DETERMINATION OF β-CAROTENE SOLUBILITY IN SUPERCRITICAL CARBON DIOXIDE BY A PIEZOELECTRIC QUARTZ CRYSTAL

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Solubility data of β-carotene is fundamental for any process application and design of supercritical fluid extraction systems. A quartz crystal microbalance was used to measure β-carotene solubility in supercritical carbon dioxide. This technique uses a piezoelectric quartz crystal and a high-pressure vessel to determine very low solubilities of thermally labile compounds at supercritical conditions. First, the behavior of the quartz crystal under supercritical CO_2 was analyzed. Then, β-carotene solubility was measured by observing the crystal's frequency change as the β-carotene dissolved in the supercritical fluid. Solubility data are presented at 313 K and pressures ranging from 80 to 200 bar.

1. Introduction

β-Carotene is a lipid-soluble compound of high molecular weight (536.9g/mol), composed of a highly unsaturated hydrocarbon chain ($C_{40}H_{56}$). It is easily degraded by light, heat and air, and has a color ranging from yellow to dark red. The main natural sources of β-carotene are palm oil, carrots, algae and fungi; however, the bulk of its production is by synthetic means. β-Carotene is an important functional food ingredient due to its antioxidant, pro-vitamin A and anti-carcinogenic properties [1].

The study of the solubility behavior of β-carotene in supercritical fluids (SCFs) is of great importance for a wide variety of applications and their use at an industrial scale. To date, limited solubility data have been reported for β-carotene. Sakaki [2], Skerget et al. [3], and Subra et al. [4] have studied β-carotene solubilities in supercritical carbon dioxide (SC CO_2). There are, however, large discrepancies in the published data, with solubilities ranging over more than one order of magnitude. This discrepancy can in part be attributed to the different experimental techniques used to measure β-carotene solubility in SCFs, such as the dynamic [2,5], static [3,6] or circulation [4,7] methods. These techniques present deficiencies in sample collection and sometimes equilibrium may not be reached [8]. Therefore, new reliable data are needed for a large range of temperatures and pressures.

In this study, a technique using a piezoelectric quartz crystal or a quartz crystal microbalance (QCM) placed in a high-pressure vessel is used for obtaining solubility data of β-carotene in SC CO_2. The advantages of the QCM technique are its compactness, simplicity for system set-up, stability for *in-situ* measurements and high sensitivity for small mass changes. Furthermore, the QCM technique does not require calibration and it allows *in-situ* observation of the diffusion and equilibrium processes [9,10,11].

The QCM technique is currently used for film deposition measurement, gas phase detection, immune sensors, DNA biosensors, drug analysis [12,13], and has been used for the determination of solubilities of metal chelates and polymers in liquid and SC CO_2 [10,14,15]. Using the QCM technique for solubility measurements in SCFs involves placing a known mass of a solute on the surface of the electrode area. This mass change on the surface of the crystal results in a frequency change according to the well established Sauerbrey equation (Eq. 1). The

mass change (Δm, kg) due to solute deposition onto the crystal can be calculated by the change in frequency (ΔF, Hz) and by knowing the other constants such as the resonant frequency of unloaded crystal (F_0, Hz), the active vibrating area (A, m^2), the quartz crystal density (ρ_q, kg/m^3), and the quartz crystal stiffness (μ_q, N/m^2).

$$\Delta F = -\frac{2F_o^2 \Delta m}{A\sqrt{\mu_q \rho_q}} \tag{1}$$

The frequency of a quartz crystal resonator in a high-pressure fluid is not only affected by a mass placed on its surface, but also by the pressure, the roughness of the crystal's surface, and the viscosity and density of the surrounding fluid [14]. The change in frequency of the quartz crystal from its inherent resonant frequency (Fo) can be expressed as:

$$\Delta F = F-Fo = \Delta F_M + \Delta F_P + \Delta F_V + \Delta F_A \tag{2}$$

where ΔF_M reflects the change in frequency due to an added mass, ΔF_P is frequency change due to the pressure of the fluid (compression effect) (Eq. 3), ΔF_V is frequency change due to the density and viscosity of the fluid and ΔF_A is frequency change due to any adsorbed mass. This equation accounts for the change in frequency caused by the interaction of SC CO_2 on an ideally smooth electrode surface [15] and therefore no correction for surface roughness is included. Both ΔF_M and ΔF_A are calculated using Eq. (1) and

$$\Delta F_p = F_o \alpha P \tag{3}$$

$$\Delta F_v = -(Fo/2t_Q \rho_Q)*(\rho\mu/\pi F_o) \tag{4}$$

where α is the proportionality constant of 1.05×10^{-5} MPa^{-1} [14], P is pressure in MPa, t_Q is the thickness of the crystal, ρ_Q is the density of the crystal, ρ is the density of the fluid, and μ is the viscosity of the fluid. The first term of Eq. (4) is also called the mass sensitivity of the quartz crystal. ΔF_A, mass adsorbed onto the QCM crystal, can be calculated by Eq. (1) [16], considering the frequency change due to pressure of the fluid, density and viscosity of the fluid and subtracting the total frequency shift observed experimentally in SC CO_2 (ΔF_{CO2}).
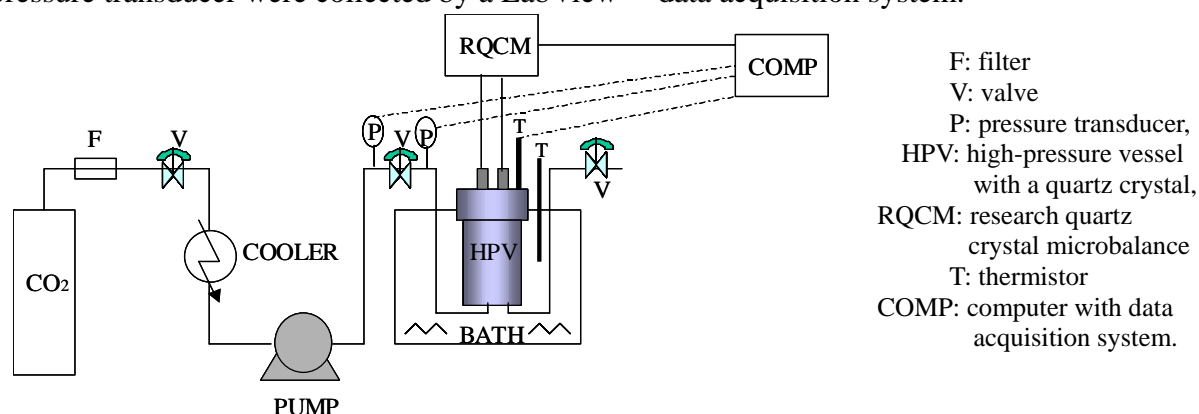
These factors (ΔF_P, ΔF_V, ΔF_A) have not been clearly examined nor quantified in previous work. Park et al. [11] investigated the behavior of QCM in high pressure CO_2 at limited conditions. In this study, the effects of pressure, fluid density and viscosity and adsorbed species on the frequency shift of a QCM in SC CO_2 are examined and discussed. Furthermore, solubilities of β-carotene in SC CO_2 at different pressures and 313 K are determined.

2. Experimental

Materials: β-Carotene (99.9% purity) and chloroform (99.98% purity) were purchased from Fluka-Sigma Aldrich (Buchs, SG, Switzerland). Supercritical fluid chromatography grade CO_2 was purchased from Praxair (Edmonton, AB, Canada). Polished quartz crystals (9MHz AT-cut) with wrap-around gold plated electrodes were purchased from International Crystal Manufacturing Co. Inc. (Oklahoma, OK, USA). The properties of the crystal are: area of 1.96×10^{-5} m^2, density of 2649 kg/m^3 and stiffness of 2.90 $\times 10^{10}$ N/m^2. A Research Quartz Crystal Microbalance (RQCM) was purchased from Maxtek (Santa Fe Springs, CA, USA).

Loading β-carotene on the crystal: β-Carotene was dissolved in chloroform to obtain a solution of known concentration (10 mg/mL). An aliquot (1μL) was placed on the quartz crystal electrode area. To obtain a uniformly distributed layer of β-carotene, the solvent was slowly evaporated under gentle nitrogen flow. The crystal frequency was recorded before and after loading to ensure that the frequency shift was in agreement with the amount of solute placed on the crystal. After loading, the crystal was placed in the high-pressure vessel, which was in turn connected to the pumps and placed into the water bath.

QCM and high-pressure apparatus: The apparatus (Fig. 1) consists of two ISCO 500D syringe pumps (ISCO Inc, Lincoln, NE, USA), an Isotemp 3013 refrigerated circulating water bath (Fisher Scientific, Pittsburgh, PA, USA), a heated water bath (Fisher Scientific, Pittsburgh, PA, USA), and a stainless steel high-pressure vessel (Frontier Valve International, Edmonton, AB, Canada). The temperature in the high-pressure vessel was registered by a YSI 406 thermistor (Dayton, OH, USA) and a thermometer in the water bath indicated the temperature in the system. A pressure transducer (Omega, Stamford, CT, USA), connected to the inlet tube, monitored the pressure of the pump and high-pressure vessel. Data from the thermistor and pressure transducer were collected by a LabView^{TM} data acquisition system.

F: filter
V: valve
P: pressure transducer,
HPV: high-pressure vessel
　　with a quartz crystal,
RQCM: research quartz
　　crystal microbalance
T: thermistor
COMP: computer with data
　　acquisition system.

Figure 1. Apparatus

Solubility experiment: The high-pressure vessel containing the loaded crystal was placed in the water bath to reach the desired temperature. After approximately 30 min, the inlet valve was opened and CO_2 was introduced into the vessel. The pressure transducer and thermistor monitored the pressure and temperature in the vessel. The frequency of the quartz crystal was recorded every 10s throughout the experiment. The experiment was continued until the frequency reading remained constant ($\pm$5Hz). Once the frequency stabilized, the inlet valve was closed and the vessel was depressurized by opening the outlet valve.

3. Results and Discussion

　　For each experiment, the resonant frequency of the 9 MHz quartz crystal was measured. The average resonant frequency was 8,993,155 Hz with a standard deviation of $\pm$77 Hz based on five measurements. This low standard deviation was observed in all the resonant frequency measurements of five different crystals.

　　Figure 2 shows the frequency change due to a loading of 1μL of β-carotene solution on the surface of the QCM. After evaporation of the solvent (chloroform), a uniformly distributed crystalline solid was observed on the crystal electrode surface. The overall frequency shift (final minus the initial frequency) was used in the Sauerbrey equation to calculate the total mass change on the surface of the electrode. In Table 1, this mass is compared to the mass that was loaded onto the QCM with the syringe. The mass placed on the crystal as calculated by the Sauerbrey equation was in accordance with the mass delivered by the syringe (Table 1). The small error between the two methods is to be expected and is due to experimental error. For example, in Table 1, the mass predicted by Sauerbrey is greater than that calculated from the volume of solution delivered by the syringe. This difference may be due to the error associated with the concentration of the delivered solution. In the case of other β-carotene loadings, the mass predicted by Eq. (1) was either higher or lower than the mass calculated from the volume of solution. In all cases, the difference between the two masses was small ($\pm$5%) and can be

attributed to experimental error.

Table 1. Mass calculation using the equation of Sauerbrey (Run N°75, Crystal C1)

Volume (mL)	Concentration (mg/mL)	Theoretical mass (mg)	Fo (Hz)	Delta F (Hz)	Sauerbrey mass (mg)	Error (%)
1×10^{-3}	10	1.0×10^{-2}	8,993,155	-9894	1.05×10^{-2}	5.0

In Figure 3, the surface morphology of the deposited β-carotene was examined by scanning electron microscopy (SEM) at 190X magnification. The small crystal formation of β-carotene as well as the even spreading of the solution on the electrode surface contributed to the precise mass reading from the QCM. The crystallization shape is uniformly irregular orthorhombic similar to Cocero et al. [17].
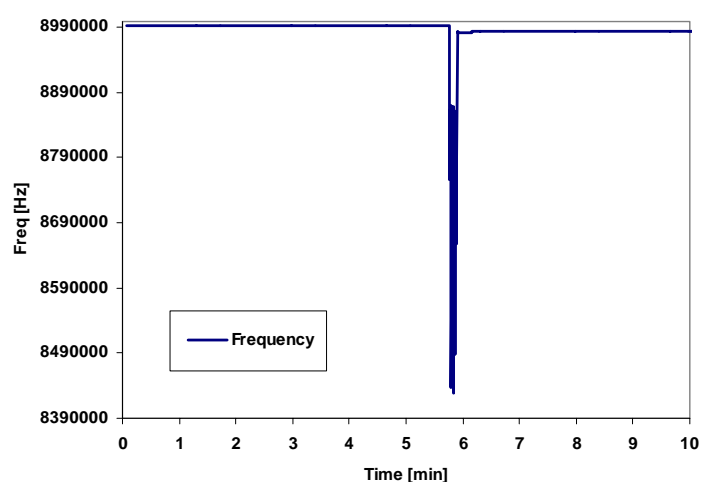
Figure 2. Frequency change during loading 1μL of 10mg/mL β-carotene solution.
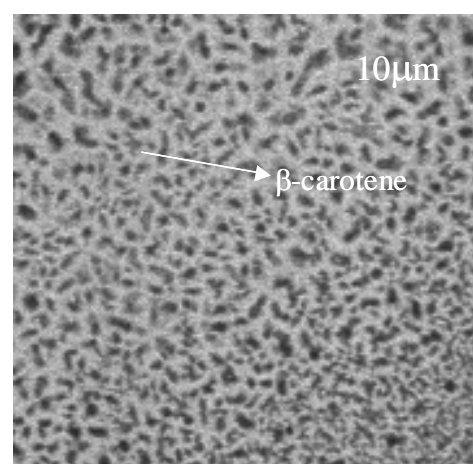
**Figure 3**. SEM of β-carotene loaded on the electrode of the QCM

Before obtaining solubility data with a QCM, it is necessary to know the behavior of the crystal due to the supercritical conditions. Figure 4 shows the frequency responses of the QCM in SC CO_2 at different pressures and constant temperature. The observed frequency shift (ΔFCO_2) depends on the frequency change from the compression effect due to pressure of the fluid (ΔF_p), the frequency change due to the density and viscosity of the solvent (ΔF_v) and the frequency change due to the mass of adsorbed species (ΔF_A) [15].

Table 2 shows the ΔFCO_2 obtained experimentally at 313 K and different pressures. Table 2 also provides ΔF_p, ΔF_v and ΔF_A calculated using Eqs.(2), (3) and (4).

Table 2. ΔFCO_2 and effect of ΔF_p, ΔF_v, ΔF_A at 313 K and different pressures

Pressure (bar)	Density (g/mL)	ΔFCO_2 (Hz)	ΔF_p (Hz)	ΔF_v (Hz)	ΔF_A (Hz)	Δm (g) (Eq. 1)
80	0.286	364.6	748	-137.9	-245.7	3.87E-07
100	0.638	472.4	935	-303.4	-159.6	5.02E-07
120	0.722	620.7	1120	-358.7	-143.0	6.59E-07
150	0.783	832.9	1400	-402.3	-167.8	8.84E-07
200	0.842	1185.1	1870	-448.8	-236.8	1.26E-06

Figure 5 shows the frequency and pressure change versus time during a β-carotene solubility experiment at 313 K and 120 bar. In the first 10 min, the frequency reading was at ambient conditions (T=297 K, P=10 bar). After that time, the temperature was increased to 313 K and at 20 min, the pressure was increased to 120 bar. This pressurization leads to a frequency drop, which can be attributed to the change of CO_2 properties and state and to the CO_2 adsorption onto the quartz crystal and the surface of the solute. Following this initial frequency drop, the frequency begins to increase due to the dissolution and diffusion of β-carotene into the SC CO_2. This dissolution and diffusion of β-carotene results in a loss of mass from the crystal surface and hence in an increase in frequency. From 20 to 80 min, dissolution is rapid due to a high driving force [18]. The rate of dissolution decreases and equilibrium is reached when the frequency stabilizes.
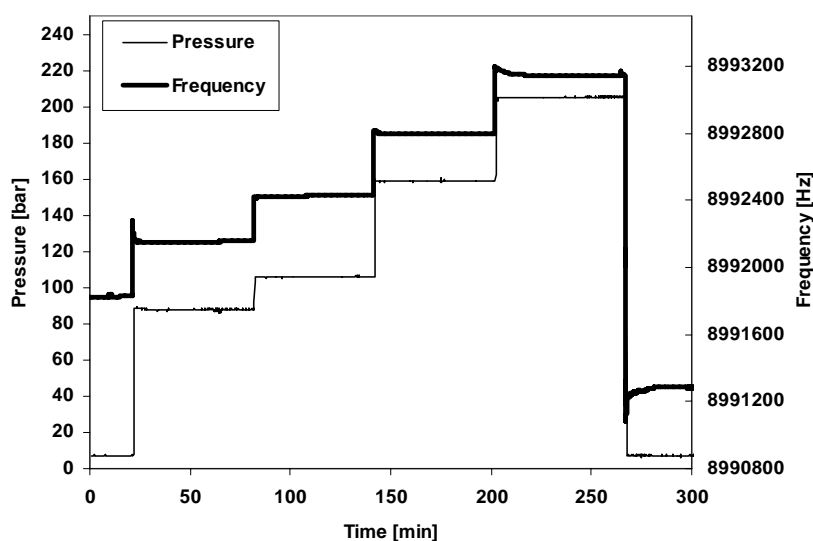
Figure 4. Frequency and pressure change versus time with $SCCO_2$ at 313 K
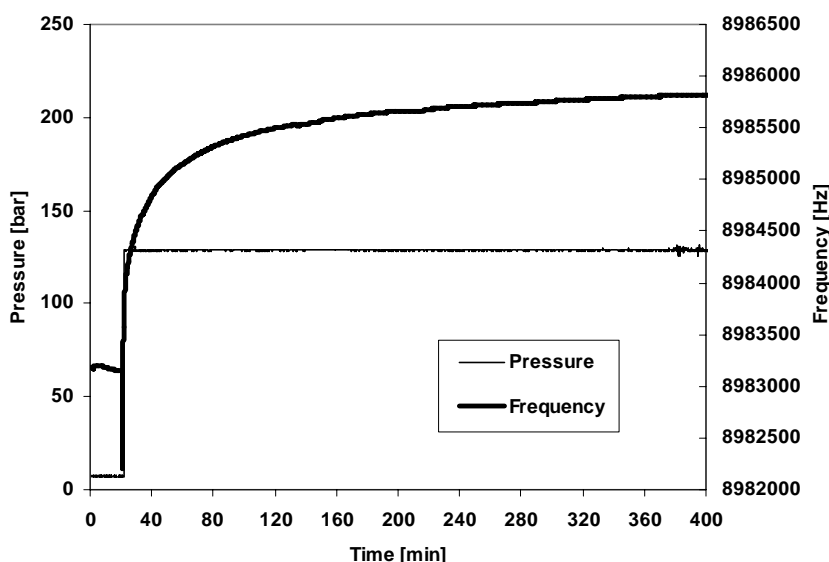
Figure 5. Frequency change during a β-carotene solubility experiment (120 bar; 313 K)

Table 3 gives the β-carotene solubilities determined in this work and compares these data to literature values. The data in Table 3 show that the solubilities measured using the piezoelectric quartz crystal technique are in accordance with the literature values.

Table 3. Solubility of β-carotene in SC CO_2 at different conditions

Pressure (bar)	Temperature (K)	Density (g/mL)	Solubility (mol/mol)	Reference
80	313	0.286	9.0×10^{-8}	this work
99	313	0.620	1.1×10^{-8}	[2]
101	310	0.691	2.2×10^{-8}	[4]
109	313	0.682	1.8×10^{-7}	[3]
120	313	0.722	2.9×10^{-7}	this work
150	313	0.783	7.9×10^{-7}	this work

5. Conclusions

This simple QCM static method provided *in-situ* data of β-carotene solubility in SC CO_2. The QCM technique allows the diffusion, dissolution and kinetics of the process to be monitored, thus ensuring that equilibrium is reached. Furthermore, some errors are eliminated with this method, as there is no need for sample collection after the process. The behavior of the quartz crystal under high pressure CO_2 was analyzed, and the factors that affect the frequency shift in the mass change were examined.

6. References

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