

Controlling the CO₂-lipid liquid phase separation via process tuning and lipid structural design

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Abstract

The volume expansion of organic solvent at near critical and supercritical CO₂ (ScCO₂) is a critical property for designing and optimizing ScCO₂-mediated process. The lipid expansion behavior in the ScCO₂ is found to be a dominant factor toward ScCO₂ ani-solvent precipitation process, liposome capsule production using ScCO₂, and ScCO₂ assisted lipid extraction process. In this work, the oleic acid, linoleic acid, and 1-octadecene are selected to perform measurement of liquid volume expansion during CO₂ pressurization at a pressure–volume–temperature (*PVT*) cell with visible window, to investigate the role of carboxyl group and double bond to CO₂ dispersion behavior in the lipid/alkene at pressure range of 0.1 MPa to 20.0 MPa, temperature range of 308 to 338K.

It is found that the dispersion behavior of CO₂ in the liquid phase is dominated by the carboxyl group on the lipid. The temperature and pressure dependent liquid expansion in CO₂ environment is reasonably fitted by Peng-Robinson equation of state combining with Wong-Sandler mixing. From analysis of molecular σ -profile, carboxyl group and double bond shows strong and weak hydrophilic character, respectively. Both functional groups are disadvantage of CO₂ dispersion into liquid lipid, leading to CO₂-lipid phase separation at high pressure region ($P > 15.0$ MPa).

Keywords : supercritical CO₂, volume expansion, thermodynamics modeling

1. Introduction

The supercritical carbon dioxide (ScCO₂) has attracted particularly interests due to the relatively mild critical conditions ($T_c = 304.2\text{ K}$, $P_c = 7.3\text{ MPa}$) for utilization[1]. Due to the advantages of inexpensive, nontoxic and nonflammable, the ScCO₂-Assisted Process is a main utilized working fluid in supercritical fluid extraction (SCFE) technology, and widely implement in food processing[2], pharmaceutical industry[3], and bioprocess[4], as known as the ScCO₂-assisted process.

Recently, the supercritical antisolvent (SAS) technics through utilizing CO₂ expanded lipid is developed for preparation of liposomes and solid lipid nanoparticles (SLN). Liposomes as one of the ideal drug carrier due to benefits of non-toxicity, good biodegradability and low immunogenicity[5]. For the above ScCO₂-assisted process, the interaction between lipid components and the pressurized supercritical carbon dioxide is very critical. Fatty acids are a major component of in the lipids (up to 70 wt%) [6], is a carboxylic acid with a long hydrocarbon chain. The double bond group on the hydrocarbon chain leads the fatty acid become unsaturated (with lower melting point). In this work, three organic compounds of oleic acid, linoleic acid, and 1-octadecen are chosen to investigate molecular functional group (carboxylic acid and double bond group) and process parameter effects to volumetric expansion of liquid lipid under high-pressure CO₂ through a pressure-volume-temperature (*PVT*) transparent apparatus. The work presented here is expected to provide fundamental inside and the suitable operation windows while utilizing CO₂-lipid expansion properties for process development.

2. Experiment and Modeling

2.1 Experimental Apparatus and Procedure

An apparatus containing a pressure-volume-temperature (*PVT*) cell was used. A schematic diagram of the *PVT* apparatus is shown in **Fig. 1**. CO₂ after liquefied by a cooler and liquid solvent were initially introduced into separated preheating coils by high pressure pumps (PU-4386, JASCO Japan). The pressure was generated with an CO₂ pump and was measured with a pressure sensor (with an accuracy of 0.1 MPa), and the temperature of the *PVT* cell was controlled by an electric heating system (with an accuracy of 0.1 °C). The visible window was attached to the front of the cell to obtain full visibility of all the contents in the cell. The volume of the fluid phase during pressurization is determined by a CCD camera-based measurement system.

Initially, a known volume of lipid or alkene was injected in 1.0 cm quartz cell (Optima, Japan), then placed in the *PVT* cell at atmospheric pressure and setting experimental temperature. After the temperature reached the experimental temperature and was kept for 20 min, V_{P0} (volume of the liquid phase at atmospheric pressure and setting temperature) was

measured by a CCD system. After determination of V_{P0} , CO_2 was injected into the cell and pressurized to target pressure, maintained for 1 hr. The volume of the liquid phase under different targeting pressure is then measured as V_{Pn} ($n= 1, 2, 3, 4$ corresponding to targeting pressure = 6.0 MPa, 10.0 MPa, 15.0 MPa, and 20.0 MPa, respectively.) The volume expansion (η) of pressurized liquid was determined by $\eta = V_{Pn} / V_{P0}$. The all observed results were averaged from three individual measurement.

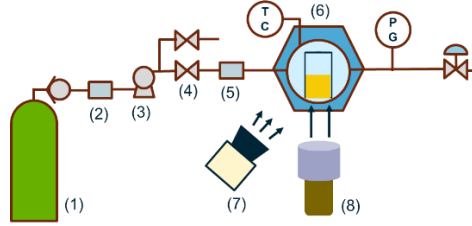


Fig. 1. PVT view cell reactor; (1) CO_2 cylinder, (2) cooler, (3) pump, (4) valve, (5) heater, (6) view cell reactor, (7) light source, (8) CCD camera.

2.2 Theoretical modeling and correlation

The Peng Robinson equation of state[7] is used to model volume expansion at vapor liquid equilibrium

$$P = \frac{RT}{\underline{V} - b(\underline{x})} - \frac{a(T, \underline{x})}{\underline{V}(\underline{V} + b(\underline{x})) + b(\underline{x})(\underline{V} - b(\underline{x}))} \quad (1)$$

where $a(T, \underline{x})$ and $b(\underline{x})$ are the pseudo one fluid parameters for mixture and can be determined from Wong–Sandler (WS) mixing rule[8]. The Wilson equations were incorporated as the excess Gibbs free energy model needed in the WS mixing rules.

2.3 Molecular σ -profile from COSMO theory

The spatial distribution of molecule surface screening charges obtained from QM/COSMO solvation calculation [9] is transformed into one dimensional σ -profile [$p_i(\sigma)$], which is the probability of finding a surface segment with screening charge density of σ through:

$$p_i(\sigma) = \frac{A_i(\sigma)}{A_i} \quad (5)$$

where $A_i(\sigma)$ is the surface area with a charge density of σ on molecule i , and A_i is the total surface area of species i . the molecular surface screening charge distribution for each conformer was determined by QM/COSMO solvation calculations at B3LYP/6-31G(d,p) level implemented in Gaussian09 (G09)[10].

Table 1. The investigated compounds, chemical structures and their critical properties for modeling in this work

No.	1	2	3	4
Compound	CO ₂ <chem>O=C=O</chem>	OA (Oleic acid) <chem>CCCCCCCC=CCCCCCCC(=O)O</chem>	LA (Linoleic acid) <chem>CCCCC=CCCCC=CCCCCCCC(=O)O</chem>	1-OD (1-octadecene) <chem>CCCCCCCC=CCCCCCCC</chem>
Tc (K)	304.2	796.3	796.0	744.2
Pc (MPa)	7.38	1.242	1.24	1.18
ω	0.239	0.924	0.776	0.807

3. Results and discussion

3.1 Volume expansion of lipid and alkene in ScCO₂

The studied compounds and the corresponding critical properties, chemical structures and acentric factors are listed in **Table 1**. The volume expansion coefficients for each CO₂ – lipid/alkene system under different conditions were shown in **Fig. 2**. It can be expected that the volume of the liquid phase is generally expanded with an increase of pressure for all lipid/alkene systems. However, for lipid systems of OA and LOA, the volume expansion coefficients keep as a constant as pressure > 10.0 MPa. The saturation of volume expansion can be understood that the existence of a LLE for CO₂ – fatty acids as pressure > 10.0 MPa, and at temperature from 308 to 338K. The similar behavior was observed for CO₂ – ethanol, the dissolved rate of CO₂ into liquid phase was suddenly reduced as increasing pressure due to occurrence of LLE[11]. By comparison, volume expansion for 1-OD shows totally different behaviors, keeping increase from $P = 0$ to 20.0 MPa. It is worth noting that the liquid phase of 1-OD is vanished at pressure > 15.0 MPa and temperature = 308 K, representing the operation condition reaching supercritical condition of CO₂ – 1-OD. The volume expansion coefficient therefore can't be determined, and no experimental points of 1-OD are listed on the **Fig. 2c**.

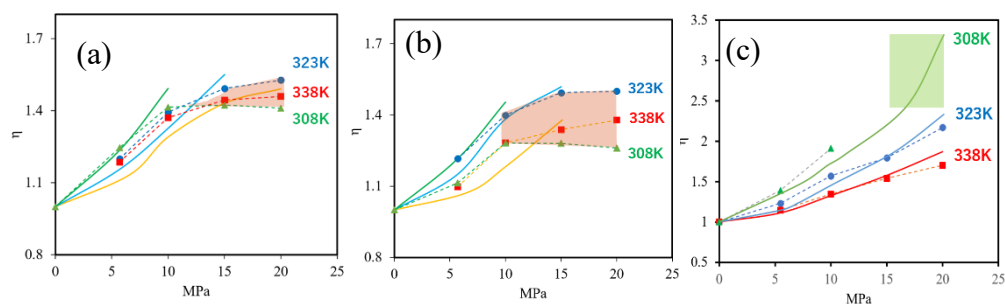


Fig. 2. Volume expansion behavior of studied chemicals as functional of CO₂ pressure. (a) CO₂ – OA, (b) CO₂ – LOA, and (c) CO₂ – 1-OD binary system.

3.2 Mechanism investigation from molecular information

The σ -profiles of OA, 1-OD, CO₂ and the excess Gibbs energy for three studied mixtures

at 308K are shown in **Fig. 3**. The σ -profiles of a molecule can be divided into three regions, representing H-bond donor ($\sigma < -0.0082 \text{ e}/\text{\AA}^2$), non-polar ($-0.0082 \text{ e}/\text{\AA}^2 < \sigma < 0.0082 \text{ e}/\text{\AA}^2$), and H-bond acceptor ($\sigma > 0.0082 \text{ e}/\text{\AA}^2$), respectively. The σ -profiles of a CO_2 shows four main peaks within $\sigma = \pm 0.006$, displaying the non-polar characters of a CO_2 molecule. The σ -profiles of the 1-OD shows one strong peak at $\sigma = 0.0 \text{ e}/\text{\AA}^2$, representing its strong non-polar characters. As known, the OA molecule is a 18-carbon unsaturated fatty acid with a substitution of carboxyl group. However, the carboxyl substitution group significantly change its molecular property, as demonstrated in **Fig. 3a.**, causing the strength of the main peak much weaker at $\sigma = 0.0$. The carboxyl substituted group also generates charge segments in both H-bond donor and acceptor region. The excess Gibbs energy for mixtures of CO_2 – OA, CO_2 – LOA, and CO_2 – 1-OD shows in **Fig. 3c**. Also indicates that carboxyl substituted functional group and double bond in OA and LOA both reduce the ideality of a mixture, explaining the observed LLE behaviors.

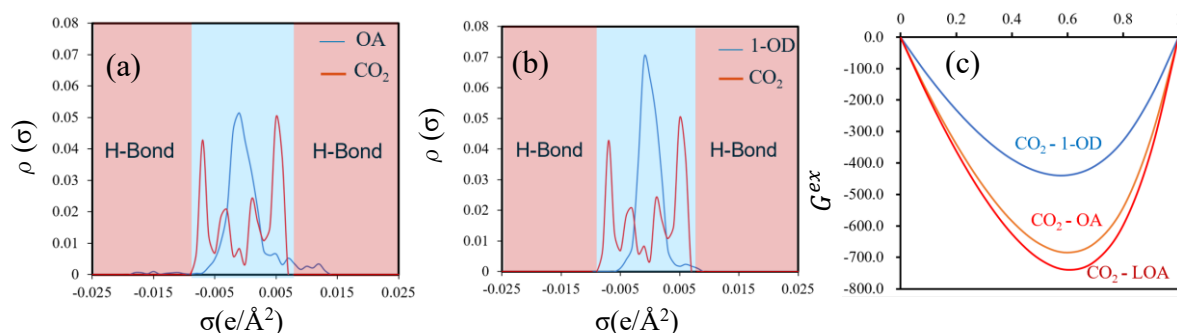


Fig. 3. σ -profiles obtained by COSMO-SAC calculation of (a) OA/ CO_2 and (b) 1-OD/ CO_2 and (c) Excess Gibbs energy for three studied mixtures at 308K.

The spatial charge distribution of OA, 1-OD and CO_2 are illustrated by **Fig. 4**. For OA and 1-OD, neutral molecular surface segments surround hydrocarbon backbones. The carboxyl substitution group on the tailed position of OA contains both positive and negative surface charge segments, corresponding to character of H-bond donor and acceptor in σ -profile. The larger non-polar surface charge segment of 1-OD therefore leads to strong London dispersion character to CO_2 , and also turn into supercritical mixture at 308 K and $P > 15.0 \text{ MPa}$ as discussed in previous section. By comparison, the carboxyl group and double bond on the OA/LOA reduces the London dispersion force to CO_2 , leading to occurrence of phase segregation while increasing CO_2 partial pressure.

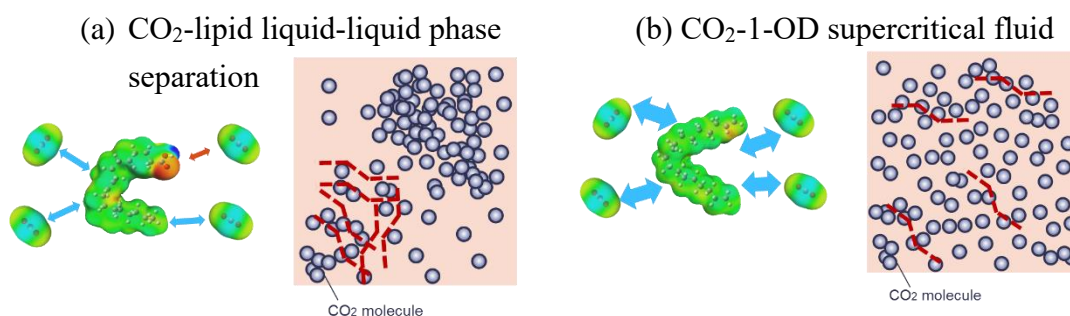


Fig. 4. Schematic illustration of (a) CO₂-lipid liquid-liquid phase separation (b) CO₂-1-OD supercritical fluid.

4. Conclusion

The volumetric expansion behavior for lipid liquid phase under near critical or supercritical conditions of CO₂ is systematically investigated at a *PVT* cell with visible window. From experimental observations, oleic acid (OA) and linoleic acid (LOA) consisted by C18 hydrocarbon backbone and tailed carboxyl functional group, leading to LLE between lipid to CO₂ while $P > 15.0$ MPa and temperature range from 308 to 338K. By comparison, no LLE is observed for mixture system of CO₂-1-octadecene (1-OD). The volumetric expansion behavior is proportional to CO₂ partial pressure, which is similar as must published alkane - CO₂ mixture systems.

From the analysis of molecular σ -profile, the carboxyl substituted functional group and double bond both reduce the non-polar properties of the lipid. Such a tiny decoration of carboxyl substitution on hydrocarbon chain leads to CO₂-lipid phase separation at high CO₂ pressure region ($P > 10.0$ MPa). The results represented in this work unveil the mechanism of CO₂-lipid volumetric expansion behavior from molecular property point of view and also can be regarded as suggested operation boundaries for CO₂-lipid expansion assisted process.

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