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Synthesis and Characterization of Soy Lecithin Coated Magnetic Iron Oxide Nanoparticles for Magnetic Resonance Imaging Applications

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ABSTRACT

In this work, we report synthesis and characterization of soy lecithin (SL) coated iron oxide nanoparticles by one step co precipitation method in an aqueous solution using ferrous and ferric salts (1:2), different values of SLP (0.0, 1.5 and 6 gr) and ammonia to adjust pH=10. Characterization of the samples (labeled as SPION, "$SPION_{1.5}$ and $SPION_6$) carried out using X-ray powder diffraction (XRD) patterns indicated formation of magnetite (Fe_3O_4) nanoparticles with a calculated average crystalline size of 25 nm for naked Fe_3O_4, 13 nm for "$SPION_{1.5}$" and 9 nm for "$SPION_6$" nanoparticles by Sherrer's equation. FT–IR spectroscopy and Thermo-gravimetric analysis (TGA) were used to investigate the presence of "SL" on the nanoparticles surface. The images and morphology of the samples were examined on scanning electron microscope (SEM). Detailed chemical analysis of the nanoparticles was obtained from energy dispersive X-ray (EDX) data. To measure magnetic properties of the prepared samples, a Vibrating Sample Magnetometer (VSM) was used and a Dynamic Light Scattering (DLS) instrument was finally used to measure hydrodynamic diameter of the nanoparticles. The results revealed that the soybean lecithin was coated on Fe_3O_4 nanoparticles surface and smaller particle size was obtained with increased concentrations of soybean lecithin.

Keywords: Water dispersed solution; Soy Lecithin; Surface modification; Iron oxide magnetic nanoparticles (SPION).

INTRODUCTION

Magnetic Iron oxide nanoparticles (SPION) with appropriate surface chemistry have been attracted a lot of interest for their numerous applications[1-3] such as magnetic resonance imaging contrast enhancement[4,5], immunoassay, detoxification of biological fluids, hyperthermia[6], drug delivery systems[7,8] and in cell separation and immobilization of biomolecules such as nucleic acids or proteins[9], etc. All of these biomedical applications require that these nanoparticles should have high magnetization values and size between 10 to 100 nm[10] with an overall narrow particle size distribution, so that the particles could have uniform physical and chemical properties and

have to be optimized for intravenous injection and most prolonged drug circulation time in the body. Furthermore, magnetic nanoparticles need to have special surface coating to be not only non-toxic and biocompatible but also to allow a targetable delivery with particle localization in a specific area[11]. To this end, to prevent formation of the large aggregates and preparation of an appropriate substrate for functional groups (amines or carboxylic acid) for bio conjugation with certain drugs we attempted to synthesize in one pot soy lecithin protein coated iron oxide nanoparticles using in situ coating co-precipitation procedure. The size, morphology, and magnetization of the prepared samples as well as, the effect of different values of SL coating on the size and magnetic properties of magnetic nanoparticles will be finally reported

MATERIALS AND METHODS

Materials

Ferric chloride hexahydrate ($FeCl_3.6H_2O$) and Lecithin from Soybean were obtained from Sigma-Aldrich. Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$), and ammonium hydroxide 25 wt%, were purchased from Fluka (Buchs, Switzerland).

Methods

X-ray diffraction patterns (PW 1800 PHILIPS), and FT-IR spectra (Perkin Elmer spectrum 100) were used to determine crystal structure of SPION and its lecithin coated products. The morphology, particle size and particle size distribution determination were carried out using Scanning electron microscopy (Philips EM208) and Dynamic Light Scattering (Otsuka, LPA-3000, 3100, Japan) analysis. The presence of lecithin on SPION was studied by Thermogravimetric analysis (TGA 931 TA Perkin-Elmer instruments) and Eltra-cs analyzer. The magnetic properties were finally evaluated using a Vibration Sample Magnetometer (VSM, Quantum Design PPMS-9).

Preparation of SL Coated Iron Oxide Nanoparticles

Soy lecithin coated iron oxide nanostructures were synthesized following a simple one-step coprecipitation approach. Initially, 2.7 g of ferric chloride hexahydrate ($FeCl_3.6H_2O$) and 1.39 g of ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) (molar ratio 2:1, respectively) were dissolved in 50 mL deionized water and the mixture, stirring vigorously under N_2 atmosphere was heated to 85°C and was drop wisely added 200 mL of ammonia solution (25%) containing 0.00, 1.50 and, 6.00 g of soy lecithin deoxygenated by dry nitrogen stirring over a period of 20 min until observing change of color from dark orange to black. Stirring was steadily continued for more than 2 h followed under rapid stirring in N_2 atmosphere. The precipitates were finally removed by magnetic decantation and washed for several times with water to make it free of any residual salts, until obtaining pH= 7. The final products were dried in a vacuum oven at room temperature for 24h.

Preparation of water dispersed solution of SL Coated Iron Oxide Nanoparticles

In order to obtain good hydration of lecithin coated iron oxide nanoparticles, considering that lecithin is a mixture of phospholipids with phosphatidylcholine (PC) as a main component (up to 98% w/w), we prepared a dispersed aqueous solution of lecithin coated iron oxide nanoparticles by dispersing our synthesized lecithin coated iron oxide nanoparticles ($SPION_6$) in water or in an isotonic aqueous solution by means of extensive mixing at temperature 40–60°C.

RESULTS AND DISCUSSION

X-ray power diffraction study

Fig. 1 represents XRD patterns of $SPION_6$ (a); $SPION_{1.5}$ (b) and naked Fe_3O_4 (c) nanoparticles. This results show clearly that, all the patterns are in good agreement with the standard diffraction spectrum (JCPDS Card No. 19-0629)[12] and the synthesized products were crystalline Fe_3O_4. The average particle size was calculated to be at about 25 nm for naked Fe_3O_4, 13 nm for $SION_{1.5}$ and 9 nm for $SION_6$ nanoparticles by using Sherrer's equation:

$$D = K\lambda/(\beta Cos\theta)$$

Where, D is the particle size and K, λ, β and θ denote Sherrer constant, X- ray wavelength, the peak width of half-maximum and the Bragg angle. These results and comparing the crystalline size obtained indicate that, SLP has served as a surfactant and coating agent in the precipitation of Fe_3O_4 nanoparticles and we found that, the size of

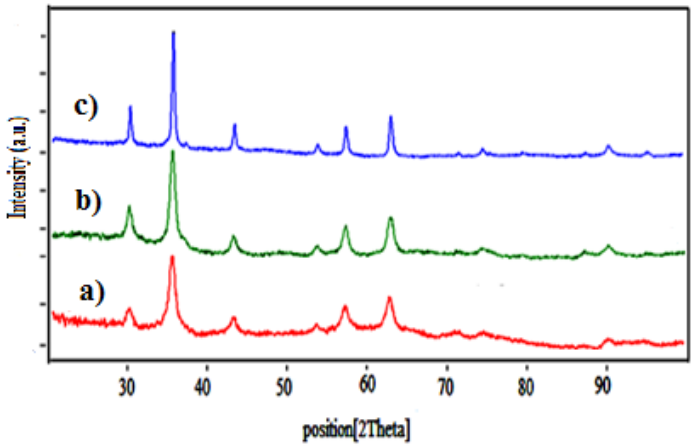

Fig. 1: X-ray power diffraction patterns of: a) $SPION_6$; b) $SPION_{1.5}$ and c) Naked- SPION

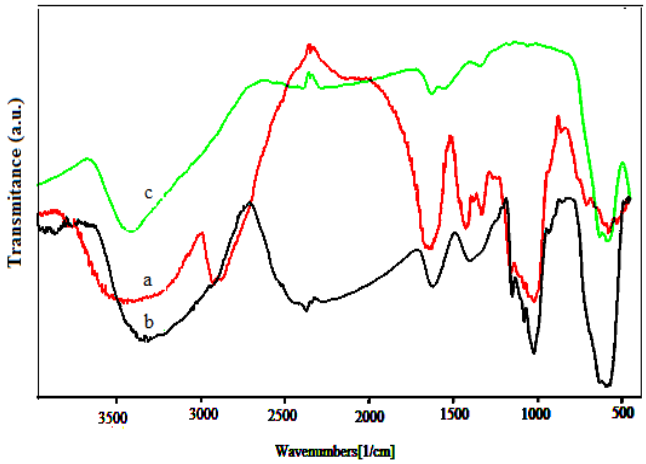

Fig. 2: FTIR spectra of: a) free Soy lecithin protein; b) $SPION_6$; c) SPION

Table 1: FTIR band assignment for naked fe_3O_4, SION6 and free Soy lecithin

Bond	Naked Fe_3O_4	SION	Soy Lecithin
υ (Fe-O)	571	580	
υ (HO-H) stretching	3412		
υ (C=O)		1624	1620
υ (P-O)		1075	1075
υ (C-H ,CH_2 and CH_3)		1322-1400	1331-1427
υ (C-O-C)		1153	1160
υ (choline-containing phospholipids)		1020	1020

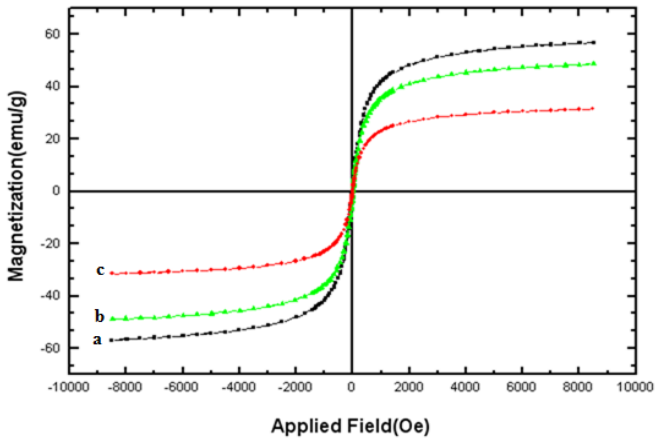

Fig. 3: Magnetic curves of: a) Naked Fe_3O_4, b), $SPION_{1.5}$ and c) $SPION_6$

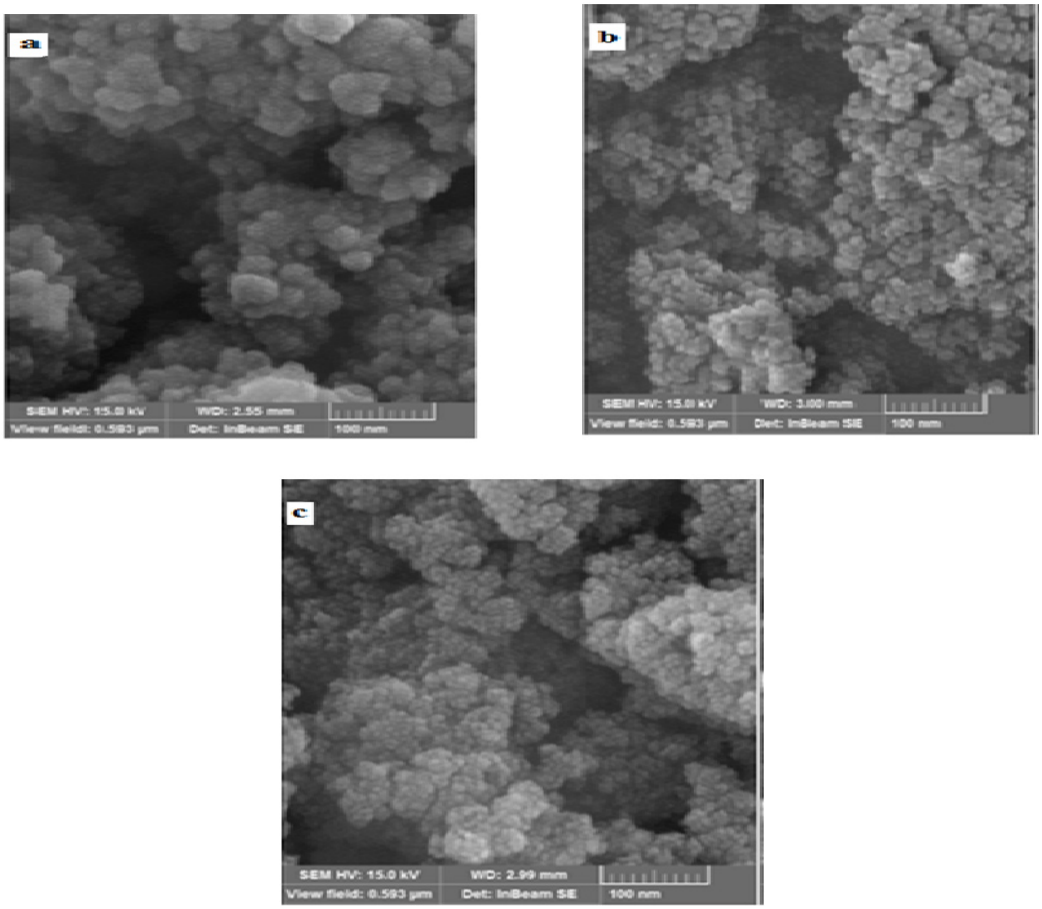

Fig. 4: SEM image of: a) Naked Fe_3O_4, b) $SION_{1.5}$ (b) and c) $SION_6$

coated Fe_3O_4 nanoparticles prepared by this method was decreased by increasing concentration of soy lecithin. This phenomena can be related to the nature of soy lecithin which is a mixture of phospholipids with phosphatidylcholine (PC) as a main component (up to 98% w/w). Noting that, phosphatidyl cholines molecules are amphiphatic (both hydrophilic and hydrophobic), and in accordance with other authors[13], we can conclude that, the SL in aqueous environment can make bilayer structure containing an aqueous phase entrapped by lipid bilayers,[14] labled as vesicles and can be encapsulate a region of aqueous solution containing ferrous, ferric and hydroxide ions within a hydrophobic membrane. As a result, iron oxide nanoparticles were formed in the limited size in the liposomes vesicles.

FTIR study

Fourier transform infrared spectroscopy can be used in order to identify the soy lecithin presence on Fe_3O_4 nanoparticles surface. The FT-IR spectra of the free soy lecithin (a), soy lecithin coated iron oxide nanoparticles $SPION_6$ (b) and Naked (SPION) (c) are given in Fig.2. In this figure, naked iron oxide nanoparticles indicate strong band at 571 cm^{-1} that is assigned to the stretch vibration mode of Fe-O bond. Similar peaks have been observed in the spectrum of the soy lecithin coated iron oxide nanoparticles (SPION) at 580 cm^{-1}. The formation of soy lecithin coating on the surface nanoparticles was confirmed through the comparison of soy lecithin and SPION spectra (Table 1). Other additional bands observed peaks at 3710 cm^{-1} and 3400 cm^{-1}, which indicated the presence of N-H stretching and O-H stretching of amino group and hydroxyl group as well as the bands at about 1245 attributed to the presence of P=O (phosphomoyl) group[15] and appearance of a new broad band at about 2400 cm^{-1} in soy lecithin coated SPION's spectrum can be clearly attributed

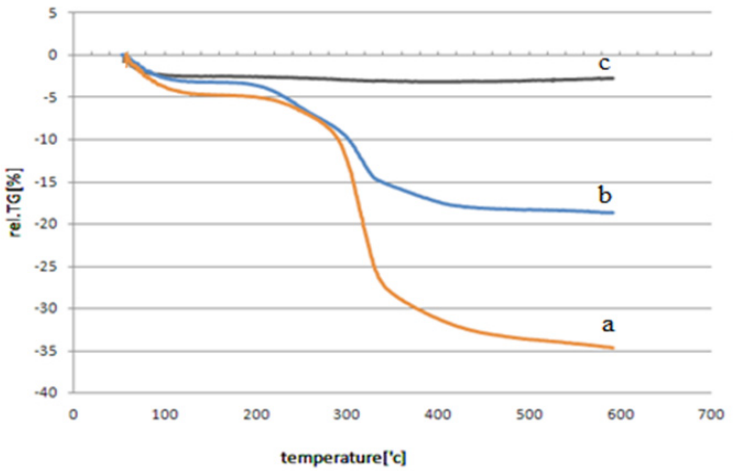

Fig. 5: Thermogravimetric analysis of Naked-Fe_3O_4(c), $SPION_{1.5}$ (b) and $SPION_6$ (a) nanoparticles

Table 2: EDX and eltra-cs analysis data for $SPION_{1.5}$ and $SPION_6$ nanoparticles

	Element	Line	Mass[%]	Atomic[%]	Formula	Mass[%]
$SION_{1.5}$	Fe	K	66.62	33.89	Fe_3O_4	90.68
	O		25.93	46.75		
	C		7.82	18.78		7.82
$SION_6$	Fe	K	57.81	25.47	Fe_3O_4	79.89
	O		22.53	34.65		
	C		19.35	39.64		19.35

to the presence of soy lecithin coats on the SPION nanoparticles.

Magnetic measurements

In order to study nanoparticles magnetic behavior and the effect of soy lecithin coating in magnetic properties, magnetization measurements for naked Fe_3O_4 and soy lecithin coated Fe_3O_4 nanoparticles ($SPION_{1.5}$ and $SPION_6$) were performed. As can be observed Fig.3, all of them have a hysteresis loop with zero coercivity and remanence values. This means that these are single domains with superparamagnetic characteristics. The saturation magnetization value of naked Fe_3O_4, $SPION_{1.5}$ and $SPION_6$ were found to be 56.8, 48.5 and 31.3 electromagnetic units per gram (emu/g) respectively. The reduction in saturation magnetization was likely due to the existence of soy lecithin on the surface of Fe_3O_4 nanoparticles. This reduction is in direct relation with the soy lecithin coating density. Nanoparticles with a denser coating, which have been synthesized in higher soy lecithin concentrations, are less sensitive to a magnetic field[16].

Scanning Electron Microscopy

The morphology of the synthesized was investigated using Scanning electron microscope. The SEM photograph of prepared samples, naked Fe_3O_4, $SPION_{1.5}$ and $SNIO_6$ is shown in Figure 4. SEM image shows that the morphology of the Fe_3O_4 nanoparticles is roughly spherical shape. spherical shapes are usually formed because the nucleation rate per unit area is isotopic at the interface between

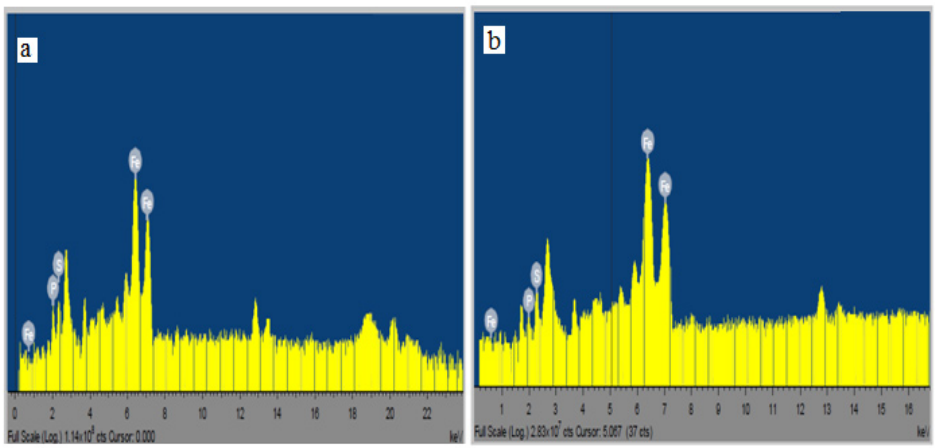

Fig. 6: EDX data curve for: a) $SION_{1.5;}$ b) $SION_6$

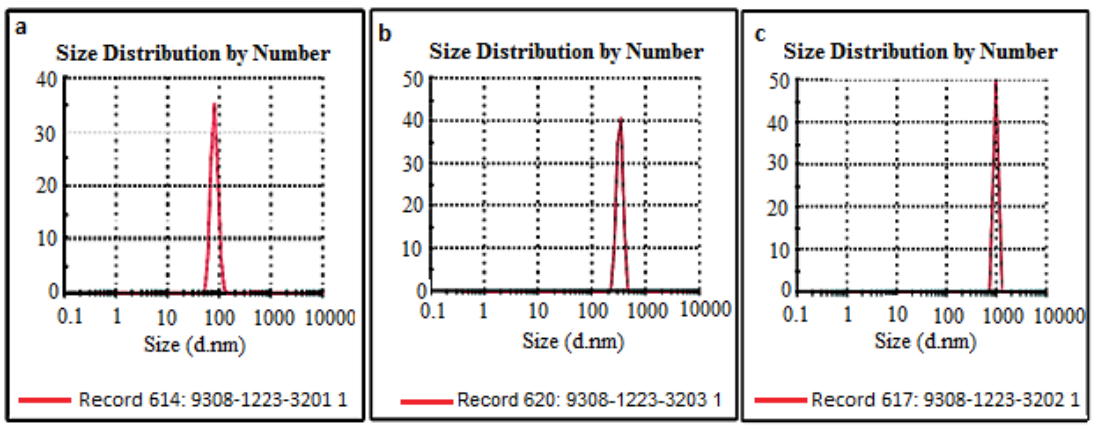

Fig. 7: Particle size distribution of: a) Naked Fe_3O_4; b) $SPION_{1.5}$ and c) $SPION_6$

the Fe_3O_4 magnetic nanoparticles[17], which the driving force for Ostwald ripening, minimization of the surface free energy by reduction of total surface area/volume, results in the equivalent growth rate along different directions of the nucleation because the sphere has the smallest surface area per unit volume of any shape[18].

Thermo Gravimetric analysis

Thermogravimetric analysis (TGA) of naked and soy lecithin coated iron oxide nanoparticles were presented in Figure 5. This analysis was carried out for powder samples (7 mg) in N_2 atmosphere with a heating rate 10 ^{0}C/min from 100 up to 700^{o}C. For naked Fe_3O_4, no signiûcant peak appeared in the TGA curve. Also, it indicates that the weight loss in the temperature ranged from 100 to 300 only around 2%. This may be justified by the loss of water remaining on surface of iron oxide nanoparticles. For soy lecithin coated iron oxide, the TGA curve shows gradual and steady weight loss in the temperature ranged from 200 to 430 ^{o}C results in the burning of soy lecithin coated. As it is shown in the figure, the further soy lecithin concentration**,** the greater amount of weight loss is observable.

Dispersive X-ray study

Fig. 6 and Table.2 represent the data obtained using X-ray energy dispersion (EDX) and Eltra-cs analyzer for as prepared samples, $SPION_{1.5}$ and $SPION_6$. These results confirm the presence of iron (Fe), oxygen (O) with iron abundance higher than oxygen. Eltra-cs analyzer was used to confirm the presence of soy lecithin on surface of Fe_3O_4 nanoparticles by the analysis of carbon. The results indicate that percentage of carbon in $SPION_6$ (19.35) is more than $SPION_{1.5.}$ (7.82). This results shows that, the amount of adsorbed soy lecithin on the nanoparticle surface is higher when we used higher soy lecithin concentration. This results will be confirmed by the thermo-grd avimetry analysis Spectrum.

Dynamic Light Scattering (DLS) study

The DLS measurements results of naked Fe_3O_4 (a), $SPION_{1.5}$ (b) and $SPION_6$ (c) dispersed in aqueous solution are shown in fig. 7. This figure shows that the hydrodynamic diameter for 25 nm particles is 78 nm while for 13 and 9 nm particles, prepared by increased soy lecithin concentration, reveal higher hydrodynamic diameter (342 and 955nm respectively). This results confirms increasing hydrodynamic diameter of the sample by increasing soy lecithin concentration.

CONCLUSION

In summary, soy lecithin coated iron oxide nanoparticles (SPION, $SPION_{1.5}$, $SPION_6$) were successfully synthesized by in situ co-precipitation method. By increasing soy lecithin concentration in the preparation process, the SPION crystalline size decreased from 25 to 9 nm. The presence of soy lecithin on the surface of iron oxide nanoparticles has been confirmed through XRD patterns and FTIR spectra respectively. The roughly spherical morphology of the soy lecithin coated SPION confirms that soy lecithin in aqueous environment has formed bilayer structures ferrous, ferric and hydroxide ions within a hydrophobic membrane. As a result, iron oxide nanoparticles were therefore formed in the limited size in the liposomes vesicles. Investigation performed by TGA and DLS analysis were finally confirmed that, SPION nanoparticles surface was protected by soy lecithin layers as a surfactant.

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