

A STUDY AND CHARACTERIZATION OF SYNTHESIS OF MAGNETIC FERRITE NANOPARTICLES WITH FEATURING POLYZWITTERIONIC COATINGS**Asha J K and Dr. Mohan Nathulal Giriya**Department of Physics, Dr. A. P. J. Abdul Kalam University, Indore, MP, India
ashagurumurthy@gmail.com and mohangiriya@gmail.com**ABSTRACT**

Magnetic nanoparticles (MNPs) that exhibit high specific loss power (SLP) at lower metal content are highly desirable for hyperthermia applications. The conventional co-precipitation process has been widely employed for the synthesis of magnetic nanoparticles. However, their hyperthermia performance is often insufficient, which is considered as the main challenge to the development of practicable cancer treatments. In particular, ferrite MNPs have unique properties, such as a strong magneto crystalline anisotropy, high coercivity, and moderate saturation magnetization, however their hyperthermia performance needs to be further improved. In this study, cobalt ferrite (CoFe₂O₄) and zinc cobalt ferrite nanoparticles (ZnCoFe₂O₄) were prepared to achieve high SLP values by modifying the conventional co-precipitation method. Our modified method, which allows for precursor material compositions (molar ratio of Fe³⁺ : Fe²⁺ : Co²⁺ / Zn²⁺ of 3:2:1), is a simple, environmentally friendly, and low temperature process carried out in air at a maximum temperature of 60 °C, without the need for oxidizing or coating agents. The particles produced were characterized using multiple techniques, such as X-ray diffraction (XRD), dynamic light scattering (DLS), transmission electron microscopy (TEM), ultraviolet-visible spectroscopy (UV-Vis spectroscopy), and a vibrating sample magnetometer (VSM). SLP values of the prepared nanoparticles were carefully evaluated as a function of time, magnetic field strength (30, 40, and 50 kA m⁻¹), and the viscosity of the medium (water and glycerol), and compared to commercial magnetic nanoparticle materials under the same conditions. The cytotoxicity of the prepared nanoparticles by in vitro culture with NIH-3T3 fibroblasts exhibited good cytocompatibility up to 0.5 mg/mL. The safety limit of magnetic field parameters for SLP was tested. It did not exceed the $5 \times 10^9 \text{ Am}^{-1} \text{ s}^{-1}$ threshold. A saturation temperature of 45 °C could be achieved. These nanoparticles, with minimal metal content, can ideally be used for in vivo hyperthermia applications, such as cancer treatments.

Keywords: specific loss power; magnetic ferrite; modified co-precipitation; hyperthermia

INTRODUCTION

For decades, magnetic nanoparticles (MNP) have been in focus within a range of scientific disciplines as they show high potential in a variety of different application fields, ranging from chemistry, biology, medicine to physics. One unifying aspect herein is surface properties of such nanomaterials. To date, there have been several reviews focusing on surface modifications of nanomaterials with polyelectrolytes, and most of them have focused on biomedical applications of these materials [1–7]. However, to our knowledge the only example specifically focusing on zwitterionic coating materials for nanomaterials was written by García et al. and here the central aspect is the behavior under in vivo conditions [4]. Within this review article, we therefore focus on the preparation and characterization of MNP featuring zwitterionic coating materials as they open up an interesting area of bio-repellent, pH responsive, and dispersion-stable hybrid materials. The magnetic core enables the selective separation of these particles for analytical issues and external magnetic fields can be used for biomedical applications like hyperthermia and drug targeting. This review aims to serve as a guide for various synthetic strategies for immobilizing polyzwitterions at the surface of magnetic nanoparticles which have been explored during the last decade and is structured as follows: we begin with a section on different magnetic core materials, followed by the synthesis of polyzwitterions, suitable methods for nanoparticle coating, and finally we discuss important characterization methods for such hybrid materials. Throughout the different chapters, we also showcase potential application fields.

The Core: Materials for Magnetic Nanoparticles

All chemical elements or compounds of our planet show under certain conditions different magnetic effects. Since we focus herein on magnetic nanoparticles for medical and technical applications, we concentrate on materials with ferro- or ferrimagnetic, superparamagnetic, and superferrimagnetic behavior at room temperature. In that regard, three classes of materials exist.

Metals—The only metallic elements showing ferromagnetism at room temperature are iron, cobalt, and nickel. The preparation of nanoparticles hereof is possible and such materials show promising magnetic behavior for medical applications [8–13]. Since such nanoparticles show a strong oxidation tendency to non-magnetic oxides (e.g., antiferromagnetic FeO, CoO, NiO), an oxidation-protective layer is necessary. Due to this fact, and also the toxicity of Ni and Co, metallic nanoparticles play only a minor role regarding their applications in medicine [14].

Alloys—The second group of ferromagnetic materials are the ferromagnetic alloys, e.g., CoPt, FePt, FeNi, or FeCo. The preparation of magnetic nanoparticles consisting of ferromagnetic alloys is described in the literature by several groups. Up to now, none of those nanostructures has found access in medical applications mainly due to two facts: First, some of the ferromagnetic alloys (e.g., AlNiCo, CoPt, FeCoCr) show a hard-magnetic behavior (a remnant magnetization and coercivity), leading to potential agglomeration of the particles due to the remanence, and exposing the patient to the risk of vessel embolism. Second, most of the alloys with promising magnetic behavior contain toxic components (e.g., Ni or Co) which inhibit the application of such materials in the human body.

Oxides—The group of magnetic oxide materials can be divided into mixed oxides with different crystal structures (e.g., the magnetic garnets and the ferrites) as well as the pure metallic oxides. Since the saturation magnetization of all garnets is very low, these materials are not suitable for application in medicine. Depending on their composition, the ferrites show soft- or hard-magnetic behavior. Despite some groups having found promising magnetic properties of soft-magnetic ferrites for certain medical applications only very few studies can be found in the literature. Representative hard-magnetic ferrites with promising magnetic behavior for medical application are barium-, strontium- or cobalt-ferrite. Since cobalt-ferrite (CoFe₂O₄) shows less toxic effects than Ba- or Sr-ferrite, nanoparticles of this material find increasing application for medical purposes, e.g., for magnetic hyperthermia as minimal invasive tumor treatment and for lab-on-a-chip applications in diagnostics. The promising magnetic properties of cobalt-ferrite can be tuned by variation of the Co/Fe-ratio and thus this material will play a major role in the future in our opinion. As Ni and Co form no oxides showing ferromagnetism at room temperature, only iron has to be considered in this case. Here, mainly four different oxides have to be mentioned: iron(III) oxide (Fe₂O₃) and iron(II,III) oxide (Fe₃O₄), as well as the rather unstable iron(II) oxide (FeO) and iron(I) oxide (Fe₂O). From Fe₂O₃ several phases exist, e.g., α -, β -, γ -, or ϵ -Fe₂O₃, which all show different magnetic behavior. Of the iron oxides only maghemite (γ -Fe₂O₃) and magnetite (Fe₃O₄) show ferromagnetic behavior or, more precisely, ferrimagnetism due to the spinell structure (a subtype of the cubic lattice). A comprehensive work on the nature of iron oxides and their properties is given by Schwertmann.

Magnetic Properties of Magnetic Nanoparticles

Beside other parameters like magnetic anisotropy or shape, the magnetic behavior of magnetic particles is determined by the particle size. For macroscopic particles in the size range of μm and above, several areas of homogeneous magnetization are formed. These so-called magnetic domains are separated by Bloch walls. Due to this domain formation, the magnetic stray field of the particle is minimized and the domain formation in the absence of an external magnetic field is energetically favorable compared to a homogeneously magnetized particle. The magnetization directions of all domains in the particle are statistically oriented, which leads to a compensation of all magnetic moments within the particle, resulting in no external magnetization of the particle without an external magnetic field. With decreasing dimensions of the magnetic particle, the relative proportion of wall energy to that of the entire particle energy increases. Due to energetic reasons, no magnetic domains are formed below a critical particle size and the whole particle shows a spontaneous magnetization in one direction. The direction of the magnetization of these so-called single domain particles is determined by the crystal lattice of

the particle and is named “the easy axis”. The critical size for the formation of single domain particles is given by the material specific magnetic anisotropy K and the form factor (ratio of particle length in different directions related to the magnetic field) of the particle. For cubic and spherical particles made of magnetite, the theoretical upper limit for the formation single domain particles is about 80 nm, which was confirmed experimentally. A further decrease of the particle size leads to a decrease of the magnetic anisotropy energy of the particles. In this case a certain probability exists, that for finite temperatures the thermal energy exceeds the anisotropy energy due to thermic variations and the particle spontaneously changes the orientation of magnetization.

A special case of magnetism can occur if small super paramagnetic particles form a larger cluster. In the absence of an external magnetic field these clusters show super paramagnetic behavior with no remnant magnetization or coercivity. If the particles are exposed to an external field, depending on the strength of the particle interactions, a collective magnetism may result and the clusters show ferromagnetic behavior with an observable hysteresis. This so-called super ferrimagnetism is typical for magnetic multicore particles and such particles show very promising properties for medical applications.

Preparation of Magnetic Nanoparticles

The following section briefly covers the main preparation routes for magnetic nanoparticles. Detailed information can be found in excellent reviews on this topic [46–49]. Magnetic nanoparticles are obtained by three different preparation routes.

(i) Bio mineralization

(ii) Physical methods

(iii) Chemical methods

(i) By means of biomineralization some living organisms prepare magnetic particles for use for their sense of direction. For example, magnetotactic bacteria are capable of preparing magnetosomes (protein coated nanosized crystals of magnetic iron oxide). The bacteria use the particles as a compass to find their preferred habitat in anaerobic areas at the bottom of the sea. Under anaerobic synthesis conditions in the lab, which are similar to the conditions of their habitat, uniform particles of 20 to 45 nm core diameter may be produced. Despite the fact that magnetosomes show excellent magnetic properties for medical application (especially hyperthermia), they have found no application in medicine until now due to their bacterial protein coating. Current recent research on magnetosomes focuses on elucidation and optimization of the biomineralization process with the aim to develop wet chemical preparation routines which emulate the biologic process, thus providing MNP with similar magnetic behavior.

(ii) The physical methods can be divided into “top down” and “bottom up” procedures. Top down methods are based on the size reduction of macroscopic magnetic materials to the nanometer range, e.g., by means of milling. A major drawback of these methods is the difficulty of adjusting the desired particle size and shape. Furthermore, the milling procedure leads to lattice defects that cause deviations in the magnetic properties compared to regular particles of the same size. Bottom up methods use the condensation of nanoparticles from either a liquid or gaseous phase. A promising bottom up method for the synthesis of MNP powders is laser evaporation. Starting materials are coarse metal oxide powders of a few μm sized particles, which are evaporated by means of a laser. As a result of the steep temperature gradient outside of the evaporation zone, a very fast condensation and nucleation takes place from the gas phase and nanoparticles are formed. The resulting mean particle sizes (20 to 50 nm) and magnetic phase are tuned by laser power and composition of the atmosphere in the evaporation chamber.

(iii) The chemical methods provide a multitude of different bottom up synthesis routes for the preparation of MNP, from which the most prominent will be described shortly.

The co-precipitation synthesis procedure is a very simple method for the preparation of MNP. Most scientific work uses aqueous media for precipitation. Very often, the magnetic iron oxides are prepared by means of a co-precipitation from aqueous Fe²⁺ and Fe³⁺ salt solutions, to which a base is added. Magnetic phase and particle size can be tuned by variation of iron salts, Fe²⁺/Fe³⁺ ratio, temperature, pH, and the type of base used. Pioneering work on this preparation route was performed by Khallafalla and Reimers and Massart. For this method, particles are in the superparamagnetic size range from 5 to 15 nm and the obtained size distribution is relatively broad. By varying the reaction conditions, the size can be increased to up to 40 nm. In this size range, the particles show single domain ferrimagnetic behavior. Different modifications of this method were reported over recent years. Upon applying high pressure homogenization during precipitation or using slower reaction conditions, superferrimagnetic clusters of single crystals of 10 to 15 nm are formed, which show very promising magnetic properties for medical applications. Furthermore, size control of the resulting magnetite nanoparticles could also be shown by reactions carried out at high temperatures. Co-precipitation is also used for the preparation of ferrites, e.g., cobalt ferrite by replacing a part of the Fe²⁺ by Co²⁺ in the starting solutions.

Recent Developments in the Synthesis of Magnetic Nanoparticles

Over the past 10 years, the major aim of magnetic nanoparticle preparation was to develop strategies for a versatile and robust protocol for the synthesis of tailor-made samples. Due to the high diversity of the required magnetic properties of the particles for the different applications outlined above and below, several structural parameters (e.g., size and size distribution) have to be tuned. For example, medical applications benefit in three ways from magnetic particles. First, magnetic particles can be manipulated mechanically by an external magnetic field (gradient), resulting in a rotation or attraction of the MNP which can find application in magnetic drug targeting. Second, due to their magnetic moment, MNP are a source of a magnetic stray field, which can be detected by appropriate sensors and might find application in medical imaging. Finally, if MNP are exposed to an alternating magnetic field, the particles are heated up due to reversal magnetization losses and the generated heat can be used for therapeutically applications, e.g., hyperthermia as an example for minimal invasive cancer therapy. To obtain MNP which show promising magnetic behavior for mechanical manipulation, MNP with a high magnetic moment are needed and quite often this is translated into a large particle volume. Several groups obtained different strategies for the preparation of so called large single domain particles (LSDP). Despite the fact that the steric stabilization of such large particles is challenging (due to the strong tendency to form agglomerates) sedimentation stable dispersions of large single domain particles exist. A possible solution for the challenging stabilization of LSDP is the use of Co-ferrites. They show magnetic properties similar to that of LSDP but much smaller diameters of about 10 to 15 nm, which enable sufficient steric stabilization.

Synthesis and Characterization of Magnetic Nanoparticles

Controlling the chemical composition and size of magnetic materials is important to enhance magnetization that effectively leads to an increase in magnetic hyperthermia heating efficiency of magnetic NPs. Cobalt ferrite nanoparticles (CF-MNPs) and zinc cobalt ferrite nanoparticles (ZCF-MNPs) were prepared by a controlled co-precipitation method, which is a simple, environmentally friendly, and low-temperature method. By shaking, the prepared nanoparticles can be dispersed in water, but after a while they tend to aggregate and sediment. As shown in Figure 1, CF-MNPs had a wide size distribution with an average particle size of 8 ± 2 nm. In the case of ZCF-MNPs, the average particle size was 25 ± 5 nm, with a wide size distribution and varied agglomeration behavior. The incorporation of Zn in the NP structure leads to an increase in the particle size and agglomeration. Magnetic particles agglomerate as a result of high surface energy between the nanoparticles and magnetic dipole-dipole interactions. The crystalline nature of the prepared nanoparticles was observed using HRTEM. The clear lattice boundary in the HRTEM image illustrates the higher crystallinity of CF-MNPs, as compared to ZCF-MNPs, which is confirmed by XRD (shown later). The corresponding selected area electron diffraction (SAED) image of nanoparticles displays the ring characteristics consistent with a structure composed of small domains with their crystallographic axes randomly oriented with respect to one another. The SAED pattern shows diffuse rings with less intensity that can be indexed to the nanoparticle plane reflections. Results indicate that our method produced

smaller nanoparticles with fewer aggregations for CF-MNPs, as compared to ZCF-MNPs, due to a progressive increase in the solubility product constant of the corresponding divalent metal hydroxides.

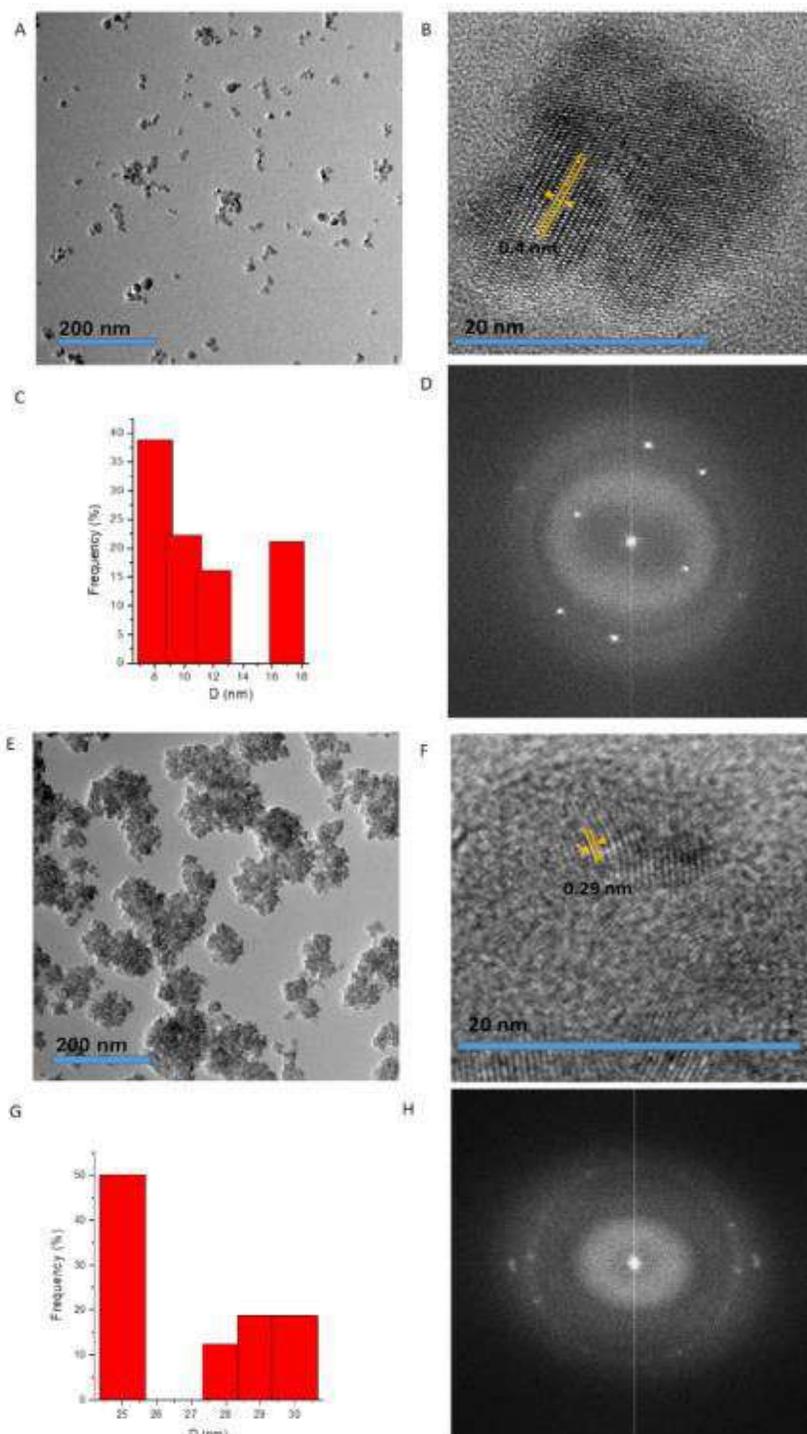


Figure 1: Transmission electron microscopy (TEM), selective area electron diffraction (SAED) and particle size distribution histograms for CF-MNPs (A–D) and ZCF-MNPs (E–H)

As shown in Figure 2, the mean hydrodynamic size obtained by DLS was 50.9 and 575 nm for CF-MNPs and ZCF-MNPs, respectively (which was higher than that obtained using TEM analysis). Wide size distribution may result from the hydrophobic nature of the prepared nanoparticles. The measurements of the zeta potential were used to assess the effects of nanoparticles in the colloidal phase and their aggregates. Higher zeta potentials indicate stable nanoparticle systems. The zeta potential values were +30.59 and +14.69 mV for CF-MNPs and ZCF-MNPs, respectively (Figure 2). Thus, the synthesized cobalt ferrite nanoparticles dispersed in water due to the large electrostatic repulsive forces between the particles. In contrast, zinc cobalt ferrite nanoparticles appeared less stable due to the low electrostatic repulsive forces between them. Obtaining stable colloidal systems is particularly important from the perspective of using synthesized nanoparticles in Nano-medicine and biomedical applications.

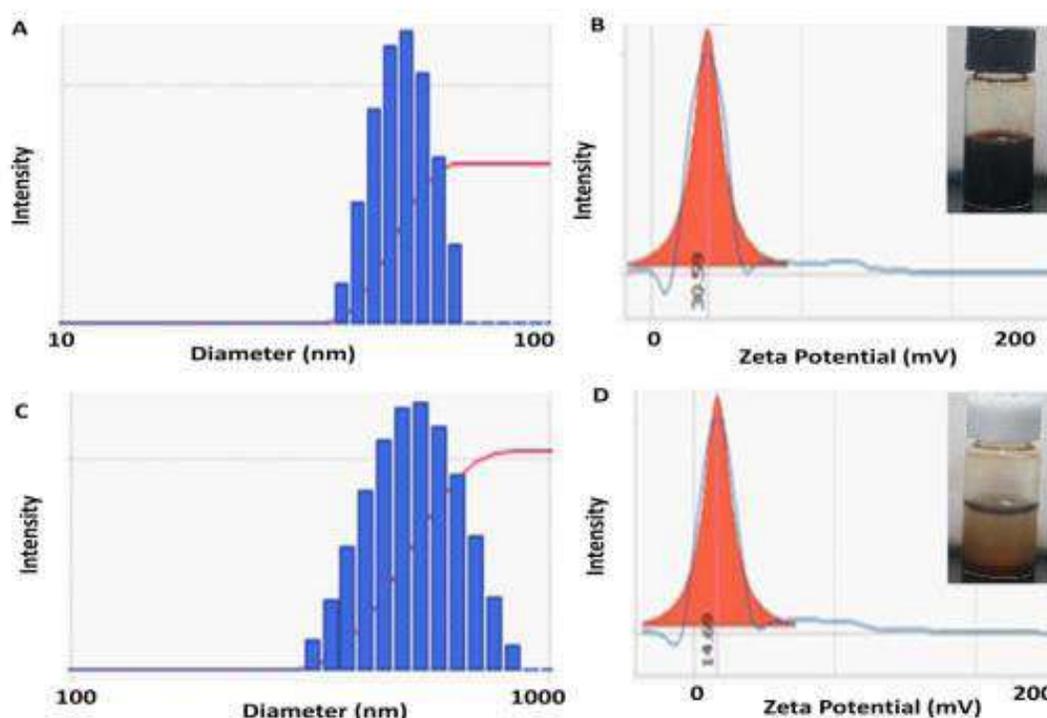


Figure 2: Dynamic light scattering (DLS) and zeta potential results for CF-MNPs (A,B) and ZCF-MNPs (C,D).

CONCLUSION

In this study, ferrite nanoparticles, namely cobalt ferrite (8 ± 2 nm) and zinc cobalt ferrite (25 ± 5 nm), were prepared using a controlled co-precipitation process. The synthesis process was a modified version of the conventional co-precipitation methods, with changes in the composition of the precursor materials (molar ratio of $\text{Fe}^{+3}:\text{Fe}^{+2}:\text{Co}^{+2}/\text{Zn}^{+2}$ of 3:2:1) and a simple, environmentally friendly, and low-temperature process carried out in air (the maximum temperature was 60°C and neither oxidizing nor coating agents were required). The prepared nanoparticles exhibited optical activity with moderate magnetic saturation (50 emu/g). SLP was enhanced with respect to time, magnetic field strength, concentration, and medium viscosity. The highest SLP obtained was 552 W/gmetal for zinc cobalt ferrite nanoparticles at a concentration of 8 mg/mL with a magnetic field of 50 kA/m and frequency of 97 kHz, while the lowest SLP was 11.57 W/gmetal, obtained at a concentration of 25 mg/mL with magnetic field of 30 kA/m and frequency of 97 kHz. The SLP values of our magnetic nanoparticles coated with sodium citrate were found to be higher than those for the commercial iron oxide materials. The nanoparticles exhibited high cell viability and none of the applied AC magnetic fields exceeded the physiologically tolerable limit of $5 \times 10^9 \text{ Am}^{-1} \text{ s}^{-1}$. The nanoparticles prepared using the presented method achieve high SLP and are promising for biomedical applications, such as hyperthermia cancer treatment.

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