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Research Article

MICROWAVE ASSISTED SYNTHESIS OF MAGNETIC NANOPARTICLES WITH HIGHER RELAXIVITIES AS CONTRAST AGENTS FOR MRI

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ABSTRACT

There is considerable interest in developing magnetic nanoparticles and their surface modifications with therapeutic agents. The study involves the synthesis of biocompatible HPPH [2-(1-Hexyloxyethyl)-2-devinyl pyropheophorbide-a], a cancer drug coated with iron oxide nanoparticles and to evaluate their efficacy as MRI contrast agents. A simple and quick microwave method to prepare Fe_3O_4n anoparticles has been developed. Microwave heating offers potential advantages over conventional heating to enhance chemical reactions. The relaxivities (r_2) of the coated magnetic nanoparticles were also measured and the results showed that r_2 of the Fe complex was higher than that of MRI contrast agent Gd-DTPA used in clinics. The drug was successfully conjugated to the Fe_3O_4n anoparticles which can be used for various applications such as photodynamic therapy, hyperthermia etc.

INTRODUCTION

Nanoparticles (NPs) are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic or molecular structures. This is typically because NPs have a greater surface area per volume as compared to larger particles which causes them to be more reactive than their bulk counterparts. The research is currently an area of intense scientific interest due to a wide variety of potential applications especially in biomedical and catalytic fields (R.Taylor *et al*., 2013). In the past several decades about 40 percent of newly designed drugs, especially those which are based on biomolecules such as peptides, oligonucleotides, proteins and DNA, often exhibit low bioavailability and are rejected by the pharmaceutical industry (J. L.Vivero-Escoto *et al*., 2010). To achieve these goals, numerous materials have been extensively investigated, such as amphiphilic block copolymers (R.Gref *et al*., 1994; B.Jeong *et al*., 1997; D. A. Hammer 1999), liposomes (V. P. Torchilin *et al*., 2005), dendrimers (C.Gao *et al*., 2004; C. C. Lee *et al*., 2005), hydrogels (N. A.Peppas *et al*., 2000; N. A. Peppas *et al*., 2006) as well as NPs (J. L.Vivero-Escoto *et al*., 2010; D. M. Jeftinija *et al*., 2003). MagneticNPs (Fe_3O_4) is one of the most attractive nanomaterial for various biomedical applications such as multifunctional theranostic agent, biological detection, magnetic resonance imaging (MRI) contrast agents, cell sorting, drug delivery and targeted therapy (F. H.Chen *et al*., 2008; R.Hergt *et al*., 1998; D. L.Huber 2005). Imaging has been widely used in scientific and technological applications due to its visual and intuitional interface. In particular, biological imaging has been a rapidly growing field, not only in fundamental biology but also in medical science (H. B.Na *et al*., 2009). In particular, MagneticNPs are excellent MRI contrast agents for non-invasive cellular and molecular imaging though metallic Fe, Co, and Ni are highly magnetic, their use in biological applications is limited due to their toxicity. Nevertheless, iron oxides are very safe in clinical use due to their excellent biocompatibility. Magnetic NPs dispersed in composites usually have strong tendency to form agglomerates for reduction of energy associated with high surface area-to-volume ratio of nano-sized particles. To avoid aggregation of magnetic NPs, protection strategies have been developed to chemically stabilize the naked magnetic NPs by grafting of or coating with organic species, including surfactants or polymer or coating with an inorganic layer, such as silica or carbon. The incorporation of these functionalized magnetic NPs in polymer or other matrices for the development of magnetic nano-composite material proved to be more effective (S.Kalia *et al*., 2014). An important medical application of polymer coated

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iron oxide NPs as imaging agents allows for conjugation of functional Ligands. Coating helps against degradation after synthesis (protection against oxidation). Surface properties of magnetic NPs are the main factors determining colloidal stability. This can be improved by controlling surface charge and specific surfactant. Increase in surface area increases material's chemical reactivity and ability to connect with additional functional materials. Magnetic NPs labeled with targeting agent (polymers) with high affinity for tumors can pass through the blood-brain barrier (BBB) and successfully target brain tumors in mouse which has been proved experimentally. This highlights one advantage of Magnetic NPs over commonly used Gd chelates (R. S.Chaughule *et al*., 2014).

Magnetic NPs (MNPs) offer some attractive possibilities in biomedicine. MRI shows high spatial resolution (~100 μm), long effective imaging window, rapid *in vivo* acquisition of images, and the absence of exposure to ionizing radiation (L.Li *et al*., 2013). Due to its low sensitivity, inclusion of contrast agents improves the quality of images and accentuates differences between normal and diseased lesions. Thus contrast agents play an important role for the analysis of the biological information and the diagnosis of the diseases (D. L.Huber 2005). The relaxation rates or the relaxivity of water proton is dependent on the given Fe_3O_4 concentration. Thus improvement in NPs size, development of magnetic core materials increases the relaxivity also it depends on the molecular structure and kinetics of the complex. To increase the number of water molecules that are in the inner sphere of the complex, or to slow down the molecular rotational correlation time, are the possibilities to improve the water relaxivity (S.Mornet *et al*., 2004). In clinical practice, it is often necessary to add a substance (contrast agent) to the system under observation in order to enhance contrast by altering its intrinsic relaxation times for MRI. The images can be enhanced by reducing the longitudinal (T_1) and transverse (T_2) relaxation times of the water protons which is often done by the use of contrast agents such as gadolinium chelate (G.Strijkers *et al*., 2007). To get the T_2 MRI contrast, the magnetic dipole moment is induced in SPIONs under an applied magnetic field. When water molecules diffuse into the outer space of the induced dipole moment, the magnetic relaxation processes of the water protons are perturbed and T_2 is shortened. This shortening of T_2 is a signal reduction, compared to the surrounding water protons, appearing dark region on a T_2 weighted MR image.One of the important criteria for the contrast agents is to have high magnetic moment, it is well documented that the coating particles controls oxidation (e.g., spin-canting) and thus the reduction in magnetization is controlled (A. G.Roca *et al*., 2009).

Synthesis of Nanoparticles

There are several publications available for the synthesis of magnetic NPs that include highly stable, shape controlled and mono dispersed NPs. These include co-precipitation, thermal decomposition, micelle synthesis, hydrothermal synthesis, laser pyrolysis etc. Though there is significant development in the synthesis procedure, maintaining the stability of these NPs for a long time without agglomeration is a prime concern. With this point in mind we tried to develop a microwave method for synthesis of NPs for good stability and dispersity.

Microwave Method

The microwave technique that we carried out in our laboratory is simple, rapid, convenient and significant for the synthesis of magnetic NPs. Microwave heating offers several potential advantages over conventional heating or enhancing chemical reactions. The formation of magnetic NPs using microwave requires only few minutes and this is much faster than other reported methods.

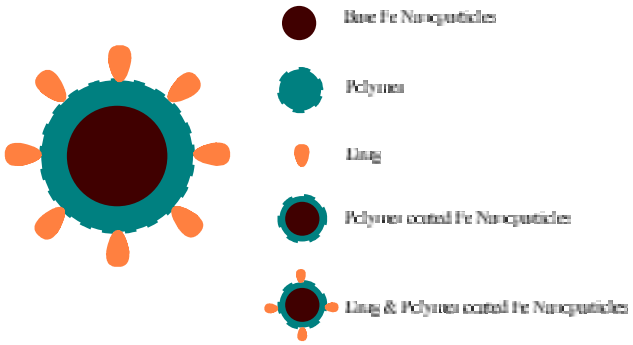
In a snap cap tube of microwave, 30 mM of Iron (III) nitrate nonahydrate [$Fe(NO_3)_3.\ 9H_2O$] dissolved in water was taken and kept for constant stirring while during constant stirring add 0.025 M ascorbic acid dissolved in ethanol (all chemicals were purchased from Sigma Aldrich and were 99 % in purity) kept the above solution for stirring for few minutes and the solution was irradiated in the Microwave Synthesizer (CEM Discover mono mode) for 3 minutes. The reaction was carried out under dynamic condition at 130^0C and 65 W of power the power max was off as the reaction mixture did not require simultaneous cooling. After the microwave process gets over we observe that there is formation of black precipitate of Fe_3O_4 NPs. Wash the above formed precipitate of Fe NPs with Chloroform for 3 to 4 times to remove impurities and then dry the above precipitate in oven at 60^0C. After drying, the Fe NPs were kept in muffle furnace for 1 hour at 500^0C.

Polymer Coating to Fe NPs

For PEG coating, iron oxide nanoparticles were washed with ethanol and dried in an oven at 100 °C for 30 min. Ten milligram iron oxide nanoparticles were mixed with 5 mL of 3 mM Polyethylene glycol (MW 1000, Sigma Aldrich) and sonicate for 1 h. The mixture was washed thoroughly with ethanol, and centrifuged for 5 min at 5000 rpm.

ConjugationofDrug

HPPH coated Fe_3O_4 magnetic NPs were prepared with concentration 4×10^{-2} mM of HPPH added with 1 mg/mL of Fe_3O_4-PEG magnetic NPs by slight stirring for 60 minutes. After stirring the negatively charged HPPH combined with positively charged Fe_3O_4-PEG thus resulted in HPPH- Fe_3O_4-PEG magnetic conjugates.

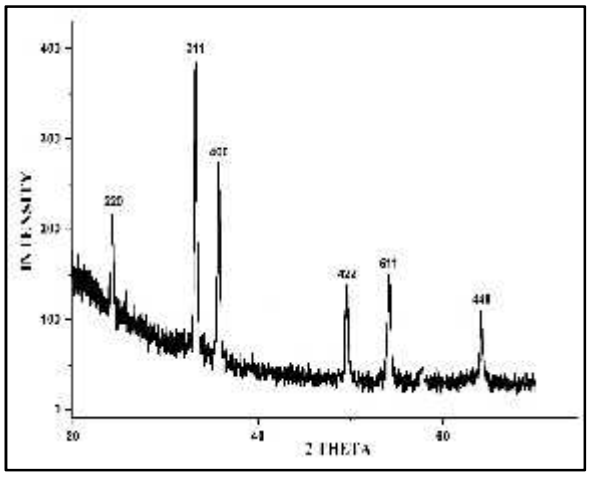


Characterization

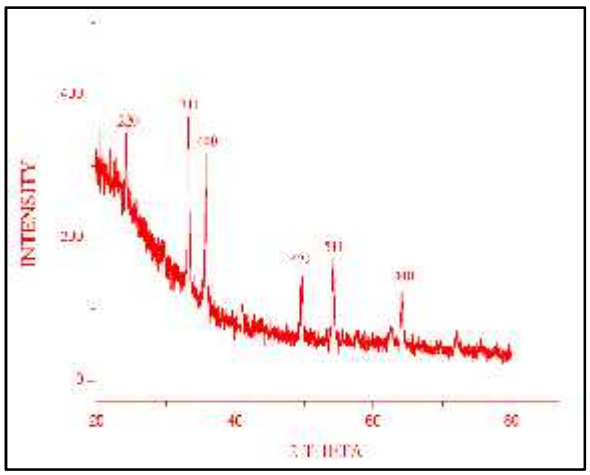
X-ray Diffraction

XRD measurement was used to identify the crystalline structure of the products. As shown in Fig.1 the X-ray diffraction pattern of bare Fe_3O_4 NPs recorded in Xpert Pro MPD operated with Cu Kα (1.5405 Angstrom)match well with

the characteristic peaks of inverse cubic spinel structure (JCPDS 19-0629), which indicate the crystalline structure of Fe_3O_4 NPs it also indicates that the crystallite size can be remained after surface modification. All the peaks are well crystallized. The broadness of the peaks indicates small crystallite size.



a



b

Fig. 1 X-ray diffraction pattern showing (a) $Fe_3O_4$and (b)Fe_3O_4–PEG NPs

The size calculation was done using Scherrer formula. It is used to calculate particle size using the following relation

$$D = K^{a} / \cos^{-}$$

Where, D is the mean size of the ordered (crystalline) domains, which may be smaller or equal to the grain size, K is a dimension less hape factor, with a value close to unity, λ is the X-ray wavelength, β is the line broadening at half the maximum intensity (FWHM), θ is the Bragg angle.

From calculations the sizes of bare Fe_3O_4 NPs were 26.47 nm and that for Fe_3O_4 -PEG NPs were 28.86 nm. The difference between bare NPs and coated NPs were 2.39 suggesting the coating of the polymer to the magnetic NPs.

Energy Dispersive X-ray Spectroscopy

The energy dispersive X-ray spectroscopy is used for the elemental analysis or chemical characterization of the sample. It relies on the interaction of source of X-ray excitation and a sample. Fig. 2 is the EDS spectrum of Fe_3O_4 NPs which show the peaks of the elements Fe and O that confirms the formation of Fe_3O_4 NPs.

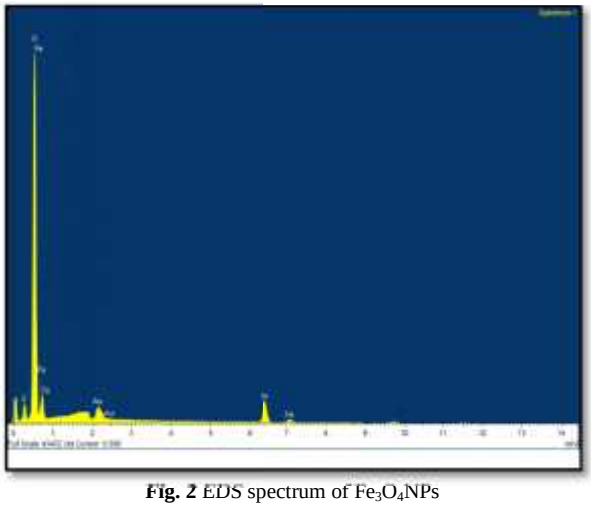


Fig. 2 *EDS* spectrum of Fe_3O_4NPs

Infrared Spectroscopy

Fig.3 shows the infrared spectrum of Fe_3O_4NPs. The analysis was done using Perkin Elmer Infrared Spectrum unit version 10.03.07. In the IR spectrum, it can be seen that the characteristic absorption of Fe-O bond is at 580 cm^{-1} and 634 cm^{-1}, while that of -OH bond is at 3398 cm^{-1}The peaks at 2855 cm^{-1} is from the vibration of long alkyl chain -CH_2 and -CH_3.The O-H stretch, C-H stretch and Fe-O stretch show the formation of Fe_3O_4 NPs with the coating of Polyethylene glycol.

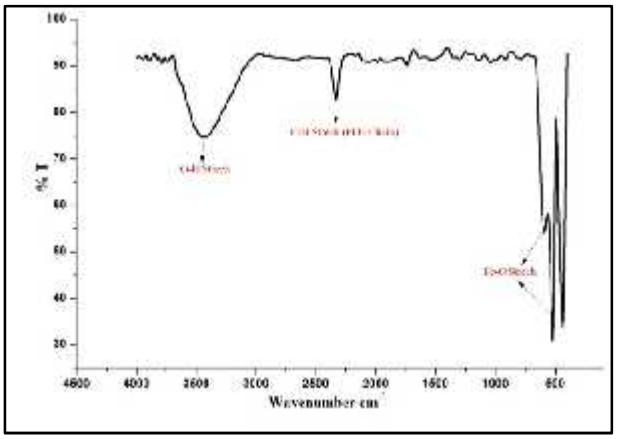


Fig 3 Infrared Spectrum Showing Fe_3O_4 and Fe_3O_4–PEG NPs

Scanning Electron Microscope (SEM) Studies

Figs. 4 and 5 show the SEM images of Fe_3O_4- PEG-DrugNPs. The analysis was carried out using model Zeiss Merlin 6073 Compact. It is seen that the obtained NPs are uniform in size. The average size of NPs from the above analysis was in the range around 30 nm.

RESULTS AND DISCUSSION

MRI is one of the most widely used and powerful tools for non-invasive clinical diagnosis due to soft tissue contrast, spatial resolution and penetration depth. Contrast agents are evaluated on the basis of their relaxivity or how much relaxation rates of

water protons are increased in the presence of the MRI contrast agent (e.g., magnetite NPs) at a given concentration.

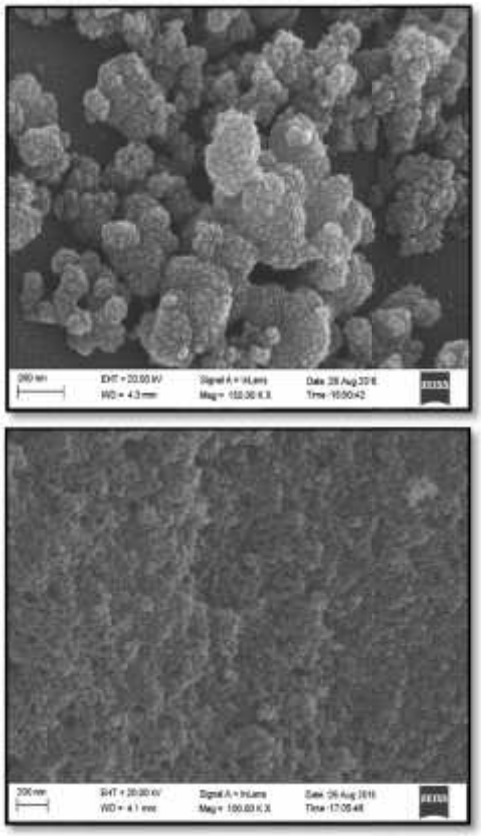


Fig. 4 & 5 zoom in and zoom out SEM images of Fe_3O_4NPs coated with PEG and Drug

A T_2 image with no contrast agent was taken as reference. Magnetite NPs were dispersed in water and diluted to various concentrations (0.0375, 0.075, 0.1, 0.2, and 0.5 mM) and pH of the solutions were adjusted to 7. A 5 ml glass sample holder was placed in the iso-center of the magnet using head coil. Spin-echo pulse sequences were utilized to obtain T_2 maps of each sample and the r_2 relaxation rates were obtained from the reciprocal of obtained T_2 results. MR imaging capabilities of the magnetite NPs were examined at 3 T with the parameters point resolution of 320×256 matrix and 4 mm of slice thickness. TE was varied between 15 to 90 ms with TR value of 3420 ms. Fig. 6 show the T_2-weighted MR images. The degree of the T_2 contrast effect is typically represented by the spin-spin relaxivity r_2 (r_2= $1/T_2s^{-1}$), where higher values of r_2 result in a greater contrast effect.

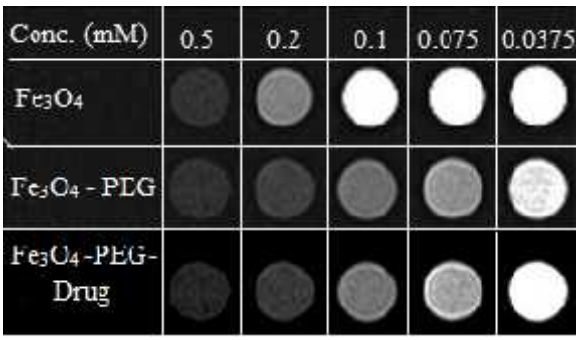


Fig. 6 MRI Images of Fe_3O_4, Fe_3O_4-PEG, Fe_3O_4-PEG-Drug For Fe conc. of 0.5, 0.2, 0.1, 0.075, 0.0375 mM in Water

The plot of relaxation rates ($1/T_2$, s^{-1}) of Fe_3O_4, Fe_3O_4-PEG and Fe_3O_4-PEG-Drug versus iron concentration in water in the magnetic field of 3 T at room temperature is shown in Fig.7. T_2 values were calculated using Free Induction Decay (FID) of the sample using relation

$$\mathbf{M^2 = M_0\, e^{2/T_2}}$$

Where Mτis the intensity of the FID signal at time τand $M_0$is the intensity of the signal at time τ = 0.

From the graph of the Fe concentration dependent relaxation times, the relaxivity r_2 was determined. T_2 is reduced by the presence of super paramagnetic nanoparticles which means that T_2 is influenced much more by the coating of magnetic NPs. From Fig. 7 it is seen that as the particle sizes of the bare Fe NPs, polymer coated Fe NPs and polymer -drug coated Fe NPs are increased, the relativities are also increasing accordingly which is in agreement with the results reported earlier (J.H.Lee *et al*., 2007).

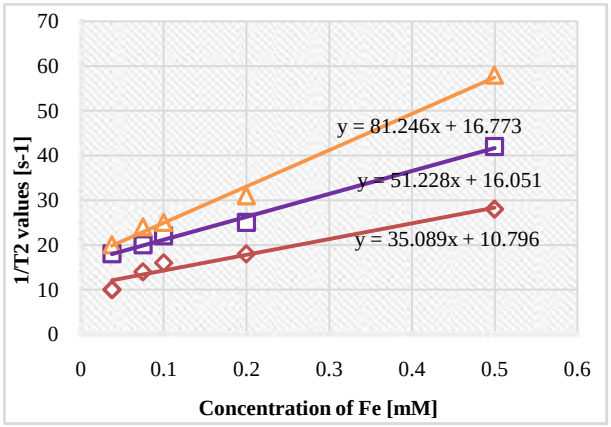


Fig. 7 Plot of $1/T_2$ versus concentration of Fe_3O_4, Fe_3O_4-PEG and Fe_3O_4-PEG-Drug △Fe_3O_4-PEG-Drug □ Fe_3O_4-PEGFe_3O_4 ◇

CONCLUSION

We synthesized iron oxide (Fe_3O_4) nanoparticles by using Microwave Synthesizer, and coated them with biocompatible Polymer PEG (Polyethylene glycol) and a photosensitizer drug HPPH [2-(1-Hexyloxyethyl)-2-devinyl pyropheophorbide-a].The sizes of microwave synthesized are Fe NPs, polymer coated Fe NPs and polymer-drug coated Fe NPs were 26.47, 28.86 and 30 nm respectively. The coated nanoparticles were found to be spherical in SEM images with a core shell structure, and showed a uniform size distribution.T_2 relaxation is mainly influenced by outer sphere processes. Relaxivity decreased with increase in overall particle size. From MRI it is seen that theT_2 relaxivity increases linearly with increase in concentration of Fe NPs in water. The T_2 relaxivity of polymer coated Fe NPs and polymer -drug coated Fe NPs also increases than bare Fe NPs due to the increase in hydrodynamic sizes.It is also concluded that increase in coating thicknesses increases the overall relaxivity.

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