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Synthesis, characterisation and biomedical applications of curcumin conjugated chitosan microspheres

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ABSTRACT

Curcumin is a diaryl heptanoid of curcuminoids class obtained from *Curcuma longa*. It possesses various biological activities like anti-inflammatory, hypoglycemic, antioxidant, wound-healing, and antimicrobial activities. Chitosan is a biocompatible, biodegradable and non–toxic natural polymer which enhances the adhesive property of the skin. Chemical conjugation will leads to sustained release action and to enhance the bioavailability. This study aims to synthesis and characterize biocompatible curcumin conjugated chitosan microspheres for bio-medical applications. The Schiff base reaction was carried out for the preparation of curcumin conjugated chitosan by microwave method and it was characterised using FTIR and NMR. Curcumin conjugated chitosan microspheres (CCCMs) were prepared by wet milling solvent evaporation method. SEM analysis showed these CCCMs were 2–5 μm spherical particles. The antibacterial activities of the prepared CCCMs were studied against *Staphylococcus aureus* and *Escherichia coli*, the zone of inhibition was 28 mm and 23 mm respectively. Antioxidant activity of the prepared CCCMs was also studied by DPPH and H_2O_2 method it showed IC_{50} esteem value of 216 μg/ml and 228 μg/ml, and anti-inflammatory activity results showed that CCCMs having IC_{50} value of 45 μg/ml. The results conclude that the CCCMs having a good antibacterial, antioxidant and anti-inflammatory activities. This, the prepared CCCMs have potential application in preventing skin infections.

1. Introduction

Curcumin is a standout amongst the most flexible mixes acquired from *Curcuma longa*. Chemically, curcumin is (1,7-bis(4-hydroxy 3-methoxyphenyl)- 1, 6-heptadiene-3, 5-dione) a characteristic polyphenol, which expo the keto-enol tautomerism having a ubiquitous keto structure in acidic medium and stable enol frame in antacid medium [1]. Curcumin have a large continuum of pharmacological activity especially antimicrobial, antiinflammatory, antioxidant, wound healing and cytotoxic activity against cancer cell lines etc. Several studies proved that curcumin have a potent activity against multi drug resistant microbes [2–5]. The real hindrance in the therapeutic utilization of curcumin is its solubility and plasma level concentration. The chemical conjuncation with biopolymer will enhance the bioavilabitity and solubility of the lipophillic drugs [6–8]. Chitosan is a biocompatible, biodegradable polymer which improves adhesive property of the skin [9,10], chitosan has responsive amino and hydroxyl group whereas the amino gathering prompts to the likelihood of a few concoction modifications. The present study aimed to develop curcumin conjugated chitosan microspheres and its antioxidant, antimicrobial and anti-inflammatory activities were studied. The main advantages of this study was the synthesised curcumin derivatives consist of free phenolic hyrdroxyl group it is essential for pharmacological action especially antimicrobial, antiinflammatory and antioxidant activity [11]. The chemical conjugation of curcumin with chitosan may enhances its solubility and adhesive property.

2. Materials and methods

All the commercial reagents procured were of GR/AR grade and the reactions were carried out in dried borosil glass vessels. The synthesized compound was characterized using TLC, IR, NMR. Curcumin ($C_{21}H_{20}O_6$) has been purchased from Merck, Germany. TLC was performed on silica gel G F_{254} (Merck aluminium plate) as stationary phase and ethyl acetate: methanol: acetic acid (6:3:1) as a mobile phase. Iodine chamber was used for detection of the spots. All microwave syntheses were carried out in Catalyst™

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Systems CATA 2 R Scientific Microwave Synthesizer with ten different power settings. The IR spectra of the synthesized compounds were recorded on a Shimadzu-FTIR spectrometer (IR Affinity 1) using potassium bromide pellet technique. ^{1}H and ^{13}C NMR of the compounds were recorded using Bruker Spectrospin 400 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Chemical shift was expressed as delta values relative to TMS in units of ppm. NIH $3T_3$ (Mouse embryonic fibroblast) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trysin were obtained from Sigma Aldrich Co, St. Louis, USA; EDTA, glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai, India and DPPH, dimethyl sulfoxide (DMSO), propanol and other chemicals and medias were procured from E. Merck Ltd., Mumbai, India.

2.1. Synthesis of curcumin conjugated chitosan

Curcumin conjugated chitosan (CCC) was synthesised by imine formation (Schiff base reaction) method. Curcumin (184 mg of (1 mol)) was dissolved in 15 mL of ethanol and mixed with Chitosan (255 mg of 2 mol) in 0.1 N acetic acid [12]. The mixture was stirred for 30 min continuously at 800 rpm and subsequently placed in a microwave oven (90 W) for 10 min at 60 °C. TLC analysis was performed to ensure the completion of reaction. The reaction mixture was cooled at 4 °C, filtered and recrystallized with ethanol and characterised by spectral analysis. (Fig. 1).

2.2. Synthesis of curcumin conjugated chitosan microspheres (CCCMs)

CCCMs were prepared by wet milling solvent evaporating technique. 10 mg of CCC was dissolved with 5 mL of ethanol. From this 1 mL of CCC was added drop wise to deionised water (50 mL) with a flow rate of 0.1 mL/min under steady blending (800 rpm). Probe sonication was performed using a vibra cell sonicator with a working force of 130 W, and a recurrence of 20 KHz. Sonication was carried out for 20 min at room temperature until a turbid was acquired. It demonstrates that the curcumin conjugated chitosan microspheres are framed in milli-Q water. The prepared microspheres were lyophilized [13].

2.3. Physiochemical characterization of prepared CCCMs

2.3.1. Particle size analysis and stability studies

Measure appropriation of CCCMs was investigated by dynamic light scattering (DLS utilizing DLS-ZP/molecule sizer Nicomp^{TM} 380 ZLS). Size and surface morphology of the microspheres were further affirmed by SEM (JEOLJSM- 6490 LA). The CCCMs suspension was weakened with water and a drop was set on the metallic stub, air dried and examined the surface charge and particle size of CCCMs was measured using a zeta potential analyser [14].

2.3.2. FT-IR analysis

FT-IR spectra of curcumin and CCCMs were recorded by KBr pelleting method (1% w/w of item in KBr) Transmittance was measured from 4000 to 400 cm^{-1}. This analysis has been performed in order to test the degradation or chemical interaction of formulation.

2.3.3. In vitro *curcumin drug release*

In vitro curcumin release of CCCMs determined by resuspending 22 mg of CCCMs in 10 mL citrate buffer (pH 5) and incubated in 37 °C shaking incubator. At different time intervals, 3 mL of the supernatant solution have been taken and centrifuged at 12000 rpm for 10 min. The released Curcumin has been evaluated at 429 nm using UV/VIS spectrophotometer. These procedures have been continued until 100% drug release has been obtained. After withdrawn of sample has been replaced with 3 mL of the buffer solution. Data obtained from *in vitro* release studies were fitted to various kinetic models to find the mechanism of drug release. The kinetic models used were Zero order, First order, Higuchi model and Korsemeyer-Peppas Kinetic models [15].

2.4. Biological evaluations

2.4.1. In vitro *antimicrobial studies*

The antimicrobial action of curcumin and CCCMs were tested against *Staphylococus aureus* (*S. aureus*) and *Escherichia coli (E.coli)* by disc diffusion and serial dilution method. About 100 μL of overnight *S. aureus* and *E.coli* cultures were spread over Muller Hinton agar plates (Composition- 2 g of beef extract, 17 g of casein hydrolysate, 1.5 g starch, 17 g agar and make upto 1000 mL with water and adjust the pH for 7.2.), respectively. 0.5 cm filter paper discs containing 50, 100, 150, 200, 250 and 300 μg of CCCMs and curcumin were located on the agar media. Following which, the plates were incubated overnight in an vertical position at 37° C and the zone of inhibition was measured. [16,17]

The minimum inhibitory concentration (MIC) of CCCMs and curcumin was determined in accordance with the Clinical and Laboratory Standards Institute guidelines. Serial two-fold dilutions of CCCMs and curcumin were prepared in triplicate followed by addition of a standard *S. aureus* and *E. coli* suspension of $1–5 \times 10^5$ CFU/ml. After 24 h at 37 °C, the opacity were determined using a microplate reader (BioTek) at 578 nm. The MIC was determined as the lowest concentration of CCCMs and curcumin with where no visible bacterial growth was observed.

2.4.2. Estimation of antioxidant activity by DPPH and hydrogen peroxide method

The antioxidant action of the were measured by 2,2−diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging method [18]. Various concentrations of curcumin, CCCMs and standard ascorbic acid (100, 200, 300, 400 and 500 μg/ml) were taken and mixed with 5 mL of 0.01 mM methanolic solution of DPPH was added, shaken well and blend was kept at 37 °C for 30 min. Absorbance was measured at 517 nm using an UV/Vis spectrophotometer. Absorbance of DPPH was taken as control. The investigation was done in triplicate. The half maximal inhibitory concentration (IC_{50}) values calculated.

About 0.04 mM Hydrogen peroxide solution (0.6 mL) in phosphate buffer was mixed with different concentration of CCCMs. The above solution was stand for 10 min and the absorbance was measured at 230 nm in UV spectrophotometer, phosphate buffer used as the blank and the percentage scavenging activity of H_2O_2 were determined and the IC_{50} Values were calculated [19].

$$\text{Percentage scavenging effect} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

2.4.3. In vitro *anti −inflammatory studies*

Curcumin and CCCMs were screened for anti-inflammatory activity utilizing hindrance of albumin denaturation strategy The response blend comprises of 1 mL of 1% aqueous arrangement of bovine serum albumin division, 1 mL of PBS at pH 6.4 and 1 mL of different concentration of curcumin and CCCMs were arranged (25, 50, 100, 250, 500, 750 and 1000 μg/ml) utilizing DMSO and distilled water individually. 10 μg/ml diclofenac sodium has been used as the standard drug and distilled water as the control. Then the mixtures were incubated at (37 ± 2) °C for 15 min and then heated at 70 °C for 5 min. After bring down the temperature of the mixture, the absorbance was measured at 660 nm. The experiment was

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