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ORIGINAL ARTICLE

Cyclodextrin-based nanosponges of curcumin: formulation and physicochemical characterization

S. S. Darandale · P. R. Vavia

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Abstract Curcumin is the source of the spice turmeric having potential application in tumor treatment but has limited therapeutic utility because of its poor aqueous solubility. Curcumin suppresses the onset of tumors as well as their growth and metastasis. Cyclodextrin-based nanosponges (NS) have been used to increase the solubility of curcumin and to control its release. The aim of the study was to formulate the complex of curcumin with β-cyclodextrin nanosponge obtained with dimethyl carbonate as a cross linker. The particle size of loaded nanosponge was found to be 487.3 nm with minimum polydispersibility index (0.476). The loaded NS have shown more solubilization efficiency (20.89 μg/ml) in comparison with plain curcumin (0.4 μg/ml) and β-CD complex (5.88 μg/ml). The zeta potential was sufficiently high (−27 mV) which indicates formation of a stable colloidal nanosuspension. The curcumin nanosponge complex (CrNS) was characterized for FTIR, XRD and DSC studies and it confirmed the interactions of curcumin with NS. The in vitro drug release of curcumin was controlled over a prolonged period of time. The in vitro hemolysis study showed that the complex was non-hemolytic. CrNS sample showed only a slight reduction in cytotoxicity against MCF-7 cells, which concludes that there is no change in molecular structure of curcumin in CrNS formulation.

Keywords Curcumin · Nanosponges · β-Cyclodextrin · Controlled release · Complexation

S. S. Darandale · P. R. Vavia (✉)
Department of Pharmaceutical Sciences and Technology,
Center for Novel Drug Delivery Systems,
Institute of Chemical Technology, N. P. Marg,
Matunga (E), Mumbai 400 019, India
e-mail: vaviapradeep@yahoo.com; pr.vavia@ictmumbai.edu.in

Introduction

Curcumin is the source of the spice turmeric having potential application in most chronic disease like tumor, pulmonary, cardiovascular and metabolic diseases [1]. It is extracted from the dried root of the rhizome Curcuma Longa. Curcumin is polyphenolic compound with low intrinsic toxicity. It is practically insoluble in water at acidic and neutral pH, and soluble in alkali [2–4]. The structural formula for curcumin is shown below (Fig. 1).

In current research progress, curcumin is an upcoming herbal active for multitargeted therapy in treatment of various fatal diseases including cancer. Curcumin is known for its potent anticancer activity against a number of cancers, including breast, prostate and colon cancer. Preclinical and clinical studies have proved its safety while administered at very high doses [5]. Though, curcumin has showed higher safety and efficacy profile, the poor aqueous solubility and low systemic bioavailability limits its therapeutic utility [6, 7]. Furthermore, for anticancer treatment the drug molecule should present at the site of tumor for longer period of time to exert its therapeutic action [8]. Many researchers are engaged in development of new strategies to improve upon the solubility of curcumin as well as to prolong its release at the site of action.

One possible way to increase its therapeutic utility is to encapsulate the curcumin as a guest within the internal cavity of nanocarrier, which improve the solubility as well as controls the rate of drug release [9]. In addition, the nanocarrier formulation should provide high drug loading capacity, small size, targeting efficiency by passive mechanism and biocompatibility.

Nanosponges are recent findings which are made up of microscopic particles with cavities a few nanometers wide. They are intended to encapsulate a variety of compounds

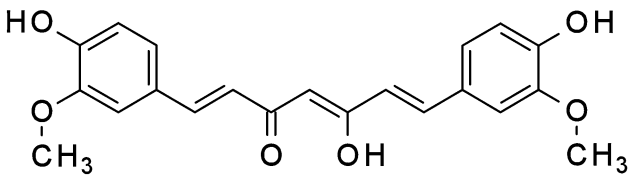

Fig. 1 Structural formula of curcumin

that can be transported through aqueous media [5]. Cyclodextrin-based nanosponges have been used in recent years as drug delivery vehicles, improving the therapeutic efficacy and bioavailability of the poorly water-soluble drugs [10–12]. Cyclodextrin-based nanosponges are the hypercrosslinked structures obtained by crosslinking different types of cyclodextrin using crosslinkers like carbonyldiimidazole, dimethyl carbonate, diphenyl carbonate etc. [11]. They are mainly used to improve the solubilization efficiency and prolong the release of hydrophobic drug molecules. Cyclodextrin-based nanosponges form inclusion and noninclusion complex with drug molecules [13].

In the present research work we describe cyclodextrin-based nanosponges of curcumin. We also present the results of its pharmaceutical and physicochemical characterizations and cytotoxic studies against tumor cells.

Materials and methods

Materials

β-Cyclodextrin (β-CD) was provided by Signet Chemical Corp. Pvt. Ltd (India). Curcumin was obtained from UNICO Pharmaceuticals (Ludhiyana, India). Dimethyl carbonate was purchased from S Define Pvt. Ltd. (Mumbai, India). All other chemicals and reagents were of analytical grade. Milli Q water (Millipore) was used throughout the studies.

Methods

Synthesis of β-cyclodextrin nanosponges

β-CD NS were prepared using dimethyl carbonate for the cross-linking as previously reported [14]. Briefly, Betacyclodextrin (2 g) was dissolved in 30 ml of Dimethyl formamide (DMF). To the solution triethyl amine (1 ml) was added. Crosslinker Dimetyl carbonate (14 ml) was then added and the solution was sonicated for 10 min. After refluxing for 3 h, the solvent is distilled out. The mass remaining was the crude nanosponges. These are then purified by washings with distilled water (25 ml) and ethanol (25 ml). The mass was then dried at 80 °C and was subjected to soxhlet extraction using ethanol (200 ml) for a period of 24 h.

Preparation of curcumin nanosponge complex (CrNS)

CrNSs were prepared by freeze drying technique. Accurately weighed quantities of nanosponges were suspended in 50 ml of distilled water on a magnetic stirrer, to it calculated amount of curcumin was added and the mixture was sonicated for 10 min. The solution was lyophilized using 2.5 Freezon freeze dryer (Labconco) at −45 °C temperature and operating pressure below 0.1 mbar to obtain drug-loaded NS formulation. The dried powder was sieved through 60# and stored in a desiccator.

Solubilization efficiency of CrNS

The excess quantity of curcumin was suspended in distilled water as well as with fixed quantities of nanosponges and β-cyclodextrin in distilled water separately. The glass vials were placed on a mechanical shaker at ambient temperature for 24 h. Obtained suspension was centrifuged at 10,000 rpm for 10 min using (RESEARCH COMPUFUGE, Remi PR-24 Centrifuge) and filtrate analyzed for curcumin concentration by HPLC method described earlier [15]. Briefly, drug analysis was performed using a C18 column (Lichrosorb 18, 5 μ, spherical, 4.6 × 250 mm, Merck KGaA, Germany) with mobile phase containing mixture of methanol and 1 % acetic acid (75/25, v/v) at a flow rate of 1 ml/min. Sample injection volumes were 20 µl and curcumin detection was performed using a Jasco PU-2080Plus at a wavelength of 428 nm.

Characterization of CrNS

Particle size and zeta potential measurement by dynamic light scattering (DLS)

Particle size analysis and polydispersibility index of CrNS were performed using dynamic light scattering (DLS) (Zetasizer ZEN 3600, Malvern, UK) with a scattering angle of 90° at 25 °C. The CrNS sample was diluted in distilled water prior to measurement.

Fourier transformed infra-red (FT-IR) spectroscopy

Pure curcumin, plain NS and CrNS were subjected to FTIR spectroscopic studies by potassium bromide disc method using Perkin Elmer system 2000 FTIR Spectrophotometer in the region of 4,000^{-1}–400 cm^{-1}.

X-ray powder diffraction

Pure curcumin, plain NS and CrNS were subjected to XRD studies using an X-ray diffraction meter (Rigaku miniflex, Japan) with Ni-filtered CuK radiation of wavelength

$\lambda = 1.54060$ Å with a graphite monochromator. The scan was taken in the 2θ range, 5–40° with a scanning speed and step size of 10/mm and 0.010, respectively. The percentage crystallinity was calculated.

Differential scanning calorimetry (DSC)

Pure curcumin, plain NS and CrNS were subjected to DSC studies using Differential Scanning Calorimeter (Pyris 6) of Perkin Elmer with 3 mg samples in standard aluminium pans. The samples were heated at a constant rate of 5 °C/min under nitrogen. The measurements were done in the temperature range from 30 to 250 °C.

In vitro release studies

The release study of curcumin from nanosponge complex was performed using direct dispersion method as explained in literature [16]. Briefly, a known quantity of curcumin loaded NSs were taken in 18 ml 10 mM Phosphate buffer saline (pH 7.4) and it was divided into 18 eppendorf tubes (10 set each having 3 tubes). The tubes were then incubated in a water bath shaker at 37 °C. At definite time intervals, one set of tubes were taken out and centrifuged at 1,200 rpm for 3 min to pelletize the released drug, leaving the entrapped drug within nanosponges, which stays in supernatant. The pellets were dissolved in methanol and amount of drug released was quantified by HPLC method as described earlier.

Hemolysis study

Hemolysis study was performed using the method reported previously [17]. Erythrocytes were isolated from heparinized human blood by centrifugation (2,800 rpm for 5 min). The supernatant was discarded and the settled erythrocytes were resuspended in Phosphate buffer saline (pH 7.4). After centrifugation and discarding of the supernatant, the washing steps were repeated three times. The purified erythrocytes were then resuspended in normal saline to obtain 2 % (v/v) of the RBC suspension. Thereafter, 1.8 ml of the erythrocyte suspension was incubated with 0.2 ml of tested samples at 37 °C for 0.5 h in an incubator shaker and then centrifuged at 2,800 rpm for 5 min. The percent hemolysis was measured by UV–Vis analysis of the supernatant at the wavelength of 545 nm. Phosphate buffer saline (pH 7.4) was used as the negative control with 0 % hemolysis, and distilled water was used as the positive control with 100 % hemolysis. The percent hemolysis was calculated using following equation:

$$\%\text{Hemolysis} = \frac{(\text{ABS} - \text{ABS}_0)}{(\text{ABS}_{100} - \text{ABS}_0)} \times 100$$

where ABS100 and ABS0 are the absorbances of the solution at 100 and 0 % hemolysis, respectively.

Cytotoxicity studies against MCF-7 Cells

The cytotoxicity of pure curcumin, plain NS and CrNS formulation against MCF-7 cells was assessed using MTT assay. Briefly, MCF-7 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % Fetal Calf Serum at 37 °C in a humidified incubator containing 5 % CO_2. Confluent cell monolayers were trypsinized and cells in exponentially growing phase were used. MCF-7 cells in culture medium were seeded in 96-well plates and incubated for adherence at 37 °C in 5 % CO_2, 100 µL of drug solution or a formulation was added into the wells. The concentrations of drug solutions were 1, 10, 50 and 100 ng/ml in different multi well plates. After 24 h incubation, 20 µL MTT solution (5 mg/mL) was added to each well and the plates were incubated for further 2 h. The solution in each well containing media, unbound MTT and dead cells was removed by suction and 100 µl of DMSO was added to each well. The plates were then shaken and the optical density (OD) was read on MULTISKAN ELISA Reader (LAB SYSTEMS Research Systems Division, Finland) at test wavelength of 570 nm. Cells incubated in culture medium alone served as a control for cell viability (untreated wells).

Results and discussion

Solubilization efficiency of CrNS

Solubilization efficiency of the CrNS was studied (Fig 2). The loaded NS showed more solubilization efficiency (20.89 µg/ml) in comparison with plain curcumin (0.4 µg/ml) and β-CD complex (5.88 µg/ml). The higher solubilization potential of CrNS was may be due to formation of

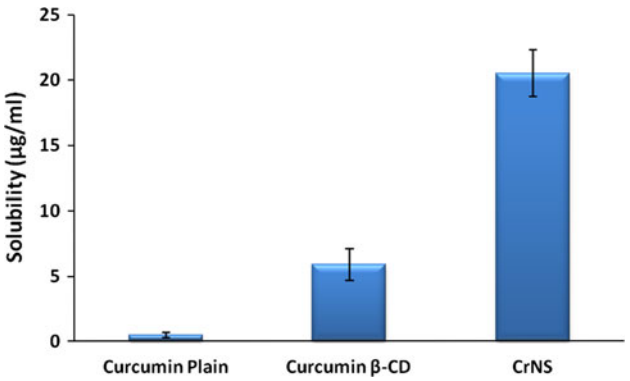

Fig. 2 Solubilization efficiency of CrNS, β-CD and plain curcumin in distilled water

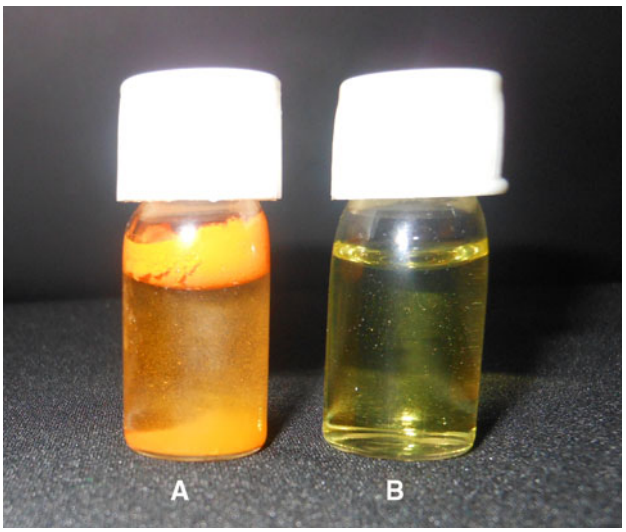

Fig. 3 Comparative photographs of solubility of curcumin plain (**a**) and with NS (**b**) in distilled water

inclusion complex with drug as well as entrapment in the matrix form. Figure 3 shows comparative photographs of solubility of pure curcumin and with NS in distilled water. The reason for more solubilization potential of CrNS than β-cyclodextrin was may be due to higher crosslinking nature of CrNS, which provides more quantity of drug to entrap in the matrix as well as cyclodextrin cavity. The primary purpose of increasing solubilization efficiency over parent cyclodextrin has been served by synthesizing the nanosponges.

Characterization of CrNS

Particle size and zeta potential measurement by dynamic light scattering (DLS)

The particle size analysis by DLS study showed that the CrNS have average particle size of 487.3 nm with a minimum polydispersibility index of 0.476. The particles were having unimodal particle size distribution with narrow range. The zeta potential of the CrNS formulation was found to be −27 mV and it is sufficiently high to form stable colloidal nanosuspension.

FT-IR

Figure 4 shows the comparison of FTIR spectra of pure curcumin, plain nanosponge and CrNS. Plain nanosponge showed a characteristic peak of carbonate bond at around 1720–1750 cm^{-1} which confirms the formation of cyclodextrin-based nanosponges. In addition, the other characteristics peak of NS were found at 2918 cm^{-1} due to the C–H stretching vibration, 1418 cm^{-1} due to C–H bending vibration and 1026 cm^{-1} due to C–O stretching vibration of primary alcohol. The main characteristics peaks of pure curcumin were found at around 3503 cm^{-1} due to the phenolic O–H stretching vibration, sharp absorption bands at 1605 cm^{-1} due stretching vibrations of benzene ring, 1508 cm^{-1} due to the C = O and C = C vibration, 1427 cm^{-1} due to olefinic C–H bending vibration, 1273 cm^{-1} due to aromatic C–O stretching vibration, and 1026/856 cm^{-1} due to C–O–C stretching vibrations of the curcumin were noticed. In FTIR spectra of CrNS, all the sharp peaks belonging to the NS have appeared and only few characteristics peak of curcumin are visible. Because of curcumin complexation with NS, the peaks related to the NS were shifted to higher/lower wave numbers, i.e., 2918–2914^{-1}; 1745–1749^{-1}; 1418–1409^{-1}; 1026–1029^{-1}. This data confirms definite interactions between curcumin and NS in CrNS formulation.

XRD

To study the physical nature of curcumin with in the cyclodextrin nanosponges, XRD pattern of pure curcumin, plain nanosponges and CrNS were investigated (Fig. 5). The characteristics peaks of curcumin demonstrated the high crystalline structure. However, there were no characteristics peak of pure curcumin were observed in CrNS. The absence of such crystalline peaks of curcumin in CrNS clearly indicates that curcumin encapsulated in NSs is in the disordered crystalline phase or amorphous or in the solid-state solubilized form in the polymeric matrix. When the drug is in amorphous or in disordered crystalline phase, the drug molecules can easily diffuse through the polymeric matrix, leading to a controlled release of the encapsulated drug [16]. When drug is in crystalline form inside the NSs, the drug release get hampers because, the large sized molecules unable to diffuse through the small pores of the NSs.

DSC

Figure 6 shows the comparison of DSC thermograms of pure curcumin, plain NS and CrNS. The physical state of drug in the polymeric matrix influences drug release. The results showed an endothermic peak of native curcumin approximately at 176 °C. This characteristic peak was not observed in the CrNS formulation, which indicates that curcumin is well complexed with NSs.

In vitro release studies

In vitro release study of curcumin from CrNS formulation showed sustained drug release (Fig. 7). A biphasic release pattern of curcumin was observed. The observed initial burst release was might be due to the curcumin, which is present in the matrix form but not associated with the

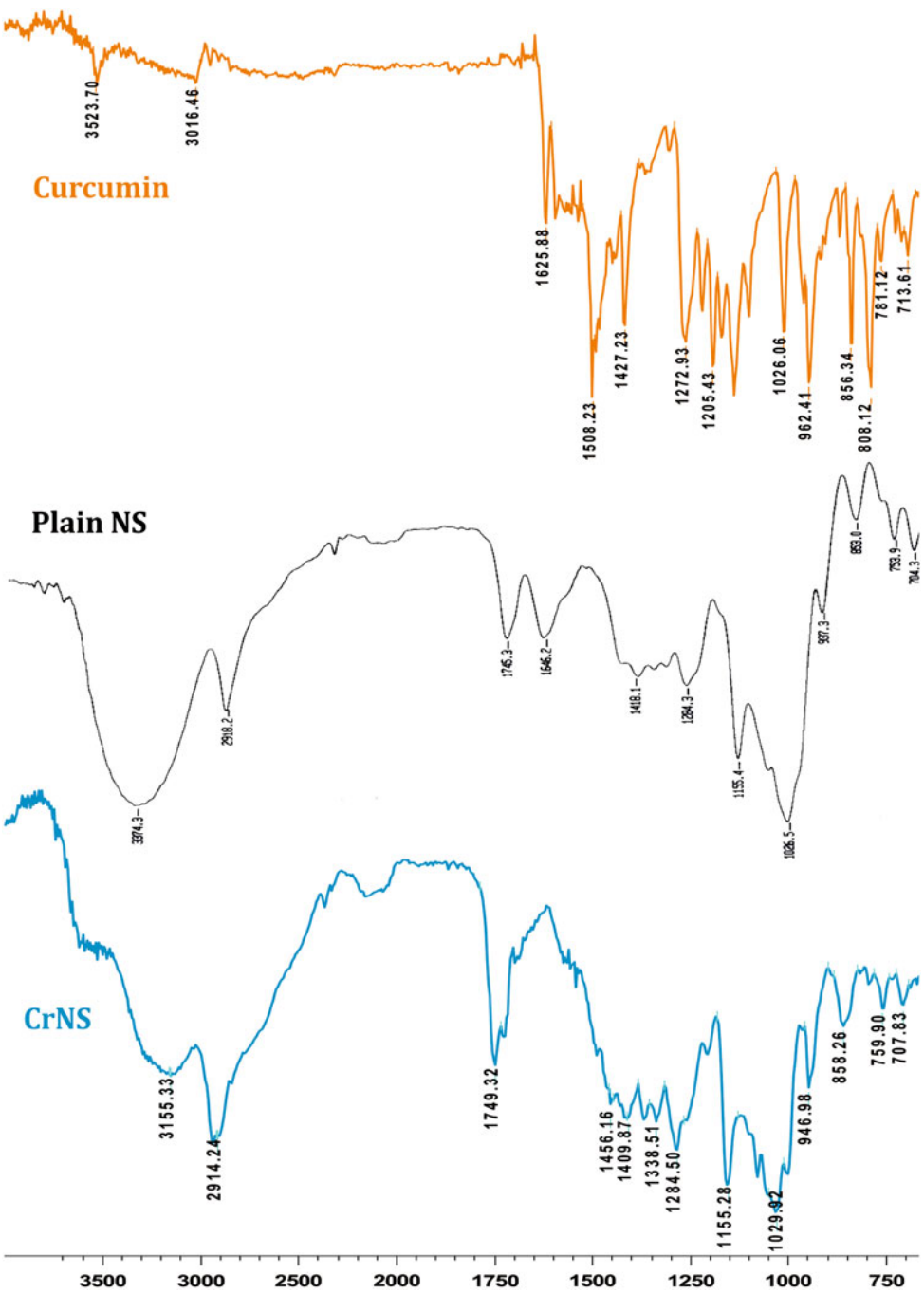

Fig. 4 FTIR spectra of curcumin, NS and CrNS

inclusion complex. Subsequently, sustained release of drug was observed due to the presence of curcumin in inclusion complex.

Hemolysis study

The non-toxicity of formulation for parenteral administration is mandatory requirement. Hence to evaluate the safety of the plain NS and CrNS, the haemolytic activity was determined. Figure 8 shows the hemolytic activity of NS and CrNS. It was found that aqueous suspension of NS was non-haemolytic up to the tested concentration of 2 mg/ml. CrNS loaded formulation also showed a good safety profile with erythrocytes. Optical microscopic study showed the intactness of the erythrocytes after incubation with NS and CrNS formulations thereby proving its safety (Fig. 9).

Cytotoxicity studies against MCF-7 Cells

Cytotoxicity of plain NS, curcumin and CrNS on MCF-7 cells were studied using MTT assay as shown in Fig. 10.

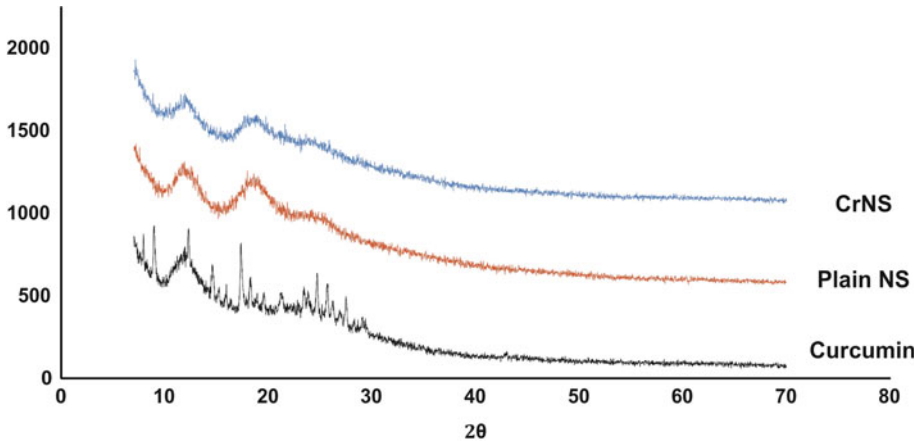

Fig. 5 X-ray diffractograms of curcumin, NS and CrNS

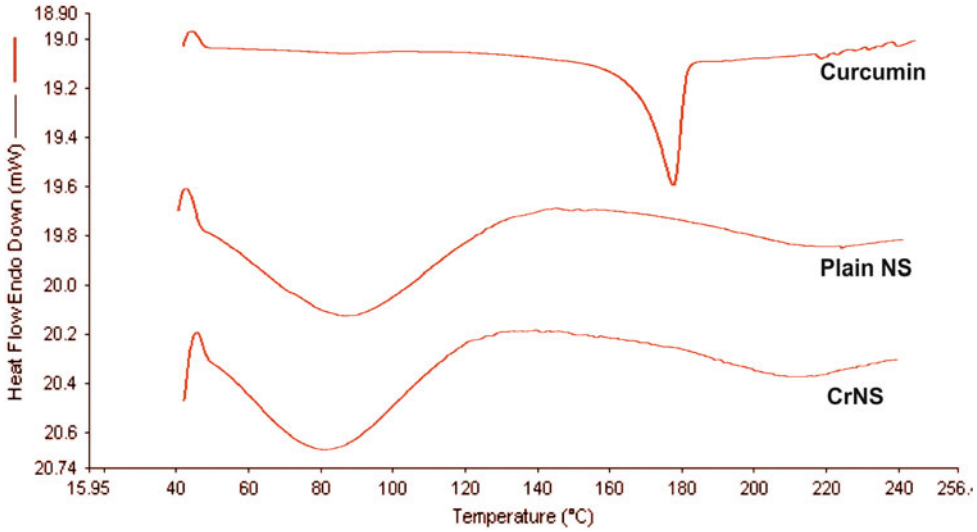

Fig. 6 DSC thermograms of curcumin, NS and CrNS

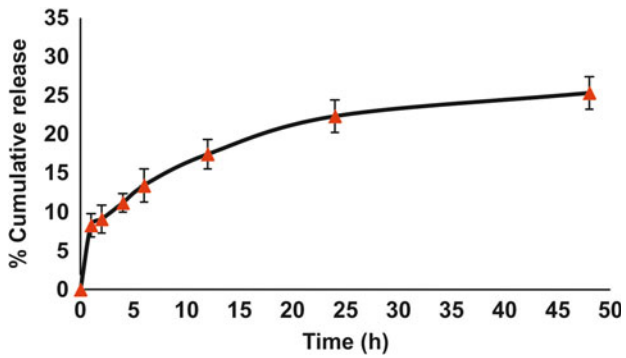

Fig. 7 In-vitro drug release of curcumin from CrNS

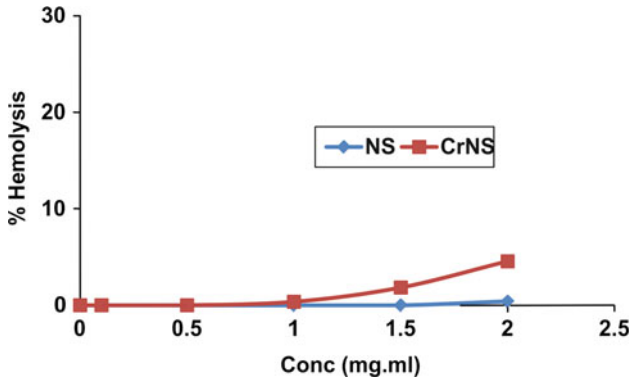

Fig. 8 In-vitro hemolysis study of NS and CrNS

Plain NS sample showed no toxicity profile whereas the CrNS sample showed only a slight reduction in cytotoxicity vs. plain curcumin. These similar cytotoxicities of curcumin and CrNS observed in the present study conclude that there is no change in molecular structure of curcumin in CrNS formulation.

Conclusions

In this study, curcumin loaded β-cyclodextrin based nanosponges were prepared by conventional inclusion complexation technique. The CrNS showed higher solubilization potential (45-fold) than plain curcumin. FTIR,

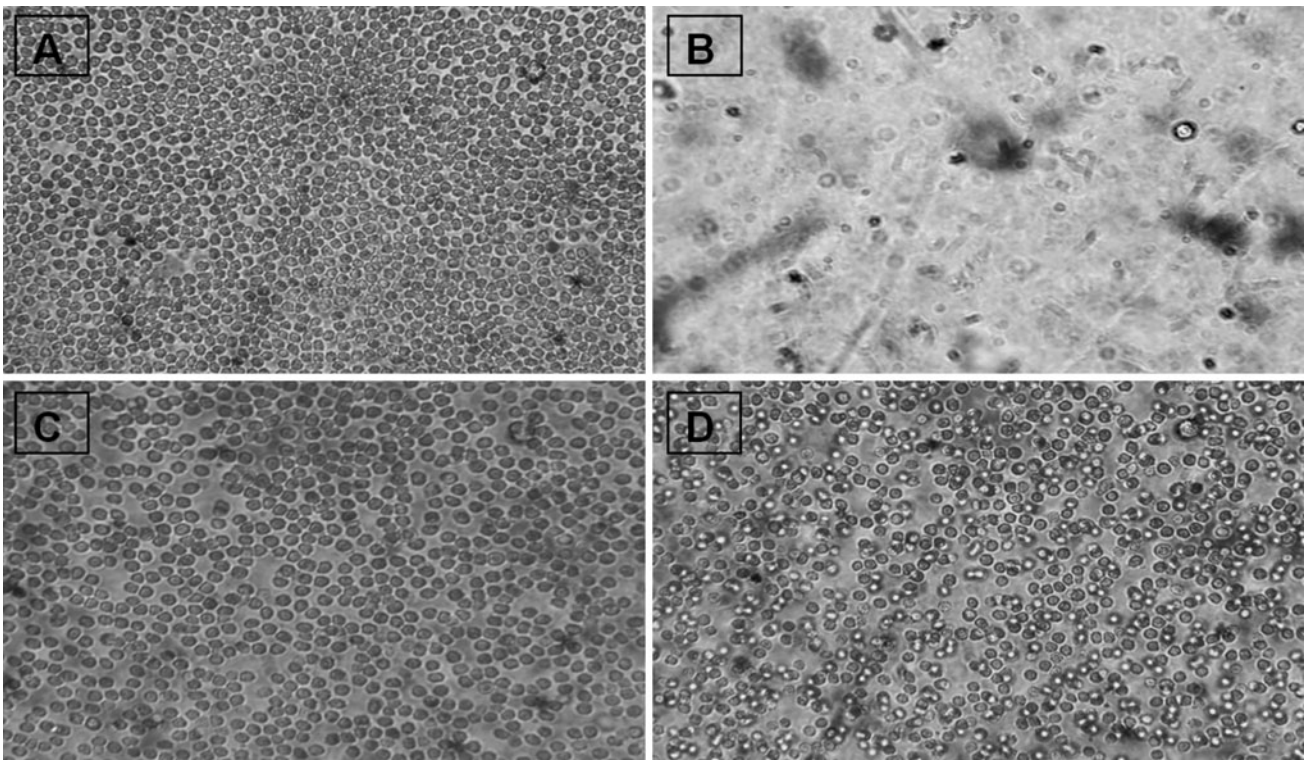

Fig. 9 Photomicrographs of erythrocytes treated with PBS (**a**), SLS (**b**), NS (**c**) and CrNS (**d**)

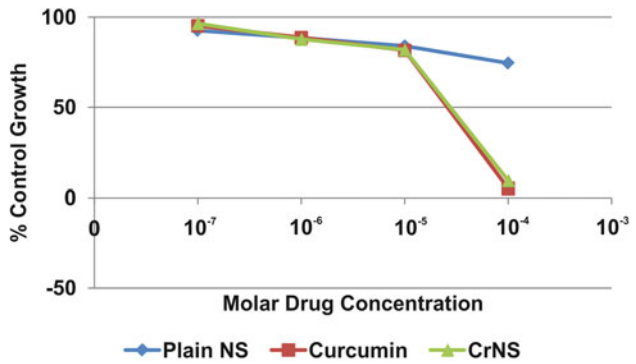

Fig. 10 In-vitro Cytotoxicity of curcumin, NS and CrNS on MCF-7 cells

DSC and XRD studies confirmed the formation of inclusion complex of curcumin with nanosponges. Hemolysis study confirmed the safety profile of plain NS as well as CrNS for parenteral delivery. The higher solubilization and prolonged release of curcumin from CrNS served the purpose of synthesizing nanosponges. Cytotoxicity study revealed that CrNS formulation is as effective as parent curcumin and there is no change in molecular structure of pure curcumin on complexation with NS. Hence, it can be concluded that cyclodextrin-based nanosponges of curcumin offers a potential drug delivery system for curcumin in cancer treatment.

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