INVESTIGATION OF THE THERMODYNAMIC PROPERTIES OF THE BINARY SYSTEM VITAMIN K₃/CARBON DIOXIDE

Article Highlights
- Solubility of vitamin K₃ in CO₂ as a function of T and P was measured
- Data at temperatures of 40, 60 and 80 °C and pressures up to 40 MPa are given
- Partial molar volumes were determined by a vibrating tube densitometer
- Partial molar volumes of vitamin K₃ in CO₂ are large and negative
- Solubility data correlation is performed by a density-based model

Abstract
The binary system of vitamin K₃ and CO₂ was investigated at temperatures of 40, 60 and 80 °C up to a pressure of 40 MPa. Solubility was measured by a static-analytic method. Partial molar volumes were determined by a method involving a vibrating tube densimeter. The solubility of vitamin K₃ in CO₂ is found as a function of pressure and temperature. The highest solubility ($3.16 \times 10^{-4}$ mol·mol⁻¹) was attained at a pressure of 25.40 MPa at temperature of 40 °C. However, at temperature of 60 °C and a pressure of 24.02 MPa, the solubility was $18.79 \times 10^{-4}$ mol·mol⁻¹. Solubility was lower at a temperature of 80 °C and a pressure of 22.06 MPa ($6.48 \times 10^{-4}$ mol·mol⁻¹). The partial molar volumes are negative and the dissolved vitamin K₃ has a minor impact on the density of the solution of K₃ in CO₂ compared to the density of the pure CO₂.

Keywords: solubility, density, partial molar volume, food industry, food processing.
The knowledge of thermodynamic properties of an investigated system is fundamental from the economic point of view, since the solubility is the critical parameter that controls the feasibility of the process.

Two different authors report solubility of vitamin $K_3$ in CO$_2$ [7,8]. In the present work, in addition to the solubility of the vitamin $K_3$ in carbon dioxide, the partial molar volumes and the density of the vitamin dissolved in the supercritical fluid are presented, which to our knowledge has not yet been investigated.

Despite the importance of these data, there is still a lack of knowledge on the interactions between the solute and solvent. Interactions between molecules are described by partial molar volumes and are important for applications of supercritical fluid technology in industry [9].

**EXPERIMENTAL**

**Materials**

Nitrogen with purity 99.999 wt.% and carbon dioxide with purity 99.995 wt.% were obtained from Messer (Slovenia). Vitamin K$_3$ (Menadione) with catalogue number CAS 58-27-5 and absolute ethanol ($\geq$99.8 wt.%) were obtained from Sigma-Aldrich. Milli-Q water was used for the calibration of the equipment. The substances were used without further purification. Vitamin K$_3$ and Milli-Q water were degassed before application.

**Methods**

**Vibrating tube densimeter**

A vibrating tube densimeter was used for density determination. The main part of the densimeter is the Anton Paar DMA 512 unit, which is connected to the DMA 60 unit. The interior of the DMA 512 unit was thermostated with a thermostatic bath. Inside the DMA 512 unit the U-tube was thermostated with a circulating water bath with an accuracy of $\pm 5 \times 10^{-3}$ K and the temperature inside the U-tube was measured with a GTH 1150 digital thermometer NiCr-Ni with accuracy 1%. The autoclave and the U-tube were equipped with a manometer Nuova Fima EN837-1 with an accuracy of 0.25% for pressures lower than 60 MPa.

The apparatus was validated by measuring solubility of systems of substances for which data is available in literature [9]. The knowledge of thermodynamic properties of an investigated system is fundamental from the economic point of view, since the solubility is the critical parameter that controls the feasibility of the process.

Two different authors report solubility of vitamin $K_3$ in CO$_2$ [7,8]. In the present work, in addition to the solubility of the vitamin $K_3$ in carbon dioxide, the partial molar volumes and the density of the vitamin dissolved in the supercritical fluid are presented, which to our knowledge has not yet been investigated.

Despite the importance of these data, there is still a lack of knowledge on the interactions between the solute and solvent. Interactions between molecules are described by partial molar volumes and are important for applications of supercritical fluid technology in industry [9].

**Determination of the vibration period and sampling procedure**

Before each measurement the entire apparatus was properly cleaned with ethanol and pressurized with CO$_2$ and N$_2$. The autoclave was filled with a sufficient amount of vitamin K$_3$, approximately 5 g, connected to the densimeter (Figure 1a).

After the components were connected, the entire system was heated to the desired temperature by the thermostatic baths and electric wires. Mixing was performed using a magnetic stirrer. After opening the valves, the hand pump was set to the minimum volume and the whole system was depressurized with a vacuum pump. Then, CO$_2$ was introduced into the system to access the desired pressure. After a one-hour wait for equilibrium, the stirring was stopped and an additional hour was required for the sedimentation of vitamin K$_3$ as it was experimentally determined. Valve V2 was then closed and the hand pump (Nova Swiss piston pump) was turned to the direction of its maximal volume, which enabled the transport of the saturated carbon dioxide with vitamin K$_3$ to the densimeter. A pressure drop occurred.

The equipment and the method are described in literature [9], but due to the pressure drop and in order to ensure saturation of CO$_2$ with vitamin K$_3$, a modification of the apparatus and measuring procedure was made.

When valve V1 was closed, valve V2 was opened and the hand pump was turned back to the beginning position to its minimal volume and the fluid with saturated vitamin K$_3$ was returned back into the autoclave to the starting conditions (Figure 1a). Then, the stirring was restarted, the equilibrium was established and the circulation of the mixture was repeated. After the third equilibrium establishment and fluid circulation, the vibration period of the fluid saturated with vitamin K$_3$ was measured with the DMA 60 unit. With further equilibrium establishment the mole fraction of vitamin K$_3$ in the mixture was constant. Fluid density was calculated from the vibration period. After determining the vibration period, the valves V6 and V7 were closed (the pressure increased for approximately 0.2 MPa), the pipeline between the valves V2 and V7 was removed and a sampling system was connected (Figure 1b). About 0.1 mL sample was taken through the sampling valve V7 into a trap with ethanol where the vitamin was solubilised. The expanding valve was purged with 5-7 mL of ethanol. The amount of CO$_2$ was measured with a disposal of water in a graduated cylinder. The content of vitamin K$_3$ in the sample was determined with a Varian 50 probe UV/Vis spectrophotometer at a wavelength of 251 nm.
Density determination with a vibrating tube densimeter

The most recent method for the determination of partial molar volume is the densimeter [10,11]. A vibrating tube densimeter is basically a spring-mass system in which the frequency of vibration of the tubing is measured (vibration period) and related to the fluid density. Eq. (1) was applied for calculating the density ($\rho$/kg·m$^{-3}$):

$$\rho = K \left( \tau^2 - \tau_1^2 \right) + \rho_1$$  \hspace{1cm} (1)

where $K$ (kg·s$^{-2}$·m$^{-1}$) is the characteristic parameter of the U-tube, $\tau$ (s$^{-1}$) the vibration period of the U-tube when filled with a fluid of an unknown density, $\rho_1$ (kg·m$^{-3}$) is the density of reference fluid 1 and $\tau_1$ (s$^{-1}$) the vibration period of the U-tube is filled with reference fluid 1.

Previously, the characteristic parameter $K$ has to be determined by Eq. (2) at each temperature and pressure by measuring the vibration period of two reference fluids of known densities:

$$K = \frac{\rho_1 - \rho_2}{\tau_2^2 - \tau_1^2}$$  \hspace{1cm} (2)

The main disadvantage of this calibration procedure is that it is time consuming. Moreover, it is recommended that the difference between the density fluids is lower than 500 kg·m$^{-3}$ and in cases when the densities of the reference fluids are not “well known” this is not the appropriate calibration method [12]. Therefore, new calibration processes with important limitations were developed to simplify the procedure, as reported by the authors [13].

In the present research, the reference fluids were Milli-Q water and nitrogen. The density data at different temperatures and pressures for calibration fluids are well known and were obtained from literature [14].

Solubility maximum

The existence of a maximum concentration of solids in supercritical fluids was observed before [6,15–17], and is of great importance, especially when employing supercritical fluids for extraction of solid...
compounds [16]. The concentration extrema of solid-
fluid phase equilibria can be derived as:

\[
\ln y_2^o = \frac{1}{RT} \int_{\rho_2}^{\rho} (V^o_{m,2} - \bar{V}_2^o) d\rho
\]

(3)

where \( y_2^o \) is the mole fraction of the low-volatility component 2 in the fluid phase [16]. The solubility extremum (minimum and maximum) is a function of pressure can be related to the partial molar volume \( (\bar{V}_2^o ) \) of the solute (component 2) in the supercritical phase (component 1). When the molar volume of the pure solid component \( V_{m,2} \) equals the partial molar volume of the dissolved solid in the fluid phase \( (V^o_{m,2} - \bar{V}_2^o) \) the concentration extrema occurs [16,17].

Partial molar volumes

The partial molar volumes \( \bar{V}_2^o \) \((m^3\cdot mol^{-1})\) were calculated after determining the densities of the system of vitamin K\(_3\) in \( \text{CO}_2 \) by the vibrating tube densimeter according to the following equation [9]:

\[
\bar{V}_2^o = \frac{-1}{\rho_{K_3/CO_2}} \left( M_2 - V_m \frac{n_2}{(n_1 + n_2)^2} \frac{d\rho}{dV_2^o} \right)
\]

(4)

where \( \rho_{K_3/CO_2} \) \((g\cdot cm^{-3})\) is the density of the binary system of \( \text{CO}_2 \) and vitamin \( K_3 \) and \( V_m \) \((cm^3\cdot mol^{-1})\) is the volume of the mixture. \( M_2 \) \((g\cdot mol^{-1})\) is the molar mass of vitamin \( K_3 \), \( n_1 \) and \( n_2 \) the number of moles of \( \text{CO}_2 \) and vitamin \( K_3 \) and \( y_2^o \) the mole fraction of vitamin \( K_3 \).

Partial molar volumes were calculated from the slope of the linear function of densities versus the mole fraction of vitamin \( K_3 \) that was plotted for the data up to the maximum solubility of vitamin \( K_3 \) in \( \text{CO}_2 \). Therefore the results of partial molar volumes are presented to the pressure of the maximum solubility (approximately 25 MPa).

Solubility data correlation

The solubility data have been correlated with a simple density model (\( \log y_2 \) against \( \log \rho \)). Solubility of a crystalline solute in a supercritical fluid as a function of solvent density is expressed by [18]:

\[
\log y_2 = C_0 - \theta \log \rho
\]

(5)

where \( y_2 \) \((mol\cdot mol^{-1})\) is the molar fraction of \( K_3 \) in supercritical fluid and \( \rho \) \((mol\cdot m^{-3})\) [14] is the density of the pure supercritical fluid, \( C_0 \) is a constant and \( \theta \) is the slope of the curve of \( \log y_2 \) as a function of \( \log \rho \).

Thermal analysis

The melting points and the degree of crystallinity based on melting points and the change in enthalpy were determined for vitamin \( K_3 \) before and after exposure to \( \text{CO}_2 \) with a Mettler Toledo DSC 20 apparatus with a temperature step of 5 K/min as:

\[
cr(\%) = 100 \frac{\Delta H_{m2}}{\Delta H_{m1}}
\]

(6)

The crystallinity degree of vitamin \( K_3 \) before and after exposure to \( \text{CO}_2 \) was compared. \( \Delta H_{m1} \) is the enthalpy of fusion of vitamin \( K_3 \) before exposure and \( \Delta H_{m2} \) the enthalpy of fusion after exposure to \( \text{CO}_2 \).

RESULTS AND DISCUSSION

Solubility comparison

The solubility data are presented in Figure 2 (and Tables under Supplementary data available from corresponding author upon request). Compared to literature values [7,8], the measured solubilities of vitamin \( K_3 \) in \( \text{CO}_2 \) obtained in the present research are on the same order of magnitude, however they are somewhat lower, probably as a consequence of the different methods used for the solubility determination [7].

The solubility difference (at the solubility maximum) between the data obtained in the present work and Knez-Škerget and Johannsen-Brunner is: at 40 °C the solubility is for 23% lower compared to Knez and Škerget and 52% lower compared to Johannsen and Brunner. At a temperature of 60 °C the solubility measured in the frame of our research is for 44% lower compared to the one of Knez and Škerget (Figure 3), and at a temperature of 80 °C and at pressure of 23 MPa for 64% lower compared to the one of Knez and Škerget (Figures 4 and 5). The discrepancy is increasing with temperature, probably due to the solubility decrease (due to the lower solvent power of \( \text{CO}_2 \)) and therefore a higher discrepancy in solubility calculations.

The non-polar \( \text{CO}_2 \) requires high pressures to dissolve even small amounts of high-molecular-mass compound [19] like vitamin \( K_3 \) and therefore sampling of low concentrated sample is difficult [7]. One of the reasons for lower solubility compared to literature could be the pressure decrease while circulating the saturated fluid with vitamin through the circuit into the vibrating U-tube. During expansion of the fluid with vitamin \( K_3 \), the equilibrium was probably disturbed and some amount of the vitamin precipitated. After the expansion probably compression took place, and it is assumed that the entire amount of precipitated vitamin was not completely dissolved in \( \text{CO}_2 \). The solubility has a clear trend (Figure 2) with some deviations, probably due to the fact that the depressoris-
Figure 2. Molar fraction ($y_2^\star$) of vitamin K$_3$ in vitamin K$_3$/CO$_2$ solution at 40 °C and comparison to the literature of Johannsen and Brunner, 1997 [7] and Knez and Škerget, 2001 [8].

Figure 3. Molar fraction ($y_3^\star$) of vitamin K$_3$ in vitamin K$_3$/CO$_2$ solution at 60 °C and comparison to the literature of Knez and Škerget, 2001 [8].

Figure 4. Molar fraction ($y_3^\star$) of vitamin K$_3$ in vitamin K$_3$/CO$_2$ solution at 80 °C and comparison to the literature of Knez and Škerget, 2001 [8].
The solubilities of vitamin K3 in supercritical CO2 in the presented research reach a maximum at temperatures of 40 and 60 °C. The isotherm at temperature of 40 °C is higher than at 60 °C and at 60 °C the isotherm is higher than at a temperature of 80 °C (Figure 2). The solubility decreases with increasing temperature. Generally, the solubility behaviour is influenced by two effects: the decrease of solvent power of the fluid with increasing temperature due to the decreasing density and the increase of solubility due to the increasing vapour pressure of the substance with increasing temperature [7]. In the present research, the decrease of solvent power dominates over the increasing vapour pressure of vitamin K3.

Another explanation for a solubility difference compared to literature could be the structure of the vitamin K3. Vitamin K3 was added into the autoclave in a crystalline and a powderous form. After equilibrium was established, the supercritical CO2 saturated with vitamin K3 was circulated through the pipelines to the U-tube. During this transport through the pipelines, by means of a hand pump, first a pressure drop occurred and small amounts of the vitamin probably precipitated, leading to change in the morphology of the solid (e.g., degree of crystallinity), resulting in the change of the amount of vitamin dissolved in CO2. The crystallinity degree, determined in air atmosphere by DSC, changed for 16%.

The melting points of the two previously mentioned forms of vitamin K3 (before and after it was exposed to CO2 in the densimeter) were measured by a gas pycnometer (Micromeritics, AccuPyc II 1340) at 294 K. The measured data are available under supplementary data).

**Solubility data correlation**

The density-based model (log y2 against log ρ) is normally used for solubility data that increase with pressure. The determined constants of the model for the vitamin K3/CO2 solutions and the AARD (%) of the model from the data are given in supplementary data).

**Method comparison**

In the literature [7,8], vitamin K3 with the same purity as in this study (98%) was used. Furthermore, CO2 with 99.995% purity was used in the present work and in the research of Knez and Škerget, while Johannsen and Brunner used CO2 with purity of 99.95% and a static analytical method with direct coupling of an equilibrium cell to a supercritical fluid chromatographic system as described in literature [20]. In addition, Knez and Škerget also used a static analytic method but with a different sampling method with a glass trap, and the samples taken from the autoclave were analysed on UV/Vis spectrophotometry [8, 21]. The results of the present research were also obtained by a static analytic method and a sampling method similar to the one of Knez and Škerget. The difference between all three methods is the sampling, consequently the pressure drop due to the sampling, and the analysis method. Also other authors reported differences in solubilities between different researches for the same system [22]. A small difference in the purity or in the protocol can provide high deviations - even more than one order of magnitude, as reported in literature [23]. A detailed description of experimental methods for phase equilibria at high pres-
sures with their specific advantages and weaknesses can be found in the literature [24,25].

Density of solution of vitamin K₃ in CO₂

The interesting aspect of supercritical fluids is their microscopic inhomogeneity, clustering and the most well-known are the long-range density fluctuations near the critical point, which can be studied by the partial molar volume. The partial molar volume measurements can explain the unusual behaviour of solutes in supercritical fluids [26]. Much research of partial molar volumes has already been done and there are many reports that indicate a large negative partial molar volume, which are dependent on particle size, shape, interaction strength, polarity and on interactions between solute-solvent and solvent-solvent molecules [26].

The densities of vitamin K₃/CO₂ solutions are presented in Figure 6 (and in Tables under Supplementary data). By comparing the densities of CO₂/vitamin K₃ solutions to the pure CO₂ densities [14] (Figure 6), it is clear that the dissolved amount of vitamin in the CO₂ has a minor impact on the density of the fluid.

With increasing temperature at constant pressure, the density of the fluid with the dissolved solid compound decreases and it increases with increasing pressure at constant temperature. In the present study, the density steps where the densities increased the fastest took place at following conditions: from pressure of approximately 7 MPa to pressure of approximately 13 MPa at temperature 40 °C, from pressure of approximately 9 MPa to pressure of approximately 21 MPa at temperature 60 °C and from pressure of approximately 10 MPa to pressure of approximately 23 MPa at temperature 80 °C.

Partial molar volumes and solubility maximum

The molar volume of the pure solid compound (vitamin K₃) was calculated from:

$$V_{m_2}^n = \frac{M_2}{\rho_2^s}$$

where $\rho_2^s$ (g∙cm⁻³) is the density of the solid vitamin K₃ determined at room temperature of 294 K and atmospheric pressure with the helium pycnometer (Supplementary data), $M_2$ (g∙mol⁻¹) is the molar mass of vitamin K₃ and is 172.18 g∙mol⁻¹. The molar volumes of the pure solid vitamin K₃ (determined with Eq. (7)) were converted to the unit 10⁻³ m³∙mol⁻¹ and are: $V_{m_2}^n$ (before exposure to CO₂) = 0.1288×10⁻³ m³∙mol⁻¹ and $V_{m_2}^n$ (after exposure to CO₂) = 0.1317×10⁻³ m³∙mol⁻¹. The difference between the vitamin before and after exposure to CO₂ is approximately 2 % and the average of $V_{m_2}^n$ before and after exposure to CO₂ was considered as $V_{m_2}^n$ and is 0.13×10⁻³ m³∙mol⁻¹.

Figure 7 (and Tables under Supplementary data) shows that all partial molar volumes at all investigated temperatures and pressures are negative. Below and near the critical point, the partial molar volumes are large and negative, which indicates that the clustering of the solvent molecules (CO₂) is a consequence of the strong attractive forces between solvent and solute particles [11]. The compressibility of supercritical solutions is high and they have large free volumes,
therefore solvent molecules move into energetically favourable locations and form clusters around the solute molecule [9]. In the supercritical region, the local density increases (due to the solvent molecules around the solutes) and a divergence of density fluctuations appears [11]. In literature, similar conclusions were made for other systems with a similar or a different partial molar volume determination methods [4,9,27-29].

Partial molar volume of vitamin K3 in CO2 decreases with increasing temperature at isobaric conditions (Figure 4). The partial molar volume at pressure of 0.34 MPa and at temperature 80 °C has an extremely negative value and is not represented in the figure.

After comparing the calculated \( V_{m,2}^{\infty} \), which is \( 0.13 \times 10^{-3} \text{ m}^3\text{mol}^{-1} \), with the absolute value of \( V_{m,2}^{\infty} \) at maximum concentration it is seen that at 40 °C the \( V_{m,2}^{\infty} \) is \( 2.45 \times 10^{-3} \text{ m}^3\text{mol}^{-1} \), at 60 °C the \( V_{m,2}^{\infty} \) is \( 19.40 \times 10^{-3} \text{ m}^3\text{mol}^{-1} \) and at 80 °C and pressure of 22.06 MPa the \( V_{m,2}^{\infty} \) is \( 59.54 \times 10^{-3} \text{ m}^3\text{mol}^{-1} \). The calculated partial molar volume depends on the solubility of vitamin K3 in CO2. The differences could be a consequence of the precipitation of vitamin K3 during the fluid circulation period, which is a result of pressure drop. Furthermore, for calculations of the \( V_{m,2}^{\infty} \) the density of the pure vitamin should be measured at the experimental temperatures 40, 60 and 80 °C by a pycnometer equipped with a heater. The inaccuracy for partial molar volume of vitamin K3 in CO2 is considerable. This was well expected due to the fact that partial molar volumes were calculated after determining densities of the system according to Eq. (4). Each segment of the equation (except constant values such as \( M_2 \) - molar mass of vitamin K3) contributes to the total inaccuracy. It is assumed that inaccuracy of density measurements is lower than 0.5% (for all isotherms), the one of the volume of the mixture is lower than 1%, amounts of moles of CO2 and vitamin K3 (\( n_1 \) and \( n_2 \)) were determined gravimetrically with an inaccuracy of approx. 1% (the values are considered by a square function, therefore the value is multiplied by two). Slope of the linear function of densities versus the mole fraction of vitamin K3 that was plotted for the data up to the maximum solubility (\( d\rho/dy_2^2 \)) contributes highest value to the total inaccuracy which ranges up to 10%. If the total inaccuracy is evaluated as a summary of uncertainties of each variable of the equation, it is estimated to be around 14.5%.

**CONCLUSIONS**

Solubility is a function of temperature - with increasing temperature the solubility of vitamin K3 in CO2 decreases at isobaric conditions due to the lower density of the supercritical fluid and lower solubility power. The calculated partial molar volumes of vitamin K3 in CO2 are large and negative, especially before and near the critical point, which indicates major interactions between the supercritical fluid and vitamin molecules. The calculated partial molar volume of the solid in the supercritical fluid at the maximum solubility point is lower than the molar volume of the pure solid compound. However, the results correspond quite well, namely that at maximum solubility the partial molar volume of the pure solid compound is equal to the partial molar volume of the solid compound in dissolved in the solvent, at temperature of 40 °C. Comparing the solubility data to literature shows that different authors report different solubility data for the same binary system differ due to different experimental equipment and analytical methods. It is appropriate to apply the density of the mixture vitamin K3/CO2 in density based models or equations of state, due to the small difference between density of pure
Acknowledgements

Special thanks to Janko Trupej for the grammatical corrections of this article, to Marko Krainer and Igor Krmelj for the technical support, to Aljana Petek and Darja Pečar for helping with the theoretical part, to the Slovenian Research Agency (ARRS) and to the research programme group P2 - 0046: Separation processes and production design for the financial support.

REFERENCES


TERMODINAMIČKA SVOJSTVA BINARNOG SISTEMA VITAMIN K3/UGLJEN-DIOKSID

Binarni sistem vitamina K3 i CO2 je analiziran na temperaturama 40, 60 i 80 °C i pritisku do 40 MPa. Rastvorljivost je merena statičko-analitičkom metodom. Delimične molske zapremine određene su metodom koja uključuje denzitometar sa i vibrirajućom cevi. Rastvorljivost vitamina K3 u CO2 je određena kao funkcija pritiska i temperature. Najveća rastvorljivost (31,16 × 10-4 mol∙mol-1) je postignuta pri pritisku 25,40 MPa na temperaturi 40 °C, dok je na temperaturi 60 °C i pritisku 24,02 MPa, rastvorljivost iznosila 18,79 × 10-4 mol∙mol-1. Rastvorljivost (6,48 × 10-4 mol∙mol-1) je bila niža na temperaturi od 80 °C i pritisku od 22,06 MPa. Parcijalni molski zapremine su negatивne, a rastvoreni vitamin K3 ima mali uticaj na gustinu rastvora K3 u CO2 u poređenju sa gustinom čistog CO2.

Ključne reči: rastvorljivost, gustina, parcijalna molska zapremina, prehramba, industrija, prerada hrane.