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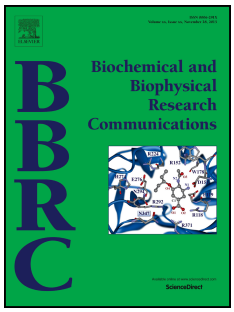

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Magnetic iron oxide nanoparticles: recent trends in design and synthesis of magnetoresponsive nanosystems

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Abstract:

Recent developments in nanotechnology and application of magnetic nanoparticles, in particular in magnetic iron oxide nanosystems, offer exciting possibilities for nanomedicine. Facile and precise synthesis procedures, high magnetic response, tunable morphologies and multiple bio-functionalities of single- and multi-core magnetic particles designed for nanomedicine applications are thoroughly appraised. This review focuses on the structural and magnetic characterization of the cores, the synthesis of single- and multicore iron oxide NPs, especially the design of the latter, as well as their protection, stabilization and functionalization by desired coating in order to protect against the corrosion of core, to prevent non-specific protein adsorption and particle aggregation in biological media, and to provide binding sites for targeting and therapeutic agents.

1. Introduction

Superparamagnetic iron oxide - magnetite and maghemite- nanoparticles are the most used constituents of magnetoresponsive nanosystems in the rapidly expanding researches and applications in nanomedicine and biology, including magnetic resonance imaging, magnetic particle imaging, magnetic drug delivery systems,

magnetic fluid hyperthermia and magnetic labeling and separation of cells [1–10]. Patient safety is an important motivation for a proper choice of magnetic particulate systems designed for both diagnosis and therapy (theranostics). To exemplify, iodine or gadolinium tracers are hazardous for patients with chronic kidney disease, therefore their use as contrast agents is a public health safety concern and requires a safer replacement, such as iron oxide nanoparticles (IONPs) [11]. Complex nanosystems of coated and multiple functionalized superparamagnetic iron oxide nanoparticles started to become the most important tools of nanomedicine [7,9,12,13], as they represent the best compromise between good magnetic properties and very reduced toxicity, evidenced by extensive *in vitro* and *in vivo* tests [14] and by quantitative evaluation of biodistribution and local therapeutic effects [12,15]. Other magnetic nanoparticles, like Co or FePt [16,17], have higher saturation magnetization and better magnetic response, but are toxic, which impedes their use in most of biomedical applications. The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved the medical use of only a few iron oxide nanoparticle formulations, most probably due to the lack of commonly accepted physicochemical practice of particle design and manufacturing, as well as of qualifying criteria. The nanoparticle systems involve the magnetic iron oxide core and the sterically and/or electrostatically repulsive shell(s) around the core to ensure colloidal stability and salt tolerance in the biological environment [18], usually a biocompatible polymer and additionally molecules fulfilling the roles of anchors, spacers and various functionalities [19]. The surface engineered iron oxide nanoparticles for biomedical applications should be highly water dispersible, i.e. the resulted IONP systems should be colloidally stable water based ferrofluids [10,19].

The synthesis procedures, physicochemical properties, toxicity and biocompatibility, as well as vectorization methods of iron oxide nanoparticle systems for biomedical applications were thoroughly reviewed along the years in several comprehensive works [1,3,5,6,8–10,20]. The basic physical properties of magnetic nanoparticles, the size and surface effects on their magnetic behavior (spin canting) and the specific composition, structure and functional coatings for biomedical applications, are summarized in [4,10,21].

The actual composition of magnetic nanoparticles in ferrofluids used in biomedical applications involves single core and multicore iron oxide particles [22–25]. The multicore particle ferrofluids have the advantage of improved magnetic response of particles, while keeping the superparamagnetic behaviour and satisfactory colloidal stability in spite of greater hydrodynamic size of dispersed particles, actually iron oxide nanoparticle clusters. Therefore the use of multicore products in magnetic targeting and hyperthermia treatment is of great importance. These multicore ferrofluids have to be distinguished from single core (''true") ferrofluids [26], both of great interest for nanomedicine.

Polymer based magnetic microspheres encapsulating magnetite nanoparticles were proposed already in 1978 by Senyei et al [27] for magnetic drug delivery and targeting. The actual high interest for water dispersible surface functionalized multi-core magnetic particles is related to the development of more facile and precise synthesis procedures, high magnetic response, tunable morphologies and multiple bio-functionalities of particles designed for nanomedicine applications. The terms "single core" and "multicore" were used more frequently in the last few years; however, they cannot be found for example in the excellent book on magnetic nanomaterials [28] or even in some recent reviews [3,29], but a review on single versus multi-core iron oxide nanoparticles has just appeared [30].

The present minireview will focus on recent results on the synthesis and properties of surface coated single core and multicore iron oxide nanoparticle systems designed for applications in nanomedicine.

2. Structure and magnetic behavior

Magnetic iron oxides - magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) - are ferrimagnetic materials below their Curie temperatures (850K and 986K respectively [31]). Ferro- and ferri-magnets in bulk state present a multidomain magnetic structure, thus without a permanent magnetic moment. Reducing the volume of the material to the size range specific to magnetic nanoparticles, ferro- and ferri-magnetic particles can be single domain with a permanent magnetic moment due to collective spin correlation at the

scale of the entire nanoparticle. The properties of usually subdomain size iron oxide nanoparticles are no longer similar to bulk materials and motivate the outstanding interest for their use in nanomedicine [3,10,13,21]. The size distribution and morphology are essential in defining the magnetic behaviour of nanoparticles. The size and shape dependent magnetic properties of nanoparticles and nanoparticulate systems, such as magnetocrystalline and shape anisotropy, interparticle interactions and magnetic relaxation processes, investigated by static and dynamic magnetometry and Mössbauer spectroscopy, were thoroughly reviewed in [21,32].

Small spherical nanoparticles made of soft magnetic materials with diameter well below the domain size show negligible magnetic anisotropy which is why their magnetic moment is free to rotate relatively to the particle (Néel effect) and are thus superparamagnetic, i.e. paramagnetic below the Curie temperature [21]. The modulus of the permanent magnetic moment is given by the product of the volume of the monodomain nanoparticle and the domain magnetization, i.e. saturation magnetization of the material [33]. The direction of the permanent magnetic moment of the nanoparticle is set by the balance between thermal fluctuation and magnetic anisotropy that tend to fix it with respect to either crystalline structure (easy magnetization axis / axes) or particle morphology (shape anisotropy main axis / axes) [34]. The energy of the crystallographic and shape magnetic anisotropy is proportional to the particle volume, therefore the smaller the monodomain particle the wider the fluctuation of the magnetic moment around the direction of the anisotropy axis. Besides volume proportionality, the energy of the shape magnetic anisotropy is increasing with increasing nanoparticle morphological anisotropy, i.e. aspect ratio, so that spherical nanoparticles show zero shape anisotropy [34]. The volume is also relevant for the strength of the nanoparticle interaction with an external magnetic field (dipole-field interaction) as well as with the magnetic field generated by another nanoparticle (dipole-dipole interaction) [35].

The saturation magnetization of ferro- or ferrimagnetic materials is lower in nanoparticle than in bulk state due to either surface or core spin disorder [36]. The surface spin disorder breaks the homogeneity of the nanoparticle magnetic structure to a magnetic core surrounded by a “dead” magnetic layer of either canted or disordered spins , the thickness of this layer being dependent on the chemical reaction between the surfactant

and iron oxide nanoparticle [37,38]. The core and surface spin disorder can originate from either amorphous or polycrystalline structure as a result of synthesis procedure and surface coating [39,40]. The surface spin canting is specific of small nanoparticles, while larger size nanoparticles show also volume spin canting. Low TEM size polydispersity of magnetic nanoparticles does not guarantee low polydispersity of the magnetic dipole moment. Crystal defects, such as twinning and dislocations, can have a highly detrimental effect on the strength and low polydispersity of the magnetic properties. Crystallite sizes from XRD, volume-averaged TEM and magnetogranulometry for iron oxide nanoparticles synthesized by aqueous coprecipitation [41,42] and thermal decomposition [43,44] processes show significant differences between geometric and magnetic sizes for “twins” and “spheres”, while for the “facets”, the “precipitates” and the 8 nm “spheres” TEM and magnetic measurements give a similar average size and polydispersity [39].

The interaction of a magnetic nanoparticle with an external magnetic field is twofold: (1) the orientation of the particle’s magnetic moment such that it becomes parallel to the applied magnetic field in order to minimize the dipole-field interaction energy [10] and (2) the translation of the particle in the direction of the field gradient, i.e magnetophoresis [45].

The orientation of MNP’s magnetic moment under the action of an external magnetic field occurs either by the movement of domain walls in multidomain particles or by the rotation in single domain particles. The rotation of the magnetic moment can occur either free with respect to the particle (Néel rotation) or together with the particle (Brown rotation) [21]. Except for particular situations, the orientation of MNP’s magnetic moment in AC magnetic fields shows hysteresis. The phenomenon of AC magnetic hysteresis is the basis of magnetic particle hyperthermia [21,46] and susceptometric granulometry of single and multicore MNPs [47]. In DC magnetic fields, due to the permanent magnetic moment of subdomain MNPs, the magnetization of diluted single core particle dispersions follows the Langevin equation which gives the theoretical framework for the magnetogranulometry of single core particles [48]. Multicore particles have no permanent magnetic moment, provided that the constituent particles are small enough such that the magnetic dipole-dipole interaction is negligible. The induced

(resultant) magnetic moment of multicore particles is parallel to the external magnetic field and follows the Langevin equation. If the constituent particles are large, i.e. the anisotropy energy overcomes the thermal energy, the multicore particles show magnetic coercivity and remanence due to dipole-dipole interactions. The induced magnetic moment of multicore particles at saturation is the sum of the constituent particles' magnetic moments [49]. Many applications of magnetic nanoparticles and nanocomposites rely on their ability to be manipulated using magnetic fields. This ability depends on the effectiveness of the magnetophoretic force, determined by the particle magnetic moment and the field gradient, to fix or to move the particles [45]. The magnetophoretic force exerted upon single core superparamagnetic nanoparticles is less effective due to their small diameter and magnetic moment implicitly, but in the case of multicore composites the resultant field induced magnetic moment is high enough in order to allow magnetic targeting already for moderate values of field intensity and gradient. Therefore, in order to assess the magnetic targeting / fixing applicability of magnetic particles, the particles' magnetic moment is more relevant than mass magnetization [45,50]. The magnetic targeting potential of multicore particles, in particular the magnetic moment and magnetic mobility were determined in recent magnetophoresis investigations [45,50-54] (Table 1).

The synergy between magnetic dipole-dipole and dipole-field interactions may lead to magnetically induced clustering in both single and multicore particle liquid dispersions. This can cause the complete separation of the magnetic phase from liquid dispersions, but most often the formation of spindle shape linear aggregates with dimensions that can extend up to several microns diameter and tens to hundreds microns length [49]. Magnetically induced linear aggregates of this size have to be avoided by adequate physical-chemical design of IONP systems for each nanomedicine application; their occurrence can have severe consequences due to the drastic decrease of specific surface or the danger of clogging blood capillaries, to mention only a few issues. Magnetically induced linear aggregates can occur in both single core [55,56] and multicore liquid dispersions [57,58]. The magnetically induced phase condensation in single core liquid dispersions can show critical behavior: below a critical temperature (Tc) the condensation occurs only at field intensities larger than a critical value

(H>Hc(T)) while above the critical temperature the condensation doesn't occur no matter how intense the magnetic field [59]. This critical temperature for a myristic acid sterically stabilized single core magnetite water based ferrofluid resulted to be 42.5ºC [55], a temperature close to the range of interest for live cell experiments. Phase condensation was found to have a negative influence on the effectiveness of magnetic nanoparticles' use as contrast agents in MRI [56] evidenced by` the influence of the magnetically induced linear chains in PEG coated single core particle dispersions on the proton transverse relaxation rates. It was found that the transverse relaxation rate diminishes with increasing particle chaining, i.e. both with increasing field exposure and decreasing polymer molecular weight due to specific surface decay. Magnetically induced linear aggregates were found to take place in multicore magnetic nanogels [49]. The aggregation susceptibility diminishes with increasing temperature. In both single core and multicore systems the magnetically induced condensation process may take minutes before reaching equilibrium [49,56]. It is worth mentioning that spontaneous clustering can also occur in both single core and multicore magnetic particle dispersions when the attractive forces (van der Waals, magnetic dipole-dipole) overcome the repulsive ones (steric, electrostatic) [56].

3. Single- and multicore iron oxide NPs

The synthesis of superparamagnetic iron oxide nanoparticles (SPIONs) is a complex process because of their colloidal nature [3]. The preparation of colloidal dispersions of IONPs, can be accomplished in two ways, (i) dispersion (size reduction to nanometer range and dispersing solid phase) of iron or iron oxides in aqueous medium considering the classical colloidal routes and (ii) condensation of precursors from either liquid or gaseous phase, well-known procedures for ferrofluid preparation [60], called as top down (mechanical attrition) and bottom up (chemical synthesis) methods in fabrication of magnetic nanoparticles, in particular of SPIONs for biomedicine [6].

The huge drawback of dispersion – a non-chemical, simple, top down – way is that it is difficult to design and control the process to produce the desired particle size and shape [61]. However, the new laser ablation method (named also laser evaporation synthesis) for example is really effective to produce enough small particles [62]. This method is

suitable for preparation of large amounts of maghemite/magnetite powder from initial materials, e.g., coarse hematite, iron powders or even from massive iron blocks. The product is a fine powder with average particle size 20–50 nm and a relatively narrow size distribution.

The bottom-up synthesis procedures [1,3,6,20], such as chemical coprecipitation, reactions in constrained environments, hydrothermal reactions, high-temperature, sol-gel reactions, polyol methods, flow injection syntheses, electrochemical, aerosol/vapor methods, sonolysis [3], thermal decomposition, solvothermal reaction [8], hydrolytic and non-hydrolytic wet chemistry methods [10], liquid phase, microemulsion and laser evaporation syntheses, biomineralization [46], proved to be the most successful ones in SPIONs manufacturing.

The in situ formed NPs spontaneously vary with time mainly in polydisperse systems, and ageing processes (Oswald ripening [1]) result in bigger coalesced or irreversible adhered NPs as mentioned sometimes in reviews [1] and showed as an example for magnetite [63]. The synthesis method most suitable for upscaling is the coprecipitation of the stoichiometric mixture of Fe(II) and Fe(III) ions under alkaline (pH>8.5) and reductive conditions [64]. The colloidal stability of precipitating systems is determined by electrostatic and steric factors [65]. The size of forming crystals is mainly guided by the ratio of reaction rates of nucleus formation and crystal growth, while the shape is controlled by complex formation on preferred surface sites. The mechanisms of nanoparticle growth from solution recently reviewed in [66] evidenced that IONPs synthesized by thermal decomposition in non-aqueous solutions follow a classical nucleation and growth path [44] and the data are well fitted in the frame of the LaMer model. At the same time, the growth of IONPs synthesized by means of chemical coprecipitation in aqueous solution follows a non-classical path: primary particles of about 1 nm diameter attach to the surface of growing larger particles (Fig.1) [67].

The alkaline coprecipitation of stoichiometric amounts of ferrous and ferric salts relies on the Massart method [41]. In this process, cheap chemicals and mild reaction conditions are used and naked magnetite is obtained even in high concentration; it is easy to scale up [10]. An excellent analysis on how the superparamagnetic IONP formation is influenced by reaction conditions has just been published [68]. The effect of

iron salt concentration, reaction temperature, ratio of hydroxide ions to iron ions and ratio of Fe(III)/Fe(II) was analyzed. It is worth highlighting that formation of particles between 3 and 17 nm with higher saturation magnetization being directly related to the bigger particle size are favored at the highest temperature, the highest iron salt concentrations, a molar ratio of Fe(III)/Fe(II) below 2:1 and a hyperstoichiometric molar ratio of hydroxide ions to iron ions of 1.4:1. Nowadays a sonochemical treatment is frequently used during the synthesis of IONPs or their hydrothermal ageing [3,69,70]. The coprecipitation under the ultrasonic irradiation results in spherical magnetite nanoparticles with higher crystallinity. The coprecipitation synthesis of naked magnetite and also that surface modified by organic compounds (oleic and mercaptopropionic acids and cetyltrimethylammonium bromide) was performed at 80^{o}C under microwave heating for 8-15 minutes [71]. The obtained magnetite particles are of ~10 nm and rather homogeneous in size, but definitely aggregated in the sample of naked magnetite.

The naked IONPs are hydrophilic; their surface is fully covered by reactive ≡Fe-OH sites, which are hydrated and prone to react with several species from aqueous medium [41]. The IONPs gain charges in the protonation/deprotonation processes reacting with H^+/OH^- ions freely available in acidic and alkaline water, respectively. Therefore, positively charged IONPs exist below a characteristic pH (called point of zero charge (PZC)), which is around pH 8 for synthetic magnetite, while above it, IONPs carry negative charges due to deprotonation reaction [63, 72]. Coprecipitation was performed in diethyleneglycol at higher temperature then the formed IONP surface (PZC~7.2) was modified by silanization, and the change of pH-dependent charging was characterized by electrokinetic or zeta potential measurements providing direct evidence of sign and relative amount of charges [73].

The in situ coating of coprecipitation product is widespread. The most often used coating agents are fatty acids, especially the oleic acid. The product of coprecipitation optimally at 80-82^{o}C is magnetite, mostly single core with diameter 5-8 nm and having hydrophobic (chemisorbed oleate monolayer) or hydrophilic (due to a physisorbed secondary oleic acid layer) coating, the particles being dispersible in nonpolar solvents or water, respectively [26,64,74]. Beside efficient stabilization of MNPs in organic non-

polar carriers, the oleic acid hydrophobic coating of IONPs organized in magnetic nanoparticle ensembles is particularly important in improving relaxivity in MR imaging, according to a recent evaluation [75]. The microwave assisted coprecipitation synthesis in the presence of in situ surface modifiers like oleic acid results in much lower degree of IONPs' aggregation than that observed in their absence [71]. Recently, the so called one pot or single step synthesis procedure is preferred, which did not result in uniform product, but the quality of cores sometimes is promising. For example, a stoichiometric Fe(II) and Fe(III) salt mixture was hydrolysed in the mixture of diethylene glycol (DEG) and N-methyldiethanolamine (NMDA) at high temperature over the longer period to allow clustering and coalescence of the preformed seeds [76]. A single step procedure resulted in a big mixture of seemingly individual and definitely adhered (coalesced flower-like nanocrystals) maghemite nanoparticles, which had to be fractionated in acidic medium by increasing the ionic strength of the suspension, utilizing the basic principles of electrostatic colloidal stability. The mixture of particles formed in the single step synthesis and two examples selected from its fractions are shown in Fig. 2 to perceive the marked difference between the single and multicore IONPs.

A similar, coprecipitation preparation strategy can be found in the latest literature [77]. However, the authors use microwave radiation to enhance the citrate mediated dissolution–recrystallisation process and state that their process yield a stable multi-core dispersion with good reproducibility and that the synthesis procedure is potentially scalable. Exceptional magnetic heating parameters and good potential for future use are reported; nevertheless, neither the purification, i.e., removal excess citrate and dissolved iron nor the possible consequences (e.g., oxidative stress in vivo) were mentioned. The multicore nanoparticles, which can be considered as clusters of single cores of about 8–10 nm, are found to be very interesting for hyperthermia purposes [46].

Preparation of naked (often called Massart-type [41]) magnetite NPs in the coprecipitation process results most probably in the mixture of single and multicore particles exclusively in all cases; however, the common purification of products involving magnetic separation (magnetic decantation) allows to remove more or less all

multicores leaving the electrostatically stabilized single cores in the acidified (e.g., [78]), or alkaline (e.g., [26]) aqueous medium.

The fact that ferrofluids may contain single or multicore particles probably influences not only their hyperthermia efficiency but also other properties. The core composition dependent flow properties of commercial biomedical ferrofluids have been started to investigate only very recently [25] and it turned out even from this preliminary study that ferrofluids of multicore particles have significant magnetoviscous effect leading to an external field dependent increase in viscosity, which is highly undesirable in vivo. Also quite recently, a study on the potential of magnetofection in delivering pDNA to cells and the effect of magnetic cell labeling on transfection efficacy has been published [54]. Multicore IONPs synthesized by common coprecipitation process followed by immediate spontaneous adsorption of organic shell components for magnetic cell labeling were proved to be effective for magnetophoretic delivery.

IONPs can form in biomineralization process. The natural product of magnetotactic bacteria, the magnetosomes are uniform particles of 20–45 nm core diameter, single domain crystals and the smaller ones are superparamagnetic [79]. Their structure is probably single core. Coprecipitation of ferrous and ferric ion at pH=9 without additives, in conditions of ultraslow crystal growth kinetics (5 to 480 minutes) similar to magnetite growth in magnetotactic bacteria, can deliver relatively large size magnetite nanoparticles (with “frozen” dipole moment) or even multi-domain particles, with non-zero hysteresis losses [80].

Thermal decomposition of organo-metallic compounds (e.g., iron carbonyl, acetate, acetylacetonate, carboxylate) in organic solvents in the presence of a mixture of surfactants results in monodisperse nanoparticles of iron and/or iron oxides, with diameters ranging from 3 to 50 nm [81]. The products are also probably single core NPs coated with organic stabilizing shell. Appropriate organic compounds such as oleic acid, oleylamine are applied as in situ coating agents in each case of thermal decomposition of organic precursors as seen in the most cited paper of this method [82], in which the decomposition of $Fe(acac)_3$ in an ether solution at higher temperature (typically first up to 200 ^{o}C and then up to 260–300 ^{o}C), in the presence of a polyol acting as reducing agent was studied. The reaction steps are monomer formation (decomposition of

organic precursor) followed by appearance of nuclei then their growth. Size of IONPs was controlled well through a seeded growth mechanism and monodisperse, hydrophobic NPs with size from 3-4 up to 20 nm have been obtained. The mixed magnetite and maghemite crystals with average sizes in the range 4−28 nm have been prepared by thermal decomposition of iron stearate in the presence of different surfactants [40]. The synthesis parameters (the quality of solvents and surfactants, the surfactant (ligand)/precursor ratio, the reaction time, the temperature) were varied, and no doubt that excellent monodisperse, single core (this term was not used in the paper) crystals were produced in different classes of size (e.g., 7-8, 10-11 nm). The formation of monodisperse NPs was explained qualitatively by LaMer nucleation model for designed synthesis of colloids. Each synthesized NP is superparamagnetic at room temperature and their saturation magnetization increases from 51 to 82 emu/g measured at 5K with increasing average TEM size of nanocrystals between 5.4 and 21 nm. Oleic acid is among the most used and efficient surfactants in thermal decomposition, which may undergo loss of unsaturation when subjected to high temperatures specific to this synthesis route [83]. Prior to their use in biomedicine, phase transfer into aqueous media of the resulted hydrophobic IONPs is required, involving ligand exchange, addition of new functional groups or new coating layers of polymers or inorganic materials [3,10,19]. Various shape core-shell type nanoparticles were synthesized by thermal decomposition by varying the ligand nature (oleic acid and/or sodium oleate), the composition of the overall magnetic core itself involving the formation of an antiferromagnetic inner core $Fe_{1-x}O$ surrounded by a ferrimagnetic $Fe_{3-x}O_4$ shell (Fig.3 a,b), a hydrophilic dendron coating ensuring good water dispersibility of the composite particles [84]. Due to the antiferromagnetic-ferrimagnetic composite nature of the magnetic core, the particles display highly improved in vitro as well as in vivo MRI properties. The significant magnetic spinel phase of the core gives high values for the magnetic moment density depending on the composite core size (Table 1).

The major disadvantages of these thermal decomposition synthesis methods are that toxic organic solvents are used, the product NPs are coated chemically bound organic molecules and so the IONPs should be transferred into the aqueous phase. The latter

can be performed by means of surfactants (e.g. fatty acid salts like Na-oleate), which form an oppositely oriented second layer due to hydrophobic interaction with the alkyl chains of first layer chemisorbed on the surface of iron oxide NPs [74]. Long-term colloidal stability of aqueous nanoparticle dispersions in non-uniform magnetic field and in physiological conditions has not been proved yet [26]. If oleic acid is the surfactant in the synthesis, another possibility is the oxidization of double bond of chemisorbed oleate using strong oxidant like potassium permanganate under acidic or alkaline conditions, which leads to the formation of azelaic acid (an organic diacid) on the surface of IONPs and good water dispersibility of carboxylated product [85]. The other way is replacing the hydrophobic surfactants by hydrophilic molecules with anchoring chemical groups (e.g. carboxylic acid, phosphonic acid, dopamine) that have higher affinity to surface sites of IONPs in a ligand-exchange process widely used recently [86].

4. Designed synthesis of multicore iron oxide nanoparticles

Iron oxide nanoparticle assemblies are receiving continuously increasing interest for diagnosis and treatment due to the newly acquired performances, especially in what concerns their magnetic response in targeting, local heating and MRI detection. The high magnetic moment of the functionalized multi-core carriers is among the most important requirements for successful applications in biomedicine, in particular for magnetic targeting [13,45]. The localization and amount of magnetic nanoparticles in the magnetic-nonmagnetic material structure, such as assembling onto the surface of a non-magnetic core [87], homogeneous distribution in a non-magnetic matrix, entrapment into a vesicle bilayer [19] or close packing into a magnetic core, which are essential for the overall magnetic response, favor the magnetic core-organic shell type nanostructures [88]. Magnetic nanoparticle clusters in a polymer shell, summing up the magnetic moments of individual nanoparticles, were obtained by in situ coprecipitation of magnetic nanoparticles in microgels as microreactors [79,80] and also by strongly polar solvent induced destabilization of a ferrofluid [53]. The controlled clusterization of magnetic nanoparticles using the well-established miniemulsion technique [91], followed

by encapsulation of the densely packed magnetic clusters in a polymer shell, is a successful joining of ferrofluid technology and oil-in-water miniemulsion procedure [92–95]. Densely packed magnetic multi-core in thermoresponsive polymer shell obtained by ferrofluid miniemulsion procedure provided high magnetization spherical particles [57,96], promising for both efficient magnetic drug delivery and temperature controlled release. The surface density of oleic acid coating of individual MNPs controls the morphology of MNP clusters resulting in the oil-in-water miniemulsion process [50]. Incomplete OA coverage reduces the hydrophobicity of MNPs which tend to accumulate at the oil-water interface and gives rise to highly non-spherical shaped magnetic nanocomposites. Multiple iron oxide cores (maghemite nanoparticles) assembled as magnetically cooperative multi-core particles were obtained by applying a single-step high temperature hydrolysis procedure [76] and also by chemical coprecipitation in a microwave reactor [77]. Loading of liposomes with iron oxide nanoparticles [97], mostly clusters preformed from aqueous [51] and organic [98] ferrofluids, gives rise to magnetoliposomes with high magnetophoretic mobility and MRI contrast, proved to be very effective in drug and gene delivery into cancer cells in vitro and in vivo. Magnetic nanocapsules containing iron oxide nanoparticles and anticancer drugs have been designed to provide highly magnetic carriers under moderate gradient magnetic fields for tumor penetration, which are responsive to remote RF field for ON–OFF switchable drug release [52]. The encapsulation of hydrophobically coated IONPs from ferrofluids together with camptothecin anticancer drug into PPO block of Pluronic vesicles, provided a scalable continuous manufacturing procedure to obtain multi-core theranostic drug delivery vehicles [99], which is a promising step in consolidating recent achievements in targeted drug delivery to cancer cells.

The great variety of magnetic multi-core particles developed during the last few years, some of them reproduced in Fig.4, illustrate the progress in design and production of these versatile magnetic vectors with adjustable physicochemical properties (e.g., size, magnetic moment, surface charge, morphology, shell thickness), taking into account the requirements of achievable magnetic field strength and gradient, as well as of colloidal stability in biorelevant media.

The synthesis procedures, characteristic sizes and magnetic properties of several types of single-core and multi-core nanoparticles designed for biomedical applications are summarized in Table 1.

5. Protection, stabilization and functionalization of magnetic nanoparticles by designed coating

The different synthesis ways mostly result in IONPs with coating shell, naked IONPs are not often prepared, probably because of their pH-dependent surface properties and strong aggregation in neutral aqueous environment as discussed above [63,73]. Good colloidal stability of IONPs, prevention of particle aggregation in biological milieu at certain pHs like pH~7.4 in blood and high salt and protein concentration in general are absolute requirements for biomedical applications [19, 72]. Therefore IONPs have to be coated either during or after their synthesis. The literature distinguishes in situ coating, post-synthesis adsorption or post-synthesis grafting [13]. In the latter polymer functional groups are anchored to the IONP's surface, forming brush-like chains. As demonstrated, for example, in the work of Rinaldi and co-authors [110,111], covalent anchoring in many instances can enhance colloidal stability as compared with the adsorption of coating molecules. If a chemical surface modification is used, all the applied chemicals and by-products should be removed in order to reduce the chemical hazard of the formulation [112].

Coatings largely influence not only the colloidal stability, but also the functionality and biological fate of IONPs. One can distinguish several diverse functions of coating, namely i) colloidal stabilization under physiological conditions (protecting against aggregation at biological pHs and salty medium), ii) inhibiting corrosion and oxidation of magnetic core (passivation reducing the iron leakage), iii) hindering non-specific protein adsorption in biological milieu, iv) providing reactive groups for grafting drugs and targeting molecules, and v) control nano-bio interfacial interactions (bio/hemocompatibility, reticuloendothelial system (RES) uptake, blood circulation time, IONP's internalization efficiency, toxicity, targeting efficiency, in vivo fate, etc. as discussed in detail [10,13,19,112]).

The colloidal stability, the interaction between particles is controlled by their collision frequency (thermal motion) and its efficiency, depending on the attractive and repulsive contributions of resulting interaction in aqueous medium. The classical theory of colloidal stability, the DLVO (Derjaguin, Landau and Verwey, Overbeek) theory describes the van der Waals and electrostatic forces. Besides these one should consider the hydration effects, the hydrophobic interactions and the steric stabilization [113]. In case of magnetic particles, the attraction between magnetic dipoles, the magnetic dipolar interaction also should be considered [114] to get theoretical stability predictions comparable to experimental results [111].

The combined steric and electrostatic, called as electrosteric stabilization seems to be enough efficient, for example by polyelectrolyte (polyacrylic or polylactic acid, polyethylenimine, etc.) coating on IONPs; however, superb salt tolerance can be reached by coating with hydrophilic polymers like dextran, which is the most widely used polysaccharide in water-based magnetic fluids [7,12,19]. Frequently used coating agents are silica materials [115], small and big, natural and synthetic organic molecules (e.g., carboxylates, phosphates, phosphonate, sulfates, amines, alcohols, thiols, etc. [7,13, 72]). Mostly chemical bonds form between the functional groups of organic agents and reactive (both charged and uncharged) sites on the surface of IONPs. For example, in the well-known citrated magnetic fluids, the ≡Fe-OH sites are complexed by citrate through its OH and COOH groups [18] or by dopamine with both phenolic OH groups in the other favored core-shell product [7,19]). The effect of surface coverage changing with the amount of coating agent is poorly studied. Trace amounts of the polyacids can destabilize IONP dispersions at pH below PZC, while their high loading masks the original surface properties of IONPs and improves both colloidal stability and salt tolerance of dispersions [13]. In the presence of greater amounts of polyacids (above the adsorption saturation), the surface coverage of IONPs becomes completed causing a sign reversal of oxide particle charge and overcharging of nanoparticles enhancing their colloidal stability at pHs above ~5 [116]. The multi-site bonding is characteristic of macromolecular adsorption, which makes the coating layer resistant against dilution and easy the removal of excess from equilibrium medium [113].

In the editorial of a very recent ACSNano issue [118], the toxicity aspect of engineered nanomaterials (ENMs) is discussed and it has been stated, beside many important facts, that we must understand the ENMs' properties (such as intrinsic material and extrinsic properties modified through interactions with the suspending medium, and dynamically emerge at the nano bio interface) responsible for the toxicological response; characterization should be performed in the relevant medium, and not simply in water, especially in case of ENMs aggregation (agglomeration) and re-engineered their physicochemical characteristics for risk prevention and safety use. Therefore colloidal stability of IONPs has to be satisfactory under physiological conditions, at biological pHs, in salty medium, in the presence of proteins and in cell culture media as well. Testing aggregation (DLS sizing, filtration test, visual observation of sedimentation) under arbitrary conditions is quite usual [19]. Coagulation kinetics experiment is a correct method to test the salt tolerance of IONPs, and so their resistance against aggregation in physiological environment can be predicted [73]. It was suggested a straightforward route of physicochemical (iron dissolution) and colloidal (pH-dependent charging and particle size, salt tolerance from coagulation kinetics) measurements to assess eligibility of IONPs for biomedical tests [18]. The biomedical use of citrated IONPs is favored in literature (e.g., the famous VSOP-C184 product in [3, 7, 28] or the very recent multi-core samples in [77]), however they coagulate much below the physiological salt concentration and even more the quantity of iron leaching from them is very high due to the reductive and complex forming ability of citric acid; the dissolved iron ions may cause oxidative stress besides the danger of particle aggregation in vivo. Amstad et al. [117] reported a similar iron dissolution effect of catechol derivatives anchored to Fe_3O_4 surfaces, especially mimosine anchoring , which has the highest affinity to Fe(III) ions leads to gradual dissolution of Fe_3O_4 nanoparticles through complexation. The other crucial point is the non-specific protein adsorption, the formation of protein corona in biological fluids [119]. To date the most widely accepted approach is to coat IONPs with hydrophilic polymers, among the several coating agents the most favored are polyethylene oxides or glycols (PEG) and carbohydrates such as dextran [10,19] or sugar derivatives [120] which are bound to NP surface chemically or by multiple hydrogen bonds and develop super hydrophilic cloud around IONPs, and so

can inhibit the surface accumulation of proteins. However, immediate protein corona formation on MNPs coated with dextran and its derivatives (carboxymethyl-dextran and diethylaminoethyl-dextran) was observed recently [121]. Coating IONPs with PEG, grafted with poly(ethylene glycol)-silane [111] or in an one pot synthesis (thermal decomposition of $Fe(acac)_3$ in the mixture of poly(ethylene glycol) and poly(ethylene imine) [122], i.e., their PEGylation can improve the pharmacokinetic profile of drug delivery systems, enhance the drug accumulation in tumor and may improve the blood-brain barrier transport of IONPs [13].

An artificial protein coating from bovine serum albumin (BSA) was designed to increase the colloidal stability of a lauric acid-coated IONPs [123]. It was revealed that BSA coating greatly reduced the toxicity of nanoparticles and that the mitoxantrone drug-loaded system exhibited excellent therapeutic potential in vitro. The surface of magnetite cores was directly coated with BSA and further cross-linked to improve colloidal stability [124]. Monoclonal antibodies against vascular endothelial growth factor (mAbVEGF) were covalently conjugated to BSA coating on IONPs, which are promising MRI contrast agents for glioma visualization in brain.

In biological environment, nanoparticles get contact with biological entities, forming nano-bio interfaces with dynamically interacting components of nanoparticle surface, its aqueous interface and the interface of contact zone with biological substrates [112]. Among these only the particle surface can be tailored in order to improve in vivo biocompatibility of NPs. In this respect, the main independent particle variables are the size, zeta potential (in relation with the sign and magnitude of surface charge) and dispersibility in aqueous media (hydrophilic/hydrophobic feature). Positively charged particles are more likely to be toxic than the larger relatively hydrophobic particles, which are rapidly removed by the RES system. Particles that promote enhanced permeation and retention (EPR) effects in biological systems generally have mid-range sizes and relatively neutral surface or weakly negative charges [112].

6 Conclusions

Magnetic iron oxide nanoparticles are the basic components of remote controlled nanoparticle systems for nanomedicine. They seem to fulfil most of biocompatibility and

specific formulation requirements related to magnetic field guided drug delivery and hyperthermia systems, as well as contrast agents. There are envisaged optimized synthesis procedures to ensure large scale and reproducible production of IONP systems with optimal surface properties, shape, size, biocompatibility and high magnetic moment. Especially targeting in depth requires focusing on the increase of the magnetic moment of particles. In this respect recent results refer explicitly to single core and multi-core magnetic particles in strong correlation with colloidal stability in physiological environments, pharmacokinetics and in vivo fate of the two kinds of IONP systems.

The main challenge is dual involving the design of magnetic core and its surface engineering to provide appropriate core-shell IONPs for theranostic purpose. Optimizing the synthesis of core with high magnetic moments, and the surface and colloid chemistry of coating as well in order to prepare feasible particles for the selected purpose in a reproducible way, is the main requirement of present researches in order to proceed further in scale up the manufacturing of various IONP systems. All of their magnetic, physical chemical and colloidal parameters (magnetic response, saturation magnetization, size, shape and chemistry of IONP crystals, hydrodynamic size and charge state at the given composition of equilibrium aqueous phase, dissolved iron and organic matter content) and propensities (hydrophilicity, pH-dependent charging and salt tolerance), which might be needed in biomedical use have to be characterized by correct laboratory methods in details before expensive in vitro and in vivo testing. More attention was paid recently to the required chemical and colloidal stability of IONPs and their nanobiointerfacial interactions in biological media, such as iron leaching from magnetic core due to its improper passivation by e.g., citrate coating, which increases oxidative stress *in vivo*, and more the leaching impurities (organic residuals, toxic ions like Cu^{2+}) from chemical synthesis (e.g., click reaction); salt induced IONPs' aggregation under physiological conditions, as well as non-specific protein adsorption and interactions with cell membranes in serum or any other biological media.

The impressive progress in optimizing the composition, structure and functional coatings of iron oxide nanoparticle systems is a great promise for the design and reproducible manufacturing of single- and multi-core magnetoresponsive nanocomposites for specific applications in nanomedicine.

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Table1. Single core (SCIO) and multi-core iron oxide (MCIO) magnetic particles systems

Magnetic particles system	Primary single-core IONP	Preparation method	Characteristics		References
			Size (nm)	Magnetic*	
naked SCIO	-	coprecipitation	3-17[a]	26-89[c]	[68]
naked SCIO	-	coprecipitation	6.7-8.1[a]	52[c] **	[63]
naked SCIO Fe_3O_4	-	room temperature coprecipitation ultraslow rate	15-47[a]	52-69[c]	[80]
SCIO Fe_3O_4/OA	-	coprecipitation microwave heating	~10 [a]	-	[71]
SCIO Fe_3O_4/OA	-	coprecipitation	6.9 [a]	50[c]	[64]
SCIO / CA obtained by fractionation	-	high temperature hydrolysis polyol approach	10.3 [a]	57.5[c]	[76]
SCIO Fe3O4 /OA and OAm	-	$Fe(acac)_3$ thermal decomposition	3 - 20 [a]	82 - 83[c]	[82]
SCIO maghemite / fatty acids and amines	-	thermal decomposition of iron stearate	5.4 - 21 [a]	51 - 82[c]	[40]
SCIO FeO_{1-x}@$Fe_{3-x}O_4$/ hydrophilic dendron	-	thermal decomposition of iron stearate	14-20 [a]	26-41[c]	[84]
MCIO/CMD	-	modified alkaline precipitation method	40 - 80 [a]	-	[22,23]
MCIO /CTAB; MCIO /PEI; MCIO /PAA	Fe_3O_4/OA-Oam in hexane	emulsion	30 - 88 [a]	60[c]	[93]
MCIO /SDS	IONP/fatty acid (commercial product)	emulsion	45 – 79 [a]	62[c]	[95]
MCIO /SDS/ hydrogel poly(NIPAM-AA)	IONP/fatty acid (commercial product)	emulsion/ precipitation polymerization	145 -177[a]		[95]
MCIO / PEG-AA	Fe_3O_4/OA in hexane (ferrofluid)	emulsion-templated	430-660 [a]	7.5 – 24.8[c] 15-43% [g]	[92]
MCIO/ PEI/PAAMA	Fe_3O_4/OA in hexane	emulsion	~1200 [a]	~ 45 [e]	[50]

	(ferrofluid)				
MCIO/ SDS-Tween 85; MCIO/CTAB-Tween 85; MCIO/Pluronic PE 6800	Fe_3O_4/OA in octane (ferrofluid)	emulsion	50-300 [a]	57[c]	[100]
MCIO / PBMA-g-C12	$MnFe_2O_4$ / OA	emulsion	80 [a]	11 – 32[c]	[101]
MCIO in soybean, corn, cottonseed, olive oil or MCT/PEG-DSPE	Fe_3O_4/OA in toluene (ferrofluid)	emulsion	30-95[b]	-	[102,103]
MCIO/SDS/ PAA MCIO/SDS/ PNIPAM-PAA	Fe_3O_4/OA toluene ferrofluid	emulsion/ radical polymerization	100-200 [a]	43 – 46.8[c]	[57]
Silica coated magnetic nanocapsules (SiMNCs)	Fe_3O_4/OA in octane	emulsion/ silica coated of Fe_3O_4-polystyrene nanospheres / polystyrene burned	100 [a]	~ 45[c]	[52]
MCIO / PAA	-	hydrothermal	30-280 [a]	30.9 – 70[c]	[58,104, 105]
MCIO / SiO_2/PNIPAM MCIO / Carbon /PNIPAM	-	solvothermal / radical polymerization	90–260 [a] 160 [a]	41.6[c] 13.75[c]	[106,107]
MCIO / CA	-	microwave assisted coprecipitation	13 - 17 [a]	65.2 – 72.9[c]	[77]
MCIO/Polymer embedded colloidal assemblies	$MnFe_2O_4$ /LA; Fe_3O_4 / OA dispersed in toluene/ tetrahydro furane	thermal decomposition/ colloid destabilization by acetonitrile	70-134 [a]	43[e] 2.3x10-16 [d]	[53]
MCIO / CA obtained by fractionation	-	high temperature hydrolysis polyol approach	19.7–28.8 [a]	65.4-81.8[c]	[76]

MCIO	-	microwave irradiation	100 [a]	38.3[c]	[108]
MCIO/Polymer vesicle (Pluronic)	IONP/OA dispersed in tetrahydro furane (ferrofluid)	IONP embedded in polymer vesicle by microfluidic mixing	~ 160 [a]	4.1-17.4% [f]	[99]
MCIO/liposomes	γ-Fe_3O_4/ citrate in water (ferrofluid)	encapsulation of IONP into the liposomes	200 [a]	24-33% [g]	[51]
MCIO / liposomes-PEG	Fe_3O_4 in water (suspension)	encapsulation of IONP into liposomes coated with PEG	90 -110 (AFM)	-	[97]
MCIO/liposomes-PEG/PEG-Folic acid/Doxorubicin	Fe_3O_4 in water (commercial ferrofluid)	encapsulation of IONP and doxorubicin into the liposomes coated with PEG and PEG+Folic acid	156+-11 [b] 361+-20 [b]	-	[109]

Size: [a] TEM; [b] DLS

Magnetic: [c] M_S-saturation magnetization (emu/g); [d] μ - magnetic moment ($A.m^2$); [e] u-magnetophoretic velocity (μm/s)

Magnetic content-[f] IONP % (w/w) ; [g] IONP% (vol/vol)

* Measurement at room temperature

** data from the authors

PCL-b-PEG : diblock copolymer of poly(ε-caprolactone)-b-poly(ethylene glycol)

CTAB : cetyl trimethylammonium bromide

PEI : poly(ethyleneimine)

PAA : poly(acrylic acid)

OA : oleic acid

OAm : oleylamine

SDS : sodium dodecyl sulfate

NIPAM : N-isopropylacrylamine

AA : acrylic acid

PEG : poly(ethylene glycol)

PNIPAM : poly(N-isopropylacrylamine)

PBMA-g-C12 : poly(isobutylene-alt-maleic anhydride) grafted with 1-dodecylamine

CA – citric acid

CMD – carboxymethyldextran

MCT - medium chain triglycerides

PEG-DSPE - distearoyl-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]

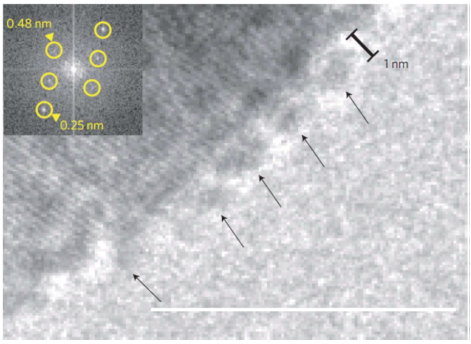

Fig.1. Cryo-TEM image of primary particles attaching to the surface of a magnetite nanoparticle (*Reproduced from Ref.[67] with permission of the Nature Publishing Group*).

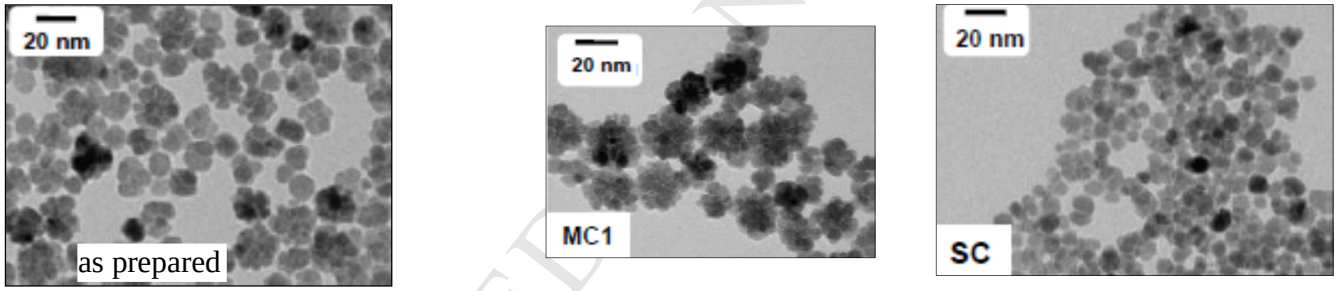

Fig. 2. TEM images of the polydisperse mixture of IONPs (as prepared left side) and its fractions containing multicore (MC1 middle) and single core (SC right side) nanoparticles (*Reprinted with permission from [76]. Copyright (2012) American Chemical Society*).

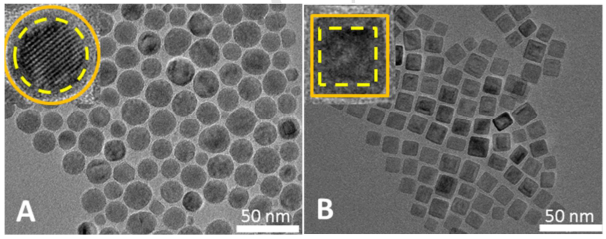

Fig.3. TEM images of antiferromagnetic-ferrimagnetic core-shell NPs: (a) nanospheres, (b) nanocubes. Insets show HRTEM images. Dashed lines indicate the $Fe_{1-x}O$ core and

solid lines indicate the $Fe_{3-x}O_4$ shell. (*Reprinted with permission from [84]. Copyright (2014) American Chemical Society*.)

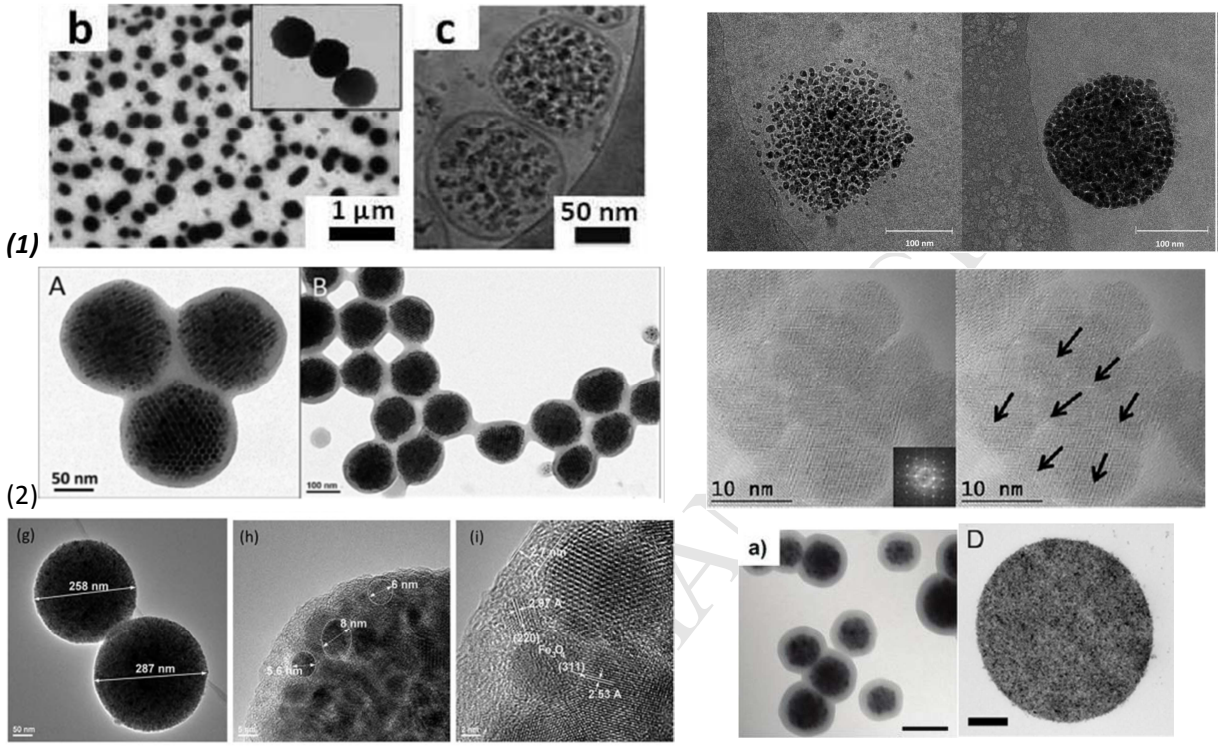

Fig. 4 Magnetic multi-core particles obtained by different synthesis procedures:
(1) encapsulation of MNPs into liposomes, polymersome: left - TEM (b) and cryo-TEM (c) micrographs of Ultra Magnetic Liposomes (UMLs) prepared by reverse phase evaporation process (REV) process. MNPs are trapped inside unilamellar vesicles (c) and dipole−dipole interaction can occur as exemplified by magnification (b) (*Reprinted with permission from [51]. Copyright (2012) American Chemical Society*); **right** - Cryo-TEM image showing iron oxide nanoparticles incorporated in the polymersome membrane with 4.1% iron oxide (left), and 17.4% iron oxide (right). (*Reproduced from Ref.[99] with permission of The Royal Society of Chemistry*);
(2) thermal decomposition: left - TEM images of polymer encapsulated colloidal ordered assemblies (polymer-COA) at higher (A) and lower (B) resolution. The dark pattern (A) results from the ordering of the closed packed assemblies within the nanobeads, while the brighter gray ring is caused by the polymer shell (lower electron density) of around 20 nm thickness. (*Reprinted with permission from [53]. Copyright (2013) American Chemical Society);* **right –** HRTEM of multi-core NP showing the continuity of the crystal lattice at the grain interfaces. The Fourier transform of this high-resolution image (see inset) shows the monocrystalline fcc structure of the multi-core nanoparticles, oriented along the [001] zone axis. (*Reprinted with permission from [76]. Copyright (2012) American Chemical Society);*

(3) miniemulsion: left – TEM images of magnetic microgel with magnetite nanoparticles cluster as a core coated with two layers of cross linked polymer shells poly-N-isopropylacrylamide-polyacrylic acid(*Reproduced from Ref.[57] with permission of The Royal Society of Chemistry*)*;* **center -** TEM image of magnetic clusters encapsulated in a copolymer hydrogel poly(N-isopropylacrylamide-acrylic acid). Scale bar: 100 nm. (*Reprinted with permission from [95]. Copyright (2011) American Chemical Society);* **right** - TEM image of cross section of superparamagnetic microparticles produced with ferrofluid nanoparticle concentrations of 1 g/L using oil-in-water emulsion-templated assembly. (*Reprinted with permission from [50]. Copyright (2013) American Chemical Society)*

Highlights:

- Synthesis procedures of iron oxide nanoparticle systems are comparatively analysed
- The properties of single core and multicore iron oxide nanoparticles are reviewed
- Biocompatible functional coatings are thoroughly discussed