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ORIGINAL CONTRIBUTION

Elaboration of hydrophilic aminodextran containing submicron magnetic latex particles

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Abstract Aminodextran containing submicron magnetic latex particles were prepared in two steps: (a) transformation of oil-in-water magnetic emulsion into structured magnetic latex particles via combination of seed and miniemulsion-like polymerization process and (b) immobilization (adsorption and chemical grafting) of prepared aminodextran onto negatively charged seed magnetic latex particles. The elaborated magnetic latex particles were characterized in terms of particle size, size distribution, morphology, surface charge density, chemical composition, magnetic properties, and also colloidal stability. The results showed that the morphology of the prepared seed magnetic latex is core–shell like and the cationic latex particles are hydrophilic and of high colloidal stability, irrespective of the aminodextran immobilization process.

Keywords Magnetic particles · Aminodextran · Adsorption · Hydrophilic particles · Core–shell

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Introduction

In recent years, magnetic particles have been studied due to their potential applications as magnetic carriers in biomedical area such as in medicine and diagnostics. Indeed, magnetic particles offered many advantages: reduction of heavy steps (i.e., centrifugation, filtration, etc…), time-consuming, concentration step, and easy to use in automated systems [1–4].

Among their numerous applications, biomedical diagnosis requires the development of magnetic submicron particles with narrow size distribution, high content of functional groups, good colloidal stability, and biocompatibility.

To reach these criteria, many approaches and roots have been investigated. The first approach is based on the precipitation of iron oxide submicron particles in the presence of water-soluble polymer such as dextran for instance [5–7]. The second approach consists in the polymerization of suitable monomers mixture in the presence of iron oxide particles stabilized by an appropriate surfactant. This polymerization has been achieved using various processes such as suspension, emulsion, and dispersion [8, 9]. Miniemulsion [10, 11] and inverse microemulsion [12] polymerization have been also investigated as reported by various authors. Finally, the processes based on chemical modification of magnetic particles in order to induce surface modification and surface functionalization [13] has been reported but not systematically used. It is interesting to notice that various works have been dedicated to stimuli-responsive magnetic particles as recently reported Suzuki and Kawaguchi [14] and thermally sensitive magnetic latexes as widely reported by various authors [15, 16].

The main objective of molecular diagnosis consists in the extraction, purification, concentration, and amplification of nucleic acids using magnetic beads or magnetic latex particles [17, 18], although various magnetic latex particles have been prepared in order (a) to obtain a sufficient surface area with a high content of magnetic material, (b) to introduce an appropriate surface functionality, and (c) to enhance the compatibility between the used enzymes and the particles via a hydrophilic polymer shell [19].

To fulfill these three criteria, some previous works have been done using radical polymerization process of styrene-based monomers in the presence of oil-in-water magnetic droplets [20]. The obtained magnetic latexes are submicron (between 200 and 300 nm), bearing carboxylic function with high magnetic content. However, these particles were not hydrophilic and cationic in order to be use in molecular diagnosis for nucleic acids adsorption and enzymatic amplification.

In order to reach such a specific application in molecular diagnosis, the aim of this work is to control the chemical composition of magnetic latex particles via oil-in-water magnetic emulsion polymerization, the surface functionality (suitable cationic character for electrostatic interaction enhancement), and no release of any potential inhibitor of the amplification step of captured nucleic acids. Then, this study reports on the elaboration of seed core–shell-like magnetic latex particles, the surface functionalization via adsorption and chemical grafting of the prepared aminodextran (AMD) and finally, the evaluation of the final magnetic latex particles in generic capture and amplification of nucleic acids.

Materials and methods

Materials

The magnetic emulsions were from Ademtech (France) and used as received. The amphiphilic polymer was kindly provided by Coatex (Mw=50,000 g/mol, Coatex, France) is a polyacrylic acid containing hydrophobic chains and was used as received. The divinylbenzene (DVB) and the potassium persulfate (KPS) were from Sigma-Aldrich and used as received.

Poly(maleic anhydride-alt-methyl vinyl ether (PMAMVE), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC), hydrochloric acid, potassium hydroxide, acetic acid, sodium acetate, and Triton X-100 are purchased from Merck and DextranT40 is purchased from Amersham Biosciences. Water is of Milli-Q grade (Millipore SA).

Methods

Magnetic emulsion preparation and compatibilization

The magnetic emulsions are prepared according to a process described by Mason and Bibette [21]. An organic ferrofluid (made of small 10 nm Fe_2O_3 nanoparticles coated with oleic acids and dispersed in octane) is emulsified in an aqueous solution of a nonionic surfactant [p-(1,1,3,3-tetramethylbutyl)phenoxypoly(ethylene glycol)] with an average of 40 oxyethylene units (Triton X-405) and sheared in a Couette emulsifier. Such precise controls of shear leads to submicron oil-in-water magnetic emulsion droplets with a rather narrow size distribution (D_h=190 nm) bearing 77% wt/wt iron oxide content [22, 23].

Preparation seed magnetic latex particles

The oil-in-water magnetic emulsion was first stabilized with the amphiphilic polymer via three washes with a fresh solution of this amphiphilic polymer at 0.5 g/L and pH 9. The last wash was carried out with deionized boiled water under nitrogen. Two grams of the stabilized oil-in-water emulsion was then induced in a 50-mL glass reactor. The dispersion was placed under nitrogen stream for a few minutes under stirring. Nine hundred milligrams of DVB was then added and the stirring was maintained for 1 h before adding the potassium persulfate initiator (18 mg) dissolved in 1 mL of water. The polymerization reaction was performed in batch conditions, at 70 °C for 20 h under mechanical stirring. Polymerization conversion was found to be generally above 70% as has been reported for styrene and DVB mixture in a previous paper [20].

Preparation of aminodextran polymer

DextranT40 (50 g, 0.308 mol) was dissolved in 250 mL of distilled water; 26.4 g (0.123 mol) of $NaIO_4$ was added to the dextran solution in order to obtain oxidized dextran solution; and 31.5 g (0.271 mol) of 1.6-hexanethylenediamine was then added. Once a homogeneous Orange solution was obtained, 18.6 g (0.493 mol) of sodium borohydride in 200 mL of 1 mM aqueous potassium hydroxide was added to the reaction mixture. The solution becomes yellow-orange. Aminodextran solution was freeze-dried over 48 h to produce 16 g (32% yield) of flaky, pale yellow crystals. Elemental analysis of the final aminodextran shows the presence of nitrogen from amine groups: C=48.75%, H=8.26%, N=6.25%, and O=36.77%. A complete study has been reported in a previous paper [24].

Cationic and hydrophilic magnetic polymer particles preparation

Adsorption of aminodextran Two milliliters of 1 wt.% seed magnetic latex particles was washed three times with Triton X-100 (1 g/L) and then 1.9 mL of buffer acetate buffer (at a given concentration and pH) was added. Then to 100 μL of aminodextran solution (ranging from 5 to 120 mg/mL) was added 1.9 mL of carboxylic magnetic latex particles. The adsorption was performed during one night under stirring condition. The magnetic particles were finally washed three times with acetate buffer.

Chemical grafting of aminodextran Two milliliters of 1 wt.% seed magnetic latex particles was washed three times with Triton X-100 (1 g/L) and then 1.9 mL of acetate buffer was added. To this suspension, 100 μL of EDAC was added and the mixture was stirred at room temperature for 40 min. The suspension was washed three times with acetate buffer. Then to 100 μL of aminodextran (concentration ranging from 5 to 120 mg/mL) was added 1.9 mL of activated carboxylic magnetic particles. After one night incubation under stirring, the magnetic particles were washed three times using acetate buffer.

Characterization of magnetic latex particles

Transmission electron microscopy Magnetic latex particles are observed under a Phillips CM120 transmission electron microscope (CMEABG, Villeurbanne) at 80 kV under vacuum. In all cases, a drop of the highly dilute sample was deposited and dried on a copper grid covered with a Formvar–carbon membrane.

Dynamic light scattering The hydrodynamic particles size (D_h) of the colloidal dispersions is determined by dynamic light scattering on a Sizer 4700 λ=633 nm from Malvern Instrument at room temperature and in 10^{-3} M NaCl concentration. The mean hydrodynamic diameter is calculated by using the Stokes–Einstein's equation:

$$D_\mathrm{h} = \frac{kT}{3\pi\eta D} \qquad (1)$$

where, k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the medium, and D is the diffusion coefficient.

Electrophoretic mobility measurements Electrophoretic mobility (μ_e) measurements of the dispersions are carried out on a Zetasizer 3000HS (from Malvern Instrument) as a function of pH at 25 °C in 10^{-3} Mol/L NaCl solution. Each reported value is the average of five measurements. Electrophoretic mobility is converted into zeta potential by using Smoluchowski's equation [25].

$$\mu_\mathrm{e} = \frac{\varepsilon}{4\pi\eta}\varsigma \qquad (2)$$

where ε is the dielectric constant, η is the viscosity of the medium, and ζ is the zeta potential.

Elemental analysis The chemical composition of magnetic particles is determined by elemental analysis (CNRS, Solaize). The amount of both ferric and ferrous is measured on dried samples to determine iron oxide content in magnetic latex particles.

Magnetization measurements Magnetization measurements of magnetic submicron particles are performed at room temperature using an Automatic Bench of Magnetic Measurements (CNRS-IRC, Villeurbanne). Magnetization of magnetic submicron particles is measured using the Weiss extraction method [26] by decreasing the magnetic field from 21 to 0 kOe. Measurements are performed on dried magnetic dispersion. For magnetic particles dispersion, the diamagnetic value of water was systematically subtracted. Specific saturation magnetization (M_s in emu/g) of a given sample is calculated by using the following relation:

$$M_\mathrm{s} = \frac{4,300 \times \delta_\mathrm{s}}{m} \qquad (3)$$

where 4,300 is the apparatus constant, m is the mass of the sample (in g), and δ_s is the experimental electric signal value (arbitrary unit) at the saturation magnetization. Iron oxide content (IOC) is determined from specific saturation magnetization of the magnetic particles.

Thermogravimetric analysis Thermogravimetry analysis (TGA) was performed on dried magnetic dispersion using a thermogravimetry analyzer apparatus from AT instruments at a heating rate of 10 °C/min under nitrogen atmosphere (LMPB-Villeurbanne).

Conductimetric titration To determine the surface charge density on the particles surface, a conductimetric back titration was performed on 25 mL of diluted magnetic latex particles with a volume fraction of 0.2% at room temperature. An excess amount of 1 M HCl solution was firstly added into the latex. Back titration was started when 0.01 M NaOH was automatically dropped into the latex (Titronic Universal; SCHOTT) under gentle stirring. The change in conductivity value of the medium was measured by combined system conductimeter and pH meter 4330 (JENWAY) equipped with glass conductivity cell (K=1).

The number of microequivalents of amino groups per gram of magnetic particles was given as follows:

$$\mathrm{NH}_3^+ = \frac{[\mathrm{NaOH}] \times V_{[\mathrm{NaOH}]}}{\mathrm{ms}} \tag{4}$$

where [NaOH] and $V_{[\mathrm{NaOH}]}$ are the concentration and the volume of NaOH (in mL) at equivalent point respectively and (ms) is the mass of sample (in g).

Chemical titration of amine groups Amine function titration was also performed via colorimetric titration process using Orange II ($C_{16}H_{12}N_2O_4S$). The titration was carried out by mixing 100 μL of magnetic latex particles (0.5 mg/mL) with 100 μL of an Orange II solution (2.10^{-4} mol L^{-1}) during 15 min at room temperature. Then after magnetic separation, 100 μL supernatant was taken out for optical density measurement at 484 nm. The amount of amine function was then determined from the standard curve.

Colloidal stability The colloidal stability of the functionalized magnetic latex particles was examined as a function salinity concentration at constant pH and temperature. The experiments were performed by measuring the aggregation rate (N_τ) via the measurement of turbidity as a function of wavelength.

$$N_\tau = -\left|\frac{\mathrm{d}\log\tau}{\mathrm{d}\log\lambda}\right| \tag{5}$$

where τ is the turbidity of sample and λ is the wavelength. A given amount of particles was added into various NaCl solutions (10^{-5} to 3 M) to obtain 0.005% wt/wt. The mixture was stored at 20 °C for 1 h before measuring the turbidity variation versus the wavelength ranging from 400 to 700 nm using a UV–Vis spectrophotometer (Monaco; SAFAS; UVmc^2).

Results and discussion

Preparation and characterization of seed magnetic latex particles

Cationic and hydrophilic magnetic latex particles were prepared from seed magnetic latex particles. These seed latex particles were prepared from submicron oil-in-water magnetic emulsion. Indeed, a new method involving the transformation of submicron oil-in-water ferrofluid emulsions into magnetic latexes has been established in order to obtain functionalized latex particles with a regular core–shell morphology structure. In this aim, various parameters such as the nature of the initiator, the use of a crosslinker, and the influence of a surface-active agent on the morphology of the latex have been studied. First, studies of emulsion polymerization in the presence of St/DVB/AIBN with 40 wt.% crosslinking agent showed that the use of DVB in the polymerization of the styrene/AIBN system allowed to rapidly crosslink the PS phase in the particles, preventing significantly the phase separation. Nevertheless, even with a large amount of DVB, iron oxide was still not properly encapsulated by the polystyrene layer. Then a water-soluble initiator KPS has been used instead of AIBN. The results showed that the combination of the effects of an ionic initiator and an oil-soluble crosslinking agent permitted to obtain particles with a core–shell-like morphology. Emulsion polymerization in the presence of St/DVB/KPS systems reduced the secondary nucleation, and consequently, the incorporation of sulfate groups onto the surface of magnetic particles was enhanced allowing a better compatibility of the polymer with the water phase and then allowing the polymeric phase to remain outside the particles. Nevertheless, the surface charge density could be affected by the formation of a polystyrene phase inside the ferrofluid droplets inducing the burial of the carboxylic charges (originated from oleic acid molecules) into the crosslinked PS matrix. To improve the latex particle functionalization, the use of a functional amphiphilic polymer adsorbed on the surface of the magnetic emulsion before polymerization was selected. TEM analyses demonstrated that the use of this polymeric surfactant to stabilize the oil-in-water magnetic droplets played a role on the interfacial tensions and therefore contributed to the formation of homogeneous core–shell particles. The increase of the amount of the crosslinking agent until 100% favored also the homogeneity of the polymer shell around the iron oxide core. The morphology of the seed magnetic latex prepared using 100% DVB is compared with the morphology of the magnetic emulsion reported in Fig. 1.

Immobilization of aminodextran onto seed magnetic latex particles

Cationic and hydrophilic magnetic submicron latex particles were prepared from seed magnetic latex particles. To obtain a homogeneous layer of cationic and hydrophilic polymer on such negatively charged magnetic latexes, two different processes were used: (a) adsorption of aminodextran on seed magnetic latex particles and (b) chemical grafting of aminodextran onto activated carboxylic containing magnetic latex particles.

As a systematic study, the influence of aminodextran/seed particles ratio from 25 to 600 mg/g on the colloidal properties of the magnetic particles has been examined. The adsorption of such cationic polyelectrolyte onto oppositely charged particles is mainly governed by attractive electrostatic interactions as below discussed. As a general

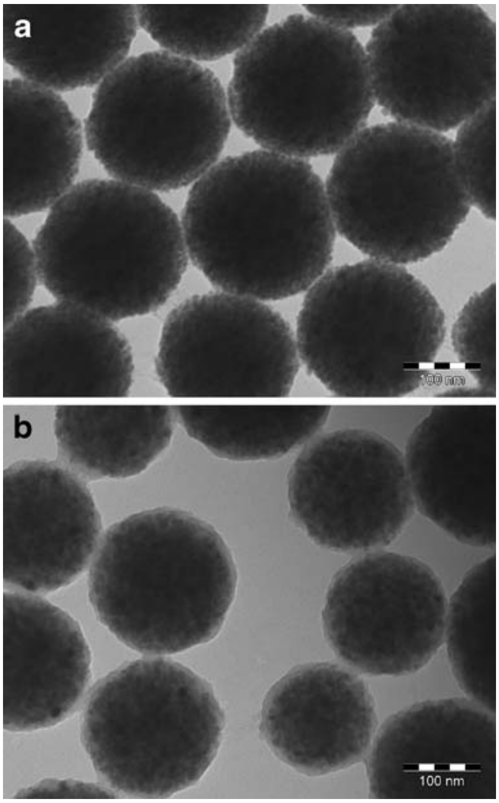

Fig. 1 TEM images of **a** magnetic emulsion and **b** seed magnetic particles 100% DVB (Cx38)

tendency, a slight aggregation phenomenon was observed when the adsorption was performed below 120 mg/g (AMD/particles) ratio. It is interesting to notice that the aggregates observed are after the washing step. This can be attributed to poor surface functionalization and saturation due to the low amount of used aminodextran. In addition, for such low aminodextran amount, the bridging flocculation phenomenon cannot be avoided.

Physico-chemical properties of the cationic magnetic latex particles

Size and size distribution determination

The hydrodynamic diameter of the aminodextran containing magnetic latex particles was examined as a function of pH, salinity, and initial aminodextran amount.

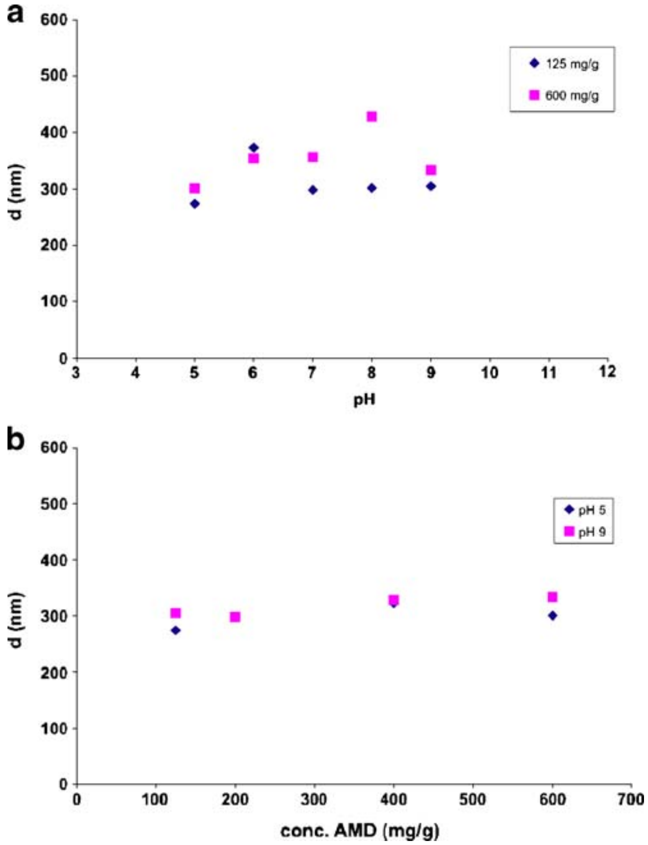

Fig. 2 The effect of pH (**a**) and the influence of aminodextran (**b**) on the hydrodynamic particles size

First, the hydrodynamic size of aminodextran magnetic latex particles was investigated as a function of the adsorption pH and for two aminodextran amounts (125 and 600 mg/g) as reported in Fig. 2a. The particle size was found to be almost constant irrespective of the pH of the adsorption medium. The average hydrodynamic particle size was in between 300 to 400 nm. The same tendency was observed when the hydrodynamic size was examined as a function of initial aminodextran concentration at pH 5 and pH 9 (Fig. 2b). Interestingly, below 120 mg/g of aminodextran amount, the magnetic latex particles aggregate irrespective of pH.

These results showed that the pH of acetate buffer has no significant influence on the size of the final magnetic latex particles whatever the initial aminodextran amount above 125 mg/g. As no significant difference was observed between 125, 200, 400, and 600 mg/g, special attention was focused on the study of the particles obtained with the minimum and the maximum initial aminodextran amount per gram of seed magnetic latex particles: 125 and 600 mg/g. In addition, the influence of salinity of the acetate buffer used for the adsorption process was studied. Acetate buffer pH 5 and pH 9 with different salinities (2, 5, 10, 30, 60, and 100 mM) was used in order to point out the effect of salinity.

The hydrodynamic particle sizes determined from washed suspensions via magnetic separation redispersion cycles in the corresponding acetate buffer and the obtained value are reported in Fig. 3. This could explain the aggregation formation with the increase of salinity due to the charge screening on the particles surface. We could notice that the closest results were obtained for 2 M acetate buffer whatever the pH (pH 5 or pH 9) and the aminodextran amount (125 or 600 mg/g).

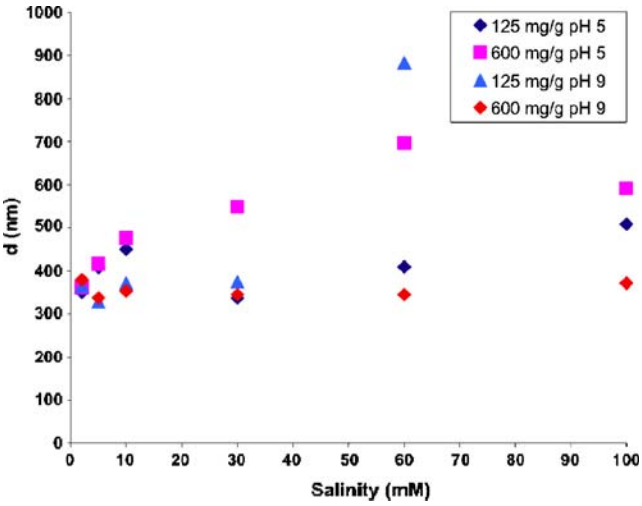

Fig. 3 Effect of salinity on the hydrodynamic particles size at pH 5 and pH 9 performed using 125 and 600 mg aminodextran/g of seed negatively charged magnetic latex particles

As no significant influence of pH have been highlighted, an example of size and size distribution of amino magnetic particles (125 and 600 mg/g) obtained by adsorption (ad) and by chemical binding reaction (cr) in 2 mM acetate buffer pH 6 compared to the size of the seed magnetic latex (Cx38) is presented Fig. 4.

The average particle size determined for each sample showed a significant increase of the hydrodynamic diameter after surface modification, from 267 nm for seed magnetic latex particles (Cx38) to 323 and 325 nm for 600 mg/g (ad) and 600 mg/g (cr), respectively. A slight increase of the diameter from 289 to 323 nm and from 311 to and 325 nm was observed for the two ratios 125 and 600 mg/g (ad & cr), respectively. The slight increase in width of size distribution can be attributed to the formation of some aggregates.

Zeta potential study

The zeta potential of the prepared cationic latexes was investigated in order to point out the real surface modification from negatively charged magnetic latex seed to cationic magnetic one. The zeta potentials of cationic latexes prepared at different pH (acetate buffer of (pH 5, 6, 7, 8, and 9) and for various aminodextran concentrations (from 125 to 600 mg/g) are reported in Figs. 5 and 6, respectively.

These results showed that the pH of acetate buffer used during the adsorption of aminodextran has no significant influence on the zeta potential of the final magnetic latex particles whatever the initial aminodextran amount above 125 mg/g of particles. As no significant difference was observed for 125, 200, 400, and 600 mg/g, special attention was focused on the study of the particles obtained using the minimum and the maximum initial aminodextran amounts (i.e., 125 and 600 mg/g). The influence of salinity (2, 5, 10, 30, 60, and 100 mM) of the acetate buffer (at pH 5 and pH 9) used for the aminodextran adsorption (for the 125 and 600 mg/g ratio) was studied and the results obtained are reported in Fig. 7.

The obtained results show that the salinity and the pH of the acetate buffer used during the adsorption of aminodextran has no effect on the zeta potential of the obtained cationic magnetic latex particles. This constancy of the zeta potential can be attributed to (a) the surface saturation of the seed magnetic latex particles by the adsorbed aminodextran irrespective of pH, salinity, and used initial amount of aminodextran; and (b) to the marginal effect of salinity and pH on the adsorption of such highly charged polyelectrolyte onto highly charged particles.

As no significant influence of pH and salinity has been highlighted, the zeta potential of cationic latexes prepared via adsorption and chemical grafting using 125 and

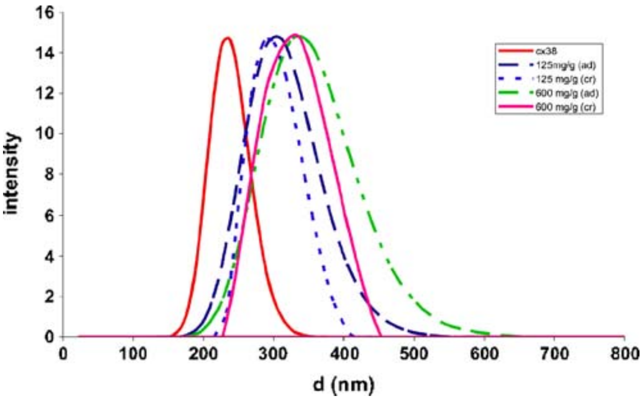

Fig. 4 Hydrodynamic particle size and size distribution of the magnetic latex particles. The cationic particles are prepared in 2 mM acetate buffer, pH 6

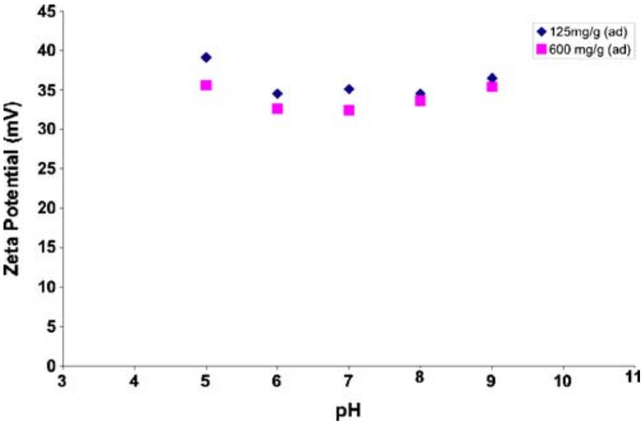

Fig. 5 Zeta potential of cationic magnetic latexes prepared at various pH

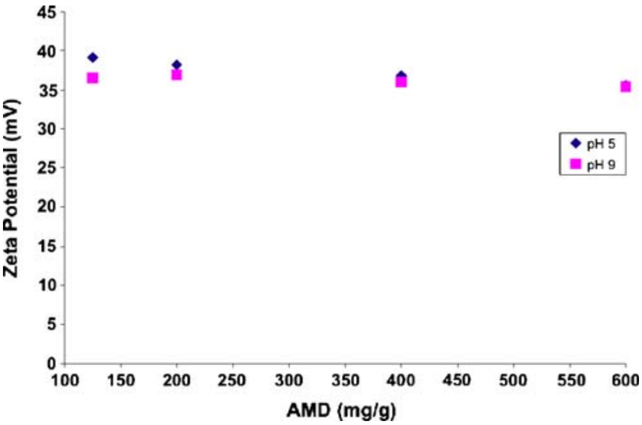

Fig. 6 Zeta potential of cationic magnetic latexes prepared using various aminodextran/particles ration (in mg/g)

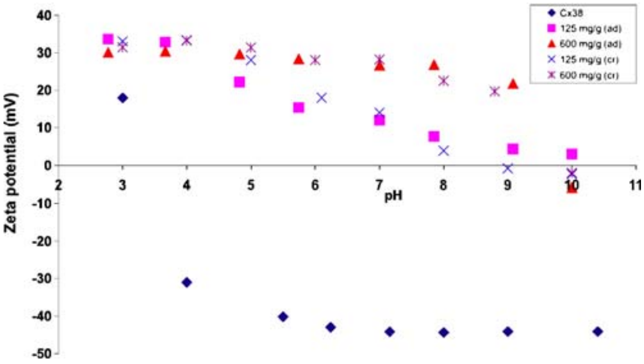

Fig. 8 Zeta potential versus pH of the used seed magnetic latex particles (Cx38) and the obtained cationic latexes via adsorption (*ad*) and via chemical grafting (*cr*) (25 and 120 correspond to 125 and 600 mg/g, respectively)

600 mg/g in a buffer acetate pH 6, 2 mM, was measured as a function of pH. The aim of the investigated study is to point out the cationic character of the final aminodextran containing latexes compared to the used negatively charged seed magnetic latex (Cx38), and the obtained results are reported in Fig. 8.

For the seed magnetic latex particles (Cx38), a decrease of zeta potential with the increase of pH from pH 4 was observed. Above pH 6, the zeta potential (−40 mV) is negatively constant. This is characteristic of a weak acid behavior, which confirms that in addition to sulfate groups from the used initiator (KPS), the carboxylic groups are also present on the particles surface. The surface carboxylic groups originated from the amphiphilic polymer used to ensure the colloidal stability of the oil-in-water magnetic emulsion.

Compared to negatively charged seed latex particles, aminodextran containing magnetic particles are positive in pH domain ranging from pH 3 to pH 9. As a general tendency, the isoelectric point (in between pH 9 and 10) of the cationic magnetic latexes is close to the p*K*a of the aminodextran. A slight difference between particles prepared in 125 and 600 mg/g of aminodextran conditions was observed. Indeed, in the case of the 600-mg/g immobilization condition ratio, the aminodextran particles either obtained via adsorption or via chemical grafting showed a high positive zeta potential value in between 20 and 30 mV for pH ranging from 3 to 9. Whereas, the zeta potential decreased regularly from 30 to 0 mV with increasing pH from 3 to 9 for both cationic latexes prepared via adsorption or via chemical grafting when 125 mg/g ratio was used. These results confirmed the influence of the initial aminodextran concentration on the cationic surface character of magnetic latex particles. This can be attributed to the possible changes in the conformation of the adsorbed polymer and also to the slight variation in the adsorbed amount.

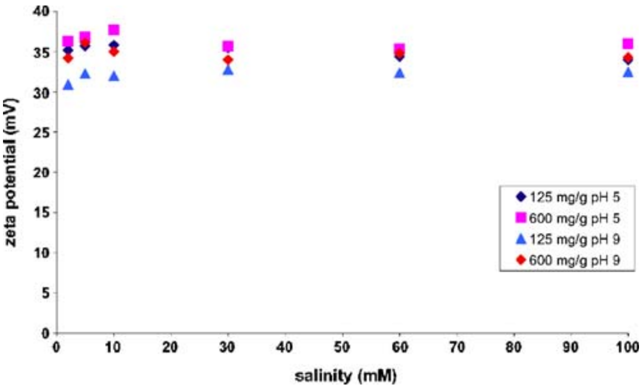

Fig. 7 Zeta potential of cationic magnetic latex particles prepared using 125 and 600 mg of aminodextran/g of particles and using acetate buffer at pH 5 and pH 9 at a given salinity

Chemical composition of the magnetic submicron particles

Determination of iron oxide content

The chemical composition of the prepared latexes was determined using an Automatic Bench of magnetic measurements, and the amount of magnetic material (i.e., IOC) was deduced using the following equation:

$$\mathrm{IOC}(\%) = \frac{\mathrm{mmm} \times 0100}{\mathrm{sm}} \tag{6}$$

with mmm is the magnetic material mass (in g) and sm is the sample mass (in g)with

$$\mathrm{mmm} = \frac{4,300 \times M_\mathrm{s}}{\delta_\mathrm{s}} \tag{7}$$

M_s corresponds to the saturation magnetization for the magnetic particles (u.a.) and δ_s is the specific magnetization of the analyzed material (for Fe_2O_3, δ_s=79.6 emu/g) [27]. The value of maghemite Fe_2O_3, which is the most stable structure of iron oxide, was used in this study for M_s measurements.

Table 1 Iron oxide content (IOC; wt.%) of magnetic submicronic particles

Sample	IOC (wt.%) (elemental analysis)	IOC (wt.%) (magnetization)	IOC (wt.%) (TGA)
Es211	73.5	77	67.2
Cx38	57	46.7	54.2
125 mg/g (cr)	57.5	42	51.5
125 mg/g (ad)	55.26	30	44.7
600 mg/g (cr)	57.8	49.3	58.5
600 mg/g (ad)	57.8	50	41.7

Values 125 and 600 represent the AMD/particles amount (in mg/g); cr and ad correspond to the elaboration process

To determine the inorganic material content (i.e. total iron oxide), TGA was used. The dried magnetic latex particles sample was heated from ambient temperature to 900 °C in order to burn organic material. As iron oxide is an inorganic material, the percentage of the remaining product after calcination corresponds to iron oxide content. The results obtained by TGA confirmed those obtained by magnetic property measurements even if these results showed a higher iron oxide content and the values obtained are reported in Table 1. Indeed, iron oxide content determined via magnetic measurements takes into account only the magnetic material part and not the total iron oxide. Consequently, iron oxide content is underestimated, whereas thermogravimetric values took into account all the inorganic species present in the sample such as iron oxide material, inorganic salts, and impurities. The obtained data are compared to values obtained from elementary analysis. The calculations of IOC from elementary analysis data were performed by considering that iron (Fe) is mainly from iron oxide Fe_2O_3 structure.

Determination of aminodextran content

Thermogravimetric analysis was also used to determine the amount of aminodextran in the magnetic latex particles. Indeed, the use of the spectrum of the magnetic seed particles before aminodextran adsorption and the spectrum of the amino magnetic particles after aminodextran adsorption are reported in Fig. 9, and the amount of immobilized aminodextran in the cationic magnetic particles can then be determined from the difference between the two data. As no difference has been observed concerning the zeta potential between the cationic magnetic particles prepared by adsorption (ad) and by chemical grafting (cr), the study has been focused on the particles obtained by adsorption only. After careful data analysis, the following results are obtained: 59.2 mg of AMD per gram of dried magnetic particles for initial AMD/particles ratio of 125 mg/g and 89.1 mg of AMD per gram of magnetic particles for initial AMD/particles ratio of 600 mg/g.

The amount of amine groups on the surface of the magnetic particles was determined by conductimetric titration [28, 29]. Figure 10 shows the conductimetric titration of amino groups on the surface of the magnetic latex particles. The amine surface concentration was calculated from the titration curve. The amount of amine equivalents function per gram of dried magnetic latex particles is found to be 0.270 meq/g for amino particles with initial AMD/particles ratio of 125 mg/g and 0.207 meq/g for amino particles for initial AMD/particles ratio of 600 mg/g.

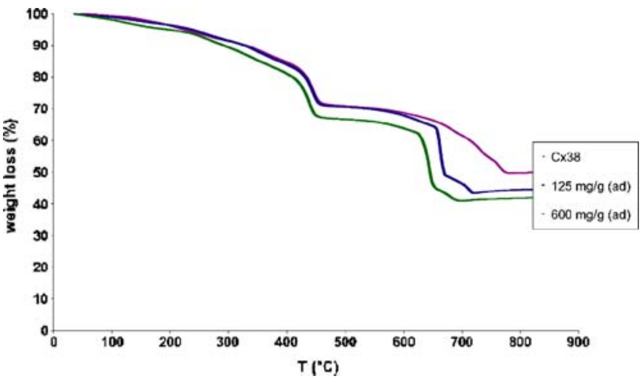

Fig. 9 TGA curves of seed magnetic latex particles (*Cx38*) and aminodextran containing magnetic latex particles prepared via adsorption (*ad*) using 125 and 600 mg/g

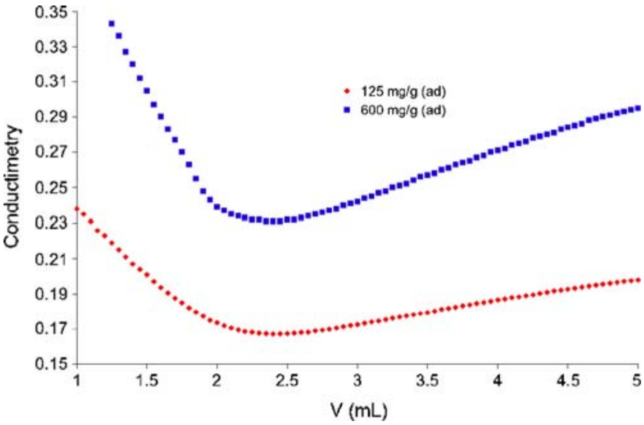

Fig. 10 Conductimetric titration of amine groups onto aminodextran containing magnetic latex particles prepared by adsorption

Table 2 Immobilized amount of aminodextran onto seed magnetic latex particles using different techniques

	$[NH_3^+]$ meq/g		Immobilized amount in mg/g		Immobilized amount in mg/m^2	
	125 mg/g	600 mg/g	125 mg/g	600 mg/g	125 mg/g	600 mg/g
ATG	0.244	0.368	59.2	89.1	7.69	11.59
Conductimetry	0.270	0.207	65.6	50.2	8.51	6.52
Orange II	0.250	0.275	60.6	66.6	7.88	8.66

Using such technique, no significant difference in terms of surface amine density was observed on magnetic latexes prepared using the 125- and 600-mg/g ratio. In order to determine more precisely the amount of immobilized aminodextran polymer, the Orange II titration method based on complexation between amine function and Orange II molecule was used. The principle of such process is to mix magnetic latex particles with an Orange II solution. Then, after a magnetic separation, the absorbance of the supernatant was determined by UV analysis method. Then from a standard calibration curve (absorbance versus Orange II concentration), the concentration of free Orange II (i.e., which has not been complexed by the amine function on the magnetic particles surface) can be determined and then the concentration of Orange II complexed on the amine surface function can be indirectly deduced. This allowed determining the amount of immobilized amine function on the magnetic particles surface and consequently the amount of aminodextran polymer. The determined values of surface amine function on the magnetic latex particles are 0.250 and 0.275 meq/g for magnetic latex particles prepared using 125 and 600 mg/g AMD/particles, respectively.

Using conductimetry titration process, the number of amine equivalents function per aminodextran mole was determined in a previous paper [24] and found to be equal to 0.813 (eq/mol). From these titration results, the amounts of immobilized aminodextran (in mg/g and in mg/m^2) were calculated as reported in Table 2. The results obtained by the Orange II method gave the closest results between 125 and 600 mg/g. Consequently, even if all the technique gave the same tendency, these techniques seem to be the not appropriate for direct titration of amine groups in the adsorbed layer. In fact, the titration may be affected by the chemical nature of the seed magnetic latex particles. In addition, we have not examined the possible release of immobilized aminodextran during the titration process.

The influence of pH and salinity on the amount of immobilized aminodextran on the seed magnetic latex particles surface was then systematically quantified by using the Orange II technique and the obtained results are reported below.

The effect of pH and salinity on the amount of immobilized aminodextran onto seed magnetic latex particles and the results obtained are reported in Figs. 11 and 12. The amount of immobilized aminodextran is found to be constant (≈8 mg/m^2) irrespective of pH and almost constant (≈9.5 mg/m^2) as a function of salinity. Such behavior can be attributed to strong attractive electrostatic interaction between the aminodextran polyelectrolyte and the highly charged seed magnetic latex particles.

Magnetic properties of the final magnetic latex particles

The magnetic properties of seed and cationic magnetic latexes were examined and the results obtained are shown in Fig. 13 in which the relative magnetization values versus an external magnetic field (*H*) are reported. For comparison study, maghemite Fe_2O_3 nanoparticles used for oil-in-water

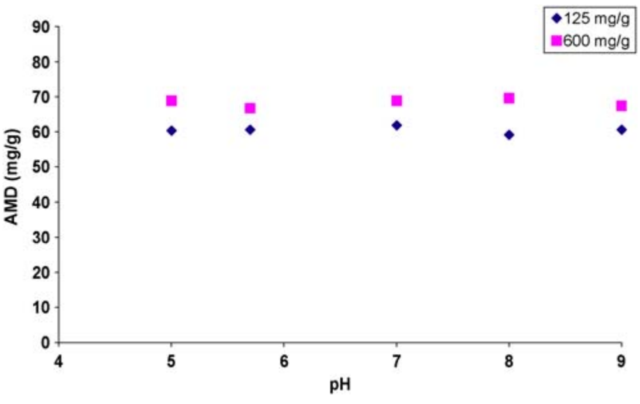

Fig. 11 Influence of pH on the amount of immobilized aminodextran

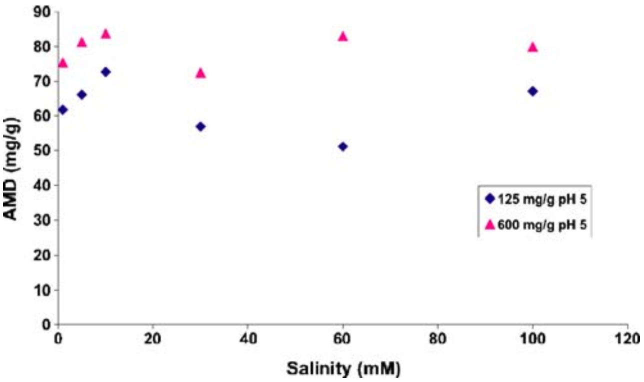

Fig. 12 Influence of salinity on the amount of immobilized aminodextran

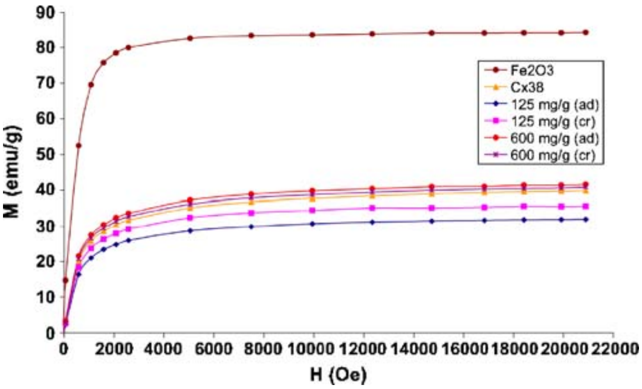

Fig. 13 Magnetization curves of iron oxide, seed magnetic latex particles, and cationic magnetic latexes at 300 K

magnetic droplets preparation, seed magnetic latex (Cx38), and for the aminodextran containing magnetic latex obtained by adsorption (ad) or via chemical grafting (cr) (prepared using two different initial aminodextran/particles ratio 125 mg/g and 600 mg/g) were analyzed. The saturation magnetization M_s for the magnetic latex is half smaller than that of the bulk maghemite (79.6 emu/g). As the seed magnetic latex was prepared from oil-in-water magnetic droplets based on maghemite (Fe_2O_3), the value of saturation magnetization of the seed magnetic latex was found to be equal to 39.8 emu/g. This low value is due to the presence of non-magnetic organic material (oleic acid, polyDVB). For cationic magnetic latex, the amount of aminodextran adsorbed on the surface of the seed latex was around 9 mg/m^2. This interfacial aminodextran amount is small enough to induce any marked difference between seed and cationic magnetic latexes in terms of saturation magnetization of latex (i.e., 36 emu/g for 125 mg/g and 41 emu/g for 600 mg/g). It is interesting to notice that the prepared magnetic latexes are superparamagnetic in nature. This is due to the superparamagnetic character of the used maghemite in all latexes. These results showed that the polymerization process and the surface modification did not induce any modification of the magnetic properties of the magnetic material.

Colloidal stability

The colloidal stability was examined as a function of salt concentration at fixed pH and particles concentration. Since no significant difference in zeta potential was observed between the cationic magnetic particles prepared by adsorption (ad) or chemical grafting process (cr), the colloidal stability study was performed on the particles obtained by adsorption only. The colloidal stability was examined by measuring the turbidity variation as a function of salt concentration and the obtained results are reported in Fig. 14. The aggregation of the particles is detected by a decrease in the magnitude of the parameter *n*. The value of the parameter *n* is constant for low salinity and suddenly decreases in instable salinity domain. For seed magnetic latex, a drastic decrease of the parameter *n* was observed at 0.01 M NaCl concentration, whereas for cationic particles (i.e., 125 mg/g), a slight decrease of parameter *n* was observed at 0.1 M NaCl concentration. For cationic magnetic particles prepared using 600 mg/g ratio, a constant parameter *n* was observed in a broad range of NaCl concentration from 10^{-5} to 3 M. The high colloidal stability of magnetic latex particles prepared using 600 mg/g can be related to the high surface concentration of aminodextran which can induce both hydration forces and sterical stability of the particles. The low colloidal stability of the 125 mg/g sample can be attributed to the low content of aminodextran as demonstrated by TGA. In brief, the aminodextran containing magnetic latex particles is of high colloidal stability compared to seed magnetic latex particles.

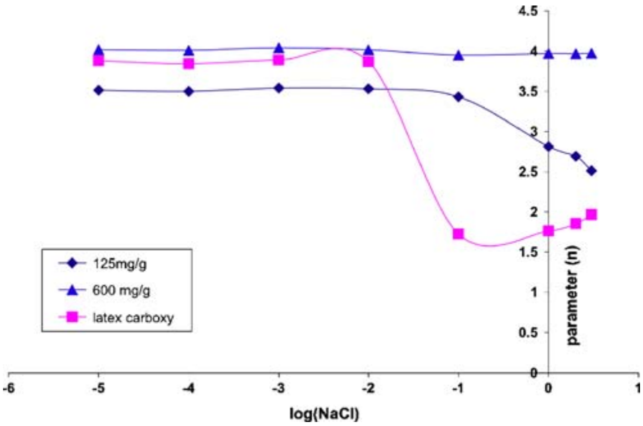

Fig. 14 Colloidal stability of seed latex and cationic latexes (125 and 600 mg/g) determined by turbidity measurement

Conclusion

The elaboration of hydrophilic cationic submicron magnetic latexes from oil-in-water magnetic emulsion was studied in this work. Firstly, seed negatively charged magnetic latex particles were prepared via oil-in-water magnetic emulsion transformation using radical polymerization in dispersed media. The functionalization step was performed using two processes: (a) via attractive electrostatic adsorption of aminodextran (cationic polyelectrolyte) onto negatively charged submicronic particles and (b) via chemical immobilization of aminodextran onto seed magnetic latex particles (via chemical grafting of amine onto activated carboxylic group of the particles). The physico-chemical and colloidal properties of the cationic hydrophilic magnetic latex particles have been studied in aminodextran ranging from 25 to 600 mg/g. It is interesting to notice that above 600 mg/g, the aminodextran solution becomes too viscous. No significant difference has been observed concerning the hydrodynamic size and the size distribution for 125, 200, 400, and 600 mg/g either by adsorption or by chemical immobilization. Below the 125-mg/g ratio, a irreversible aggregation phenomenon has been observed. Above 125 mg/g, modest stable cationic magnetic latex particles have been observed. For 600 mg/g, highly stable magnetic particles have been prepared.

In terms of quantitative aspect, the amount of immobilized aminodextran has been determined using various techniques. From these results, it was observed that the amount of immobilized aminodextran onto seed magnetic latex particles was almost constant (≈8 mg/m^2) irrespective of the aminodextran/particles ratio in between 125 and 600 mg/g, pH, and salinity. The chemical composition of the aminodextran containing magnetic latexes has been examined and showed 50% iron oxide constant whatever the elaboration process and the initial aminodextran concentration. The colloidal stability of the cationic latex particles was found to be higher compared to classical particles since the particles are stable until 0.1 M NaCl concentration. The aminodextran particles are positively charged until pH 9, which demonstrates the high cationic character of the elaborated magnetic hydrophilic latex particles.

Therefore, aminodextran submicronic magnetic particles containing magnetite present promising properties to be used as a magnetic carrier in molecular diagnosis.

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