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SYNTHESIS, SURFACE MODIFICATIONS AND BIOMEDICAL APPLICATIONS OF SUPER PARAMAGNETIC IRON OXIDE NANOPARTICLES (SPIONs)

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Abstract

This review mainly discussed about the synthetic chemistry, functionalization of iron oxide nanoparticles with various biologically compatible molecules i.e. polymeric molecules such as PEG, PVA, gelatin, dextran, inorganic molecules such as silica, gold, gadolinium & biological molecules such as proteins, antibodies & targeting ligands & applications of these iron oxide nanoparticles in various fields such as cellular labeling, tissue repair ,drug delivery, MRI, Hyperthermia, magnetofection, detoxification of biological fluid & removal of carbon monoxide.

Keywords: SPIONs, Synthesis, Surface Modification and Biomedical Applications.

1. Introduction

Super paramagnetic Iron Oxide Nanoparticles (SPIONs)

Iron oxide exists in many forms in nature, with magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), and hematite (α- Fe_2O_3) being probably the most common. These three oxides are also very important technologically and they are the subject of this review. Hematite is the oldest known of the iron oxides and is widespread in rocks and soils. It is also known as ferric oxide, iron sesquioxide, red ochre, specular iron ore, kidney ore or martite. Super paramagnetic iron oxide nanoparticles (SPIONs) with appropriate surface chemistry have been widely used experimentally for numerous in vivo applications such as magnetic resonance imaging contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery and cell separation etc. [1]

SPIONs which are biocompatible such as magnetite have been widely used for in vivo biomedical applications including magnetic resonance imaging (MRI) contrast enhancement, tissue specific release of therapeutic agents, hyperthermia, and magnetic field assisted radionuclide therapy [2]. Their slower renal clearance and higher relaxation values compared to the gadolinium-based contrast agents make them more attractive for imaging purposes.

Some SPIONs with core sizes of 3– 6 nm and dextran coating (with 20– 150 nm hydrodynamic sizes) such as Endorem, Combidex, Feridex and Sinerem are approved for MRI in patients. Similarly, SPIONs loaded with drug can be guided to the desired target area using an external magnetic field while simultaneously tracking the bio-distribution of the particles [2].

Recently, formation of nanochains structures made of iron oxide (γ-Fe_2O_3)/Fe_3O_4) nanoparticles inside polymers films, obtained by applying the magnetic field (0.160 T) during film drying [2]. The chains (or wires) are micrometers long (Fig.1), whereas maintaining the super paramagnetic character of the constituent particles. Moreover, we have notices that films with these wires exhibit anisotropic behavior of magnetization under weak fields as revealed by SQUID magnetic measurement, opening the possible application of these plastic films in magnetic sensors, or magnetic shields [3].

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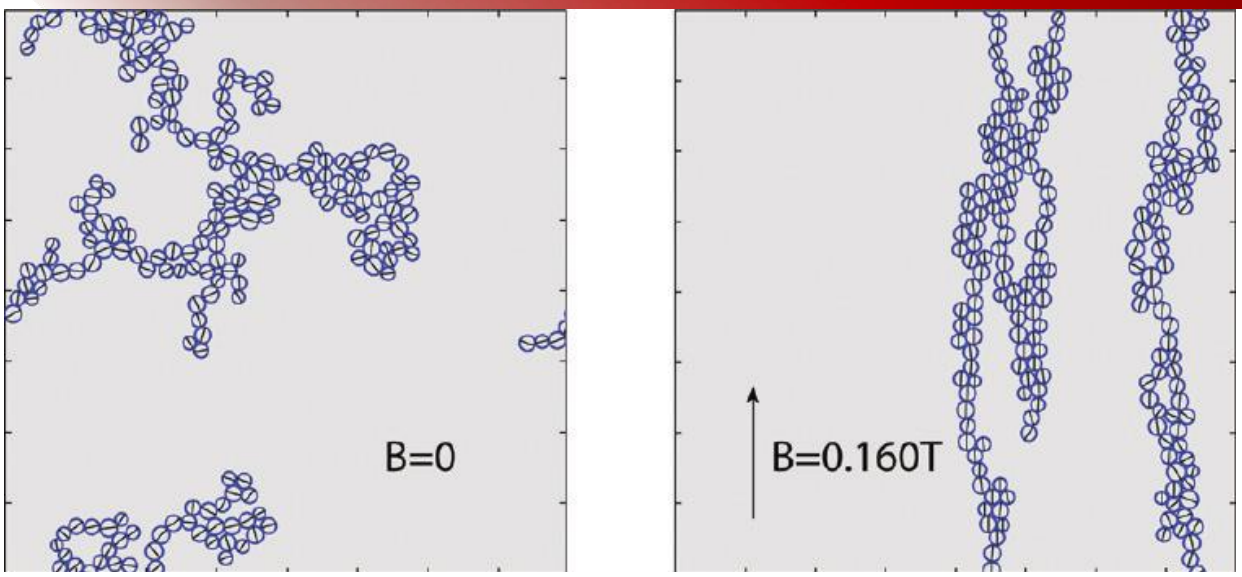

Fig. 1 Formation of nanochains structure of iron oxide nanoparticle in PEMMA polymer after application of a magnetic field of 0.160 T during polymer film drying. (reproduced from the reference no. 3 of this review article).

All these biomedical and bioengineering applications require that these nanoparticles have high magnetization values and size smaller than 100nm with overall narrow particle size distribution, so that the particles have uniform physical and chemical properties. [3]

In addition, these applications need special surface coating of the magnetic particles, which has to be not only non-toxic and biocompatible but also allow a targetable delivery with particle localization in a specific area [3]. Magnetic nanoparticles can bind to drugs, proteins, enzymes, antibodies, or nucleotides and can be directed to an organ, tissue, or tumour using an external magnetic field or can be heated in alternating magnetic fields for use in hyperthermia [3].

Over the past decade, magnetic nanoparticles, like magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), have received intensive interest because of their unique properties, such as easy handling, low cytotoxicity, good biocompatibility, relatively low cost, and eco-friendly performance. Due to these characteristics, the application of magnetic nanoparticles ranges across the areas of data storage, magnetic resonance imaging (MRI), bioseparation, drug delivery, heavy metal removal and catalysis [3]. Especially, the recyclable and localizing properties of magnetic nanoparticles by applied magnetic field can facilitate efficient target-therapeutic delivery. [3]

Functionalized magnetic nanocomposites based on iron oxide have been extensively used in biomedical applications due to their significance in several applications, such as targeted drug delivery, cancer treatment, and MRI imaging. Nanotechnology is currently one of the leading areas in applying such magnetic nanocomposites in various applications because of their enormous technological advancements, especially in chemistry, medicine, biology, and pharmacy. [4,5]

In general, nanotechnology is covering materials in the size range of 1– 100 nm, which implies to one billionth of a meter (10^{-9}) although there are many examples of nanoparticles having size in few hundreds of nanometers in size made up of inorganic or organic materials which have many novel properties compared with bulk materials. On this basis SPIONs have many unique magnetic properties such as superparamagnetism, low curie temperature, high coercivity and high magnetic susceptibility etc. Iron oxide nanoparticles are of great interest for researchers from a broad range of disciplines, including data storage, magnetic fluids, catalysis and bioapplications. In the last decade, there is increased

investigation in the field of magnetic iron oxide nanoparticles (mostly includes Fe_3O_4 magnetite, $Fe^{II}Fe^{III}_2O_4$, ferromagnetic, superparamagnetic when the size is less than 15 nm), α-Fe_2O_3 (hematite, weakly ferromagnetic or antiferromagnetic), γ-Fe_2O_3 (maghemite, ferrimagnetic), FeO (Wustite, antiferromagnetic), ε-Fe_2O_3 and β-Fe_2O_3). [6,7]

M. Leitgeb et. al studied that Superparamagnetic Iron Oxide Nanoparticles (SPIONs) have numerous technological applications which can be applied in the field of nanoscience and nanotechnology. The properties of these nanoparticles include flexibility, designed modulation and having low toxicity which is primary requirement for the biological systems. These nanoparticles have excellent applications in drug delivery systems, biosensors and contrast agents for sensors as well as environment sciences and green chemistry. [8,9]

P. Guardia et. al studied about the magnetic properties of iron oxide nanoparticles and found that these properties depend upon the morphology, composition, size of particles, particle size distribution and degree of crystallinity. The most preferred size in most of their application is in the range of 10-50 nm. Normally, nanoparticles in this range of size behave as single magnetic domain and when the temperature is above the blocking temperature, they show the superparamagnetic behaviour. In the state of superparamagnetic, these nanoparticles have very high magnetic moments and alignment of all the magnetic domains are in the same direction and magnetism is maximum on the application of applied magnetic field. These features make the superparamagnetic nanoparticles (SPIONs) highly attractive for biomedical applications. [10, 11]

Y. Yang et. al studied that the characteristic properties of magnetic iron oxide nanoparticles such as magnetization, surface area and microstructure of these materials largely depend on their bulk structure. All the important characteristics of nanomaterials are mainly determined by their shape, size, and surface area. The shape of magnetic iron oxide nanoparticles is mainly related to shape and geometries of specific composites which can be spherical, cubic or shell-core structures while the surface of iron oxide nanoparticles is mainly depends upon the presence different materials bound to the surface such as polymers, metals, carbon-based materials and rare metals etc. The presence of specific materials on the surface of these iron oxide nanoparticles modify the surface properties of these iron oxide nanoparticles play important role in preventing agglomeration and aggregation and also increased magnetic properties of their different composites. In the absence of surface modifications of these iron oxide nanoparticles, surface energy and dipole interactions are increased which lead excessive aggregation and agglomeration of these nanoparticles. [12,13]

In recent years, nanotechnology has gained immense advantages in drug delivery. Especially, MNPs are becoming materials that are extremely popular to be used as nanocarriers with their many advantages, such as ease of preparation, tolerability, biocompatibility, flexibility in accommodating different drugs and bioactive molecules, their control of drug release, high drug accumulation in targeting tissues, and many more. Magnetic nanoparticles probes are evolved as a class of novel and tracking agent in magnetic resonance imaging. When used as a contrast agent for MRI,

superparamagnetic iron oxide nanoparticles allow researchers and clinical scientists to enhanced or increased the tissue contrast of an area of study by increasing relaxation rate of water. By variations of particles size, coating thickness and targeting ligands, these nanoparticles probes can be tailored to target specific cells, organs and even molecular markers for different types of diseases in the biological systems. [14]

On the other hand, there are major drawbacks in the application of magnetic nanoparticles originating from the magnetically-induced aggregation, surface oxidation, and

deficiency of functional groups. In order to solve these problems, designative preparation of polymer-coated magnetic nanoparticles has been researched in many potential application areas. The polymer shell provides a flexible functional group to magnetic nanoparticles and protects them from surface oxidation and aggregation, increasing their stability. [15]

In this review paper, we present a comprehensive overview of the physical and morphological characteristics of SPIONs that influence their applications in biological environments, as well as examine the various applications of SPIONs in cellular labelling, tissue repair, drug delivery, magnetic resonance imaging, hyperthermia, magnetofaction, catalysis, photo catalysis and removal of carbon dioxide and additionally, we provide a critical analysis of the essential properties of SPIONs, their surface chemistry, and the conjugation methods employed in various bio-applications. [15]

2. Synthesis of magnetic iron oxide nanoparticles

2.1 Co-precipitation method

Physical methods such as gas phase deposition and electron beam lithography are elaborate procedures that suffer from the inability to control the size of particles in the nanometer size range [15]. The wet chemical routes to magnetic nanoparticles are simpler, more tractable and more efficient with appreciable control over size, composition and sometimes even the shape of the nanoparticles.

Iron oxides (either Fe_3O_4 or γ-Fe_2O_3) can be synthesized through the co precipitation of Fe^{2+} and Fe^{3+} aqueous salt solutions by addition of a base [15]. The control of size, shape and composition of nanoparticles depends on the type of salts used (e.g. chlorides, sulphates, nitrates, per chlorates, etc.), Fe^{2+} and Fe^{3+} ratio, pH and ionic strength of the media [15].
Conventionally, magnetite is prepared by adding a base to an aqueous mixture of Fe^{2+} and Fe^{3+} chloride at a 1:2 molar ratios [15]. The precipitated magnetite is black in colour. The chemical reaction of Fe_3O_4 precipitation is given in Fig. 2.

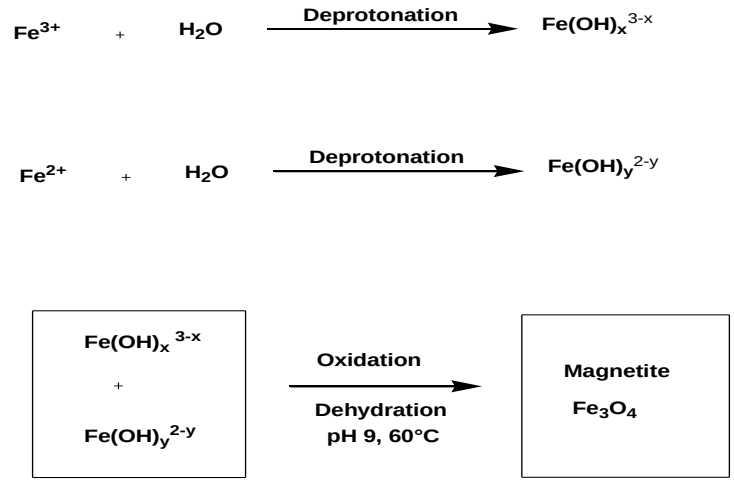

Fig. 2 Scheme showing the reaction mechanism of magnetite particle formation from an aqueous mixture of ferrous and ferric chloride by addition of a base. (reproduced from the reference no. 15 of this review article). The precipitated magnetite is black in colour.
The overall reaction may be written as follows:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \longrightarrow Fe_3O_4 + 4H_2O \quad (1)$$

According to the thermodynamics of this reaction, a complete precipitation of Fe_3O_4 should be expected between pH 9 and 14, while maintaining a molar ratio of Fe^{3+}: Fe^{2+} is 2:1

under a non-oxidizing oxygen free environment [15]. Otherwise, Fe_3O_4 might also be oxidized as

$$Fe_3O_4 + 0.25O_2 + 4.5\,H_2O \longrightarrow 3\,Fe(OH)_3. \quad (2)$$

This would critically affect the physical and chemical properties of the nanosized magnetic particles [9]. In order to prevent them from possible oxidation in air as well as from agglomeration, Fe_3O_4 nanoparticles produced by reaction (1) are usually coated with organic or inorganic molecules during the precipitation process. To control the reaction kinetics, which is strongly related with the oxidation speed of iron species, the synthesis of particles must be done in an oxygen-free environment by passing N_2 gas [16].

Bubbling nitrogen gas through the solution not only protects critical oxidation of the magnetite but also reduces the particle size when compared with methods without removing the oxygen [16]. Genesis of the particles in the solution under optimum synthetic conditions takes place by the formation of tiny crystalline nuclei in a supersaturated medium, followed by crystal growth. The latter process is controlled by mass transport and by the surface equilibrium of addition and removal of individual monomers, i.e., Atoms, ions, or molecules.

Hereby, the driving force for monomer removal (dissolution) increases with decreasing particle size. Thus, within an ensemble of particles with slightly different sizes, the large particles will grow at the cost of the small ones. This mechanism is called Ostwald ripening and is generally believed to be themain path of crystal growth.

The disadvantage of these bulk solution syntheses is that the pH value of the reaction mixture has to be adjusted in both the synthesis and purification steps. As a result, the production of significant quantities of narrowly dispersed [16], nanometer sized magnetic particles remains a significant challenge through these methods.

The critical difficulty is that these particles form aggregates and grow to minimize the overall surface free energy, so that free precipitation is not a viable technique [17]. Advancement in the use of magnetic particles for biomedical applications depends on the new synthetic methods with better control of the size distribution, magnetic properties and the particle surface characteristics. Synthesis of MNPs by co-precipitation method is widely used and promoted in the biomedical field due to its enormous advantages, such as high product yields, crystalline sizes with narrow size distribution of the particles, and environmentally friendly solvents in which the particles are synthesized, as they are normally dissolved in aqueous medium. However, the main disadvantage is the continuous washing and drying, and other limitations that might occur with the co-precipitation method include particle aggregation as a consequence of small particle, size, shapes as well as poor dispensability.[17] For example, SPIONs were synthesized by co-precipitation method to investigate their antitumor activity on Ehrlich carcinoma as reported by Abd- Elghany et al. [18], while Chiarelli et al. developed the synthesis of SPIONs by co-precipitation method for tumor-targeted delivery to intensify the transfer of energy for conventional photon radiotherapy on cellular basis.[19]

2.2 Microemulsion method: -

A micro emulsion is defined as a thermodynamically stable isotropic dispersion of two immiscible liquids, since the micro domain of either or both liquids has been stabilized by an interfacial film of surface – active molecules. In water-in-oil micro emulsions, the aqueous phase is dispersed as micro droplets (typically1– 50 nm in size) surrounded by a monolayer of surfactant molecules in the continuous hydrocarbon phase [19, 20].

When a soluble metal salt is incorporated in the aqueous phase of the micro emulsion, it will reside in the aqueous micro droplets surrounded by oil. These micro droplets will continuously collide, coalesce, and break again [19, 20].

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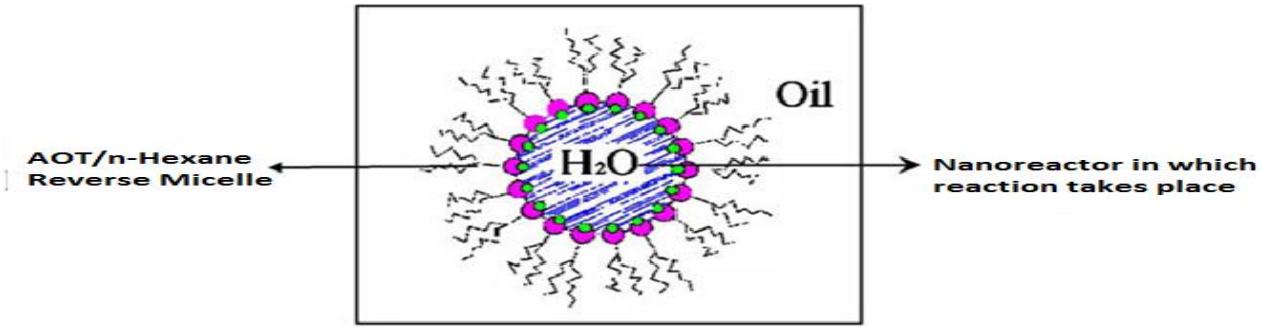
Conceptually, when reactants A and B are dissolved in two identical water-in-oil micro emulsions, they will form an AB precipitate on mixing. The growths of these particles in micro emulsions can be conceptualized as a progress of inter droplet exchange and nuclei aggregation. The finely dispersed precipitate so produced can be extracted from the surfactants [19, 20].

Recently, we have utilized water-in-oil micro emulsions to synthesize super paramagnetic iron oxide nanoparticles in narrow size range with uniform Chemical and physical properties [19,20]. Highly monodispersed iron oxide nanoparticles were synthesized by using the aqueous core of aerosol-OT (AOT)/n-hexane reverse micelles (w/o micro emulsions) (Fig.3).



FFig. 3. Structure of reverse micelles formed by dissolving AOT, a surfactant, in n-hexane (reproduced from the reference no. 15 of this review article). The inner core of the reverse micelle is hydrophilic and can dissolve water-soluble compounds. The size of these inner aqueous droplets can be modulated by controlling the parameter Wo (Wo= [water]/ [surfactant]).

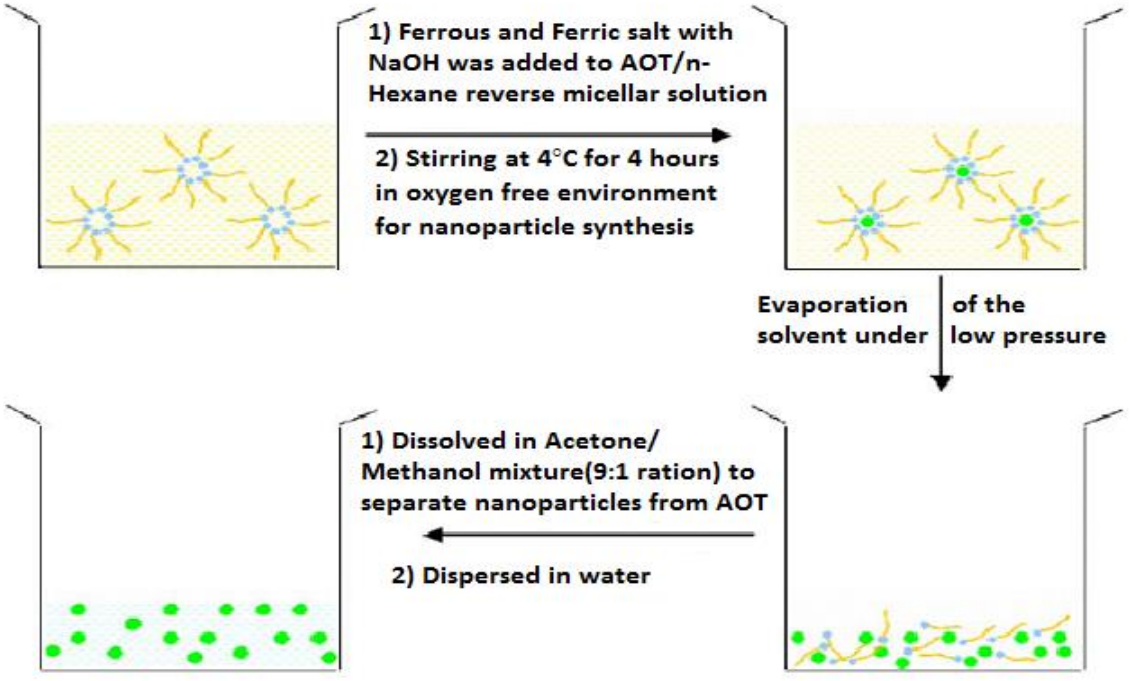


Fig.4. Strategy of preparing highly monodispersed iron oxide nanoparticles inside the w/o micro emulsion droplets. Iron salts were dissolved inside the aqueous cores of reverse

micelles and precipitated using alkali solutions to get the particles of desired size (reproduced from the reference no. 15 of this review article).

The reverse micelles have aqueous inner core, which can dissolve hydrophilic compounds, salts, etc. A deoxygenated aqueous solution of the Fe^{3+} and Fe^{2+} salts (molar ratio 2:1) was dissolved in the aqueous core of the reverse micelles formed by AOT in n-hexane [20]. Chemical precipitation was achieved by using a deoxygenated solution of sodium hydroxide. Smaller and more uniform particles were prepared by precipitation of magnetite at low temperature in the presence of nitrogen gas [19, 20] (Fig. 4).

The size of the inner aqueous core of reverse micelles is in nanometer range, so the magnetic nanoparticles prepared inside these nano reactors were found to be very small in size (less than 15 nm) with narrow size distribution [20]. The colloidal nanoparticles exhibit super paramagnetic behavior with high magnetization values. The principle advantage of utilizing this type of micro emulsion system for nanoparticle formation is that the size of nanoparticles can be controlled by modulating the size of aqueous micellar core.

Yaacob et al. have reported the production of magnetic nanoparticles by precipitation within spontaneous generated vesicles from mixtures of single-tailed Cationic (cetyl trimethyl ammonium bromide (CTAB)) and anionic (dodecyl benzene sulphonic acid (DBSA)) surfactants [20].

The CTAB/DBSA molar ratio was 7:3 and the magnetic particles were produced by gently heating the Fe^{2+} hydroxide precipitate [20] that formed under the room temperature conditions used in the experiments.

Since solution pH is an important factor controlling the stability of metal hydroxides precipitated in aqueous media use of the acid form of the anionic surfactants afford an opportunity to tune to the pH.

2.3 Hydrothermal Synthesis: -

Iron oxide NPs with controlled size and shape are technologically important due to strong correlation between these parameters and magnetic properties. The micro emulsion and thermal decomposition methods usually lead to complicated process or require relatively high temperatures [21]. As an alternative, hydrothermal synthesis includes various wet chemical technologies of crystallizing substance in a sealed container from the high temperature aqueous solution (generally in the range from 130 to 250 °C) at high vapor pressure (generally in the range from 0.3 to 4 MPa).

This technique has also been used to grow dislocation-free single crystal particles, and grains formed in this process could have a better crystallinity than those from other processes, so hydrothermal synthesis is prone to obtain the highly crystalline iron oxide NPs. Several authors have reported the synthesis of iron oxide NPs by hydrothermal method [21].

For example, Wang et al. have reported a one-step hydrothermal process to prepare highly crystalline Fe_3O_4 nanopowders without using the surfactants. The nanoscale Fe_3O_4 powder (40 nm) obtained at 140 °C for 6h possessed a saturation magnetization of 85.8 $emug^{-1}$, a little lower than that of the correspondent bulk Fe_3O_4 (92 $emug^{-1}$) [22]. It is suggested that the well-crystallized Fe_3O_4 grains formed under appropriate hydrothermal conditions should be responsible for the increased saturation magnetization in nanosized Fe_3O_4.

On the contrary, Zheng et al. reported a hydrothermal route for preparing Fe_3O_4 NPs with diameter of ca. 27 nm in the presence of a surfactant, sodium bis (2-ethylhexyl) sulfosuccinate (AOT) [21,22]. The magnetic properties of the NPs exhibited a super paramagnetic behavior at room temperature. Moreover, hydrothermal treatment is one of the successful ways of growing crystals for iron oxide NPs.

2.4 Sonochemical Synthesis: -

As a competitive alternative, the sonochemical method has been extensively used to generate novel materials with unusual properties. The chemical effects of ultrasound arise from acoustic capitation, that is, the formation, growth, and implosive collapse of bubbles in liquid. The implosive collapse of the bubble generates a localized hotspot through adiabatic compression or shock wave formation within the gas phase of the collapsing bubble [21, 22].

The conditions formed in these hotspots have been experimentally determined, with transient temperatures of 5000 K, pressures of 1800 atm, and cooling rates in excess of 1010 K/s. These extreme conditions were beneficial to form the new phase, and have a shear effect for agglomeration [21,22], which is prone to prepare the highly monodispersive NPs.

For instance, magnetite NPs can be simply synthesized by sonication of iron (II) acetate in water under an argon atmosphere. Vijaya kumar et al. reported a sonochemical synthetic route for preparing the pure nanometer-size Fe_3O_4 powder with particle size of ca. 10 nm. The prepared Fe_3O_4 NPs are super paramagnetic and its magnetization at room temperature is very low (1.25 emu g^{-1}).

Recently Pinkas et al. developed a sonochemical synthetic method for preparing the amorphous nanoscopic iron oxide by sonolysis of $Fe(acac)_3$ under Ar with a small amount of added water [21,22]. The organic content and the surface area of the Fe_2O_3 NPs can be controlled with an amount of water in the reaction mixture, and it increases from 48 m^2 g^{-1} for dry solvent up to 260 m^2 g^{-1} when wet Ar is employed. Such surface modified ultra-small (1– 2 nm) NPs exhibit an unrecorded low magnetic transition temperature of about 25 K, below this temperature they behave as speromagnetic, highly magnetically disordered systems with a high contribution of the surface anisotropy and this speromagnetic behavior can be observed by Mossbauer spectra. [21,22].

2.5 Microwave irradiation method: -

Since 1986, microwave irradiation as a heating method has found a number of applications in chemistry. The microwave heating technique has been developed. The microwave synthesis has been widely used to zeolites. Compared with the usual method, microwave synthesis has the advantages of very short time, small particle size, narrow particle size distribution and high purity [23].

Janse et.al. suggested that these advantages could be attributed to fast homogeneous nucleation and the easy dissolution of the gel [23]. Unfortunately, the exact nature of the interaction of the microwave with the reactants during the synthesis of materials is somewhat unclear and speculative.

In this method, amorphous Fe_2O_3 nanoparticles were successfully prepared by microwave irradiation, by means of the hydrolysis of $FeCl_3$ H_2O in aqueous solution containing polyethylene glycol and urea [24].

The nanoparticles were characterized by X-ray diffraction XRD, Transmission electron microscopy TEM, etc. Using the same method, amorphous Cr_2O_3 nanoparticles were also successfully prepared.

The five above mentioned synthetic methods have several advantages and disadvantages for preparing iron oxide NPs, respectively [24]. In terms of size and morphology control of the iron oxide NPs, thermal decomposition and hydrothermal synthetic route seems the optimal method. For obtaining the water-soluble and biocompatible iron oxide NPs, co-precipitation often was employed, but this method presents low control of the particle shape, broad distributions of sizes and aggregation of particles [24].

As a time competitive alternative, sonochemical route can also be used to synthesis iron oxide NPs with unusually magnetic properties. In addition, it is noteworthy that some

green chemical synthesis routes and biological synthesis routes have been reported for environment protection purposes.

For example, recently Bharde et al. reported the bacterium Actinobacter sp. is capable of synthesizing maghemite under aerobic conditions when reacted with the ferric chloride precursors [25]. Moreover, maghemite NPs show super paramagnetic characteristics as expected. Therefore, as the environmental protection and eco friendliness competitive alternative, green chemistry and biological methods such as bacterially induced synthesis based synthesis of iron oxide NPs are important advances [25].

3. Surface modification of magnetic nanoparticles for biomedical applications and their effect on stability and magnetization in the preparation and storage

In the preparation and storage of nanoparticles in colloidal form, the stability of the colloid is of utmost importance. Nanoparticle coatings may be comprised of several materials including both inorganic and polymeric materials.

Polymeric coating materials can be classified into synthetic and natural polymers based on poly (ethylene-co-vinyl acetate), poly (vinylpyrrolidone)(PVP), poly (lactic-co-glycolic acid) (PLGA), poly (ethylene glycol) (PEG), poly (vinyl alcohol) (PVA), etc. are typical examples of synthetic polymeric systems [26].

Natural polymer systems include use of gelatin, dextran, chitosan, pullulan, etc. Various surfactants, e.g. sodium oleate, dodecylamine, sodium carboxy methylcellulose, are also usually used to enhance discernibility in an aqueous medium [20].

3.1. Surface modification with non-polymeric organic stabilizers

Oleic acid and stearic acid are similar surfactants which, however, lead respectively to stability and to precipitation of ferro fluid suspensions: to understand this, the forces between layers of oleic-like surfactants and between layers of stearic-like surfactants across a hexadecane (HD) medium were measured using a surface force balance (SFB), the force versus distance profiles between layers of oleic-tailed and of stearic tailed surfactants, and the wet ability of these layers by HD. Ferro fluid dispersions are stabilized by oleic acid but not by stearic surfactant[19].

The main property of these small molecules is to produce a homogeneous coating of the entire iron oxide core that is able to inhibit the protein absorption.

Phosphoryl choline (PC)-derived polymers are known to protect prosthesis against protein contamination, but pure PC coatings do not allow colloidal stability at physiological pH [4].

3.2. Surface modification with polymeric stabilizers

Polymeric coatings on magnetic nanoparticles offer a high potential in several areas of applications. Precipitation of inorganic particles in a cross-linked polymer matrix or network of gel often prevents coagulation of particles, giving rise to monodisperse particles.

The pioneering work of Ugelstad et al., based on the preparation of hydrophobic mono sized polystyrene magnetic particles, has stimulated the research in this domain. The methodology used is basically based on direct precipitation of iron salts inside in the pores of the porous polystyrene seed. The particles obtained exhibit large particle sizes (i.e. 2.8 and 4.5 mm) with a good magnetic separation [26].

The hydrophilic temperature-sensitive latexes have been obtained by encapsulating adsorbed iron oxide nanoparticles onto oppositely charged polystyrene-core/poly (N-isopropyl acrylamide) shell. The encapsulation has been performed using water-soluble monomers such as N-isopropyl acrylamide, N-N’ methylene bis acryl amide, itaconic acid etc. The final particles exhibited thermal-sensitive property.

Lee et al. have modified nanoparticle' s surface with PVA by precipitation of iron salts in PVA aqueous solution to form stable dispersion. They found that the crystallinity of the particles decreased with increasing PVA concentration, while the morphology and particle size remained almost unchanged [26].

For better dispersion, magnetite particles are often modified after precipitation. In a recent publication, we have shown that the synthesis of hydrophilic magnetic polymeric nanoparticles with magnetite core and polymeric shell is possible using an inverse micro emulsion polymerization process. The strategy of utilizing inverse micro emulsion approach to modulating the surface of magnetic nanoparticles with
PEG is based on the following prior observations:

(i) Preparation of hydrophilic nanoparticles is possible in the aqueous cores of reverse micellar droplets.
(ii) The size of the particles can be modulated down to 10 nm diameter by regulating the size of the aqueous core of reverse micelles.
(iii) Since the cross-linking and polymerization reactions take place in the aqueous core of reverse micelles, it is possible to coat the magnetic particles inside these nanoreactors.

The results obtained have demonstrated that the inverse micro emulsion is a superior method over other bulk precipitation methods to synthesize magnetic polymeric nanoparticles with a good control over iron oxide amount and magnetic properties [26,16].

When considering the coating materials for drug delivery applications, it is usually required that particles should have sufficient hydrophilic surfaces and size less than 100nm so that they can evade the reticuloendothelial system [26].

However, nanoparticles have large surface area/volume ratios and tend to agglomerate and adsorb plasma proteins. When the nanoparticles agglomerate, or are covered with adsorbed plasma proteins, they are quickly cleared by macrophages in the reticuloendothelial system before they can reach target cells.

One possible approach, therefore, to increasing the circulation time of nanoparticles in the blood stream is to coat the particles with hydrophilic polymers such as PEG to disperse them and minimize or eliminate the protein adsorption [26].

3.3. Surface modification with inorganic molecules

Metallic core shell types of iron oxide nanoparticles have been investigated by several researchers. These nanoparticles have inner iron oxide core with an outer metallic shell of inorganic materials. The iron oxide nanoparticles have been coated with silica, gold or gadolinium, etc. These coatings provide not only the stability to the nanoparticles in solution but also help in binding the various biological ligands at the nanoparticle surface for various biomedical applications [26].

The Au also provides a good surface for subsequent functionalization with chemical or biological agents. The Au coating is not sufficiently thick to keep the particles from aggregating, though. Ionic capping ligands, which bind to the particles' surface, must also be added during nanoparticle synthesis. The ligand's electrostatic charge causes the particles to repel, countering the magnetic attraction pulling them together [26].

The larger the magnetic particle, the stronger the force it can exert against blood flow when delivering its pharmaceutical tag. But particles must be sufficiently small to rule out any risk of clogging small capillaries, which could be just a few microns wide? Magnetic nanoparticles designed for drug delivery must also be completely biocompatible. Iron oxide particles are known to be non-toxic, and are eventually broken down to form blood hemoglobin.

Carpenter prepared metallic iron particles coated by a thin layer of gold via a micro emulsion. The gold shell protects the iron core against oxidation and also provides functionality, making these composites applicable in biomedicine. Zhou et al. prepared gold coated iron core-shell structure nanoparticles (Fe/Au), synthesized using reverse micelles characterized by transmission electron microscopy (TEM). The average nanoparticle size of the core-shell structure is about 8 nm, with about 6nm diameter core and 1– 2 nm shell [26].

The magnetic measurement of the nanoparticles also proved successful synthesis of gold-coated iron core shell structure. The nanoparticles were then assembled under 0.5 T magnetic field and formed parallel nanobands about 10 mm long. Several authors have reported the magnetic iron oxide nanoparticles coated with silica [26]. An advantage of having a surface enriched in silica is the presence of surface silanol groups that can easily react with alcohols and silane coupling agents to produce dispersions that are not only stable in non-aqueous solvents but also provide the ideal anchorage for covalent bounding of specific ligands. The strong binding makes desorption of these ligands a difficult task. In addition, the silica surface confers high stability to suspensions of the particles at high volume fractions, changes in pH or electrolyte concentration. [26]

A w/o micro emulsion method has also been used for the preparation of silica-coated iron oxide nanoparticles. Three different non-ionic surfactants (Triton X-100, Brij-97 and Igepal CO-520) have been used for the preparation of micro emulsions and their effects on the particle size, crystallinity, and the magnetic properties have been studied. By using this method, magnetic nanoparticles as small as 1– 2 nm and of very uniform size (standard deviation less than 10%) have been synthesized. A uniform silica coating as thin as 1 nm encapsulating the bare nanoparticles is formed by the base-catalyzed hydrolysis and the polymerization reaction of TEOS in the micro emulsion. [26]

3.4. Surface functionalization with targeting ligands

Various biological molecules such as antibodies, proteins, targeting ligands, etc., may also be bound to the polymer surfaces onto the nanoparticles by chemically coupling via amide or ester bonds to make the particles target specific [25].

The possibilities of targeting protein coatings are numerous linker molecules such as 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide hydrochloride (EDCI), N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), N-hydroxysuccinimide or N, N’ methylene bis acryl amide (MBA) are usually used to attach the initial hydrophilic coated molecules to a protein coating aimed at cell surface attachment [25].

3.5. Magnetic properties of iron oxide nanoparticles

Iron oxide particle materials are classified by their response to an externally applied magnetic field. Description of orientations of the magnetic moments in a particle helps to identify different types of magnetism observed in nature. The magnetic properties of these particles can be described by the dependence of the magnetic induction B on the magnetic field H.

Some materials such as iron exhibit ferromagnetism, in that they can be permanently magnetized. In most materials the relation between B and H is linear: $B=\mu H$, where μ is the magnetic permeability of the particles [27]. Iron oxide particles exhibit paramagnetism if $\mu>1$, and diamagnetism if $\mu<1$. In vacuum, $\mu= 1$. Alternatively, the magnetic susceptibility $\chi=\mu-1$ is used. Hence, paramagnetic nanoparticles have $\chi >0$; diamagnetic particles $\chi <0$; and in vacuum $\chi=1$.

One important advantage for the magnetic nanoparticle is their super paramagnetism that enables their stability and dispersion upon removal of the magnetic field as no residual magnetic force exist between the particles. Below approximately 15 nm, such particles are so small that the cooperative phenomenon of ferromagnetism is no longer observed and no permanent magnetization remains after the particles have been subject to an external magnetic

field. However, the particles still exhibit very strong paramagnetic properties (hence the name of the phenomenon) with a very large susceptibility [27].

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Particles whose unpaired electrons spins align themselves spontaneously so that the material can exhibit magnetization without being in a magnetic field are called ferromagnetic particles. Ferromagnetism is a so-called cooperative phenomenon, as single atoms cannot exhibit ferromagnetism, but once a certain number of atoms are bound together in solid form, ferromagnetic properties arise. When the ferromagnetic particles are removed from the field, they exhibit permanent magnetization [27].

Ferromagnetic materials, which are ground down to particle dimensions smaller than a particular domain, are no longer ferromagnetic but exhibit super paramagnetism. Particles made from iron oxide usually behave differently in magnetic field depending on their size.

It was reported previously by several researchers that abrupt changes in magnetic properties take place when the size of the particles is reduced from micrometer to nanometer range. For example, particles have super paramagnetic behavior when the size are sufficiently small (i.e. 6– 15 nm) and they behave as ferromagnetic when the grain size is in micrometer range.

It was shown by Chatterjee et al. that the magnetic behavior is dependent on the blocking temperature of the particles (blocking temperature is the transition temperature between the ferromagnetic and super paramagnetic state and is directly proportional to the size of the particles), which in turn is dependent on the size of the particles. Particles with lower blocking temperature exhibited super paramagnetic properties, whereas the higher blocking temperature of the particles showed the ferromagnetic behavior of the particles [28].

Gomez-Lopera et al. have found that surface coverage of poly (lactideco-glycolide) polymer on the iron oxide nanoparticles decreased the Ms of particles to about one-half of that of the pure magnetite and the initial magnetization– magnetic field dependence is steeper in this case. Voit et al. also observed similar results. They studied the magnetic behavior of super paramagnetic iron oxide nanoparticles in ferrofluids coated with different polymers such as sodium oleate, polyvinyl alcohol (PVA) or starch [28].

They found that surface coverage of the iron oxide with either of these polymer resulted in decreased saturation magnetization values of the particles. They also observed that the values of particle sizes calculated from the magnetization data are found to be lower than the values calculated by XRD and TEM measurements, which may be attributed to a magnetically ineffective layer on the particle surface. [28]

4. Applications of magnetic nanoparticles: -

4.1. Cellular labeling/cell separation

Cell labelling with ferro/paramagnetic substances is an increasingly common method for in vivo cell separation as the labelled cells can be detected by MRI. Most current labelling techniques utilize either of two approaches:

(a) Attaching magnetic particles to the cell surface

(b) Internalizing biocompatible magnetic particles by fluid phase endocytosis receptor-mediated endocytosis or phagocytosis. One strategy for efficient and specific cell labelling of magnetic particles is to modify the nanoparticle surface with a ligand that is efficiently taken up by target cells via receptor-mediated endocytosis [29].

Targeting agents such as transferrin, lactoferrin, albumin, insulin and growth factors, etc., have been demonstrated to preferentially target cell surface, because the receptors for these ligands are frequently over expressed on the surface of mammalian cells.

The derivatized nanoparticles act as cellular markers that are targeted at the surface receptors expressed on human fibroblasts surface without being internalized. These receptors are not only cellular markers, but also have been shown to efficiently internalize molecules coupled to these receptors [29].

In the absence of any system to inhibit endocytosis, most nanoparticles are endocytocysed by cells and eventually sequestered in digestive vacuoles in the cell.

Once the particles are endocytocysed, they are probably removed from contact with specific cell surface receptors and become effectively ineffective. As a result of these events, the cells are at high risk of apoptosis from overload with particles. If the particles can be prevented from leaving the cell surface, they will remain in contact with their specific receptors and would be expected to leave the cell in a state of prolonged stimulation while protecting the cells from side effects due to endocytosis [29].

In recent studies, we have discovered a route to derivatizing super paramagnetic nanoparticles with various targeting proteins such as lactoferrin, transferrin, ceruloplasmin, etc. that binds strongly to surface receptors such that phagocytosis is inhibited (Fig.5). We have prepared lactoferrin, ceruloplasmin and insulin derivatized super paramagnetic nanoparticles with cubic shape and size less than 20nm having high magnetization values.

These particles are characterized in vitro and their influence on human dermal fibroblasts is assessed in terms of cell adhesion, viability, morphology and cytoskeleton organization using various techniques to observe cell– nanoparticle interaction, including light, fluorescence, scanning and transmission electron microscopy [30].

In these studies, we showed that the protein derivatization on nanoparticle surfaces makes the nanoparticles cell surface adhesive and inhibits the endocytosis. Adhesion is the basis behind many techniques for detecting immunospecific molecules on surfaces of cells and adhesive interactions can be investigated by using immunospecific particles as research tools.

In another approach, the cell-bound magnetic particles can be detected by measuring the magnetization of individual cells. The magnetism of cells can be determined by measuring the velocity of cells by the method of magnetophoresis in which the number of particles bound to a cell can be determined by measuring the velocity of the cell in magnetophoresis. Using the method of magnetophoresis and Langmuir absorption theory, Tchikov et al. have evaluated the ligand– receptor interactions resulting from the adhesion of immunomagnetic particles to surfaces of target cells by counting the number of magnetically labelled cells.

Recently, Nam et al. have developed an ultrasensitive method for detecting protein analytes using magnetic particle probes. This system is based on magnetic microparticle probes with antibodies that specifically bind a target of interest such as prostate specific antigen (PSA) and nanoparticle probes that are encoded with DNA that is unique to the protein target of interest and antibodies that can sandwich the target captured by the micro particle probes [30].

4.1.1. Detoxification of biological fluids

In an attempt to isolate living cells from biological fluids containing toxic substances by using cell surface antigens for cell nanoparticle binding, magnetic beads were coated with antibodies against epithelial surface antigens, specifically in nearly all these cases with epithelial specific antigens [29-30].

The size of these particles varied from 50 nm to a few microns, the matrix material was mostly silica and in some cases polystyrene. During incubation of a blood sample with beads coated with an epithelial specific antibody, the beads bind to the epithelial cells. The

rosetted cells can then be purified by washing steps on a magnet rack. In all cases, the purity, recovery rate and condition of the isolated tumour cells depend on the number of washing steps, composition of used buffers and specification of the beads [30].

4.2. Tissue repair

Tissue repair using iron oxide nanoparticles is accomplished either through welding, apposing two tissue surfaces then heating the tissues sufficiently to join them, or through soldering, where protein or synthetic polymer-coated nanoparticles are placed between two tissue surfaces to enhance joining of the tissues. Temperatures greater than 50 °C are known to induce tissue union [31]. This is believed to be induced by the denaturation of proteins and the subsequent entanglement of adjacent protein chains.

Nanoparticles that strongly absorb light corresponding to the output of a laser are also useful for tissue-repairing procedures. Specifically, gold- or silica-coated iron oxide nanoparticles have been designed to strongly absorb light [31]. The nanoparticles are coated onto the surfaces of two pieces of tissue at the site where joining was desired. This technique affords methods to minimize tissue damage by using the least harmful wavelengths of light and/or lower powered light sources.

The super paramagnetic nanoparticles could be coupled to the cells and used to target these cells at the desired site in the body. In addition, various proteins, growth factors, etc., could be bound to these nanoparticles that might be delivered at the damaged tissue, where it would play a role in tissue development. While there is no doubt that the use of stem cells in the form of cell-based therapies offers tremendous potential for disease treatment and cures for many common diseases including diabetes, cancer, heart disease, Alzheimer’ s and Parkinson’ s disease, central to this process would be the ability to target and activate these stem cells at required sites of injury and repair using magnetic particle technology [31].

4.3. Drug delivery

Another possible and most promising application of these colloidal magnetic nanoparticles is in drug delivery as carriers of drug for site-specific delivery of drugs. 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.

Particles ranging from ca. 10 to 100 nm are optimal for intravenous injection and demonstrate the most prolonged blood circulation times. The particles in this size range are small enough both to evade RES of the body as well as penetrate the very small capillaries within the body tissues and therefore may offer the most effective distribution in certain tissues.Super paramagnetic iron oxide nanoparticles of narrow size range are easily produced and coated with various polymers, providing convenient, readily targetable magnetic resonance imaging agents. Because of the large surface area to volume ratio, the magnetic nanoparticles tend to agglomerate and adsorb plasma proteins [32].

The body’ s reticuloendothelial system (RES), mainly the kupffer cells in the liver, usually takes up these nanoparticles due to the hydrophobic surface. Surface coverage by amphiphilic polymeric surfactants such as poloxamers, poloxamines and poly (ethylene glycol) (PEG) derivatives over the nanoparticles significantly increases the blood circulation time by minimizing or eliminating the protein adsorption to the nanoparticles [33].

Fine ferromagnetic particles have been coated with poly (ethylene glycol)/amino or carboxyl groups to permit the covalent attachment of proteins, glycoproteins, and other ligands with the retention of proteins, glycoproteins and ligand with the retention of biological activity. Ferromagnetic particles have also been used for various in vivo applications such as a tracer of blood flow, in radionuclide angiography, and for use in inducing clotting in artervenous malformations. During drug delivery, the care must be taken with the particles size of the carriers: any fraction larger than 5 μm must be avoided in order to prevent capillary blockade.

Gomez-Lopera et al. have described a method for preparing colloidal particles formed by a magnetite nucleus and a biodegradable poly (DL-lactide) polymer coating. The method is based on the so-called double emulsion technique, employed to obtain polymeric spheres loaded with therapeutic drugs, to be used as drug delivery vectors. The aim of this work was to obtain, in a reproducible and rather simple way, colloidal particles that were both magnetic field responsive, and useful as drug delivery systems [33].

In order to investigate to what extent this target is achieved; they have compared the structure, chemical composition, and surface properties of the composite particles with those of the nucleus and the coating material, simple molecules to macromolecules, cells, or other colloids. Considerable work that demonstrates that biodegradable polymers are ideal as drug carriers because of their minimum toxicity and immunological response has been performed.

The attachment of drugs to magnetic nanoparticles can be used to reduce drug doses and potential side effects to healthy tissues and the costs associated with drug treatment. Most iron oxides have a relatively short blood half-life and their primary application is for imaging of liver, spleen and the GI tract. Surface modified iron oxide nanoparticles having long blood circulation times, however, may prove very useful for imaging of the vascular compartment (magnetic resonance angiography), imaging of lymph nodes, perfusion imaging, receptor imaging and target specific imaging [34].

4.4. Magnetic resonance imaging

Super paramagnetic iron oxide nanoparticles play an important role as MRI contrast agents, to better differentiate healthy and pathological tissues. Recent developments in MR imaging have enabled in vivo imaging at near microscopic resolution. In order to visualize and track stem and progenitor cells by MR imaging, it is necessary to tag cells magnetically. Tat protein-derived peptide sequences have recently been used as an efficient way of internalizing a number of marked proteins into cells [35].

Lewin et al. hypothesized that biocompatible magnetic particles could be derivatized with similar sequences and that entire particles could be efficiently ferried into hematopoietic and neural progenitor cells in quantities upto 10– 30 pg of super paramagnetic iron per cells.

Iron incorporation did not affect cell viability, differentiation, or proliferation of CD 34 + cells homed to bone marrow per gram of tissue samples. Following intravenous injection into immune deficient mice, 4% of magnetically CD34+ cells homed to bone marrow per gram of tissue, and single cells could be detected by MRI in tissue samples. In addition, magnetically labelled cells that had homed to the bone marrow could be recovered by magnetic separation columns.

Weissleder et al. have presented the evidence that transgene expression can be visualized non- invasively by MRI in vivo. The authors have conjugated human holo-transferrine to iron oxide nanoparticles and showed that increase in receptor levels at the cell surface can cause considerable changes in MRI signals [35].

These super paramagnetic iron oxide nanoparticles are relatively non-toxic when administered intravenously, and similar preparations are in clinical use and as the iron oxide core is biodegradable, iron oxide nanoparticle degradation theoretically will allow multiple imaging of transgene expression over time.

Magnetic nanoparticles have been used to detect apoptosis by MRI by Zhao et al. Apoptosis is an active process of cellular self-destruction that plays an important role in number of disorders including neurodegenerative diseases, cerebral and myocardial ischemia and organ rejection following transplant. Therapeutic treatment of tumour cells in vivo results in changes in MR image contrast that are thought to reflect the morphological features of apoptosis, such as cell shrinkage and membrane blebbing [26].

The C2 domain of synaptotagmin I, which binds to anionic phospholipids in cell membranes, was shown to bind to the plasma membrane of apoptotic cells by both flow

cytometry and confocal microscopy. Administration of C2-SPION can lead to significant increases in image contrast in those regions of a tumour containing relatively large number of apoptotic cells. The authors showed that conjugation of the protein to SPION allowed detection of this binding using MRI. Specific detection of apoptotic cells using this contrast agent was demonstrated both in vitro, with isolated apoptotic tumour cells and in vivo in a tumour treated with chemotherapeutic drugs [36].

The MRI technique can detect apoptosis at an early stage in the process and has the advantages over other methods such as magnetic resonance spectroscopy (MRS) and radionuclide techniques, that it can detect apoptotic regions with relatively high spatial resolution. The SPION label is highly sensitive to MR detection and is also relatively non-toxic. SPION has been approved for clinical use as a blood pool agent for MRI [36].

4.5. Hyperthermia

Magnetic induction hyperthermia, one of the therapies for cancer treatment, means the exposition of cancer tissues to an alternating magnetic field. Magnetic field is not absorbed by the living tissues and can be applied to deep region in the living body. When magnetic particles are subjected to a variable magnetic field, some heat is generated due to magnetic hysteresis loss. The amount of heat generated depends on the nature of magnetic material and of magnetic field parameters. Magnetic particles embedded around a tumour site and placed within an oscillating magnetic field will heat up to a temperature dependent on the magnetic properties of the material, the strength of the magnetic field, the frequency of oscillation and the cooling capacity of the blood flow in the tumour site [37].

Cancer cells are destroyed at temperature higher than 43°C, whereas the normal cells can survive at higher temperatures. Heat could be generated applying an appropriate magnetic field. The size of the magnetite crystals is sub micrometric, so the powders or bulk of these biomaterials have comparable properties. These materials are not only biocompatible, but also bioactive and could be useful for bone tumours. [37]

Choosing high-power magnetic particles combined with appropriate external magnetic field, very small amounts of magnetic fine particles in the order of tenth of milligram may easily be used to raise the temperature of biological tissue locally up to cell necrosis. It was shown that hyperthermia greatly enhances cytotoxicity of radiation and drug treatment with brain tumour cell lines, which were also confirmed by multimodel hyperthermia studies with rat, rabbits and dogs [37]. Toxicity studies revealed a maximum tolerable thermal dose of normal brain in dogs to be 44°C, 30 min, using interstitial microwave antennas [38]. Known side effects of hyperthermia in animal experiments are cerebral necrosis, oedema, focal haemorrhage and infarction.

A breakdown of the blood– brain barrier is observed at temperatures of 42.5– 43°C, 60 min. Clinical studies performed so far have shown that interstitial brain hyperthermia is feasible and that toxicity is acceptable under careful control of the heating and limitation of the target volume.

Wada et al. have proved the usefulness of dextran magnetite (DM) for the oral cancer hyperthermia. DM suspension was locally injected into the tumour-bearing tongue and tongues were heated up to 43.0– 45.0°C, by an AC magnetic field of 500 kHz. They found that the inhibition of the growth of tongue carcinoma in the four-time heating group was significantly greater than in the control group. Moreover, the survival rate was significantly higher in the heated groups than in the control group. Histological examination revealed a brown uniform DM accumulation at the stroma in the margin of the tumours. Many of the tumour cells disappeared at the site adjacent to this accumulation [38].

Heat-induced therapeutic gene expression is highly desired for gene therapy to minimize side effects. Furthermore, if the gene expression is triggered by heat stress, combined therapeutic effects of hyperthermia and gene therapy may be possible. Ito et al.

combined TNF-a gene therapy driven by the stress-inducible promoter, gadd 153, with hyperthermia using magnetite cationic liposomes (MCLs). In nude mice, MCLs induced cell death throughout much of the tumour area on heating under an alternating magnetic field. This heat stress also resulted in a 3-fold increase in TNF-a gene expression driven by the gadd 153 promoter as compared with that of non-heated tumour. The combined treatment strongly arrested tumour growth in nude mice over a 30-day period, suggesting the potential for cancer treatment [39].

4.6. Magnetofection

Magnetofection (MF) is a method in which magnetic nanoparticles associated with vector DNA are transfected into cells by the influence of an external magnetic field. For this purpose, magnetic particles might be coated with the polycation polyethylenimine. These complexes readily associate with negatively charged DNA since the magnetic particles are positively charged due to the polyethylenimine.

Whether viral or non-viral vectors, MF has been shown to enhance the efficiency of the vectors up to several thousand times [40,41]. For magnetically enhanced nucleic acid delivery, MF is universally applicable to viral and non-viral vectors, because it is extraordinarily rapid, simple and yields saturation level transfection at low dose in vitro.

Further, since these magnetic particles do not rely on receptors or other cell membrane-bound proteins for cell uptake, it is possible to transfect cells that normally are non-permissive.

Krotz et al. have recently used MF to enhance gene transfer to cultured primary endothelial cells. MF of human umbilical vein endothelial cells (HUVEC) increased transfection efficiency of a luciferase reporter gene up to 360-fold compared to various conventional transfection systems. In contrast, there was only 1.6-fold increase in toxicity caused by MF, suggesting that the advantages of MF outbalanced the increase in toxicity. The authors suggested that MF could be an effective tool for pDNA transfection of endothelial cells allowing high efficiencies of transfection [40,41].

In another study, the same authors have utilized the technique of MF to enhance ODN delivery at low toxicity and procedure time in vitro and in vivo. In vitro, target knockout was assessed at protein and mRNA levels and by measuring superoxide generation after antisense MF against the p22 (phox) subunit of endothelial NAD (P) H-oxidase [40.41]. Antisense MF against p22 (phox) significantly decreased basal and prevented stimulated superoxide release due to loss of NAD(P)H-oxidase activity by mRNA knockout as assessed after 24 h.

4.7 Catalysis: -

Super paramagnetic nanoparticles were synthesized following a micro emulsion method, and functionalized with Schiff-base groups on the surface to form immobilized bidentate ligands. The functionalized nanoparticles were complexed with palladium acetate, affording the immobilized palladium complex catalyst [42] with a palladium loading of 0.24 mmol/g.

The catalyst was characterized by X-ray powder diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), vibrating sample magnetometer (VSM), thermo gravimetric analysis (TGA), Fourier transform infrared (FT-IR), atomic absorption spectrophotometry (AAS), and nitrogen physisorption measurements.

The immobilized palladium complex was used as an efficient catalyst for the Sonogashira reaction of iodobenzene and phenyl acetylene to form diphenyl acetylene as the principal product without added phosphine ligands [42].

Recovery of catalyst was facilely achieved by simple magnetic decantation. The immobilized palladium complex catalyst could be reused several times without significant degradation in catalytic activity. No contribution from homogeneous catalysis of active palladium species leaching into reaction solution was detected [43].

Magnetite and hematite have been used as catalysts for a number of industrially important reactions, including the synthesis of NH_3 (the Haber process), the high temperature water gas shift reaction, and the desulfurization of natural gas [43].

Other reactions include the dehydrogenation of ethyl benzene to styrene, the Fisher-Tropsch synthesis for hydrocarbons, the oxidation of alcohols, and the large scale manufacture of butadiene [44].

Magnetite and hematite are semiconductors and can catalyze oxidation/reduction reactions. Hematite has also been used as a support material for gold in catalysts for the oxidation of carbon monoxide at low temperature. Iron oxides can be used as acid/base catalysts and to catalyze the degradation of acrylonitrile-butadiene-styrene copolymer into fuel oil [44].

4.8 Photo catalyst: -

Hematite has been used as photo catalyst for the degradation of chlorophenol and azo dyes. The photo catalytic detoxification of wastewater is a process that combines heterogeneous catalysis with solar technologies. Semiconductor photo catalysis, with a primary focus on TiO_2, has been applied to a variety of problems of environmental interest in addition to water and air purification. The diameter of TiO_2 should be in 10 nm [45].

Organic compounds such as alcohols, carboxylic acids, amines, herbicides and aldehydes, have been photo catalytically destroyed in laboratory and field studies [30]. The photo catalytic process can mineralize the hazardous organic chemicals to carbon dioxide, water and simple mineral acids (Ahmed et al., 1984).

Kormann et al. (1989) also examined the suitability of α-Fe_2O_3 as photo catalysts using transparent α-Fe_2O_3, 3–20 nm in size. They also compared the photo catalytic activity of hematite to the activities of colloids and suspensions of ZnO and TiO_2. While ZnO and TiO_2 were found to be quite active photo-oxidation catalysts in the formation of hydrogen peroxide and in the degradation of chlorinated hydrocarbon molecules, only negligible photo catalytic activity was found for α-Fe_2O_3 [46].

4.9 Removal of Carbon monoxide: -

Superfine Fe_2O_3 nanoparticles were evaluated both as a catalyst and as an oxidant for carbon monoxide oxidation. It was found that the nanoparticles are much more effective as carbon monoxide catalysts than the non-nano oxide powder. For the Fe_2O_3 nanoparticles, the reaction order is first-order with respect to the partial pressure of carbon monoxide, and zero-order with respect to the partial pressure of oxygen. The apparent activation energy was 14.5 kcal mol^{-1} and the normalized reaction rate was 19 s^{-1} m^{-2} at 300°C [47].

In the absence of oxygen, Fe_2O_3 nanoparticles oxidize carbon monoxide directly as an oxidant. The resulting reduced forms of Fe_2O_3 also catalyze a disproportionation reaction for a considerable amount of carbon monoxide [46]. The significant amount of carbon monoxide it can remove through the catalytic oxidation, direct oxidation, and the disproportionation reaction make it a very promising material in certain special applications, such as removing the carbon monoxide from a burning cigarette, where the potential toxicity of other, more conventional catalysts would be undesirable [47].

The higher activity of Fe_2O_3 nanoparticles over non-nano Fe_2O_3 powders was attributed to a small particle size (3 nm), [46] the presence of an hydroxylated phase of iron oxide (FeOOH), as revealed by both high resolution transmission electron microscopy (HRTEM) and a comparable study of FeOOH (goethite) powder.

4.10 Remediation of uranium contaminated environmental water: -

An emerging technology for the treatment of contaminated land and water is the use of zero-valent iron nanoparticles (here after nano-Fe^0) [34]. This technology, whilst still in its relative infancy, has the potential to become widely adopted as a rapid, highly effective and low-cost alternative to conventional remediative technologies.

Compared to bulk scrap metal (granular or powdered Fe^0, >0.1 mm in diameter) more commonly used in permeable reactive barriers, nano-Fe^0 particles have a significantly greater surface area to volume ratio, higher surface energy and, resultantly, a significantly improved reactivity with regard to contaminants. Their colloidal size also makes their deployment flexible due to their conceptually high mobility through porous media and their potential for injection at almost any location and depth in terrestrial ground water systems [48].

Through laboratory testing, nano-Fe^0 have been proven as being highly effective for the removal of a wide range of aqueous chemical species, including chlorinated organics, inorganic anions and a range of heavy metals, including Pb, Cr, Cu, As, Ni, Zn, Cd and Ag. The application of nano-Fe^0 for the removal of radionuclides, however, remains less widely researched with studies limited to radioisotopes of barium and TcO_4 and studies for U carried out at the University of Bristol [48]. Limited to Dickinson and Scott (2010) within the afore mentioned studies is the application of nano-Fe^0 for the removal of U from a chemically complex solution, using an industrial waste effluent from the Atomic Weapons Establishment, Aldermaston, UK. Results concluded nano-Fe^0 as highly effective despite any competitive reactions that may have occurred.

It is well recognized that scrap/bulk Fe and Fe-based minerals are highly effective scavengers of U^{VI}. U^{VI} removal is attributed to a combination of two processes: the association of U^{VI} with Fe corrosion products via adsorption or structural incorporation and the reductive precipitation of U^{IV} oxide (UO_2) from electron transfer reactions between Fe^{II} and U^{VI} at the surface of the material [33]. As Fe^0 is
typically considered a stronger reducing agent than $Fe^{II}(aq)$, it was previously considered that contaminant reduction by Fe^0 was driven by the oxidation of Fe^0 to Fe^{II}.

However, it is now well recognized that: (i) structurally bound Fe^{II} may be a comparable reducing agent to Fe^0; and (ii) Fe^0 surfaces, including the nano-Fe^0 used in the present work, will have a ubiquitous layer of surface oxide that may prevent direct interaction between Fe^0 and aqueous oxidants [48]. Furthermore, nano-Fe_3O_4 of a known surface area was selected such that the observed reactivity of the two particulates could be compared in relation to total surface area.

The current study presents a comparative and site specific study of sorption and corrosion data for the application of nano-Fe^0 and nano-Fe_3O_4 to remediate U^{VI}-contaminated water collected from the Lisava valley in Banat, Romania. The waters are observed to be chemically complex, with high conductivity and multiple inorganic species present. The site is valley confined and bounded by limestone ridges which contribute significant concentrations of dissolved carbonate ($(CO_3)^{2-}$) to ground and surface waters, a chemical species that is well recognized to significantly enhance U^{VI} mobility in terrestrial water systems [48].

The water is used for mining purposes and is pumped from approximately 200m below sea level, a depth significantly below the water table. It initially contains low concentrations of dissolved oxygen (DO) (<3 mg L^{-1}), however quickly equilibrates with the atmosphere to reach oxygen concentrations more typical for that of vadose and/or surface waters (w=12 mg L^{-1}), changing its redox potential and associated U^{VI} transport properties in the process.

Nano-Fe^0 particles have been shown to be highly effective for the removal of U from water collected from Lisava, in Banat District, Romania [34]. U was removed to <2% of its initial concentration (0.484mgL^{-1}) within the first hour of the reaction period in both oxygen rich and oxygen poor conditions, and remained stable on the surface of the nano-Fe^0 for 48 h. The principle mechanisms of U removal in both systems, evidenced by XPS analysis of extracted solids, were indicated to be sorption followed by coupled Fe-U redox reactions resulting in the chemical reduction of U^{VI} to U^{IV} on the surfaces of the nano-Fe^0.

Following a period where U is retained on the surfaces of the reacted nano-Fe^0 as a partially reduced oxide (UO_{2+x}), a period of U re-release was observed and ascribed to oxidative dissolution [47]. By comparison, nano-Fe_3O_4 was shown to have limited remedial effect on U at any period. This difference in behavior is attributed to the presence of Fe^0 within nano-Fe^0, providing an additional and active source of electrons for aqueous reaction and associated contaminant removal [48].

Conclusion

The concept of drug delivery using magnetic nanoparticles greatly benefit from the fact that nanotechnology has developed to a stage that it makes possible not only to produce magnetic nanoparticles in a very narrow size distribution range with super paramagnetic properties but also to engineer particle surfaces to provide site specific delivery of drugs. Magnetite due to its strong magnetic properties was used first in biology and then in medicine for the magnetic separation of biological products and cells as well as magnetic guidance of particle systems for site-specific drug delivery. The size, charge and surface chemistry of magnetic particles could strongly influence their biodistribution. The most relevant biomedical applications of iron oxide nanoparticles are Cell Separation, MRI, drug delivery, hyperthermia & magnetofection etc. In addition to biological application, Iron oxide nanoparticles have applications in catalysis, photo catalysis, removal of carbon monoxide & remediation of uranium contaminated environmental water.

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