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Original Research Article

Study The Interaction between LH, FSH, and TSH with New Synthesized Magnetic Nanoparticles Coated with Dextran

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Abstract

Nanoparticle (NP) was used *in vivo* and *in vitro* in contact with the body fluids that contain different types of proteins. The proteins have the ability to adsorb on NPs surface, and alter its activity due to the modification of the secondary and tertiary structures. In this study, the interaction between coated magnetic nanoparticles (MNP@Dextran) and the hormones (LH, FSH, and TSH) were studied in details. The possible change in the secondary and tertiary structure of these parameters is discussed. The (MNPs) were prepared using co-precipitation method of Fe3O4 followed by reacted these MNPs with Dextran under an optimum conditions to produce coated MNP@Dextran. Different concentrations of each hormone were incubated with a known amount of MNP@Dextran and the quantity of the hormones adsorbed was calculated. The experiments were repeated at different temperatures to calculate the thermodynamic parameters. Secondary structures of hormones were examined using circular dichroism (CD).Tertiary structures of protein were measured using fluoroscopy technique.The adsorption process obeyed Freundlich adsorption isotherm indicating heterogeneity of the MNP@Dextran surface,and it associated with the changing in the secondary and tertiary structure of hormone after adsorption on MNP@Dextran.

Key words: Magnetic nanoparticles, protein adsorption, LH, FSH, TSH and protein structure.

الخلاصة

تم استخدام الجسيمات النانوية (NP) بالتماس مع مختلف البروتينات الموجودة في سوائل الجسم) داخل الجسم وخارج الجسم.(تمتلك البروتينات القدرة على الامتصاص على سطوح الجسيمات النانوية مغيرة بذلك فعاليتها ونشاطها بسبب التحوير في تركيبها ألثانوي والثالثي .في هذه الدراسة، تم دراسة التاثر بين الجسيمات النانوية المغناطيسية المغلفة بالدكسترات (MNP @ ديكسترات (وهرمونات LH) ،FSH،و (TSH بشيء من التفصيل, ونوقشت ألتغيرات ألمحتمله في التراكيب ألثانويه والثالثية .حضرت الجسيمات المغناطيسيه النانوية (MNPs) بطريقة الترسيب المشارك لل Fe3O4 ثم غلفت بالديكسترات تحت الظروف المثلى مكونا (MNP@ ديكسترات . (حضنت تراكيز مختلفة من كل الهرمون مع كمية محددة من (MNP@ ديكسترات (وحسب كمية الهرمونات الممدصة. اجريت التجارب في درجات حراريه مختلفة لحساب ألمعاملات الحرارية . تم دراسة التركيب الثانوي للهرمونات باستخدام تقنية ألازدواج ألوني ألدائري .(CD). قيست التراكيب الثالثي من البروتين باستخدام تقنية التفلور. وجد ان عملية الامتزاز تطيع معادلة فروندليتش الأيسوثرم مما يدل عدم تجانس سطح ال MNP@دكسترات (،وهذا راجع الى ألتغيرات في التراكيب الثانوية والثالثية للهرمونات, ان هذه التغييرات والتي اثبتت عمليا من خلال دراستنا انها ممكن ان تعطي صوره واضحه في المستقبل كعلاجات تعطى لمرضى السرطان.

Introduction

Colloidal dispersions of magnetic nanoparticles (MNPs) exhibit a unique combination of capability and fluidity to interact with external magnetic fields, which make them interesting for biological and medical applications. Such as particle size, magnetic structure, surface charge and surface chemistry typically determine the magnetic response and chemical or physical interactions of the nanoparticles with biological entities during application. Emerging novel applications are focused on the development of MNPs as targeted magnetic carriers for drug delivery, the usage of magnetic nanoparticles as specific contrast enhancement agents for in vivo magnetic resonance imaging (MRI, protein immobilization, magnetic cell sorting, and biocompatible magnetic nanoparticles for cancer treatment mediated by hyperthermia [1]. Surface modification of MNPs with organic molecules have been widely used for biomedical and biotechnological applications, such as separation processes [2]. The modified surfaces of the MNPs not only stabilize the magnetic core, but they can also provide functional groups for further modification. Those functional groups can bind to different molecules, such as targeting agents, optical dyes, therapeutic agents, enzymes, and nucleic acids. When the MNPs functionalized with more than one type of molecule, they are termed multifunctional. These molecules can be conjugated on the surface or incorporated within MNPs. They also can bind to the modified surface by chemical bonds, These chemical bonds include amide, ester, hydrogen and other bonds [3]. Dextran has been widely used as a polymeric coating mostly because of it is biocompatibility, no. and polar interactions. (chelation and hydrogen bonding) that give dextran a high affinity towards MNPs surfaces. Dextran bind to MNPs surface by covalent bond between oxygen and iron II and III ions along the MNPs surfaces [4]. Dextran a mono-dispersity in particle size and mesoporosity in these agglomerates are considered advantageous if this coated NPs is to be used as drug delivery vehicles or adsorbents or catalysts for various chemical reactions with potential environmental importance [5]. MNPs carrier coated with dextran had good biocompatibility due to its sugar components. The increase in cytotoxicity might be because of some MNPs lost their dextran coating, consequently exposing MNPs to the cells [6], the application of MNPs *in vivo* would require surface modification that would ensure that particles are non-toxic, biocompatible and stable like hydrophilic compounds [7]. Hydrophilic compounds such as dextran polymer . Dextran will be degraded into sugar components and MNPs will be degraded into free iron II and III. Free Iron in the hepatocytes cytoplasm can be used in essential physiological functions, such as mitochondrial adenosine triphosphate (ATP) generation and DNA damage repair. Meanwhile, unused iron is stored in the iron-binding protein such as ferritin, hemosiderin, and transferrin. Iron might exit the cells by exocytosis, bind to transferrin in blood, and get transported throughout the body as di-ferric transferrin. Iron homeostasis is preserved by the loss of about 1 mg of iron per day through the sloughing of intestinal mucosa and skin. On the other hand, 20–25 mg of iron is essential each day for the hemoglobinization of new erythrocytes [8]. The interaction between proteins and NPs have many applications covered by different newly published papers [9]. NPs can interact with human proteins by different forces including adsorption process [10]. The formation of protein corona has been found to depend largely on the protein properties and physicochemical characteristics of the NPs [11], Human plasma proteins of can be adsorbed rapidly on the surface of NPs leading to the formation of a protein “corona.[12]. The adsorption of proteins on NPs occurs mainly due to specific or nonspecific interactions between the NP surface and the proteins [13] , Many factors were found to be determinant in the quantity of protein that adsorbed on the NPs particles and on the quality of adsorption process [14].

Furthermore, the biological activity of some proteins were found to be increased or decreased [15] after interaction with nanoparticles in comparison with their free state. The fact that Protein-NPs interaction may retain or modify the secondary and tertiary protein structure after adsorption on the NPs is important for the explaining of the change in the bioactivity after adsorption [16]. Furthermore, some protein-NPs interaction may leads to biodegradation of NPs by proteins. In this study of hormones interaction with MNPs is less studied. Luteinizing hormone (LH), follicular stimulating hormone (FSH) and thyroid stimulating hormone (TSH) are hetero dimeric glycoprotein hormones contain differ in beta chain and same alpha chain [17]. The interaction between these proteins and magnetic nanoparticles (MNPs) is not studied yet.

Materials and Methods

1-Synthesis of Fe3O4 nanoparticles:

The magnetic particles were prepared by co-precipitation using ferrous and ferric salts and ammonium hydroxide [18] .

2-Synthesis of MNP@dextran:

To prepare MNPs @Dextran, The Dextran solution was added to the solid Fe3O4 MNPs. The reaction mixture was stirred at 298 K for 1.5 minutes and then. it was washed three times with de-ionized (DI) water, then dried at 350 K. Dry powders were finely ground and characterized for their composition, morphology, thermal and magnetic properties ,"Transmission electron microscopy (TEM) was used to visualize the morphology and size of the prepared MNP@Dextran. TEM image was obtained on a Zeiss-Em10c transmission electron microscope with an accelerating voltage of 80 kV. Particle size distribution and Zeta potential of the particles also was assessed using Malvern Zetasizer".[19]

3-Interaction of hormones with MNP@ Dextran:

To study the interaction of hormones with MNP@Dextran, 100µl of LH or FSH hormone solution at different concentrations (0, 5, 10, 25, 50 & 100mIU/ml for LH and 0, 5, 25, 50, 100, & 200 mIU/ml) was added to 25mg of MNP@Dextran in the Eppindroff tubes. For TSH, the concentrations added were (0, 0.5, 2.5, 5, 10, 20, & 40 mIU/ml) to 25mg of MNP@Dextran in the Eppindroff tubes. The units of hormones concentration were converted into ng/ml and µg/ml according to WHO standards; the tubes were incubated with shaking for one hour which is more than the equilibrium time obtained from previous experiments. The mixture then centrifuged at 4000rpm for 20 minutes. The hormone concentration in the aspirated supernatant was estimated by the Enzyme Linked Immunosorbent Assay (ELISA) using Kits supplied by Monobind Co. USA. The amount of hormone adsorbed on the surfaces was calculated using the following equation:

$$Qe = X/m = [V(Co-Ce)]/m \quad [2]$$

X: The quantity adsorbed (ng), V: Volume of solution (ml), Co: Initial concentration (ng/ml), Ce: Equilibrium concentration (ng/ml), m: Weight of adsorbent (compound 1 or 2).

The Qe values were plotted versus the equilibrium concentrations (Ce) and the resulting figures representing the adsorption isotherms that required for understanding and interpreting the systems under investigation. The linear form of Freundlich isotherm is $LogQe=LogK_f + 1/nLogCe$ which is found to be the most applicable adsorption isotherm equation for the adsorption process of the hormones on MNP@Dextran. It used to interpret the interaction process where Kf is a function of energy of adsorption and temperature and is a measure of adsorptive capacity of the adsorbent (mg1-(1/n)·L1/n·g -1) and n is an empirical constant related to the magnitude of the adsorption driving force (intensity of adsorption) [20].

4-Desorption process:

To study the strength of the interaction of hormone with MNP@Dextran, the desorption experiments were carried out [20]. In each case, hormone was adsorbed from solution onto the surface of MNP@Dextran using a protocol similar to that for the adsorption

using one initial hormone concentration (100µl) for each compound. After incubation and centrifugation as described above, the supernatant was removed and replaced by 100µl of normal saline solution (0.9%NaCl) to prevent hormone denaturation. The samples were further incubated at the room temperature to allow desorption to occur. Before measurement, the tubes were centrifuged at 3000rpm for 5 minutes to precipitate the MNP@Dextran and the hormone level was estimated in the aspirated supernatant by ELISA technique. The desorption percentages were calculated from the ratio between the hormone quantity released into the solution on the hormone quantity desorbed initially on the MNP@Dextran i.e., weight of hormone in 100µl of normal saline solution divided by the weight of hormone in 25mg of MNP@Dextran:

%Desorbed= [(Ce/0.1) / (Qe/25)] *100% ..... [7]

5-Fluorescence Spectrophotometer

The instrument was adjusted to zero using phosphate buffer 50mM, then the spectra of free hormones solution (40mIU/ml) was measured, also the spectra of Hormone-MNP@Dextran were measured.

6- Circular Dichroism (CD Spectra):

The Circular Dichroism of free hormones and hormone-MNP@Dextran composites after adsorption were obtained by using the following concentrations: 40mIU/ml free TSH or TSH-MNP@Dextran, 50mM potassium phosphate, pH=7.5. For LH and FSH, 100mIU/ml were used. The free hormones were put in 0.1cm Spectrosil quartz cuvette (Starna Cells) and the cuvette was put in the Jasco CD Spectrometer at 20°C. Three scans were averaged by using Chirascan Pro-Data Viewer software. Equipment parameters consisted of a 20.0–99.0°C gradient with stepped ramping, 10°C per step, with a 60s equilibration time, and a 20s read at each step subsequent to equilibration. Ellipticity profiles were fitted to a two-state model. The results were explained using K2D3 program by listing the results online to the web site (*http://cbdm-01.zdv.uni-mainz.de/~andrade/k2d3/*) that designed according to the research of Louis-Jeune *et al* (2012) [21].

Results and Discussion

1- Characterization of the synthesized Dextran@MNPs.

TEM images of the MNPs and MNP@Dextran are presented in Figure 1. The images showed a distortion

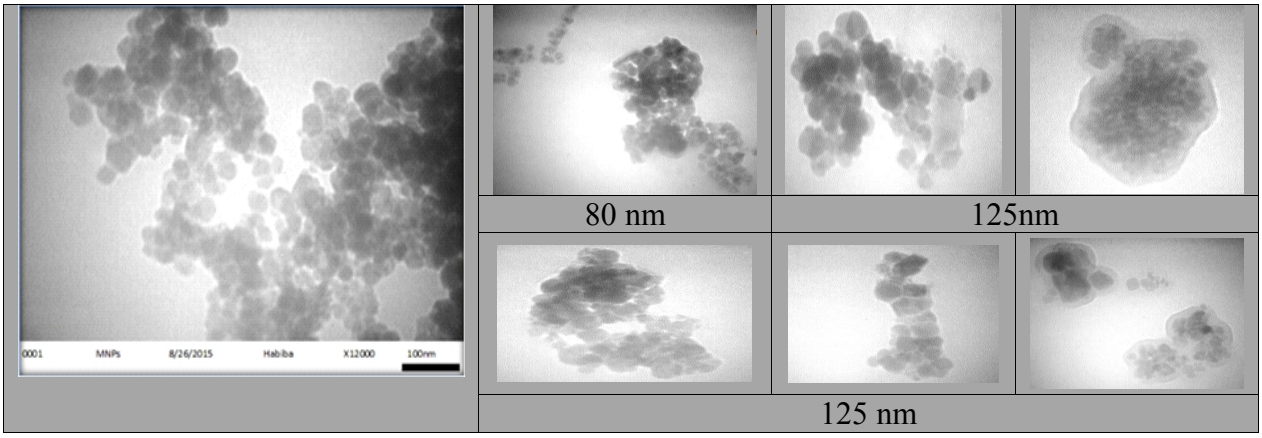

Figure 1: TEM images of the MNPs (Left) and MNP@Dextran (Right)

in the MNPs sphere structures after coating with Dextran. Figure 2 represent the AFM images of the MNPs and MNP after interaction with dextran. These images are differ in the surface roughness due to an increase in the particles after coating with dextran.

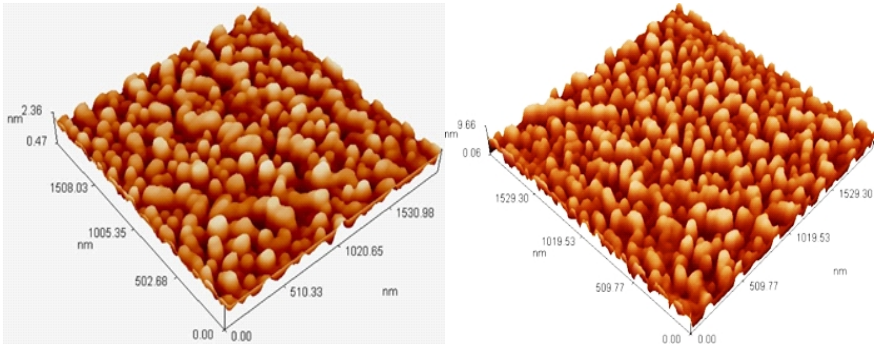

Figure 2: AFM images of the MNPs (Left) and MNP@ Dextran (Right).

In Figures 1 & 2 indicated the formation of MNP@ Dextran due to the change in the particle shapes and the surface of the MNPs after coating with Dextran.

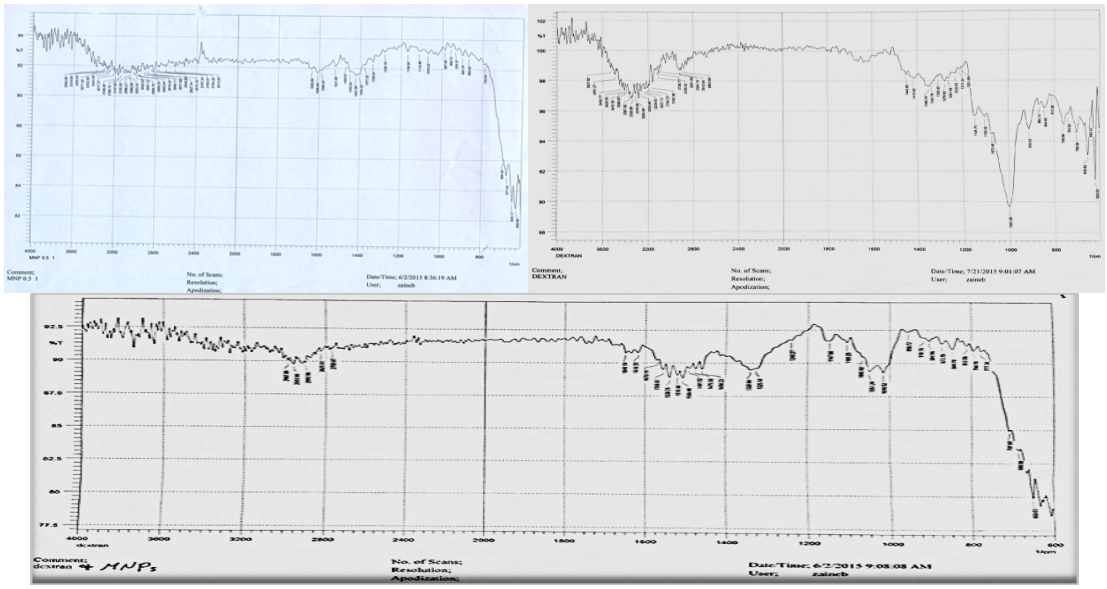

Figure 3: FTIR spectra of A: free Fe3O4(MNPs) (Left), pure Dextran (Right) and B: MNP@Dextran (down)

Free MNPs, dextran coated MNPs are shown in *Figure (3:A&B)* . Comparing spectra A and B , some new absorption bands are appeared: The spectra present vibrational modes characteristic of the organic structure of dextran, such as the α-glucopyranose ring of dextran at 910 cm-1. The α-glucopyranose ring in a pure dextran is also represented by two bands at 759 and 844 cm-1. Furthermore, bands of pure dextran at 1276 and 1348 cm-1 are due to C-H vibrational modes, A band at 1145 cm−1 confirms the presence of glycosidic linkage (C–O–C) in dextran. The band in the region of 1001 cm−1 is due to the C–O stretching. A broad peak present at 3429 cm−1 and is due to the stretching of –OH. And observed that the sample functionalized with dextran exhibits a vibrational mode at 626 cm-1 which is related to the Fe-O bond, present in magnetite. the band at 626cm-1 which is assignable to the absorption of ν (Fe–O), and the broad absorption peak appeared at about 3319 cm−1 can be related to the presence of hydroxyl groups. Due to hydrophilic nature of Dextran, a peak shows at 1446 cm−1 is due to bending of water molecule. These data proved that the surface of MNPs has been covered with dextran polymer. It is believed that different interactions between dextran and Fe2+ and Fe3+ ions in the MNPs surfaces such as hydrogen bond, van der Waals force, and

electrostatic interactions keep dextran on the surface of MNPs [23].As seen in Figure 4- The comparison between these spectra and the spectra of unbound MNP@dextran revealed the formation of new system consists of MNP@dextran-hormone system as discussed in details below. That indicating binding between coated MNPs and hormones leading to formation of new systems. FTIR spectra were taken for the free and the adsorbed hormones.

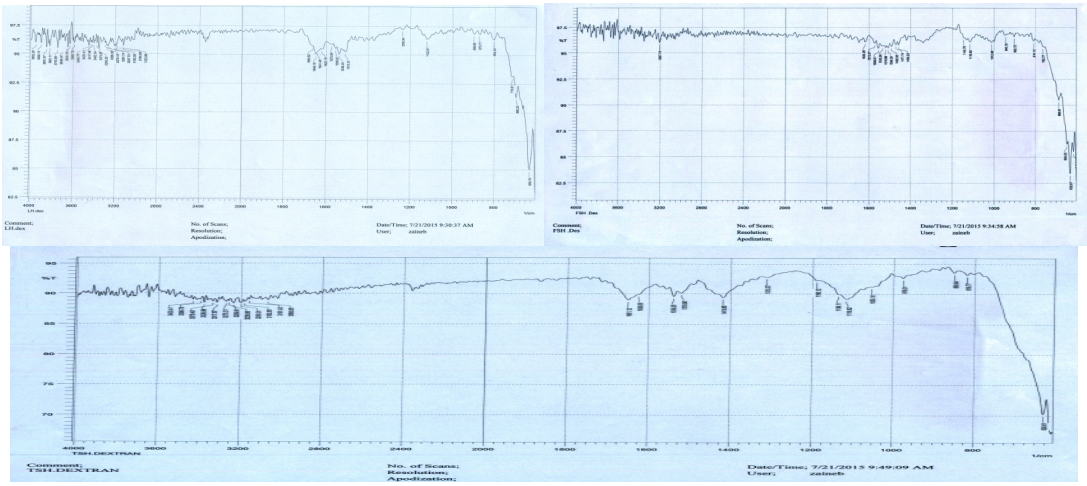

Figure 4: FTIR spectra of LH-MNP@ Dextran (Above), FSH-MNP@ Dextran (Middle), an TSH-MNP@Dextran (Below).

The analysis of the secondary structure of the proteins by FTIR spectroscopy revealed evidences about the possible secondary structural changes that undergo upon adsorption. FTIR studies showed The particles exhibited typical amide bond signatures at around 1640 cm–1 as seen in other work. The intensity of this peak is decreased and shifted to a longer wave number after adsorption of these hormones on the surface of MNP@Dextran. Furthermore, the following peaks of the amide groups behave as previous peak; beta sheets between 1630-1638 cm–1, α-helix between 1645-1658 cm–1, and beta sheets between 1669-1696 cm–1 . While the peaks of amide groups that belong to the random coils between 1642- 1652cm–1 was spread and fused indicating an increase in that structure. These results were further confirmed by CD studies as explained in the next paragraphs. The absorption bands below 1620 cm–1 can be due to the aggregation usually associated with formation of strong beta-sheet structures [24]. To evaluate the change in the particle size and zeta potential of the MNP@Dextran after adsorption of the hormones on its surface, Table 1 revealed the particle size by DLS and zeta potential of free and bound nanoparticles. The ZP is an indicator of the stability of NP suspensions. A higher electric charge on the surface of the NPs will prevent aggregation of the NPs in buffer solution because of the strong repellent forces among particles [25]. Tween 80 also provides a steric stability for maintaining the stability of single layer nanoemultion (SLN). As a rule of thumb, absolute ZP values above 30 mV provide good stability [25] and above 60 mV excellent stability. About 20 mV provide only short term stability, values in the range -5 mV to +5 mV indicate fast aggregation. This is valid for low molecular weight surfactants and pure electric stabilization, but not for higher or large molecular weight stabilizers, which act mainly by steric stabilisation. In this case ZP values of only 20 mV or much lower can provide [25]. TSH-MNP@Dextran has the lowest zeta potential, while particle size is the higher among all the coated nanoparticles. By reason of Zeta potential is an important physicochemical parameter that influences the

stability of nanosuspensions. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensures easy re dispersion [25].

Table 1: Zeta potential and particle size by DLS of free and bound nanoparticles

Nanoparticle	Zeta potential mV	Particle size by DLSnm (%)
MNPs	12.9±4.3	433±83,4 (94.3%)
MNP@Dextran	−20.0 ± 5.16	204±81.8 (95.3%)
LH-MNP@Dextran	-18.2	251.9±158(97.5%)
FSH-MNP@Dextran	-21.1	288.5±110 (100%)
TSH-MNP@Dextran	3.63	420.6 ±97.8(100%)

In the case of a combined electrostatic and steric stabilization, a minimum zeta potential of ± 20 mV is desirable. The size of the MNPs decreased from 433 ± 83.4 to 204 ± 81.8 nm, and a significant change of the zeta-potential from positive (12.9±4.3 mV) to negative (−20.0 ± 5.16 mV) was observed, indicating that most of the positively charged in free MNPs was closely covered and successful dextran conjugation to the MNPs surface. Coating also plays a role in the colloidal stability of the particles against aggregation and gravitational settling by reducing the inter-particle dipole-dipole forces. The most common coatings are derivatives of dextran [26]. In general, the particle size of hormone-bound nanoparticles is increase due to the coating of the original nanoparticles by more than one layers of the protein. Zeta potential value of FSH-MNP@Dextran is the higher among all the coated nanoparticles. UV-visible spectra showed significant peaks in the far UV at around 200nm. Maximum wave lengths of the MNP@Dextran and the MNP@Dextran coated with hormones obey the following sequence. (LH-MNP@Dextran (210nm) > FSH-MNP@Dextran (205nm) >MNP@Dextran (203 nm) > TSH-MNP@ Dextran (201 nm)). The peak value is changed significantly when the MNP@Dextran bound with TSH. Figure 4 presents the spectra of the synthesized nanoparticles.

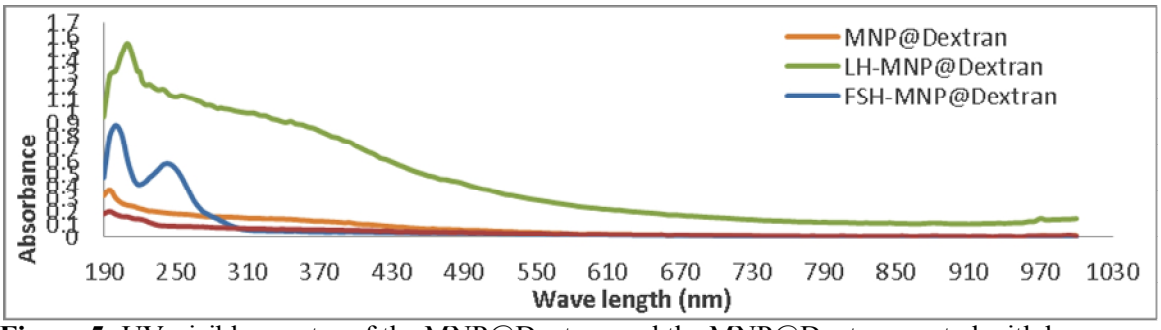

Figure 5: UV-visible spectra of the MNP@Dextran and the MNP@Dextran coated with hormones

However, absence of such features does not indicate an absence of protein aggregates [27]. These aggregation were owed to the formation of more than one layer of the adsorbed molecules. This fact are further confirmed by the Freundlich adsorption isotherm. The adsorption of the hormones on the surface of MNP@Dextran showed that the maximum quantity adsorbed on the surface of MNP@Dextran at 25°C is as following: LH (97.66µg/g) > FSH (44.12µg/g) > TSH (21.63µg/g). These quantities are lower than the quantities adsorbed on the free MNPs (uncoated) [28] , indicating that the coating with Dextran leads to decrease the adsorption activity of MNPs. These results indicate a proper capability of MNP@Dextran to adsorb these hormones efficiently at their relatively very low concentration. The reason about why LH showed higher capability for adsorption and TSH showed a lower adsorption extent cannot be explained by the difference in molecular weights or to the number of amino acids residues. Alpha subunit is identical in all hormones. While beta chain contain 120 amino acid in LH , 111 amino acid in FSH and 118 amino acid in TSH. Because there is only small difference in the number of amino acids and molecular weights, these parameters cannot predict the results of the adsorption.

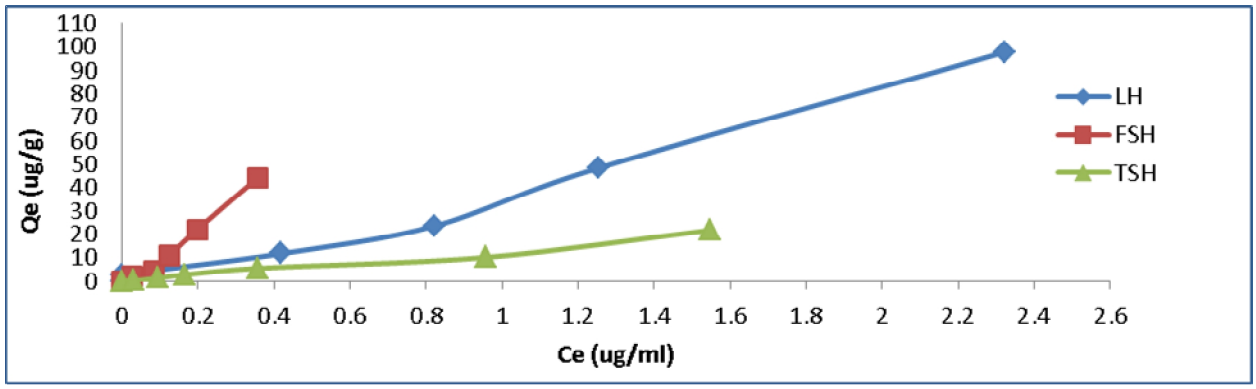

Figure 6: Adsorption isotherms of the adsorption of hormones on the MNP@Dextran surface at 298°K.

The type and sequence of amino acids in the hormone chains, secondary and tertiary structure can explain the results. In the large molecules such as hormones under study, charged domains can greatly contribute to how that protein interacts with surfaces, as well as its own tertiary structure (protein folding). The surface modified Dextran magnetic nanoparticles were developed as effective magnetic affinity adsorbent for Bovine serum albumin from aqueous solutions, Magnetic Dextran Nano composites showed an excellent absorption of substances that contain free amino and hydroxyl groups. The charged amino acids are located on the outer surface of proteins, where they are able to interact with surfaces . As it will be explained in the next discussions, it seems that LH molecule has more domains or groups that can interact with the surface of MNP@ Dextran than other hormones. Therefore, is the most adsorbable protein in comparing with the other two hormones .The results in Figures 6 & 7 revealed the applicability of Freundlich equation on the adsorption process of hormones on the surface of MNP@dextran indicating the heterogeneity of a surface. This type of adsorption isotherm is widely used to describe the adsorption on MNPs [29]. Freundlich isotherms imply different forces on the surface or on the hormones molecules to produce heterogeneity by non-covalent protein-MNP@dextran conjugation. The adsorption isotherms showed the following types according to Giles Classification of the adsorption isotherms [30], TSH-MNPs@dextran=S1, LH-MNPs@ dextran=S3, and FSH-MNPs@ dextran=H2. According to the Giles et al [30], in the initial part of the S curves, the initial direction of

curvature shows that adsorption becomes easier as concentration rises and more and more adsorbate will be adsorbed over the first monolayer of protein. This implies a cooperative adsorption that is molecules do interacts with the neighbors side by side leading to hold them more to the surface. There is also opposite force due to the attraction of the protein molecules with water draws this molecules as far as possible into the water phase, and thus the net of these forces represent the adsorption intensity [30]. FSH-MNPs@dextran obey the H2 type on Giles classification. In this type, the solute has such high affinity that in dilute solutions it is completely adsorbed, or at least there is no measurable amount remaining in solution. The initial part of the isotherm is therefore vertical.

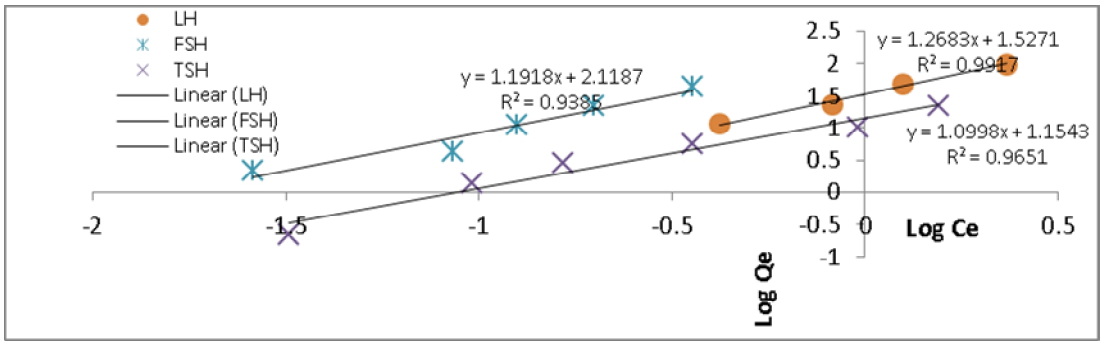

Figure 7: Freundlich lines of the adsorption of the hormones on the surface of MNP@Dextran

The adsorbed species are often large units, *i.e.,* ionic micelles or polymeric molecules, but sometimes they are apparently single ions which exchange with others of much lower affinity for the surface. Protein molecule attached to the surface using different forces, however the solvation power of water resist the adsorption process. If the adsorption forces is higher than the solvation forces, then the protein will be adsorbed favorably. Some papers [31]. Represent protein as a solid that attached irreversibly with the solid surface. This not the case it is vibrate, rotate, change the folding and tertiary structures as seen by different techniques used in the present work by CD, fluoroscopy, and desorption process.

The Table 2: contains the Freundlich constants of the hormone-MNP@dextran systems. FSH-MNPs@dextran showed the highest kf and n values in comparing with LH-MNPs@dextran and TSH-MNPs@dextran. These results are further confirmed by the order of the adsorption maximum capacities of these hormones on the surface of MNP@dextran. The value of n is a measure of the intensity of adsorption .Therefore, the release of the hormone from the MNP@dextran-hormone system is predictable from the value of n.If n<1 Then adsorption is (a chemical process) and if n>1the adsorption is (a physical process). The value in Freundlich equation was found to be 1.10–2.11 [32].

Table 2 : Freundlich constants of the hormone-MNP@Dextran systems.

	LH-MNP@Dextran	*FSH-MNP@Dextran*	*TSH-MNP@Dextran*
n	*0.79*	*0.84*	*0.91*
Kf	*1.53*	*2.12*	*1.15*

The percentage of the quantities of hormones desorbed from the surface of MNP@Dextran is presented in Figure 8. In general, when the quantity desorbed increases, the adsorption forces are weak leading to easily breaking of the linkage between the adsorbent and the adsorbate. The results of desorption in the Figure 8 revealed that the TSH has the highest desorption percentage (26.12%) leading to the conclusion that the adsorption forces between FSH (14.43%) and MNP@Dextran are stronger than LH (16.67%) and TSH. TSH showed the highest desorption percentage due to the weak forces with MNP@Dextran.

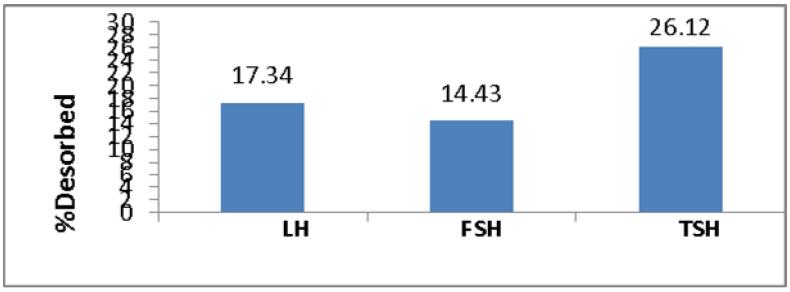

Figure 8: Desorption percentages of hormones from MNP@Dextran surface

The results of CD of free and adsorbed LH, free and adsorbed FSH, and free and adsorbed TSH are presented in CD diagrams (Figure 9 & Table 4) indicated that there are many changes in the secondary protein structure of proteins adsorbed on the surface of MNP@Dextran. While the random coil percentages were increased indicating the involvement of the domains the contain these structures (alpha helix and beta sheet) in the interaction with the surface. The high noise in the charts is partially due to background absorption, and partially due to the low CD intensity of the hormones under study with a very little helix structure percentages [33] .The results confirm that FSH contains significantly more α-helix than LH [33]. This table also showed significant changes in secondary structures of protein as adsorbed on the surface of MNP@Dextran. Alpha chains of the hormones have been reduced while beta chains percentages have been increased. The changes in the secondary structure of these hormones as the adsorption on MNP`s surface occurs was analyzed via CD spectroscopy, which is particularly efficient in determining the protein folding and in characterizing the secondary structure of protein [34].The aromatic CD spectrum displays several characteristic bands and shoulders. The almost conservative nature of the CD spectrum suggests that at least part of the spectrum is due to an interacting pair of aromatic residues. The relatively high intensity of the signal suggests that these residues must be in close contact with the surface active sites [35].The results of CD in general indicating that the attachment of the proteins with the surface of MNP@Dextran was occur at the domains that rich in α-helix structure. After attachment, these structures tend to loss their order and turns into random coils. Furthermore, the distortion of the protein molecules at all, due to the attraction with the MNP@Dextran surface and the attraction with the water molecules in the bulk solution, leads to other changes in the secondary protein structure. Hence the adsorption of these hormones is associated with changes in the secondary protein structures.

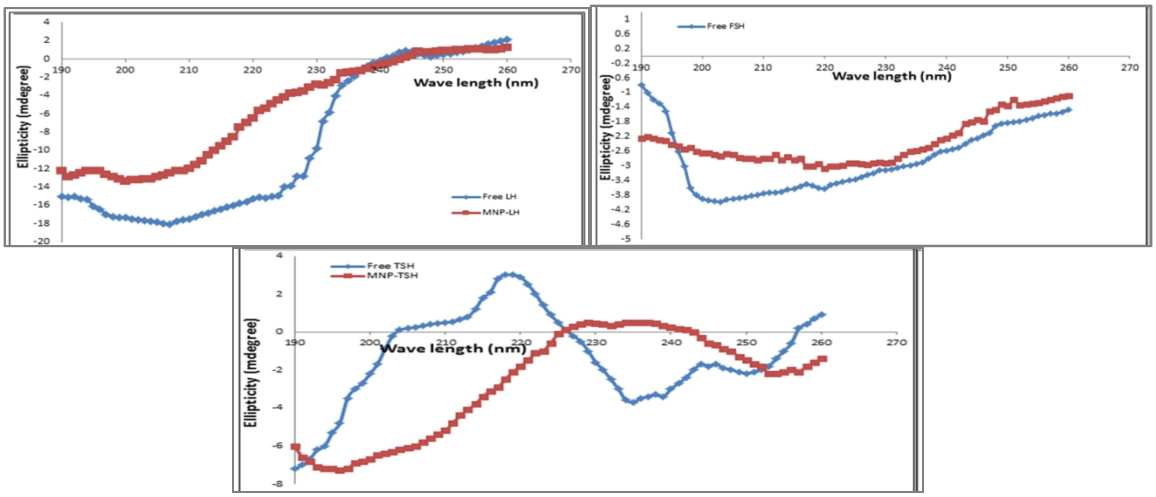

Figure 9: CD for the free LH and LH-MNP@Dextran, free FSH and FSH-MNP@Dextran, and free TSH and TSH-MNP@Dextran

The CD spectra analysis reveals that the secondary structure of the other proteins like BSA was more deviated from the native protein structure after adsorption [36] .These changes are due to the decrease in the degree of freedom as the molecules retained to the surface of MNP@Dextran. The change in the tertiary protein structure after adsorption on different surfaces are recorded previously [36] .The entropically favored interaction between Hormones and MNP@Dextran leads to substantial loss in protein secondary and tertiary structures with the release of a significant amount of bound water, as indicated by CD, FTIR and fluorescence spectroscopies [30].

Table 4: The percentages of secondary protein structure components of free and immobilized hormones on MNP@ Dextran surface

Secondary Structure	LH		FSH		TSH	
	LH	Bound LH	FSH	Bound FSH	TSH	Bound TSH
α-Helix %	5.73	3.75	9.35	2.42	8.27	1.94
β-Sheet %	37.81	26.84	26.57	30.44	39.73	30.22
Random coil %	56.46	69.41	64.08	67.14	52.00	67.84

The fluorescence spectroscopic studies show that MNP@Dextran quenches the fluorescence of the hormones under study both statically and dynamically, and induces some perturbations on the conformation of the hormones as well as the microenvironments around the Trp and Tyr residues as cited previously for other hormones [24].

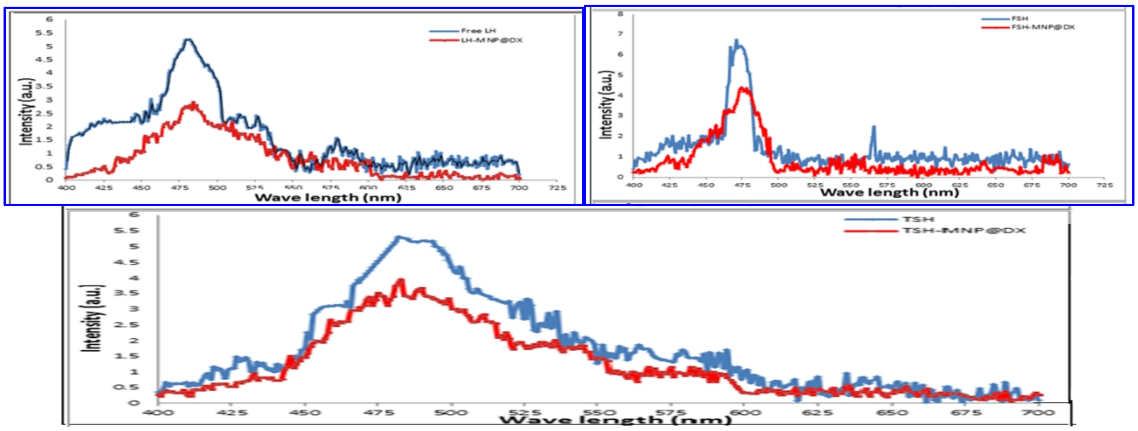

Figure 10: Fluorescence spectra of free LH and LH- MNP@Dextran, free FSH and FSH- MNP@Dextran and free TSH and TSH-MNP@Dextran.

The formation of large assemblies (aggregates) suggest that MNP@Dextran induces protein unfolding at its surface ,Whereas the adsorption system shows distortion in CD spectra at lower wavelengths, strengthening the perception of protein unfolding. Large surficial interaction that may lead to protein unfolding [28]. The strong forces between FSH and TSH and the active sites on the MNP@Dextran leads to reduction in the quantities desorbed from the surface. Different forces are involved in the attachment of protein on the surface of NPs ranging from reversible physical adsorption and ionic linkages to stable covalent bonds [37]. The secondary structure was determined via CD spectroscopy in the far-UV spectral region (200–260 nm), the chromophore at this wavelength is the peptide Bond. And the analysis of these figures was explicated using K2D3 program by listing the results online. The results obtained are listed in Table 4. The overall CD results involve a reduction in the alpha-helix and beta-sheet percentages of the proteins after adsorption on the surface of MNP@Dextran. In figure 10 revealed changes in the fluorescence spectra after adsorption of the hormones on the surface of MNP@Dextran. The intensities were reduced and the peaks were shifted toward the longer wave length as adsorption occurs. For LH, the shift from 482.66nm to 487.95nm; for FSH, from 472.36 nm to 479.33nm; and for TSH from 480.93nm to 485.72nm as the hormones adsorbed on the MNP@Dextran.

Conclusions

The synthesized MNP@Dextran has the ability to adsorb the hormones LH, FSH, and TSH on its surface spontaneously. This adsorption is concomitant with alteration in the secondary and tertiary protein structures. The effect of the MNP@Dextran on the protein structures leads to subsequent alteration in the protein activity when the nanomaterial used in contact with the body fluid.

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