P-099	**Thymoquinone @ β-cyclodextrin nanoencapsulation system: a preliminary study**	

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INTRODUCTION AND OBJECTIVES

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

Previous studies reported that TQ exhibit strong anti-oxidant, anti-inflammatory, anti-neoplastic, analgesic effects both *in vitro* and *in vivo*. There is 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 adernocarcinoma, uterine sarcoma and prostate cancer cells while it is minimally toxic to normal cells. Additionally, TQ and *Nigella sativa* could also be potent chemopreventive agents (Khader M *et al.*. 2009).

The inclusion of molecules in the cavities of cyclodextrins (CD) has been intensively used in the industry (pharmaceutical, food, cosmetic etc.) to produce more stable preparations with improved solubility (Astray, G. *et al.* 2009).

CDs are cyclic oligosaccharides, macrocycles with hydrophilic external and hydrophobic internal surface, consisting of six (α-CD), seven (β-CD), and eight (γ-CD) D-glucopyranose residues linked by α-1,4 glycosidic bonds that can be represented as a truncated cone structure (Figure 1). Theirs inner cavity diameters are about 0.57, 0.78, and 0.95 nm, respectively. As a drug carriers, the fundamental advantages of natural cyclodextrins are (1) complete known chemical structure, with many potential sites for chemical modification or conjugation; (2) availability of different cavity size; (3) low toxicity and pharmacological activity; (4) certain water solubility; (5) protection of included/conjugated drugs from biodegradation (Moreira da Silva, 2009)..

So, one of the most important applications of cyclodextrins in pharmaceutical fields is to enhance aqueous solubility of drugs through inclusion complexation at molecular level (nanoencapsulation).

To study the properties of the inclusion compounds based on cyclodextrins (Figure 1) and thymoquinone, TQ (Figure 2) in aqueous medium and solid state through Fourrier Transformed Infrared (FTIR) and Utraviolet-Visible Spectroscopies and also, calorimetric studies by Differential Scanning Calorimetry (DSC).

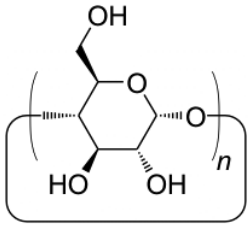

Figure 1: Cyclodextrins structure: n = 6, 7 or 8 glucose units is α-CD, β-CD and γ-CD, respectively

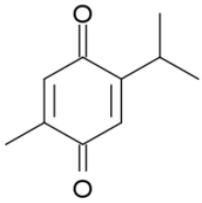

Figure 2: Thymoquinone, TQ, structure (2-isopropyl-5-methyl-p-benzoquinone)

MATERIAL AND METHODS

β-CD was a gift from Wacker-Chemie and TQ was purchased from Sigma.

The inclusion compounds were prepared by the co-precipitation method (Moreira da Silva, 2009)

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

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 ºCmin^{-1} between 30 ºC and 300 ºC.

The inclusion compound stoichiometry determination was done by the Continuous Variation Method - Job plots (Job 1928) followed by visible-ultraviolet spectrophotometry using the equipment Kontron Uvikon (Serial 922) at λ_{max}=331 nm.

RESULTS AND DISCUSSION

FTIR studies The inclusion compounds FTIR spectra of thymoquinone@ß.CD in crystalline state were analysed.

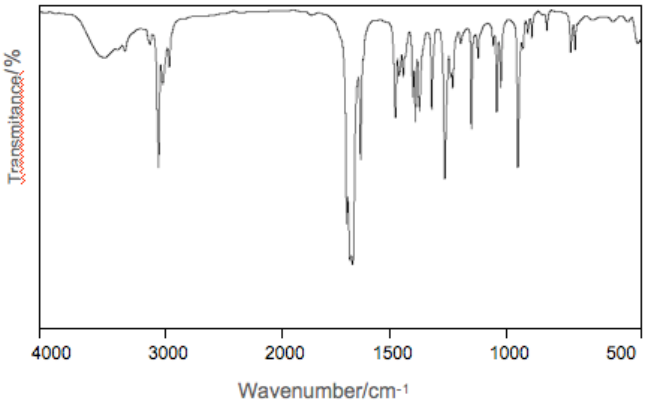

Figure 3: FTIR thymoquinone molecule spectrum.

Thymoquinone molecule (Figure 3) provide group frequencies for probing the guest perturbed by mixing it with CD´s or by complex formation with CD´s. In particular, the following Infrared (IR) bands occurring in 1560-1800 cm^{-1} region were found to be good structural probes.

Calorimetric studies Has showed in Figure 4 DSC has proven the presence of true complex formation as the thymoquinone melting band (a) disappears on the thermogram.

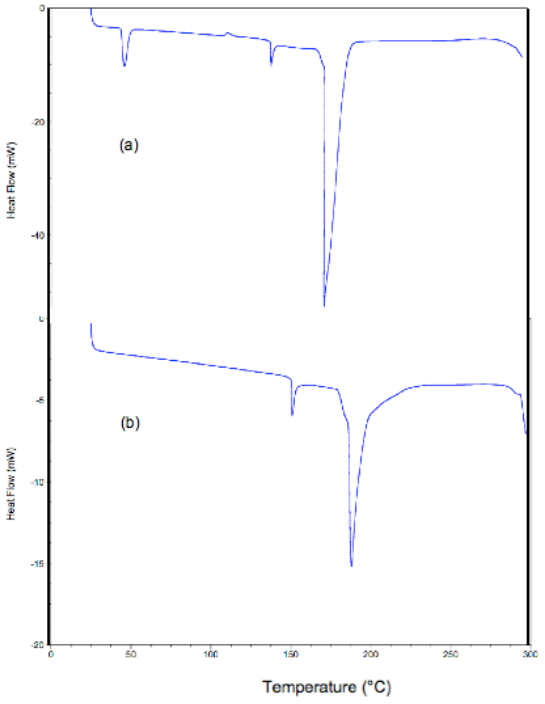

Figure 3: DSC thermograms of the (a) the physical mixture of thymoquinone and β-CD and (b) TQ@β−CD

Stoichiometry of the Host-Guest System: Job Plots Visible and ultraviolet spectroscopic studies, were used to apply the continuous variation method (Job Plots).

Plotting $\Delta\lambda\ [\beta\text{-CD}]_0$ (λmax at 331nm) against r (r= =[β-CD]/ ([β-CD].[TQ]) leads to maxima at $r \approx 0.5$ (Figure 5), pointing to 1 : 1 stoichiometry [Job, 1928]. In fact, these distributions are roughly symmetrical, suggesting the presence of associations with a single stoichiometry, in this case, of the 1 : 1 type.

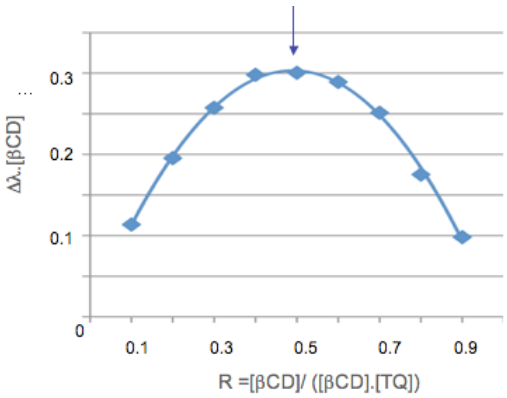

Figure 5: Continuous Variation Plots. The value of r=0.5 is assigned pointing a 1:1 TQ@β−CD stoichiometry

CONCLUSIONS

The experimental results reported indicate the formation of 1:1 cyclodextrin inclusion compounds with thymoquinone. DSC measurements provided evidences of complexation such as the absence of the endothermic peak assigned to the melting of the thymoquinone.
Other studies are underway and further studies will explore the potencial use of these systems in pharmaceutical formulations and/or new food products formulations as functional/novel foods.

ACKNOWLEDGEMENTS

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