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Enrichment of Omega-3 Polyunsaturated Fatty Acid Methyl Esters by Ionic Liquids Containing Silver Salts

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Abstract: In this paper, we demonstrate that the hydrophobic ionic liquid (IL) such as 1-hexyl-3-methyl imidazolium hexafluorophosphate, [hmim][PF_6], with silver salt, e.g. $AgBF_4$, is an excellent extraction phase to separate and enrich omega-3 polyunsaturated fatty acid methyl esters (PUFAMEs) from the mixed solution containing closely related saturated, monounsaturated or diunsaturated fatty acid methyl esters. With this silver salt-ionic liquids extraction phase, the health-beneficial omega-3 PUFAMEs such as methyl ester of *all-cis*-5,8,11,14,17-eicosapentaenoic acid (20:5 or EPA) and methyl ester of *all-cis*-4,7,10,13,16,19-docosahexaenoic acid (22:6 or DHA) were largely enriched from 18% (wt %) in the original cod liver oil to greater than 80% in the 1-hexene stripping solvent. The unique properties of nonvolatility and adequate polarity allow ILs to dissolve or suspend silver salts and to be conveniently adopted as extraction phase in separating PUFAMEs. The ILs with different hydrophobicities and different silver salts were screened to obtain an optimal combination of IL and silver salt with the highest extraction capability and selectivity. The screening results showed that $AgBF_4$ exhibited high extraction capability in the hydrophobic ILs but little or no extraction capability in the hydrophilic ILs. Furthermore, the high extraction capability of $AgBF_4$ in hydrophobic ILs was much greater than that of $AgBF_4$ in traditional silver-water or silver-alcohol extraction systems. Pretreating the silver-ILs extraction phase with steric hindered short chain olefins could significantly enhance its extraction selectivity for PUFAMEs. Nine runs of the IL-silver extraction phase showed no obvious decrease in its extraction capabilities and selectivities.

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Keywords: Fish oil; Ionic liquids; Liquid phase extraction; Omega-3 fatty acid methyl ester; Silver salt

INTRODUCTION

Omega-3 polyunsaturated fatty acids and their derivatives (commonly referred to as omega-3 fatty acids) are recommended by the American Heart Association for all people because of their benefits against cardiovascular disease (1). It has been shown that omega-3 fatty acids decrease the risk of arrhythmias, which can lead to sudden cardiac death. They also decrease triglyceride levels and the growth rate of atherosclerotic plaque. OMACOR (Omega-3 fatty acid ethyl esters), a concentrated form of omega-3 fatty acids (as ethyl esters), is approved by Food and Drug Administration for treating patients with high triglycerides. Omega-3 fatty acids are also widely used in infant formula as they are essential for infant growth (2). Humans, however, do not have the capacity for biosynthesis of omega-3 fatty acids, due to the absence of Δ-15 and Δ-12 desaturase enzymes (3). Consequently, omega-3 fatty acids have to be obtained from external sources. Omega-3 fatty acids are known to exist in fish, microalgae, krill, and flax (4). For example, fish oils are frequently used as omega-3 fatty acid supplements. However, more concentrated forms of omega-3 fatty acids are desirable, as the amount of fish oil required to achieve the desired biological effects carries the risk of vitamin A and D overdose and increases intake of saturated fatty acids (5). In addition, the concentrated form is also needed for baby food additives and prescription medicines such as OMACOR mentioned earlier. Therefore, efficient enrichment methods for omega-3 fatty acids are needed. Such efficient enrichment method could also make omega-3 fatty acids more readily available, as more natural sources could be used to produce omega-3 fatty acids economically (6). In designing an efficient separation method, one challenge is the separation of omega-3 fatty acids from saturated fatty acids, which exist in large quantity and have undesirable health effects. Due to the similarity of molecular size, polarity, and structure, separation of omega-3 fatty acid from their saturated analogues is difficult. Here we report an efficient enrichment method for omega-3 fatty acids based on extraction using room temperature ionic liquids with dissolved silver salts. With the same extraction system, the concentration of omega-3 fatty acids such as 20:5 and 22:6 from cod liver oil was also achieved.

A diverse range of methods have been reported for the separation and enrichment of PUFAMEs. These methods included urea inclusion complexation (7), low temperature fractional crystallization (8), salt solubility methods (9), liquid-liquid extraction using aqueous silver nitrate solution (10), liquid-solid phase extraction (11), and supercritical fluid extraction of essential fatty acids from rice bran oil or seed oil (12,13). Chromatographic techniques (14) have also been utilized to separate and purify PUFAs or PUFAMEs but they have been limited to a small scale. Liquid-liquid extraction has been a favored process for the development of separation process owing to its high separation capacity and simple manipulations. However, in traditional solvent extraction, highly toxic, flammable, volatile, and sometimes expensive organic solvents pose challenges to the green chemistry.

Room temperature ionic liquids have attracted significant attention as substitutes for traditional organic solvents in organic synthesis, catalytic transformations, and separations, as they possess unique properties such as polarity and solubilizing ability (15–17). They have been utilized as "green" solvents in the extraction of a variety of substances, including metal ions (18), organics (19), and organosulfur and nitrogen compounds from transportation fuels (20), and gases (21).

From earlier on, people have known that silver ions (or other metal ions such as cuprous) could form reversible π-bond complexation with double bond (22). Olefin molecules donate π electrons from their occupied 2p orbitals to the empty s orbitals of the silver ions to form σ-bonds. Back donation of electrons from the occupied d orbitals of the silver ions into the empty π^*-2p antibonding orbitals of the olefin molecules results in π-bonding. This reversible and specific interaction between silver ions and olefinic double bonds has been widely utilized to separate olefins from saturated paraffin (23,24). In these studies, the extraction solvents used to dissolve silver salts were limited to traditional organic solvents such as alcohol or water. The hydrolyzation of silver salts by water and consequently, the decrease in complexation ability is unavoidable in the aqueous phase. Therefore, to minimize the hydrolyzation of silver ions and increase the extraction capability of silver salts, higher concentrated silver solution was often utilized or polar co-solvent such as methanol was added. However, by dissolving silver salts in ionic liquids, the hydrolyzation of silver ions could be largely decreased and consequently, lower amount of silver salts can be used to achieve a high extraction capability. Using ionic liquids with suspended silver or cuprous salts, Munson and coworkers (25,26) developed a novel liquid extraction method for separating olefins from saturated paraffins.

In our view, ionic liquids and metal ions provide a unique combination for the extraction of hydrophobic organic compounds such as omega-3 fatty acids, as this combination can take advantage of the unique selectivity of metal ions effectively. Despite the unusual selectivity of metal ions towards different classes of organic compounds, metal ions have not been widely used for the selective extraction of hydrophobic organic compounds partially due to the poor solubility of their complex with hydrophobic organic compounds in organic solvents. With their amphiphilic structural feature, ionic liquids are well-positioned to dissolve metal organic complexes, which are also amphiphilic in nature.

Using this rationale, we successfully achieved the separation and concentration of omega-3 fatty acid methyl esters by extraction with ionic liquids containing silver salts. As ionic liquids and silver salts are of relative high cost, using them to separate commercially valuable omega-3 fatty acid methyl esters may promise a more cost-effective application.

EXPERIMENTAL

Materials

$AgBF_4$, $AgNO_3$, $AgClO_4$, CF_3SO_3Ag, CF_3COOAg, 1-methylimidazole, pyridine, 1-hexyl chloride, 1-butyl chloride, ethyl acetate, ethyl bromide, $NaBF_4$, $Li(CF_3SO_2)_2N$ from Aldrich (Milwaukee, WI, USA), 4-picoline, HPF_6 from Fluka (Ronkonkoma, NY, USA), and hexane, chloroform, dichloromethylene from Fisher Scientific (Pittsburgh, PA, USA, all the solvents are HPLC grade) were used without further purification. Cod liver oil was acquired from J. R. Carlson Lab Inc. (Arlington Hts. IL, USA). For FAMEs (Scheme 1), 20:5 (EPA), methyl all-cis-5,8,11,14,17-eicosapentaenoic acid; 22:6 (DHA), methyl all-cis-4,7,10,13,16,19-docosahexaenoic acid; 18:3, methyl all-cis-9,12,15-octadecatrienoic acid; 18:2, methyl all-cis-9,12-octadecadienoic acid; 18:1, methyl cis-9-octadecaenoic acid; 18:0, methyl octadecanoate; 16:0, methyl hexadecanoate;

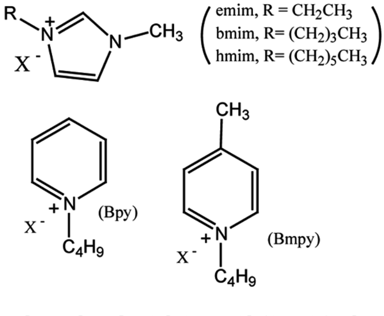
EPA or 20:5 $CH_3(CH_2CH{=}CH)_5(CH_2)_3CO_2CH_3$

DHA or 22:6 $CH_3(CH_2CH{=}CH)_6(CH_2)_2CO_2CH_3$

18:2 $CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7CO_2CH_3$

18:1 $CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2CH_3$

16:0 $CH_3(CH_2)_{14}CO_2CH_3$ 18:0 $CH_3(CH_2)_{16}CO_2CH_3$



Scheme 1. Structures of fatty acid methyl esters and ionic liquids studied. 20:5 and 22:6 are the omega-3 polyunsaturated fatty acid methyl esters.

and 19:0, methyl nonadecanoate were obtained from Supelco (Milwaukee, WI, USA). In the above FAME abbreviations, the first number indicates the numbers of carbons in the fatty acid; the second indicates the number of double bonds in that chain. These same designations were used for their corresponding methyl esters.

Silver trimethylacetate was prepared according to a published method (27).

Synthesis of Ionic Liquids

In a typical synthetic procedure, using the quaternization reaction, a mixture of 1-methylimidazole (or 4-picoline or pyridine) and n-alkyl chloride (or ethyl bromide) was heated at 70°C for 3 d under argon to form N,N-disubstituted imidazolium chloride or bromide, or N-butylpyridinium chloride, or 1-butyl-4-picolinium chloride as previously reported (28). Then these pale-yellow viscous liquids or pale-white solids were washed three times with anhydrous ethyl acetate. In some cases, an additional decoloration by activated carbon was used when the colors of ionic liquids were dark. Excess ethyl acetate was evaporated at 90°C for 4 h. Subsequently, the water soluble 1-butyl-3-methyl imidazolium chloride was metathesized with silver nitrate or silver trifluoroacetate in water at room temperature to afford the hydrophilic ionic liquids. The N,N-disubstituted imidazolium chlorides or bromide, or N-butylpyridinium chloride, or 1-butyl-4-picolinium chloride were anion-exchanged with sodium tetrafluoroborate, lithium trifluoromethanesulfonimide or potassium hexafluorophosphate in acetone at room temperature. According to their miscibilities with water, the ILs were purified by a short silica column filtration or cold water washing. Their identities are confirmed by ^1H NMR. The complete removal of halides in the ionic liquids after purification was checked by silver nitrate tests. The cations of ionic liquids (Scheme 1) are referred to as: [emim]:1-ethyl-3-methylimidazolium, [bmim]: 1-butyl-3-methylimidazolium, [hmim]:1-hexyl-3-methylimidazolium, [bmpy]:4-methyl-1-butylpyridinium, [bpy]:N-butylpyridinium.

Preparation of Fatty Acid Methyl Ester Feed Mixture Solution

Mixture of FAMEs (saturated 16:0 and 18:0, monounsaturated 18:1, diunsaturated 18:2 and polyunsaturated 22:6) were dissolved in hexane (1 ml) and extracted as the standard mixture solution. Because of the thermal lability and light sensitivity of PUFAMEs, we only made the feed mixture solution sufficient to be used in one series of tests. This means the feed solutions we used in different series of tests might differ slightly in

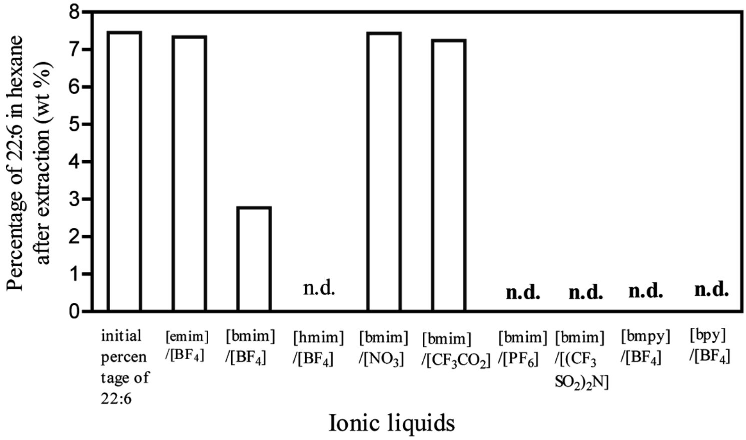


Figure 1. Ionic liquids screening tests. Hexane solution (1 mL) containing fatty acid methyl ester mixture was extracted by $AgBF_4$ (80 mg) in 1 mL of ionic liquids. Initial fatty acid methyl ester composition (wt %): 16:0 = 22.49%, 18:0 = 18.91%, 18:1 = 25.21%, 18:2 = 26.02%, 22:6 = 7.36%.

their respective FAMEs concentrations (as shown in Figs. 1–4). In all the feed mixture solutions, the mass concentrations of 16:0, 18:0, 18:1, and 18:2 are around 1.5 mg/ml, and the concentration of polyunsaturated 22:6 is around 0.3 mg/ml.

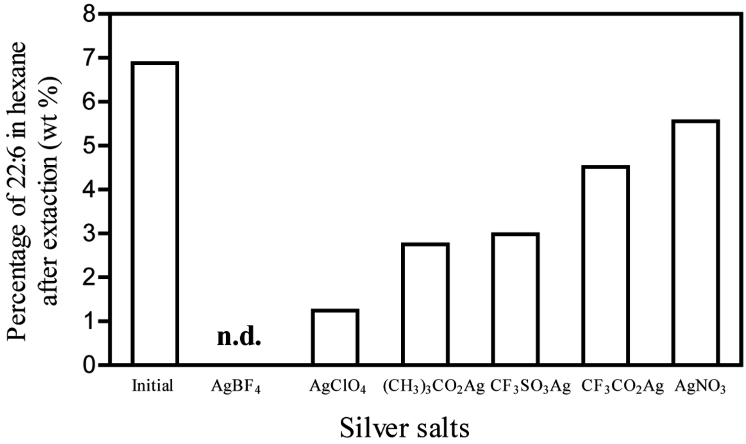


Figure 2. Silver salts screening test. Initial FAMEs composition (wt %): 16:0 = 24.21%, 18:0 = 23.12%, 18:1 = 24.76%, 18:2 = 21.05%, 22:6 = 6.87%. Silver salts (0.05 mmol) in $[hmim][PF_6]$ (1 ml) were used as the extraction phases.

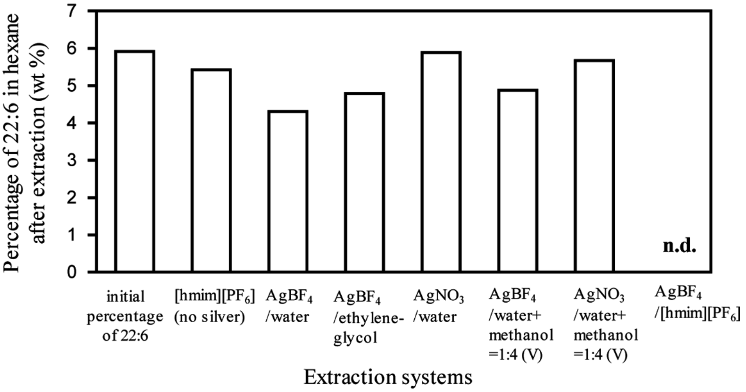


Figure 3. Comparison of various extraction systems. Initial FAMEs composition in hexane (wt %): 16:0 = 21.70%, 18:0 = 22.66%, 18:1 = 25.50%, 18:2 = 24.25%, 22:6 = 5.95%. Silver salt $AgBF_4$ or $AgNO_3$ (0.05 mmol) was used.

Extraction and Desorption (Stripping) Manipulations

The feed mixture solution was extracted by IL (1 ml) containing $AgBF_4$ or other silver salts (extraction phase). The extractions were performed by quick vibration on a shaker (Mistral Multi-Mixer) for 30 min at 20°C

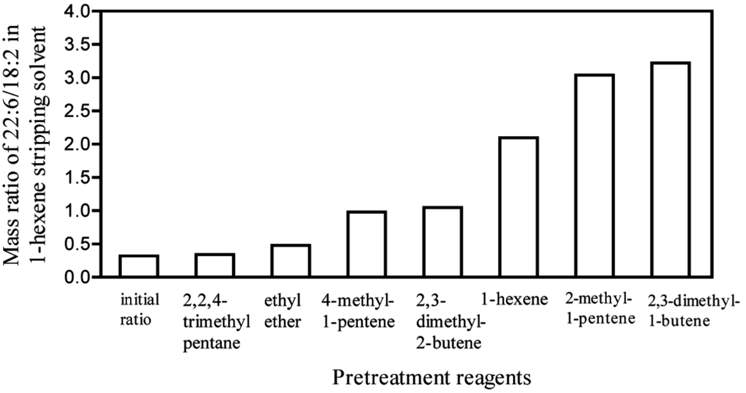


Figure 4. Effects of pretreatment reagents on the extraction selectivity for PUFAME 22:6. Initial FAMEs compositions (wt %): 16:0 = 24.66%, 18:0 = 23.06%, 18:1 = 25.25%, 18:2 = 20.67%, (22:6) = 6.36%. $AgBF_4$ (0.05 mmol) in [hmim][PF$_6$] (1 ml) was used as the extraction phase.

in a capped amber-colored vial. The extraction and desorption time were preliminarily studied. We found that the extraction equilibrium could be reached in 30 min when no further change in the concentration of PUFAMEs was found. Such quick equilibrium is due to the chemical complexation between silver ions and double bonds. Also as PUFAMEs are thermal-labile and light sensitive, we selected this short time (30-min) as the standard extraction time. After standing and centrifuging, the upper hexane phases were analyzed by GC-FID to assess the extraction capabilities of the silver salt-IL extraction phases. In the stripping step, 1-hexene (1 ml) was added and vibrated for 30 min to strip and recover the PUFAMEs from IL phase after a syringe carefully pipetted the hexane out. Then the 1-hexene phases were analyzed by GC-FID to determine the extraction selectivities for PUFAMEs.

Because the extraction of PUFAMEs (20:5 and 22:6) is our major concern, the variations of percent concentration (wt %) were determined and shown in Figs. 1–4 to indicate the extraction results. However, the mono- or diunsaturated FAMEs may also be partially extracted. The saturated FAMEs were nearly not extracted.

It should be mentioned here that in the previous work, the desorption of PUFAMEs from the aminopropyl bonded silica (29) or $AgNO_3$/bonded benzenesulfonic acid column (30) adsorbents were commonly conducted by washing the adsorbents with high polar organic solvents such as methanol, dichloromethylene, or acetonitrile. According to our observations, however, these high polar organic solvents usually lead to severe leaching of $AgNO_3$ from the adsorbents. Therefore, the reusability of the silver adsorbent is impaired. In our work, for the first time, 1-hexene with low polarity but high desorption ability was selected as an excellent stripping solvent. Consequently, high recoveries of PUFAMEs were obtained when the silver-ionic liquid extraction phase was stripped by 1-hexene. The recovery of PUFAMEs was determined by GC-FID analysis using an internal standard (19:0) method. The average recovery of 22:6 was about 85%.

Recycle Tests of the Silver Salt-ionic Liquid Extraction Phase

$AgBF_4$ (10 mg) in ionic liquid [hmim][PF_6] (1 ml) was used as the extraction phase. Other manipulations were the same as in the above-mentioned extraction and stripping procedures. After the upper 1-hexene phase was pipetted out, the lower extraction phase was dried under vacuum for 15 min at room temperature to regenerate the $AgBF_4$-[hmim][PF_6] extraction phase.

Analysis of Fatty Acid Methyl Esters by GC-FID

An HP 5890 series II gas chromatography (Hewlett Packard, Palo Alto, CA) equipped with an autoinjector (model HP6890) and a flame ionization detector (FID) was used. The separation was done on a Supelco Omegawax 320 ($30\,\text{m} \times 0.32\,\text{mm}$, d_f 0.25 μm) fused silica capillary column. The oven temperature was programmed as follows: held constant at 190°C for 10 minutes; and then increased at 3°C/minute to 230°C; and kept it constant for 20 min. The internal standard (nonadecanoic acid methyl ester, 19:0) method was used to quantify the composition of FAMEs according to the equation $Ci = Cs(Ai/As)$ where Ci is the amount of any methyl ester I, Cs is the amount of 19:0, Ai is the area under the chromatographic peak of i, and As is the peak area of internal standard 19:0.

Preparation of FAMEs from Cod Liver Oil

FAMEs were synthesized by the one-step extraction-transesterification method described by Indarti and coworkers (31) with some appropriate modifications. Cod liver oil (330 mg) was weighed into a clean, amber-colored glass vial (10 ml), to which a fresh solution (7 ml) containing methanol, concentrated sulfuric acid, and chloroform (17:3:20, volume ratio) was added. This solution was heated at 100°C for 45 min. After cooling to room temperature, distilled water (1.3 ml) was added and this mixture was then thoroughly vortexed for 2 min. Two phases were formed. The lower phase containing FAMEs was transferred to another brown bottle and dried with anhydrous Na_2SO_4. The FAMEs product distribution from cod liver oil was analyzed quantitatively by GC-FID using nonadecanoic acid methyl ester (19:0) as the internal standard.

RESULTS AND DISCUSSION

Screening of Ionic Liquids

Since silver ions could form reversible complexes with olefin molecules through interactions with the double bonds, we investigated the extractive separation of omega-3 fatty acid methyl esters from their saturated and less unsaturated analogues using several ionic liquid and silver salt combinations. As seen in Fig. 1, for the imidazolium ionic liquid $[C_n\text{mim}][BF_4]$ ($n = 2, 4, 6$) series, the extractive adsorption capacity of silver-ionic liquids increases with the increase in alkyl chain length on the 1-position of imidazolium cation. $AgBF_4$ in $[\text{emim}][BF_4]$ ($n = 2$)

showed almost no extraction ability, as there was little change in PUFAME 22:6 percent even when the extraction time was prolonged to more than 2 h. On the other hand, $AgBF_4$ in [hmim][BF_4] ($n = 6$) had such a strong extraction ability that even diunsaturated methyl linoleate (18:2) was completely adsorbed along with 22:6 after 5 min extraction. $AgBF_4$ in [bmim][BF_4] ($n = 4$) showed moderate capacity with a small amount of PUFAME 22:6 left in the feed stock solution after the extraction. The two pyridinium ionic liquids, [bmpy][BF_4] and [bpy][BF_4] showed very strong extraction capacities, as both 22:6 and 18:2 completely vanished in hexane phase after extraction.

As also indicated in Fig. 1, for $AgBF_4$ in [bmim][X] series, the extraction capacity of these ionic liquids falls into three groups. The first group includes [bmim][NO_3] and [bmim][CF_3CO_2], both containing hydrophilic anions. The extraction capacities of $AgBF_4$ in these two ionic liquids were very poor. The second group includes [bmim][PF_6] and [bmim][$(CF_3SO_2)_2N$], both containing hydrophobic anions. The extraction capacities of these two were very strong; they not only extracted all 22:6 and 18:2 but also some of monounsaturated fatty acid methyl ester 18:1. The third group includes the moderately hydrophobic [bmim][BF_4], which exhibits moderate extraction capacity. These anion dependence behaviors correlate well with the guidelines established by Seddon and coworkers (32) on the choice of anions for miscibility with water. They found that imidazolium salts with halide, nitrate and trifluoroacetate anions tend to be miscible with water, [PF_6^-] and [$(CF_3SO_2)_2N^-$] imidazolium salts tend to be immiscible with water, and that [BF_4^-] imidazolium salts can be totally miscible or immiscible depending on the substituents on the cation.

These cation/anion studies indicate that the extraction capacities of $AgBF_4$ in ionic liquids increase with the increase in the hydrophobicity of ionic liquids, which helps to solubilize the hydrophobic chain of omega-3 fatty acid methyl esters.

Screening of Silver Salts

Six silver salts with different counter anions in an identical ionic liquid, [hmim][PF_6], were screened to study their extraction capabilities for PUFAME 22:6. With the same molar amount of silver salts, the extraction capabilities decreased in the following order: $AgBF_4 > AgClO_4 \gg (CH_3)_3COOAg \approx CF_3SO_3Ag > CF_3COOAg > AgNO_3$ as seen in Fig. 2. $AgBF_4$ showed exceedingly strong extraction capability for PUFAME 22:6 in ionic liquids. After extraction with $AgBF_4$ for 30 min, 22:6 completely disappeared from the feed mixture solution and even the less unsaturated 18:2 nearly vanished, too. $AgClO_4$ showed a strong extraction capability as well. $(CH_3)_3COOAg$, CF_3SO_3Ag and CF_3COOAg showed moderate extraction capabilities. Although $AgNO_3$ is widely used in the selective adsorption of unsaturated compounds due to its relatively lower cost and readily availability, it showed the lowest extraction capability for 22:6 among the six silver salts studied. Kang and coworkers (33) reported that in the separation of olefin/paraffin by facilitated silver transport membrane, the separation performances were in the order of $AgBF_4 > AgCF_3SO_3 \gg AgNO_3$. A silver ion interacts weakly with an anion which has large size and low lattice energy such as BF_4^-, $CF_3SO_3^-$, $(CH_3)_3COO^-$ or ClO_4^-, and thus favorably interacts with double bonds by forming reversible double bond/silver complexes. Silver ions with this type of anions can be used as a good extraction agent for PUFAMEs. On the other hand, small-size and highly electronegative anions having high lattice energies such as F^-, Cl^-, or NO_3^- obstruct the double bond/olefin complexation due to the strong electrostatic interaction between the silver ion and the anions (34). Therefore, this type of silver salts showed lower extraction capabilities for PUFAMEs such as $AgNO_3$.

Comparison of $AgBF_4$-[hmim][PF_6] with other Liquid Extraction Systems and Blank Test

The extraction capability of $AgBF_4$ in ionic liquid [hmim][PF_6] for PUFAME 22:6 was compared with other extraction systems. These extraction systems included the pure ionic liquids themselves without silver salt, $AgBF_4$ in water, $AgBF_4$ in ethylene glycol, $AgBF_4$ in water/methanol (1/4, volume ratio), $AgNO_3$ in water, and $AgNO_3$ in water/methanol (1/4) (Figure 3). In all these tests, the same molar amount of silver salts was used.

Only a minimal amount of PUFAME 22:6 could be extracted into [hmim][PF_6], one of the most effective ionic liquid in the presence of $AgBF_4$, as the concentration of 22:6 decreased slightly to 5.42% (column 2) from 5.92% (column 1, the initial percentage of 22:6 in the hexane feedstock solution). Therefore, the minimal extraction capability of pure ionic liquid confirms that the extraction of PUFAMEs predominantly results from the coordinative interaction between silver ions and the double bonds in PUFAMEs. Actually, it is this silver-double bond complexation effect that provides a rationale for the highly selective concentration of PUFAMEs from the closely-related less unsaturated analogues. Without using ionic liquids, the amount extracted by the silver salts is quite modest. For example, with $AgBF_4$ in water, the percent of

PUFAME 22:6 decreased to 4.31% (column 3). With $AgBF_4$ in ethylene glycol, the percent of 22:6 decreased to 4.79% (column 4). For $AgBF_4$ in water/methanol (1/4), the percent of 22:6 decreased to 5.67%. In addition to $AgBF_4$, we also examined the extraction ability of $AgNO_3$ in water and in aqueous methanol, as the latter two have been studied for the extraction of ethyl esters of omega-3 fatty acids (10). Under our conditions, $AgNO_3$ in water showed nearly no extraction effect for PUFAME 22:6 probably because we used a much lower concentration of $AgNO_3$ than Teramoto et al. used. The extraction ability of $AgNO_3$ increased moderately when methanol was added to water to increase the hydrophobicity of the extraction solution, as the percent of 22:6 decreased from 5.92% to 4.88%. As a sharp comparison, however, the complete extraction of 22:6 was observed with $AgBF_4$ in ionic liquid [hmim][PF_6] system, as shown in column 8 (Fig. 3).

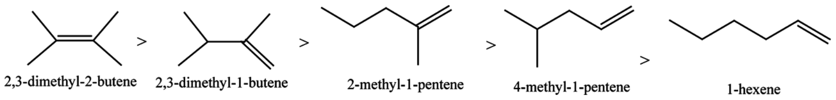
These experiments clearly demonstrate the important synergy between the silver salts and the ionic liquids for the efficient extraction of omega-3 fatty acids methyl esters. Kang and co-workers (35) also found that the activation of Ag^+ in silver polymer electrolytes in the presence of ionic liquids is principally due to the reduction of the interactions between Ag^+ and NO_3^- produced by the interaction between $BMIM^+$ and NO_3^- (ionic liquid, [bmim][NO_3], was used in this paper), and this effect does not occur in the presence of common salts such as $NaNO_3$.

USING THE PRETREATMENT REAGENT TO INCREASE THE EXTRACTION SELECTIVITY

It has been proved that the more double bonds the unsaturated FAMEs contain, the more readily the FAMEs can be extracted by silver ions in ILs. Furthermore, this suggests that if a less unsaturated compound has coordinated with the silver ions, a more unsaturated compound which has a stronger coordination ability with silver ions can replace the less unsaturated compound. Our idea is that the $AgBF_4$-ionic liquids extraction phase can be pre-mixed with an unsaturated compound referred to as a pretreatment reagent for 3 min, then the pretreatment reagent is pipetted out and the FAMEs feedstock mixture solution is introduced and extracted. It is expected that this unsaturated pretreatment reagent will block or impede the less unsaturated FAMEs (18:2 and 18:1) coordination, but the PUFAMEs 22:6 and 22:5 can replace the unsaturated pretreatment reagent and coordinate with silver ions. Consequently, the extraction selectivity for PUFAMEs should be enhanced. The results listed in Fig. 4 confirmed this idea.

The initial ratio between PUFAME 22:6 and diunsaturated 18:2 is 0.32 (column 1) in the feedstock solution. The extraction selectivity is enhanced if the ratio between 22:6 and 18:2 in 1-hexene is increased. The extraction selectivities estimated by 22:6/18:2 ratio exhibited only minimal changes (column 2 and 3) when the saturated 2,2,4-trimethylpentane and a low polar diethyl ether solvents were used as pretreatment reagents. These results proved that the saturated and low polar solvents used as the pretreatment reagents have very small influences on the coordination ability of silver ions. On the contrary, the selectivities increased significantly when unsaturated olefins were used as pretreatment reagents. Because of their low boiling points, short chain olefins were selected. The excessive olefins can be easily purified from ionic liquids by vacuum evaporation at room temperature.

It is obvious that when the PUFAMEs replace the pretreatment reagent, short-chain olefins, to coordinate with silver ions, the steric hindrance around the double bond of olefins should play a major role. The steric hindrance around the double bonds decreases in the sequence as:



The bulky α-olefins with a methyl group branch at C_2-position have significantly positive effects on the extraction selectivity. When the $AgBF_4$-[hmim][PF_6] extraction phase was pretreated by the highly steric hindered olefin 2,3-dimethyl-1-butene, the extraction selectivity (estimated by 22:6/18:2 ratio) increased to the largest value of 3.22 (column 8) from 0.32 in feed solution. With 2-methyl-1-pentene, the extraction selectivity also stood at a fairly higher value of 3.04. For the 4-methyl-1-pentene and 1-hexene whose methyl group branches are not in the immediate vicinity of double bonds, the extraction selectivities showed relatively lower values of 0.98 and 2.10, respectively. It seems that a higher 22:6/18:2 ratio in the 1-hexene stripping solution could be obtained by pre-mixing the extraction phase with olefins possessing large steric hindrance around the double bonds. However, the most steric hindered internal olefin, 2,3-dimethyl-2-butene, when used as pretreatment reagent, exhibited only a moderate influence on the selectivity: the ratio of 22:6/18:2 was 1.05. This relatively lower extraction selectivity could be attributed to the excessively large steric hindrance around the internal double bond of 2,3-dimethyl-2-butene. If the hindrance is too large, the double bond has difficulty in coordinating with the silver ions, therefore, it shows a smaller positive influence on the extraction selectivity.

Recycle Tests of the $AgBF_4$-[hmim][PF_6] Extraction Phase

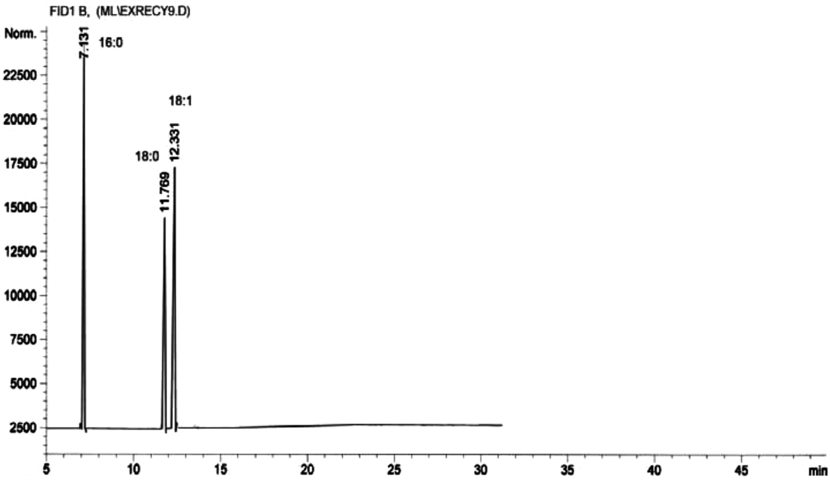
Ionic liquids and silver salts are relatively expensive. The excellent reusability of the extraction phase is undoubtedly highly desirable in its scale-up test and potential industrial application. Nine successive runs were done to investigate the recycle performances of the $AgBF_4$-[hmim][PF_6] extraction phase. After each extraction, PUFAME 22:6 disappeared in the hexane phase indicating that the high extraction capability of $AgBF_4$ in [hmim][PF_6] was well retained. The percentage (wt %) of PUFAME 22:6 was increased from 5.58% to 75.07% in the first recycle and to greater than 80%, even to around 90% in the next eight recycles. The extraction capability was retained very well after nine runs in one month without additional supply of silver salts or ionic liquids during the entire recycle tests. It is worth noting that the ionic liquids with silver salts should be kept in the dark during the test and in storage due to the light-sensitivity of silver salt.

The percentage (wt %) of PUFAME 22:6 was increased from 5.58% to 94.37% in the last recycle. The relatively higher extraction selectivity in the last recycle resulted from the fact that the stripping solvent of 1-hexene dissolved in the ionic liquids after the first run might enhance the extraction selectivity for PUFAMEs. This result corroborated the discussion that pretreating the extraction phase by unsaturated 1-hexene could increase the extraction selectivity for PUFAMEs. The typical GC chromatograms of the FAMEs present in the extraction phase and stripping phase were shown in Fig. 5. PUFAME 22:6 disappeared in the hexane phase after extraction (Fig. 5a); while, 22:6 was recovered and largely enriched in the 1-hexene phase after stripping (Fig. 5b).

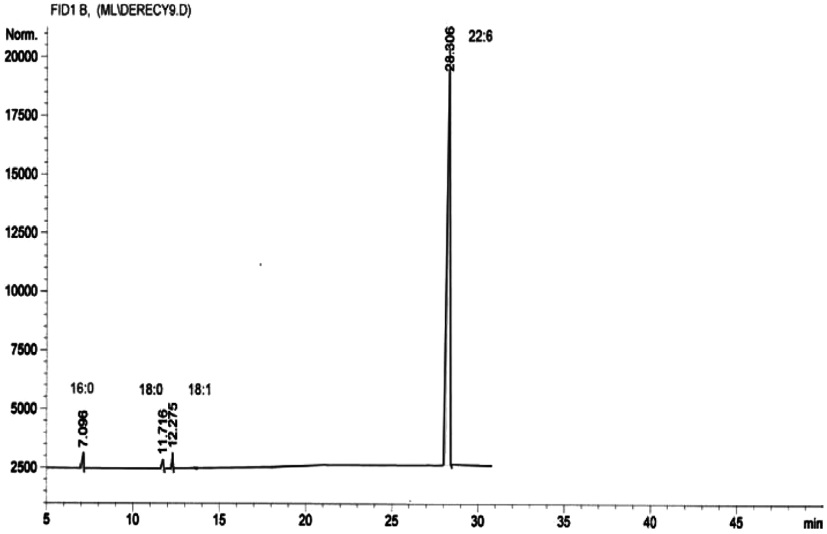
EXTRACTION AND ENRICHMENT OF PUFAMES 20:5 AND 22:6 FROM COD LIVER OIL

The extraction method was tested for the enrichment of the omega-3 PUFAMEs 20:5 and 22:6 from cod liver oil. For this purpose, the cod liver oil was converted to fatty acid methyl esters with a conversion yield of 94% using the transesterification method. Using GC-FID analysis, the total percentage (wt %) of 20:5 and 22:6 is 18.11% (the percentages of 20:5 and 22:6 are 7.35% and 10.76%, respectively) in the resulting fatty acid methyl esters mixture.

The fatty acid methyl esters obtained above were dissolved in hexane and extracted with 1 mL of [hmim][PF_6] containing 85 mg of $AgBF_4$. The percentages of 20:5 and 22:6 in 1-hexene stripping solvent were determined by GC-FID. The total percentage of 20:5 and 22:6



(a)



(b)

Figure 5. Typical GC chromatograms of FAMEs present in the hexane solution after extraction (a) and in the 1-hexene solution after stripping (b).

was enhanced from 18.11% in the original cod liver oil to as high as 74.15%. Moreover, if the extraction phase was pretreated by 2,3-dimethyl-1-butene prior to the extraction, the final total percentage of 20:5 and 22:6 was significantly enhanced to 82.11%. According to the GC-FID analysis, the other components in the 1-hexene phase were other polyunsaturated fatty acid methyl esters such as 22:5, 20:4, 18:4, and 18:3. The saturated fatty acid methyl esters 14:0, 16:0, and 18:0 were almost completely removed. Moreover, the percentages of monounsaturated fatty acid methyl esters 16:1, 18:1, 20:1, 22:1, and the diunsaturated fatty acid methyl esters 16:2, 18:2 were greatly reduced.

CONCLUSION

In summary, we have described a novel and efficient method which demonstrates the separation power of ionic liquids with dissolved silver salts. The example clearly demonstrates the synergy between silver compounds and ionic liquids. The screening of ILs showed silver ions in hydrophobic ILs exhibited higher extraction ability than those in hydrophilic ILs did. Compared with other liquid-liquid biphasic extraction systems, e.g. water or ethylene glycol, $AgBF_4$ in ILs showed the highest extraction ability. $AgBF_4$ with low crystalline lattice energy showed higher extraction capacity than $AgNO_3$. The extraction selectivity for PUFAMEs (20:5 and 22:6) can be enhanced by the pretreatment of silver salt-ILs extraction phase with steric hindered short chain olefins. Nine runs of the silver salt-ILs extraction phase showed no obvious decrease in extraction capacity and selectivity. The extraction and concentration of health-benign omega-3 PUFAMEs from cod liver oil also performed well. Future efforts include expansion of this system to the separation of other class of organic compounds.

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