

*Dedicated to the memory of
Professor Petru Spacu (1906–1995)*

SYNTHESIS AND CHARACTERIZATION OF COBALT FERRITE NANOPARTICLES COATED WITH DEHYDROCHOLATE ANIONS

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Cobalt ferrite nanoparticles were obtained by the complexation method using dehydrocholate sodium salt. The polynuclear coordination compounds precursors for magnetic nanoparticles and the cobalt ferrite were characterized by FTIR and UV-VIS spectroscopy, X-ray diffraction and magnetic measurements.

INTRODUCTION

Biocompatible nanometric superparamagnetic particles have been extensively investigated in the last four decades due to their potential applications (especially for *in vivo* applications). Nanoparticles that possess magnetic properties offer new opportunities including hyperthermic treatment for tumor cells,¹⁻⁴ improving the quality of magnetic resonance imaging (MRI),⁵⁻⁷ target drug delivery.⁸⁻¹⁰ The major beneficial factor of using magnetic nanoparticles is the use of localized magnetic field gradients to attract the particles to a chosen site, to hold them there until therapy is complete and then to remove them. The particles may be injected directly into the general area where treatment is desired or intravenously and then blood circulation will transport them to the region of interest for treatment. The investigations have demonstrated that the particles of about 5-10 nm diameter are suitable for the most forms of therapy. Larger particles of about 50-200 nm cannot be transported and remain attached to the walls of the vascular system increasing the risk of thromboses.

One notices that some of the material properties can significantly change when particles size reduce from bulk to nanoscale. Magnetism is one of these properties. Single domain magnetic nanoparticles with high saturation magnetization, high magnetic susceptibility and low or zero coercivities, are suitable for drug delivery purposes. The ratio of surface atoms to that of interior atoms increases with decreasing particle size and the surface effect will predominate the material properties. So, surface coating of the nanoparticles will also affect the material properties. Coating with ligands can induce an interparticle repulsion that counteracts the magnetic attraction between particles.

For biomedical applications, the absorbed ligand layer should completely cover the particles and be as dense as possible in order to protect the iron oxide core against contact with blood proteins and phagocytosis – associated receptors.

Most of the intravenously injected particles are recognized by the body as foreign and subsequently removed from the blood circulation by the macrophages of the mononuclear phagocytic system. This process leads to a high uptake of particles in liver, spleen, lungs, kidneys, heart and bone marrow. In order to minimize cytotoxic problems in these organs, the nanoparticles must be assessed for compatibility with biological environment. For this reason it was investigated the citotoxicity of the various ferrites: Fe₃O₄, BaFe₁₂O₁₉, SrFe₁₂O₁₉, CoFe₂O₄, Co_{0.9}Ni_{0.1}Fe₂O₄, Li_{0.5}Fe_{2.5}O₄, Ni_{0.65}Zn_{0.35}Cu_{0.1}Fe_{1.9}O₄. The results showed that

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Fe_3O_4 , $\text{SrFe}_{12}\text{O}_{19}$, CoFe_2O_4 , $\text{Co}_{0.9}\text{Ni}_{0.1}\text{Fe}_2\text{O}_4$ are non-cytotoxic, $\text{Ni}_{0.65}\text{Zn}_{0.35}\text{Cu}_{0.1}\text{Fe}_{1.9}\text{O}_4$ mildly cytotoxic and $\text{BaFe}_{12}\text{O}_{19}$ cytotoxic.²

Among the various methods for producing nanoparticles, wet chemical routes have the advantages of being relatively simple and providing good control over particle properties.¹¹⁻¹³

The aim of this work was to obtain and characterize cobalt ferrite nanoparticles in the presence of dehydrocholic acid sodium salt.

Dehydrocholic acid (3,7,12-triketo-5 β -cholan-24-oic acid, shown in Figure 1) is an unnatural bile acid having the cholanoic acid structure. This derivative usually obtained from cholic acid *via* oxidation of the C3, C7 and C12 hydroxyl group, possesses important therapeutic and chemical applications.¹⁴ From a pharmaceutical point of view, dehydrocholic acid is used as a choleric and for the treatment and prevention of gallstones.¹⁵

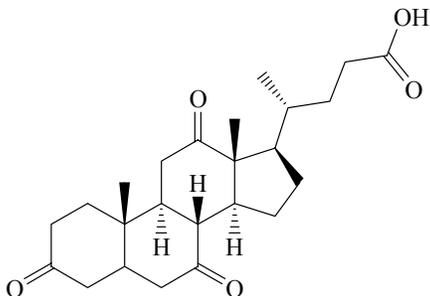


Fig. 1 – Dehydrocholic acid.

EXPERIMENTAL PART

Iron(III) nitrate nonahydrate, cobalt(II) nitrate hexahydrate, ammonium hydroxide (25% NH_3 in water), dehydrocholic acid sodium salt (DHCNa) were obtained from Merck. All chemicals were used as received.

[Fe^{III}Co(DHC)₆(OH)₂]-6H₂O (I) 0.404 g Iron nitrate and 0.145 g cobalt nitrate were dissolved in 30 ml distilled water at room temperature, with vigorous stirring, such that $\text{Fe}^{3+}/\text{Co}^{2+} = 2$. As the solution was heated, a solution of 0.5 g sodium dehydrocholate in water was added. A brown precipitate was formed which was stirred for one hour. The pH of the reaction medium was ~6. After this time, the precipitate was filtered, washed with water for five times in order to remove the excess sodium dehydrocholate and dried over P_4O_{10} . Anal. Calcd./Found Fe% 4.12/3.51, Co% 2.17/2.02, C% 63.55/63.45, H% 7.79/7.76.

[Fe₂Co(DHC)(OH)₇] (II) The same quantities of iron nitrate and cobalt nitrate were dissolved in distilled water. Then a solution of 0.5 g sodium dehydrocholate in water was added under stirring and heating. After one hour, a small volume of NH_4OH (25%) was added until the pH of the reaction medium became ~8. The brownish mixture was stirred for an additional hour and then the precipitate formed was filtered, washed for five times with water and dried over P_4O_{10} . Anal. Calcd./Found Fe% 16.21/17.00, Co% 8.54/9.20, C% 41.68/41.67, H% 6.22/5.78.

Fe₂CoO₄. The same quantities of iron nitrate, cobalt nitrate and sodium dehydrocholate were dissolved in distilled water and after one hour of stirring and heating to about 50°C, a large amount of NH_4OH (25%) was added until the pH was greater than 10 unit and the black suspension of Fe_2CoO_4 formed was heated to 80°C. After two hours, the suspension was filtered, washed with water to remove the excess of sodium dehydrocholate and NH_4OH and dried over P_4O_{10} .

The metal content for all of the three samples was determined by atomic absorption spectrometry. The C, H analysis was performed by microcombustion method.

The IR spectra (KBr pellets) of the compounds were recorded in the range 400–4000 cm^{-1} using a Bio-Rad FTS-135 spectrophotometer.

Data about the stereochemistry of the metal ions were obtained from UV-VIS spectra (diffuse reflectance technique) recorded on an UV4 Unicam Spectrophotometer in the range 200 – 900 nm.

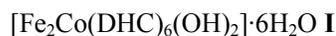
The magnetic susceptibilities of the complex compounds and the saturation magnetizations of the cobalt ferrite, at the room temperature, were determined with a Faraday balance using $\text{HgCo}(\text{SCN})_4$ as calibrant ($\chi = 16.44 \cdot 10^{-6}$ cgs units).

The crystalline phases in the black compounds and, also, in the calcined powders were identified by XRD powder methods using a Rigaku – Multiflex X-ray diffractometer, with $\text{CuK}\alpha$. For quantitative analysis a step scanning technique was applied in the 2θ range 20 – 80, with a step of 2°/min.

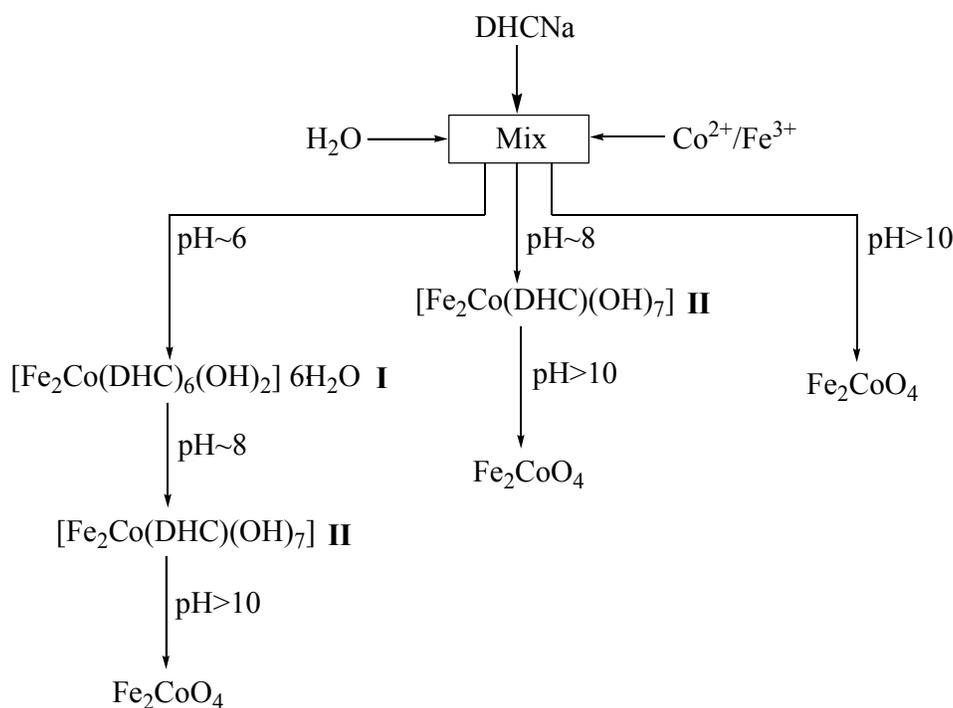
RESULTS AND DISCUSSION

In order to establish the optimum conditions for obtaining the magnetic nanoparticles in the presence of dehydrocholate anion, we studied the behaviour of the system:

$2\text{Fe}^{\text{III}} : \text{Co}^{\text{II}} : \text{C}_{24}\text{H}_{33}\text{O}_5^-$ ($\text{C}_{24}\text{H}_{33}\text{O}_5^-$ is the anion of the dehydrocholic acid) on various values of pH (pH \sim 6, pH \sim 8 and pH $>$ 10). The following types of compounds were obtained (Scheme 1):



The formula of the complex compounds **I** and **II** were established correlating elemental chemical analysis with physico-chemical measurements (IR, UV-VIS spectra, magnetic measurements). The thermal decomposition of the compounds **I** and **II** at 800°C for one hour lead to the cobalt spinel ferrite with α - Fe_2O_3 impurities. The cobalt ferrite obtained by chemical coprecipitation of Fe(III) and Co(II) ions in the presence of sodium dehydrocholate is single phase. Both types of ferrites exhibit positive magnetic behavior in the presence of a permanent magnet.



Scheme 1.

Figure 2 shows the FTIR spectra of sodium dehydrocholate (DHCNa), complex compounds **I**, **II** and cobalt ferrite nanoparticles coated with dehydrocholate anion. The 1709 cm^{-1} peak assignable to the C=O vibration on the steroid moiety is present in spectrum of the compounds **I** and **II**, but not appear in spectrum of the cobalt ferrite coated with dehydrocholate anion. The spectra of the compounds **I** and **II** exhibit two strong bands at $\sim 1586\text{-}1538 \text{ cm}^{-1}$ and $\sim 1430\text{-}1402 \text{ cm}^{-1}$ assignable to the carboxylate anion asymmetric vibration (ν_{asymOCO}) and symmetric vibration (ν_{symOCO}) and are also present in the spectrum of ferrite nanoparticles as a broad bands. Carboxylate group of dehydrocholate anion complexes with the Fe and Co atoms on the ferrite surface and render a partial single bond character to the C=O bond, weakening it and shifting the stretching frequency to a lower value. It is proposed that dehydrocholate anion binds to the ferrite surface by chemisorbtion of the carboxylate group. The $\Delta\nu$ ($\nu_{\text{asymOCO}} - \nu_{\text{symOCO}}$) values for the compounds **I** and **II** are lower than the $\Delta\nu$ value in DHCNa (coresponding to a ionic bond). This observation suggests a bidentate chelation of dehydrocholate anion to the metal ions. The print region in the spectrum of cobalt ferrite, recorded at wave numbers less than 800 cm^{-1} , revealed the vibrations of the metal oxide skeleton.

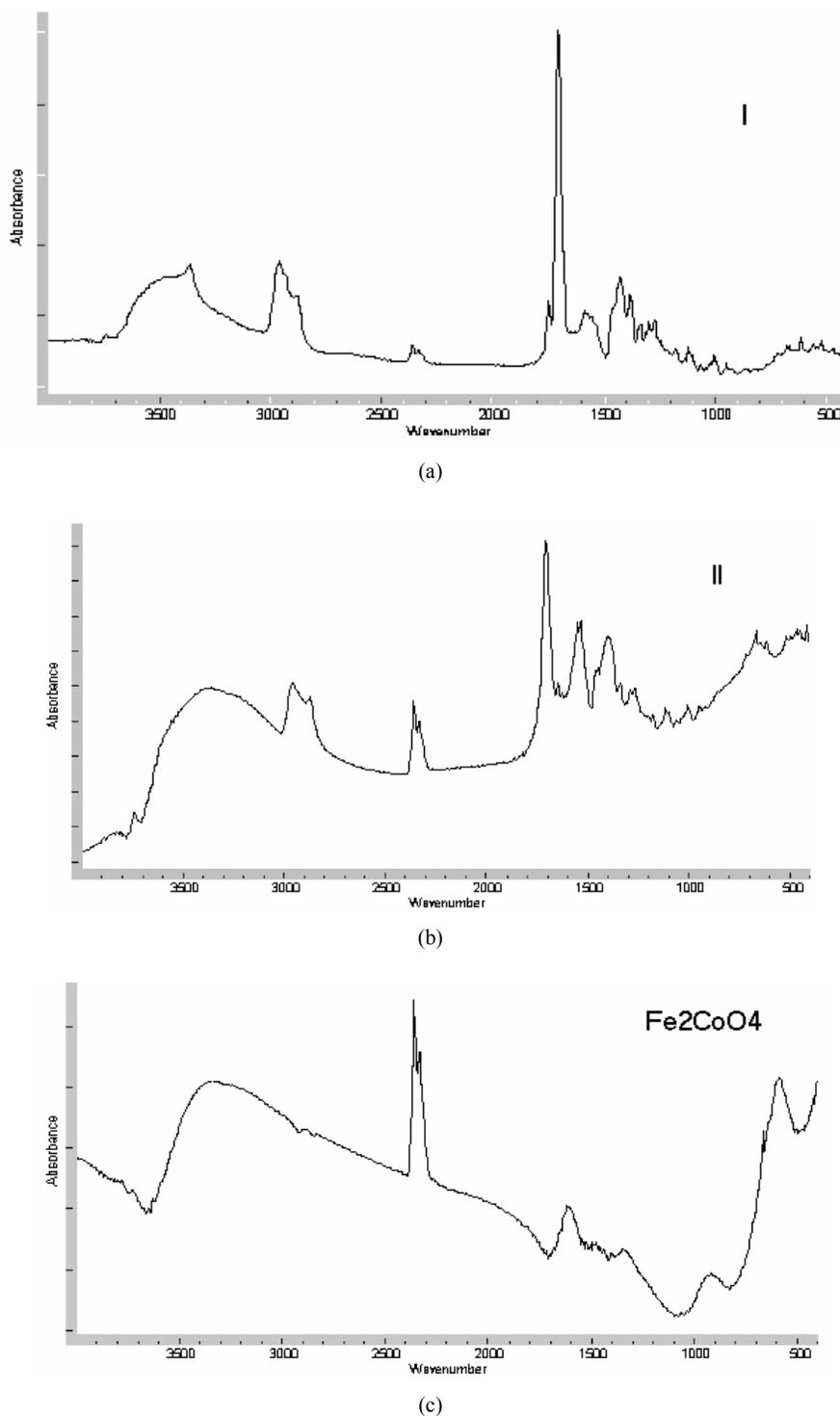
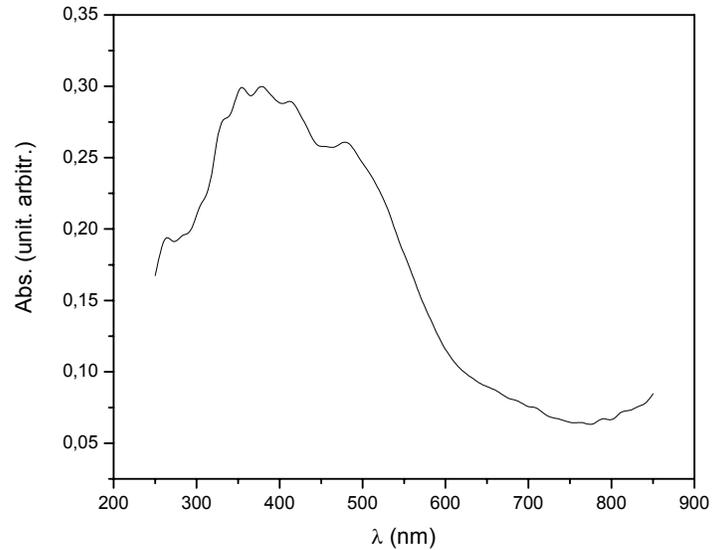


Fig. 2 – IR spectra of: (a) $[\text{Fe}_2\text{Co}(\text{DHC})_6(\text{OH})_2] \cdot 6\text{H}_2\text{O}$ **I**; (b) $[\text{Fe}_2\text{Co}(\text{DHC})(\text{OH})_7]$ **II**; (c) Fe_2CoO_4 .

The electronic spectrum of the compound **I** shows the bands characteristic for an octahedral environment of both the metallic ions, high spin Fe(III) (d^5) and Co(II) (d^7): the forbidden transition band ${}^4A_{1g}, {}^4E_g(\text{G}) \rightarrow {}^6A_{1g}$ (~ 400 nm) and ${}^4T_{1g}(\text{G}) \leftarrow {}^6A_{1g}$ (~ 500 nm) for Fe(III) (d^5) and ${}^4T_{1g}(\text{P}) \leftarrow {}^4T_{1g}(\text{F})$ (ν_3) for Co(II) (d^7). The last band of Fe(III) (~ 500 nm) is overlapped by the band of Co(II) (d^7) (ν_3) (Figure 3).

Fig. 3 – UV-VIS spectrum of compound I.



In Figure 4 are shown the nanocrystals' X-ray diffraction patterns corresponding to cobalt ferrite obtained by thermal decomposition of the compound I (a) and by coprecipitation in the reaction medium (b). The XRD shows a finite broadening of the diffraction lines that provides an estimate of particle sizes from the Scherrer formula: $d_{\text{domain}} = 0.9\lambda/\beta\cos\theta$. The average diameter of particles calculated from the full width at half maximum of the peaks is in the range 26 – 41 nm in case (a) and 5 – 7 nm in case (b).

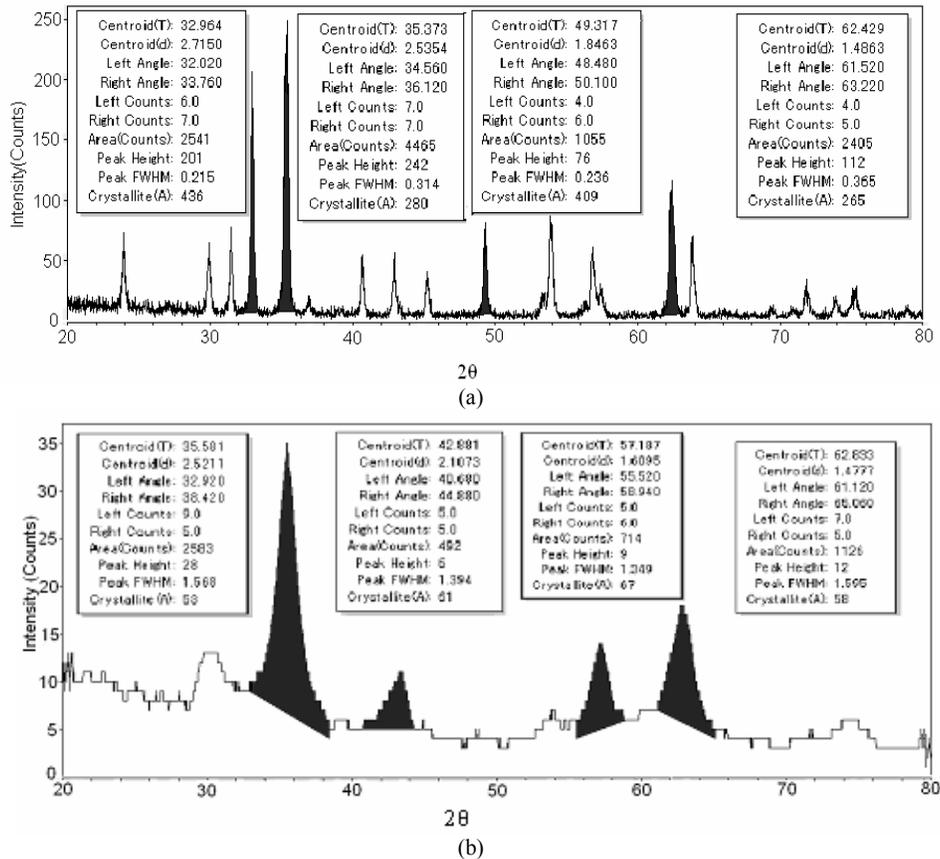


Fig. 4 – XRD spectrum of cobalt ferrite obtained: (a) by thermal decomposition of the compound I; (b) by coprecipitation in the reaction medium.

In order to observe the magnetic behaviour of these cobalt ferrites, magnetization vs. H/T were performed (Figure 5). The saturation magnetization value of cobalt ferrite obtained by coprecipitation in the reaction medium (18.3 emu/g – curve (a)) is much smaller than that of the cobalt ferrite obtained by thermal decomposition of the compound **I** (62.56 emu/g – curve (b)) because its nanostructural character (particle size 5–7 nm) which induces superparamagnetic effects.

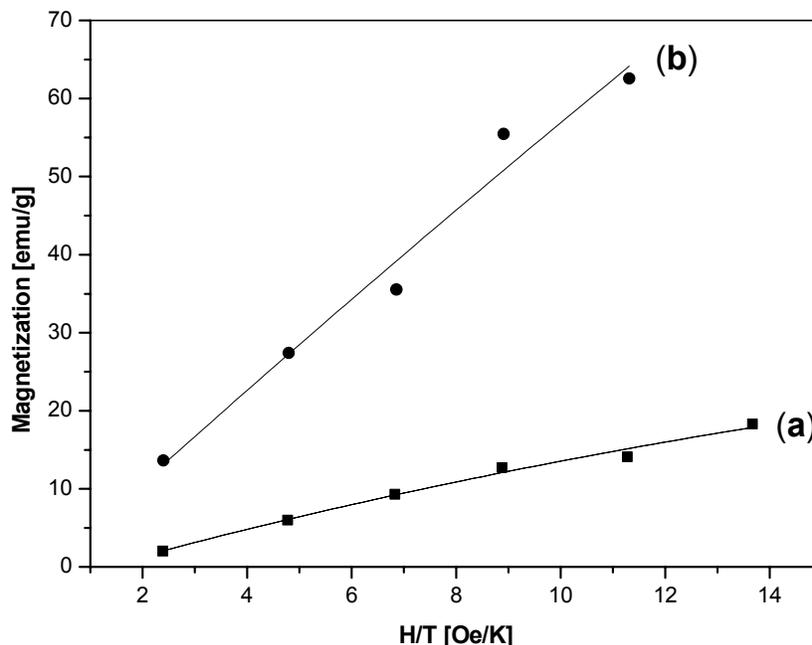


Fig. 5 – Magnetization vs. H/T for: (a) Fe_2CoO_4 obtained by thermal decomposition of the compound **I**; (b) Fe_2CoO_4 obtained by coprecipitation in the reaction medium.

CONCLUSIONS

In conclusion, relatively monodisperse cobalt ferrite nanoparticles were synthesized from iron(III) and cobalt(II) ions in the presence of sodium dehydrocholate and characterized by IR and UV-VIS spectroscopy, X ray diffraction and magnetic measurements. The FTIR spectra allow us to verify the type of attachment of the dehydrocholate anion to the particles. Finally, because of the small sizes of the nanoparticles, these should be of considerable value in numerous areas of biomedical research including bioingestion and bioimaging.

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