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pH-sensitive nano carriers for oral-curcumin delivery

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Abstract: The aim of this study is to utilize the nano carriers for oral delivery of curcumin (Cur). In the first, Modified Nano Silica particles (MNS) and Nano Chitosan (NCs) were synthesized, and then the curcumin natural compound covalently attached to these carriers. The composition of the nano carriers containing curcumin was determined by FTIR spectroscopy. The in vitro curcumin release profiles were established separately in both enzyme-free simulated gastric and intestinal fluids (SGF, pH=1) and (SIF, pH=7.4), respectively. Detection of hydrolysis solution by UV-Vis spectroscopy at selected intervals showed that the curcumin can be released by hydrolysis of the imine bond between the curcumin and nano carriers in low rate. This study showed us, the degree of hydrolysis in MNS-Cur and NCs-Cur increases in acidic pH because of carriers' high surface.

Keywords: Turmeric, Curcuma Longa, Curcumin, Drug delivery, Nano carriers.

Introduction

Curcumin (Cur), a natural yellow-orange dye polyphenol, found in the powdered rhizomes of Curcuma longa (turmeric), exhibits a spectrum of pharmacological properties such as anti-inflammatory, antineoplastic, anti-oxidant and chemopreventive activities [1–3] and it has also been used as a photodynamic agent useful for the destruction of bacteria [4] and tumor cells [5].

Oral drug delivery is the most popular method for drug delivery. The goal of oral delivery systems is to protect the sensitive drug from proteolytic enzyme degradation in the stomach and upper portion of the small intestine [6]. The ideal drug delivery system should be inert, biocompatible, bioadhesive, comfortable for the patient, and capable of achieving high drug loading [7]. The drug delivery systems, which can deliver precise quantities of therapeutic drugs to the targeted cells or tissues in a tailored release manner to enhance drug efficiency and reduce toxicity, has been one of the major thrust areas of pharmaceutical research these days [8-10]. As the acidic tumor microenvironment is most common in solid tumors, the pH targeting approach is regarded as a more general strategy than many other targeting approaches [11-15]. Nano carriers have important potential applications for the administration of therapeutic molecules [16]. The excellent protection and hydrophilicity of silica make them good candidates for controlled drug delivery due to their low toxicity and high drug-encapsulated efficiency. The drug molecules can be easily coated in the silica nanoparticles (SN) through the sol–gel polymerization. In addition, the silica can be used as a stable and biocompatible matrix to incorporate drug molecules into the silica shell to avoid a side effect on cells [17, 18].

Chitosan (Cs) is a versatile natural polymer. The development of new applications for Cs is mainly due to the fact that it is renewable source of natural biodegradable polymer. Cs is hydrophilic in nature, thereby it has the ability to form gels at acidic pH. These gels can be used as a drug-delivery system [19]. Nanochitosan (NCs) is a natural material with excellent

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physicochemical properties. It is environmentally friendly and bioactive. It is frequently used as a controlled-release drug carrier for gene transfer in artificial organs and for immuneprophylaxis [20]. NCs can be prepared in several ways from Cs [21].

Results and discussion

In order to study potential application of nano carriers (MSN and NCs) containing curcumin (Cur-MSNs and Cur-NCs) as pharmaceutically natural compound, we have studied the hydrolysis behavior of the carriers under physiological conditions. Although the carriers were not soluble in water, they were dispersed in a buffer solution (HCl/KCl for pH=1 or KH_2PO_4/Na_2HPO_4 for pH=7.4), and the hydrolysis was evaluated as a heterogeneous system. The release percentage of the carriers containing curcumin as a function of time is shown in Figure **3**. The release curves appear that the degree of hydrolysis depends on particles size of carriers. We saw nano carriers containing curcumin in pH=1 have a high degree of hydrolysis but the degree of hydrolysis in chitosan containing curcumin increases in increased pH. There are more curcumin compound on nano carriers for their high square and for this reason, the degree of hydrolysis increases in pH=1 comparing to pH=7.4. As the acidic tumor microenvironment is most common in solid tumors, the nano carriers containing curcumin are best for oral delivery. As we show in Figures **1** and **3**, the release percentage of curcumin increased in NCs-Cur comparing to MSN-Cur because low size of NCs (Table **1**).

Table 1: Comparison of carriers' efficiency.

	Loading Efficiency (%)	$Release_{max}$ (%) after 6h at pH 1	$Release_{max}$ (%) after 6h at pH 7.4
MSN-Cur	52	85	28
Cs-Cur	32	65	70
NCs-Cur	42	96	43

SNs: IR (neat, cm^{-1}): 3422 (stretching O-H), 1427 (stretching Si-C), 1084 (Si-O-Si) and 799 (bending Si-C).

MSN: IR (neat, cm^{-1}): 3420 (stretching O-H and NH_2), 1628 (bending NH_2), 1489 (stretching Si-C), 1061 (Si-O-Si) and 802 (bending Si-C).

NCs: IR (neat, cm^{-1}): 3422 (stretching O-H and NH_2), 2925 (stretching CH_{SP3}), 1637 (bending NH_2), 1386 (stretching C-N) and 1063 (stretching C-O).

Cur-MSNs: IR (neat, cm^{-1}): 3418 (stretching O-H), 2968 (stretching CH_{SP2}), 2921 and 2862 (stretching CH_{SP3}), 1660 (stretching imine), 1650 (stretching aromatic ring), 1256 (stretching Si-C), 1121 (stretching C-O) and 1082 (stretching Si-O).

Cur-Cs and Cur-NCs: IR (neat, cm^{-1}): 3461 (stretching O-H of Cs or NCs and stretching CH_{SP2} of Cur), 2870 (stretching CH_{SP3}), 1643 (stretching imine), 1633 (stretching aromatic ring), 1258 (stretching C-N) and 1117 (stretching C-O).

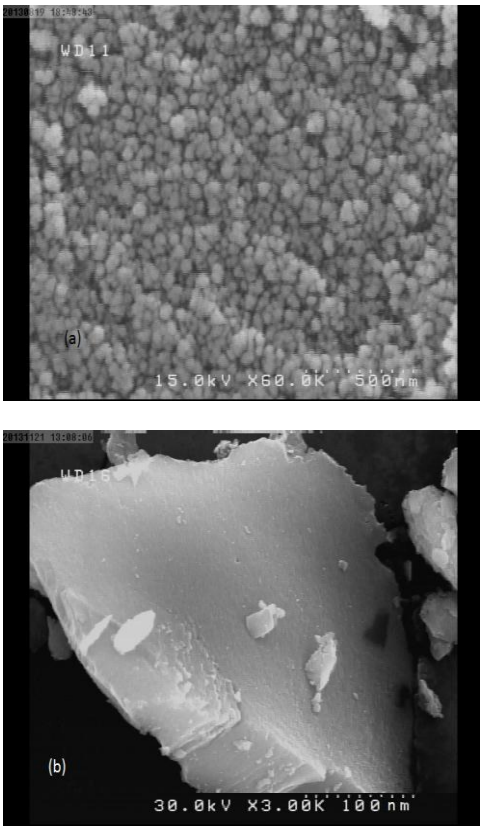


Figure 1: SEM images of (a) MSNs, (b) NCs.

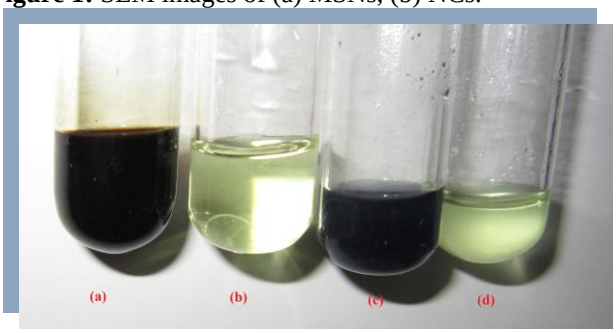


Figure 2: After adding ninhydrine to (a) trimethoxy silylpropylamine; (b) trimethoxy silylpropylchloride; (c) modified nanosilica; (d) silica nanoparticle.

Conclusion

In conclusion, the curcumin release behavior of the as prepared nano particles is studied to reveal their

potential use in drug delivery system. At acidic pH because of the high surface of nano carriers, the release rate of the bonded curcumin molecules increased. These nano carriers take advantage of the curcumin-controlled-release in our physiological buffer (pH=1). Hence, these carriers could be designed as oral drug delivery systems that improve curcumin release kinetics to anti-tumor purposes.

Experimental

Materials:

The curcumin, ethanol, toluene, acetic acid, HCl, H_2O_2 and sodium polyphosphate were obtained from Merck. Chitosan, 3-aminopropyltriethoxysilane (3-APTS), tetraethylorthosilicate (TEOS), ammonia solution (37%), absolute methanol, dioxane and n-hexane were purchased from Sigma-Aldrich. All the solvents were distilled and stored over a drying agent.

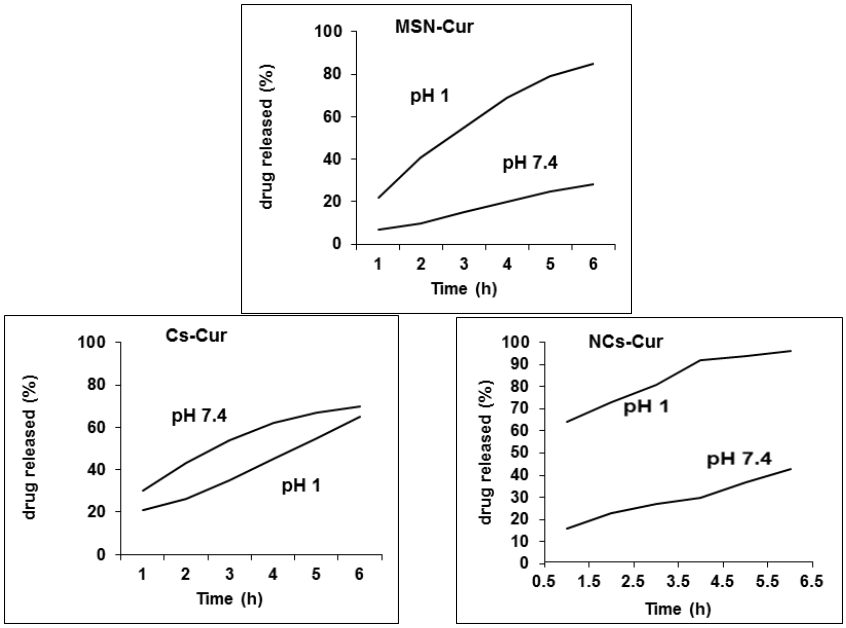


Figure 3: The in vitro curcumin release profiles.

Measurements:

Infrared spectra were recorded with a 4600 Unicam FT-IR spectrophotometer as KBr pellets. The concentration of curcumin released at selected time intervals was determined on a Philips PU 8620 UV spectrophotometer at the absorption maximum of the free drug in aqueous alkali (λmax = 423 nm) using a 1-cm quartz cell.

General procedure for the synthesis of SNs:

SNs are produced by Sol-Gel process (Scheme **1**). A sol is composed of two phases: a solid phase of suspended particles usually in colloidal sizes (1-100 nm), and a liquid phase in which the particles are suspended. A sol is usually stable as a solution due to the small size of the particles, but if the particles aggregate, they may precipitate. When this phenomenon occurs, the sol has formed into a solid gel [22, 23]. The colloidal size silica nanoparticles were obtainable under acidic conditions from silicon alkoxide precursors. The mechanism for particle formation was hydrolysis of the silicon alkoxide precursor. This hydrolysis generated silicic acid Si(OH)4, which condensed into polymers of SiO2 networks.

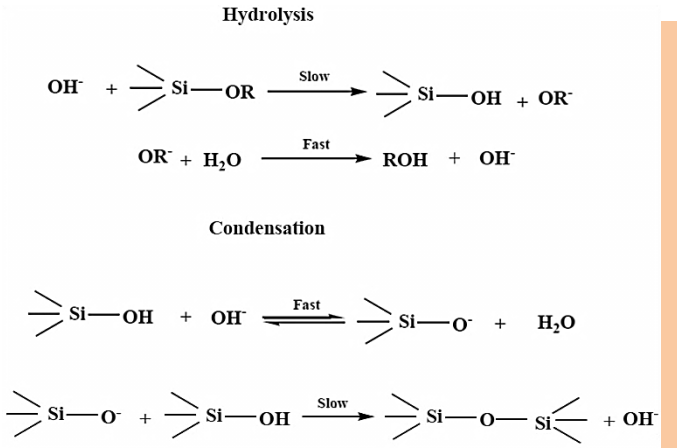
For preparing of silica nanoparticles, 100 mL methanol, 750 µL NH_3 (25%) and 2 g water were added together in a flask. The mixture was stirred at room temperature for 5 min. Then 500 mmol TEOS was added to flask and was stirred for 3 days. After reaction time, petroleum ether antisolvent was added to gel. After 15 min, the mixture was centrifuged at 7,000 rpm for 10 min in order to collect the SNs, and then was dried at 45 ºC for about 15 h.

Chemical Modification of Silica Surface with 3-APTS: MSN:

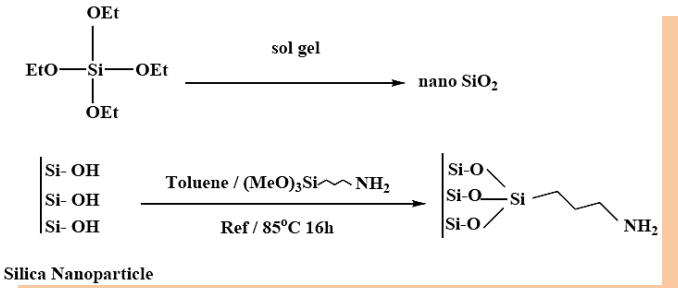
The nanosilica surface modification with organofunctional groups is an important step to increase the affinity between the organic and inorganic phases [27–30]. Nanosized amino group functionalized

silica particles can be obtained through a modified Stober method by using 3-APTS in ethanol (Scheme **2**) [31, 32]. In a typical experiment, MSNs was prepared as protocol method. The process involves hydrolysis and condensation of tetraethylorthosilicate in the presence of mineral base (NH_3) as catalyst [24–26].

For chemical modification of silica surface, 3 g SNs, 10 mL toluene and 1.5 g 3-APTS were added together in a flask. The flask was heated to 85 ºC with stirring for 16 h. Toluene removed by rotary evaporation. The product was collected and washed with toluene and dried under vacuum. The SEM analysis showed that the pure silica nanoparticles size is about 500 nanometer (Figure **1a**).



Scheme 1: NCs synthesis with sol-gel method.



Scheme 2: Mechanism of silica surface chemical modification with 3-APTS.

One drop of ninhydrine added in four test tubes containing silica nanoparticle, modified nanosilica, trimethoxysilylpropylchloride and trimethoxysilylpropylamine for amino group identification (indication of successful modification of SNs). The amino group functionalized compounds colour converted to purple but other compounds colour didn't convert (Figure **2**).

Preparation of NCs:

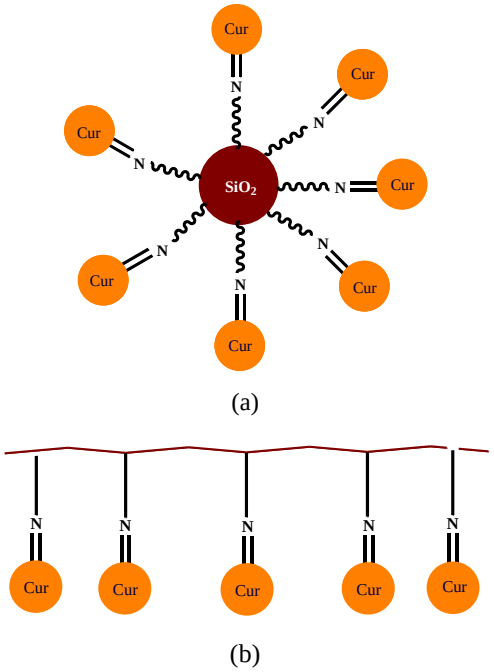
0.6 g of chitosan were dissolved in 50 ml 0.1 M HCl and stirred for 1 h. Then, 30 ml H_2O_2 was added in 30% concentrations. The mixture was heated and stirred for 2 h at 60 degree centigrade and then 20 ml distilled water was added. The impurities filtered from main solution. The solvent evaporated by rotary technique. The residue was baked, and weighed. The product was dissolved in 10 ml distilled water and then 60 ml ethanol was added to the solution, which was left for 24 h to precipitate, after which it was filtered, dried, and weighed. This gave low molecular-weight water-soluble chitosan. After H_2O_2 treatment, the molecular weight of the chitosan decreased. This was due to the degradation of the chitosan molecular chain by H_2O_2. Then, low molecular-weight chitosan was dissolved in 50 ml of 10% acetic acid and stirred for 30 min. Then, the solution was added to 50 ml of sodium polyphosphate (0.5 g/L), stirred for 2 h at room temperature and then centrifuged at high speed. The isolated NCs was rinsed with distilled water, freeze-dried [33, 34]. The SEM analysis showed that the NCs size is lower than 100 nanometer (Figure **1b**).

Curcumin loading on MSNs, Cs, NCs and their in vitro release studies:

For 0.4 g of each drug platforms (MSNs, Cs and NCs), 20 mg curcumin soluble in 1, 4-dioxane and 20 ml deionized water (with 3-4 acetic acid drops for Cs and NCs) in tube added together, then the mixture was stirred at room temperature for 24 h by Scheme **3**. After this time for the determination of loading efficiency, the mixture was centrifuged at 14,000 rpm for 5 min in order to collect the curcumin-loaded. The supernatant contains unloaded curcumin which was then removed and the absorbance of resulting solution was measured using a UV-Vis Spectrophotometer at 423 nm for the loading efficiency calculating. The calculation showed 52%, 32% and 42% curcumin loaded on MSNs, Cs and NCs respectively. The curcumin loaded determined by FT-IR technique.

To measure the releasing, curcumin loaded on each bed divided to two parts and they were immersed in various buffer solutions (pH 7.4 and pH 1) at 37 °C. The release rate of curcumin from beds was determined using UV-Vis Spectrophotometer. The dissolution was performed using 10 mL of buffer solutions having pH=7.4 or pH=1 at 37 ± 0.5°C and 100 rpm. A sample (3 mL) of the solutions was withdrawn from the dissolution tubes hourly for 6 hours and the samples were replaced with fresh dissolution medium. The absorbance of these samples was measured at 423 nm using a UV/ Vis double-beam spectrophotometer.

Cumulative percentage curcumin release was calculated by using an equation obtained from a standard curve. Results for in vitro curcumin release study are shown in Figure **3**.



Scheme 3: Structures of (a) MSN-Cur, (b) Cs-Cur and NCs-Cur.

Acknowledgments

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