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RESEARCH PAPER

Synthesis and Characterization of Novel Modified and Functionalized Silica Nano-particles for Protein Delivery Applications

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ABSTRACT

In this study, the synthesis, characterization and controlled release behavior of new Hollow Silica Nano particles (HSNPs) and Magnetic Silica Nano Particles (MSNPs) were studied. Magnetic Silica Nano particles (MSNPs), as drug delivery vehicles, were synthesized through the coating of Fe_3O_4 nano-crystals with silica layers. The HSNPs were obtained by removal of Fe_3O_4 templates with hydrochloric acid and then calcinated at the high temperature. In the first step, MSNPs and HSNPs were modified and the amine and aldehyde functional groups were introduced on the silica surfaces. In the next step, positively charged silica nano particles were synthesized through the quaternization of Aminated Silica Nano Particles (ASNP) with sodium monochloroacetate (SMCA). Following characterization, insulin was loaded into the nano particles as a model system which demonstrated a notable sustained drug release. The study shows that the HSNPs could load a higher amount of drug. The release rates from the HSNPs were faster than those of MSNPs.

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INTRODUCTION

Controlled drug release systems began in the 1970s and have continued to develop so rapidly that there are now a lot of products, both on the market and in development. Furthermore, the ideal drug delivery system should be inert, bioadhesive, biocompatible, comfortable for the patient and capable of achieving high drug loading. Owing to the large surface area, large pore volume, highly ordered pore structure, and adjustable pore size, nano silica material have wide and interesting applications in the biotechnology field. For instance, hollow mesoporous silica materials can be used as a drug carrier for the controlled release of pre-loaded therapeutic agents [1-8].

Hollow nano particles can be synthesized with various materials such as silicates, organic polymer, carbon, titanium, etc. To produce the cavity in the nano particles, various removable templates, such as polymeric micelles or surfactants, gold nano particles, silicates and luminescence semiconductors have been used. In particular, biocompatible silica nano particles have been extensively used because of facile formation and a convenient surface modification procedure through functionalization of silanol groups. To achieve sustained release with a hollow structure, a surface modification process seems to be crucial [9-12].

Herein, magnetic and hollow silica nano particles with a large cavity were synthesized as nano containers for drug delivery vehicles. For this purpose, magnetic assemblies (MAs) were prepared by clustering of Fe_3O_4 magnetic

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nanocrystals with a surfactant binder and then these templates were coated with silica layers. The resulting magnetic silica nano particles (MSNPs) were treated with hydrochloric acid and calcinated at high temperature to form a large core cavity [12-16].

Then, aminated silica nano particles were synthesized through attaching 3-aminopropyltriethoxy silane (APS) on the surface of silica nano particles. These aminated silica nano particles were modified through a secondary chemical reaction: first, they were activated with glutaraldehyde, and then pH-sensitive positively-charged silica nano particles were prepared via the covalently attachment of sodium monochloroacetate to the aminated silica nano particles (the quaternization reaction). Insulin was chosen as a model drug, being loaded on these surface-modified silica nano particles, and in vitro release profiles was established at pH 7.

It is worth mentioning that , in previous related researsch studies, simple nano silicas were considered as a drug delivery systems, yet, the present study tries to modify nanosilica layers employing OH, NH2 functional groups (with &without cavity) and Cationtic functional groups (with & without cavity). It also tries to prepare polymerized and nanosilical layers with different functional groups (modified with glutaraldehyde functional groups, chitosan, ….) in order to investigate the effects of different functional groups and cavities and megnatic functions in loading and delivering insulin drug. The other purpose of this study is to prepare and investigate imprinted and non-imprinted nanosilic layers in order to analyze loading and delivering Metatroxide in different (4, 7) assimilated gastric and intestine.

MATERIALS AND METHODS

Insulin was recombinant human insulin (AK2U Nobel France; lot # 821156, Batch L-00023822). Iron (III) acetylacetonate, 1, 2-hexadecanediol, dodecanoic acid, dodecylamine, 3-aminopropyltrimethoxy silane (3-APTMS), sodium monochloroacetate (SMCA), tetraethylorthosilicate (TEOS), and poly vinyl alcohol (PVA; Mw, 15-20 kDa) were purchased from Sigma-Aldrich. All solvents were obtained from Acros Co. All other chemicals and reagents were analytical grade and used as received.

Measurements

The IR spectra were recorded by a Bruker Vector-22 FT-IR spectrometer. The amount of released drug was measured by a JACSO 7850 UV spectrophotometer at the absorption maximum of the free drug in pH 7 (λ_{max}=272 nm) using a quartz cell. XRD spectra of samples were recorded on a Bruker AXS model D8 Advance diffractometer using CuKα radiation (λ=1.542 A˚), with the bragg angle ranging from 2–70°. Morphology of the samples were studied using SEM images recorded by a Scanning Electron Microscopy (Cam Scan MV 2300).

Preparation

Prepared nanoparticles and their synthesis route are illustrated in Fig. 1.

Synthesis of Magnetic Nanocrystals (MNCs)

A mixture of iron (III) acetylacetonate (2.0 mmol), dodecanoic acid (6.0 mmol), 1, 2-hexadecanediol (10 mmol), and dodecylamine (6.0 mmol) were stirred in 20 ml of benzyl ether under ambient nitrogen atmosphere. The reaction mixture was preheated to 145 °C for 25 min and then heated to reflux at 300 °C for 30 min. The mixture was cooled down to room temperature and then products were purified through washing with excess ethanol. Magnetic nanocrystals were synthesized via the seed mediated growth method.

Synthesis of Magnetic Silica Nano particles (MSNPs)

To prepare magnetic assemblies (MAs), magnetic nanocrystals (5.0 mg) was dispersed in chloroform (5.0 mL) then the organic phase was added to a 11 mL of aqueous phase containing 200 mg of PVA. After saturation of the phases, the mixture was emulsified for 12 min using an ultrasonic apparatus at 200 W. The organic solvent was evaporated and the products were purified by filtration and centrifuged at 19000 rpm. The MSNPs containing MAs (as templates) were synthesized by the sol-gel method. The particles were prepared in a mixture of alcohol and water using MA seeds. A solution of MA (5.0 mg/mL) was prepared and 1.0 ml of the prepared solution was diluted with 4.0 mL of ethanol and 0.1 mL of 28% ammonia solution. In order to introduce an outer silica shell, 60 µL of TEOS was added dropwise to the suspension and stirred for 15 h then, washed with water and ethanol respectively and separated

by means of external magnet.

Synthesis of Hollow Silica Nano Particles (HSNPs)

In order to prepare hydroxyl terminated HSNPs (HSNPs-OH), MSNPs (50 mg) was dispersed in 5.0 ml of deionized water and then 4.0 mL of HCl (37%) was added to the reaction mixture. The dark brown solution turned to a bright yellow color. After removing of templates (magnetic nanocrystals) by HCl etching, the residual product was precipitated by centrifugation at 11000 rpm. The precipitate was washed several times with water and the products were frozen and vacuum dried. The nano particles were calcinated at 310 °C to remove the organic compounds.

Amination of magnetic silica and hollow silica nano particles (General Procedure)

10 mg of silica nano particles was added to 0.05 mL of 3-APTMS in deionized water. The mixture was vigorously stirred at 65 °C for 24 h. The products were washed several times with water and then precipitated using centrifugation.

Activation of aminated silica nano particles by glutaraldehyde

1 g of aminated silica nanoparticle was slowly poured into the 10 ml of glutaraldehyde solution (5%) at 25 °C for 1 h then washed with water until there was no residual glutaraldehyde solution.

Activation of aminated silica nano particles by sodium monochloroacetate

Sodium monochloroacetate (4.0 g, 42 mmol) was dissolved in 30 ml methanol under stirring, then the aminated silica nano particle (0.5 g) was slowly added and the mixture was heated to 60 °C for 24 h. The precipitates were washed several times with water and dried under vacuum overnight.

Insulin Loading

First, 1.0 g of each silica nano particles was placed in 25 ml of solution containing 200 IU of insulin in 4 °C to suck up the total amount of drug solution. After 48 h, the product was filtered off, washed with distilled water, and dried under vacuum. The supernatant was kept for calculating the drug loading percentage. The amount of drug loaded in the silica nano particles was assessed by UV-Spectrophotometer. The difference between the amount of insulin initially employed and the drug content in the supernatant was taken as an indication of the amount of entrapped drug. The Entrapment efficient was calculated using the equation (1):

$$\text{Entrapment efficient (\%)} = \frac{\text{IAD} - \text{SFAD}}{\text{IAD}} \times 100 \qquad (1)$$

where IAD: Initial amount of drug and SFAD: Supernatant free amount of drug.

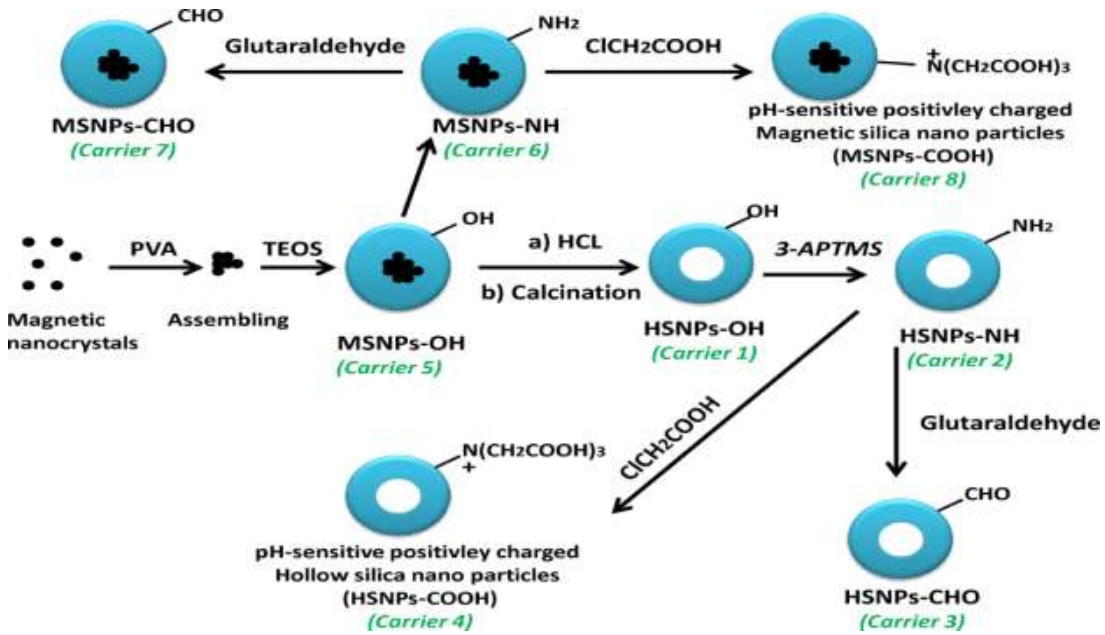

Fig. 1. Illustrations of procedures for preparing porous hollow silica nano particles

Insulin Release

The 10 mg of each insulin loaded silica nano particles was poured into 5.0 mL of aqueous buffer solution at 37±1 °C and in vitro release profiles were established at pH 7. In order to maintain a constant volume, the measured aliquot was added back to the solution. The amount of released drug was determined on UV spectrophotometer by using a standard calibration curve obtained under the same conditions.

RESULTS AND DISCUSSION

The schematic process for the preparation of nano carriers is shown in Fig 1. In the first step, the magnetic assemblies (MAs) of Fe_3O_4 nano particleswere synthesized via the nano-emulsion route using PVA as a surfactant. The hydrophobic magnetic nano crystals were well encapsulated by amphiphilic PVA and then the outer silica layer on MAs was formed by hydrolysis and condensation of the TEOS. Therefore, MSNPs with a core-shell design were successfully prepared. To synthesize HSNPs-OH, magnetic nano crystals (templates) and PVA components were removed via acid etching and calcination, respectively. The large hollow core was obtained by Fe_3O_4 (template) elimination with HCl (37%). The polymeric compounds (PVA were removed by the calcinations of nano particles at 310 °C.

The Silica nano particles without any modification as drug carriers show rapid drug release. To tackle the fast release, a surface modification process seems to be essential. Eight types of modified silica nanocomposites as drug delivery vehicles, [HSNPs-OH, HSNPs-NH_2, HSNPs-CHO, MSNPs-OH, MSNPs-NH_2, MSNPs-CHO] and pH-sensitive positively charged silica nano particles (MSNPs-COOH and HSNPs-COOH)] are prepared (illustrated in Fig. 1).

XRD Pattern

After HCl treatment and then calcination of the MSNPs, the removal of magnetic component was confirmed via XRD studies (Fig. 2). Fig 2a shows the XRD spectrum of pure Fe_3O_4. The XRD pattern of the MSNPs in Fig. 2b shows both silica and inverse spinel structure of Fe_3O_4. After the removal of magnetic templates from the MSNPs, peaks related to Fe_3O_4 were absent in the XRD pattern of HSNPs (Fig. 2c).

FT-IR Studies

The FT-IR analysis shows that, the Fe-O band (around 600 cm^{-1}, Fig. 3a) was not observed at HSNPs-OH, HSNPs-NH_2, HSNPs-CHO and HSNPs-COOH spectra due to elimination of magnetic

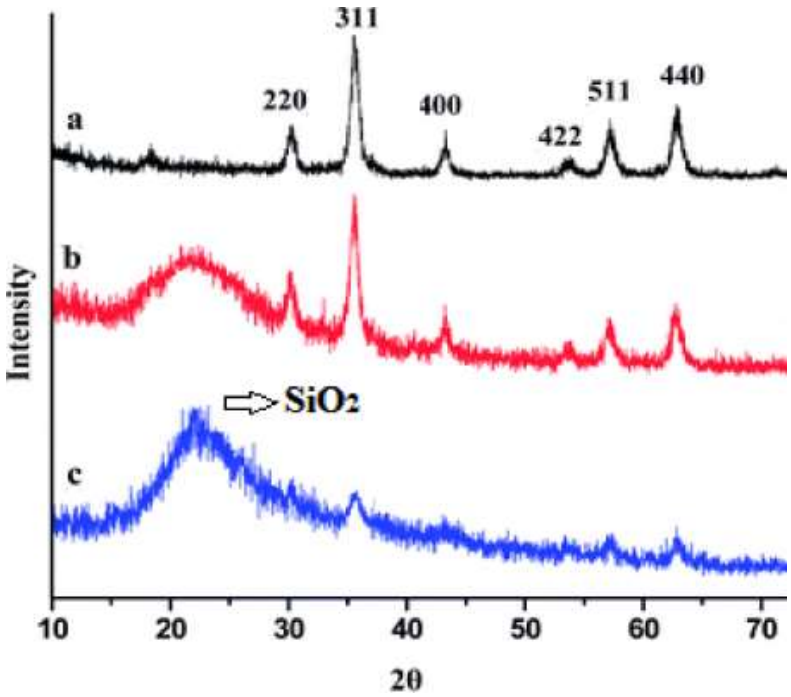

Fig. 2. XRD spectra of (a) pure Fe_3O_4, (b) silica coated Fe_3O_4 (MSNPs), (c) HSNPs

components. The strong peaks at around 1100 cm^{-1} (which is not seen in the spectrum of MAs) are corresponding to silica layers and confirms that the silica layers have been formed (Fig. 3 b, c, d, e). Comparing of FT-IR spectrum of HSNPs-OH, HSNPs-NH_2 (Fig. 3b and 2c) shows some differences such as peaks at around 2900 cm^{-1} (related to stretching vibration of aliphatic –C-H). It confirms that the particles have been functionalized with 3-APTMS. In the spectrum of HSNPs-CHO (Fig. 3d), peaks at 2900 cm^{-1} are strengthened and also the sharp peak around 1700 cm^{-1} appears that corresponding to existing of aliphatic –C-H and –CHO groups, respectively. For pH-sensitive positive-charge silica nano particles (HSNPs-COOH), due to the strongly hydrogen-bonded hydroxyl group in -COOH, this band is spread over the range of 3500–2800 cm^{-1}. The peak related to stretching vibrations of the carboxylic group appears at 1710 cm^{-1}. The spectra of other magnetic carriers (MSNPs-OH, MSNPs-NH_2, MSNPs-CHO and MSNPs-COOH) were similar to their hollow analogues except that the peaks related to MAs can be seen in the spectrum of magnetic carriers.

SEM and EDX Analysis

The structure and morphology of the silica nano particles could be observed in the SEM images, Fig. 4 shows the SEM micrograph of the MSNPs and HSNPs. The average diameters of the particles are about 100 nm and the particle shapes are also uniform. The SEM images of both MSNPs-COOH and HSNPs-COOH shows that the surfaces of these nanocarriers seem smoother than those of other nano carriers. The presence of elements in the nano particles can be confirmed via EDX spectrum. EDX spectra of both HSNPs-NH_2 and HSNPs-CHO (Fig. 5b and Fig. 5c) show the additional peaks which are related to carbon and nitrogen atoms and they confirm that the functional groups has been attached onto the silica nano particles. The spectrum of HSNPs-COOH (Fig. 5d) also shows the peak of chlorine confirms that the functional group attached and the Cl is the counter ion for this positively charged nano carrier.

In vitro Studies

Drug loading efficiency usually relies on the affinity between the nano carrier and the drug molecules. When a drug molecule is loaded inside of the non-functionalized silica matrix through a weak attraction (e.g. hydrogen bonding), a low loading capacity and a fast release profile are usually observed. The drug loading content for two systems HSNPs-OH and HSNPs-NH_2 are close together. In case of these two systems, hydrogen bonding is the key contributor to the specificity of intramolecular and intermolecular interactions since insulin molecules have a tendency to attach to polar groups via hydrogen bonding (because of

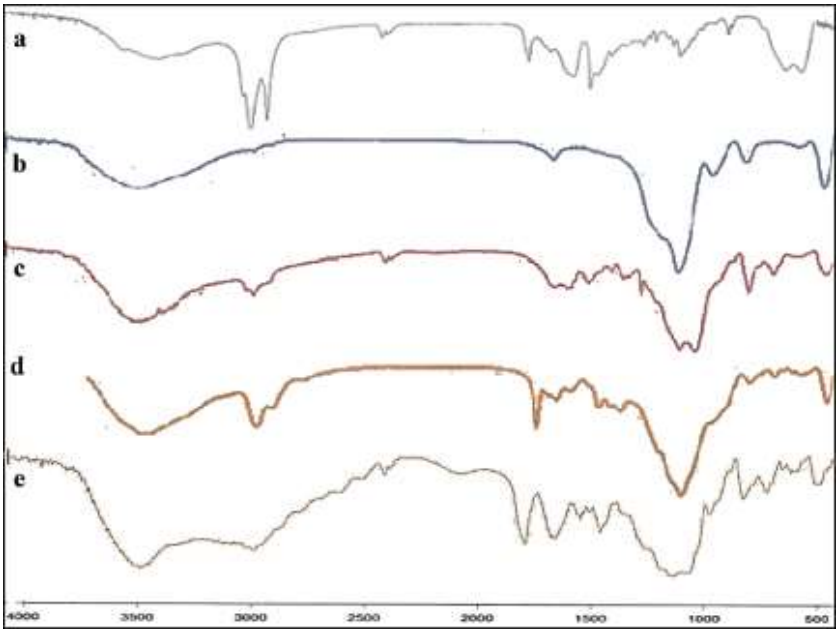

Fig. 3. FT-IR spectra of (a) MAs (Magnetic Assembles), (b) HSNP-OH, (c) HSNP-NH_2, (d) HSNP-GLU and (e) HSNPs-COOH

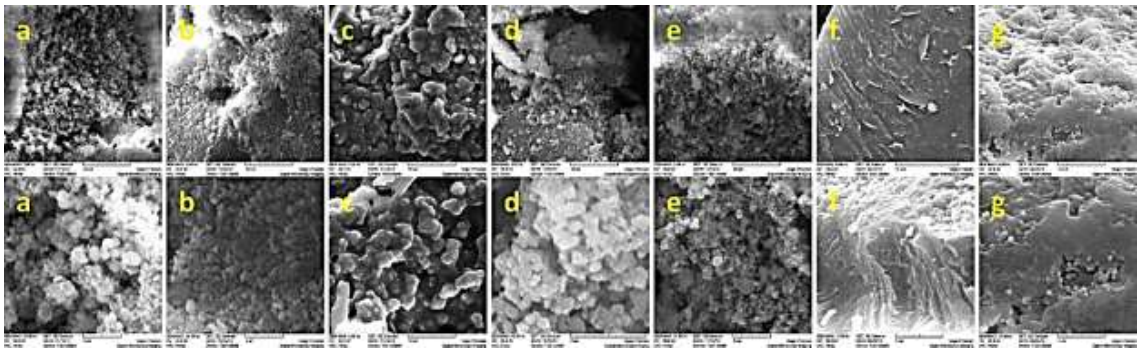

Fig. 4. SEM images of (a) HSNPs-OH, (b) MSNPs-OH, (c) HSNPs-NH_2, (d) MSNPs-NH_2, (e) HSNPs-CHO, (f) HSNPs-COOH, (g) MSNPs-COOH

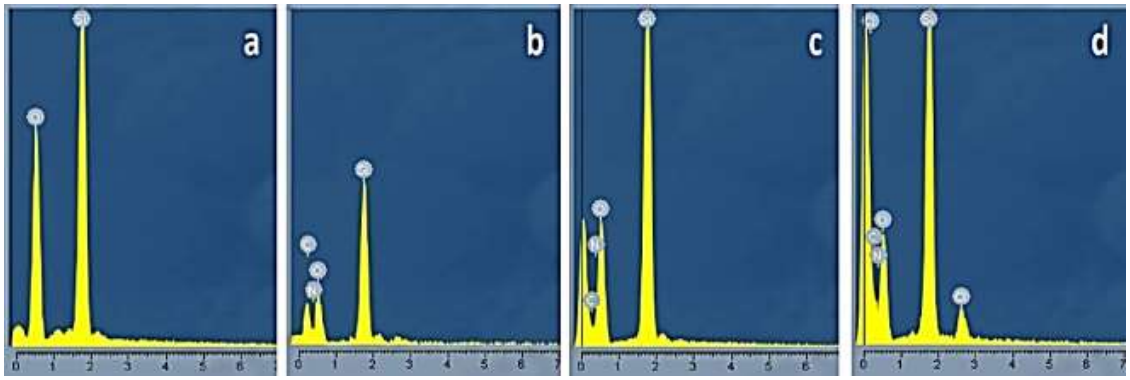

Fig. 5. EDX spectra of (a) HSNPs-OH, (b) HSNPs-NH_2, (c) HSNPs-CHO, (d) HSNPs-COOH

Table 1. The loading percentages of nanocarriers

Nanocarriers	MSNPs-OH	HSNPs-OH	MSNPs-NH_2	HSNPs-NH_2	MSNPs-CHO	HSNPs-CHO	MSNPs-COOH	HSNPs-COOH
Drug Loading%	36	40	40	45	40	78	40	40

having many polar groups). The increases in drug loading and entrapment efficiency for HSNPs-CHO, was due to the chemical attachment of insulin to silica nanocomposites. The loading percentages are summarized in Table 1 and it shows that loading percentages for HSNPs are higher than those of MSNPs.

The in vitro release profiles were established separately at pH 7 and insulin release as a function of time are shown in Fig. 6. The HSNPs (i.e. Fig. 6a, 6b, 6c, 6d) demonstrated a rapid drug release from the nano structure in comparison with their magnetic counterparts (i.e. Fig. 6e, 6f, 6g, 6h). Briefly, these HSNPs could load a higher amount of drug; however, the release rates from the HSNPs were faster than those of MSNPs. This is thought to be caused by additional available space into the nano particles for drug encapsulation from removal of Fe_3O_4 nano particles provide more available space into the nano particles for drug encapsulation, therefore the drug loading percentages in HSNPs are higher than those of MSNPs. In addition, this has effects on the release rates. According to Fig. 6, the HSNPs would release drugs faster than their magnetic mates. Most of the extra amount of drug loaded into the HSNPs, do not have any chemical or physical interaction with carriers so they can be released faster. Most of drug molecules in MSNPs are loaded on the surface of carriers containing polar functional groups, so these drugs have chemical or physical interaction with carriers thus MSNPs would release drugs slowly.

The amine-modified carrier (HSNPs-NH_2)

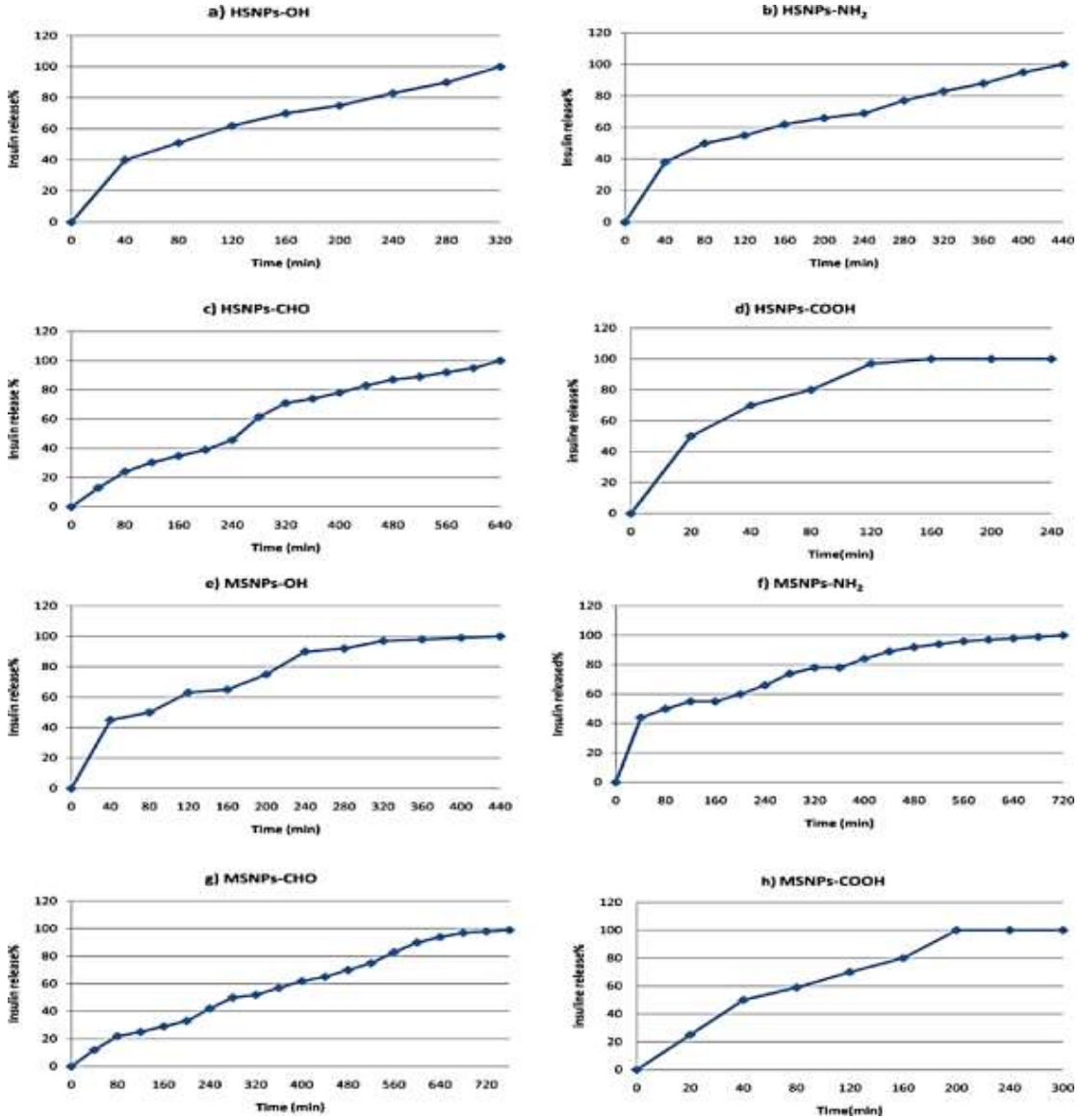

Fig. 6. Release of insulin from different carriers as a function of time at 37±1 °C. (a) HSNPs-OH, (b) HSNPs-NH_2, (c) HSNPs-CHO, (d) HSNPs-COOH, (e) MSNPs-OH, (f) MSNPs-NH_2, (g) MSNPs-CHO, (h) MSNPs-COOH

showed a better drug loading content and the insulin release was slower than that of HSNPs-OH, which can be attributed to the strong interactions of insulin with the amine groups of HSNP-NH_2. Notably, the release rate of insulin from the HSNPs-OH was almost two times higher than that of HSNPs-CHO (i.e. HSNPs-CHO<HSNP-NH_2<HSNPs-OH). The HSNPs-CHO nano carrier demonstrated a remarkable sustained release profile. Therefore, retardation of drug release from HSNPs-CHO was successfully accomplished by covalent attachment of insulin to the surface of nano carrier.

In MSNPs-COOH and HSNPs-COOH, at physiological buffer (pH around 7), the silanol groups (Si–OH) and -COOH in the nano carriers would become de-protonated, and a strong electrostatic repulsion between the negative charges of (SiO^- and $–COO^-$ groups) and the negative charge of insulin molecule (pI of 5), would be generated and consequentlypromote the release rate.

CONCLUSION

In this work, two groups of modified silica-

based nanoparticles as carriers in drug delivery systems have been introduced. The first group contains magnetic nano silica materials (MSNPs). These nano materials were modified and some functional groups (-OH, -NH_2, -CHO, -$N(COOH)_3^+$) were attached on the surface of nano particles using the sol-gel method.

To prepare the second group, MSNPs were treated with HCl to remove the Fe_3O_4 nano particles then the hollow structured silica nano particles (HSNPs) were obtained. The release rates from the HSNPs were faster than those of MSNPs.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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