

Application of MNPs-IHSPN nanoparticles in really stabilization of biomolecules bio drugs In-vitro environment

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Research

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Abstract

All scientists are working on biomedicine and Nano-biotechnology. So, one of the most important issues is the study of magnetic nanoparticles (MNPs-IHSPN). The purpose of this project is to examine the approach of using a common buffer to determine the degree of stabilization and release of two molecules of drugs that have been analyzed In-vitro. The structures as a SEM and FT-IR instruments and were used in the amount of 25 mg. After the reaction with biomolecules, the absorption rate was about 60-80%. The release of biomolecules was done by a buffer PBS by spectrophotometer analysis. It is noteworthy that these biomolecules was tested in two forms of covalent and electrostatic bonding. EDX analysis and electrophoresis were used to stabilize the absorption rate in the electrostatic transplant and FT-IR analysis for fixation of covalent bonding. The overall result of this project is to use the common characteristics of some biomolecules for important drug exchange in the living environment of cells.

Introduction

Nanotechnology is a key element in understanding the nature of the coming decade. Interdisciplinary research collaboration, special training and transfer of ideas from people in the industry; including the benefits of nanotechnology in the future. Nanocatalysts nanotechnology industry had been made significant progress. Nanocatalysts types are designed, among them, iron oxide nanoparticles (based on magnetic properties) had been many applications especially in the field of drug delivery, the proteins and biodrug (MTX biomolecule drug) are necessary. One of the most important and most widely used magnetic nanoparticles in which a variety of materials to create their own unique characteristics compared to other Nano-specific applications screwed. These particles are applied in various branches. But the role of them in life_medicine and, as mentioned, are significant in terms of its delivery to the inherent magnetism gives them a lot of things, including facilitating spotter the delivery of these are very important [1].

Application and structure of magnetic nanoparticles

Over the past few years, efforts have been devoted to the magnetic Functionalized nanoparticles as the level of cover will gain significant benefits from it. However, there are many types of materials available in magnetic coatings Nanoparticles, such as metal oxides, metal, and plastic; Silica is still considered to be the best candidate surfaces Functionalization because it is highly stable against degradation. In addition, the silica to improve the biocompatibility, hydrophobicity profile as well as the availability of high-level performance Group silanol (-SiOH) on the surface [2], that makes a promise Materials for a variety of biological applications. Silica-coated magnetic nanoparticles are used for various applications in recent years, such as separation of protein and enzyme immobilization [3, 4].

TEOS-MPTES (3-mercaptopropyl triethoxy silane), SH-polymer

Although silica coating enhances the surface performance of magnetic nanoparticles, the surface can be modified to increase this performance as well as to increase the biomolecule uptake at the nanoparticle

surface. For this purpose, another coating called mercapto (with a sulfur functional group derived from the green chemical composition of imidazole ion) was used which results showed that the size of the magnetic nanoparticles decreased after adding the mercapto functional group and The stability of the reaction is increased. Nowadays, chemical-organic compounds known as green solvents without harmful effects on the environment are used, as indicated by imidazole ion combining ionic liquids with two cationic and anionic components to provide stability and enhance the performance of nanoparticles used. It should be noted that in this complex, the imidazole ion ($C_3N_2H_4$) as a cationic portion and a variety of halides such as chloride and bromide ion as the anionic portion provided the complex composition for the mercapto (sulfur functional group) on the surface of the magnetic nanoparticles [5].

Stabilization and stabilization of methotrexate by Magnetic Nanocomposite Magnetite

Methotrexate conjugated with large microparticles made of gelatin or polyglutaraldehyde improves methotrexate levels when interacting with tumor cells. This is called a targeted pharmacologist and its therapeutic efficacy has improved. The size of the compound (the molecule conjugated to the microparticles) should be small, rather than the movement of the drug molecule in the flow of blood circulation (in the veins) to the target location is easy. Thus, nanoscale nanoparticles with small particle sizes are used at the nanoscale, for example, a drug substance (methotrexate) conjugate to the surface of the magnetite magnetic nanoparticles that are being studied [6]. Magnetic nanoparticles have been prioritized to Nano-compounds due to their stability, their small particle size, and their controllability by external magnetism for targeted drug delivery, and antibacterial properties [7-8]. There are many papers and researchers to stabilize biomolecules on magnetic nanoparticles. The main goal of this project is to cover the SiO_2 activation of Fe_3O_4 magnetic nanoparticles for the stabilization of biomolecules (protein (BSA) (serum albumin)) and $Fe_3O_4@SiO_2/SH/NH_2$ for the stabilization of biomolecules methotrexate (MTX) drug molecule. In this study, they sought to stabilize and release them *In-vitro* according to the result, the basic point is the stabilization of our electrostatic bond (for protein biomolecules) and covalent bond (for MTX drug) between them (by electrophoresis and EDX analyses) at room temperature ($25^\circ C$) for protein and $37^\circ C$ for MTX (Figure 1).

Experimental

Materials

All solvents and chemicals are purchased from commercial Suppliers. The structure of materials was provided by Transmission electron microscope (Philips CM-200 and Titan Krios TEM, derivative in the University of the Shahid mohaggege Ardabil). BSA were obtained from Sigma (St. Louis, MO). Materials such as; ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), ferric nitride Nona hydrate ($Fe(NO_3)_3 \cdot 9H_2O$) and sodium hydroxide (NaOH) were purchased from Merck KGaA (Darmstadt, Germany). And, phosphate buffered saline (PBS (pHs 6.0–8.0)), argon gas, HCl, methanol, TritonX100, EDTA, Boric acid, NaCl, glutaraldehyde and Salmon sperm (protein) sodium salt is purchased from Sino-pharm Chemical Reagent Co. (Shanghai, China). Methotrexate (Ibo, Canada) with a molecular weight (454.44 g/mol, NHS

(n-hydroxysuccinimide) molecular weight (115.09 ppm), EDC (1-ethyl-3-(3-(dimethyl)-amino)-propylene (carbon dioxide) with molecular weight (155.25 g/mol), imidazole ($C_3N_2H_4$, molar mass 68.077 g/mol), (3-chloropropyl) triethoxysilane or CPTES, (molecular weight 198.72, 97% purity), (3-mercaptopropyl) triethoxysilane or MPTES, (molecular weight 196.34 g/mol, 95% purity), APTES (3-amino-propyl tri-methoxysilane), DMSO (dimethyl sulfoxide), sodium hydroxide and chloride-containing acid with a concentration of 0.1 molar, TEOS (tetraethyl-orthosilicate), Hydrazine (34% by weight aqueous solution, reducer) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Protein used in the lab is models (Maragheh, Iran). Deionized water was used in each experiment.

Synthesis of silica-coated with Fe_3O_4 magnetic nanoparticles

Chemical Co-precipitation also one of the easiest and most convenient methods of synthesis of magnetic nanoparticles with core/shell structure. So, in this way, sample container iron salts with amounts of 1 to 2 (1.5mg of $FeCl_2 \cdot 4H_2O$ and 3mg of $Fe(NO_3)_3 \cdot 9H_2O$) were dissolved in distilled water. The reaction temperature was 25° C and high-intensity spinning under inert nitrogen gas. After 3 hours to prevent additional oxidation and increasing the absorption of biomolecules for biological targets of 3 ml tetraethyl-orthosilicate was used. Finally, the yellowish-brown product was obtained in the same magnetic nanoparticles. In the read more, the solution was washed repeatedly with methanol and water and then dried in the oven, the powder was gathered.

Synthesis of $Fe_3O_4@SiO_2/SH/NH_2$ magnetic nanoparticles

After synthesizing $Fe_3O_4@SiO_2/SH$ magnetic nanoparticles, weigh out 100 mg of same magnetic nanoparticles and dissolve in distilled water 4 ml to completely dissolve in water. In another part, it prepared 3 ml of the APTES solution (3-aminopropyl tri-methoxy-silane), and then the magnetic nanoparticles $Fe_3O_4@SiO_2$. After synthesizing $Fe_3O_4@SiO_2$ magnetic nanoparticles weigh out 100 mg of same magnetic nanoparticles and dissolve in double distilled water 4 ml to completely double dissolve in water. After complete dissolution of sample, place in reflux conditions at 100°C, and the amount 250 μ l (CPTES) linker is added to the solution. Then 24 hours, 250 μ l of (MPTES) was added to the solution. On another 36 hr, 250 μ l of N-methyl imidazole was added to remove chloride from the first linker. Which are completely dissolved (in water) in the case of a heater and powered by a magnet the solution was completely dissolved in a drop-drop of APTES solution to magnetic nanoparticles and placed in a non-temperature condition for one night. Finally, we wash the magnetic nanoparticles $Fe_3O_4@SiO_2/SH/NH_2$ prepared by distilled water twice ionized and placed in the oven. Finally, magnetic nanoparticles containing a yellowish-brown amine agent group was prepared.

Magnetic $Fe_3O_4@SiO_2/SH$ nanoparticle structure with amine group agent for stabilization of MTX drug molecule

The concentration of 25 mg of nanoparticles (dissolved in water) in 100 μ l of methotrexate, in a dimethyl sulfoxide solvent and a reaction temperature of 37° C under vigorous stirring (300 at a speed of rotation

per second) for 36hr of times. The synthesis was carried out under conditions where the magnetic nanoparticles were completely dissolved in the water solvent to distribute the particles uniformly; in another test tube, the methotrexate particles were dissolved in a solution of dimethyl sulfoxide and the reason for using this solution (dimethyl sulfoxide) was therefore to reduce the solubility of methotrexate in water. When methotrexate was conjugated with magnetic nanoparticles, two materials: (1-ethyl-3- (3-(dimethylamino) propyl) carbonyl amide (7.5 μ l)) and N-hydroxy succinimide (15 μ l)). Both of them, as we have mentioned, play an important role in the process of stabilizing methotrexate at the surface of the nanoparticles and creating a link between the two. Finally, after completion of the reaction, the nanoparticles are detached by a magnetic magnet and the prepared solution is prepared from the samples with a mixed mass (consisting of nanoparticles and conjugated molecules conjugated to their surface) for absorption analysis which is then fully investigated has been.

BSA adsorption from aqueous solution

BSA adsorption experiments were carried out in batch-wise. Approximately 25 mg of magnetic silica nanoparticles were mixed with 1ml of various concentrations of BSA solution in water. The mixture was shaken at room temperature for 1 h, which proved to be sufficient period to reach equilibrium. Then the magnetic particles were separated with help of the permanent magnet and the supernatant was assayed for remaining protein concentration by the UV-Vis spectrophotometer at 595 nm. The adsorbed amount of protein was calculated by mass balance.

Results

Synthesis and characterization of magnetic nanoparticle coated with silica

In this section, we will try to use the aforementioned magnetic nanocomposite first to prepare its core / shell structure and then to evaluate it by highly specialized analyzers. For this purpose, various analytical devices are used which we have been able to record by the SEM analyzer, the orderly structure of the nanocatalyst with the active reactant surface. As it appears from the nanocatalyst formulation, it provides a suitable surface for conducting a variety of chemical-biological reactions with most biomolecular biomolecules. In the range of 1-100nm magnetic nanoparticles should be made, such as the size of the magnetic nanoparticles in chemical reactions and medical procedures are important. The structure of magnetic nanoparticles coated with silica by analytical SEM is shown in (**Figure 2a**).

The FT-IR spectrum of Fe₃O₄@SiO₂/SH/NH₂ (MNPS-IHSPN)

In order to show the bond between the functional groups to make the covalent bond, FT-IR analysis is used, the range of frequencies is 500-4000 cm^{-1} , the absorption band for the 808 SiO_2 functional group and 1.222 cm^{-1} Si-O-Si. In the present magnetic nanocatalysis, the amount of silica coating at 879 and 694 and for mercapto (-SH functional group) was at 1.212 cm^{-1} and so, 2930-3390 cm^{-1} is about amino linker (-NH₂ functional group, which after the addition of sulfur functional group, the peak rate became

narrower ie more regular. This would indicate an increase in the level of performance of the magnetic nanoparticles (**Figure 2b**).

Discussion

Results of protein and MTX loaded onto magnetic nanoparticles $Fe_3O_4@ (SiO_2/SH$ or $SiO_2/SH/NH_2)$ by spectrophotometry

The purpose of this section is to investigate the absorption of drug biomolecules on the magnetic nanoparticle bed. Therefore, using the equation [7-8], we can examine the absorption rate. Under standard conditions, the amount of 30 micrograms per μ l of the bio-molecule protein and drug are dissolved in 2 ml of sterile water, and then twice 25 milligrams of magnetic nanoparticles weigh in two separate dishes and on each One of the bio-molecule proteins and drug (60min, 36h) were solved separately.

The instant of dissolution of the two mixtures was continued at the instant of zero minutes to about an hour for each of the mixtures and summarized the obtained data. The results showed that the absorption rate of the protein in the first half hour was about 60% and the absorption rate of the drug on the nanoparticles were about 80%. The results obtained in the future did not change later, and the results showed that the absorption of nanoparticles in non-co-valcanic and covalent magnetic nanoparticles is approximately equal to one. After methotrexate has been stabilized on a surface of $Fe_3O_4@SiO_2/SH/NH_2$ magnetic nanoparticles with different methods, we will now consider the methotrexate release rate. This is done with a phosphate buffer solution which is a mixture of 4 K_2HPO_3 , Na_2HPO_3 , KCl, and NaCl salts in a specified amount of distilled water, which is ready for use in the reaction after the autoclave. Using phosphate buffered saline, sodium hydroxide, and chloride acid adjust the pH to 8.8 to adjust the pH of the pH in the presence of phosphate buffer of methotrexate on the surface of nanoparticles and with sodium hydroxide and chloride. And the rate of release has been measured over a period of 2 hr to 72 hr at pH of 7.28-8.0. Looking at the data in the chart, it can be concluded that after 24 hr, the release of the methotrexate drug molecule from the surface of nanoparticles is about 80% at pH 7.8. With these results, can be said that is a full stabilization of MTX drug molecule on MNPs-IHSPN *In-vitro*. The results are shown in (**Figure 3**). All Error bars are selected as standard, indicating the accuracy of the data presented in the chart.

The effect of time in absorption

Reaction time for absorption, 1hr and 36hr in this test as the standard time for this research is very important. At the beginning of the reaction, patterns were to study the magnetic nanoparticles containing silica-protein-MTX absorption studies conducted showed that the uptake protein the concentration (30 μ g/ml) and 10 μ g/ml for MTX, in The wavelength of 595nm for 30min (absorption wavelength protein) is 60% and the uptake MTX the concentration (10 μ g/ml), in The wavelength of 304nm for 36h (absorption wavelength MTX) is 80%, and after 1hr and 36hr absorption be stable over time because the absorption process of protein and MTX was complete on the surface of magnetic nanoparticles. The absorption of

biomolecules (MTX, protein) within a half-hour and twenty four-hour episodes were tested and the results were observed in **(Figure 4)**. And according to the results, we find that over time gradually increased uptake, and it been stable after 1h for protein and 36h for MTX drug molecule.

The stabilization of methotrexate and protein to the surface of the magnetic nanoparticles $Fe_3O_4@SiO_2/SH/NH_2$ by EDX, FT-IR

In this discussion, by W% of elements perceived whichever were dependent to the reactants of MTX and protein and magnetic nanoparticles $Fe_3O_4@SiO_2/SH$, $Fe_3O_4@SiO_2/SH/NH_2$ and EDX analysis showed that both of the reactants bonded together been in the product. Also, elements of Fek_α and Fek_β with elements Si (with a strong peak) and O are shown in $Fe_3O_4@SiO_2$ product. This analysis may be demonstrative bond between magnetic nanoparticles and MTX or BSA. The results of the EDX analysis show that binding of agent N in 750 keV and agent of O 1100 keV and agent N in 750 keV and agent of C of 600 keV because they are in a line so, it may be stated this approaching is electrostatic bonding same for N-O and covalent bonding same for C-N. Element O is in the agent group O_2 of coated silica (SiO_2) and element N of the agent NH_2 of MTX or protein. Evidence of EDX analyses is a Spectrophotometer seconder for this tissue. The result of absorption, and the link between magnetic nanoparticles and MTX (down picture (B)) or BSA (up picture (A)) protein by EDX analysis, shown in **(Figure 5, left picture)**.

In the FT-IR spectrum **(Figure 5, right picture)**, by factorizing the nanoparticles with silica coating, we observe no change in the structure of the nanocomposite of magnetism. By examining the pixels of the four samples, the following results were obtained: A) the structure of magnetic nanoparticles with coating silica, B) the structure of magnetic nanoparticles with amine linker consisting of 2930 cm^{-1} ppm for the (-CH) group, 1407 and 1550 cm^{-1} , for the $-NH_2$ group and 1100 cm^{-1} for the Si-O group and furthermore so, 1112 cm^{-1} is for -SH group, C) Magnetic nanoparticle structure with SH- NH_2 linker and fixed methotrexate on it includes 1606 and $1644\text{ pixels cm}^{-1}$ for covalent bond between carboxyl group (-COOH) methotrexate and amine group ($-NH_2$), and D) methotrexate structure including peak 3391 cm^{-1} related the group (-OH) and peaks of and 1603 and 1644 cm^{-1} correspond to poor absorption of methotrexate on surface of an amine-bonded magnetic nanoparticle. -SH linker present in the ionic complex in the structure of the magnetic nanoparticles improves the covalent bond between the amine linker of the nanoparticles with the carboxyl linker of the methotrexate bio-drug and is a sharp and regular peak that shows drug absorption and strong bonding with the nanoparticles. So it can be said that the presence of ionic liquid composition increases the rate of drug absorption on the surface of the nanoparticles.

Stability of magnetic nanoparticles in repeated use after recycling

The results of the magnetic nanoparticles with silica coating for MTX or BSA adsorptions were analyzed by spectrophotometric analysis over a period of 12-170 h for 10 periods for protein and 4 days for MTX, and the results showed that the efficiency of nanoparticles in the application again and again the

stabilization of biomolecules, MNPs-MTX, BSA even decreased by 10 percent over the course of 15 percent. Magnetic nanoparticles are very important for sustainability under favorable reaction conditions and having the ability to re-use these magnetic nanoparticles. On the picture 6, the magnitude of this stability has been investigated in 7 days (the reaction process and optimal conditions are the same as in the discussion and conclusion). The results are shown in **(Figure 6)**.

The results of the absorption of protein by electrophoresis

In this section, protein is analyzed by the electrophoresis. Electrophoresis analysis is based on absorption at absorption times. Here, vertical electrophoresis to measure protein absorption. With respect to **(Figure 7)**, it can be seen that the amount of stained specimens in the range of 0 to 60 mins, the absorption of protein on the magnetic nanoparticles have gradually dimmed, which this fading stain shows that biomolecules are absorbed on surface of magnetic nanoparticles. Therefore, spectrophotometric absorption and electrophoresis of both devices showed acceptable results for this absorption. First, in 5 different patterns for protein in three separate tests at a dose of **30 µg/ml** and at **0-60min** on a sol-gel plate (Different mixed supernatant solution and biomolecules (protein)-MNPs-IHSPN, and eventually the last point of the mass of magnetic nanoparticles containing biomolecules). In the first line, the ladder is first point, the second line of staining for protein and without nanoparticles (sample 1), the third line contains any protein mixed in nanoparticles (without catalytic) at zero time (sample 2), the fourth line contains either fixed protein in nanoparticles at 5 minutes (sample 3), fifth line at 15 minutes for protein (sample 4) and line six for 30 minutes for protein (Example 5). Then, sol-gel was placed on the electrophoresis and began to scan and stained. After 2-5 hours, results in same spots of about **60%** of the apparently weaker spots were observed separately from the specimens containing the pure amount of the protein, and the staining smoothed showed that the absorption rate the nanoparticle surface is at least 60% for protein. Biomolecules protein is absorbed on surface of the magnetic nanoparticles, which confirms the stability of the biomolecules (protein) in the nanoparticles, which are shown in **(Table 1** (down picture), **Figure 7** (up picture)) evidence for this stability.

Conclusion

In this project, the maximum capacity of magnetic nanoparticles (MNPs-IHSPN) with silica coating was used to stabilize and liberate biomolecules (MTX and protein). To do this, the MNPs were originally synthesized using a chemical co-precipitation method and their structure was identified with tools such as SEM and FT-IR. To do this, the amount of 25 mg of MNPs in 1 ml of solution of each biomolecules (protein, with an optimal concentration of $30 \mu\text{g}\cdot\text{ml}^{-1}$ and MTX, with an optimal concentration of $10 \mu\text{g}\cdot\text{ml}^{-1}$) at 25°C (room temperature) for protein and 37°C for MTX in a water solvent for protein and water with DMSO for MTX in the specimens were then isolated for 60 minutes for the protein and 36 hour for MTX, after the reaction (these times are optimal) and tested in the spectrophotometer and UV-Vis apparatus to measure their absorbance. The results showed that the absorption rate a protein higher than 60% and 80% for MTX, which indicates a high percentage of absorption of biomolecule (protein or MTX) on the surface of magnetic nanoparticles, addition of biomolecule (protein or MTX) from the surface of the

nanoparticles in the presence of external magnetic field and PBS buffer was shown to be more than 90%. After the experiments, magnetic nanoparticles were extracted by external magnetic field. Overall results showed that biomolecules (MTX, protein) were almost completely stabilized on surface of magnetic nanoparticles. With the endured studies, this project can be a way of stabilizing biological and drug-mental molecules by MNPs- (MTX, protein) systems.

Declarations

Acknowledgments

All the information obtained from this research work is the result of an effort made in the laboratory over many years (under the supervision of Maragheh University, 2015-2019).

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data's and values are fully measurable and availability.

Consent for publication

That is applicable to this work.

Ethical approval and consent to participate

Not applicable.

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Figures

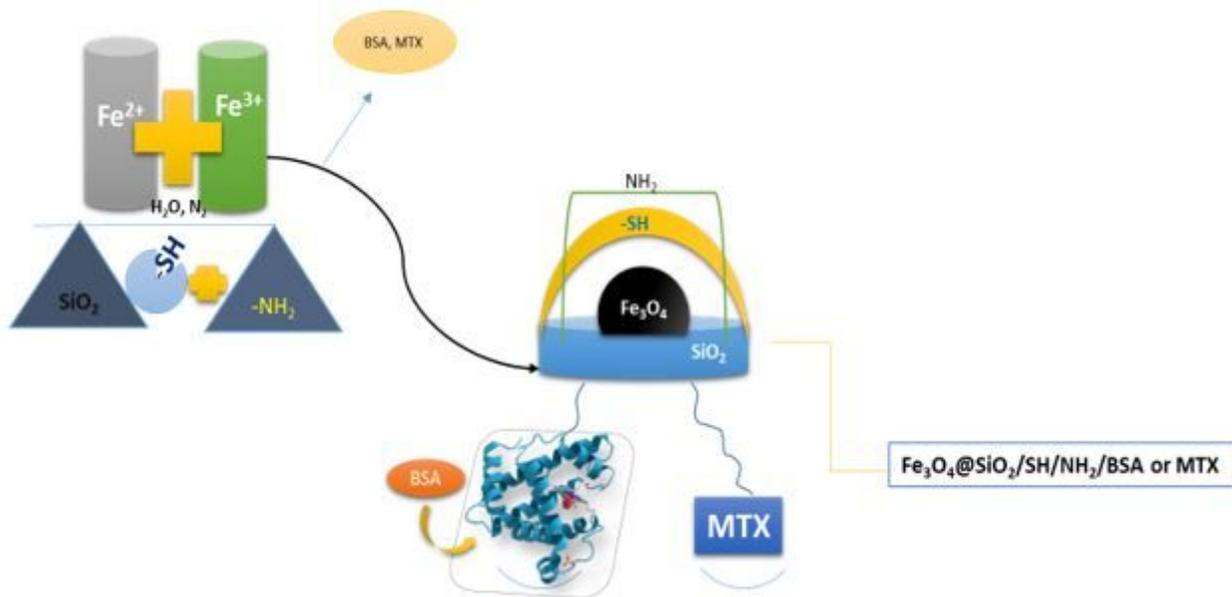


Figure 2

The general process of MNPS-ISP nanoparticles formation and its reaction with biomedical biomolecules

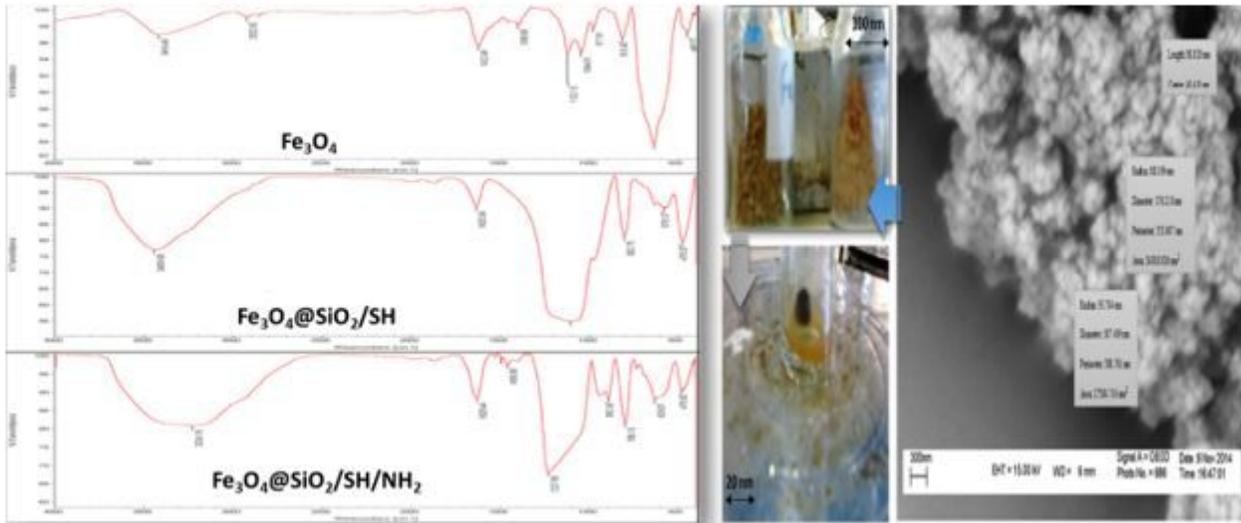


Figure 4

Left picture (figure 2b) is FT-IR analysis of nanoparticles (of up to down) and so, right picture (figure 2a) is SEM analysis of Fe₃O₄@SiO₂ nanoparticles.

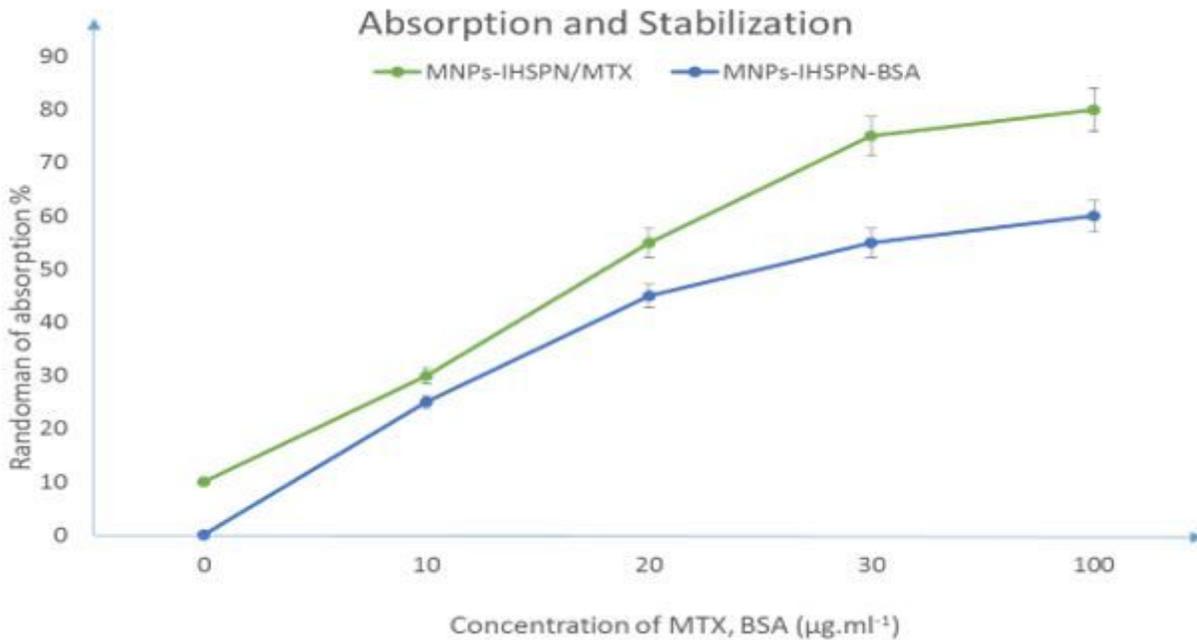


Figure 6

Results of absorbed MTX, BSA biomolecules on MNPs-IHSPN nanoparticles in concentration (10-100 µg/ml)

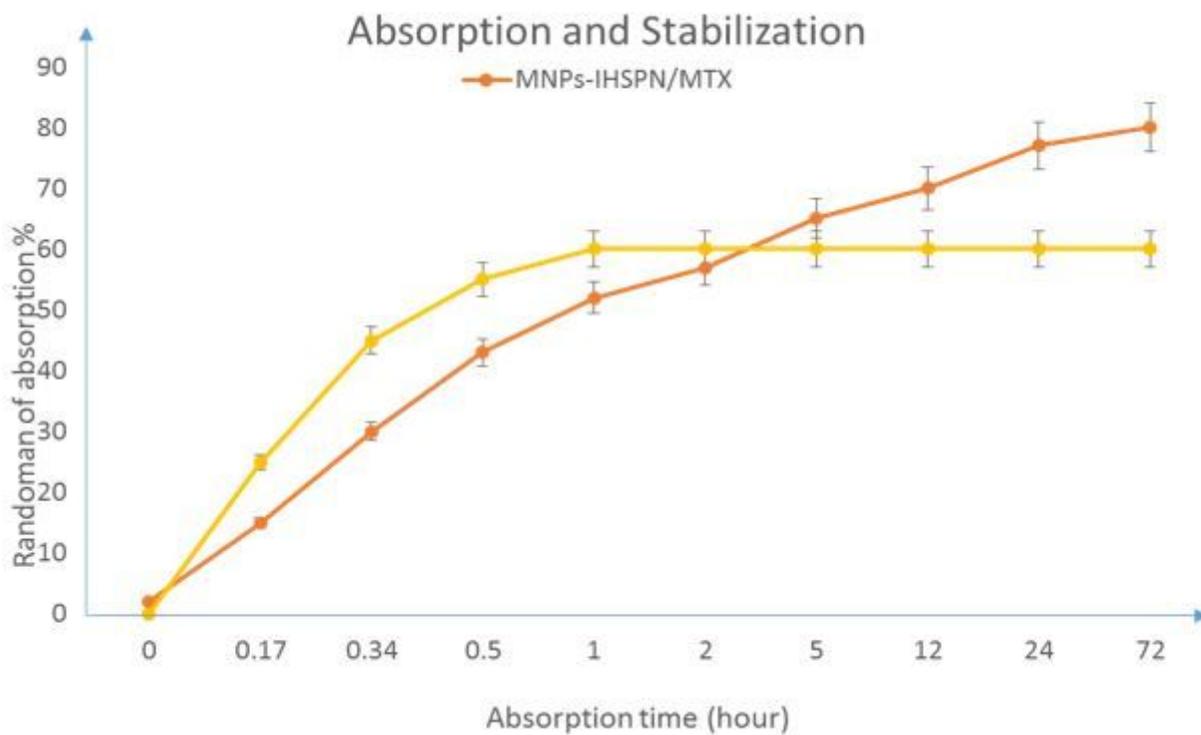


Figure 8

Results of stabilization of MTX, BSA biomolecules on MNPs-IHSPN nanoparticles at time (hour)

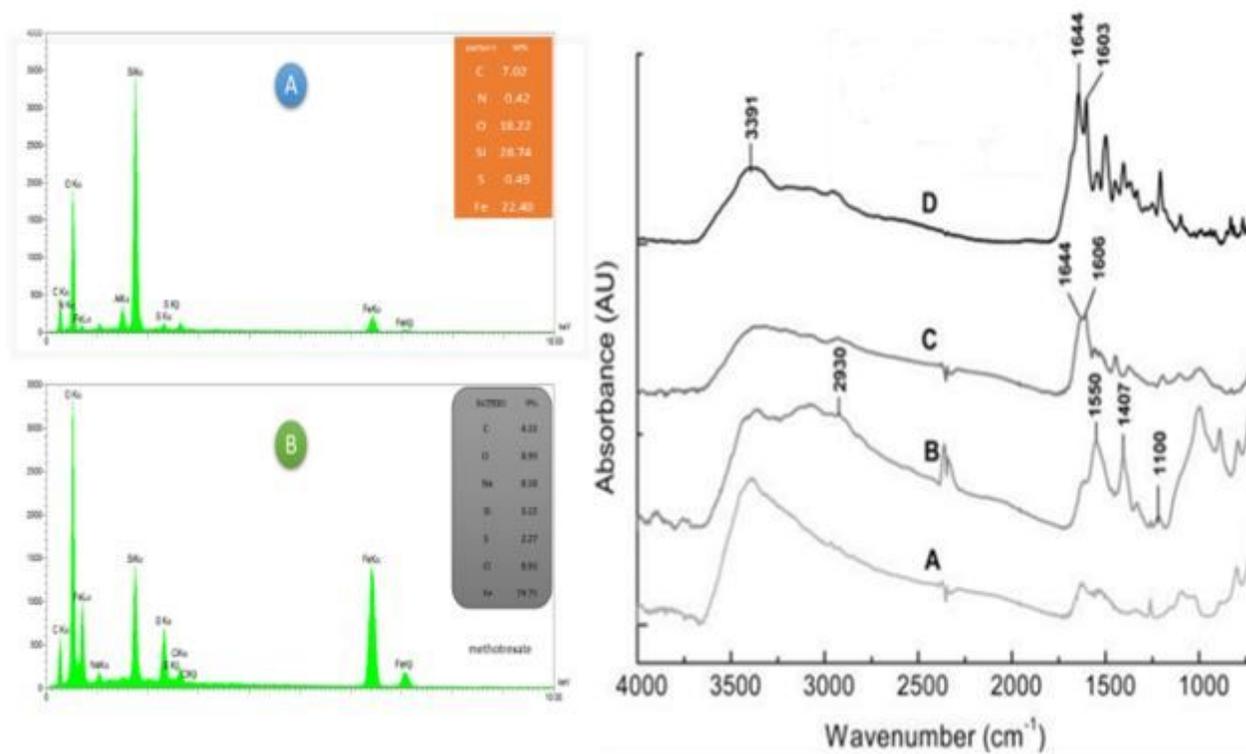


Figure 10

Results of EDX analysis. Up picture is for BSA protein and, down picture for MTX biodrug, left pictures. And so, result of FT-IR analysis for absorption of MTX biomolecules on MNPs-IHSPN nanoparticles, right picture

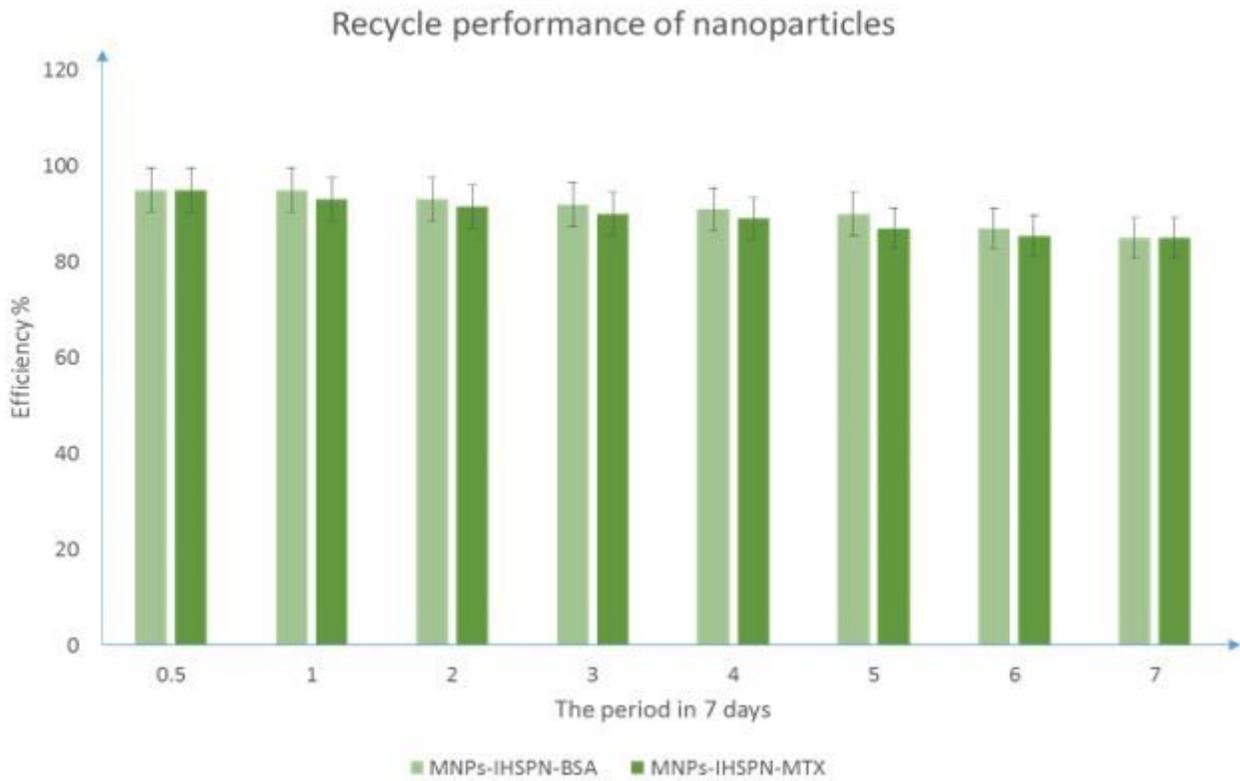


Figure 11

Efficiency of MNPs-IHSPN nanoparticles in stabilization of MTX, BSA biomolecules for repeated use over a period of 7 days

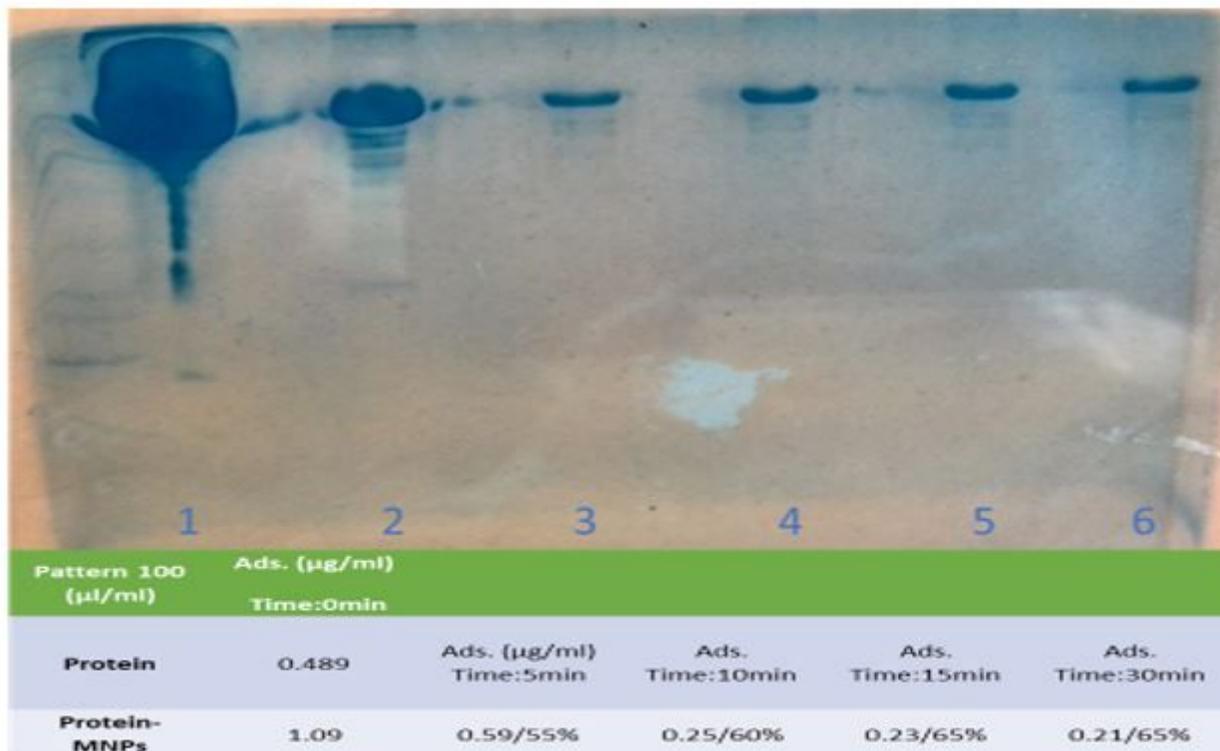


Figure 14

Electrophoresis results to stabilize the amount of protein adsorbed on the surface of the nanoparticles, up picture and so, the data obtained is categorized in the table 1, down picture