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SHORT COMMUNICATION

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Different methods for the fractionation of magnetic fluids

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Abstract Magnetic fluids are used in many fields of application, such as material separation and biomedicine. Magnetic fluids consist of magnetic nanoparticles, which commonly display a broad distribution of magnetic and nonmagnetic parameters. Therefore, upon application only a small number of particles contribute to the desired magnetic effect. In order to optimize magnetic fluids for applications preference is given to methods that separate magnetic nanoparticles according to their magnetic properties. Hence, a magnetic method was developed for the fractionation of magnetic fluids. Familiar size-exclusion chromatography of two different magnetic fluids was carried out for comparison. The fractions obtained and the original samples were also magnetically characterized by magnetic resonance and magnetorelaxometry, two biomedical applications. The size-exclusion fractions are similar to those of magnetic fractionation, despite the different separation mechanisms. In this respect, magnetic fractionation has several advantages in practical use over size-exclusion chromatography: the magnetic method is faster and has a higher capacity. The fractions obtained by both methods show distinctly different magnetic properties compared to the original samples and are therefore especially suited for applications such as magnetorelaxometry.

Key words Magnetic fluids · Magnetic nanoparticles · Magnetic fractionation · Size-exclusion chromatography · Magnetic relaxation

Introduction

Magnetic fluids are usually colloidal solutions of stabilized ferro- or ferrimagnetic single-domain nanoparticles. They are widely applied in industry, for example, for material separation or as damping materials for loudspeakers [1]. In addition, they are used in biomedical applications such as magnetic cell separation [2] or as injectable contrast agents in magnetic resonance imaging [3]. Magnetorelaxometry was recently introduced as a method for the evaluation of immunoassays [4]. All these applications utilize the magnetic properties of the nanoparticles in magnetic fluids, an example being the magnetic moment. Furthermore, they depend on the hydrodynamic size. Magnetorelaxometry also depends on the core size. All these parameters exhibit a broad distribution in common magnetic fluids; thus, in many cases only a small portion of particles contributes to the desired magnetic effect. The relative amount of these particles can be increased by the fractionation of magnetic fluids. Up to now this has been achieved mainly by centrifugation [5] and size-exclusion chromatography (SEC) [6]. These methods separate on the basis of the nonmagnetic properties of the particles. In contrast, the separation of magnetic fluids on a magnetic basis has so far been used to remove aggregates (magnetic filtration) [7] or nonmagnetic material [8]. These were mainly separations that divide a source into only two fractions for each step, whereas fractionation splits it up into several fractions.

A technique for the magnetic fractionation (MF) of magnetic fluids has therefore been developed and used for the optimization of these fluids. For comparison, the magnetic fluids were also fractionated with familiar SEC, which is size-selective. The fractions yielded by both methods are characterized and compared in terms of their particle size and magnetic properties measured by magnetic resonance and magnetorelaxometry, two biomedical applications of magnetic nanoparticles.

Experimental

Two different samples of magnetic fluids were studied. Magnetic fluid 1 was an aqueous solution of iron oxide nanoparticles stabilized with dextran, a polymeric carbohydrate. It came from a single-step synthesis [9]. Investigations by electron microscopy showed that these particles had a mean core diameter of 5–6 nm. The saturation magnetization estimated from magnetization curves amounted to approximately 5 $Am^2 mol^{-1}Fe$.

Magnetic fluid 2 consisted of iron oxide nanoparticles in water, the particles being coated with poly(ethylene glycol) in a second step after core synthesis according to the method in Ref. [10]. The nanoparticles had a mean core diameter of 8 nm, whereas the saturation magnetization was about 6 $Am^2 mol^{-1}Fe$.

Unless otherwise stated, fractionations and measurements were made at room temperature.

The experimental setup for MF consisted of an electromagnet with pole shoes and a source of variable direct current (Oxford Instruments, Oxford, UK). It generated a maximum induction of 1.2 T. Since the retention of magnetic nanoparticles requires high magnetic field gradients, columns filled with soft magnetic iron spheres of 0.3-mm diameter were placed between the pole shoes (MACS columns, Miltenyi Biotech, Bergisch Gladbach, Germany). For fractionation the magnetic fluid was poured into the column with the highest magnetic field strength applied. The column was then washed with approximately 500 $mm^3 s^{-1}$ deionized water until the collected feed-through was almost colorless, i.e. it contained only a few magnetic particles. The current, and thus the magnetic field, was then gradually reduced. The columns were washed again with deionized water until the feed-through was colorless. This procedure was repeated stepwise until the field had been decreased to zero.

For comparison, SEC was performed with a preparative liquid chromatographic system (Pharmacia, Uppsala, Sweden) consisting of a P-500 pump, a UV-M UV monitor, a FRAC-100 fraction collector and a LCC-500 controller. The chromatographic conditions were chosen so that there was no undifferentiated total exclusion of larger particles in the samples by the pores of the column material. A suitable column material was Sephacryl S-500 HR (Pharmacia), which contains relatively large pores. The eluant was an aqueous solution of 50 mol m^{-3} $NaHCO_3$ (p.a.) pumped at a flow rate of 17 $mm^3 s^{-1}$.

The total size of the coated magnetic nanoparticles was measured at about 297 K with photon correlation spectroscopy using a Zetasizer 3000 (Malvern Instruments, Malvern, UK) with a He–Ne Laser at 633 nm. The results are given as so-called *Z*-averages of the hydrodynamic diameter, weighted by the sixth power of the size.

The iron content was measured using an ICP-OES 3560 B analyzer (ARL, Offenbach, Germany).

Magnetic fluids alter the magnetic field in nuclear magnetic resonance and thus shorten the relaxation times of the nuclear spins, particularly the transverse relaxation time, T_2. This effect increases with the concentration of the magnetic fluid. A plot of T_2^{-1} as a function of the concentration yields a straight line. Its slope is called relaxivity r_2. The water proton relaxation times of liquid samples were recorded with a minispec pc 120 (Bruker, Karlsruhe, Germany) at 0.47 T (20 MHz) and 313 K. The Carr–Purcell–Meiboom–Gill sequence was used for T_2 measurements.

The magnetorelaxometry mentioned previously observes the relaxation of the net magnetic moment of single-domain particles after they have been exposed to a magnetizing field. Only freeze-dried samples were investigated here. In this case, the magnetic relaxation can only result from rotation of the internal magnetization vector within the core as described by Néel [11]. The samples were magnetized in a field of 1.6 kA m^{-1} for 1 s. After removal of this magnetizating field the decreasing magnetic fields of the samples were recorded with a multichannel superconducting quantum interference device magnetometer at the Physikalisch-Technische Bundesanstalt [12]. The difference in magnetic flux density between 5 and 995 ms was determined for evaluation after the signal of an empty vial had been subtracted. This parameter is referred to as the Néel relaxation amplitude.

Results

First, magnetic fluid 1 was fractionated by the magnetic method. The fractions were collected at different magnetic flux densities and investigated by photon correlation spectroscopy, magnetorelaxometry and nuclear magnetic resonance. It was found that the hydrodynamic diameter of the fractions increases with the retention of the particles on the magnetic column and with a decreasing magnetic field. Former magnetorelaxometric investigations of freeze-dried magnetic nanoparticles containing different amounts of iron showed that, for a magnetic fluid, the Néel relaxation amplitude is directly proportional to the iron content of the sample; therefore, the Néel relaxation amplitude is related to the iron content in order to compare different samples in the following. The Néel relaxation amplitude and r_2 of the magnetic fractions are shown as a function of the hydrodynamic diameter in Fig. 1. Both magnetic parameters increase with the hydrodynamic size.

Furthermore, magnetic fluid 1 was fractionated by SEC. Photon correlation spectroscopy shows that large particles elute before small particles. The Néel relaxation amplitude and r_2 of the size-exclusion fractions were measured and are shown in Fig. 2 as a function of the hydrodynamic diameter. Both magnetic parameters

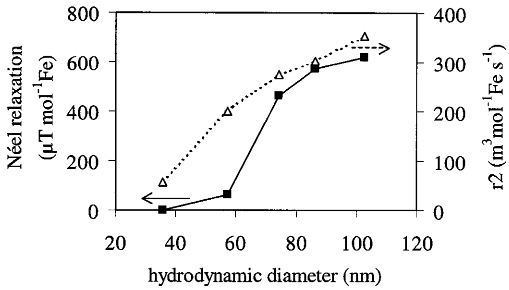

Fig. 1 Magnetic fractions of magnetic fluid 1: Néel relaxation amplitude and relaxivity r_2 as a function of the hydrodynamic diameter

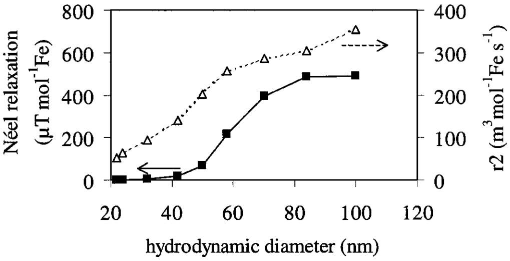

Fig. 2 Size-exclusion fractions of magnetic fluid 1: Néel relaxation amplitude and relaxivity r_2 as a function of the hydrodynamic diameter

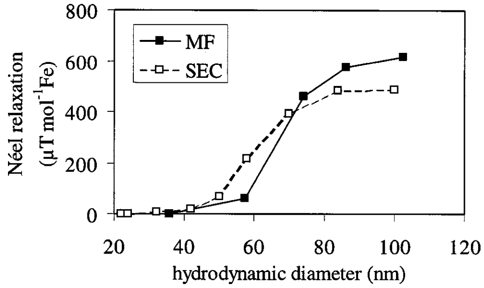

Fig. 3 Magnetic fractions (*MF*) (—) and size-exclusion chromatography (*SEC*) fractions (- -) of magnetic fluid 1: Néel relaxation amplitude as a function of the hydrodynamic diameter

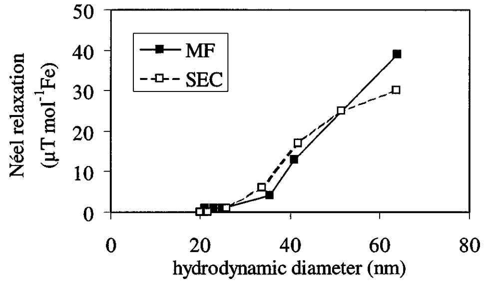

Fig. 4 MF (—) and SEC fractions (- -) of magnetic fluid 2: Néel relaxation amplitude as a function of the hydrodynamic diameter

increase with the hydrodynamic diameter, as found for the magnetic fractions. The particle diameter varies from below 40 to above 100 nm, whereas the Néel relaxation amplitude and r_2 vary from 0 to 600 μT mol^{-1}Fe and from 50 to 350 m^3 mol^{-1}Fe s^{-1}, respectively. The original magnetic fluid 1 has a mean hydrodynamic diameter of about 60 nm, whereas the Néel relaxation amplitude and r_2 are 84 μT mol^{-1}Fe and 121 m^3 mol^{-1}Fe s^{-1}, respectively.

In order to compare MF and SEC, the Néel relaxation amplitude of fractions yielded by both methods is plotted in Fig. 3 as a function of the hydrodynamic diameter. The curves of the two methods are similar. The same is valid for the largest particles yielded by both methods.

In addition, magnetic fluid 2 was fractionated by the magnetic method as well as by SEC. The fractions yielded by both methods were investigated by photon correlation spectroscopy, magnetorelaxometry and nuclear magnetic resonance. While MF yielded fractions whose particle size grows with the retention of the particles on the magnetic column, SEC produced fractions whose particle size decreases with increasing elution time. The Néel relaxation amplitudes of the fractions yielded by (MF) and SEC are shown as a function of the hydrodynamic diameter in Fig. 4. The Néel relaxation amplitude increases with the particle diameter for both methods and the two curves are similar. The particle diameter and the Néel relaxation amplitude vary from 20 to 60 nm and from 0 to 40 μT mol^{-1}Fe, respectively; however, the original magnetic fluid 2 has a mean hydrodynamic diameter of 40 nm and a Néel relaxation amplitude of 1 μT mol^{-1}Fe.

Discussion

MF of magnetic fluids shows that the particle sizes of the fractions grow with a decreasing magnetic field. The Néel relaxation amplitude increases with the hydrodynamic diameter and with a decreasing magnetic field [13]. It should be mentioned that the Néel relaxation amplitude is a measure of the number of magnetic cores that magnetorelaxometry can detect. These magnetic cores are relatively large for low anisotropic magnetic materials such as the iron oxide used. They represent only a small part of the core distribution. This part and thus the Néel relaxation amplitude differ from magnetic fluid to magnetic fluid. A growing Néel relaxation amplitude corresponds to an increasing number of bigger magnetic cores and, in cases of fractions of a magnetic fluid, to an increasing mean core size. Additionally, r_2 increases with the hydrodynamic diameter and a decreasing magnetic field. It is known that r_2 increases with the magnetic core size of single-domain particles [14]. Therefore, the Néel relaxation amplitude and r_2 are correlated with the magnetic core size. The core size thus appears to increase with a decreasing magnetic field. Furthermore, the magnetic moment of a particle is proportional to the core size. This indicates that MF separates the particles on the basis of their magnetic moments. The increases in the Néel relaxation amplitude and in r_2 with the hydrodynamic diameter of the fractions indicate that the core size correlates with the hydrodynamic size for the magnetic fluids investigated. It should be noted that, in cases of magnetic nanoparticles coated with polymers such as those here, the hydrodynamic size of a particle is clearly larger than its core size.

SEC uses nanoporous column materials for separation. With this method large particles are partly kept out by the pores of the column material and therefore elute before the small particles, which penetrate the pores. The SEC of the magnetic fluids confirms this elution order and yields fractions with different particle sizes. The same was reported for the SEC of other dextran-stabilized magnetic nanoparticles [15]. Magnetic investigations of the fractions yielded in the present work show that the Néel relaxation amplitude and r_2 increase with the hydrodynamic diameter. Since the Néel relaxation amplitude and r_2 are correlated with the magnetic core size, the core appears to grow with the overall particle size in the case of the magnetic fluids investigated. Fractionation by SEC was described for magnetic nanoparticles stabilized by surfactants [16]. Electron microscopy and magnetization curves showed that the core size also decreases with increasing elution time. It should be mentioned that the hydrodynamic and core diameters of surfactant-stabilized nanoparticles are relatively similar in contrast to those of polymer-coated nanoparticles.

The fractionation of magnetic fluids by the magnetic method and by the well-known SEC is compared below. SEC separates nanoparticles on the basis of their hydrodynamic sizes, whereas MF utilizes magnetic properties, which are determined by the magnetic cores of the polymer-coated particles. Nonetheless, it was found that the Néel relaxation amplitude and r_2 of the fractions are similar with respect to the hydrodynamic diameter, regardless of the method used. Hence, MF and SEC not only complement each other but can also be used as alternatives to each other. The two techniques are therefore compared with respect to preparative fractionation of magnetic fluids. One difference is the fact that big particles elute first in SEC, whereas small particles do so in MF. One disadvantage of SEC is the necessary addition of electrolyte for defined separation conditions; this electrolyte often destabilizes the magnetic fluid and then has to be removed. Another handicap is the small range of available column materials for the separation of large particles. Conversely, the magnetic method accomplishes this easily at low magnetic field strengths. Moreover, it is easier to scale up MF than SEC. A size-exclusion chromatogram also takes much longer than MF. Finally, the removal of a certain particle fraction requires a complete chromatographic pass, while two magnetic field strengths suffice for collection on and drainage from the column. For these reasons MF is preferable to SEC.

Since the largest hydrodynamic diameters yielded by MF and SEC are similar, the high magnetic forces of the magnetic method do not appear to produce any aggregation of the particles. As mentioned, the mean core size of both magnetic fluids is relatively low, the particles are coated with polymers and the hydrodynamic size increases with the core size. The polymer shells prevent the approach of magnetic cores. Through the low magnetic interaction there is no particle aggregation.

The results presented here are not only found for one magnetic fluid but for two magnetic fluids synthesized differently and with different coating material; therefore, the results do not seem to be restricted to the magnetic fluids described but appear to be more generally valid. The hydrodynamic diameter, Néel relaxation amplitude and r_2 of the fractions yielded cover a relatively broad range for both magnetic fluids investigated. Hence, a broad distribution in size as well as in magnetic parameters of the original samples is implied. The Néel relaxation amplitude and r_2 of the fractions are distinctly different compared to the original samples; therefore, the fractions are especially suitable for applications such as magnetic resonance or solid-phase assays for magnetorelaxometry.

Conclusion

A simple magnetic method for the fractionation of magnetic fluids is presented here. It is based on interaction with the magnetic moments of the nanoparticles in contrast to other separation techniques. The magnetic properties of the fractions yielded by MF are distinctly different compared to the original sample. The particles obtained are very suitable for a number of applications involving magnetic fluids, for example, magnetic resonance and solid-phase assays for magnetorelaxometry; hence, MF can optimize magnetic fluids considerably with respect to their applications. Besides preparative purposes, MF can also be used for analytical purposes.

For comparison, SEC was performed. The fractions yielded are similar to those of MF, despite the different separation mechanisms. In this respect, MF has several advantages in practical use over SEC: the magnetic method is faster, has a higher capacity and does not require electrolyte solutions.

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