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Chitosan Functionalized Fe_3O_4@Au Core-Shell Nanomaterials for Targeted Drug Delivery

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***Abstract*—**Chitosan functionalized Fe_3O_4-Au core shell nanoparticles have been prepared using a two-step wet chemical approach using $NaBH_4$ as reducing agent for formation of Au in ethylene glycol. X-ray diffraction studies shows individual phases of Fe_3O_4 and Au in the as prepared samples with crystallite size of 5.9 and 11.4 nm respectively. The functionalization of the core-shell nanostructure with Chitosan has been confirmed using Fourier transform infrared spectroscopy along with signatures of octahedral and tetrahedral sites of Fe_3O_4 below 600cm^{-1}. Mössbauer spectroscopy shows decrease in particle-particle interaction in presence of Au shell (72% sextet) than pure oleic coated Fe_3O_4 nanoparticles (88% sextet) at room temperature. At 80K, oleic acid coated Fe_3O_4 shows only sextets whereas the Chitosan functionalized Fe_3O_4 and Chitosan functionalized Fe_3O_4@Au core shell show presence of 5 and 11% doublet, respectively.

***Keywords*—**Magnetic nanoparticles, Fe_3O_4@Au core shell, iron oxide, Au nanoparticles.

I. Introduction

NANOMATERIALS assembled together can result in multifunctional materials leading to exploration of new industrial and biomedical applications [1]. Unlike single-component nanomaterials which usually contain only one unique property of the active ingredient, the ingredients of multi-component materials determine the versatile properties and applications of these materials [2]. Although these materials possess attractive and versatile applications, controlled synthesis of these materials is a difficult task. Magnetic iron oxide nanoparticles (Fe_3O_4 and γ-Fe_2O_3) are leading the way in medical research because of its strong magnetic property [3]. Super paramagnetic iron oxide nanoparticles with appropriate surface chemistry can be used for numerous *in vivo* applications, such as MRI contrast enhancement, tissue repair, detoxification of biological fluids, hyperthermia, drug delivery and cell separation. Fe_3O_4 NPs have been used as negative contrast agents for T2-weighted magnetic resonance (MR) imaging due to their high reflexivity, excellent contrast enhancement and low toxicity. On the other hand, gold nanoparticles provide attractive scaffolds for the creation of transfection agents [4]. Gold colloids are bio-inert and nontoxic. They also provide a multifunctional platform for both therapeutic and diagnostic purpose. Through functionalization, these particles can be engineered to accumulate preferentially at tumor sites using targeting ligands, providing a powerful tool for cancer gene therapy.

Gold coated iron oxide nanoparticles (Fe_3O_4@Au and γ-Fe_2O_3@Au) are known to have a wide range of promising applications in biomedical imaging because they exhibit facile functionalization, enhanced chemical stability, excellent biocompatibility, superparamagnetic capability and strong X-ray attenuation property [2], [5]. Gold-coated nanoparticles have great biocompatibility with the human body with the ability to interact with polypeptides, DNA, and polysaccharides. Chitosan [poly-b-(1-4)-2-amino-2-deoxy-D-glucose] has many favorable characteristics such as low toxicity and high biocompatibility [6]. It has been widely used in many fields, such as waste water treatment, biomedical applications as a drug carrier, therapy for repairing spinal damage and for preserving nervous cell and mitochondrial membranes from harmful reactive oxygen species. Gold nanocomposites utilizing Chitosan offer several potential benefits using the magnetic core for controllability, as well as the immobilization of biomolecules and other optical properties through their gold shell. Li et al. [1] demonstrated that Fe_3O_4@Au core shell nanostars provide a unique multifunctional the ranostic platform for multi-mode imaging and photothermal therapy of tumors, which may find applications in theranostics of different types of cancer. Fe_3O_4@Au core shell nanomaterials have also been used as a base material for development of sensitive and selective hydrolase biosensor based on self-assembly of methyl parathion hydrolase for detecting methyl parathion [5]. Li et al. demonstrated that oligonucleotide-gated silica shell-coated Fe_3O_4–Au core shell nanotrisoctahedra can act as magnetically targeted and near-infrared light-responsive theranostic platform [7].

In the present study, Chitosan functionalized iron oxide-Au core shell has been prepared using a simple wet chemical method by using $NaBH_4$ as reducing agent. The structural and magnetic studies were performed using XRD, FTIR and Mössbauer measurements.

II. Material Synthesis and Experimental Details

Materials Synthesis: Chemicals used for synthesis of Chitosan functionalized Fe_3O_4@Au core shell nanomaterials were gold acetate (CH_3COOAu), ferric chloride ($FeCl_3.6H_2O$), ferrous sulphate ($FeSO_4.7H_2O$), oleic acid ((9Z)-Octadec-9-enoic acid), Chitosan (poly-b-(1-4)-2-amino-2-deoxy-D-glucose), hydrochloric acid (HCl) and acetone(CH_3CHO). All the chemicals were of analytical grade and procured from Merck. Distilled water with resistivity of 15MΩ cm was used for all the experimental process. The core-shell nanostructure

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has been prepared in a two-step process.

(a) Synthesis of oleic acid coated magnetic nanoparticles: The first step involves synthesis of fine grained Fe_3O_4 nanoparticles prepared through a conventional co-precipitation approach in presence of oleic acid as surfactant. Freshly prepared aqueous solution of $FeCl_3.6H_2O$ (0.4 M) and $FeSO_4.7H_2O$ (0.2 M) were mixed in 1:1 volumetric ratio at a constant stirring speed of 600 rpm at 90 °C. The solution was allowed for 15 minutes of vigorous stirring for uniform mixing. 30 ml of NaOH (2M) solution was added rapidly to above precursor solution for precipitation of Fe_3O_4 nanoparticles. 2 ml of oleic acid was added to the reaction mixture after 5 minutes of addition of NaOH to restrict the growth of nucleated nanoparticles. The nanoparticles were separated using a permanent magnet and washed several times with water and acetone for removal of inorganic and organic impurities.

(b) Synthesis of Chitosan functionalized Fe_3O_4@Au core shell nanostructure: 150 mg of oleic acid capped Fe_3O_4 nanoparticles were dispersed in ethylene glycol by 30 minutes of ultra-sonication. After the dispersion was stabilized, gold acetate was added to the mixture along with 15 mg of Chitosan. The molar ratio of Fe_3O_4 to Au was maintained at 1:2. Dilute

(c) HCl solution was added to the above solution for providing slight acidic condition for dissolution of Chitosan. The solution was kept under vigorous stirring at 180˚C for 3 hrs for adsorption of Au^+ ions on the surface of Fe_3O_4 nanoparticles. $NaBH_4$ (2M) was added to the above solution drop wise for precipitation of Au shell over the Fe3O4 nanoparticles in presence of Chitosan. The reaction mixture was vigorously stirred for 30 minute to allow subsequent growth of the formed nanoshells. Initially, the particles were separated by magnet and then washed several times with acetone and water using centrifugation. Finally, obtained Chitosan functionalized Fe_3O_4@Au core shell nanostructures were dispersed in ethylene glycol.

III. Results and Discussion

Fig. 1 confirms formation of pure spinel Fe_3O_4 nanoparticles with diffraction from (220), (311), (400), (511) and (440) crystal planes. The crystallite size was calculated using Scherrer's formula to be 5.9 nm. The lattice parameter was calculated to be 8.39Å which is in agreement with the reported value of Fe_3O_4 [8]. The diffraction pattern of Chitosan coated Fe_3O_4 shows pure cubic iron oxide phase and the crystallite size was calculated to be 6.6 nm.

Diffraction pattern of Chitosan functionalized Fe_3O_4@Au core shell nanostructure shows presence of both Au and Fe_3O_4 nanoparticles. The presence of Au diffraction patterns along with Fe_3O_4 confirms either formation of a core-shell or hybrid structure. The size of the gold nanoparticles calculated to be 11.4 nm which is 5.5 nm higher than that of the core Fe_3O_4. So the expected shell thickness formed over the iron oxide core is 5.5 nm. The lattice parameter of Au found to be 4.08Å which is in agreement with the reported cubic gold structure [9].

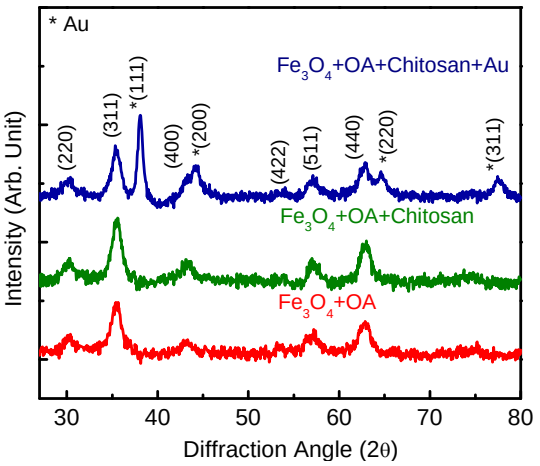

Fig. 1 X-ray diffraction patterns of oleic acid coated Fe_3O_4, Chitosan functionalized Fe_3O_4 and Chitosan functionalized Fe_3O_4@Au core shell nanoparticles

Rietveld analysis of as prepared core Fe_3O_4 and Fe_3O_4@Au core-shell structure were performed using MAUD (Materials Analysis Using Diffraction) software and shown in Fig. 2. Oleic acid coated Fe_3O_4 nanoparticles are confirmed to be pure with little higher error parameters (Rwp=16%, GOF=1.8). This can be attributed to the low intensity and broad peaks of fine grained nanoparticles. Refinement of functionalized Fe_3O_4@Au core shell nanostructure confirms presence of Au and Fe_3O_4 nanoparticles with weight parameters (Rwp=2.55%, Rexp=1.51% and GOF=1.7) in accepted range.

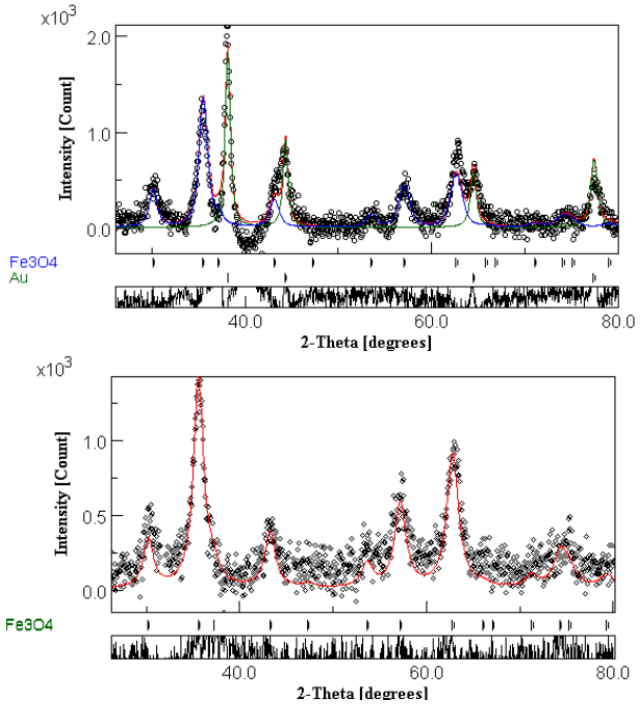

Fig. 2 Rietveld refinement of oleic acid coated Fe_3O_4 and Chitosan functionalized Fe_3O_4@Au core shell nanoparticles

Structural and bonding information of capping have been studied using Fourier Transform Infrared Spectroscopy (FTIR) studies (Fig. 3). In pure oleic acid coated Fe3O4 nanoparticles, absorption bands near 3415cm^{-1} are due to presence of –OH groups of adsorbed water molecules. Absorption bands near 2900 and 2850cm^{-1} can be attributed to the aliphatic –CH bonds of oleic acids.

The bands at 2360, 1553 and 1429cm^{-1} are due to the CO_2, -COO^- and $–CH_2$ groups respectively. Metal oxide (Fe-O) absorption bands from Fe_3O_4 were also observed at 585 and 425cm^{-1} corresponding to tetrahedral and octahedral sites respectively. An additional peak observed at 609cm^{-1} can be attributed to the bonding of Fe items with the O atoms of the carboxylic group in oleic acid. FTIR spectra of Chitosan coated Fe_3O_4 and core shell nanoparticles show a decrease in aliphatic absorption band near 2900cm^{-1} along with other high intense absorption band at 1050cm^{-1} due to –CHOH groups of cyclic ring in Chitosan. The peak near 1550cm^{-1} shift towards higher wave number is due to the –CONH group of Chitosan. However, absorption bands due to metal-oxide bonds below 600cm^{-1} remains intact irrespective of functionalization.

Least square fitted Mössbauer spectra as prepared samples at 300 and 80K are depicted in Fig. 4. Pure oleic acid coated Fe_3O_4 nanoparticles show presence of 12% of doublet due to presence of superparamagnetic particles along with 88% of surface components due to particle interactions. The area of doublet increases to 24% after functionalization of the oleic acid coated Fe_3O_4 nanoparticles with Chitosan as the particle-particle interactions decreases due to presence if Chitosan coating (76% surface components). Decrease in surface components to 72% has been observed upon addition of Au to the system due to further decrease in particle-particle interaction in presence of gold shell.

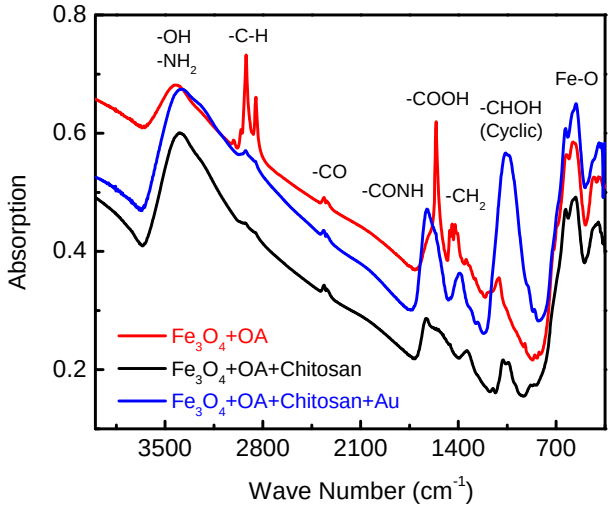

Fig. 3 FTIR spectra of oleic acid coated Fe_3O_4, Chitosan functionalized Fe_3O_4 and Chitosan functionalized Fe_3O_4@Au core shell nanoparticles

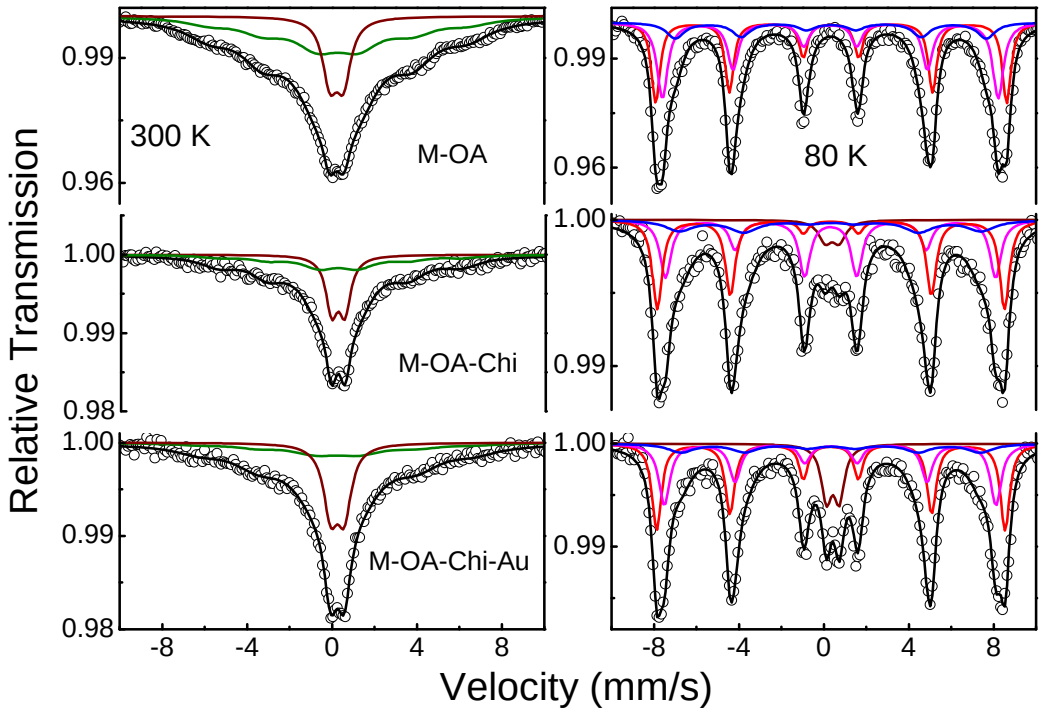

Fig. 4 Mössbauer spectra of oleic acid coated Fe_3O_4, Chitosan functionalized Fe_3O_4 and Chitosan functionalized Fe_3O_4@Au core shell nanoparticles at 300 and 80K

IV. Conclusions

Chitosan functionalized Fe_3O_4-Au core shell nanoparticles have been prepared using a two step wet chemical approach using $NaBH_4$ as reducing agent for formation of Au in

ethylene glycol. X-ray diffraction studies shows individual phases of Fe_3O_4 and Au in the as prepared samples with crystallite size of 5.9 and 11.4 nm respectively. The functionalization of the core-shell nanostructure with Chitosan has been confirmed using Fourier transform infrared spectroscopy along with signatures of octahedral and tetrahedral sites of Fe_3O_4 below $600cm^{-1}$. Mössbauer spectroscopy shows decrease in particle-particle interaction in presence of Au shell (72% sextet) than pure oleic coated Fe_3O_4 nanoparticles (88% sextet) at room temperature. At 80K, oleic acid coated Fe_3O_4 shows only sextets whereas the Chitosan functionalized Fe_3O_4 and Chitosan functionalized Fe_3O_4@Au core shell show presence of 5 and 11% doublet, respectively. Our results show an efficient and simple method to functionalize Fe_3O_4@Au core shell nanostructures with thin shell thickness. Further, the method provides an opportunity to control the shell thickness by controlling the precursor solution.

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