Chapter 2

SURFACE FUNCTIONALIZATION OF MNPS:
THEROTICAL BACKGROUND

“Optimism is the faith that leads to achievement.
Nothing can be done without hope and confidence”

- Helen Keller
2.1. Introduction

$\text{Fe}_3\text{O}_4$ MNPs have proved their tremendous potential to be used for various biomedical applications. They offer attractive possibilities in biomedicine, research and separation processes due to such notable properties as superparamagnetism and biocompatibility. Additionally, the ease of synthesis and subsequent coating and functionalization, and the high surface area to volume ratios of NPs provide unparalleled versatility. These applications include biosensing, targeted drug delivery, hyperthermia therapy, cell labelling, MRI etc. Out of these, \textit{in vivo} applications of MNPs need to consider some parameters like biocompatibility and cytotoxicity of them. However, these properties primarily depend on their surface characteristics. Nature of surface charges and relative functional groups present on the surface of MNPs determine their colloidal stability, biocompatibility and cytotoxicity.

The bare SPIONs are eager to reduce their surface energy via formation of clusters and agglomerates. The formation of magnetic aggregates is not suitable for biomedical applications because of very fast detection by immune system. A suitable coating should be applied on the SPIONs in order to achieve NPs with hydrophilic surfaces that have high colloidal stability and dispersability without deterioration of their magnetic properties. Colloidal stability of SPIONs in the biological environment is also very important for \textit{in vivo} applications and particularly for intravascular injection [1, 2, 3]. If particles lose their stability in biological fluids, they could end to atherosclerosis and thus would lose their functionality and application. Since
the isoelectric point of SPIONs is around pH = 7 [4, 5], colloidal stability of SPIONs in the biological environment is a major shortcoming. Surface modification of the NPs by coating materials is thus became a suitable route to improve the colloidal stability of SPIONs. In fact, the physical and chemical properties of the applied coating materials influence the colloidal stability in turn [6, 7]. Ability to escape from reticuloendothelial system (RES) and consequent increasing in the blood circulation half-life for NPs is regarded as another important role which coatings may play in biomedical applications [8-11]. Finally, coatings should be able to isolate the magnetic core from in vivo environment, improving the biocompatibility of the NPs. As a matter of fact, the type and amount of coating can be recognized as a critical matter in biological application of magnetic materials.

Biocompatible polymers are widely used as coating materials for SPIONs to accomplish multiple purposes including colloidal stabilization, delivering biologically active agents with a controlled release profile, and targeting capability to specific tissues via conjugation with specific ligands [12]. The polymeric coatings can be induced either during [13, 14] or after synthesis [15-19] dependent on the properties required. Biopolymers are made of simple biological compounds and are produced by living organisms. They are renewable materials, generally non-toxic and biodegradable, thus combining excellent functional properties with environmental friendliness and sustainable development features.
2.2. Surface functionalization

When MNPs are coated with a polymer, it forms an interface. This interface is nothing but a heterojunction or organic-inorganic interface. A heterojunction is defined as the interface formed between two dissimilar materials usually two semiconductors having different band gaps. The nature of junction interface is classified as abrupt and graded. When transition from one material to other takes place within a few atomic distances (< or equal to 1 micron), it is called abrupt type. In graded type, the interface is of the several diffusion lengths. The two semiconductors forming heterojunction having similar type of conductivity are isotype heterojunction otherwise anisotype. The three main factors affect properties of interface, viz. lattice mismatch, thermal mismatch and cross diffusion or interdiffusion between two materials.

A) Lattice mismatch

The materials should be closely matched in lattice spacing. The lattice mismatch (L.M) for the materials with lattice parameters \(a_1\) and \(a_2\) given by,

\[
\text{Lattice mismatch} = \frac{2 (a_2 - a_1)}{a_2 + a_1} \quad \text{......... (2.1)}
\]

Lattice mismatch is zero for ideal junction and \(a_1 = a_2\).

B) Thermal mismatch

Due to different thermal expansion coefficients, mismatch may occur at high temperature, resulting in cracking or peeling of interface. This mismatch
can be minimized by fabrication of junction at low temperature or room temperature and by controlling thickness of both materials.

C) Interdiffusion

The diffusion coefficient of two materials should be small as one material may diffuse to other material at high temperature. Interdiffusion can be avoided by fabricating heterojunction at low temperature.

2.3. Surface functionalization of MNPs with chitosan

Considering biodegradability and toxicity, much attention has been paid to chitosan, since it possesses interesting properties such as gelation characteristics, biodegradability, biocompatibility, film forming ability and bioadhesion. Another advantage of CS is that the amine groups can offer a variety of active sites for further biofunctionalization.

2.3.1. Chitosan

Chitosan is a linear polysaccharide. It is composed of randomly distributed deacetylated units i.e. β-(1-4)-linked D-glucosamine and acetylated units i.e. N-acetyl-D-glucosamine. Chitosan is produced commercially by deacetylation of chitin (Fig. 2.1). Chitin is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi. Chitosan is an alkaline, nontoxic, hydrophilic, biocompatible, and biodegradable polymer. The amino group in chitosan has a pKa value of ~6.5,
which leads to a protonation in acidic to neutral solution with a charge density dependent on pH and the %DA-value. This makes chitosan water soluble and a bioadhesive in nature, which readily binds to negatively charged surfaces like mucosal membranes. Chitosan boosts the transportation of polar drugs across epithelial surfaces and is biocompatible and biodegradable.

![Fig 2.1: Structures of chitosan and its parental molecule, i.e. chitin.](image)

### 2.3.2. suitability of chitosan for functionalization

Several studies have been conducted with the purpose of developing chitosan coated magnetic nanoparticles. Chitosan is a biocompatible, biodegradable polymer with antibacterial activity and affinity for many biomacromolecules. Alcohol, amine, amide, and ether functions present in the
chitosan structure can be involved in the formation of hydrogen bonds with substrates by inter- or intramolecular hydrogen bonding. Such groups give the molecule important characteristics such as bioadhesion (prolonged interaction with biological tissue). Also, it has been hypothesized that chitosan directly interacts with cell membranes. Such characteristics have motivated interest in the development of iron oxide–chitosan nanoparticles.

There are much attention on using chitosan as a coating of SPION due to its specific biological properties such as biocompatibility, biodegradability, antibacterial, wound healing activity and mucoadhesive properties (causing high affinity for cell membranes) \[20-22\]. It has been approved that chitosan enhances the contact between drug and ocular mucosa due to their high mucoadhesive properties \[23-27\]. Another advantage of CS is that the amine groups can offer a variety of active sites for further biofunctionalization.

### 2.3.3. Mechanism of coating of chitosan

Incorporation of chitosan into the structure of matrix phase induced pronounced pH sensitivity upon the presence of ionizable amino functional groups on its backbone. Reports indicate that the hydrogen of primary amino group (\(-\text{NH}_2\)) in CS from strong hydrogen bonding with the oxygen in \(\text{Fe}_3\text{O}_4\) \[28\]. The details are shown in Fig. 2.2.
Fig. 2.2: Coating mechanism of CS on the surface of Fe₃O₄.

Fig. 2.3: Cross-linking of CS molecules using glutaraldehyde as cross-linking agent.

Some reports suggest use of glutaraldehyde as a cross-linker for the stability of coating. Glutaraldehyde crosslinks chitosan by a Schiff base
formation which is confirmed by presence of imine (C=N). Fig. 2.3 shows Schiff base formation in CS and glutaraldehyde molecules [29].

2.4. Surface functionalization of MNPs with acrypol

Carbomer is a term used for a series of polymers primarily made from acrylic acid. They are white, fluffy powders but are commonly used as gels in personal care products and cosmetics. They can be found in ample variety of product types including hair, nail, skin and makeup products.

2.4.1. Acrypol

Acrypol, i.e. carbomer is polymers of acrylic acid cross-linked with a polyalcohol allyl ethers. It is slightly acidic polymer. The pH value of carbomer in water is changes between 2.8 to 3.2 according to the content. The higher of carboxylic acid groups level, the lower of pH value.

Carbopol polymers are offered as fluffy white, dry powders (100% effective). The carboxyl groups provided by acrylic acid backbone of the polymer are responsible for many of the product benefits. Carbopol polymers have an equivalent weight of 76 per carboxyl group. The general structure of polyacrylic acid can be demonstrated with Fig. 2.4.
2.4.2. **Suitability of acrypol for functionalization**

Carbopol polymers readily absorb water, get hydrated and swell. In addition to their hydrophilic nature, their cross-linked structure and their essential insolubility in water makes them a potential candidate for use in controlled release drug delivery system. Carbopol polymers form hydrogels that change their swelling behavior upon exposure to an external stimulus such as change in temperature, light, pH or electric field and are called as "smart gels" or "environmentally responsive polymers". They have recently attracted considerable interest in the field of drug delivery as a means of providing an on-off release by shrinking and swelling in response to the change in pH. In stomach, the carbopol polymer forms the hydrogen bond with the drug and also with the proteins or polysaccharides of mucosa, which is possibly the major mechanism for bioadhesion. Additionally, in alkaline conditions of the intestine, their gels are very highly swollen. Their presence in mucoadhesive formulation can provide a gastric retention system by swelling in stomach and inducing a pseudofed state, thus reducing peristaltic contraction. This
observable fact is dependent on viscosity, the higher the viscosity, lower is the contraction. In this study design, the polymer used is carbopol-934 (C934) which consists of chains of polyacrylic acid. The hydrophilic polymers may form a complex with the low solubility drug-like norfloxacin.

2.4.3. **Mechanism of coating of acrypol**

Acrypol (AP) is reported to be adsorbed on the surface of $\text{Fe}_3\text{O}_4$ MNPs [30]. Originally, AP is a highly coiled cross-linked polymer before contact with water (Fig. 2.5). Acrypol is used as a suspension media by few researchers. MNPs suspended in acrypol solution show tremendous stability as acrypol forms a very viscous solution. Wet acrypol gets swollen and increase in its size in which MNPs are suspended. This can be shown properly shown as in Fig. 2.6. The actual mechanism by which AP forms hydrogen bonding with magnetite molecule is revealed in Fig. 2.7.

![Fig. 2.5: Tightly coiled Cross-linked polyacrylic acid before contact with water.](image)
Fig 2.6: Suspension of MNPs in swollen acrypol.

Fig 2.7: Oxygen atoms of Fe₃O₄ form hydrogen bonding with hydrogen atoms of acrypol.

2.5. Surface functionalization of MNPs with oleic acid

OA is supposed to decrease particle size of MNPs when added in the reaction mixture. Furthermore, OA is a biomolecule and hence, naturally, is
biocompatible. Therefore, whenever MNPs with smaller sizes are prepared for in vivo biomedical applications, OA coating is mostly preferred.

2.5.1. Oleic acid

OA is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is odourless, colourless oil, even though commercial samples may be yellowish coloured. In chemical terms, OA is categorized as a monounsaturated omega-9 fatty acid, abbreviated with the lipid number of 18:1 cis-9. It has the formula \( \text{CH}_3(\text{CH}_2)_{7}\text{CH}=\text{CH}(\text{CH}_2)_{7}\text{COOH} \) (Fig. 2.8). The term "oleic" signifies related to or derived from oil or olive, the oil that is principally composed of OA.

![Fig. 2.8: Structure of oleic acid.](image)

2.5.2. Suitability of OA for functionalization

Oil-soluble type functionalization employed in order to prevent or decrease the agglomeration of iron oxide MNPs and increase the stability give
rise to the monodispersity, for instance, iron oxide MNPs frequently dispersed in long chain substance of hexadecane, the classic example being OA $[\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}]$, which has a C-18 tail with a cis-double-bond in the middle, forming a kink. Such kinks have been assumed to be necessary for effective stabilization.

Fig. 2.9: Schematic of OA coating on Fe$_3$O$_4$ MNPs and its advantages.

Additionally, OA is widely used in ferrite nanoparticle synthesis because it can form a dense protective monolayer, thereby producing highly uniform and monodispersed particles. Capping agents such as oleic acid are often used because they form a protective monolayer, which is strongly bonded
to the surface of nanoparticles. This is necessary for making monodisperse and highly uniform MNPs. All the advantages concerned with OA coating on the surface of the MNPs are shown in Fig. 2.9.

2.5.3. Mechanism of coating of OA

In one-pot synthesis of Fe₃O₄ MNPs, oleic acid plays an important role in controlling the particle size. When the nucleation starts during the reaction of MNPs synthesis, OA gets coated on them and prevent further growth of the particle size. COO’ of OA molecules form hydrogen bonding with oxygen atoms of the Fe₃O₄ particles [31]. The resulting structure of OA coated MNPs is given in Fig. 2.10.

![Fig 2.10: Mechanism of OA coating on surface of Fe₃O₄ MNPs.](image)
2.6. Rendering hydrophilicity to oleic acid-coated MNPs

Coating of Fe$_3$O$_4$ MNPs with OA makes the particles dispersible only in organic solvents and consequently limits their use for biomedical applications [6]. For biomedical applications in aqueous environments, this hydrophobic coating has to be substituted with a hydrophilic coating. Lattuada and Hatton [7] reported that the oleic groups initially present on the above nanoparticle surfaces were replaced via ligand-exchange reaction with various capping agents bearing reactive hydroxyl moieties (Fig. 2.11).

![Diagram of rendering hydrophilicity to hydrophobic OA coated Fe$_3$O$_4$ MNPs by further functionalization with CS.]

**Fig. 2.11:** Rendering hydrophilicity to hydrophobic OA coated Fe$_3$O$_4$ MNPs by further functionalization with CS.
This route was proposed a flexible methodology for the preparation of various types of monodisperse, water-soluble magnetic Fe$_3$O$_4$ NPs coated by different polymer brushes.
REFERENCES


