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Thymoquinone β-Cyclodextrin Nanoparticles System: A Preliminary Study

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Abstract. Thymoquinone is a natural product, the main constituent of *Nigella sativa* seeds, which exhibits anti-inflammatory and anticancer activities. Among several existing molecules capable of forming an inclusion compound structure, cyclodextrins are applied in the pharmaceutical industry either to increase solubility of hydrophobic molecules or to protect molecules from inactivation or degradation. β-Cyclodextrin is currently the most common cyclodextrin in pharmaceutical formulations and probably the best studied in humans. In order to study the properties of inclusion compounds based on cyclodextrins and thymoquinone Fourier Transform Infrared (FTIR), Ultraviolet-Visible, Positron Annihilation Lifetime (PAL) Spectroscopies and calorimetric studies by Differential Scanning Calorimetry (DSC) were used. The obtained results indicate the formation of a 1 : 1 inclusion compound between cyclodextrin and thymoquinone. PALS and DSC measurements also provided evidence of the inclusion compound's activity.

Keywords: β-cyclodextrin, DSC, FTIR, inclusion compounds, PALS, thymoquinone

1. Introduction

Thymoquinone (TQ) is the main constituent of *Nigella sativa* seeds, commonly known as black seeds. The *Nigella sativa* essential oil has been traditionally employed in folk medicine due to various pharmacological effects.

Previous studies reported that TQ exhibits strong antioxidant, anti-inflammatory, antineoplastic, and analgesic effects, both *in vitro* and *in vivo*.

There is a growing interest in the therapeutic potential of TQ in different research fields, including diabetes, but, particularly, in cancer therapy. TQ was found to be a potent inhibitory drug in colon cancer cell, leukemia cells, laryngeal carcinoma cells, pancreatic cells, ovarian adenocarcinoma, uterine

sarcoma, and prostate cancer cells, while it is minimally toxic to non-neoplastic cells. Additionally, TQ and *Nigella sativa* are also promising chemopreventive agents [1].

Most drugs, especially those of hydrophobic nature, have failed in human clinical trials due to either lack of safety or poor efficacy, which may in part be a consequence of their poor bioavailability [2]. Although natural products have served as leads for the majority of clinically used drugs, poor oral bioavailability has greatly hindered their development.

The inclusion of molecules into cyclodextrin (CD) cavities has been intensively used in pharmaceutical, food, and cosmetic industries, in order to produce more stable preparations with improved hydrophilicity [3].

CDs are cyclic oligosaccharides, macrocycles with a hydrophilic external surface and a hydrophobic internal moiety, consisting of six (α-CD), seven (β-CD), or eight (γ-CD) D-glucopyranose residues linked by α-1,4 glycosidic bonds, that can be represented as a truncated cone structure (Figure 1). Their inner cavity diameters are about 0.57, 0.78, and 0.95 nm, respectively. As drug carriers, the fundamental advantages of natural cyclodextrins are (i) their complete known chemical structure, with several potential sites for chemical modification or conjugation; (ii) the availability of different cavity sizes; (iii) their low toxicity and pharmacological activity; (iv) their significant water solubility; (v) the protection of included/conjugated drugs from biodegradation [4].

The possible guest list for molecule encapsulation in cyclodextrins includes compounds such as straight or branched chain hydrocarbons, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatic compounds, gases (e.g., 1-methylcyclopropene), and polar compounds such as halogens, oxyacids, and amines [5]. The hydrophobic cavity of cyclodextrins provides an environment into which appropriately sized nonpolar molecules can enter and form stable inclusion species. During inclusion no covalent bonds are broken or formed [6]. One of the most important applications of cyclodextrins in the pharmaceutical field is the enhancement of the aqueous solubility of drugs through CD inclusion (nanoencapsulation).

2. Objectives

To study the properties of the inclusion compounds based on cyclodextrins (Figure 1) and thymoquinone (TQ) (Figure 2), in both aqueous medium and the solid state, using Fourier Transform Infrared (FTIR), Ultraviolet-Visible (UV-vis), and Positron Annihilation Lifetime (PAL) Spectroscopies, as well as Differential Scanning Calorimetry (DSC) experiments.

3. Material and Methods

β-CD was a gift from Wacker-Chemie and TQ was purchased from Sigma. The inclusion compounds were prepared by the coprecipitation method [4].

The FTIR spectral studies were carried out using a Nicolet 170SX FTIR spectrometer, employing a KBr pellet method.

The inclusion compound stoichiometry determination was carried out by the continuous variation method (Job plots [7]), followed by visible-ultraviolet spectrophotometry using the equipment Kontron Uvikon (Serial 922) at $\lambda_{\max} = 331$ nm.

Adequate samples were prepared for carrying out the PALS measurements [8]. This was achieved by pressing the powders under $\sim$ 70 bars into disks of 10 mm diameter and about 0.5 mm thickness.

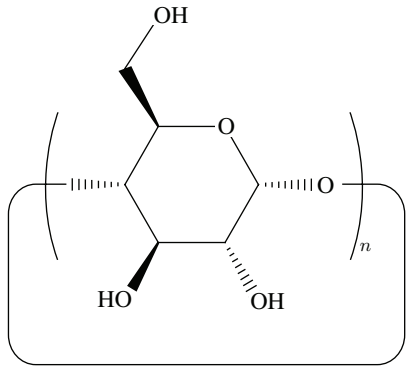

Figure 1: Cyclodextrins structure, $n = 6, 7$, or 8 glucose units is α-CD, β -CD, γ-CD, respectively.

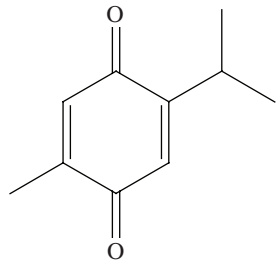

Figure 2: Thymoquinone (TQ) structure (2-isopropyl-5-methyl-p-benzoquinone).

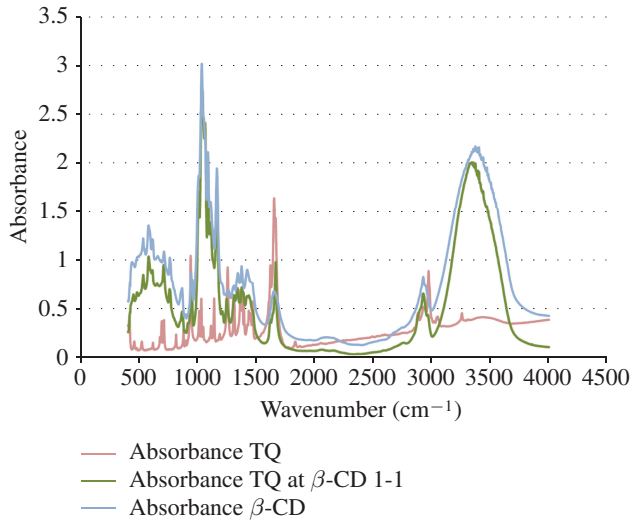

Figure 3: FTIR spectra of thymoquinone (TQ), β-cyclodextrin (β-CD), and TQ @ β-CD inclusion compound.

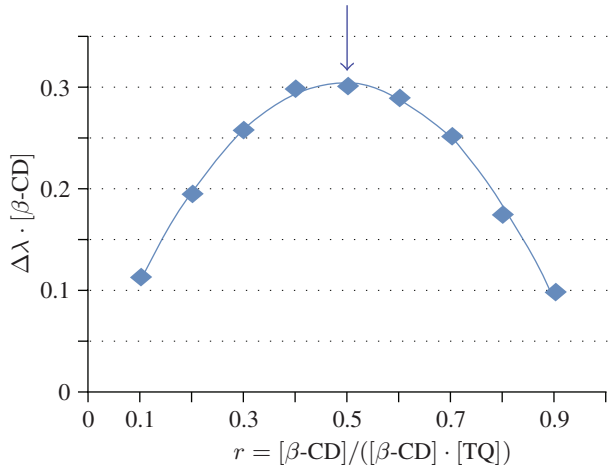

Figure 4: Continuous variation plots. The value of $r = 0.5$ is assigned pointing a 1 : 1 TQ @ β-CD stoichiometry.

The positron ^{22}Na source (ca. 7×10^4 Bq, closed between Kapton foils) was sandwiched between two identical sample disks. As the thickness of the specimens was not sufficient to stop all the positrons, "backing" of SS316 stain steel was used and the set finally wrapped in Al-foil. All measurements were performed at 298 K with the sample assembly placed into an evacuated stainless steel tube.

The lifetime (LT) spectra were recorded in our fast-fast coincidence PALS setup (featuring Pilot-U scintillators and XP 2020 photomultipliers) with a time resolution of 270 ps [8]. Each sample was measured four times with individual lifetime spectra having ca. 2.5×106 integral counts and the analysis was performed with the LT (version 9) code [9].

The Differential Scanning Calorimetry (DSC) thermograms were performed by a Mettler TA 4000 apparatus equipped with a DSC 25 cell. Samples were weighted in aluminium pans with a perforated lid and scanned at 10°C min^{-1} between 30°C and 300°C.

4. Results and Discussion

4.1. FTIR Studies

The FTIR spectrum of the thymoquinone @ β-CD inclusion compound, in the crystalline state, was analysed (Figure 3). The thymoquinone molecule (Figure 2) contains vibrational bands (namely, in FTIR) frequencies quite suitable for probing the guest perturbation by either mixing or inclusion with CD's. In particular, the infrared (IR) bands in 1560–1800 cm^{-1} region were found to be good structural probes (Figure 3).

The infrared spectrum of the inclusion compound (Figure 3) reflects a substantial variation of bond strength and length. The most interesting signals are those due to the polar functional groups of thymoquinone and β-CD, suggesting that the polar interaction can act as a stabilizing force in the inclusion compound formation.

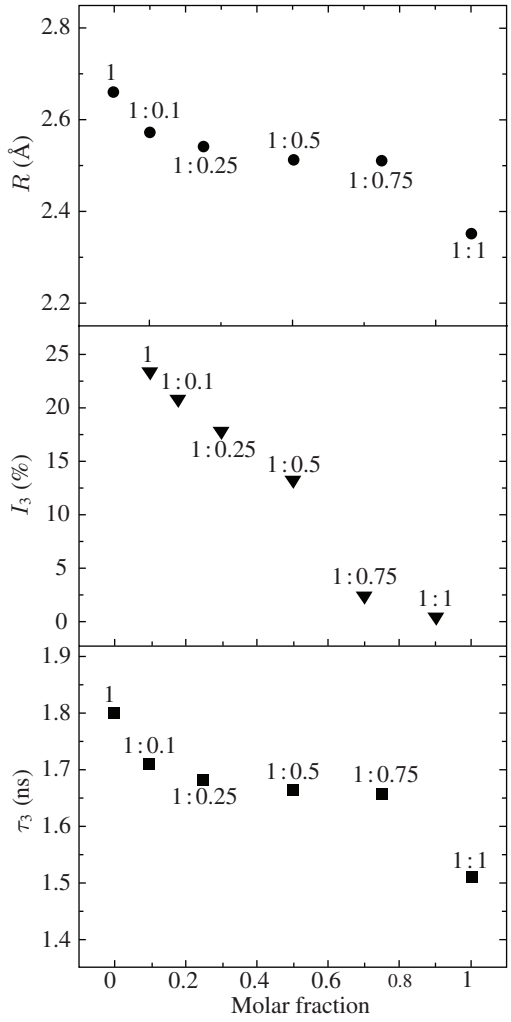

Figure 5: PALS parameter and calculated radii of o-Ps trapping sites for complexes of β-CD and TQ where 1 represents pure β-CD.

The quite complete disappearance of the thymoquinone signals is also a confirmation of the inclusion compound formation. In fact, a "shield" effect on the guest molecule by cyclodextrins has been detected for some CD inclusion compounds by NMR spectroscopy. Probably this is due to a dipole moment variation upon inclusion, that can be explained considering that the original crystalline host molecule has been quite completely rearranged in the inclusion complex [10, 11].

4.2. Stoichiometry of the Host-Guest System: Job Plots

Visible-ultraviolet spectroscopic studies were used to apply the continuous variation method (Job Plots) [7] to the thymoquinone and β-CD inclusion systems.

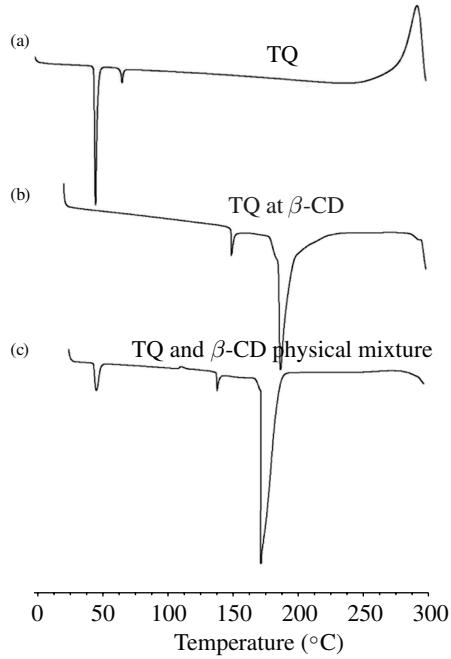

Figure 6: DSC thermograms of the thymoquinone (a), TQ @ β-CD (b), and the physical mixture of thymoquinone and β-CD (c).

Plotting $\Delta\lambda$ [β-CD]$_0$ ($\lambda_{\max}$ at 331 nm) against r ($r = [\beta\text{-CD}]/([\beta\text{-CD}] \cdot [\text{TQ}])$ leads to maxima at $r \approx 0.5$ (Figure 4), pointing to a 1 : 1 stoichiometry [7]. In fact, these distributions are exactly symmetrical, suggesting the presence of associations with a single stoichiometry, in this case, of the 1 : 1 type.

4.3. Positron Annihilation Lifetime Spectroscopy (PALS)

Figure 5 represents the observed lifetimes (τ_3) and intensities (I_3) for various samples, including pure β-CD as reference and the resulting radii according to the semiempirical model of Tao-Eldrup [8] that establishes the correlation between free-volume hole radius R (in Å) and o-Ps pick-off lifetime, τ_3. Values for pure β-CD are in agreement with previous results [9]. For the thymoquinone inclusion compound, τ_3 (and thus R_3) decreases with increasing thymoquinone concentration. However, for the 1 : 0.5 and 1 : 0.75 guest : host ratios there is a slight decrease, in contrast to the decrease obtained in 1 : 1. The common behavior of I_3 agrees well with the reduction of free volumes in the hollow truncated cone of the β-CD due to the inclusion of guest molecules, which decreases drastically with an increasing guest concentration. The results show that cyclodextrin molecule has few free volumes, just 0.41%, for 1 : 1 concentration which means that almost all sites are occupied.

4.4. Calorimetric Studies

As evidenced from Figure 6, DSC shows the presence of a true inclusion compound, as the thymoquinone melting band (a) disappears on the thermogram.

5. Conclusions

The reported experimental results indicate the formation of 1 : 1 cyclodextrin inclusion compounds with thymoquinone. Regarding the FTIR spectrum of the inclusion compound, it is very similar to that of pure β-CD. The final crystal structure of this species should be at least similar to that of β-CD, implying that TQ would be included and not interposed between β-CD molecules. This is, indeed, verified by the present experimental results, which is a good indication of the guest-host complex formation.

The PALS assays show a decrease of the β-CD free volumes upon an increase in the TQ molar fraction, reflecting an inclusion process between the two compounds.

Differential scanning calorimetry (DSC) measurements, in turn, provide evidence of inclusion compound such as the absence of the endothermic peak assigned to TQ melting.

Other studies are underway in our laboratory, namely, on the potential use of these systems in pharmaceutical formulations and/or on new food products formulations such as functional/novel foods.

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