

# Free volumes studies in Thymoquinone and Carvone $\beta$ -cyclodextrin nanoparticles by positron annihilation lifetime spectroscopy

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**Abstract.** Positron annihilation lifetime spectroscopy is used to study free volume in  $\beta$ -cyclodextrin with the encapsulation of thymoquinone and S-carvone, in samples covering the guest to host fraction range from 1:0.1 to 1:1. The results clearly indicate the presence of long lifetime components related to Ps-formation. Although the behavior of the two guests is different, in both cases the results indicate the formation of 1:1 cyclodextrin inclusion compounds. Data show that the addition of carvone to  $\beta$ -cyclodextrin results in a decrease of the *o*-Ps lifetime corresponding to a reduction of the average radius of cavities from 2.41 Å to 2.29 Å, whereas the addition of thymoquinone decreases the radius from 2.57 Å to 2.35 Å. In turn, the intensity varied from 20.55 to 19.20% and from 20.83 to 0.41%, respectively.

## 1. Introduction

$\beta$ -cyclodextrin ( $\beta$ -CD) is a cyclic oligosaccharide consisting of seven glucopyranose units linked by  $\alpha$ -1,4 glycosidic bonds in normal chair conformations. The steric arrangement of glucose units in the CD molecule results in the shape of a hollow truncated cone with a hydrophilic outer surface (which makes CD water soluble) and a hydrophobic internal cavity. This enables CD to form inclusion complexes with various hydrophobic guest molecules [1]. Due to their extensive applicability in different fields (food and pharmaceutical industry, agriculture, etc.) CDs, capable of hosting a wide variety of molecules, are in the forefront of structural investigations [1,2,3].

The present work aims at studying by positron annihilation lifetime (PALS) the encapsulation in  $\beta$ -CD of two different molecules: thymoquinone (ThQ), the main constituent of *Nigella sativa* seeds, traditionally employed in folk medicine due to various pharmacological effects, and (4S)-(+)-Carvone (S-Crv), present in the essential oil of caraway seeds *Carum carvi* L., that acts as a sprouting inhibitor agent for potato tubers in storage. PALS became a powerful technique for the investigation of microstructures, with applicability in the determination of free volume size and volume fractions [4,5], as it allows one to obtain both the lifetimes ( $\tau_i$ ) and the relative abundances (*i.e.*, the intensities,  $I_i$ ) of



the various annihilation channels. In order of increasing lifetimes, one normally finds the singlet (*p*-Ps, *i*=1), free positrons (*i*=2) and triplet Ps state (*o*-Ps, *i*=3). In samples where no positronium (Ps) formation takes place, positron trapping provides the annihilation channel which could sense structural imperfections. In turn, in media facilitating Ps-production the trapping of Ps reveals characteristic parameters of internal voids and cavities. The *o*-Ps channel constitutes the most useful probe, since the associated lifetimes are long and distinct. The primary annihilation mechanism of this long-lived *o*-Ps is the “pick-off” process, in which the positron in *o*-Ps annihilates with one of the surrounding spin opposite electrons, which obviously depends on the local physicochemical properties. The lifetime and intensity of this component can be translated into hole size (radius) and density of holes, respectively, as a probe to characterize various properties of matter [5].

## 2. Experimental

The  $\beta$ -CD, kindly offered by Wacker-Chemie, Munich (Germany), was recrystallized by cooling concentrated aqueous solutions in a Dewar flask from *ca.* 80°C to room temperature. The S-carvone (2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one or (4S)-(+)-Carvone) was purchased from Aldrich Chemical Co, and the Thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone) from Sigma-Aldrich. The preparation of the inclusions of S-carvone and of thymoquinone in  $\beta$ -CD followed previous description [6]. Starting with those powders, samples were pressed under ~70 bar into disks of 10 mm diameter and about 0.5 mm thickness. The positron  $^{22}\text{Na}$  source (*ca.*  $7 \times 10^4$  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 assemblies placed into an evacuated stainless steel tube.

The lifetime (LT) spectra were recorded in a fast-fast coincidence PALS setup (featuring Pilot-U scintillators and XP 2020 photomultipliers) with a time resolution of 270 ps. Each sample was counted three times. The lifetime spectra had a total number of *ca.*  $2.3 \times 10^6$  integral counts and were evaluated through the LT (version 9) code [7].

## 3. Results and discussion

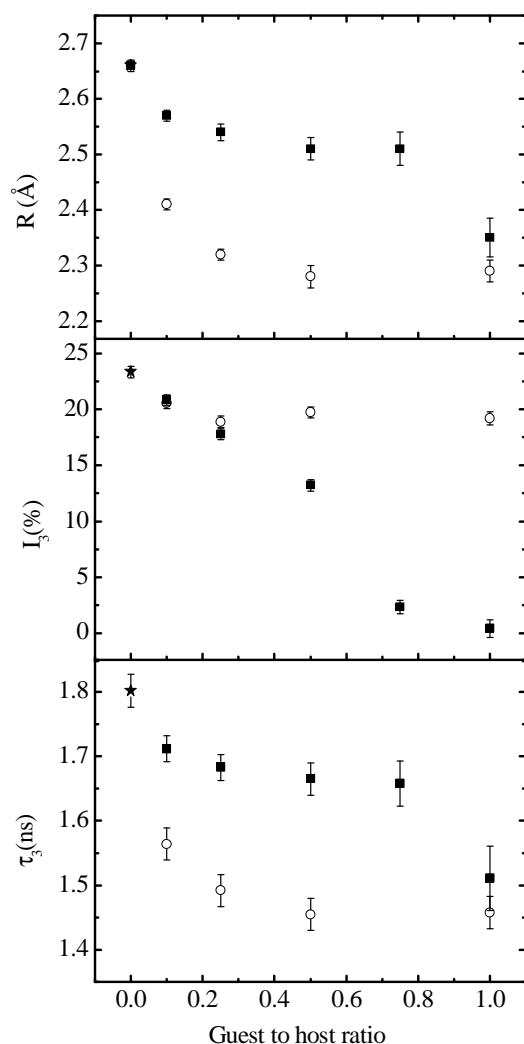
All spectra measured are well fitted with three lifetime components, the longest one ( $\tau_3$  and  $I_3$ ), being associated with pick-off annihilation of *o*-Ps in the CDs free volume.

For an *o*-Ps confined in an infinitely deep spherical potential well there is a correlation between the free volume hole radius, *R*, and the long-lived lifetime, according the semi-empirical model of Tao-Eldrup [8,9] that establishes the correlation between free-volume hole radius, *R* (in Å), and *o*-Ps pick-off lifetime,  $\tau_3$ ,

$$\tau_3 = 0.5 \left[ 1 - \frac{R}{R_0} + \frac{1}{2\pi} \sin \left( \frac{2\pi R}{R_0} \right) \right]^{-1} \quad (\text{in ns}) \quad (1)$$

where  $R_0 = R + \Delta R$  and  $\Delta R = 1.656$  Å is the thickness of a homogeneous layer for the pick-off annihilation [10]. The average radius *R* of the free volume holes in polymers and the average volume of the holes, taken as  $V_f = (4/3)\pi R^3$ , can be calculated from the measured *o*-Ps lifetime. Despite of its principal limitation which cannot be circumvented (the apparent free volume integrates only the sites seen by the *o*-Ps [11]), the model has been successfully used in PALS studies on a wide class of substances including polymers, membranes [11,12] and cyclodextrins [6,13]. Although equation (1) was derived for spherical holes, which is not the case of CDs, the value of *R* extracted from  $\tau_3$  can be considered as an “average” radius and it is most often further translated into an “average” free volume. Given the chemical interaction of *o*-Ps with the surrounding medium, it must not be expected that the assessed volumes coincide accurately with those determined by other techniques.

Figure 1 shows the lifetimes ( $\tau_3$ ) and intensities ( $I_3$ ) observed in samples of complexes of S-Crv and of ThQ in  $\beta$ -CD, also including pure  $\beta$ -CD as a reference. The radii obtained with equation 1 are also plotted. Previous results [6] for pure  $\beta$ -CD are in agreement with the present one and with those



**Figure 1.** PALS parameter and calculated average radii of *o*-Ps trapping sites for the different samples studied:

- ★ pure  $\beta$ -cyclodextrin ( $\beta$ -CD)
- $\beta$ -CD with ThQ
- $\beta$ -CD with S-Crv

reported in literature [14].

For the inclusion of S-Crv in  $\beta$ -CD, the value of  $\tau_3$  (and thus  $R$ ) decrease until 0.25 guest to host ratio and then remains practically constant, the resulting free volume holes varying from 78.8 (pure  $\beta$ -CD) to 52.9 Å<sup>3</sup>.  $I_3$ , in turn, shows an initial slight decrease remaining practically constant above 0.25 guest to host fraction.

For the ThQ inclusion compound, the value of lifetime  $\tau_3$  remains roughly constant within the error bars with increasing guest concentration, except for the 1:1 guest to host ratio. The computed average free volumes vary from 78.8 Å<sup>3</sup> (pure  $\beta$ -CD) to 66.4 Å<sup>3</sup> for 0.75 and then to just 27.7 Å<sup>3</sup> for 1:1 ratio. However the intensity  $I_3$  for this 1:1 ratio is just 0.41%, and such a low value is actually compatible with no Ps formation. Parameter  $I_3$ , related with both the density of free volume elements and the chemical environment that determines the probability of *o*-Ps formation, shows a continuous decrease with increasing guest to host ratio. The behavior of  $I_3$  agrees well with the reduction of free volume on the hollow truncated cone of the  $\beta$ -CD due to the inclusion of the guest molecules, implying that the holes become fully occupied, thus pointing to a 1:1 stoichiometry.

Furthermore, this latter observation is in agreement with results of Differential Scanning Calorimetric (DSC) and Fourier Transform Infrared Spectroscopy (FTIR) measurements [15]. DSC

provides evidence for the inclusion of the guest, revealing the absence of the endothermic peak assigned to ThQ melting for the highest guest to host fraction. In turn, the FTIR spectrum for 1:1 guest to host ratio with ThQ is very similar to the pure  $\beta$ -CD, implying that ThQ would be included and not interposed between  $\beta$ -CD molecules [15].

The behavior of  $I_3$  with increasing guest content is similar for both S-Crv and ThQ up till 0.25 guest to host ratio. However, whereas above this concentration the inclusion of S-Crv does not cause changes in  $I_3$ , for the ThQ case a continuous decrease is observed and the smallest values are reached.

The product of  $\tau_3$  and  $I_3$  is an interesting quantity, since it can reflect the free volume of the sample [16]. In the present case such quantity reveals a different behavior for the two guests: whereas for ThQ it shows a linear decrease, for S-Crv it presents a linear decrease until 0.25 guest to host ratio (similar to the thymoquinone) and for higher guest fractions it remains practically constant. As stated above, the different behavior observed for the two guests may be associated with distinct chemical interaction between  $\beta$ -CD and guests with diverse molecular structures.

#### 4. Conclusions

The present study of  $\beta$ -cyclodextrin and their complexes by PALS provides a strong assessment of way the free volume is used, proving that the technique is adequate for gathering micro structural information in such systems.

Although the difference between the inclusion of ThQ and S-Crv was made evident, in order to clarify the mechanisms involved, further systematic studies must be made. Particular attention should also be devoted to clarifying the evolution with the time elapsed since encapsulation, an aspect of utmost practical importance. For the characterization of S-Crv inclusion in the  $\beta$ -CD, DSC and FTIR have already proven to be important. However, in order to achieve a better understanding of the encapsulation mechanisms, the use of other complementary techniques like Raman, NMR and Doppler broadening is foreseen.

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