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Curcumin complexation with cyclodextrins by the autoclave process: Method development and characterization of complex formation

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ABSTRACT

One approach to enhance curcumin (CUR) aqueous solubility is to use cyclodextrins (CDs) to form inclusion complexes where CUR is encapsulated as a guest molecule within the internal cavity of the water-soluble CD. Several methods have been reported for the complexation of CUR with CDs. Limited information, however, is available on the use of the autoclave process (AU) in complex formation. The aims of this work were therefore to (1) investigate and evaluate the AU cycle as a complex formation method to enhance CUR solubility; (2) compare the efficacy of the AU process with the freeze-drying (FD) and evaporation (EV) processes in complex formation; and (3) confirm CUR stability by characterizing CUR:CD complexes by NMR, Raman spectroscopy, DSC, and XRD. Significant differences were found in the saturation solubility of CUR from its complexes with CD when prepared by the three complexation methods. The AU yielded a complex with expected chemical and physical fingerprints for a CUR:CD inclusion complex that maintained the chemical integrity and stability of CUR and provided the highest solubility of CUR in water. Physical and chemical characterizations of the AU complexes confirmed the encapsulated of CUR inside the CD cavity and the transformation of the crystalline CUR:CD inclusion complex to an amorphous form. It was concluded that the autoclave process with its short processing time could be used as an alternate and efficient methods for drug:CD complexation.

1. Introduction

Combining curcumin (CUR) with the current treatment protocols for the Head and neck squamous cell carcinoma (HNSCC) was reported to alleviate inflammatory responses and reduce harmful side effects for patients (Kim et al., 2011). Unfortunately, due to its poor aqueous solubility (Prasad et al., 2014) clinical studies have required the administration of high doses of CUR to achieve a suppressive effect on IKKβ kinase activity and on the expression of inflammatory cytokines (Kanai et al., 2011). A common approach to enhance CUR solubility in water is to form cyclodextrin (CD) inclusion complexes where CUR is encapsulated as a guest molecule within the internal cavity of the water-soluble host (Chi et al., 2015).

Several techniques have been reported for the preparation of the CUR:CD inclusion complexes, among which the most commonly used are co-precipitation, freeze-drying (FD), and solvent evaporation (EV) methods (Jahed et al., 2014; Marcolino et al., 2011; Mohan et al., 2012; Rocks et al., 2012). The choice of the complexation method and its effect on complexion efficiency has been discussed by Moyano et al. (1995). Although few studies have reported on the thermal stability of CDs when using heat for complex formation (Badilli et al., 2014; Kurkov et al., 2010), complexation with CDs by the autoclave process (AU) is not widely reported. Traditionally autoclaves are used to provide a physical method for disinfection and sterilization by controlling temperature, pressure, and time. Furthermore, to our knowledge, very little information is available on the solution and solid-sate characteristics of inclusion complexes that have been prepared by the AU process (Badilli et al., 2014; Wolzen and Pipkin, 2015; Kurkov et al., 2010). Therefore, the aims of this work were (a) to investigate the autoclave process as a method for complex-formation to enhance CUR solubility; (b) to examine the thermostability of CUR and its complexes under the extreme autoclaving conditions by nuclear magnetic resonance (^{1}H-NMR) and Raman spectroscopy, differential scanning calorimetry (DSC), and X-ray Powder diffraction (XRD); (c) to compare the efficacy of the AU process with FD and EV as alternate complexation techniques; and (d) to contrast the efficacy of Kleptose® HPB (Hydroxypropyl-β-cyclodextrin) and

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CAPTISOL® SBEβCD (sulfobutylether-β-cyclodextrin) in the preparation of CUR:CD inclusion complexes

2. Materials and methods

2.1. Materials

Curcumin (Curcumin C3 Complex) was a gift sample from Sabinsa Corp. (East Windsor, NJ). Kleptose® HPB (Hydroxypropyl-β-cyclodextrin) was provided by Roquette Pharma. (Keokuk, IA). CAPTISOL® SBEβCD (sulfobutylether-β-cyclodextrin) was provided by CyDex Pharmaceuticals. (Lawrence, KS). Water used throughout the study was double distilled. All other products and reagents were of analytical grade.

2.2. Quantitative analysis of curcumin

CUR was quantified by HPLC analysis using a SpectraSystem HPLC system equipped with a UV/Visible variable wavelength detector (Thermo Electron Corp., San Jose, CA). A previously developed HPLC method was used with minor adjustments (Heath et al., 2003). Briefly, CUR was dissolved in the mobile phase, which consisted of 40% v/v acetonitrile, 23% v/v methanol, 36% v/v water, and 1% v/v acetic acid. Samples (20 μl) were then injected into Kinetex™ C18 (5 μm, 250 × 4.6 mm) analytical column (Phenomenex Inc., Torrance, CA). The flow rate of the mobile phase was 1 ml/min and the analytes were detected at 262 λ_{max}. Data acquisition and analysis was performed using ChromQuest™ chromatography software version 4.2 (Thermo Electron Corp., San Jose, CA).

2.3. Phase Solubility studies

The phase solubility studies of the CUR and CD molecules were carried out using the Higuchi and Connors method (Higuchi and Connors, 1965). Briefly, different concentrations of Kleptose® HPB and CAPTISOL® SBEβCD (0, 100, 200, 300, 400 and 500 mM) were prepared in double distilled water in glass capped vials. To each vial 20 mg CUR was added to attain saturation. These vials were then sealed and placed on a horizontal shaker at 25 ± 2 °C until equilibrium was reached. After 72 h samples were centrifuged at 1500 rpm for 7 min to remove the undissolved CUR. The supernatant was then filtered through a 0.45 μm Supor® hydrophilic polyethersulfone membrane (Pall Corporation., Ann Arbor, MI). The amount of dissolved CUR was quantified by HPLC. All measurements were performed in triplicates. Samples were protected from light throughout the experiment and analysis. The phase solubility diagram was constructed by plotting the concentration of dissolved CUR *vs.* the concentration of CD. Stability constant for A_L-type diagram (K1:1) was calculated to compare the affinity of CUR to the cyclodextrin derivatives using the following equation Eq. (1):

$$K1:1 = \frac{slope}{Sinter\,(1 - slope)} \quad (1)$$

S_{Inter} (the intercept) is the equilibrium solubility of CUR in the absence of CD. The value for the slope was obtained from the linear portion of the diagram as described by Higuchi and Connors (Higuchi and Connors, 1965).

Complexion efficiency (CE) was also calculated as a more accurate parameter than the stability constant (K_s) in formulation work because it is less sensitive to error arising when estimating intrinsic solubility (S_{Inter}) from the intercept (Brewster and Loftsson, 2007). CE was calculated using an intrinsic solubility (S_0) of 0.5 μg/ml, as reported in the literature (Cutrignelli et al., 2014), and from the slope of the phase solubility diagram according to Eq. (2).

$$CE = S_0 Ks = \frac{slope}{S_0\,(1 - slope)} \quad (2)$$

The CUR:CD molar ratio in aqueous CD solutions saturated with CUR was calculated using the following equation Eq. (3) (Loftsson et al., 2005):

$$Molar\ ratio = 1 : (CE + 1)/CE \quad (3)$$

2.4. Preparation of CUR:CD inclusion complexes

2.4.1. Solvent evaporation method

Inclusion complexes were prepared by the solvent evaporation method as reported by Mangolim et al. with minor adjustments (Mangolim et al., 2014). Briefly, 20 mg of CUR in acetone was added to an aqueous solution containing 600 mg of CD in 1.5 ml of water. Mixtures were allowed to stir in open vials at room temperature. After overnight stirring, mixtures were centrifuged for 5 min and the supernatant was filtered through a 0.45 μm Supor® hydrophilic polyethersulfone membrane filters to remove undissolved CUR. The saturation solubility of CUR in the filtered solutions was measured by HPLC. Clear CUR solutions were then freeze-dried using a FreeZone 6 Liter Console Freeze Dry System (Labconco., Kansan City, MO) to obtain the dry CUR:CD complex.

2.4.2. Freeze-drying method

Inclusion complexes were prepared by the Freeze-drying method as reported elsewhere (Mohan et al., 2012). Similar to the solvent evaporation method, 20 mg of powdered CUR was added to glass vials containing 600 mg of CD dissolved in 1.5 ml water. Vials were protected from light and allowed to shake for 5 days at 180 rpm. At the conclusion of the experiment, mixtures were centrifuged for 5 min and the supernatant was filtered through a 0.45 μm Supor® hydrophilic polyethersulfone membrane filters to remove undissolved CUR. The saturation solubility of CUR in the filtered solutions was measured by HPLC. Clear CUR solutions were then freeze-dried to obtain the dry CUR:CD complex.

2.4.3. Autoclaving method

Heating, or enthalpy driven devices such as an autoclave or an ultrasonic bath have been used for complexation (Hassan et al., 1990; Kurien et al., 2007; Loftsson and Hreinsdottir, 2006). Autoclaving cycle starts by charging the chamber with steam, which is held until it reaches set parameters of pressure, temperature, and time. During autoclaving samples are exposed to a wet and dry phase. In this study the autoclave cycle was 90 min-long of which the chamber is heated during the first 15 min to reach 121 °C. The samples then undergo a 60 min sterilization cycle followed by 15 min of cooling to reach room temperature. The sterilization cycle consists of a 30 min steaming phase followed by a 30 min drying phase.

To fully evaluate the AU for complex formation, samples were prepared similarly to the other methods where 20 mg of powdered CUR was added to 5 ml glass serum vials containing 600 mg of CD dissolved in 1.5 ml of water. Vials were crimped with rubber stoppers and aluminum seals, and then sonicated for 2 min in a water bath at room temperature to de-agglomerate any particles and to attain a homogenous suspension. Immediately thereafter, vials were autoclaved as described above using a Tuttanuer Autoclave-Steam sterilizer (model 2540E). At the end of the autoclave cycle CUR suspensions were centrifuged for 5 min and the supernatant was filtered through a 0.45 μm Supor® hydrophilic polyethersulfone membrane filters to remove undissolved CUR. The saturation solubility of CUR in the filtered solutions was measured by HPLC.