Cobalt ferrite nanowhiskers as $T_2$ MRI contrast agent†

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A novel, one-step synthesis of one-dimensional cobalt ferrite nanowhiskers (CfW) is reported. SQUID studies confirmed that the mono-dispersed surfactant-free CfW exhibit superparamagnetic behaviour. This characteristic makes CfW an ideally suited contrast agent for $T_2$-weighted MRI, thus proving to be very effective for biomedical applications.

One-dimensional (1D) nanostructured materials like nanowires, nanowhiskers and nanorods have attracted enormous interest because of their small dimension, thermodynamic stability, physicochemical properties and great potential for various applications such as drug-delivery, optics, optoelectronics and biosensors.1–3 Some magnetic nanostructures, especially cobalt ferrite, are known hard magnetic materials with high saturation magnetization4 and high coercivity, having good chemical and physical stability and high magneto-crystalline anisotropy,5 gaining utilization in MRI6 and hyperthermia.7 Magnetic spinel ferrites are efficient MRI contrast agents (CA) as they accelerate spin–spin relaxation of adjacent water molecules ($T_2$ or transverse relaxation) rather than spin–lattice relaxation ($T_1$ or longitudinal relaxation). The efficiency of such agents is influenced by shape, size, composition, atomic structure and surface properties.8 The greatest concern for MRI CA is that they must be hypersensitive even at low dosage, allowing high contrast MR images.9 CoFe$_2$O$_4$ (CF) is an efficient $T_2$ weighted CA as compared to magnetite, since CF has same saturation magnetization due to iron oxide, but possesses a higher order of crystalline anisotropy thus causing slower magnetic moment relaxation in comparison to magnetite.10

In this communication, a novel and reproducible one-pot synthesis route for CfW preparation is reported using coprecipitation method13 without any surfactants. Magnetic studies showed that CF was highly superparamagnetic and cytotcompatible in nature. Hence these nanowhiskers were used as MRI agents, thus proving to be an efficient $T_2$-weighted CA.

CoFe$_2$O$_4$ was obtained by using 4 mM of ferric(iii) chloride (FeCl$_3$·6H$_2$O) (97%) and 2 mM of cobalt(II) nitrate hexahydrate (Co(NO$_3$)$_2$·6H$_2$O) (99.999%) as precursors. 10 ml of 1.5 M solution of NaOH was used as a reducing agent which was kept under nitrogen atmosphere at 80 °C for 1.5 h (pH 12). The reaction is as follows:

$$2\text{Fe}^{3+} + \text{Co}^{2+} + 8\text{OH}^- \rightarrow \text{CoFe}_2\text{O}_4 + 4\text{H}_2\text{O} \quad (1)$$

The black solid powders were separated by using a strong magnet then washed with distilled water and ethanol for 3 times. The black precipitate was redispersed in isopropanol and used for characterization studies.

X-ray diffraction patterns (in ESI Fig. S1a†) illustrate the crystalline structure of CfW. It is clear that whiskers are highly crystalline and all characteristic peaks for the cubic spinel cobalt–ferrite phase14 (JCPDS card no. 22-1086) match well with the crystal planes of pure CF as demonstrated by selected area electron diffraction (SAED) pattern (in ESI Fig. S1b†).

A low-magnification TEM image of CfW in Fig. 1a depicts individual nanowhisker with an aspect ratio (length/diameter) of 40–90. HRTEM image (Fig. 1b) indicates diameter of nanowhisker to be 15 nm. The inset shows Inverse Fast Fourier Transform (IFT) diffractogram displaying well crystallized CfW with well-defined lattice fringes showing d-spacing of 0.25 nm which corresponds to the (311) spinel planes of the ferrite crystal phase with high regularity and negligible disorderliness.
Magnetic properties of CfW were evaluated using SQUID. Magnetization field-dependent curve obtained at 5 K (in ESI Fig. S2†) displays characteristics attributable to a soft ferromagnet, showing coercivity, $H_c = 146$ Oe, remanence, $M_r = 7.5$ emu g$^{-1}$, and saturation, $M_s = 74$ emu g$^{-1}$ (see inset). However, the remanence ratio, $M_r/M_s = 0.1$, was lower than that expected for a soft ferromagnet ($M_r/M_s = 0.8$) and even lower than that observed on nanostructure systems with uniaxial anisotropy ($M_r/M_s = 0.5$). This feature suggests that there is other source of anisotropy apart from magnetocrystalline anisotropy, controlling the relaxation of magnetic moment of nanostructures. This may originate from whisker-like morphology and close coupling of individual CfW.

Fig. 2a shows the magnetization field-dependent curves of the CfW measured at 300 and 312 K. As it was observed, the CfW lack $H_c$, although there is a measurable $M_r$ of 0.39 and 0.24 emu g$^{-1}$ at 300 and 312 K, respectively (see inset). The existence of a measurable remanence along with its sudden waning off at low coercive field suggests demagnetizing interaction among particles at these temperatures.

Fig. 2b shows zero field cooling (ZFC) and field cooling (FC) measures of CfW. It states that ZFC curve describes a continuous increase in magnitude of magnetization as temperature increases. Moreover, there is a remarkable irreversibility between ZFC and FC, as FC magnetization is well above that observed in ZFC curve. These features explain the departure of ZFC curve from a state where magnetic moments are randomly oriented. However, as temperature increases certain amount of magnetic moments are de-blocked, thus contributing to magnetization of the sample during ZFC measurements. Furthermore, the reorientation of magnetic moments is gradually blocked as the temperature decreases.\(^{10}\)

From the Fig. 2b it is clearly observed that the blocking temperature of $T^\text{ZFC}$ CfW is 320 K. However, the rather broad and flat peak is due to the particle size distribution and the interactions makes the determination of $T^\text{ZFC}$ rather unreliable.

The role of CfW as a CA for MRI was investigated. Nanowhiskers (Fig. 3a) showed dark $T_2$-weighted MR images in concentration dependent manner. These measurements depend on local de-phasing of the spins following the application of transverse energy pulse, leading to $T_2$ relaxation process. The MR contrast capability of nanowhiskers was tested using a 7T clinical MRI system by employing spin echo sequence using fixed repetition time (TR) of 2000 ms and varying echo time (TEs), (from 15 ms to 250 ms) for the $T_2$ and $T_1$ measurements (in ESI†). Suspensions of nanowhisker at varying concentrations (0.01–0.5 mM) of CfW were prepared in distilled water (Dw) in 1.5 ml microcentrifuge tubes with Dw as a control. On evaluating the $T_2$-weighted images of nanowhiskers in Dw, a noticeable darkening is seen with decreasing signal intensity and thereby negative contrast was observed with increasing CfW concentrations. This negative contrast is mainly due to the magnetic field causing inhomogeneity, which affects the microenvironment and cause dephasing of the magnetic moment of protons. However, the $T_2$ signal intensity of nanowhiskers was relatively strong because of CfW concentration, thus proving to be efficient MRI CA. The MR imaging data obtained in this experiment was also in agreement with the magnetization data recorded using SQUID, indicating that nanowhisker showed excellent superparamagnetic characteristics.

The calculation of $r_2$, transverse relaxation rate from the linear plot slope of the inverse of $T_2$ against the CfW concentration (Fig. 3b) was determined to be approximately 256.3 mM$^{-1}$ s$^{-1}$.\(^{11}\)
Recently, it is proposed that the nanoparticles exhibit different role as a contrast agent in intracellular and perfusion phase. So, the relaxivity of CfW labelled L6 cells with similar concentration (0.01–0.5 mM) were studied using agarose phantom gel. Surprisingly, the $r_2$ value of CfW labelled cells is decreased to 177.59 mM$^{-1}$s$^{-1}$. This is due to reduction of proton diffusion coefficient inside the cell, by means of enhanced viscosity and the interactions with the cellular membrane. For instance, the internalization of magnetic nanoparticles inside the cell especially in endosomes decreases the $r_2$ value from 357.3 mM$^{-1}$s$^{-1}$ to 248 mM$^{-1}$s$^{-1}$.

The obtained $r_2$ of CfW is higher as compared to the reported $r_2$ value of Resovist (164 mM$^{-1}$s$^{-1}$) and also much higher than that recently reported by Nidhin et al., for flower-shaped assembly of cobalt ferrite nanoparticles. The $r_1$, longitudinal relaxation rate, was found to be 2.367 mM$^{-1}$s$^{-1}$ for CfW in Dw and 1.4024 mM$^{-1}$s$^{-1}$ in agarose gel. This decrement in $r_1$ relaxivity can be due to reduced hydration effect, causing diminished propinquity of water molecules to CfW. Therefore, these relaxivity measurements make CfW a potential candidate for $T_2$ MRI CA.

There are two different behaviors existing for $R_{1,2}$-CfW concentration plots obtained in water as well as in agarose gel:

1. $R_{1,2}$-CfW concentration plots obtained in water

The $R_2$-CfW graph shows linearity until 0.3 mM, but after that, the behaviour of the graph turns out to be sublinear. This may be due to clumping or aggregation and heterogeneous distribution caused by whisker shaped morphology. This is reciprocated from the zeta potential results, which confirms the value of $-$13.3 mV at pH 7.4. Previous reports indicated that iron aggregation greatly amplifies local diffusion gradient leading to increased transverse relaxivity. Similar behaviour was observed for $R_1$-CfW plots.

2. $R_{1,2}$-CfW concentration plots obtained in agarose gels

The $R_2$-CfW plots exhibit linearity up to 0.1 mM and then it shows a curvilinear relationship. This may be due to the random particle distribution in and outside the cells causing inter-cellular and intra-cellular anisotropy as well as whisker shaped morphology. It also arises because static refocusing causes insensitivity of $R_2$ at higher CfW concentrations. Similar trend was seen for $R_1$-CfW plots also.
Towards the comprehension of physiological stability of CfW, zeta potential (ζ) has been measured by incubating CfW in distilled H₂O, Phosphate buffered saline and Dulbecco’s Modified Eagle’s medium (DMEM)-2%Fetal Bovine Serum (FBS) at pH 7.4. The iso-electric point (IEP) of CfW in PBS was 4.4 which is higher than the IEP in distilled H₂O (2.8) (in ESI Fig. S3†). This is due to electrostatic and steric repulsion existing in physiological buffer. Further, ζ values for DMEM-2% FBS was −35.9 which is considered to be higher as compared to PBS (−29.6) and d/w H₂O (−13.3) as shown in ESI Table 1.† Higher ζ values correspond to higher stability which can be corroborated by the adsorption of proteins on to the CfW. There is no drastic change in ζ values even after 7 days which was confirmed in ESI Fig. S4.†

The cytocompatibility of CfW was demonstrated by analysing cell viability [[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] MTT assay] using L6 (skeletal muscle cell lines) cells. These cells were exposed to CfW at different concentrations ranging from 100–1000 μg ml⁻¹ for 24 h. L6 cells demonstrates more than 75% of viability even at very high concentrations of 1000 μg ml⁻¹ (Fig. 4a). Fig. 4b depicts the confocal image of Phalloidin-Rhodamine and Hoechst staining which doesn’t show any disruption of cellular and nuclear membranes up to 500 μg ml⁻¹ but as the concentration increases, there is gradual rise in the mortality of the cells.

Conclusions

CoFe₂O₄ nanowhiskers were synthesized by a facile co-precipitation method. The smooth nucleation and regulated growth of whisker led to anisotropic nanostructures exhibiting superparamagnetic features at room temperature. From MR imaging it proves to be a potential contrast agent. Moreover, such as-synthesized nanostructures exhibit high stability and cytocompatibility, which suggests that it can be used for biomedical applications. Further investigations of the various properties of these nanowhiskers are underway.

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References


