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Research Article

SYNTHESIS AND CHARACTERIZATION OF POLYMERIC NANOCAPSULES AS COLON-SPECIFIC DRUG DELIVERY SYSTEM

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ABSTRACT

Silica-poly (methacrylic acid) (PMA) core–shell nanoparticles (NPs) were successfully prepared via graft copolymerization of methacrylic acid (PMA) onto vinyl-bond-modified silica NPs. The grafting procedure consisted of surface activation with 3- trimethoxysilyl propyl methacrylate (3-TMSM) or trimethoxy(vinyl) silane (TMVS), followed by free-radical graft polymerization of methacrylic acid (MAA) in ethyl acetate with 2, 2′-azobis-isobutyronitrile (AIBN) initiator and in both presence and absence of ethylene glycol dimethacrylate (EGDMA) as a crosslinking agent. The polymeric nanocapsules (PNC) were achieved after the etching of the silica nanoparticle templates with hydrofluoric acid. The polymeric nanocapsules were characterized by FTIR and SEM. A model drug, naproxen was entrapped in these carriers and the in-vitro release profiles were established separately in both enzyme-free simulated gastric and intestinal fluids SGF, pH 1 and SIF, pH 7.4, respectively. Because of their pH-sensitive character and small size, nanostructure devices designed from the smart polymeric nanocapsules have potential applications as colon-specific drug delivery system.

Keywords: Silica nanoparticles, Nanocapsule, pH-sensitive, Naproxen, Oral drug delivery

INTRODUCTION

Environmental-stimuli-responsive polymers that are able to alter their conformation and properties in response to environmental stimuli have been used in sensors, drug delivery devices, selective separation membranes, bioactive carriers, and as biomimetic engineering materials.[1-4] The external stimulus could be temperature, pH, ionic strength, solvent composition, enzyme, light, or an electrical field.[5-9] On the other hand, colloidal nanoparticles (NPs), especially silica NPs, are also important materials due to their potential application as drug and catalyst carriers.[10-14] Encapsulation of colloidal NPs with these smart polymers has attracted great interest. In addition to enhanced nanoparticle dispersion, encapsulation has been used to control release, and manipulate the optical and magnetic properties of NPs.[15-17] As a typical smart polymer, poly (methacrylic-acid) (PMAA) is an anionic polyelectrolyte which has frequently been employed as a scaffold for immobilization of biologically active molecules. The high density of carboxylic acid moieties along its backbone can be used to immobilize molecules containing amine groups, such as proteins.[18, 19] Thus; the preparation of PMAA-bearing surfaces constitutes one of the promising approaches to the generation of biofunctional surfaces for the use of silica NPs in biological fields.

To the best of our knowledge, no attention has been paid to the preparation of PMAAc-grafted silica NPs using free radical copolymerization to date. It is well-known that copolymerization is an efficient approach for immobilizing polymers onto inorganic particle surfaces with high graft yield and high graft density. Xu et al. have synthesized TiO_2–SiO_2/poly (methyl methacrylate) (PMMA) core–shell nanocomposite particles via free radical copolymerization with a microphase-inversion method.[20] Zeng et al. have prepared silica-PMMA core–shell structure composite nanoparticles via emulsifier-free emulsion copolymerization.[21] Wu et al. have synthesized a series of SiO_2 / PMMA composite particles with different morphologies via conventional emulsion polymerization.[22]

In recent years, significant progress has been made in the design and fabrication of polymeric micro- and nanocapsules, attracting great attention due to variety of applications such as delivery vesicles for drugs.[23-25]

In this paper, we synthesized stimuli-responsive silica nanoparticles by copolymerizing vinyl-functionalized silica nanoparticles and methacrylic acid monomers. The nanocapsules were achieved after the etching of the modified silica nanoparticle template with hydrofluoric acid. The naproxen was entrapped in these nanocarriers and the in-vitro release profiles were established separately in both enzyme-free simulated gastric and intestinal fluids SGF, pH 1 and SIF, pH 7.4, respectively.

Experimental

Methacrylic acid (MAA), Ethylene glycol dimethacrylate (EDMA), tetraethoxysilane (TEOS, 99 wt%), trimethoxy(vinyl)silane, 3-trimethoxysilyl propyl methacrylate (3-TMSM, 97 wt%), 2, 2-azobisisobutyronitrile (AIBN) and other chemicals were purchased from Aldrich. Methacrylic acid was purified by distillation under vacuum. Initiator of 2, 2′-azobisisobutyronitrile (AIBN) was purified by crystallization of methanol.

The IR spectra were recorded on a Shimadzu FTIR-408 spectrophotometer. The amount of released naproxen was determined using a Philips PU 8620 UV spectrophotometer at the maximum adsorption of the free drug in aqueous buffered solutions (λmax=315 nm) using a 1 cm quartz cell. Surface characteristic studies of the prepared polymeric nanocapsules were performed by scanning electron microscopy (SEM) model LEO 440I (UK).

Silica nanoparticles loaded with coupling agent (SNPV)

Silica NPs modified by 3-TMSM or TMVS were prepared by a modified Stöber method[26], which comprises the base-catalyzed hydrolysis of TEOS and in-situ coupling agent modification in ethanol. More specifically, 3.6 ml of TEOS, 0.25 ml of 3-TMSM or TMVS, and 88 ml of pure ethanol were mixed in an Erlenmeyer flask under magnetic stirring at 20 ºC. Then, 12 ml of ammonia was added quickly under vigorous stirring. Gentle stirring was continued for at least 24 h to ensure that the reaction was complete. The silica NPs was washed extensively with absolute ethanol by centrifuging at a rate of 4000 rpm to remove excess reagent.

Synthesis of PMAA-grafted silica NPs: (PSNP)

The polymeric nanoparticles were synthesized by graft polymerization of silica modified NPs, MAA and ethylene glycol dimethacrylate (EGDMA) as crosslinking agent (%4) (with variable feed ratio as shown in Table 1) in a solution of dried dioxane. Polymerization was carried out in the presence of 2, 2′-azobis isobutyronitrile (AIBN) as initiator (0.01 $molL^{-1}$) at 60-70 ºC in a thermostatic water bath. All experiments were carried out in Pyrex glass ampoules sealed off under vacuum. After polymerization, the grafted silica NPs were separated from the suspension by centrifugation and then washed several times by centrifuging/re-suspension in deionized water. Finally, the PMAA-grafted silica NPs

was dried at 80 ºC for 6 h in vacuum to perform further characterization. IR (KBr): 3350-2550 (broadened, -COOH group), 1725, 1675, 1610, 1420, 1240, 1225 cm^{-1}.

Synthesis of polymeric nanocapsules: (PNCs)

The polymeric nanoparticles were converted to hollow capsules by soaking composite in an aqueous solution of %12 wt. HF for 24 h. The resulting nanocapsules were collected by centrifugation technique, washed thoroughly with ethanol, and dried under the vacuum (Scheme 1).

Drug loading in polymeric nanocapsules

200 mg of each polymeric nanocapsule were dispersed with stirring in 5 ml of solution containing 20 mg of naproxen in order to suck up the total amount of the drug solution. After approximately 240 min, the completely swollen PNCs loaded with drug were placed in desiccators and dried under the vacuum at room temperature.

Determination of the amount of entrapped drug

The amount of the entrapped drug in the PNCs was determined by an indirect method. After loading, washings were collected and tested using UV-Vis spectroscopy. The difference between the amount of initially employed drug and the drug content in the washings is taken as an indication of the amount of entrapped drug. The corresponding values are given in Table 2.

The In-situ Bioadhesivity Studies

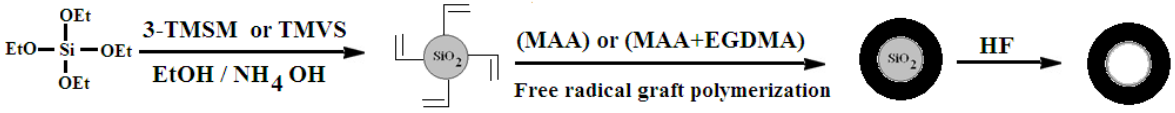
Bioadhesivity testing was done by a novel in-situ method as described by Ranga Rao and Buri.[27] A fresh-cut 5–6 cm long piece of small rat intestine was obtained and cleaned by washing in isotonic saline. The piece was cut open and the mucosal surface was exposed. Known weights of hydrogels were added evenly on the mucosal surface. The intestinal piece was maintained at %80 relative humidity for 30 min in a desiccator. The piece was taken out and phosphate buffer of pH 6 was allowed to flow over the intestinal piece for about 2 min at a rate of 20 ml/min. The perfusate was collected and dried to get the non-adhered particles. The percentage of bioadhesion was estimated by the ratio of the amount required to adhere hydrogels. The values are given in Table 2.

Table 1: Composition of copolymers

Polymeric modified silica NPs	Molar composition in the feed			
	NPSM[1]	NPVS[2]	MAA	EGDMA
PSNP -1	1	---	3	---
PSNP -2	1	---	3	10%
PSNP -3	---	1	3	---
PSNP -4	---	1	3	10%

1) Modified silica NPs with 3-TMSM: NPSM

2) Modified silica NPs with TMVS: NPVS



Scheme 1: Schematic illustration of production steps for the polymeric nanocapsules (PNCs)

Table 2: The percentage of particles adhered onto rat intestine and drug loading numbers

Polymeric nanocapsules	Percentage of naproxen loading (%)	Adherence percentage
PNC-1	97	64
PNC-2	98	69
PNC-3	99	57
PNC-4	99	59

RESULTS AND DISCUSSION

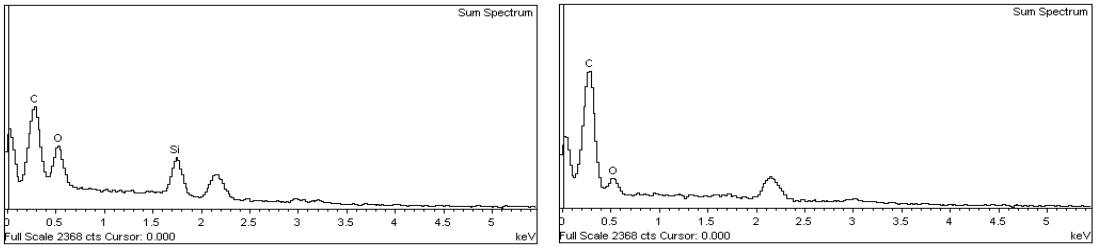
The chemical structures of the modified silica NPs and PMAA-grafted silica NPs were studied by the FTIR spectroscopy. The absorption bands at 1105, 1655, and 3435 cm^{-1}, which are attributed to Si–O–Si bond stretching of the silica and vinyl bond stretching of the TMVS, and O–H bond stretching, are present in the SNPV samples. Comparing the spectrum of the PSNP with that of the TMVS, the absorption band at 1730 cm^{-1} was present. The results suggest that the PMAA chains were grafted onto the silica NPs by free radical copolymerization.

Subsequently the cross-linked polymer grafted silica nanoparticles (CP-SNs) were dispersed in HF. In order to validate the complete etching of the silica templates, the FTIR technique was used. In the FTIR spectrum of the products treated with HF, the absorption bands at 1105 cm^{-1} of the Si–O–Si symmetric stretching mode and Si-O at 464 cm^{-1} were absent. This indicated that the silica nanoparticle templates encapsulated in the cross-linked polymer shell were etched completely. This also accounts for the minimum swelling of the PNC in a medium of pH 1. However, when the sample was placed in a medium of pH 7.4, the almost complete ionized–COOH groups present within the PNC, increased the ion osmotic swelling pressure to a great extent and this factor ultimately resulted in a greater increase in the water uptake.

All the matrices with the presence of CA and increase in the content of MAA had shown increased bioadhesivity (see Table 2). Because of the presence of a network of three-dimensional structure that acts as a framework the bioadhesivity will increase. The binding with sialic acid residues makes a prolonged contact of the drug with the epithelium.

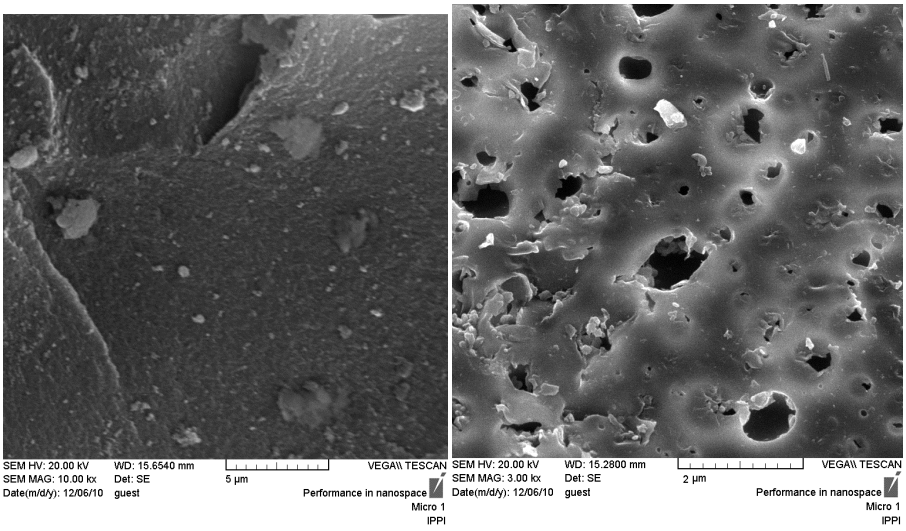
SEM/EDX

SEM-EDX is the name of the energy-dispersive X-ray spectroscopy analysis conducted by means of SEM. It allows determining the chemical composition of nanocomposites. The comparison between scanning electron micrographs of PSNP and PNC (Figure 1) shows that after treating with HF, the peak intensity of Si is extremely ~~is~~ reduced or completely removed.



A **B**

Fig. 1: The EDX spectroscopy analysis of (A) PSNP and (B) PNC



(A) **(B)**

Fig. 2: The SEM images of (A) PSNP and (B) PNC

The hollow structure of the obtained polymeric nanocapsules (PNC) could be observed in the SEM analysis. Figure 2 shows the resulting polymeric nanocapsules. The particles have an average diameter of about 200 nm.

The in-vitro release studies

As shown in Figure 3, for PNC-2 and PNC-4 with cross-linking structures diffusion of the hydrolyzing agents is reduced and the hydrolysis rate becomes slower. In all of the samples drug release are proceeds more efficiently at higher pH (SIF).

In the simulated gastric fluid (pH 1), the existence of hydrogen-bonding interactions between –COOH groups in the PNC matrix results in a complex structure in the silica network, and consequently the movement of PNC segments becomes much more restricted.

In used pH regime, naproxen is entrapped in this fixed structure and has a tendency to attach to polar silanol and –COOH groups due to hydrogen-bonding causing a decrease in the release rate. At physiological buffer (pH around 7.4), the silanol groups Si–OH and -COOH in the PNC would become deprotonated, and a strong electrostatic repulsion between the negative charges of SiO- and –COO- groups and the negative charge of naproxen molecule would cause, the pH value of 7.4, to be promoted to the releasing rate.[28]

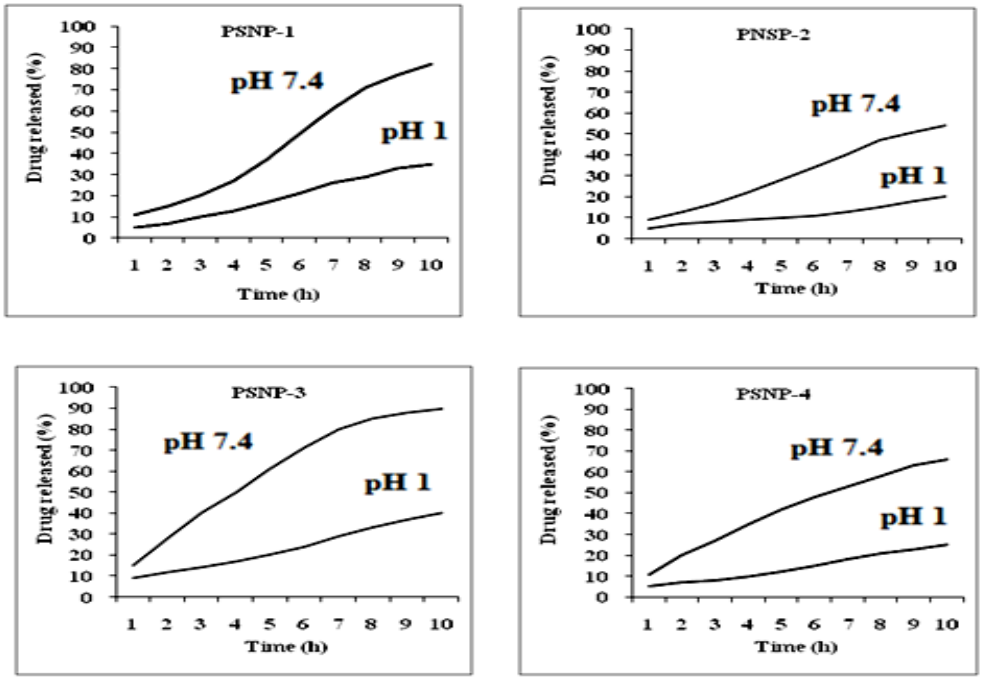
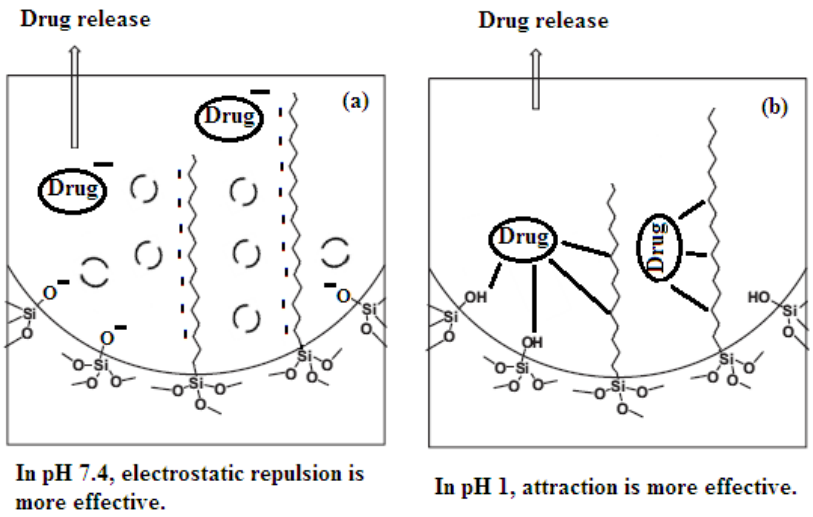


Fig. 3: Release of naproxen from nanocarriers as a function of time at 37 ºC

The residual drug molecules may be occluded in the channels prohibiting the overall release. The mechanism of naproxen release from PNC is shows in (Scheme 2).



Scheme 2: The representation of the release mechanism of naproxen adsorbed in PNC sample. a) Drug released by electrostatic repulsion at pH 7.4. b) Drug released at pH 1

CONCLUSIONS

A pH-responsive controllable drug release system has been synthesized by graft copolymerization of methacrylic acid (PMA) onto vinyl-bond-modified silica NPs. The polymeric nanocapsules (PNC) were achieved after the etching of the silica nanoparticle templates with hydrofluoric acid. The naproxen molecules can be efficiently adsorbed inside ~~of~~ the nanochannels with minimal release under acidic pH value. At pH 7.4 due to the deprotonation of -COOH and silanol groups, giving strong electrostatic repulsion, and the release rate of the adsorbed drug molecules increases. This controlled-release mechanism takes advantage of the changing pH value and ionic strength in our physiological buffer. Due to the significant differences in hydrolysis rate at pH 1 and 7.4, these nanocarriers appear to be good candidates for colon-specific protein delivery.

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