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The effect of the water on the curcumin tautomerism: A quantitative approach

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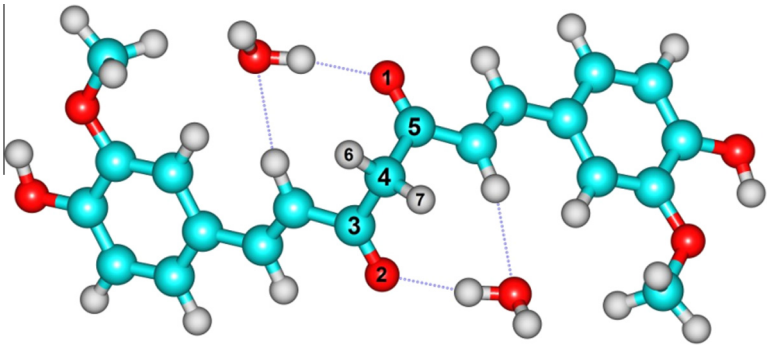
HIGHLIGHTS

- First time, when curcumin tautomeric mixture is analyzed quantitatively.
- Molar fractions of curcumin tautomers and their pure spectra have been determined.
- It has been shown that in ethanol the enol–keto tautomer only is presented.
- The addition of water leads to appearance and stabilization of the diketo tautomer.
- The observed shift in the equilibrium is clarified by quantum chemical calculations.

GRAPHICAL ABSTRACT



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ABSTRACT

The tautomerism of curcumin has been investigated in ethanol/water binary mixtures by using UV–Vis spectroscopy and advanced quantum-chemical calculations. The spectral changes were processed by using advanced chemometric procedure, based on resolution of overlapping bands technique. As a result, molar fractions of the tautomers and their individual spectra have been estimated. It has been shown that in ethanol the enol–keto tautomer only is presented. The addition of water leads to appearance of a new spectral band, which was assigned to the diketo tautomeric form. The results show that in 90% water/10% ethanol the diketo form is dominating. The observed shift in the equilibrium is explained by the quantum chemical calculations, which show that water molecules stabilize diketo tautomer through formation of stable complexes. To our best knowledge we report for the first time quantitative data for the tautomerism of curcumin and the effect of the water.

Introduction

Turmeric, derived from *Curcuma longa* plant, is a gold-colored spice commonly used in Asia for health care, food preservation and yellow dye for textiles. During the centuries numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders [1]. Extensive research within the last half century has proven that most of these activities, once associated with turmeric, are due to curcumin (**1**), which gives its yellow color. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer activities and thus has a potential against various malignant

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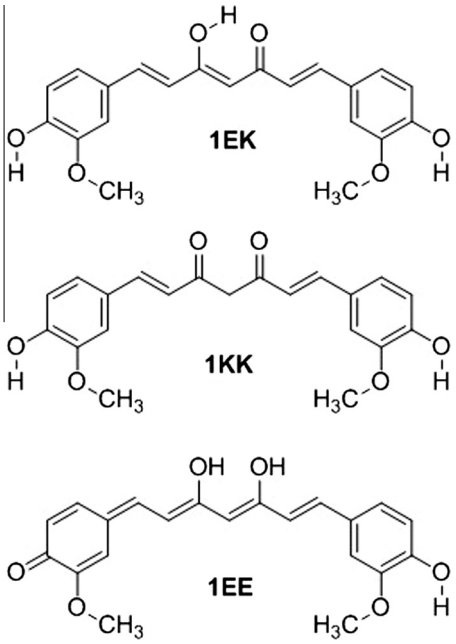
diseases, diabetes, allergies, arthritis, Alzheimer's disease, and other chronic illnesses [1–3]. In the light of these promising properties, broad spectrum of activities, and of course – natural origin, several questions need to be answered in order to improve its systematic applicability. These pertain to the solubility and stability of curcumin, its optimum dose, pharmacokinetics, mechanism of action of curcumin for a given disease, bioavailability profile, and intricacies of prevention and cure of an identified disease [1].

Curcumin is a tautomeric compound and it is naturally that the tautomerism influences its activities [4]. As shown in Scheme 1, it potentially exists as three main tautomers, each of them with a number of isomers [5–8]. There is substantial number of theoretical studies, showing the strong predominance of the enol–keto form (**1EK**) in **1** and curcumin-like structures. Additionally, **1EK** is the tautomeric form existing in solid state [9]. In solution the tautomerism of **1** has been studied by NMR [9,10], IR [4,10], UV–Vis absorption [11], and emission [13,12,14–16] spectroscopies, showing that in most solvents the enol–keto tautomer is the only existing one. However, under some structural and environment conditions the diketo form (**1KK**) could be observed as well [17,18]. Stabilization of this form is reported upon addition of surfactants [8,19] as a result of specific interaction between them and the carbonyl groups in **1KK**.

Quite surprisingly, as it is related to the curcumin bioavailability, there is no deep investigation of the tautomerism of **1** in water containing mixtures. Taking into account the solubility problem and the fact that the keto/enol equilibrium in β-dicarbonyl compounds is fast in the NMR time scale [17], the UV–Vis spectroscopy remains the only method that can register tautomers as individual species in water containing mixtures. The difficulty in the quantitative analysis of tautomeric mixtures, due to the impossibility for physical isolation of the individual tautomers, has been recently solved by using advanced data processing procedures [20].

Therefore, the aim of the current study is to prove that the diketo tautomer is stabilized by addition of water. This will be achieved by providing for the first time quantitative data for the molar fractions of the tautomers, obtained by chemometric analysis of the absorption spectra of **1** in water/ethanol binary solvent mixtures. The effect of the water will be explained by using DFT calculations.



Scheme 1. Possible tautomeric structures of **1**.

Experimental part

Curcumin was purchased from Fluka as primary pharmaceutical reference standard and was used without additional purification.

Spectral measurements were performed on Jasco V-570 UV–Vis–NIR spectrophotometer, equipped with thermostatic cell holder (using Huber MPC-K6 thermostat with precision 1 °C), in spectral grade solvents at 25 °C. The derivative spectra were calculated using the "step-by-step filter" as described in the literature [21]. The spectra in the binary ethanol/water mixtures were analyzed by a quantitative procedure based on resolution of overlapping bands, which yields tautomeric molar fractions in each solution and the individual spectra of the single tautomers [22,23].

Quantum-chemical calculations were performed by using the Gaussian 09 program suite [24] using M06-2X fitted hybrid meta-GGA functional [25] with def2-TZVP basis set [26]. It is worth to mention that this method has been recently shown as very suitable for describing tautomeric state in azonaphthols and related Schiff bases [27] as well as for prediction of the absorption spectra of organic dyes [28]. The tautomeric forms of curcumin were optimized without restrictions in gas phase and in solvent medium under normal optimization conditions by using ultrafine grid in the computation of the two-electron integrals and their derivatives. The optimized structures then were characterized as true minima by vibrational frequency calculations. In all cases the solvent medium was described by using the PCM model, namely IEFPCM [29], as implemented in Gaussian 09. The specific effect of the water was modeled by addition of water molecules and optimizations of the overall complex in water medium without any restrictions.

Results and discussion

The tautomerism of curcumin has been object of several theoretical studies [5–7], each of them considering the main tautomers as shown in Scheme 1 and substantial number of possible isomers. Therefore we are concentrated in the most probable tautomers **1KE** and **1KK** shown as the most stable isomers (Fig. 1) according to our calculations. As seen, the enol–keto tautomer is linear, bearing intramolecular hydrogen bonding, while in the diketo tautomer the carbonyl groups are in *anti*-position, forming two non-conjugated fragments separated by the C(4) atom.

It should be reminded that in most of the cases the energy difference between isomers of the corresponding tautomer are negligible as already shown in [5]. For instance the difference between *cin* and *anti* OMe group in respect of the tautomeric OH and C=O in **1KE** is less than 0.24 kcal/mol in gas phase and less than 0.8 kcal/mol in water environment (Table S1, Supplementary Information).

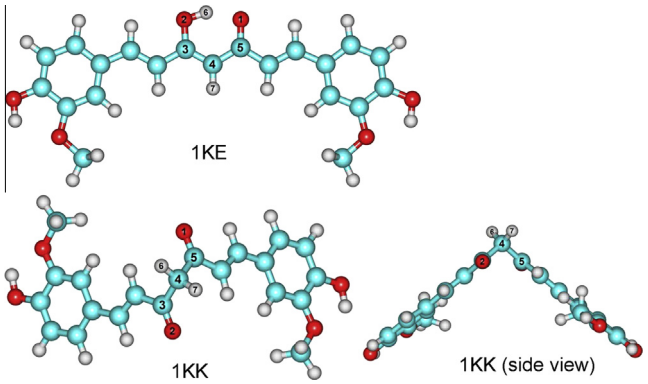


Fig. 1. The most stable tautomers **1KE** and **1KK** in gas phase.