Reactive and Highly Submicron Magnetic Latexes for Bionanotechnology Applications

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Summary: Colloidal particles are largely used in biomedical applications as a solid support, as a carrier, as nanoreactors and as labels for target molecule detection. With the recent development of bionanotechnology, more appropriate colloidal particles should be elaborated. In this direction, new specification are listed in order to develop reactive nanoparticles to be use in microsystems, microfluids and all combined systems in which we can conduct sample preparation, specific capture, purification, concentration and detection in small volume (generally less than 100 μl). Then the aim of this short review is to give to the readers some recent orientations of reactive magnetic latex particles for in vitro bionanotechnology applications.

Keywords: bionanotechnology; diagnosis; latexes; magnetic

Introduction

Various kind of colloidal particles are used as solid support in numerous biomedical applications such as immunoassay,[1] cell sorting,[2,3] nucleic acids capture and detection.[4] Such applications require various steps in which particles' separation is absolutely needed. In this area two separations are often used: particles filtration or centrifugation. Then, to avoid such heavy separation processes, which limit the automation of biomedical diagnosis methodologies and time consuming, magnetic colloids are of great interest.

Various steps involved in biomedical applications are reduced by the development of magnetic nanoparticles (Fe_3O_4: magnetite, γ-Fe_2O_3: maghemite).[5] In fact, the main advantage in using magnetic particles and particularly magnetic latexes is related to the fast particles separation upon applying even low permanent magnetic field. The magnetic particles separation under magnetic field is related to various parameters: iron oxide chemical nature (γ-Fe_2O_3, Fe_3O_4...), amount of magnetic domains in the final composite microspheres, particle size, physical density, viscosity of the medium and finally, the intensity of the applied magnetic field.[6] Consequently, each biomedical application needs well-defined and appropriate magnetic carrier.[7]

Since the first elaboration of iron oxide nanoparticles (ferrofluid solution), various magnetic colloids have been prepared[6] and evaluated in numerous biomedical diagnosis applications.[8]

In fact, increasing interest has been focused on the preparation of magnetic latex particles and their use in biomedical diagnostic based. All elaborated magnetic latexes are principally used as a solid support of biomolecules involved in specific capture of the targeted biomolecules such as antigens in immunoassays and in molecular biology for specific nucleic acid detection.[8,9]

Nowadays, the main objective in biomedical diagnosis is to enhance the sensitivity, to reduce time consuming, price and volume by elaborating new submicron reactive magnetic latexes for in vitro biomedical diagnosis based on bionanotechnology methodologies. The general problem in this field is related to the

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limited sensitivity level. One possibility to partially solve this problem is to increase the concentration of the captured targets (antigens, viruses, bacteria, nucleic acids etc...) before specific detection step. Then, the use of appropriate magnetic colloids particles and methodologies (micro system, lab-on-chip, biosensors)[10–12] for extraction and concentration of biomolecules is of extreme interest. The objective of this contribution is to present and to discuss the elaboration of reactive submicron magnetic latexes from oil in water magnetic droplets and their use for specific and non-specific extraction, concentration and detection of biomolecules using recent biotechnology based methodologies.

Elaboration of Submicron Magnetic Latex Particles

In the state of art, numerous interesting approaches have been reported regarding the encapsulation or iron oxide nanoparticles in polymer matrix in order to prepare magnetic latexes. The developed approaches are ranging from classical heterogeneous polymerization processes; emulsion, suspension, dispersion, miniemulsion inverse emulsion or inverse microemulsion as well as some multi-step synthesis procedures.[6]

Briefly, two major strategies leading to submicron particles have been reported, one using miniemulsion polymerization, the other is based on the use of oil in water ferrofluid droplets as seed in emulsion polymerization.

Miniemulsion Polymerization Process

Miniemulsion has been largely explored in order to prepare submicron hybrid latex particles.[13,14] But such process has shown various undesirable limitations such as: heterogeneous distribution of magnetic nanoparticles in the polymer matrix and low magnetic content in the polymer particles and finally low particles size which lead to low magnetic separation under classical magnetic field.[15]

Via such approach, various interesting works have been recently reported in order to target the one step elaboration of functionalized mondisperse magnetic latex particles.[16–19]

To overcome the above-mentioned limitations, Joumaa et al.[18] prepared magnetic polystyrene nanoparticles via miniemulsion polymerization (Figure 1). The process consists in using a nonconventional phosphate-based poly(propylene glycol) methacrylic monomer as stabilizer of iron oxide nanoparticles in oil (octane). This surfmer has a phosphate group well known to strong link to iron oxide surface.[26] The methacrylic function was used to favor irreversible incorporation of iron oxide inside the polystyrene particles through copolymerization with styrene. As a general tendency of the obtained results, the authors report a broad particle size distribution but associated with a high iron oxide loading ($\sim$30 wt %).

Transformation of Oil in Water Magnetic Ferrofluid Droplets into Magnetic Latex Particles

The use of oil in water as seed in emulsion polymerization has been first reported by Montagne et al.[20] This new approach is based on the transformation of magnetic droplets into magnetic latex particles via radical emulsion polymerization using hydrophobic monomers (i.e. styrene). The used magnetic seeds are submicron droplets of highly stable magnetic emulsion (Figure 2). The particle size distribution was controlled by the size distribution of the initial magnetic emulsion. It was evidenced that homogeneous encapsulation of the iron oxide nanoparticles was efficiently achieved using styrene/DVB monomer mixture; potassium persulfate as initiator. High iron oxide content of about 60% wt% and high carboxylic surface charges were reported.[20]

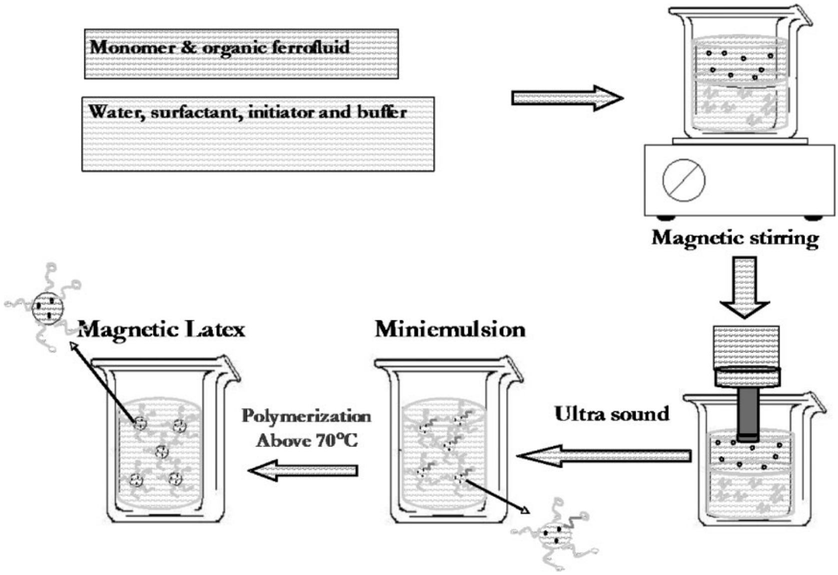

Figure 1.
Schematic illustration of miniemulsion polymerization process leading to submicron magnetic latex particles. The oil in water emulsion was prepared by using for instance iron oxide nanoparticles stabilized by phosphate containing surfmer and dispersed in styrene monomer as reported by Joumaa et al.[18]

In addition to direct emulsion polymerization, inverse process has also been explored in order to obtain reactive and suitable magnetic latex particles. Such process has been reported to be appropriate to elaborate highly magnetic latexes for in-vitro biomedical application as reported recently by Horak et al.[21]

It is interesting to notice that inverse emulsion has been expended to various polymerization in dispersed media processes, such as that inverse microemulsion[22] as well as some multi-step processes as reported.[23–25]

Some Fine Applications in Bionanotechnology

In recent years, increasing interest has been given to the preparation of functionalized latex particles for diagnostic applications.[26] All elaborated latexes are principally used as a solid support for covalent immobilization of biomolecules involved in specific capture of a given target. In fact, the magnetic colloids are largely used in immunoassays (i.e. ELISA Enzyme linked Immuno Sorbent Assay) and nucleic acid detection (ELOSA, Enzyme Linked Oligo Sorbent Assay). The main objective in biomedical diagnosis is the elaboration of new polymer supports and tested methodologies in order to enhance analysis sensitivity and specificity. The problem encountered in this field, and particularly in the area of nucleic acid probes, is the level of sensitivity. One possibility to partially solve this problem is to increase the concentration of the target biomolecules such as (RNA and/or DNA), proteins and viruses in the considered medium before specific detection of the target probe. Then utilization of appropriate colloidal particles for adsorption of biomolecules would be of extreme interest.[26,27]

Magnetic particles have been used in various biomedical applications. In fact, they have been used in as a carrier in conventional biomedical diagnostic in order to replace the heavy process related to the centrifugation step. They are also

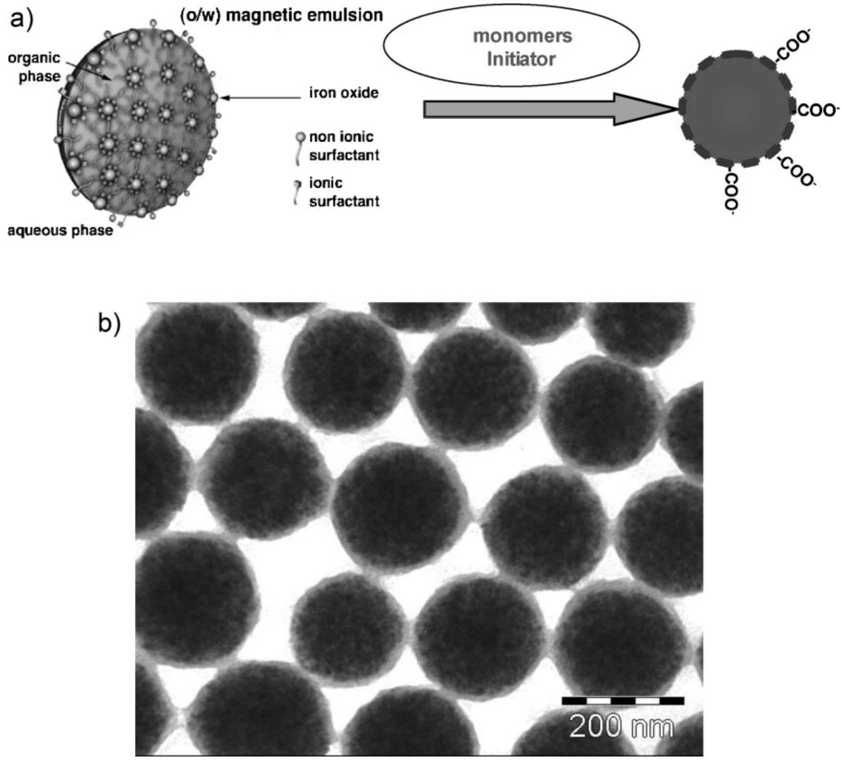

Figure 2.
(a) Turn (o/w) magnetic emulsion into magnetic latex via radical polymerization and (b), Transmission microscopy analysis of magnetic latex particles.[20]

used for in vivo diagnostic as in MRI for cancer disease detection.[28–30] Actually, magnetic particles are used in different in vitro biomedical and biotechnological applications such as in automated micro-system, microfluidic[3,31] based technology and also bionanotechnology[32] which combines capture, purification, concentration and detection of the targeted biomolecules or pathogenic or toxic molecules as below listed:

(i) Specific and non specific extraction and concentration of nucleic acids.[8,33]
(ii) Cell sorting and identification using a combination of fluorescent antibody and magnetic particles[34] (Figure 3).
(iii) Specific bacteria isolation and identification
(iv) Viruses extraction, purification and detection.[35]
(v) Immunoassays for rapid capture and detection of antigens and antibodies.[36,37]

The specificity and the sensitivity of the targeted application efficiency are directly related to the surface properties of the

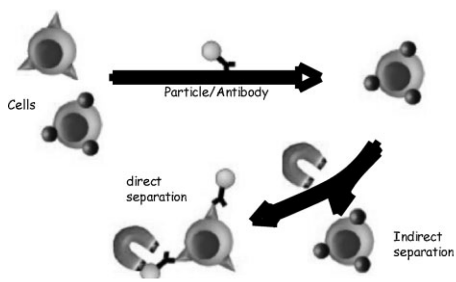

Figure 3.
Schematic illustration of direct and indirect cells sorting.

particles and to the accessibility of the immobilized biomolecules or reactive compounds on the particles surface. The interactions between biomolecules and reactive particles are strongly dependent on the colloidal and surface properties of the dispersion, and the physico-chemical properties of the used or targeted biomolecules. In this direction, considerable attention has been paid to the preparation of magnetic latex particles for automated Microsystems based on bionanotechnologies applications.[38] The main advantage of colloidal magnetic particles is due to their separation upon applying an external magnetic field. For more information regarding the use of magnetic particles in automated systems, micrsystems, microfluidic, readers can be consulted with the following recent book.[26]

Conclusion

To enhance the sensitivity of biomedical diagnosis, the specific and non-specific extraction, the purification and the detection of targeted biomolecules are being attracted special attention of all industries involved in life science. The only way to reach such objective is to combine submicron magnetic particles, microsystems and biosensing in order to perform all steps rapidly and in small volume. The biosensor should be of high sensitivity so as to enhance the sensitivity. In this direction, the use of colloidal magnetic particles and principally magnetic latex particles has been reported to be promising way to enhance the sensitivity and to avoid time consuming compared to conventional techniques. The use of magnetic particles is well appreciated to replace centrifugation, filtration and sedimentation.

[1] S. Avraemas, B. Guilbert, *C.R.Acad.Sc.Paris* **1971**, *273*, 2705–2707.
[2] G. Kögler, A. Bru Capdeville, M. Hauch, H. T. Brüster, U. Göbel, P. Wernet, S. Burdach, *Bone Marrow Transplant.* **1990**, *6*, 163–168.
[3] V. I. Furdui, D. J. Harrison, *Lab Chip* **2004**, *4*, 614–618.
[4] R. Boom, C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van Dillen, J. Van der Noordaa, *J. Clin. Microbiol.* **1990**, *28*, 495–503.
[5] J. C. Bacri, R. Perzynski, D. Salin, V. Cabuil, R. Massart, *Journal of Magnetism and Magnetic Materials* **1986**, *62*, 36–46.
[6] A. Elaissari, F. Sauzedde, F. Montagne, C. Pichot, *in Colloidal Polymers, Synthesis and Characterization, Edited by Elaissari, Marcel Dekkert Edition* **2003**, *Surfactant Science Series Volume 115*, 285–318.
[7] U. Häfeli, W. Schütt, J. Teller, M. Zborowski, Plenum Press, New York 1997.
[8] A. Elaissari, R. Veyret, B. Mandrand, J. Chatterjee, *in Colloidal Biomolecules, Biomaterials, and Biomedical Applications, Edited by Elaissari, Marcel Dekker Edition* **2003**, *Surfactant Science Series, Volume 116*, 1–26.
[9] A. Spanova, D. Horak, E. Soudkova, B. Rittich, *Journal of Chromatography B* **2004**, *800*, 27–32.
[10] L. Bao, L. Nie, S. Yao, W. Wei, *Biosens.Bioelectron.* **1996**, *11,n°12*, 1193–1198.
[11] D. R. Baselt, G. U. Lee, M. Natesan, W. Metzger, P. E. Sheehan, R. J. Colton, *Biosensors & Bioelectronics* **1998**, *13*, 731–739.
[12] P. Bataillard, F. Gardies, N. Jaffrezic-Renault, C. Martelet, B. Colin, B. Mandrand, *Anal.Chem.* **1988**, *60*, 2374–2379.
[13] B. Erdem, D. Sudol, V. L. Dimonie, M. S. El-Asser, *Journal of Polymer Science:Part A: Polymer Chemistry* **2000**, *38*, 4441–4450.
[14] B. Erdem, E. D. Sudol, V. L. Diamonie, M. S. El-asser, *Journal of Polymer Science: Part A: Polymer Chemistry* **2000**, *38*, 4431–4440.
[15] L. P. Ramirez, K. Landfester, *Macroml. Chem. Phys* **2003**, *204*, 22–31.
[16] S. Lu, J. Forcada, *Journal of Polymer Science Part A: Polymer chemistry* **2006**, *44*, 4187–4203.
[17] S. Lu, J. Ramos, J. Forcada, *Langmuir* **2007**, *23*, 12893–12900.
[18] N. Joumaa, P. Toussay, M. Lansalot, A. Elaissari, *Journal of polymer science* **2007**, *46*, 327–340.
[19] R. Faridi-Majidi, N. Sharifi-Sanjani, *Journal of Magnetism and Magnetic Materials* **2007**, *311*, 55–58.
[20] F. Montagne, O. Mondain-Monval, C. Pichot, A. Elaissari, *Journal of Polymer Science: Part A: Polymer Chemistry* **2006**, *44*, 2642–2656.
[21] H. Mackova, D. Kralova, D. Horak, *Journal of Polymer Science Part A: Polymer chemistry* **2007**, *45*, 5884–5898.
[22] P. A. Dresco, V. S. Zaitsev, R. J. Gambin, B. Chu, *Langmuir* **1999**, 1945.
[23] J. Ugelstad, L. Kilaas, O. Aune, J. Bjorgum, R. Herje, R. Schmid, P. Stenstad, A. Berge, In *Advances in biomagnetic separation*, M. Uhlén, E. Hornes, O. Olsvik, Eds., Eaton, Stockholm, Oslo, Atlanta 1993, pp 1–19.
[24] K. Nakamae, S. Tanigawa, T. Tsujiguchi, S. Okamoto, K. Yamaguchi, *Colloid.Surface A.* **1993**, *80*, 85–92.

[25] J. Lee, T. Isobe, M. Senna, *J.Colloid Interface Sci.* **1996**, *177*, 490–494.

[26] *Colloidal Nanoparticles in biotechnology*, A. Elaissari, Ed., **2008**.

[27] A. Elaissari, W. Yang, *Smart Nano and Microparticles, The MML serie, Volume 7, Kenji Kono, Reza Arshady, (Editors), Kentus Books, United Kingdom* **2006**, *7*.

[28] S. Pauser, R. Reszka, S. Wagner, K. J. Wolf, H. I. Buhr, G. Berger, *Anticancer Drug Des* **1997**, *12*, 125–135.

[29] C. Chouly, D. Pouliquen, I. Lucet, J. J. Jeune, P. Jallet, *Journal of microencapsulation* **1996**, *13*, 245–255.

[30] J. W. M. Bulte, T. Douglas, S. Mann, R. B. Frankel, B. M. Moskowitz, R. A. Brooks, C. D. Baumgarner, J. Vymazal, M. P. Strub, J. A. Frank, *JMRI* **1994**, *4*, *n°3*, 497–505.

[31] J. choi, K. W. Oh, J. Thomas, W. Heineman, H. Haslsall, J. Nevin, A. Helmicki, H. Henderson, C. Ahn, *Lab Chip* **2002**, *2*, 27–30.

[32] J. M. Laval, J. Chopineau, D. Thomas, *TIBTECH* **1995**, *13*, 474–481.

[33] R. Veyret, *These Université Claude bernard-Lyon 1* **2003**, *157-2003*.

[34] A. Rembaum, R. C. K. Yen, D. H. Kempner, J. Ugelstad, *J. Immunol. Methods* **1982**, *52*, 341–351.

[35] R. Veyret, A. Elaissari, P. marianneau, A. A. Sall, T. Delair, *Annalyical Biochemistry* **2005**, 59–68.

[36] M. H. Charles, M. T. Charreyre, T. Delair, A. Elaissari, C. Pichot, *S. T. P. Pharma. Sciences* **2001**, *11*, 251–263.

[37] M. Meza, In *Scientific and chinical applications of magnetic carriers*, U. Häfeli, W. Schütt, J. Teller, M. Zborowski, Eds., Plenum Press, New York 1997, pp 303–309.

[38] Arshady *Citus Books* **2001**, *3*.

