# Purification of Hydroxy Ethyl Starch with Supercritical Fluids: Application of reverse micelles

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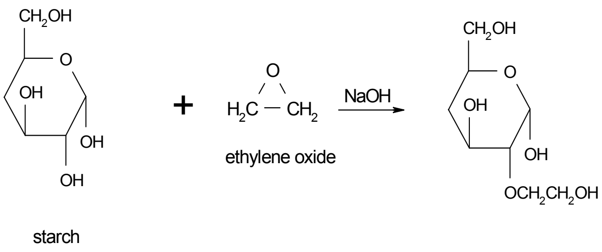
## 1. Introduction

Hydroxy Ethyl Starch (HES) is a plasma volume expander (PVE) used for the compensation of surgical blood loss. It is commonly produced in a batch reactor from the reaction of cornstarch with ethylene oxide in the presence of a base (Figure 1)[1]

Figure 1**:** HES based reaction.



After the reaction, the polymer follows a series of purification steps. This includes acetone and water washings, acetone stripping, filtration, drying, etc. Although the goal of producing a "pure" HES product is achieved (1-10 mg/kg), several challenges remain in the separation processes. Some of them include:

i) Time required to purify one batch due to the separation challenges (≈ 30 or more hours of processing)

ii) Unacceptable residual amounts of undesired substances (*e.g.,* ethylene glycol (EG) and ethylene chlorohydrin (ECH)).

Supercritical fluid extraction (SFE) is an environmentally benign separation technique for obtaining compounds with a high degree of purity and has become an industrial alternative for solvent extraction processes. As an alternative to the numerous separation processes for the polymer purification, the use of a SFE provides the tools for the precipitation and separation of the polymer directly from the reaction mixture in a single step. In addition to the increased efficiency, one also replaces the use of Volatile Organic Compounds (VOC's, *e.g.,* acetone) and the reduction of the processing time. This research has focused on the separation of ethylene glycol using supercritical $CO_2$ and to purify HES. This approach is even more appealing, considering that higher temperatures are not viable options for the stability of the polymer. Carbon dioxide ($CO_2$) is an ideal solvent for the extraction of lipophilic, non-polar and slightly polar compounds. Besides, it is inexpensive, non-combustive, non-toxic, readily available, and easily to remove; thus, it is eligible for many industrial processes. However, $CO_2$ shows low solvency capacity for high molecular weight or hydrophilic compounds due to its low dielectric constant and polarizability per volume. One strategy to increase the solvent power of $scCO_2$ is through the addition of cosolvents that increase its polarizability. Cosolvents are usually small polar organic compounds that modify the fluid phase generating hydrogen bonding (HB) acceptor or HB-donor capabilities on the non polar $CO_2$. The addition of small amounts of a cosolvent with intermediate volatility can increase the solvent capacity of the primary solvent (e.g., $CO_2$), while retaining the favorable mass transport properties (high molecular diffusivities and low viscosities). There are many studies that use cosolvents to increase the solvating power of $CO_2$. Koga et al. measured the influence of certain cosolvents (i.e., ethanol, and octane) on solubilities of fatty acids and higher alcohols, concluding that ethanol is more effective on the solubility enhancement for fatty acids in $scCO_2$[2]. Even though there has been an extensive amount of research done in the use of cosolvents, the mechanisms of these entrainer effects has not been clarified yet. Thus, a more reasonable choice of entrainer may be possible by understanding the microscopic structure in $scCO_2$.

Other strategy for increasing the $scCO_2$'s solvent power is using surfactants to aggregate in $scCO_2$ and to form microemulsions or reverse micelles. The formation of microemulsions and emulsions in $scCO_2$ has been verified by visual inspection of phase behavior[3,4]; spectroscopic techniques (e.g., SANS[5], SAXS[6] were also used. Furthermore, it has been demonstrated that the inner core of the reverse micelle is able to trap $H_2O$[7,8] , and organic compounds[9]. This investigation attempts to remove impurities from HES using $scCO_2$, using different purification strategies (e.g., pure $scCO_2$, cosolvent, and surfactants). An FT-IR spectroscopy study was done to understand the quantitative results to the intermolecular interactions. This is an interesting approach to use commercial surfactants in $scCO_2$.

## 2. Experimental

### *2.1 Materials*

The HES used in this investigation was provided by BBRAUN PR, their principal characteristics are presented in Table 1. It should be pointed out, that the two different lots correspond to two different stages of their manufacturing process. Lot 2 has undergone further purification and thus, has a smaller amount of impurities.

Table 1: Characteristic of HES used in the investigation

| | HES lot 1 | HES lot 2 |
|---|---|---|
| Batch number | 0060278528 | 9700048362 |
| Molecular weight | 259 883 | 201 100 |
| Ethylene Glycol [ppm w/w] | 42 236 | 788.5 |
| 2-Chloroethanol [ppm w/w] | 27.9 | 13.30* |

* Detection limit

Pluronic surfactants (e.g., L31, and 17R2), Gele and Zonyl, are briefly described in Table 2. They have been used to extract EG and ECH from the HES polymer.

Table 2: General characteristics of Surfactants[10-13].

| Name<br>Formula | Mw | Characteristics |
|---|---|---|
| Pluronic L31<br>$(EO)_1(PO)_{16}(EO)_1$ | 1056 | Transparent viscous<br>1w% soluble in $H_2O$ at 37°C |
| Pluronic 17R2<br>$(PO)_{15}(EO)_{10}(PO)_{15}$ | 2125 | Transparent viscous<br>1w% soluble in $H_2O$ at 35°C |
| Zonyl FSN<br>$C_6H(EO)_{11}F_{13})$ | ≈ 950 | Turbid liquid<br>$n^{20}$/D: 1.382 (lit)<br>Fp: 72°F<br>Density: 1.06 g/mL |
| Gele<br>$C_{14}H_{26}O_2(EO)_{2-5}$ | ≈ 360 | Stabilizer for emulsions. $n^{20}$/D: 1.45 (lit). Fp: >230°F<br>Density: 1 g/mL |

Cosolvents (e.g., ethanol, acetone, and acetonitrile) used were bought from Sigma-Aldrich all were HPLC grade.

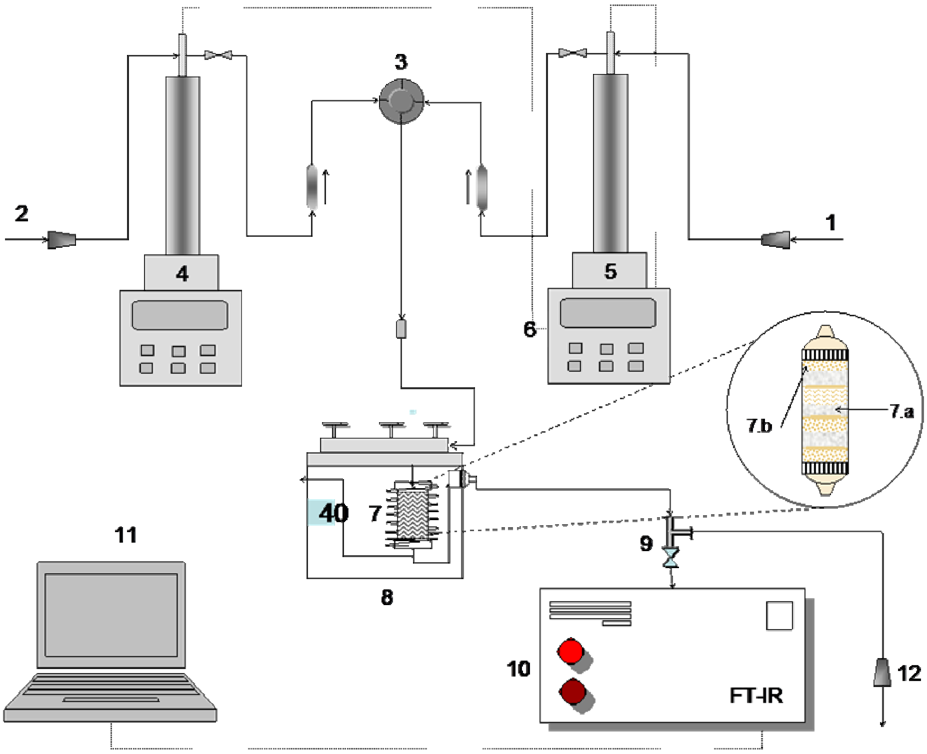
### 2.2 *Procedure*

We used a SFE adequate for this kind of processes. The polymer (HES) was supplied by BBRAUN PR and the analysis of the final product was done in B BRAUN Crissier Switzerland.

The experimental set up is showed in Figure 2. The extraction with $scCO_2$ pure was described elsewhere[14], for all the experiments dynamic extraction was performed. The extraction time, depending on the flow used, range from 2 to 5 hours. For the cosolvent-$scCO_2$ extraction, two syringe pumps were used; one was loaded with cosolvent, and the other with $CO_2$. These pumps are adequately controlled with a single controller unit which regulates the flow of both fluids ($CO_2$ and cosolvent) into the extraction camera.

Previously to the extraction, the cell was prepared loading with 2.6±0.2 g of HES supported in glass wool fiber, simulating a distillation column. This distribution was determined to be adequate to avoid channeling and minimizing the mass transfer limitations. The cell was then put on the extraction camera, which was already heated to the extraction temperature. Several considerations for SFE were taken to assure thermodynamic equilibrium[1014]. For the decompression of the SCF a heated capillary restrictor was used, but previous to the restrictor a bifurcation tee was connected. A nearby valve allowed the extraction fluid to flow into the FT-IR cell and therefore be able to take the spectrum every time it was required.

Figure 2: Experimental set-up. (1) $CO_2$ from tank. (2) Cosolvent from deposit. (3) Te of mixing. (4) Cosolvent syringe pump. (5) $CO_2$ syringe pump. (6) Dual pump controller. (7) Extraction cell. (7.a) Glass wool fiber. (7.b) Solute. (8) Extraction camera. (9) Bifurcation Te. (10) Thermo Nicolet FT-IR (11) Acquisition data computer. (12) Expansion nozzle.



When surfactant was used, static extractions were performed; approximately 30 min were enough to fill up the extraction cell, allowing contact between the load of the cell (HES and surfactant) and the $scCO_2$. The mixture HES-surfactant was prepared previously mixed with a magnetic stirring. The same extraction procedure described above was followed.

## 3. Results and Discussion

### *3.1. Extraction with scCO$_2$*

After performing extraction in a range of pressures (100–300 bar) and temperatures (40–60°C), the conditions of maximum solubility were found; these are at 200 bar and 50°C. The extractions were performed using 241.7 ± 13.6 [mL] of $scCO_2$, a flow of 1.3 ± 0.3 [mL/min] during an average time of 3 hours (Table 3).

**Table 3:** Characteristic of HES after extraction with $scCO_2$

| | HES lot 1 | HES lot 2 |
|---|---|---|
| Batch number | 0060278528 | 9700048362 |
| Molecular weight | 264 600 | 199 400 |
| Ethylene Glycol [ppm w/w] | 21 902 (48%*) | 31.61 (96%) |
| 2-Chloroethanol [ppm w/w] | 12.70 (54%) | 13.30** |

(*) Percentage of extraction (**) Detection limit

### *3.2. Extraction with cosolvent-scCO$_2$*

The alcohols used as cosolvents have comparatively large α and β values in the liquid phase, and act as HB-donors as well as HB-acceptors. They form HB bonds with organic compounds that often contain polar organic groups. The results obtained of the extraction of HES lot 1 is summarized in Table 4.

Table 4: Extraction of EG and ECH with cosolvents (HES lot 1)

| | Composition [mg/kg] | Extraction [%] |
|---|---|---|
| Acetone-scCO$_2$ | | |
| Ethylene Glycol | 14658.80 | 65 |
| 2-Chloroethanol | 25.0 | 10 |
| Ethanol-scCO$_2$ | | |
| Ethylene Glycol | 6187.99 | 85 |
| 2-Chloroethanol | 13.30 | ----- |
| Acetonitrile-scCO$_2$ | | |
| Ethylene Glycol | 5486.35 | 87 |
| 2-Chloroethanol | 13.30 | ----- |

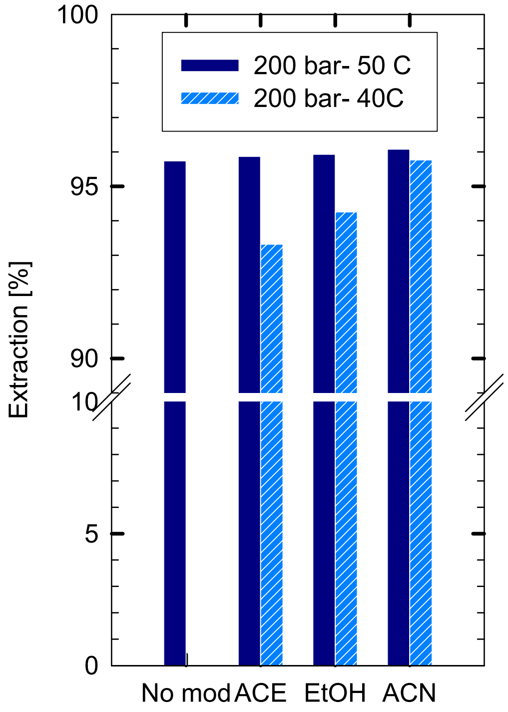
The removal obtained from the extraction performed with the HES lot 2 is summarized in Table 5, and compared with the results obtained with pure scCO$_2$ (Figure 3).

Table 5: Results of extraction of EG with cosolvents (HES lot 2)

| | EG [mg/kg] | Extraction [%] | Mw [Da] |
|---|---|---|---|
| 200 bar – 50°C | | | |
| No modifier | 33.61 | 95.74 | 199400 |
| Acetone | 32.54 | 95.87 | 200500 |
| Ethanol | 32.1 | 95.93 | 199700 |
| Acetonitrile | 30.89 | 96.08 | 198600 |
| 200 bar – 40°C | | | |
| Acetone | 52.7 | 93.32 | 199100 |
| Ethanol | 45.24 | 94.26 | 197900 |
| Acetonitrile | 33.39 | 95.77 | 200200 |

Two isotherms were obtained at 200 bar. There are no great differences among the cosolvents used in the extraction process. The higher percentage of extraction is obtained when acetonitrile is used at 200 bar and 50°C. One important characteristic of this lot is its low water content.

Figure 3: Percentage of extraction (HES lot 2).



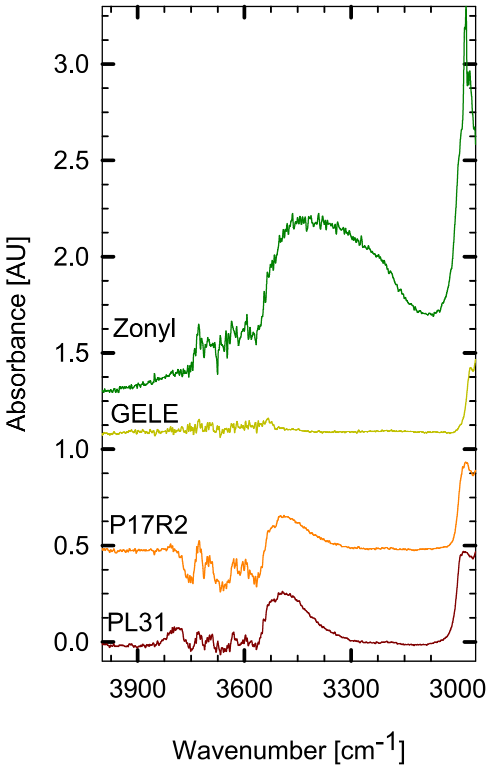
### *3.3. Extraction with surfactants-scCO$_2$*

The process intents to trap those polar organic molecules in the inner core of the aggregates formed in the $scCO_2$ media. The IR spectrum of the surfactants used in this investigation in $scCO_2$ are well described in [15].

### 3.3.1. Pluronic-$scCO_2$

The IR spectrums of pluronic surfactants in the extraction process (e.g., L31 and 17R2) did not show great differences. The vibration modes in the region 2300-2200 $cm^{-1}$ are intensified when pluronic 17R2 is used rather than L31. Both surfactants present broaden bands in the region 3400-3500 $cm^{-1}$ corresponding to the existence of ν(OH), and therefore denoting the presence of intermolecular bonded OH (Figure 4).

Figure 4: IR spectrum of removal with surfactants-$scCO_2$.



### 3.3.2. Zonyl-$scCO_2$.

The principal features of the Zonyl-$scCO_2$ IR spectrum are the presence of a broaden peak from the ν(OH) vibration at 3338 $cm^{-1}$ approximately, corresponding to $H_2O$ and EG at 2976 and 2893 $cm^{-1}$, the symmetric and asymmetric ν($CH_2$) (Figure 4). In the region 2200-2400 $cm^{-1}$, a very intense band is attributed to the interaction between stretching of the $CO_2$ and the fluorinated surfactant because of its high electron-donating capacity of the fluoroether functional groups[15,16]. At lower frequencies, the region of C–F vibration modes is identified.

Visible variations in the spectrums can be seen in the region corresponding to C-F deformation around 1250 $cm^{-1}$ which undergoes red shift to 1240 $cm^{-1}$ as extraction is performed, this could also indicate a changing environment due to the removal of EG, $CF_3$ at 1340-1360 $cm^{-1}$ and the high intensity at around 1000-1100 $cm^{-1}$ that is overlapped with the $CF_2$ vibration. $H_2O$ bonded in the system can be seen at 1635 $cm^{-1}$ corresponding to the δ(OH) vibration.

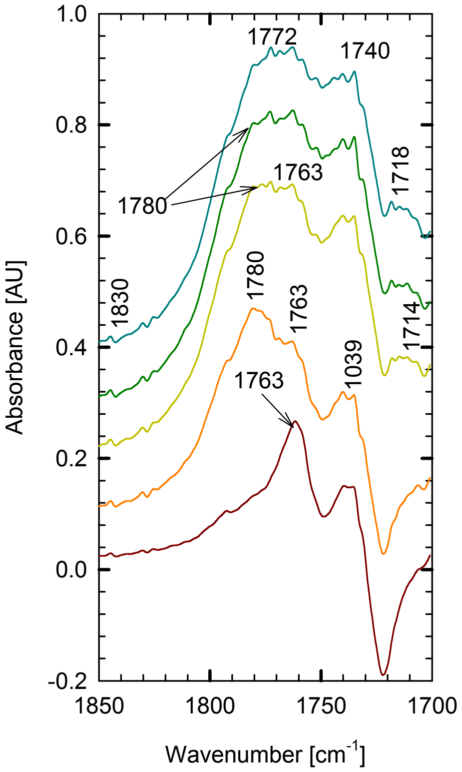
### 3.3.3. Gele-$scCO_2$

In the IR spectrum of Gele surfactant there are variations along extraction in some vibration modes. Shift of the ν($CH_3$) asym from 2930 to 2916 $cm^{-1}$, the increase in intensity of the vibration ν($CH_2$) asym at 2860 $cm^{-1}$. The peak at 1126 $cm^{-1}$ shifts to

1108 $cm^{-1}$ and broadens of the peak along extraction. There is a variation at the vibration C=O, from 1762 to 1773 $cm^{-1}$. Also, the appearance of shoulders at lower frequencies, denote interaction with other molecules or the formation of dimers. According to the IR spectrum of Gele[15], the C=O peak is detected at 1780 $cm^{-1}$, with a shoulder at 1740 $cm^{-1}$. In the extraction IR spectrum, there is a peak at 1763 $cm^{-1}$ and a shoulder at 1740 $cm^{-1}$. This shift Δv equal 17 $cm^{-1}$ could indicate the existence of formation of hydrogen bond with some other species. According to Yamamoto, et al.[2], a shift equal to 23 $cm^{-1}$ was detected in the system acetic acid monomer-ethanol-$scCO_2$; this peak was dependent on the ethanol concentration.

In Figure 5, we can observe that the peak at 1763 $cm^{-1}$ increases throughout the extraction, to finally overlap with the peak at 1780 $cm^{-1}$. This could be because of the presence of EG that initially is at high concentration generating the vibration at 1763 $cm^{-1}$, and as long as EG is being removed, the monomer configuration is favored. Obviously the dimerization of the surfactant occurs through the extraction process, this can be observed through the increase of the peak at 1740 $cm^{-1}$. The appearance of peaks at lower frequencies suggests the existence of another species. Those can be linear dimers, based on the behavior of $scCO_2$ to act as a Lewis acid, as well as Lewis base[17] or a dimer interacting with EG2.

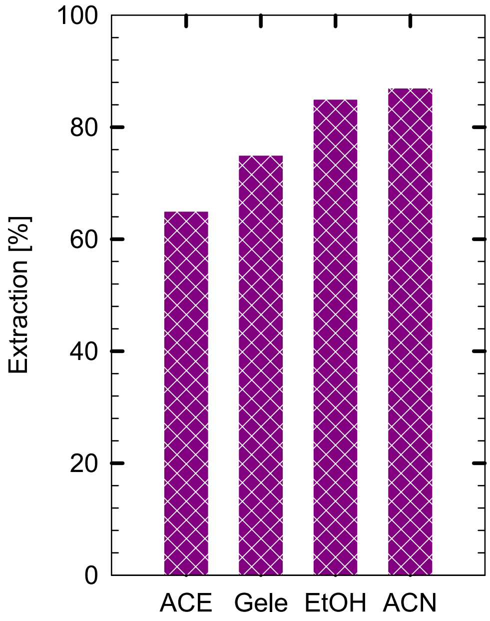
Figure 5: Carboxylic region Gele-scCO2 (200 bar, 40°C).



Results show that it was possible to remove EG with Gele surfactant. The concentration of EG was reduced from 42,236 to 10,500 ppm, approximately 75% of extraction. ECH was reduced until detection limit (13.30 ppm), and a molecular weigh of 271000 Da.

Comparison of the percentage of extraction with the cosolvents used can be seen in Figure 6.

Figure 6: Comparison of extraction with cosolvents and Gele.



As we can observe in Figure 4 the principal difference is at the v(OH) vibration. Zonyl surfactant seems to have the major capacity to trap $H_2O$, followed by the pluronic surfactants and finally Gele that showed little evidence of HB bonding formation. The broaden peak in Zonyl also corresponds to the v(OH) vibration of EG, thus we can say that it removed more EG.

## 4. Conclusions

Pure $scCO_2$ was able to remove polar organic compounds like EG and ECH from the drug HES. Differences in extraction were found between the two lots of HES treated. For HES lot 1, the maximum extraction of 48% (reducing from 42 236 to 21 902 ppm) of EG was achieved at 200 bar and 50°C, in a 3 hours single extraction process. For HES Lot 2 the reduction in EG concentration was about of 96% (reducing from 788 to 31 ppm). Although the high polarity of EG, it was possible to describe some intermolecular interaction with $CO_2$. This solubility can be attributed to a quadrupole/dipole interaction between the hydroxyl group and the $CO_2$. Because of the low tendency to accept protons of $CO_2$ the interaction through the carbonyl group of $CO_2$ and the non interacting oxygen of the glycol could occur.

This could be proven if we were able to detect the asymmetric bending vibration ($v_2$) of $CO_2$, which occurs at a frequency 667 $cm^{-1}$, but we were limited by the IR windows range of transparency. We can not discharge another mechanism where $CO_2$ acts as an hydrogen bond acceptor, even its low capacity to behave like that.

In both cases, the red shift of the frequency should be observed. In the same way the asymmetric stretching vibration of $CO_2$ is detected in the region 2200-2600 $cm^{-1}$ and it is a broad intense band, where not precise conclusion could be made. Due to the low concentration of EG it was not possible to detect intermolecular bonded EG; thus, the interaction with intramolecular and free EG will be more appealing. Unfortunately these vibration modes are detected in the 3900-3400 $cm^{-1}$ and overlap with the broad band of $scCO_2$.

$H_2O$ has an important factor in the extraction of EG. Because of EG has great affinity for $H_2O$, in a hydrated system the EG will prefer to interact with $H_2O$ rather than $CO_2$. Thus, a competition between $CO_2$ and $H_2O$ for EG association will limit the extraction. The extraction is not totally limited, because $H_2O$ is partially soluble in $CO_2$. This can be observed in the red shift that the δ(OH) vibration mode (from 1652 to 1609 $cm^{-1}$) undergoes during extraction of EG upon hydrogen bonding interaction. In a previous work[18], it was found that when $H_2O$ was removed by drying from the polymer, the extraction of EG with pure $scCO_2$ was about 79% (from 42 236 to 8 757 ppm). In the same way when HES lot 2 was used the extraction was 96% (from 788 to 31 ppm). It is important to point it out that HES lot 2 corresponds to a more treated product (e. g., drier and cleaner).

In any case, the stretching vibration ν(OH) was not possible to detect because it was overlapped with the overtone/combination bands of $CO_2$ in the region 3400-3900 $cm^{-1}$.

$CO_2$ has different interactions with each cosolvent used in the present research. Ethanol is hydrogen bonding donor as well as hydrogen bonding acceptor. It is obvious that the interaction for EG will be strong, because of its high polarity and infinite dilution in $H_2O$. The blue shift of the peak at 1452 $cm^{-1}$ to 1470 $cm^{-1}$ corresponding to the asymmetric δ($CH_3$) vibration overlapped with the δ($CH_2$) scissoring, it could be attributed to the strengthening of electrostatic/dispersion or repulsive contribution due to compression. This also occurs with EG that shift from 1461 to 1470 $cm^{-1}$ in extraction. The symmetric δ($CH_3$) vibration is slightly affected by the EG as well as the stretching C–O vibration mode that undergoes a blue shift (from 1053 to 1076 $cm^{-1}$). Because of this shift, we can state that the hydrogen bond donor is preferred. Obviously, the presence of ethanol favors the solubility of EG increasing to 85% (from 42 236 to 6 200 ppm) of extraction.

Acetone is an aprotic solvent with hydrogen bonding acceptance capacity. The shape of the region around the asymmetric δ($CH_3$) vibration suggests an enhancement of the rotation freedom of the molecular group. Also, the blue shift is observed decreasing from 1442 to 1470 $cm^{-1}$. Conversely to ethanol, the stretching C–O vibration mode undergoes a red shift from 1093 to 1076 $cm^{-1}$; this would favor the mechanism stated above. The use of acetone increases the extraction of EG to 65% (from 42 236 to 14 275 ppm).

Acetonitrile is an hydrogen bonding acceptor solvent, the interaction with EG could be through the nitrogen and the OH of the glycol. This interaction is detected in the strong intense band in the region 2200-2400 cm$^{-1}$. We observe the increase in the asymmetric δ($CH_3$) vibration as well as the symmetric δ($CH_3$) vibration that undergoes to red shift from 1393 to 1378 cm$^{-1}$. Also the shift of the stretching C-C vibration would support this mechanism. The presence of acetonitrile increases the removal of EG to 87% (from 42 236 to 5 490 ppm).

Based in the percentage of extraction we ranked the performance of the cosolvents from best to worst (acetonitrile>ethanol>acetone). This behavior agrees with their dielectric constants. This is expected because acetonitrile has a dielectric constant similar to EG (37 at 68°F).

When HES lot 2 is processed, there is not difference in extraction for any of the cosolvents used. The extraction is comparable to the extraction with pure $scCO_2$.

The interactions between $scCO_2$ and the surfactants were studied in a previous investigation[15]. We mainly focused on the capacity of the surfactant to trap the organic compounds in the core on the microemulsion. This was verified in the stretching v(OH) vibration of the glycol. According to the extraction results, we can conclude that zonyl has the major ability to trap organic compounds.

The surfactant approach seems to be adequate for the extraction of EG, or to any other system were the ppm-level concentration does not compromise the phase behavior of the system.

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