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K Hervé, L Douziech-Eyrolles, E Munnier, S Cohen-Jonathan, M Soucé, et al.. The development of stable aqueous suspensions of PEGylated SPIONs for biomedical applications. Nanotechnology, Institute of Physics, 2008, 19 (46), pp.465608. 10.1088/0957-4484/19/46/465608 . hal-03407065

HAL Id: hal-03407065

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Submitted on 28 Oct 2021

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Nanotechnology **19** (2008) 465608 (7pp) doi:10.1088/0957-4484/19/46/465608

The development of stable aqueous suspensions of PEGylated SPIONs for biomedical applications

K Hervé[1,4], L Douziech-Eyrolles[1], E Munnier[1], S Cohen-Jonathan[1], M Soucé[1], H Marchais[1], P Limelette[2], F Warmont[3], M L Saboungi[3], P Dubois[1] and I Chourpa[1]

[1] 'Nanovecteurs Magnétiques pour la Chimiothérapie', EA 4244 'Physicochimie des Matériaux et des Biomolécules', Université F. Rabelais, Faculté de Pharmacie, 37200 Tours, France
[2] Laboratoire LEMA, UMR 6157 CNRS/CEA, Université F. Rabelais, Parc de Grandmont, 37200 Tours, France
[3] Centre de Recherche sur la Matière Divisée, CNRS Université d'Orléans, UMR 6619, 1B rue de la Férollerie, 45071 Orléans cedex 2, France

E-mail: katel.herve@univ-tours.fr

Received 10 July 2008, in final form 24 September 2008
Published 22 October 2008
Online at stacks.iop.org/Nano/19/465608

Abstract
We report here the development of stable aqueous suspensions of biocompatible superparamagnetic iron oxide nanoparticles (SPIONs). These so-called ferrofluids are useful in a large spectrum of modern biomedical applications, including novel diagnostic tools and targeted therapeutics. In order to provide prolonged circulation times for the nanoparticles *in vivo*, the initial iron oxide nanoparticles were coated with a biocompatible polymer poly(ethylene glycol) (PEG). To permit covalent bonding of PEG to the SPION surface, the latter was functionalized with a coupling agent, 3-aminopropyltrimethoxysilane (APS). This novel method of SPION PEGylation has been reproduced in numerous independent preparations. At each preparation step, particular attention was paid to determine the physico-chemical characteristics of the samples using a number of analytical techniques such as atomic absorption, Fourier transform infrared (FT-IR) spectroscopy and Raman spectroscopy, transmission electron microscopy (TEM), photon correlation spectroscopy (PCS, used for hydrodynamic diameter and zeta potential measurements) and magnetization measurements. The results confirm that aqueous suspensions of PEGylated SPIONs are stabilized by steric hindrance over a wide pH range between pH 4 and 10. Furthermore, the fact that the nanoparticle surface is nearly neutral is in agreement with immunological stealthiness expected for the future biomedical applications *in vivo*.

1. Introduction

Aqueous suspensions of iron oxide nanoparticles (commonly called ferrofluids) are being increasingly used in biosciences and medicine [1, 2]. Considerable efforts are currently devoted to the use of ferrofluids in *in vivo* biomedical applications including magnetic resonance imaging (MRI) contrast enhancement [3, 4], and cancer therapy by hyperthermia [5, 6] and/or by targeted drug delivery [7, 8].

For drug delivery, the magnetic properties of nanoparticles could be used to increase the antitumour efficacy and to reduce the systemic side-effects of anticancer drugs. In fact, with the application of an external localized magnetic field, drug-loaded magnetic nanoparticles could be retained in a tumour site and consequently release the drug in the tumour.

Numerous recent investigations indicate that magnetic nanoparticles composed of magnetite (Fe_3O_4) and maghemite

[4] Address for correspondence: Laboratoire de Chimie Analytique, UFR de Pharmacie, 31 avenue Monge, 37200 Tours, France.

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(γ-Fe_2O_3) are promising for drug targeting, mainly because they have rather high magnetic saturation [9] and negligible toxicity [10]. Superparamagnetic iron oxide nanoparticles (SPIONs) smaller than 10 nm are particularly interesting for these applications since their magnetization does not persist when the magnetic field is switched off.

In addition to the magnetic properties of the iron oxide core, the behaviour of the nanoparticles is dominated by the properties of their surface. For *in vivo* application two conditions are essential [11]. First of all, the suspensions have to be stable against agglomeration under physiological conditions. Generally, the stability of suspensions is favoured by the surface charge of the nanoparticles. However, for iron oxide nanoparticles an isoelectric point of about 7 is observed, which means that the particles aggregate and precipitate at physiological pH.

For administration of ferrofluids by intravenous injection, another requirement is immunological stealthiness, i.e. prevention of the nanoparticle elimination from the blood circulation by the immune system. Indeed, many studies have highlighted rapid nanoparticle capture and removal from the bloodstream by the mononuclear phagocyte system (MPS). Vonarbourg *et al* [12] have recently reviewed the physico-chemical parameters influencing the stealthiness of colloidal drug delivery systems. These authors have concluded that in order to have a prolonged circulation time in blood, particles in colloidal suspension should preferentially be small, composed of natural compounds and must present a neutral and hydrophilic surface.

These properties (stability and stealthiness) can be modulated by coating particles with polymers or dendrimers [13]. Long polymer chains on the particle surface can inhibit aggregation by steric hindrance. Several hydrophilic and biocompatible polymers, such as dextran [14] and poly(ethylene glycol) (PEG) [15], have been employed to modify the surface of the iron oxide nanoparticles. PEG seems to be one of the most appropriate ones and it has been shown to improve biocompatibility and blood circulation times [16–19].

Many studies have presented PEG coating of polymeric nanoparticles; however, only very few authors have reported stable covalent binding of PEG on the iron oxide surface [15, 20–22].

Here, we report a preparation of aqueous suspensions of PEGylated ferrofluids by functionalization of iron oxide nanoparticle surfaces with poly(ethylene glycol) molecules by an intermediate of silanes. The first part of this study is focused on the preparation of stable initial ferrofluids based on magnetite/maghemite nanoparticles and on the surface modification of those nanoparticles with silane groups. In the second part, we successfully anchored PEG on the silanized nanoparticles through a covalent bond. Their multistep synthesis and their characterization by FT-IR and Raman spectroscopy, transmission electron microscopy (TEM), magnetic measurements, photon correlation spectroscopy (PCS) and zeta potential measurements are discussed below.

2. Experimental details

2.1. Materials

Iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, 99%) and hydrochloric acid (37%) were purchased from Acros Organics (Noisy le Grand, France). Anhydrous iron (III) chloride ($FeCl_3$), ferric nitrate nonahydrate ($Fe(NO_3)_3{\cdot}9H_2O$) and nitric acid (65%) were furnished by Fisher Bioblock Scientific (Illkirch, France). 3-aminopropyltrimethoxysilane (APS) and methoxypoly(ethylene glycol) 5000 propionic acid *N*-succinimidyl ester (activated PEG, aPEG) were obtained from Sigma Aldrich (Saint-Quentin-Fallavier, France). All other reagents were of analytical grade. In all the experiments, water was previously deionized (18 MΩ). Dialysis tubing (cellulose, molecular weight cut off 8000 Da) was obtained from Interchim (Montluçon, France).

2.2. Initial ferrofluids preparation protocol

The initial ferrofluids were prepared according to a method described previously [14, 23]. Briefly, 5.12 g of $FeCl_3$ and 3.14 g of $FeCl_2{\cdot}4H_2O$ were dissolved respectively in 350 ml of water and 17 ml of hydrochloric acid (1.5 M). Black magnetite nanoparticles were precipitated by addition of 30 ml concentrated ammonia solution. After washing with water, the nanoparticles were treated with 20 ml of nitric acid (2 M) and then oxidized with 60 ml of a 0.33 M ferric nitrate nonahydrate solution at 100 °C. The brown nanoparticles were peptized with 20 ml nitric acid (2 M) by stirring for 15 min. After precipitation and washing three times with acetone, the ferrofluids were dispersed in 100 ml of water, and acetone traces were evaporated under vacuum at 60 °C. Finally, the total volume was brought to 100 ml by adding water.

2.3. Preparation of silanized ferrofluids

2.20 ml (12.4 mmol) of APS in 10 ml of methanol were added to a mixture of 20 ml (8.8 mmol of iron) ferrofluid and 10 ml of methanol. The mixture was stirred at room temperature for 12 h. To the resulting solution, 20 ml of glycerol was added and methanol then water were removed with a rotary evaporator. After evaporation, the solution was dehydrated in vacuum at 100 °C for 2 h. The treated nanoparticles were washed three times with 40 ml of water/acetone: 30/70. Following the addition of 40 ml of water, peptization was performed by slowly decreasing pH to 3 with nitric acid under vigorous stirring.

2.4. Preparation of PEGylated ferrofluids

A mixture of aPEG (600 mg, 0.12 mmol) and 3.0 ml (0.41 mmol of iron) of silanized ferrofluids in 15 ml of deionized water was stirred at room temperature for 24 h. The solution was then purified by dialysis against water; the dialysis procedure was repeated every 4 h for a total of 5 times.

2.5. Analytical methods

2.5.1. Transmission electron microscopy. The morphology of various particles was examined using an Philips CM20 electronic transmission microscope, operating at 200 kV. The samples were deposited on a carbon-coated copper TEM grid, the excess of solvent was then removed with filter paper, and the samples were left to air-dry before TEM viewing.

2.5.2. FT-IR spectrometry. Measurements were carried out on a Bruker Vector 22 FT-IR spectrometer (Bruker, Germany). Air-dried samples were mixed and compressed with KBr to obtain pellets for FT-IR analysis. Each spectrum presented is an average of at least three independent measurements.

2.5.3. Raman spectrometry. Raman spectra of the particles were measured with a LabRam confocal scanning microspectrometer (HORIBA Jobin Yvon, France) equipped with the 632.8 nm line of a helium–neon laser. To avoid sample photodegradation, the laser power under the microspectrometer objective (Olympus, 100×) was limited to 0.4 mW. Prior to the Raman measurements, a 20 μl drop of ferrofluids was deposited on a glass cover slide and dried at ambient temperature in the dark. Each spectrum presented is an average of at least three independent measurements.

2.5.4. Nanoparticle size determination. The mean hydrodynamic diameter of nanoparticles in suspension was determined by photon correlation spectroscopy (PCS) using a Malvern Autosizer 4700 (Malvern Instruments, Malvern, UK). Before measurement, the ferrofluids were diluted in deionized water to 0.15–0.2 g l^{-1}. Each measurement was done in triplicate.

2.5.5. Surface charge. The ferrofluids diluted as described above were characterized with respect to the zeta potential of nanoparticles by using a Malvern NanoZ (Malvern Instruments, Malvern, UK). The zeta potentials of different samples were determined as a function of pH ranging from 4.0 to 10.0. Each measurement was done in triplicate.

2.5.6. Iron concentration. The total iron concentration in the suspensions was determined by atomic absorption spectrophotometry (AAS) measurements at 248 nm (SpectrAA-10 plus, Varian, France). Prior to the AAS measurements, 1 ml of suspension or a weighed sample of freeze-dried nanoparticles were digested by 5 ml of hydrochloric acid (6 M). The digestion was maintained overnight and the resultant solution was diluted with hydrochloric acid (1%). A calibration curve was obtained by treating an acidic solution of $FeCl_3$ (2 g l^{-1}) in the same conditions.

2.5.7. Magnetization measurements. A Quantum Design physical property measurement system magnetometer was used to investigate the magnetic properties of coated magnetite nanoparticles. The magnetization (M, emu g^{-1}) of these different particles was measured as a function of the magnetic field (H, Oe) at 300 K.

3. Results and discussion

PEG-stabilized nanoparticles were synthesized in three stages: initial, silanized, and PEGylated ferrofluids. Throughout the whole process, the nanoparticles remain in suspension, avoiding the risk of agglomeration. Each step is followed by a characterization of the product including spectroscopy, magnetization, size, and surface charge measurements.

3.1. Initial ferrofluids

The initial aqueous suspensions of iron oxide nanoparticles were prepared according to a method previously presented by Mornet [14]. This method consists in nanoparticle formation by co-precipitation from iron II and iron III salts in alkaline medium. The nanoparticles resulting from this protocol are known to consist of magnetite (Fe_3O_4) oxidizable to maghemite (γ-Fe_2O_3), at least at superficial particle layers [24]. In order to prevent further uncontrollable oxidation, the nanoparticle surface can be intentionally oxidized from the very beginning. For this reason, we realized an oxidation step by successive treatment of the nanoparticle surface with nitric acid and ferric nitrate nonahydrate (see section 2).

We confirmed the chemical composition of the nanoparticles by FT-IR and Raman spectroscopy, both applied to air-dried samples. In the FT-IR spectrum of the nanoparticles (figure 1), maghemite was detected by two bands at about 590 and 630 cm^{-1}, the band at 590 cm^{-1} being increased due to the presence of magnetite [25]. The distinct vibrational bands of magnetite and maghemite in the Raman spectra at about 671 and 721 cm^{-1}, respectively (inset in figure 1), confirm the simultaneous presence of the two oxides according to the method we reported previously [26]. Moreover, the IR spectrum displays an intense band at around 1400 cm^{-1} which is attributed to nitrate ions [14]. According to our ferrofluid protocol, the resulting ferrofluids were obtained by peptizing nanoparticles into a cationic aqueous sol using nitric acid; thus nitrate ions were present at the nanoparticle surface. The colloidal stability of nanoparticles depends on the surface charge. In aqueous environment of neutral pH, the magnetite/maghemite nanoparticles flocculate, since their surface presents FeOH groups that have no charge. In contrast, their suspensions can be stabilized in an acidic or alkaline aqueous medium as cationic ($FeOH_2^+$) or anionic (FeO^-) sols, respectively. In both cases, the colloidal stability is provided by electrostatic repulsion [11]. The cationic ferrofluids obtained in our study were stable against flocculation for several months, in spite of rather high iron concentrations of 20.1 ± 1.9 g l^{-1} as determined by AAS (see the summary of nanoparticle characteristics in table 1). At this concentration, the ferrofluids appeared as a dark brown liquid that readily moved up and down following a stationary magnet, without forming any precipitate. At room temperature, the magnetization curves of these samples showed no hysteresis opening and were completely reversible (data not shown), thus indicating superparamagnetic properties. This behaviour has led to the term SPIONs used in the literature for superparamagnetic iron oxide nanoparticles. In our study, the saturation

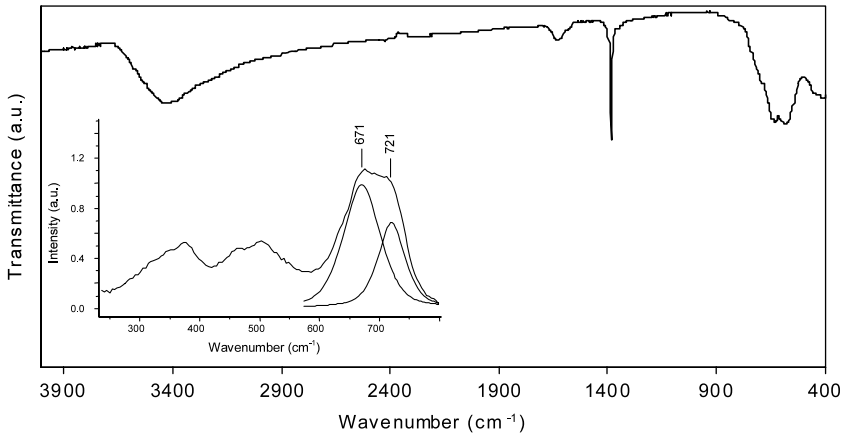

Figure 1. FT-IR and Raman (inset) spectra of the initial ferrofluids.

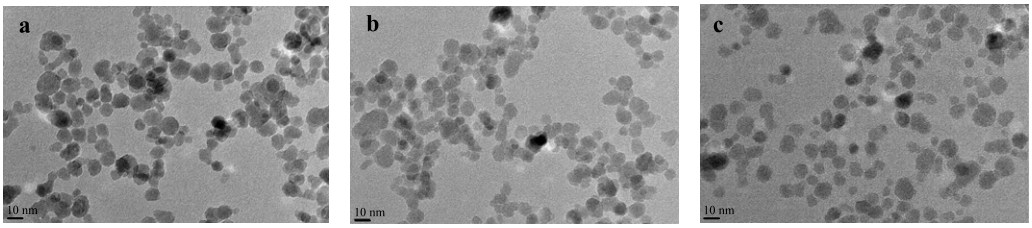

Figure 2. TEM images of initial (a), silanized (b), and PEGylated (c) ferrofluids.

Table 1. Physico-chemical properties of nanoparticles.

	Initial ferrofluids	Silanized ferrofluids	PEGylated ferrofluids
Iron concentration			
Suspension	20.1 ± 1.9^{a} g l^{-1}	7.7 ± 0.6^{a} g l^{-1}	0.16 and 0.17^{b} g l^{-1}
Dried sample	0.59 g/g	0.57 g/g	0.046 g/g
Z average mean (PCS)	56.3 ± 9.5^{a} nm	57.1 ± 8.1^{c} nm	82.4 ± 7.9^{d} nm
Particle size (TEM)	8.0 ± 1.9^{e} nm	7.5 ± 1.4^{e} nm	7.5 ± 1.4^{e} nm
Isoelectric point (IEP)	pH 7.0	pH 9.0	pH 4.8
Saturation magnetization			
per g of dried sample	57.4 emu g^{-1}	55.8 emu g^{-1}	4.5 emu g^{-1}
per g of iron	97 emu g^{-1}	98 emu g^{-1}	98 emu g^{-1}

Averages: number of batches synthesized. [a] $n = 6$, [b] $n = 2$, [c] $n = 12$, [d] $n = 4$, [e] $n = 3$, 20 particles from five images were evaluated for each batch.

magnetization σ_s for SPIONs was 57.4 emu g^{-1} of dried sample. Although this value is close to the values reported in the literature (56 emu g^{-1} in [27] for nanoparticles of unknown magnetite/maghemite ratio), the direct comparison with the literature data requires knowledge of the maghemite/magnetite content in those studies.

The stability of all the suspensions was also confirmed by PCS techniques in terms of mean hydrodynamic diameter and zeta potential. The cationic sols exhibited the Z-average mean diameter of 56.3 ± 9.5 nm, whereas the mean diameter of solid nanoparticles in TEM images was 8.0 ± 1.9 nm (table 1 and figure 2(a)). The hydrodynamic diameter did not increase within a period of several months, which indicated good colloidal stability. The electrostatic nature of the stability of the initial cationic ferrofluids was confirmed by pH-dependent changes in the zeta potential (figure 3) and by an isoelectric point (IEP) found at pH 7.0, which is concomitant with literature data [28, 29].

Although the pH can be used to maintain the colloidal stability of the initial ferrofluids, further biomedical applications of magnetic nanoparticles require pH-independent stability. This can be obtained if the particle surface is modified with neutral molecules like PEG to provide steric repulsion. To bind PEG to the SPION surfaces, we developed protocols using silane molecules as the binding intermediate.

3.2. Silanized ferrofluids

Silanized ferrofluids were prepared by bonding of trialkoxysilane molecules on the surface hydroxyl groups of the SPIONs (figure 4(a)). We used 3-aminopropyltrimethoxysilane with the amino terminal group available for further binding of polymer or drug molecules. The silane coating protocol includes

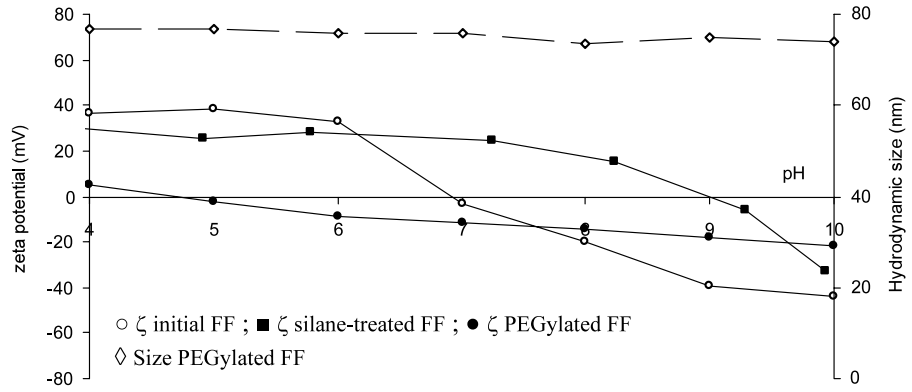

Figure 3. Zeta potential curves as a function of pH for ferrofluids (FFs) and variation of hydrodynamic size with pH for PEGylated ferrofluids.

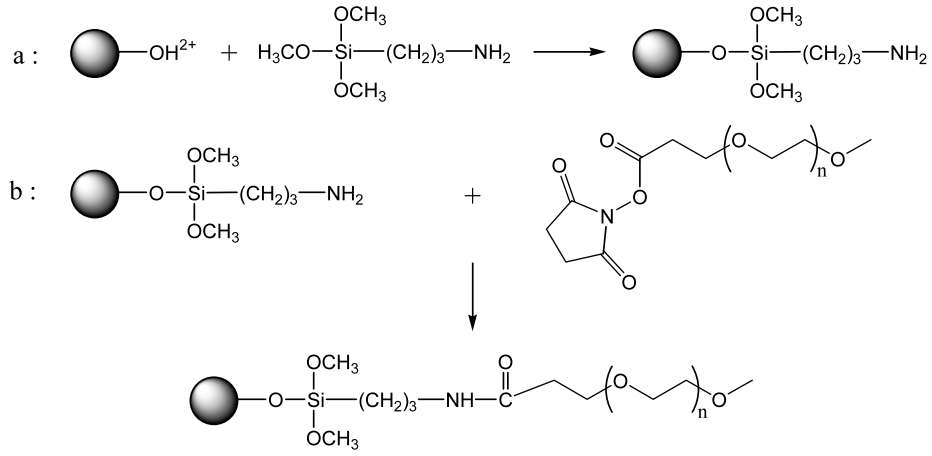

Figure 4. Synthesis of silanized (a) and PEGylated (b) ferrofluids.

a dehydration step under vacuum for the amino group of the silane to turn towards the exterior [30, 31]. The silane-coated nanoparticles were finally peptized with nitric acid. After triplicate washing with a water/acetone mixture, air-dried samples were analysed by FT-IR (figure 5). The presence of silanes was revealed by IR bands at 1199, 1434, 2868 and 2914 cm^{-1} assigned to Si–CH_2 and methylene groups, in agreement with the literature [14]. Furthermore, the polycondensation of silanes was confirmed by the presence of the strong IR absorption bands at 1040 and 1108 cm^{-1} assigned to siloxane Si–O–Si bonds [32].

Successful silanization was also confirmed by zeta potential measurements. After the APS coating, the IEP of ferrofluids changed from pH 7.0 to pH 9.0. This value is somewhat lower than the pK_a 10.4 of the aminopropyl group, possibly because in addition to amines, silanol groups might also be present on the SPION surfaces. In general, a colloidal suspension with electrostatic repulsion is considered stable when the zeta potential is more positive than +30 mV or more negative than −30 mV [33]. From this point of view, the zeta potential curves of silanized ferrofluids show that this colloidal suspension is stable at pH between 4.0 and 7.0, i.e. within a larger pH range than the initial ferrofluids.

As expected, the other characteristics of the silanized ferrofluids were quite similar to those of the initial ferrofluids (table 1 and figure 2(b)).

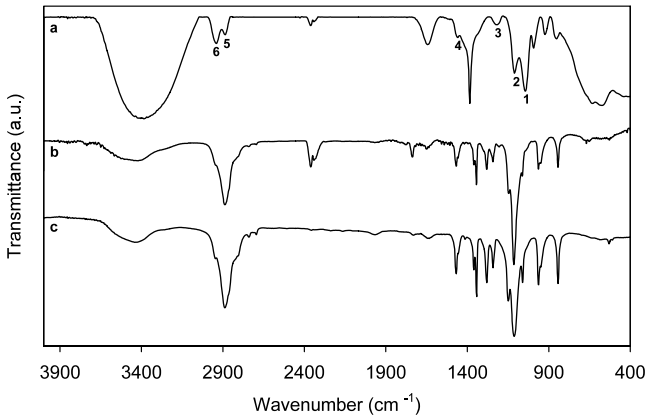

Figure 5. FT-IR spectra of silanized ferrofluids (a) (significant IR bands (1) 1040 cm^{-1}; (2) 1108 cm^{-1}; (3) 1199 cm^{-1}; (4) 1434 cm^{-1}; (5) 2868 cm^{-1}; (6) 2914 cm^{-1}), pure PEG (b) and PEGylated ferrofluids (c).

3.3. PEGylated ferrofluids

PEGylated ferrofluids were prepared by mixing silanized ferrofluids with methoxypoly(ethylene glycol) 5000 propionic acid *N*-succinimidyl ester. The binding reaction consists in the formation of an amide bond between the amino group of the silane and the activated ester group of aPEG (figure 4(b)).

The reaction was held at pH below 6.0 where the silanized nanoparticles were prevented from flocculation. The samples were used for characterization after eliminating excess PEG by multiple dialysis steps.

The FT-IR spectrum of an air-dried PEGylated ferrofluids sample (figure 5) shows the characteristic bands of the aPEG molecule. The FT-IR bands of the amide bond (C=O band at 1647 cm^{-1} and CNH band at 1561 cm^{-1}) were not observed in the spectra due to the low fraction of these bonds with respect to PEG–SPION samples. Unfortunately, the FT-IR bands of iron oxide were not detectable either, since the composite nanoparticles contain less than 10% of iron oxide. Nevertheless, the presence of the iron fraction was detectable by AAS, and the total iron content found for two batches of PEGylated ferrofluids was 0.16 and 0.17 g l^{-1}, respectively. The magnetization versus H (magnetic field strength) measurements at room temperature (figure 6) showed that the PEGylated samples still have superparamagnetic properties. The saturation magnetization for PEGylated SPIONs was equal to 4.5 emu g^{-1} (calculated per total solid mass). This value is related to the presence of the low mass fraction of iron oxide compared to that of the polymer. If we calculate the saturation magnetization according to the iron mass, all samples (initial, silanized and PEGylated) have the same magnetization equal to 98 emu g^{-1}. Finally, the magnetic sensitivity of PEGylated SPIONs also made them move toward a magnet.

The average hydrodynamic size of PEGylated nanoparticles was found to be 82.4 ± 7.9 nm (table 1). This value is higher than those of the initial and silanized SPIONs. The increase in hydrodynamic size of nanoparticles is consistent with PEG coating. For comparison, the nanoparticles PEGylated using covalent surface binding by Xie *et al* [21] exhibited a size distribution with a narrow maximum at 70 nm as measured by DLS. As expected, the polymeric coating layer was undetectable in the TEM images (figure 2(c)).

Finally, the most pertinent results confirming successful PEGylation of SPIONs were the data of zeta potential and hydrodynamic size measurements as a function of pH. The IEP value for PEG-coated nanoparticles was different from that of silanized particles. The isoelectric point for PEG was shifted to below pH 5, which is consistent with the fact that the majority of the amino groups were transformed into neutral amide groups by the PEG coating. The zeta potentials for PEGylated nanoparticles were very low (−11.1 mV at pH 7.0) and nearly insensitive to pH change. At higher pH, the zeta potential decreases to −20 mV, which can be explained by the presence of residual free silanol groups. These results are in close agreement with related parameters reported for PEGylated nanoparticles [34, 35].

Since aggregation of suspensions leads to increased particle diameters, the hydrodynamic diameter of PEGylated SPIONs was measured as a function of pH. It did not show any significant increase (figure 3), which clearly confirmed that the colloid remained stable over the whole pH range from 4.0 to 10.0. Moreover, the stability of our nanoparticle suspensions was confirmed at pH 7.4 in the presence of NaCl (0.9%), and no significant increase of hydrodynamic diameter was observed. Indeed, for most biomedical applications, the most important feature should be the colloidal stability of ferrofluids at physiological pH 7.4.

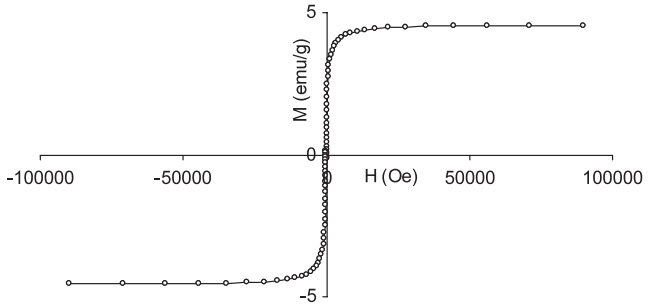

Figure 6. Magnetization M versus H plot at room temperature of PEGylated ferrofluids.

4. Summary

The original PEGylation protocol developed in this study provides aqueous suspensions of superparamagnetic iron oxide nanoparticles (SPIONs) stable within a wide range of pH (4.0–10.0). This stability and relative surface neutrality of these ferrofluids made them compatible with systemic administration *in vivo*. Further studies of drug incorporation and biological evaluation of resulting vectors are in progress in our laboratory. Finally, the developed nanosystem should be particularly useful for a wide spectrum of biomedical applications in which the SPIONs' magnetic properties are intended to improve the diagnostics and drug delivery.

Acknowledgments

We are grateful to Pr Etienne Duguet and to Dr Stephane Mornet (ICMCB, Université de Bordeaux 1) for fruitful collaboration and discussions.

This work was supported by Région Centre, France, and by Ligue Nationale contre le Cancer, Loir-et-Cher, France.

References

[1] Gupta A K and Gupta M 2005 *Biomaterials* **26** 3995
[2] Mornet S, Vasseur S, Grasset F, Veverka P, Goglio G, Demourgues A, Portier J, Pollert E and Duguet E 2006 *Prog. Solid State Chem.* **34** 237
[3] Wilhelm C, Lavialle F, Péchoux C, Tatischeff I and Gazeau F 2008 *Small* **4** 577
[4] Cheng F Y, Su C H, Yang Y S, Yeh C S, Tsai C Y, Wu C L, Wu M T and Shieh D B 2005 *Biomaterials* **26** 729
[5] Hergt R and Dutz S 2007 *J. Magn. Magn. Mater.* **311** 187
[6] Habib A H, Ondeck C L, Chaudhary P, Bockstaller M R and McHenry M E 2008 *J. Appl. Phys.* **103** 07A307
[7] Lübbe A S, Bergemann C and Riess H 1996 *Cancer Res.* **56** 4686
[8] Alexiou C, Arnold W, Klein R J, Parak F G, Hulin P, Bergemann C, Erhardt W, Wagenpfeil S and Lübbe A S 2000 *Cancer Res.* **60** 6641
[9] Bean C P and Livingston J D 1959 *J. Appl. Phys.* **30** 120S

[10] Neuberger T, Schöpf B, Hofmann H, Hofmann M and von Rechenberg B 2005 *J. Magn. Magn. Mater.* **293** 483
[11] Douziech-Eyrolles L, Marchais H, Hervé K, Munnier E, Soucé M, Linassier C, Dubois P and Chourpa I 2007 *Int. J. Nanomed.* **2** 541
[12] Vonarbourg A, Passirani C, Saulnier P and Benoit J P 2006 *Biomaterials* **27** 4356
[13] Boas U and Heegaard P M H 2004 *Chem. Soc. Rev.* **33** 43
[14] Mornet S, Portier J and Duguet E 2005 *J. Magn. Magn. Mater.* **293** 127
[15] Zhang Y, Kohler N and Zhang M 2002 *Biomaterials* **23** 1553
[16] Quaglia F, Ostacolo L, De Rosa G, La Rotonda M I, Ammendola M, Nese G, Maglio G, Palumbo R and Vauthier C 2006 *Int. J. Pharm.* **324** 56
[17] Mosqueira V C, Legrand P, Gulik A, Bourdon O, Gref R, Labarre D and Barratt G 2001 *Biomaterials* **22** 2967
[18] Gbadamosi J K, Hunter A C and Moghimi S M 2002 *FEBS Lett.* **532** 338
[19] Gref R, Domb A, Quellec P, Blunk T, Muller R H, Verbavatz J M and Langer R 1995 *Adv. Drug Deliv. Rev.* **16** 215
[20] Butterworth M D, Illum L and Davis S S 2001 *Colloids Surf.* A **179** 93
[21] Xie J, Xu C, Kohler N, Hou Y and Sun S 2007 *Adv. Mater.* **19** 3163
[22] Mondini S, Cenedese S, Marinoni G, Molteni G, Santo N, Bianchi C L and Ponti A 2008 *J. Colloid Interface Sci.* **322** 173
[23] Massart R 1981 *IEEE Trans. Magn.* **17** 1247
[24] Tang J, Myers M, Bosnick K A and Brus L E 2003 *J. Phys. Chem.* B **107** 7501
[25] Tartaj P, Morales M P, Veintemillas-Verdaguer S, Gonzales-Carreno T and Serna J C 2003 *J. Phys. D: Appl. Phys.* **36** R182
[26] Chourpa I, Douziech-Eyrolles L, Ngaboni-Okassa L, Fouquenet J F, Cohen-Jonathan S, Soucé M, Marchais H and Dubois P 2005 *Analyst* **130** 1395
[27] Mohapatra S, Pramanik N, Mukherjee S, Ghosh S K and Pramanik P 2007 *J. Mater. Sci.* **42** 7566
[28] Vayssières L, Chanéac C, Tronc E and Jolivet J P 1998 *J. Colloid Interface Sci.* **205** 205
[29] Fauconnier N, Bée A, Roger J and Pons J N 1999 *J. Mol. Liq.* **83** 233
[30] Vrancken K C, Van Der Voort P, Possemiers K, Grobet P and Vansant E F 1994 *Chemically Modified Surfaces* ed J J Pesek and I E Leigh (Cambridge: Royal Society of Chemistry) p 46
[31] Lesniak C, Schiestel T, Nass R and Schmidt H 1997 *Mater. Res. Soc. Symp. Proc.* **432** 169
[32] Flesch C, Joubert M, Bourgeat-Lami E, Mornet S, Duguet E, Delaite C and Dumas P 2005 *Colloids Surf.* A **262** 150
[33] Vyas T K, Babbar A K, Sharma R K, Singh S and Misra A 2006 *J. Pharm. Sci.* **95** 570
[34] Kumagai M *et al* 2007 *Colloids Surf.* B **56** 174
[35] Miyamoto D, Oishi M, Kojima K, Yoshimoto K and Nagasaki Y 2008 *Langmuir* **24** 5010