Solubility of α-Tocopheryl Succinate in Supercritical Carbon Dioxide Using Offline HPLC-MS/MS Analysis

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The solubility of the vitamin E-related compound α-tocopheryl succinate in supercritical carbon dioxide was measured at pressures ranging from (15.0 to 30.0) MPa and temperatures of (40 and 50) °C using a simple microsampling type apparatus with a 100.5 μL sample loop to remove aliquots and collect them in ethanol for off line analysis. α-Tocopheryl succinate concentrations in the collected samples were measured using HPLC-MS/MS analysis. The solubility of α-tocopheryl succinate in supercritical carbon dioxide ranged from mole fractions of 0.28×10^{-5} at 15.0 MPa and 50 °C to 2.56×10^{-5} at 30.0 MPa and 50 °C.

Introduction

α-Tocopheryl succinate has been shown to have selective toxicity and apoptosis-inducing activity toward transformed and cancer cells both in vitro and in animal model experiments, creating interest in its use in preclinical and translational cancer studies.[1−5] α-Tocopheryl succinate inhibits the growth of a wide variety of cancer cell types both in vivo and in vitro.[5−8] These findings, along with evidence from cell culture studies and in vivo studies indicating that α-tocopheryl succinate is not toxic to normal cells, have created interest in examining the possible use of α-tocopheryl succinate alone or in combination with other agents in cancer prevention and treatment.[2]

Prior research has indicated that the relatively low cost, low environmental impact, and tunable solvent properties of supercritical fluid carbon dioxide may make it an advantageous medium for processing a wide variety of substances.[9−12] Effective utilization of supercritical carbon dioxide as a processing solvent is facilitated by measurements of the solubilities of chosen solutes in supercritical carbon dioxide.[9,10,13,14] We have interest in using supercritical carbon dioxide to process α-tocopheryl succinate and study its use as an experimental lung cancer therapy[15−17] and have therefore sought to determine the solubility characteristics of α-tocopheryl succinate in supercritical carbon dioxide. In prior work by Cortesi et al., the solubility of α-tocopheryl succinate in supercritical carbon dioxide was examined,[18] but it was below the detection limit of their study and so was simply estimated to be a mole fraction of less than 10^{-6}. In the present work, solubilities of α-tocopheryl succinate in supercritical carbon dioxide were measured for (40 and 50) °C and (15 to 30) MPa using highly sensitive and selective high-performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS/MS) for quantitation.

Experimental Section

Materials. Solid, crystalline α-tocopheryl succinate (CASRN 4345-03-3, shown in Figure 1) was obtained from Sigma Chemical Co. (St. Louis, MO) and used as received. Pure carbon dioxide (Coleman grade, 99.99 %) was obtained from General Air Service & Supply (Denver, CO). Absolute ethanol was obtained from Aaper Alcohol and Chemical Co. (Shelbyville, KY). All other chemicals were obtained from Sigma.

Apparatus and Procedure. A schematic of the experimental apparatus is shown in Figure 2. It is a microsampling system with a high-pressure sample loop and valve system adapted from the system described by McHugh and Paulaitis[19] and is similar to the apparatus used in many previous reports in which the static sampling strategy has been used.[9,20] Such systems utilize a sample loop to remove small portions of a saturated supercritical fluid solution for analysis. This system was previously validated in our laboratory by determining the solubility of vanillin in carbon dioxide and comparing it to published values. Then it was used to determine the solubility of the sesquiterpene alcohol patchoulol in supercritical carbon dioxide.[21] Briefly, a high-pressure syringe pump (Isco model 260D, Lincoln, NE) was used to pressurize the carbon dioxide (P = 15.0 to 30.0 MPa); the pressures delivered by the syringe pump at each set point were measured using a mechanical Bourdon tube-type gauge rated at ± 0.25 % accuracy (Helicoid Gage, ACCO, Bridgeport, CT), and the precision of the set pressures was verified to be ± 0.5 %. The pressurized carbon dioxide was directed through a coil of 1/16 in. stainless steel tubing as a preheater, contained within a recirculating, temperature-controlled water bath (VWR Scientific model 1131, West Chester, PA), and then into the bottom of a 7.5 mL stainless steel extraction vessel as a saturator (Keystone Scientific, Bellefonte, PA) packed with α-tocopheryl succinate and 3 mm glass beads, oriented vertically, and using glass wool at each end to position the solid solute in the center of the vessel. The fittings on the inlet and outlet of the saturator vessel contained sintered stainless steel frits to act as filters and prevent undissolved solute from being swept into the sample loop. The effluent out the top of the saturator was directed through a nominally 100 μL sample loop (actual volume 100.5 ± 2 μL) attached to a six-port, two-way valve (Rheodyne model 7000, Rohnert Park, CA, referred to here as valve A), and then vented at a low flow rate through an adjustable valve to waste (fill position). The preheater, saturator, and sampling loop were all contained within the circulating bath and maintained at a

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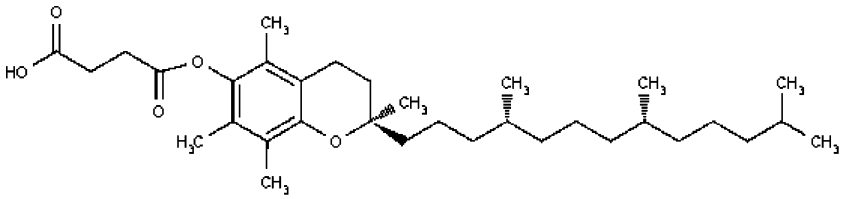

Figure 1. Structure of α-tocopheryl succinate (vitamin E succinate).

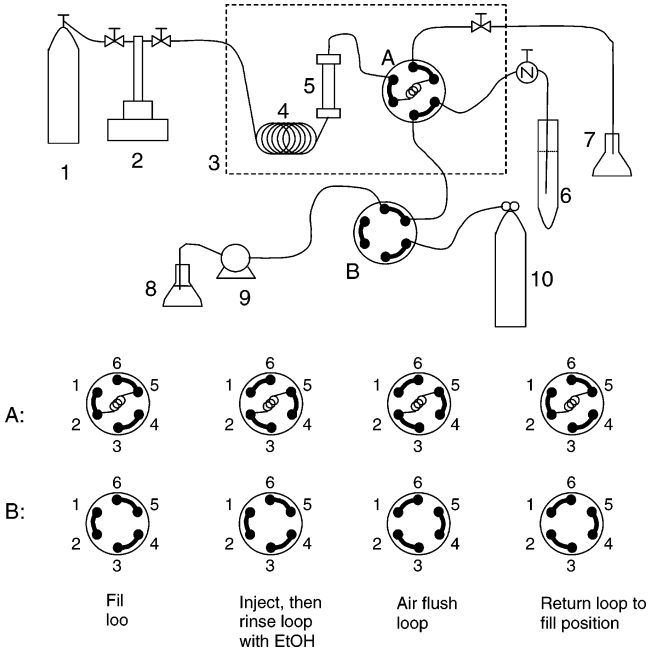

Figure 2. Schematic diagram of the apparatus used: 1, carbon dioxide cylinder with dip tube; 2, syringe pump; 3, thermostated water bath; 4, preheater coil; 5, saturator; 6, off line sample collection tube; 7, waste collection flask; 8, ethanol reservoir; 9, HPLC pump; 10, pressure-regulated air supply; A, six-port valve with sample loop; B, six-port valve.

constant temperature (T = (40.0 or 50.0) °C, calibrated with a Hg thermometer and controlled to within ± 0.5 °C). When filled with saturated solution, the valve was turned to the inject position, which allowed the loop to depressurize through a needle valve into a preweighed collection vial outside of the temperature-controlled bath into which 2 mL of ethanol solvent was added to trap the solute, and then the loop was flushed with additional 3 mL of ethanol into the collection vial using a HPLC pump (Waters model 501, Milford, MA) connected to a second Rheodyne six-port, two-way valve (valve B, rinse position), on which only three of the ports were utilized. The total amount of ethanol solvent used to collect the solute was determined gravimetrically (± 0.0001 g). Before switching valve A back to the fill position for the next sample collection, valve B was switched (air flush position), and the lines and sample loop were flushed with compressed air to remove ethanol otherwise trapped within, and then valve B was switched back to rinse position with flow from the HPLC pump turned off. Three samples were collected at each pressure and temperature combination.

Quantitation of Carbon Dioxide. The carbon dioxide solution densities were estimated to be the same as those for pure carbon dioxide, which has been shown to be accurate for solutions with mole fraction < 1 %.[20,22] To determine the molar amount of solvent contained within the known loop volume, the carbon dioxide densities at each pressure and temperature used were determined using an equation of state.[23] To accomplish this, the online version of software from the U.S. National Institute of Standards and Technology was used (http://webbook.nist.gov/cgi/fluid.cgi?ID=C124389&Action=Page), which allows calculations of the density and other thermophysical properties of carbon dioxide at specified temperatures and pressures.

Quantitation of Solutes. The solute collected in a gravimetrically determined amount of ethanol was quantitated by HPLC-MS/MS. Standard dilutions of α-tocopheryl succinate were prepared from (5 to 100) ng·mL^{-1} in 80 % methanol/20 % water. Quantitative analyses were performed with a PE Sciex 200 Autosampler and HPLC system (Applied Biosystems, Framingham, MA) with detection using a PE Sciex API-3000 triple quadrupole mass spectrometer fitted with a turbo ionspray source to interface with the HPLC. The HPLC system utilized a Luna 5 μm C-18 column, 50 × 2 mm (Phenomenex, Torrance, CA), and the mobile phase was isocratic 98 % methanol. The flow rate was 200 μL·min^{-1}, and the injection volume 20 μL. The mass spectrometer settings were as follows: turbo ion spray temperature, 300 °C; needle voltage, −4000 V; declustering potential (DP), −10 V; focus plate (FP), −80 V; collision energy (CE), −15 V; needle position, 5; and collision gas (N_2) density (CAD), 10. The instrument was operated in the selected reaction monitoring mode (negative ion), monitoring the ion transition from m/z 529.3 to 429.4, depicted in Figure 3. The α-tocopheryl succinate eluted at 4.3 min with a total analysis time of 6 min. The standard curve was generated using peak area of α-tocopheryl succinate, and it was linear over a range of (5 to 100) ng·mL^{-1} with an r^2 of 0.994 (using $1/x$ weighting). The acceptance criteria for the lower and upper limit of quantitation (LLOQ and ULOQ) are that the mean value should have a minimum accuracy of 80 % and minimum precision (% RSD) of 20 %.[24] The LLOQ and ULOQ of this method are 5 and 100 ng·mL^{-1}. Each of the three samples collected at each P and T combination was analyzed by HPLC-MS/MS in duplicate.

Using the α-tocopheryl standards, accuracy and precision were calculated in the common manner using the following equations:

$$\frac{\text{accuracy}}{\%} = 100\cdot\left(1 - \left|\frac{(\text{theoretical} - \text{measured})}{\text{theoretical}}\right|\right)$$

$$\frac{\text{precision}}{\%\ \text{RSD}} = 100\cdot\left(\frac{\text{SD}}{\text{mean}}\right)$$

Accuracies ranged from (80 to 99) %, and precisions ranged from (1.8 to 9.0) %; both were acceptable according to the acceptance criteria above.

Results and Discussion

The microsampling solubility measurement system used for the present study was previously validated by measuring the solubilities of vanillin in supercritical carbon dioxide and was previously used to determine the solubilities of patchoulol in supercritical carbon dioxide at (40 and 50) °C and (10.0 to 25.0) MPa,[21] indicating that the apparatus used in the present study is appropriate for supercritical carbon dioxide solubility determinations of α-tocopheryl succinate, a lipophilic, crystalline solid.

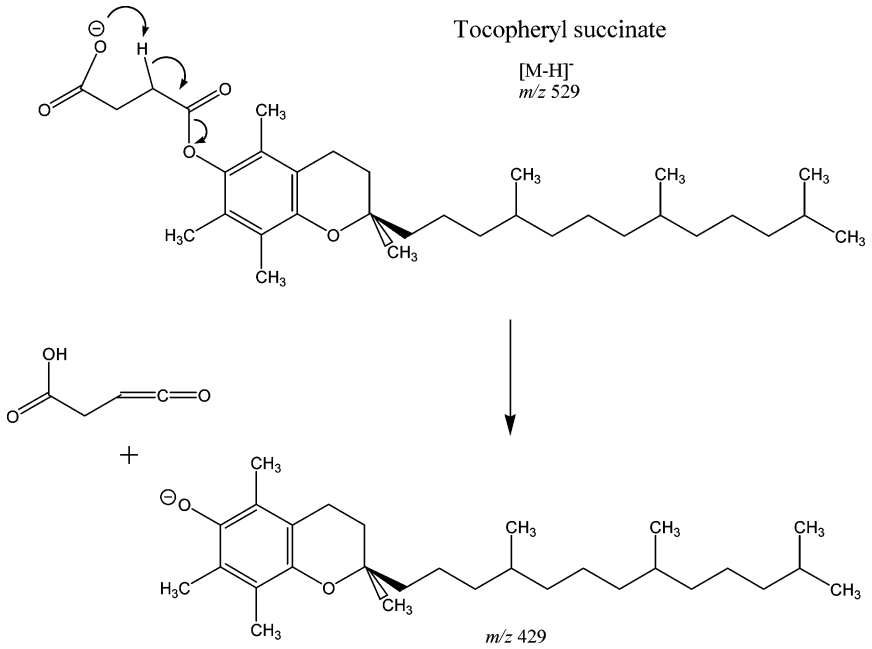

Figure 3. Depiction of the α-tocopheryl succinate $[M - H]^-$ ion and the detected product ion, *m*/*z* 429, generated under the described mass spectrometric conditions.

Table 1. Solubility of α-Tocopheryl Succinate in Supercritical Carbon Dioxide[a]

T/°C	*P*/MPa	ρ/kg•m^{-3}	y ($\times 10^5$) ± SEM	C/mg•L^{-1} ± SEM
40.0	15.0	780.2	0.38 ± 0.02	35.4 ± 1.5
40.0	20.0	839.8	0.91 ± 0.04	92.1 ± 3.7
40.0	25.0	879.5	1.41 ± 0.03	149.9 ± 3.7
40.0	30.0	909.9	2.26 ± 0.12	247.6 ± 12.9
50.0	15.0	699.8	0.28 ± 0.01	23.4 ± 0.8
50.0	20.0	784.3	0.93 ± 0.08	87.6 ± 7.3
50.0	25.0	834.2	1.67 ± 0.02	168.0 ± 2.2
50.0	30.0	870.4	2.56 ± 0.12	268.6 ± 12.5

[a] *T*, temperature; *P*, pressure; ρ, supercritical carbon dioxide density (from ref 23); *y*, mole fraction; and *C*, concentration.

The solubilities of α-tocopheryl succinate in supercritical carbon dioxide are presented in Table 1 and Figure 4. Each point is the average of three separate samples ± the standard error of the mean. The solubility of α-tocopheryl succinate in supercritical carbon dioxide in the conditions tested ranged from mole fractions of 0.28×10^{-5} at 15.0 MPa and 50 °C to 2.56×10^{-5} at 30.0 MPa and 50 °C. As shown in Figure 4, the mole fraction of α-tocopheryl succinate in supercritical carbon dioxide increased with increasing pressure at constant temperature and decreased with increasing temperature at the low-pressure tested (15 MPa), but increased with increasing temperature at the higher pressures tested (25 and 30) MPa, indicating that a crossover point exists at approximately 20 MPa.[25]

In previous work, Cortesi, et al. attempted to measure α-tocopheryl succinate solubility in supercritical carbon dioxide by using a dynamic system and determining collected solute amounts gravimetrically.[18] They determined that the α-tocopheryl succinate solubility was too close to their detection limit to report and instead estimated a mole fraction solubility of less than 1×10^{-6} in supercritical carbon dioxide under conditions of (35 to 60) °C and (13 to 25) MPa.[18] The present work indicates mole fraction solubilities for α-tocopheryl succinate in supercritical carbon dioxide that are several-fold higher than this previous estimate of $< 1 \times 10^{-6}$ with similar pressure and temperature conditions, probably due to the use of a more sensitive detection system.

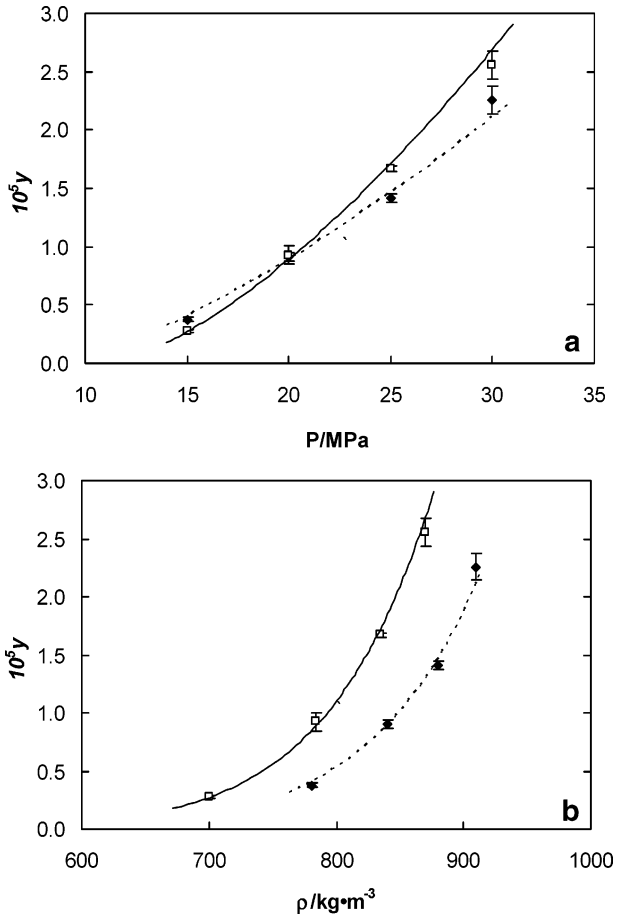

Figure 4. Solubilities of α-tocopheryl succinate in carbon dioxide plotted as a function of applied pressure (a) and as a function of the supercritical carbon dioxide density (b). Experimental: ◆, 40 °C; □, 50 °C; — and - - -, calculated by eq 3, the Mendez-Santiago−Teja model. The error bars on the plotted points are the SEMs of the experimental data.

A solubility correlation model proposed by Mendez-Santiago and Teja was used to correlate the experimental solubility data,[26] as shown in Figure 5. The model utilizes a simple

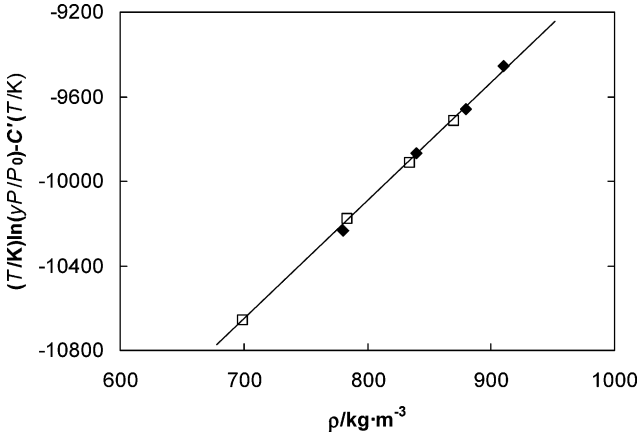

Figure 5. Correlation of the solubility of α-tocopheryl succinate in carbon dioxide at (40 and 50) °C by the method of Mendez-Santiago−Teja.[26] Experimental: ◆, 40 °C; □, 50 °C; —, best-fit line to the experimental data.

linear expression to correlate the solubility of solids in supercritical fluids according to the following equation, modified according to Hansen et al., to include a reference pressure P_0 = 1 MPa to make the argument of the natural logarithm dimensionless:[27]

$$T \ln\left(\frac{yP}{P_0}\right) = A' + B'\rho + C'T$$

where A', B', and C' are constants that are independent of the system temperature T, y is the mole fraction of the solute in supercritical carbon dioxide, P is the pressure of the system, and ρ is the density of the carbon dioxide. This modeling approach has proven to be effective for fitting experimental results in several recent studies.[27−29] In the present work, it can be seen that the model fits the experimental data well (Figure 5), independent of temperature, with multiple linear regression yielding A' = −14633 K, B' = 5.665 K·m^3·kg^{-1}, and C' = 22.902 (with units for T in K, P in MPa, and ρ in kg·m^{-3}). As usual, the modeling results should not be extrapolated beyond the range of experimental conditions applied in the study. The difference between the measured experimental mole fraction solubilities (y_{meas}) and the calculated mole fraction solubilities (y_{calc}) according to the Mendez-Santiago−Teja model have an average absolute relative deviation (AARD) of 4.16 %, calculated according to

$$\frac{\text{AARD}}{\%} = \left(\frac{100}{n}\right)\sum\left|\frac{(y_{calc} - y_{meas})}{y_{meas}}\right|$$

Conclusions

The solubilities of α-tocopheryl succinate in supercritical carbon dioxide were determined at (40 and 50) °C in the pressure range of (15.0 to 30.0) MPa. The observed solubilities ranged from mole fractions of 0.28 × 10^{-5} at 15.0 MPa and 50 °C to 2.56 × 10^{-5} at 30.0 MPa and 50 °C, with close fit to the solubility correlation model proposed by Mendez-Santiago−Teja. Under these experimental conditions, the α-tocopheryl succinate solute was a crystalline solid and the mole fraction in supercritical carbon dioxide increased with increases in pressure at constant temperature and increased with increases in temperature at constant pressure for pressures above 20 MPa. The results support the idea that supercritical carbon dioxide may be useful for the processing of α-tocopheryl succinate.

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