Chapter 2

Surface Functionalization Of Magnetic Nanoparticles

1. Magnetic core
2. Hydrophilic, biocompatible shell
3. Fluorophores
4. Targeting ligands
5. Responsive elements
6. Drugs
2.1 Introduction

Nowadays, NPs are the significant material for science and technology such as in materials, medicals, and cosmetic areas. Controlling the dispersion stability of NPs in different liquid media is an essential issue to control the properties of the final products. Since NPs possess different surface structures and surface interactions compared to the sub-micron sized particles, NPs have an extremely high tendency of adhesion and aggregation [1]. Therefore, it is indispensable to develop the techniques to control the dispersion/aggregation phenomena of NPs to apply them into functional materials and products. Surface functionalization of NPs is very important in determining the NP’s stability under physiological conditions. Due to the strong magnetic dipole-dipole interactions, the MNPs tend to agglomerate without a hydrophilic coating layer. Surface for those MNPs can made hydrophilic with PEG, PVA, and PVP, etc. as the capping agents. Because of the advantage of a larger surface area to volume ratio as well as homogeneity in aqueous solution, biomolecule-coated NPs have exposed promising applications in complicated bio-systems.

The surface characteristic of NPs is a vital issue that not only decide the biocompatibility of these magnet materials, but also plays an important role in cell adhesion on biomaterials. Through a combination of the unique optical, electronic, or magnetic properties of NPs with various probes, functionalized NPs have become an usable field for biosensing and diagnostic applications [2]. Type of surface coatings and their subsequent geometric arrangement on the NPs establish the overall size of the colloid as well as play a significant role in biokinetics and biodistribution of NPs in the body. The types of specific coating must be chosen by keeping a particular application in mind, or derivatization, for these NPs depend on the end application and whether it is expected at inflammation response or anti-cancer agents. MNPs used to bind to drugs, proteins, enzymes, antibodies, or nucleotides and can be
directed to a disease organ, tissue, or tumour using an external magnetic field or can be heated in alternating magnetic fields for use in hyperthermia [3].

Tailoring of surface is also an effective manner of preventing the dissolution and release of the core materials that may cause toxicity to biological system [4]. Furthermore, the steric hindrance of coating can affect the fate of NPs in biological systems, such as cellular uptake and accumulation, circulation and clearance from body [5-6]. Also, the surface can affect the maintenance of the intrinsic nanocrystal characteristics such as fluorescence and magnetic behaviour. Moreover, appropriate surface functionality is the prerequisite for conjugating biomolecules to NPs for biomedical applications.

Fig. 2.1 Schematic representation of NP surface functionalization via a) surfactant exchange and (b) surfactant addition [7].
For applications in biomedicine, surface of the NPs should be hydrophilic in nature which can be achieved through surfactant addition or surfactant exchange, as illustrated in Fig. 2.1. Surfactant addition is accomplished through the adsorption of amphiphilic molecules that contain both a hydrophobic segment and a hydrophilic component. The hydrophobic segment creates a double layer structure with the original hydrocarbon chain, while hydrophilic assemblies are exposed to the outside of the NPs, turning into water soluble while surfactant exchange is the direct replacement of the original surfactant with a new bifunctional surfactant. This surfactant which is bifunctional has a single functional group competent of binding to the NP surface firmly via a strong chemical bond and the second functional group at the other end has a polar character so that the NPs can be dispersed in water or be further functionalized. Theoretically, four types of forces can contribute to the interparticle potential in the structure of materials. van der Waals forces make strong short-range isotropic attractions. Therefore, by adding salt to the suspension the electrostatic repulsive forces can be partially screened. The theoretical description of these two forces is recognized as the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory. For magnetic suspensions, magnetic dipolar forces between two particles must be added which is a significant issues. These forces encourage anisotropic interactions, which are found to be globally potential if the anisotropic interparticle potential is integrated over all directions. Ultimately, steric repulsion forces have to be considered for non-naked particles [8]. There are several types of materials that can be chosen for functionalization of NPs which is discussed in this chapter.
2.2 Need of surface functionalization of NPs

2.2.1 Stabilization against aggregation

Capping agents are important on the surface of NPs since, they enable the growth of small seeds and circumvent the formation of agglomerates. The repulsive force between particles can in principle be due to electrostatic repulsion, steric exclusion or a hydration layer on the surface. Depending on the particle system, i.e. the core material, and the aqueous media in which the particles are dispersed, the choice of the right surfactant or capping agent might yield to stable particles. First the coating agents have to be bound to the particle surface by some attractive interactions, either chemisorptions, electrostatic attraction or hydrophobic interaction. Different chemical functional groups possess a certain affinity to inorganic surfaces; the most famous example is being thiol to gold. In some cases, this principle is already exploited during synthesis of NPs. In organic solvents, the NPs surface is covered by hydrophobic ligand molecules that prevent aggregation of the particle cores. However, the bonds between the inorganic NPs surface and e.g. an electron-donating end group of a ligand molecule, such as thiol [9-11], amine or phosphine [12], undergo dynamic binding and unbinding processes [13-14]. This yields to the important consequence that the ligand molecules can get off, e.g. by excessive washing or mass action by another incoming ligand, which might compromise the stability of the NPs that ultimately aggregate and precipitate.

Usually, due to attractive van-der-Waals forces, unstabilized NPs tend to agglomerate. The attractive van-der-Waals potential can in a first approximation be stated as,

\[
U_{\text{vdw}} = -\frac{A}{6} \left( \frac{2r^2}{D(4r+D)} + \frac{2r^2}{(2r+D)^2} + \ln \left( \frac{D(4r+D)}{(2r+D)^2} \right) \right) \]

(2.1)
Where, $r$ is the radius of the sphere, $D$ the interparticle distance and $A$ the Hamaker constant [15]. One can see from equation (2.1), smaller NPs result in weaker van-der-Waals attraction potentials and thus are, at a first glance, less prone to agglomeration compared to larger counterparts. However, the lower van-der-Waals potential has to be associated with the higher surface to volume ratio of the former NPs. The higher surface to volume ratio is energetically expensive and, depending on the dispersion media, adds additional strong attraction potentials. There are two different strategies to decrease NPs agglomeration. NPs can be electrostatically or sterically stabilized. Optionally, the two stabilization methods can be combined which are discussed below.

2.2.1.1 Electrostatic Nanoparticle Stabilization

![Fig. 2.2 Schematic of Electrostatic Nanoparticle Stabilization [16].](image)

In the absence of a steric stabilization layer, the interparticle interaction potential can be described by the DLVO theory, that consists of an attractive van-der-Waals potential and a repulsive electrostatic potential [17]. In pH dependent zeta potential measurement because of electrostatic stabilization
relies on charged surfaces, NPs can be electrostatically stabilized at pH < < than the isoelectric point (IEP) and pH > > IEP [18]. Conversely, NPs start to agglomerate if the pH approaches the IEP as zeta potential of NPs becomes too small. Moreover, the electrostatic repulsion potential is screened if ions are added. Thus, electrostatic NPs stabilization is effective at low salt concentrations and at pHs far above or below the IEP of NPs. Schematic (2.2) represents electrostatically stabilized NPs.

2.2.1.2 Steric Nanoparticle Stabilization

![Schematic for Steric NPs Stabilization](image)

**Fig. 2.3** Schematic for Steric NPs Stabilization [19]

NPs intended for applications that require NP stability under high salt concentrations and over a wide pH range have to be sterically stabilized. Steric stabilization relies on polymers, so-called dispersants, that surround NP cores. If two sterically stabilized cores approach each other, polymer brushes are confined. This reduces the entropy of dispersants and increases the
osmotic pressure between NPs. The resulting repulsive potential critically depends on the dispersant density, profile packing density, binding reversibly and the solvent quality with respect to the dispersants.

### 2.2.2.3 Electrostatic vs. Steric Stabilization

NP agglomeration can only be prevented if the sum of the attractive van der Waals potential, for MNPs additionally the attractive magnetic potential, the repulsive electrostatic and steric potentials result in an overall repulsive potential barrier at a given interparticle distance that is high compared to kBT. If this condition is fulfilled, NPs do not agglomerate. Better NP stability at high salt concentration is a stringent requirement, especially for biomedical applications. NP stability under these conditions can only be ensured if these particles are sterically stabilized. Additionally, dispersants allow to chemically modify surfaces such that they become, for example protein resistant. This possibility, however, is only applicable for sterically and not for electrostatically stabilized NPs. Thus, NPs intended for biomedical use are sterically stabilized [20].

According to DLVO theory, the stability of a particle is dependent upon its total potential energy function $V_T$,

$$V_T = V_S + V_R + V_A$$  \hspace{1cm} (2.2)

Along with the theory, $V_T$ is the balance between several factors which are solvent potential energy $V_S$, attractive forces $V_A$ and repulsive forces $V_S$. With DLVO theory, stability of the colloidal NPs calculated by the balances between the van der Waals ($V_A$) and electrical double layer repulsive ($V_R$) forces exist between NPs. If the repulsive forces between the particles are sufficient, the dispersion will oppose to flocculation and it will
Chapter 2

stabilize the colloidal NPs [21]. Here, bare or uncoated NPs have not enough repulsive forces so that ultimately aggregation takes place [22].

Additionally, in the presence of salts or any other electrolytes in the biological media, electrostatic stabilization arising from the MNPs surface charge falls behind the adequate level to overcome the attractive forces between two NPs and leads larger aggregates which can easily be removed by RES and/or by opsonisation. The most used surfactant types of steric stabilization of particles are polymers [23]. These polymers are able to offer stability against agglomeration and opsonization. Also, it provides surface groups which can be applied for biological functionalization of NPs with proteins, peptides and hyaluronic acids. PEG and dextran are most common polymer types for not only coating of MNPs but also all NPs due to their better biocompatibility. PEG also reveals antifouling property that reduces their uptake by macrophages and extends their blood circulation time [24].

2.2.2 Estimation of Surface charge with colloidal stability

Biomedical applications require that MNPs are to be placed in liquid conditions. The corresponding systems are known as magnetic fluids (or ferrofluids). For colloidal stabilization of magnetic fluids, the particles are coated with special shells of surfactants or polymers. Despite the fact that the magnetic fluids are actively used in technical applications for a rather long time, still it is not an easy task to place MNPs in highly polar water without aggregation. The main problem is a strong interaction of surfactants with water, which is competitive with their adsorption on MNPs. At the moment, there are several ways to stabilize water-based ferrofluids, but what concerns the biological media it is not possible to prevent aggregation completely.
In biomedical context the formation of aggregates has side effects relating to the difficult elimination of NPs from organisms, the possible appearance of blood clots, as well as the reduction in the therapeutic efficiency. Thus, knowledge of aggregation regimes in magnetic fluids is a key point for their development in biomedical applications. In this connection, an important goal is a reliable diagnostics of aggregation and determination of the aggregation regimes and their control in biocompatible magnetic fluids.

Another most promising application with the rapid development of nanotechnology, colloidal NP-based drug carriers have been emerging as effective tools for drug delivery in cancer therapy. The first preclinical experiments using magnetic albumin microspheres loaded with doxorubicin for cancer treatment in rats were reported by Widder et al. [27]. There are many different kinds of macromolecular structures designed for drug delivery.
systems, such as micelles, liposomes, NPs, dendrimers and polymers. In these methods, the drug is entrapped, attached, adsorbed, or encapsulated into or onto nano-matrices. Ideally, they could bear on their surface or in their bulk a pharmaceutical drug that could be driven to the target organ and released there. For these applications, the size, charge and surface chemistry of the magnetic particles are particularly important and strongly affect both the blood circulation time as well as bioavailability of the particles within the body. In addition, magnetic properties and internalization of particles depend strongly on the size of the magnetic particles [28]. For instance, following systemic administration, larger particles with diameters greater than 200 nm are generally sequestered by the spleen as a result of mechanical filtration and are ultimately taken out by the cells of the phagocyte system, resulting in decreased blood circulation times. SPM iron oxide NPs of narrow size range are easily produced and functionalised with different polymers, providing convenient, readily targetable MRI agents. Because of the large surface area to volume ratio, the MNPs tend to agglomerate and adsorb plasma proteins. The body’s RES, mainly the kupffer cells in the liver, typically take up these NPs due to the hydrophobic surface. Surface coverage by amphiphilic polymeric surfactants such as poloxamers, poloxamines and PEG derivatives over the NPs importantly, increases the blood circulation time by minimizing or eliminating the protein adsorption to the NPs [3].

The dispersibility and stability of the NPs are strongly dependent on their surface chemistry which are the primary issues in biomedicine. The colloidal stability of NPs in dispersions that strongly depends on the net surface charge is also affected by the pH. Not only ionic groups more hydrophilic than their protonated forms, but also the coulombic interaction between these charged groups will prevent aggregation. For example, ABA or dopamine coated NPs can be dispersed in acidic conditions and form stable
solutions because a net positive charge will be induced on the amine groups and generate electrostatic repulsions.

Usually, zeta potential is used as an indication of the colloidal stability of the system. Schematic representation of zeta potential is as shown in Fig. 2.4. In NPs dispersion, the surface of the particle is typically stabilized by capping ligand molecules that are able to generate charge or steric hindrance. If the surfactants contain carboxyl molecules, for example, NPs carry many negative charges on their surface, which will attract positive ions to the particle surface. This strongly bound ion layer is referred as stern layer. Outside the stern layer, positive ions are less firmly linked which form a diffuse layer. The ions within the diffuse layer form a stable entity with the particles, in which ions move together with particle while ions beyond the diffuse layer stay in the bulk dispersant. The potential at this plane is defined as the zeta potential. The magnitude of zeta potential indicates the stability of the particles dispersion. Generally particles in dispersion media are considered to be stable in between -30 mV to +30 mV. The particles larger value of zeta potential (absolute value) suggests that particles are strongly positive or negative charged at the boundary of diffuse plane and the electrostatic repulsion can separate particles and prevent agglomeration. However, lower zeta potential implies that there are not enough repulsive forces between particles, and they tend to aggregate easily to produce an unstable colloidal dispersion [29].

2.3.3 Particle size and size distribution

Depending on the size of MNPs it offers some attractive possibilities in biomedicine. MNPs should have controllable sizes ranging from a few nanometres up to tens of nanometres, which places them at dimensions that are smaller than or comparable to those of a cell (10–100µm), a virus (20–450 nm), a protein (5–50 nm) or a gene (2 nm wide and 10–100 nm long).
makes them get close to a biological entity of interest. If coated with suitable biological molecules, they can interact with or bind to a biological entity, providing a controllable means of tagging for the biological entity. In the design of MNPs, some aspects should be taken into account. First, MNPs should be crystalline, and each particle consists of only one domain [30]. Most of the biomedical applications of MNPs need that the NPs are monodisperse so that each individual NP possesses nearly identical physical and chemical properties for controlled biodistribution, bio-elimination and contrast effects. Such requirements have motivated many research groups to develop increasingly advanced synthetic approaches to MNPs with controlled size, size distribution, composition and magnetics. The synthesis strategies have also led to several surface modification processes to generate biocompatible MNPs for biomedicine [31]. As MNPs show remarkable new phenomena such as superparamagnetism, high field irreversibility, high saturation field, extra anisotropy contributions or shifted loops after field cooling. These phenomena arise from finite size and surface effects that dominate the magnetic behaviour of individual NPs [18].

2.2.4 Biocompatibility and Toxicity of the MNPs

Nanotechnology has been instrumental in the development and translation of basic research to the clinically relevant therapies. In particular, MNPs have been applied to tag, track and activate stem cells, cell labelling, cell targeting offering an effective means of monitoring in vitro and in vivo behaviour. MNPs are comprised of magnetic oxide core with a biocompatible coating. Safety is an issue of constant concern and emphasis on the importance of investigating the issue of toxicity. Any indication of toxicity can ultimately limit the therapeutic efficiency of the therapy. Toxicity is mainly dependent on the physical, chemical and structural properties of the MNPs itself as well as dose and intended use. Some in vitro studies have
reported, adverse effects of MNPs on cells at *in vitro* in therapeutic doses. However, long term *in vivo* studies have not been reported as extensively \[32\].

Recently, LSMO MNPs have been used for biomedical applications in order to overcome difficulties produced by iron oxide MNPs. Since last some years, the MNPs which have been used in biomedical applications are mainly iron oxide particles such as magnetite (Fe₃O₄) and its two oxidized products, tetragonal maghemite (γ-Fe₂O₃) and hexagonal hematite (α-Fe₂O₃). These natural materials are found in many biological systems. Metallic magnetic materials such as iron, cobalt and nickel are toxic, and may susceptible to oxidation. Therefore, it is essential to chemically stabilize the uncoated MNPs against degradation which occurs due to oxidation (corrosion) and acid erosion. As *in vivo* applications require MNPs to be biocompatible, stable, nontoxic, and monodispersed which requires controlling particle material, size and coating properties. For NPs to be able to approach and interact with the biological molecules they have to be able to evade the RES. Instantly, after being injected into the blood stream, the NPs are coated with blood plasma proteins. This process, is referred as opsonization, makes the NPs susceptible for detection and later removal by phagocytic cells. Hydrophilic NPs (due to their coating chains such as derivatives of dextran and PEG) may be able to resist opsonization and therefore increase their circulation time and enhance the possibility of reaching their target cells \[33-35\]. Some of the possible approaches to enhance the biocompatibility are coating the NPs with organic species (including surfactants or polymers) and coating the NPs with an inorganic layer (such as silica or gold).

Even though the extensive use of MNPs in biomedical applications, questions arise relating to the influence of NPs size and coating on NPs cytotoxicity. Yu et al. \[36\] studied NPs uptake and cytotoxicity by exposing porcine aortic endothelial cells to 5 and 30 nm diameter iron oxide NP coated with either the polysaccharide dextran, or the polymer PEG. Bare NPs of both
sizes induced a more than 6 fold increase in cell death at the highest concentration (0.5 mg/mL) and led to significant cell elongation, whereas the cell viability and morphology remained constant with functionalized NPs. Significant reactive oxygen species (ROS) formation was induced with only the uncoated large (30 nm) NPs. NPs are more toxic to cells at lower concentrations when cells were cultured within 3D gels and toxicity could be reduced by using coating, however, several mechanisms have been reported for NPs in different sizes.

2.3 Stabilization of MNPs in solvents

![Schematic of common surfactants for stabilizing NPs in water](image_url)

**Fig 2.5** Schematic of common surfactants for stabilizing NPs in water [37].

Stabilization of MNPs in solvents against aggregation is achieved through different stabilizers such as monomeric stabilizer, polymeric
stabilizer, Inorganic Materials, and ligand exchange. Fig. 2.5 shows common coating material used for stabilization of MNPs.

2.3.1 Monomeric Stabilizers

Various functional groups, including carboxylates, phosphates, amines and hydroxyls are used to modify the surface of MNPs. Additionally, this stabilization can be tailored for dispersibility into oil/hydrocarbon carrier fluids or aqueous media.

2.3.1.1 Carboxylates

The surface of MNPs can be functionalized in an aqueous dispersion by the adsorption of citric acid. This acid adsorbed on the surface of the MNPs by the process of coordinating via one or two of the carboxylate functionalities, depending upon the steric necessity and the curvature of the surface. This leaves at least one carboxylic acid group exposed to the solvent, which should be responsible for making the surface negatively charged and hydrophilic. Carboxylates have important effects on the growth of iron oxide NPs and their magnetic properties. Liu et al. studied the influence of the presence of citric acid during iron oxide synthesis. They reported that increasing concentrations of citric acid caused significant decreases in the crystallinity of the iron oxides formed. It means, the presence of citrate led to changes in the surface geometry of the materials formed [38].

2.3.1.2 Phosphates

The numbers of researchers have focused on phosphate surfactant for stabilization of MNPs. Researchers have studied the possibility of using alkanesulphonic and alkanephosphonic acid surfactants as efficient binding ligands on the surface of ferrite NPs and as stabilizers for particle dispersion in organic solvents [39]. Now days, SPM nanosized particles have been
prepared by controlled coprecipitation method in the presence of highly hydrophilic poly(vinylalcohol phosphate) (PVAP). The impacts of the polymer concentration on the particle size, size distribution, colloidal stability, and magnetic property have been studied. The aqueous suspension of MNPs, prepared using 1% PVAP solution, has been found to be stable for 4 weeks at pH 5-8 [40]. The acceptable biocompatibility of phosphonate and phosphate ligands may advance toward the use of encapsulated MNPs in medical applications, such as MRI and other biophysical purposes.

2.3.1.3 Glycine

Glycine is a simplest amino acid and is quite abundant in various proteins and enzymes. The carboxylic acid group present in the glycine donates its proton to the amino group to form the structure \( \text{NH}^+ \text{CH}_2\text{COO}^- \). Thus, in solid state, glycine exists as a dipolar ion in which carboxyl group is present as a carboxylate ion and amino group is present as ammonium ion. Due to this dipolar nature, glycine has a high melting point. To our knowledge, the study indicates the incorporation of amino acids in the elaboration of magnetic colloids deal normally with the synthesis of magnetite cores followed by its functionalization with different amino acids or even synthesizing MNPs in the presence of amino acids, in one step [41]. Particularly for glycine, Viota et al. studied the electrokinetic characterization of MNPs functionalized with several amino acids, including glycine [42] and Barick and Hassan reported a single-step approach for the synthesis of glycine-passivated iron oxide NPs both for biomedical applications. Glycine has a higher affinity to the surface of ultrafine MNPs so that agglomeration of small particles is reduced [43]. In present work LSMO NPs have coated with glycine.
2.3.2 Polymer Stabilizers

There are several strategies have been developed to coat MNPs including in situ coatings and post-synthesis coatings. In the first step, NPs are coated during the synthesis. Generally, in coprecipitation method NPs have been coated during the synthesis. The two step synthesis coating method consists of grafting the polymer on the magnetic particles once synthesized. The most common coating polymers in the literature are dextran, carboxymethylated dextran, carboxydextran, starch, arabinogalactan, glycosaminoglycan, sulfonated styrene-divinylbenzene, PEG, PVA, poloxamers, and polyoxamines, PVP etc. [39].

2.3.2.1 Dextran

Dextrans are polysaccharides obtained by the chemical attachment of hydrocarbons to dextran, a polymer of anhydrogulococe monomers. Dextran has been used often as a polymer coating mostly because of its biocompatiability [39]. They exhibit spontaneous associative properties in aqueous dispersion due to sequestering of the hydrophobic chains away from the water. Since dextrans are biocompataible, have a zero net charge, and can undergo a variety of chemical coupling reaction, dextrans are attractive candidates to create a water- stable colloids from fatty acid coated MNPs. Though a single hydrogen bond is relatively weak, total bonding energy of hydrogen bonds over the length of the polysaccharide molecule can be very high because of the large number of hydroxyl groups per molecule. Even though, several approaches for surface functionalization of NPs have been studied a procedure for coating NPs with a stable and reactive coating possessing aldehyde group capable of conjugating amino containing molecules was not reported. Bhayani et al. described for the first time, biocompatiable molecules such as BSA and dextran can be effectively immobilized onto LSMO NPs which showed lower cytotoxicity and enhanced
hyperthermia effect [44]. Thorat et al. studied the dextran coated LSMO NPs for hyperthermia application. [45].

2.3.2.2 PVP

PVP is a hygroscopic, amorphous and in powder form. It is nontoxic, neutral and has enormous applications in pharmaceutical, medicine, cosmetics and industry. It is well known that PVP is additive to the coating of the NPs possesses very good wetting property which can easily forms film. Recently, Lee et al. studied PVP-coated iron oxide NPs as an MRI contrast agent. From in vitro and in vivo MRI studies, they observed that the reduction of $T_2$ MRI intensity is comparable to or more obvious for the PVP-IO as compared to commonly used Feridex (ferumoxides injectable solution) at the same concentration of iron oxide NPs [46]. In earlier research work, we have reported the PVP coated LSMO for cancer hyperthermia therapy application [47]. Very few reports are available in literature of LSMO NPs coated with amphiphilic polymer PVP for biomedical application. Results showed better colloidal stability of PVP coated LSMO NPs suspended in phosphate buffer solution for hyperthermia cancer therapy.

2.3.2.3 Acrypol

Recently, acrypol (AP) has been used as potential agent for suspension medium as well as a coating material. AP is a synthetic high molecular weight, cross linked water soluble polymer of acrylic acid, which is also known as ‘Carbomer’. It is broadly employed in cosmetic, pharmaceutical and household industries. In water at neutral pH, AP is an anionic polymer, i.e. many of the side chains of AP will lose their protons and obtain a negative charge. This outcome in the AP polyelectrolyte, with the ability to absorb and retain water and swell to many times their original volume. Especially, AP is an aqueous soluble polymer with a high density of reactive functional groups,
i.e. -COOH, which makes it very attractive in biomedicine mainly due to its capability to form flexible polymer chain-protein complexes through electrostatic, hydrogen bonding or hydrophobic interactions. In earlier research work, we have reported the AP coated LSMO for MFH or application [48]. In this research work, we have reported the influence of acrypol coating on structural, magnetic and colloidal properties of LSMO manganite and heating mechanism. This study mainly focused on optimization of surface coating for cancer hyperthermia. These coated NPs showed good colloidal stability and enhanced biocompatibility and enhanced hyperthermia effect for particular coating concentration (4% w/v).

2.3.2.4 PEG

PEG is widely used as surfactant due to its hydrophilicity, non-immunogenicity, non-antigenicity and nontoxicity. PEG coating on the surface of the MNPs can reduce protein and cell adsorption onto the particles and reduce the rate of clearance (through organs such as kidney) of “PEGylated” materials. Therefore, PEG coating increases the particle circulation time for in vivo applications [49]. Kim et al. reported that PEG coated MNPs are internalized into the cytoplasm to a greater extent because of their high affinity for phospholipid bilayer membranes and also they are internalized via fluid phase endocytosis without cellular membrane stress [50]. Feruglose (Clariscan) can be regarded as true “stealth NPs”, because of the pegylation of the coating starch, that are hardly recognized by the macrophage-monocytic system and probably not suitable for macrophage imaging. Recently, novel SPM iron oxide NPs coated with polymerized polyethylene glycolylated bilayers were prepared [51]. The PEG-coated NPs revealed excellent solubility and stability in aqueous solution as well as in physiological saline.
2.3.2.5 PVA

PVA is a unique synthetic polymer that can transform into a polymer gel that is a class of macromolecular network with unique properties. PVA is a hydrophilic, biocompatible polymer. PVA coating onto the particle surface prevents their agglomeration, giving rise to monodisperse particles. For example, Lee et al. modified the surface of NPs with PVA by precipitation of iron salts in PVA aqueous solution to form stable dispersion [52]. Albornoz et al. reported the synthesis of an aqueous ferrofluid and the preparation of a magnetic gel with PVA and glutaraldehyde (GTA). They reported a good stability of its properties versus time. The magnetic gel was dried to generate a biocompatible film [53].

2.3.3. Inorganic Materials

Mostly, silica and gold are used for protecting MNPs against agglomeration. MNPs can be coated with silica, gold, or gadolinium (III). These surfactants not only enhance the stability to the NPs in solution, but also help in binding various biological ligands to the NPs surface. These NPs show an inner magnetic core with an outer metallic shell of inorganic materials.

2.3.3.1 Silica

Silica has been utilized as a surfactant for MNPs. The Stöber method is a well-known and prevailing approach for coating MNPs, a process in which silica is formed in situ through the hydrolysis and condensation of a sol-gel precursor (e.g. Tetraethyl orthosilicate (TEOS)) [54]. The coating thickness can be controlled by changing the amount of TEOS. The silica coatings have several advantages due to their ability to protect the magnetic cores and to prevent aggregation via variation of the shell thickness. Moreover, the presence of surface silanol groups can enable the covalent attachment to the
surface of NPs. For example, amino groups or fluorescent groups have been introduced on the surface of silica-coated MNPs by hydrolysis and condensation of 3-aminopropyltriethoxysilane (APTES) or fluorescein isothiocyanate-linked APTES on the surface of MNPs, respectively [55]. Recently, mesoporous silica shell coated MNPs obtained after acid etching have been applied for controlled drug release [56]. Usually, an inert silica coating on the surface of MNPs prevents their aggregation in liquid, improves their chemical stability, and provides better protection against toxicity. This coating stabilizes the MNPs in two different ways. One is by shielding the magnetic dipole interaction with the silica shell. On the other hand, the silica NPs are negatively charged. Therefore, the silica coating enhances the coulomb repulsion of the MNPs.

2.3.3.2 Gold

Gold (Au) is another inorganic coating which is appropriate for protecting the iron core against oxidation. Since, Au can form strong bonds with sulfur, the Au shell also allows facile conjugation with a variety of biomolecules, making these composites useful in biomedical applications. For example, Au -coated MNPs with thiolated PEG masks them from the intravascular immune system. Moreover, gold nanocrystals exhibit an absorption band in the visible region due to its surface plasmon phenomenon, allowing the Au -coated MNPs to be used for optical applications. It was suggested that gold-coated MNPs could be promising for applications in biomedicine [57].

2.3.4 Ligand exchange

For applications in biomedicine, ligand exchange of NPs usually involves a process that the initial hydrophobic ligands are replaced by other more strongly bonding hydrophilic ligands that allow the transferring of NPs
from the organic phase to aqueous solution. In order to enhance the colloidal stability of given NPs, the ligand molecules on the surface can be exchanged by others that can possibly offer new properties or functionality to the particles. A number of hydrophilic ligands such as thiol, carboxyl, amine, phospine group, etc., have been reported to exchange the nature ligand on the surface of NPs and bring them to an aqueous solution, which includes small molecules with functional headgroups (e.g. PEG derivatives and biological molecules [58]. Small molecules with high affinity head functional group are primary candidates for generating water-soluble particles, as they produce NPs with a smaller hydrodynamic radius, which promotes in vivo trans membrane permeation and excretion of NPs. General examples of small molecules are alkylthiol terminated molecules that can strongly bind to the inorganic surface of NPs, e.g. Au and Ag [59] or CdSe QDs, by substituting weaker ligands. However, the colloidal stability of the resulting NPs in buffer solution is often poor, which is partly ascribed to the desorption of ligands from NPs. In order to overcome this problem, bidentate ligands such as dihygrolipoic acid (DHLA) and dithiocarbamate ligands are used to stabilize the NPs by increasing the number of anchor points on the particle surface.

In most cases, study showed the incoming ligand molecule is bound more strongly to the inorganic NPs surface. Additionally, alkyl thiol terminated ligand molecules; many molecules with other functional headgroups have also been developed to transfer NPs from the organic phase to aqueous solution [60]. However, one drawback is that the small ligands rely on electrostatic interaction to stabilize NPs. Therefore, when the solution condition such as pH and salt concentration changed, the NPs may be “salting out” and forming aggregation. These considerations imply that for ligand exchange, the new ligand molecules should have an affinity as strong as possible to the inorganic core in order to quickly and effectively replace the original surfactant molecules. In addition, the molecular geometry of the
Chapter 2

ligands in relation of the particle diameter is a factor that influences how densely the molecules are packed around the particles, which in turn influences ultimately the colloidal stability of the particles. However, ligand-coated NPs differ from simple micelles consisting of the ligand molecules alone, which are only held together by intramolecular forces, in that on NPs, ligand molecules are additionally attached to the NPs surface, in most cases of a chemical functional group.

2.4 Overview on biomedical applications of surface functionalised LSMO NPs

MNPs, especially SPM LSMO NPs, are potential magnetic probes for biomedical applications. These MNPs with a small size, narrow particle size distribution and high magnetization values had been used in many biomedical applications, such as targeted drug delivery contrast agents in MRI and hyperthermia treatment of cancers. [47-48], [61-63]. At a core diameter of less than 20 nm and overall hydrodynamic diameter of less than 50 nm, these NPs are of a size that is comparable to the nuclear pore size (50 nm) and are much smaller than a cell (normally 10–100mm). Under the normal range of magnetic field strengths used in MRI scanners (usually higher than 1 tesla), these SPM NPs in the targeted area can be magnetically saturated, establishing a substantial locally perturbing dipolar field that leads to a marked shortening of proton relaxation (T₂ relaxation) in the MRI process and giving a “darker” image of the targeted area over the biological background.

Kacenka et al. have studied first time dual imaging probes for MRI and fluorescence microscopy based on LSMO perovskite MNPs [62]. The group indicated that the relaxometric study of fluorescent coated LSMO NPs at 20°C carried out at magnetic fields of \(B₀=0.5\), 1.5 and 3T, which are similar values of \(T₂=580, 540\) and \(520\ s^{-1}\ \text{mmol (Mn)}^{-1} \text{L}\), respectively, far exceeding the relaxivities reported for clinically used iron oxide NPs. These values belong to
the highest ever reported $T_2$ relaxivities of experimental contrast agents. They showed that the difference of $T_2$ relaxivities could originate both from the different nature of the coating layers around the magnetic cores and the distinct size distributions of the magnetic cores in the compared samples (as a consequence of differences in the mechanical processing of the as grown products and different size fractionation connected with the encapsulation procedures). Haghniaz et al. have reported for the first time by *in vivo* experimentation that Dextran stabilized-LSMO NPs can also be used as a new type of contrast agent in MRI [63]. Interestingly, they observed that the Dex-LSMO NPs (average size = 50 nm) exhibit both positive and negative contrast properties with $T_1$ relaxivity value equal to 6.741/s mg mL and superior $T_2$ value of 778 /s mg mL. More, significantly, when injected at the site of a tumor (viz. melanoma), Dex-LSMO NPs exhibit strong magnetomotive signals.

Louguet et al. indicated first time thermoresponsive hybrid system for drug delivery purposes which is designed by modifying the surface of silica-coated magnetic LSMO NPs with block copolymers following a non-covalent approach [61]. The physical adsorption of polyether-bpoly(L-lysine) copolymers onto LSMO MNPs embedded in a silica layer affords the opportunity to finely tune the grafting density of the polyether blocks at the particle surface. In their work importantly, it is shown that using a short segment of PLL is of interest to have a dense brush of polyether blocks with stabilizing properties. This work focuses specially, binary brushes of Polyethylene oxide (PEO) and P(EOx-co-POy) and is achieved with a good control over their thermoresponsiveness and their drug loading capacity by varying a single parameter, the PO content [61]. Researchers have also focused on toxicity studies of LSMO NPs which is the primary issues for biomedical application. Recently, our group studied cytotoxicity assays with different cell lines and with different assays and compared the toxicity of
coated and uncoated materials. These results show that coated particles were more biocompatible than the bare particles [45], [47-48], [64-65].

Furthermore, under an alternating (AC) magnetic field with controlled field amplitude and field reversal frequency, magnetization of the SPM NPs attached to the bio-entity can be switched back and forth. Under these conditions, these MNPs show heating ability which is the required for cancer hyperthermia therapy [63]. As like in vitro study, the selected and important contributions of LSMO NPs in the field of magnetic hyperthermia are discussed in the given table 2.1.
Table 2.1 Overview on different LSMO compounds used for hyperthermia application.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Synthesis method</th>
<th>Average size of MNPS nm</th>
<th>Coating material</th>
<th>Magnetic property At RT</th>
<th>AMF (Am$^{-1}$)</th>
<th>Frequency (kHz)</th>
<th>SAR W/g</th>
<th>Cell line</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSMO $(0.23 \leq x \leq 0.45)$</td>
<td>Microwave refluxing technique</td>
<td>20–30</td>
<td>Acrypol suspension</td>
<td>Ms=38 emu/g</td>
<td>425</td>
<td>99</td>
<td>P66, P9, and P102</td>
<td>[66]</td>
<td></td>
</tr>
<tr>
<td>La$<em>{0.7}$Sr$</em>{0.3}$MnO$_3$</td>
<td>Sol-gel</td>
<td>20-25 nm</td>
<td>TEOS</td>
<td>Ms=17 emu/g, Hc=0</td>
<td>230 kHz</td>
<td>156</td>
<td>LE cells</td>
<td>[67]</td>
<td></td>
</tr>
<tr>
<td>La$<em>{0.7}$Sr$</em>{0.3}$MnO$_3$</td>
<td>Citrate-gel</td>
<td>60-210 nm by SEM</td>
<td>BSA</td>
<td>Ms=14 emu g$^{-1}$, Hc=&lt;100 Oe</td>
<td>20000 kHz</td>
<td>0.15 to 16.5</td>
<td>-</td>
<td>[68]</td>
<td></td>
</tr>
<tr>
<td>La$<em>{0.75}$Sr$</em>{0.25}$MnO$_3$</td>
<td>Sol-gel</td>
<td>25-45 nm by SEM</td>
<td>Dextran</td>
<td>Ms=30 emu/g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>La$<em>{0.75}$Sr$</em>{0.25}$MnO$_3$</td>
<td>Sol-gel</td>
<td>20 nm</td>
<td>silica</td>
<td>-</td>
<td>8.7 kA/m</td>
<td>480kHz,</td>
<td>130</td>
<td>A549 cells, HeLa cells, Saos-2 cells and HepG2 cells</td>
<td>[69]</td>
</tr>
<tr>
<td>Formula</td>
<td>Method</td>
<td>Size</td>
<td>Additive</td>
<td>Binder</td>
<td>Ms</td>
<td>H</td>
<td>Cell Line</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
<td>-------</td>
<td>----------</td>
<td>------------</td>
<td>------------</td>
<td>---</td>
<td>----------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>La$<em>{0.82}$Sr$</em>{0.18}$MnO$_3$</td>
<td>Combustion</td>
<td>9 nm</td>
<td>Silica</td>
<td>-</td>
<td>88 mT</td>
<td>108 kHz</td>
<td>36</td>
<td>[70]</td>
<td></td>
</tr>
<tr>
<td>La$<em>{0.7}$Sr$</em>{0.3}$MnO$_3$</td>
<td>Citrate-gel</td>
<td>-</td>
<td>Dextran</td>
<td>-</td>
<td>88 mT</td>
<td>264 kHz</td>
<td>13 ± 3 A375 cell line</td>
<td>[71]</td>
<td></td>
</tr>
<tr>
<td>La$<em>{0.75}$Sr$</em>{0.25}$MnO$_3$</td>
<td>Planetary ball mill</td>
<td>10.3</td>
<td>Silica</td>
<td>-</td>
<td>-</td>
<td>125</td>
<td></td>
<td>[67]</td>
<td></td>
</tr>
<tr>
<td>La$<em>{0.7}$Sr$</em>{0.3}$MnO$_3$</td>
<td>Combustion</td>
<td>~25 nm.</td>
<td>OA–betaine HCl</td>
<td>M$_s$=35-70 emu/g</td>
<td>335.2 Oe</td>
<td>265 kHz</td>
<td>45 HeLa and L929 cells</td>
<td>[72]</td>
<td></td>
</tr>
<tr>
<td>La$<em>{0.7}$Sr$</em>{0.3}$MnO$_3$</td>
<td>Combustion</td>
<td>30 nm</td>
<td>Oleic acid</td>
<td>Ms=29em u/g</td>
<td>335.2 Oe</td>
<td>265 kHz</td>
<td>40.22 HeLa and L929 cells</td>
<td>[64]</td>
<td></td>
</tr>
<tr>
<td>La$<em>{0.7}$Sr$</em>{0.3}$MnO$_3$</td>
<td>Combustion</td>
<td>25 nm</td>
<td>-</td>
<td>Dextran</td>
<td>Ms=29emu/g,</td>
<td>335.2 Oe</td>
<td>265 kHz</td>
<td>51 HeLa and L929 cells</td>
<td>[65]</td>
</tr>
<tr>
<td>La$<em>{0.7}$Sr$</em>{0.3}$MnO$_3$</td>
<td>Combustion</td>
<td>23 nm</td>
<td>PVP</td>
<td>-</td>
<td>Ms=36.4 4 emu/g,</td>
<td>335.2 Oe</td>
<td>265 kHz</td>
<td>62.3 -</td>
<td>[47]</td>
</tr>
<tr>
<td>La$<em>{0.7}$Sr$</em>{0.3}$MnO$_3$</td>
<td>Combustion</td>
<td>22 nm</td>
<td>Acrypol</td>
<td>-</td>
<td>Ms=43.1 7 emu/g,</td>
<td>335.2 Oe</td>
<td>265 kHz</td>
<td>80.82 HeLa cells</td>
<td>[48]</td>
</tr>
</tbody>
</table>
References


Chapter 2


[37] “Surface functionalization and bioconjugation of nanoparticles for biomedical applications” Ph.D. thesis submitted to The School of Graduate and Postdoctoral Studies, the University of Western Ontario London, Ontario, Canada by L. Chen (2013) 25.


[64] N. D. Thorat, V. M. Khot, A. B. Salunkhe, A. I. Prasad, R. S. Ningthoujam


