Acetylation of Soybean Lecithin and Identification of Components for Solubility in Supercritical Carbon Dioxide

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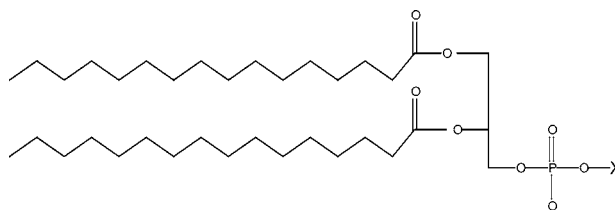
There is a growing interest to develop environmentally friendly surfactants for utilization with supercritical carbon dioxide (scCO$_2$), which is a "green" solvent with many industrial applications. The goal of the present work was to separate the commonly used soybean lecithin into a phospholipid-rich fraction, acetylate this fraction, and then test its solubility in scCO$_2$ to gauge its suitability as a surfactant for potential scCO$_2$-based applications. Soybean lecithin was first purified by fractionation using acetone and ethanol and then acetylated with acetic anhydride. The acetylated lecithin was further purified by fractionation with acetone to separate the acetylated fraction from the nonacetylated fraction. High-performance liquid chromatography and electrospray ionization mass spectrometry were utilized to characterize these fractions. The various acetylated phospholipid fractions were then tested for solubility in scCO$_2$ under various pressures and temperatures using both a cloud-point and a Fourier transform infrared apparatus. Acetylation was found to increase the solubility of the phospholipids in scCO$_2$, and N-acetylated phosphatidylethanolamine (NAc-PE) was found to be the most soluble component of the acetylated phospholipids.

KEYWORDS: Acetylation; carbon dioxide; lecithin; phase equilibria; phospholipids

INTRODUCTION

Soybean lecithin is a complex mixture of phospholipids, triglycerides, and other substances derived from various soybean oil-refining processes (*1, 2*). Because of its abundant availability and excellent properties, including emulsifying behavior, color, and taste, soy lecithin is an important industrial additive. Phospholipids, mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA), are the main components of crude soy lecithin with other minor components being present. Phospholipids find many applications in pharmaceuticals, cosmetics, and the food industry as effective biocompatible emulsifiers, stabilizers, biosurfactants, antioxidants, and wetting agents (*3−6*).

The basic phospholipid structure and its important head groups are shown in **Figure 1**. Phospholipids are polar lipids having surface-active properties. They are amphipathic molecules with hydrophilic heads and hydrophobic tails, which allows self-assembly of these molecules into various structures in solvents such as water. For example, self-assembly into bilayers is important in the formation of membranes (*1, 7*). As such, they support the formation of cells and cell compartments. By virtue of their surface activity, they prevent surfaces from

Figure 1. Structures of the main phospholipids from soybean lecithin. X represents the polar head groups shown below the general structure.

| Phospholipid	Abbreviation	Head group (X)
Phosphatidyl choline	PC	$CH_3CH_2N(CH_3)_3$
Phosphatidyl ethanolamine	PE	$CH_3CH_2NH_2$
Phosphatidyl serine	PS	$CH_3CH (NH_2) COO$
Phosphatidyl inositol	PI	$C_6H_6(OH)_5$
Phosphatidic acid	PA	H
Phosphatidyl glycerol	PG	$CH_3CH (OH) CH_2OH$

sticking together, e.g., in the lungs of infants, where they act as lung surfactants (*1*).

Increasing health concerns caused by the industrial use of organic solvents, such as hexane and methylene chloride, by way of either environmental emissions and/or trace residues in products, have propelled research efforts aimed at developing environmentally benign or "green" processing techniques that either eliminate or significantly mitigate pollution at the source. Supercritical carbon dioxide (scCO$_2$) has emerged as a viable alternative to organic solvents for several applications, including

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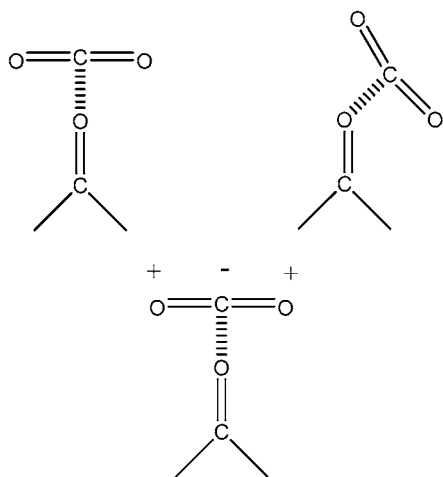
Figure 2. Schematic of chemical interations between the CO_2 solvent and the carbonyl group.

extraction of nutraceuticals, polymerization, and nanoparticle preparation (*8−11*). Carbon dioxide is inexpensive, nontoxic, nonflammable, and environmentally and chemically benign. In the supercritical state, it can have unique properties such as a liquidlike density and gaslike diffusivity, with these properties being tunable by varying the pressure and/or temperature (*12*). However, lack of solubility of polar materials in scCO2 has held back several commercial applications of this technology. This hurdle has led to the investigation of CO2-philic groups that enhance the solubility of otherwise scCO2-insoluble derivatives. It has been reported that the addition of acetate side chains to polymers, silicones, or sugars may lead to high solubility of these compounds in liquid and scCO2 (*13−16*) through the possible binding of CO2 with the carbonyl group by Lewis acid−base type interactions as shown in **Figure 2** (*15−18*). While scCO2 is an excellent solvent for nonpolar lipids such as triglycerides and fatty acids, polar lipids such as phospholipids have shown only limited solubility in scCO2 (*19, 20*). For this reason, there has been only limited work performed on lecithin solubility in scCO2, with ethanol as an entrainer (*13, 20*). The only work reported for phospholipids with scCO2 without using organic modifier is that of egg phospholipids at very high pressures (*8*).

Acetylation of lecithin, especially the PE head group, is a well-established process (*1, 4, 17*). Acetylation of lecithin is used for improved properties, such as increasing its resistance to heat. In addition, acetylation allows some compounds to be soluble in acetone, so that they can be separated from other nonacetylated compounds with simple batch solvent extraction and precipitation (*4, 21, 22*). Hence, the aim of this work was to (i) modify the polar head group, especially ethanolamine of soybean phospholipids, and separate these molecules from other phosphatides with a simple inexpensive batch solvent extraction and precipitation process and (ii) evaluate the solubility of the acetylated lecithin compounds in scCO2 to determine suitability for scCO2-soluble surfactants.

MATERIALS AND METHODS

Materials. Crude bleached soybean lecithin (hereafter call lecithin) was obtained from CanAmera Foods (Hamilton, Ontario). All solvents and reagents used in this work were of analytical or higher grade from EM Science or Fisher Chemicals. PE, PC, and PI standards for high-performance liquid chromatography (HPLC) analysis were purchased from AvantiPolar Lipids, Inc. (Alabaster, AL).

Table 1. Chemical Properties of Soybean Lecithin

acetone insoluble	63.9%
acetone soluble	34.7%
moisture	0.88%
hexane insoluble	0.03%
acid value	32.0

Methods. *Analytical Procedures.* The acetone-insoluble content (Ja-4-46), the moisture content (Ja-2b-87), the acid value (Ja-6-55), and the hexane-insoluble matter (Ja-3-87) of lecithin were determined according to the Official and Tentative Methods of the American Oil Chemists' Society (*23*). These methods were repeated three times, with the average value reported in **Table 1**, and a relative error of ±4%.

Lecithin Deoiling. The process of fractionation and acetylation of lecithin fractions is summarized in **Figure 3**. Briefly, lecithin (25 g) was deoiled by dispersion in cold acetone (150 mL) and stirred with a magnetic stir bar for approximately 1 h. The solvent containing neutral lipids was decanted into a separate beaker, and the process was repeated until the solvent was color free. The acetone wet polar lipid material, e.g., deoiled lecithin (I), was dried under vacuum and stored below 0 °C until further processing. All procedures were repeated at least three times.

Ethanol Fractionation. Dry deoiled lecithin (I; 15 g) was transferred to a beaker and extracted with ethanol (150 mL) using magnetic stirring for 1 h. The solvent containing the ethanol-soluble fraction was decanted into a separate beaker. This process was repeated four times to ensure quantitative separation. Both fractions, ethanol-soluble (II) and ethanol-insoluble (III), were dried under vacuum and stored below 0 °C until further processing.

N-Acetylation. Free amino groups were N-acetylated using acetic anhydride according to Doig and Diks (*4*). A weighed amount of sample (e.g., I, II, and III) was dissolved in an excess amount of hexane, and either 1.0 or 1.5 mol equivalent of acetic anhydride was added (based on PE content), along with triethylamine (TEA) (2× volume of acetic anhydride). The solution was stirred at 45 °C for 1 h, and the resultant solution was dried under vacuum. Acetylated compounds were dissolved in acetone, separated from residual insoluble material by centrifugation, and subsequently vacuum-dried. The acetone-soluble acetylated fractions from I, II, and III were termed, I-A, II-A, and III-A, respectively, where **Scheme 1** shows, as an example, the acetylation of the phospholipid PE.

HPLC Analysis. Phospholipids were analyzed on a Beckman HPLC system (model 126 pump, model 168 PDA detector, and model 507e autosampler) according to Beare-Rogers et al. (*24*). Phospholipids (10 μL) were injected into a Microsorb MV 100 5-Si column (4.6 mm × 250 mm, 5 μm spherical silica) and eluted isocratically with *n*-hexane: 2-propanol:acetic acid (8:8:1) at 2 mL min⁻¹. The eluent was monitored at 206 nm. System control and data analysis were performed using 32 Karat System (V3.0) Software (Beckman-Coulter). Phospholipids were identified using authentic phospholipid standards (Avanti Polar Lipids).

Electrospray Ionization Mass Spectrometry (ESI-MS). ESI-MS analyses were performed using a Micromass Quattro Micro mass spectrometer with ESI (Micromass, Manchester, England) in both negative and positive ion modes. The samples (run with/without internal standards) were introduced into the mass spectrometer by infusion of the samples mixed with CHCl3 at a ratio of 1:1 or 2:1 and flow rate of 10 μL min⁻¹. For negative ion ESI, NH4OH was added to a final concentration of 2%. The following parameters were utilized for all experiments: data range, 200−1200 *m/z*; cone voltage, 60 V; source temperature, 80 °C; and desolvation temperature, 150 °C. Calibration was performed using a NaI solution and verified with standard DMPC and DMPG purchased from Avanti Polar Lipids. MassLynx (V4.0) software was used for instrument control, data acquisition, and data handling. To 200 μL of each sample, 4 or 8 μg of DMPC or DMPG was added for quantification (with the intensity of peaks corresponding to DMPC or DMPG kept less than 5% of the most intense peak), assuming that all of the phospholipids have a similar response factor upon ESI-MS (see the Supporting Information).

Solubility and Phase Equilibria. The solubility experiments were performed using a high-pressure view cell (25 mL capacity) constructed

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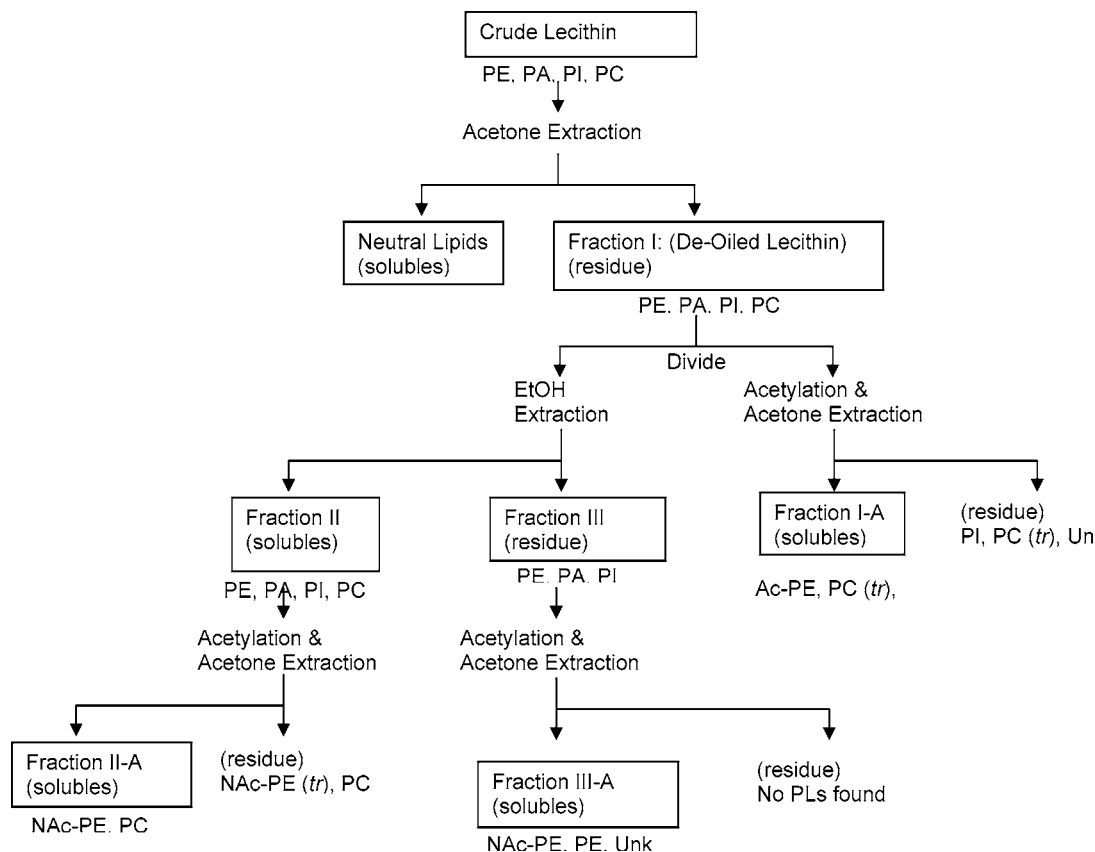
Figure 3. Fractionation of crude soybean lecithin. Crude lecithin was first extracted with acetone, and the residue (fraction I) containing the phospholipids was divided into two subsamples. The first subsample was further extracted with EtOH, with both soluble (fraction II) and insoluble (fraction III) components subsequently acetylated and extracted with acetone to yield acetone-soluble fractions II-A and III-A, respectively. The second subsample of fraction I was acetylated and extracted with acetone to yield acetone-soluble fraction I-A. The main phospholipids observed in each fraction (HPLC) are indicated. NAc, N-acetyl; tr, trace; and unk, unknown.

Scheme 1. Overall Acetylation Reaction for the Synthesis of NAc-PE
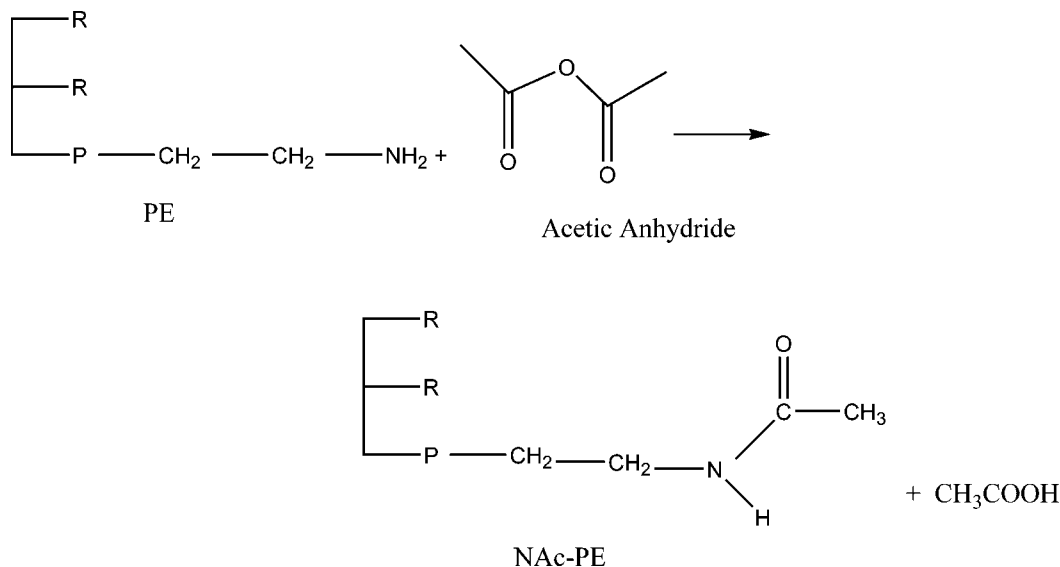
from 316 SS and fitted with 10 mm thick sapphire windows (Insaco). Temperature and pressure in the view cell were measured and controlled by means of a T type thermocouple (Omega), a heating tape, and a pressure transducer (Omega). The CO$_2$ liquid was added to the cell using a high-pressure syringe pump (Isco 260D). Typically, the cell was purged several times with CO$_2$ (99.99% BOC Gases, Mississauga, ON, Canada) to remove any traces of air, after loading the lecithin sample (300−400 mg). The cell contents were stirred using a magnetic stir bar controlled by a magnetic stirrer placed beneath the cell. The

contents of the cell were monitored visually for phase separation by decreasing pressure at a rate of 30−50 psig/min. Each cloud-point experiment was conducted at least three times under the same conditions, and the average values were provided with an associated relative error of ±5%.

Fourier Transform Infrared (FTIR) Spectra. In situ FTIR monitoring of solute concentration was performed in a stirred 100 mL high-pressure autoclave (Parr Instruments 4842) using a high-pressure diamond immersion probe rated to 5000 psig (Sentinel-ASI Applied Systems).
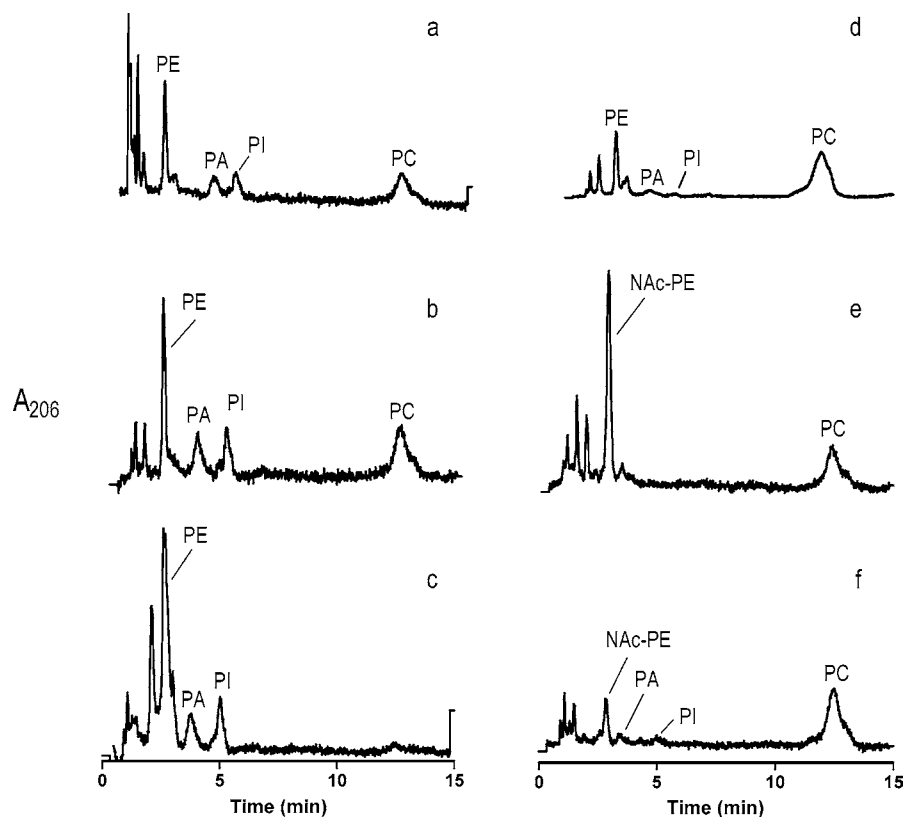
Figure 4. HPLC analysis of soybean lecithin fractions. The different lecithin fractions were generated as depicted in **Figure 3** and analyzed by normal phase (see Materials and Methods for details). Representative chromatograms of (**a**) crude lecithin, (**b**) deoiled lecithin (fraction I), (**c**) fraction III, (**d**) fraction II, (**e**) fraction II-A, and (**f**) fraction II residue remaining after acetylation are shown. Chromatograms are not normalized.

Table 2. Phospholipid Composition of Crude Lecithin[a]

phospholipid	composition (%)	mol composition
PC	16	2
PE	14	2
PI	12	2
PA	6	1

[a] Values provided by CanAmera Foods. Phospholipids account for 48% of the crude lecithin.

The probe was attached to an attenuated total reflectance (ATR)-FTIR spectrometer (ASI Applied System ReactIR 4000), connected to a computer, and supported by ReactIR software (ASI). Spectra were recorded at a resolution of 2 cm^{-1}, and the absorption spectra were the results of 64 scans. Approximately 1 g of acetylated lecithin (I-A, II-A, and III-A) was introduced into the autoclave, which was subsequently pressurized by CO_2 at a controlled rate using a syringe pump (Isco 260D) monitored by a pressure transducer (Parr Instruments ± 50 psig) with the temperature maintained at 40 °C by a temperature controller (Parr Instruments ± 0.5 °C).

RESULTS AND DISCUSSION

The chemical properties of the crude lecithin starting material used for this work are shown in **Table 1**. **Figure 3** provides the methodology used for fractionation of crude soybean lecithin. HPLC analysis of this crude material revealed the presence of PA, PC, PE, and PI (**Figure 4a**), in agreement with the composition reported by the manufacturer (**Table 2**). Deoiling of the crude lecithin produced a white powder with increased relative phospholipid content (by removal of triglycerides and free fatty acids) with the same relative ratio of phospholipids as crude lecithin (**Figure 4b**). Deoiled lecithin was further

separated into an ethanol-insoluble fraction (III) lacking PC (**Figure 4c**) and an ethanol-soluble fraction (II) comprised of all four phospholipids but enriched in PC (**Figure 4d**). These results are similar to those reported in the literature (*25, 26*).

N-acetylation was carried out using fractions I, II, and III, subsequently producing fractions termed I-A, II-A, and III-A, as also summarized in **Figure 3**. A 1.5 mol equivalent of acetic anhydride (based on PE content) was used in the reaction to ensure maximum acetylation of PE (*4, 27, 28*) along with TEA (2× acetic anhydride) to hasten the reaction between the free amino group and the acetylating agent (*27*). The acetylated mixture was further fractionated using acetone, where the acetone-soluble fraction contains the acetylated compounds (**Figure 4e**).

Acetylation resulted in the formation of a new compound with a slightly earlier retention time than PE in our HPLC system (**Figure 4e**), which was identified as N-acetylated PE (NAc-PE) by comparison to an authentic standard and ESI-MS (see below). Both acetylated PE and PI standards were prepared by acetylation of the HPLC standards in microscale experiments to ensure HPLC peak identification. In all three fractions (I-A, II-A, and III-A), the acetone-soluble components account for more than the estimated NAc-PE (*26*) indicating that some additional acetylated compounds dissolved in acetone, including NAc-PC, NAc-PI, and others. However, NAc-PE was found to be the major compound in the acetone-soluble fraction for all three acetylated samples, accounting for 84% of fraction I-A, 81% of fraction II-A, and 69% of fraction III-A, based on the HPLC results. The presence of the peak in **Figure 4e** at a similar retention time to the PC peak from **Figure 4d** is presumed to be acetylated-PC, as only acetylated phospholipids will dissolve in acetone, and PC is known to be acetylated under similar conditions (*29*). The ESI-MS results (discussed below) are also

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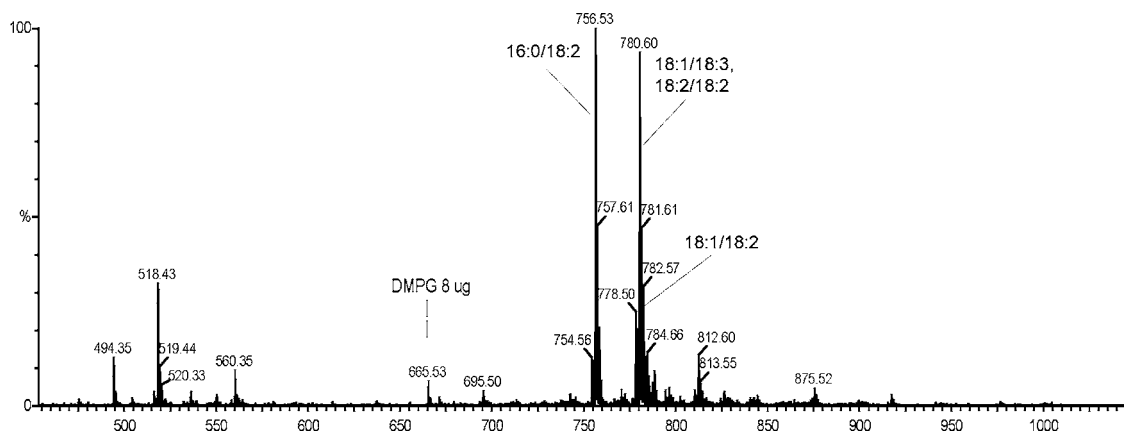
Figure 5. Electrospray ionization mass spectrum of fraction II-A. The acetone-soluble fraction derived from the acetylation of fraction II was analyzed by ESI-MS in the negative ion mode as described in the Materials and Methods. On the basis of HPLC analysis, the predominant component in the mixture is NAc-PE with smaller amounts of PC (see **Figure 4e**). In the ESI-mass spectrum, strong signals representing NAc-PE with 16:0 and 18:2 acyl chains (*m/z* 756.53), 18:1 and 18:3 (or two 18:2) acyl chains (*m/z* 780.60), and 18:1 and 18:2 acyl chains (*m/z* 782.57) were present. Because the head groups on NAc-PE and PC have the same mass, contributions by PC to the major mass signals at *m/z* 756.53, 780.60, and 782.57 cannot be ruled out.
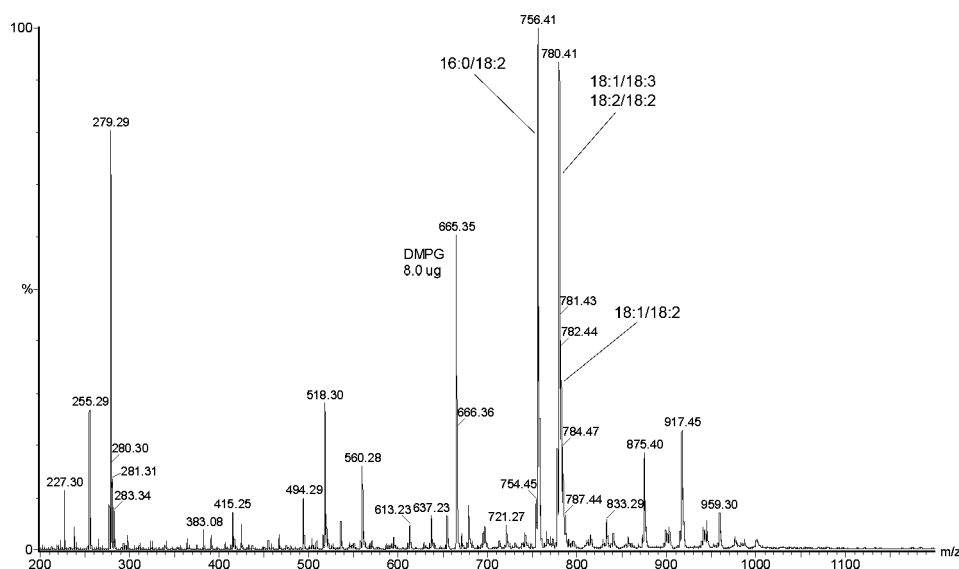
Figure 6. Electrospray ionization mass spectrum of fraction III-A. The acetone-soluble fraction derived from the acetylation of fraction III was analyzed by ESI-MS in the negative ion mode as described in the Materials and Methods. On the basis of HPLC analysis, NAc-PE is the predominant phospholipid in this fraction. In the ESI-mass spectrum, strong signals representing NAc-PE with 16:0 and 18:2 acyl chains (*m/z* 756.53), 18:1 and 18:3 (or two 18:2) acyl chains (*m/z* 780.60), and 18:1 and 18:2 acyl chains (*m/z* 782.57) were present.

consistent with this. There were also some unknown minor peaks in the HPLC chromatograms that were not identified. These components could be sphingolipids or lyso-phosphatidyl derivatives, which are formed (or generated) due to hydrolysis of the main phospholipid components (*30*). Several of the unknown peaks in some chromatograms were found to elute before PE, likely corresponding to nonpolar lipids or/and ceramides (*31*).

Because of both the lack of commercially available HPLC standards of acetylated phospholipids and the superior structural information provided, identification of the acetylated compounds was determined by ESI-MS. For example, the negative ion ESI-MS spectra for fractions II-A (**Figure 5**) and fraction III-A (**Figure 6**) show intense ions from negatively charged acetyl lecithin components. The main components in both **Figures 5** and **6** are NAc-PE with fatty acids totaling 34 and 36 carbon atoms. Soybean lecithin usually contains palmatic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) fatty acids. Thus, the main peaks in **Figure 5** correspond to NAc-PE with attached palmitic and linoleic acids ([M]$^-$ = 756), oleic

and linolenic acids ([M]$^-$ = 780), two linoleic acids ([M]$^-$ = 780), and oleic and linoleic acids ([M]$^-$ = 782). These assignments are supported by the positive ion spectra (data not shown) having signals at [M]$^+$ = 758, 782, and 784 with corresponding signals at [M]$^+$ = 859/883 (+1 TEA) and 960/984 (+2 TEA). Smaller peaks in **Figures 5** and **6** represent fragment ions from the breakdown of parent phospholipids during analysis (*32*). Sample III-A was found to have more peaks from ESI-MS analysis than sample II-A, likely due to the higher acetic anhydride ratio used for the acetylation reaction (1.5 as compared to 1.0), which provided acetylation of a larger variety of phospholipid species.

In addition to PE being acetylated, the ESI-MS results indicate that some other minor components also reacted. In **Figures 5** and **6**, the peak at [M] = 875 likely corresponds to partially acetylated PI (with palmitic and lenoleic acids attached) (*32, 33*). This is in agreement with the HPLC results of PI being in both the ethanol-soluble (II) and the ethanol-insoluble (III) lecithin fractions. In **Figure 5**, the peak at [M] = 813 likely

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corresponds to acetylated PC (acetylated PC with stearic and oleic acids attached) (*32, 33*). This is also in agreement with the HPLC results (**Figure 4e**), which shows acetylated PC in the acetone-soluble fraction only.

Solubility of the different fractions of acetylated lecithin, e.g., I-A, II-A, and III-A was studied in $scCO_2$ using both a solubility apparatus and an in situ FTIR. Using the solubility apparatus, where contents of the high-pressure cell were observed visually, temperatures ranging from 40 to 70 °C (to prevent denaturation of lecithin) and pressures up to 58.6 MPa were studied. Both a soluble and a nonsoluble component were observed for the three acetylated samples when studied up to 59 MPa without a cosolvent. The soluble fraction of the three acetylated samples gave cloud-point pressures of 12.4, 13.4, and 14.1 MPa at temperatures of 40, 60, and 70 °C, respectively. The fact that all three acetylated samples gave the same cloud-point pressure of the soluble component(s) at a given temperature indicates a similar structure of the soluble component(s), as analyzed further below by ESI-MS. For the three fractions at a fixed pressure of 41 MPa, there was a significant increase in solubility as determined visually when increasing the temperature from 30 to 40 °C and no significant increase from 40 to 50 °C in independent experiments. Increasing the temperature above 50 °C to 60 and 70 °C resulted in a decreased solubility of the fractions, likely due to the decrease in the CO_2 density, thus decreasing the solubility of acetylated lecithins, similar to the effect observed with nonacetylated lecithin (*2, 13*). Experiments performed with the three acetylated fractions with an increase in pressure from 9 to 58.6 MPa at 40 °C gave a steady increase in solubility, likely due to the increase in solvent density with pressure (*2*).

In addition, ethanol was used as a cosolvent with CO_2, as ethanol is generally considered as safe (gcas) and is miscible in CO_2 under the experimental conditions. The three acetylated lecithin fractions were studied using 4−8% ethanol (90, 95, and 100%) with 95% ethanol providing the best solubility of the three studied ethanol solutions. It was previously reported by Montanari et al. that nonacetylated lecithin could not be dissolved with less than 10% ethanol (*13*). The addition of ethanol (95%) as a modifier to CO_2 at 8% (w/w) lowered the cloud point of the three acetylated fractions to ~9 MPa psig at 40 °C, hence significantly increasing the solubility of acetylated lecithin in CO_2.

While venting CO_2 out of the view cell after the solubility experiments without the presence of ethanol, the soluble fraction would accumulate under the view cell outlet during depressurization (**Figure 7**). This material, which had a high affinity for $scCO_2$, was further analyzed by ESI-MS as shown in **Figure 8**. Peaks at $[M]^- = 756$ (NAc-PE with palmitic and linoleic acids attached) and $[M]^- = 780$ (NAc-PE with either oleic and linolenic or two linoleic acids attached) predominated, with few other fragments in the negative ion spectrum. These results were supported by the positive ion spectrum of the same extract (data not shown) in which appropriate TEA conjugates (i.e., at $[M]^+ = 859, 883, 960,$ and 984) were observed, in addition to the expected peaks at 758 and 782. Hence, the ESI-MS results indicate that the major portion of $scCO_2$ soluble components is NAc-PE, in addition to NAc-PE bound with TEA.

To further analyze the solubility of the acetylated material, ATR-FTIR was utilized. ATR-FTIR is highly suitable to determine solubility, as only the component soluble in $scCO_2$ will be detected by the system, while any insoluble content will not interfere with the analysis (*34, 35*). **Figure 9a** (with CO_2) and **b** (with CO_2 subtracted) shows the spectra of the E-soluble
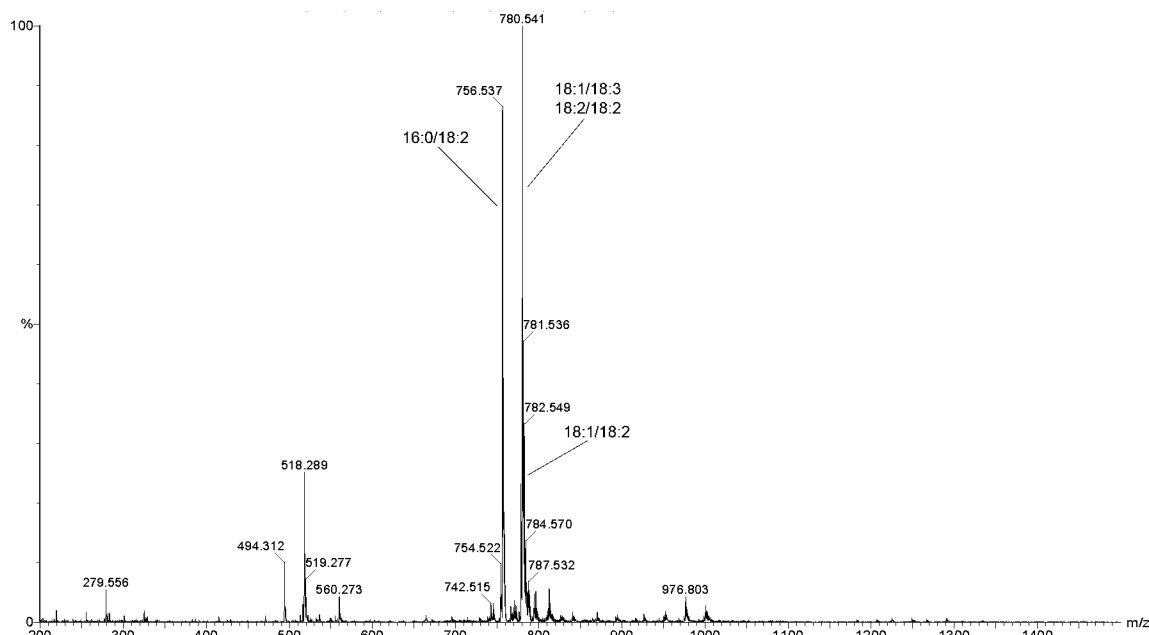

Figure 7. Recovery of $scCO_2$ soluble components extracted from fraction II-A. The photo shows the residue (indicated by arrow) remaining under the outlet of the cell after the venting of CO_2.

lecithin (II-A), while the spectra of the other acetylated fractions (I-A and III-A) were similar. No change was observed in these spectra during pressurization between 12 and 33 MPa at 40 °C, indicating that the soluble fraction dissolved at 12 MPa, and no other materials dissolved as the pressure was increased. This corresponds to the solubility experiments, where the soluble fraction had a cloud-point pressure at 12 MPa at 40 °C.

To distinguish between PE, PC, and PI by FTIR, both the three bands in the 3010−2850 cm^{-1} region and the PO$_2$ group vibration at 980 cm^{-1} are used (see **Figure 1** and **Scheme 1** for the structures of the phospholipids and NAc-PE) (*36, 37*). The peak shapes for the three peaks in the 3010−2850 cm^{-1} region and the absence of a strong peak at 980 cm^{-1} are indicative of the PE phospholipid (*36, 37*), which corresponds with the ESI-MS results of the CO_2-soluble fraction. The characteristic peaks at 1054 and 1171 cm^{-1} in the spectrum are from the phospholipid phosphate group (single bond P−O and double bond stretching bands) (*36, 37*). Acetylation of the lecithin is indicated from the IR peaks at 1737 cm^{-1} (carbonyl C=O stretching of ester), 1372 cm^{-1} of [C−H in −O(C=O)−CH$_3$], and 1235 cm^{-1} (C−N stretching of acetyl group) (*38*). No absorption was observed in the spectral region from 1840 to 1760 cm^{-1} indicating that the acetylated products are free of unreacted acetic anhydride, and the absence of a peak at 1700 cm^{-1} indicates that the acetylated products are free of acetic acid byproducts (*38*).

The increased solubility of acetylated materials in $scCO_2$, in particular NAc-PE, is presumably due to the molecular binding between the CO_2 molecule and the carbonyl oxygen by Lewis acid−base type interactions, as depicted in **Figure 2** (*15−18*). It is likely that not all acetylated phospholipids can bind to CO_2 through the carbonyl group due to steric hindrance. NAc-PE is a terminal acetylated material, which should be relatively accessible to the nonpolar CO_2 solvent. When considering the nonsoluble component of the acetylated material in $scCO_2$, the presence of sphingolipids and/or lyso-phosphatidyl derivatives in acetylated lecithin, as shown by our HPLC and ESI-MS analysis results, is due to losing one or two fatty acids from the phospholipids. After losing fatty acids, the sphingolipids and/or lyso-phosphatidyl derivatives become more polar as compared to the main phospholipids. In addition, both the length of the fatty acid chains and the number of double bonds in the chains

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Figure 8. Electrospray ionization mass spectrum of the $scCO_2$-soluble components from fraction II-A. The acetone-soluble fraction derived from the acetylation of fraction II was extracted with $scCO_2$, recovered after solvent evaporation, and analyzed by ESI-MS in the negative ion mode as described in the Materials and Methods. In the ESI-mass spectrum, strong signals representing NAc-PE with 16:0 and 18:2 acyl chains (*m/z* 756.53), 18:1 and 18:3 (or two 18:2) acyl chains (*m/z* 780.60), and 18:1 and 18:2 acyl chains (*m/z* 782.57) were present.
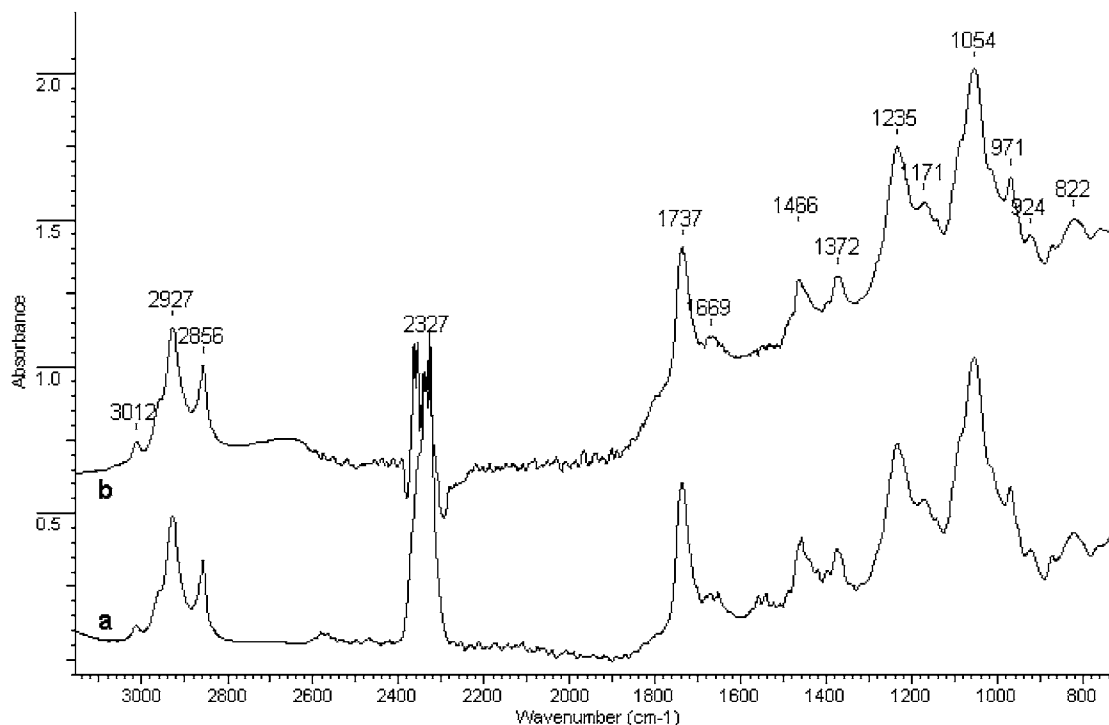
Figure 9. FTIR spectra of fraction II-A in situ in $scCO_2$. The acetone-soluble fraction derived from acetylation of fraction II was analyzed by in situ ATR-FTIR as described in the Materials and Methods. On the basis of this analysis, NAc-PE is the predominant phospholipid in this fraction.

are known to influence the elution order of phospholipids in HPLC (*39*), hence influencing the solubility of the materials in $scCO_2$.

Because of its relatively low cloud-point pressure of 13 MPa at 40 °C, NAc-PE is potentially attractive for industrial applications, which are preferably below 35 MPa in $scCO_2$. Now that NAc-PE has been identified to have affinity for potential applications in CO_2, future work will explore techniques to isolate this material and perform detailed cloud-point solubility and light-scattering studies to determine how acetylated phos-

pholipids can be harnessed as benign surfactants and as stabilizers for nanoparticles and nanosuspension formulations using $scCO_2$ and other green solvents.

Hence, in conclusion, acetylation of the head group in the phospholipids, primarily PE, rendered them soluble in acetone and allowed them to be separated from the phospholipid mixture. The acetylated acetone-soluble fraction was found to contain N-acetyl PE, which showed good solubility in $scCO_2$, using both a solubility and an in situ FTIR apparatus, as compared to the nonacetylated fraction. ESI-MS spectra of CO_2-soluble acety-

lated lecithin in **Figure 8** shows N-acetyl PE with 34 and 36 carbons fatty acids. The presence of acetyl group in PE lowered the cloud-point pressure to 13 MPa at 40 °C, which is attractive for potential industrial applications.

ACKNOWLEDGMENT

Supporting Information Available: Spectrum of PG in ES⁻ and spectrum of PC in ES⁺. This material is available free of charge via the Internet at http://pubs.acs.org.

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