A New Complex of Curcumin with Sulfobutylether-β-Cyclodextrin: Characterization Studies and *In Vitro* Evaluation of Cytotoxic and Antioxidant Activity on HepG-2 Cells

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ABSTRACT: Curcumin (CR) is a natural polyphenol with antioxidative, anti-inflammatory, and anticancer properties but its therapeutic potential is substantially hindered by the rather low-water solubility and bioavailability. Thus, in this work, a new soluble inclusion complex of CR with sulfobutylether-β-cyclodextrin (SBE-β-CD) was prepared in solution and at the solid state using different preparation techniques and characterized by Fourier transform infrared, nuclear magnetic resonance, differential scanning calorimetry, scanning electron microscopy, phase solubility studies, and Job's plot method. Results clearly indicate that CR reacts with SBE-β-CD to form a host–guest complex with an apparent formation constant of 1455 M^{-1}. Moreover, SBE-β-CD strongly increases water solubility of CR (from 0.56 to 102.78 μg/mL, at 25°C), and lyophilization method seems to be the best preparation technique to obtain the complex at the solid state. Finally, an *in vitro* test on a human hepatic cancer cell line (HepG-2) shows that complexation positively influences CR anticancer and antioxidant activity. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: curcumin; sulfobutylether-β-cyclodextrin; complexation; solubility; solid state; cell culture; cytotoxicity; antioxidant activity; UV-VIS spectroscopy

INTRODUCTION

Curcumin (CR), or 1,7bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, is a low-molecular-weight polyphenol compound derived from the rhizome of the plant *Curcuma longa*. It has been widely used as a yellow pigment, as spice in the food industry, and in traditional Ayurvedic and Chinese medicine since the second millennium. CR has a wide range of pharmacological applications because it possess potent anti-inflammatory, liver antifibrotic, antiangiogenic, antineoplastic, chemoprotective, and antioxidative (free radical scavenging activity) properties with low or no intrinsic toxicity.[1–3]

Despite all these extraordinary properties, CR suffers from low solubility in aqueous solution and undergoes rapid degradation at physiological pH[4] that results in low systemic bioavailability, poor pharmacokinetics, and greatly hampers its *in vivo* efficacy. To improve CR application as therapeutic agent, it would be necessary to improve its stability and solubility. In this regard, several efforts have already been made and different formulation techniques have been employed for CR, including nanoparticles,[5] liposomes,[6] self-microemulsifying drug delivery systems,[7] micelles,[8] phospholipid complexes,[9] and cyclodextrins (CDs)-based delivery systems.[10–14]

In particular, researchers have recently been studying the inclusion complex of CR with CDs, cyclic oligosaccharides that provide an interesting organic host system, as they have a hydrophobic inner cavity available to form noncovalent host–guest inclusion complexes with a wide variety of organic molecules of appropriate shape and size. This property has attracted considerable attention in the field of the encapsulation of molecules because some physicochemical properties of the guest, such as water solubility and stability, can be notably changed by its inclusion.[15]

Data reported in literature show an increase of CR water solubility and stability,[13,14] and an enhancement of the antioxidant, anti-inflammatory, and antiproliferative activities of the drug, particularly when it is complexed with natural CDs or with their hydroxypropyl (HP-α-CD, HP-β-CD, and HP-γ-CD) and methyl (CH_3-β-CD) derivatives.[10–12]

Complexation of CR with sulfobutylether-β-cyclodextrin (SBE-β-CD) has been less studied and to our knowledge there are very few studies on this complex in literature,[14,16,17] and no detailed characterization in solution and at the solid state has been performed. Thus, the aim of this work was to prepare a new CR/SBE-β-CD inclusion complex, to evaluate the influence of this on the water solubility of the drug and to perform a complete characterization of the same.

The complex was prepared in the solid state by freeze-drying, kneading, and coprecipitate methods and characterized by nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and FT-IR spectroscopy. In solution, solubility phase studies and Job's plot were performed in order to investigate the stoichiometry of the inclusion complex. Moreover, the influence of

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complexation with SBE-β-CD on anticancer and antioxidant activities of CR was tested on a human hepatic cancer cell line (HepG-2).

MATERIALS AND METHODS

Materials

Curcumin [1,7bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a product of Sigma–Aldrich Chemie® (Milan, Italy). SBE-β-CD (CAPTISOL®, average degree of substitution 6.2; average MW 2115) was kindly supplied by CyDex Pharmaceutical (Lenexa, Kansas City, Missouri). They were all employed without any further purification. The water used throughout the study was double-distilled and deionized, and filtered through 0.45-μm Sartorius Stedim polyamid filters (Biotech Gmbh, Wiesbaden,Germany). All other products and reagents were of analytical grade.

Methods

Quantitative Analysis of CR

Quantitative analysis of CR was performed through UV–visible (Vis) spectroscopy using a PerkinElmer double-beam UV–visible spectrophotometer Lambda Bio 20 (Milan, Italy), equipped with 10 mm path-length-matched quartz cells. Standard calibration curves were prepared at a wavelength of 421 nm using methanol as solvent and were linear (r^2 = 0.999) over the range of tested concentration (from 0.336 to 33.6 μg/mL).

Phase Solubility Analysis and Solubility Studies

Phase solubility studies were carried out according to Higuchi and Connors.[18] An amount of CR exceeding its solubility was added to unbuffered aqueous solutions of SBE-β-CD (0.0–8.32 $\times$ 10^{-2} M) in 5-mL capped vials and sealed to avoid changes because of evaporation. Then, the mixtures were vortexed for about 5 min and placed in the dark in a thermostated shaker bath for 5 days at 25 ± 0.1°C (Memmert WB 10, Labexchange, Germany). After equilibrium was reached, an aliquot of the aqueous phase of each mixture was transferred in a 5-mL glass syringe and filtered through a 0.22-μm cellulose acetate membrane filter (Millipore®, Milan, Italy). The filtrate was allowed to stand at the appropriate temperature until analyzed through UV–Vis spectroscopy at 421 nm. All measurements were repeated at least three times. The water solubility value (S_0) of CR was determined in the same way and resulted in good agreement with that reported in literature (≈0.60 μg/mL at 25°C).[19] The data obtained were used to determine the binding constant of the CR/SBE-β-CD inclusion complex, according to Higuchi and Connors equation. No degradation of CR was observed under experimental conditions.

Job's Plot Method

The stoichiometry of the inclusion complex CR/SBE-β-CD in aqueous solution was determined by a method known as the continuous variation method or Job's method.[20] Briefly, equimolar (2×10^{-5} M) methanol–water solutions (55/45, v/v) of CR and SBE-β-CD were mixed to a fixed volume by varying the molar ratio from 0.1 to 0.9, keeping the total molar concentration of each final solution constant. After stirring for 1 h, the absorbance (abs) of each solution was measured by UV–Vis spectroscopy at 421 nm and Δabs was determined as the difference between abs with and without SBE-β-CD. Then, Δabs × [CR] was plotted versus r, where:

$$r = \frac{[\mathrm{CR}]}{[\mathrm{CR}] + [\mathrm{SBE} - \beta - \mathrm{CD}]}$$

Preparation of the Inclusion Complex CR/SBE-β-CD in the Solid State

The CR/SBE-β-CD solid inclusion complex was prepared using three different techniques, that is, freeze-drying, kneading, and coevaporation, in order to evaluate the influence of the method of preparation on the characteristics of the final product. The physical mixture (PM) CR/SBE-β-CD (PM) in the mole ratio 1:1 was also prepared and characterized.

In detail, in preparing the solid freeze-dried inclusion complex (LIO–CX), an amount of CR exceeding its intrinsic solubility was added to a solution of SBE-β-CD (200 mg in 10 mL of water). The mixture was vigorously vortexed for about 5 min, stirred for 5 days in a dark place in order to prevent the photo degradation of CR, filtered through a 0.22-μm cellulose acetate membrane filter (Millipore®) and the clear filtrate was finally freeze dried (Lio 5P, Milan, Italy).

In the kneading procedure (KN–CX), SBE-β-CD and CR in the mole ratio 1:1 were first mixed in a mortar and then 500 μL of acetone were added in order to obtain a paste that was dried under vacuum until a dry powder was obtained.

For coevaporation technique (CO–CX), SBE-β-CD and CR were taken in the mole ratio 1:1 and individually dissolved in a water–acetone (1/2, v/v) mixture. Both solutions were mixed together and the solvent was evaporated under reduced pressure at 40°C.

All dry powders obtained were pulverized into a mortar to obtain a fine powder, sealed in an aluminum foil to protect them from light and stored in a desiccator until their further manipulation.

Determination of CR Content in CR/SBE-β-CD Solid Complexes

The amount of CR present in each CR/SBE-β-CD complex was determined by dissolving 5 mg of each sample in 5 mL of a water–methanol (1/1, v/v) mixture; samples were stirred for 2 h at room temperature and UV abs was measured at 421 nm after filtration through a 0.45-μm cellulose acetate membrane filter (Millipore®) and suitable dilution with the same water–methanol mixture.

Characterization Techniques

FT-IR Spectroscopy

Fourier transform infrared spectroscopy spectra of pure CR, PM, and CR/SBE-β-CD solid complexes were obtained on a Perkin-Elmer 1600 FTIR spectrometer using KBr discs (2 mg sample in 200 mg of KBr). The scanning range was 400–4000 cm^{-1} and the resolution was 1 cm^{-1}. Four scans were performed for each IR spectrum and calibration of the instrument was repeated periodically.

DSC Analysis

Differential scanning calorimetry measurements were carried out in a Mettler Toledo DSC 822e Star^e 202 system (Mettler Toledo, Greifensee, Switzerland) equipped with a thermal analysis automatic program. Aliquots of about 5 mg of each