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ANALYSIS

SOLID STATE CHARACTERIZATION OF α-TOCOPHEROL IN INCLUSION COMPLEXES WITH CYCLODEXTRINS

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Abstract: The alternative for a pure soluble, sensible for physical and chemical conditions oil form of α-tocopherol (α-T) is its complexation with cyclodextrins. A different influence of cyclodextrins on the included substance demands a stability investigation of the substance enclosed in a host-guest complex. Hence, the thermal stability of α-T in inclusion complexes (InCs) with cyclodextrins (CDs) was studied. The inclusion complexes were obtained by two different methods: a lyophilization and a kneading method, and their formation was examined by IR spectroscopy, differential scanning calorimetry and ^{1}H-NMR spectroscopy. The inclusion complexes were subjected to the test of accelerated aging at 323 K, 333 K, 338 and 343 K, for comparison α-T as a substance and physical mixtures (PhM) of α-T with CDs were used. Changes in α-T concentration during the experiment were followed by HPLC method and next, the products of thermal decomposition were studied by LC-ESI-MS/MS method. The reaction of α-T decomposition in inclusion complexes with CDs was found to be of the first order. The same order of a decomposition reaction was observed in a sample of α-T as a substance. It seems that cyclodextrins protect α-T against thermal decomposition, moreover, the protective effect of natural β-cyclodextrin (β-CD) appears to be greater than that of 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD). However, the CDs do not influence the type of a formed product of decomposition. This product, i.e., the dimer of α-T (m/z 859 Da), was found in all tested samples. The protective effect of CDs and transformation from the liquid state to the solid state of α-T can be used to create a new pharmaceutical form – tablets with α-T.

Keywords: inclusive complexes, cyclodextrins, tocopherol, solid state, ^{1}H-NMR, mass spectrometry

For over a decade inclusion complexes of therapeutic substances with CDs have been of great interest to pharmaceutical industry due to their ability to modify the solubility, stability, bioavailability and even to diminish the toxicity of many therapeutic drugs (1, 2). CDs are oligosaccharides consisting of glucopyranose molecules linked by α-1,4-glycoside bonds to form a conical structure, with a hydrophobic cavity and hydrophilic outside part (3). This specific conformation enables them to form host-guest complexes (4). The ability of forming inclusion complexes with various molecules depends on the size and polarity of the included substance and the type of CD (3, 5). Cyclodextrins can stabilize the guest molecules but in some cases they can accelerate their degradation as well, e.g., bis(4-hydroxyphenyl)ethane (6–9). Hence, a different influence of CDs on the included substance demands the stability investigation of the substance enclosed in the host-guest complex.

A widely known significance of tocopherol in living organisms is the ability to sweep out free radicals and protect the cell lipid membranes against autocatalytic peroxidation. The following issues of its function have been analyzed so far: antioxidant-(anti-radical) activity, structure-function regulation of cell membranes and regulation of enzyme activity (10). The protection of cell membranes against oxidation is vital and essential to fight sclerosis of blood vessels, cardiac muscle ischemia or cardiac infarcts (11). The novelty of a tocopherol family application are anti-cancer properties which do not relate to their anti-radical activity (12, 13). This new discovered action by pro-apoptotic mechanisms, e.g., in human breast cancer cells, results in further finding a new form of tocopherol more stable and better soluble in water (14). So far, chemically stable have been tocopherol esters (acetate or succinate), but in these forms their bioavailability and effectiveness were not better than the present mole-

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cule (15–17). For this reason a new form of α-T has still been searched and the inclusion complexes with different derivatives of CDs have been proposed. The replacement of the natural β-CD in complexes with tocopherol by branched derivatives (e.g., HP-β-CD or HP-γ-CD) has not influenced the antioxidative properties of vitamin E, but considerably enhanced its light-induced decomposition, hence the selection of the CD's derivative during preparation of inclusion complexes is significant (18). The aim of this study was to choose the best way of inclusion complex preparation and to evaluate the stability of α-tocopherol (α-T) in inclusion complexes with β-CD and 2-HP-β-CD in conditions of storage for diet supplements. Moreover, the purpose of the study was to discuss whether the protecting effect of natural cyclodextrin and its 2-hydroxypropyl derivative exists and if these structures can be used in a new pharmaceutical form.

MATERIALS AND METHODS

Chemicals

α-T was purchased from Sigma-Aldrich Chemie (Germany). β-CD and 2-HP-β-CD were supplied by Fluka Sigma-Aldrich Chemie. Methanol LiChrosolv was purchased at Merck (Germany). All reagents were analytically examined by HPLC.

Preparation of inclusion complexes and physical mixtures

Inclusion complexes

Kneading method

2-HP-β-CD (1.380 g) was dissolved in distilled water and α-T (0.431 g) was dissolved in ethanol (760 g/L). Both saturated solutions were mixed and transferred quantitatively to the agate mortar. The mixture was kneaded for about 2.5 h to obtain dry powder. The product was dried at room temperature to a constant mass.

Lyophilization method

For the solid inclusion complex preparation, a saturated solution of α-T was added to an aqueous solution containing the β-CD. The molar ratio of the guest molecule to CD was 1 : 1. The dispersion of α-T in aqueous β-CD solution was shielded from light and stirred for 24 h at room temperature to achieve equilibrium of the complexation reaction. Next, after filtration through a 0.2 μm nylon membrane, the solvent was removed and the remaining coprecipitate was collected. These solid complexes were frozen at –20°C and then lyophilized in a laboratory freeze-dryer (Alpha 1-2 Christ, Germany).

Physical mixtures

Physical mixtures were simply obtained by thorough mixing of testing substances.

Samples identification

Infrared spectroscopy (IR)

IR absorption spectra were measured using FT-IR Bruker IFS 66v/S spectrometer. The samples of the solid substances were previously mixed thoroughly with potassium bromide (KBr) and next, the KBr disks were prepared by compressing the powders under force of 10 ton in a hydraulic press. The measurement of α-T was performed using KBr discs due to its oily form. Scans were obtained at a resolution of 2 cm^{-1}, from 4000 to 500 cm^{-1}.

Differential scanning calorimetry (DSC)

DSC measurements were performed on a Shimadzu DSC-50 differential scanning calorimeter with a thermal analyzer. All accurately weighed samples (2.0 mg) were placed in aluminium pans before heating under nitrogen flow (30 mL/min) at a scanning rate of 10°C/min from 20 to 400°C. An aluminium pan with accurately weighed Al_2O_3 (2.0 mg) was used as a reference standard.

Nuclear magnetic resonance spectroscopy (^{1}H-NMR)

The solid-state cross-polarization magic angle spinning (CP/MAS) NMR experiments were performed on 400 MHz Bruker Avance III spectrometer, equipped with a MAS probe head using 4-mm ZrO_2 rotors at a frequency of 400.13 MHz for ^{1}H. The conventional ^{1}H MAS spectra were performed using the following parameters: measurements temperature of 296 K, spinning rate of 8 kHz, proton 90° pulse length of 5 μs, repetition delay of 3 s, spectral width of 40 kHz and time domain size of 16 k data points. In ^{1}H-NMR measurements α-T was excluded due to its oily form.

Stability studies

The study was performed during 29 weeks in the atmosphere of dry air at 323, 333, 338 and 343 K. Accurately weighed samples of α-T (2.5 mg), InC with β-CD and InC with 2-HP-β-CD (10.0 mg, respectively) were placed in glass vials, which afterwards were placed in sand baths and closed in thermostats set at appropriate temperature. Prior to initiating studies, the sand baths were heated to required temperature during one day. Next, at certain intervals samples were taken and analyzed by HPLC method. On the basis of a diminished concentration of α-T in time the kinetic decomposition equation was set.

HPLC method

Equipment and chromatographic conditions

Chromatographic analyses were made on the HPLC apparatus equipped with a DAD detector, made by Agilent Technologies series 1200. Separation was performed in the reversed phase system using a LiChroCART 250-4 column filled with LiChrospher 100 RP-18 (5 µm). Results were analyzed on a computer integrated with the chromatograph using ChemStation software for LC 3D System.

Analyses of α-T, its inclusion complexes and physical mixtures with β-CD and 2-HP-β-CD were performed in the following conditions: 100% methanol as the mobile phase, at the flow rate of 1.3 mL/min, column thermostated at 25°C, injection volume of 20 µL, detection at 292 nm.

Sample preparation

α-T (2.5 mg), InCs (10.0 mg) were transferred quantitatively into a measuring flask (10.0 mL) and the volume was supplemented with methanol. Prior to injection on the column all the solutions were filtered.

Validation

The method was validated according to the ICH recommendations (19). The method's selectivity, linearity, precision, accuracy and recovery were determined. The optimization and validation conditions for the HPLC method have already been published (20).

LC-ESI-MS/MS method

The analysis was carried out by using a system consisting of a liquid chromatograph Agilent

Table 1. Kinetic parameters of á-tocopherol decomposition.

Temp. [K]	(k ± Δk) [s^{-1}]	(a ± Δa) [h^{-1}]	$t_{0.5}$ [h]	$t_{0.1}$ [h]	S_a	r	n
343	$(4.976 \pm 0.298) \cdot 10^{-7}$	$(-1.79 \pm 0.11) \cdot 10^{-3}$	386.9	58.8	$4.65 \cdot 10^{-5}$	–0.997	10
338	$(4.189 \pm 0.342) \cdot 10^{-7}$	$(-1.51 \pm 0.12) \cdot 10^{-3}$	459.5	69.9	$4.43 \cdot 10^{-5}$	–0.997	10
333	$(3.352 \pm 0.219) \cdot 10^{-7}$	$(-1.21 \pm 0.08) \cdot 10^{-3}$	574.5	87.4	$3.22 \cdot 10^{-5}$	–0.998	9
323	$(2.081 \pm 0.082) \cdot 10^{-7}$	$(-7.49 \pm 0.29) \cdot 10^{-4}$	924.6	140.6	$1.30 \cdot 10^{-5}$	–0.998	11

k – rate constant of reaction, a – slope, S_a – standard deviation for a, r – correlation coefficient.

Table 2. Kinetic parameters of decomposition of α-T in inclusion complex with β-CD.

Temp. [K]	(k ± Δk) [s^{-1}]	(a ± Δa) [h^{-1}]	$t_{0.5}$ [h]	$t_{0.1}$ [h]	S_a	r	n
343	$(1.019 \pm 0.069) \cdot 10^{-7}$	$(-3.67 \pm 0.25) \cdot 10^{-4}$	1891.0	287.6	$1.13 \cdot 10^{-5}$	–0.995	11
338	$(7.798 \pm 0.304) \cdot 10^{-8}$	$(-2.80 \pm 0.11) \cdot 10^{-4}$	2468.9	375.5	$4.97 \cdot 10^{-6}$	–0.998	13
333	$(5.334 \pm 0.188) \cdot 10^{-8}$	$(-1.92 \pm 0.06) \cdot 10^{-4}$	3608.9	548.9	$3.04 \cdot 10^{-6}$	–0.998	12
323	$(2.812 \pm 0.174) \cdot 10^{-8}$	$(-1.01 \pm 0.06) \cdot 10^{-4}$	6845.7	1041.2	$2.87 \cdot 10^{-6}$	–0.996	12

k – rate constant of reaction, a – slope, S_a – standard deviation for a, r – correlation coefficient.

Table 3. Kinetic parameters of decomposition of α-T in inclusion complex with 2-HP-β-CD.

Temp. [K]	(k ± Δk) [s^{-1}]	(a ± Δa) [h^{-1}]	$t_{0.5}$ [h]	$t_{0.1}$ [h]	S_a	r	n
343	$(3.033 \pm 0.175) \cdot 10^{-8}$	$(-1.09 \pm 0.06) \cdot 10^{-3}$	634.7	96.6	$2.67 \cdot 10^{-5}$	–0.998	9
338	$(2.095 \pm 0.089) \cdot 10^{-7}$	$(-7.54 \pm 0.32) \cdot 10^{-4}$	918.9	139.8	$1.39 \cdot 10^{-5}$	–0.998	11
333	$(1.607 \pm 0.121) \cdot 10^{-7}$	$(-5.78 \pm 0.43) \cdot 10^{-4}$	1197.9	182.2	$1.94 \cdot 10^{-5}$	–0.995	11
323	$(9.538 \pm 0.742) \cdot 10^{-8}$	$(-3.43 \pm 0.27) \cdot 10^{-4}$	2018.5	307.0	$1.16 \cdot 10^{-5}$	–0.995	11

k – rate constant of reaction, a – slope, S_a – standard deviation for a, r – correlation coefficient.

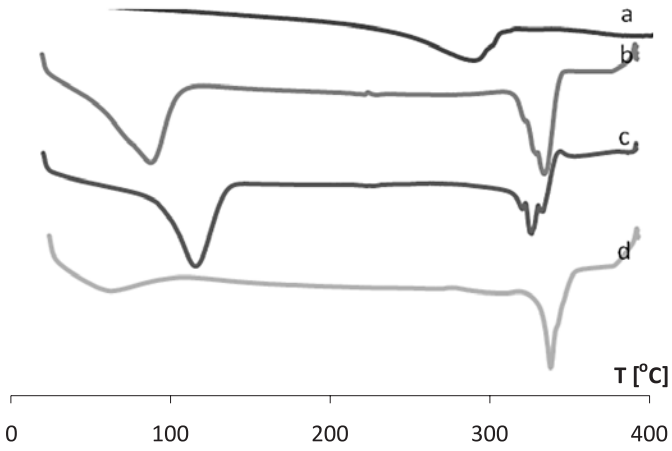

Figure 1. DSC thermograms of: a – α-tocopherol, b – β-CD, c – physical mixture of α-T-β-CD, d – inclusion complex of α-T-β-CD

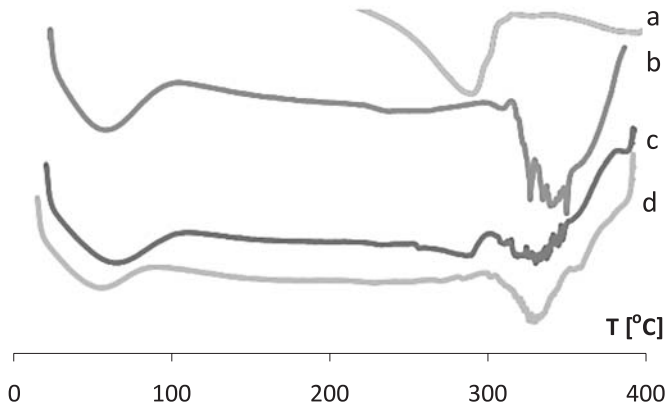

Figure 2. DSC thermograms of: a – α-tocopherol, b – 2-HP-β-CD, c – physical mixture of α-T-2-HP-β-CD, d – inclusion complex of α-T-2-HP-β-CD

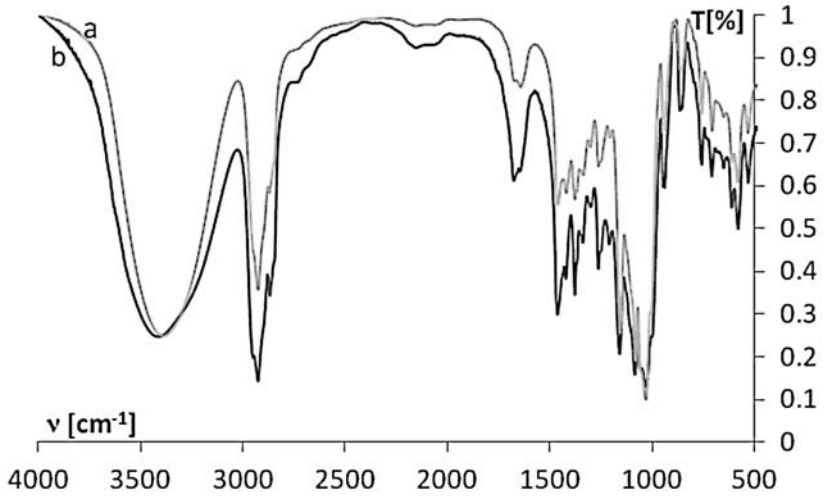

Figure 3. IR spectra of: a – inclusion complex of α-T-β-CD, b – physical mixture of α-T-β-CD

Infinity 1260 Series coupled with triple quadrupole QTRAP 4000 mass spectrometer equipped with electrospray ionization (ESI). LC separation was achieved using a LiChroCart (250 × 4 mm) LiChrospher 100 RP-18 (5 µm) analytical column and a solution of 5.0 mM ammonium acetate in methanol 100% as a mobile phase was used. The flow rate was set at 0.5 mL/min and the volume injected was 10 µL. The column temperature was thermostated at 25°C. The analytes were ionized by a positive electrospray ionization mode. The standard solution of α-T (1 µg/mL) was used for optimization of the mass spectrometer parameters, which was carried out using the commands and protocols incorporated in the instrument software. The final ionization conditions were set at 5.5 kV – capillary voltage, 350°C – desolvatation temperature. Nitrogen was used as both the desolvatation gas (70 psi) and as the cone gas (30 psi). The mass spectra were acquired between 100 and 900 at scan rate of 2 s per spectrum (full-scan mode).

RESULTS

Identification of samples

The products obtained by the kneading and lyophilization methods of α-T with β-CD and 2-HP-β-CD were analyzed by the thermal method (DSC), IR spectroscopy and ^{1}H-NMR spectroscopy. The three identification methods confirmed obtaining the inclusion complexes of α-T with β-CD and 2-HP-β-CD. The results of studies were illustrated graphically in Figures 1–6.

Kinetic parameters of decomposition of α-T and its inclusion complexes with CDs

Kinetic parameters of α-T decomposition in the uncomplexed form and in the inclusion complexes with CD were found from the semilogarithmic plots of $c = f(t)$, where c is the concentration of the substrate at time t. The regression coefficients a and b were calculated by the least squares method. The time after which 10% or 50% of the initial substrate concentration is decomposed was obtained from the formulae $t_{0.5} = 0.693/k$ and $t_{0.1} = 0.1054/k$.

The kinetic parameters of α-T decomposition in the uncomplexed form and in inclusion complexes with CDs are given in Tables 1–3.

Thermodynamic parameters of decomposition of α-T and in inclusion complexes with CDs

The effect of temperature on the stability of substances is described by the Arrhenius equation, illustrated graphically in Figure 7.

On the basis of the rate constants of α-T decomposition, the thermodynamic parameters were calculated and the results were subjected to the statistical analysis. Enthalpy and entropy were calculated from the equations: $\Delta H^{\#} = Ea - RT$ [J/mol]; $\Delta S^{\#} = R\cdot(lnA - ln(kT/h))$ [J/(K·mol)], where k is the Boltzmann constant ($1.3806\cdot10^{-23}$ [J/K]), h is the Planck constant ($6.6262\cdot10^{-3}$ [J·s]), R is the gas constant (8.314 [J/mol/K]), see Table 4.

Products decomposition of α-T in inclusion complexes with CDs

Products decomposition of α-T in inclusion complexes were checked by LC-ESI-MS/MS method in a full scan mode. As illustrated in Figure 8, in the full scan mode the spectrum showed an ion at m/z 431. Moreover, for samples treated with an elevated temperature the spectrum showed an ion at m/z 859. The result is given in Figure 9. The same situation takes place for the second inclusion complex of α-T with 2-HP-β-CD.

DISCUSSION

The inclusion complexes of α-tocopherol and cyclodextrins prepared by the kneading and lyophilization methods were identified by the thermal method (DSC), IR spectroscopy and NMR spectroscopy and obtaining of inclusion complexes was confirmed. The kneading method was ineffective in the case of preparation the inclusion complex with the hydroxypropyl derivative of cyclodextrin.

The thermogram of α-T shows a single endothermic peak at about 268.6°C, which corresponds to decomposition of this compound. The thermograms of β-CD and 2-HP-β-CD show two endothermic peaks at 91.6°C, 329.9°C and 56.6°C, 339.9°C, respectively. The peaks at lower temperatures were assigned to dehydration of cyclodextrins (21), whereas the peaks >300°C correspond to the melting point of the substance. The thermograms of the InCs (Fig 1, 2) do not exhibit a signal at about 268°C, which is assigned to α-T, but show peaks assigned to cyclodextrins shifted towards higher temperatures. This is the main opposite of the thermograms of physical mixtures where the endothermic peak assigned to α-T appears. The changes of the thermograms seem to testify the occurrence of physicochemical interactions of CDs and α-T, accompanied by the formation of inclusion complexes in a solid phase. Similar changes have been observed for substances such as sparfloxacin or tolbutamide (5, 21). The analogical situation takes places for both InCs with β-CD and 2-HP-β-CD.

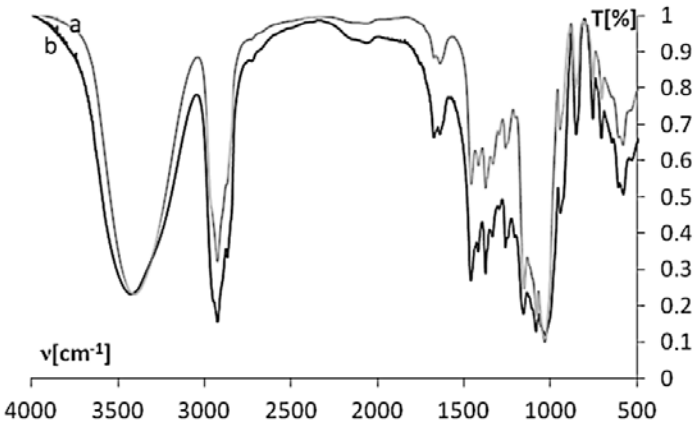

Figure 4. IR spectra of: a – inclusion complex of α-T-2-HP-β-CD, b – physical mixture of α-T-2-HP-β-CD

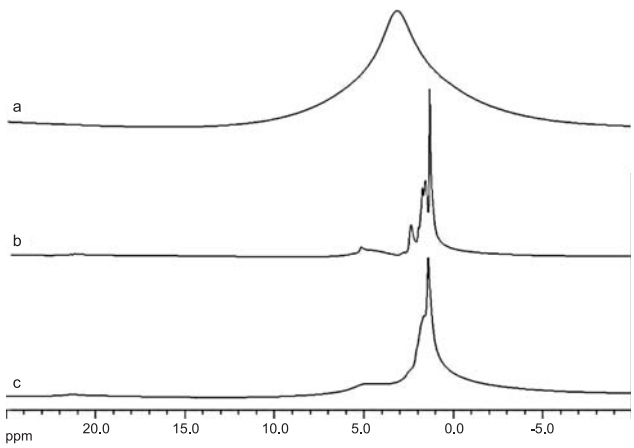

Figure 5. ^{1}H-NMR spectra of: a – β-CD, b – physical mixture of α-T-β-CD, c – inclusion complex of α-T-β-CD

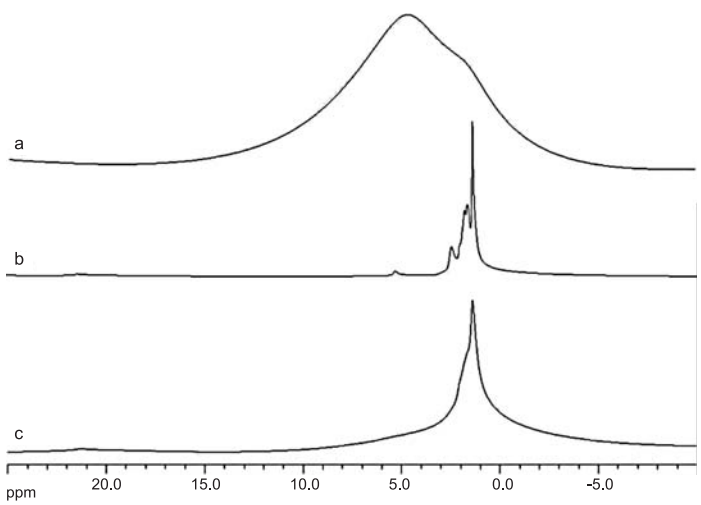

Figure 6. ^{1}H-NMR spectra of: a – 2-HP-β-CD, b – physical mixture of α-T-2-HP-β-CD, c – inclusion complex of α-T-2-HP-β-CD

The IR spectra of all tested substances were standardized to stretching vibrations of the secondary group OH (ν C-O 1038 cm^{-1}) band. The comparison of the inclusion complexes spectra and their physical mixtures (Fig. 3, 4) points to the formation of the hydrogen bond between the phenol OH group of α-T and the primary OH group of CDs. This is confirmed by shifts of the CDs (β-CD, 2-HP-β-CD) bands corresponding to their OH group stretching vibrations in the physical mixtures (3451, 3457 cm^{-1}, respectively) and in inclusion complexes (3442, 3438 cm^{-1}, respectively). The spectra of inclusion complexes exhibit lower intensities of the bands in the ranges corresponding to stretching vibrations of the aliphatic chain (2950-2720 cm^{-1}) and benzopyrene ring (1500–1000 cm^{-1}) of α-T. The disappearance of the bands in these ranges seems to be the result of the tocopherol incorporation into the cyclodextrin cavities, thus confirming these complexes formation for both situations.

More evidence of the complex formation was obtained from ^{1}H nuclear magnetic resonance study in the solid state. The spectra of InCs are very similar to the spectra of pure CDs. Spectra of PhMs of both CDs with α-T exhibit well-resolved five signals at δ 1.39; 1.63; 1.78; 2.45; 5.26 ppm, and at 1.39; 1.64; 1.78; 2.45; 5.31 ppm for mixture with β-CD and with 2-HP-β-CD, respectively. In turn, the spectra of InCs exhibit one singlet at δ 1.39 ppm and two broad defused signals at δ 1.69; 5.02 ppm for InC with β-CD, whereas one broad defused signal at δ 1.39 ppm for InC with 2-HP-β-CD. Interestingly, well resolved signals which appeared in PhMs spectra seem to come from α-T protons that are not involved in the formation of hydrogen bonds, although should not be visible in NMR in solid state studies due to the liquid oil form of α-T. The proposed explanation of this phenomenon is the absorption of α-T on the surface of the CDs resulting in giving dimensions on the spectra of mixtures. Whereas the engagement of CDs and tocopherol protons in interaction results in significant changes in signal multiplicity, only one diffused broad signal is observed in InCs spectra. The disappearance of the signals and their significant overlapping suggest the formation of inclusive complexes between α-tocopherol and β-CD or 2-HP-β-CD. The ^{1}H-NMR spectra are presented in Figures 5 and 6.

The stability of α-T and α-T in inclusion complexes with β-CD and 2-HP-β-CD in the solid state was evaluated on the basis of kinetic and thermodynamic parameters of α-T decomposition at a certain temperature. The decomposition was accompanied by a change in the color from yellow to brown for α-T and from cream to yellow for α-T in InCs.

As follows from the semi-logarithmic character of the time dependence of concentration, the thermal decomposition of α-T, both uncomplexed and in inclusion complexes, can be described by a simple equation of a first order reaction $\ln[C] = [C_0] - kt$, where [C] is the concentration of decomposition products upon heating, $[C_0]$ – the concentration of decomposition products at $t_0 = 0$, t – time of heating, k – the rate constant of a first order reaction. The character of the decomposition reaction was the same as that reported by Chung (22), who studied tocopherol decomposition in temperatures 100–200^{o}C and claimed that it was the first order reaction. The reaction rate constants and activation energies may also indicate the same mechanism of

Table 4. Thermodynamic parameters of α-T and α-T in inclusion complexes with CDs decomposition.

	α-Tocopherol	α-T/β-CD InC	α-T/2-HP-β-CD InC
Statistical evaluation of lnk = f(1/T)			
a ± Δa	–4874.10 ± 770.81	–7207.10 ± 1070.00	–6262.17 ± 1808.57
S_a	179.13	248.66	420.30
b ± Δb	–0.28 ± 2.31	4.92 ± 3.20	3.20 ± 5.41
S_b	0.54	0.74	1.26
r	–0.999	–0.999	–0.996
Thermodynamic parameters			
Ea [kJ/mol]	40.53	59.92	52.07
$\Delta H^{\#}$ [kJ/mol]	38.09	57.49	49.63
$\Delta S^{\#}$ [J/(K·mol)]	–247.14	–203.85	–218.21

lnk – logaritm of rate constant of reaction; a – slope of Arrhenius plot; S_a – standard deviation for a, b – intercept of Arrhenius plot; S_b – standard deviation of b; Ea – activation energy, $\Delta H^{\#}$ –activation enthalpy; $\Delta S^{\#}$ – activation entropy.

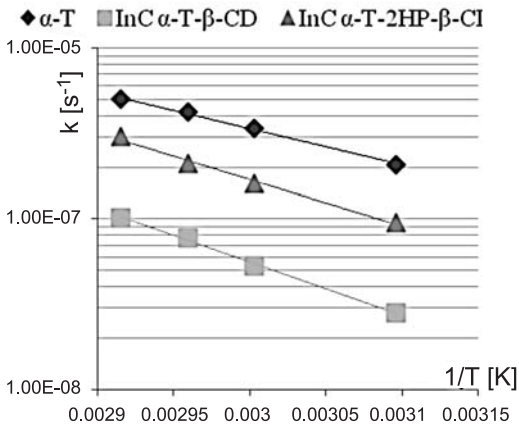

Figure 7. Arrhenius plots of the decomposition of: α-tocopherol, inclusion complex of α-T-β-CD (InC α-T-β-CD), inclusion complex of α-T-2-HP-β-CD (InC α-T-2-HP-β-CD)

α-T uncomplexed and complexed decomposition. The reaction rate constants were found to increase along with temperature, which means that temperature has an important effect on tocopherol decomposition (23). The lower rates constants of α-T decomposition in InCs with CDs than that for α-T indicate that in the solid phase α-T in inclusion complexes is more stable. The comparison of the $t_{0.1}$ and $t_{0.5}$ times for α-T and α-T in complexes with β-CD and 2-HP-β-CD showed that β-CD brings a stronger stabilizing effect, which confirms the values of $t_{0.1}$ time increased about 64% for 2-HP-β-CD and about 400% for β-CD at the highest temperature (Tables 1–3). The results obtained differ from those reported by Cho et al. (24), who studied the stability of tocopherol in the inclusion complex in several solvents e.g., distilled water. These authors reported

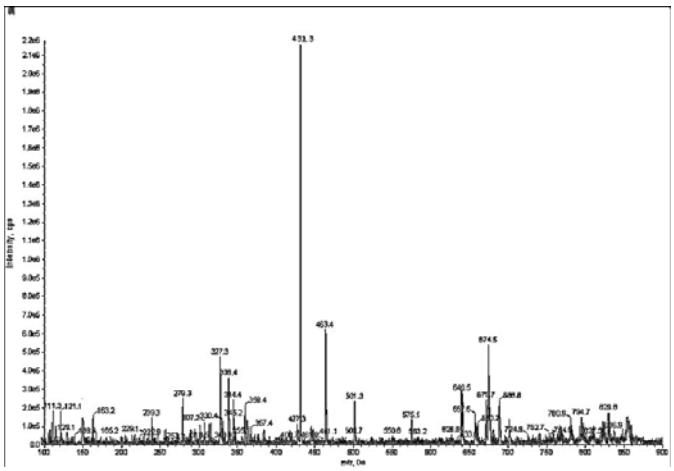

Figure 8. MS spectra of α-tocopherol (m/z 431 Da)

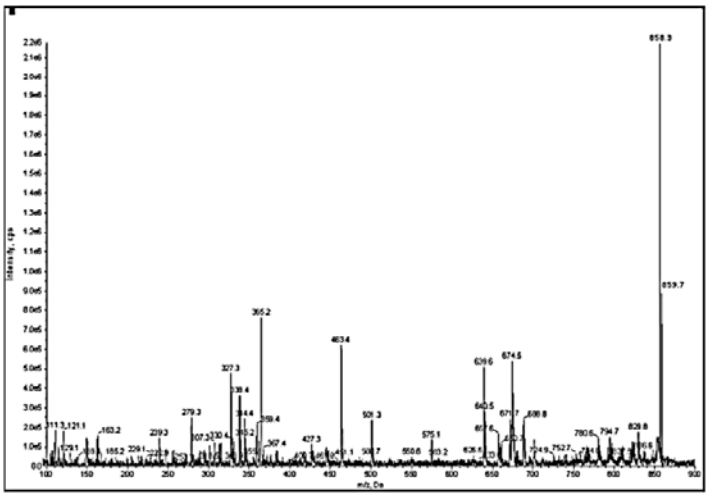

Figure 9. MS spectra of α-tocopherol in inclusion complex after decomposition (m/z 859 Da)

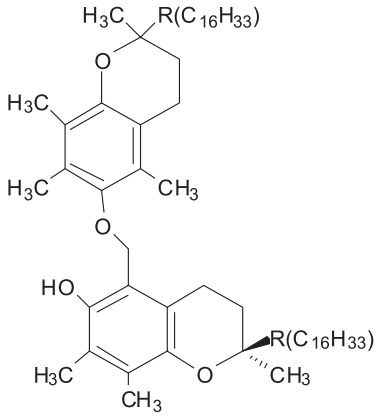

Figure 10. Proposed structure of α-tocopherol dimer

greater stability of tocopherol in the inclusion complex with 2-HP-β-CD than with β-CD. Most likely, the difference related to the greater hygroscopicity of HP-β-CD, which is an advantage from the viewpoint of water solubility but it is a disadvantage from the viewpoint of a given substance stabilization in the solid phase.

On the basis of the reaction rate constants and the Arrhenius relation it was possible to find a dependence between temperature and stability of α-T and α-T in inclusion complexes. This dependence was linear for α-T in InCs with both CDs. The energy of activation and changes in enthalpy and entropy were found from the parameters of the Arrhenius formula $\ln k = \ln A - (Ea/R)\cdot(1/T)$. The activation energy and enthalpy of α-T in inclusion complexes were higher than the corresponding values for the α-T (Table 4), which means that the uncomplexed α-T is more susceptible to temperature. Positive values of $\Delta H^{\#}$ and negative values of $\Delta S^{\#}$ confirm that the reaction of α-T decomposition both uncomplexed and in inclusion complexes is endothermic and the activation energy is greater for α-T in complexes.

Next, the samples of uncomplexed α-T and α-T in InCs with β-CD and 2-HP-β-CD treated with elevated temperature were studied by LC-ESI-MS/MS. The samples of α-T, and α-T in InCs with CDs not subjected to thermal heating were used as standards and the peak was found at m/z 431. However, on spectra of samples subjected to thermal decomposition the peak was detected at m/z 859 and was formed by the connection of two molecules of tocopherol. The same situation takes place for α-T as a substance and α-T in InCs with both CDs. The most probable structure of the dimer is presented in Figure 10 (25).

The results discussed above indicate that the α-T in inclusion complexes with CDs are more stable than the uncomplexed substance. The thermal decomposition of α-T in inclusion complexes with cyclodextrins proceeds according to the first order reaction. Cyclodextrins have a protective effect on α-T in the solid phase, which is higher for natural β-CD and lower for its 2-hydoxypropyl derivatives. The main decomposition product obtained in elevated temperature is α-tocopherol dimer. The improved stability of the tested substance in the obtained form results in new possibilities of a new pharmaceutical form of tablets with α-T.

CONCLUSIONS

1. In a stable phase inclusion complexes of α-T with β-CD and 2-HP-β-CD were produced by kneading and lyophilization. The identification of extracted products was carried out by a DSC, IR and ^{1}H-NMR. The identity of both inclusion complexes has been confirmed.

2. A method of denoting the amount of α-T in inclusive complexes was HPLC, which had been subjected to validation previously. The method turned out to be the most useful.

3. The decomposition of α-T follows according to the simple first order reaction. The kinetic parameters like $t_{0.1}$ and $t_{0.5}$ indicate that cyclodextrins, both β-CD and 2-HP-β-CD, exhibit the protective influence on the α-T. $T_{0.1}$ for temperature 343 K increased by 400% for α-T in InC with β-CD and 64% for 2-HP-β-CD derivative.

4. Both β-CD and its 2-HP-β-CD derivative demonstrate the protective effect for α-T, after its inclusion to complexes, but β-CD effect was significantly greater.

5. The main product of α-T in inclusion complexes decomposition treated with an elevated temperature is α-tocopherol dimer.

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