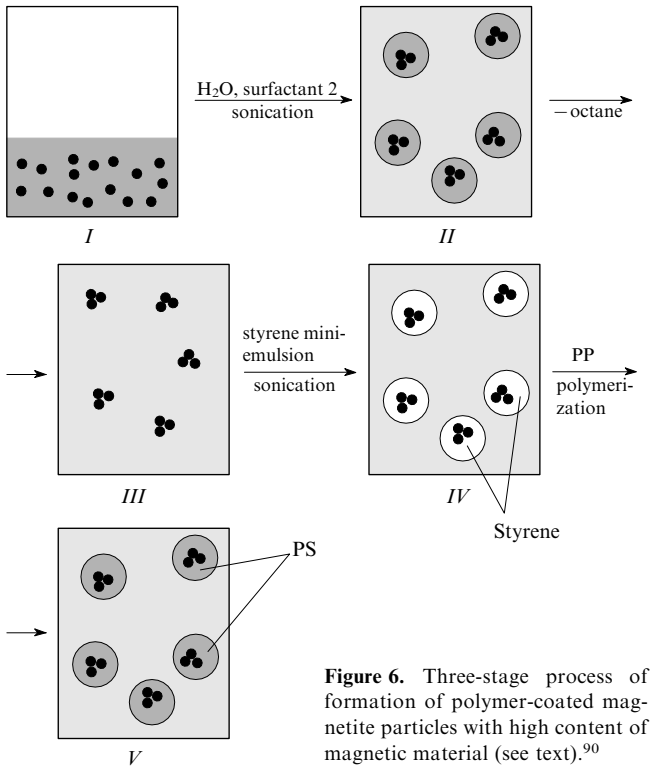


Figure 6. Three-stage process of formation of polymer-coated magnetite particles with high content of magnetic material (see text).[90]

was polydisperse, the average particle size was 1 – 3 μm, particles had the core – shell structure and comprised 13 mass % – 23 mass % of iron.

Recently, polymer microspheres filled with inorganic material were prepared by miniemulsion polymerization[88] carried out in two stages. In the first stage, a stable dispersion of magnetite particles in styrene was obtained in the presence of a surfactant. Then, the addition of new surfactants transformed it into a dispersion in water. Hexadecane was introduced as a hydrophobic additive into the monomer (styrene) phase to prevent the Ostwald ripening process. The magnetite content in the thus prepared polymer microspheres was 20 mass %.

The higher magnetite content in polymer particles was achieved by applying another procedure.[89] The monomer (styrene) and magnetite were dispersed independently in water in the presence of sodium dodecyl sulfate (SDS) as the surfactant and gradually mixed in different monomer : magnetite ratios with the aim of manufacturing magnetic polymeric microspheres with different concentrations of inorganic nanoparticles. Then, the mixture was subjected to sonication to reach the necessary degree of dispersion in this system after which the polymerization process was initiated.

The homogeneous distribution of magnetite, at its high content, in polymeric microspheres, could be achieved by a three-stage preparation route as illustrated in Fig. 6.[90]

Magnetite particles with the average diameter of 10 nm were prepared in the co-precipitation[91] as a result of quick addition of an ammonia solution to a solution of Fe^{II} and Fe^{III} salts.

In the first stage, using oleic acid as surfactant 1, at a temperature above its melting point, hydrophobization of magnetic particles occurred. Evaporation of water and removal of non-adsorbed oleic acid resulted in the formation of a dry powder easily dispersible in octane (*I*, Fig. 6); the average particle size was 20 nm. Insofar as microemulsions prepared in the presence and in the absence of hexadecane were stable, it was concluded that oleic acid could replace the hydrophobic additive that prevented the Ostwald ripening.

In the second stage, the resulting dispersion in octane was mixed with an aqueous solution of sodium dodecyl sulfate (surfactant 2). As a result of sonication, an oil-in-water microemulsion was formed (*II*). Evaporation of octane afforded the aqueous magnetic fluid containing magnetite particles covered with a surfactant 1/surfactant 2 bilayer, where the external SDS layer provided the stabilization of particles in water (*III*).

In the third stage, a microemulsion of a monomer (styrene) in water was prepared and the aqueous magnetic fluid prepared in the previous stage was added. Then, this mixture was co-sonicated in order to destroy the aggregates and liquid droplets [*i.e.*, to form miniemulsions (*IV*)], the initiator was added and polymerization was started. The resulting magnetic polymeric microspheres (*V*) are characterized by the uniform distribution of the magnetic material.

The preparation of ultrafine magnetic polymer microspheres with the narrow particle size distribution and the high content of magnetic material was carried out.[92]

The manufacture of magnetic polystyrene microspheres involved the use of a styrene-based magnetic fluid prepared by the dispersion medium exchange. In the first stage, a stable aqueous suspension was prepared by chemisorption of sodium oleate on the surface of magnetite nanoparticles. Then acetic acid, which makes the nanoparticle surface hydrophobic, and styrene were added to the system; sonication was employed throughout the process.

The stable suspension of magnetite nanoparticles in styrene was added to an aqueous SDS solution, which gave rise first to a microemulsion and then to polymerization.

This afforded ultrafine magnetic polystyrene microspheres with the average diameter of 80 – 100 nm, a narrow

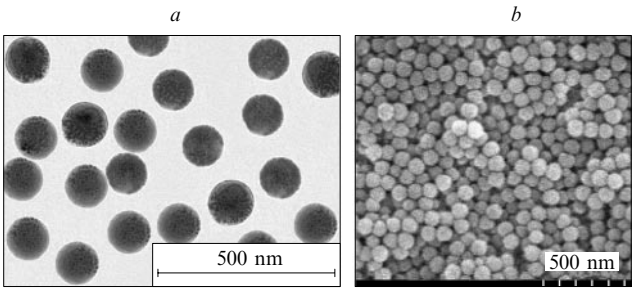


Figure 7. Microimages of magnetic polymer microspheres obtained by transmission electron microscopy (*a*) and scanning electron microscopy (*b*).[92]

size distribution and the magnetite content in the polymer microsphere approaching ~40 mass % (Fig. 7).

Functionalization of the surface of magnetic polymer microspheres could be effected by seed polymerization of chloromethylstyrene on polystyrene seed microspheres at 40 °C using the potassium persulfate–sodium bisulfite redox system.[93]

b. Suspension polymerization

Suspension polymerization of styrene in the presence of a magnetic fluid was studied.[94] Magnetic nanoparticles were dispersed in styrene containing an oil-soluble initiator and mixed with an aqueous solution of a surfactant to form magnetic monomer droplets. The size of magnetic polymeric particles produced varied in the range of 0.03–5 μm and the magnetic material content approached 45 mass %.

Suspension co-polymerization of styrene and divinylbenzene afforded magnetic polymer microspheres 100–300 μm in diameter.[95] Seed magnetic particles 20 μm in diameter were first dispersed in the organic phase with sonication, the dispersion was mixed with the water phase, and polymerization was carried out at 80 °C in the presence of an oil-soluble initiator. After the end of polymerization, the polymer suspension was subjected to UV radiation. As a result of photooxidation of the polystyrene shell, carbonyl groups emerged on the surface of microspheres, which was confirmed by IR spectroscopy. The amount of iron oxide incorporated into the polymer matrix was low and amounted to 1.25% of the monomer mass.

The fabrication of magnetic polyacrylamide microspheres by suspension co-polymerization of acrylamide, methylenebisacrylamide and *N*-acryloyloxysuccinimide in the presence of sorbitan monolaurate (Tween 20) in carbon tetrachloride was induced by a redox system Fe^{2+}–potassium persulfate.[96] However, the iron oxide content in particles was rather low.

c. Dispersion polymerization

Dispersion polymerization was employed to obtain poly(glycidyl methacrylate) microspheres with the diameter of 1.6 μm containing superparamagnetic particles (up to 12 mass % of magnetite).[97] Ferrite nanoparticles were prepared in glycidyl methacrylate and dispersed in a water–alcohol solution containing a surfactant and an initiator. The degree of inorganic material encapsulation was very high and only insignificant number of empty microspheres were formed.

Magnetic polymer microspheres were also obtained by dispersion co-polymerization of styrene and hydroxyethyl methacrylate. Preliminarily, ferrite nanoparticles measuring 0.3–0.4 μm were dispersed in poly(ethylene glycol) (PEG) with sonication.[98] It was assumed that the presence of PEG on the surface improved the affinity between ferrite and the monomers. Dispersion polymerization was induced with potassium persulfate. Magnetic polymer particles with surface hydroxyl groups had the diameter from 50 to 500 μm. A fraction with the diameter of 50–60 μm was used for the covalent immobilization of bacterial proteinase.

d. Polymerization on the surface of nanoparticles

The use of different polymerization methods and different monomers makes it possible to vary the nature and the properties of magnetic polymer microspheres.

A relatively old method of polymerization in the presence of ferrite particles is the polymerization of methacrylate derivatives induced by cobalt-60 γ-radiation[99] or a redox reaction.[100] The particle diameter was in the range of up to 50 nm, and the ferrite suspension retained its original magnetic properties.

Surface modification of inorganic materials substantially affected the process of magnetite encapsulation into polymer upon polymerization. These processes often utilized surfactants; however, this is not the only approach. For example, according to a patent,[101] magnetite nanoparticles were first dispersed in methanol and then trimethoxysilane derivatives [*p*-aminophenyltrimethoxysilane, 3-aminopropyltrimethoxysilane, *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane] were deposited on the surface of ferrite nanoparticles. Polymerization of silane by the condensation reaction afforded particles with magnetic cores and polysilane shells. Polysilanes form covalent bonds with surface OH groups of ferrites through dehydration. Actually, silanization is carried out at elevated temperature (90 °C) in an acidic medium containing a surfactant. The presence of the latter made it possible to prevent aggregation of particles following polymerization when removing excess of water, silane and organic solvent by evaporation at 160 °C. Average diameters of magnetic polymer microspheres were in the range of 0.1–1.5 μm.[101]

The surface of magnetic particles can be modified with other inorganic compounds. Thus deposition of silica prepared by controlled hydrolysis of sodium silicate onto magnetite nanoparticles transformed them into silica-coated particles.[102] Then polymerization of pyrrole was carried out in the presence of the magnetite–silica particles using the following redox systems: $H_2O_2-Fe^{3+}-HCl$ or $H_2O_2-(NH_4)_2S_2O_8-HCl$. The particles obtained contained from 10 mass % to 17 mass % of magnetite but had a broad size distribution.

Manufacture of biodegradable magnetic polymeric microspheres by other heterophase polymerization methods is documented. Thus anionic polymerization of isobutyl cyanoacrylate in the presence of ferrite particles was described to afford polymer microspheres with the diameter of 0.22 μm containing 28 mass % of ferrite. Polymerization occurred at the interface between the magnetic fluid and the monomer; capsules were obtained as a result of gel formation or interface precipitation.[103, 104]

A method of preparation of aqueous dispersions of magnetic nanoparticles in the presence of a polymeric stabilizer, namely, a co-polymer of maleic anhydride with vinyl monomers, was proposed.[103] Adsorption of the co-polymer on the surface of inorganic nanoparticles was followed by cross-linking as a result of the temperature

increase to 90 °C and acidification of the medium. In this case, a dense polymer shell was formed around magnetic nanoparticles, which efficiently prevented their oxidation thus retaining their magnetic properties.

4. Preparation of multilayer composite particles

Multilayer composite particles consist of an organic core surrounded by a magnetic layer of iron oxide nanoparticles and a polymer shell. There are two methods of their preparation.

The first method consists of carrying out low-temperature seed polymerization of styrene in the presence of ferrites and polystyrene microspheres, potassium persulfate as the initiator and a surfactant being added. However, in the course of styrene seed polymerization, new polymer particles appear in the aqueous phase.[105] The synthesized polymer microspheres had the size of ~4 μm, a broad size distribution, a rough surface and a low ferrite content.

Another method of manufacturing multilayer composite particles was proposed,[106] this is illustrated in Fig. 8.

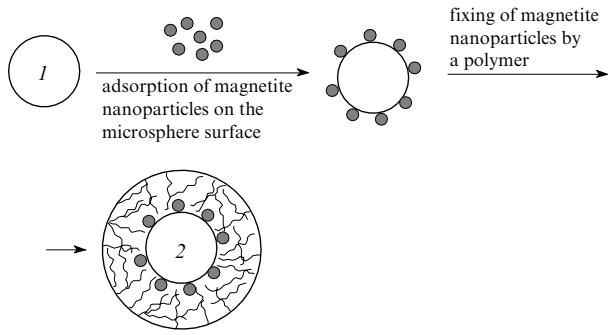


Figure 8. Scheme of preparation of multilayer composite microspheres (see text).[106]
(*1*) Polystyrene microsphere with charged surface, (*2*) multilayer composite microsphere.

Positively charged particles of a magnetic material ($NiO \cdot ZnO \cdot Fe_2O_3$ powder) 20 nm in diameter were adsorbed on the surface of negatively charged polystyrene microspheres (containing sulfonic or carboxylic surface groups) due to electrostatic interaction to afford complexes. The electrostatic interaction was determined by the ionic strength of the medium, the nature of solvent and pH. For pH > 9, oxide particles had the same charge as the polymer microspheres so that the amount of inorganic nanoparticles adsorbed on the surface of polymer particles was very small due to electrostatic repulsion. To prevent the oxide release and provide its interaction with biomolecules, polymeric microspheres with adsorbed inorganic nanoparticles were covered with a polymer layer.

An ionogenic surfactant (*e.g.*, sodium oleate) should be present for stabilization of composite particles. However, its concentration should be limited to avoid the formation of microspheres of surfactant micelles containing no iron oxide.[106] This method allowed the control over the diameter of magnetic polymeric microspheres by varying the size of seed particles. However, this publication provided no information on the size distribution and the metal oxide content in the composite particles.

Later, it was proposed to form hydrophilic thermosensitive polymer suspensions[107, 108] by using polymer microspheres with amine and amide groups, for example, based on a co-polymer of styrene and aminoethyl methacrylate or vinylbenzylamine,[109, 110] poly(*N*-isopropylacrylamide)[111] or a co-polymer of styrene and *N*-isopropylacrylamide.[112, 113] These hydrophilic thermosensitive magnetic microspheres were monodisperse and had surface carboxyl groups. Such composite microspheres were used in covalent immobilization of antibodies.[114]

To form monodisperse composite microspheres of controllable morphology, Fe_3O_4 particles were deposited on preliminarily prepared monodisperse microspheres of poly(methyl methacrylate-*co*-acrolein) (PMMAAC).[115] Then polymerization of styrene was carried out in the presence of the resulting dispersion to form a polymeric layer coating the magnetite layer.

As a basis for the preparation of composite particles, monodisperse polystyrene microspheres with surface carboxyl groups were tested[116] as well as PMMAAC with carboxyl and aldehyde groups.[117] As a result, monodisperse three-layer composite particles were obtained. They represented regular spheres and consisted of a PMMAAC core, a magnetite interlayer and a PS shell with surface carboxyl groups for binding immunoreagents. The thermogravimetric analysis has shown that the magnetite content in particles was ~17 mass %.

* * *

Thus, several methods for the manufacture of magnetic polymeric microspheres are currently available; however, each has its advantages and drawbacks. From our viewpoint, the most promising method for the preparation of magnetic polymer microspheres is the monomer polymerization in the presence of magnetic nanoparticles. Its merits include the efficient protection of magnetic nanoparticles from the environment and the possibility of varying widely the size of magnetic polymeric microspheres and the content of magnetic nanoparticles in them.

The use of different methods of heterophase polymerization makes it possible to obtain magnetic polymer microspheres with different morphology, different sizes (both in the nanometre range and ~4 μm in diameter) and different amounts of the magnetic material in the microsphere. These magnetic polymer microspheres have the extremely wide application field varying from the magnetic separation of different technological media to the preparation of polymeric suspensions for biomedicinal purposes.

This study was carried out with financial support from the Russian Foundation of Basic Research (Project Nos 08-03-00624-a and 09-03-00888-a).

References

1. A Ito, M Shinkai, H Honda, T Kobayashi *J. Biosci. Bioeng.* **100** 1 (2005)
2. N Shamim, L Hong, K Hidajat, M S Uddin *Separ. Purif. Technol.* **53** 164 (2007)
3. K E Scarberry, E B Dickerson, J F McDonald, Z J Zhang *J. Am. Chem. Soc.* **130** 10258 (2008)
4. U O Hafeli *Int. J. Pharm.* **277** 19 (2004)
5. C H Reynolds, N Annan, K Beshah, J H Huber, S H Shaber, R E Lenkinski, J A Wortman *J. Am. Chem. Soc.* **122** 8940 (2000)
6. T N Nagaraja, R L Croxen, S Panda, R A Knight, K A Keenan, S L Brown, J D Fenstermacher, J R Ewing *J. Neurosci. Methods* **157** 238 (2006)

7. T K Jain, J Richey, M Strand, D L Leslie-Pelecky, C A Flask, V Labhasetwar *Biomaterials* **29** 4012 **(**2008)
8. D-L Zhao, H-L Zhang, X-W Zeng, Q-S Xia, J-T Tang *Biomed. Mater*. **1** 198 (2006)
9. G Degré, E Brunet, A Dodge, P Tabeling *Lab Chip* **5** 691 (2005)
10. *Colloidal Biomolecules, Biomaterials, and Biomedical Applications (Surfactant Science)* Vol. 116 (Ed. A Elaïssari) (Basel: Marcel Dekker, 2004)
11. *Colloidal Polymer. Synthesis and Characterization (Surfactant Science)* Vol. 115 (Ed. A Elaïssari) (Basel: Marcel Dekker, 2003)
12. S K Ghosh *Functional Coatings: by Polymer Microencapsulation* (Weinheim: Wiley-VCH, 2006)
13. G Kickelbick *Hybrid Materials: Synthesis Characterization, and Applications* (Weinheim: Wiley-VCH, 2007)
14. P Gémez-Romero, C Sanchez *Functional Hybrid Materials* (Weinheim: Wiley-VCH, 2004)
15. B A Trofimov, B G Sukhov, in *Nauka i Nanotekhnologii. Materialy Nauchnoi Sessii Prezidiuma SO RAN* (Science and Nanothechnologies. Proceedings of the Scientific Session of Presidium of the Siberian Branch of the Russian Academy of Sciences) (Novosibirsk: Siberian Branch of the Russian Academy of Sciences, 2007) p. 155
16. B A Trofimov, B G Sukhov, G P Aleksandrova, S A Medvedeva, L A Grishchenko, A G Mal'kina, L P Feoktistova, A N Sapozhnikov, V I Dubrovina, E F Martynovich, V V Tirskii, A L Semenov *Dokl. Akad. Nauk* **393** 634 (2003) [a]
17. US P. 5164297 (1992)
18. Z Ma, H Liu *Chin. Particuology* **5** 1 (2007)
19. G E Zil'berman *Elektrichestvo i Magnetizm* (Electricity and Magnetism) (Dolgoprudnyi: Intellekt, 2008)
20. R M Cornell *The Iron Oxides: Structure, Properties, Reactions, Occurrence and Uses* (Weinheim: Wiley-VCH, 2003)
21. J L Dormann *Adv. Chem. Phys*. **98** 283 (1997)
22. J S Miller *Magnetism, Nanosized Magnetic Materials (Magnetism: Molecules to Materials)* (Weinheim: Wiley-VCH, 2002)
23. J D Patterson *Solid-State Physics: Introduction to the Theory* (Berlin: Springer, 2007)
24. M Mikhaylova, D K Kim, N Bobrysheva, M Osmolowsky, V Semenov, T Tsakalakos, M Muhammed *Langmuir* **20** 2472 (2004)
25. C Yang, J Xing, Y Guan, J Liu, H Liu *J. Alloys Compd*. **385** 283 (2004)
26. H Iida, T Nakanishi, H Takada, T Osaka *Electrochim. Acta* **52** 292 (2006)
27. N Bao, L Shen, Y Wang, P Padhan, A Gupta *J. Am. Chem. Soc*. **129** 12374 (2007)
28. J Park, K An, Y Hwang, J-C Park, H-J Noh, J-Y Kim, J-H Park, N-M Hwang, T Hyeon *Nat. Mater*. **3** 891 (2004)
29. J P Chen, K M Lee, C M Sorensen, K J Klabunde, G C Kadjipanayis *J. Appl. Phys*. **75** 5876 (1994)
30. D Ganguli *Inorganic Particle Synthesis via Macro- and Microemulsions: a Micrometer to Nanometer Landscape* (Berlin: Springer, 2003)
31. O A Platonova, L M Bronstein, S P Solodovnikov, I M Yanovskaya, E S Obolonkova, P M Valetsky, E Wenz, M Antonietti *Colloid Polym. Sci*. **275** 426 (1997)
32. D L Leslie-Pelecky, X Q Zhang, R D Rieke *J. Appl. Phys*. **79** 5312 (1996)
33. J Osuna, D de Caro, C Amiens, B Chaudret, E Snoeck, M Respaud, J M Broto, A Fert *J. Phys. Chem*. **100** 14571 (1996)
34. M H Khedr, A Omar, S A Abdel-Moaty *Mater. Sci. Eng., A* **432** 26 (2006)
35. V T Liveri *Controlled Synthesis of Nanoparticles in Microheterogeneous Systems* (Berlin: Springer, 2006)
36. C H Griffiths, M P O'Horo, T W Smith *J. Appl. Phys*. **50** 7108 (1979)
37. C N R Rao, A K Cheetham, A Müller *The Chemistry of Nanomaterials: Synthesis, Properties and Applications* (Weinheim: Wiley-VCH, 2006)
38. J-P Jolivet *Metal Oxide Chemistry and Synthesis: from Solution to Solid State* (Chichester: Wiley, 2000)
39. R Vijayakumar, Yu Koltypin, I Felner, A Gedanken *Mater. Sci. Eng. A* **286** 101 (2000)
40. S P Gubin, Yu A Koksharov, G B Khomutov, G Yu Yurkov *Usp. Khim*. **74** 539 (2005) [*Russ. Chem. Rev*. **74** 489 (2005)]
41. K Butter, A P Philipse, G J Vroege *J. Magn. Magn. Mater*. **252** 1 (2002)
42. L Vekas, D Bica, M V Avdeev *Chin. Particuology* **5** 43 (2007)
43. E Blüms, A O Cebers, M M Maiorov *Magnetic Fluids* (Berlin: Walter de Gruyter, 1997)
44. M Shinkai, H Honda, T Kobayashi *Biocatal. Biotransform*. **5** 61 (1991)
45. V Pillai, P Kumar, M S Multani, D O Shah *Colloids Surf. A: Physicochem. Eng. Aspects* **80** 69 (1993)
46. E M Natanson *Kolloidnye Metally* (Colloid Metals) (Kiev: Academy of Sciences of Ukr. SSR, 1959
47. I Nakatani, T Furubayashi, T Takahashi, H J Hanaoka *J. Magn. Magn. Mater*. **65** 261 (1987)
48. S Dutz, W Andrä, R Hergt, R Müller, C Oestreich, C Schmidt, J Töpfer, M Zeisberger, M E Bellemann *J. Magn. Magn. Mater*. **311** 51 (2007)
49. O Mykhaylyk, D Vlaskou, N Tresilwised, P Pithayanukul, W Möller, C Plank *J. Magn. Magn. Mater*. **311** 275 (2007)
50. L A Garcia-Cerda, M U Escareno-Castro, M Salazar-Zertuche *J. Non-Cryst. Solids* **353** 808 (2007)
51. M J Bonder, Y Zhang, K L Kiick, V Papaefthymiou, G C Hadjipanayis *J. Magn. Magn. Mater*. **311** 658 (2007)
52. I Dumazet-Bonnamour, P Le Perchec *Colloids Surf. A: Physicochem. Eng. Aspects* **173** 61 (2000)
53. C Alexiou, R J Schmid, R Jurgons, M Kremer, G Wanner, C Bergemann, E Huenges, F G Parak *Eur. Biophys. J*. **35** 446 (2006)
54. E Pollert, K Knížek, M Maryško, K Závěta, A Lančok, J Boháček, D Horák, M Babič *J. Magn. Magn. Mater*. **306** 241 (2006)
55. M Maeda, C S Kuroda, T Shimura, M Tada, M Abe, S Yamamuro, K Sumiyama, H Handa *J. Appl. Phys*. **99** 103 (2006)
56. V S Zaitsev, D S Filimonov, I A Presnyakov, R J Gambino, B Chu *J. Colloid Interface Sci*. **212** 49 (1999)
57. P A Dresco, V S Zaitsev, R J Gambino, B Chu *Langmuir* **15** 1945 (1999)
58. US P. 4358388 (1982)
59. US P. 5356713 (1994)
60. K Wormuth *J. Colloid Interface Sci*. **241** 366 (2001)
61. Z Z Xu, C C Wang, W L Yang, Y H Deng, S K Fu *J. Magn. Magn. Mater*. **277** 136 (2004)
62. Y Deng, L Wang, W Yang, S Fu, A Elaïssari *J. Magn. Magn. Mater*. **257** 69 (2003)
63. J Kandzia, W Scholz, M J D Anderson, W Mueller-Ruchholtz *J. Immunol. Methods* **75** 31 (1984)
64. D Müller-Schulte, F Fmss, M De Cuyper, in *Scientific and Clinical Applications of Magnetic Carriers* (Eds U Häteli, W Q Schütt, J Teller, M Zborowski) (New York: Plenum, 1997) p. 93
65. B Hertzog, T Mottl, D Yim, E Mathiowitz, in *Scientific and Clinical Applications of Magnetic Carriers* (Eds U Häteli, W Q Schütt, J Teller, M Zborowski) (New York: Plenum, 1997) p. 77
66. I Safarik, M Safarikova *BioMagn. Res. Technol*. **2** 7 (2004)
67. J Krizova, A Spanova, B Rittich, D Horak *J. Chromatogr. A* **1064** 247 (2005)
68. Y Wu, J Guo, W Yang, C Wang, S Fu *Polymer* **47** 5287 (2006)
69. US P. 4774265 (1986)
70. J Ugelstad, A Berge, T Ellingsen, R Schemid, T N Nilsen, P C Mork, P Stenstad, E Hornes, O Olsvik *Prog. Polym. Sci*. **17** 87 (1992)
71. J Ugelstad, P C Mork, R Schmid, T Ellingsen, A Berge *Polym. Int*. **30** 157 (1993)
72. J Ugelstad, P C Mork, K H Kaggerud, T Ellingsen, A Berge *Adv. Colloid Interface Sci*. **13** 101 (1980)

73. S A C Gould, B Drake, C B Prater, A L Weisenhorn, S Manne, Kelderman, H-J Butt, H Hansma, P K Hansma, S Maganov, H J Cantow *Ultramicroscopy* **33** 93 (1990)
74. B I Haukanes, C Kvam *Biotechnology (NY)* **11** 60 (1993)
75. J Ugelstad, O Olsvik, R Schmid, A Berge, S Funderud, K Nustad, in *Molecular Interactions in Bioseparation* (Ed. T Ngo) (New York: Plenum, 1993) p. 229
76. *Advances in Biomagnetic Separation* (Eds M Uhlén, E Hornes, O Olsvik) (Natick, MA: Eaton, 1994) p. 209
77. H Kawaguchi, K Fujimoto, Y Nakazawa, M Sakagawa, Y Ariyoshi, M Shidara, H Okazaki, Y Ebisawa *Colloids Surf. A: Physicochem. Eng. Aspects* **109** 147 (1996)
78. R Tannenbaum *Inorg. Chim. Acta* **227** 233 (1994)
79. H Tamai, H Sakura, Y Hirota, F Nishiyama, H Yasuda *J. Appl. Polym. Sci.* **56** 441 (1995)
80. J Richardson, P Hawkins, R Luxton *Biosens. Bioelectron.* **16** 989 (2001)
81. Y Zhang, N Kohler, M Zhang *Biomaterials* **23** 1553 (2002)
82. X Wang, C Zhang, X Wang, H Gu *Appl. Surf. Sci.* **253** 7516 (2007)
83. M Klokkenburg, J Hilhorst, B H Erne *Vib. Spectrosc.* **43** 243 (2007)
84. A K Gupta, M Gupta *Biomaterials* **26** 3995 (2005)
85. J L Viota, F Gonzales-Caballero, J D G Duran, A V Delgado *J. Colloid Interface Sci.* **309** 135 (2007)
86. H Noguchi, N Yanase, Y Uchida, T Suzuta *J. Appl. Polym. Sci.* **48** 1539 (1993)
87. L C Santa Maria, M A S Costa, F A M Santos, S H Wang, M R Silva *Mater. Lett.* **60** 270 (2006)
88. D Hoffmann, K Landfester, M Antonietti *Magnetohydrodynamics* **37** 217 (2001)
89. W Zheng, F Gao, H Gu *J. Magn. Magn. Mater.* **288** 403 (2005)
90. L P Ramirez, K Landfester *Macromol. Chem. Phys.* **204** 22 (2003)
91. J-H Wu, S P Ko, H-L Liu, S Kim, J-S Ju, Y K Kim *Mater. Lett.* **61** 3124 (2007)
92. A Yu Gervald, Candidate Thesis in Chemical Sciences, Moscow State Academy of Fine Chemical Technology, Moscow, 2008
93. R Faridi-Majidi, N Sharifi-Sanjani *J. Magn. Magn. Mater.* **311** 55 (2007)
94. X Liu, H Liu, J Xing, Y Guan, Z Ma, G Shan, C Yang *Chin. Particuology* **1** 76 (2003)
95. J Hong, D Xu, J Yu, P Gong, H Ma, S Yao *Nanotechnology* **18** 1356 (2007)
96. A Elaïssari, F Sauzedde, F Montagne, C Pichot, in *Colloidal Polymer Synthesis and Characterization (Surfactant Science)* Vol. 115 (Ed. A Elaïssari) (Basel: Marcel Dekker, 2003) p. 285
97. E B Altintas, L Uzun, A Denzli *Chin. Particuology* **5** 174 (2007)
98. X Li, Z Sun *J. Appl. Polym. Sci.* **58** 1991 (1995)
99. R S Molday, A Rembaum, S P S Yen *Nature (London)* **268** 437 (1977)
100. J S Nunes, C L de Vasconcelos, F A O Cabral, J H de Araujo, M R Pereira, J L C Fonseca *Polymer* **47** 7646 (2006)
101. US P. 4695393 (1987)
102. M D Butterworth, S A Bell, S P Armes, A Q Simpson *J. Colloid Interface Sci.* **183** 91 (1996)
103. M C Levy, D Poncelet *Biofutur* **132** 16 (1994)
104. S Neveu-Prin, V Cabuil, R Massart, P Escaffre, J Dussaud *J. Magn. Magn. Mater.* **122** 42 (1993)
105. J Lee, M Senna *Colloid Polym. Sci.* **273** 76 (1995)
106. K Furusawa, K Nagashima, C Anzai *Colloid Polym. Sci.* **272** 1104 (1994)
107. F Sauzedde, A Elaïsari, C Pichot *Colloid Polym. Sci.* **277** 1041 (1999)
108. F Sauzedde, A Elaïsari, C Pichot *Colloid Polym. Sci.* **277** 846 (1999)
109. F Sauzedde, F Ganachaud, A Elaïsari, C Pichot *J. Appl. Polym. Sci.* **65** 2331 (1997)
110. F Ganachaud, F Sauzedde, A Elaïssari, C Pichot *J. Appl. Polym. Sci.* **65** 2315 (1997)
111. F Meunier, A Elaïssari, C Pichot *Macromol. Symp.* **150** 283 (2000)
112. D Duracher, F Sauzedde, A Elaïssari, C Pichot, L Nabzar *Colloid Polym. Sci.* **276** 920 (1998)
113. D Duracher, F Sauzedde, A Elaïssari, A Perrin, C Pichot *Colloid Polym. Sci.* **276** 219 (1998)
114. F Sauzedde, A Elaïssari, C Pichot *Macromol. Symp.* **151** 617 (2000)
115. Yu O Skurkis, A Yu Men'shikova, T G Evseeva, N A Chekina, in *Struktura i Dinamika Molekulyarnykh Sistem, Kazan', 2003* (Structure and Dynamics of Molecular Systems, Kazan, 2003) No. 10, Pt. 3, p. 77
116. A Yu Men'shikova, T G Evseeva, B M Shabsel's, I E Il'ina, G P Vlasov *Kolloid. Zh.* **59** 671 (1997) [b]
117. A Yu Men'shikova, T G Evseeva, N A Chekina, Yu O Skurkis, S S Ivanchev *Zh. Prikl. Khim.* **74** 1677 (2001) [c]

[a] — *Dokl. Chem. (Engl. Transl.)*
[b] — *Colloid J. (Engl. Transl.)*
[c] — *Russ. J. Appl. Chem. (Engl. Transl.)*