

Curcumin loaded in bovine serum albumin–chitosan derived nanoparticles for targeted drug delivery

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Abstract. The main aim of this study is to prepare biocompatible polymeric nanoparticles for targeted delivery of curcumin to human colorectal adenocarcinoma (DLD-1) cells. Curcumin has an ability to block proliferation of cancer cells by suppressing the nuclear transcription factor NF-KB hence, it is chosen as drug in the current study. To avoid its low bio-availability, high dosage and poor aqueous solubility, curcumin nanoparticles are prepared and loaded in naturally available biopolymers like chitosan and bovine serum albumin (BSA) by nanoprecipitation method at pH 6.3. The prepared nanoformulation was then characterized for surface morphology, particle size, polydispersity index, FT-IR spectra, UV–Visible spectrometer, confocal microscopy and *in vitro* cytotoxicity studies. Results showed that sizes of the prepared nanoparticles were ranged between 181 and 363 nm and curcumin-loaded particles were selectively targeting colorectal carcinoma cells effectively when concentration gets increased. So this study proved that BSA–chitosan based nanoparticles can be used as an efficient vehicle for effective curcumin delivery in treatment of cancer cells.

Keywords. Nanoparticles; passive targeting; chitosan; curcumin; cancer.

1. Introduction

A typical drug delivery system involves delivering therapeutic agents to the targeted sites. To achieve the desired therapeutic response and to reduce the toxic side effects, the drug must be encapsulated within the nanoparticles [1]. An effective drug delivery system must be incorporated in polymeric nanocarriers for increasing the circulation time of the therapeutic drugs and also to control the high dosage levels [2,3]. Natural polymeric carriers are the widely chosen candidates for the targeted drug delivery systems due to their versatile aspects of nature like biocompatibility, biodegradability, reduced toxicity and low immunological properties.

Biodegradable polymeric nanoparticles hold great promise in targeted drug delivery due to their drug targeting specificity, improved bioavailability and sustained release of drugs with minimal toxicity and immunogenicity [4]. Chitosan, a natural polysaccharide, obtained from chitin is the widely used polymer of choice due to its hydrophilic characteristics. These polysaccharides are highly stable, non-toxic and biodegradable. Antitumoral, antibacterial and mucoadhesive aspects of chitosan make it an ideal candidate for drug delivery [5–8]. Bovine serum albumin (BSA), a natural biocompatible protein having non-toxic and non-antigenic characteristics can be used as a carrier of Paclitaxel for liver cancer treatment. Also, the BSA nanoparticles find applications in treating cerebral ischaemia by delivering Tanshinone

IIA (TIIA). The nanoparticles loaded with 5-FU are prepared from chitosan and BSA which can be effectively used for liver cancer treatment [9–11].

Curcumin, an extract obtained from the rhizome of the plant *Curcuma longa* (Zingiberaceae) can be used as a model drug. The biological attributes of curcumin include anti-oxidant, anti-inflammatory and anti-cancer properties. Curcumin blocks the cell proliferation, induce apoptosis in tumour cells and block the nuclear kappa B (NF-KB) in various human cancer cell lines. Also faster degradation of curcumin at basic pH finds its applications for treating cancer at GI tract [12,13]. Nevertheless, the limiting factor of curcumin is its poor water solubility, which in turn reduces its bioavailability. And hence, to improve the level of low availability, polymeric nanocarriers were chosen.

The present work reveals the synthesis of curcumin-loaded BSA–chitosan nanoparticles by nanoprecipitation method. The prepared nanoparticles were characterized for particle size distribution based on intensity, polydispersity index, scanning electron microscopy, FTIR spectroscopy UV–Visible spectrophotometer and confocal microscopy. The biocompatibility of the drug-loaded nanoparticles was evaluated using *in vitro* cytotoxicity studies.

2. Materials and methods

Chitosan, bovine serum albumin, Tween-20 and Tween-80 were purchased from SRL Chemicals, India. Curcumin was purchased from Sigma-Aldrich, India.

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2.1 Nanoparticles preparation

BSA–chitosan nanoparticles were prepared by nanoprecipitation method. Initially, 2.5% (w/v) of chitosan is dissolved in 1% acetic acid and 2% (w/v) BSA dissolved in distilled water. The solvent phase containing chitosan–BSA solutions, tween-20 and acetone were added with drug curcumin (100 mg) under continuous stirring. Solvent phase was added into non-solvent phase containing tween-80 and 50 ml distilled water under magnetic stirring. As solvent gets diffused into the aqueous phase, the chitosan gets precipitated and results in the instantaneous formation of nanoparticles. The BSA forms the core with encapsulated curcumin and 10 ml of the prepared nanoparticle solutions were centrifuged at 16,000 rpm for 30 min at 4°C. The supernatant was discarded and pellet was washed three times with distilled water and lyophilized for 24 h. The nanoparticles were then prepared from polymer loaded with and without drug in different combinations (see table 1).

2.2 Surface morphology and particle size

Scanning electron microscopy was performed to characterize the surface morphology of the curcumin loaded BSA–chitosan nanoparticles. The particle size distribution based on intensity and polydispersity index of the drug-loaded nanoparticles were determined by dynamic laser scattering (DLS) using a Malvern system (Malvern Instruments).

2.3 Fourier transform-infrared (FTIR) spectroscopy

FTIR spectroscopy was done to study the interaction between the encapsulated drug and the nanoparticles. The samples (chitosan, BSA, curcumin, curcumin-loaded BSA–chitosan nanoparticles) were analysed using FTIR spectrometer. Spectral scanning was performed in the wavelength region between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} with a scan speed of 2 mm s^{-1} .

2.4 Drug distribution within the nanospheres

The distribution of curcumin within the nanospheres was examined using confocal microscope. Free nanoparticles, nanocurcumin, and PEGylated nanocurcumin were used in

this study. Since curcumin is naturally fluorescent in the visible green spectrum, no further labelling was used for confocal imaging. The samples were mounted on cover slips and visualized in a fluorescein isothiocyanate (FITC) channel and propidium iodide (PI) channel with excitation wavelength at 488 and 555 nm.

2.5 UV–Vis spectroscopy

UV–Vis spectra of curcumin were recorded using a double beam Perkin Elmer–Lambda 35 instrument. A drop of the sample was taken and then added to acetone and placed in quartz cuvette which was considered as sample cuvette. In the other quartz cuvette, only the acetone was taken which was considered as reference cuvette.

2.6 In vitro cytotoxicity studies: MTT assay

The biocompatibility of the curcumin-loaded nanoparticles was evaluated using MTT assay. The cultured cells (H9C2 cardiomyocytes and DLD1 colorectal adenocarcinoma cells) were seeded in 96-well plates with cell density of 10,000 cells per well. Then, the cells were treated with different formulations (curcumin, BSA–chitosan nanoparticles and nanocurcumin) for 24 h. Later, the cells were incubated with 10 μl of MTT (5 mg ml^{-1}) for 4 h at 37°C. The media was removed and 100 μl of DMSO was added to each well. Viable cells cause the reduction of yellow tetrazolium salt to violet formazan crystal. Finally, the absorbance was measured at 590 nm using ELISA reader. Based on the cell viability percentage, the cytotoxic nature of the drug-loaded nanoparticles was evaluated [15,16].

3. Results and discussion

3.1 Surface morphology and particle size

The drug-loaded nanoparticles exhibited a spherical morphology with mean diameter between 200 and 300 nm (figure 1). The result of the study was in agreement with the result obtained by Fan Yuan *et al* [8]. It ensured that prepared nanoparticles can efficiently reach the leaky tumour vasculature passively (EPR effect). The average size of the nanocurcumin was in agreement with the DLS results (nanoparticles with aggregates).

Polydispersity index values increases with the increase in concentration of polymers and drug. PDI values confirmed that the homogeneity of the drug-loaded nanoparticles decreases when compared with free nanoparticles (table 2).

3.2 FTIR spectroscopy analysis

FTIR spectra of chitosan, BSA, curcumin and curcumin-loaded BSA–chitosan nanospheres were recorded by a FTIR

Table 1. Concentrations of chitosan, BSA and curcumin (w/v).

Model	PEGylated chitosan (mg)	BSA (mg)	Curcumin (mg)
1	250	—	—
2	—	200	—
3	250	200	—
4	250	200	100

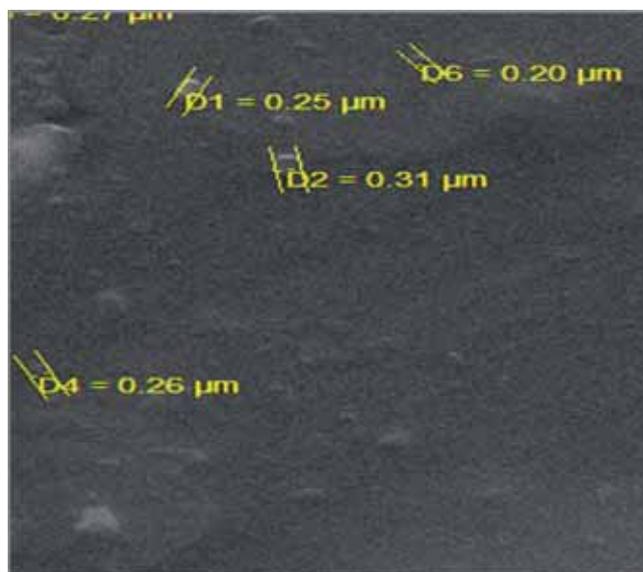


Figure 1. SEM image of curcumin-loaded nanoparticles (magnification 200 nm).

Table 2. Average particle size and PDI.

Model	Average particle size (nm)	PDI
1	181.9	0.28
2	149.4	0.44
3	241.8	0.45
4	363.6	0.61

spectrometer (figures 2–6). The FTIR spectra of chitosan showed strong peaks at 3456.0, 1632.3, 1485.2 and 1415.3 cm^{-1} which correspond to O–H stretching and hydrogen bonding, N–H bonding of primary amines, C–C aromatic stretch, respectively. The FTIR spectra of BSA showed characteristic peaks at 3447.0, 2918.2, 2849.6, 2103.3, 1636.4, 1459.5, 1292.8 and 716.6 cm^{-1} due to O–H stretching and hydrogen bonding, C–H stretch, $\text{C}=\text{C}$ alkynes stretch, N–H bonding of primary amines, C–C aromatic stretch and C–H bond, N–O symmetric stretch, C–H bonding vibrations, respectively. The FTIR spectra of curcumin showed characteristic peaks at 3621.6, 3570.2 and 3405.9, 2920.6 and 2850.1, 1597.0, 1292.2, 1157.2 cm^{-1} that correspond to strong O–H stretch, O–H stretching and H-bonded, C–H stretch, C–C stretch in aromatics (strong), C–O stretching vibrations.

In the FTIR spectrum of drug-loaded nanoparticles (nanocurcumin), a shift from 3456.0 to 3399.0 cm^{-1} was observed and the valley of 3399.0 cm^{-1} became wider, thereby indicating enhanced hydrogen bonding. In nanocurcumin, the peak at 1636.4 cm^{-1} of N–H bonding vibration shifted to 1597.0 cm^{-1} . It was due to the interaction of aromatic group of curcumin with BSA–chitosan nanoparticles.

3.3 Drug distribution within the nanospheres

Free nanoparticles do not exhibit any fluorescence in FITC and PI channels. Curcumin-loaded nanoparticles (nanocurcumin and PEGylated nanocurcumin) exhibits green fluorescence (by curcumin auto-fluorescence) in FITC channel

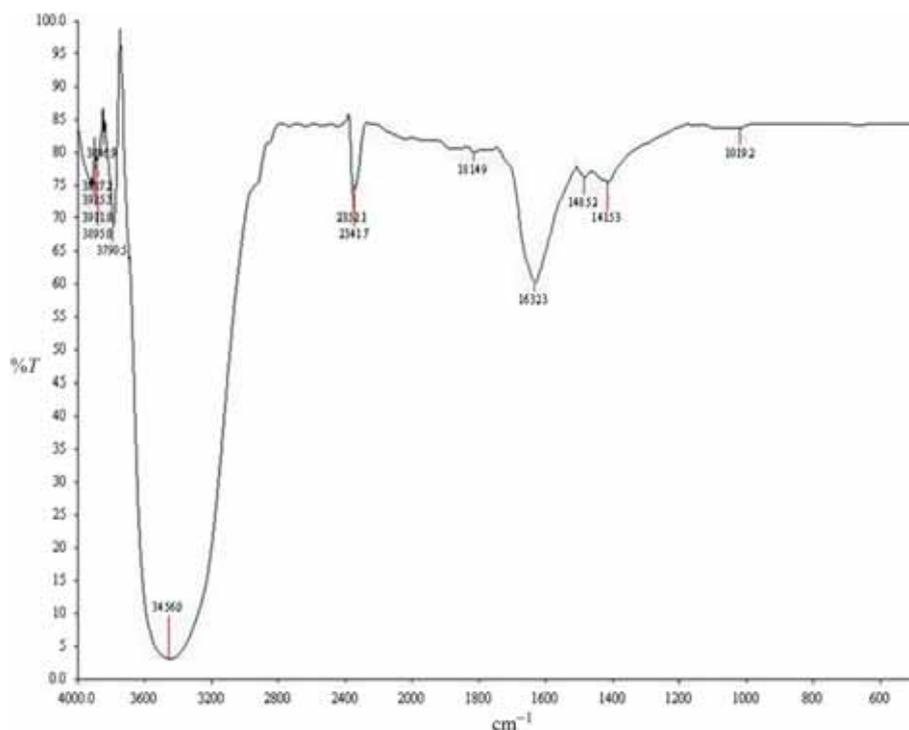


Figure 2. FTIR spectra of chitosan.

(488 nm). But no fluorescent image obtained from PI channel for all the three samples (figure 7). This confirms the auto-fluorescence property of curcumin showing the green

fluorescent image in FITC channel. The green fluorescent image confirms that curcumin is encapsulated within the nanoparticles.

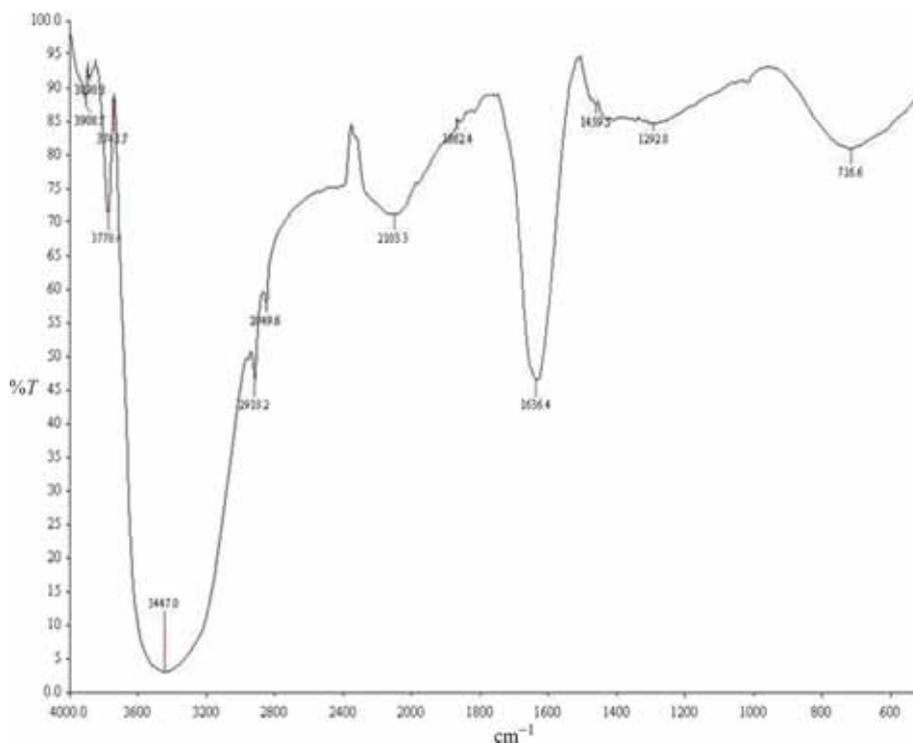


Figure 3. FTIR spectra of BSA.

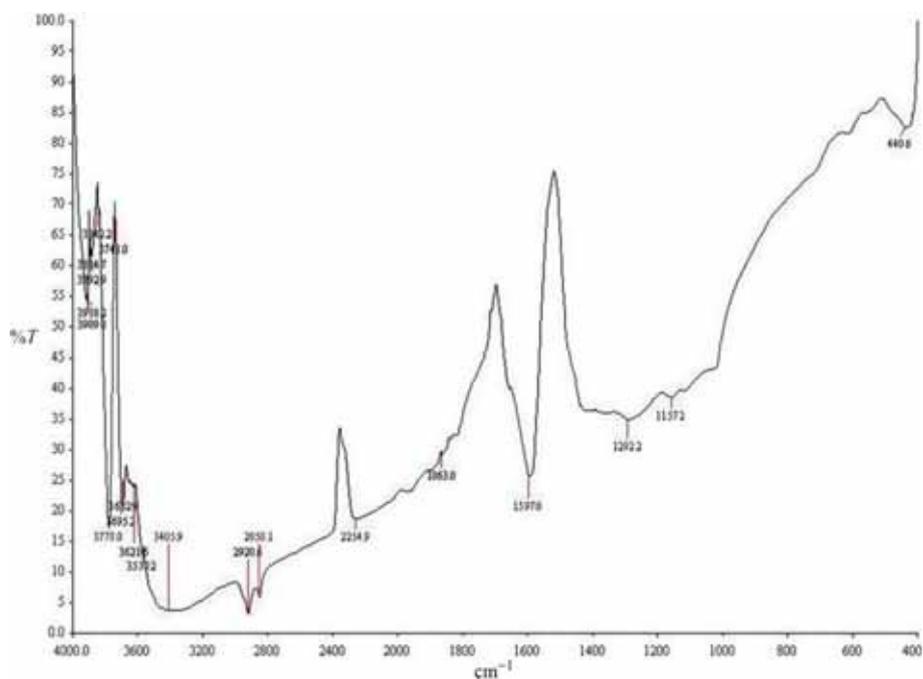


Figure 4. FTIR spectra of curcumin.

3.4 UV-Vis spectroscopy

Curcumin (red) exhibits intense absorption in the visible region of UV-Vis spectra (422 nm). BSA–chitosan

nanoparticles (blue) and PEGylated BSA–chitosan nanoparticles (green) showed similar absorption in the visible region with absorbance wavelength at 419.4 and 419.9 nm. This confirms the existence of curcumin within the nanospheres.

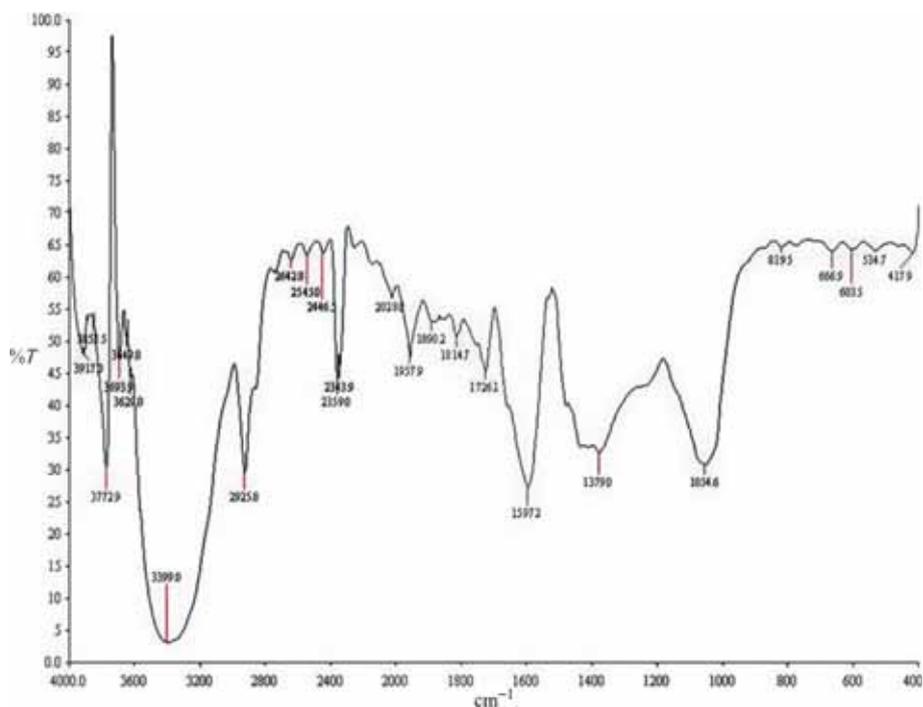


Figure 5. FTIR spectra of curcumin-loaded nanoparticles.

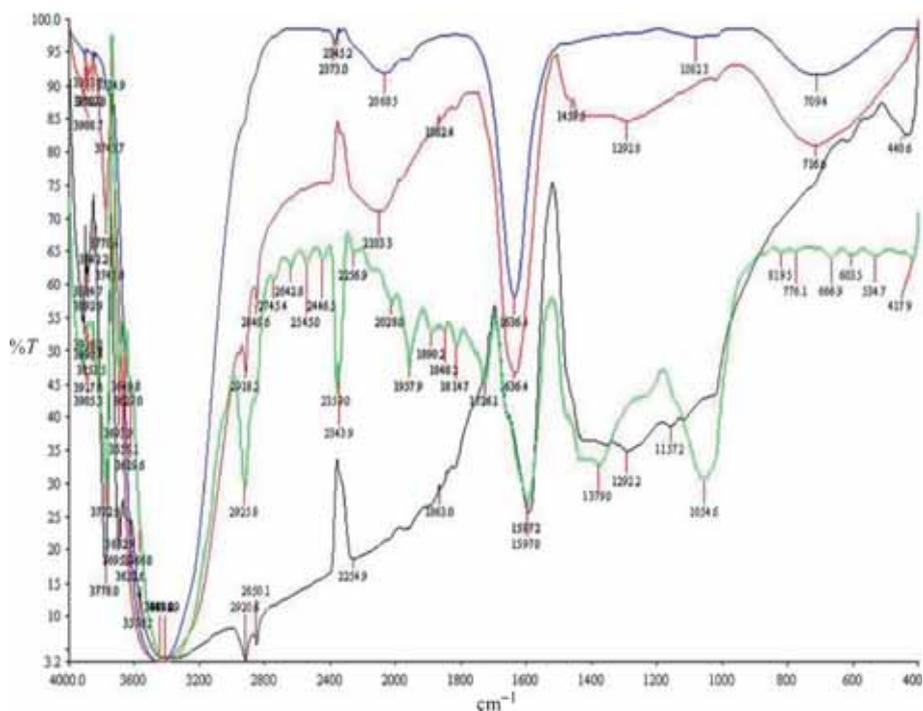


Figure 6. FTIR spectra overlay.

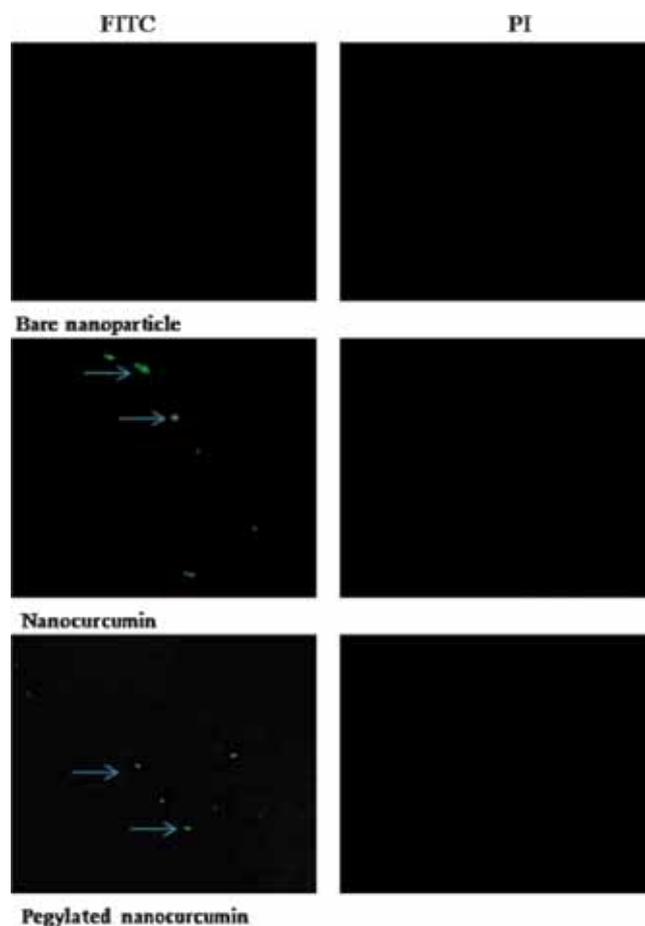


Figure 7. Curcumin-loaded nanoparticles exhibiting green fluorescence (by curcumin auto-fluorescence) in FITC channel.

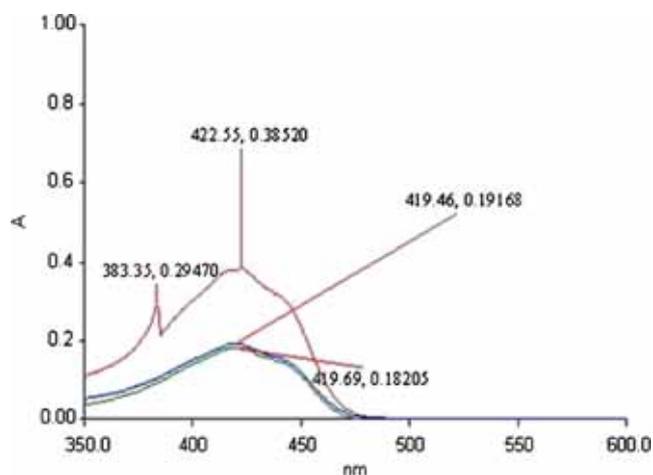


Figure 8. UV-Vis spectra of curcumin (red), nanocurcumin (blue) and PEGylated nanocurcumin (green) at pH 6.3.

Blank nanoparticles do not exhibit any absorbance in the visible region of UV-Vis spectra (figure 8).

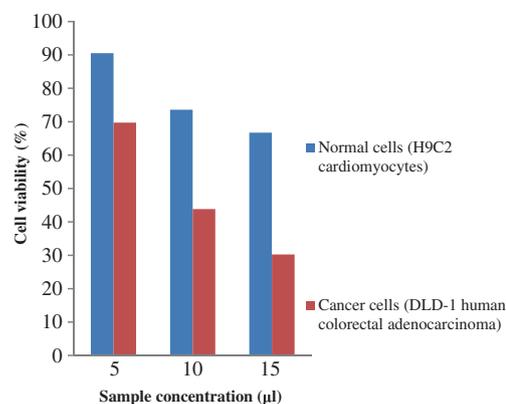


Figure 9. *In vitro* cytotoxicity studies using MTT assay after 24-h treatment.

3.5 Cytotoxicity studies

The biocompatibility of the prepared samples was evaluated by *in vitro* cytotoxic studies. MTT assay showed the viable nature of the living cells by the conversion of yellow tetrazolium salt into violet formazan crystal.

After 24 h treatment, the prepared PEGylated nanocurcumin showed cell viability of 91% on normal H9C2 cardiomyocytes and 70% of viability in colorectal adenocarcinoma cells for 5 µl concentration. The viability percentage decreases with an increase in concentration of the nanoparticles. These results confirmed that the cytotoxicity was dosage-dependent and prepared nanoparticles were selectively toxic to DLD-1 cancer cells (figure 9).

4. Conclusion

This work proposes a new method of preparing curcumin-loaded nanoparticles using chitosan (2.5%) and BSA (2%) by nanoprecipitation method. Nanoparticles prepared by this method have particle size in the range of nanometres (200–300 nm) can be used for passive method of drug targeting. Polydispersity index in the range of 0.2–0.6. PDI values confirmed that the nanoparticles became poly-dispersive in nature with the increase in the concentration of polymers and drug.

Scanning electron microscopy results confirmed that the prepared nanoparticles had spherical morphology with an average diameter of the nanoparticles (Z average = 258 nm). FTIR spectral studies showed that the drug reacts with the polymers through enhanced intermolecular hydrogen bonding and interaction of amino and aromatic groups. Also, intermolecular hydrogen bonding reduces intramolecular hydrogen bonding of phenolic hydrogen. Confocal microscopy results proved that the auto-fluorescence (green fluorescence in FITC channel) property of curcumin which are loaded in BSA–chitosan nanoparticles. UV-Vis spectral studies confirmed the presence of curcumin in nanocurcumin ($\lambda_{\max} = 419.4$ nm) and PEGylated nanocurcumin

(λ_{\max} = 419.6 nm). *In vitro* cytotoxicity studies revealed that the prepared nanocarrier was biocompatible (at low concentration of 5 μ l for 24 h treatment) showing greater viability for normal H9C2 cells (91%) when compared to DLD-1 cancer cells (70%). MTT assay showed that curcumin would not exhibit any cytotoxic effect on normal H9C2 cardiomyocytes even at high drug concentrations. Thus, BSA–chitosan based nanocarriers could be used as a pertinent vehicle to deliver curcumin.

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