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Review

A review of the methods of magnetic nanocomposites synthesis and their applications as drug delivery systems and immobilization supports for lipases

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ABSTRACT

Iron oxide-based magnetic nanoparticles (IONP) that display high saturation and high magnetic susceptibility are of great interest for biotechnological and biomedical applications. Magnetite displays strong ferrimagnetic behaviour and is less sensitive to oxidation than other magnetic transition metaloxides. It is then not surprising that IONP include magnetite in their formulation. The role of IONP technology in the cited areas requires specific characteristics with regard to the particle size and surface functionality. Therefore the incorporation of polymeric moieties appears as a valuable tool to achieve the desired properties. On this context surface-modified IONP and magnetic nanocomposites (MNC) can be obtained depending on the role of the polymeric network in the formulation process. The methods to prepare each kind of magnetic-polymeric composite are reviewed in this work. First, the different kinds of IONP are carefully analyzed and second, the techniques available to incorporate polymer and other modifiers, are addressed. An investigation of the relationship between the nature of the modifier and the tailored application of the generated particles is also included with the focus mainly in the effect of some experimental variables on the nanoparticle size. Finally, two specific applications of magnetic nanocomposites in novel and relevant fields will be discussed. The role of magnetic nanoparticles as drug delivery systems (DDS) is of high significance and consequently, the available information is rich, but it is mostly dedicated to the sustained release of anticancer drugs. Here we will show other drugs, able to be delivered from magnetic DDS. MNC have recently emerged as effective supports for lipase immobilization. The reported data concerning to this topic is still quite limited and therefore novel published advances are reviewed here.

KEYWORDS: magnetic nanocomposites, magnetite, co-precipitation, magnetic supports, magnetic nanocarriers

1. INTRODUCTION

Magnetic particles in the nanosize level have been of scientific and technological interest due to their unique physical and chemical properties [1, 2]. The nanoparticles derived from iron(III) oxide are especially attractive because they present paramagnetic properties. These materials are single-domain particles with discrete randomly oriented magnetic moments. When placed in an external magnetic field, their magnetic moments rapidly rotate into the direction of the magnetic field and enhance the magnetic flux. When the

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external magnetic field is removed, Brownian motion is sufficient to cause the magnetic moments and particles themselves to randomize, hence their magnetic properties are not present [3].

The development of the technology associated to magnetic nanoparticles (MNP) has been accelerated due to their wide range of applications, in particular those related to the biotechnological and biomedical fields. The application of nanotechnology to the health market is noteworthy considering the extensive research developed in this area during the last decade. Cell labelling with MNP is an increasingly common method for *in vivo* cell separation since the labelled cells can be detected by MRI (magnetic resonance imaging) [4, 5]. Another possible and most promising application of MNP is as drug delivery systems (DDS) for site-specific release of medicaments. The MNP could bear a pharmaceutical drug that could be driven to the target organ and be released there. Anticancer therapeutic agents often cause serious damage to organs/tissues different from the target place. Therefore the use of MNP technology for this purpose appears as a valid alternative to solve this drawback and could be extended to other medicaments that require to be localized in a specific site, for instance some anti-inflammatory drugs.

The use of magnetic technology has been extended to diverse fields. Among others, enzyme immobilization [6] and adsorption of contaminants have become attractive topics being extensively investigated in the last years [7-9].

For any of the mentioned applications it is essential to conveniently adjust the characteristics of the MNP, such as particle size, charge and surface chemistry, since these parameters strongly affect the performance of the particles with regard to the possibility of interaction with other agents and, more important, with regard to the feasibility to preserve the paramagnetic behaviour [10]. Therefore the coating of magnetic particles is required in order to achieve suitable functional materials. A series of reasons that justifies the coating of MNP may be summarized as follows:

1. the high surface energy of metal nanoparticles, that often requires the surface to be passivated with a protective layer of another species.
2. to avoid particle agglomerations due to magnetic attractive forces. In this case, the particles are usually coated with stabilizers/ surfactants.
3. to improve biocompatibility while maintaining the high magnetization and also to enable efficient excretion and protection of the body from toxicity, in the case of MNP intended for biomedical purposes. In such cases, non-polymeric but hydrophilic organic coatings such as albumin, dextran, or hydroxyethylmethacrylate could also be employed to enhance biocompatibility [10, 11].

A polymeric template is able not only to solve all these drawbacks but also may turn the MNP into completely versatile materials useful not only in biomedicine and biotechnology but also in electronics, opticoelectronics, magnetic storage fields, besides other uses. Particularly the coating of polymeric network on /with MNP offers the possibility to design the material as a function of the desired applications. On this way, one may fabricate MNP:

- as DDS, by coating magnetite with biodegradable polymers;
- for separation of biological species or contaminants, coating the magnetite with functionalized polymers able to interact with the corresponding agent by physical or chemical linkages;
- for immobilization of lipases, employing usually hydrophobic polymers;
- for purification of proteins, if properly selected.

Table 1 summarizes the most commonly used polymers to achieve the desired applications of the MNP, with the corresponding references. It is important to note that this review is centered in the polymeric coating of MNP, but certainly it is only a kind of modification that could be performed on magnetic particles. Almost similar results could be expected using other modifiers, as it will be shown later.

In spite of the vast information and the advance of this research area, the reported literature about MNP seems to be quite ambiguous with regard to some issues. For example, after a revision of the available articles it is not easy to distinguish between the different kinds of magnetic

Table 1. Potential applications of magnetic nanocomposites as a function of the used polymeric templates (iron oxides are mostly magnetite and maghemite).

Polymer	Application	Reference
Polyetilenglycol (PEG)-methyl ether and polycaprolactone	Drug delivery systems (DDS)	[12]
Alginate	DDS	[13]
Poly(lactic-co-glycolic) acid (PLGA)	DDS	[14-16]
Poly lactic acid (PLA)	Immobilization of proteins	[17]
PLGA	MRI contrast agent	[18]
PLA	DDS	[16, 19, 20]
Polysterene	Adsorption of water contaminants	[21]
Chitosan (CS)	DDS	[22]
Poly(N-isopropylacrilamide)(PolyNIPAM)	Cell separation	[23]
CS	Lipase immobilization	[24]
Polyethylene deoxythiophene	Support to catalyst	[25]
Gelatin	DDS	[26, 27]
PEG	DDS	[28]
Polyglycidyl Methacrylate-co-methacryloxyethyl trimethyl ammonium chloride	Lipase immobilization	[29]
poly(diallyldimethylammonium chloride)	enzyme, heavy and noble metal extraction, and petroleum sorption	[30]
Polydimethylsiloxane (PDMS)	Biomedical applications	[31]
Polyvinyl alcohol (PVA)	DDS	[32]
poly_methylmethacrylate (PMMA)	Lipase immobilization	[33]

nanoparticles. In fact it is not clearly discernible if such particles are composed only by iron oxide or by other substrates such as polymers, fatty acids, proteins, etc. since in general they are called magnetic particles or nanoparticles in any case.

The function of different compounds added during formulation of MNP is not clear enough in many of the reviewed publications. Although it is common to find articles reporting the addition of oleic acid (OA), polyvinylalcohol (PVA), proteins or polymeric moieties, it is not generally well established in such articles the role or function of the added compounds, and their influence on the iron oxide characteristics.

Another point that generates confusion is the method of the MNP preparation since it is not well distinguished between the techniques employed to synthesize pure iron oxide and those used to obtain iron oxide/polymer (or other additives) composites. In the last case information regarding the nature of interactions or linkages originated between the iron oxide and the added substrate is rarely provided.

The main objective of the present research is to clarify the uncertain points cited above. Although there are many published reviews about this topic in general, our manuscript differs from the existent ones basically in two ways:

1. A general description of iron oxide nanoparticles is presented, however greater attention is devoted to the polymeric materials and the available obtention methods.
2. Specific applications of magnetic nanoparticles as drug delivery systems (biomedical area) and in the protein/enzyme immobilization (biotechnological area) are presented and discussed.

In this context, the current review has many goals. First, it tries to clarify the differences between the kinds of iron oxide based nanoparticles that are commonly enclosed under the denomination of "magnetic nanoparticles". Second, it addresses in detail all techniques available for preparation of the different magnetic nanoparticles. Special emphasis is given to polymeric magnetic nanoparticles and the methods to prepare them. Third and last, a different view of the applications of such materials is proposed. The requirements that the MNP must accomplish to be suitable DDS will be described. The reported literature deals with the use of these kinds of carriers mainly to the sustained release of anticancer medicaments, here the use of other drugs such as anti-inflammatory and/or proteins will be presented. Due to the fact that the research on immobilization of lipases on magnetic supports is currently in expansion, not enough published information is actually found. Hence a state of the art of the recent published contributions is included. The benefits associated to the use of MNP as supports for enzymes (in the preparation of biocatalysts) are also addressed.

2. Definitions

In several publications it is not obvious to distinguish between iron oxide nanoparticles, modified iron oxide nanoparticles and magnetic nanocomposites. The knowledge of the identity of the magnetic particles is highly useful looking at the kind of linkage, chemical or physical, between the modifier agent and the iron oxide. These data could be relevant to assure the success of the prepared particles in the selected applications.

In the Introduction section, the magnetic materials were named as MNP, a generic term. Different kinds of MNP can be found according to their composition, function and obtention method.

2.1. Iron oxide nanoparticles

Materials of interest to magnetically guidable systems are iron oxides. Magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are the most common and intensely researched ones. Since the ferromagnetic properties of these materials only appear when they are reduced to sizes on the order of the nano, special interest exists in the preparation of iron oxide-based particles with sizes lower than the micrometer [34].

The aqueous precipitation of hydrated iron ions from bulk solutions creates sphere-like nanoparticles of various sizes depending on reaction conditions. The compounds obtained through this route are basically IONPs. Later in this manuscript we will review their obtention methods.

2.2. Ferrofluids

These materials are constituted by IONP dispersed in water or in an organic solvent. In this last case the addition of hydrophobic stabilizers is required. Hence, nanoscale magnetic particles prepared with surface stabilizers and dispersed in an appropriated media constitute magnetic fluids, also known as ferrofluids. These magnetic fluids display superparamagnetic properties and their importance is related to the fact that they are single domain particles with discrete randomly oriented magnetic moments. When placed in an external magnetic field their moments rapidly rotate into the direction of the magnetic field and enhance the magnetic flux. When the external magnetic field is removed they have no magnetic remanence [35-37].

2.3. Surface modified IONP

The surface Fe atoms, which were not bound to oxygen atoms, may act as Lewis acids and therefore to coordinate with molecules that donate lone pair electrons (Lewis bases). Therefore in aqueous systems the coordinatively unsaturated sites (CUS) (Fe ions at the surface) coordinate

water, which dissociates readily to leave the iron oxide surface hydroxyl-functionalized. Surface hydroxyl groups are amphoteric and may therefore react with either acids or bases [38].

Oleic acid, a C18 fatty acid is a common example of functionalized species bound to the surface of iron oxides, creating magnetic dispersion in non-polar hydrocarbon solvents (Ferrofluids). Then, the surface-modified IONP are constituted by the IONP covered by a layer of the modifier agent. The thick of such coating as well as the identity of the modifier agent will define the characteristics of the obtained particles.

In general, surface-modified IONP are intended for applications under moderate conditions in order to avoid the modifier agent to be removed from the iron oxide surface. The surface modification is the preferred treatment aiming to avoid/reduce the aggregation tendency of IONPs.

2.4. Magnetic nanocomposites (MNC)

The materials resulting from the inclusion of magnetite into a polymer matrix are usually named nanocomposites [39]. The term nano-composite is originated from the nanotechnology field and defines a mixture of two different materials compounding a new one with improved properties; while the term composite may refer to a blend of several and different materials. The principle of existence of these hybrids is that an organic material (polymer) is used to limit particle growth and it is isolated with the magnetic material. An inorganic-organic nanocomposite - an hybrid- can, on this way, be obtained.

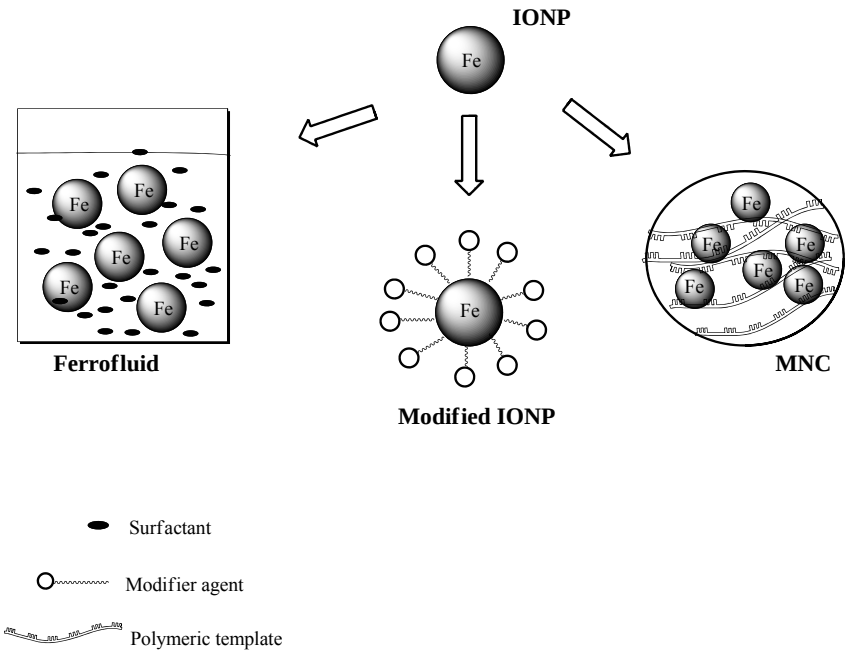
To better visualize the similarities and differences between the defined magnetic nanoparticles, in the Scheme 1 each kind of magnetic particle is represented.

3. Methods of synthesis of IONPs

Undoubtedly the most broadly employed method for the preparation of super-paramagnetic iron oxide is the co-precipitation of hydrated divalent and trivalent iron salts in the presence of a strong base [40-48]. In spite of this, a growing number of novel techniques have emerged with the goal of improving the characteristics of the obtained materials, specially regarding particle size and size distribution. Here we review the most relevant as well as the most recent ones.

3.1. Co-precipitation

Conventionally, magnetite is prepared by the addition of a base to an aqueous mixture of



Scheme 1. Classification of magnetic nanoparticles.

Fe^{+2} and Fe^{+3} chlorides whose molar ratio is commonly 1:2, under inert atmosphere at moderate temperatures (between 50 and 80°C). The precipitation of magnetite is evidenced by the appearance of a dark black solid [49]. Despite its long history, the exact mechanism of magnetite formation is still not well understood. One general mechanistic theory proposes that the Fe^{2+} salt hydrolyzes to $Fe(OH)_2$ and reacts with hydrous oxides to form magnetite [50]. Another perspective offered by Farley *et al.* suggests that the formation occurs via surface adsorption of cations, which leads to a balance between surface complexation reactions and precipitation [51]. Several researchers ascertain that the IONP are formed by nucleation and crystal growth mechanisms. Nucleation of the ions in solution must be a discrete first step followed by crystal growth to obtain monodisperse particles [47].

Although this method seems to be the simplest one, a lack of size control is frequently associated to it. Therefore, certain experimental parameters have to be adjusted in order to reach a better size control of the obtained particles.

Kim *et al.* studied the effect of the pH, concentration of base and presence of N_2 during the co-precipitation synthesis of IONP [52]. Therefore they have performed the synthesis employing NaOH with concentrations of 0.9, 1.0, 1.1 and 1.5 M; pHs ranged between 11.5 and 14 while compared the effect of N_2 atmosphere. The experimental results have shown that N_2 flow acts not only protecting against oxidation but also reducing the particles size when compared with methods involving the presence of oxygen and agree with most of the published works in the need of the avoidance of oxygen during all steps of the precipitation. They found an increment of the particles size from 13 to 30 Å when the concentration of precipitating NaOH solution increased from 0.9 to 1.5 M at a fixed pH = 14. On the other hand, decreasing the pH value from 14 to 11.5 and fixing the NaOH concentration, resulted in increasing particle size from 30 to 60 Å.

Dutz *et al.* have evaluated the effect of the temperature of the preparation procedure on the particle size of the magnetite. They observed magnetite sizes on the order of 14 nm when the temperature was set at 25°C while the diameter increased to 34 nm employing a temperature of 90°C. However they have not noticed a significant variation of the magnetic properties [53]. Other investigations that support the theory of nucleation and growth of the IONP, indicate that the temperature of formation should not exceed 70°C, since the quality of the particles could be negatively affected [47].

The concentration of the aqueous Fe^{+2}/Fe^{+3} solutions have also an influence on the IONP characteristics. Researchers speculate that if the reagents are too concentrated during magnetite formation, water is not able to participate in the hydrolysis and limits the di- and trivalent iron ion species mobility and hence their ability to polycondense [47]. Also, the rate of the base addition should be high (1 - 2 s) with intense agitation. A slow base addition creates in homogeneous regions of the hydrated iron species, which leads to nonmagnetic iron compounds [47].

Taking advantage of the simplicity of the co-precipitation method, many authors have attempted to introduce a number of modifications to improve the iron oxide properties regarding the desired applications. For instance, Lee *et al.* added ammonia oxalate to the ammonia alkaline solution during the precipitation step. Oxalate on the particle surface, was then replaced with Sodium dodecyl sulphate (SDS) by mixing an aqueous suspension of oxalate-magnetite with SDS at the molar ratio of 10:1 in an acidic environment (pH 5), working under a nitrogen atmosphere at 75ºC [54]. In general, the surface modification of IONP is performed with the aim to stabilize the particles, avoiding the particle aggregation. On this way, aggregation due to the attractive forces associated with magnetite nanoparticles are prevented by creating an electrical double layer, use of a surfactant functioning as a steric stabilizer, or by the modification of the isoelectric point of the oxide with an adequate coating. More details about the surface modification and stabilization of IONP will be supplied in next sections.

3.2. Electrochemical method

Between the most common disadvantages associated to the co-precipitation, the lack of control in the particles size and the particle size

polydispersion are undoubtedly the most evident. Hence, alternative methods for the synthesis of IONP, have emerged to solve these drawbacks.

Within this technique, the iron oxide is obtained by an electrochemical process through the induction of oxidation-reduction reactions. A sacrificial iron anode and an iron cathode were used for these purposes, and the separation between them plays a key role concerning the selectivity of the reaction in the sense that magnetite was the main product, between the other possible oxides [55].

Cabrera *et al.* have utilized this method for the fabrication of magnetite nanoparticles aiming to ensure an adequate control in the size and size distribution of the prepared particles. They found that the iron oxide particle size control was achieved by conveniently adjusting the imposed electrooxidation current density (*i*) or potential (*E*) to the system. Therefore they varied the current intensity between 50 and 150 mA and the potential between 3 and 5 V; and observed the formation of pure Fe_3O_4 when potentials lower than 6 V were applied. Although the increase in current density or potential promotes higher size homogeneity of the nanoparticles, metallic iron is present as an impurity. The optimum distance to produce magnetite was determined [57]. Advantages associated to electrochemical method are the control over the particle size and the obtention of relatively large particles comparing to conventional methods-i.e. co-precipitation [56, 58].

3.3. Oxidative alkaline hydrolysis (OAH)

This is a chemical method based on the oxidative alkaline hydrolysis of a Fe(II) solution. Briefly, a Fe(II) salt (usually $FeSO_4$ or $FeCl_2$) is dissolved in distilled water and KOH (alkaline medium) and KNO_3 (oxidizing agent) solutions are added drop-wise under stirring. The formation of magnetite is evidenced by the appearance of a black precipitate.

Dutz *et al.* have employed this procedure to obtain magnetic nanoparticles for heating purposes in biomedicine [53]. The sizes of the particles prepared by these authors were around 50 nm. In the cited publication the oxidative alkaline method was compared with the traditional co-precipitation. The data presented suggest that bigger IONP were formed using OAH technique while the BET area of such particles was lower than the corresponding to the particles prepared by co-precipitation. They also observed that the magnetic properties were strongly dependent on the iron oxide particle's size.

Bruice *et al.* have used the same method to prepare magnetite nanoparticles that were later modified with silica to be used in the separation of nucleic acids. They have used several methods to obtain the particles and found the best results, in terms of purity of the iron oxide, using OAH. The data supplied by this research indicate that OAH ensured magnetite particles with a narrow apparent size range distribution and mean particle size on the order of 55 nm [59].

3.4. Miscellaneous methods

The open literature includes a number of non-conventional, novel and less used methods that lead to the formation of magnetite and other iron oxides (or mixtures of them). Most of them are summarized in Table 2, with a brief description of their fundaments and the corresponding references.

3.5. Surface Modification of IONP

In general, three types of interactions may be distinguished among magnetic nanoparticles: London-Van der Waals interactions, magnetic forces and interactions of the electrical double layer. The preceding two attractive interactions among the particles must be counteracted by the later repulsive force to make them exist stably, for instance in a solvent. But this kind of repulsive force is very small. Generally, in order to get well-dispersed IONP in a solvent, the nanoparticles must undergo surface modification to make them able to adsorb one layer of surfactant on the surface. Thus, the repulsive force from the surfactant-coated particles can overcome the attractive forces between the particles. Because of the same reasons and due to the surface modification of the particles the IONP or MNP dispersion will be better.

Besides the stabilization, the surface of IONP could be modified through the creation of few atomic layers of a wide variety of materials, organics (i.e. polymers, proteins) and inorganics

Table 2. Miscelaneous methods of IONP obtention.

Method	Characteristics	References
Continuous hydrothermal processing (CHP)	$Fe(NO_3)_3$ is used as precursor, employing a special device to perform the reaction. The reaction was conducted under N_2. Diameter of particles between 7 and 27 nm.	[48, 60]
Aerosol synthesis	Based on the pyrolysis of aerosols generated from ethanol/water solutions containing iron inorganic salts and mono- or polysaccharides. Sizes between 5 and 60 nm with broad distribution. Formation of aggregates. Pyrolisis method in aqueous solution has also been reported, using self-made iron oleate precursor as starting material.	[61, 62]
From $Fe(CO)_5$	Formation of iron clusters by the loss of at least one CO followed by a cascade of ligand dissociation giving rise to coordinatively unsaturated, multinuclear iron complexes highly reactive, generating nucleation and growth processes that finally form the IO nanocrystals.	[63]
From $Fe(aca)_3$	From iron(III) acetylacetonate, dissolved in benzyl alcohol and treated in autoclave between 175 and 200ºC. This approach leads to monocrystalline magnetite particles with sizes ranging from 12 to 25 nm.	[64-67]
Sol-gel method	Based on the reaction of ferric nitrate and ethylene glycol under vacuum in the temperature range of 300-550ºC. Formation of highly porous materials with sizes ranged between 20-200 nm.	[68]

(silica, gold), intended for potential functionalization by the attachment of diverse bioactive molecules.

The surface of nanoparticles could be coated with a number of different functionalities, most commonly, amines, aldehydes or carboxylic acids. These are incorporated to the nanoparticle surface with the goal of promoting the desired reaction with different substrates/ligands.

Therefore IONP with improved characteristics could be achieved by a well-selected modifier agent. From the available literature, long chain surfactants appear as classic and useful modifier agents and between them oleic acid (OA) has been undoubtedly the most broadly utilized [69-71]. The goals with the incorporation of OA are basically two:

1. to give stability to nanoparticles avoiding aggregation.
2. to generate a hydrophobic surface able to interact with non-polar substrates; or to make the IONP dispersible in non-polar solvents.

OA exhibits a C18 tail with a *cis* double bond in the middle, forming a kink. It is hypothesized that such kinks are the responsible for the stabilization of the IONP [72]. Therefore OA is a more effective stabilizer than, for instance, stearic acid. Korolev *et al.* have compared the performance of oleic, stearic, and linoleic acids on the stabilization of magnetite in CCl_4 and hexane. A higher amount of oleic acid was adsorbed on the magnetite surface for both solvents as compared to stearic and linoleic acids. OA was the most suitable stabilizer in the studied conditions. The nanoparticle size obtained in this investigation varied between 5.7 and 9.3 nm, depending on the reaction temperature [73].

Polymeric macromolecules (amphiphilic) can form stable micelles due to the hydrophobic-hydrophilic interactions with the two phases. The major used surfactants are poly(vinyl alcohol) (PVA), polyethylene glycol (PEG), poloxamer and poloxamines family, pluronic family (F68, F127, and others) and sodium cholate [74].

PVA was generally chosen as a protective polymer because it has the desired solution properties in water and it contains many isolated hydroxyl functional groups, which can adsorb and complex with metal ions [60]. Chunbao Xu *et al.* have evaluated the influence of the concentration of PVA during the synthesis of maghemite nanoparticles using the Continuous hydrothermal method. They found that maghemite nanoparticles had average particle sizes of about 22 nm and were nearly independent of PVA concentration in the range of PVA concentrations studied in their work. They observed, however, that the size distribution (standard deviation) decreased with increasing PVA concentrations. The particles were mostly spherical, and, as expected, more aggregated at lower PVA concentrations. The same authors proposed that weak hydrogen-bonding in the hydrated PVA also plays a role in promoting homogeneous physical entrapment between the (–OH) hydroxyl groups and magnetite surface, thereby hindering aggregation and resulting in a relatively uniform particle size distribution [60]. Pardaoe *et al.* have found that IONP nanoparticles formed in the presence of aqueous PVA (30-40 kg/mol) created necklace-like chain ordering ~100 nm in length. The particle sizes of the PVA- modified oxide particles was 5.78 nm with a Fe content of 7.7 wt. % [75].

PEG is an hydrophilic, water-soluble, biocompatible polymer that is widely used in biological research, as it protects surfaces from interacting with cells or proteins. Several authors have reported the use of PEG-based surfactants to increase the biocompatibility of iron oxide dispersions. Therefore special attention was devoted to the use of pegylated iron oxides in biomedical/pharmaceutical applications. The stabilizer effect of PEG coating IONP has been evaluated by Grupta and Well [76]. These researchers have modified the surface of IONP with PEG and MA-PEG. They have performed the modification in microemulsion. The colloidal solution of IONP coated with PEG showed very high stability at neutral pH and no sedimentation was observed even after two months of storage at room temperature, whereas uncoated magnetic particles did not form a stable colloidal suspension and sedimented within a week. The registered particles sizes, measured by TEM, were ranged between 40 and 50 nm. The magnetic measurements revealed that PEG-coated IONP do not retain any magnetism after removal of a magnetic field, which make them especially attractive for drug targeting systems.

The performance of PEG in surface-modified IONP as surface coating was evaluated looking at their application in biomedicine. In this case, Gupta and Curtis [77] studied the effect of PEG-coated magnetite on human fibroblasts cells and found that the cellular uptake was improved when compared with unmodified magnetite. The particle's sizes measured in this work are in agreement with those reported by Grupta and Wells [76].

Bonder and co-workers [78] have synthesized IONP in the presence of different varieties of PEG as a possible route to produce highly magnetic polymer-modified nanoparticles for biomedical applications. The results indicated that the formation and agglomeration of the nanoparticles are controlled by the polymer concentration, and the geometry and/or terminal group of PEG.

Particle sizes were between 70 and 300 nm and a correlation between PEG concentration and Fe content was observed. As the polymer concentration increased the overall saturation magnetization of the surface-modified IONP nanoparticles initially increased due to the increased iron content with a maximum of 70 emu/g for nanoparticles with the largest Fe content. According to the final characteristics of the prepared particles, the authors concluded that they were suitable for biomedical applications.

SDS is a common non-polymeric stabilizer. Its hydrophobical nature is highly convenient to change the hydrophilic character of the IONP. The advantage of the use of SDS as stabilizer over the polymeric ones is its low molecular weight. Less voluminous modifier leads to smaller surface-modified IONP. Hydrophobic IONPs are required for instance in the case of the immobilization of lipases. SDS has been employed to generate a hydrophobic surface around the IONP destined to lipase immobilization. The IONP were prepared by co-precipitation and firstly modified with oxalate. In a second step the reaction between oxalate and SDS was performed originating SDS surface-modified IONP. The so obtained particles had a mean diameter of 10 ± 2 nm and were efficiently employed as lipase supports [54].

Besides the usual surface modifiers (namely OA, PVA and SDS) that play a role also as stabilizers, several compounds have been employed with the aim to provide adequate functionality to the IONP with regard to the desired applications. As an example, Mondini *et al.* [79] managed to synthesize hydroxyl-decorated magnetite nanosized particles in one step by high-temperature decomposition of iron(III) ω-hydroxy-carboxylates. These IONPs bear on their outer surface terminal hydroxyl groups that can be easily transformed into a variety of chemical groups and bound to other molecules. Hydroxyl-decorated IONP sizes ranged between 5 and 7 nm and were highly reactive as it was demonstrated by the reaction of the OH group (from the IONP) with succinic anhydride.

Del Campo *et al.* developed a simple and effective procedure for the surface modification of pure magnetite and silica-coated magnetite with an $-NH_2$ linker using aminopropyltriethoxysilane as the surface-modification agent. The modified particles were used to immobilize oligo-nucleotides [80]. Other article reports a simple and inexpensive method to synthesize stable magnetite nanoparticle aqueous dispersions by the precipitation of ferrous ions in the presence of an ionic solution containing acrylic acid anion. FTIR and TGA analysis confirmed that the acrylic acid anion of the ionic solution was attached to the nanoparticle surface in the chelating bidentate configuration. This configuration favoured the obtention of stable magnetite-nanoparticle dispersions [81].

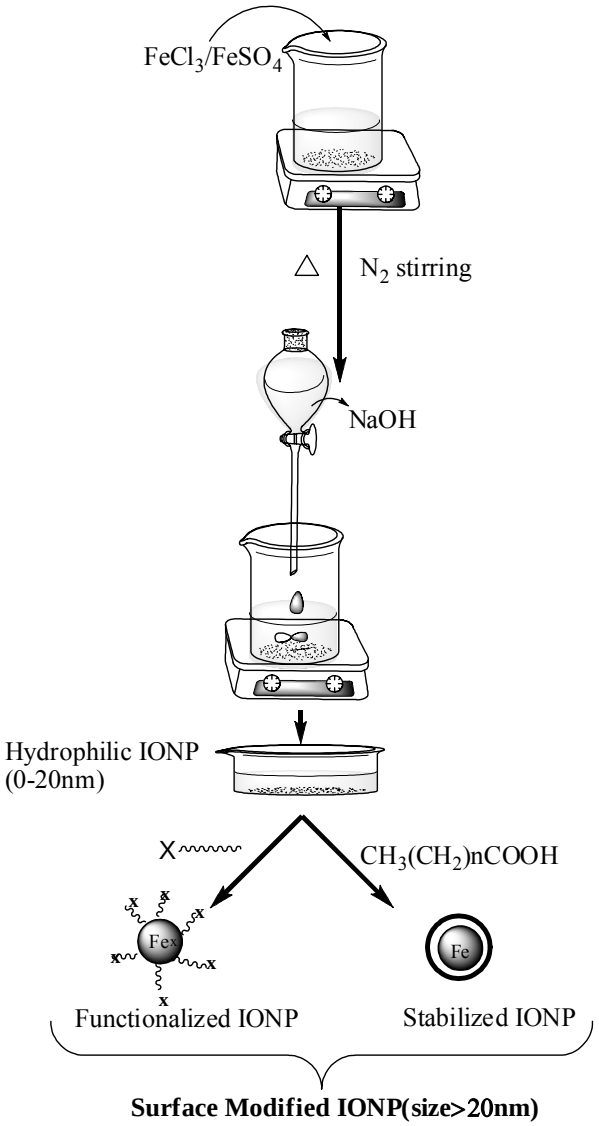
Recent studies have confirmed the protein matrix-mediated-modification of IONP with tailored surface chemistry that made them highly suitable for numerous *in vivo* applications [82-84]. For instance, a variety of potential ligands such as transferrin, lactoferrin, albumin, insulin, growth factors, etc. have been conjugated to IONP surfaces to facilitate receptor-mediated endocytosis of the particles. Ajay Kumar Gupta [77] reported the coupling of insulin to the nanoparticle surfaces to preferentially target the human fibroblasts. The derivatization of the nanoparticles was performed with the goal to generate a stronger link between the protein and the IONP surface. The results from SEM and TEM characterization indicated that the surface-functionalised nanoparticles with insulin showed high affinity for cell surface receptor mainly due to ligand-receptor interactions. Their specific attachment to cell surface offers the opportunity to label the cells with magnetic particles while reducing non-specific phagocytosis [84]. A similar investigation with almost similar concluding remarks was reported by Grupta and Curtis, using lactoferrin and ceruloplasmin as ligands or surface modifiers.

A key factor for IONP stabilization appears to be the coater/stabilizer concentration compared to the amount of magnetic iron oxide. The amount of stabilizer has great impact on the size, the dispersion and the magnetic properties of IONP [49]. Then, fundamentally in the case of polymeric stabilizers, it is feasible to prepare surface-modified IONP or nanocomposites depending on the amount of stabilizer and the

order or addition during the formulation process. The experimental steps generally involved in the surface modification of IONP are represented in the Scheme 2. It is clear from the Scheme that starting by a conventional co-precipitation the coating of the IONP with the desired moieties could be achieved in a few numbers of simple steps.

The main characteristics (in terms of the particles size and content of paramagnetic moieties) of surface-modified IONP are listed in the Table 3, in order to compare them with the properties of the IONP and MNC achieved by the methods described later in this manuscript. Hence, Table 3, contains the main characteristics of the different kinds of magnetic particles as a function of the preparation methods. The goal of the Table is to compare:

1. the kind of magnetic particle that could be obtained with the different methods,
2. the properties of each magnetic particles regarding the size, the content of paramagnetic component (highly associated to the magnetic properties) and the hydrophobic/hydrophilic nature.



Scheme 2. Representation of the experimental steps involved in the surface modification of IONP.

The surface-modification and functionalization of IONP has become an area of incipient development, hence the literature on this issue is plentiful. It is not the aim of this review to provide detailed information about surface modification of IONP. To see more in-deep description and analysis related to surface modification of IONP see references [74] and [105].

4. Methods of Synthesis of MNC

4.1. Polymerization methods

As it has been commented above, different techniques have been proposed for the fabrication of magnetic polymeric composites. The desire for robust encapsulation, control over the loading of magnetic nanoparticles within the polymer particles, and control over the size of the resulting magnetic polymer particles drives the interest in the incorporation of the iron oxides directly during the polymerization reaction. Thus precipitation, suspension, and mostly, emulsion and seed polymerization have been developed as fabrication methods of MNC. The nano-composites prepared from these routes are, then, constituted by a magnetic nucleus surrounded by a polymeric coating, being the polymeric network the major component of the resultant particles [106].

The nanocomposites prepared from these techniques are preferred mainly for applications where MNC are exposed to rigorous conditions that may affect the Fe/polymeric linkage. In this section the most widely employed polymerization

Table 3. Kind of magnetic nanoparticle, loading of entrapped IO and average size of the magnetic particles as a function of the preparative technique.

Method of preparation	Kind of particle	Loading of iron oxide (%)	Average Size of the magnetic particles	Observations	Ref.
Co-precipitation	IONP	100	30 Å to 14 nm	-Too small sizes -High aggregation -Lack of functional groups -High magnetic properties	[47, 53]
Surface modification					
Modifier Agent	Modified IONP				
Fatty acids [(1)]		10-30	10-20 nm	Modified IONP hydrophobical in nature	[70, 85, 86]
Polymeric modifiers[(2)]		Less than 10	4-7 nm	Near spherical and well dispersed nanoscale particles	[75, 87, 88]
Proteins		Not reported	200 nm-1 μm	Especially suitable to bind charged moieties to the modified IONP surface.	[82, 89]
Others[(3)]		Highly variable depending on the modifier	40-200 nm	-	[69, 90]
Polymerization based methods					
EP	Nano and micro composites	19-55	100-600 nm	High polydispersity and presence of residual polymerization reactives	[29, 91]
SP	Nano and micro composite	12-48	36-300 nm	High polydispersity	[97-100]
Pre-formed polymer and magnetite					
Chemical attachment	Nano and micro composite	8-50	21-61 nm	Use of crosslinkers or toxic additives	[32, 39, 63]
Physical attachment	Nano and micro composite	12 (DE) 50-70 (NP)	75 nm-10 μm (DE) 7-40 nm (NP)	Huge differences in sizes and characteristics depending on the chosen method	[12, 14, 16, 101-104]

[(1)]Mainly OA, stearic acid
[(2)]PVA, PEG, dextran, etc.
[(3)]SDS, Silica

methods - i.e. emulsion and seed polymerization- will be described. General issues about these techniques are provided below. Specific characteristics such as the MNC sizes and the percentage of loading iron oxides are included in Table 3 and compared with other preparative protocols analysed within this work.

Emulsion polymerization (EP)

The technique basically consists in the direct polymerization of emulsion droplets of the monomer/s in presence of an aqueous dispersion of a surfactant-coated magnetic nanoparticles to yield magnetic latex [92, 93, 98, 106-110]. The system is then composed of the monomers, the initiator, the appropriate solvent and the iron oxide as the extra component. Several monomers have been used to form the shell surrounding magnetic nanoparticles, while the most common are styrene and acrylate derivatives such as methyl methacrylate, hydroxyethyl methacrylate, etc. Hydrophobic monomers, such as styrene, commonly require a double-layer of surfactant to facilitate the transfer of the inherently hydrophilic magnetic particles into the non-polar monomer styrene, allowing the encapsulation of the iron oxides.

Although the conventional EP appeared earlier at the end of the last decade, many modifications of the original method have been recently performed to entrap the magnetic moieties and to enhance the properties of the resulting nanoparticles. In conventional EP, monomer-filled droplets coated with surfactant float in a continuum containing surfactant micelles. Monomers diffuse from the droplets to the micelles, which catalyse the polymerization. As a difference, in "miniemulsion" polymerizations, little or no surfactant coats the monomer droplets, the continuum contains no micelles, and polymerization proceeds, roughly speaking, within the droplets [111]. Wourmouth *et al.* [95] have employed poly(ethylene-co-butylene)-*b*-poly(ethylene oxide block copolymer as emulsifiers to carry out the polymerization of 2-hydroxyethyl-methacrylate (HEMA) and methyl methacrylate (MMA) in the presence of coated-magnetic particles employing ABIN as initiator. They found that block copolymers steer the process: PEO-PMMA facilitated synthesis and dispersion of superparamagnetic iron oxide, and PBE-PEO block copolymer stabilizes emulsion droplets to coalescence. In addition, the "osmotic stabilization" achieved by loading the droplets with PEO-PMAA coated magnetic particles, which are "insoluble" in the oil continuum, likely aids the encapsulation process by hindering Ostwald ripening of the droplets. X. Liu *et al.* have developed a protocol for the surface modification of magnetic nanoparticles after their synthesis by miniemulsion polymerization using MMA and divinylbencene (DVB) as monomers. They succeed in the preparation of uniform nanoparticles with sizes in the order of 102 nm [96]. DVB is a usual component of the EP systems. It is conveniently used as co-monomer, providing hydrophobic character to the resultant polymer. However DVB participates also as a cross-linker since the crosslinking of the polymer/iron oxide nanocomposites is commonly performed to reinforce the nanomaterials and to enhance the interaction oxide/polymeric networks.

In EP, the surfactant plays an important role in the stability, rheology and control of the microsphere size of the resulting latexes. Although SDS appears as the most common and effective one, other researchers have explored new alternatives in view of the requirements. For instance Pollert *et al.* have employed the Na salt of poly(oxyethylene) alkylaryl ether sulfate (commercially Disponil AES 60) as surfactant in the EP of glycidyl methacrylate (GMA) in the presence of dextran-coated magnetic nanoparticles using 4,40-azobis(4-cyanovaleric acid) (ACVA). They selected this surfactant because of its high hydrophilicity (due to its poly(oxyethylene) chain) on one site and the alkylaryl group with affinity for the organic liquid on the other site. Moreover, its anionic charge from SO_3^- boosts the emulsifier efficiency [94].

From the examination of the available literature about EP; it was noted that the data related to IO/monomer concentration (wt% or molar ratio) are not usually provided. These data are fundamental not only to understand the mechanism of formation of MNC, but also with regard to the possibility of the repetition of experiments by other researchers. The work of Pollert *et al.* [94], accounted a magnetite/GMA (w/w) ratio of

0.05 in the formulation of MNC from GMA and magnetite by EP, as it was commented above. These authors indicated that the ratio IO/monomer plays a relevant role in the resultant magnetic properties of the MNC, hence they have fixed the monomer concentration while varied the IO one. They observed, as expected, the increase of the saturated magnetization with increasing content of the IO phase in the magnetic PGMA microspheres.

Seed polymerization (SP)

SP is carried out in an organic solvent, commonly ethyl acetate, where magnetite is dispersed. The system is completed with the addition of the monomers, the initiator and a crosslinking agent to the organic solution. Since the polymerization is performed in organic solvent, the iron oxide previously synthesized has to be conveniently modified. For instance, Fe_3O_4 nanoparticles were modified using thiodiglycolic acid as the primary surfactant and 4-vinylaniline as the secondary surfactant. These double surfactant coated-magnetic particles were then used for SP using *N*-isopropylacrylamide as the main monomer, *N*,*N*-methylene bis acrylamide as the crosslinker and potassium persulfate as the initiator [97]. The so-prepared MNC showed paramagnetic behaviour, adequate size and high percentage of IO loading (see Table 3). The MNC obtained as such were further used in the adsorption and desorption of bovine serum albumin (BSA) showing high adsorption capacity.

Similarly, Lee and Senna [100] described the SP of styrene in the presence of sodium oleate stabilized magnetite. Sodium oleate coated-magnetite was added to an emulsion of styrene monomer and surfactant. The solution was mixed with seed polystyrene particles and the heterogeneous polymerization of polystyrene was initiated. The core shell morphology of the particles consists of a polystyrene centre with magnetite/polystyrene composite shells. The researchers suggest that this heterogeneous polymerization method may be used to prepare a wide variety of magnetic composite microspheres.

The concentration of monomers and crosslinker was a matter of analysis since these parameters may affect the properties of the obtained nanoparticles. Zaitsev *et al.* have analyzed the effect of these variables in the SP of methacrylic acid and HEMA monomers in the presence of a magnetite nanoparticles dispersion using AIBN and polymerizing at 65ºC under N_2. The concentration of monomer ranged between 0.5 - 2.5 wt% and the crosslinker concentration was between 1 and 10 mol% (related to the monomer). The authors determined that an inclusion of the magnetite particle into a hydrophilic polymeric shell increased the stability of the dispersion and decreased the influence of the stabilizing agent on the magnetic and structural properties of MNC. Under these conditions, the particles sizes oscillated between 340 and 400 nm in diameter [99].

Interesting magnetic composites may be also fabricated by anionic polymerization. The magnetic composites are generally prepared by anionic initiation of the monomer by covalent bases. In this process a magnetite suspension in HCl solution was added to the polymerization medium. The monomer was added drop wise to the stirring solution where polymerization occurred. The size of the polymer-coated particles and their internal magnetic cores are strongly dependent on the experimental parameters [112].

Although the polymerization techniques assure the formation of reinforced materials where the magnetic core is strongly bonded to a surrounded polymeric shell, the work-up procedure involving the synthesis and mainly the purification of the resultant particles appear as a difficult task from the experimental point of view. The current literature on this topic does not include enough details about the wide variety of possible by products formation associated to the use of these techniques and the required strategies to purify the obtained MNC.

4.2. Preparation of MNC using pre-fabricated polymers

The methods described in this section involve the incorporation of preformed polymers during the magnetite synthesis or similarly the entrapment of previously prepared magnetite onto the polymer chain. In this last case conventional techniques to obtain polymer nano and microparticles may be adapted for the entrapment of IO [113].

The MNC obtained from these methodologies can be classified according to the kind of linkage formed between the iron oxide and the polymeric substrate as follows:

a) Chemical attachment of magnetite in polymeric networks
b) Physical attachment of magnetite in polymeric networks

a. Chemical attachment of magnetite in polymeric networks

The formation of MNC is performed by a chemical reaction between the polymeric and the magnetic moieties. Several articles report different routes to achieve the chemical linkages, employing diverse reactants and strategies. Nanoparticles bearing a strongly bound polymer coating were designed by Burke *et al.* They have used polyisobutylene, polyethylene, or polystyrene functionalized with tetraethylenepentamine, a short polyethyleneimine chain, as polymeric dispersants. The iron oxide nanoparticles were formed by the thermal decomposition of iron pentacarbonyl in the presence of ammonia and polymeric dispersants, conducting the reaction in an appropriated solvent at 190°C during 2 h. The obtained MNC exhibited metallic cores, each of which was coated with a strongly bound polymer layer. The authors found a strong relation between the nanoparticles tendency to aggregation and the system solvent/dispersant employed in the formulation [39].

The synthesis of iron oxide (γ-Fe_2O_3) nanoparticles from the decomposition of iron pentacarbonyl ($Fe(CO)_5$) carried out in two different types of polymeric media was reported by *Dan et al.* [63]. They used PS and PMMA as substrates to perform the reaction. PS is known to adsorb onto metal surfaces through weak dipole-dipole interactions, while PMMA interacts with the metal surface through the coordination of the carbonyl groups on the acrylate side group, resulting in a relatively strong bond compared with the magnetic nanoparticles prepared by physical interactions. Although Dan *et al.*'s work was centered in the kinetic of formation of the nanoparticles the authors determined that the average particle's sizes were near 65 nm for PS-based MNC and 20 nm in the case of PMMA-based MNC. They found that the sizes were independent of the reaction temperature and the precursor concentration.

Vijaya Kumar *et al.* have reported the preparation of magnetite-embedded PVA nanoparticles by a novel ultrasound radiation method. Sonochemistry arises from acoustic cavitation phenomena, that is, the formation, growth, and implosive collapse of bubbles in a liquid medium. The effects of ultrasound radiation on chemical reactions are due to the very high temperatures and pressures, which develop during the collapse of a cavity, formed in the liquid. Thus, Fe pentacarbonyl was used as starting material in contact with a solution of PVA. As a result, uniformly dispersed amorphous nanoparticles of magnetite in a PVA matrix have been obtained with average sizes between 20 and 30 nm depending on the decomposition temperature [114].

Alginate/PVA iron oxides composites have been prepared by Nishio and col. with the adaptation, in a suitable way, of the conventional *in situ* synthesis method aiming to generate stronger polymer/oxides linkages. In brief, an aqueous solution of alginate (Alg) was contacted with a ferrous sulfate solution at room temperature during 24 h, allowing the formation of ferrous alginate gels (Fe-Alg). These gels were then oxidized by alkaline earth metallic hydroxides (Ca, Sr or $Ba(OH)_2$). They found paramagnetic behavior only when Ca^{2+} hydroxide was employed and was attributed to the difference in the cation exchanging manner affecting the dimension of the reaction space for the oxide synthesis in the polymeric network. This investigation was mainly centered in the paramagnetic properties of the prepared composites; an analysis on the morphology and the sizes of the Alg/PVA magnetic particles was not included [115]. A similar procedure was described by Alizade *et al.* using gelatin as polymeric material. The results of Alizade's investigation suggested that magnetite particles were rigidly bonded to the matrix that acts as a protective layer against the oxidation remaining their properties unchanged for a considerable period of time [102].

About the analysis of the available literature on the chemical preparation of magnetic

nanocomposites, one may infer that there are not available in-deep mechanistic studies on the chemical reactions occurring during magnetic nanoparticles formation. Hence, it becomes very difficult to discern if the iron oxide is finally adsorbed or chemically linked to polymeric templates.

b. Physical attachment of magnetite in polymeric networks

In general, in the physical entrapment techniques, the starting materials are the polymer and magnetite (or the selected iron oxide in any case). No chemical reactions are involved in the process. Magnetite is entrapped into the polymeric matrix by hydrophobic-hydrophilic, electrostatic, or steric interactions. In such cases, two sets of methods can be employed:

i) The conventional techniques commonly used to prepare polymeric nano- and microparticles have been modified regarding the addition of the iron oxide. From such techniques, the double emulsion (DE) and the Nanoprecipitation (NP) are so far the most widely employed even for the preparation of magnetic nanoscale particles [6, 102, 103]. The information concerning DE and NP as methods to fabricate polymeric nano and microparticles is huge; hence it is not the goal of this review to describe such techniques with a high level of detail. Complete information about them and others can be found in reference [113].

The DE method has been intended for the encapsulation of numerous active principles on polymeric nano- and microparticles. Although there are several variants on this method, it basically involves the use of an organic solution containing the polymer that is added drop wise to an aqueous solution containing the magnetite. The resultant mixture is then emulsified on an aqueous solution containing an adequate surfactant. It has been found in the open literature that the described conventional procedure is, in many cases, well adapted to improve the properties of magnetic nanoparticles proposed for specific applications. For instance Patel *et al.* have dispersed magnetite in chloroform and PLGA in ethyl lactate and treated with NH_4OH to raise the pH to 11. By adding the polymeric solution drop wise to the magnetite dispersion, all components were mixed. After ultrasonic and vigorous stirring, the mixture was poured into an aqueous solution of a surfactant, Pluronic F-127, whose concentration was higher than its critical micellar concentration (cmc). After the synthesis, the surface modification of IONP took place by facile replacement of the surfactant(s) such as OA and/or oleylamine by PLGA, which bears both the alcoholic OH and carboxylate as the end groups (carboxylate group is known to have stronger interaction with the surface of the iron oxide nanoparticle) [18]. Jeong *et al.* have also entrapped magnetite into a preformed polymer (PLGA) by the DE method. They used a solution of the polyester in ethyl acetate (2%) and a ferrofluid as starting materials. The mixture of both solutions was emulsified in an aqueous solution containing 5 wt% F-127 Pluronic surfactant [101]. The use of sonication allowed the formation of MNC of near 120 nm in size. Lee *et al.* have investigated the entrapment of magnetite in PLGA employing a similar procedure than Jeong *et al.* They evaluated the effects of homogenizer and agitator speed on the nanoparticles formation and the magnetic properties of the resulting MNC [116].

Wassel *et al.* have investigated the same system than Jeong (i.e magnetite/PLGA), working also with DE method. In this case both magnetite and polyester were dissolved in chloroform and mixed with water to form an “oil in water” emulsion that was then emulsified in an aqueous solution of PVA, to form the double emulsion. Special emphasis was given to the effect of the experimental variables (ultrasound time, concentration of polymer and magnetite, etc.) on the particle size and the efficiency to entrap the iron oxide. They found that higher amounts of incorporated magnetite lead to saturation magnetization and that the amount of entrapped magnetite was readily controlled by adjusting the amount of iron oxide in the feed [14].

PLGA-coated magnetite/maghemite nanoparticles have been prepared by Okassa *et al.* employing the DE method [16]. They have used methylene chloride as the solvent for the polymer solubilization being the organic phase, poured in the aqueous phase containing the selected iron oxide. Then the resulting mixture was emulsified

in an aqueous solution of PVA. In Okassa *et al.* the hydrophobic/hydrophilic character of the iron oxide was analyzed with regards to the final percentage of loaded oxide. They determined that the iron oxide nanoparticles previously treated with OA were entrapped more efficiently in the PLGA templates; presumably due to the increment of the affinity as a consequence of the hydrophobic nature of OA coated iron oxide particles. Several experimental parameters have been analyzed in this work with the aim to optimize the formulation procedure in terms of percentage of loaded iron oxide, particle size and particle aggregation tendency [16].

It is important to establish the role of each polymer used in the formulation process using the DE method. Although it is rather common to find certain polymeric substrates such as PVA, dextran, gelatin, polyvinyl pirrolidone, etc. as part of the systems during fabrication of magnetic nanoparticles through DE, it is worth mentioning that they act as surfactants. Their function is to form a protecting thin layer around the oil drops, polymer and magnetite, with the aim to reduce the coagulation and to stabilize the emulsion. Then, the surfactants/stabilizers are compounds related to the DE method and should be eliminated during the purification step of the fabricated particles. Therefore, it is essential to distinguish between the polymer/s selected to form the MNC and the ones employed as surfactants [115, 117]. The reviewed available literature suggests that biodegradable polymeric substrates are preferred to encapsulate IONP using DE. As a consequence this method could be chosen to prepare MNC destined to biomedical applications.

The traditional NP technique used to formulate polymeric nanoparticles has been also adapted for the entrapment of magnetite onto several polymeric templates [113]. It basically consists in the precipitation of an organic solution of the polymer (generally hydrophobic) in an aqueous solution of the magnetite, performing the addition at a controlled rate [118, 119]. The so-obtained nanoscale particles are recovered after solvent evaporation and commonly sonication treatment is performed to disperse the obtained MNC. Introducing some modifications to the NP simple procedure, it is possible to entrap the magnetite onto hydrophilic polymers. For instance, Meerod *et al.* (reference) have used this methodology to entrap magnetite onto caprolactone-co-PEG copolymers. Due to the fact that the copolymer was amphiphilic in nature, the hydrophilic component of the NP system was the polymer templates while the magnetite was modified with OA to generate the hydrophobic character. Then, the IONP constituted the organic phase dissolved in hexane and was added in a controlled way to an aqueous solution containing the polymer. The mixture was then ultrasonicated to transfer the particles from the hexane top layer to the aqueous bottom layer. The authors found that experimental variables such as the concentration of polymer, the concentration of stabilizer (OA) and the time of ultrasonication, affected considerably the size of the obtained particles. The effect of the mentioned parameters on the size and other MNC properties will be addressed in next sections [12].

The ultrasonic treatment is a common task in NP procedure. It is included for the effective entrapment of magnetic moieties on polymeric networks, as a way to avoid the aggregation of the particles due to the electrostatic forces, considering the lack of surfactant/stabilizers.

Timko *et al.* have also adapted the conventional NP to prepare PLA-based magnetic nanoparticles. These authors have dissolved the polyester in an organic solvent. The organic phase was added drop-wise into an aqueous phase containing dispersed magnetite and phosphate buffer of pH 7.4. After this, the solution was stirred at room temperature until complete evaporation of the organic solvent. Hence, the magnetic nanoparticles were obtained as an aqueous suspension. The generated MNC were successfully employed as carriers for the sustained release of Indomethacin [20].

Chitosan (CS) and highly substituted *N*-trimethyl chitosan loaded magnetite nanoaparticles were fabricated by Bellesi *et al.* using NP based method. The simple procedure consisted in mixing up a solution of CS (in acetic acid) with an aqueous solution containing the magnetite dispersion. The magnetic-chitosan mixture was diluted with water, stirred at room temperature and then the acidic solution was neutralized with a solution of $NaHCO_3$. In this research variables

associated to the formulation process such as CS/magnetite ratio, concentration of CS, etc. have been studied regarding to the size, the super-paramagnetic behaviour and the agglomeration of the resultant particles. The authors concluded that the nanoparticles were non-aggregated. The sizes (ranged between 10 and 40 nm) were highly appropriate for biomedical purposes. Furthermore, they observed very high saturation magnetization reachable at low applied field [104].

ii) The co-precipitation method used to generate iron oxide nanoparticles, described in Section 3.1 has also been tailored to prepare MNC *in situ* during the synthesis of the IONP. In general terms, the porous pre-synthesized polymer (in bulk or in solution) is processed by salts of iron +2 and +3 followed by sedimentation of magnetite nanoparticles by alkali treatment. There are some publications that report the addition of the polymeric template to the alkaline solution [26].

In any case, the function of the polymer template is to provide a constrained environment for the formation or stabilization of iron oxide nanoparticles. Due to its simplicity and versatility, co-precipitation has quickly emerged as one of the most used techniques to fabricate magnetic nanocomposites employing a wide variety of polymeric networks. Especially suitable are water-soluble polymeric substrates since a monophasic system is created by contacting a polymer with the iron oxide.

The order of addition of the polymeric template in the co-precipitation procedure deserves special attention. MNC with different characteristics may be obtained by addition of the polymer at the beginning or after the reaction. Based on literature reports, polymeric templates incorporated after the magnetite synthesis generally resulted in polymer weakly bonded to the magnetite. These polymeric templates were the above described as stabilizers meaning that their function is limited to avoid the particles aggregation. As a difference when the polymer is in the reaction media, as starting material in contact with the IO precursors, the resulted interactions polymer/iron oxide are more resistant and could serve for instance as connectors for different active molecules according to the desired applications [17]. Although the mechanism of formation of polymer-coated magnetic nanoparticles by this route is not well established, many hypotheses have been postulated and it seems to be strongly dependent on the studied system. For instance, Chu *et al.* have proposed that physical association of carboxylated polymers with iron oxide particles was due to hydrogen bonds between the particle surface oxide and hydroxyl groups [120]. It was suggested that free carboxyl groups on these structures could act as mineral nucleation sites, and therefore provide templates for the growth of nanoparticles [121]. Another published article proposed that the PLA-pendant carboxyl groups might form strong chelating bonds with ferric or ferrous ions providing the nucleation point for iron oxide formation. At the same time, the highly branched polymer chains may limit the rapid growth of the crystals and blocked the aggregation of different crystals. Compared to the loose polymeric coating after iron oxide co-precipitation, the PLA matrix with free carboxyl groups became complexed with iron oxide nanocrystals during the process of synthesis resulting in a much tighter association (and more stable nanoparticles) [17]. The crosslinking during the co-precipitation has been studied as a way to reinforce the generated IO/polymer linkage. Sepúlveda *et al.* used styrene (ST)-co-N-4-carboxybutylmaleimide copolymers, with different amount of DVB as crosslinker, and goethite as iron oxide to prepare styrene-based MNCs. The TEM characterization of the obtained materials revealed that the nanoparticles with the lower crosslinking degree showed a higher number of spherical goethite particles whereas in the nanocomposite of higher cross-linking, the goethite needle-like shape was dominant. The authors justify this achievement in terms of the mechanism of formation of the nanoparticles. They proposed that a surface-located precipitation would appear to be the prevailing mechanism in the case of higher cross-linking degrees. As a difference when lower crosslinking levels were registered, the polymer template appeared to control the shape, degree of aggregation and size of the particles [122].

Another modification to the conventional co-precipitation technique involves the use of a

porous gel structure as polymeric matrix, where iron oxide nanoparticles are formed in the constrained architectures of a polymer gel [115, 123]. Pores in the gel act as nanoreactors where iron oxides nanoparticles are formed *in situ*.

It is well-known that the NH_2 group on CS molecules may interact with Fe^{2+} in aqueous solution. Wu *et al.* have prepared CS nanoparticles previously cross-linked with sodium tripolyphosphate in HCl solution, resulting in porous CS-gel nanoparticles. Then Fe^{2+} was added into the polymeric solution and adsorbed by the CS nanoparticles. NaOH was used to adjust pH and to precipitate $Fe(OH)_2$, and a small amount of O_2 was used to oxidise the $Fe(OH)_2$ into Fe_3O_4. As the Fe_3O_4 nanoparticles were present in the pores of the CS gel, the monodispersion was good. According to the authors' description, nanomagnetic gels were obtained with this procedure [24]. Comparative studies between nanoprecipitation/DE and *in situ* co-precipitation techniques have been reported in the available literature [102]. As an example, Morales *et al.* have compared *in situ* magnetite co-precipitation on a gel with nanoprecipitation, using Ca-alginate beads. From the size point of view, the iron oxide produced before alginate gelation generated particles that were significantly larger than the iron oxide produced after alginate gelation in which polymer is present during IO the synthesis. Hence they concluded that the presence a polymeric gel *in situ* during the synthesis of IONP is highly effective to inhibit the growth of the formed particles, leading to a narrow MNC sizes distribution [13].

In order to complete the information detailed in Table 3 and to better visualize the experimental steps involved in the formation of MNC, a representation of the polymerization, nanoprecipitation and *in situ* co-precipitation methods is included in Scheme 3. Although any of the three procedures lead to MNC different characteristics are expected for each "differently prepared" MNC.

Considering the similarity of the available methods to fabricate MNC and to help the researcher in the selection of the most appropriated one in relation to the desired application, Table 4 summarize the advantages and disadvantages associated to each preparative method.

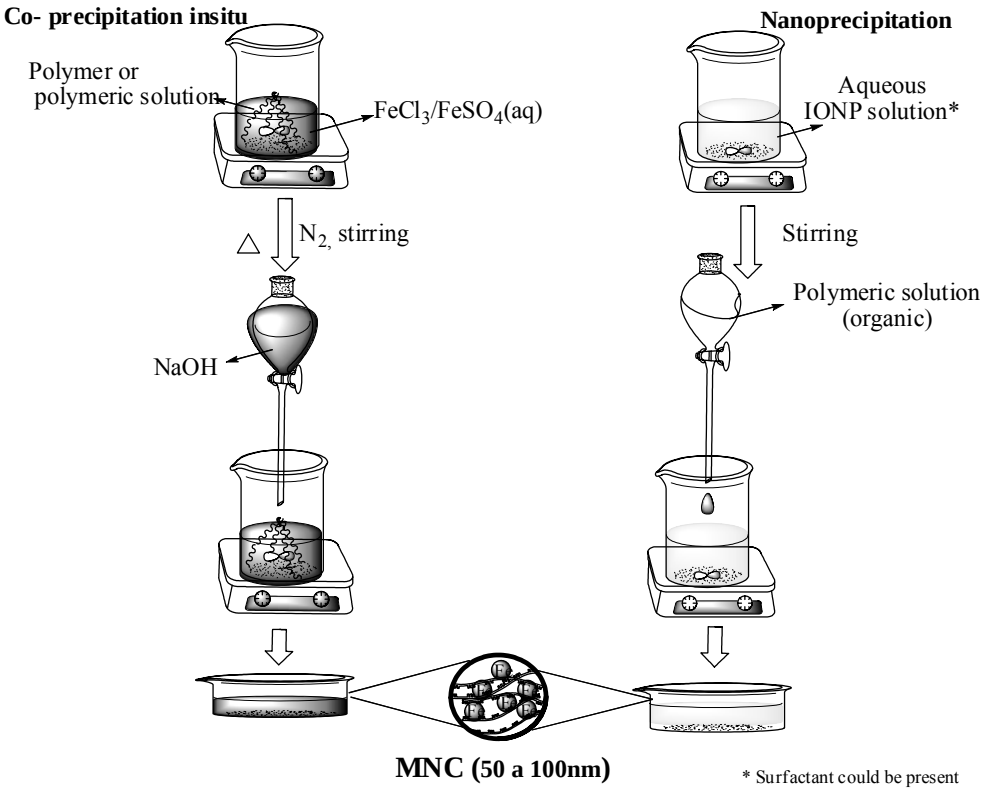
b. 1. Experimental variables associated with the physical entrapment methods

A rapid examination of the available literature suggests that extremely different nanoparticles properties may be achieved (in terms of their size, aggregation and amount of loading iron oxide) by adjusting the experimental conditions involved in the "Physical entrapment methods". While the concentration of the polymeric template and magnetite appear to be the critical factors; other less discussed in the current reports seem to have an effect not only on the characteristics but also in the potential applications of the nanocomposites. In this section the most relevant variables affecting the properties and performance of MNC prepared though the physical entrapment methods will be discussed.

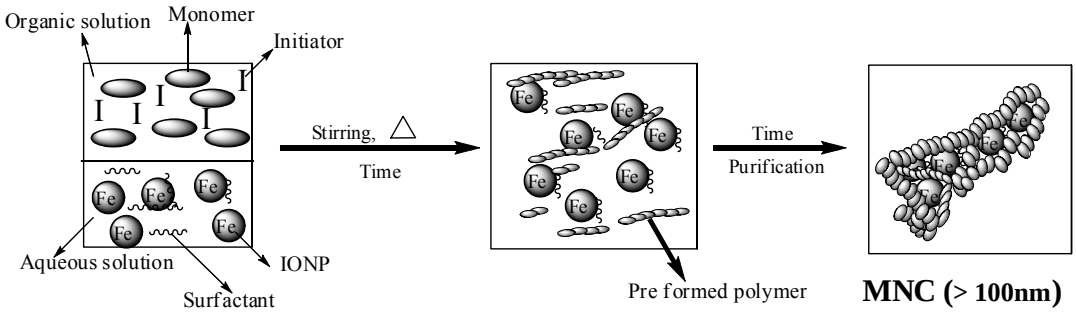
Polymer concentration

Polymer concentration is an important parameter to consider when nanoparticles are synthesized. As it was previously commented, the concentration of the polymeric template, combined with its nature and the order of addition during synthetic procedure, might define its role as stabilizer or as a component of the magnetic nanocomposite. Even more important is the influence of the polymeric concentration on the size of the prepared particles, hence it has been broadly analysed in the current literature independently of the preparation method. Okassa *et al.* employed the DE technique to prepare MNC from maghemite/magnetite and PLGA. The amount of polymer was increased from 100 to 200 mg, and the effect on the nanoparticles characteristics (mainly on diameter and polydispersity index, zeta potential and magnetite incorporation efficiency) was assessed. The results of increasing the amount of polymer did not significantly affect the MNC size distribution. In contrast, when the polymer amount was increased up to 200 mg, the percentages of entrapped magnetite were approximately divided by two [16].

Meerod *et al.* agreed with Okassa in that the size of magnetic nanoparticles was independent on the polymer concentration in the solutions. These



Polymerization method (EP)



Scheme 3. Comparison between polymerization and physical protocols able to prepare MNC.

authors have investigated the influence of the copolymer concentration on the encapsulation of magnetite on PEG-co-CL copolymers using the nanoprecipitation method and employing polymeric solutions of 0.5 and 1%. They found that copolymers concentration played an important role on dispersability of the nanoparticles since they observed a critical reduction of the aggregates by increasing the amount of copolymers in organic solution [12].

The influence of the polymeric template amounts has also been evaluated regarding the co-precipitation method. Border *et al.* have employed stock solutions containing 1.0 to 5.0 mg/ml of PEG (with Mw 8000). The data on this research indicated that increasing PEG concentration, increased agglomeration and coating. For the lowest concentration (1.0 mg/ml) the reaction produced well-separated 70 nm nanoparticles reminiscent of the smallest nanoparticles produced

Table 4. Comparison between polymerization and physical methods for the preparation of MNC.

Preparative Method	Advantages	Disadvantages
Polymerization methods	-Forming well-controlled magnetic core polymeric shell nanoparticles as compared to other methods. -Higher protection of the magnetic core from oxidation. -Lead to enhance compatibility with organic ingredients -Reduce susceptibility to leaching.	-Incomplete and non-uniform encapsulation, in particular using EP. -MNC obtained are not suitable for applications as DDS: 1. because the polymeric shell, in some cases, is not thick enough to transport an appropriate amount of drug. 2. because of the presence of residual toxic by products and additives.
Physical attachment of magnetite in polymeric networks from preformed polymer and IONP	-Low cost, simple and scalable -The use of non-highly toxic solvents -Additives can be used for nanoparticle size reduction. -IONP without modification could be used since these methods are suitable for both hydrophilic (by DE) and hydrophobic (NP) active components	-High consumption of energy by the necessity of high stress shear (i.e. sonication or microfluidization). -Weak linkage IONP-polymeric coating; if the monolayer of polymers desorbs, the magnetic particles become exposed to the continuum for instance for MNC as DDS.
Physical attachment of magnetite in polymeric networks by *in situ* co-precipitation of IONP.	-Simple and not time consuming procedure -Higher magnetic susceptibility because of the higher magnetic content	-Difficult to control particle size and size distribution because of the lack of homogeneity of the reaction in the solution.

in the reaction without PEG. By increasing the PEG concentration to 2.5 mg/ml, the 70 nm nanoparticles coexist with larger agglomerates; and finally using 5.0 mg/ml stock solution, the fraction of larger agglomerates (near 300 nm) is more prominent [78]. On the contrary, Shupeng Liu *et al.* have observed that the stabilization effect of the polymer is concentration dependent for the synthesis of MNC using PLA and the *in situ* co-precipitation method. They determined a minimum polymer concentration (50 mg/ml) related to the effective stabilization and similarly, a maximum concentration (400 mg/ml) at which no more particles could be formed. They observed a decrease in the magnetic nanoparticle's size with the increase of the polymeric concentration. The analysis was performed using a fixed amount of Fe^{3+} ion concentration (37 mg/ml) [17].

Concentration of iron oxide

The amount of iron oxide incorporated during the formulation procedure appears to be crucial with regard to the magnetic properties and hence, to the final applications of the resulted MNC.

As high saturation magnetization is required, an optimal concentration of magnetite is needed to produce the movement of the particles to a specific location [124]. Low concentrations of IONP are strongly preferred because of the effect that high concentrations of IONP might have on the loading efficiency and/or activity of the bioactive agent. Besides, the toxicity of the remaining magnetism should be considered regarding the applications of MNC in the biomedical field.

Wassel *et al.* have studied the effect of magnetite content in the formulation of nanoparticles coated with PLGA using DE. They varied the amount of magnetite from 1 to 5 mg/ml and found that using lower concentration of magnetite fewer IONP were incorporated into the polymer and their appeared to be non aggregated. From the viewpoint of the magnetic properties, they observed an almost linear trend between magnetism and amount of magnetite in the organic phase. In any case, encapsulation of the nanoparticles to form the MNC, does not seem to affect the super paramagnetic nature of the IONP. In this case, it was noted that the weight fraction of magnetite incorporated inside the PLGA was readily controlled by adjusting the amount of magnetite in the feed. The initial amount of the magnetic component of the generated MNC was directly proportional to the quantity of IONP initially added to the reaction mixture. These authors registered 12% (w/w) as maximum amount of magnetite bonded to the PLGA nanoparticles [14].

The influence of the IO content in the size and size distribution of the MNC has also been evaluated in the open literature. It has been reported that increasing the internal aqueous phase, an increment in the iron loading could be achieved, when DE method was the selected preparative technique. However, a four-fold rise in the volume of the magnetite aqueous suspension (i.e. from 50 to 200 µl) gives particles with similar characteristics to the ones obtained at higher iron loading, with respect to their mean diameter and polydispersity index [16].

The magnetite/CS ratio was varied in the range 1:4–1:1 (g/g) by Denkbas *et al.* in the fabrication of CS loaded magnetite microparticles using the DE method. A reduction in the particle size with the increment of the magnetite content was registered [6]. On the contrary and as it was expected, improved magnetometric properties were recorded using higher magnetite contents. Lee *et al.* have reported the decrease of the size of PLGA based MNC with the increase of the magnetization and magnetic susceptibility. This observation was explained considering that the volume fraction of embedded ferromagnetic particles to PLGA matrix was increased as the size of encapsulated nanoparticles decreased [116].

Ultrasound

As it was stated above, working with DE or NP methods, ultrasonic treatment of the organic/aqueous mixture of iron oxide and polymer is strongly required to avoid the aggregation and to control the size of MNC. Therefore, ultrasonic treatment has an effect on the properties of the fabricated particles, especially on their size. In general terms, longer sonication times and higher sonication powers led to smaller particles, as it was showed by Wassel *et al.* [14]. They evaluated this parameter by changing the sonication time and power from a few seconds to 5 min and from 3 to 15 W, respectively. They observed that the size was reduced from near 300 to 150 nm as a result of changes in the experimental conditions.

Besides the particle sizes, magnetic properties and also the percentage of loaded iron oxide could be affected by the sonication conditions, as it was demonstrated by Meerod *et al.* in the fabrication of magnetic PLGA nanoparticles by DE [12]. The data achieved in this research reveal that the incorporation of magnetite to the polymeric templates (between 66 and 71%) was not dependent on the sonication time (from 1 to 4 h). Furthermore, a lower magnetic response was detected using sonication time of about 1 h, therefore ultrasonication time periods of at least 2h were required to obtain particles with good magnetic response. The authors justified these results in terms of the particle's size. They proposed that relatively small particles migrated to the aqueous phase when only 1h sonication was applied as indicated by the average particle size of 7.8 nm. Increase of ultrasonication time might further enhance phase-migration efficiency of the particles resulting in migration of larger particles (9.0 nm) to aqueous phase and thus particles with high enhanced magnetic properties could be obtained.

Alkalinization rate and pH

Besides the parameters cited above there are other variables inherent to the *in situ* co-precipitation that are able to affect the properties of the

originated MNC. One of them is the nature of the cation of the hydroxide used to precipitate the IONP during the MNC formation [47]. The time of base addition should be 1-2 s with intense agitation. A slow base addition creates in homogeneous regions of the hydrated iron species, which leads to non-magnetic iron/ polymer blends [47].

The reaction pH is also important in co-precipitation methods since it may affect the kinetics of nucleation and crystal growth. Data on the open literature suggest that strong alkaline bases such as KOH and NaOH shift the pH of the mixture to ~14, which creates iron hydrate complexes that do not form magnetite. Gribanov *et al.* informed that using ammonium hydroxide in the pH range of 8.5 to 10 ensure the exclusive formation of magnetite without non-magnetic iron oxide forms [47]. Furthermore, no solid particles are formed at pH lower than 8, while evident precipitation results when pH approaches 12. Therefore, based on the literature reports, pH of 10 appears to be the optimal in order to obtain nanosized stable magnetic composite particles [17]. Jiang *et al.* agree in that the optimal pH to fabricate dextran- based MNC ranged between 10 and 11. In this study urea was incorporated to the reaction mixture to assure a uniform increase in the pH value of the solution, and a homogeneous environment during the procedure. As a result the size distribution of magnetic Fe_3O_4 nanoparticles could be significantly narrowed down. In addition, by controlling the decomposing amount of urea via adjustment of the decomposing time, the average hydrodynamic diameters of magnetic Fe_3O_4 particles could be manipulated in the range comprised between 8 and 50 nm [14, 125].

5. Applications of the MNC

Although the application fields of MNC and surface modified IONP is growing and extending over several areas, the impact of the magnetic technology in biomedicine is undoubtedly the most illustrative example. Biomedical applications under current investigation include several fields of medicine [126, 127], cell separation methods [128, 129, 130,], improved MRI diagnostic contrast agents [131-133] and magnetic field-guided carriers for localizing drugs or radioactive therapies. The nanomagnetic particles properties can influence both material durability in biological environments and toxicity issues. In view of the varied literature on this topic we attempt to focus specifically in their use as DDS, providing the need of information regarding the design and functionality of MNC.

The other issue analyzed in this article, related to biotechnology, is the use of magnetic supports for the immobilization of enzymes (to be used as biocatalysts). As it was commented before this is an emerging topic, therefore the literature antecedents are more reduced in number but the promising of the recent published data and the scarce information encourage us to include them in this review.

5.1. Applications of MNC as DDS

Regarding to this function of MNC, the selection of the right polymer (in the case of MNC) or modifier (in the case of surface-modified IONP) is relevant to assure the success of these biomaterials, as it has been stated above. Besides this, and considering the available literature, MNC must fulfil certain characteristics to be able to act as DDS. Here, the most relevant are presented.

(i) Size and size distribution

Controversial opinions are found regarding the most adequate size of the magnetic particles. Many authors stated that small particle sizes (of less than 50 nm) allow capillary distribution at the desired target site. Particles with diameter greater than 200 nm are usually sequestered by the spleen as a result of mechanical filtration and are eventually removed by the cells of the phagocyte system, resulting in decreased blood circulation times. On the contrary too small particles on the order of 10 nm are rapidly removed. Particles ranged from 20 to 100 nm appear to be optimal for intravenous injection and exhibited the most prolonged blood circulation times [134].

On the other hand other researchers reported that controlled-release microspheres formulated for parenteral administration should be less than 250 μm and ideally less than 125 μm in the diameter [135]. The desired size of microspheres

to be injected mainly depends on the route of administration. In general, a size lower than 5 μm is used for intravenous route, lower than 125 μm is used for intra-arterial and intra-articular route. Particles whose size range near 120 μm can be administered easily by suspending in a suitable vehicle and injecting them using a conventional syringe with an 18- or 20-gauge needle [135].

(ii) Magnetic properties

Appropriate magnetic response to technically achievable local field in physiological systems is required using MNC as DDS. Advances in nanoparticles technology may lead to the ability to magnetically deliver higher concentrations of drugs, for instance, to a tumor located in a particular position in the body, which is not easily accessible by other means. On this way, side effects that occur with systemic cancer therapies could be reduced.

Consequently, MNC or surface-modified IONP should present magnetism that turns "on" upon application of a strong magnetic field and turns "off" upon removal of the field. Superparamagnetic particles fulfil this requirement, but super-paramagnetism only occurs with IONP or MNC particles smaller than 100 nm in diameter. Therefore there is a compromise between the adequate size (in terms of the physiological behaviour) and the magnetic properties with regard to the efficiency of the magnetic particles as DDS.

It has been suggested that at the arterio-capillary blood flow rate of 0.005 - 0.1 cm/s, 20% w/w magnetite is sufficient to achieve 100% retention of the magnetic carrier using 8000 G magnet [136]. In an *in vitro* experiment it was demonstrated that 28% w/w of magnetite in MNC is necessary for their effective targeting.

Low concentrations of magnetic iron oxide are strongly preferred because of the impact that high concentrations of iron oxide might have on the bioactive agent with regard to its activity and stability [14].

(iii) Kind of therapeutic agent and release kinetic

MNC must have the ability to carry numerous active drugs at sufficient loadings and at controllable release rates of the drug from the carrier. Although anticancer medicaments have been mainly entrapped in MNC or surface-modified IONP, the design of the magnetic particles could be adapted to perform the entrapment of diverse therapeutic agents [137]. For example Saravanan *et al.* have prepared MNC loaded with Diclofenac, employing double emulsion method and gelatine as the polymeric substrate. They prepared MNC with a content of magnetite of 28.7% w/w, which was sufficient to retain the microspheres at the site of targeting by using a magnet of 8000 G, with sizes of roughly 2.4 μm. The results revealed that satisfactory loading and entrapment efficiency were reached (8.9 and 89%, respectively). The *in vitro* release profile demonstrated the ability of the microspheres to prolong the drug release for more than 18 days. *In vivo* assays indicated that the quantity of injected magnetic microspheres localized at the target site was only 4-5% and the majority of microspheres was localized in liver, spleen and lungs [27].

Proteins have also been studied regarding the feasibility to formulate magnetic DDS. Co-encapsulation of IONP with insulin to form liposomes or microparticles for oral delivery has shown beneficial results since increase drug retention and absorption has been reported [138]. Teplay *et al.* formulated insulin-containing PLGA microparticles separately from the IONP; both particles were then coupled by making use of their opposite surface charges. Then the MNC or complexes were prepared through electrostatic interactions. An externally applied magnetic field could then be used to retain the microparticle/ micromagnet complexes in the intestine after oral dosing, providing an opportunity for long-term efficacy and high bioavailability of orally delivered protein drugs [15].

(iv) Toxicity

There are a number of requirements that have to be considered regarding the toxicity of MNC formulations. Many of them are inherent to the selected method to synthesize the particles such as the surfactants chosen in DE or the organic solvent in NP, while there are others directly associated with the magnetic component. Among the scarce numbers of works published in the

open literature discussing this topic, dissimilar conclusions were achieved. For example, Lacava *et al.* have investigated the toxicity of water-dispersible and citrate-based iron oxide nano-particles aiming to access their biocompatibility [139]. The researchers analyzed the toxicity of uncoated and citrate-coated magnetic nano-particles. The citrate did not produce a significant biological response. However, both of the magnetic fluids caused severe inflammatory reactions of spleen and kidney, and diarrhea. Another literature source suggests that the presence of magnetite has a minimal effect on the acute toxicity of the materials investigated (while it was reported that the toxicity of magnetite nanoparticles (LD50 in rats) was 400 mg/kg) [140, 141]. Other investigations indicated that iron oxide based-nanoparticles will require a biocompatible sheath to prevent toxic interactions in biological media [139]. Therefore, the benefits related to the incorporation of polymeric moieties on magnetic microdomains are also evidenced from the safety of magnetic carriers intended for DDS. Considering the literature context, the important thing about MNC as DDS is the content of magnetic component: it must be enough to reach the desired characteristics but minimum with respect to the toxicity limit [15, 20, 22, 97].

5.2. Immobilization of lipases

Immobilization to solid carriers is perhaps the most used strategy to improve operational stability of biocatalysts, better operation control and easier product recovery without catalyst contamination. Further, decreased inhibition by reaction substrates and products, selectivity towards non-natural substrates and better functional properties compared to the corresponding soluble enzymes, make immobilization one of the most preferred methods of enzyme improvement towards stabilization [142].

Since enzymes are expensive, catalyst reuse is critical for many processes. Product purity is usually improved, and effluent handling problems are minimized by immobilization. In addition, enzyme immobilization onto magnetic supports such as MNC or surface-modified IONP has an additional merit compared to other conventional supporting materials, which is the selective and easy enzyme recovery from the medium under a magnetic field. Hence there is no need for expensive liquid chromatography systems, centrifuges, filters or other equipment.

The practical advantages associated to the use of magnetic technology for lipase immobilisation turn them very attractive for researchers, but the investigation about this topic is nearly starting. As a consequence, literature references are currently limited and to the best of our knowledge it has not been earlier reviewed. The magnetic materials intended for these purposes should exhibit a hydrophobic nature aiming to increase the affinity between the lipase and the magnetic support. Therefore, non-polar polymeric templates are preferred to cover IONP or to form MNC. Lee *et al.* have prepared surface modified IONP using firstly oxalate as coupling agent, which was then replaced by SDS. Pancreas Porcine lipase (PPL) was immobilized in the MNC by physical adsorption. Briefly, near 200 mg of modified IONP were added to 0.85% (w/v) sodium chloride solution containing lipase. The mixture was incubated for 4 h and washed with a sodium chloride solution. The immobilized PPL was separated by magnetic decantation of the supernatants. The attachment of the lipase to the IONP surface was confirmed by FTIR and TGA assays, while test reactions were performed aiming to determine the evolution of the lipase activity. The reported data indicate that immobilized PPL showed higher specific activity and thermal stability than the free one. After an initial drop following the first use at 37ºC, the activity of the immobilized PPL remained constant over the subsequent five uses and recoveries. The stable reuse as well as the convenience in the recovery offered by magnetic separation ensures that a surface-modified IONP is a good supporting material for enzyme immobilization [54].

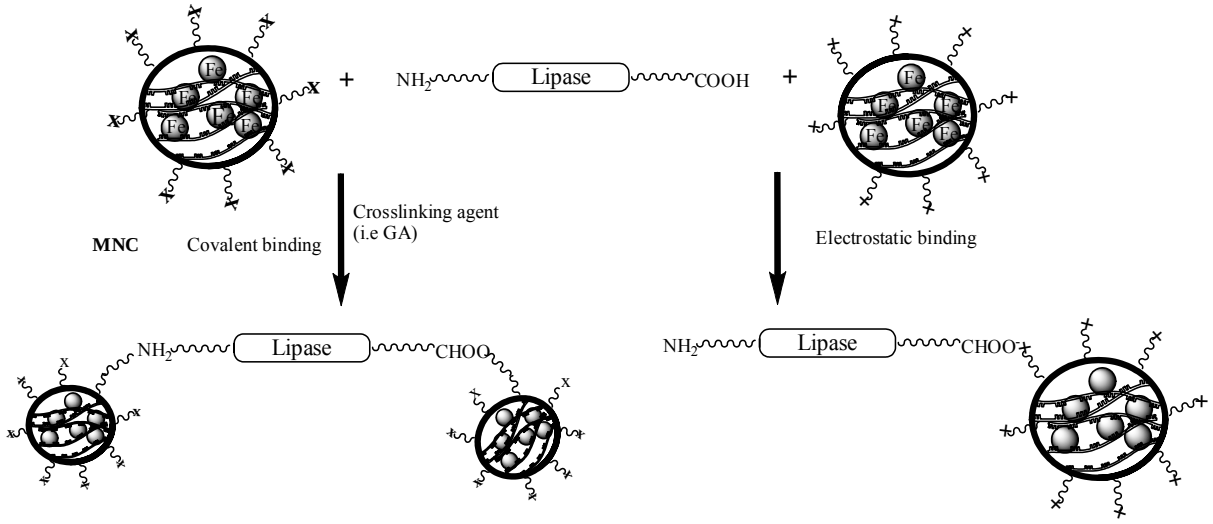
CS based MNC were employed for the immobilization of *Candida rugosa* lipase type VII by Wu *et al.* In this case a covalent binding between MNC and the lipase was generated using glutaraldehyde (GA) as coupling and crosslinking agent. The immobilization was carried out in phosphate buffer solution (PBS, pH 7) containing the lipase and MNC. The mixture was magnetically

stirred at room temperature for 12 h. After the GA addition; the mixture was stirred for another 20 min. The immobilization of lipase onto the particles showed good loading ability, and was strongly dependent on the initial amount of lipase. The adsorption capacity could reach as high as 129 mg/g, using 350 ppm of free lipase/10 ml of solution. Lower adsorptions were recorded using 200 ppm of free lipase. Little loss of enzyme activity was detected, and the stability of the catalyst was satisfactory. The biocatalyst only lost 12% of the initial enzyme activity after five reuses [24].

In Scheme 4 the two possible ways for the interactions between MNC and lipases are illustrated. It is clear that in both cases, the surface functional groups on MNC are essentially important to induce the interaction MNC/lipase.

A requirement of MNC intended as supporting materials is that they provide a large surface area suitable for enzyme reactions, and substrate and product transport with the least diffusional restriction. Arica *et al.* [33] have employed MNC based on PMMA and magnetite to immobilize glucoamylase. The effect of the immobilization process on the enzyme activity, the kinetic parameters, thermal and storage stabilities of the enzyme were investigated. Two different coupling agents were explored for the immobilization of glucoamylase on magnetic PMMA microspheres: carbodiimide, that was attached to the lipase through carboxylic groups, and cyanogen bromide (CNBr). The immobilization of glucoamylase through amino groups via CNBr coupling onto magnetic microspheres resulted in a higher protein loading (4.35 mg protein/g microspheres) and a higher activity yield (262.4 U/g microspheres) than the immobilization of protein through carboxyl groups via carbodiimide coupling, (2.56 mg protein/microspheres). SEM analysis suggested that the microspheres have a rough surface due to the abrasion of magnetic crystals during the coating procedure. These surface properties of the magnetic microspheres would favour higher immobilization capacity for the enzyme due to the increase of the surface area. Within this work some variables associated to the immobilization procedure were analysed such as pH, temperature, and storage stability. Hence they found that using CNBr coupling method yielded suitable residual activity, and a high operational, thermal, and storage stability [33].

Yong *et al.* used polymer-grafted magnetic nanoparticles for lipase immobilization, with the loading ability of the particles reaching 105.2 mg/g, and losing 30% of the initial activity after 5 times of use. The MNC were composed of magnetite and both GMA and methacryloxyethyl trimethyl ammonium chloride. The polymeric template provides epoxi and NH_2 groups,



Scheme 4. Representation of lipase immobilization on the MNC.

respectively, as available reactive groups. The authors postulated an electrostatic interaction as the mechanism for *Candida rugosa* lipase type VII immobilization in the initial stages. Then, a covalent reaction between epoxy groups of the particles and the amino groups (or thiol and hydroxyl groups) of the lipase appears to be the responsible for the lipase immobilization. The enzyme loading on the particles was 105.2 mg protein/g MNC. The immobilized lipase still retained 70% of its initial activity after five reuses. This result confirmed that the immobilized lipase on MNC has not only good durability but also good magnetic recovery [29].

Other published works support the hypothesis for the electrostatic origin of the interactions between MNC and lipases. Carboxylated PEG was employed to fabricate MNC with terminal COOH groups to promote the coupling of the lipase through the amino groups of the enzyme and the carboxyl group of the polymer bound to the magnetite. Surprisingly, the size range of these particles was 30 to 70 nm, which appears to be aggregated in TEM micrographies [143.].

6. CONCLUDING REMARKS

The utilization of magnetic technology became a prominent area of research due to its high adaptability and feasibility to be applied in relevant fields such as biomedicine and biotechnology. A variety of magnetic materials, i.e. IONP, surface-modified IONP and MNC could be obtained according to the requirements.

The available techniques to produce such kind of materials demonstrated to be very versatile since they are useful to produce specific magnetic compounds on the nanoscale with the needed characteristics by the selection of the appropriate polymeric template and the proper control of the experimental conditions. Advantages and disadvantages of each one should be considered fundamentally regarding the last properties of the materials and their applications.

The increase in the number of reported studies of MNC and IONP as DDS and, most recently, their biotechnological uses as enzymes (particularly lipases) supports, are clear examples of the high impact of the magnetic technology in the most diverse fields and the potential further growth of the discipline.

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REFERENCES

1. Shen, L., Stachowiak, A., Fateen, S. E. K., Laibinis, P. E., and Hatton, T. A. 2001, Langmuir, 17, 288.
2. Pankhurst, Q., Connolly, J., Jones, S., and Dobson, J. 2003, Phys. D. J. Appl. Phys., 36, 167.
3. Rosensweig, R. E. 1985, Ferrohydro-dynamics, Cambridge University Press, Cambridge.
4. Olsvik, O., Popovic, T., Skjerve, E., Cudjoe, K., Hornes, E., Ugelstad, J., and Uhlen, M. 1994, Clin. Microbiol. Rev., 7, 43.
5. Yeh, T., Zhang, W., Ldstad, S., and Ho, C. 1993, Magn. Reson. Med., 30, 617.
6. Denkbas, E., Kilicay, E., Birlikseven, C., and Ozturk, E. 2002, Reactive & Functional Polymers, 50, 225.
7. Zeng, L. 2003, Water Research, 37, 4351.
8. Chang, F., Lin, P., and Holl, W. 2006, Colloids and Surfaces A: Physicochem. Eng. Aspects, 280, 194.
9. Luiz, C., Oliveira, R., Rios, V., Fabris, J, Sapag, K., Garg, V., and Lago, M. 2003, Applied Clay Science, 22, 169.
10. Chouly, C., Pouliquen, D., Lucet, I., Jeune, P., and Pellet, J. 1996, J. Microencapsul., 13, 245.
11. Klee, D., and Hocker, H. 1999, Adv. Polym. Sci., 149, 1.
12. Meerod, S., Tumcharern, G., Wichai, U., and Rutnakornpituk, M. 2008, Polymer, 49, 3950.
13 Morales, M., Finotelli, P., Coaquira, J., Rocha-Leão, M., Diaz-Aguila, C., Baggio-Saitovitch, E. M., and Rossi, A. M. 2008, Materials Science and Engineering C, 28, 253.
14. Wassel, R., Grady, B., Kopke, R., and Dormir, K. 2006, Colloids and Surfaces A: Physicochem. Eng. Aspects, 292, 125.

15. Teply, A., Tong, R., Jeong, S., Luther, G., Sherifi, I., Yim, C., Khademhosseini, A., Farokhzad, O., Langer, R., and Cheng, J. 2008, Biomaterials, 29, 1216.
16 Okassa, L., Marchais, H., Douziech-Eyrolles, L., Jonathan, S., Souce, M., Dubois, P., and Chourpa, I. 2005, International Journal of Pharmaceutics, 302, 187.
17. Liu, S., Wei, X., Chu, M., Peng, J., and Xu, Y. 2006, Colloids and Surfaces B: Biointerfaces, 51, 101.
18 Patel, D., Moon, J., Chang, Y., Kim, T., and Lee, G. 2008, Colloids and Surfaces A: Physicochem. Eng. Aspects, 313-314, 91.
19. GÓmez-Lopera, S., Plaza, R., and Delgado, A. 2001, Journal of Colloid and Interface Science, 240, 40.
20. Timko, M., Koneracka´, M., Tomasovicova´, N., Kopcansky´, P., and Zavisova, V. 2006, Journal of Magnetism and Magnetic Materials, 300, 191.
21. Machado, L., Lima, F., Paniago, R., Ardisson, J. D., Sapag, K., and Lago, R. M, 2006, Applied Clay Science, 31, 207.
22. Zhu, A., Yuan, L., Jin, W., Dai, S., Wang, Q., Xue, Z., and Qin, A. 2008, Acta Biomaterialia., 5, 1489.
23. Chen, G., and Hoffman, A. 1993, Bioconjug. Chem., 4(6), 509.
24. Wu, Y., Wang, Y., Luo, G., and Dai, Y. 2009, Bioresour. Technol., doi:10.1016/ j.biortech.2009.02.018.
25. Shin, S., Yoon, H., and Jang, J. 2008, Catalysis Communications, 10, 178.
26. Gaihre, B., Khil, M., Lee, D., and Kim, H. 2008, Journal of Pharmaceutics, 365, 180.
27. Saravanan, M., Bhaskar, K., Maharajan, G., and Pillai, K. 2004, International Journal of Pharmaceutics, 283, 71.
28. Zhang, J., Rana, S., Srivastava, R. S., and Misra, R. D. K. 2008, Acta Biomaterialia, 4, 40.
29. Yong, Y., Bai, Y., Li, Y., Lin, L., Cui, Y., and Xi, Ch. 2008, Journal of Magnetism and Magnetic Materials, 320, 2350.
30. Kekalo, K., Agabekov, K., Zhavnerko, G., Shutava, T., Kutavichus, V., Kabanov, V., and Goroshko, N. 2007, Journal of Magnetism and Magnetic Materials, 311, 63.
31. Zhao, Y. X., Zhuang, L., Shen, H., Zhang, W., and Shao, Z. J. 2009, Journal of Magnetism and Magnetic Materials, 321, 377.
32. Albornoz, C., and Jacobo, S. 2006, Journal of Magnetism and Magnetic Materials, 305, 12.
33. Arica, M., Yavuz, H., Patir, S., and Denizli, A. 2000, Journal of Molecular Catalysis B: Enzymatic, 11, 127.
34. Cornell, R. M., and Schertmann, U. 1996, The Iron Oxides: Structure, Properties, Reactions, Occurrence and Uses, VCH Publishers, Weinheim.
35. Rosensweig, R. E. 1985, Ferrohydro-dynamics, Cambridge University Press, Cambridge.
36. Babincova, M., Babinec, P., and Bergemann, C. 2001, Z. Naturforsch., (Sect. C), 56, 909.
37. Wang, Y. X., Hussain, S. M., and Krestin, G. P. 2001, Eur. Radiol., 11, 2319.
38. Cornell, R. M., and Schertmann, U. 1991, Iron Oxides in the Laboratory; Preparation and Characterization., VCH, Weinheim.
39. Burke, H., and Dawson, F. 2002, Chemistry of Materials, 14, 4752.
40. Blum, E., Cebers, A., and Maiorov, M., 1997, Magnetic Fluids, Walter de Gruyter, Berlin.
41. Sato, T., Iijima, T., Sekin, M., and Inagaki, M. 1987, Journal of Magnetism and Magnetic Materials, 65, 252.
42. Zins, D., Cabuil, V., and Massart, R. 1999, Journal of Molecular Liquids, 83, 217.
43. Qui, X. 2000, Chinese Journal of Chemistry, 18, 834.
44. Bacri, J., Perzynski, R., Salin, D., Cabuil, V., and Massart, R. 1990, Journal of Magnetism and Magnetic Materials, 85, 27, 118.
45. Massart, R. 1981, IEEE Transactions on Magnetics, Mag-17, 1247.
46. Shen, L., Laibinis, P., and Hatton, T. 1999, Langmuir, 15, 447.
47. Gribanov, N. M., Bibik, E. E., Buzunov, O. V., and Naumov, V. N. 1990, Journal of Magnetism and Magnetic Materials, 85, 7.

48. Wu, K. T., Kuo, P. C., Yao, Y. D., Tsai, E. 2001, IEEE Transactions on Magnetics, 37, 2651.
49. Yan, H., Zhang, J., You, Ch., Song, Z., Yu, B., and Shen, Y. 2008, Materials Chemistry and Physics, 113, 46.
50. Blesa, M., and Matijevic, E. 1989, Advances in Colloid and Interface Science, 29, 173.
51. Farley, K. J., Dzombak, D. A., and Morel, F. M. M, 1985, Journal of Colloid and Interface Science, 106, 226.
52. Kim, D. K., Zhang, Y., Voit, W., Rao, K. V., and Muhammed, M. 2001, Journal of Magnetism and Magnetic Materials, 225, 30.
53. Dutz, H., Murbe, J., Muller, R., Zeisberger, M., Andra, W., Topfer, J., and Bellemann, M. E. 2007, Journal of Magnetism and Magnetic Materials, 308, 305.
54. Lee, D., Ponvel, K., Kim, M., Hwang, S., Ahn, I., and Lee, Ch. 2008, Journal of Molecular Catalysis B: Enzymatic, 57, 62.
55. Ying, T-Y., Yiacoumi, S., and Tsouris, C. 2002, J. Dispersion Sci. Technol., 23, 569.
56. Franger, S., Berthet, P., and Berthon, J. 2004, J. Solid State Electrochem., 8, 218.
57. Cabrera, L., Gutierrez, S., Menendez, N., Morales, M. P., and Herraste, P. 2008, Electrochimica Acta, 53, 3436.
58. Tsouris, C., DePaoli, D.W., and Shor, J. T. 2001, US 6,179,987 B1.
59. Bruce, I., Taylor, J., Todd, M., Davie, M., Borioni, E., Sangregorio, E., and Sen, T. 2004, Journal of Magnetism and Magnetic Materials, 284, 145.
60. Xu, Ch., and Teja, A. 2008, J. of Supercritical Fluids, 44, 85.
61. Tartaj, P., Gonzalez-Carreño, T., Rebolledo, A., Bomatí-Miguel, O., and Serna, C. 2007, Journal of Colloid and Interface Science, 309, 68.
62. Chiu, W. S., Radiman, S., Abdullah, M. H., Khiew, P. S., Huang, N. M., and Abd-Shukor, R. 2007, Materials Chemistry and Physics, 106, 231.
63. Dan, N., Zubris, M., and Tannenbaum, R. 2005, Macromolecules, 38, 9243.
64. Haddad, P., Martins, T., D'Souza, L., and Konradin Metze, L. 2008, Materials Science and Engineering, C 28, 489.
65. Li, Z., Chen, H., Bao, H., and Gao, M. 2004, Chem. Mater., 16 (8), 1391.
66. Pinna, N., Grancharo, S., Beato, P., Bonville, P., Antonietti, M., and Niederberger, M. 2005, Chem. Mater., 17, 3044.
67. Sun, S., and Zeng, H. 2002, J. Am Chem. Soc., 124, 8204.
68. Xu, J., Yang, H., Fu, W., Du, K., Sui, Y., Chen, J., Zeng, Y., Li, M., and Zou, G. 2007, Journal of Magnetism and Magnetic Materials, 309, 307.
69. Landfester, K., and Ramírez, L. 2003, J. Phys. Condens. Matter, 15, S1345.
70. Montagne, F., Mondain-Monval, O., Pichot, C., Mozzanega, H., and Elaissari, A. 2002, Journal of Magnetism and Magnetic Materials, 250, 302.
71. Wooding, A., Kilner, M., and Lambrick, D. B. 1990, Journal of Colloid and Interface Science, 144, 236.
72. Tadmor, R., Rosensweig, R. E., Frey, J., and Klein, J. 2000, Langmuir, 16, 9117.
73. Korolev, V., Ramazanova, A., and Blinov, A., International Edition, 51 (11).
74. Kumar Gupta, A., and Gupta, M. 2005, Biomaterials, 26, 3995.
75. Pardoe, H., Chua-anusorn, W., St. Pierre, T., and Dobson, J. 2001, Journal of Magnetism and Magnetic Materials, 225, 41.
76. Kumar Gupta, A., and Wells, S. 2004, IEEE transactions on nanobioscience, 3, 1.
77. Gupta, A. K., and Curtis, A. S. 2004, Journal of Materials Science: Materials in Medicine, 15, 493.
78. Bonder, T., Zhang, Y., Kiick, K. L., Papaefthymiou, V., and Hadjipanayis, G. C. 2007, Journal of Magnetism and Magnetic Materials, 311, 658.
79. Mondini, S., Cenedese, S., Marinoni, G., Molteni, G., Santo, N., Bianchi, C., and Ponti, A. 2008, Journal of Colloid and Interface Science, 322, 173.
80. del Campo, A., Sen, T., Lellouche, J., and Bruce, I. 2005, Journal of Magnetism and Magnetic Materials, 293, 33.
81. Yongai Zhai, Fengqi Liu, Qing Zhang, Ge Gao, 2008, Colloids and Surfaces A: Physicochem. Eng. Aspects xxx xxx–xxx.
82. Kumar Gupta, A., Berry, C., Gupta, M., and Curtis, A. 2003, IEEE transactions on nanobioscience, 2, 4, 255.

83. Sinha, A., Nayar, S., Nath, B., Das, D., and Mukhopadhyay, P. K. 2005, Colloids and Surfaces B: Biointerfaces, 43, 7.
84. Chatterjee, J., Haik, J., and Chen, P. 2001, Journal of Magnetism and Magnetic Materials, 225, 21.
85. Landfester, K., Korolev, V., Ramazanova, A., and Blinov, A. 2002, Russian Chemical Bulletin, International Edition, 51 (11), 2044.
86. Hong, R.Y., Pana, T., and Li, H. 2006, Journal of Magnetism and Magnetic Materials, 303, 60.
87. Lee, J., Isobe, T., and Senna, M. 1996, Journal of Colloid and Interface Science, 177, 490.
88. Ding, X. B., Sun, Z. H., Wan, G. X., and Jiang, Y. Y. 1998, Reactive & Functional Polymers, 38, 11.
89. Berry, C. B., and Curtis A. S. G. 2003, Journal of Physics D: Applied Physics, 36, R198-R206.
90. Goodarzi, A., Sahoo, Y., Swihart, M. T., and Prasad, P. N. 2004, Materials Research Society, 789, N6.6.1-N6.6.6.
91. Betancouit-Galindo, R., Saldivar, R., Rodriguez-Fernandez, O.S., and Ramos-de Valle L. F. 2004, Polymer Bulletin, 51, 395.
92. Sunderland, K., Brunetti, P., Spinua, L., Fang, J., Wang, Z., and Lu, W. 2004, Materials Letters, 58, 3136.
93. Dresco, P., Zaitsev, V., Gambino R., and Chu, B. 1999, Langmuir, 15, 1945.
94. Pollert, E., Knızeka, K., Maryskoa, M., Zaveta, K., Lancoka, A., Boha´cek, J., Hora´kd, D., and Babic, M. 2006, Journal of Magnetism and Magnetic Materials, 306, 241.
95. Wormuth, K. 2001, Journal of Colloid and Interface Science, 241, 366.
96. Liu, X., Guan, Y., Ma, Z., and Liu, H. 2004, Langmuir, 20, 10278.
97. Shamim, N., Hong, L., Hidajat, K., and Uddin, M. S. 2007, Colloids and Surfaces B: Biointerfaces, 55, 51.
98. Lu, S., Qu, R., and Forcada, J. 2009, Materials Letters, 63, 770.
99. Zaitsev, V., Filimonov, D., Presnyakov, I., Gambino, R., and Chu, B. 1999, Journal of Colloid and Interface Science, 212, 49.
100. Lee, L., and Senna, M. 1995, Colloid and Polymer Science, 273, 76.
101. Jeong, J. R., Lee, S. J., Kim, J. D., and Shin, S. C. 2004, IEEE Transaction on Magnetics, 40 (4), 3015.
102. Ali-zade R. 2005, Colloids and Surfaces A: Physicochem. Eng. Aspects, 255, 111.
103. Koo, H., Chang, S., Choi, W., Park, J., Kim, D., and Velev, O. 2006, Chem. Mater., 18, 3308.
104. Belessi, V., Zboril, R., Tucek, J., Mashlan, M., Tzitzios, V., and Petridis, D. 2008, Chem. Mater., 20, 3298.
105. McCarthy, J., and Weissleder, R. 2008, Advanced Drug Delivery Reviews, 60, 1241.
106. Bourgeat-Lami, E., and Lang, J. 1998, J. Colloid Interface Sci., 197, 293.
107. Yanase, N., Noguchi, H., Asakura, H., and Suzuta, T. 1993, J. Appl. Polym. Sci., 50, 765 .
108. Noguchi, H., Yanase, N., Uchida, Y., and Suzuta, T. 1993, J. Appl. Polym. Sci., 48, 1539.
109. Kondo, A., and Fukuda, H. 1999, Colloid Surf. A, 153, 435.
110. Kondo, A., Kamura, H., and Hagashitani, K. 1994, Appl. Microbiol. Biotechnol., 41, 99.
111. Landfester, K., Willert, M., and Antonietti, M. 2000, Macromolecules, 33, 2370.
112. Arias, J. L., Gallardo, V., Gomez-Lopera, S. A., Plaza, R. C., and Delgado, A. V. 2001, Journal of Controlled Release, 77, 309.
113. Lassalle, V., and Ferreira, M. L. 2007, Macromolecular Bioscience, 7, 767.
114. Vijaya Kumar, R., Koltypin, Y., Cohen, Y., Cohen, Y., Aurbach, D., Palchik, O., Felner, I., and Gedanken, A. 2000, J. Mater. Chem., 10, 1125.
115. Nishio, Y., Yamada, A., Ezaki, K., Miyashita, Y., Furukawa, H., and Oiré, K. 2004, Polymer, 45, 7129.
116. Lee, S. J., Jeong, J. R., Shin, S. C., Kim, J. C., Chang, Y. H., Chang, Y. M., and Kim, J. D. 2004, Journal of Magnetism and Magnetic Materials, 272-276, 2432.
117. Novakova, A. A., Lanchinskaya, Y., Volkov, A. V., Gendler, T. S., Kiseleva, T., Moskvina, M., and Zezin, S. 2003, Journal of Magnetism and Magnetic Materials, 258-259, 354.

118. Wilson, Goff, J. D., Riffle, J. S., Harris, L. A., and St. Pierre, T. G. 2005, Polym. Adv. Technol., 16, 200.
119. Baker, C., Ismat Shaha, S., and Hasanainc, S. K. 2004, Journal of Magnetism and Magnetic Materials, 280, 412.
120. Chu, W. J. 1995, Magn. Reson. Imaging, 13 (5), 675.
121. Ryan, M. K., Chester, L., Daniel, C., Morley, O., and Rajesh, R. 2004, J. Am. Chem. Soc., 126, (41), 13282.
122. Sepulveda-Guzman, S., Perez-Camacho, O., Rodrıguez-Fernandez, O., and Garcıa-Zamora, M. 2005, Journal of Magnetism and Magnetic Materials, 294, e47-e50.
123. Saenko, E. V., Khokhlov, A. R., Volkov, V. V., Dembo, K. A., Klechkovskaya, V. V., Shtykova, E. V., and Zanaveskina, I. S. 2003, Microelectronic Engineering, 69, 324.
124. Kopke, R., Wassel, R., Mondelek, F., Grady, B., Chen, K., Liu, J., Gibson, D., and Dormer, K. 2006, Audiol. Neurotol., 11, 123.
125. Jiang, W., Yang, H. C., Yang, S. Y., Horng, H. E., Hung, J. C., Chen, Y. C., and Hong, C. 2004, Journal of Magnetism and Magnetic Materials, 283, 210.
126. Phillips, J., Li, C., Dailey, J., and Riffle, J. 1999, Journal of Magnetism and MagneticMaterials, 194, 140.
127. Rutnakornpituk, M., Thompson, M., Harris, L. A., Framer, K. E., Esker, A. R., Riffle, J. S., Connolly, J., and St. Pierre, T. G. 2002, Polymer, 43, 2337.
128. Molday, R. S., and MacKenzie, D. 1982, Journal of Immunological Methods, 52, 353.
129. Roath, S. 1993, Journal of Magnetism and Magnetic Materials, 122, 329.
130. Jordan, A., Scholz, R., Wust, P., Schirra, H., Schiestel, T., Schmidt, H., and Felix, R. 1999, Journal of Magnetism and Magnetic Materials, 194, 185.
131. Papisov, M., Bogdanov, Jr. A., Schaffer, B., Nossiff, N., Shen, T., Weissleder, R., and Brady, T. 1993, Journal of Magnetism and Magnetic Materials, 122, 383.
132. Kim, S., Zhang, Y., Kehr, J., Klason, T., Bjelke, B., and Muhammed, M. 2001, Journal of Magnetism and Magnetic Materials, 225, 256.
133. Babes, L., Denizot, B., Tanguy, G., Le Jeune, J. J., and Jallet, P. 1999, Journal of Colloid and Interface Science, 212, 474.
134. Pratsinis, S. E., and Vemury, S. 1996, Powder Technol., 88, 267.
135. Tice, T. R., Mason, D. W., Eldridge, J. H., and Gilley, R. M. 1990, Clinical use and future of parenteral microsphere delivery system. In: Prescott, L. F., Nimmo, W. S. (Eds.), Novel Drug Delivery and Its Therapeutic Application. Wiley, Chichester, England, pp. 223-236.
136. Gupta, P. K., and Hung, C. T. 1989, Life Sci., 44, 175-186.
137. Jain, T. K., Morales, M. A., Sahoo, S. K., Leslie-Pelecky, D. L., and Labhasetwar, V. 2005, Mol. Pharm., 2(3), 194.
138. Cheng, J. J., Teply, B. A., Jeong, S. Y., Yim, C. H., Ho, D., and Sherifi, I. 2006, Pharm. Res., 23, 557.
139. Lacava, Z. G. M., Azevedo, R. B., Martins, E. V., Lacava, L. M., Freitas, M. L. L., Garcia, V. A. P, Rebula, C. A., Lemos, A. P. C., Sousa, M. H., Tourinho, F., Da Silva, M., and Morais, P. 1999, Journal of Magnetism and Magnetic Materials, 201, 431.
140. Ibrahim, A., Couvreur, P., Roland, M., and Speiser, P. 1982, Journal of Pharmaceutics and Pharmacology, 35, 59.
141. Iannone, A., Magin, R., Walczack, T., Federico, M., Swartz, H., Tomasi, A., and Vannini, V. 1991, Magnetic Resonance in Medicine, 22, 435.
142. Mateo, C., Palomo, J. M., Fernandez-Lorente, G., Guisan, J. M., and Fernandez-Lafuente, R. 2007, Enzyme Microb. Technol., 40, 1451.
143. Tamaura, Y., Takahasi, K., Kodera, Y., Saito, Y., and Inada, Y. 1986, Biotechnology Letters, 8, 877.