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Curcumin Conjugated Silica Nanoparticles for Improving Bioavailability and Its Anticancer Applications

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ABSTRACT: Curcumin, a yellow bioactive component of Indian spice turmeric, is known to have a wide spectrum of biological applications. In spite of various astounding therapeutic properties, it lacks in bioavailability mainly due to its poor solubility in water. In this work, we have conjugated curcumin with silica nanoparticles to improve its aqueous solubility and hence to make it more bioavailable. Conjugation and loading of curcumin with silica nanoparticles was further examined with transmission electron microscope (TEM) and thermogravimetric analyzer. Cytotoxicity analysis of synthesized silica:curcumin conjugate was studied against HeLa cell lines as well as normal fibroblast cell lines. This study shows that silica:curcumin conjugate has great potential for anticancer application.

KEYWORDS: *Curcumin, silica nanoparticles, bioavailability*

1. INTRODUCTION

Curcumin, a natural component of the rhizome of turmeric (*Curcuma longa*), has recently attracted the attention of researchers due to its unique ability to work through so many pathways with its astonishing antioxidant, anti-inflammatory, anticarcinogenic, chemopreventive, antiangeogenic, antidiabetic, antiviral and antibacterial properties[1−5] making it a potential candidate for curing almost every known disease.[4,5] Extensive research has been carried out on curcumin around the globe to demonstrate its great potential as a therapeutic agent and has shown the way toward clinical trials for a variety of diseases.[6−8] However, one of the greatest challenges in developing curcumin for clinical efficacy is its low oral bioavailability due to its hydrophobic nature. Curcumin's poor bioavailability leads to poor activity, low absorption, high rate of metabolism within the living system and rapid elimination from the system.[9,10] This has been a major obstacle in its progress from the lab to clinic as a drug. In this view, curcumin's chemoprevention and therapeutic potential has not been fully exploited for the prevention and treatment of various diseases.

In recent years, several research groups have focused to explore the potent applications of curcumin with the aid of modern nanotechnology.[11−13] Nanoparticles,[14] liposomes,[15] micelles,[16,17] soy protein isolate,[18] oil body encapsulation[19] and phospholipid complex[20] are being used to enhance the dispersibility and bioactivity of curcumin for clinical trials. Recently Kang Pan et al. encapsulated the curcumin in casein nanoparticles to enhance its dispersibility and bioactivity.[21] Further, this encapsulation was studied against HCT-116 for cytotoxicity analysis. C. Gong et al. have suggested that combination of bioactivity of curcumin and thermosensitive hydrogel in the in situ gel-forming composite promoted tissue reconstruction processes, indicating that curcumin encapsulation in polymeric micelles loaded with thermosensitive hydrogel (Cur-M-H) composite is a potential wound dressing for cutaneous wound healing.[22] M. Li et al. and P. T. Ha et al. have explored the binding of curcumin to β-lactoglobulin[23] and polymer encapsulated curcumin nanoparticles[24] for antioxidant and anticancer activities respectively. H. Yu et al. and M. Yang et al. have used organogel-based nanoemulsions[25] and bovine serum albumin[26] for improving the bioavailability of curcumin and its implications on the stability and antioxidant property. S. Manju et al. have reported synthesis of water-soluble gold nanoparticles in curcumin−polymer conjugate and studied it for blood compatibility and targeted drug delivery onto cancer cells.[27] They have conjugated curcumin with hyaluronic acid (HA) and then synthesized gold nanoparticles within the solution by reducing the chloroauric acid. In this and many other reports, it has been observed that, though the bioavailability improves upon conjugation, the main therapeutic group of this molecule gets engaged in the conjugation thereby killing its therapeutic activity to any biological system.[28] Therefore, there is a need for extensive research in this regard which would not only improve the bioavailability of the molecule but also keep the therapeutic activity high and impart multifunctional properties to the conjugated system. In our previous study, we have conjugated curcumin with PVP capped gold nanoparticles for improving bioavailability, and the conjugate was studied against TZM-bl cell lines.[29]

Silica nanoparticles have also been immensely explored due to their interesting properties such as hydrophilic surface favoring protracted circulation, versatile silane chemistry for

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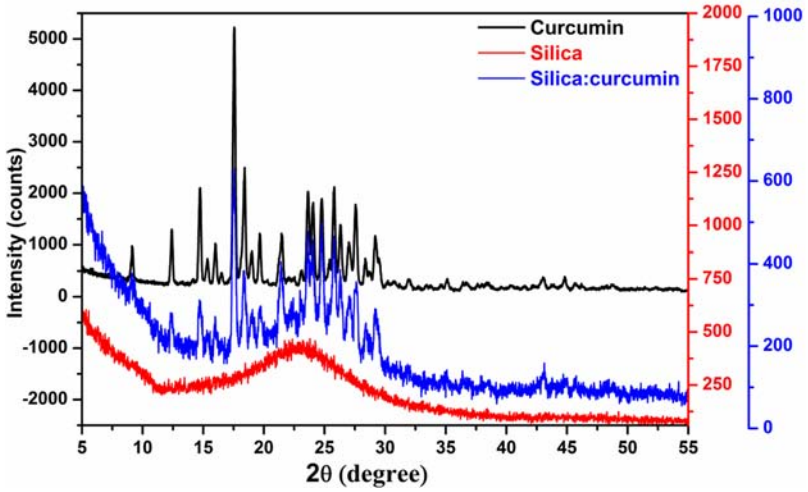

Figure 1. XRD patterns of pristine curcumin (black), silica nanoparticles (red) and silica:curcumin conjugate (blue).

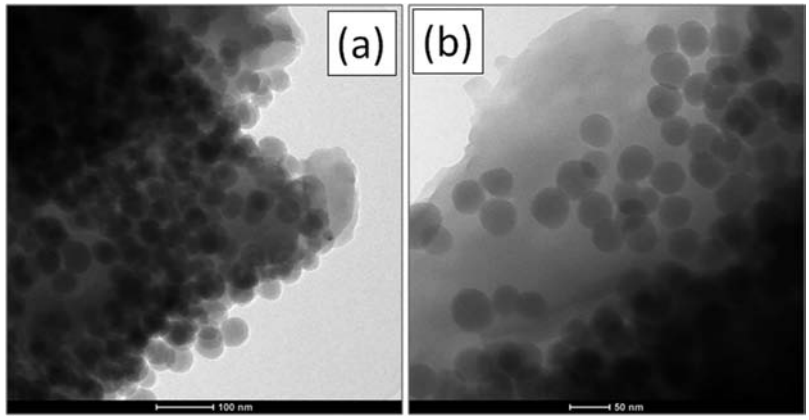

Figure 2. (a, b) TEM images of synthesized silica:curcumin conjugate at two different scales.

surface functionalization, excellent biocompatibility, ease of large scale synthesis and porosity.[30,31] These have been projected to be one of the safest (nontoxic) candidates for DNA conjugation, drug delivery and many other applications.[32,33]

In this paper, we report a neat protocol which delineates the conjugation of curcumin with silica nanoparticles by using a simple wet chemical method. This protocol envisages good bioavailability of the curcumin molecule, keeping its therapeutic activity intact. Further, this conjugate was studied against cancerous and primary cell lines. This work shows potential application of such a conjugate in the biomedical domain.

2. EXPERIMENTAL SECTION

2.1. Materials and Methods. Tetraethyl orthosilicate (TEOS, 99%), ammonia solution (25%), absolute ethanol (C_2H_5OH, 99.9%), sodium hydroxide (NaOH), potassium dihydrogen ortho phosphate (KH_2PO_4) and curcumin crystalline (MW 368.39) were used as received without further purification. Deionized water (DI water, ≈18 MΩ) was used in the experiment.

For the synthesis of silica:curcumin conjugate, first a modified Stober method[34] was followed for the synthesis of silica nanoparticles, and curcumin was added during the reaction. In a typical synthesis process, 40 mL of ethanol was mixed with 10.0 mL of DI water and 2.0 mL of ammonia solution in the reaction flask. The solution was then placed on a magnetic stirrer hot plate at 300 rpm for 20 min at 60 °C. The initial color of the solution was transparent. Afterward, the solution was heated to the desired temperature; 2.0 mL of tetraethyl orthosilicate (TEOS) was added dropwise into the heated solution within two minutes. 50 mg of curcumin was added to the reaction mixture immediately after the addition of TEOS. The solution was continuously stirred and heated at the same temperature for next 60 min. The final color of the solution was reddish yellow. After this, the reaction flask was placed in an oven at 40 °C for 24 h to evaporate the solvent. Powder was collected and washed three times in DI water. The color of the powder was found to be yellow. Silica nanoparticles were also synthesized for comparison with the same procedure except the addition of curcumin.

2.2. Characterization. Synthesized silica:curcumin conjugate and silica nanoparticles were characterized by UV−vis spectroscopy (Ocean Optics, HR4000), Fourier transform infrared spectroscopy (FTIR, Perkin-Elmer), thermogravimetric analyzer (TGA, Perkin-Elmer STA 6000), X-ray diffractometer (XRD, Bruker AXS D8 Advance) and transmission electron microscopy (TEM, FEI-Tecnai G^2 20). For TEM characterization highly diluted specimens were drop cast on carbon coated copper grid and dried under the IR lamp. Toxicity analysis was done using MTT assay as described elsewhere.[29]

3. RESULTS AND DISCUSSION

Figure 1 shows the XRD patterns of silica nanoparticles, curcumin and synthesized silica:curcumin conjugate using Cu Kα radiation (λ = 1.5406 Å). Measurements were performed at a voltage of 40 kV and 40 mA within the 2θ range of 5−55°. The XRD pattern of pristine curcumin showed several characteristic peaks due to its crystalline nature, while the typical amorphous pattern was observed for silica nanoparticles.

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It can be observed from this figure that curcumin retains its crystallinity after conjugation, however the intensity of the corresponding peaks has been reduced. Therefore, one can conclude that the curcumin gets coated on the silica nanoparticles, also evident from the TEM data. Figure 2a,b shows TEM images of synthesized silica:curcumin conjugate at two different scales. It is observed from this figure that silica nanoparticles are spherical in shape, having diameter of 50 ± 5 nm. It can be seen from this figure that silica nanoparticles are completely surrounded by curcumin as a shell. This is in good agreement with XRD, FTIR and UV−vis analysis discussed further.

Figure 3 shows FTIR spectra of (i) silica nanoparticles, (ii) curcumin and (iii) silica:curcumin conjugate. Here spectra (i) and (ii) exhibit the typical signatures of both silica nanoparticles and curcumin. These signatures may be recognized as asymmetric vibration of Si−O (1093 cm^{-1}), asymmetric vibration of Si−OH (950 cm^{-1}) and symmetric vibration of Si−O (798 cm^{-1}) in silica nanoparticles. Signatures of curcumin are free O−H group (3508 cm^{-1}), C═O and C═C (enol) (1450−1630 cm^{-1}), C−H (methyl) (2930 cm^{-1}), C−H (aryl) (3017 cm^{-1}) and C−O−C (1000−1300 cm^{-1}) typically attributed to symmetric and asymmetric configurations of C−O−C chains. Spectrum (iii) illustrates the conjugation of curcumin with silica nanoparticles. This spectrum reveals signatures of both silica nanoparticles and curcumin molecule. Curcumin molecule may attach to silica nanoparticles with enolic hydroxyl group, which results in a shift of Si−O stretching from 1093 cm^{-1} to 1082 cm^{-1}, whereas the basic diaryl heptanoid group, which is the chromophore group of curcumin, remains intact.[35]

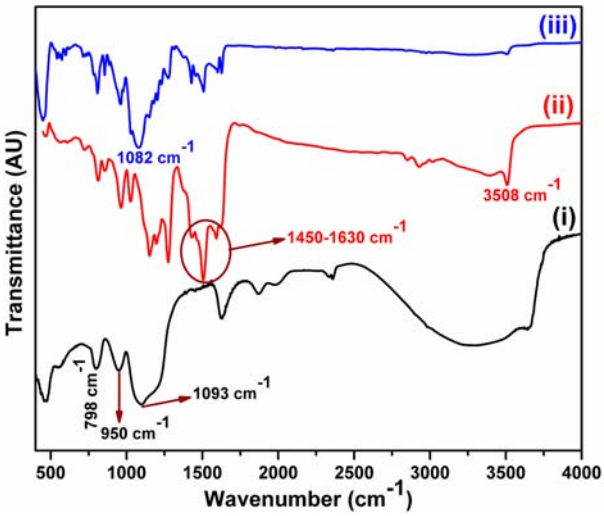

Figure 3. FTIR spectra of (i) silica nanoparticles, (ii) curcumin and (iii) silica:curcumin conjugate.

Figure 4a shows possible schematic for the conjugation of curcumin molecule with silica nanoparticles. Curcumin gets attached to the silica nanoparticles with enolic hydroxyl group, which results in a shift of Si−O stretching from 1093 cm^{-1} to 1082 cm^{-1} without affecting the basic diaryl heptanoid group of curcumin. This is also supported by UV−vis spectroscopy explained below. Figure 4b shows the UV−vis spectra of (i) silica nanoparticles, (ii) curcumin and (iii) synthesized silica:curcumin conjugate. Spectrum (ii) shows an absorption peak at ∼415 nm, which is the signature of the basic diaryl heptanoid chromophore group of curcumin.[26] The UV−vis spectra of silica:curcumin conjugate (iii) clearly reveals that absorption band has broadened due to conjugation while characteristic peak of curcumin remains intact after conjugation. Hence, both the UV−vis data along with FTIR results show that curcumin retains its diaryl heptanoid chromophore group after conjugation. Therefore, curcumin with the improved solubility in aqueous medium due to conjugation and signature peak in UV−vis retaining its high therapeutic activity was studied further for its anticancer capabilities.

Thermal analysis of neat silica nanoparticles, curcumin and silica:curcumin conjugate was investigated using TGA as shown in Figure 5. It can be observed from this figure that neat silica nanoparticles (black curve) do not exhibit any significant weight loss up to 800 °C, except for a small loss at 80−100 °C due to the removal of absorbed water. The TGA curve for pure curcumin (red curve) shows rapid weight loss in the range of 270−445 °C, which may be attributed to the degradation of curcumin. Synthesized silica:curcumin conjugate also begins to

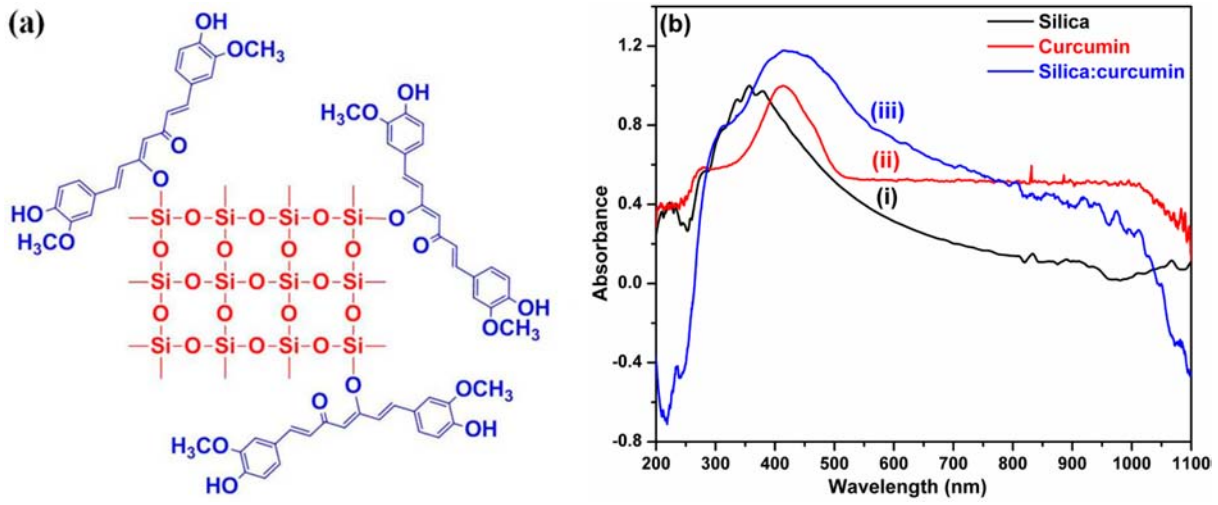

Figure 4. (a) Schematic for probable conjugation of curcumin with silica nanoparticles and (b) UV−vis spectra of (i) silica nanoparticles, (ii) curcumin and (iii) silica:curcumin conjugate.

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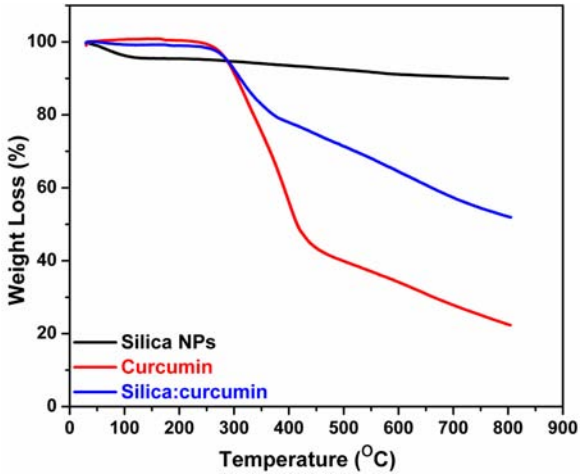

Figure 5. TGA curves for silica nanoparticles (black), curcumin (red) and silica:curcumin conjugate (blue).

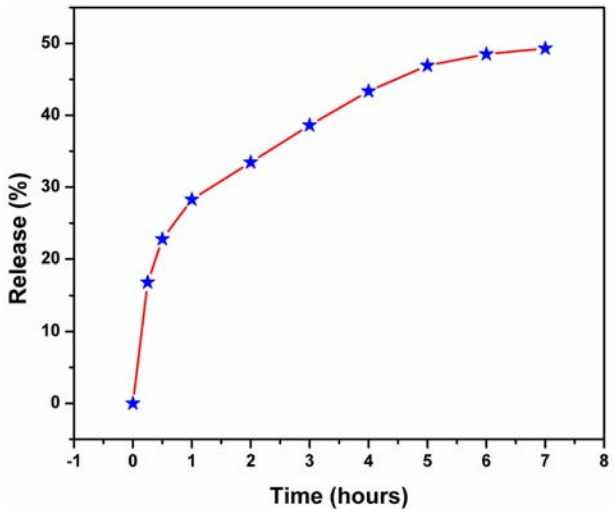

Figure 7. Graph of in vitro release kinetics of curcumin at 35 °C in buffer solution (pH = 7.4).

lose weight at 270 to 300 °C with high rate, and afterward it becomes slower. The TGA results show that ∼40% of curcumin gets loaded on the silica nanoparticles.

The cytotoxicity effect of silica:curcumin conjugate was studied against HeLa cell lines as well as normal fibroblast cell lines. Figure 6 shows the cytotoxicity results of the cell lines after incubation with the conjugate for 48 h with increasing concentration. This figure indicates ∼1.4% cytotoxicity of the compound on the HeLa cells even at a low concentration of 2 μg/mL, which was nontoxic to the normal cells. However with increasing concentration of the compound there is a steep rise in cytotoxicity toward HeLa cells. At 20 μg/mL, compound was found to be ∼73% and ∼40% cytotoxic toward HeLa and normal cell lines respectively. In the case of normal cell lines, 73% cytotoxicity was observed when concentration was increased to 4-fold, i.e., 80 μg/mL. Thus the conjugate can be further analyzed for in vivo studies for its effectivity.

In vitro release kinetics of curcumin from silica nanoparticles was studied using UV−vis spectroscopy and is shown in Figure 7. For this purpose, silica:curcumin conjugate was dispersed (2 mg/mL) in the phosphate buffer solution (PBS: 7.4 pH) and an experiment was performed at 35 °C. It is observed from this figure that ∼28% of loaded curcumin was released within one hour and then the rate of release became slow. Net release of curcumin was observed up to 50% within 7 h. These results are quite encouraging owing to the applications of curcumin as antimicrobial and antiviral agents.

In conclusion, we have conjugated curcumin with silica nanoparticles by using a simple wet chemical method. Synthesized silica:curcumin conjugate was well dispersed and stable in aqueous medium at room temperature for 2−3 weeks. The diaryl heptanoid chromophore group of curcumin which is a much needed group in biomedical applications remained intact after conjugation as observed from FTIR and UV−vis spectroscopy analysis. Nearly 40% of curcumin was getting loaded on silica as observed from TGA analysis. Further, the conjugate was studied against HeLa as well as normal fibroblast cell lines. Cytotoxicity analysis revealed that conjugate was more toxic to HeLa cell lines compared to primary cell lines. Release kinetics was also studied and found to be ∼50% in 7 h.

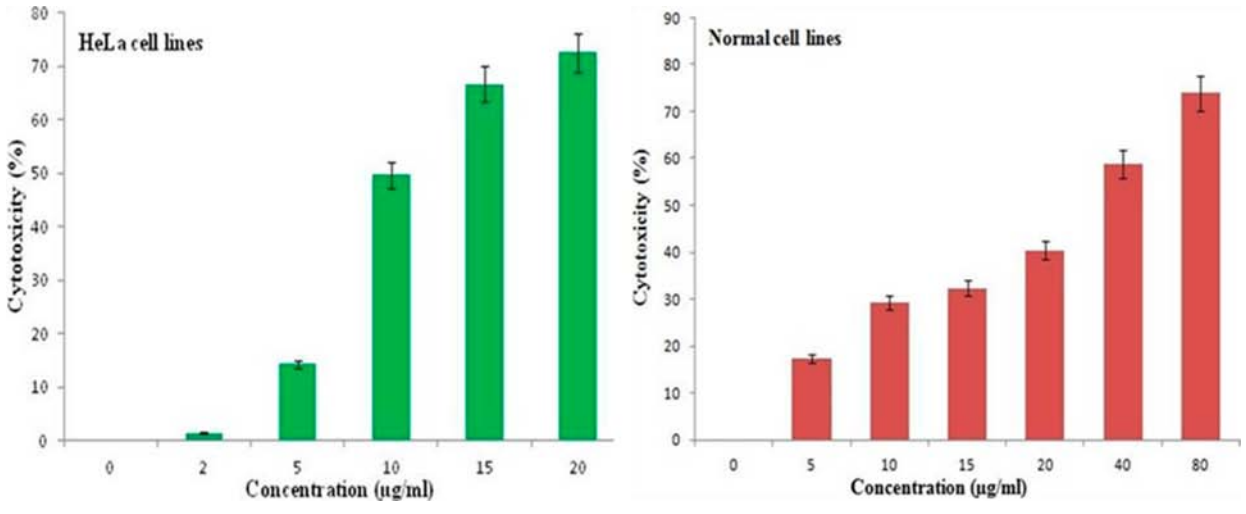

Figure 6. Cytotoxicity (%) of synthesized silica:curcumin conjugate against HeLa cell lines and normal fibroblast cell lines.

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Notes

The authors declare no competing financial interest.

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