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Separation and determination of phospholipids in plant seeds by nonaqueous capillary electrophoresis

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Abstract

A method has been developed for the separation and determination of phospholipids by nonaqueous capillary electrophoresis in a separation medium of acetonitrile–2-proponol (3:2, v/v), 0.3% acetic acid and 60 mM ammonium acetate. To optimize the separation conditions, the composition of separation medium including alcohols, acetic acid, *n*-hexane and ammonium acetate was studied. The solvation interaction and ion–dipole interaction were also investigated. The contents of phospholipids in soybean, sunflower, peanut, apricot kernel, filbert and walnut were determined by the recommended method. The results obtained by the nonaqueous capillary electrophoreses were in good agreement with those determined by micellar electrokinetic chromatography.
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Keywords: Nonaqueous capillary electrophoresis; Phospholipids; Dielectric constant; Ion–dipole interaction; Plant seeds

1. Introduction

Phospholipids are lipids that contain phosphoric residues, polar head groups and non-polar lipid chains [1]. The groups include choline, ethanolamine, glycerol, inositol, and hydrogen, etc. The corresponding phospholipids are named phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidic acid (PA), respectively (Fig. 1) [2]. Due to their wide occurrence in foods and their pro- and antioxidant effects, phospholipids have the potential as a multifunctional additive in food, pharmaceutical and industrial applications [1]. Thin-layer chromatography (TLC) [3,4] and high-performance liquid chromatography (HPLC) [5,6] have been used in the analysis of phospholipids of animal tissues, plants and plant seed oil extracts [2–6]. Compared with TLC and HPLC, capillary electrophoresis (CE) has its unique features of higher resolution, minimal sample volume required, short analysis time and high separation efficiency, thus CE is used for the analysis of phospholipids [7–11]. Considering the poor aqueous solubility of phospholipids, micellar electrokinetic chromatography (MEKC) was commonly adopted in the analysis of phospholipids [7–10]. Although the lack of chromophores was a problem for identification and quantification of phospholipids, ultraviolet (UV) detection of phospholipids at 200–214 nm was frequently applied to the determination of phospholipids due to the presence of unsaturated groups, such as carbonyl, carboxyl and phosphate [7,8]. Methyl-β-cyclodextrin modified MEKC combined with laser-induced fluorescence (LIF) has been adopted in the analysis of aminophospholipids [9] and their molecular species [10]. However, the MEKC methods for the separation of phospholipids were often carried out at temperature of 45 °C or even higher [7–11]. Generally, it is difficult to keep such high temperature stable during the CE analysis. Therefore, the development of a method at ambient temperature is required.

Because the different substituted groups in the phosphate have different proton donating-accepting capabilities, nonaqueous capillary electrophoresis (NACE) could be a good alternative to MEKC. Raith et al. [11] measured the migration

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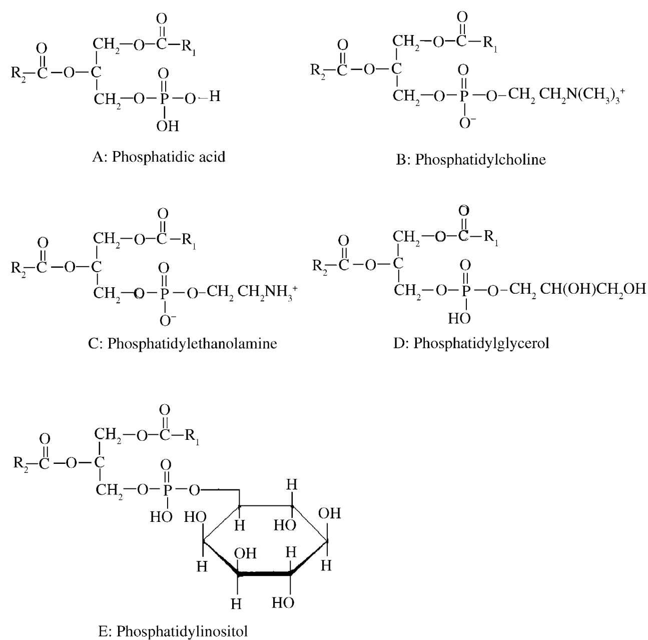

Fig. 1. Molecular structure of phospholipids redrew based on ref. [2].

time of four phospholipid standards by NACE using electrospray ionization mass spectrometer (ESI-MS) as a detector. However, it is worthy of establishing a method feasible for the analysis of phospholipids in real samples.

NACE offers excellent selectivity, thus NACE has been successfully applied for the analysis of organic environmental pollutants [12–14], drugs and pharmaceuticals [15–18], plant extracts [19], chirals [20,21], inorganic anions [22,23], organic acids [24] and oligomers [25]. The selectivity of NACE was often realized by manipulating separation medium [26], the additives and their concentrations [27], and electrolyte concentrations [28–31]. The mechanisms of NACE seem to be more complicated than those of capillary zone electrophoresis (CZE). In a series of papers, the effects of viscosity [32,33], dielectric constant [34–37], apparent pH [38], pK_a [38] and electrostatic interactions (such as ion–ion, ion–dipole and dipole–dipole interactions) [28–31] on the mobility of analyte were studied. Based on Hückel law [32], Walden's rule [39], Hubbard–Onsager theory [34–37], Debye–Hückel–Onsager theory [32] and affinity capillary electrophoresis (ACE) theory [28–31], various theoretical models have been used to evaluate those influences on the mobility. Although these theoretical models were thought to be more useful in studying the NACE theory for some of over-simplified systems, there were very few reports on the migration behaviors of analytes in the multi-component solvent system [40]. Therefore, the research to the migration behavior of analytes in mixed solvent system is of great importance, because the mixed solvents are more often used than only one component solvent in the NACE studies.

In this work, a practical NACE method was developed for the separation of phospholipids at room temperature. Phospholipids of six plant seeds were determined, and the results determined by the recommended method were compared with those obtained by MEKC. In addition, an effort was made to study the relationship between solvent composition and mobility of phospholipids.

2. Experiment

2.1. Equipments and chemicals

MEKC and NACE separations were performed on a Beckmann P/ACE MDQ capillary electrophoresis system equipped with UV detector (Beckman, Fullerton, CA, USA). A fused silica capillary of 75 μm I.D., 375 μm O.D. with a total length of 50 cm (40 cm to detector) was used. Apparent pH of nonaqueous solvent and pH of micellar buffer were measured by a DELTA 320 pH meter (Mettler-Toledo, Shanghai, China) at 25 °C. The viscosity of separation medium was measured by a capillary viscosity meter.

The phospholipids standards were purchased from Sigma (St. Louis, MO, USA) and used without further purification. Mixed phospholipids standards were dissolved in the separation medium, and used for the optimization of separation conditions. The main components of mixed phospholipids, PC, PE, and PI, were identified by using a single standard phospholipid. However, the minor components of the mixed phospholipids were not identified due to the lack of the corresponding standard phospholipids available. The buffer for MEKC method [8] was composed of 75 mM cholic acid (sodium salt) (Acros, Gell, Belgium), 6 mM boric acid, 10 mM phosphate and 30% (v/v) 1-propanol (pH 8.5). All chemicals used were of analytical reagent or chromatographic grade.

2.2. Extraction of phospholipids from plant seeds

Soybean, sunflower, peanut, apricot kernel, filbert and walnut seeds were purchased from local market. These plant seeds were mechanically ground to fine particles. Phospholipids were extracted from 1 g of ground seeds using 25 mL chloroform/methanol (2:1, v/v) by shaking end-over-end for about 30 min and filtered through nylon membrane (0.45 μm). Solvent of filtrate was blown off by N_2 stream and the residue was referred as to raw phospholipids. The raw phospholipids were dissolved in 10 mL cooled acetone and the acetone insoluble component was taken as purified phospholipids because of the low solubility of phospholipids in acetone [41].

One mg of the purified phospholipids were dissolved in 1 mL of the separation medium of acetonitrile–2-propanol (3:2, v/v), 0.3% acetic acid and 60 mM ammonium acetate for NACE or in the running buffer of 75 mM cholic acid (sodium salt), 6 mM boric acid, 10 mM phosphate and 30% (v/v) 1-propanol (pH 8.5) for MEKC. The dissolved phospholipids were filtered using a 0.45 μm nylon membrane.

2.3. NACE conditions

For NACE mode samples were hydro-dynamically introduced by applying pressure of 0.5 psi for 3 s; UV detection was carried out at 200 nm; separation voltage was 30 kV or 10 kV; the capillary was thermo-stated at 25 °C.

Prior to use or change operation mode from MEKC to NACE, the capillaries were successively rinsed with 1 M sodium hydroxide for 20 min, distilled water for 10 min, methanol for 10 min and separation medium for at least 15 min. Between analyses, the capillaries were flushed with the separation medium for 3 min.

2.4. MEKC conditions

Samples dissolved in MEKC buffer were hydro-dynamically introduced by applying pressure of 0.5 psi for 3 s; UV detector was used at 200 nm; separation voltage was 20 kV; the capillary was thermo-stated at 45 °C.

Prior to use the capillaries were sequentially rinsed with 1 M sodium hydroxide for 20 min, distilled water for 10 min and MEKC buffer for at least 15 min. After each determination the capillary was flushed with the running buffer for 3 min.

3. Results and discussion

Alcohols and acetonitrile are often used as background electrolytes in NACE separations. To optimize the separation of phospholipids, a mixed solvent of acetonitrile–alcohol was adopted in this study and the separation efficacy was tested. Especially, the effects of various alcohols with different chain length (from methanol to 1-octanol), acetic acid fraction, *n*-hexane as non-dipole additive and ammonium acetate concentration on the separation of phospholipids were studied, the details were discussed below.

3.1. Effects of different alcohols on the separation of phospholipids

The mismatch between sample zone and separation medium in NACE often brings forth a high resistance at the interface of the zones. Bubbles will form because of boiling of organic solvent by Joule heat at the interface, thus influencing or breaking down the NACE separation when the separation voltage is applied. In order to eliminate the formation of bubbles during the NACE separation, the samples were also dissolved in the same separation medium in the present study.

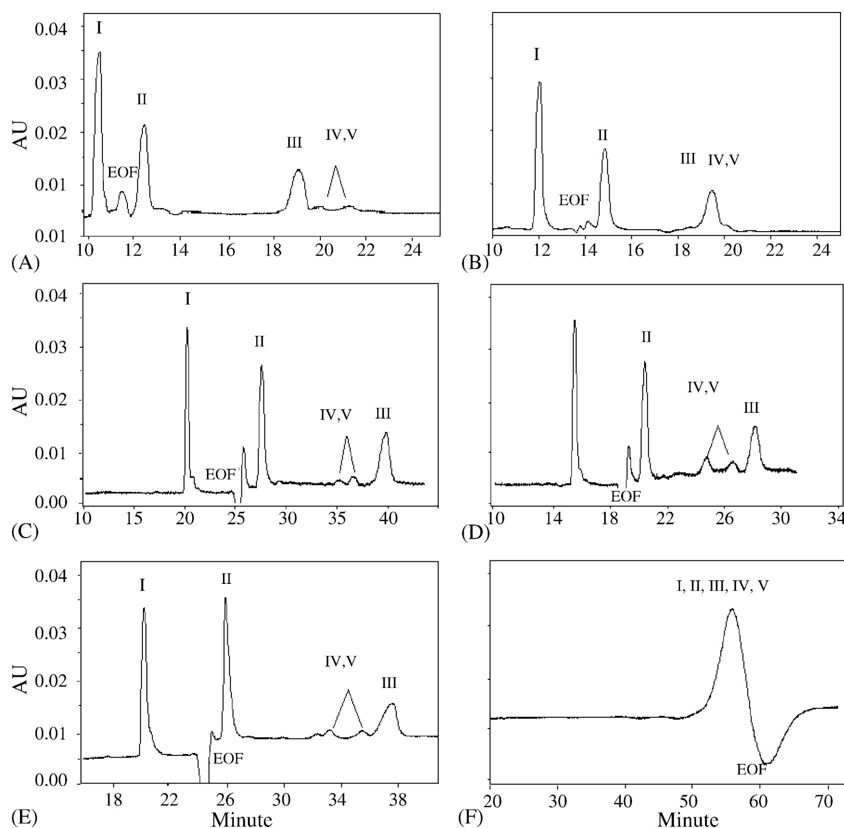

Fig. 2. Electropherograms of phospholipids in acetonitrile:various alcohols (3:2, v/v), 0.3% acetic acid and 60 mM ammonium acetate: (A) methanol, (B) ethanol, (C) 1-propanol, (D) 2-propanol, (E) 1-butanol, (F) 1-octanol. Fused-silica capillary, 50 cm (40 cm to the detector), 75 μm I.D, 10 kV, UV detection at 200 nm, temperature of 25 °C. Peaks I–III were referred to as PC, PE and PI, respectively. Peaks IV and V were not identified, which maybe PA and PG, and referred to as PL_1 and PL_2 hereafter.

Table 1
Viscosity, apparent pH and Walden products ($\mu\eta$) of phospholipids in the separation medium containing different alcohols[a]

	Alcohol					
	Methanol	Ethanol	1-Propanol	2-Propanol	1-Butanol	1-Octanol[b]
Viscosity (cP)	0.473	0.513	0.545	0.566	0.57	0.699
pH^*	7.54	8.1	8.11	8.42	8.01	7.74
Analyte	$\mu\eta$ (10^{-8}cP m^2 s^{-1} v^{-1})					
PC	0.397	0.689	0.749	0.666	0.584	0.137
PE	−0.322	−0.191	−0.168	−0.163	−0.159	0.137
PL_1	−2.002	−0.941	−0.680	−0.659	−0.729	0.137
PL_2	−1.854	−1.155	−0.837	−0.726	−0.856	0.137
PI	−1.735	−1.08	−0.962	−0.864	−0.961	0.137

[a] Separation medium was composed of acetonitrile–various alcohol (3:2), 0.3% acetic acid and 60 mM ammonium acetate. Separation voltage was 10 kV.
[b] Phospholipids in the solvent of acetonitrile:1-octanol is poorly separated.

The separation of phospholipids was studied using acetonitrile–alcohols (3:2), 0.3% acetic acid and 60 mM ammonium acetate, and the electropherograms are presented in Fig. 2. As shown in Fig. 2A, peaks III–V were poorly separated in the separation medium of acetonitrile–methanol. In the solvent of acetonitrile–ethanol, peaks III–V were unable to be separated (Fig. 2B). When 1-propanol, 2-propanol or 1-butanol was used in the separation medium, satisfactory resolutions were obtained (Fig. 2C–E). On the contrary, no separation was obtained in the mixed solvent of acetonitrile:1-octanol (Fig. 2F). The peaks I–III were referred to as PC, PE and PI, respectively. Because there was no other single standard phospholipid available, no effort was made to identify peaks IV and V, which might be PA and PG, and were referred to as PL_1 and PL_2 in the remainder of the experiments. The above comparison indicated that the separation medium of acetonitrile:2-propanol (3:2), 0.3% acetic acid and 60 mM ammonium acetate was super to other components and was employed in the real sample analysis.

EOF decreased when the alcohols are changed from methanol to 1-octanol in the solution owing to increase of viscosity of the separation medium (Table 1). The relationship between the viscosity and mobility is intuitionally presented in the Walden's rule, a deduction from Hücke law [42]:

$$\mu\eta = \text{const.} \tag{1}$$

where μ and η are the mobility and viscosity, respectively. Walden product should be a constant, which was independent of the solvent composition by assuming that the effective charges and Stoke radii of ions are less changed during the change of composition of separation medium. However, in Table 1, the Walden products of phospholipids in the solvent of alcohols of different chain length are different from one to another, especially to that of methanol and 1-octanol. The decreased dielectric constant also affected EOF and the migration behaviors of phospholipids. The ionization of phospholipids decreased with the decrease of dielectric constant; the magnitude of electrostatic interactions is greater in medium of lower dielectric constant; and dielectric friction, which arises from the non-instantaneous relaxation of the solvent dipoles around the moving ion, opposes the motion of the ion. However, the interpretation of the effect is hampered by the inadequate of the exact dielectric constants of the separation mediums and the unexpected disturbance from the interactions between phospholipid molecules and solvent.

As shown in Fig. 2, the differences in electrophoretic behaviors of phospholipids in methanol, ethanol, 1-octanol and

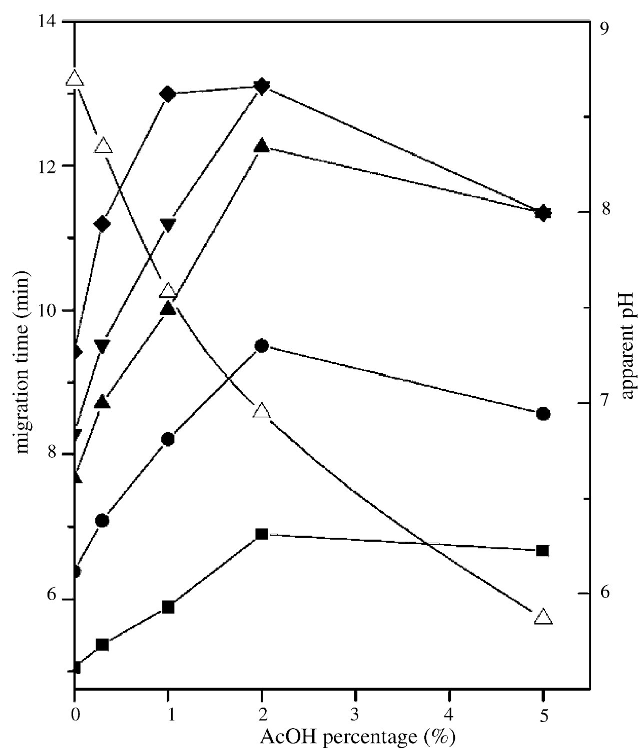

Fig. 3. Relationship between the migration time of phospholipids, or apparent pH, and the percentage of acetic acid in the separation medium. The separation medium was acetonitrile–2-propanol (3:2, v/v), 60 mM ammonium acetate containing acetic acid at different concentration levels. Other conditions were the same as Fig. 2 except the separation voltage of 30 kV. (■) PC, (●) PE, (▲) PL_1, (▼) PL_2, (♦) PI, and (△) apparent pH.

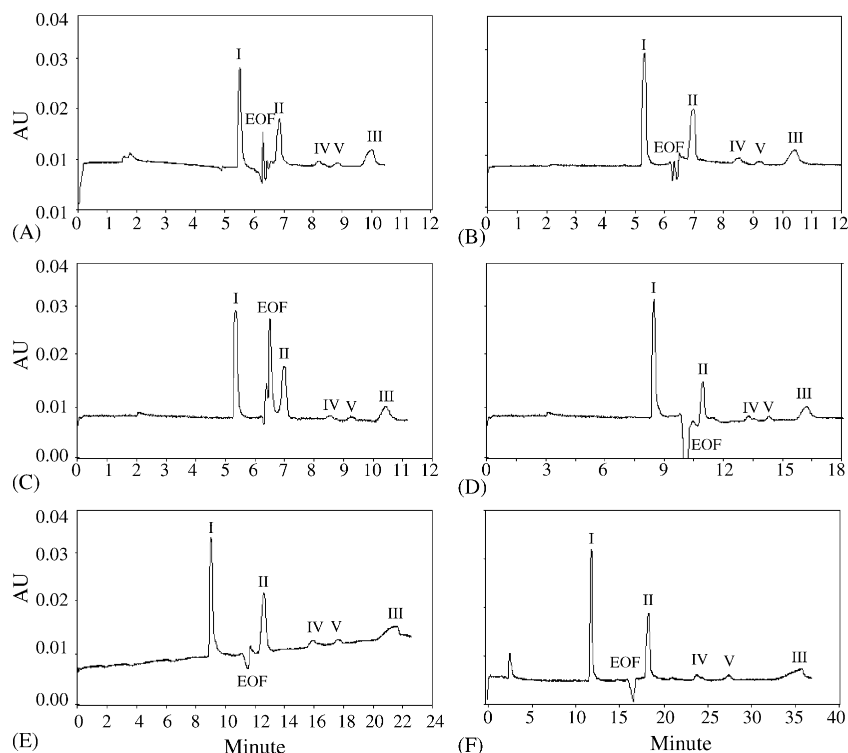

Fig. 4. Electropherograms of phospholipids in the separation medium of acetonitrile–2-propanol (3:2, v/v), 0.3% acetic acid, and 60 mM ammonium acetate containing (A) 0%, (B) 1%, (C) 2%, (D) 5%, (E) 10%, and (F) 20% *n*-hexane. Other conditions were the same as Fig. 3.

other alcohols cannot be ascribed merely to the viscosity and dielectric constant. The other important factor is the different capability of the solvent mixture to solvate analytes. Solvation is influenced by many parameters, such as dielectric constant of medium, coordinating numbers and ability, the polarity and steric structures of both analytes and solvents [17]. The solvation influences the ionization and solvated shapes of analytes and electrolytes. Both the Stoke radii of the solvated analytes and the magnitude of pK_a values are affected by solvating effects.

At last, the apparent pH (pH*) of the separation medium was changed when alcohols were changed (Table 1). The proton concentration in the nonaqueous solvent affects the ionization of phospholipids as that does in aqueous solvent. The proton concentration could further influence the charge property of silicic groups on the capillary wall and induce the changes in the EOF.

3.2. Effect of acetic acid

Since acidic–basic property is one of the most important parameters influencing the selectivity of NACE separation [12,29], the effect of apparent pH on the migration time of phospholipids was studied in acetonitrile: 2-propanol (3:2, v/v) and 60 mM ammonium acetate with various fraction of acetic acid from 0% to 5%. The results are shown in Fig. 3. It

Table 2
Viscosity, apparent pH and Walden products ($\mu\eta$) of phospholipids in the separation medium containing *n*-hexane at different levels[a]

	Hexane (%)					
	0	1	2	5	10	20
Viscosity (cP)	0.545	0.539	0.529	0.528	0.499	0.485
pH*	8.40	8.34	8.34	8.32	8.29	8.32
Analyte	$\mu\eta$ (10^{-8} cP m^2 s^{-1} v^{-1})					
PC	0.316	0.318	0.319	0.231	0.179	0.232
PE	−0.144	−0.140	−0.126	−0.092	−0.071	−0.054
PL_1	−0.425	−0.416	−0.393	−0.283	−0.216	−0.175
PL_2	−0.521	−0.503	−0.479	−0.354	−0.268	−0.229
PI	−0.657	−0.634	−0.606	−0.448	−0.364	−0.310

[a] Separation medium was composed of acetonitrile–2-propanol (3:2), 0.3% acetic acid and 60 mM ammonium acetate with different percentage of *n*-hexane. Separation voltage is 30 kV. Other conditions are the same as that of Table 1.

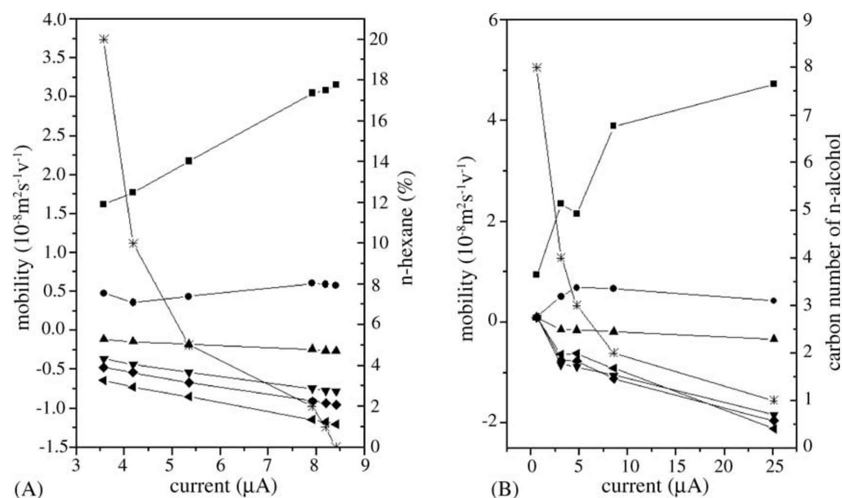

Fig. 5. Relationship between current and mobility of phospholipids in (A) acetonitrile–2-propanol (3:2, v/v), 0.3% acetic acid and 60 mM ammonium acetate with *n*-hexane at different percentage levels, other conditions were the same as Fig. 4; (B) acetonitrile–various *n*-alcohol (including methanol, ethanol, 1-propanol, 1-butanol and 1-octanol) (3:2), 0.3% acetic acid and 60 mM ammonium acetate, and other conditions are the same as Fig. 2. (■) EOF, (●) PC, (▲) PE, (▼) PL_1, (♦) PL_2 and (◄) PI and (*) percentage of hexane in A and carbon number of *n*-alcohol in B.

can be seen clearly that the resolution of PI, PL_1 and PL_2 slightly increased with increasing acetic acid from 0% to 1%. However, three acidic phospholipids PI, PL_1 and PL_2 were unable to be separated when the concentration of acetic acid was ranged from 2% to 5%. Therefore, 0.3% acetic acid (approximately 100 mM) was chosen in the real sample analysis.

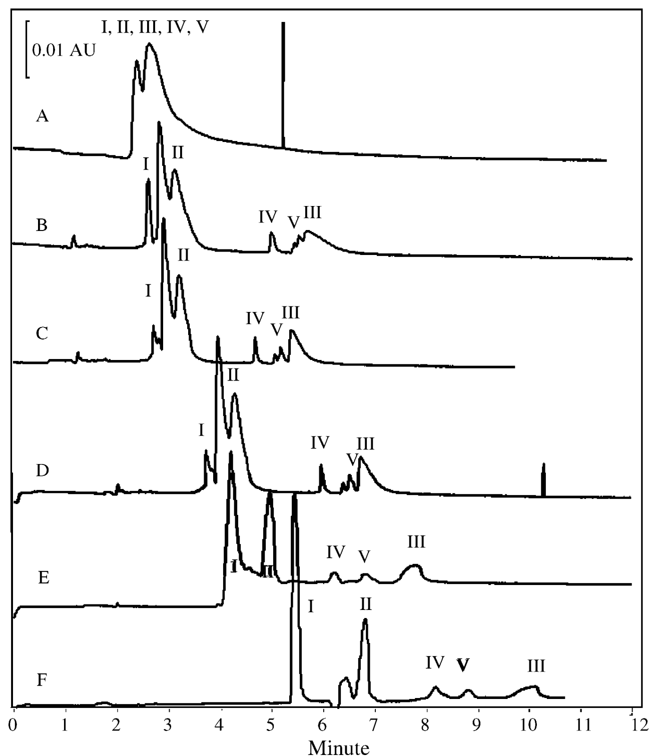

Fig. 6. Electropherograms of phospholipids in acetonitrile–2-propanol (3:2, v/v) and 0.3% acetic acid: (A) 0, (B) 5, (C) 20, (D) 30, (E) 45, and (F) 60 mM ammonium acetate. Other conditions were the same as Fig. 3.

3.3. Effect of n-hexane

As a non-polar solvent, *n*-hexane is often used to manipulate EOF and to enhance the solubility of non-dipolar analytes in the separation medium. Raith et al. [11] used a solvent containing *n*-hexane for the analysis of phospholipids. In our research, when *n*-hexane increased from 0%

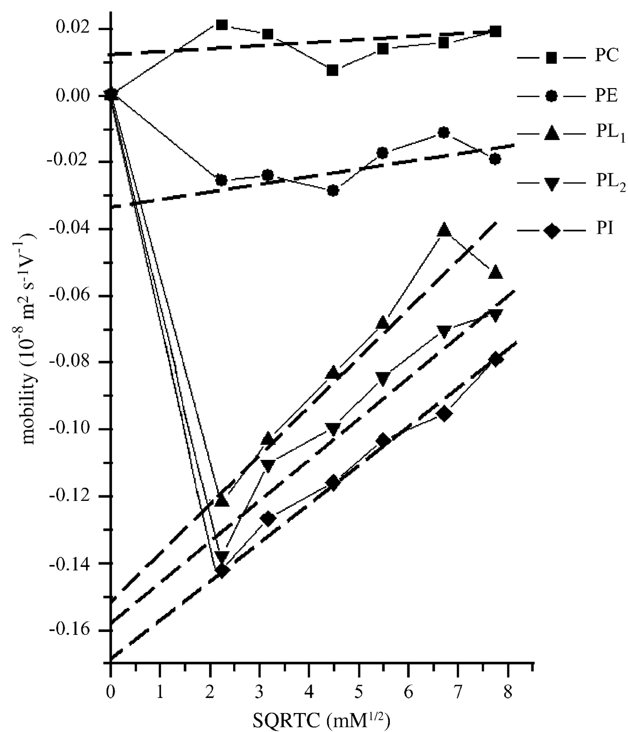

Fig. 7. Relationship between the square root of ammonium acetate concentration and the mobility of phospholipids. Data are experimental values, and dashed lines are their regression curves.

to 20% in acetonitrile–2-propanol (3:2, v/v), 0.3% acetic acid and 60 mM ammonium acetate, the analysis time increased from about 10 min to nearly 40 min (Fig. 4). However, the separation was not improved by the addition of *n*-hexane.

Table 2 gives the viscosity, apparent pH and Walden products of phospholipids in the separation medium containing n-hexane at different levels. The viscosity decreased from 0.545 to 0.485 cP when the content of *n*-hexane increased from 0% to 20%. However, the apparent pH remained almost the same. The absolute values of the Walden products of each phospholipid decreased with increasing *n*-hexane in the separation medium.

Since the viscosity remained almost unchanged over the range of *n*-hexane concentration from 0% to 5%, and even decreased over the range of 5–20%, the dielectric constant was the dominant factor affecting EOF and mobility of phospholipids. The absolute values of mobility and EOF were decreased linearly with the decrease of the current when the percentage of *n*-hexane in the medium increased as shown in Fig. 5A. The R^2 of the regression lines ($\mu = aC + b$, where μ is the absolute mobility, and C is the current during separation) between current and mobility of EOF and phospholipids were above 0.99 except PC. The relationship between current and mobility is a certain deduction of the Ohm law and the ion independently migrating theory [42]. Because the change of properties of the separation medium by alcohols was much more complicated than that by *n*-hexane, the relationships were deviated from linearity when alcohols in the separation mediums was changed (Fig. 5B).

Based on the above study, one may conclude that *n*-hexane had no positive effect on the separation efficiency, but prolonged the analysis time. Therefore, *n*-hexane was excluded from the separation medium in the following quantitative study.

3.4. Effect of ammonium acetate and ion–dipole interaction

Not only the separation time increased, but also separations were improved when the concentration of ammonium acetate increased from 0 to 60 mM (Fig. 6). As shown in Fig. 6, phospholipids could not be separated in the nonaqueous medium if there was no ammonium acetate. The resolution was drastically increased with the increase of the concentration of ammonium acetate. A satisfactory separation was obtained when the concentration of ammonium acetate was 60 mM.

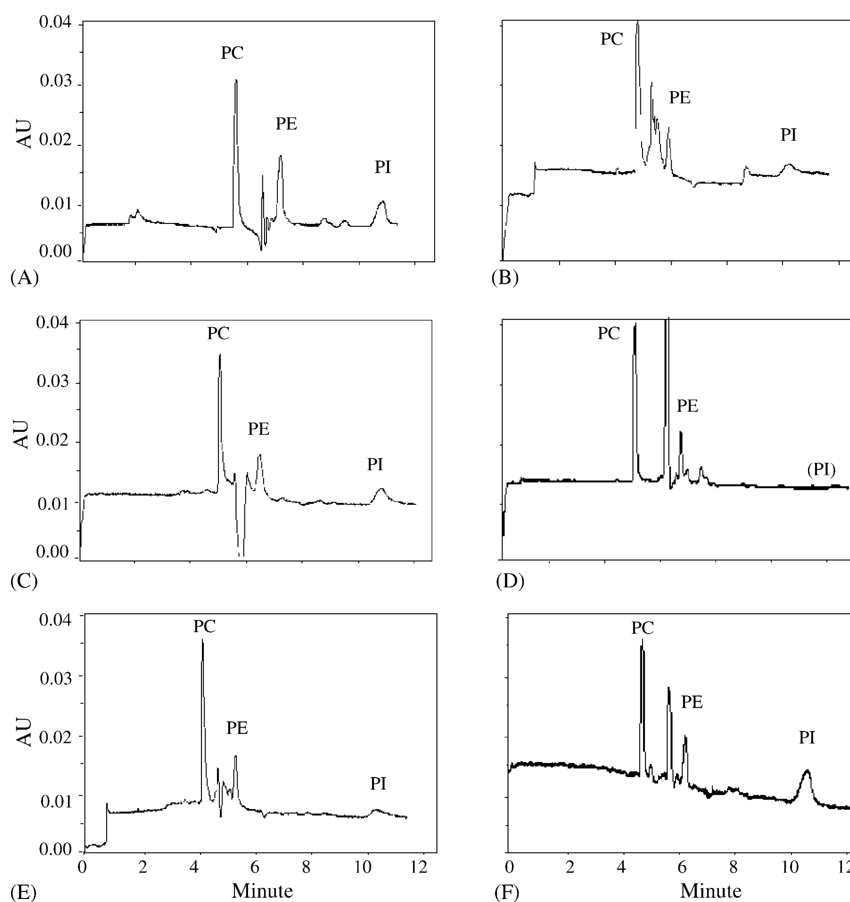

Fig. 8. Electropherograms of phospholipids in the separation medium of acetonitrile–2-propanol (3:2, v/v), 0.3% acetic acid and 60 mM ammonium acetate: (A) soybean, (B) walnut, (C) sunflower, (D) peanut, (E) apricot kernel, and (F) filbert. Other conditions were the same as Fig. 4.

Table 3
Linearity range, correlation coefficient and limits of detection (L.D.) (S/N = 3) of different phospholipids[a]

Analyte	Migration time (min)	Linearity range (mg/mL)	Regression equation ($\times 10^5$)[b]	Correlation coefficient	L.D. (mg/mL)	Peak area RSD (%)
NACE						
PC	5.45 ± 0.04	0.03–3	$2.82x + 0.18$	0.990	0.013	3.1
PE	6.73 ± 0.06		$2.67x + 0.25$	0.985	0.018	2.2
PI	10.6 ± 0.10		$3.38x - 0.13$	0.982	0.023	4.5
MEKC						
PC	12.1 ± 0.08	0.03–3	$2.52x - 0.22$	0.993	0.007	1.5
PE	12.3 ± 0.01		$2.75x + 0.40$	0.986	0.011	2.7
PI	15.7 ± 0.18		$3.69x + 0.30$	0.990	0.031	4.0

[a] Precision of migration time and peak area was determined at phospholipids concentration of 0.2 mg/mL ($n = 5$) under the optimized NACE and MEKC conditions.

[b] Regression equation is expressed as $y = ax + b$, where 'x' represents the concentration of corresponding analyte (mg/mL).

The mobility decreased with the increase of ammonium acetate in the separation mediums by the ion atmosphere theory. According to Debye–Hückel–Onsager theory, the mobility decreased linearly with the increase of square root of ion strength of the electrolyte solution [42] and the intercepts of the lines should be the mobility of the analyte when electrolyte concentration was 0. However, in Fig. 7, the relationship between square root of ammonium acetate concentration and the mobility of phospholipids was deviated from linearity when the concentration of acetic ammonium increased from 0 to 60 mM and there was a gigantic difference between the mobility obtained based on Debye–Hückel–Onsager theory (the intercepts of the regression lines) and that obtained by the experiments when the ammonium acetate concentration was 0 (Fig. 7).

The deviation of Debye–Hückel–Onsager theory and the improvement of separation by the ammonium acetate were caused by the ion–dipole interaction in the NACE separation of phospholipids, although there were no reports in the literatures on this topic. In solvent of poor hydrogen-bond donors, the molecules of weak acids such as phosphoric residues of phospholipid molecules are not dissociated or poorly dissociated, the anions of electrolyte tend to accept hydrogen from the acidic groups [28–30], as a result, a negatively charged complex formed. On the other hands, the weak alkali groups such as choline and ethanolamine groups of PC and PE will interact with the cation in the solvent, thus a positively charged complex formed. The formation of ion–dipole complexes improved the separation of poorly dissociated acidic or basic analytes by NACE. So, it is understandable that phospholipids are poorly separated at lower concentration of ammonium acetate and are well separated at higher concentration of ammonium acetate (Fig. 6); the mobility obtained based on Debye–Hückel–Onsager theory were quite different from that obtained by the experiments in the absence of ammonium acetate (Fig. 7). For PC and PE, the ion–dipole interactions are more complicated because they have both acidic and basic groups, which could interact with both the negatively charged acetate anion and positively charged ammonium ion, so the regressing curves of PC and PE are more deviated from linearity (Fig. 7).

Based on the above study a separation medium composed of acetonitrile–2-propanol (3:2, v/v), 0.3% acetic acid and 60 mM ammonium acetate was finally chosen for the separation and determination of phospholipids in real plant samples.

3.5. Determination of phospholipids by NACE and MEKC

Fig. 8 shows the electropherograms of phospholipids of six plant seeds. Considering the lack of chromophores of phospholipids, the detection wavelength was 200 nm and the detection limits of phospholipids are relatively high (Table 3). The results determined by the recommended NACE were also compared to those obtained by MEKC (Tables 3 and 4). For the MEKC determination of phospholipids, the method

Table 4
Contents of phospholipids in six plant seeds determined by NACE and MEKC (mg/g, $n = 8$)

Plant seeds	NACE	MEKC
Soybean		
PC	2.64 ± 0.032	2.76 ± 0.048
PE	1.46 ± 0.048	1.46 ± 0.054
PI	0.82 ± 0.022	0.70 ± 0.032
Walnut		
PC	0.68 ± 0.016	0.62 ± 0.008
PE	0.24 ± 0.012	0.28 ± 0.011
PI	0.54 ± 0.021	0.50 ± 0.012
Sunflower		
PC	1.82 ± 0.048	1.88 ± 0.020
PE	0.58 ± 0.032	0.70 ± 0.022
PI	0.60 ± 0.036	0.52 ± 0.021
Peanut		
PC	2.82 ± 0.021	2.62 ± 0.054
PE	0.84 ± 0.020	0.98 ± 0.058
Apricot kernel		
PC	2.34 ± 0.012	2.10 ± 0.030
PE	0.52 ± 0.012	0.82 ± 0.024
Filbert		
PC	1.36 ± 0.012	1.36 ± 0.008
PE	0.60 ± 0.022	0.76 ± 0.016
PI	1.64 ± 0.008	1.52 ± 0.016

of Sztics et al. [8] was applied except that 75 mM sodium cholate was used instead of 35 mM sodium deoxycholate. A good agreement was obtained between the data determined by the recommended NACE method and MEKC method.

4. Conclusion

The composition of separation medium plays an important role in the NACE separation of phospholipids due to their integrated effects of viscosity, dielectric constant, solvation interactions and ion–dipole interactions. Among these parameters, the solvation effects and ion–dipole interactions are the key factors. Compared with MEKC method, separation of phospholipids can be achieved in relatively short time at room temperature by NACE method, while the MEKC method needed to be performed at higher temperature and a longer separation time. Therefore, NACE is a good alternative to MEKC for the analysis of phospholipids.

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