Encapsulated magnetite particles for biomedical application

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Abstract
The process of miniemulsification allows the generation of small, homogeneous, and stable droplets containing monomer or polymer precursors and magnetite which are then transferred by polymer reactions to the final polymer latexes, keeping their particular identity without serious exchange kinetics involved. It is shown that the miniemulsion process can excellently be used for the formulation of polymer-coated magnetic nanoparticles which can further be used for biomedical applications. The use of high shear, appropriate surfactants, and the addition of a hydrophobe in order to suppress the influence of Ostwald ripening are key factors for the formation of the small and stable droplets in miniemulsion and will be discussed. Two different approaches based on miniemulsion processes for the encapsulation of magnetite into polymer particles will be presented in detail.

1. Introduction

The formulation and application of polymer particles and hybrid particles composed of polymeric and magnetic material are of great interest for biomedical applications. For such applications, it is necessary that the materials—especially the surfaces of the particles—are biocompatible and non-toxic, and sometimes also biodegradable. Many different approaches are used for the generation of nanoparticles in order to obtain the required properties. For the preparation of polymer particles, processes such as the microemulsion technique [1] and the technique of emulsion polymerization [2] are used, which are based on kinetic control during the preparation: the particles are built from the centre to the surface, and the particle structure is governed by kinetic factors. Because of the dictates of kinetics, serious disadvantages such as lack of homogeneity and restrictions on the accessible compositions have to be accepted. Therefore, there is a desire to take advantage of

  (i) potential thermodynamic control for the design of nanoparticles and
 (ii) the concept of 'nanoreactors' where the essential ingredients for the formation of the nanoparticles are already present from the outset [3].
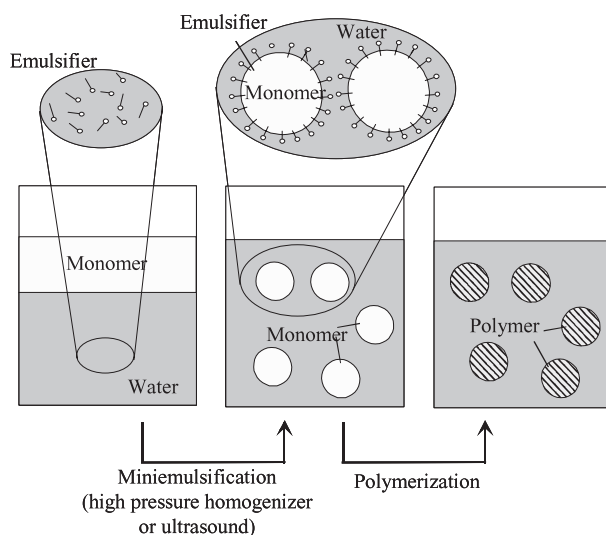
Figure 1. The principle of miniemulsion polymerization.

It is underlined that particle formation in nanoreactors takes place in a highly parallel fashion, i.e. the synthesis is performed in 10^{18}–10^{20} nanocompartments which are separated from each other by a continuous phase. However, previous systems present serious restrictions and failure mechanisms, as recently discussed in [4].

The idea of polymerization in a nanoreactor is technically realized to a high degree of perfection in suspension polymerization, where droplets in the micrometre range are created which can be polymerized without change of particle identity [5]. The suspension principle was utilized to obtain smaller droplet sizes by Ugelstad [6], who scaled down the droplet size to several hundred nanometres by shearing the system.

It is the aim of this paper to describe a recent development where the availability of high-shear devices such as ultrasound and high-pressure homogenizers has decreased the droplet or nanoreactor diameter down to 30–100 nm and allows one to formulate magnetite hybrid particles for biomedical applications.

2. The miniemulsion process

A system where small droplets with high stability in a continuous phase are created by using high shear [7–9] is classically called a 'miniemulsion'. One of the tricks for obtaining stability of the droplets is the addition of an agent which dissolves in the dispersed phase, but is insoluble in the continuous phase. The small droplets can be hardened by either a subsequent polymerization or by decreasing the temperature (if the dispersed phase is a low-melting-point material). For a typical oil-in-water miniemulsion, an oil, a hydrophobic agent (or several), an emulsifier and water are homogenized by high shear (see figure 1) to obtain homogeneous and monodisperse droplets in the size range of 30–500 nm [3].

In a first step of the miniemulsion process, small stable droplets in the size range between 30 and 500 nm are formed by shearing a system containing the dispersed phase, the continuous phase, a surfactant and an osmotic pressure agent. In a second step, these droplets are polymerized without changing their identity.

On the basis of the principle on miniemulsion, the preparation of new nanoparticles which could not be prepared in heterophase processes is now possible. That also includes, in

particular, the encapsulation of inorganic materials, e.g. magnetite, which will be the focus of this paper.

For creating a miniemulsion, the step of homogenization is of great importance since fairly monodisperse small droplets have to be achieved. The homogenization can be achieved by using an ultrasonifier (for the miniemulsification of small quantities in a laboratory scale batch process) or a high-pressure homogenizer (for larger scales). At the beginning of the homogenization, the polydispersity of the droplets is still quite high, but as a result of continuous fusion and fission processes induced by the high shear, the size and polydispersity decrease until the miniemulsion reaches a steady state [10]. The process of homogenization was followed by different methods, e.g. by means of turbidity and surface tension measurements. A constant value indicating the steady state is reached in both of the latter experiments. The surface tension reaches high values, indicating that the coverage of the droplets by surfactant molecules is very low. Indeed, incomplete coverage of droplets by surfactant molecules is an important characteristic of miniemulsions and shows that the surfactant is being very efficiently used. It was observed that the coverage of surfactant depends on the droplet size: the smaller the droplets, the higher the coverage for obtaining stable droplets. The exact size of the droplet can be selectively adjusted by changing the type and amount of surfactant used for the stabilization. Anionic and cationic surfactants allow the formation of monodisperse droplets between about 30 and 200 nm; non-ionic oligomeric or polymeric surfactants are suitable for the formation of droplets between about 100 and 800 nm.

Such minidroplets were previously regarded as a rather unstable dispersion state of matter for two growth mechanisms for the droplets: growth by Ostwald ripening and growth by collisions (and subsequent coalescence). The suppression of both processes is desirable for the formulation of a stable miniemulsion. Coalescence can be controlled by the effective use of a surfactant. Ostwald ripening can be efficiently suppressed by the addition of a hydrophobic agent to the dispersed phase. This agent cannot diffuse from one droplet to another and is trapped in each droplet; this provides an osmotic pressure inside the droplets which counteracts the Laplace pressure. The effectiveness of the hydrophobe increases with decreasing solubility in the continuous phase.

This mechanism was already used for the stabilization of fluoroalkane droplets by addition of the ultrahydrophobe perfluorodimorphineopropane which results in an effective and stable blood substitute [11]. As hydrophobes, a variety of molecules can be used and they can be chosen so as to add a useful property to the final product, i.e. the molecule can be a dye, a plasticizer or a cross-linker. For biomedical applications, this component could be also a fluorescent marker or a drug.

The addition of an ultrahydrophobe does not completely block droplet growth (due to a still finite solubility, the existence of droplet collisions and surfactant-assisted transport), but it slows it down remarkably. The final state to be expected is given by the balance of the osmotic pressure and Laplace pressure. Since the Laplace pressure immediately after miniemulsification is usually larger than the osmotic pressure, the miniemulsion tends to grow on the timescale of days to weeks before reaching an equilibrium state. Due to the timescale involved, this growth is usually not relevant for synthesis application, but it is also possible to handle it in a thermodynamic fashion. This can be done by increasing the amount of osmotic agent, increasing the particle size or adding a second dose of surfactant after dispersion (to lower the surface tension and the related Laplace pressure) [10].

The extremely high stability of those nanodroplets as well as the absence of exchange of material between the droplets (in the case of low solubility in the continuous phase) have been graphically illustrated by classical colour reactions such as the formation of Prussian blue or nickel murexide in inverse miniemulsion systems [12, 13]. In the case of nickel murexide,

one miniemulsion with droplets containing a murexide solution and another miniemulsion containing a Ni^{2+} solution are mixed and the mixed miniemulsion (represented at 0 s) stays red and no nickel–murexide complex is formed. This indicates that droplets with murexide and droplets with Ni^{2+} coexist and no fusion/fission process takes place. If one performed, as a comparison, the same experiment with two *micro*emulsions, an immediate reaction would take place because the low interfacial tension of close to zero leads to highly dynamic processes in the system. The droplets in microemulsions do not retain their identity, while those in miniemulsions do. This is because in a miniemulsion higher energy is required to perform this process, e.g. ultrasonication. In this case, fusion and fission processes are induced and it can be seen that with increasing ultrasonication time the miniemulsion turns yellow. The overall droplet size does not change.

Because of this, each miniemulsion droplet can indeed be treated as a small nanoreactor; the stabilization of each of the nanoreactors can be achieved by using surfactants. This enables a whole set of new reactions to be induced, which lead to nanoparticles as well as the synthesis of nanoparticle hybrids which were not accessible before.

3. Magnetic fluids

Magnetic fluids are stable dispersions of ultrafine magnetic particles or encapsulated magnetic particles in an organic or aqueous carrier medium. The stabilization of these particles can be achieved by surfactants which hinder particle flocculation or sedimentation. In ideal cases, the particles also maintain their stability under exposure to magnetic fields.

Magnetic polymer nanoparticles, which are usually dispersed in a carrier liquid, can be tailor-made for the final application [14, 15]. Several kinds of magnetic polymer nanoparticle have been produced from both natural and synthetic polymers with the intention of incorporating groups on the surface or treating their surfaces in order to perform, for instance, selective separations. In special cases, magnetic nanoparticles with or without polymer encapsulation can be used in magnetic drug targeting [16, 17], tissue engineering, magnetic resonance imaging [18] and hyperthermia [19].

In early publications, magnetic fluids were produced by grinding magnetite with heptane or long-chain hydrocarbon and a grinding agent, e.g. oleic acid [20]. Later, magnetic fluids were produced by precipitating an aqueous Fe^{3+}/Fe^{2+} solution with a base, coating these particles with an adsorbed layer of oleic acid and then dispersing them in a non-aqueous fluid [21]. Both processes result in tiny magnetite particles, a surfactant coating these magnetite particles and a non-aqueous liquid carrier in which the hydrophobic magnetite particles are dispersed. Obviously, the latter process is more feasible for application in the production of more homogeneous magnetite particles.

Other applications of magnetic fluids rely on water as the continuous phase. Kelley [22] produced an aqueous magnetic material suspension by conversion of iron compounds to magnetic iron oxide in an aqueous medium under controlled pH conditions in the presence of a petroleum sulfonate dispersant. Shimoiizaka *et al* [23] developed a water-based magnetic fluid from the oleic-acid-coated magnetite particles dispersed in an anionic or non-ionic surfactant solution which is suitable for forming a second surfactant layer.

Polymer-covered magnetic particles can also be produced by precipitation *in situ* of magnetic materials in the presence of a polymer which acts as a stabilizer. In this way, magnetic polymer nanoparticles are produced in the presence of water-soluble dextran [24], poly(ethylene imine) [25], poly(vinyl alcohol) [26], poly(ethylene glycol) [27], sodium poly(oxyalkylene di-phosphonates) [28] and amylose starch [29]. In all cases, the magnetic particles are surrounded by a hydrophilic polymer shell.

Another method for producing magnetic polymer particles consists in separately synthesizing magnetic particles and polymer particles and then mixing them together to enable either physical or chemical adsorption of the polymer onto the magnetic material to be achieved. The polymer material can be produced in different ways, for instance by means of emulsion or precipitation polymerization [30].

It is also possible to use a strategy comprising polymerization in a heterophase in the presence of magnetic particles. The magnetic material, preferably having a surfactant coating, is embedded into a polymer using processes such as suspension, emulsion or precipitation polymerization. Magnetic particles have been encapsulated in hydrophilic polyglutaraldehyde by suspension polymerization resulting in particles with an average diameter of 100 nm [25]. Magnetite-containing nanoparticles of 150–200 nm have also been synthesized by seed precipitation polymerization of methacrylic acid and hydroxyethyl methacrylate in the presence of magnetite particles containing tris(hydroxy methyl)aminomethane hydroxide in ethyl acetate medium [31]. Polymethacrylate/poly(hydroxy methacrylate)-coated magnetite particles have also been prepared by a single inverse microemulsion process, leading to particles with a narrow size distribution, but with a magnetite content of only 3.3 wt% [32].

Daniel and co-workers [33] obtained magnetic polymer particles by dispersing a magnetic material in an organic phase which consists of an organo-soluble initiator, vinyl aromatic monomers and/or a water-insoluble compound. The mixture was emulsified in water by using an emulsifier and then polymerization took place, producing polymer particles with a magnetite content between 0.5 and 35 wt% with respect to the polymer. However, the resulting particle size distribution was rather broad (between 30 and 5000 nm). Charmot and Vidil [34] used a similar method to produce magnetizable composite microspheres of a hydrophobic cross-linked vinylaromatic polymer, but they obtained a mixture of magnetizable particles and non-magnetizable blank microspheres. Wormuth [35] used the inverse miniemulsion process [36] to encapsulate magnetic particles by a hydrophilic polymer.

Magnetic polymer nanoparticles should meet some criteria for further biomedical application: no sedimentation, uniform size and size distribution, high and uniform magnetic content, superparamagnetic behaviour, no toxicity, no iron leakage, high selectivity in the case where the particles are used for hyperthermia purposes and sufficient heat generation at lower frequencies to enhance selective heating [19]. Therefore, magnetite particles homogeneously encapsulated in a hydrophobic polymer which keep water-soluble components from contacting the magnetite particles are of great interest.

There are several reasons for using polystyrene as the hydrophobic encapsulation material in biomedical applications [37], e.g. it is inexpensive and it is a hydrophobic polymer which allows physical adsorption of antibodies or proteins; it can also be functionalized e.g. by carboxylic groups, which enables covalent binding of antibodies, proteins or cells to be achieved.

Polymerization in direct miniemulsion can be used for the efficient encapsulation of water-insoluble materials in hydrophobic polymers to obtain hybrid particles which are homogeneous in size and inorganic material content, as was recently shown for the encapsulation of hydrophobized $CaCO_3$ [38].

4. Encapsulation of magnetite by the miniemulsion process

Recent work shows that the miniemulsion process allows the encapsulation of hydrophobized calcium carbonate [38] and carbon black [39]. Magnetite, however, is hydrophilic and requires an effective surface treatment before the encapsulation process can be performed. This is preferably done by means of the adsorption of a secondary surfactant on the magnetite surface
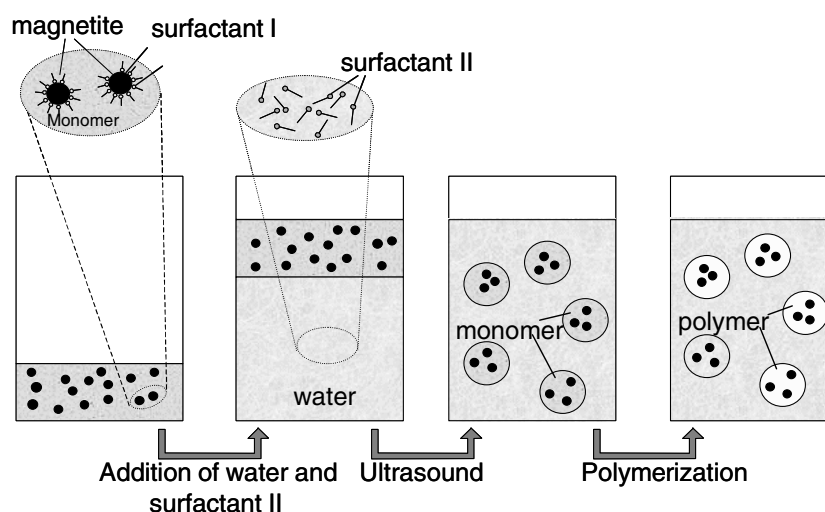
Figure 2. The principle of the encapsulation of magnetite in polymer particles by the miniemulsion process.

which does not interfere with the primary surfactant system needed to stabilize the polymer particles.

Magnetite particles were encapsulated in a polystyrene matrix by a miniemulsion polymerization process, as shown in figure 2 [40]. In a first step, a stable dispersion of magnetite particles in styrene is required. For that purpose, an effective surfactant system has to be used in order to make the particles hydrophobic and to prevent aggregation. Oleoyl sarcosine acid and the more efficient oleic acid were used as the first surfactant system to handle the magnetite/oleic acid interface. That allows one to efficiently stabilize magnetite particles down to 10 nm also in styrene and related monomer mixtures. These lipophilic dispersions could be miniemulsified in water by using sodium dodecylsulfate (SDS) as a second surfactant system forming a stable emulsion. It is important to note that the simple magnetite particles remain well dispersed in the monomer droplets, as shown by electron microscopy. This means that the acid stays at the magnetite/styrene interface and is not redistributed towards the monomer/water interface, an important prerequisite for keeping such double dispersions stable.

Since hexadecane was added to the monomer phase as an ultrahydrophobe to prevent Ostwald ripening, the monomeric double miniemulsion was already kinetically stable. The polymerization was then started by raising the temperature. The final dispersion was free of coagulum and stable. The brown colour did not change during the polymerization, which proves that the radical polymerization process did not significantly interfere with the oxidation state of the magnetite colloid. A simple test with a magnet showed that the dispersion is a ferrofluid, i.e. is magnetic. Thermogravimetric measurements revealed that the magnetite loading in the final particles was up to 15 wt%, indicating that no change of composition by selective loss of inorganic material occurred throughout the reaction.

In figure 3, a transmission electron micrograph (TEM) of polystyrene particles with encapsulated magnetite is shown. Particles with diameters of about 100 nm are found.

This reaction however still showed some imperfections, since the distribution of magnetite between the particles and within each particle was still rather heterogeneous, which was presumably due to the interaction between the magnetite moieties and a related size- and
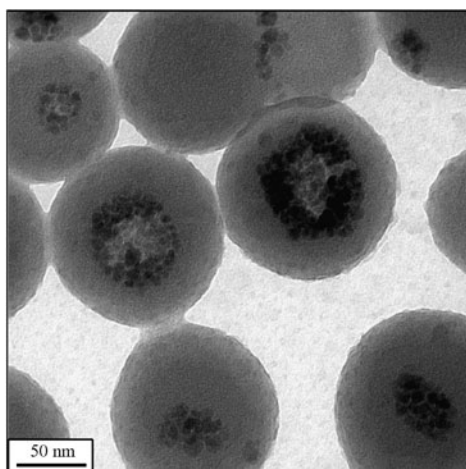
Figure 3. Encapsulation of Fe$_3$O$_4$ in polystyrene particles.

content-specific destabilization of the miniemulsion droplets, and the magnetite content in the polystyrene matrix was limited to about 15 wt%. Also the influence of the pH seemed to be crucial, which was attributed to both the pH dependence of the surface charge of magnetite and the protonation of the oleoyl sarcosine acid and the coupled interface energy.

5. Encapsulation of large amounts of magnetite

In order to be able to increase the magnetite content in the polymer particles, another process had to be developed. Also, limitation of the encapsulating inorganic material was observed in the case of carbon black encapsulation. Here another approach was developed for the encapsulation of large amounts of carbon black [39]: both the monomer and the carbon black were independently dispersed in water using SDS as a surfactant and they were mixed afterwards in any ratio of the monomer and carbon. Then, this mixture was cosonicated, and the controlled fission/fusion process characteristic of miniemulsification destroyed all aggregates and liquid droplets; only hybrid particles composed of carbon black and monomer remained due to their higher stability [39]. In order to obtain homogeneous encapsulation with a high magnetite content, a three-step process had to be developed [41].

In the first step, hydrophobized magnetite particles with a diameter of about 10 nm were synthesized in a classical co-precipitation procedure. In a second step, these magnetite particles were transformed to magnetite aggregates of about 40–200 nm in water by using a miniemulsion process. In a third step, the magnetite aggregates were encapsulated with a monomer by an ad-miniemulsification process and, after polymerization, polymer particles with high magnetite loading were obtained.

The first step is already well known from the literature [21]. The importance of the second step was shown previously [41]. If the small hydrophobized magnetite particles obtained in the first step were dispersed in water as separate magnetite particles, in the following fusion/fission process only a very low number of these small magnetite particles can collide with the monomer droplets, resulting in a very low encapsulation efficiency. Therefore, for a successful fusion/fission process, larger magnetite aggregates are necessary, which could be obtained in the second step of the synthesis route by using the miniemulsion process. The influence of reaction parameters on the magnetite aggregate size will be discussed in detail.
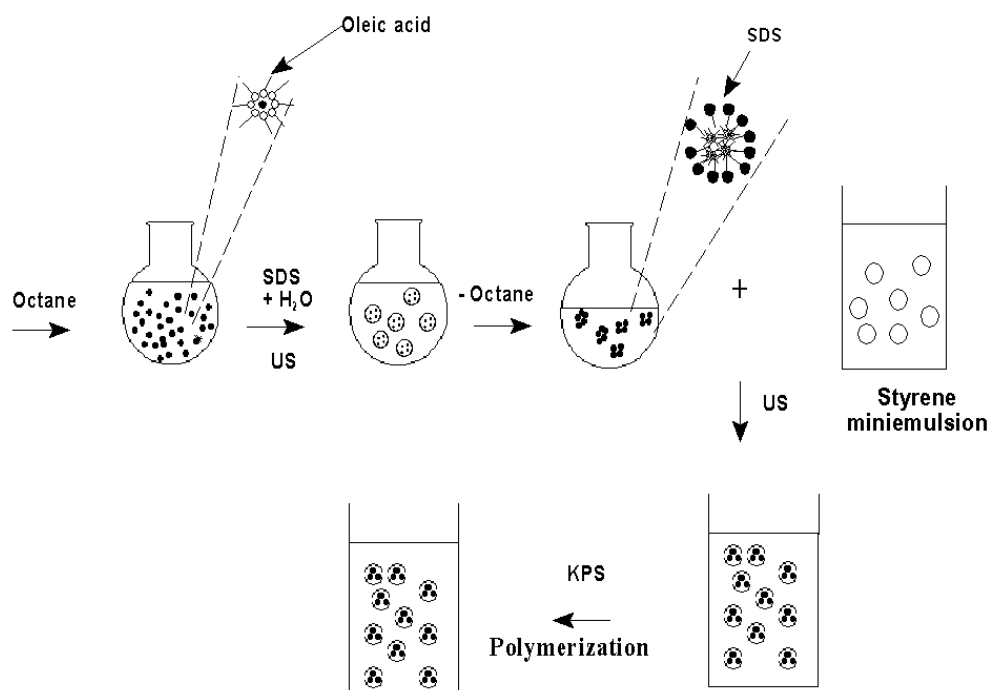
Figure 4. Formulation of polymer-coated magnetite particles with a high magnetic ratio. In the first step, hydrophobized magnetite particles are produced and, in a second step, they are transferred to magnetite aggregates in water by using the miniemulsion process. In a third step, the principle of co-miniemulsion is used. The controlled fission/fusion process in the miniemulsification realized by high-energy ultrasound or high-pressure homogenization treatment destroys all aggregates and liquid droplets, and only hybrid particles composed of magnetite and monomer remain due to their higher stability.

The third step, involving the influence of the aggregate size and the presence of acrylic acid on the encapsulation of the aggregates, will be described. The three-step synthesis route is shown in figure 4.

5.1. Hydrophobic magnetite nanoparticles

Magnetite particles with a mean diameter of 10 nm were obtained in a co-precipitation process [21] by quickly adding a concentrated ammonium solution to a solution of Fe^{2+}/Fe^{3+} with a molar ratio of 3:2 which allows compensation for the oxidation of some iron II to iron III during the co-precipitation in an open vessel [21].

By adding oleic acid (22.2 or 44.4 wt% with respect to the magnetite) at temperatures above its melting temperature, the magnetic particles were hydrophobized. After evaporation of the water and washing out the non-adsorbed oleic acid, a dry powder was obtained. The oleic acid content was determined by thermogravimetric measurements of the powder as shown in figure 5. In the case where 22.2 wt% oleic acid was added for hydrophobization, between 200 and 400 °C a one-step mass loss of about 20% is detected, indicating that most of the oleic acid is indeed adsorbed on the particles and could not be washed out. A calculation with a mean diameter of 10 nm and a magnetite density of 5.16 g cm^{-3} leads to an area occupied by one oleic acid molecule of 0.27 nm^2. When 44.4 wt% oleic acid was added for hydrophobization, a two-step mass loss was detected. The first mass loss of 15% was obtained between temperatures of 200 and 280 °C; the second mass loss of about 18% was observed
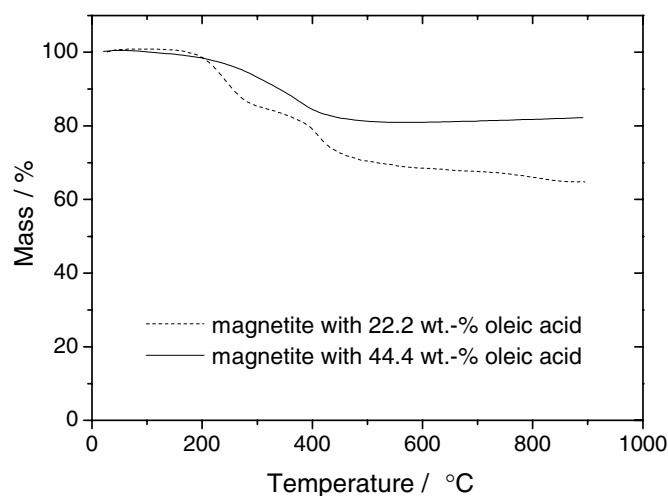
Figure 5. Thermogravimetric curves of magnetite particles coated with oleic acid.
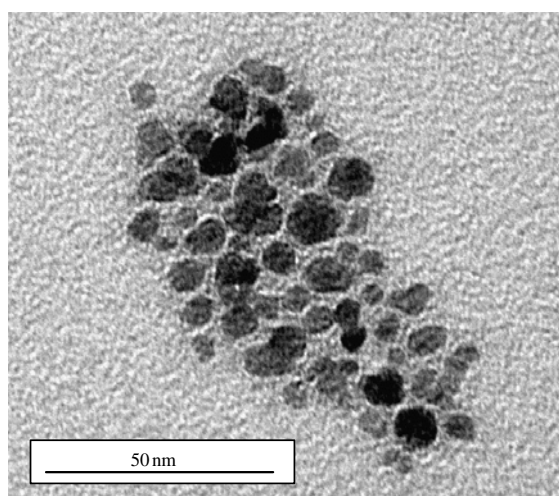

Figure 6. Oleic-acid-coated magnetite particles in octane.

between temperatures of 380 and 480 °C. The two-step decay indicates that different species of oleic acid are present in such a sample. The area which could, at maximum, be occupied by one oleic acid molecule was calculated to be about 0.16 nm^2, which suggests that a multi-layer arrangement is realized.

The hydrophobized magnetite particles could be easily dispersed in octane; the particle size of the oleic-acid-coated particles in octane was about 20 nm (see figure 6). A stable dispersion with a magnetite content of 14 wt% was prepared for further use.

5.2. Aqueous magnetite aggregate dispersion

In a next step, the hydrophobic magnetite particles in octane as the dispersion medium are reformulated to stable water-based magnetic fluids for the encapsulation process. To that end, a miniemulsion process was carried out to produce magnetite/octane-in-water dispersions. Stable aqueous miniemulsions with droplets consisting of the magnetite/octane dispersions
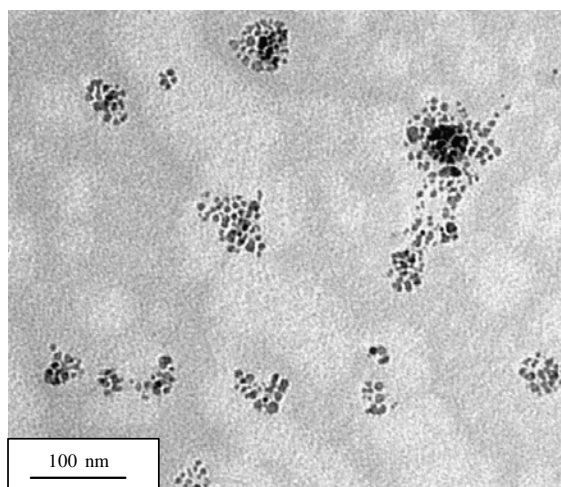
Figure 7. Magnetite aggregates obtained after a miniemulsion process in water.

(as obtained above) were obtained by using as little as 1.0 wt% of the anionic surfactant SDS. For the formulation of stable miniemulsions, the addition of a hydrophobic agent is required to provide osmotic stabilization. However, the magnetite/octane-in-water miniemulsions with hexadecane and without hexadecane both show high stability, indicating that oleic acid not only acts as a coating agent but also can replace the osmotic pressure agent.

After evaporation of the octane, a water-based ferrofluid consisting of oleic-acid-coated aggregated magnetite dispersed in a water phase is obtained (for an example, see figure 7). In other words: the magnetite aggregates must have a surfactant double layer; the first layer is oleic acid, which provides hydrophobicity of the particles for later encapsulation, and the second layer is SDS, which promotes the stabilization in water. The size of these aggregates depends on both the oleic acid (introduced in the first step) and the SDS concentration and can be adjusted between 40 and 200 nm. The higher the SDS concentration, the smaller the magnetite aggregate sizes. Doubling the amount of oleic acid in the magnetite particles leads to smaller magnetite aggregates.

5.3. Encapsulation of the magnetite particles in polymer

In the third step of the synthesis route, a monomer miniemulsion with 20 wt% dispersed phase is prepared and added to the water-based ferrofluid containing the magnetite aggregates as obtained above. Then, this mixture is cosonicated and the controlled fission/fusion process which is characteristic of miniemulsification is expected to destroy all aggregates and liquid droplets; only hybrid particles composed of magnetite and monomer are expected to remain, since this species shows the highest stability, as was already shown for the encapsulation of carbon black in styrene [39]. The idea is to obtain another stable miniemulsion from two stable miniemulsions by cosonication and to take advantage of the possibility of using different magnetite and styrene concentrations.

Then, polymerization of the monomer was started for all the samples presented here by adding the initiator potassium peroxodisulfate (KPS) at 72 °C. Whereas in a typical styrene miniemulsion polymerization, the polymerization using KPS at 72 °C is usually completed after 2 h, in the case of the magnetite/styrene miniemulsion, between 18 and 24 h was required for a full conversion of the styrene. Then stable dispersions without the formation of any coagulum were obtained.

For the synthesis of the particles, different sized aggregates can be used. The aqueous dispersion with the smallest magnetite aggregates of 40 nm and a styrene miniemulsion were mixed in a ratio of the two dispersed phases of 1:1. This means that the particles consist of 50% styrene and 50% magnetite/oleic acid/SDS, which results in a theoretical magnetite content of 19.4%. After 40 min of the reaction, acrylic acid was added in order to functionalize the particles for further possible reactions (e.g. for binding antibodies onto the particle surface). After completion of the polymerization, the latex particles have a particle size of about 60 nm. The weight-average molecular weight of the polymer formed in the particles was determined to be about $210\,000$ g mol^{-1}, which indicates that the presence of magnetite in the polystyrene does not have any effect on the final weight-average molecular weight of the polymer. However, the amount of oleic acid is not negligible in the particles and also contributes to the polymer, since double bonds of the oleic acid are also converted. In the control miniemulsion polymerization of styrene/oleic acid of a miniemulsion without any magnetite, about 50% of the double bonds have been converted.

TGA measurements show a two-step decay: between 250 and 350 °C a mass loss of about 20% is detected; then a second mass loss between 400 and 450 °C is observed, resulting in a remaining mass of 20% which is attributed to the magnetite, a value corresponding well to the expected value.

The structural homogeneity of the encapsulation process is characterized by sedimentation experiments in a preparative ultracentrifuge. In a density gradient, it is simple to differentiate between the low-density polymer phase, the high-density magnetite phase and all intermediates, which are the encapsulated species with different layer thicknesses and degrees of layer perfection. For the experiment, sucrose solutions of different densities were layered, building up a density gradient from 1.0 to 1.3 g cm^{-3}.

In the case of this latex, only one particle fraction with a narrow density distribution (1.2 g l^{-1}) is detected (see figure 9; latex: 19.4% magnetite, with AA), indicating that all particles formed show very similar magnetite/polymer compositions or degrees of encapsulation. At the same time, the absence of pure polystyrene particles or pure magnetite aggregates is clearly proven.

The corresponding latex without acrylic acid (see figure 9) shows a rather similar density distribution of the particles. This means that the encapsulation of the small magnetite aggregates is not influenced very much by the presence of acrylic acid.

This structural or composition homogeneity is also observed by electron microscopy. As shown in figure 8, full encapsulation of the magnetite particles is achieved. It is found that the small magnetite particles are well separated, which means that each is presumably completely coated with a thin layer of oleic acid and then the entire aggregate is covered with a layer of polymer. Due to the oleic acid, the polymer formed is rather soft.

With increasing aggregate size, the density distribution becomes larger, indicating that homogeneous encapsulation of larger particles is more difficult. This is because of statistical factors operative during the fusion/fission process: the magnetite aggregates should not be too large because then only a few large aggregates are faced with many styrene droplets. Please also note that the 10 nm magnetite particles are too small to be efficiently encapsulated. A situation where there are similar numbers of magnetite aggregates and styrene droplets is therefore favoured for a homogeneous encapsulation process.

Using the ferrofluid with the smallest magnetite aggregate particles (40 nm) for encapsulation, the magnetite content could easily be varied between 20 and 34% as determined by TGA; the densities were rather homogeneous (figure 9). Note that the average density (see the arrows) is increasing with increasing magnetite load.
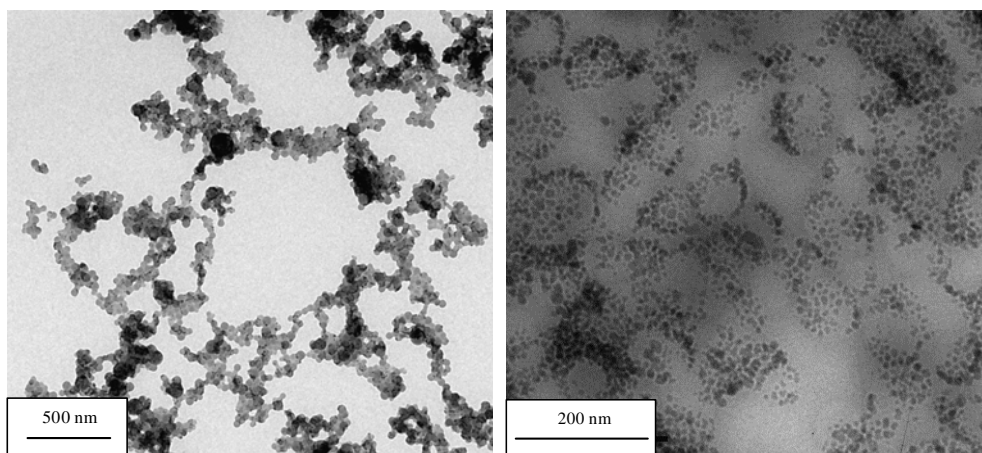
Figure 8. TEM images for magnetite polystyrene particles at different magnifications.

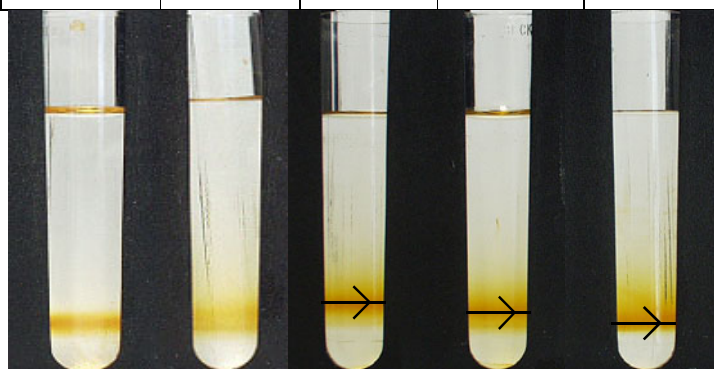| with AA	without AA	with AA	with AA	with AA
19.4 %	19.4 %	25.4 %	29.5 %	34.7 %
magnetite	magnetite	magnetite	magnetite	magnetite

Figure 9. Encapsulation of polystyrene/magnetite particles studied by ultracentrifuge experiments in a density gradient: samples with 40 nm magnetite aggregates and with or without acrylic acid were used at different magnetic contents. Note that the average density (arrows) is increasing with increasing magnetite load.

(This figure is in colour only in the electronic version)

5.4. Magnetic properties

The magnetic properties of synthesized ferrofluids with polymer-encapsulated magnetite particles are of great interest for further applications. The magnetic properties, including the saturation magnetization (M_s), the susceptibility (χ), the specific saturation magnetization (σ, values extrapolated to $H \rightarrow \infty$), the relative saturation remanence ($m_r = M_r/M_s$) and the coercivity (H_c), of some of the ferrofluids prepared are presented in figure 10. All of the ferrofluids show typical superparamagnetic behaviour at room temperature without any hysteresis loop. The superparamagnetic behaviour is also reflected in the low M_r/M_s ratio

and in the small H_c-values. This clearly indicates that the magnetite primary particles with a diameter of 10 nm are still well separated in the polymer particles.

In figure 10(a), the magnetization curves for the samples at different stages of the synthesis are compared, namely

(a) the oleic-acid-coated magnetite particles dispersed in octane (magnetite particles in octane),
(b) the water-based ferrofluid containing the 40 nm magnetite aggregates (after octane evaporation) and
(c) the polymer-encapsulated magnetite aggregates.

For the oleic-acid-coated magnetite particles in octane, a saturation magnetization value of 87 emu g^{-1} magnetite is obtained which is close to the saturation magnetization of the bulk material (89 emu g^{-1} magnetite). The 40 nm magnetite aggregates in water have a saturation magnetization of 68 emu g^{-1} magnetite; the loss of magnetization may be due to oxidation processes taking place during the sonication, which lead to the formation of some non-magnetic iron oxide (Fe_2O_3). For the polystyrene magnetite particles, the saturation magnetization is still as high as 53 emu g^{-1} magnetite, which is about 60% of the magnetization of the bulk material. This means that during the polymerization, again probably some of the magnetite converts to Fe_2O_3 due to the presence of the oxidizing initiator fragments.

Figure 10(b) shows that the amount of magnetite in the particles does not influence the saturation magnetization. In all cases, the saturation magnetization was between 51 and 54 emu g^{-1} magnetite indicating that the magnetization is quite constant after the polymerization process.

6. Use of biosurfactants

The majority of the recipes described in the literature are based on the anionic sodium dodecylsulfate (SDS) as a model system. The possibility of using cationic surfactants such as octadecyl pyridinium bromide for the preparation of miniemulsions was first exploited in 1976. However, the emulsions were prepared by stirring and the resulting emulsions showed broadly distributed droplet sizes [42, 43]. Recent work on steady-state miniemulsions showed that cationic and non-ionic surfactants form well-defined miniemulsions for further miniemulsion polymerization processes, resulting in narrowly size-distributed stable cationic and non-ionic latex particles [44]. Non-ionic miniemulsions can be made by using 3–5% of a poly(ethylene oxide) derivative as the surfactant, resulting in larger, but also very well-defined latexes [44].

However, all these surfactants show low biocompatibility. Therefore, the use of other surfactants is required if the magnetic latex particles are to be used in biomedical applications.

Lecithin can be used in an efficient way as a biocompatible surfactant for the preparation of miniemulsions. Lecithin is usually used as synonym for phosphatidylcholine, which is the major component of a phosphatide fraction which is frequently isolated from either egg yolk or soya beans. The structure of lecithin, which is given in figure 11(a), is variable and dependent on the fatty acid substitution. Unlike the synthetic alternatives, lecithin can be totally biodegraded and metabolized, since it is an integral part of biological membranes, making it virtually non-toxic, whereas other emulsifiers can only be excreted via the kidneys. The natural origin of lecithin produces, however, a rather complex composition, while in pharmacy in general well-defined singular excipients are favoured. Lecithin is regarded as a well-tolerated and non-toxic compound, making it suitable for long-term and large-dose infusion. As an emulsifier of intravenously administered fat emulsions, its composition and behaviour determine the structure and stability of the emulsion in a decisive way.
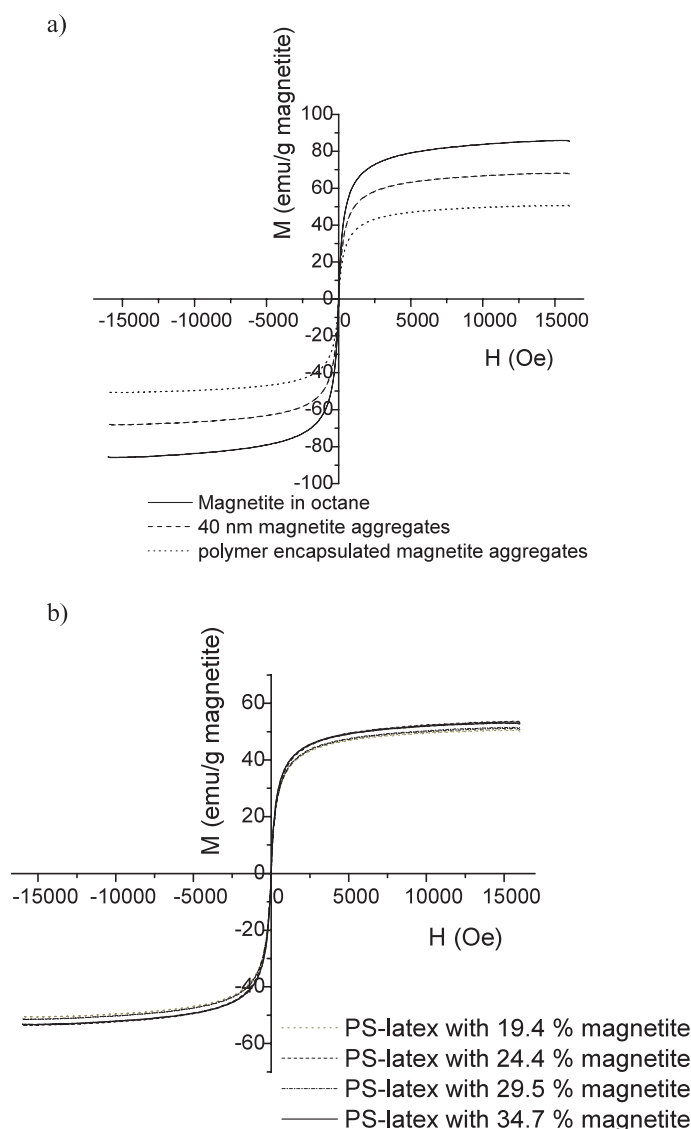
a)

b)

Figure 10. The magnetic field dependence of the magnetization: (a) of magnetite in octane, the 40 nm magnetite aggregates in water and the polymer-encapsulated magnetite particles; (b) of different encapsulated magnetite particles.

The salt of the *cholic acid* (3α, 7α, 12α-trihydroxy-5β-cholan-24 acid) is also excellent for use in the formulation of miniemulsions and for the stabilization of magnetic aggregates in water (see figure 12) or, after encapsulation, for the stabilization of magnetic polymer particles. Cholic acid is composed of a steroid unit with a carboxylic acid group and three hydroxyl groups, which are all located on one side of the steroid nucleus (figure 11(b)). Cholic acid is one of the bile acids and its salt is found as a natural constituent of bile. The nuclei of the bile acids are closely related to cholesterol, from which they are formed in the liver, and this conversion depends on their relative concentrations. Due to their amphiphilic character, bile salts affect the absorption of fats, fat-soluble vitamins and various ions. *Tween*
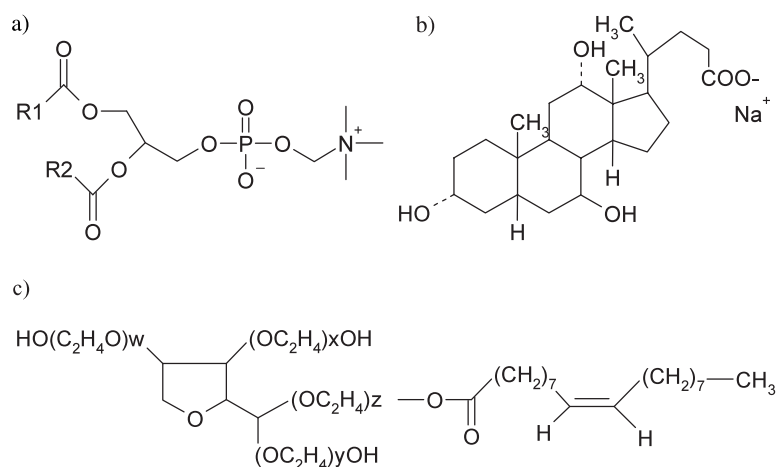
a)

b)

c)

Figure 11. The chemical structure of (a) lecithin; R1, R2: typically linear aliphatic rests with 15 or 17 carbon atoms with up to four *cis* double bonds, (b) the sodium salt of cholic acid and (c) Tween 80. The sum of w, x, y, z is 20.
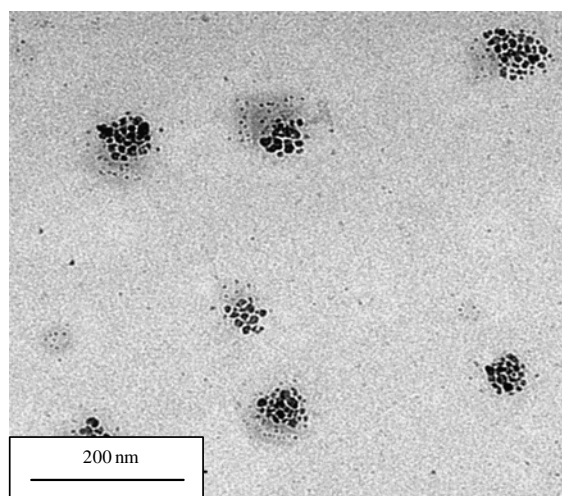

Figure 12. Magnetite aggregates in water with cholic acid as the surfactant.

80 (polyethoxysorbitan mono-oleate) is a non-ionic surfactant comprised of a sorbitan ring and about twenty ethylene oxide units (figure 11(c)). The surfactant is excellent for use in the formulation of miniemulsions. The surfactant is known as a non-toxic surfactant with excellent physiological properties and is widely used in biochemical applications including emulsifying and dispersing substances for pharmaceutics, cosmetics and food products.

7. Conclusions

Our main aim was to demonstrate the possibility of using the miniemulsion process for formulation of magnetic nanoparticles which can be used in biomedical applications. The use of high shear, appropriate surfactants and the addition of a hydrophobe in order to suppress

the influence of Ostwald ripening are key factors for the formation of small and stable droplets in miniemulsions. It was shown that the strength of the miniemulsion approach lies in its ability to form polymeric nanoparticles consisting of polymers or polymer structures, which are hardly accessible by other types of heterophase polymerization route.

The encapsulation of magnetite particles into polystyrene particles can be achieved efficiently by a miniemulsion process using oleoyl sarcosine acid for the magnetite/styrene interface and SDS for the styrene/water interface, thus creating a polymer-coated ferrofluid. This reaction however still shows some imperfections, which are presumably typical for highly magnetic nanoparticles. The distribution of magnetite among the particles and within each particle is still rather heterogeneous, which is presumably due to the interaction between the magnetite moieties and a related size- and content-specific destabilization of the miniemulsion droplets.

The encapsulation of large numbers of magnetite particles into polystyrene particles can be achieved efficiently by a new three-step preparation route including two miniemulsion processes. In the first step, oleic-acid-coated magnetite particles in octane are prepared. In the second step, a dispersion of the magnetite in octane is miniemulsified in water by using SDS as the surfactant. After evaporation of the octane, the magnetite aggregates which are covered by an oleic acid/SDS bilayer are mixed with a monomer miniemulsion and, in the third step of the synthesis route, an ad-miniemulsification process is used to obtain final and full encapsulation. Here, a fusion/fission process induced by ultrasound is only effective for the monomer droplets, whereas the monomer-coated magnetite aggregates remain intact. In that way, all monomer droplets are split and heteronucleated onto the magnetite aggregates to form a monomer film. After polymerization, polymer-encapsulated magnetite aggregates were obtained. Characterization by thermogravimetry, preparative ultracentrifugation and transmission electron microscopy showed that up to 40% magnetite could be encapsulated, resulting in particles with a high homogeneity of the magnetite content.

Magnetometry measurements reveal that the magnetite aggregates still consist of separated superparamagnetic magnetite particles, which is due to the coverage by the oleic acid. During the encapsulation process, 60% of the magnetization compared to bulk magnetite is preserved.

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