In the name of God

Characterization of bis(indolyl) methane dye loaded human and bovine serum albumins imobolized magnetic nanoparticles for drug delivery systems



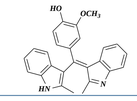
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Introduction

Nanoparticles are one of the most interesting materials in different areas of life. The capability of targeting specific locations in the body, reduction in the amount of drug needed to achieve a certain concentration in targeted diseased area and reduction in concentration of the drug at non-target sites minimizing side effects, are of significant advantages of drug delivery systems based on the use of nanoparticle [1]. Surface functionalized, superparamagnetic iron oxide nanoparticles (SPIONs), became more and more fascinating due to an increasing variety of biological applications such as targeted drug/ gene delivery, magnetic resonance imaging, bio separation, tissue repairing, and thermal tumor therapy [2]. Serum albumins are large multi-domain all α-helical plasma proteins that are the most abundant proteins in plasma. They are responsible for transport of small molecules such as metals, hormones, fatty acids, and drugs to specific tissues, regulate osmotic pressure, maintain blood pH, and serve as predominant plasma antioxidants [3]. Albumin immobilized nanoparticles have several specific advantages which biodegradability, easy preparation and reproducibility are of them [4]. Indole is a π-excessive heterocyclic ring system that is ubiquitous in many biological systems commonly found in nature. This class of compounds has shown wide range of applications in medicinal and many other areas of chemistry. Bis(indolyl)methanes (BIM) are indole derivatives that display diverse biological properties. They are of immense interest because of their pharmacological properties, such as inhibitory activities against cancer cells (bladder cancer, renal cell carcinoma, lung cancer cells, colon cancer, mammary tumor, breast tumor, prostate cancer cell lines, etc.), antibacterial activity, antiangiogenic activity, and acting as cytotoxic agents [5]. In this work, super paramagnetic iron oxide nanoparticles (SPIONs) were prepared by the chemical co-precipitation of Fe(III) and Fe(II) ions. Synthesized nanoparticles were modified by aminosilane agent (APTES) and then the amino-functionalized magnetic SPIONs were activated using trichlorotriazine (TCT). Human and bovine serum albumins were bound covalently to activated nanoparticles. Then, albumin immobilized SPIONs were reacted with BIM (Scheme 1) as an anticancer drug.

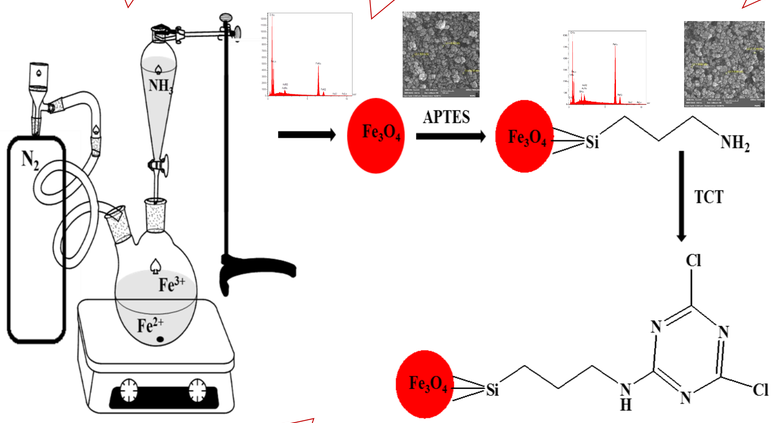
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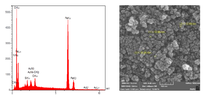
0.37 g of $FeCl_3 \cdot 4H_2O$ () and 1 g of $FeCl_3 \cdot 6H_2O$ were dissolved in 30 mL of deionized water under N_2 gas. Then, the ammonia solution (10 ml) was slowly added to the mentioned solution under magnetic stirring. The SPIONs precipitates were separated magnetically and washed several times with deionized hot water until the supernatant reached pH 7.

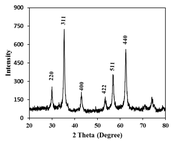
FESEM images of SPIONs showed that the structures of the SPIONs are spherical and particle dimensions are mainly less than 15 nm. Although the particles are agglomerating however the boundaries between single crystallites are observable. Purity and composition of the products were studied using EDX (Energy Dispersive X-ray). The EDX traces revealed the presence of Fe and O peaks for SPIONs.

FESEM images of APTES modified SPIONs are shown in figure. The particle size of APTES modified SPIONs are identical to SPIONs size. The EDX pattern of APTES modified SPIONs, in this pattern indicated the SPIONs functionalized with APTES. The EDX traces revealed the presence of Fe and O peaks for SPIONs, Si, C and N for APTES agent. It is clear that the as-APTES modified SPIONs are extremely pure.



Particles in TCT modified SPIONs are identical to that in the SPIONs, particles sizes are larger in the former cases. It is clear that with increasing TCT on the surface of SPIONs, particles size incensed. The EDX pattern of TCT modified SPIONs indicated that SPIONs are functionalized with TCT. The EDX traces revealed presence of Fe and O peaks for SPIONs, Si, C and N for APTES agent and Cl for TCT agent. It is clear that prepared nanoparticles are extremely pure.

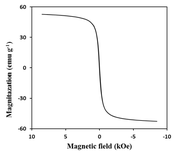




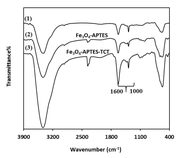
The XRD pattern of TCT modified SPIONs is shown in this figure, the diffraction peaks are indexed to a cubic crystalline phase of magnetite (JCPDS-01-1111). The average particle size, *D*, was calculated using the Debye–Scherrer equation:

$$D = \frac{K\lambda}{B\cos\theta}$$

The particle size for as-prepared modified SPIONs is approximately 19 nm.

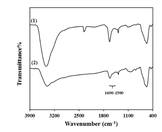


The magnetic properties of TCT modified SPIONs were analyzed by VSM. The magnetization value of TCT modified SPIONs was found to be equal to 52.7 emu/g. . As can be seen these particles are superparamagnetic.

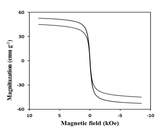


FT-IR spectra of the SPIONs (1), APTES modified SPIONs (2) and TCT modified SPIONs (3) are shown in above figure. Fe — O bond of SPIONs shows a strong absorption band at 570 cm-1. C — H stretching vibration depict at around 2923 cm-1. Amid group represented a bands around 3400 and 1630 cm-1. TCT connection with SPIONs were demonstrated fine peaks between 1000 and 1600 cm-1. Si — O and Si — H stretching vibrations of APTES modified SPIONs represented two bands at 1000 and 2300 cm-1, respectively.

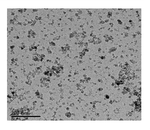
BIM loaded HSA imobolized magnetic nanoparticles

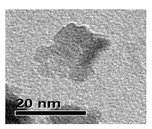


FT-IR analysis of TCT modified SPIONs (1) and HSA coated TCT modified SPIONs (2) are shown in fig. The FTIR bands at low wave numbers (≤700 cm−1 come from vibrations of Fe O bonds of iron oxide.
sharp peaks in the range of 1500-1600 cm^{-1} confirmed binding of HSA on the surface of SPIONs. Amide I and amide II bands (1450-1600 cm^{-1}) certified covalent binding of α- and β-caseins on the surface of SPIONs.



The magnetic properties of TCT modified and HSA coated SPIONs were analyzed by VSM. The magnetization value of TCT modified and HSA coated SPIONs were 52.7 and 44.9 emu/g respectively. The magnetization of HSA coated SPIONs was smaller than that of TCT modified one because of large amount of protein on the surface of SPIONs. As shown in the figure, no reduced remanence and coercivity being zero were detected, indicating both of them are superparamagnetic.





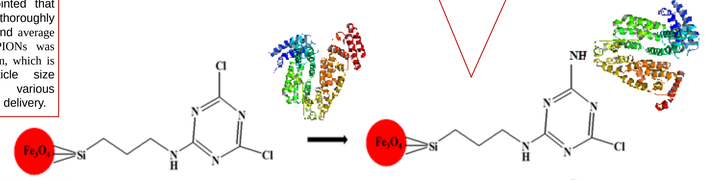
TEM results appointed that the SPIONs were thoroughly coated with HSA and average particle size of SPIONs was found to be ~ 20 nm, which is the desired particle size appropriate for various applications in drug delivery.

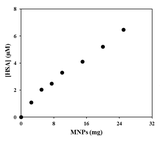
HSA and BSA were covalently immobilized onto the surface of modified SPIONs. 4.0 mg of the triazine functionalized MNPs was dispersed in 500 μL of PBS (pH = 7.4) and 500 μL of BSA and HSA solution (0.01 mM) was added to MNPs. The mixture was shaken at room temperature for 4 hours. The amount of BSA immobilized on MNPs was determined using the Bradford method

BIM standard solutions (0.1 mM) was prepared by dissolving it in a solvent mixture of PBS (pH: 7.4) and acetonitrile (90%-10%, v/v). To obtain loading content of BIM on the albumins immobilized nanoparticles, several implosions were made by weighting nanoparticles in the range of 0-25 mg/mL and mixing with dye solutions. Binding of BIM was calculated using Uv-vis instrument and by calibration regression equation.
BIM loading content and entrapment efficiency were determined by the following equations:

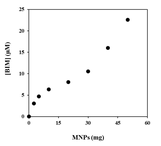
$$BIM\ loading contents = \frac{\mu g\ of\ BIM\ in\ nanoparticle}{mg\ of\ nanoparticle}$$

$$BIM\ entrapmentefficiency(\%) = \frac{Weight of\ BIM\ in nanoparticle}{weight of\ BIM\ fed\ initially} \times 100$$

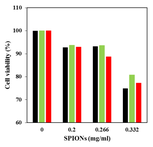




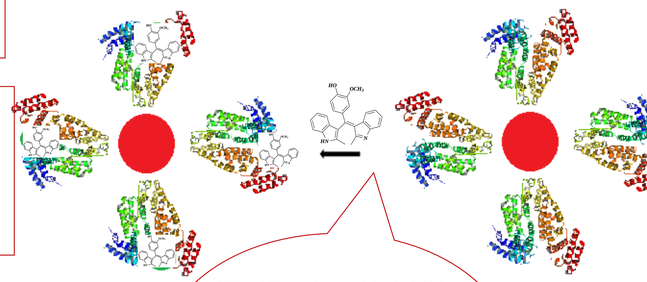
Results of Bradford method revealed that with increasing the mg of SPIONs, amount of protein binding increased by maximum percentage of 64.55% For HSA.



entrapment of BIM on HSA coated SPIONs measured using UV-Vis technique. As can be seen increasing the mg of HSA coated SPIONs first resulted in increas in the amount of BIM binding (%) by maximum amount of 18 (μg/mg) and maximum entrapment efficiency of 22.61%.

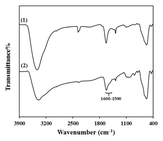


The in vitro cell viability of the TCT coated SPIONs (black), HSA Immobilized TCT coated SPIONs (green) and BIM-loaded HSA Immobilized TCT coated SPIONs (red plot) was evaluated to determine the selective cancer therapy activity of the drug-loaded nanoparticles in different concentrations on the MCF7 cell lines by MTT assay.
A cell viability assay revealed that immobilization of SPIONs by HSA decreased toxicity on MCF7 cells. In contrast, the BIM-loaded HSA Immobilized TCT coated SPIONs exhibited cytotoxicity towards MCF7 cells.

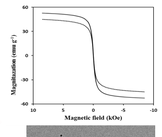


FTIR and TEM results showed that the SPIONs were coated with HSA and BSA with desired particle size appropriate for various applications in drug delivery. The results of VSM analysis showed that despite immobilization of SPIONs with albumins, their magnetic properties were not changed. UV-Vis spectroscopy data showed good binding and entrapment capacity of albumin coated SPIONs with BIM. The Cell culture study suggested that these albumin coated SPIONs are biocompatible and appropriate for biomedical applications.

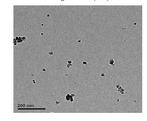
BIM loaded BSA imobolized magnetic nanoparticles

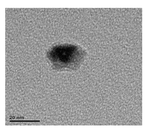


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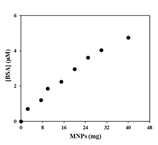


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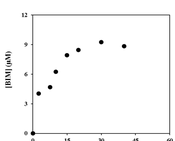




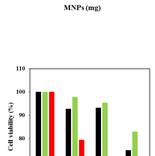
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entrapment of BIM on BSA coated SPIONs measured using UV-Vis technique. As can be seen increasing the mg of BSA coated SPIONs first resulted in increase in the amount of BIM binding (%) by maximum amount of 11 (μg/mg) and maximum entrapment efficiency of 12.61%.



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References

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[3] Dezhampanah, H., Firouzi, R., & Hasani, L. (2016). Spectroscopy and Molecular Modeling Study on Binding of Nickel Phthalocyanine to Human Serum Albumin. Protein and peptide letters, 23(9), 800-807.
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