Magnetic Nanoparticles for Diagnosis and Medical Therapy

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Abstract Magnetic nanoparticles (MNPs) reveal promising opportunities for biomedical applications, potentially allowing minimally invasive diagnosis and therapeutic usage at several levels of human body organization (cells, tissue and organs). An increasingly broad collection of MNPs has been recently developed not only at the research level but also in some specific cases for medical applications. Superparamagnetic iron oxide (SPIO) nanoparticles are commonly used in clinical practice as contrast agents for magnetic resonance imaging (MRI) of liver and angiography. Carbon nanotubes (CNTs) are another type of nanomaterials with great potential for biomedical applications. Filled with ferromagnetic materials, an ensemble of aligned CNTs displays a highly non-linear, anisotropic and hysteretic magnetization behaviour due to their extremely high aspect ratio (length/diameter >100). The intrinsic properties of such ferromagnetic nanoparticles can potentially improve diagnosis and therapy of numerous diseases. Combining tailored biocompatible ferromagnetic nanomaterials with dedicated detection technology can provide a new approach leading to the exciting perspective of accurate medical imaging and medical therapy (magnetic hyperthermia, targeted drug delivery, etc.) at the cellular level. Elongated Fe-filled CNTs (Fe-CNTs) are foreseen as potential nanotools leading to minimally invasive, highly sensitive, and cost effective novel investigation routes for complete human body systems.

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1 Introduction

During the last two decades dedicated magnetic nanoparticles (MNPs) have been developed for application in various fields in science and engineering. Different MNPs have been developed for various purposes, for example for immunoassays, cell separations, biosensing, and protein binding studies [1]. For several clinical procedures in vivo application of MNPs is approved. The fact that the human body is almost non-magnetic is crucial for these medical applications. These particles are used in a growing number of MRI applications such as angiography and liver imaging [2–4]. MNPs have been developed with a wide range of magnetic and hydrodynamic properties. The particles are designed to be easily movable in liquids, excited magnetically or detected inside non-magnetic tissue. Nanoparticles functionalised with a wide range of bio-compatible molecules have been internalized in cells. Most of the research has been focussed on one specific type of nanoparticles called SPIO nanoparticles. Most of these SPIO particles are spherical and consist of one or multiple magnetic cores and a shell with further functionalisations designed for specific biomedical applications. These MNPs display superparamagnetic properties, which render them useful and efficient for diagnosis of diseases. SPIO nanoparticles are also foreseen as a promising tool for in vitro immunoassays [5]. Another noteworthy type of MNP is gadolinium based nanoparticles, which show paramagnetic properties and are mainly used as contrast agents [6].

In the case of in vivo diagnosis as an MRI technique, SPIO nanoparticles are used as so-called contrast agents. The contrast agent can be used to image the blood flow immediately for angiography, for example to detect stenosis. The SPIOs can also be used for diagnosis of other health-related problems. While flowing in the bloodstream of a patient, the SPIO nanoparticles are recognized by the reticuloendothelial system, and after opsonisation, internalised by macrophages. This path will eventually transport the particles to the liver and spleen [5, 7]. This way, SPIO nanoparticles are successfully used to image accurately the lymphatic system and cancerous lesions in the liver or spleen of patients. Unfortunately, this passive route restricts the use of SPIO nanoparticles to a few organs, i.e. liver and spleen and limits the time window for angiography.

Active targeting of SPIO nanoparticles as a tool for magnetic drug delivery has not been successful in the past decades [8–15]. Due to their spherical shape, SPIO nanoparticles are subject to strong drag forces when injected into the blood flow of patients, while the magnetic response of the particles to clinically applicable magnetic fields is rather low. The particles can therefore not be easily trapped by a magnetic field at a specific area in the body. The spherical shape is a main drawback of SPIO nanoparticles and these have proven their limits [16]. To increase the potential of the MNP research has focused on the development of elongated MNPs; so-called magnetic nanowires or nanorods. Among these magnetic nanorods one new and unique type of MNPs is magnetic carbon nanotubes (CNTs). CNTs are quasi one dimensional anisotropic structures of rolled sheets of graphene, that have shown their potential in various biomedical fields [17–21]. The carbon shell can be used for functionalisations that optimise the properties of the nanoparticles for the desired biomedical applications and render them biocompatible. In magnetic CNTs the carbon shell also protects the magnetic content from the external environment. The high potential of magnetic CNTs in medicine lies in the fact that the shape, size, surface chemistry and magnetic properties can be controlled.

2 Ferromagnetic Carbon Nanotubes

In most biomedical applications, the CNTs used do not contain any magnetic content, except for a small quantity of catalyst remaining from their synthesis process. These unfilled CNTs do therefore not show significant magnetic properties but only reflect the magnetism of the remaining catalyst (unfilled Fe-cat-CNTs). The absence of natural magnetism of carbon denotes that significant magnetization can only be accomplished through hybridization with magnetic materials, e.g. Co, Fe, Ni and alloys like Fe_3Co [22]. This hybridization can be achieved by encapsulation, incorporation within the walls or depositing the material on the outer surface of the nanotubes, both during the synthesis or in a subsequent process [23–25].

Due to the extremely high aspect ratio (length/diameter >100) of CNTs, an ensemble of aligned magnetic CNTs shows a highly non-linear anisotropic and hysteretic magnetisation curve [26]. Furthermore, individual magnetic CNTs have a strong magnetic moment with a preferred direction along their axis [27]. Exactly this property makes these magnetic CNTs exceptionally interesting for novel hyperthermia treatments, local temperature sensing and drug delivery [28, 29].

Fe-Filled Carbon Nanotubes

As other proposed methods will alter the properties of the CNTs, filling the CNTs is considered the most favourable method to add magnetic properties to the particles. Filling a CNT with magnetically susceptible liquids can be achieved by opening the CNTs and subsequent filling with magnetic materials. Another method is by simultaneous growth of CNTs with the chemical vapour deposition technique and growing a metallic nanowire within the CNT, rendering so-called Fe-filled multi-walled CNTs (Fe-MWCNTs or Fe-wire-filled MWCNTs) [30, 31].

When evaluating the magnetic properties of a sample of Fe-MWCNTs many aspects have to be taken into account as they influence the magnetic properties. First the length and diameter of the inner nanowire will determine the shape of the magnetization curve. Secondly, the number of walls will influence the mechanical stresses and therefore the magnetic properties. Furthermore, the alignment of the

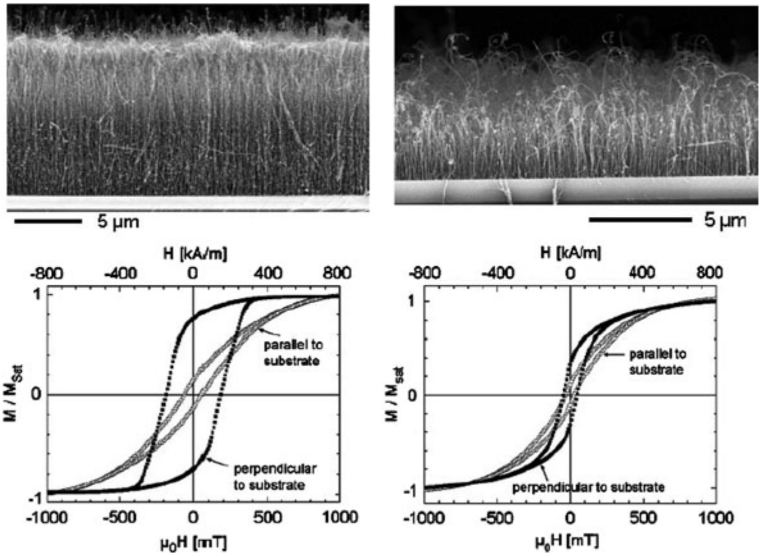


Fig. 1 Comparison of hysteresis loops of strong (*left*) and suboptimal (*right*) aligned filled CNTs at $T = 300$ K [26]

nanotubes will have a large influence on the magnetization curve (Fig. 1) as randomly orientated nanotubes will be partly in a non-favourable orientation for magnetization.

The magnetic properties of the particles will also change when the particles are suspended in different media. The relaxation characteristics will change when particles are in suspension and able to show Brownian relaxation, or fixed in a matrix, such as frozen or in agar gel when only Néel relaxation is possible. These variations in relation to the medium are studied in our laboratory and will be discussed below. In this chapter, magnetisation characterisation of MWCNTs has been performed using a vibrating sample magnetometer (Model 10 Mark II VSM, Microsense). As for complex magnetic susceptibility a homemade set-up has been used [32].

Finally, not only the amount of filling but also the phase of the filling of the nanotube is important. For the Fe-MWCNTs mentioned in this work, the main component of the CNT filling is ferromagnetic α-Fe (bcc phase). Nevertheless, γ-Fe (fcc phase) and paramagnetic Fe_3C can also be found in insignificant proportions [31, 33, 34].

Pristine Fe-Filled Carbon Nanotubes

Prior to further studies on magnetic properties of elaborated Fe-MWCNTs based suspensions and phantoms, magnetisation of pristine Fe-MWCNTs and unfilled

Fe-cat-MWCNTs has been studied. It is noteworthy that unfilled MWCNTs have similar physical properties (average length, diameter, etc..) as Fe-MWCNTs, but they do not contain a magnetic filling with nanowires; remaining catalyst material is nevertheless present in measurable quantities (3 wt%) and causes a weak magnetisation of the unfilled Fe-cat-MWCNTs.

At room temperature ($T = 300$ K) magnetisation at saturation of pristine Fe-MWCNTs is determined to be 9 emu/g; whereas unfilled MWCNT magnetisation at saturation is a factor of 5 lower (Fig. 2). The magnetisation curve clearly indicates a ferromagnetic behaviour of pristine Fe-MWCNTs due to the highly non-linear anisotropic characteristics.

Suspensions of Fe-Filled Carbon Nanotubes

Pristine Fe-MWCNTs and unfilled MWCNTs were used as a starting material to obtain CNT suspensions. These suspensions are of great interest for the fundamental understanding of the properties and the behaviour of individually dispersed Fe-MWCNTs in biological media.

Pristine Fe-CNTs are difficult to disperse in water-based solutions. Furthermore, when brought into suspension, individual Fe-CNTs are hardly kept into stable suspensions over a significant period of time necessary to any biotechnological use. This is mainly due to the fact that individual Fe-CNTs are subject to attractive interaction (Van der Waals and magnetic dipolar forces) when they are dispersed in aqueous solutions. A way to prevent flocculation of Fe-MWCNTs is to create a repulsive (electrostatic or steric) interaction which will hinder

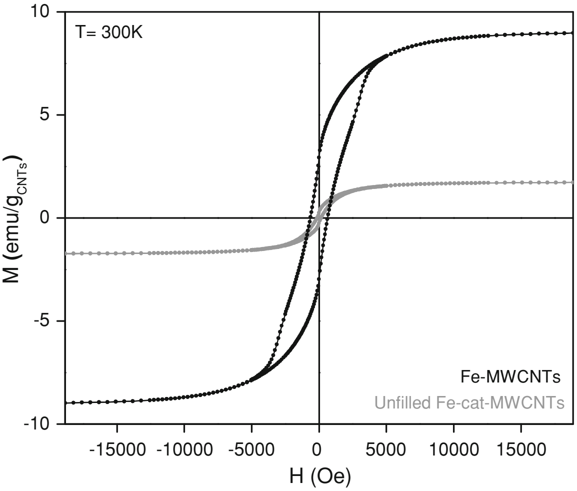


Fig. 2 Magnetisation curves of pristine Fe-MWCNTs and unfilled Fe-cat-MWCNTs at $T = 300$ K

aggregation of Fe-CNTs in large clusters and consequently their sedimentation due to gravity.

Stable suspensions of individually dispersed Fe-CNTs can be obtained using non-covalent functionalisation. This latter has been implemented for both pristine Fe-MWCNTs and unfilled MWCNTs (as a control). Several different biologically compatible dispersants (CMC-Na salt, polyphenylalanine–lysine (Lys:Phe, 1:1), PL-PEG-NH_2 and RNA) have been selected for this purpose. In aqueous media, all these dispersants feature a total electrostatic charge which generates a repulsive interaction between individually dispersed CNTs when non-covalently functionalised.

Stability of these different Fe-MWCNT suspensions was verified over several months using reliable and quantitative UV/Vis spectroscopy (data not shown). This analysis in combination with AFM and TEM imaging (Fig. 3) and studies of chemical properties [ζ-potential (data not shown)] of the suspensions indicated carboxymethylcellulose (CMC) Na salt as the dispersant leading to the most stable suspensions. For all following experiments, all the suspensions were prepared using CMC-Na salt as dispersant.

The magnetic properties of nanowire-filled Fe-MWCNT suspensions were studied at room temperature ($T = 300$ K). Suspensions of Fe-MWCNTs clearly possess a superparamagnetic-like (Fig. 4) behaviour, with a magnetization at saturation of 4.40 emu/g mL^{-1}. For comparison, unfilled Fe-cat-MWCNTs (Fig. 4) show a magnetization at saturation of 0.80 emu/g mL^{-1}. Therefore, the solubilisation process does not alter the ratio of the mass magnetization at saturation between both types of MWCNTs. Nevertheless, the solubilisation process that involves selection of individually dispersed MWCNTs reduces the mass magnetization at saturation by a factor of 2 for both types of MWCNTs.

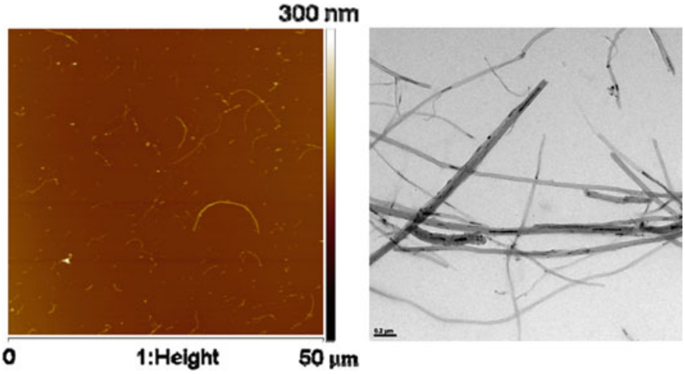


Fig. 3 AFM (*left*) and TEM (*right*) images of Fe-MWCNT suspensions using CMC-Na salt as dispersant

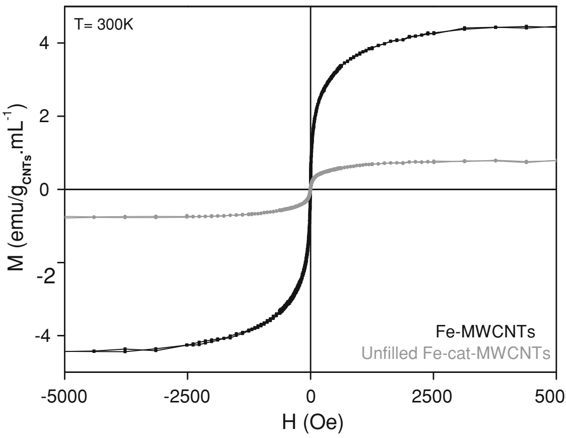


Fig. 4 Magnetisation curves of nanowire-filled Fe-MWCNTs and unfilled Fe-cat-MWCNTs suspensions at $T = 300$ K

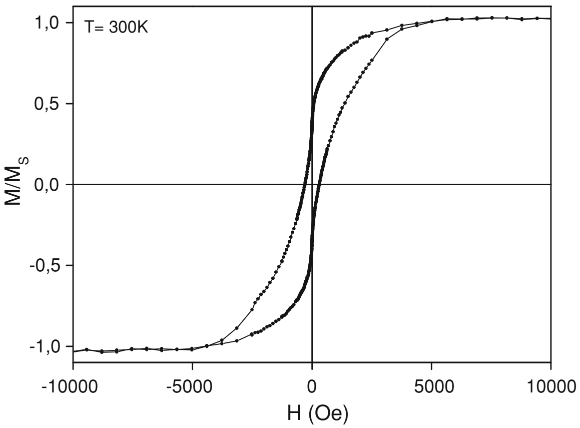


Fig. 5 Magnetisation curve of nanowire-filled Fe-MWCNTs in agar based phantoms at $T = 300$ K

Fe-Filled Carbon Nanotubes in Phantoms

Stable nanowire-filled Fe-MWCNT suspensions display superparamagnetic-like behaviour at $T = 300$ K as seen previously (Fig. 4). When brought into agar based phantoms (from suspension) in order to mimic embedded Fe-CNTs in tissue, magnetic response is significantly changed when excited by an external magnetic field at $T = 300$ K. The main characteristic of the agar based phantoms is the high viscosity of the sample (gel-like sample), restricting freedom of movement of Fe-MWCNTs embedded in the phantom. Figure 5 shows how phantoms affect the magnetisation of Fe-MWCNTs. In this case, Fe-MWCNTs clearly display a ferromagnetic curve when they are homogeneously dispersed in phantoms. This is established by the data in Fig. 5 which shows the magnetisation curve of Fe-MWCNTs embedded in a phantom. The data clearly show a hysteretic behaviour of Fe-MWCNTs at $T = 300$ K which is similar to the magnetisation curve of pristine Fe-MWCNTs in powder form (Fig. 2).

Mobility of Fe-Filled Carbon Nanotubes in Aqueous Media

As seen previously, nanowire-filled Fe-MWCNT suspensions display superparamagnetic-like behaviour when exposed to an external magnetic field. This is due to Brownian relaxation of the Fe-MWCNTs. The experimental data shown in Fig. 6 demonstrate that the complex magnetic susceptibility ($\chi = \chi' - j\chi''$) of Fe-filled MWCNTs goes to zero at frequencies above 1,000 Hz. Above 5 Hz, the real and imaginary components are the same, whereas below 5 Hz, the real component keeps increasing at decreasing frequency and the imaginary component possibly reaches a plateau around 0.3 Hz. The response is due to the rotational motion of the particles in the solvent, particles that apparently have a permanent magnetic dipole moment with a component along the long axis of the particles. Then, a frequency of the order of 0.3 Hz seems plausible for Fe-MWCNTs having a length of several micrometers.

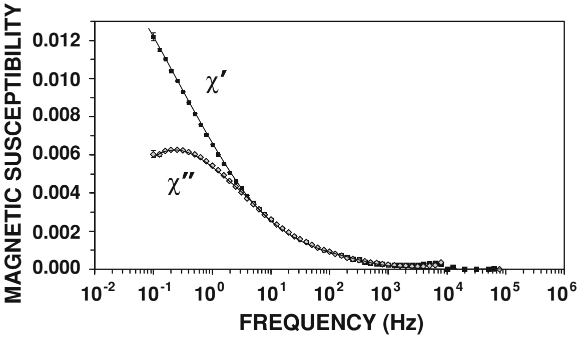
On the basis of

$$f_{\mathrm{char}} = \frac{12k_{\mathrm{B}}T\ln r}{\eta L^3} \quad [35]$$

where r is the aspect ratio, η is the viscosity (0.001 Pa s), and L is the length of the rods, assuming a diameter of 60 nm, the length corresponding to 0.3 Hz is 2.5 μm. This does assume infinite dilution, which is not the case, giving an order of magnitude of several micron long Fe-MWCNTs.

The analyses of the complex magnetic susceptibility and relaxation of the magnetic particles can be used in biomedical applications especially non-invasive sensing. The variations in temporal response of Fe-MWCNTs to a rapidly changing magnetic field can be used to analyse interactions between the particles and the environment.

Fig. 6 Complex magnetic susceptibility of nanowire-filled Fe-MWCNTs in suspension at $T = 300$ K



3 Conclusion

Magnetic nanoparticles show promise for biomedical applications. Potential applications include minimally invasive diagnosis and therapy. Nanowire-filled Fe-MWCNTs are proposed as unique nanocontainers applicable for magnetic sensing and therapy in near future. The high potential of CNTs in medicine lies in the fact that these particles show anisotropic magnetic behaviour. Furthermore, their shape, size, magnetic properties and surface chemistry properties can be controlled potentially rendering CNTs biocompatible and inert to the environment.

Thus, these nanoparticles show a highly non-linear anisotropic and hysteretic magnetisation behaviour due to their extremely high aspect ratio. This behavior changes when the particles are brought into stable suspension. In this case, the suspensions show superparamagnetic-like behaviour with a mass magnetization at saturation approximately half of the value measured on the pristine MWCNTs. This decrease in magnetization at saturation is attributed to the fact that in the solubilisation process a selection is made on individually dispersed Fe-MWCNTs, which are likely to be smaller and less filled with Fe. On the other hand we analysed samples mimicking tissues. In these measurements the Fe-MWCNTs were not able to move or rotate with the applied magnetic field. Therefore the hysteresis curves are more open compared to the measurements on powder and especially suspensions.

These magnetic measurements were done as a first step potentially leading to new non-invasive sensing methods using Fe-MWCNTs in in vivo diagnosis. The application of nanotechnology and dedicated magnetic detection technique may permit cost efficient diagnosis and treatment of various diseases.

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