Yuanyuan Ma[1,2]
Tianxiang Chen[2]
Muhammad Zubair Iqbal[2]
Fang Yang[2,3,4]*
Norbert Hampp[4]
Aiguo Wu[2,3]
Liqiang Luo[1]*

[1]Department of Chemistry, College of Sciences, Shanghai University, Shanghai, P. R. China
[2]CAS Key Laboratory of Magnetic Materials and Devices & Division of Functional Materials and Nanodevices, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Ningbo, P. R. China
[3]Cixi Institute of Biomedical Engineering, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Ningbo, P. R. China
[4]Fachbereich Chemie, Philipps Universität Marburg, Marburg, Germany

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Review

Applications of magnetic materials separation in biological nanomedicine

As a result of their advantages for superparamagnetic properties, good biocompatibility, and high binding capacity, functionalized magnetic materials became widely popular over the past couple of decades, being applied on large scale in various processes of sample preparation for biomedicine. In this work, we perform an in-depth review on the current progress in the field of magnetic bead separation, discussing in detail the physical basis of this process, various synthesis methods and surface modification strategies. We place special focus of attention as well on the latest applications of magnetic polymer microspheres in cell separation, protein purification, immobilized enzyme, nucleic acid separation, and extraction of bioactive compounds with low molecular weight. Existing problems are highlighted and possible trends of magnetic separation techniques for biomedicine in the future are proposed.

Keywords:
Cell separation / Magnetic materials / Magnetic separation / Nucleic acid separation / Protein purification
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1 Introduction

Science 1970s, magnetic separation technology [1–10], as a physical processing technology [1–3, 11–14], has been widely used in the fields of decolorizing and whitening kaolin, coal desulfurization, ore selection, bioengineering, enzyme reaction engineering and so on. It has been successfully applied to the treatment of industrial wastewater [15–19] and domestic sewage [20]. Recently, separations using external magnetic fields have become mainstream in biotechnology, some of which are used for both protein purification as well as for flow cytometry [21–24]. The application of magnetic separation technology in biological field was first proposed by Robinson et al. in 1973 [25], who applied such strategies to cellulose magnetic microspheres to immobilize enzymes. Guesdon and Avrameas described a sandwich non-competitive enzyme-immunoassay procedure using antigen or antibody covalently linked to magnetic polyacrylamide-agarose beads [26]. The breakthrough in producing magnetic materials with a highly uniform size came in 1979 when John succeeded in making perfectly spherical monosized particles in a size range from 0.5 to 100 microns [27, 28].

With the development of nanotechnology, nanoscale magnetic materials have attracted considerable attention. This is because nanoscale materials manifest properties that are often different from their bulk state, which presents a series of advantages that can be exploited for various applications. Specifically, nanoscale magnetic materials exhibit properties among others that can reflect in-surface and quantum confinement, which can be used to finely tune their chemical reactivity as well as their mechanical, optical, electrical, and magnetic properties [29–31].

Magnetic nanoparticles (MNPs) present some attractive possibilities in biological nanomedicine due to many of their outstanding properties and the recent progress in the development of various synthetic methods [32–34]. Firstly, MNPs have controllable sizes ranging from a few nanometers up to

Correspondence: Professor Aiguo Wu, CAS Key Laboratory of Magnetic Materials and Devices & Division of Functional Materials and Nanodevices, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Ningbo, P. R. China
E-mail: aiguo@nimte.ac.cn

Abbreviations: **FIS**, Flow injection synthesis; **HGM**, high gradient magnetic; **IONPs**, iron oxide nanoparticles; **MBs**, magnetic beads; **MNPs**, magnetic nanoparticles; **NP**, nanoparticle; **QMS**, quadrupole magnetic flow sorter

*Additional Corresponding Author: Professor Liqiang Luo and Dr. Fang Yang **Email**: luck@shu.edu.cn; yangf@nimte.ac.cn

Color online: See the article online to view Figs. 2, 5–7, 9, 13, 16, 18–20 in color.

tens of nanometers, which make them active targeting agent when they are coated with biological molecules. Usually, NPs are smaller than or comparable to those of a cell (10–100 μm), a virus (20–450 nm), a protein (5–50 nm), or a gene (2 nm wide and 10–100 nm long) [35]. Secondly, magnetic properties of these MNPs translate to the fact that they obey Coulomb's law, and can hence be manipulated by an external magnetic field gradient and therefore by external magnetic fields [36–38]. Furthermore, the MNPs which exhibit superparamagnetism called 'magnetic beads' (MBs), the superparamagnetism behavior happens when the size is below certain critical dimensions depending on materials, for example, 35 nm for Fe_3O_4 [39]. One of the most important characteristic of superparamagnetic MNPs is no hysteresis, which provides a strong response to an external field and their magnetic moment vectors relax to random directions (i.e., an unmagnetized state) in the absence of an applied magnetic field. In this case, MNPs have no attraction for each other, thereby reducing the risk of particle aggregation [39, 40]. In summary, the above mentioned characteristics provide distinct features for their wide use at the time being in various applications belonging to the field of biomedical separation and purification, for instance, they are used as carriers for cell separation [41–45], nucleic acids isolation [46–51], protein purification [52–54], enzyme immobilization [55, 56], blood detoxification [57, 58], and rare cancer cell detection [59–61].

In this review, the history and recent advances in the development of magnetic separation, synthesis methods and surface modification of magnetic materials, physical and chemical properties of MBs, and their applications in nanomedicine are discussed in detail. The advantages and disadvantages of MBs separation technique in bio-nanomedicine are summarized. Finally, a summary and the future of MBs separation in bio-nanomedicine are given along with current challenges and possible solution is proposed.

2 The physical properties of magnetic separation in biological nanomedicine

The primary physical properties of MBs (magnetization curve, susceptibility, etc.) are very promising to explain the physical interpretation of magnetic separation process. MBs abbreviated as immunomagnetic beads represent, a newly developed technique in the field of immunology [62–64]. When a magnetic material is placed in a magnetic field of strength $\boldsymbol{H}$, the individual atomic moments in the material contribute to its overall response, and the magnetic induction can be expressed as [35, 65, 66]:

$$B = \mu_0 (H + M) \tag{1}$$

where $\boldsymbol{\mu_0}$ is the permeability of the free space, and $\boldsymbol{M}$ is the magnetization. Whereas $\boldsymbol{M}$ is:

$$M = \chi H \tag{2}$$

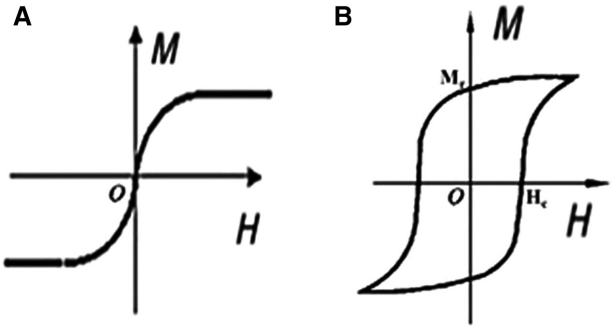


Figure 1. (A) Schematic magnetization loops of an ensemble of superparamagnetic particles and (B) ferromagnetic particles. This Figure is adapted with permission.

$\boldsymbol{\chi}$ is the magnetic susceptibility; the physical quantity that characterizes the properties of the magnetic medium [67–70]. For paramagnetic substances, $\boldsymbol{\chi} > 0$ (the range is about 10^{-6} to 10^{-1}), and for diamagnetic substances, the susceptibility $\boldsymbol{\chi} < 0$ (the range is about -10^{-6} to -10^{-3}). For ferromagnetics, $\boldsymbol{\chi}$ is large and is also related to $\boldsymbol{H}$ (i.e., there is a complex nonlinear relationship between $\boldsymbol{M}$ and $\boldsymbol{H}$). For isotropic magnetic media, $\boldsymbol{\chi}$ is a scalar and for anisotropic magnetic media susceptibility is a second-order tensor. Thus, the most of the material will be magnetized under the action of a magnetic field and exhibit certain characteristics of magnetism. This magnetism is not only represented by the magnitude of magnetization or magnetic induction, but also by the variation of magnetization with external magnetic field [35, 71].

In the state of magnetized saturation of ferromagnets, if the magnetic field intensity (H) gradually decreases from the maximum value, the magnetic induction intensity (symbolized as B) decreases along a slightly higher curve instead of original path [72, 73]. When H is equal to zero but B is not equal to zero, the change of B in the magnet lags behind the change of H. The phenomenon is called hysteresis. The retentivity of a substance is the maximum value (H_c) at which the residual flux density can be attained [74, 75] and the residual magnetization is referred to as M_r [76]. (See Fig. 1)

At small sizes (of the order of tens of nanometers or less), a material will embody a superparamagnetic state. The magnetization curve of an ensemble of such superparamagnetic particles in Fig. 1A is hysteresis-free (at least, for frequencies that are not too high). In the field of biological nanomedicine, the biomaterials tagged by superparamagnetic particles can be removed from a matrix using magnetic field and it does not causes agglomeration. Hence, it is a good phenomenon, an external magnetic field to control the presence or absence of magnetic interaction [77, 78].

Biocompatible MB towards magentic separation is a good way to separate specific biological entities from their native environment, so that concentrated samples can be prepared for subsequent analysis or other further uses [35]. The separation process can be divided into two steps: (1) functionalization of MBs to target biological entities, (2) using a magnetic separation device in which there is a magnetic field gradient

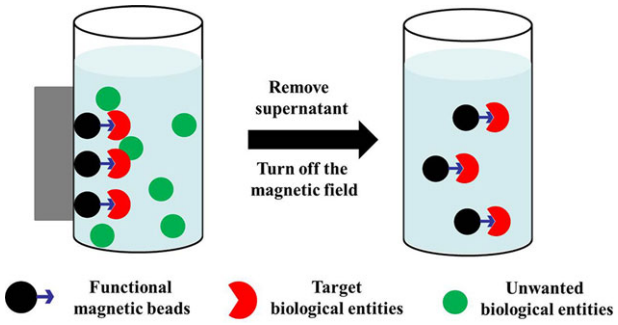


Figure 2. The standard method of magnetic separation used in biological nanomedicine.

based on fluid isolated from these labeled target biological entities. In this device, the magnetic force equation can be expressed as:

$$F_m = V_m \Delta\chi \nabla\left(\frac{1}{2}B \cdot H\right), \quad (3)$$

in which the magnetic force is related to the differential of the magnetostatic field energy density, and $\frac{1}{2}B \cdot H.\Delta\chi = \chi m - \chi w$ is the effective susceptibility of the particle relative to the water [79], and $\boldsymbol{V_m}$ is the volume of the particle, equating hydrodynamic drag and magnetic forces, and writing $V_m = \frac{4}{3}\pi {R_m}^3$.$R_m$ is the radius of the magnetic particle.

This force $\boldsymbol{F_m}$ needs to overcome the hydrodynamic drag force acting on the magnetic bead in the flowing solution, according to the following formula:

$$F_d = 6\pi\eta R_m \Delta v, \quad (4)$$

where $\boldsymbol{\eta}$ is the viscosity of the medium surrounding the target biological entities (e.g., water), and $\Delta v = v_m - v_w$ is the difference in velocities of the target biological entities and the water. In Fig. 2, we depict the standard method of using magnetic separation in biological nanomedicine. A solution containing target and unwanted biological entities flows continuously through a region of strong magnetic field gradient, often provided by packing the column with steel wool, which captures the target particles [79]. Thereafter, the target particles are recovered by removing the field and flushing through with water [80,81]. Therefore, these physical properties of MBs are meaningful to design different instruments for magnetic separation in nanomedicine.

3 Synthesis and surface modification of magnetic materials

3.1 Synthesis of magnetic materials

Magnetic materials include various elements such as Fe, Co, Ni powder or oxide and their alloys [82–85]. Iron-based materials such as Fe_3O_4 and α-Fe_2O_3 are widely used in the field of biological nanomedicine because of their facile synthesis, controllable magnetization, superparamagnetism, and low toxicity [86–88].

Ever since magnetic micro-/nanoparticles (NPs) have been discovered, the development of novel preparation methods and applications has been the key points of connected research. The preparation methods that are currently available can be divided into physical and chemical methods [89]. Therefore, in the following sections, we will summarize some important examples regarding the synthesis of iron-based MBs.

3.1.1 Physical methods for the preparation of magnetic micro- and NPs

The main physical method for the preparation of magnetic micro- and NPs mainly revolve around mechanical attrition; the method by which magnetic materials are being ground to a nanometer size. It is easy to operate but has long production cycle [90–92].

Among the notable efforts for physical preparation, in 2004, Janot and co-workers used a planetary ball mill equipped with stainless steel bottles to grind iron powder into water at different times, according to the following reaction [93]:

$$2\,Fe + 3H_2O \longrightarrow \gamma\,Fe_2O_3 + 3H_2 \quad (5)$$

In 2004, Goya et al. mixed magnetite powder (99.99%, mean particle size about 0.5 μm) with methanol in a closed-loop environment protected by hydrogen. Fe_3O_4 particles with an average diameter of 7–10 nm were obtained by controlling the content of methanol and milling time. In Fig. 3, we illustrate the schematic diagram of the ball milling experiment [94], where handling the particle size and distribution of Fe_3O_4 NPs through ball milling are depicted.

In 2009, Hajra et al. subjected magnetite powder of 99% purity taken in steel vials of 80 ml volume to grinding operation in a Fritsch Pulverisette 5 Planetary ball Mill under ordinary atmosphere, and the NPs comprising of magnetite (Fe_3O_4) core – hematite (α-Fe_2O_3) shell with mean diameter around 9 nm were obtained [95]. (see Fig. 4)

Ball milling is the dominant synthesis technique to prepare MNPs. The principle of MNPs preparation is based on mechanochemistry, i.e., introducing mechanical energy through different modes of action to change the physicochemical properties and structure of the object under stress. The mechanical force provides energy to break the chemical bonds, produces new surfaces, and creates lattice defects inside the crystal structure. It also increases the internal energy of the substance to stimulate the chemical reaction by developing an unstable chemical active state. Generally, the nanoscale ferrite powder is obtained by grinding metal iron powder under different conditions. Goya et al., [94] obtained a series of Fe_3O_4 NPs with different sizes by argon protection and controlling the methanol content and milling time. While Hajra et al. ensured the diffusion of atmospheric oxygen in the pores between the particles in the air atmosphere,

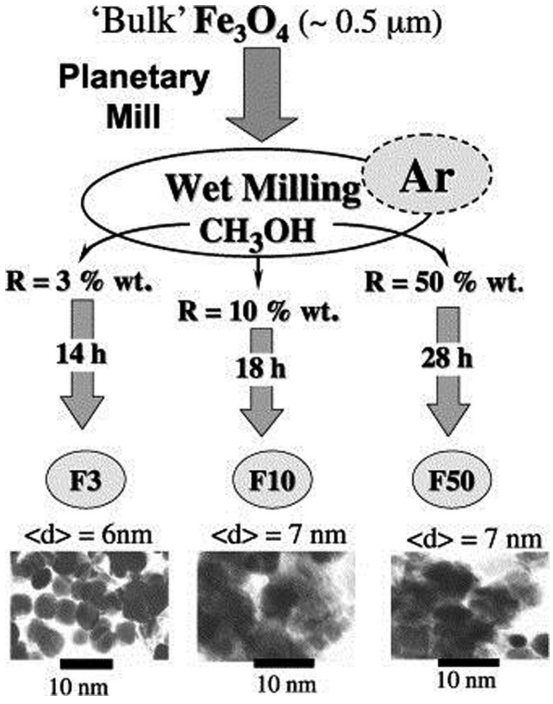


Figure 3. Schematic diagram of the ball milling experiment performed to obtain series of magnetite-based dispersions with different average crystallite sizes and concentrations (scale bar: 10 nm). This figure is adapted with permission [94].

resulting in phase transition and the formation of α-Fe_2O_3 shell [95].

3.1.2 Chemical methods for the preparation of magnetic micro- and NPs

Various chemical methods for the synthesis of iron oxide nanoparticles (IONPs) primarily involve co-precipitation [96–100], thermal decomposition [101–106], hydrothermal/solvothermal synthesis [107–110], microemulsion/nanoemulsion-based synthesis [107–114], flow injection method [115, 116], and aerosol/vapor-phase method, but also other strategies.

The equipment used for synthesizing MBs by chemical methods are simple to utilize, and these synthetic method can fabricate controllable, highly stable, and monodispersed MNPs [1–3, 6]. In the following paragraphs several chemical synthesis methods are discussed.

3.1.2.1 Coprecipitation method

The co-precipitation method is a simple method to synthesize metal NPs by liquid phase chemical reaction. It can be used to achieve highly dispersed Fe_3O_4 NPs in large quantities, and it has the advantages of simple operation, low cost, and well established and popular synthesis method and technique [117–119]. This fabrication technique is based on the chemical reactions carried out in an aqueous solution with the process of both the nucleation and growth of iron hydroxide nuclei. The type of precursor, the temperature of the medium, the pH value, and the ionic strength of the medium affect the size, shape, and composition of MNPs [30]. The principle of chemical co-precipitation can be expressed as follows:

$$M^{2+} + 2Fe^{3+} + 8OH^- \longrightarrow MFe_2O_4 + 4H_2O \quad (6)$$

where M can be Fe^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+}, and Ni^{2+}. The complete precipitation should be expected at pH levels between 8 and 14, with a stoichiometric ratio of Fe^{3+} and M^{2+} is 2:1 [120]. Fe_3O_4 NPs are not very stable under ambient conditions, and are easily oxidized to and become maghemite NPs or dissolve in an acidic medium.

Michael et al. prepared the superparamagnetic iron oxide NPs with different core sizes and polymer content (see Fig. 5) using the co-precipitation approach [121]. Shen and coworkers [122] developed a co-precipitation synthesis method with precise size control of exceedingly small magnetic iron oxide NPs below 5 nm (i.e., 1.9, 2.6, 3.3, 3.6, 4.2, 4.8, and 4.9 nm) and found that 3.6 nm is the optimal particle size for

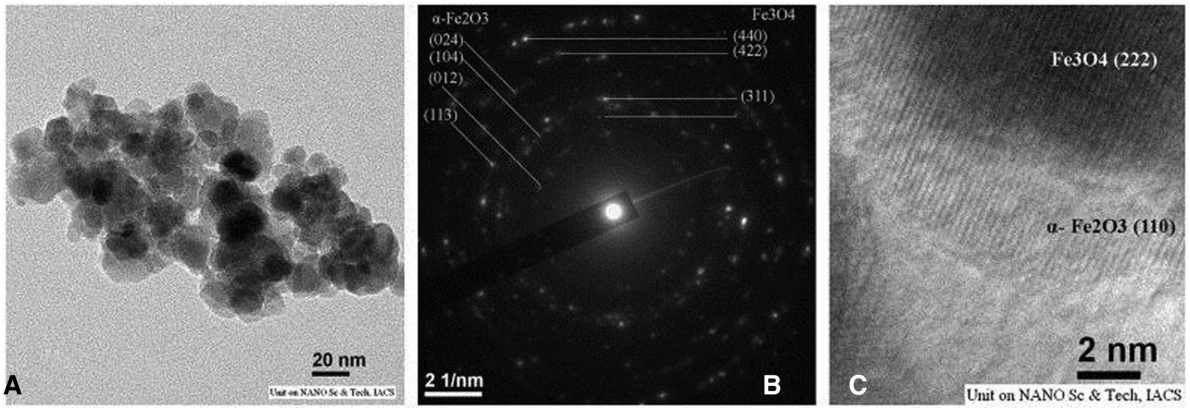


Figure 4. (A) Transmission electron micrograph for a specimen subjected to a milling operation for 6 h (scale bar: 20 nm). (B) Electron diffraction pattern obtained from Figure 4(A). (C) High resolution electron micrograph for a specimen grinding for 6 h (scale bar: 2 nm). This figure is adapted with permission [95].

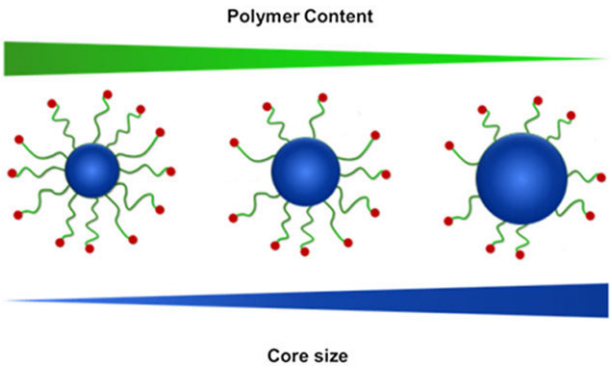


Figure 5. Decreasing amount of polymer used in a co-precipitation reaction results in superparamagnetic iron oxide NPs with similar hydrodynamic diameter. Larger iron oxide core size and less polymer in the outer shell. This figure is adapted with permission [121].

exceedingly small magnetic iron oxide NPs to be utilized as T_1-weighted MRI contrast agent.

Coprecipitation method is generally based on the mixture of chemical raw materials in a solution by addition of appropriate precipitating agent. Therefore, the various precursors in the solution are mixed uniformly and co-precipitated according to stoichiometric ratio, or calcination and decomposition after reaction precipitation of intermediate products in the solution. This method has advantages of simple synthesis steps, mild conditions, and high yield. Water soluble NPs can be obtained directly which are valuable in biomedical applications. But how to prevent the agglomeration of NPs is the major concern of this method. Monodispersed NPs can be obtained by using stabilizers. Shen et al. used poly acrylic acid as stabilizer to improve the stability of ultra-small ferric oxide synthesized by co-precipitation method [122]. Michael et al. explored the effects of fluorescein isothiocyanate diethylaminoethyl and the amount of precursors on the physical and chemical properties of ultra-small ferric oxide [121].

3.1.2.2 Thermal decomposition method

This method yields individuall and dispersible IONPs with excellent monodispersity, good size control, narrow size distribution, and good crystallinity. The reaction temperature for the preparation of IONPs through thermal decomposition using iron compounds or iron precursors (such as $Fe(CO)_5$ and $Fe(acac)_3$) is usually 300°C equal to the boiling temperature on the surfactant, which is critical to control the nucleation and growth of NPs from solution [30, 123]. Moreover, the high temperature can increase the solubility of most ionic species, which can result in uniform particles with narrow size distribution.

Cheon et al. used a one-pot thermal decomposition method, involving a metal chloride (MCl_2, M = Zn^{2+}, Mn^{2+}, and Fe^{2+}) and iron ttris-2,4-pentadionate [$Fe(acac)_3$] in the presence of oleic acid, oleylamine, and octyl ether as shown in Fig. 6 [124].

Hyeon and co-workers investigated the thermal decomposition reaction of the precursor compound and fitted it to an autocatalytic process [125]. (see Fig. 7)

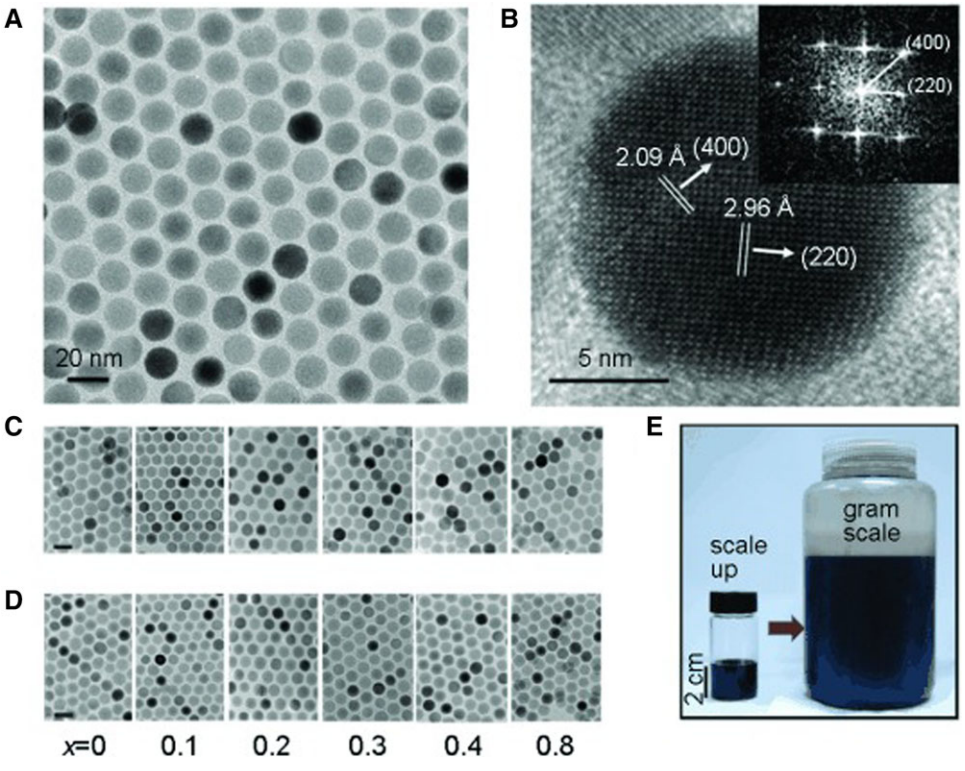


Figure 6. (A) TEM image of 15 nm $(Zn_{0.4}Fe_{0.6})Fe_2O_4$ NPs. (B) High-resolution TEM image of 15 nm $(Zn_{0.4}Fe_{0.6})Fe_2O_4$ NPs. The inset shows the FFT pattern.(c,d) TEM images of 15 nm$(Zn_xMn_{1-x})Fe_2O_4$ (C) and $(Zn_xFe_{1-x})Fe_2O_4$ (D) NPs (scale bar: 20 nm). (E) Photograph showing that the synthesis of 15 nm $(Zn_{0.4}Fe_{0.6})Fe_2O_4$ NPs can be scaled up to ca. 10 g. This figure is adapted with permission [124].

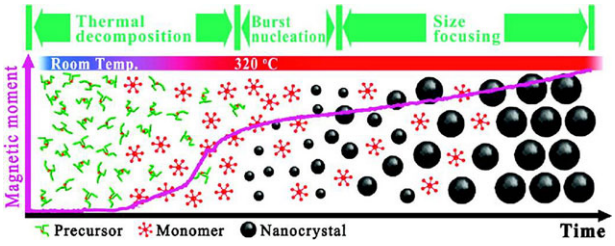


Figure 7. Effect of reaction temperature and reaction time on size, morphology and magnetic properties of iron oxide. This figure is adapted with permission [125].

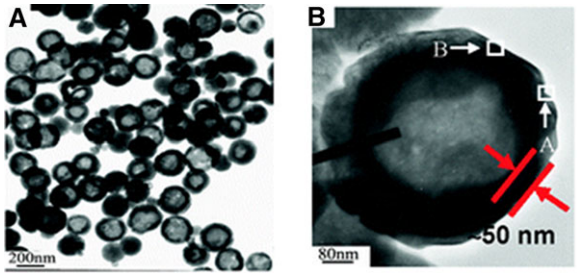


Figure 9. (A) TEM image of Fe_3O_4 hollow spheres (scale bar: 200 nm). (B) Enlarged TEM image of an individual hollow sphere (scale bar: 80 nm). This figure is adapted with permission [133].

The high crystallinity, homogeneity, and uniform distribution of IONPs obtained by thermal decomposition are considered to be the best among all methods. The saturated magnetization of iron oxide obtained by pyrolysis is higher than that of other methods with the same particle size. The 15 nm $(Zn_{0.4}Mn_{0.6})$ Fe_2O_4 NPs synthesized by Cheon et al. have very high saturation magnetization (175 emu/g) at 300 K [124]. However, pyrolysis at high temperature is not widely used as compared with other synthetic methods because of the harsh synthetic conditions, high cost, and complicated steps. The reaction requires high temperature which is also one of the serious drawbacks of this method.

3.1.2.3 Hydrothermal and solvothermal synthesis

Hydrothermal reactions are performed in aqueous media in reactors or autoclaves with high pressure and temperature, and the solvothermal synthesis is developed by hydrothermal synthesis, generally in the medium of organic solvents or mixed solutions of organic solvents and water [126–130]. Among the notable efforts for hydrothermal and solvothermal synthesis, Yin et al. had successfully developed a high-temperature solution-phase hydrolysis procedure for the synthesis of magnetite NPs with good controllability and size distribution, high crystallinity, and high water solubility [131]. (see Fig. 8)

Fu et al. used a template-free solvothermal system to synthesize uniform-sized, monodisperse, and single-crystal magnetite hollow spheres with a diameter of 200-300 nm and a shell thickness of ~50 nm [132] (see Fig. 9).

Hydrothermal method is a very popular synthesis process for obtaining high crystallinity, controllable particle size, and uniform particle size distribution after high temperature pyrolysis. This method is the most widely used for the preparation of promising NPs. Hydrothermal approach is superior to other mentioned methods due to its facile manipulation, less expensive, safe handling, and simple equipment.

3.1.2.4 Microemulsion/nanoemulsion-based synthesis

Emulsions are examples of kinetically stable multiphase colloids with a droplet size ranging from 20 nm to tens of millimeters. When the entire size distribution of an emulsion is below 80 nm, it gains advanced properties compared to conventionally sized emulsions including: optical transparency, high colloidal stability, and a large interfacial area to volume ratio. Such emulsions are often called nanoemulsions or miniemusions [133].

A microemulsion media is formed on addition of an aliphatic alcohol (co-surfactant) to an ordinary emulsion. There are three types of microemulsions: water-in-oil (w/o), oil-in-water (o/w), and bicontinuous microemulsion [134].

In a different approach, Housaindokht and co-workers synthesized hematite NPs via a reverse microemulsion route at room temperature. The microemulsion system, which contained water, chloroform, 1-butanol, and surfactant, was combined with iron nitrate solution to result in IONPs precipitation [134] (see Fig. 10). In this system of monomer emulsifier dissolving in diluted or oil solution, when its concentration

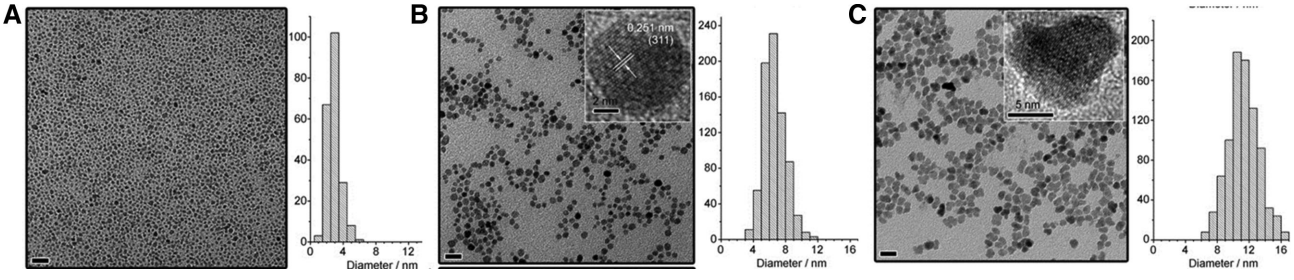


Figure 8. Representative TEM images and the corresponding measured size distributions of Fe_3O_4 nanocrystals with an average diameter of (A) 2.9 nm, (B) 6.6 nm, and (C) 11.3 nm, respectively (scale bar: 20 nm).This figure is adapted with permission [131].

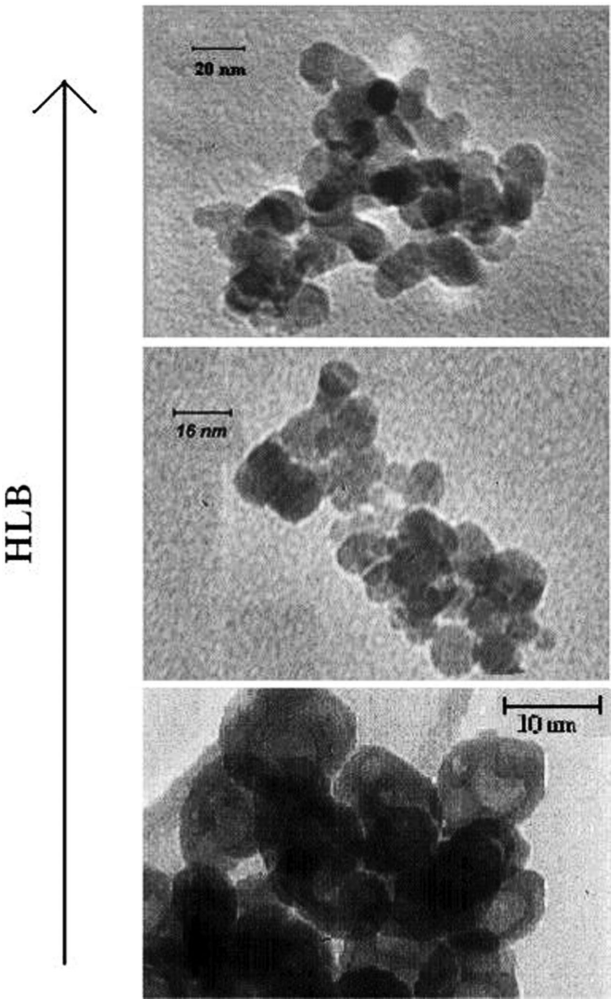


Figure 10. TEM images of prepared iron oxide NPs. The effect of HLB of the surfactant on the morphology of the samples was shown (scale bars: 20, 15, and 10 nm, respectively). This figure is adapted with permission [134].

exceeds a certain limit which means CMC, the molecules of the emulsifier are spontaneously associated with aggregate.

3.1.2.5 Flow injection synthesis (FIS)

FIS represents a novel method based on continuous or segmented mixing of reagents under a laminar flow regime in a capillary reactor for preparation of NPs with narrow size distribution. FIS takes advantages of a flow-injection analytical technique [22].

Flow injection technique is a new synthesis method. It has the advantages of control over morphology and size with high reproducibility. Zagorodni and co-workers developed a novel synthesis method, whose concept was demonstrated by generating magnetite NPs [115] (see Fig. 11). They proved that the size distribution can be manipulated and reduced by changing the concentration and velocity of reagent.

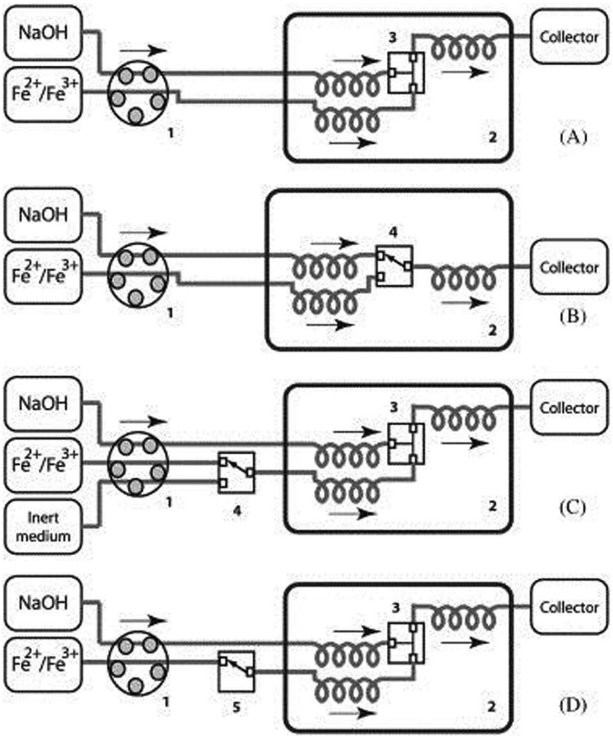


Figure 11. Different flow-injection schemes: (A) continuous injection; (B) flow segmentation by injection of reagents in turn; (C) flow segmentation with an inert medium; (D) flow segmentation by injection of one reagent in continuous flow of second reagent. (1) multi-channel peristaltic pump; (2) Thermostat; (3) t-injector; (4) computer-operated switching valve; (5) Computer-operated on–off valve. Helixes indicate thermostated capillaries. This figure is adapted with permission [115].

3.2 Surface modification of magnetic materials

Novel types of MNPs exhibiting excellent properties in terms of high specific surface activity and biocompatibility have been reported. In order to preserve their specific magnetic properties, and to protect these NPs from both oxidation and agglomeration, the application of the encapsulation procedure has been proposed [135]. A MNP can be copolymerized and modified to give a variety of reactive functional groups(such as -OH,-COOH,-CHO,-NH_2) on their surface, and then coupled with many biological macromolecules such as cells, enzymes, proteins, antibodies and nucleic acids, so that it can be specifically targeted to separate substances [136–143].

In the following part of this review, we will summarize the research progress in surface modifications of MBs containing the following three categories: i) modifications by organic small molecules such as coupling agents and surfactants; ii) modifications by organic polymers including natural biopolymers, synthesized polymers and the compounds of the two kinds of polymers; iii) modifications by inorganic nanomaterials such as SiO_2, Au, and Ag.

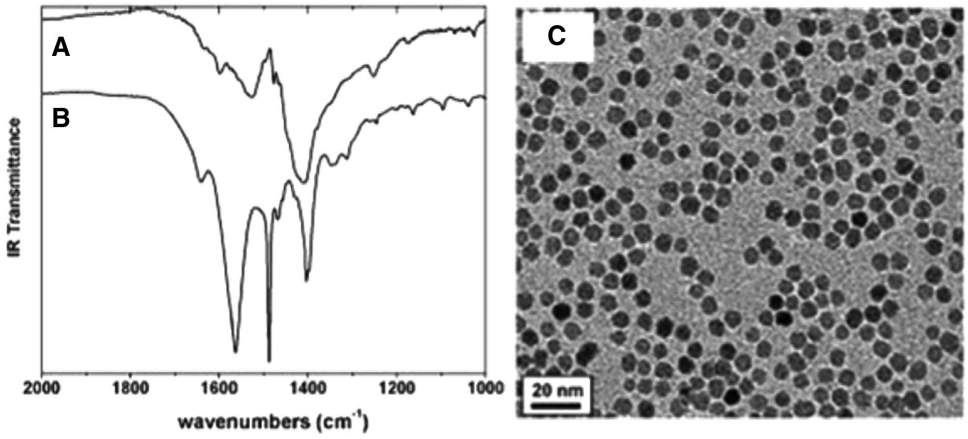
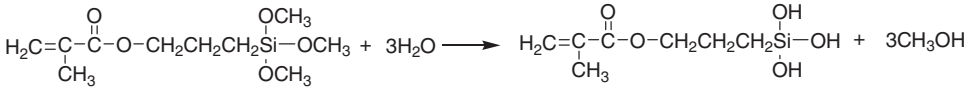


Figure 12. (A) Infrared spectrum of the as-synthesized hydrophobic 6 nm Fe_3O_4 NPs, (B) infrared spectrum of tetramethylammonium 11-aminoundecanoate-coated 6 nm Fe_3O_4 NPs, and (C) TEM bright field image of the 6 nm Fe_3O_4 NPs (scale bar: 20 nm) in (B) deposited from water dispersion on amorphous carbon-coated copper grid. This figure is adapted with permission [143].

3.2.1 Small organic molecule modification

The small organic molecules for modifying MBs mainly consist of agents and surfactants [144]. In the case of the unstable MBs prepared by the co-precipitation method, these can be first dispersed in water, and then modified by adding organic small molecules. Another approach to couple MNPs with small organic molecule consists of adding such molecules in the process of preparing MBs; for the lipophilic MBs, they can be modified by further special interactions between ornaments or ligands exchange reactions are utilized to achieve water-solubility and biocompatibility of NPs [145,146]. In the following paragraphs, we discuss a series of approaches for modifying MNPs to include small organic molecules.

MBs with an average diameter of 18 nm were prepared by Sheng et al. using co-precipitation method and treated with silane coupling agent KH570 [142]. The C=C group was introduced into the surface of MBs, which could be further copolymerized with other unsaturated monomers. The underlying reactions can be expressed as below:



(7)

(8)

Sun et al. substituted oleic acid and oleamine on the surface of NPs with a bipolar surfactant to obtain MNPs with good dispersibility and solubility [143]. (see Fig. 12)

3.2.2 Organic polymer modification

Biocompatible polymers used to modify MBs can be classified into the two categories: (i) natural macromolecules (such as glucan, chitosan, and amino acids); (ii) synthetic macromolecules (such as PEG, PVP, PS, PMMA, and PLA). At present, the research on organic polymer modified MBs mainly focuses on the two aspects: (i) the synthesis of polymer MBs with high content and uniform size; (ii) the development of polymer/MBs composite particles with a clear core-shell structure [147–151]. Notable efforts concerning these research directions are discussed in the following paragraphs.

Hyeon et al. developed chitosan oligosaccharide-stabilized ferromagnetic iron oxide nanocubes (Chito-FIONs) as an effective heat nanomediator for cancer hyperthermia [152]. (see Fig. 13)

The NPs system (NP-CP-PEI) is made of a superparamagnetic iron oxide nanoparticle by Zhang et al., which enables magnetic resonance imaging, coated with a novel copolymer (CP-PEI) comprised of short chain polysaccharide, chitosan (CP), which allows efficient loading and protection of the nucleic acids [153].

3.2.3 Modification of inorganic materials

Most common inorganic materials to modify MBs include silicon dioxide [154–156], gold [157–159], and silver [160–162]. These materials are used to obtain composite MNPs with a core-shell structure. The formation of MNPs can exhibit richer and better physical and chemical properties compared to other MNP variants. Further on we discuss a series of remarkable efforts in this direction.

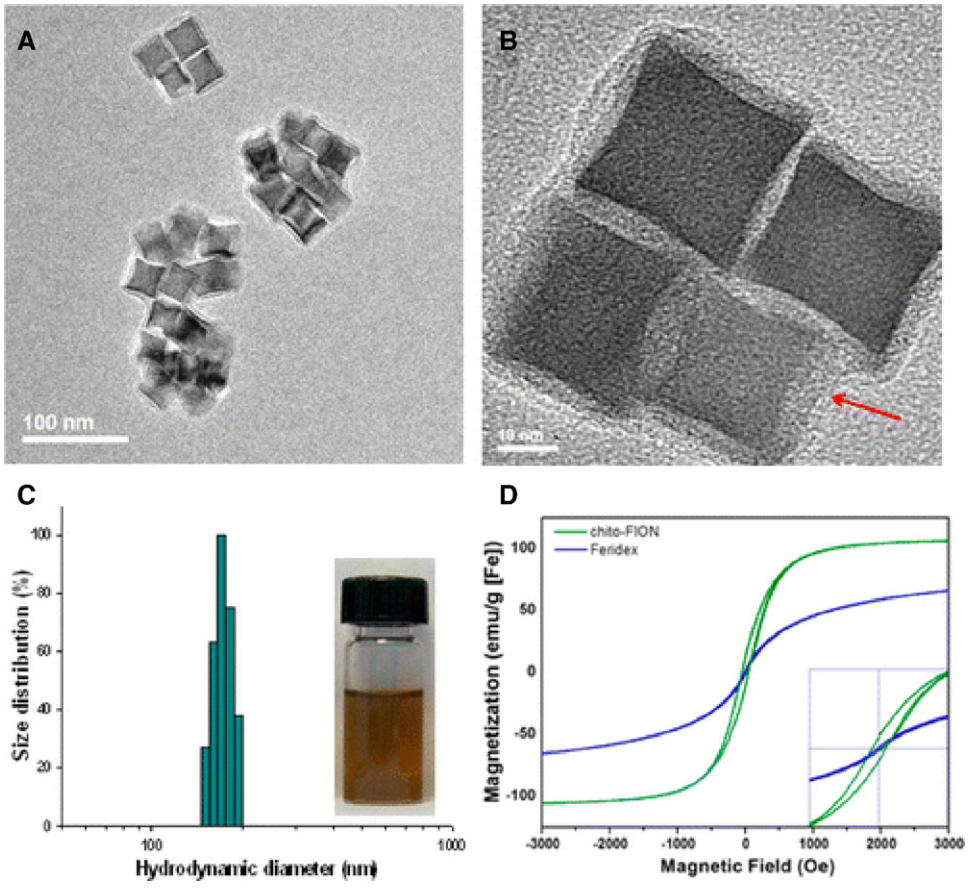


Figure 13. (A-B) TEM images of Chito-FIONs (red arrow indicates the presence of the polymer coating layers (scale bars: 100 nm and 10 nm, respectively). (C) Hydrodynamic diameters of Chito-FIONs in phosphate-buffered saline solution. Inset: Photograph showing the aqueous dispersion of Chito-FIONs. (D) Field-dependent magnetization curves of Chito-FIONs and Feridex. This Figure is adapted with permission [152].

Hyeon et al. presented discrete, monodisperse, and precisely size controllable core-shell mesoporous silica NPs smaller than 100 nm by using single Fe_3O_4 nanocrystals as core (designated as Fe_3O_4@mSiO_2) [163]. (see Fig. 14)

Silva et al. obtained such core-shell composite MNPs by dispersing the Fe_3O_4 MNPs exhibiting a particle size of about 9 nm into an emulsion firstly, followed by a reduction of the Ag^+ by glucose. The approach resulted in a new class of silver-coated MNP [164] (see Fig. 15).

4 The applications of magnetic materials as separation vectors in biological separation

MBs own small particle size and large specific surface area, and therefore they exhibit good suspension stability and large ability for efficient desire targeting to be coupled with the aim of products efficiently [21–24]. Because of their superparamagnetism, in the case of MBs, the separation of solid and liquid under the action of external magnetic field is very simple, which can save time without involving complicated operations such as centrifugation and filtration [165, 166]. Therefore, MNPs have broad-application prospects in cell separation [167–170], classification, protein purification [171–174], nucleic acid separation [175–178], and extraction of bioactive compounds with low molecular weight. In the following sections, we perform a brief survey of these topics.

4.1 Cell separation

The separation and sorting of biological cells is critical for variety of biomedical applications including diagnostics, therapeutics, and fundamental cell biology. As in many applications, the samples of interest consist of heterogeneous cellular populations that are in culture or that comprise a tissue, techniques of isolating specific cells are essential for understanding how cells function and respond to various stimuli [178].

Biologically active adsorbents or other ligands (such as antibodies, exogenous coagulins) attached to MBs can be specifically bound to target cells by means of external magnetic fields [21–24]. Compared with the commonly used cell separation methods, these approaches are simple, fast, efficient, and safe.

There are two main ways to isolate cells from MBs: one is to isolate target cells directly from the mixture of cells, which is called 'positive phase separation' or 'positive selection'; the other is to remove unrelated cells by MBs and enrich and purify target cells, which is called 'negative phase separation' or 'negative selection' (see Fig. 16).

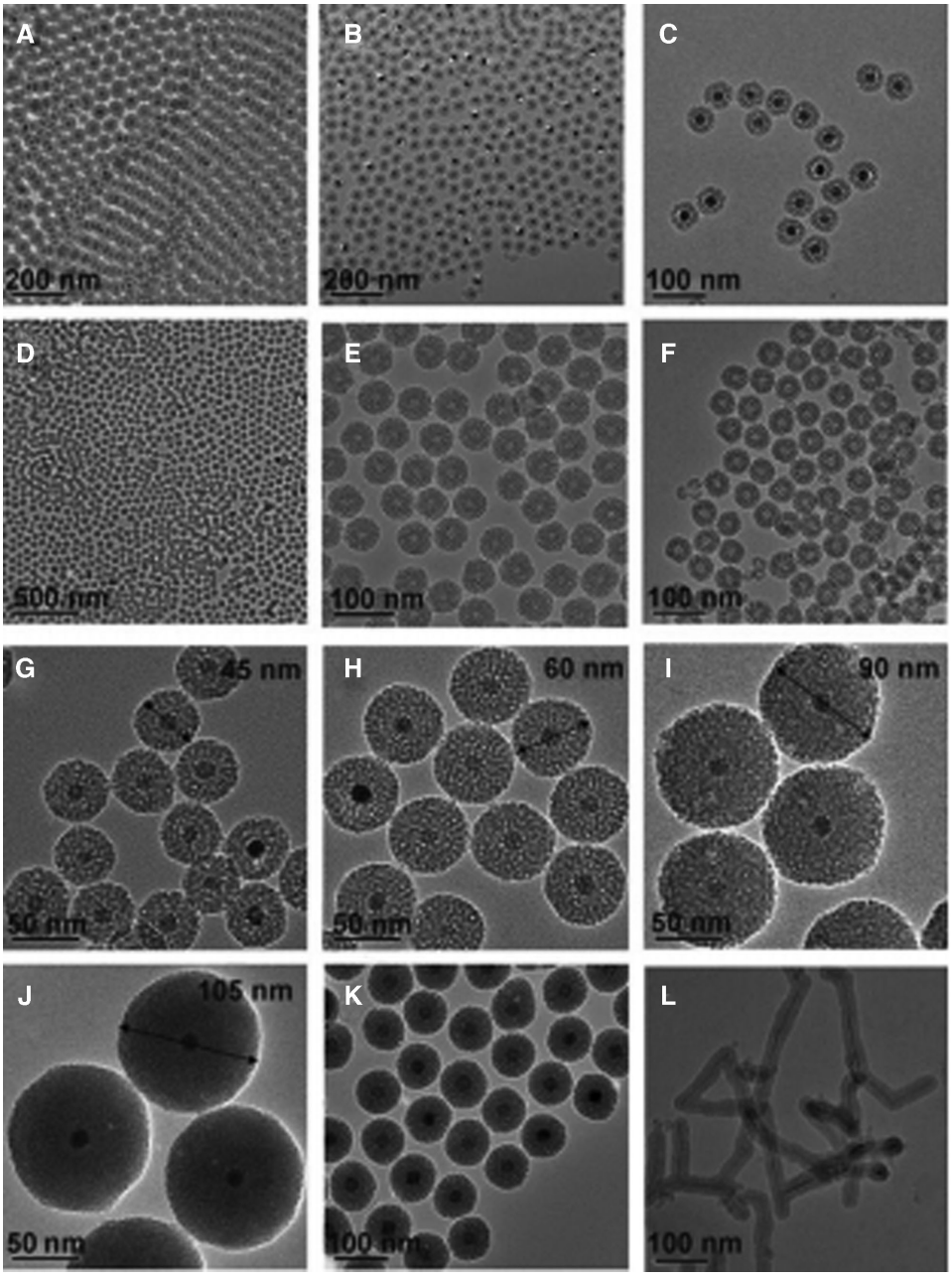


Figure 14. TEM images of core–shell and hollow mesoporous silica NPs. (A) 53 nm Fe_3O_4@$mSiO_2$ with 15 nm core (scale bar: 200 nm). (B, C) 45 nm Fe_3O_4@$mSiO_2$ with 22 nm core (scale bars: 200 and 100 nm, respectively). (D, E) H-$mSiO_2$ (scale bar:100 nm) and 100 nm from (A). (F) H-$mSiO_2$ from (scale bar: 100 nm) (B, C). Different sized uniform 15 nm Fe_3O_4@$mSiO_2$ of (G) 45 nm (scale bar: 50 nm), (H) 60 nm (scale bar: 50 nm), (I) 90 nm (scale bar: 50 nm), and (J) 105 nm (scale bar: 50 nm). (K) MnO@$mSiO_2$ (scale bar: 100 nm). (L) a-FeOOH@$mSiO_2$ (scale bar: 100 nm). This figure is adapted with permission [163].

Molday and co-workers were the pioneers of separating cells with MBs. They labeled magnetic polymer microspheres containing carboxyl groups on the surfaces of NPs with fluorescent dyes, activated by carbodi-imide, coupled with antibodies or foreign lectins on their surfaces, and successfully isolated red blood cells and B lymphocytes [179].

Other related efforts are connected to magnetic-activated cell sorting which is a high gradient magnetic (HGM) cell separator specially designed for antibody conjugated nanomaterials (20–100 nm). This system was first developed by Biotec in Germany in 1990. In this approach cells stained sequentially with biotinylated antibodies, fluorochrome-conjugated avidin, and superparamagnetic biotinylated-microparticles (about 100 nm diameter) are separated on HGM columns. Unlabeled cells pass through the column, while labeled cells are retained. Magnetically separated cells can be analyzed by fluorescence microscopy, flow cytometry, or sorted by fluorescence-activated cell sorting without further treatment. Magnetic tagging and separation do not affect the cell viability and proliferation [180]. In a HGM field, generated in a column of steel wool which is inserted into an external magnetic field, cells labeled with superparamagnetic heads will attach to the matrix. (see Fig. 17A and Fig. 18). Unlabeled cells are eluted. The labeled cells can be eluted when the column is demagnetized by removal from the external magnetic field.

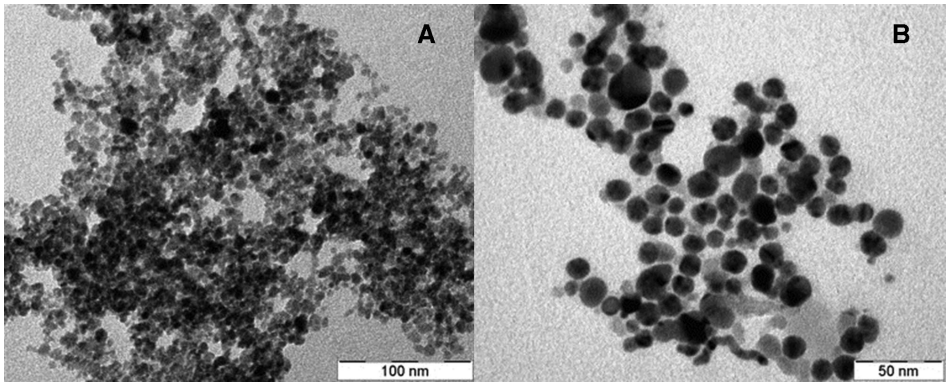


Figure 15. TEM images of (A) Magnetite NPs (scale bar: 100 nm) and (B) NPs of Fe_3O_4@Ag (scale bar: 50 nm). This figure is adapted with permission [164].

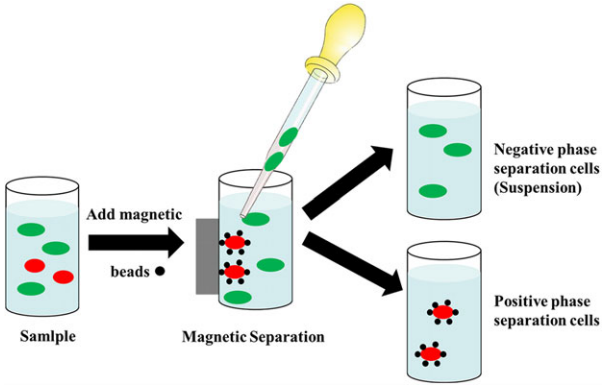


Figure 16. Positive and negative methods for magnetic separation of cells.

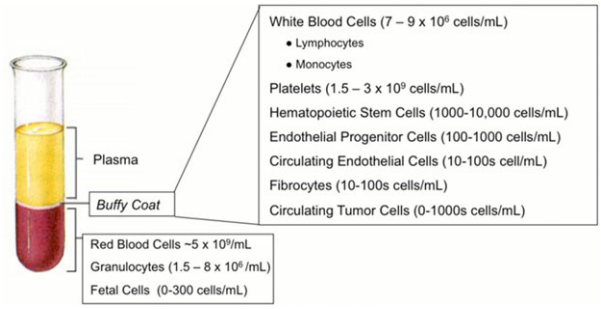


Figure 18. Blood is a rich source of cells for tissue engineering, diagnostics, and fundamental biology, containing several rare populations. This figure is adapted with permission [181].

Another application of MBs in biomedicine refers to blood which represents a biological tissue extremely rich in information yet easily accessible, a complex blend of cells. The accurate analysis of blood character and condition requires isolation of a few desired cells.(see Fig. 18). So cell separation in blood is a very important application in biological nanomedicine. Masayuki Nakamura et al. developed a quadrupole magnetic flow sorter (QMS) to facilitate high-throughput binary cell separation in order to separate a breast cancer cell line from human blood. The QMS was designed specifically for high-throughput cell separations, whereas the dipole magnetic flow sorter was designed to fractionate positively labeled cells into subfractions on the basis of their differences in mobility. In addition to high-throughput cell sorting, other advantages of the QMS are the feasibility of scale-up and the relatively low cost. A schematic drawing of the QMS is shown in Figure 17B. The cell suspension is fed from the inlet (a') by a syringe pump. The cells are subjected

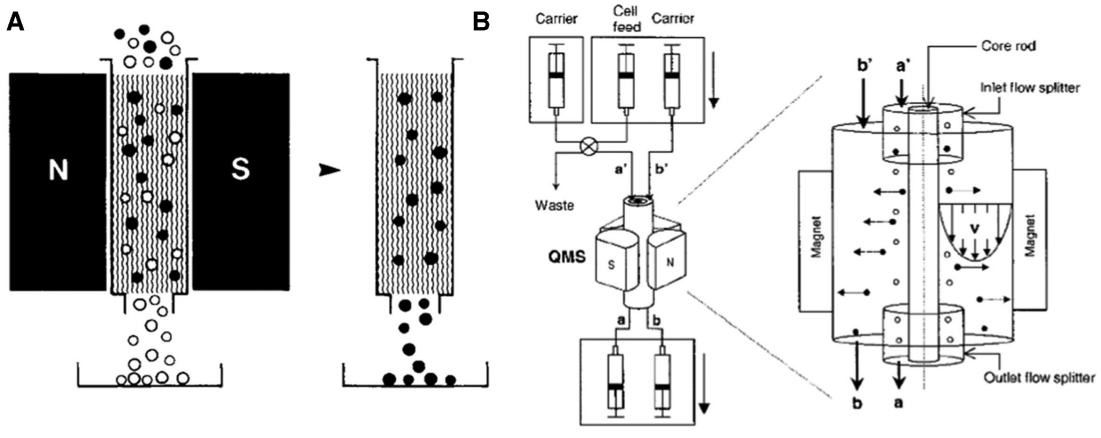


Figure 17. Conventional magnetic-activated cell sorting platform (A) standard quadrapole magnetic flow sorting and (B) deflection of magnetic moieties within a continuous stream flow stream. This figure is adapted with permission [180].

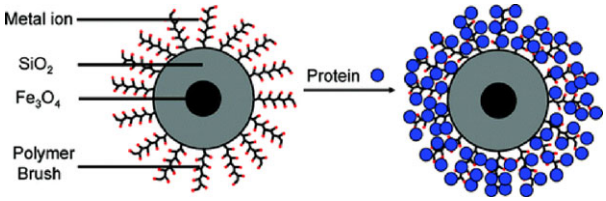


Figure 19. Schematic diagram of brush-modified materials and protein binding to these materials. This figure is adapted with permission [185].

to a magnetic energy gradient in the annular flow channel, resulting in the migration of immunomagnetically labeled cells toward the outer wall. At the outlet, the cells are sorted into depleted (a) and enriched (b) fractions [181].

Magnetic materials have large specific surface area and strong polarity. The application of magnetic materials in cell separation can be categorized into two parts: non-specific adsoption and specific adsorption. Under normal physiological conditions, cells are usually charged. Magnetic materials and cells can be combined by electrostatic interaction. This type of adsorption is generally not selective, hence it is called non-specific adsorption. However, the surface of magnetic materials modified by specific molecules conjugate with the homologous cells in order to separate them from primary biological entities. Primary technical indicaton for evaluating a cell separation system is based on capture efficiency and purity of targeted cells. The capture efficiency is the percentage of captured desired cells from the required target cells in the blood, whereas the capture purity is the percentage of captured desired cells in the total number of targeted cell. Therefore, surface modification of magnetic materials is an important way to improve the capture purity and efficiency.

4.2 Protein purification

Unlike cell separations where antibody conjugated adsorbents predominate, protein separations usually employ more cost-effective and robust synthetic ligands exploring affinity, ionic, hydrophobic, or mixed-mode interactions [165].

The field of proteomics attracted much attention in the post-genomic era because of its pertinence to functional genomics [182]. The enrichment and purification of proteins/peptides is an important field in proteomics. A great deal of research has been done on the qualitative and quantitative analysis of endogenous peptides in complex biological samples.

Many protein-purification methods employ a separation step based on specific interactions between immobilized ligands and affinity tags on the protein. The most common affinity tag is polyhistidine, which binds to immobilized Ni^{2+} or Co^{2+} complexes [183, 184].

Xu et al. [185] grew polymer brushes[poly(2-hydroxyethyl methacrylate)] on silica-coated Fe_3O_4 to produce stable MNPs (see Fig. 19), which bound an order of magnitude more proteins than typical commercial magnetic particles. (see Table 1)

His-tagged proteins can cover the surface of MNPs selectively and quickly, reducing nonspecific adsorption of undesired entities. Recently, various magnetic materials have been successfully developed for the specific separation of His-tagged proteins [186]. Shao and co-wokers demonstrated an in situ growth method for preparation of Fe_3O_4@SiO_2@LDH microspheres with large surface areas and uniform mesoporous channels. The Ni^{2+} cations in the NiAl-LDH shell provided docking sites for His and the materials exhibited excellent performance for separation of a His-tagged fluorescent protein [187].

Enzymes are proteins produced in living cells with specificity and catalytic activity, also known as biocatalysts. However, their advanced structures are unstable to heat, strong acid, strong alkali, and organic reagents. In addition, it is difficult to separate a free enzyme from the substrate product, and it is also difficult to recycle and reuse enzymes, resulting in product pollution and increased production costs. In order to overcome these difficulties, enzyme immobilization technology has been developed. With the development of MBs, these materials have been widely used as a carrier for enzyme immobilization. Teresa and co-wokers proposed a method for preparing superparamagnetic iron oxide/silica nanocomposites with ordered mesoscopic porosity, high BET (Brunauer, Emmett, and Teller) specific surface area, and high pore volume for lysozyme immobilization [188].

The applications of magnetic materials have been rapidly developed in the field of separation and purification of proteins due to its continously modified technique. The target protein was isolated by reversible binding of functional groups modified by magnetic materials to target proteins. The application of magnetic globin separation is mainly dependent on the preparation of magnetic materials with high adsorption capacity, good selectivity, reusability, and low cost. Therefore, the rational preparation of magnetic materials for protein separation is one of the advanced separation method. In addition, with the rapid development of smart polymer materials, the synthesis of smart magnetic materials is also one of the development directions for protein separation.

4.3 Separation of nucleic acids

The traditional methods (boiling, cracking, protease K digestion, etc.) of nucleic acid separation and purification are complicated, time-consuming, inefficient, contact with toxic reagents, and difficult to achieve automatic operation. Magnetic bead separation is a simple, rapid, efficient, safe, and low-cost method for nucleic acid separation (see Fig. 20). The whole separation process does not requires centrifugation or column separation, and can process multiple samples simultaneously. It is easy to realize automatic operation. Because of its excellent efficiency, this method is especially suitable for nucleic acid extraction of micro samples.

Comparing with other nucleic acid separation techniques, magnetic separation has several advantages. The

Table 1. Features of commercially available beads that bind His-Tagged proteins. This table is adapted with permission [185]

Material Name	Company	Size/μm	Incubation time (on ice) /min	Capacity	Matrix
PopCulture His Mag purification kit	Novagen	3	5	5 mg/mL	agarose
MagneHis Ni-Particles	Promega	——	2	1 mg/mL	——
Ni-NTA magnetic agarose beads	QIAGEN	20—70	30	0.25—1 mg/mL	agarose
Dynabeads TALON	Dynal Biotech	1.1	10	40 mg/g	——
HIS-Select nickel MBs	Sigma	20—75	30	≥10 mg/mL	agarose
uACS His isolation kit	Miltenyi Biotec	0.05	30	——	——
Ni^{2+}-NTA-SA-PHEMA-initiator-SiO_2—Fe_3O_4	N/A	~0.1	5	220—245 mg/mL	polyme

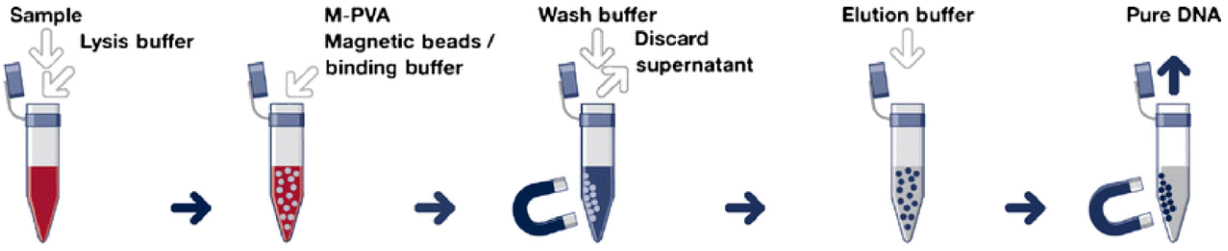


Figure 20. Schematic procedure for nucleic acid purification by magnetic bead technology (illustration by chemagen Biopolymer-Technology AG, Germany). This figure is adapted with permission.

isolation of nucleic acids represents a crucial step before many biochemical and diagonistic processes. Using magnetic separation method, nucleic acids can be directly isolated from crude biological samples without any restrictions with respect to the sample volumes. Many downstream applications such as determination, amplification, cloning, hybridisation, sequencing, and synthesis can not be carried out with the crude samples [189]. The development of functional magnetic materials and appropriate buffer systems, are possible to extract target substances from crude cell extracts and purify them directly and efficiently. The centrifugal steps that may lead to degradation of nucleic acids are avoided in the magnetic separation process [189]. In addition, nucleic acids can be separated from viscous suspensions because of their controllable magnetic properties of the solid matters. Moreover, it is proved that magnetic separation method is one of the possible ways to recycle tiny particles from the mixture of biological waste and other fouling materials with similar sizes. Furthermore, the efficiency of magnetic separation is especially suited for large-scale purifications [190–192], because it only needs to apply a magnet to the side of the vessel containing the sample mixture for magnetic separation of the required particles.

Nargessi et al. discovered that cellulose-coated magnetic particles could adsorb nucleic acid by adding salt (NaCl) and PEG [193]. Probst and coworkers showed the potential of magnetic particles functionalized with DNA probes in the simultaneous purification of multiple targets. Starting with a magnetic particle functionalized with three different DNA probes, they were able to simultaneously purify three independent targets, each recognized by a specific antibody-DNA conjugate, from a four component mixture containing a negative control [194].

4.4 Extraction of bioactive compounds with low molecular weight

Magnetic separation is also an effective and non-destructive method for selective extraction of low molecular weight bioactive compounds such as drugs and pharmacologically active compounds from biological samples [22]. As one of the main tasks in the field of analytical toxicology, this method is used to analyze drugs, especially drug abuse (DOA), such as antibiotics and hormones. Therefore, people are committed to develop methods for extracting DOA from biological organisms for further analysis.

The screening of cellular or botanical extracts as potential sources for ligands (or complexes) of known or orphan receptors is known as ligand fishing [22]. Many researches have demonstrated that macromolecule (protein, enzyme, receptor, DNA, etc.) functionalized MNPs may serve as baits to recognize bioactive small molecules in natural products. Thus, ligand fishing based on biologically functionalized MNPs has been proven powerful, effective, and convenient for identification and isolation of pharmacologically active compounds from natural products [195].

5 Concluding remarks

Magnetic materials combine the unique advantages of solidifying reagents with highly specific immunological reactions. Based on immunology, magnetic materials have penetrated into pathology, physiology, pharmacology, microorganisms, biochemistry, and molecular genetics. MBs have been widely used in immunoassay, cell separation, purification of biological macromolecules, and molecular biology. These are mainly

because of: (1) the whole process of magnetic microsphere separation is relaxed to ensure the structural integrity of active components; (2) the separation and purification steps are simple; (3) no expensive large-scale equipment, such as centrifuge, chromatographic system, and ultrafiltration device; (4) simple and rapid elution, high product concentration magnetic separation technology makes it easy to automate separation analysis.

Magnetic separation techniques are relatively new and still under development. Moreover, up to now, magnetic separation techniques in small scale prevail, but the potential of these techniques is far from fully exploited. How to improve the binding efficiency and specificity of biomacromolecule on magnetic microspheres, the innovation of magnetic separation methods and the extension of its application will be the focus of future research in this field.

Traditional magentic separation technology needs to modify the surface of magnetic materials and target specificity antibodies (proteins) or cell incubation. Therefore, by adjusting the surface chemistry of magnetic materials for specific antibody can combine standardly and easily purify samples under the action of external magnetic field. On the other hand, the unspecific and non-required targeted proteins were washed out. However, most of the conventional magnetic separation operations are carried out in the centrifugal tube, and non-specific binding resulting unwanted precipitation with magnetic materials. In other words, due to the protein corona effect, even non-target visual cells can be easily "surrounded" by a large number of magnetic materials, resulting in eventually being retained. The microfluidic chip can improve the traditional magnetic separation method, in this situation, the microarray structure which can control the gradient distribution of magnetic field can be prepared in the micro channel to improve the magnetic separation efficiency. In addition, as the whole system is in a dynamic environment, non-specific binding can be improved to a great extent, thus enhancing the final separation purity [196, 197].

While the physics of magnetic separation is very well understood, and the chemistry and material science of magnetic materials is a mature and established field, the biochemistry and biology specific to separation is still a developing topic. Its application is limited due to high costs of antibody, and of the limited and sophisticated experimental conditions required to keep the activity of antibodies. On the other hand, different separation strategies require different particle sizes and shapes of magnetic materials, so this field is still constrained by the need for novel types of MBs, which require the development of more controllable magnetic materials synthesis methods.

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