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Research Article

Synthesis and Characterization of Novel Positive-Charge Functionalized Silica Nanoparticles for Oral Drug Delivery

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ABSTRACT

We synthesized silica nanoparticles (SN) with different densities of surface positive charges. In the first time, the azide was covalently attached to the 3-trimethoxysilylpropyl chloride with replacement of all the chlorine atoms. Then, silica nanoparticle was modified by (3-trimethoxysilylpropyl) azide and convered to an azido-terminated surface. One-pot reduction of azide to N-monomethylamine accrued and then quaternary ammonium compounds with positive charges are prepared by methylation of N-monomethylamine. Naproxen as a model drug was entrapped in these positive-charge silica nanoparticles (PCSN) and the in vitro release profiles were established separately in both (SGF, pH 1) and (SIF, pH 7.4). In pH 7.4, a partial negative surface charge on the PCSN was generated due to the deprotonation of silanol groups, and the strong electrostatic repulsion triggered a sustained release of the loaded molecules.

Keywords: positive-charge silica nanoparticles, 3-trimethoxysilylpropyl chloride, pH-sensitive, naproxen.

INTRODUCTION

Colon-specific drug delivery is recognized to be advantageous in the treatment of disorders of the large intestine, such as irritable bowel syndrome, colitis, Crohn's disease, colon cancer and infectious diseases where it is necessary to attain a high concentration of active agent in the large intestine. Colon delivery can also offer a benefit over orally administered drugs which exhibit poor uptake in the luminal or mucosal enzymes. To achieve successful colonic delivery, a drug needs to be protected from absorption of the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon; this is considered the optimum site for colon-targeted delivery of drugs. One strategy for targeting orally administered drugs to the colon includes coating drugs with pH-sensitive carriers[1-7]. Our response to this challenge was to design a porous nanocarrier with a pH-sensitive properties and controlled-release function that decreased not only the degradation but also the non-specific release of drug molecules in the GI tract.

Mesoporous silica materials have generated vast interest ever since they were synthesized by Beck and co-workers in 1992[8], because of the large surface area, large pore volume, highly ordered pore structure, and adjustable pore size, mesoporous silica material have wide and interesting applications in the fields of chemical catalysts[9] and biotechnology[10]. For example, mesoporous silica material can be used as a drug carrier for the controlled release of pre-loaded therapeutic drugs[11,12]. Recently, mesoporous silica nanoparticles (MSN) have been synthesized. Silica has abundant silanol groups (Si–OH) on the pore surface, which facilitate their conjugation with different functional groups to increase the adsorption and conjugation of relevant biological molecules[13]. Drug loading efficiency usually relies on the affinity between the nanocarrier and the drug molecules. When a drug molecule is loaded inside of the non-functionalized silica matrix through a weak attraction (i.e., hydrogen bonding), a low loading capacity and a fast releasing profile are usually observed. For charge carrying drug molecules, we propose to increase the drug-loading efficiency by strengthening the electrostatic attraction through a modification of the silica material's surface to bear more opposite charges[14].

In this paper, we report a synthesis of positively charged MSN by preparation of quaternary ammonium compounds on surface of MSN. The high positive charge density of quaternary

ammonium groups generated a strong electrostatic attraction between the surface of MSN and the negative groups of the drug molecule. Naproxen as an anionic drug molecule was employed as loading compound to examine the capability of controlled release in simulated gastric fluid (pH 1) and intestinal fluid (pH 7.4). The effects of quaternary ammonium concentrations, loading concentrations, and solution pH on the loading efficiency were investigated. The pH triggered release is mainly due to the change of the surface charges in PCSN sample (PCSN carry positive charges in acidic pH and negative charges in neutral condition), the negative charges of drug molecules could have strong attraction when placed with PCSN in a weak acid solution and have strong repulsion (spontaneous and sustained release) when the pH of the solution was changed to be neutral. This vehicle can be used for the delivery of anionic drugs into the colon. The pH-triggered release of mechanism from drug loaded PCSN samples demonstrated their potential to be used in tissue specific delivery systems for oral drug administration.

EXPERIMENTAL

Tetraethoxysilane (TEOS), 3-trimethoxysilylpropyl chloride and sodium azide were purchased from Fluka Co. All the other chemicals used were of analytical reagent grade. ^{1}H-NMR spectra was recorded on a Brucker 400 AC spectrometer in $CDCl_3$. The IR spectra were recorded on a Shimadzu FT IR-408 spectrophotometer. The amount of released naproxen was determined by a Philips PU 8620 UV spectrophotometer at the maximum adsorption of the free drug in aqueous buffered solutions using a 1-cm quartz cell.

Synthesis of silica nanoparticles (SN)

In a 250 mL round bottom flask, 60 mL (10 mmol) ammonia solution (32%) and 1.98 g (110 mmol) water are added to 100 mL absolute methanol. The solution is stirred for 5 min before adding dropwise 10.41 g (500 mmol) TEOS. The final solution is stirred for three days at ambient temperature.

Synthesis of (3-trimethoxysilylpropyl) azide (TMSA)

The synthesis is carried out under argon atmosphere. Sodium azide (3.5 gr, 50 mmol) is dissolved in 30 mL absolute acetone. 3-trimethoxysilylpropyl chloride (9.93 gr, 50 mmol) is added dropwise under stirring. The mixture is heated under stirring overnight. The precipitate, sodium chloride, is filtered off under argon atmosphere. Then the product is distilled at 80 ºC under vacuum (1 mbar). The resulting (3-trimethoxysilylpropyl) azide is a transparent liquid (Scheme 1). ^{1}H NMR($CDCl_3$): d (ppm) 4.2 (t, 2H, ≡N–CH_2–CH_2–); 3.6 (s, 9H, Si(OCH_3)$_3$); 1.9 (q, 2H, =N–CH_2–CH_2–CH_2–Si); 0.6 (t, 2H, –CH_2–Si).

Preparation of azide-functionalized silica nanoparticles (SN-N_3)

1 gr of the previously prepared silica nanoparticles was suspended in two freshly prepared solution of SN-N_3 (5 and 10% (v/v) in 50 mM acetate buffer, pH 4.0 (Table 1). The solution is stirred under argon, at room temperature overnight.

One-pot conversion of SN-N_3 to N-monomethylamines (SN-NHMe)

SN-N_3 (0.3 gr) in CH_2Cl_2 (2 mL) was added a solution of $(CH_3)_3P$ in toluene (1.0M, 3 mL) at room temp. After stirring for 1.5 hr, paraformaldehyde (22.6 mg, 0.753 mmol) was added. The mixture was stirred for an additional 6 hr at room temp then reaction was cooled to 0 ºC and MeOH (2.0ml) and $NaBH_4$ (28 mg, 0.74 mmol) was added. After 6 h, the product was filtered off, washed thoroughly with distilled water and dried under vacuum at room temperature.

Preparation of positive-charge silica nanoparticles (PCSN)

The each of SN-NHMe (1.9 g) was dissolved in a mixture of DMF (15 mL) and methyl iodide (2.27 gr, 16.0 mmol) and agitated for 15 h at 20 ºC. The solution was precipitated by icy-cold Et_2O. The PCSN (with different densities of surface positive charges) was filtered and dried in vacuum (Scheme 2).

Drug loading in positive-charge functionalized silica nanoparticles

10 mg of positive-charge silica nanoparticles was dispersed with stirring in 3 ml of drug solution (1.0 mg/mL) in water and mixed for 3 days at room temperature to suck up the total amount of the drug solution. The obtained yellow solid was washed several times with an excess of CH_2Cl_2 and dried.

Determination of amount of drug entrapped

The amount of drug entrapped in the hydrogels was determined by an indirect method. After the drug loading, the washings with CH_2Cl_2 were collected and tested using UV-Vis spectroscopy. The difference between the amount of drug initially employed and the drug content in the washings is taken as an indication of the amount of drug entrapped.

Effect of pH on drug adsorption

An effect of environmental pH (from 1 to 10) on the adsorbed amounts of naproxen onto PCSN sample is investigated. Naproxen has high loading efficiency in the pH range from 5.5 to 6.5. The high loading ability in this pH range was due to the strong electrostatic attraction. Besides, a slight decrease in the loading capability at lower pH values (pH 1.0) was resulting from the protonation of the acidic groups in the naproxen molecule.

Once the functional group was protonated, the electrostatic attraction between the PCSN groups and the naproxen molecules would decrease and cause the low loading efficiency. The decrease in the loading capability from pH 6 to 10 was resulted from the presence of unmodified silanol (Si–OH) groups on the surface of the PCSN sample. The silanol groups underwent deprotonation at high pH value. The strong negative repulsion between the surface of PCSN and the loaded molecules caused a decreasing adsorption in this pH range.

Interaction between naproxen and PCSN

The type of interaction between drug and PCSN was studied using Fourier transform infrared. A comparison of the spectrum of pure PCSN and PCSN-drug adduct before and after washing with water showed that the drug was not covalently bonded to the PCSN, only a physical interaction with the PCSN occurred. This is attributed to the fact that, after washing the drug from the PCSN matrix, the spectrum of resultant PCSN-drug adduct was similar to the original pure PCSN spectrum.

In vitro release studies

The powdered PCSN-drug (10 mg) was poured into 3 mL of aqueous buffer solution (SGF: pH 1 or SIF: pH 7.4). The mixture was introduced into a cellophane membrane dialysis bag. The bag was closed and transferred to a flask containing 20 mL of the same solution maintained at 37° C. The external solution was continuously stirred, and 3 mL samples were removed at selected intervals. The volume removed was replaced with SGF or SIF. Triplicate samples were used. The sample of hydrolyzate was analyzed by UV spectrophotometer (λmax=315 nm), and the quantity of drug was determined using a standard calibration curve obtained under the same conditions.

RESULTS AND DISCUSSION

To achieve successful colonic delivery, a drug needs to be protected from absorption of the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs. These requirements have prompted the development of polymeric systems that swell minimally under acidic conditions but extensively in basic intestinal medium.

The loading numbers in Table 1 shows existence of polar functionally groups as silanol need not only for loading drug on the polymer but also for pH-sensitive properties of PCSN.

The pH dependent release systems is based on the different pH values in various parts of the GI tract, which increases from the stomach (pH 1.0–3.0), to the small intestine (pH 6.5–7.0), and to the colon (pH 7.0–8.0) [15], although successful passage through stomach can be expected, this design may result in an early release in the small intestine due to the insignificant pH variation between the small intestine and colon. Therefore, in the current study we designed a nano delivery vehicle to advantages of pH-dependent release.

Figure 1 shows the resulting nanoparticles. The particles shown have an average diameter of about 200 nm. The functionalization of the silicon nanoparticles makes use of organosilane chemistry. The organosilane of choice for this project is (3-trimethoxysilylpropyl) azide. As seen in scheme 2, (3-trimethoxysilylpropyl) azide reacts with the oxide layer on silicon through a known mechanism. The azide group present on this organosilane allows for further modification.

It can be seen that the peak at 960 cm^{-1} corresponding to Si-OH was enhanced with increasing silica content, revealing that silica was successfully introduced into PCSN and the condensation of Si-OH were not complete during the thermal treatment. Typical absorption bands for Si-O-Si near 1150 cm^{-1} also become stronger with increasing addition of silica indicating the formation of a more compact silica network.

Drug Release

The drug release behavior of the as prepared positive charged porous silica particles is studied to reveal their potential use in drug delivery system. In order to better mimic the vitro release of naproxen from the as prepared porous silica, simulated intestinal fluid (SIF, phosphate buffersolution, pH=7.4), and simulated gastric fluid (SGF, HCl aqueous solution, pH=1) are chosen as the release fluids.

The degree of hydrolysis of naproxen as a function of time is shown in figure 2. In pH 1 and 7.4, outcome both repulsion and attraction effects in drug delivery is effective. At physiological buffer (pH around 7.4), the silanol groups (Si–OH) in the positive charge of porous silica surface would become deprotonated, and a strong electrostatic repulsion between the negative charges of (SiO^- groups) and the negative charge of naproxen molecule (Scheme 1) would be generated. The electrostatic attraction between the drug molecules and the positive charge surface was stronger than the hydrogen bonding ($–NH_2$ $^{\delta-}\ldots$ $^{\delta+}$ HOOC–) and the weaker ionic attraction ($–NH_3^{+\ldots}$OOC–). Because of the large number of deprotonated silanol groups the amount of repulsion is a far more attractions. Consequently, the pH value of 7.4 in the physiological buffer promoted the releasing rate of the anionic molecules. The residual drug molecules may be occluded in the channels and therefore could not achieve the overall release.

In the simulated gastric fluid (pH 1), the silanol groups in the positive charged porous silica surface were fully protonated, so the surface predominantly carried positive charges. The existence of hydrogen-bonding interactions between polar silanol and –COOH groups in the naproxen matrix results in a complex structure within the silica network, and so the movement of naproxen segments is restricted. Because, in this pH, drug molecules have a tendency to attach to polar silanol and –COOH groups due to hydrogen-bonding caused a decrease in the release rate. The mechanism drug release from positive charged porous silica is shows in (Scheme 3).

CONCLUSIONS

A pH-responsive controllable drug release system has been designed by incorporating positive charges in the framework of silica nanoparticles so that anionic molecules can be efficiently adsorbed inside of the nanochannels with minimal release under acidic pH value. At neutral pH because of the deprotonation of surface silanol groups, while giving strong electrostatic repulsion, the release rate of the adsorbed drug molecules becomes much increased. This controlled-release mechanism takes advantage of the changing pH value and ionic strength in our physiological buffer. Hence, this kind of the carrier could be designed as oral drug delivery system that improves site specificity and release kinetics to accommodate different therapeutic purposes.

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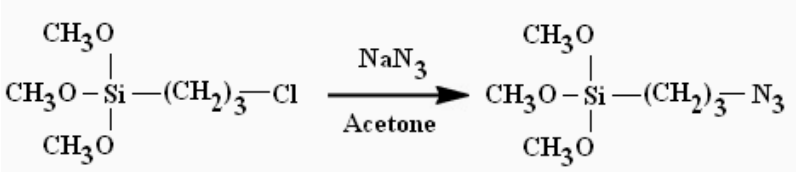
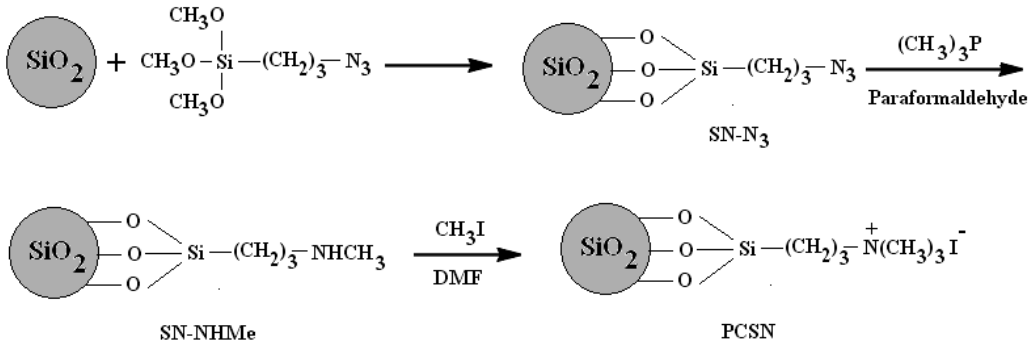


Scheme 1: Synthesis of (3-trimethoxysilylpropyl) azide



Scheme 2: Synthesis of positive-charge silica nanoparticle

Table 1: The molar composition of composite and drug loading numbers

functionalized silica nanoparticles	Molar composition in the feed		Percent of drug-loading (%) (pH=6)
	SN	$SN-N_3$	
SN-N3-A	1 gr	5%	85
SN-N3-B	1 gr	10%	94

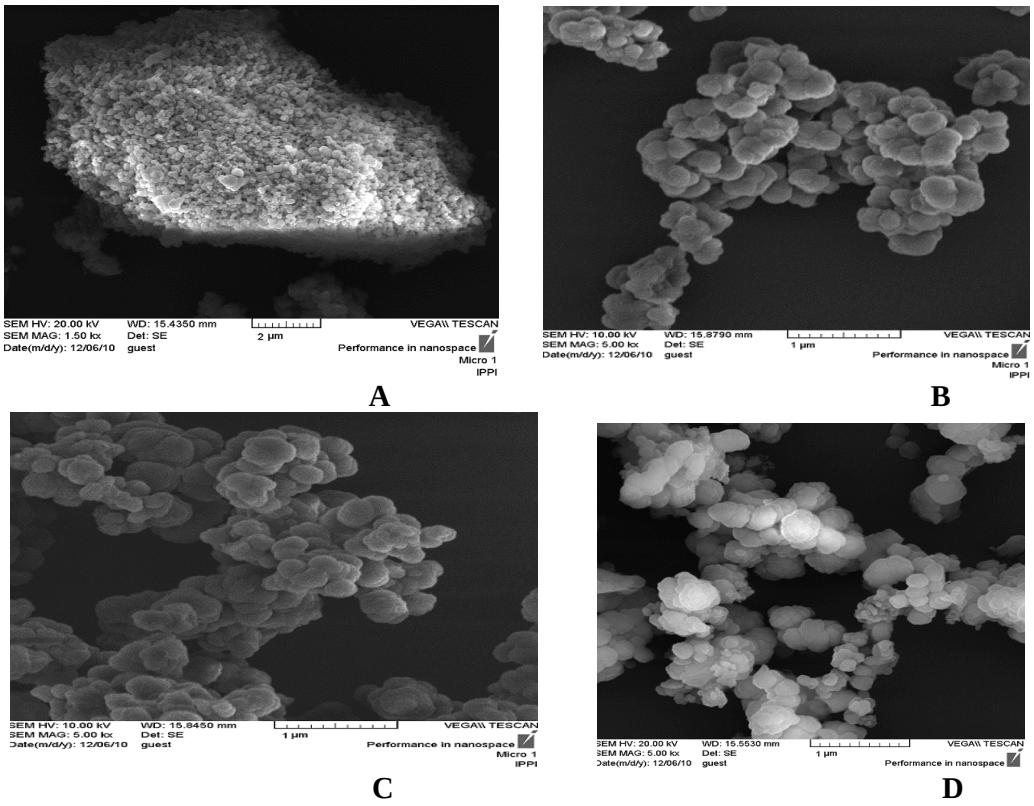


Fig. 1: Scanning electron microscopic (SEM) images of (A) SN (B) SN-N_3 (C) SN-NHMe (D) PCSN

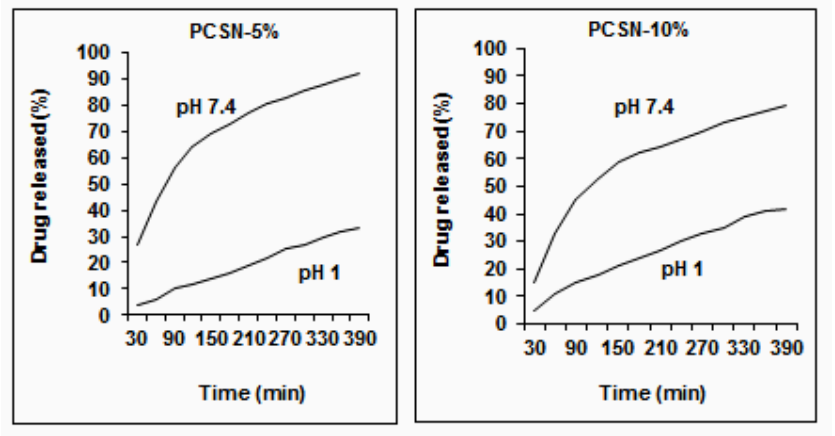
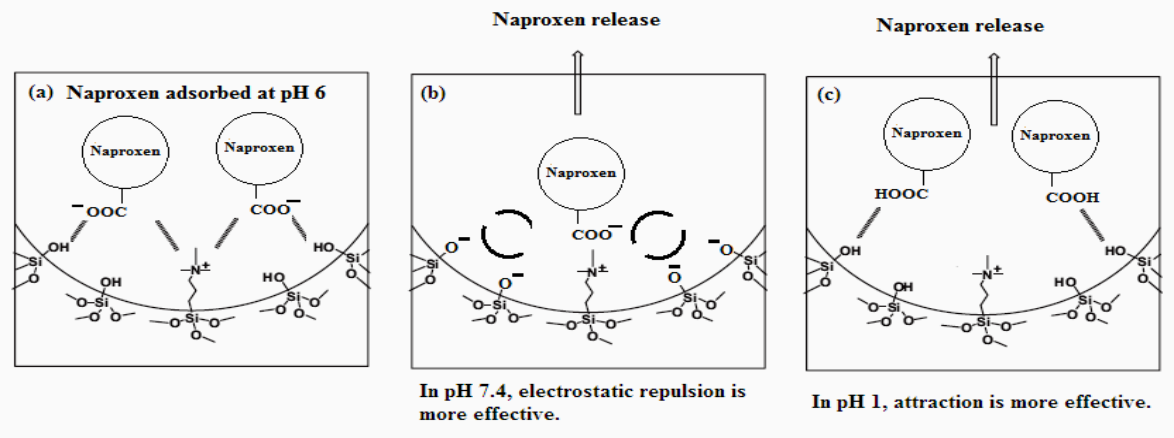


Fig. 2: Release of naproxen from different densities positive charges carriers as a function of time at 37 °C



Scheme 3: The representation of the release mechanism of naproxen adsorbed in PCSN sample. a) Loading of naproxen to PCSN, b) Drug released by electrostatic repulsion at pH 7.4. c) Drug released at pH 1

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