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Cross-linked, cyclodextrin-based nanosponges for curcumin delivery - Physicochemical characterization, drug release, stability and cytotoxicity

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ABSTRACT

Curcumin (CUR) is a poorly water-soluble and photoreactive drug having potent anticancer activity. The purpose of the study is to fabricate cyclodextrin nanosponges, for the delivery of curcumin using two crosslinkers, diphenyl carbonate (DPC) and pyromellitic dianhydride (PMDA) and to study the influence on drug solubility, stability and cytotoxicity. Solubility studies were performed with assorted cyclodextrin to crosslinker ratio and with selected drug nanosponge stoichiometric complex. The drug-loaded nanosponges were characterized using DSC, FTIR, XRD and SEM. Pore size and surface topology of nanosponges confirmed the nanochannels available for the drug to get entrapped. *In vitro* drug release revealed 16 fold increase in dissolution by CUR-PMDA-CDNS compared to the pure drug against 5 fold increase by CUR-DPC-CDNS. The photostability of CUR was enhanced to 1.7 fold in PMDA crosslinked nanosponges. *In vitro* cytotoxicity study revealed increased toxicity of drug nanosponge complex to MCF-7 cells at a lower concentration. IC_{50} value of the drug (22.51 μg/ml) was reduced by 2.2 fold (10.44 μg/ml) by CUR-PMDA-CDNS as against 1.4 fold (15.92 μg/ml) decrease by CUR-DPC-CDNS. In conclusion, PMDA-CDNS was found to be a potential nanocarrier compared to DPC-CDNS for curcumin.

1. Introduction

Most of the drugs explored today are based on natural products. One such is curcumin, the major active phytoconstituent of *Curcuma longa*, reported for antibacterial, antifungal anti-inflammatory, anti-HIV, antidiabetic, antioxidant, anticarcinogenic and Alzheimer's disease. It is highly photoreactive and exhibits a solubility of less than 1 μg/ml. Although it has no adverse effect on the intake of up to 12 g/day, it exhibits poor absorption and rapid metabolism resulting in poor bioavailability [1]. Various studies to deliver curcumin in nanoplatform including nanocrystals, lipid-based nanospheres, polycationic liposome complex, polymer-based delivery systems using poly(lactic-co-glycolic acid), polycaprolactone, chitosan, polymer conjugates with polyethylene glycol and polycatocol were reported to improve the solubility, stability and pharmacokinetics [2,3].

Inclusion complexation with cyclodextrins and modified cyclodextrins were reported to improve the solubility and dissolution rate of poorly soluble drugs [4–7]. Cyclodextrins are cyclic oligosaccharides having the hydrophilic outer surface and lipophilic inner cavity. They form an inclusion complex with the drug wherein the lipophilic drug moiety binds to the inner cavity, while the hydrophilic outer surface helps in solubilizing the drug. Curcumin beta-cyclodextrin inclusion complex prepared using co-precipitation, freeze-drying and solvent evaporation method were reported to improve the solubility and stability of the drug [8]. Curcumin complexed with beta-cyclodextrin and hydroxypropyl-beta-cyclodextrin exhibited enhanced antiangiogenic activity with a significant increase in solubility compared to pure curcumin [9]. Curcumin beta-cyclodextrin complex formulated as nanomagnetoliposomes exhibited synergistic antioxidant potential of curcumin compared to conventional curcumin liposomes [10].

Cyclodextrin nanosponges are crosslinked cyclodextrin polymers having a three-dimensional network containing nanosized porous structure. These cyclodextrin-based nanosponges can be prepared by reacting the cyclodextrin with crosslinkers such as carbonyl-diimidazole, diphenyl carbonate, hexamethylene diisocyanate and pyromellitic anhydride [11,12]. They are nontoxic, porous and stable at high temperature [11]. They form both inclusion and non-inclusion complexes with the drugs rending higher interaction sites and higher drug encapsulation compared to plain cyclodextrin. These are explored as a promising drug delivery system to improve drug solubility, prevent drug degradation, to enhance the permeability and to control the drug release [12]. They are used as a nanocarrier for various drugs including camptothecin, paclitaxel, telmisartan, tamoxifen, quercetin, erlotinib, acetylsalicylic acid, meloxicam, nifedipine, ibuprofen, captopril, enalapril, and lansoprazole [13–24]. Cyclodextrin nanosponges prepared using dimethyl carbonate as a crosslinker and loaded with curcumin

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exhibited controlled release of the drug with no significant changes in cytotoxicity compared to the plain drug [25].

Cyclodextrin-based nanosponges prepared using diphenyl carbonate as a crosslinker, when complexed with camptothecin prolonged the drug release and increased the stability of the drug. Camptothecin loaded formulation showed increased cytotoxicity against HT-29 colon carcinoma cells [14]. The solubility of telmisartan was improved from 9.9 μg/ml to 45.92 μg/ml due to complexation with cyclodextrin nanosponges prepared with diphenyl carbonate as crosslinker having 1:4 cyclodextrin, crosslinker ratio [15]. The aqueous solubility of quercetin was improved up to 20-fold upon complexation with nanosponges. Cyclodextrin nanosponges fabricated for quercetin using diphenyl carbonate as a crosslinker, enhanced the DPPH radical scavenging activity of quercetin by 569 fold and decreased the photodegradation of the drug by encapsulating the drug in nanosponge matrix [17]. Nifedipine loaded nanosponges fabricated using diphenyl carbonate crosslinked cyclodextrins, increased the *in vitro* drug release with greater drug entrapment efficiency of 78.4% and increased the oral bioavailability by 3.63 fold increase in C_{max} against the pure drug [22].

Cyclodextrin nanosponges prepared using pyromellitic dianhydride as cross-linker in the molar ratio of 1:8, enhanced the solubility of meloxicam from 9.45 μg/ml to 36.61 μg/ml. These nanosponges loaded with the drug at 1:1 w/w proportion increased the anti-inflammatory and analgesic activity of meloxicam [20]. Pyromellitic dianhydride crosslinked cyclodextrin at the different molar ratio of 1:2,1:4, and 1:8, showed different *in vitro* release pattern for acetylsalicylic acid depending upon the degree of crosslinking. The crystallinity of acetylsalicylic acid was reduced upon complexation with nanosponges [19]. Lansoprazole nanosuspension prepared using pyromellitic dianhydride crosslinked cyclodextrin nanosponges with 1:4 crosslinker ratio and 1:1w/w drug nanosponge ratio, exhibited 40% increase in solubility of the drug [24].

The objective of the present work was to study the influence of crosslinker type used in nanosponge fabrication on curcumin by synthesising and characterizing beta-cyclodextrin based nanosponges of curcumin with two different type of crosslinker namely diphenyl carbonate (DPC-CDNS) and pyromellitic dianhydride (PMDA-CDNS) and to evaluate the influence on drug solubility, dissolution, photostability and *in vitro* cytotoxicity on MCF-7 cell line.

2. Materials and methods

2.1. Materials

Curcumin, beta-cyclodextrin, diphenyl carbonate (DPC) were purchased from Himedia. pyromellitic dianhydride (PMDA), triethylamine (TEA), dimethylsulphoxide (DMSO), dichloromethane, acetone were obtained from Sigma Aldrich. All other reagents and chemicals used were of analytical grade.

2.2. Preparation of cyclodextrin nanosponges

2.2.1. Synthesis of DPC crosslinked beta-cyclodextrin nanosponge (DPC-CDNS)

Cyclodextrin nanosponges using diphenyl carbonate (DPC) as crosslinker was prepared as previously reported [26]. 1.89 g of diphenyl carbonate (0.088 M) was melted at 90 °C and 5 g of cyclodextrin (0.0044 M) was added to it and left to react for 5 h (Scheme 1 in Fig. 1). The resultant product was washed with water and Soxhlet extracted with acetone for 24 h to remove unreacted ingredients. The purified DPC-CDNS were dried at 60 °C for 24 h. They were stored at ambient temperature until further use. Four types of DPC-CDNS were prepared using crosslinker at the different molar ratio with respect to cyclodextrin namely, 1:2, 1:4,1:6 and 1:8 (CD: DPC).

2.2.2. Synthesis of PMDA crosslinked beta-cyclodextrin nanosponge (PMDA-CDNS)

Cyclodextrin nanosponges using pyromellitic dianhydride (PMDA) as crosslinker was prepared as previously reported [11,27]. 6.1 g of cyclodextrin (0.0054 M) and 2.34 g (0.01073 M) of pyromellitic dianhydride (PMDA) were added to 20 ml of DMSO containing 0.7 ml of triethylamine and left to react at room temperature for 3 h [11]. Scheme 2 in Fig. 1 illustrates the synthesis of PMDA-CDNS. The resultant product was Soxhlet extracted with acetone for 24 h. The purified PMDA-CDNS were dried at 60 °C for 24 h and stored airtight at ambient temperature until further use. Four types of PMDA-CDNS were prepared with the molar ratio of 1:2, 1:4, 1:6 and 1:8(CD: PMDA).

2.3. Preparation of curcumin loaded CDNS

Curcumin was dissolved in dichloromethane. To this solution, nanosponges were added and triturated until the solvent was evaporated [28]. The obtained product was dried at 50 °C for 24 h. The dried formulation was stored airtight at ambient temperature. The dried formulation is abbreviated as CUR-DPC-CDNS and CUR-PMDA-CDNS prepared using DPC-CDNS and PMDA –CDNS respectively.

2.4. Solubility

The solubility of curcumin alone and in the presence of beta-CD, DPC-CDNS, and PMDA-CDNS of different crosslinker molar ratio was determined in water by saturation solubility method. Excess quantity of curcumin was added to a light protective container having 10 ml of water along with fixed quantities of nanosponges/cyclodextrin. The containers were shaken at 100 rpm in a rotary shaker (LARK) for 24 h, in dark at ambient temperature. The samples were centrifuged at 5000 rpm for 10 min, supernatant filtered through the 0.45 μm syringe filter and analysed for curcumin concentration by UV –VIS Spectrophotometer (Systronics) at 425 nm (n = 3). Phase solubility studies were performed on drug-loaded nanosponges using a fixed quantity of drug to nanosponge concentration by above saturation solubility method.

2.5. Characterization

2.5.1. Determination of particle size and zeta potential

Particle size, polydispersity index (PDI) and zeta potential were measured using Malvern Zetasizer Nano ZS 90 using water dispersion method.

2.5.2. Differential scanning calorimetry (DSC) and TGA

Thermal analysis was carried out using simultaneous thermal analyzer NETZSCH STA 449F3. The samples were scanned at the heating rate of 20 °C/min under nitrogen between 30C° and 350 °C.

2.5.3. Fourier transform infrared spectroscopy (FTIR)

Curcumin, cyclodextrin nanosponges and their formulation were subjected to FTIR studies between 4000 cm^{-1} to 650 cm^{-1} using Agilent Cary 630 FTIR spectrometer operated with Agilent Resolutions Pro software.

2.5.4. X-ray diffraction studies (XRD)

Samples were studied for Powder X-ray diffraction pattern using INEL EQUINOX 1000 X-Ray Diffractometer between 8 and 40° 2θ.

2.5.5. NMR

^{13}C NMR of DPC and PMDA nanosponge was examined using Bruker-NMR-300MHz using deuterated-DMSO.

2.5.6. Scanning electron microscopy (SEM)

Scanning electron microscope JEOL JSM-5610LV, 30 kV was