Magnetic Latex Particles in Nanobiotechnologies for Biomedical Diagnostic Applications: State of the Art

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Summary: The main objective of this short state-of-the art is to report on the preparation of reactive magnetic latexes for biomedical applications. The main advantage of colloidal magnetic particles is their separation upon applying an external permanent magnetic field. Then magnetic particles bearing reactive groups or specific receptors are used in biomedical diagnosis such as immunoassay, molecular diagnosis for specific capture and detection of nucleic acids, viruses extraction and detection, cell sorting and more recently in numerous biotechnological applications. The preparation of magnetic latexes requires the use of well-defined iron oxide nanoparticles in appropriate medium. In this direction, aqueous ferrofluids were largely used to prepare functional magnetic latexes, whereas few approaches have been devoted to the use of organic ferrofluids.

Keywords: biomedical; composite particles; diagnosis; dispersion; emulsion; in vitro; inversion emulsion; magnetic particles; miniemulsion; radical polymerization; thermally sensitive polymer

Introduction

For many years, latexes (polymer-based particles) have been used in the biomedical field such as in therapy[1,2] as carrier and delivery of encapsulated active molecules and in vitro biomedical diagnosis[1–3] (i.e. immunoassay tests, cells separation and analysis, nucleic acids concentration) or as solid-phase supports for immobilization of biomolecules. Regarding in vitro diagnosis applications, the phase separation steps are incontestably required and usually achieved by centrifugation, precipitation or filtration.

The main advantage of magnetic particles over classical latexes consists in the rapid and easy separation upon applying an external magnetic field. The magnetic particles should be particularly superparamagnetic in nature in order to avoid residual magnetization when the magnetic field is suppressed.[4–6]

Since the early work reported by Khalafalla at al.[7] and Massart et al.[8,9] on the preparations of iron oxide nanoparticle based ferrofluids, numerous efforts have been devoted to the preparation of magnetic latexes and microgels for potential applications in the biomedical field.[10–12] The iron oxide preparation is below illustrated by the chemical reaction of ferric and ferrous salts which leads to coprecipitation of the product in highly basic medium. Such process leads to magnetite Fe_3O_4 or maghemite γ-Fe_2O_3 or mixture of the two structures

$$2FeCl_3 + FeCl_2 + 8NH_4OH \rightarrow Fe_3O_4 + 8NH_4Cl + 4H_2O$$

When considering biomedical diagnostics and food analysis, the ''ideal'' magnetic latex particles should possess: (i) diameters below 1 μm in order to offer a high specific area for immobilization of large amounts of biomolecules and receptors, (ii) narrow size distribution allowing a homogenous separa-

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tion of the particles in the magnetic field, (iii) appropriate surface functionalities for adsorption or covalent attachment of biomolecules, (iv) high iron oxide (superparamagnetic) content for rapid separation in the magnetic field, (v) the ability to encapsulate the magnetic material by a polymeric matrix so as to avoid direct contact with some sensitive molecules and biomolecules (e.g. enzymes) and (vi) good colloidal stability in aqueous medium of ionic strength around 0.15 mol/L (average value for biological medium).

Various interesting approaches have been reported in the literature for the preparation of magnetic latexes. Those approaches are based on classical polymerization in dispersed media, such as, emulsion, miniemulsion, suspension, dispersion, and also combination of various polymerization-based processes. In order to avoid the citation of the exhaustive number of published papers in the area, this short review will mainly focus on some commonly explored roots, the pioneering works and some recently published results on magnetic latexes obtained via polymerization in dispersed media only. For exhaustive literature in the area of preparation of magnetic particles for both in vitro, ex vivo and in vivo applications, the readers can consult the following references of Arshady[11], Hafeli[10] and Elaissari.[2]

Dispersion and Multisteps Processes

In this field, Ugelstad et al.[12] have first described interesting methodology leading to monodisperse magnetic latex particles. This pioneering process includes various steps: (i) preparation of seed polymer particles, (ii) swelling of the seed with a monomer and porogenic agent, (iii) radical polymerization in order to increase the size, (iv) iron oxide incorporation in the pores (or in situ precipitation in the pores), (vi) encapsulation and functionalization step of the intermediate composite seed (Figure 1). Such multistep process, leads incontestably to monodisperse magnetic latex particles of micrometer size (1, 2.8 and 4.5 μm), containing around 20% iron oxide material and exhibiting different surface functionality (OH, COOH, SH, NH_2). It is interesting to notice, that the magnetic latex particles originated from Ugelstad's process are manufactured and used in in vitro and in ex vivo biomedical applications.[12] Using dispersion polymerization process interesting work has been recently reported by Horak et al. on the preparation of magnetic poly(glycidyl methacrylate) microspheres[13] and hydrophilic and thermally stimuli-responsive magnetic latexes.[14] The obtained temperature-sensitive microspheres contained around 8% of iron and the presence of magnetic nanoparticles did not have any significant effect on the temperature sensitivity of the composites thermally-sensitive magnetic particles.

Miniemulsion Polymerization Process

Miniemulsion[15] polymerization process has been routinely explored to encapsulate inorganic material as reported by various authors.[16,17] This process has been extended to the encapsulation of iron oxide and organic ferrofluid (iron oxide nanoparticles bearing oleic acid surfactant and dispersed in octane) as reported by

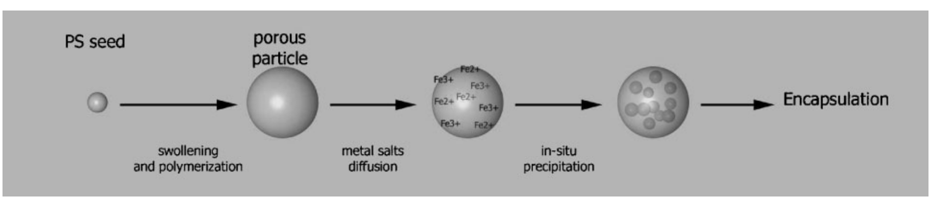


Figure 1.
Preparation of magnetic latex particles using a multistep process based on dispersion, swelling and seed polymerization processes.

Ramirez et al.[18] This organic ferrofluid is then dispersed in styrene monomer or in the presence of styrene miniemulsion for instant as illustrated below (Figure 2). In such approach, various interesting works have been recently reported in order to target the one-step preparation of functionalized mondisperse magnetic latex particles.[19–22]

Using miniemulsion polymerization, the obtained latexes are generally below 200 nm size and exhibit low iron oxide constant. Consequently, the magnetic separation velocity under applied magnetic field is low in and some cases above one 30 minutes.

Inverse Emulsion Polymerization

Inverse emulsion polymerization process has been explored in order to prepare functionalized magnetic latex particles. Such process was found to be efficient and appropriate for obtaining highly magnetic latexes for in vitro biomedical application as reported by Wormuth et al.[23] and more recently by Horak et al.[14] It is interesting to notice, that inverse emulsion has been expended to various polymerizations in dispersed media processes, such as that inverse microemulsion[24] as well as some multistep processes as reported.[25–27]

Multilayer and Stepwise Elaboration Processes

Stepwise process based on the adsorption of iron oxide nanoparticles onto oppositely charged polystyrene followed by seed encapsulation process has been first reported by Furusawa et al.[28] The final magnetic composite microspheres were narrowly size distributed and exhibited interesting magnetic properties such as acceptable separation velocity under applied magnetic field due to 15 wt. % iron oxide encapsulation.

Based on pioneering work reported by Furusawa et al.[28], Sauzedde et al.[29,30], this approach has been extended to the preparation of monodisperse thermosensitive magnetic latexes using the heterocoagulation concept as illustrated in Figure 3. Thus, hydrophilic latexes were obtained via adsorption of anionic superparamagnetic iron oxide nanoparticules on preformed cationic poly(*N*-isopropylacrylamide) (NIPAM) - polystyrene core-shell particles. In order to avoid the release of adsorbed iron oxide nanoparticles, the encapsulation

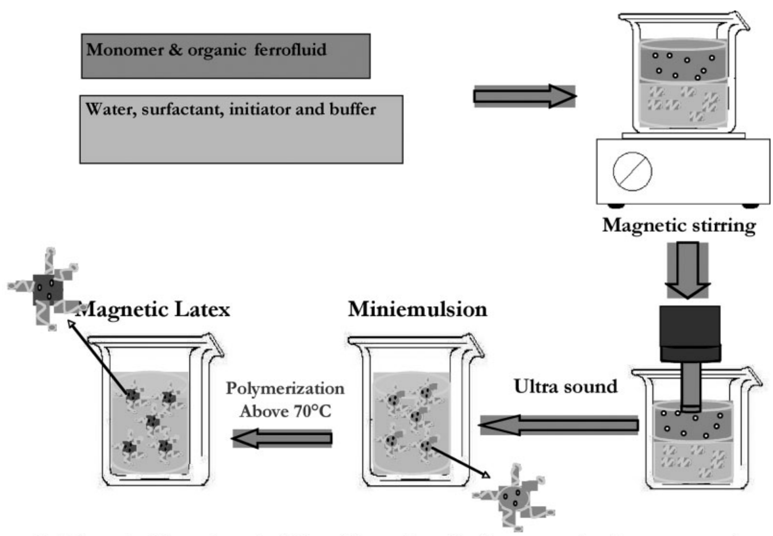


Figure 2.
Schematic illustration of miniemulsion polymerization process leading to magnetic latex particles. In this case, organic ferrofluid is dispersed in monomer solution before preparing miniemulsion.

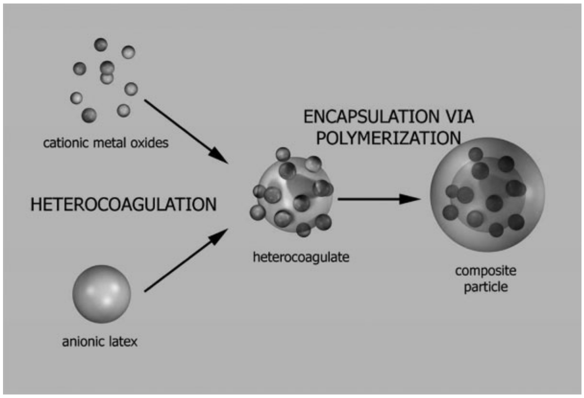
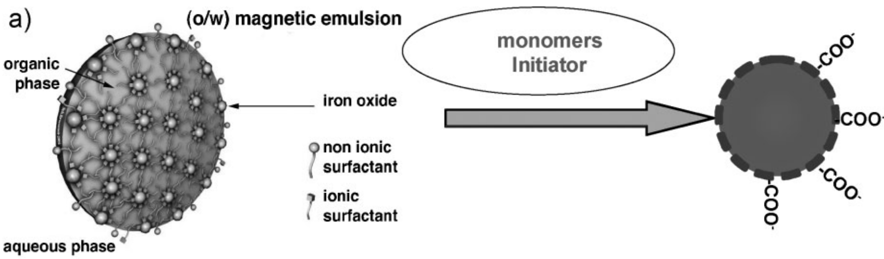


Figure 3.
Stepwise preparation of structured magnetic latex particles: Adsorption of iron oxide nanoparticles onto seed latex particles. The obtained heterocoagulates are encapsulated using monomers and crosslinkers.

has been performed via seeded radical polymerization using NIPAM monomer and a water-soluble crosslinker, *N,N′*-methylenebisacrylamide.

Seed Emulsion Polymerization

Recently, new process has been reported by Montagne et al.[31], who have been focussed on the preparation of magnetic latexes from



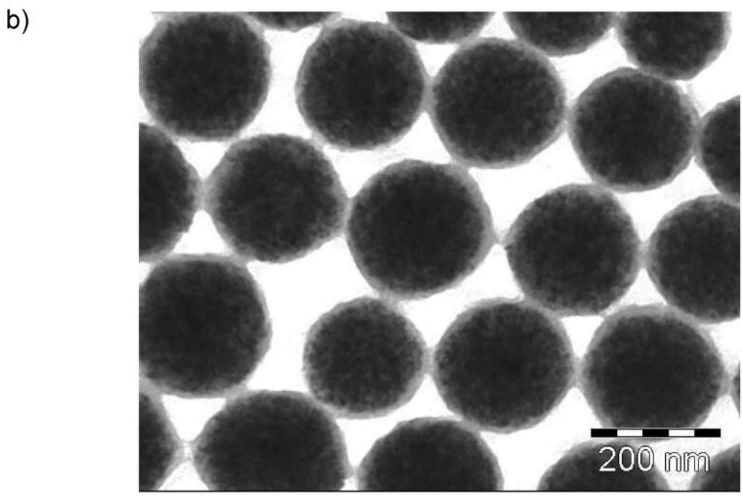


Figure 4.
(a) Conversion of o/w magnetic emulsion into magnetic latex via radical polymerization; (b), transmission microscopy analysis of magnetic latex particles.

oil-in-water (o/w) magnetic emulsion (Figure 4a). This work consists in the transformation of oil-in-water magnetic droplets into magnetic submicrometer latex particles via seeded emulsion-based radical polymerization. This process leads to highly magnetic latex particles (above 50 wt.% iron oxide content) bearing functionalized polymer shell. Their size distribution is controlled by the size distribution of the used o/w magnetic emulsion seed. The obtained magnetic latex particles have core-shell like morphology (Figure 4b). The functionalization of the particle surface has been performed during the encapsulation step using functionalized initiator or can be induced via second seed polymerization.

It is interesting to notice that, such process is able to prepare narrowly size distributed submicrometer and micrometer magnetic latexes.

Conclusion

Nowadays, to enhance the sensitivity of biomedical diagnosis, it is frequently required to extract, purify, and concentrate specifically targeted biomolecules often at low concentrations in a biological sample. Magnetic particles and particularly magnetic latexes are of paramount importance in biomedical diagnosis in vitro. The magnetic property is used to replace the centrifugation and filtration step (used with classical polymer-based particles). This property is also interesting for bionanotechnological applications based on microsystems and microfluidics. For these purposes, magnetic colloids appeared as the colloidal carrier of choice, exhibiting high specific area and easy use. A single magnet allows a fast and efficient separation of the particles from the continuous medium (total particles separation less than 5 minutes). Consequently, cells, proteins, DNA, RNA, bacteria, viruses have been adequately extracted using magnetic latex particles.

The preparation of magnetic latexes has challenged many research groups. Two major strategies have been reported: (i) using preformed non-magnetic particles as seed, and (ii) the incorporation of magnetic nanoparticles during the polymerization leading to the latex particles formation.

Required specifications of the ''ideal'' magnetic latex particles for bionanotechnological applications are the following:

- submicrometer particles of a high specific area (size generally 200 nm–1 μm);
- high magnetic properties for rapid magnetic separation (i.e. high magnetic content). The separation velocity should be less than 5 min;
- reactive or functionalized surface particles for biomolecules or receptor immobilization;
- high colloidal stability in biological medium;
- high chemical stability irrespective of pH and salinity of the medium;
- compatibility with the used biomolecules for a given application.

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