Preparation and biomedical application of layer-by-layer encapsulated oil in water magnetic emulsion

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Abstract

Hydrophilic submicron magnetic colloids were prepared via layer-by-layer encapsulation of oil in water (o/w) magnetic emulsions. The encapsulation explored in this work is based on sequential adsorption of oppositely charged polyelectrolytes onto magnetic emulsion. The functionalization was induced by the final polyelectrolyte layer. The elaborated magnetic particles were evaluated in the specific capture of nucleic acids.
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1. Introduction

The utilization of colloids and particularly magnetic particles as a solid support in biomedical field has attracted increasing interest. In fact, magnetic colloidal particles can be easily separated by applying an appropriate magnetic field [1,2]. Subsequently, the phase separation process leads to concentrate and purify captured biomolecules in a small volume. Magnetic particles are also being used as a carrier in various biotechnological applications such as microsystems based on microfluidic aspects.

The elaboration of magnetic colloids and principally magnetic latexes has been widely reported [1,3]. Various kinds of magnetic latexes have been prepared using numerous approaches. The pioneering work in this direction has been reported by Ugelstad et al. [4] on the elaboration of monodisperse magnetic micrometer size particles. The obtained latexes are of large particle size (above 2 μm) and fast sedimentation velocity. The second work has been reported by Charmot et al. [5] by investigating the emulsion polymerization of styrene in the presence of organic ferrofluid. The final latexes are polydispersed in the presence of magnetic (free and encapsulated inorganic material) and non magnetic particles (polymer particles). Several process and magnetic latexes have been reported since these works. The recent interesting

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work in the preparation of submicron magnetic latexes has been reported by Ramirez et al. [6] by performing miniemulsion polymerization of styrene containing stabilized iron oxide nanoparticles. However, the preparation of reactive, narrowly size distributed and low particle size (diameter lower than 500 nm) highly magnetic colloidal particles is always challenging. Recently, very interesting process based on albumin magnetic microspheres has been elaborated as reported by Chatterjee et al. [7,8]. These microspheres have a wide size distribution, though most of them are in the submicron range. The mount of incorporated iron oxide was found to be around 30 wt%. Due to limiting polymerization methodology in the encapsulation process, new approaches have been explored and developed. The layer-by-layer adsorption of polyelectrolytes onto colloidal particles has been reported to be well recognized method leading to particles encapsulation, surface modification and functionalization [9,10].

In this paper, the encapsulation and the functionalization of o/w magnetic emulsion using sequential adsorption of oppositely charged polyelectrolytes is presented and discussed. Firstly, the properties of magnetic emulsion are summarized. Secondly, the adsorption of each polyelectrolyte is reported and discussed. Finally, the biomedical use of the encapsulated o/w magnetic emulsion in specific capture of nucleic acids is briefly evaluated.

2. Materials and methods

Magnetic emulsion preparation. The magnetic emulsions used in this work is the courtesy of Ademtech SA (Parc Scientifique Unitec 1, 4 allée du Doyen Georges Brus, 33600-Pessac, France). The details concerning the preparation and the full colloidal characterization of such magnetic emulsion have been recently reported by Bibette [11] and Montagne et al. [12]. The chemical composition of the used magnetic emulsion is; 23 wt% charged surfactant and 77 wt% iron oxide content. The iron oxide is mainly composed of maghemite (γ-Fe_2O_3). The amount of organic phase in the ferrofluid droplets is lower than 5 vol%. The

magnetic emulsion can be schematically presented as described in scheme 1.

Polyelectrolytes. Polyethyleneimine (PEI) polymers ($M_W = 25,000$ g/mol) was from Aldrich and used as such. The PEI is prepared in Milli-Q water and (10 g/l, pH 11). Poly(maleic anhydride-alt-methyl vinyl ether) (PMAMVE) ($M_W = 67,000$ g/ mol given by the supplier) (Polysciences Inc.) was used as received. The PMAMVE is solubilized in Milli-Q water and incubated at room temperature for at least 12 h in order to allow the full hydrolysis of the polymer (scheme 2).

Encapsulation process. The encapsulation was performed via layer-by-layer polyelectrolytes adsorption process. The first cationic polyelectrolyte was adsorbed onto magnetic emulsion using well appropriate polycation concentration. After washing step using Milli-Q water, the obtained cationic magnetic particles were then introduced in high
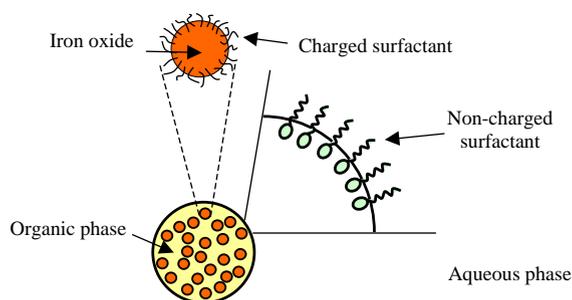
Scheme 1. Schematic illustration of an o/w magnetic emulsion droplet.
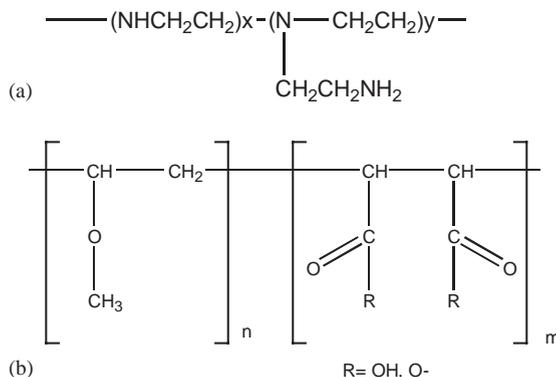
Scheme 2. Chemical structure of (a) PEI and (b) hydrolyzed PMAMVE ($n = m = 0.5$).
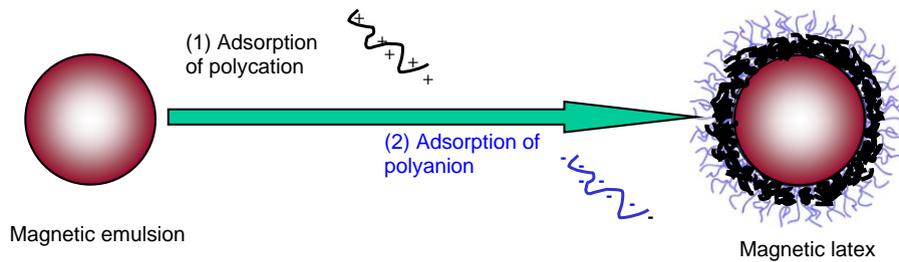
Scheme 3. Schematic illustration of layer-by-layer encapsulation process of magnetic emulsion.

concentrated polyanion solution. The final magnetic particles were washed using Milli-Q water before any colloidal characterization. The preparation details are below reported.

Cationic magnetic emulsion preparation. The amino-containing magnetic emulsion was prepared by adsorbing the PEI onto negatively charged magnetic nanodroplets. Five ml of the magnetic dispersion (5% w/v) were added to 10 ml of PEI (50 g/l) and stirred 15 min. To remove the excess of PEI, the dispersion was washed using Milli-Q water.

Anionic magnetic emulsion preparation. The adsorption of anionic polyelectrolyte onto amino-containing magnetic nanodroplets was performed as follows (scheme 3). 15 ml of the hydrolyzed PMAMVE (11 g/l) solution and 20 ml of Milli-Q water was first mixed and 45 ml of cationic magnetic emulsion (~0.5%) was introduced dropwise along with mechanical stirring. The obtained magnetic particles were washed via magnetic separation and redispersion in Milli-Q water before any characterization and utilization.

Electrokinetic properties. The electrophoretic mobility of the magnetic emulsion was measured using highly diluted dispersion. The measurements were performed on a Zetasizer 3000HS (from Malvern Instrument, England) as a function of pH and at 25 °C. In this study, the zeta potential calculated from Smolochowski's equation was used [13].

Particle size analysis. The particle size and size distribution were determined using highly diluted magnetic dispersion in water at 1 mM NaCl salt concentration and at 25 °C. The measurements were performed using Malvern Zetasizer 3000HS.

Morphology analysis. The magnetic particles morphology was analyzed using transmission electron microscopy (TEM) (Phillips CM120 at CMEABG, Villeurbanne, France). Highly diluted magnetic dispersion was deposited and dried on copper grid coated with a formvar-carbon layer.

3. Results and discussion

3.1. Magnetic emulsion properties

The size distribution of magnetic emulsion was investigated using TEM and the obtained micrograph revealed the slight polydispersity of the magnetic emulsion as illustrated in Fig. 1.

The particle size and size distribution were also analyzed using quasi-elastic light scattering. The magnetic emulsion was highly diluted in 10^{-3} M NaCl and the obtained hydrodynamic diameter and size distribution are reported in Fig. 2. The average diameter size is 240 nm with a standard deviation around 30 nm.

The general properties of the used magnetic emulsion are summarized as follow: (i) the amount of iron oxide is found to be at less 65% (wt/wt), (ii) the specific magnetization of the magnetic emulsion (under dried state or in dispersed medium) is in between 40 and 50 emu/g and (iii) the amount of surfactants in the magnetic emulsion is found to be around 25% (wt/wt) as recently reported [12].

3.2. Polycation adsorption

Various experiments were performed in order to point the optimal conditions leading to stable
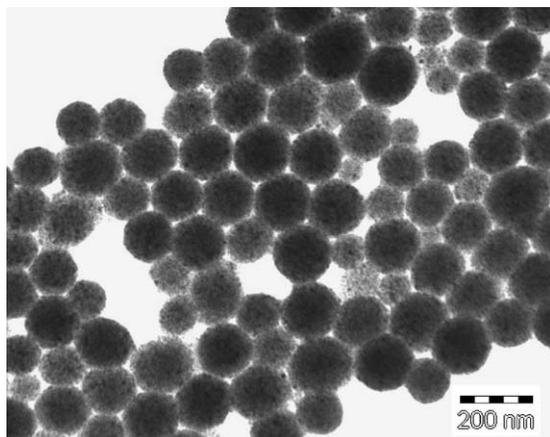
Fig. 1. TEM micrograph of the magnetic emulsion.

Fig. 3. The zeta potential of bare, PEI coated and polyanion-PEI coated magnetic emulsion at acidic pH (10 mM phosphate buffer, pH 4.5).
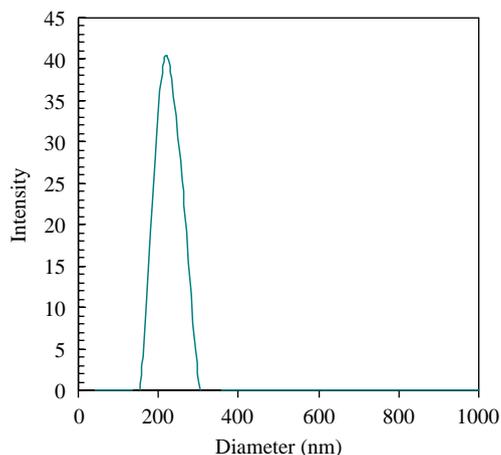
Fig. 2. Hydrodynamic diameter and size distribution of bare magnetic emulsion

cationic magnetic emulsion. For low PEI concentration (below 2–5 mg of PEI/mg of solid magnetic emulsion), the magnetic emulsion flocculates rapidly, whereas for high PEI concentration (above 5 mg of PEI/mg of solid magnetic emulsion), stable magnetic emulsion was obtained. The observed aggregation can be attributed to the bridging flocculation process induced by the PEI. The colloidal stabilization observed for high PEI concentration is due to sterical stabilization process. The adsorption here was investigated at a fixed pH by the PEI solution (i.e. pH 10). The

adsorption process of such polymer (low or no-charged at pH 10) onto negatively charged magnetic emulsion can be attributed to combination of hydrogen bounding between carboxylic group on the magnetic emulsion and amine groups of the PEI and the forced adsorption due to high polymer concentration.

The zeta potential of coated magnetic emulsion after a few washing with Milli-Q water, revealed the cationic character of the particles surface. In fact, the zeta potential measured at acidic pH was found to be positive, whereas, the zeta potential of bare emulsion at the same pH is negative as illustrated in Fig. 3.

3.3. Polyanion adsorption

The adsorption of polyanion (hydrolyzed PMAMVE) was performed via dropwise addition of cationic magnetic emulsion in highly concentrated polyanion polymer solution. This approach was selected and optimized in order to avoid aggregation of magnetic particles via bridging flocculation process. The adsorption of polyanion onto positively charged magnetic emulsion is due
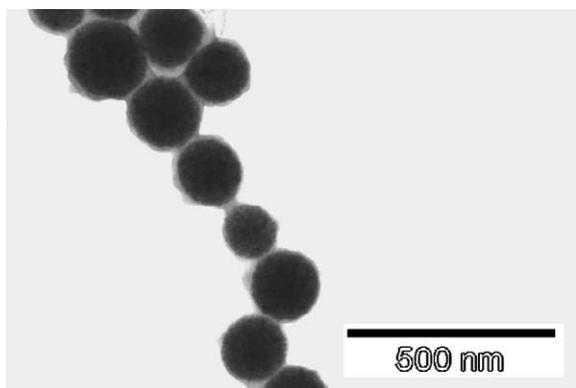
Fig. 4. Transmission electron micrograph (from TEM analysis) of double layer encapsulated magnetic emulsion.

Scheme 4. Schematic illustration of specific capture of targeted HRP-oligonucleotide onto biotinylated oligonucleotides containing streptavidin magnetic particles.

to the attractive electrostatic interaction. The adsorption of the polyanion onto elaborated cationic magnetic emulsion was evidenced by investigating the zeta potential and the obtained result is reported in Fig. 3. The zeta potential was found to be inversed as expected. The observed zigzag profile of zeta potential versus the adsorbed polyelectrolyte layer revealed the surface modification. The thickness layer of the adsorbed polycation is too thin to be evidenced by TEM analysis. Whereas, the thickness layer of polycation–polyanion shell is thicker as evidenced from the micrograph analysis as illustrated in Fig. 4. The observed behavior revealed the encapsulation efficiency. Due to the slight polydispersity of the seed magnetic emulsion, the hydrodynamic thickness cannot be easily deduced from the hydrodynamic diameters measured by quasi-elastic light scattering.

3.4. Enzyme linked oligo-sorbent assay evaluation

The elaborated carboxylic magnetic particles were evaluated in specific capture of targeted single stranded nucleic acid using enzyme linked oligo-sorbent assay (ELOSA). The magnetic particles were first functionalized by chemical grafting of streptavidin molecules (at 10 mM pH 6.8 phosphate buffer) using dicyclohexyl carbodiimide (DCC) as activating agent [14,15]. The streptavidin containing magnetic particles were then modified via immobilization of a model oligonucleotides

(dT_{25}) bearing biotin aliphatic spacer arm at its $5'$ position.

The specific capture of single stranded DNA fragment was performed using complementary oligonucleotide sequence bearing Horse Radish Peroxydase (HRP) as a target model (scheme 4). The specific capture was evaluated by measuring the optical density (OD) of the supernatant at 492 nm after o-phenylenediamine, dihydrochloride (OPD) addition (Fig. 5).

The capture efficiency was evaluated as a function of dT_{25}-magnetic particle conjugates for a fixed HRP-oligonucleotide amount. The measured OD was found to increase as a function of added dT_{25}-magnetic particle amount as expected and as reported in Fig. 5. The obtained preliminary results are encouraging since the chemical immobilization of the capture probe and the amount of the oligonucleotides-magnetic particles were not optimized in this study.

4. Conclusions

The layer-by-layer encapsulation process of oil in water submicronic magnetic emulsion was performed via sequential polyelectrolytes adsorption. The adsorption at high polyelectrolytes concentration leads to stable and functionalized magnetic particles. The encapsulation efficiency as examined by transmission electron microscopy was found to be core-shell-like structure and homogeneous in nature. The zeta potential versus the polymer adsorbed layer revealed zigzag profile as expected. In fact, the initial negative zeta potential of bare magnetic emulsion was reversed
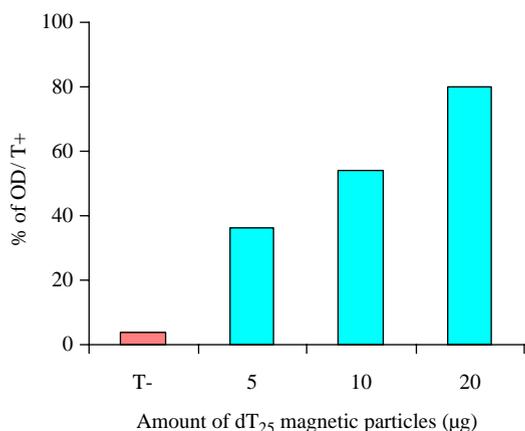
Fig. 5. Effect of dT_{25}-magnetic particles amount used for the specific capture of the nucleic acid target. The (T+) is the positive standard from Seradyn (SeraMag bearing immobilized dT_{25}). The (T−) is the anionic magnetic nanodroplets only. The target model is the HIV sequence bearing HRP-dA at 3′ position.

after polycation adsorption and then to negative value after the immobilization of polycation layer. The evaluation of the encapsulated magnetic oil in water magnetic emulsion was performed using the simplified ELOSA test. The preliminary obtained results revealed the specific capture of the targeted nucleic acids and appreciable specificity and sensitivity.

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