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INVITED REVIEW PAPER

Magnetic nanoparticles for bioseparation

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Abstract−Magnetic bioseparation has been an essential process for decades in all areas of biosciences. A variety of separation processes are being developed. Recently, much attention is being paid to applying magnetic nanoparticles for magnetic bioseparation. The purpose of this review paper is to show the importance of magnetic bioseparation by magnetic nanoparticles. Several synthesis and modification methods of magnetic nanoparticles are documented along with interactions involved in binding of the biomolecules with magnetic nanoparticles. Some practical examples of magnetic bioseparation processes are also discussed to show the efficiency of magnetic bioseparation technique.

Keywords: Magnetic Nanoparticles, Surface Modification, Separation Processes, Isolation, Magnetic Bioseparation

INTRODUCTION

The rapidly growing field of biotechnology has a critical need for simple, fast and high-throughput processes for the extraction of biomolecules from suspended solution. Isolation and purification of various biomolecules such as proteins, DNAs, antibodies, nucleic acids and antigens in highly purified form is a challenging task [1]. Several bioseparation techniques have been proposed as advanced alternatives to the classical separation methods: precipitation, centrifugation, chromatography and ligand fishing. Among these, ligand fishing is a technique where a target is immobilized on a solid support and used for fishing out ligands from the complex mixtures [2,3]. For example, magnetic nanoparticles (MNPs) coated with protein A or protein G were used to isolate proteins expressed in cellular extracts [4]. However, in most of the reported ligand fishing studies, ligand fishing has been followed by preparative stage isolation and identification separately, those are elusive, time-consuming and hamper structural information [3]. These challenges can be overcome by magnetic bioseparation technique, like using MNPs. Magnetic bioseparation is simple, robust and versatile in operation, receiving tremendous attention in biomedical applications due to important properties of magnetic nanoparticles such as low toxicity, biocompatibility and large surface to volume ratios [5]. In addition, all the separation steps of magnetic separation can take place in a single test tube [6]. The interaction between MNPs and targeted molecule with a magnetic force enables separation of targeted molecules, and this is the major advantage of magnetic bioseparation [7].

New functionalized MNPs were developed for separation processes in the field of life sciences. Due to the possibility of controlling the surface properties and their magnetic characteristics, they can be easily withdrawn out of the solution. Magnetic nanoparticles adsorb the desired product on the surface and selectively separate it from the solution. Among several MNPs, super-paramagnetic Fe_3O_4 nanoparticles are potentially employed because of their on-off nature of magnetization with and without an external magnetic field. This may help biomolecule transport. In addition, they are found to be non-toxic with well-established synthetic methods [8]. The size of MNPs can also be controlled easily, ranging from few nanometers up to tens of micrometers, comparable to those of cells (10-100 μm), proteins (5-50 nm) and genes (2 nm wide and 10-100 nm long) etc. [9].

The surface of MNPs is often modified with numerous biocompatible molecules such as polymers, organic and inorganic materials to provide colloidal stabilities to MNPs [10]. In the absence of a magnetic field, modified MNPs show no sedimentation or aggregation in solution. Coating of MNPs can also be designed to facilitate conjugation of MNPs with the desired biomolecule. Specific targeting can possibly be done by the conjugation of specifically tagged antibodies, antigens or other molecules. For example, immunospecific MNPs have been successfully developed to separate red blood cells, bacteria, lung cancer cells and breast cancer cells etc [8].

The purpose of this review is to summarize magnetic bioseparation process using MNPs. Advantages and disadvantages of numerous synthesis techniques for MNPs are discussed along with modification processes. Furthermore, driving forces involved in the binding of MNPs with biomolecules such as protein and DNA have also been documented. A broad list of magnetic purification and isolation processes are described in detail, which will help the readers to select the optimal MNPs and the separation procedures.

BASIC WORKING PRINCIPLE OF MAGNETIC BIOSEPARATION

Bioseparation processes for specific biomolecules are found to be useful in almost all areas of biosciences and currently become one of the most valuable applications of MNPs. The basic working principle of magnetic bioseparation is comparatively simple compared to other conventional methods. The simplified magnetic separation system (Fig. 1), includes the following steps; [11]

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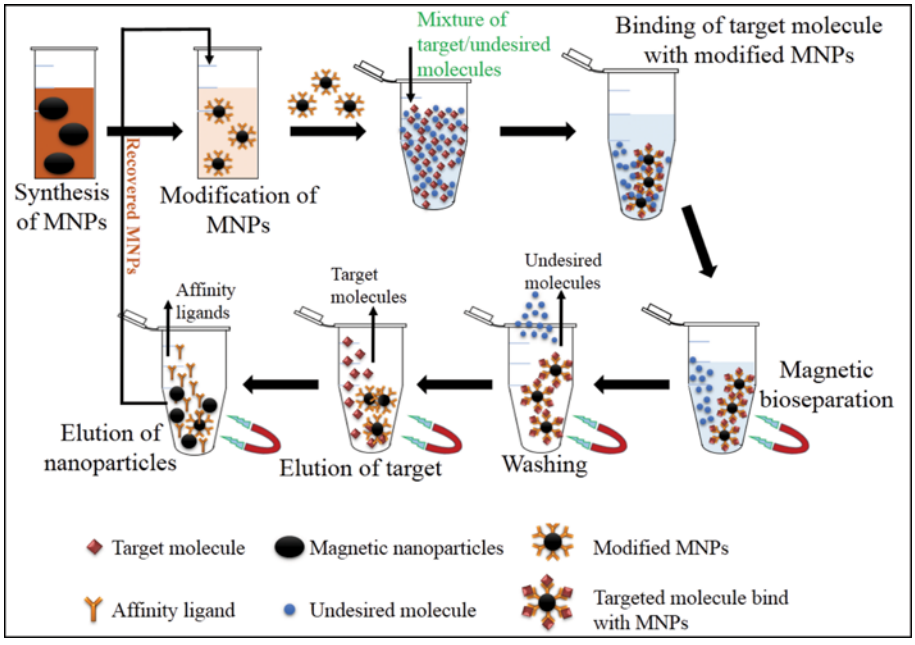

Fig. 1. Schematic diagram of bioseparation by magnetic nanoparticles.

1) Synthesis of MNPs: First step for magnetic separation is the synthesis of MNPs that involve specific synthesis steps to get specified MNPs based on the target molecules. Several synthesis methods are discussed in section 3.

2) Modification of MNPs: After synthesis, MNPs are modified to inhibit MNPs reactions and agglomeration in the aqueous phase, which is a precondition for medical applications. Surface modifications endow MNPs with multifunctional properties such as biocompatibility, colloidal stability, and biotargeting.

3) Adsorption step: Modified MNPs (mMNPs) are mixed well with the target molecules present in the sample solution and the mixtures are incubated for few minutes. The mMNPs will bind with target molecules.

4) Separation step: Switching on the magnetic field retains the mMNPs along with target molecules, while undesired molecules are separated.

5) Several washing steps: Washing buffer is allowed to enter into the column. The mixture is passed through several on-off magnetic field cycles. During the off-period, mMNPs with target molecules again suspended, while during the on-period, they are recollected from the washing buffer.

6) Elution steps: After washing, the target molecules are recovered by the addition of elution buffer.

7) Recycling step: After each separation, the MNPs are then incubated with a specified solution to introduce fresh binding sites for the next separation, i.e., washing of magnetic core-shell Fe_3O_4@SiO_2@poly(styrene-alt-maleic anhydride) spheres enriched with Ni-NTA using EDTA solution release Ni^{2+}. The particles are then incubated with $Ni(CH_3COO)_2$ solution to introduce fresh Ni-NTA sites for the next separation [12].

Table 1. Comparison of physical, chemical and microbial synthesis methods for MNPs

	Methods	Advantages	Disadvantages	References
Physical methods	Gas phase deposition	Easy to synthesize MNPs	Difficulty in particle size control	[15]
	Electron beam lithography	Good control over inter-particle spacings	Expensive	[16]
Chemical methods	Sol-gel synthesis	Precisely controlled size and aspect ratio	Weak bonding and high permeability	[19]
	Coprecipitation	Simple in operation and efficient	Low purity	[20]
	Hydrothermal synthesis	Controlled particle size and shapes	High reaction temperature and pressure	[21]
	Oxidation method	Uniform size and narrow size distribution	Small sized colloids	[22]
	Electrochemical method	Control over particle size	Reproducibility	[23]
	Sonochemical decomposition reactions	Narrow particle size distribution	Synthesis mechanism still not understood	[24]
Microbial methods	Microbial incubation	Low cost, high yield and good reproducibility	Required large time	[18]

SYNTHESIS OF MAGNETIC NANOPARTICLES

Because of the pronounced potential of MNPs in several applications, a wide range of synthetic methods have been developed to synthesize MNPs with the pronounced control over the size, shape, composition and a wide size distribution [13]. We provide here an extensive review of different synthesis methods (Table 1) [14]. Physical methods for MNPs are easy to perform [15] along with good control over the inter-particle spacing of nanoparticles [16]. Several chemical methods are also being developed for the synthesis of MNPs, such as sol-gel synthesis, chemical coprecipitation, hydrothermal and thermal decomposition. Of all existing methodologies, coprecipitation of iron salts in aqueous medium presents the most simple and efficient method. In a typical method, a base is added to precursor solution (i.e., solution of ferric and ferrous salt). Stable suspensions are formed by washing the flocculates with acidic and basic solutions. Size and shape of nanoparticles can be adjusted by controlling the experimental conditions: nature of salt, pH of the solution, ionic strength and reaction temperature [17]. Microbial method to synthesize MNPs of particular shape and size ensures high yield at low cost along with good reproducibility. However, it is a time-consuming process including fermentation processes [18].

MODIFICATION OF MAGNETIC NANOPARTICLES

The biomedical applications of MNPs depend on several factors related to size, shape, magnetization and biocompatibility [25]. Hence, modification of MNPs is required to protect them from agglomeration and to sustain their desired properties [26]. For modifications, different coating materials have been studied such as polymers, organic and inorganic materials [10]. Weissleder worked in different groups to demonstrate the functionality of dextran-coated monocrystalline [27] and cross-linked iron nanoparticles [28]. They found that these particles have the potential to be used as multifunctional imaging agents [29]. Bae et al. (2010) developed effective heat mediator for cancer hyperthermia by chitosan coated oligosaccharide-stabilized ferrimagnetic iron oxide nanocubes [30]. These particles showed efficient antitumor efficacy without serious toxicity through caspase-mediated apoptosis. Heating ability of these particles was found to be higher than commercial superparamagnetic iron nanoparticles. Chitosan has several biological applications due to high charge density, biocompatibility, ability to improve dissolution and low toxicity. Chitosan-embedded MNPs show relatively low cytotoxicity due to whole surface coverage of particles [31], while the dextran-coated nanoparticles cause cell death as naked nanoparticles [32].

Häfeli et al. [33], who developed polyethylene oxide (PEO) coated MNPs, found that PEO tail block length is inversely correlated with toxicity [33]. Tail length above 2 kDa is suitable for in vivo applications. Mahmoudi et al. (2009) reported polyvinyl alcohol-coated MNPs that showed much lower toxicity than uncoated MNPs because of the substitution with surface saturated particles [34]. Poly (lactide-co-glycolide) (PLGA) is an approved polymer coating material for a variety of drug delivery systems [35]. PLGA-coated MNPs have much superior r_2 relaxivity than normal superparamagnetic iron nanoparticles [36]. Dimercaptosuccinic acid coating eliminates the toxicity of particles by preventing direct contact of human dermal fibroblast with these particles [37]. Polyethylene glycol (PEG) coating produces a shielding effect which reduces the toxicity [38]. PEG coating may also decrease the uptake of MNPs by macrophages to increase their circulation time in the bloodstream for in vivo analysis [39].

Surface silanization is the most commonly used modification methodology to impart functionalized group over the surface of bare MNPs to maintain several properties such as increased stability, low toxicity, inertness to redox reaction and high responsiveness. Various silane molecules are shown in Fig. 2. The main advantage of silane coating is that coating process can be carried out in both organic and aqueous medium at medium temperature with no particular reaction conditions. Silane molecule first being activated by hydrolyzation follows a condensation reaction between the Si-OH groups of the silanol and the OH groups on the surface MNPs, which forms a stable bond to the surface [40]. Wu et al. (2008) synthesized silane-coated 25±5 nm MNPs [41]. A schematic of silane-coated MNPs is shown in Fig. 3. Hydroxyl group present on the surface of MNPs reacted with methoxy groups of the silane molecules to form Si-O bonds while leaving the exposed terminal group for the attachment of other molecules. Results show that silane coating increases the hydrodynamic size of the synthesized particles while maintaining all other properties such as saturation magnetization constant.

Physical and chemical nature of MNPs has decisive effects for the selection of coating material. Nowadays, inorganic coating by silica is most widely gaining attention due to several advantages of

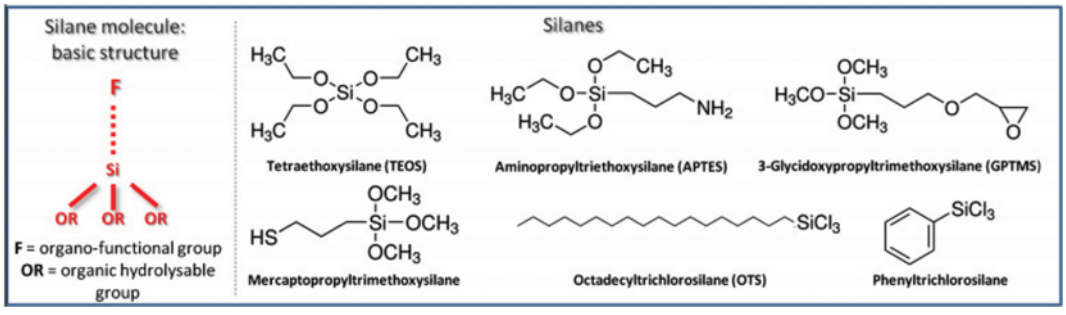

Fig. 2. (Left) Basic chemical structure of silane molecule for silanization reaction with MNPs, (Right) Organo-functional silane molecule structures contain two different basic reactive groups: an organic hydrolyzable group (OR) and an organo-functional group (F) [40].

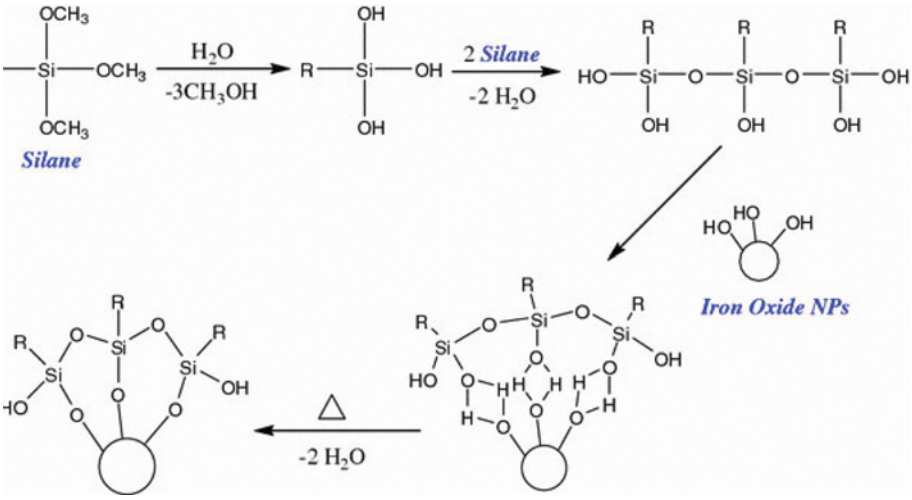

Fig. 3. Physicochemical mechanism for modifying the silane agents on the surface of iron oxide NPs [41].

silica such as colloidal stability, water solubility and photostability [42,43]. Ding et al. (2012) developed Fe_3O_4@SiO_2 core-shell nanoparticles by controlled synthesis via reverse microemulsion method [43]. They demonstrated that by controlling the size of the aqueous domain, the thickness of silica shell can be controlled. At present, Stöber methods and sol-gel method are the principal choices to coat iron magnetic nanoparticles with silica. Naeimi et al. (2014) synthesized Fe_3O_4@SiO_2 nanoparticles by a simple and low-cost method. First, magnetite nanoparticles were synthesized by coprecipitation, which follows the silica deposition (to further improve the stability) by the ammonia-catalyzed hydrolysis of tetraethylorthosilicate (TEOS). This work leads to the development of efficient and reusable catalyst by further synthesis of Fe_3O_4@silica sulfonic acid, core-shell composite (Fig. 4) [44]. Moreover, silica thickness can be tuned from 5-200 nm by controlling the amount of ammonia and TEOS.

For further extended functionality of MNPs@silica, Ma et al. (2006) reported the synthesis of Fe_xO_y@silica by modified Stöber method and sol-gel method [45]. Superparamagnetic iron oxide nanoparticles were first coated with silica to isolate the magnetic core from the surrounding, which follows the incorporation of the dye molecule. This complex showed the improved photostability and versatile surface functionalities. After silica coating, a profound decrease in saturation magnetization (M_s), (about 35 emu·g^{-1}), coercivity and blocking temperature was observed. It can be seen that MNPs@silica is a facile coating process [46]. Silica-coated MNPs can easily attach with several biomolecules as these agents have a siloxy group at one terminal and another end is free to attach a biocompatible group. On the other hand, silane coupling agents inhibit this type of further attachment process [47].

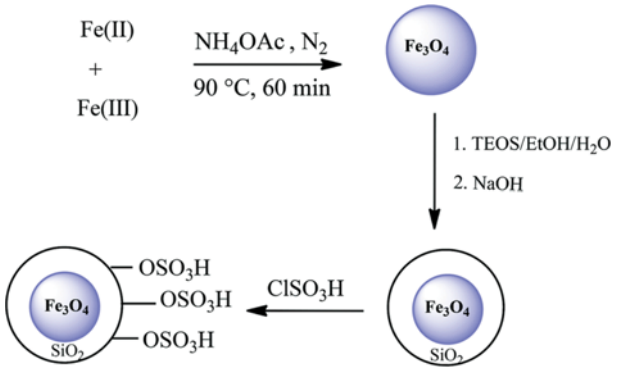

Fig. 4. Preparation steps for fabricating sulfonic acid-functionalized magnetic Fe_3O_4 nanoparticles [44].

BINDING OF MNPs WITH TARGET BIOMOLECULE

Proteins are copolymers of twenty-two different amino acids [48] which vary in the extent of hydrophobicity, hydrophilicity, polarity and nonpolarity [49]. The nature of amino acids defines the reference to improve the interactions between biomolecules and MNPs [50], for further improvement in purification and separation processes [51]. Immediately, after the addition of MNPs into the biological solution, MNPs start binding with protein complexes which describe the biological identity of nanoparticles [52]. The adsorption behavior of protein and peptides on the surface of MNPs is the result of their several interactions such as electrostatic, hydrophobic and ligand binding interactions. Other driving forces such as van der Waals forces may also influence the adsorption of protein on surfaces of MNPs.

Electrostatic properties of amino acids are important for the adsorption of zwitterion molecules, which is highly dependent on the pH of surrounding medium (Fig. 5(a)) [53]. With the increase in pH, there is an increase in anionic character, which increases the net negative charge of protein, while the decrease in pH increases net positive charge along with increased cationic character [54]. The electrostatic interaction may also generate due to the presence of side chain groups along the length of the peptide chain. These interactions help to orient the protein complexes in specific directions to form a connection with MNPs to ease their separation [55]. Adsorption of protein with MNPs can be controlled effectively by controlling the surface charges of the protein complexes, as protein

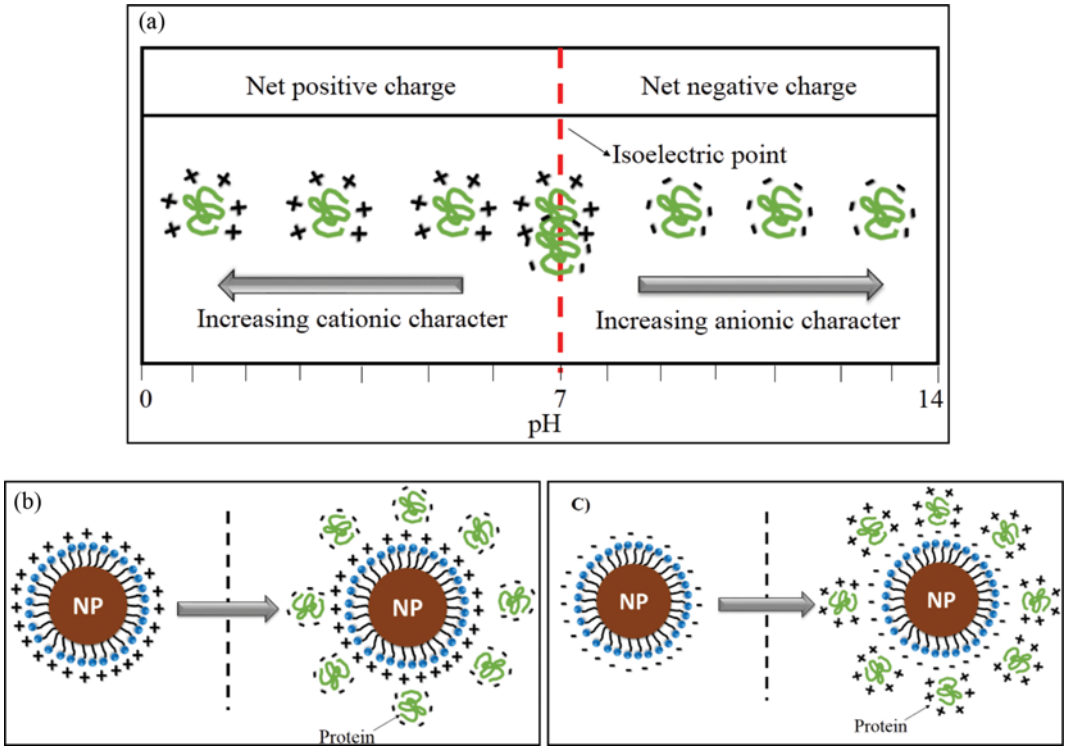

Fig. 5. (a) Effect of surrounding environment pH on the nature of protein, (b) and (c) Schematics of electrostatic interaction between negatively/positively charged magnetic nanoparticle and counter charged protein molecule.

complexes have both positive and negative charges. Based on the net charge of protein molecule, nanoparticles are being selected to study their binding behavior. Negative or positive charged protein will bind with counter charged MNPs as shown in Fig. 5(b) and (c) [55].

Calatayud et al. (2014) studied the role of surface charge of MNPs to bind with proteins [56]. They synthesized colloids of opposite charge with same hydrodynamic size; (Fe_3O_4) core of 25-30 nm functionalized with (a) positive polyethyleneimine (PEI-MNPs) and (b) negative poly(acrylic acid) (PAA-MNPs). They found an increase in the hydrodynamic size of particles after a few seconds of incubation due to the wrapping of MNPs by protein adsorption. These results were also found to be consistent with large sized protein [56]. Surface charge may also denature the adsorbed protein. Lynch et al. (2008) demonstrated the interaction behavior of protein with a positive, negative and neutral ligand attached nanoparticles [57]. Owens et al. (2006) confirmed the denaturation of the protein molecule in the presence of charged ligand, while neutral ligand retains the structure of protein [58].

Protein complexes ensemble themselves to incorporate hydrophobic residues towards the inside, while hydrophilic groups occupy outer surfaces. Increasing the hydrophobicity increases the adsorp-

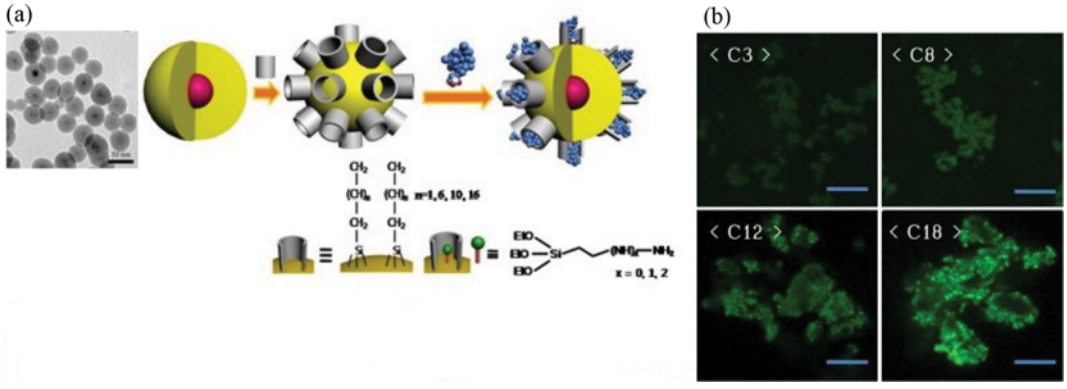

Fig. 6. (a) Scheme of hydrophobic partitioning modification on the Si-MNPs with various alkyl groups (for *n*-propyl (C3), *n*-octyl (C8), *n*-dodecyl (C12), and *n*-octadecyl (C18) groups) as a hydrophobic pocket and various amine groups (x=0, 1 and 2 denotes monoamine, diamine, and triamine, respectively) as a receptor. TEM image shows the core-shell structured Si-MNPs with 40 nm of average particle size. (b) Confocal microscopic images of various hydrophobic pocket sized Si-MNPs at pH 4.65 (The scale bar is 4 μm) [60].

tion mechanism [59]. Chang et al. (2010) showed efficient separation of protein molecules such as bovine serum albumin (BSA) by hydrophobic partitioning effect [60]. They demonstrated modified MNPs having hydrophobic pockets over the silica-coated MNP surface with various alkyl groups. The separation efficiency was strongly influenced by salt concentration, pH level and alkyl chain lengths (Fig. 6). Hydrophobic nanoparticles demonstrate more adsorption efficiency in comparison with hydrophilic nanoparticles. This suggests the presence of higher number of binding sites in hydrophobic nanoparticles. This may also happen due to clustering phenomenon of hydrophobic particles, which forms a distinct "island' (act as binding sites for protein) [61].

Furthermore, specific affinity ligand attached nanoparticles also showed the tendency to bind with the protein complexes, i.e., by dendrimers [62], protein complex binding to quantum dots by replacing mercaptoacetic acid on the nanoparticle surface [63] and thiol-containing peptides for gold nanoparticles [64]. Khng et al. (1998) investigated the behavior of functionalized magnetic particles attached to the carboxylic acid group [65]. Resulting material exposed higher surface in addition to the higher density of functional groups, which make them ideal candidates for protein isolation. However, ligand attached molecule must have an affinity as strong as possible to interact with the target molecules [64,66].

Binding of DNA molecules with MNPs follows the same mechanism as with protein complexes. The peculiar interaction between DNA molecules and MNPs is driven through several interactions such as electrostatic interaction, hydrophobic interaction, and hydrogen bonding. These interactions can be explained by charge density distribution of DNA molecule. Highest negative charge density is distributed over the phosphate group that is present on the surface of DNA molecule and in the inner grove [67,68]. Nanoparticles bind to DNA through Fe-O-P bond. [69]. Nanoparticle surfaces and DNA molecules are mainly deprotonated at the pH values 7.2-7.4 [70]. The reduction in pH values may cause protonation of phosphate groups of both MNPs surface and DNA, which increases the grafting density [71]. In addition, the lengthening of DNA molecule increases the number of phosphate groups, which ultimately increases the potential of DNA binding with MNPs [72]. Furthermore, the electrostatic interaction between the cationic nanoparticles and DNA molecules leads to forming extended composite aggregates through surface recognition [73]. One more strategy to form DNA@nanoparticle binding involves the formation of the pre-silanized surface of poly(amidoamine) MNPs, which forms a stable complex with negative charged DNA via strong electrostatic interactions [74].

DNA molecules consist of both hydrophobic and hydrophilic surfaces. Phosphate forming the backbone of DNA is readily available in water, hence it is considered to be hydrophilic, while nucleotides tend to repel water molecules by invading themselves and are found to hydrophobic. Double stranded DNA molecule exposes the apolar surface to the water molecules and whole DNA molecule remains fully hydrated. The relative strength of hydrophobicity for nucleotides is dependent on the H-bonds between nucleotides and water molecules. Some bases such as adenine, thymine, and cytosine form a lesser number of H-bonds with water molecules. Ghosh et al. (2007) demonstrated that hydrophilic nanoparticles can accommodate more DNA on their surfaces than hydrophobic surfaces. Furthermore, electrostatic interactions dominate over the hydrophobic interactions [75,76]. High-affinity interactions are often used to form noncovalent specific binding of MNP to DNA by modifying the surfaces of MNPs through affinity ligands such as biotin, avidin etc. [74].

MAGNETIC BIOSEPARATORS

The basic geometry of magnetic bioseparators is very simple. The most conventional magnetic system consists of a small permanent magnet that is placed near a batch column. A magnetic gradient is adjusted by controlling the distance between magnet and column as well as the shape of magnetic poles. Laboratory scale, commercially available batch separators are usually fabricated by embedding magnets within the disinfectant-proof material. Some of the magnetic systems also have separable magnetic plates to facilitate the washing of suspended solids. Low separation volume and low magnetic field gradient generation are the main limitations of these separators. These problems tend to exert too small a force on individual particles and very small accumulation rate. However, the application of a magnetic field is easy for such type of separation systems.

Alternatively, flow through magnetic separators can generate high magnetic force on individual particles. A typical example of such systems is high gradient magnetic separators (HGMS). For such systems, the magnetic field can be generated by using different coils, wires or tapered electrons [77-81]. These separators are characterized by the flow of suspended solution through the separation system. Characteristically, separation efficiency is increased by providing a high magnetic region. Typical design of flow through separators consists of a large column containing magnetic steel wool or beads inside to create the gradient field as shown in Fig. 7. Steel wool or beads act as dipole magnets and flux lines enter them essentially perpendicular to them, creating intensive field lines. These flux lines direct the target magnetic materials towards steel wool or

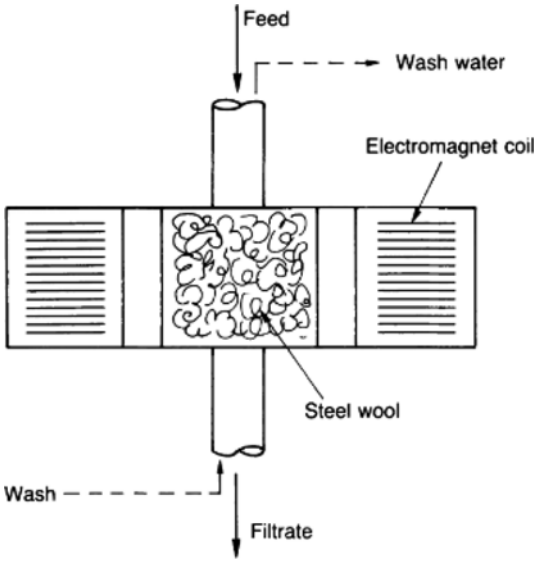

Fig. 7. Principle of high gradient magnetic separation [83].

beads, while nonmagnetic materials pass through the column. In this way, target materials are retained with magnetic beads, while nonmagnetic entities pass through the column. The recovery process of target products through washing or little vibration without applying magnetic field follows [82]. A typical example of HGMS is the separation of red blood cells [83]. Many HGMS systems have been developed based on the position of the magnet across the separation column, such as positioning of the magnet along the side column [84], or both sides [85] or bottom of the column. These separators are found to be more expensive due to their complicated design, but they give fast separation [86].

WASHING AND ELUTION OF PRODUCTS/ NANOPARTICLES

The possibility of increasing the selectivity by magnetic bioseparation through the use of mild conditions in washing and elution steps is a well-established practice. After magnetic separation, the product stream is passed through a number of elution steps. Both the adsorption and elution mechanisms are primarily governed by the surface properties such as electrostatic, hydrophobic, hydrophilic and H-bonding between MNPs and biomolecules. For adsorption, these interactions may result in attractive forces, while for elution, repulsive forces are activated.

Chen et al. (2014) reported the performance of protein extraction using hydroxy functional ionic liquids modified on the surface of silica-coated Fe_3O_4 MNPs. After separation, they recovered the target protein by adding different concentrations of NaCl solution. Results shows that with the increase in salt concentration, there is an increase in desorption efficiencies. They also recovered MNPs in each run [87]. Fang et al. (2014) developed Fe_3O_4@SiO_2@poly(styrene-alt-maleic anhydride) with or without nitrilotriacetic acid (NTA) to study their separation efficiency for His-tagged protein (Fig. 9) [12]. Green fluorescent protein (GFP) was used as a model protein to demonstrate the separation efficiency of developed MNPs. They incubated the desired amount of both synthesized MNPs for 10 min and allowed to separate target molecule from the solution by applying the magnetic field. A decrease in fluorescence intensity was observed for both Fe_3O_4@SiO_2@polymer/Ni-NTA and Fe_3O_4@SiO_2/Ni-NTA (8.2% and 2.2%, respectively) due to binding of GFP on both types of NTA-modified magnetic spheres. The recyclability of both NTA-modified magnetic spheres was also investigated. After each run of separation, the magnetic spheres were washed with EDTA solution to clean the exposed surfaces and to release Ni^{2+}.

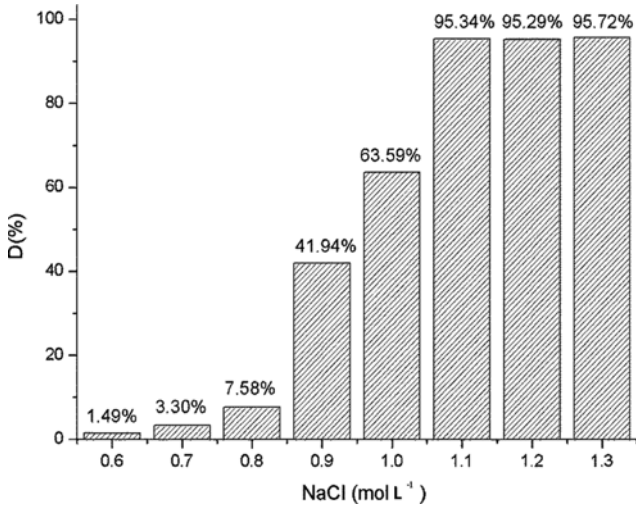

Fig. 8. Desorption BSA from the surfaces of (2-hydroxyethyl)-*N*,*N*-dimethyl-3-(triethoxy) silyl propyl-ammonium chloride-MNPs experiment [87].

EXAMPLES OF MAGNETIC BIOSEPARATION

In the field of biology, it is vital to detect, purify and analyze biomolecule or other applications, often needing to separate it from its environment. Among all existing methodologies, magnetic separation has proven to be convenient for selective separation [88]. Recent studies of MNPs show several successful methods to control and probe interactions between synthesized MNPs and biological molecule [89]. Generally, two methods are common for isolating protein: conjugation of MNPs with a specific ligand molecule, and conjugation of MNPs with antibodies. The following section describes protein isolation in detail by both methods [90, 91]. The early achievement in the separation of protein was done by employing nickel-nitrilotriacetic (Ni-NTA) functionalized MNPs to isolate His-tag proteins from cell lysate [92]. Sun et al. (2000) used mercaptoalkanoic acid to decorate FePt MNPs with the Ni-NTA complex as shown in [93]. The synthesized MNPs separate

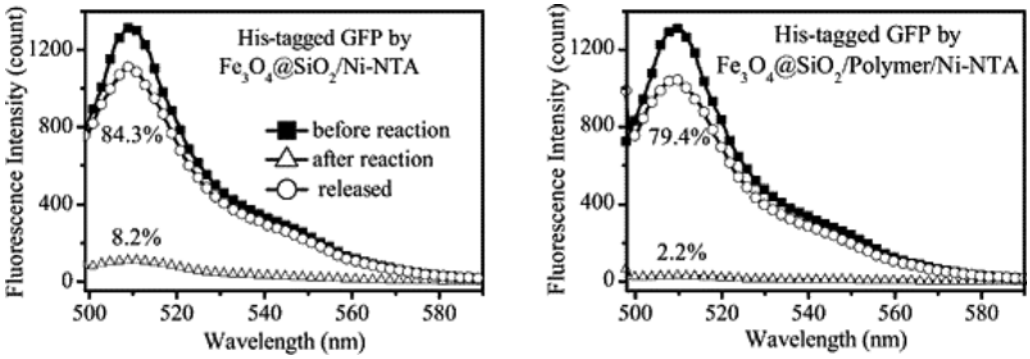

Fig. 9. Fluorescence spectra showing the change of emission intensity of the solutions of His-tagged GFP [12].

out the His-tag protein from E. coli lysate with high capacity and selectivity. Overall, the separation protocol is divided into three steps; 1) addition of synthesized functionalized MNPs into the suspension, 2) attraction of MNPs from the suspension by using small magnet, washing them with deionized water to remove unbound protein molecules, 3) washing with concentrated imidazole solution to separate MNPs from target protein to get purified protein. Target protein covers the exposed surface of MNPs rapidly to diminish generally abandoned surface zone that may cause nonspecific targeting. Xu et al. linked the dopamine and NTA on iron oxide surface by forming a strong dopamine-NTA bond [94]. The octahedral geometry of oxygen coordinated iron facilitates this bonding. The subsequent product reacts with $NiCl_2$ to form MNP-dopamine-NTA-Ni^{2+}, which facilitates ultimate separation of His-tag protein with high selectivity. This complex also shows stability under high salt concentration and heat. In another study, Xiaolan et al. (2015) developed Ni^{2+}-NTA-functionalized magnetic-submicron-particle for site-specific immobilization of 6His-tagged targets to screen out ligands from the mixture [95]. Zheng et al. (2015) synthesized phenylboronic acid-Fe_3O_4@polydopamine (Fe_3O_4@PDA-PBA) magnetic microspheres to evaluate the selectivity and binding capacity of the Fe_3O_4@PDA-PBA magnetic microspheres by using standard glycoproteins and nonglycoproteins. They showed that adsorption capacity of standard glycoproteins, i.e. ovalbumin and catalase from bovine liver, was 160 mg/g and 140 mg/g, which was 3-8 times higher than for nonglycoproteins, lysozyme, myoglobin and ribonuclease A and bovine hemoglobin, which were of 25, 57, 47 and 20 mg/g, respectively [96]. Bucak et al. (2003) developed phospholipid-coated colloidal MNPs to recover protein from protein mixtures. These particles showed high adsorptive capacities up to 1,200 mg protein/mL adsorbent [97].

Other kinds of separation include antibody attached MNPs, and these MNPs are especially used for immunoassay. Matsunaga et al. (2000) found antibody@MNPs for fully automated immunoassay to determine human insulin [98]. They suggested antibody-protein A-bacterial magnetic nanoparticles for exact identification of human insulin by forming guaranteed automated sandwich immunoassay. Ouyang et al. produced hemoglobin-functionalized MNPs to improve human serum amyloid P part (SAP), vitamin D-binding protein, and serine peptidase inhibitor [99].

DNA separation also uses the same separation processes similar to protein. Binding of DNA with MNPs is dependent on the surface properties of both DNA molecule and MNPs. Biao et al. (2009) developed silica-coated biocompatible nanoparticles for the isolation of bacterial plasmid DNA from bacterial culture. These particles have positive surface charge at neutral pH, which facilitates easy binding of DNA with MNPs. MNPs respond efficiently under the influence of a magnetic field to give high separation yield and high purity of plasmid DNA [100]. Zhao et al. (2013) synthesized water-dispersible salicylic acid-coated magnetic nanoparticles. The synthesized MNPs were first bound with mammalian cells to separate them from the native solution and further to bind with genomic DNA by completing one step extraction and another step separation processes [87]. Yongjun et al. (2013) gave a rapid detection methodology for *Pseudomonas aeruginosa*, based on magnetic separation. They successfully amplified biotin-dUTP-labeled DNA fragments of the gyrB gene by polymerase chain reaction (PCR) and detected *Pseudomonas aeruginosa* with a detection limit as low as 7.5 fM of gyrB fragments [101]. They further improved the *Pseudomonas aeruginosa* detection limits as low as 10 cfu/mL [102]. Bin et al. (2013) improved efficiency in SNP genotyping experiments using SNP detection system based on MNPs separation [103]. Xi et al. (2015) used DNA aptamers to construct a chemiluminescence aptasensor based on magnetic separation system and immunoassay to detect hepatitis B surface antigen (HBsAg) from pure protein (detection limit: 0.1 ng/mL), contributing to better detection of hepatitis B virus infection [104].

SUMMARY

The concepts of magnetic bioseparation using MNPs have been developed rapidly due to benefits of advanced nanotechnology, which enable us to produce specifically modified nanoparticles. Different kinds of nanoparticles can be synthesized and modified by various modification processes. Such magnetic nanoparticles are stable in an acidic environment, not to form agglomerates by the reduction in associated energy and to possess high surface area to volume ratio. These modifications provide biocompatible surfaces to nanoparticles for further attachment of biomolecules effectively such as protein, DNA, oligonucleotide, enzymes, and antibodies. However, the type of modification material should be carefully selected.

Magnetic bioseparation by magnetic nanoparticles exhibits opportunities to separate and purify biomolecules to further analyze their

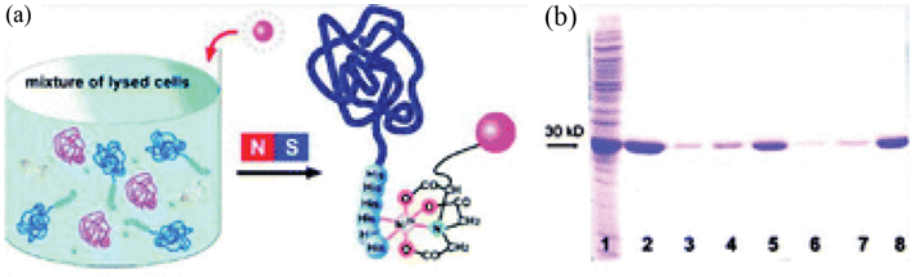

Fig. 10. Histidine-tagged protein purification using biofunctional FePt MNPs: (a) NTA-terminated MNPs selectively binding to histidine-tagged proteins; (b) SDS/PAGE analysis of the fraction of proteins washed off the MNPs by imidazole solution at 10 (lane 3), 80 (lane 4), and 500 mM (lane 5) and the fractions washed off the reused nanoparticles using imidazole solution at 10 (lane 6), 20 (lane 7), and 500 mM (lane 8) [93].

functionalities in diagnostics. A high gradient magnetic field is guided externally to isolate these types of modified magnetic nanoparticles in addition with attached biomolecule and bioaffinity. Once the magnetic particles are concentrated, it can release the targeted product for further analysis, while the nanoparticles are recovered for reuse. A combination of magnetic nanoparticles and external magnetic field has the potential to develop a strategy to diagnose different diseases such as infectious diseases. However, it is still a great challenge to develop specifically designed nanoparticles controlled by the surface modification of magnetic nanoparticles and specific ligand attachment. The ligand attached to the surface of MNPs possesses the affinity to interact with target product, which facilitates their easy separation. However, the use of MNPs is not only limited to magnetic bioseparation, but these MNPs can also have their applications in molecular imaging (e.g., MRI) for diagnostic and hyperthermia for therapy. In the near future, new bioseparation techniques can be developed with novel magnetic separation processes by utilizing the proper positioning of MNPs under a magnetic field to use proper magnetic susceptibility of materials.

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