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Solubilizing amino acids and polypeptides in supercritical CO_2 via reverse micelle formation

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Abstract

Water-in-CO_2 reverse micelles stabilized by ionic perfluoroalkyl and non-ionic perfluoropolyether surfactants were used to host amino acids and polypeptides in supercritical (SC) CO_2. The minimum pressure at which micellization occurs (P_{trans}) was found to be affected by the surfactant concentration and H_2O/CO_2 ratio. Due to the differences in hydrophilic/CO_2 balance, the two surfactants exhibited different phase behaviors at 19.3 MPa and 40 °C. At certain H_2O/CO_2/surfactant compositions, while coagulation was more evident when using perfluoroalkyl surfactant, flocculation was found to dominate the system containing perfluoropolyether surfactant. The presence of amino acid and polypeptide in reverse micelles was found to increase the P_{trans} of the system due to enthalpic and entropic changes. In perfluoroalkyl reverse micelles, the P_{trans} was found to increase with increasing hydrophilicity of the amino acid added. The presence of hydrophobic moieties in amino acids promotes interfacial solubilization that eventually resulted in lower P_{trans}. On the other hand, the nature of the amino acid was not found to affect P_{trans} in perfluoropolyether reverse micelle. Overall, this work demonstrates that polypeptides with varying molecular weight could be solubilized in water-in-CO_2 reverse micelles using perfluoropolyether while perfluoroakyl surfactants were ineffective.

Keywords: Amino acid; Fluorinated surfactant; Polypeptide; Reverse micelle; Supercritical carbon dioxide

1. Introduction

The search for more environmentally sound technologies to replace the extensive use of organic solvents has led to the utilization of supercritical fluids (SCFs), particularly carbon dioxide. CO_2 aside from being renewable is non-toxic and non-flammable; hence, it is the most widely used solvent in SCF technologies. Moreover, CO_2 attains supercritical (SC) state at relatively lower temperature and pressure (31.1 °C, 7.38 MPa), making it more suitable for thermally labile materials. Its early applications include decaffeination of coffee and tea, manufacture of hop products, and extraction of pharmaceuticals and nutraceuticals from agricultural materials [1,2]. SC CO_2 has also been shown to be a good environment for dispersion and microemulsion polymerization [3]. However, the low polarizability of CO_2 limits its applications to hydrophilic substances. Adding a small amount of a polar modifier or cosolvent such as methanol, ethanol and acetone in the system has been demonstrated to solubilize a number of polar solutes in both solubility and extraction experiments [4–9]. For highly polar solutes like metals and proteins, the addition of a modifier is normally not enough to obtain the desired polarity of the SC solvent. This limitation is resolved by using a fluorinated surfactant to stabilize the water-in-CO_2 reverse micelles through the formation of hydrophilic domains such as microemulsions [10], miniemulsions [11] and macroemulsions [12,13], which can host the polar solutes in CO_2. The droplet size of water-in-CO_2 reverse micelles is governed by temperature, pressure, nature of the surfactant, and the relative amounts of water, CO_2 and surfactant in the system. Microemulsions are thermodynamically stable dispersions of high interfacial area, generally containing

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nanometer-sized droplets stabilized by adsorbed surfactant monolayers [12,14]. They typically consist of dispersed phase droplets from 2 to 50 nm in diameter [12]. Reverse micelles with droplet size that fall in the 50–500 nm range are considered miniemulsions, which are able to emulsify a much larger volume of water [11]. Even larger than miniemulsions are macroemulsions that contain droplets with size greater than 0.1 μm [12].

The surfactants used to stabilize water-in-CO_2 reverse micelles normally consist of non-polymeric or polymeric fluoroalkyl or fluoroether tails with non-ionic or ionic head groups such as sulfonates, carboxylates, sulfates, ammonium and others. Fluorinated tails are CO_2-philic because, like CO_2, they exhibit weak van der Waals forces [10]. Proteins and organic dyes, which are both insoluble in neat CO_2, have been solubilized in SC CO_2 through the formation of water-in-CO_2 reverse micelles and microemulsions in the presence of fluorinated surfactants [10,13,15–18]. Formation of stable CO_2-in-water emulsions has also been reported using polymeric surfactants with alkylene, oxide, siloxane and fluorocarbon-based tails [19,20], which is as attractive as water-in-CO_2 reverse micelles as a host for polar solutes in SC CO_2.

As anticipated, amino acids due to their polar nature exhibit insignificant solubility in SC CO_2 unless derivatized with benzyloxy carbonyl group in the N-terminal. Without derivatization, the solubility of amino acids in unmodified SC CO_2 is only in the order of 10^{-8} mol fractions at 40 °C and 13.8 MPa [21]. In this work, we are interested in solubilizing amino acids and polypeptides in SC CO_2 via the formation of water-in-CO_2 reverse micelles. Since it has been well documented that fluorinated surfactants exhibit good compatibility with CO_2, we used ionic perfluoroalkyl and non-ionic perfluoropolyether surfactant in stabilizing water-in-CO_2 reverse micelles. Since surfactant concentration and H_2O/CO_2 ratio are known to affect the stability of micelles, the optimum conditions to obtain stable micro- and miniemulsions were determined and employed in solubilizing amino acids and proteins in SC CO_2. Moreover, different amino acids were used to establish a correlation between the nature of amino acids and pressures above which a single-phase solution was observed.

2. Materials and methods

2.1. Surfactants

Sodium perfluorododecanoate (PFD) was prepared by adding stoichiometric amounts of the perfluorododecanoic acid (Matrix Scientific, USA) and sodium hydroxide in water. The mixture was stirred for an hour and then freeze-dried overnight. The surfactant was further dried in a vacuum oven for 24 h prior to use. The perfluoropolyether glycol (FlourN 2900; 4300 Ave. MW) (PFPEG) was provided by Cytonix (Virginia, USA) and was used without further purification.

2.2. Amino acids and polypeptides

Amino acids (at least 98% purity, Sigma, USA) and polypeptides (Sigma, USA) used include phenylalanine, alanine, threonine, asparagine, methionine, lysine, serine, aspartic acid, glycine, cystein, polyalanine ($1–5 \times 10^3$ MW), polyglycine ($2–5 \times 10^3$ MW), polyisoleucine ($5–15 \times 10^3$ MW).

2.3. P_{trans} measurements

In a typical experiment, a known amount of surfactant solution was stirred with a small magnetic stirring bar in a custom built 2-ml high-pressure view cell equipped with two sapphire windows. For P_{trans} measurements in the presence of amino acids and polypeptides, 1.5 and 1.0 mg of amino acid or polypeptide, respectively was also charged in the view cell. Using a thermal tape connected to a temperature controller, the cell was heated to 40 °C and was equilibrated for several minutes. CO_2 was then introduced into the cell using an Isco syringe pump (260D) while stirring with a magnetic bar driven by an outside magnetic stirrer. The pressure was gradually increased while maintaining the temperature at 40 °C with 0.7–1.4 MPa increments, allowing 5–10 min of equilibration between successive incremental pressure increases. P_{trans} was recorded by visual observation as the pressure at which a clear or a somewhat cloudy single phase was obtained. All observations were performed at least twice.

2.4. Phase behavior

The phase behavior of the water/CO_2/surfactant system using PFD and PFPEG with different compositions was observed at 19.3 MPa and 40 °C. Different amounts of surfactant solution (20–60 μl) with different concentrations (about 0.02–0.06 g/ml for PFD and 0.1–0.4 g/ml for PFPEG) were loaded in the 2-ml view cell together with a small magnetic bar. The cell was pressurized in the same manner as described in Section 2.2 to 19.3 MPa. The phase behavior of the system was then recorded based on visual observations. The H_2O/surfactant and H_2O/CO_2 ratios were calculated for each run from the actual weight of CO_2, surfactant and H_2O in the system. The actual weight of CO_2 was obtained from its density at a given temperature and pressure as given by the National Institute of Standards and Testing (NIST). On the other hand, the actual weight of the surfactant added was calculated based on the concentration, volume and density of the surfactant solutions. The densities of the surfactant solutions were determined by accurately weighing known volume of the solutions.

3. Results and discussion

3.1. Water-in-CO_2 reverse micelles

Fig. 1 shows the schematic structure of water-in-CO_2 reverse micelles stabilized by fluorinated surfactant. The surfactant having a CO_2-philic tail tends to extend in the CO_2 continuous phase while orienting its head toward the water core. Stabilization takes place when surfactant molecules form a dense monolayer at the interfacial region minimizing the interaction of the dispersed water core [22]. The free energy required to promote emulsions is supplied either by dispersion through external

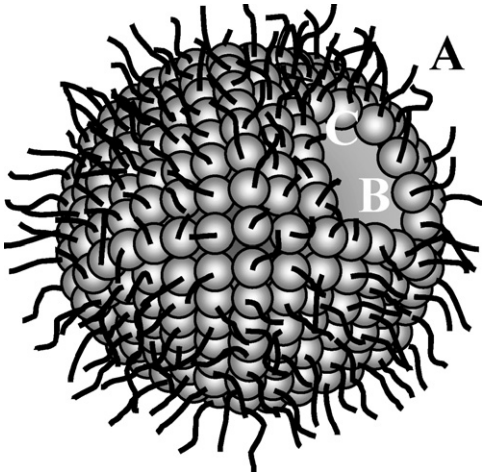

Fig. 1. Schematic structure of water-in-CO_2 reverse micelle stabilized by fluorinated surfactant ((A) CO_2 continuous phase, (B) water core and (C) amphiphilic interfacial region).

mechanical energy or condensation through the utilization of the chemical energy liberated when the components of the system come into contact [22]. Thus in our system, which only involved physical interaction, efficient mixing plays a vital role. The efficiency to promote micellization for the fluorinated surfactant mainly depends on its CO_2-philicity, CO_2 pressure (density) and temperature, which can be described by P_{trans} and critical micelle pressure (CMP). P_{trans} is defined as the minimum pressure at which the dispersion remains a stable single transparent phase at a given temperature [14]. Below the P_{trans}, the CO_2 density is insufficient to support CO_2–surfactant interactions that favor micellization leading to phase separation. The CMP on the other hand is defined as the pressure at which micelles start to form at a given temperature [15]. Liu et al. used UV–vis spectroscopy to determine the CMP of AOT/water/CO_2 systems in the presence of organic dyes by plotting absorption intensity of the microemulsion system against pressure. Similar to P_{trans}, CMP indicates a pressure below which the density of CO_2 is not sufficient enough to stabilize water-in-CO_2 reverse micelles.

3.2. Ionic perfluoroalkyl versus non-ionic perfluoropolyether surfactant

Water exhibits very limited solubility in CO_2 and its solubility is affected both by temperature and pressure. For instance the solubility of water-in-CO_2 at 15 °C, 25 MPa is only about 0.14% [14]. In the presence of fluorinated surfactant, CO_2 tends to take up water molecules several fold higher than the actual solubility of water in neat CO_2. Such a phenomenon suggests that in water/CO_2/surfactant systems, water molecules are incorporated in another portion or domain, not the bulk CO_2 phase, supporting the formation of water-in-CO_2 reverse micelles [17].

The effect of varying H_2O/CO_2 ratio and H_2O/surfactant ratio on P_{trans} is shown in Figs. 2 and 3 using the ionic perfluoroalkyl and non-ionic perfluoropolyether surfactants, respectively. The H_2O/surfactant ratios given in Fig. 2 are based on the total amount of water present in the system. While we expect that

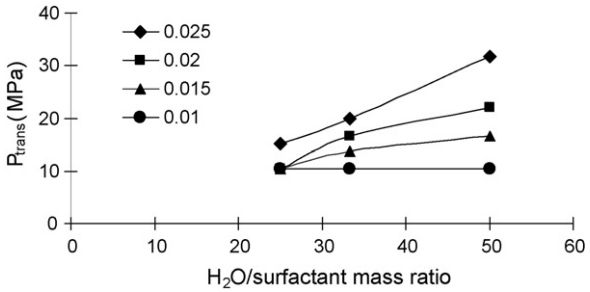

Fig. 2. Effect of H_2O/surfactant ratio on P_{trans} at 40 °C with varying H_2O/CO_2 ratio (0.01–0.025 v/v ratio) using PFD.

most of the water molecules participate in micellization, some water is dissolved in the CO_2 continuous phase. For a given H_2O/surfactant ratio, increasing the amount of the water loading (increasing H_2O/CO_2 ratio) increases the P_{trans} (Fig. 2). The increase in P_{trans} is more dramatic at higher H_2O/surfactant ratio. In the presence of higher amounts of water, more CO_2 molecules are required to counteract the attractive forces between the polar cores as a result of increased micellar size and aggregation number [17]. Thus, higher pressures are required to strengthen surfactant tail–solvent interactions to prevent phase separation [23]. In the same manner, the decrease in P_{trans} values with decreasing H_2O/surfactant ratio, especially at higher water loadings (Fig. 2), can be explained in terms of micellar size and aggregation number. At a constant amount of water, an increase in surfactant loading would result in an increase of the total number of micelle of smaller size and lower aggregation number. Since the free energy of the micellar aggregate is mainly governed by micellar size, higher solvating power of CO_2 (that is at higher pressures) is not necessary to solvate smaller sized-micelles [17] leading to lower P_{trans} values. This is true even if the total number of micelles is higher than in systems containing larger micellar aggregates.

As for the PFPEG (Fig. 3), a similar relationship exists between H_2O/CO_2 ratios and P_{trans}—that is, P_{trans} increases with increasing H_2O/CO_2 ratio. Regardless of the H_2O/CO_2 ratio, P_{trans} decreases with decreasing H_2O/surfactant ratio until a minimum point is reached. Further decreasing H_2O/surfactant ratio resulted in increases in P_{trans}. For both sets of data, this increase in P_{trans} occurred when the surfactant concentration had reached 0.004 g/ml CO_2. This suggests that at a certain surfactant concentration, tail–tail interaction becomes stronger

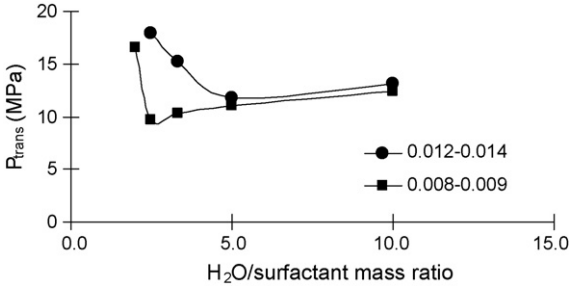

Fig. 3. Effect of H_2O/surfactant ratio on P_{trans} at 40 °C with varying H_2O/CO_2 ratio (0.008–0.014 v/v ratio) using PFPEG surfactant.

and the system would need higher density of CO_2 to strengthen the CO_2–tail interactions and thereby sustain a stable interface. In fact at H_2O/CO_2 ratio of 0.012–0.014, further decrease in H_2O/surfactant ratio to 2 corresponding to surfactant concentration of 0.006 g/ml CO_2 resulted in phase separation (aggregation of surfactant molecules) even at a pressure as high as 31 MPa.

Aside from the difference in surfactant concentration ranges where PFD and PFPEG formed reverse micelles, the two exhibit opposite trends in the relationships between P_{trans}, H_2O/CO_2, and H_2O/surfactant ratios. In the case of PFD, the increase in P_{trans} of the system became more apparent as H_2O/surfactant ratio (low surfactant concentration) increases (Fig. 2), which is the opposite when using PFPEG (Fig. 3). This could be explained by the differences in their hydrophilic/CO_2 balance and therefore by the phenomenon by which these two systems are destabilized. Whereas the system with PFD is destabilized by coagulation, the system containing PFPEG is destabilized more by flocculation. Coagulation refers to aggregation as a result of van der Waals attractive forces between the droplets excluding tails, while flocculation refers to droplet aggregation due to an attractive force between the tails [12]. In the case of PFD, the relatively shorter tails cannot easily provide a sufficient distance between droplets so as to prevent coagulation. Hence the system requires higher CO_2 solvating power (higher density) in the presence of higher amount of water. On the other hand, the longer tails in polymeric surfactant such PFPEG promote strong tail–tail interaction and therefore would require higher CO_2 density in the presence of higher amount of surfactant so as to prevent phase separation due to flocculation.

3.3. Phase behavior of H_2O/CO_2/surfactant systems using PFD and PFPEG

The phase behavior of H_2O/CO_2/PFD system with different compositions were observed at 19.3 MPa and 40 °C (Fig. 4). Within the composition range used, we observed either a one-phase or a two-phase system depending on the relative amounts of H_2O, CO_2 and PFD. The single-phase region is characterized by a clear to a somewhat cloudy mixture, which is most stable when H_2O/surfactant ratio ranges from 25 to 33 and H_2O/CO_2 ratio up to about 0.020. Once the balance between tail–tail, CO_2–tail and H_2O–head interactions were interrupted due to the presence of too much H_2O or surfactant molecules in the system, phase separation occurs. At higher H_2O/surfactant and H_2O/CO_2 ratios, the droplets assume macro-size that immediately settle once stirring is stopped. This suggests strong attractive micelle–micelle interactions, which tends to control the droplet size. With increasing pressure, however, these droplets may break into more numerous fine droplets. It is worth mentioning that at high H_2O loadings, while we observed the formation of macro-size droplets, the system remained dry in that no bulk H_2O phase was observed suggesting the absence of the Winsor II type of water-in-CO_2 emulsion, which is characterized by the presence reverse micelles in oil (CO_2 in our case) in equilibrium with an aqueous phase [24].

Fig. 5 shows the phase behavior of H_2O/CO_2/PFPEG systems with different compositions at 19.3 MPa and 40 °C. Unlike in the case of PFD, a one-phase, a two-phase or a three-phase system is observed depending on the relative amount of H_2O, CO_2 and surfactant present. Moreover, the two-phase system is characterized by white aggregates in CO_2 continuous phase suggesting flocculation rather than coagulation. Again, this could be attributed to the strong tail–tail interactions between long polymeric tails of the PFPEG. At even higher H_2O/surfactant and H_2O/CO_2 ratios aside from the white surfactant aggregates, a white-milky liquid phase was observed once stirring is stopped to give a three-phase system: CO_2-rich phase, white aggregates and white liquid phase. The appearance of the white-liquid phase at higher H_2O loadings suggests the formation of CO_2-in-water emulsion. This is in agreement with the findings of Ghenciu and co-workers [13] that perfluoroether may form either water-in-CO_2, CO_2-in-water, or three-phase emulsions depending on H_2O/CO_2 ratio and surfactant concentration. The most stable single-phase system for PFPEG lies in the region of 3.3–5.0 H_2O/surfactant ratio with up to about 0.013 H_2O/CO_2 ratios.

3.4. Amino acids and polypeptides solubilized in SC CO_2

From the phase behavior of H_2O/CO_2/surfactant systems in Figs. 4 and 5, we obtained the most stable system with lowest P_{trans} for solubilizing amino acids and polypeptides in SC CO_2. For PFD, we used the system with H_2O/surfactant and H_2O/CO_2 ratios of 25 and 0.010, respectively whereas for PFPEG, we used the system with H_2O/surfactant ratio of 3.3 and H_2O/CO_2 ratio of 0.008. Amino acids and polypeptides, which are insoluble

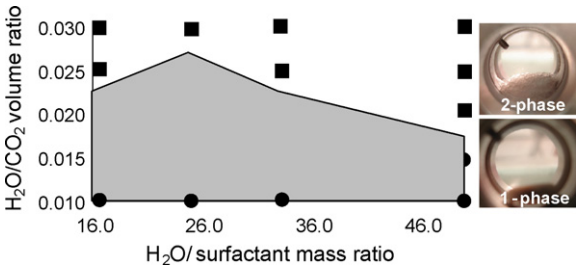

Fig. 4. Phase behavior of H_2O/CO_2/PFD systems with different compositions at 19.3 MP and 40 °C ((●) one-phase; (■) two-phase). The clear and shaded areas represent the two-phase and one-phase system, respectively. The inset photos show the clear transparent one-phase system at P_{trans} and the two-phase system characterized the formation macro-sized droplets. A stirring bar exists at the bottom of the cell.

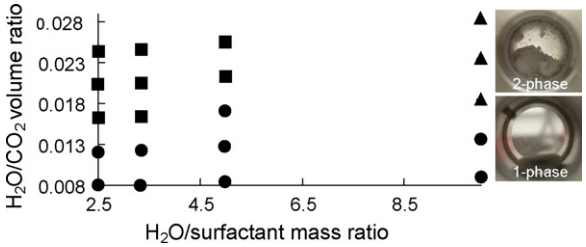

Fig. 5. Phase behavior of H_2O/CO_2/PFPEG systems with different composition at 19.3 MPa and 40 °C ((●) one-phase; (■) two-phase; (▲) three-phase system). The inset photos show the clear transparent one-phase system at P_{trans} and the two-phase system characterized by significant flocculation. A stirring bar exists at the bottom of the cell.

in neat SC CO_2, exhibit a stable clear transparent to a slightly cloudy solution in SC CO_2 in the presence of PFD and PFPEG. In both cases however, the presence of amino acid and polypeptide in the system increases the P_{trans} depending on the nature of the solute. The presence of any charged or polar solute such as amino acids and proteins in reverse micelles brings forth enthalpic and entropic changes that may affect the P_{trans}. These include the free energy change due to the interactions between the head groups of the surfactant and the solute as well as interfacial energy changes. There also exists an entropy change as a result of solute entrapping and the reconstitution of the reverse micelles [25].

Due to the formation of carbonic acid, the aqueous core in water-in-CO_2 reverse micelles is believed to be acidic [14,26]. At this pH amino acids exist in its cationic form, which favorably interact to the surfactant head group. Solubility studies of amino acids and proteins in reverse micelles, both in SC CO_2 or ordinary organic solvents suggest a strong electrostatic interaction between the surfactant head group and solute through complex formation [27] or ion-pairing type of mechanism [13]. In a more recent study, Feng and co-workers [25] proposed a multi-complex model to explain the solubilization of bovine serum albumin in H_2O/AOT/isoocatane reverse micelle under compressed CO_2. They suggested that the stability of the reverse micelle only affects the capacity of the micelle to solubilize proteins if the protein–surfactant interactions be strong.

Table 1 shows the P_{trans} of H_2O/CO_2/PFD system at 40 °C in the presence of amino acids. Amino acids bearing side chains such as methyl and phenyl group did not cause a dramatic increase in P_{trans}. In the presence of more polar amino acids such as glycine, serine, aspartic acid and tyrosine, P_{trans} of the system had increased significantly from 10.3 to 24.1–34.5 MPa. It is clear therefore, that as the amino acid hydrophobocity increases, its contribution in increasing P_{trans} becomes less dramatic. Similar to what has been observed and demonstrated in solubilizing amino acids using AOT in ordinary organic solvents, the relatively lower P_{trans} in the presence of less polar amino acids can be explained in terms of hydrophobic solubilization at the interface [28]. Leodidis and Hatton [28] observed that more polar amino acids were taken up only inside the water core, while the more hydrophobic amino acids existed in both the interfacial region and the water core. This hydrophobic effect that promotes interfacial solubilization is counter acted upon by the ability of the solute to H-bond with H_2O molecules as in the case of serine, aspartic acid and tyrosine. Owing to the removal of hydrophobic moiety from the water core, the entropic changes are expected to be minimal in the presence of more hydrophobic amino acids promoting solubilization at relatively lower pressures. Moreover in their succeeding work, Leodidis and Hatton [29] suggested the co-surfactant effect of hydrophobic amino acids in the same system. While we still observed higher P_{trans} in the presence of either hydrophobic and hydrophilic amino acids, we may infer that the presence of hydrophobic amino acids such as alanine and phenylalanine, cause minimal enthalpic and entropic changes that eventually lead to lower P_{trans} values relative to more polar amino acids.

Table 2 shows the P_{trans} of H_2O/CO_2/PFPEG systems at 40 °C in the presence of amino acids. Except in the presence of lysine and glycine whose P_{trans} are both higher than 19.3 MPa, the P_{trans} values of the systems in the presence of eight different amino acids range only from 12.4 to 15.2 MPa. This is only slightly higher than the P_{trans} of the system in the absence of amino acid, which is at 12.4 MPa. Perfluoropolyether promotes lower P_{trans} than perfluoroalkyl surfactant suggesting the presence of more stable micelles. Moreover, P_{trans} seemed to be not affected by the nature of amino acids present in the system. PFPEG owing to its longer tail compared to PFD, may exhibit greater ability to stabilize water-in-CO_2 reverse micelles and therefore give lower P_{trans} values. Surfactants with longer tail lengths exhibit more CO_2-philicity resulting in increased interfacial activity, resistance to aggregation and fusion droplets [30]. Its significantly longer tails may also explain why P_{trans} is not affected by the nature of amino acids in this system. The hydrophobic and co-surfactant effects observed with the use of PFD in the presence of less polar amino acids is not as effective with the use PFPEG because of significant differences in the length of the surfactant and amino acid side chains.

Finally, the solubilization of polypeptides in water-in-CO_2 reverse micelles stabilized by PFD and PFPEG using the same systems mentioned above for amino acids was investigated. Systems containing PFD as surfactant were unable to solubilize the polypeptides even up to 34.5 MPa (40 °C) resulting in a mixture with significant cloudiness. This could be attributed to the shorter tail of PFD and longer chain length and high MW of

Table 1
P_{trans} of H_2O/CO_2/PFD system at 40 °C in the presence of amino acids

Amino acid	Side chain	P_{trans} at 40 °C
No amino acid	–	10.3
Glycine	–H	34.5
Alanine	$–CH_3$	17.2
Serine	$–CH_2OH$	24.1
Aspartic acid	$–CH_2COOH$	27.6
Phenylalanine	$–CH_2$–phenyl	13.8
Tyrosine	$–CH_2$–phenyl–OH	27.6

H_2O/surfactant mass ratio: 25; H_2O/CO_2 v/v ratio: 0.01; amount amino acid: 1.5 mg amino acid in a 2-ml view cell.

Table 2
P_{trans} of H_2O/CO_2/PFPEG system at 40 °C in the presence of amino acids

Amino acid	P_{trans} at 40 °C
No amino acid	12.4
Glycine	Two phases at 19.3 MPa
Alanine	13.8
Serine	13.8
Aspartic acid	15.2
Phenylalanine	13.8
Methionine	13.8
Threonine	12.4
Cysteine	13.8
Lysine	Two phases at 19.3 MPa
Asparagine	15.2

H_2O/surfactant mass ratio: 5; H_2O/CO_2 v/v ratio: 0.008; amount amino acid: 1.5 mg amino acid in a 2-ml view cell.

Table 3
P_{trans} of H_2O/CO_2/PFPEG system at 40 °C in the presence of polypeptide

Polypeptide	MW (10^3)	P_{trans} at 40 °C (MPa)
No polypeptide	–	12.4
Polyglycine	2–5	19.3
Polyalanine	1–5	16.5
Polyisoleucine	5–15	15.2

H_2O/surfactant mass ratio: 5; H_2O/CO_2 v/v ratio: 0.008; amount amino acid: 1.0 mg polypeptide in a 2-ml view cell.

the polypeptide. Using PFPEG on the other hand, a transparent solution in the presence of either polyalanine, polyglycine or polyisoleucine at 40 °C was observed (Table 3). A slight difference in P_{trans} was also observed and was in the range of 15.2–16.5 MPa. This difference in P_{trans} may suggest that it is the nature of polypeptide side chains rather than the length or MW that dictates the P_{trans} of the system.

4. Summary and conclusions

The utilization of SC CO_2 as an alternative to organic solvents is gaining attention for various applications. The formation of water-in-CO_2 reverse emulsion using fluorinated surfactants resolves the limitations of SC CO_2 to host highly polar solutes such as amino acids and proteins. We have demonstrated that PFD and PFPEG were able to stabilize water-in-CO_2 reverse micelles to give a transparent to slightly cloudy micro- and miniemulsion. The P_{trans} of H_2O/CO_2 system in the presence of these surfactants was dictated by the surfactant concentration and H_2O/CO_2 ratio. In general the higher the water loading, the higher the P_{trans}. However when PFPEG was used, the increase in P_{trans} was more dramatic at lower H_2O/surfactant ratio, which is the opposite when PFD was used. This was attributed to the differences in hydrophilic/CO_2 balance of the two surfactants. At certain H_2O/CO_2/surfactant composition, while PFD reverse micelle was significantly destabilized by coagulation, PFPEG reverse micelles were predominantly destabilized by flocculation, presumably due to its longer tails.

The reverse micelles formed using PFD and PFPEG were demonstrated to solubilize amino acids to give a stable clear transparent single phase. Due to enthalpic and entropic changes, P_{trans} was always higher in the presence of solute. Interfacial solubilization and the capability of amino acid to H-bonding appeared to dictate the P_{trans} of the system when PFD was used as surfactant. On the other hand, P_{trans} of systems in the presence of amino acid using PFPEG was not affected by the nature of amino acids. Moreover, only reverse micelles stabilized by PFPEG was able to host polypeptides in SC CO_2 with varying MW.

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