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Chitosan-Iron Oxide Nanoparticle Preparation and Performance as a Lipid-Lowering Agent in Serum Patients with Nephrotic Syndrome

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Abstract
Objective: The main objectives of this study was to prepare and study the structural features of chitosan/iron oxide nanoparticles CS-FeO NPs and to examine the applications of chitosan nanoparticles as a lipid-lowering agent in children with nephrotic syndrome
Method: The preparation processes for nanoparticles are described in depth. Chitosan nanoparticle applications are shown. X-ray diffraction patterns (XRD) indicated that the magnetic Fe_3O_4 nanoparticles were pure Fe_3O_4 with a spinel structure and the coating of chitosan did not result in a phase change.
Results: The coating of CTS onto the Fe_3O_4 nanoparticles was also demonstrated by the measurement of Fourier transform infrared (FTIR) spectra. Magnetic measurement revealed that the saturated magnetization of the Fe_3O_4–chitosan nanoparticles reached +18.3 mV and the nanoparticles showed the characteristics of superparamagnetic.
Conclusion: Magnetic Fe_3O_4–chitosan nanoparticles were prepared successfully by the covalent binding of CTS on the Fe_3O_4 nanoparticles. These microspheres was apply to the magnetic-field-assisted lipid lowering processes.
Keywords: Nanoparticles, chitosan, iron oxide

Introduction

In recent years, the use of nanotechnology in medicine has increased significantly. Applications include safer and more effective tumor targeting, tissue engineering, therapy, vaccine delivery, and diagnostic imaging using nanosensors.[1] Tremendous progress has been made in nanotechnology, particularly in the fields of material science and medicine. Medical applications of nanotechnology are often referred to as the "nanomedicine".[2]

NPs must have high bioavailability, stability, and compatibility with the active compounds used to bind and transport them. Furthermore, NPs should provide selectivity and specificity and release the load upon reaching their target site. The physicochemical properties of the nanoparticles can largely affect the characteristics mentioned above.[3]

Chitosan is a linear polysaccharide formed from chitin, an abundant natural polymer found in crustaceans, insects, arthropods, and fungal cell walls. Most commercially available chitosan is generated from marine chitin collected from shrimp, lobster, and crab shells. Chitosan is the N-deacetylate form of chitin composed of D glucosamine and N-acetyl glucosamine monomers (Figure 1).[4]

Synthesis of Chitosan/Iron Oxide Nanoparticles

In recent years, metal oxide nanoparticles coated by bio-organic polymers or ligands have attracted the attention of researchers due to their various applications in biomedical and pharmaceutical industries.[5] Surface modification of metal oxide nanoparticles with organic polymers can improve their physical, chemical, and biological properties.[6] Among the various polymers, chitosan has received a lot of importance due to its unique characteristic features such as low toxicity, biodegradability, biocompatibility, and antimicrobial properties.[7] Surface modifications of metal oxide nanoparticles with chitosan enhance their biocompatibility and biomedical properties (Figure 2).[8]

Materials and Methods

Materials

Chitosan and 5% FeNPs were purchased from Sigma-Aldrich.

Synthesis of Chitosan-Iron (Cs-FeO_3) NPs by Two Steps

1. Chitosan and 5% FeNPs were mixed in equimolar ratios and a condensation reaction takes place using the Dean–Stark (Clevenger) apparatus in the presence of xylene until the theoretical amount of water was separated. Chitosan amide product was separated by filtration, washed several times with methanol, hot distilled water, ethanol, and then dried in an electric oven at 50°C and weighed.[9]
2. Cs-FeNPs (5 mg/ml) was dissolved to gather in acetic acid solution (1% w/v) until the solution is clear then, TPP solution was added to Cs-FeNPs solution with ratios; 1:2.5 (w/w%) with continuous stirring at ambient temperature for 6h. The production of Cs-FeNPs/TPP nanoparticles started via the TPP-initiated ionic gelation mechanism. These nanoparticles were separated and washed several times then the supernatant layer was removed and the precipitate was re-suspended in water and dried.[10]

Characterization of Chitosan-Iron (Cs-FeO_3) NPs

NP was characterized by Philips XL30 SFEG scanning electron microscope (SEM) was used for Observation-the surface morphology of NPs. X-ray diffraction (XRD) patterns of all NPs were analyzed by PAN analytical Empyrean diffractometer. The zeta potentials of NPs were examined by a Micromeritics

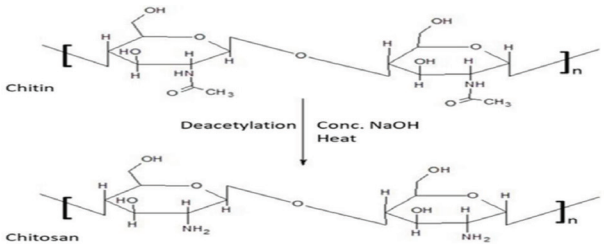


Fig. 1 **Preparation of Chitosan from chitin by deacetylation. Chemical structures of chitin and its fully deacetylated derivative Chitosan.**

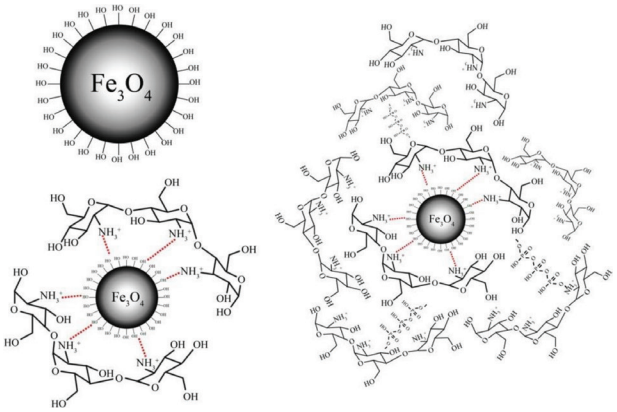


Fig. 2 **Schema of in situ synthesized chitosan-coated magnetic nanoparticles (CS MNPs).**

Zeta/Nano Particle Analyzer (Nano Plus-3). FTIR spectra were recorded via Perkin Elmer FTIR Spectrometer (Spectrum Two). The UV–vis spectra of the samples were attained via UV–Vis Spectrophotometer (Shimadzu UV-2600, Japan).

Application of Chitosan-Iron (Cs-FeO$_3$) NPs

The effect of different concentrations of chitosan-iron (Cs-FeO$_3$) nanoparticles as an active lipid-lowering agent in children with nephrotic syndrome was studied. blood samples of fifty-two nephrotic syndrome patients (33 male, 19 female) were collected from the kidney diseases unit/pediatric teaching hospital, Kerbala/Iraq, with ages ranging between 1–12 years. For biochemical evaluation, a pool of samples was pre- and post-tested for lipid profiles (Total cholesterol, Triglyceride, low-density lipoprotein) before and after the application of chitosan-iron (Cs-FeO$_3$) nanoparticles using a Roche diagnostics/Cobas c111 Auto analyzer/Chemistry System/Germany.

The study's ethical approval was confirmed by the Kerbala College of Medicine, Kerbala University, Kerbala Health Directorate, and the administration of the pediatric teaching hospital.

Results

The Iron oxide-loaded chitosan nanoparticles (CS-FeO NPs) were synthesized according to a previous study. The nanoparticles were produced by interactions between the positively charged chitosan and the negatively charged phosphate groups of TPP in the ionic gelation technique.

The microstructures of synthesized nanoparticles were investigated by SEM, as can be seen in Figure 3, CS MNPs structures were successfully fabricated and within the particle sizes around (33–55) nm. Scanning electron microscope analysis of prepared chitosan nanoparticles shows the distribution

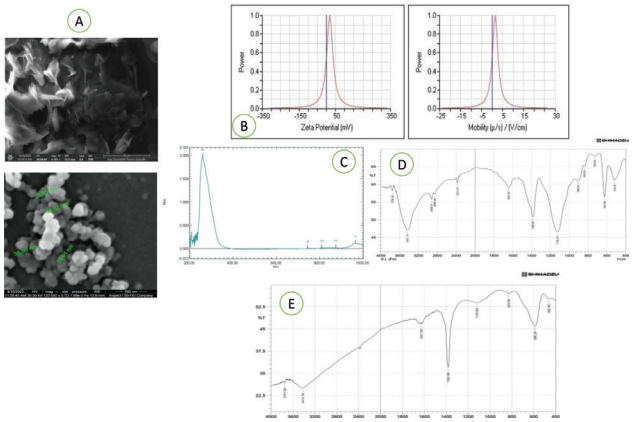


Fig. 3 **(A) Scanning electron microscopy (SEM) shows the morphology and size of biogenic nanoparticles (CS-FeO NPs), (B) Zeta Potential of (CS-FeO), (C) spectrum peak of chitosan iron oxide in UV-Vis, (D) FTIR OF (FeO nanoparticles), (E) FTIR of (CS-FeO$_3$) nanoparticle.**

and nanoparticles size. The results of SEM images show that synthesized CS-FeO NPs were smooth, spherical particles, singular or in aggregates with particle sizes in the range of (35.85), (36.99), and (55.91 nm).

The interaction and structural changes of functional groups that were used to prepare the nanoparticles were examined by FTIR spectroscopy and the results are shown in Figure 3. The FTIR spectrum of FeO NPs and CS-FeO nanocomposite indicates that the CS-FeO was synthesized purely.

The surface charges of the prepared nanoparticles were determined by zeta potential measurements.

Atomic Force Microscopy (AFM) analysis provides images with near-atomic resolution for measuring surface topography. It was used for producing the size distribution as shown in Figure 4. AFM analysis of CS-FeO$_3$ showed that the nanoparticles were well dispersed and the large ratio for small size (53%) below 56 nm.

The SEM images of CS-FeO nanocomposite also revealed agglomerated spherical-shaped grains. EDX spectrum of prepared CS-FeO nanocomposite showed the presence of Fe, O, and N. The presence of nitrogen (N) was supposed to arise from the amine (-NH2) group of chitosan. The detection of N in the spectrum also supports the presence of chitosan polymer on prepared nanocomposite.

Application of CS-FeO NPs as a Lipid-Lowering Agent

Estimation of mean levels of determination pool of serum lipid profile with and without the prepared nanoparticles (**CS-FeO NPs**) pediatric nephrotic syndrome patients was illustrated in Figure 5.

CS-FeO NPs have shown a good effect as a lipid-lowering agent. The pool level of serum lipid profile in the presence of CS-FeO NPs was decreased positively with increasing concentration of the CS-FeO NPs after incubation at 37°C as compared to the pre-test, data were presented in Figure 6.

Discussion

Chitosan nanoparticles are important materials that are widely used in many biological, engineering, and food industries. Magnetic Fe_3O_4–chitosan nanoparticles were prepared by the

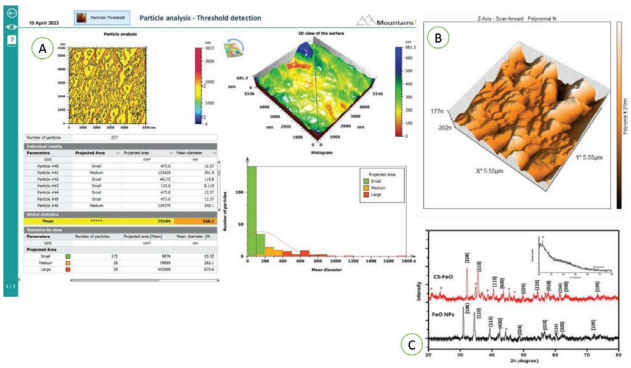
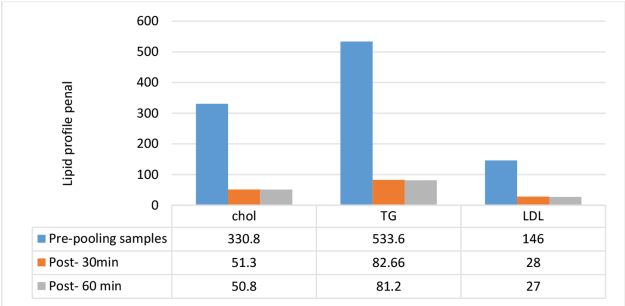
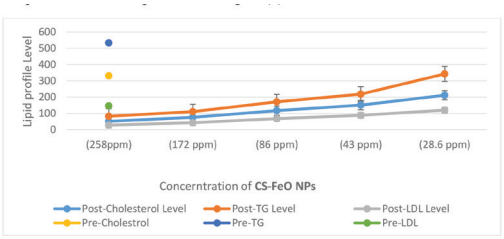


Fig. 4 **(A) Atomic force microscopy, (B) roughness of the nanoparticles, (C) XRD for chitosan iron oxide nanoparticle.**



	chol	TG	LDL
Pre-pooling samples	330.8	533.6	146
Post- 30min	51.3	82.66	28
Post- 60 min	50.8	81.2	27

Fig. 5 **Mean level of pre-post test of serum lipid profile with and without the prepared nanoparticles (CS-FeO NPs).**



Pool sample +(Cs- FeO_3) NPs	Conc.1 (258ppm)	Conc.2 (172 ppm)	Conc.3 (86 ppm)	Conc.4 (43 ppm)	Conc.5 (28.6 ppm)
Cholesterol Level	51.3	75.8	116.7	150.8	211.5
TG Level	82.66	110.6	170.9	218.3	342.5
LDL Level	28	43	68	88	120

Fig. 6 **Estimation plot of determination pool level of serum lipid profile with and without different concentrations of the prepared nanoparticles (CS-FeO NPs) in sera patients of pediatric nephrotic syndrome.**

covalent binding of chitosan (CTS) onto the surface of magnetic Fe_3O_4 nanoparticles. Interesting characteristics of chitosan include its polycationic nature, biodegradability, biocompatibility, bioactivity, and nontoxicity were the main motivation behind this work.

The formation of FeO NPs and CS-FeO nanocomposite was monitored by SEM images and UV-Visible spectroscopy. The spectral analysis was carried out in the range of 200–1000 nm and. The synthesized FeO NPs exhibited a spectral absorbance shift at 268 nm. It was reported earlier that the spectral absorbance around 250 nm is a characteristic feature of FeO NPs.

In the CS-FeO nanocomposite FTIR spectrum, the highest FTIR peaks obtained at 3437 cm^{-1} were attributed to O-H and C-H stretch vibrations of phenols and alkynes. FTIR spectral bands at 1643, 1366, 1512, 1126, 906, and 839 cm^{-1} respectively correspond to N-H bend, N-O, C-H, O-H, and C=O stretching of amino (-NH2), nitro compounds, alkyl groups phenols (O-H stretch) and carboxylic (–COOH) groups).[11] The other short peaks observed from 500 to 800 cm^{-1} were assigned to the presence of a metal-oxygen (Fe-O).[12] The Presence of other functional groups like amino, nitro, and alkyl groups may be derived from chitosan ($C_{56}H_{103}N_9O_{39}$).[13]

While the synthesized chitosan iron oxide nanoparticles exhibit positively charged surfaces with zeta potentials, the surface charge was dramatically changed to positive values (+ 18.3 mV). This confirms the presence of -NH2 groups on the surface of CS-FeO nanocomposite in a protonated form, and thus establishing the presence of chitosan on the prepared nanocomposite.[13] Similar findings were documented by Shi et al. who have reported positive zeta potential surface charge of CS-FeO NPs.[14]

The prepared chitosan iron oxide nanoparticles were examined by XRD. The **(CS-FeO)** pattern, the characteristic diffraction peaks observed around 2θ = 32°, 36°, 41°, 50°, 54°, 57°, 62°, 64°, and 74° respectively corresponding to (104), (110), (113), (024), (116), (018), (214), (300), and (109) orientation planes and were indexed to a rhombohedral crystalline structure, similar finding was reported by Shi et al.[14] The 2θ peak obtained at nearly 23° in the given XRD data may be due to the crystallization of the chitosan phase in the prepared CSFeO nanocomposite and the other extra peaks (*) may be due to the crystallization of the bioorganic phase in the prepared FeO NPs and CS-FeO nanocomposite. The average crystallite size of FeO NPs was found to be 46 nm whereas the crystallite size of CS-FeO nanocomposite was calculated to be 55 nm.

CS-FeO NPs have shown a good effect as a lipid-lowering agent. The pool level of serum lipid profile in the presence of CS-FeO NPs was decreased positively with increasing concentration of the CS-FeO NPs after incubation at 37°C as compared to the pre-test. It was found that **CS-FeO NPs** have the ability to lower lipid content indeed reducing the cholesterol accumulation in the pool samples by ~ 85%, reducing the TG level in the pool samples by 84.5%, and reducing the LDL level in the pool samples by 81%. These results confirmed that **CS-FeO NPs** might be used as cholesterol sinks for enhancing the magnetic charge of cholesterol. Our findings support the importance role of such nano-particles in hyperlipidemia which would be a potent cholesterol absorber and scavenger and might be a potential platform for treating such complications in pediatric nephrotic syndrome and other cholesterol-burden diseases.

Conclusion

Magnetic Fe_3O_4–chitosan nanoparticles were fabricated by the covalent binding of CTS on the Fe_3O_4 nanoparticles. The analyses of TEM and XRD indicated that the Fe_3O_4–chitosan nanoparticles were monodispersed and regular spheres with a range diameter of (33–55) nm. These microspheres may apply to the magnetic-field-assisted lipid lowering processes.

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