Solubility Study of Sulfamethazine and Sulfadimethoxine in Supercritical Carbon Dioxide, Fluoroform, and Subcritical Freon 134A

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An on-line SFE/HPLC system incorporating a recirculating pump to achieve efficient mixing under static extraction conditions was developed to measure the solubility of different analytes in supercritical fluids. To test the system and method, the solubility of anthracene in supercritical CO_2 was measured and the results were quantitatively similar to those reported previously. The solubilities of neat sulfamethazine (SMZ) and sulfadimethoxine (SDM) were measured in supercritical carbon dioxide, supercritical fluoroform, and subcritical Freon 134A. Results showed that both drugs have significantly higher solubility in subcritical Freon 134A than in supercritical fluoroform or CO_2.

1. Introduction

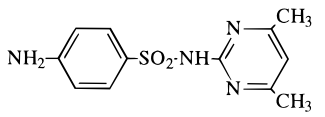
In the past several years, supercritical fluids have received widespread interest on both the process and analytical scale as an alternative solvent for extraction of nonpolar and intermediate polar analytes from both solid and liquid matrices. One of the most common process scale applications of supercritical fluids is described in the patent by Vitzthum and Hubert (1975) for removal of caffeine from green coffee beans using moist supercritical CO_2. Other applications of supercritical fluid extraction (SFE) include removal of additives from polymers (Ashraf-Khorassani and Levy, 1990), deasphalting heavy residual oils (Solomon, 1971), and extraction of pesticides from both solid and liquid matrices (Barnabas et al., 1994; Daneshfar et al., 1995). In recent years supercritical fluids have been used as a solvent for cleaning of precision parts (Purtell et al., 1993) and for forming uniform finely-divided energetic particulates (Cotton et al., 1993).

The key factor in the successful development of a process involving supercritical fluids in both the laboratory and on the industrial scale is the availability of accurate and reliable solubility data. Numerous research groups have measured the solubility of organic analytes in different supercritical fluids. Francis (1954) measured the solubility of more than 200 organic compounds including naphthalene in liquid phase carbon dioxide (25 °C and 850 psi). Since 1954, numerous authors have measured the solubility of naphthalene in CO_2 at different supercritical conditions (McHugh and Paulaitis, 1980; Kurnik et al., 1981; Dobbs et al. 1986; Sako et al., 1988; Bartle et al., 1990; Hansen and Bruno, 1993). The solubilities of many other polynuclear aromatic hydrocarbons (e.g., phenanthrene, anthracene, 2,3-dimethylnaphthalene, 2,6-dimethylnaphthalene, fluorene, and pyrene) in supercritical fluids have also been reported (Kurnik and Reid, 1982; Hampson, 1996).

Solubility measurements of organic materials in supercritical fluoroform (CHF_3) and 1,1,1,2-tetrafluoroethane ($C_2H_2F_4$) are very limited. Stahl and co-workers (Stahl and Willing, 1981) determined solubilities of different alkaloids in N_2O, CO_2 and CHF_3. Their results showed that under similar conditions alkaloids are more soluble in CHF_3 than in CO_2 or N_2O. We found no reported analyte solubilities in $C_2H_2F_4$ at either supercritical or subcritical conditions. The objective of this research was to evaluate an on-line SFE/HPLC system employing a recirculating pump to efficiently mix solubilized analyte under static conditions. Such a system is envisioned to have a number of advantages. First, the analysis of the solubilized analytes would not be in a supercritical medium where molar absorptivities are a function of pressure and temperature. Second, thermodynamic equilibrium can be truly established while uniform mixing of the fluid and analyte are achieved. And third, repeated sampling of the saturated supercritical medium is possible.

Following validation of the experimental system we present measurements and a comparison of the solubility of sulfamethazine (SMZ) and sulfadimethoxine (SDM) in three different fluids (CO_2, CHF_3, and $C_2H_2F_4$) at various pressures and 40 °C.

Sulfamethazine



Sulfadimethoxine

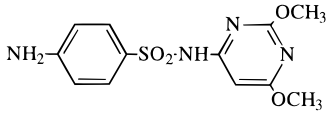


Figure 1. Molecular structure of sulfamethazine and sulfadimethoxine.

2. Experimental Section

A Suprex 200A syringe pump, a Waters (Milford, MA) 6000 high-performance liquid chromatographic pump, a Micropump (Concord, CA) recirculating pump, a 6 port−2 position valve, a 4 port−2 position (Valco, Houston, TX) valve, and a Kratos (Ramsey, NJ) Spectroflow 757 UV

S0021-9568(96)00402-5 CCC: $14.00

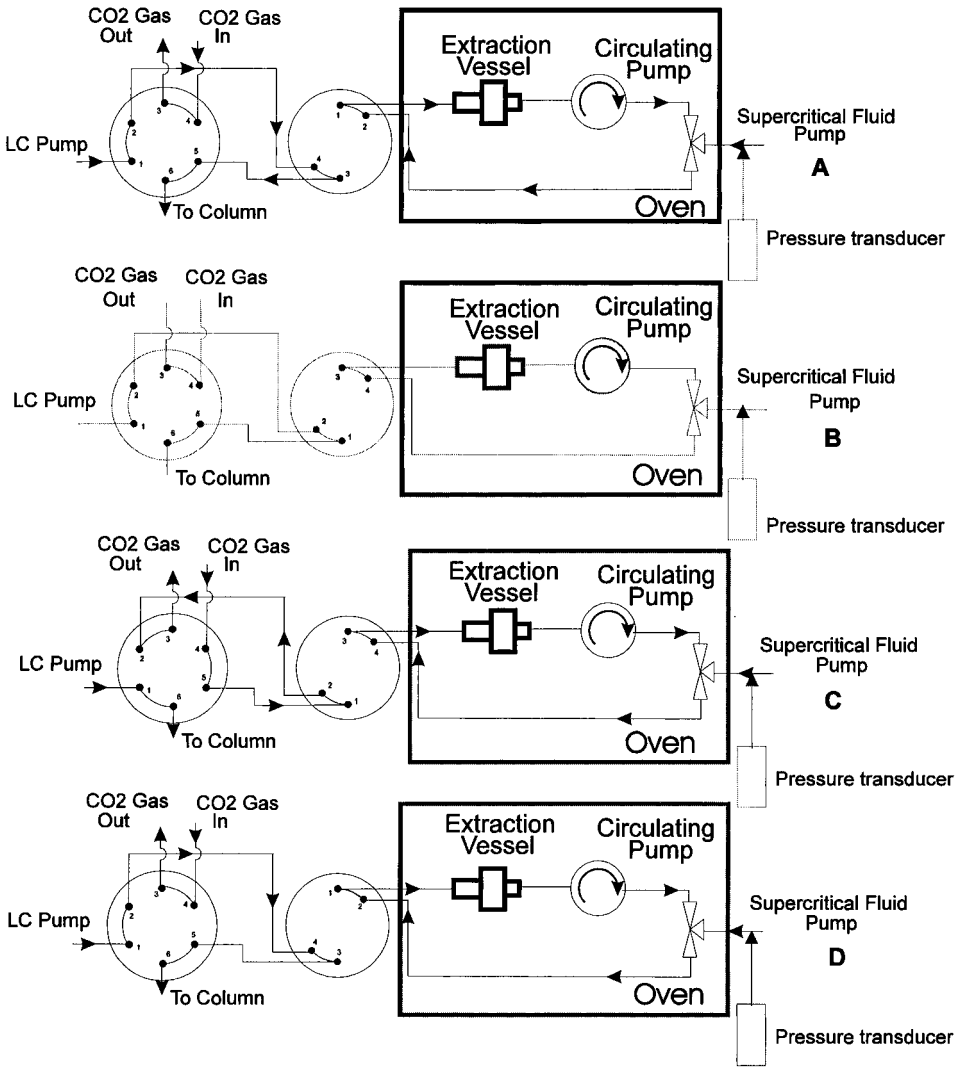


Figure 2. Schematic of solubility measurement device at various stages in the solubility measurement.

absorbance detector were used for all on-line solubility measurements. A 0.5 mL stainless steel vessel was used to contain the analyte whose solubility was to be determined.

Both sulfonamide (Figure 1) drugs were obtained in pure form from the USDA/ARS in Philadelphia, PA, courtesy of Robert Maxwell. The mobile phase for all HPLC analyses of solubilized analyte was 50/50 (v/v) CH_3CN/8 mM ammonium acetate. HPLC grade methanol and water were purchased from EM Science (Gibbstown, NJ). Ammonium acetate, ACS reagent (97% purity) grade, was obtained from Aldrich (Milwaukee, WI). SFE/SFC grade CO_2, fluoroform (CHF_3), and 1,1,1,2-tetrafluoroethane ($C_2H_2F_4$), all padded with 2000 psi helium (Air Products and Chemicals Co., Allentown, PA), were used for all solubility studies.

2.1. Solubility Measurement Apparatus. Figure 2 shows the apparatus and positioning of the valves at each step for measuring the solubility. In each measurement, a 0.5 mL extraction vessel was filled with analyte. Air was removed from the system by passing a stream of the test fluid through the vessel. The system was then pressurized to 100 ± 1 bar. After pressurization of the system and heating both the vessel and lines (40 ± 0.5 °C), the three-way valve was closed and the recirculating pump was activated (Figure 2A). The total recirculation volume was approximately 5.4 mL. The function of the recirculating pump was to ensure complete mixing and saturation of the supercritical fluid with the analyte of interest. After 30 min of equilibration time, the 4 port−2 position valve with a 1 μL internal sample loop was rotated to allow the sample in the supercritical fluid to be transferred from the 1 μL loop to the liquid chromatographic (LC) system (Figure 2B). The LC solvent flow then washed the analyte from the injection loop through the column to a variable wavelength UV absorbance detector operated at 254 nm for anthracene and 265 nm for sulfonamides. By employing a second valve (6 port−2 position), a stream of air was passed through the 1 μL sample loop to remove the chromatographic mobile phase from the loop (air was used as a convenient gas), thus avoiding modification of supercritical fluid composition (Figure 2C). The 4 port−2 position valve was then rotated back to the load position and the procedure repeated (Figure 2D). Replicate (four) determinations were made on each sample to ensure reproducibility of the system. After the solubility of the analyte was measured at 100 bar, the system pressure was increased and the same experiments were repeated.

Because of the poor thermal stability of sulfonamides, all solubility measurements were performed at 40 ± 0.5 °C, a lower temperature than the critical temperature of $C_2H_2F_4$ (t_c = 101 °C). Therefore, the solubility of each drug in $C_2H_2F_4$ was measured under subcritical conditions rather than supercritical conditions, as was the case for CO_2 and CHF_3.

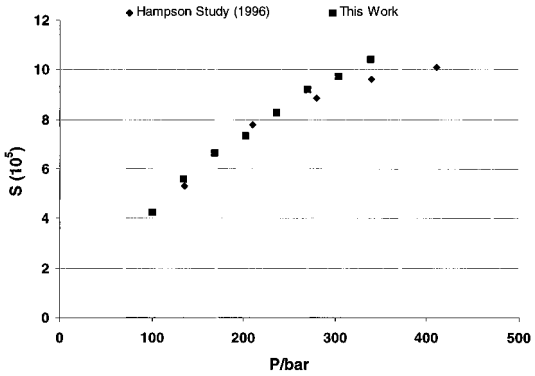


Figure 3. Solubility (mole fraction) of anthracene in supercritical CO_2 at different pressures.

Table 1. Mole Fraction Solubility of Anthracene at 40 °C versus CO_2 Pressure

P/bar	lit.[a] *S* (10^5)	this work *S* (10^5), (% RSD)
100		4.26 (5.0)
134		5.58 (3.6)
135	5.33	
168		6.64 (3.2)
202		7.35 (1.2)
210	7.77	
236		8.28 (2.4)
270		9.19 (3.4)
280	8.85	
304		9.72 (1.8)
338		10.4 (3.6)
340	9.60	
410	10.1	
470	10.3	

[a] Hampson (1996).

3. Results and Discussion

Solubility values are presented here as the mean of four samplings of the recirculating saturated fluid stream. Relative standard deviations of the measurements were in the range of 1−12%. The amount of analyte dissolved was obtained from the liquid chromatographic elution peak area (see Experimental for details) of the solubilized component relative to an external calibration curve. The solubility of sulfamethazine (SMZ) and sulfadimethoxine (SDM) are presented in two units, mole fraction of analyte in the solvating medium vs pressure of the solvating medium and moles of analyte per liter of solvating fluid vs pressure of the solvating fluid. All fluid density values were provided by Air Product and Chemicals Inc.

First, experiments were conducted to ensure that the novel system would provide reliable solubility data. Figure 3 shows the measured solubility of anthracene in supercritical CO_2 at 40 °C in the pressure range 100−340 bar. Results of this study compared favorably with previously reported data in the literature by Hampson (1996). Hampson compared the existing anthracene solubility, and it is believed to be a good diagnostic for our system. Table 1 lists the specific solubility data for anthracene in supercritical CO_2 at 40 °C, as determined by both laboratories.

Next, the solubility of sulfamethazine (SMZ) and sulfadimethoxine (SDM) was measured. Tables 2−4 show the solubility data for SMZ and SDM in supercritical CO_2, supercritical CHF_3, and subcritical $C_2H_2F_4$ at various pressures. The critical parameters of CO_2 and CHF_3 are quite similar in spite of the fact that CHF_3 is considerably more polar than CO_2 (e.g., $T_c = 31$ °C, $P_c = 73$ bar, 0 dipole moment for CO_2; $T_c = 26$ °C , $p_c = 47$ atm, and 1.6 Debye dipole moment for CHF_3). A plot of the solubility expressed in mole fraction of SMZ in CO_2, CHF_3, and $C_2H_2F_4$ vs pressure is shown in Figure 4. We observed that the mole fraction solubility of SMZ in supercritical CO_2, within experimental error, did not vary much as a function of pressure. The mole fraction solubility of SMZ in CHF_3 increased slightly with increasing pressure. The higher solubility of SMZ in CHF_3, compared to CO_2, at all pressures may be due to the fact that the CHF_3 has a larger dipole moment, and thus greater solvating power than CO_2. Previously, we reported results concerning the extraction efficiency of SMZ from different matrices using these two fluids (Combs et al., 1996). The solubility of SMZ in $C_2H_2F_4$ was approximately 1 order of magnitude higher than that obtained in CO_2 or CHF_3. The higher solubility of SMZ in $C_2H_2F_4$ can be possibly explained by the higher polarity and/or density of $C_2H_2F_4$ compared to either CO_2 or CHF_3. Figure 5 shows the solubility of SMZ expressed in moles per liter in all three fluids as a function of pressure. The SMZ solubility expressed in this manner reveals no difference in CO_2 and CHF_3.

Although the solubilities of SMZ in CO_2 and CHF_3 as a function of moles of analyte per liter of solvating fluid vs pressure were basically the same, an interesting observa-

Table 2. Mole Fraction Solubility (*S*) of Sulfamethazine in Different Fluids and Densities (g cm^{-3})

	density			solubility		
P/bar	CO_2	CHF_3	$C_2H_2F_4$	CO_2 (10^6)	CHF_3 (10^6)	$C_2H_2F_4$ (10^5)
100	0.638	0.806	1.22	2.23 (7.3)	2.43 (7.3)	1.71 (4.7)
150	0.785	0.91	1.24	2.18 (6.4)	2.52 (1.5)	1.74 (3.3)
200	0.845	0.97	1.27	2.04 (6.5)	2.52 (3.5)	1.79 (7.2)
250	0.854	1.006	1.29	2.09 (12)	2.80 (5.8)	1.87 (1.4)
300	0.914	1.038	1.31	2.08 (7.0)	2.83 (2.4)	1.85 (4.8)
400	0.96	1.094	1.34	2.11 (8.0)	3.17 (3.6)	1.91 (3.8)

[a] Values in parentheses are % RSD for four replicate measurements.

Table 3. Mole Fraction Solubility (*S*) of Sulfadimethoxine in Different Fluids and Densities (g cm^{-3})

	density			solubility[a]		
P/bar	CO_2	CHF_3	$C_2H_2F_4$	CO_2 (10^6)	CHF_3 (10^6)	$C_2H_2F_4$ (10^5)
100	0.638	0.806	1.22	2.69 (5.1)	3.64 (6.6)	1.11 (4.4)
150	0.785	0.91	1.24	2.40 (2.4)	3.47 (3.6)	1.12 (6.0)
200	0.845	0.97	1.27	2.32 (6.0)	3.37 (4.5)	1.11 (10)
250	0.854	1.006	1.29	2.27 (4.1)	4.06 (1.7)	1.02 (1.3)
300	0.914	1.038	1.31	2.14 (5.6)	4.00 (4.0)	0.953 (8.3)
400	0.96	1.094	1.34	2.26 (8.6)	3.96 (6.3)	0.785 (6.3)

[a] Value in parentheses are % RSD for four replicate measurements.

Table 4. Solubility (*S*/mol L^{-1}) of Sulfamethazine and Sulfadimethoxine in Supercritical CO_2, CHF_3, and Subcritical $C_2H_2F_4$

pressure, atm	CO_2 SMZ (10^5)	CHF_3 SMZ (10^5)	$C_2H_2F_4$ SMZ (10^4)	CO_2 SDM (10^5)	CHF_3 SDM (10^5)	$C_2H_2F_4$ SDM (10^4)
100	3.23	2.80	2.05	3.90	4.19	1.33
150	3.88	3.28	2.12	4.29	4.51	1.36
200	3.92	3.49	2.23	4.45	4.67	1.39
250	4.41	4.02	2.37	4.45	5.83	1.29
300	4.31	4.20	2.37	4.45	5.93	1.22
400	4.60	4.96	2.51	4.93	6.19	1.03

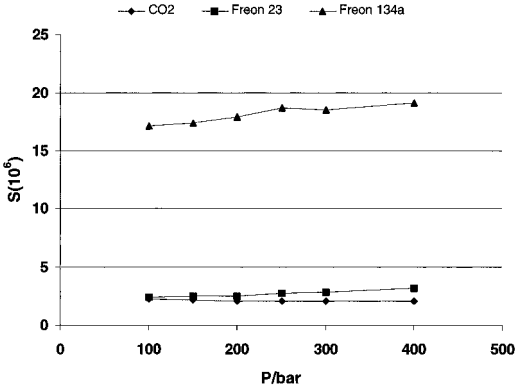


Figure 4. Solubility (mole fraction) of sulfamethazine in supercritical CO_2, CHF_3, and subcritical $C_2H_2F_4$ at different pressures at 40 °C.

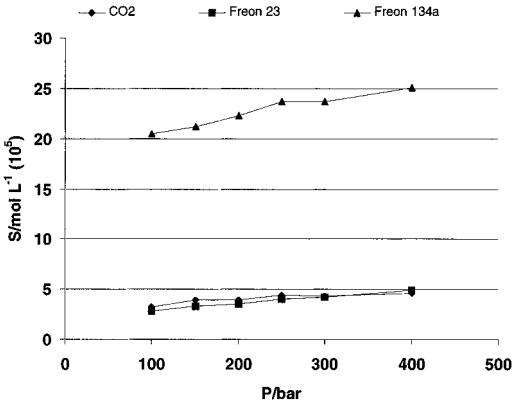


Figure 5. Solubility (moles/liter) of sulfamethazine in supercritical CO_2, CHF_3, and subcritical $C_2H_2F_4$ at different pressures at 40 °C.

tion can be made. At 100 bar up to 300 bar, the SMZ solubility (moles per liter) in CO_2 was slightly higher than in CHF_3 (Figure 5), while at 400 atm the SMZ solubility in CHF_3, was slightly higher. The lower solubility of SMZ in CHF_3, as expressed in moles per liter of analyte vs pressure (Figure 5) can be explained by the fact that at constant density the number of moles in a gram of fluid is less for CHF_3 (e.g., due to the larger molecular weight) than for CO_2.

Figure 6 shows the solubility (mole fraction vs pressure) of SDM in supercritical CO_2, CHF_3, and $C_2H_2F_4$. Results for CO_2 and CHF_3 were similar to those reported for SMZ. The solubility of SDM in $C_2H_2F_4$ was again almost 1 order of magnitude higher than the solubility in CO_2 or CHF_3. Likewise, the SDM solubility in $C_2H_2F_4$ increased slightly in going from 100 to 200 bar. A dramatic decrease in SDM solubility surprisingly commences after 200 atm in $C_2H_2F_4$. An obvious explanation for this particular compound's decrease in solubility is not presently available. The data were found to be reproducible in independent measurements of solubility. SDM and SMZ have similar molecular compositions and structures. SDM is more basic, however, by a couple of orders of magnitude. SDM is also less soluble in $C_2H_2F_4$ than SMZ. While the raffeinate from

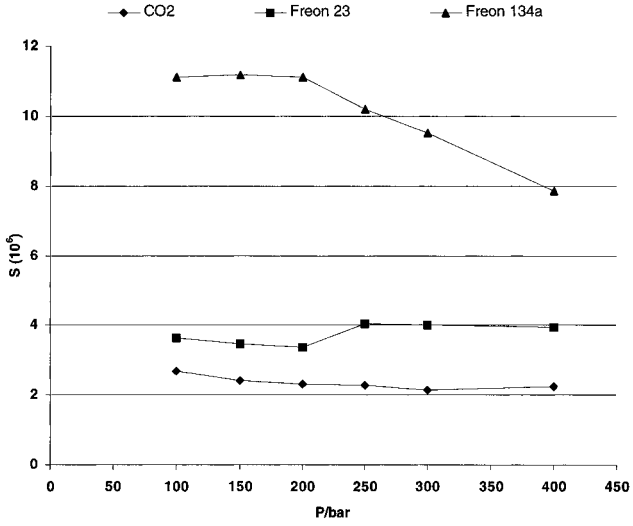


Figure 6. Solubility (mole fraction) of sulfadimethoxine in supercritical CO_2, CHF_3, and subcritical $C_2H_2F_4$ at different pressures at 40 °C.

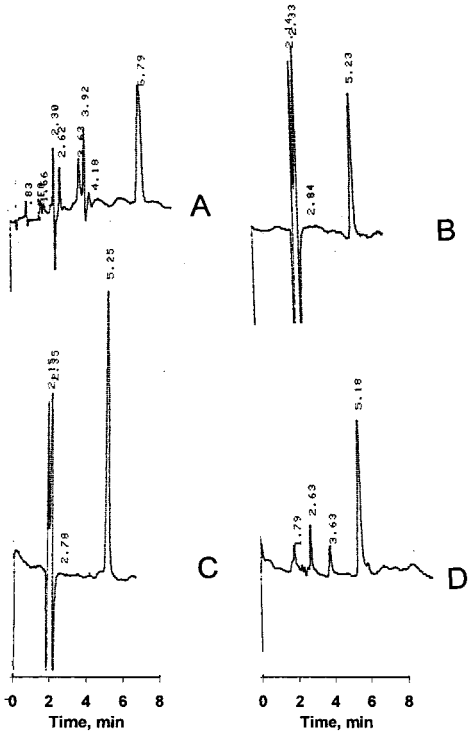


Figure 7. On-line supercritical fluid/liquid chromatogram of SDM dissolved in CO_2 (A), CHF_3 (B), and $C_2H_2F_4$ (C) injected into the column with 50/50 CH_3CN/8 mM ammonium acetate as a mobile phase. SDM was deposited from supercritical CO_2 into an empty loop filled with air (D) and then injected into the column with 50/50% CH_3CN/8 mM ammonium acetate as a mobile phase.

both SMZ and SDM solubility studies darkened somewhat, the effect with SDM was considerably more apparent.

Since sulfonamides are quite reactive, we analyzed the nature of the species that was being solubilized. For

example, salts of sulfonamides are converted to neutral species in the presence of CO_2. The effect of CHF_3 and $C_2H_2F_4$ on sulfonamide chemistry was expected to be minimal. In this regard, we observed that the liquid chromatographic retention behavior for SDM dissolved in CO_2 was different from SDM dissolved in CHF_3 or $C_2H_2F_4$. Figure 7 shows the chromatogram of SDM dissolved in CO_2 (A), CHF_3 (B), and $C_2H_2F_4$ (C) injected into the 50/50% CH_3-CN/8 mM ammonium acetate mobile phase. It was interesting to observe that the elution time for the SDM dissolved in CO_2 was approximately 1.5 min longer than the SDM dissolved in either CHF_3 or $C_2H_2F_4$. A study was performed to determine if SDM reacted with CO_2. For this purpose 1 μL of SDM dissolved in CO_2, at high pressure, was injected into a loop containing only air (atmospheric pressure) and allowed to depressurize (see schematic in Figure 2C). After the CO_2 was allowed to escape from the second loop, the second valve was rotated and the HPLC mobile phase was allowed to wash the SDM deposited in the second loop into the LC column (Figure 2D). The elution time for SDM deposited in an empty loop (Figure 7D), without the presence of CO_2, was similar to the elution time of SDM dissolved in both CHF_3 and $C_2H_2F_4$, as well as the external standard dissolved in methanol. It is believed that since the liquid chromatography mobile phase pH is approximately 6.5−7.0, SDM could be deprotonated (pK_a 6−7). When CO_2 is dissolved in the mobile phase, a localized area of low pH is formed which reprotonates the drug, thus forming the neutral species. Chromatographically speaking, it is reasonable to assume that if a neutral compound is formed, the retention time should increase, as was observed experimentally. Therefore, it is important to note that the reported solubility data may not present the true binary system of SDM−CO_2. As a consequence, the reported solubility data, for the binary mixture of SDM−CO_2 may not be completely valid. The elution time of the less basic SMZ was not affected by the CO_2 or any other fluids.

Conclusion

It was demonstrated that an on-line SFE/HPLC system with a recirculating pump can be used for direct solubility measurement of different analytes in supercritical fluids. Anthracene was used as a test compound to evaluate the system reliability. Our measured solubilities of anthracene in supercritical CO_2 at different pressures were similar to those reported previously in the literature. Solubility of sulfamethazine and sulfadimethoxine in supercritical CO_2 and CHF_3 and subcritical $C_2H_2F_4$ were measured. Results showed that both SMZ and SDM have much higher solubilities in subcritical $C_2H_2F_4$ than in the supercritical CO_2 or CHF_3. Also, the liquid chromatographic elution behavior of SDM uniquely demonstrated that the drug can undergo structural changes in supercritical CO_2 as opposed to subcritical $C_2H_2F_4$ and supercritical CHF_3.

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