European Journal of Pharmaceutical Sciences 80 (2015) 26–32

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Characterization and antimicrobial phototoxicity of curcumin dissolved in natural deep eutectic solvents

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ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form 7 September 2015
Accepted 23 September 2015
Available online 26 September 2015

Chemical compounds studied in this article:
Curcumin (PubChem CID: 969516)
Glucose (PubChem CID: 107526)
Sucrose (PubChem CID: 5988)
Maleic acid (PubChem CID: 444266)
Choline chloride (PubChem CID: 6209)

Keywords:
Curcumin
Diketo conformer
Natural deep eutectic solvents
Solubility
Antimicrobial photodynamic therapy
Escherichia coli

ABSTRACT

Natural deep eutectic solvents (NADES) are a novel class of eutectics which show a unique potential as solubilizer of water insoluble compounds. The purpose of the current study was to evaluate the potential of NADES as a solvent for the hydrophobic photosensitizer curcumin for use in antimicrobial photodynamic therapy (aPDT). Two of the seventeen NADES initially prepared (i.e., NADES GS and MC3) solubilized >0.05 mg/ml curcumin and were further characterized. The hydrolytic stability (i.e., $t_{1/2}$) of curcumin in NADES was comparable to or up to 2–10 times higher than previously reported results in cyclodextrins and up to >1300 times higher than results reported in buffer at pH 8. The photolytic stability increased by a factor 5.6–10 in GS compared to the most photostable cyclodextrin and surfactant preparations reported previously. This NADES seemed to lock curcumin in its colorless diketo conformer, resulting in higher photostability than in ethanol and in the NADES MC3. The curcumin-NADES preparations dissolved rapidly in aqueous media and formed supersaturated solutions of curcumin. Precipitation of curcumin was observed after ≤1 h depending on the dilution factor (pH < 8). The NADES MC3 containing curcumin photoinactivated *Escherichia coli* at a lower curcumin concentration (1.25 μM) than in any previously investigated preparations of curcumin. The ability of NADES to lock curcumin within one specific molecular conformation and also to potentiate the phototoxic effect of this photosensitizer emphasizes the unique properties of the NADES as a solvent.

1. Introduction

Natural deep eutectic solvents (NADES) form a third class of liquids, different from water and lipids, which is present in all living cells (Choi et al., 2011). NADES consist of two or more solid crystalline compounds that, when mixed together at specific molar ratios, form a eutectic solvent with unique properties. Deviation from the specific molar ratios either results in rapidly forming solid precipitation or no eutectic solvent at all. The components are naturally occurring compounds, mainly plant metabolites, such as certain sugars, simple organic acids and amino acids (Dai et al., 2013). The composition of the eutectic solvent differs from commonly used ionic liquids (IL) and deep eutectic solvents (DES) which in most cases are prepared by mixing a quaternary ammonium salt with metal salts, although DES also can be obtained from non-ionic species (Zhang et al., 2012). NADES are simple to make, cheap and do not pose a threat to the environment. In a pharmaceutical context these liquids may be applied e.g., to enhance the aqueous solubility of poorly soluble drugs, to stabilize dissolved biomolecules, or in drug delivery (Choi et al., 2011; Dai et al., 2013).

Curcumin is a hydrophobic polyphenol present in the rhizomes of *Curcuma longa* L. It is used as a photosensitizer (PS) in experimental antimicrobial photodynamic therapy (aPDT) (Tønnesen et al., 1987; Dahl et al., 1989; Haukvik et al., 2009). aPDT combines a PS and optical radiation in the presence of oxygen to produce reactive oxygen species (ROS), free radicals and/or other reactive photoproducts (Tegos and Hamblin, 2006). The photoproducts may cause damage to bacterial cell structures resulting in bacterial inactivation. aPDT is mainly applied topically, e.g., in the treatment of bacterial infections in skin and soft tissue, and in oral infections. Bacterial resistance to aPDT is less likely as ROS and free radicals have a broad spectrum of action (Hamblin and Hasan, 2004; Maisch, 2015). aPDT is therefore a promising treatment modality in the battle against antibiotic resistance.

The clinical application of curcumin is complicated by its low solubility in water at acidic (above pH 1) and physiological pH, its rapid hydrolysis under alkaline conditions and its susceptibility to photochemical

Abbreviations: aPDT, antimicrobial photodynamic therapy; DES, deep eutectic solvents; GS, glucose/sucrose (1:1); IL, ionic liquid; NADES, natural deep eutectic solvents; NR, Nile Red; MC3, maleic acid/choline chloride (1:3); PS, photosensitizer; ROS, reactive oxygen species.

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http://dx.doi.org/10.1016/j.ejps.2015.09.013
0928-0987/

degradation (Tønnesen and Karlsen, 1985a,b; Tønnesen et al., 1986). The solubility of curcumin in water is approximately or less than 3×10^{-8} M (Tønnesen et al., 2002; Esmaili et al., 2011). These issues have been addressed through drug encapsulation, complexation and interactions with nanoparticles such as cyclodextrins (CDs), micelles and with polymers (Tønnesen et al., 2002; Tønnesen, 2002, 2006; Tang et al., 2002; Tomren et al., 2007; Meng et al., 2015; Wegiel et al., 2014; Chaurasia et al., 2015; Wikene et al., 2014). Several studies have specifically been carried out with the aim to prepare and characterize curcumin-formulations for aPDT (Wikene et al., 2014; Haukvik et al., 2010; Hegge et al., 2011, 2012a,b, 2013; Vukicevic et al., 2014). Close contact between the PS and the target bacteria is important to achieve high phototoxicity. Free curcumin molecules in solution interact better with the bacterial cell surface than molecules encapsulated in a carrier (e.g., CD, micelle) which would prolong its release time from the vehicle (Hegge et al., 2012b). We have previously demonstrated that supersaturated aqueous solutions of curcumin combined with blue light induced high phototoxicity (>6 log reductions) towards Gram-positive and Gram-negative bacteria (Wikene et al., 2014; Hegge et al., 2012b).

The aim of the present study was to create a hydrophilic eutectic solvent (i.e., NADES) that could dissolve, stabilize and maintain the phototoxic potential of the hydrophobic photosensitizer curcumin.

2. Materials and methods

2.1. Preparation of NADES

The two or three components of each deep eutectic solvent investigated (Table 1) were dissolved in warm water (~50 °C) and evaporated at 45 °C for 10 min with a rotatory evaporator. The various NADES and the molar ratios of the components of each NADES were selected based on Choi et al. (2011) and Dai et al. (2013). The liquid obtained after evaporation was transferred to polypropylene tubes with a tight cap. The water content of each NADES was determined by Karl Fischer titration (C20 Coulometric KF Titrator, Mettler Toledo Inc., Schwerenbach, Switzerland). The pH was measured in the undiluted NADES that were selected for further studies, and after dilution of these NADES 1:10 and 1:50 with MilliQ water, 1:10 and 1:100 with phosphate buffered saline without Ca^{2+} and Mg^{2+} (PBS, Lonza, Verviers, Belgium) and 1:10 in 1/15 M phosphate buffer pH 8 (composed of 3.7% 1/15 M monopotassium phosphate and 96.3% 1/15 M disodium phosphate) using a pH 526 MultiCal® pH meter (WTW GmbH, Weilheim, Germany).

Table 1
Constituents of the natural deep eutectic solvents investigated.

Component 1	Component 2	Component 3	Molar ratio	Acronym
Sucrose	D-(−)-Fructose	D-(+)-glucose	1:1:1	SFG
Citric acid	D-(+)-Glucose		2:1	
Citric acid	Sucrose		1:1	CS
D-(+)-Glucose	Sucrose		1:1	GS
Sucrose	D-(−)-Fructose		1:1	
D-(+)-Glucose	D-(−)-Fructose		1:1	GF
DL-Malic acid	D-(+)-Glucose		1:1	
DL-Malic acid	D-(−)-Fructose		1:1	
DL-Malic acid	Sucrose		1:1	
Citric acid	D-(+)-Trehalose		2:1	
Maleic acid	D-(+)-Glucose		4:1	
Maleic acid	Sucrose		1:1	
Citric acid	Choline chloride		1:2	
Citric acid	Choline chloride		1:3	
DL-Malic acid	Choline chloride		1:1	
DL-Malic acid	Choline chloride		1:3	
Maleic acid	Choline chloride		1:1	
Maleic acid	Choline chloride		1:3	MC3
Choline chloride	Glycerol		1:2	

2.2. Solubility test

The NADES were mixed with an excess amount of curcumin (Fig. 1) synthesized by the method of Pabon (1964). The dry powder was given 1 h to sink into the viscous liquid before the tubes were agitated horizontally on an Edmund Bühler shaker (at 250 rpm) protected from light at ~22 °C for 24 h. The tubes were centrifuged (6918 *g*, 75 min, 22 °C; Centrifuge 5430R, Eppendorf AG, Hamburg, Germany) before visual evaluation of the solutions. The NADES in which curcumin was solubilized to form clear, colored (yellow) samples, or solubilized to form colorless samples without the presence of undissolved curcumin particles, were selected for further characterization. Based on the visual evaluation, five NADES were selected for quantitative analysis and measurement of pH, water content and polarity, namely SFG, CS, GS, GF and MC3 (cf. Table 1). For quantification of curcumin were samples withdrawn from these preparations and diluted at least 1:1 (or up to 500 times) with methanol followed by 1:1 dilution with the mobile phase prior to quantification by HPLC at detection wavelength 420 nm. A reversed-phase HPLC analysis was conducted with isocratic elution with a mobile phase consisting of 0.5% (w/v) citric acid buffer (pH 3.0 adjusted with KOH using a pH 526 MultiCal® pH meter) and methanol (38:62). A C_{18} column (Nova-pak®, Waters Corporation, Milford, Massachusetts, USA) was used. The retention time of curcumin at 0.8 ml/min flow was approximately 12 min under the given experimental conditions. The lower detection limit of the system (i.e., 3 times the noise level) was 3×10^{-8} M. All quantitative experiments were performed in triplicate unless otherwise stated. The two NADES with the highest curcumin content, GS and MC3 (Table 1), were studied further.

2.3. Absorption spectroscopy

Absorption spectra were recorded between 190 and 700 nm on a Shimadzu UV-2101 PC UV–Vis scanning spectrophotometer (Shimadzu Corp., Kyoto, Japan) using a quartz cuvette with a path length of 1 cm. Absorption spectra were recorded in triplicate for samples of 0.011 mg/ml curcumin dissolved in GS and MC3. The absorbance was also measured in the curcumin-MC3 samples diluted 1:10 or 1:50 in MilliQ water, 1:10 or 1:100 in PBS, 1:10 in 1/15 M phosphate buffer pH 8 or after dilution with ethanol to a final concentration of 10–60% (v/v) ethanol. Further, absorption spectra were recorded for curcumin-GS samples after the addition or ethanol to a final concentration of

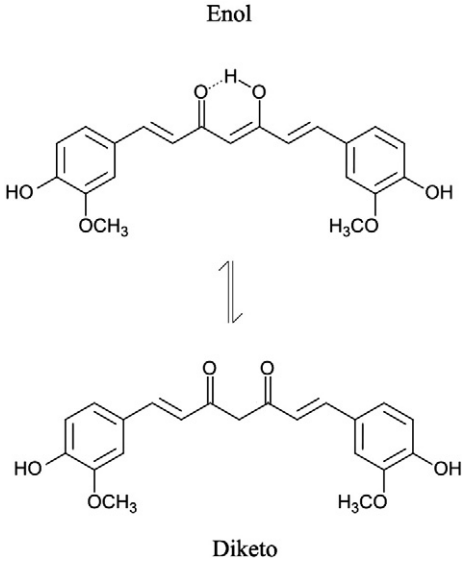

Fig. 1. Curcumin in its closed enol and diketo form.