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Study on a host–guest interaction of β-cyclodextrin with tebuconazole in water

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ABSTRACT

In order to assess the effect of β-cyclodextrin (β-CD) on the tebuconazole (TEB) solubility in water, UV–VIS spectroscopic measurements of the tebuconazole solutions with β-cyclodextrin were examined, followed by a VP-ITC calorimetric titration of the solutions of tebuconazole with β-cyclodextrin. The stoichiometry of the tebuconazole–β-cyclodextrin inclusion complex formed and the physical and chemical parameters describing the complex were determined. The effect of fungicide included inside the macromolecule of β-cyclodextrin on the fungal culture (hyphae) of *Aspergillus fumigatus* was also assessed.

1. Introduction

Cyclodextrins belonging to organic cyclic compounds are natural chiral polysaccharides. The external surface of these sugar polymers is formed of primary and secondary hydroxyl groups imparting a hydrophilic character to the molecule, whereas the torus interior, in which hydrogen atoms combined with carbon atoms and glycoside oxygen atoms with free electron pairs are located, possesses a hydrophobic character [1,2]. The specific structure of cyclic sugar polymers allows one to include in the hydrophobic cavity sparingly water soluble non-polar organic molecules. The fact that cyclodextrins show no cytotoxicity has made it possible to use them as nano-containers of drugs [3–7], substances used in cosmetics [8] and foodstuff (as a food additive, β-cyclodextrin has been marked as E 459). Cyclodextrins have also found their use as receptors that include hydrophobic organic molecules of pesticides [9,10]. The formation of a complex consisting of receptor (cyclodextrin)–ligand (non-polar molecule) can not only influence an increase in the solubility of hydrophobic substances in water and conceal unpleasant taste and smell of drugs [11,12], but it also allows one to control the release of included substances into the environment [13].

The development of agricultural production depends on the use of pesticide on a great scale. The group of these compounds includes also fungicides. Most of the currently used biologically active substances as components of commercially used fungicides are hardly soluble in water, therefore a great portion of the ready-made products of this kind contain non-polar toxic organic solvents that are hazardous to the environment and the health of men and animals.

In order to determine how the solubility of fungicide in water increases in the presence of β-cyclodextrin, it seems appropriate to examine the interactions of β-cyclodextrin with selected fungicide such as tebuconazole (TEB) [14]. Such investigations can contribute to a reduction in the amount of toxic organic solvents used in agriculture. Moreover, the fungicide included inside cyclodextrin can prolong the action of pesticides and increase their capacity. Particularly, this concerns the use of fungicide in closed spaces, such as greenhouses, foil tunnels or warehouses. The tebuconazole selected for investigations is a fungicide from the group of triazoles used for the control of ascomycetes, basidiomycetes and fusarium type fungi [15,16]. In several research laboratories also the inclusive complexes of cyclodextrin with antifungal medications (e.g., econazole and miconazole) were investigated [17–20].

The aim of the present study was to determine the number of tebuconazole molecules included inside β-cyclodextrin, as well as the formation constant and physical–chemical parameters of the fungicide–cyclodextrin complex formed, using isothermal titration calorimetry, UV–VIS and NMR spectroscopic examinations. The effect of β-cyclodextrin on the water solubility increase of the fungicide was

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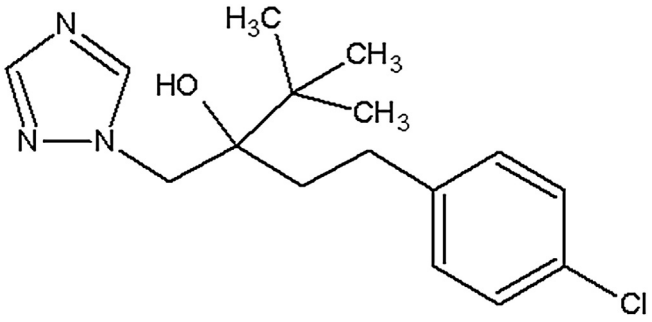

Fig. 1. Chemical structure of tebuconazole (TEB).

assessed. The action of the inclusion of tebuconazole–β-cyclodextrin complex and the fungicide itself on the survival rate of fungi was also tested.

2. Experimental

2.1. Materials

β-Cyclodextrin (β-CD) 98% pure, dimethylsulfoxide (DMSO) 99.5% pure and tebuconazole (TEB) (Fig. 1) (all Sigma-Aldrich) were used. The test substances were dried in a vacuum drier at a temperature of 333 K. Water used for calorimetric and spectrophotometric measurements (UV–VIS) was distilled three times and degassed. ^{1}H NMR measurements were carried out with the use of heavy water D_2O 99.8% (Sigma-Aldrich).

2.2. Isothermal titration calorimetry (ITC)

Calorimetric measurements were carried out in an isothermal calorimeter for VP-ITC titrations (MicroCal — USA) at a temperature of 298.15 K. A measurement cell with a volume of 1.4275 ml contained degassed solution of tebuconazole (TEB) in water. The aqueous solution of tebuconazole with a concentration of 0.26 mM was titrated with aqueous solutions of β-cyclodextrin with a concentration of 7 mM from a syringe. Dilution measurements of aqueous solutions of β-cyclodextrin in water were taken. Aqueous solutions of tebuconazole were also diluted with water.

All the measurements were carried out at a temperature of 298.15 K. The titrant solutions in all the mentioned measurements were injected from a syringe with 50 ratios, 5 μl each. The duration of injection was 10 s, and was carried out at 400 s intervals and a stirrer revolution rate of 307 rpm.

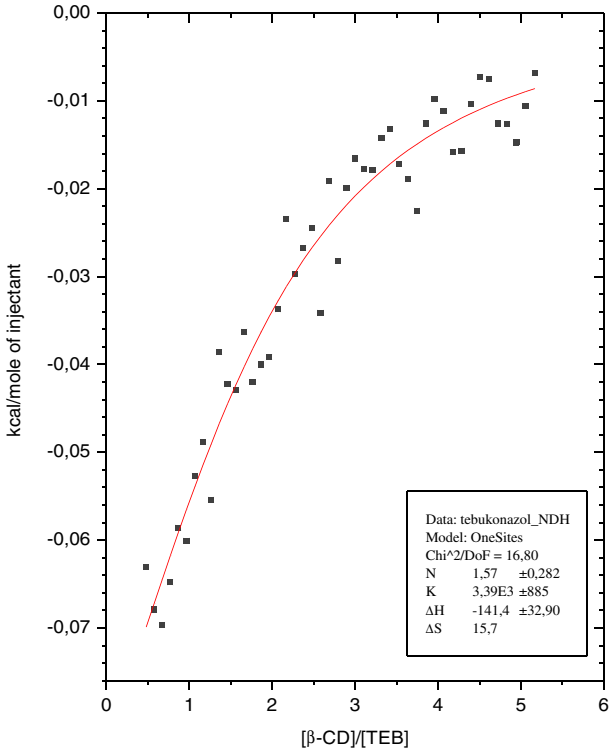

Fig. 3. Energetic effects of interaction between tebuconazole (0.26 mM) and β-cyclodextrin (7 mM) in water.

2.3. UV–VIS spectrophotometry

Changes in the absorbance of TEB in water caused by the presence of β-cyclodextrin were examined with the use of a single-beam SPECORD 50 spectrophotometer (Analytic Jena). Maximum of tebuconazole absorption in aqueous solutions has been designated for the wavelength of 222 nm. A series of measurements was made for TEB solutions with concentrations from 3.5×10^{-6} M to 52×10^{-6} M, which was used for the determination of molar absorption coefficient. The value of this coefficient determined amounts to $\varepsilon_o = 6155\ M^{-1}\ cm^{-1}$ (Fig. 2).

To determine the increase in TEB solubility in water caused by the presence of β-cyclodextrin, aqueous solutions of β-CD with concentrations from 0.5 mM to 12 mM were prepared and the excess of solid TEB was added to them. The experimental solubility of β-cyclodextrin in water at a temperature of 298.15 K amounts to 14 mM. The aqueous solutions of β-CD with TEB, after seven days of storage at constant

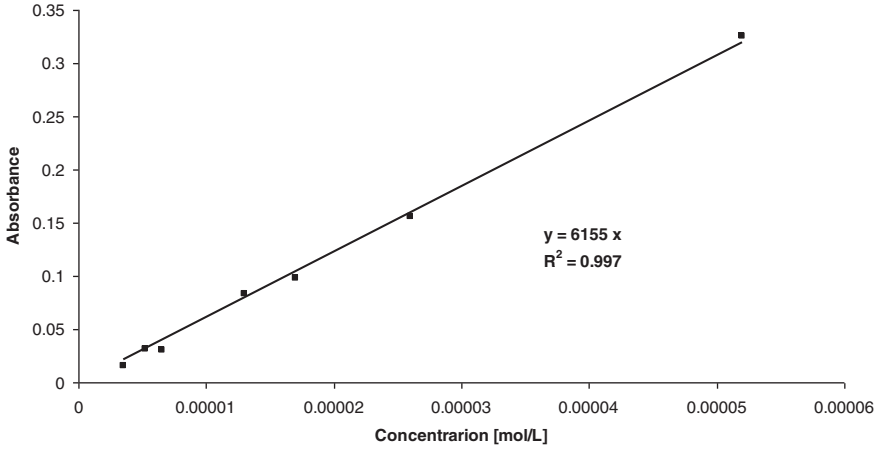

Fig. 2. Calibration curve for aqueous solutions of tebuconazole at wavelength 222 nm.