Biophysical Characterization of Curcumin Nanoparticles and Its Role in Cervical Cancer

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Abstract

Curcumin is a potent anticancer agent and has great potential efficacy against different types of cancers. A major disadvantage of curcumin, however, is its poor solubility and bioavailability. The aim of the present work is to synthesize chitosan and curcumin-loaded chitosan nanoparticles and their characterization through various physicochemical methods and cellular uptake in cervical cancer cell line SiHa. Chitosan nanoparticles were nanoformulated by a method of tripolyphosphate (TPP) cross-linking with chitosan. The influence of fabrication conditions on the physical properties and drug loading and release properties was investigated by dynamic light scattering, dynamic light scattering, FT-IR, LCMS, fluorescence and UV–vis spectroscopy. The nanoparticles could be prepared only within a zone of appropriate chitosan and TPP concentrations. The studies presented demonstrate that curcumin-loaded chitosan nanoparticles showed increased uptake in the SiHa cells as compared to free curcumin and chitosan nanoparticles did not show any significant uptake in SiHa cell line. The curcumin-loaded chitosan nanoparticles released more lactate and lower ATP as compared to native curcumin in cervical cancer lines such as SiHa, CaSki and HeLa. Thus, chitosan based curcumin nanoparticles could be used as a potent vector / delivery agent for drug targeting in the treatment of cervical cancer.

Keywords: *Curcumin, Chitosan nanoparticles, Cervical cancer, Cellular uptake, LDH assay*

1. Introduction

A novel drug delivery carrier involves delivering therapeutic compounds to the targeted specific sites *in vitro* and *in vivo* system. To obtain the desired therapeutic response and to minimize the toxic effects, the drug must be loaded within the nanoparticles (Yadav et al., 2012). An effective drug delivery system can be incorporated into polymeric nanocarriers for not only enhancing the circulation time of the drugs but also to control the drug release (Mukerjee & Vishwanatha, 2009; Dudhania & Kosaraju 2010). Natural polymeric carriers are the better chosen for the targeted drug delivery systems due to their interdisciplinary aspects of nature including, solubility, biocompatibility, biodegradability, minimal toxicity and immunogenicity (Wilson et al., 2012). Curcumin is one of the natural polyphenol compounds present in the rhizomes of turmeric (*Curcuma longa*) and has been reported to exhibit promising activities like: antioxidant, anti-inflammatory, anti-proliferative, anti-invasive and anti-angiogenic activities (Kunnumakkara et al., 2008; Anand et al., 2007). Curcumin (Figure 1) usually occurs in three major molecular form namely: curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, demethoxy curcumin (1,6-Heptadiene-3,5-dione,1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) and bis demethoxy curcumin {(1E,6E)-1,7-bis (4-hydroxy phenyl) hepta-1,6-diene-3,5-dione)}.

Recently, curcumin has also shown to have antitumor efficacies in many cancer cell lines (Sahu et al., 2008) and the clinical trials' effects are being studied in animal models on various conditions and numerous myeloma and even in human (Shimatsu et al., 2012; Carroll et al., 2011). In addition, biomedical properties, free curcumin suffered from poor solubility, bioavailability and clinical efficacy (Chin et al., 2009). Furthermore, many reviewers' have tried to increase water solubility and bioavailability of curcumin by entrapment of curcumin in biodegradable polymeric nanoparticles. However, curcumin loaded chitosan nanoparticles were synthesized and idt is used for the delivery in cervical cancer treatment (Khan et al., 2016; Anitha et al., 2011). Chitosan is a well-known and less expensive natural homo-polysaccharide which has been used as noble drug deliveries as well as they are hydrophilic, biodegradable and biocompatible with cells and tissue (El-Hag & Al-Arifi, 2009; Wang et al., 2010). Chitosan nanoparticles with particle

sizes in the range of 10–500nm have been extensively studied as controlled drug release carriers. For instance, chitosan nanoparticles loaded with drugs (curcumin, flufenamic acid, and caffeine) showed enhanced effectiveness upon permeation studies on human skin cancer and cervical cancer cell lines (Khan et al., 2016; Santander-Ortega et al., 2010). In addition, chitosan nanoparticles loaded with 5-fluorouracil (5-FU) and curcumin were found to have enhanced breast cancer cells (MCF-7) and cervical cell lines (CasKi, HeLa, SiHa, C33A) inhibition *in vitro* as compared to free 5-FU (Xiao et al., 2012) and curcumin (Khan et al., 2016).

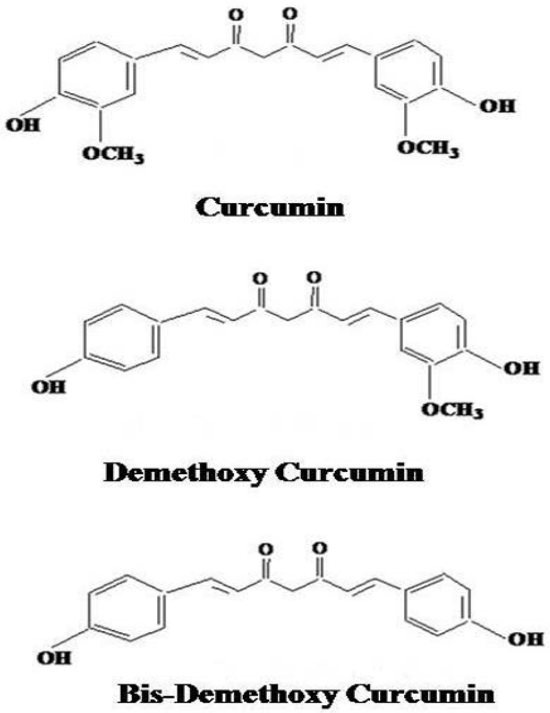

Figure 1 Chemical structure of curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin)

In our previous study, we have evaluated and optimized the best formulation of chitosan nanoparticles (CsNPs) and curcumin loaded chitosan nanoparticles (CLCsNPs) by electrostatic interaction. In addition, the biophysicochemical techniques such as particle size with surface potential by dynamic light scattering (DSC), morphology by TEM, entrapment and loading efficiencies were used for characterization in our previous article (Khan et al., 2016). The curcumin entrapment on chitosan nanoparticles properties were demonstrated by HPLC & MALDI-TOFMS and crystal structure were also measured by XRD. Furthermore, the loading and releasing study of curcumin from CLCsNPs *in vitro* were investigated to provide research foundation for pharmacokinetics and cell distribution *in vivo* (Khan et al., 2016). The aim of our studies, we have revealed a physicochemical characterization for loading of curcumin on chitosan nanoparticles by UV- spectrophotometer, fluorescence spectroscopy, LCMC, LDH and cellular uptake of free curcumin under fluorescence microscopy. The potential application of these native curcumin, chitosan nanoparticles and controlled release of curcumin from chitosan loaded nanoparticles were studied.

2. Objectives

1. Formulation of curcumin loaded chitosan nanoparticles.
2. Characterization of curcumin loaded chitosan nanoparticles by various biophysical techniques.
3. Implication of curcumin loaded chitosan nanoparticles in cervical cancer.

3. Materials and methods

3.1 Materials

Chitosan (Cs) and Curcumin (C) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol was purchased from CDH-laboratory chemical (India). Tissue culture medium, antibiotic, trypsin were purchased from Himedia (India) and fetal bovine serum from Gibco (South America). All other chemicals were of high analytical grade and Millipore grade distilled water was used in all experimental work.

3.2 Synthesis of chitosan nanoparticles and curcumin loaded nanoparticles

Chitosan nanoparticles were synthesized through the method of ionic gelation of chitosan with sodium tripolyphosphate (TPP) as described previously (Khan et al., 2016; Calvo et al., 1997). Briefly, chitosan was solubilized in 1% acetic acid with the concentration of 2 mg/ml. On magnetic stirrer at room temperature, mixing 1 mg/ml of (w/v) TPP solution for 24 hrs and then centrifuged at 8,000×g, for 15 min at 4°C using a (Sigma Centrifuge, USA). Opalescent suspension was prepared spontaneously. The particle's size and zeta potential of chitosan nanoparticles were determined by a dynamic light scattering Zeta sizer respectively. Curcumin loaded chitosan nanoparticles were prepared upon incorporation of 8 ml of TPP solution containing curcumin into 17.5 mL chitosan solutions. The curcumin loaded chitosan nanopaticles were separated from the aqueous medium containing non-associated curcumin by centrifugation at 8,000g, 4°C for 15 min and then lyophilized. The entrapment and loading efficiency of curcumin loaded chitosan nanopaticles were determined as described earlier (Khan et al., 2016). In addition, the internal structure of chitosan nanoparticles and curcumin loaded chitosan nanoparticles were monitored by TEM and also characterized by HPLC, MALDI-TOFMS, FT-IR, X-ray diffraction as described in detail (Khan et al., 2016).

3.3 Biophysicochemical characterization of curcumin and curcumin-loaded chitosan nanoparticles

3.3.1 Particles size and zeta potential analysis

Particle size and polydispersity index were determined by DynaPro-TC–04 dynamic light scattering equipment (Protein Solutions, Wyatt Technology, Santa Barbara, CA) worked on quasi-elastic light scattering (Khan et al., 2016). Briefly, 1 mg/ml of chitosan nanoparticles (CsNPs) and curcumin loaded chitosan nanoparticles (CLCsNPs) were prepared in milliQ water and sonicated for 25 sec in an ice bath (Toshcon, India). Zeta potential was measured in the same instrument at 25°C using the same procedure. All measurements were performed in triplicates and the average value was taken.

3.3.2 Absorbance spectroscopy study

The absorption studies of native curcumin, chitosan nanoparticles and curcumin-loaded chitosan nanoparticles were recorded on Shimadzu Spectrophotometer (UV 1700model, Japan) in the wavelength range of 380–470nm in a quartz cuvette of 1 cm path length.

3.3.3 Fluorescence spectra

The fluorescence spectra of the samples were taken by using a Shimadzu Spectrofluorometer (RF 5301, Japan). The pure curcumin, chitosan nanoparticles and curcumin loaded chitosan nanoparticles samples were excited at a wavelength of 430 nm. The spectra were recorded in the wavelength region of 440 nm to 600 nm.

3.3.4 Differential scanning calorimetry (DSC) studies

The thermal nature of the chitosan, curcumin, chitosan nanoparticles and curcumin-loaded nanoparticles were evaluated by differential scanning calorimetry (Microcal, USA). An approximate amount of samples (2-4mg) were weighed and scanned in a temperature range from 0^0C to 200^0C with a heating rate of 10^0C/min per cycle. The inert atmosphere was maintained by purging nitrogen at a rate of 360 cm^3/ min (Khan et al., 2017).

3.3.5 Liquid Chromatography Mass Spectroscopy (LCMS) studies

The LC–MS/MS analysis was conducted in positive ion ESI mode on a Quattro Micro API–Waters hexapole mass spectrometer connected to a liquid chromatograph (Waters Alliance). The result was conducted on a Phenomenex Luna C18 100A column (250 mm × 4.6 mm, 5 μm). The mobile phase consisted of methanol and 0.05% acetic acid solution (80:20, v/v) at a flow rate of 1.0 mL/min. The sheath gas and auxiliary gas were tuned to give an optimum response as necessary. The needle voltage was 4.5 kV. Curcumin was used as internal standard control (Liu et al., 2006). Argon was used as the collision gas at collision energy of 15eV (curcumin). The collision energy was individually tuned for each analyte to obtain an optimum value. The analytes were quantified using selected ion reaction monitoring (SRM). The ion transitions m/z 369.3→285.0 was used for the determination of curcumin. The auto-sampler cooler was maintained at 4°C.

3.3.6 Cell lines and cellular uptake studies

All cervical cancer cell lines were procured from NCCS Pune, India. HPV positive cervical cancer cell lines (Caski, HeLa and SiHa) and HPV negative cells line (C33a) were cultured and maintained in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum and antibiotics in a humidified atmosphere of 5% CO_2 at 37°C in culture dishes/flasks. Stock culture was maintained in the exponential growth phase and passage as monolayer culture using 0.02% EDTA. The dislodged cells were suspended in complete medium and reseeded routinely.

To visualize the intracellular uptake of curcumin released from curcumin loaded chitosan nanoparticles in SiHa cells (1×10^5) were seeded in a 6-well plate. Cells were treated with curcumin (24μM) and same amount of curcumin-loaded chitosan nanoparticles dissolved in 10% aqueous ethanol and chitosan nanoparticles used as blanks. After incubation of 48 hrs, the cells were washed with PBS and images were captured through fluorescence microscope at 40X.

3.3.7 Lactate dehydrogenase (LDH) assay

LDH, a cytoplasmic enzyme occurs in most eukaryotic cells, releases into culture medium upon cell death due to leakage of plasma membrane. The LDH released in supernatant can be monitored by colorimetrically by addition of suitable substrates. Lactate was measured in cell culture media using a lactate assay kit (G-Biosciences). Cells were seeded to confluence in 6-well culture plates and treated with an equal amount of curcumin loaded chitosan nanoparticles and native curcumin (24μM) for 48hrs. Media were removed and washed with PBS, deproteinized and tested for lactate concentration. The cells were removed by trypsinization and counted. The catalytic action of lactate dehydrogenase (LDH) was measured spectrophotometrically at 340 nm of lactate in terms of the generation of NADH, using Shimadzu Spectrophotometer (UV 1700 model).

4. Results

4.1 Synthesis and Characterization of curcumin nanoparticles

The preparation and synthesis of curcumin-loaded chitosan nanoparticles were performed using the same method as described earlier (Khan et al., 2016). The synthesized CLCsNPs and CsNPs had good physical and chemical stability, could be stored at 4°C and exhibited decent solubility in 10% ethanol. CsNPs revealed that the average mean diameter of the particles was 189±11.8 nm (Figure 2 (a) and (b)) with zeta potential +76±5.6 mV and CLCsNPs was found to be the mean diameter of 197±16.8 nm (Figure 2 (c) and (d)) with zeta potential +71±6.4 mV respectively by Dynamic light scattering (DLS).

Both, particle size and zeta potential were critical determinants for the fate of delivered nanoparticles (Khan et al., 2016). The purity of CLCsNPs and intactness of its chemical structure was confirmed by MALDI-TOFMS, FT-IR and XRD described earlier (Khan et al., 2016). The morphology and particle size of CLCsNPs and CsNPs were determined by TEM.

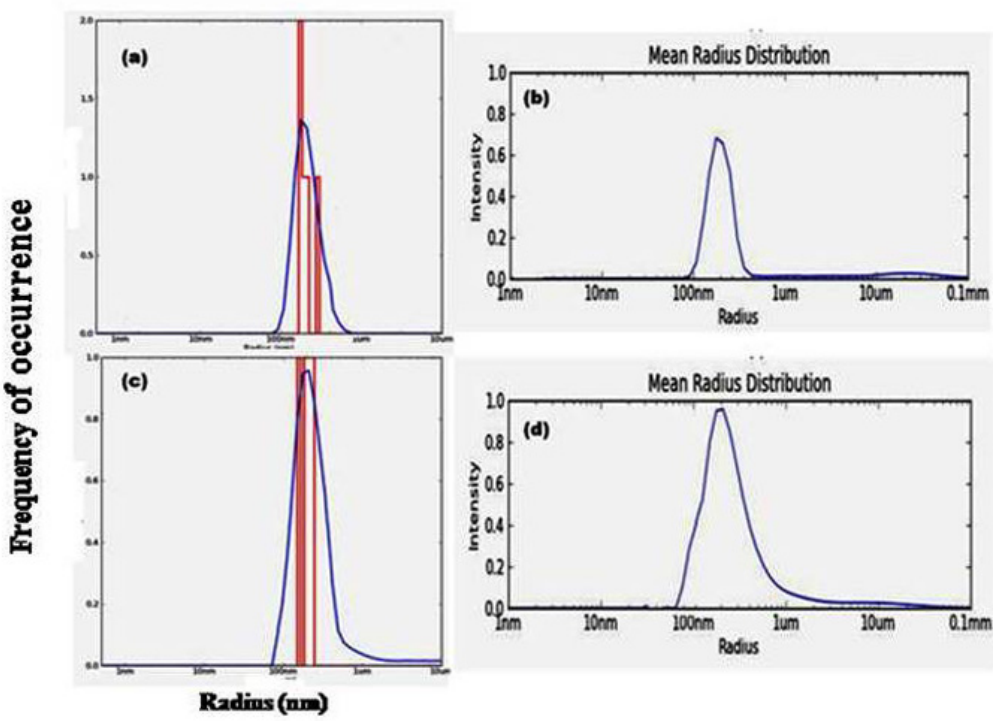

Figure 2 Size characterization by DLS of (a&b) Chitosan nanoparticles and (c&d) curcumin loaded chitosan nanoparticles

4.2 UV and fluorescence characterization of native and loaded curcumin

Commercially available curcumin was solubilized in a buffer (10% aqueous ethanol, pH 5.0). Native curcumin (8µM) showed a characteristic absorption peak at 430nm (Fig. 3).

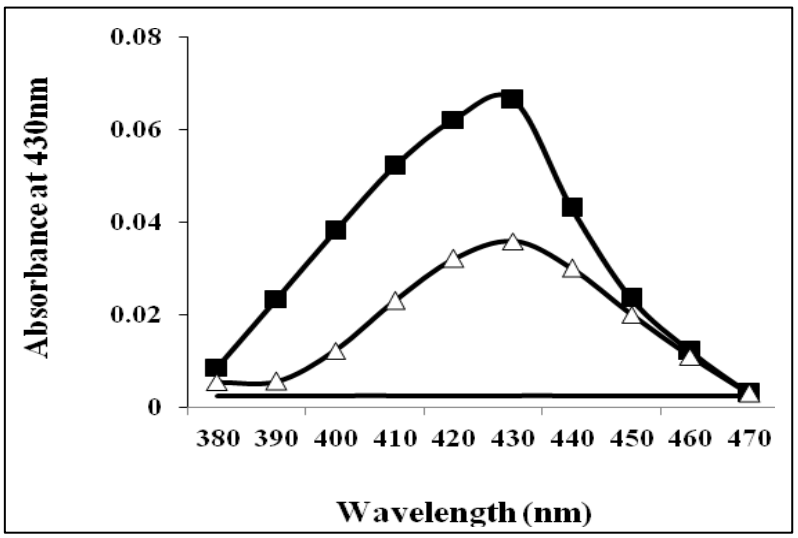

Figure 3 UV profile of chitosan nanoparticles (—), (8µM) of each native curcumin (─Δ─) and curcumin loaded chitosan nanoparticles (─■─)

Upon entrapment of curcumin on chitosan nanoparticles, there observed to be similar absorption peak at wavelength 430 nm with an increase of 5nm of wavelength to represent a red shift compared to curcumin abosorption peak. The red shift in the absorbance peak of curcumin loaded chitosan nanoparticles (CLCsNPs) might be probably attributed to the formation of intermolecular hydrogen bonding between curcumin and chitosan nanoparticles (Huong et al., 2011). The chitosan nanoparticles (CsNPs) did not show any peak under identical experimental conditions. This result clearly showed that curcumin has loaded on chitosan nanoparticles. Curcumin is a polyphenol compound and contains a chromophore group, which contribute significantly to intrinsic fluorescence and after excitation at 430 nm its maximum emission spectra was observed at 540 nm. Native curcumin (8μM) and an equal amount of curcumin-loaded chitosan nanoparticles (8μM) recorded the emission spectra at the same wavelength, using excitation wavelength at 430 nm but with change in peak intensity (Figure 4).

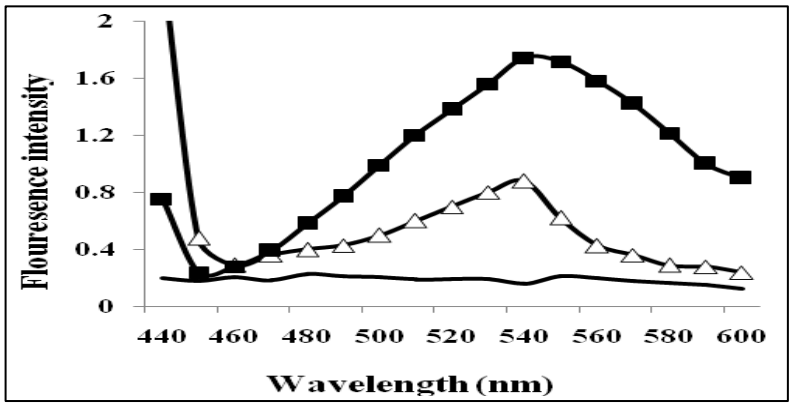

Figure 4 Fluorescence emmision profile of chitosan nanoparticles (—), (8μM) of each native curcumin (─Δ─) and curcumin loaded chitosan nanoparticles (─■─)

The peak intensity of the curcumin-entrapped chitosan nanoparticles was higher than that of native curcumin. In aqueous medium, the increased intensity of the emission spectra obtained for curcumin-loaded chitosan nanoparticles indicated that a higher number of curcumin molecules was entrapped (Rajeswari et al., 2011; Chin et al., 2014).

4.3 Differential scanning calorimetry of curcumin and loaded curcumin

Differential scanning calorimetry (DSC) was performed to evaluate any changes in crystalline properties of curcumin due to loading on chitosan nanoparticles. The melting behavior of chitosan, curcumin, CsNPs and CLCsNPs are demonatrated in Figure 5. The DSC curves of (A) chitosan, the melting points was found to be generally between 60-80°C and (B) chitosan nanoparticles (CsNPs) was found to be a similar melting peak. However, Curcumin alone exhibited a melting peak of approximately 176-180°C (Figure 5D). The physical mixture with curcumin loaded chitosan nanoparticles showed a melting point of approximately 60-80°C for chitosan and 179°C for curcumin (Figure 5C). Furthermore, Figure 5C result indicated that an interaction of curcumin with chitosan nanoparticles, which was in an amorphous state in the nanoparticles. Meanwhile, the loaded drug molecules might be disrupted in hydrogen bonds spanning adjacent head-groups, thereby alter the specific structural arrangement of a particular polar head group region, further reducing the melting point of the chitosan nanoparticles component curcumin previously (Khan et al., 2017; Sainz et al., 1993).

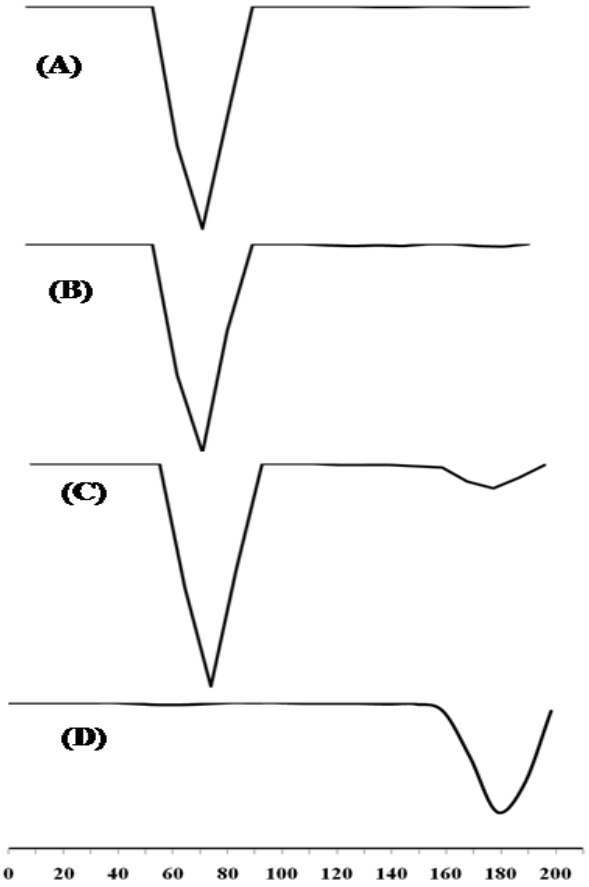

Figure 5 LC chromatographs of (A) native chitosan (B) chitosan nanoparticles (C) curcumin loaded chitosan nanoparticles (D) native curcumin

4.4 Liquid Chromatography Mass Spectroscopy studies of curcumin-loaded chitosan nanoparticles and native curcumin

Liquid Chromatography Mass Spectroscopy (LCMS) method was used for the determination and quantitation of standard curcumin and curcumin-extracted from CLCsNP. The retention time was approximately 6.11 min for standard curcumin and the total run time was 10 min. Specificity of the method was evaluated by comparing the chromatograms of curcumin (standard) and CLCsNP (sample) respectively. Representative chromatograms as shown in Figure 6, present the characterization of standard curcumin whose retention time at 6.11 min (Figure 6(a)) by LC. The mass analysis of native curcumin by LC-MS showed mass at 369.6 for standard curcumin (Figure 6(b)) and similar retention time and mass were also observed for curcumin-extracted from CLCsNP (Figure 6 (c)&(d)) as a standard curcumin. As curcumin is a highly hydrophobic in nature, when dissolved in an aqueous solution. The nano-sized crystals are formed inside the matrix of the nanoparticles (NPs) hindering drug elution from the NPs and the release profile was irregular.

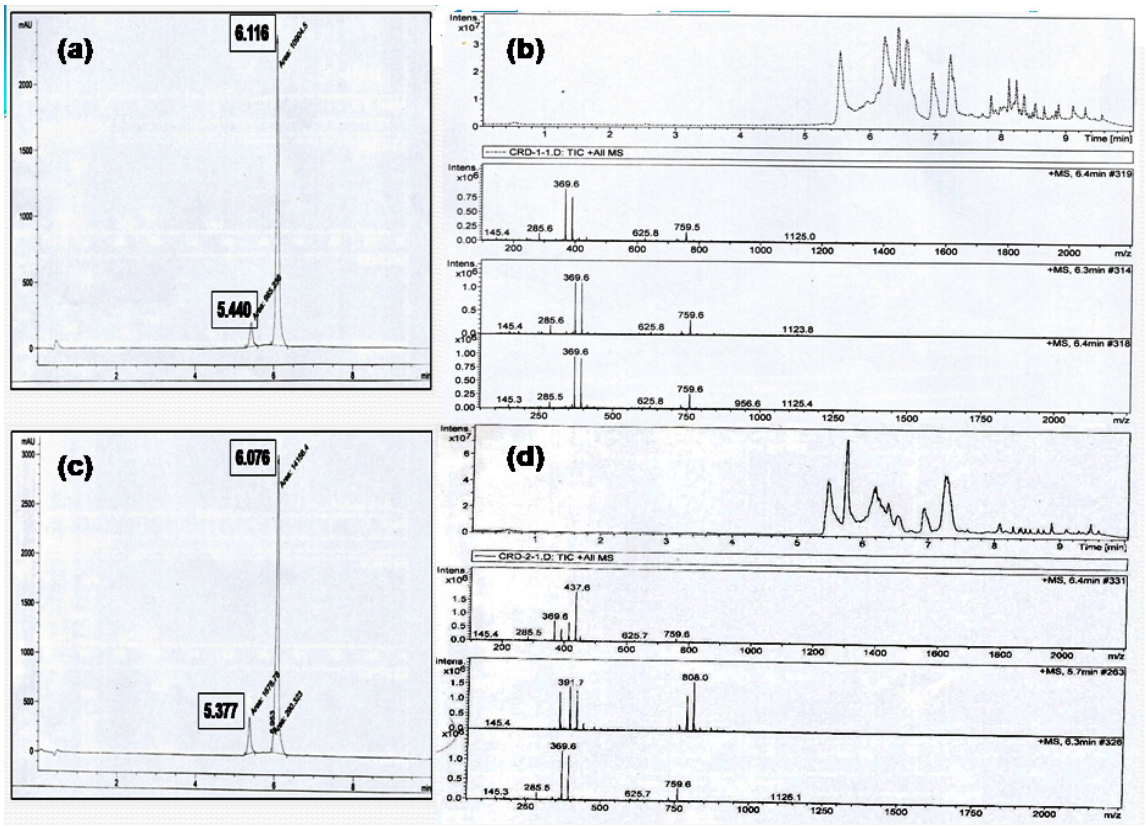

Figure 6 LC-MS analysis of (a&b) native curcumin and
(c&d) Curcumin extracted from curcumin loaded chitosan nanoparticles

4.5 Cellular uptake of curcumin-loaded chitosan nanoparticles

Initially, our study was first evaluating whether or not curcumin-loaded chitosan nanoparticles were taken up by the cancer cell lines SiHa. As shown in Figure 7d, CLCsNPs was robustly taken up by cervical cancer cells, indicated by the fluorescence emitted from the accumulated intra-cytoplasmic drug under fluorescence microscope. Uptake of CURN was also evaluated LDH assay. In a cellular uptake experiment, cervical cancer cell lines (SiHa) have grown in 6 wells culture plates and treated with cucumin-loaded chitosan nanoparticles. The cells were incubated and analyzed under fluorescence microscopy. We observed higher cellular uptake of extracted curcumin in cells treated with cucumin-loaded chitosan nanoparticles than native curcumin (Figure 7). This indicates that the strong intracellular uptake of curcumin released through curcumin-loaded chitosan nanoparticles (Figure 7(d)) than native curcumin (Figure 7(c)). The cells treated with chitosan nanoparticles, had no cellular uptake under a fluorescence microscopy (Figure 7(b)) and Figure 7(a) was served as a control without treatment with nanoparticles. The loading of curcumin on chitosan nanoparticles occurred *in situ* as previously described (Peltonen et al., 2002) in order to further confirm the loading of curcumin on chitosan nanoparticles. Thus, it is well established that curcumin extracted from CLCsNPs can elicit apoptosis or cell death *via* cell cycle arrest in human cancer cell lines (Cao et al., 2006).

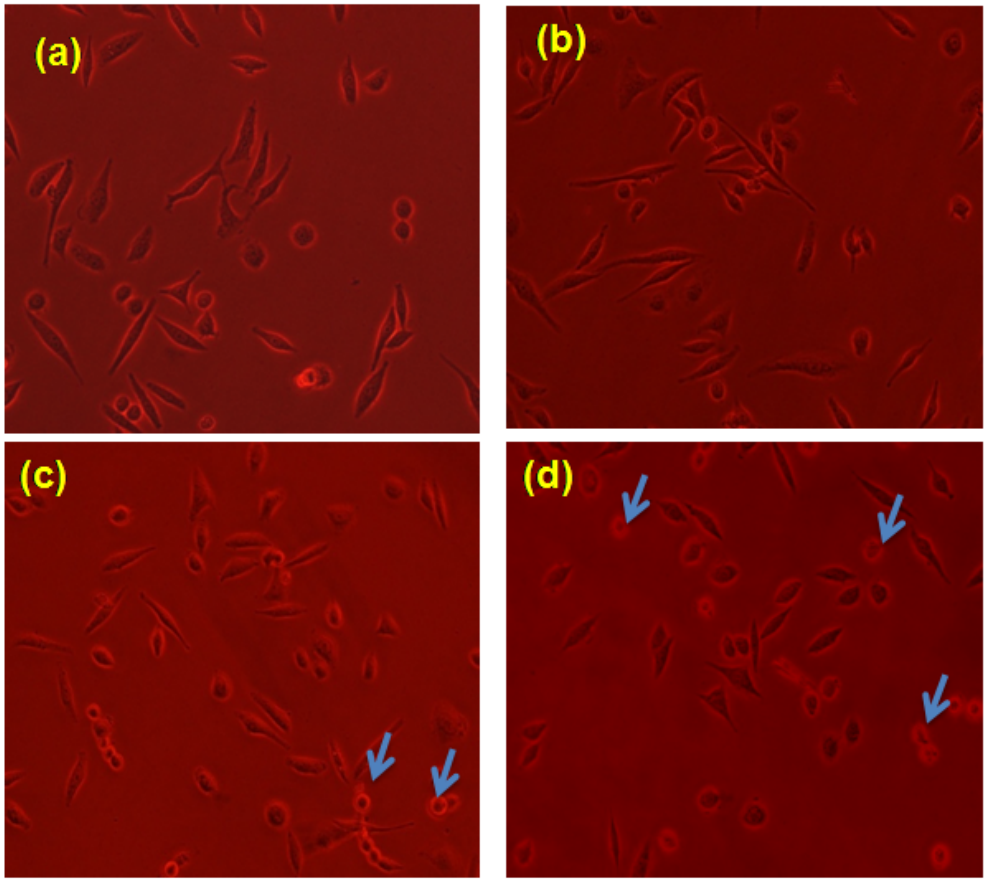

Figure 7 Cellular uptake in SIHa Cell line (c) native curcumin and (d) curcumin extract from curcumin-loaded chitosan nanoparticles. SiHa cells were treated with equivalent concentrations (24 µM) of curcumin and curcumin-loaded chitosan nanoparticles for 48 hrs (arrow indicates uptake of curcumin). (a) Control SiHa cell line and (b) treated with chitosan nanoparticles did not show a cellular uptake. Curcumin uptake was shown by fluorescence microscopy images taken at magnification 40X.

4.6 ATP depletion after the treatment of curcumin-loaded chitosan nanoparticles cervical cell line

To confirm the results of proliferation assay, *in vitro* LDH assay was performed in all four cervical cancer cell lines (C33A, HeLa, SiHa and CasKi). Human cancer cells require both glycolysis and mitochondrial oxidative phosphorylation for the production of ATP (Pedersen, 2007). We observed an increased formation of ATP and lower production of lactate in C33A cells treated with curcumin-loaded chitosan nanoparticles while curcumin treatment are due to higher glucose utilization by these cell lines and production of lesser ATP and higher lactate *via* only glucose metabolism (glycolysis), the amount of lactate, produced from glucose catabolism to pyruvic acid and conversion to lactate was measured spectrophotometrically at wavelength 340 nm in term of NADH (Figure 8). Results of these data suggested significantly higher levels of lactate (lower level of ATP) in Caski cells as compared to other cervical cell lines like, SiHa, HeLa and particularly C33A, consistent with the notion that enhanced glucose metabolism rates can protect cells against curcumin-loaded chitosan nanoparticles induced ATP depletion as compared to native curcumin (Fingrut et al., 2005). LDH assay also revealed that CLCsNPs exhibited better efficient anti-proliferative activity against the cancer cell lines. Apoptosis caused by CLCsNPs was comparable to

that caused by native curcumin. However, the results re-emphasized the fact that curcumin-loaded chitosan nanoparticles had a maximum anticancer effect on C33A cell line when compared to the other three cell lines. The cell death in cervical cancer cell lines *via* curcumin-loaded chitosan nanoparticles was further confirmed by TUNEL assay in our previous study (Khan et al., 2016).

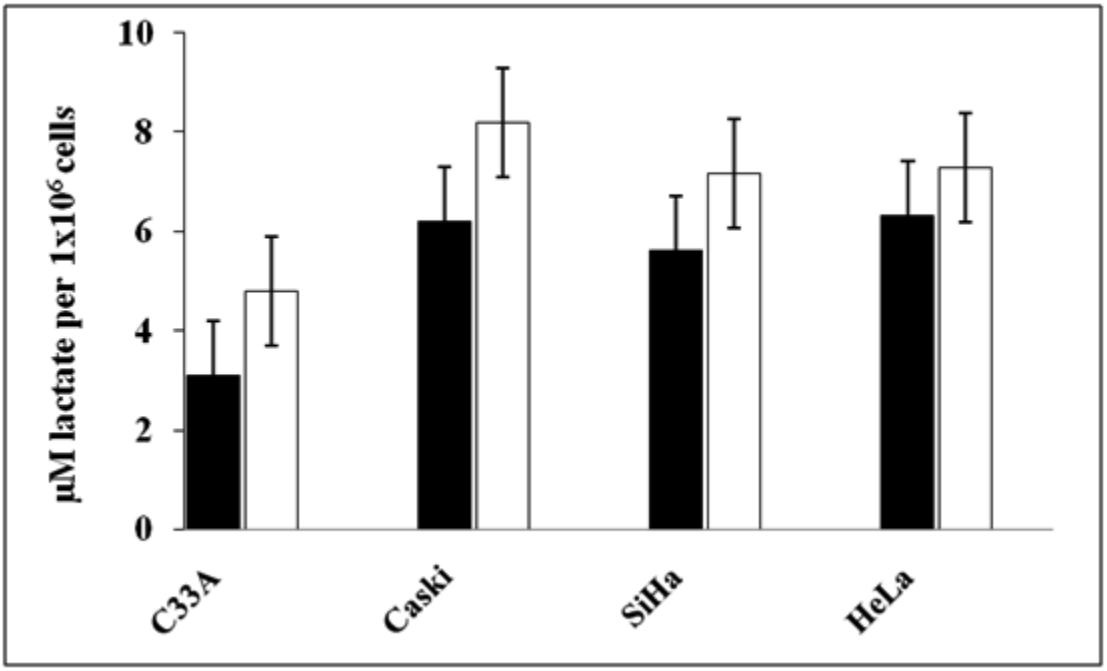

Figure 8 Lactate levels in supernatants of cervical cancer cells with the treated of native curcumin (─■─) and curcumin loaded chitosan nanoparticles (─□─).Levels of lactate were presented as micromole lactate per $1x10^6$ cells

5. Discussion

The curcumin-loaded chitosan nanoparticles were developed to minimize the problem associated with curcumin delivery including poor solubility, bioavailability and degradation. After successful preparation, synthetization and physicochemical characterization of a drug delivery system (curcumin loaded chitosan nanoparticles) were taken, it influenced physical stability, cellular uptake, bio-distribution and release of encapsulated drug (Acharya et al., 2009). In this regard, the small particle size, spherical shape and zeta potential of nanoparticles were advantageous for passive targeting to tumor tissue by increased permeability, retention effect (Das et al., 2009), cellular uptake and intracellular trafficking (Acharya et al., 2009). Hence, we could have anticipated these physical properties of our synthesized curcumin-loaded chitosan nanoparticles increased circulation times as well as escapes in human cervical cancer (Khan et al., 2016). Furthermore, the HPLC, MALDI TOFMS, FTIR and XRD analysis clearly observed the chemical nature of curcumin and its encapsulation inside the nanoparticles (Khan et al., 2016). The amount of entrapped curcumin in the nanoparticles is an important factor for determining the therapeutic efficacy of a drug delivery system. In this regard, our spectroscopic results also revealed the successful loading of curcumin in our synthesized chitosan nanoparticles. Native curcumin in ethanolic solution showed absorbance maxima at around 430 nm. The absorbance intensity of our formulated curcumin-loaded chtosan nanoparticles showed a similar absorption peaks close to the absorbance peak of native curcumin. This result proved the successfully loading of curcumin within chitosan nanoparticulate. Similarly, consistent to curcumin absorbance spectra, the fluorescence emission profile also exhibited similar trends. While revealing the fluorescence emission spectra of curcumin with excitation wavelength at 430 nm, we examined that native curcumin showed a clear fluorescence emission peak at 540 nm, similar

the fluorescence emission spectrum of curcumin-loaded chitosan nanoparticles (λemission =535nm) was shifted towards a blue spectrum (Sahu et al., 2008). This blue shift could be due to binding of curcumin with in hydrophobic domain present in curcumin-loaded chtosan nanoparticles. The curcumin-loaded chitosan nanoparticles was further confirmed by LCMS. In addition, intracellular uptake study is an important parameter that needs to be clearly explained for drug delivery of our synthesized curcumin-loaded chitosan nanoparticles to cancer tissue. In vitro cellular uptake study demonstrated native curcumin treated SiHa cell showed less uptake when compared to curcumin released from curcumin loaded chitosan nanoparticles after 48hrs of incubation. In contrast, we demonstrated the better uptake (as studied qualitative and quantitative experiments) of curcumin from our formulated curcumin-loaded chitosan nanoparticles than native curcumin by a cervical cancer SiHa cell.

In LDH studies, CaSki cells that showed the highest sensitivity to the curcumin-loaded chitosan nanoparticles showed significantly smaller decreases in ATP levels as compared to the other cervical cancer cells (Caski, HeLa & SiHa). This could be demonstrated by the higher glycolytic pathway in CaSki cells, indicated by the higher production of lactate that compensate for mitochondrial ATP loss by ATP generation from glucose metabolism. It means that, the switch from apoptosis to necrosis is less effective and cell death is mostly via apoptotic. As demonstrated from our *in vitro* studies, we further anticipated our synthesized curcumin-loaded chitosan nanoparticles will be a best suitable delivery carrier for curcumin to the experimental animals. Hence, the explained comprehensible results successfully justified curcumin-loaded chitosan nanoparticles hold better chemopreventive and chemotherapeutic activities than native curcumin due to its better solubility, biodegradable, bioavailability and it consequently elicit induction of apoptosis in tumor cells, advocating their potential use as a better strategy for cancer prevention.

6. Conclusion

The studies revealed that synthesized curcumin-loaded chitosan nanoparticles showed enhanced depletion of ATP when compared to native-curcumin in HPV positive (SiHa, CasKi and HeLa) and negative (C33A) human cervical cancer cell lines. Thus, chitosan-based nanocarriers could be used as a pertinent vehicle for the controlled delivery of curcumin in the treatment of cervical cancer.

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