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Capillary Electrophoretic Application of 1-Alkyl-3-methylimidazolium-Based Ionic Liquids

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Ionic substances with melting points at or close to room temperature are referred to as ionic liquids. Interest in ionic liquids for their potential in different chemical processes is increasing, because they are environmentally benign and are good solvents for a wide range of both organic and inorganic materials. In this study, a capillary electrophoretic method for resolving phenolic compounds found in grape seed extracts is reported. The method, in which 1-alkyl-3-methylimidazolium-based ionic liquids are used as the running electrolytes, is simple and reproducible. The separation mechanism seems to involve association between the imidazolium cations and the polyphenols. The role of the alkyl substituents on the imidazolium cations was investigated and will be discussed.

Ionic substances with melting points considerably lower than typical for ionic salts are often referred to as ionic liquids. Typically, ionic liquids consist of nitrogen-containing organic cations and inorganic anions. Bulky organic cations such as *N*-alkylpyridinium and 1-alkyl-3-methylimidazolium (shown in Figure 1)[1] are combined with inorganic anions such as Cl⁻, Cl⁻/ AlCl3, NO_3^-, PF_6^- (HFP) and BF_4^- (TFB). Less common anions include bis(trifluoromethanesulfonyl)imide $(CF_3SO_2)_2N^-$ and trifluoromethanesulfonate $(CF_3SO_3^-)$.[2,3] The combination of such cations and anions can lead to a large number of ionic liquids that provide considerable flexibility in the selection of the most suitable pair for a specific chemical application.

In the past several years, there has been growing interest in ionic liquids for their potential in different chemical processes (e.g., catalysis for clean technology,[4−6] electrochemistry,[7−9] and
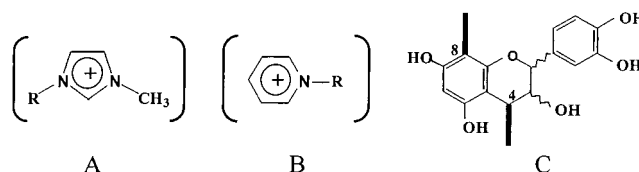

Figure 1. Structures of 1-alkyl-3-methylimidazolium cation (A), *N*-alkyl-pyridinium cation (B), flavan-3-ol monomer unit (C). R = alkyl group.

separations[10,11]) because ionic liquids present a variety of desirable properties. They are environmentally benign, nonvolatile, and nonflammable with a high thermal stability and are good solvents for a wide range of both inorganic and organic materials. The miscibility of ionic liquids in water is, to a large extent, dictated by the inorganic anion. One anion shown to provide high miscibility of ionic liquids in water is the TFB anion (e.g., 1E-3MI-TFB, 1B-3MI-TFB). In contrast, the HFP anion has shown to provide low (1E-3MI-HFP) or no solubility in water (1B-3MI-HFP).

Wilkes and Zaworotko[12] concluded that 1-ethyl-3-methylimidazolium (1E-3MI) was a generally useful cation. Fuller and co-workers[13] fully characterized the low-melting salt, 1-ethyl-3-methylimidazolium hexafluorophosphate (1E-3MI-HFP, mp 58− 60 °C). They reported that its interionic interactions were dominated by cation−anion Coulombic attraction with minimal hydrogen bonding. Like 1-ethyl-3-methylimidazolium tetrafluoroborate (1E-3MI-TFB, mp 15 °C),[14] it is air- and water-stable.

Currently, there is considerable interest in the analysis of phenolic mixtures that are derived from various food substances associated with a variety of health benefits in humans,[15] including anticarcinogenic,[16] anticariogenic,[17] and hepatoprotective activities.[18] It is thought that naturally occurring antioxidants in the

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form of polyphenols may be responsible for some of this therapeutic effect; however, studies on related compounds (bioflavonoids)[19,20] have shown that excessive consumption by expectant women could elevate the risk for leukemia in their infants.

In general, polyphenols are encountered in various biological samples[21] as complex mixtures of homologues and isomers with differing degrees and sites of polymerization (see Figure 1) and thus represent a truly challenging separation problem. Most of the methods currently employed to separate polyphenols use high performance liquid chromatography (HPLC)[22−24] and, to a lesser extent, gas chromatography.[25] More recently, capillary electrophoresis (CE) has been used for the analysis of polyphenols;[26−30] however, none of the current methods can provide complete resolution of all of the constituents in these very complicated samples.

In some CE studies, alkylammonium salts have been used as electroosmotic flow (EOF) modifiers.[31−33]. Recently, however, Yanes and co-workers[34] reported the development of a fairly robust capillary electrophoretic method for the separation of polyphenols found in grape seed extracts that uses tetraethylammonium tetrafluoroborate (TEA-TFB) as the only electrolyte in the background electrolyte. In this study, the cation not only acted as an EOF modifier but also played an active role through association with the polyphenols. The excellent reproducibility that was achieved was attributed to the coating of the capillary wall by the tetraalkylammonium cations with a permanent charge group not subject to pH-induced variations in ionization.

The purpose of the present study is to investigate the potential application of ionic liquids to the capillary electrophoretic separation of polyphenols. Currently, the majority of studies pertaining to ionic liquids focus on their characterization and their potential use in industrial processes. However, given the similarity of structure and properties of the TEA-TFB salt to the so-called "green chemistry" solvents of the ionic liquids, the extension of the previous results that were obtained using TEA-TFB in the separation of polyphenols to ionic liquids seems a natural progression. The present work describes a simple and reproducible electrophoretic method for the analysis of phenolic compounds found in grape seed extracts. The method primarily involves the use of 1-alkyl-3-methylimidazolium-based ionic liquids as the running electrolyte solutions.

EXPERIMENTAL SECTION

Materials. The (−)-catechin, (−)-epicatechin, (−)-catechin gallate, (−)-gallocatechin gallate, (−)-epicatechin gallate, gallic acid, and resveratrol were purchased from Sigma Chemical Co. (St, Louis, MO). The 1E-3MI-TFB, 1E-3MI-HFP, 1-ethyl-3-methylimidazolium nitrate (1E-3MI-NT) and 1-ethyl-3-methylimidazolium trifluoromethanesulfonate (1E-3MI-TFMS) were purchased from Fluka Chemical Corp. (Ronkondoma, NY). Sodium hydroxide, hydrochloric acid, and nitromethane were purchased from Fisher Scientific (Fair Lawn, NJ). All other chemicals were purchased from either Aldrich Chemical Co. or Fisher Scientific and used as is unless otherwise specified in the procedures. Filters (0.2 μm) were obtained from Nalge Nunc International Corporation (Rochester, NY). The fused-silica capillaries were obtained from Bio-Rad Laboratories, Inc. (Hercules, CA).

The 1B-3MI-TFB and 1B-3MI-HFP were prepared from 1-butyl-3-methylimidazolium chloride (1B-3MI-Cl) according to the following procedures.

1B-3MI-Cl. The 1B-3MI-Cl was prepared by a slight modification of a literature procedure.[35] A 50-mL aliquot of 1-methylimidazole (dried over $MgSO_4$) was combined with 250 mL of distilled 1-chlorobutane and allowed to reflux for 24 h. The excess 1-chlorobutane was removed by rotary evaporation, leaving a clear, yellow, viscous oil. This oil was heated (100 °C) under vacuum for 8 h to remove any remaining solvent, then placed in a freezer overnight. As the oil warmed to room temperature, a hard off-white precipitate formed. This solid was dissolved with heating and stirring in a mixture of acetonitrile (∼30 mL) and ethanol (∼10 mL) that had been dried over $MgSO_4$. The solution was layered with anhydrous diethyl ether (∼10 mL) and placed into the freezer overnight. The next day, a white precipitate had formed. It was isolated by vacuum filtration and placed under vacuum for 24 h. (mp = 65−69° C).

1B-3MI-TFB and 1B-3MI-HFP. The ionic liquids were prepared by a slight modification of a literature procedure.[36] The chloride salt (1B-3MI-Cl) was added to ∼50 mL of acetone, forming two layers. One equiv of the appropriate salt ($NaBF_4$ or KPF_6) was added, and the mixture was stirred at room temperature for 24 h. The resulting NaCl or KCl precipitate was then filtered off, and the solvent was removed by rotary evaporation to leave a yellowish, clear liquid that was filtered through activated charcoal to lighten the color. (fp = −76 °C for BF_4 and −8 °C for PF_6, both within the reported ranges).[2,14]

Equipment. UV spectra were obtained using a Hewlett-Packard model 8453 UV−vis spectrophotometer and a 1-cm quartz cell. Solutions for absorbance measurements were prepared in 30-mL plastic bottles. All of the CE experiments were carried out using either a Bio-Rad BioFocus 2000 or a Bio-Rad BioFocus 3000

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automated capillary electrophoresis system (Bio-Rad Laboratories, Inc., Hercules, CA) equipped with a UV detector and interfaced with a personal computer. The multiwavelength detection and high-speed UV scanning capability of the Bio-Rad Biofocus 3000 was used to determine the best wavelength for the detection of polyphenols with specific running electrolytes.

The bare fused-silica capillary (50 μm i.d.) had a total length of 50 cm (45.4 cm to the detector) when using all imidazolium ionic liquids except 1E-3MI-TFB. In this case, two columns having different lengths were used, one having a length of 50 cm and the other having a total length of 60 cm (55.4 cm to the detector). The longer column was used for the characterization and identification of polyphenols.

Daily, at the beginning of each experimental session, the capillary was conditioned by purging with 0.5 M sodium hydroxide for 10 min and distilled deionized water for 3 min, followed by running electrolyte for 4 min. Between electrophoretic runs, the capillary was rinsed with only electrolyte for 40 s. Separation conditions were found to be optimal using an applied voltage of 20 kV (anodic detection) for the long capillary and 16 kV for the shorter column, at a constant temperature of 20 °C. Detection was achieved at 240 nm. Injection was performed hydrodynamically at 5 psi for 0.4 s.

Grape Seed Extract Sample Preparation. The extracts used in the present study were obtained from Givaudan Flavors Corporation (Cincinnati, OH). A portion (0.03 g) of the grape seed extract was weighed into a 15-mL sample vial. Water then was added to bring the total weight to 15 g, giving a final concentration of 2 mg mL^{-1}. Before injection, a 600-μL aliquot of the sample was diluted to a total volume of 1600 μL with the electrolyte solution to bring the concentration to 0.75 mg mL^{-1}. Sample and running electrolytes were degassed and filtered using a 0.2 μm filter.

Standards and Running Electrolytes Preparation. For the absorbance measurements, equimolar solutions of 20 μM for catechin and 1E-3MI-TFB were prepared in water. The working CE standard solutions were prepared daily by dissolving the standards in a drop of methanol and were further diluted using the running electrolyte solution. Electrolytes for the electrophoretic runs included 1E-3MI-TFB [50−400 mM] and 1E-3MI-HFP [50−150 mM]. Electrolyte systems of 1E-3MI-nitrate, 1E-3MI-trifluoromethanesulfonate, and 1B-3MI-TFB were also prepared [50, 100, and 150 mM]. Unfortunately, 1B-3MI-HFP was not miscible in water. In all cases, reproducibility was improved by briefly shaking the background electrolyte solutions just prior to use in CE.

RESULTS AND DISCUSSION

UV Spectra. UV spectra were obtained for equimolar solutions of 1E-3MI-TFB and catechin as a representative phenol. Catechin was chosen because it was a known constituent of the sample. Figure 2 presents the spectrum of (A) 2.0×10^{-5} M 1E-3MI-TFB and (B) catechin. The 1E-3MI-TFB exhibited an absorbance maximum at 212 nm, and catechin showed a maximum absorbance at 203 nm. From the spectral data and by using the high-speed UV-scanning feature provided by the Bio-Rad Biofocus 3000, a wavelength of 240 nm was found to be optimal for polyphenol detection in the presence of the ionic liquid.
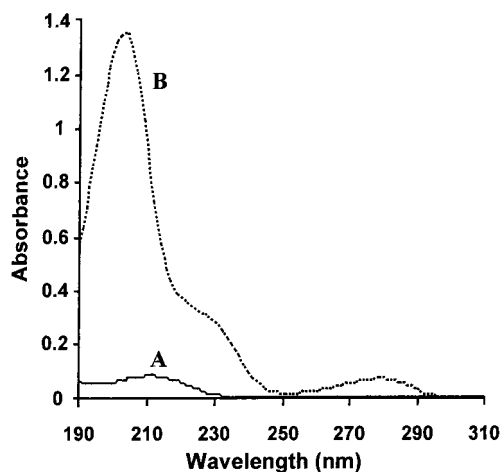
Figure 2. UV spectra of the ionic liquid 1-ethyl-3-methylimidazolium tetrafluoroborate (A) and catechin (B) at equimolar concentrations (20 μM).

1-Alkyl-3-methylimidazolium-Based Ionic Liquids as Running Electrolytes. Six 1-alkyl-3-methylimidazolium-based ionic liquids were evaluated with respect to resolution of polyphenols in grape seed extracts. All of them are differentiated from each other either by the inorganic anion (TFB$^-$, HFP$^-$, NO$_3^-$, or TFMS$^-$) or the alkyl group (ethyl or butyl) on the imidazolium cation. Some of their properties are summarized in Table 1.

In general, the presence of ionic liquids as running electrolytes provided an acidic environment in which the polyphenols should be neutral ($pK_a \sim 9.5-10.5$ for polyphenols).[37] In addition, the imidazolium ions coat the capillary walls, thus engendering anodic EOF. The polyphenols migrated after the neutral marker. These results are entirely consistent with those obtained by Yanes and co-workers[34] using TEA-TFB as the running electrolyte.

As illustrated in Figure 3, the delay in the migration time of the polyphenols may be attributed to two factors. The polyphenols may associate either with the positively charged imidazolium groups coating the capillary wall or with the free imidazolium ions in the bulk solution. This association could be partially driven by hydrophobic, hydrogen bonding or ion−dipole/ion-induced−dipole interactions between the polyphenols and the imidazolium cations. It should be noted that Figure 3 is not intended to imply any sort of ordering on the fused-silica surface.

To examine the imidazolium−polyphenol association, the migration time of the neutral marker (EOF measurement) and the effective electrophoretic mobility of catechin were plotted versus the concentration of the 1E-3MI-TFB electrolyte solution. From Figure 4, it can be seen that the EOF magnitude (nitromethane migration time) increased when the concentration of 1E-3MI-TFB was increased from 50 to 250 mM. Although the increase in EOF slowed between 250 and 400 mM, the attainment of a maximum EOF was less abrupt than was observed previously with TEA-TFB. However, it should be noted that the EOF observed using 1E-3MI-TFB was higher at all concentrations than that obtained using equal concentrations of TEA-TFB. The higher EOF observed with 1E-3MI-TFB relative to that obtained with

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Table 1. Ionic Liquid Properties[2,14]

name	mp, °C	ρ, g mL^{-1}	fw
1-ethyl-3-methylimidazolium tetrafluoroborate[a]	15	1.29	197.98
1-ethyl-3-methylimidazolium hexafluorophosphate	58−60	solid	256.13
1-ethyl-3-methylimidazolium nitrate	38−41	solid	173.17
1-ethyl-3-methylimidazolium trifluoromethanesulfonate		1.39	260.24
1-butyl-3-methylimidazolium tetrafluoroborate[a]	−76	1.05	227.04
1-butyl-3-methylimidazolium hexafluorophosphate	−8	1.33	285.20

[a] Glass transition.
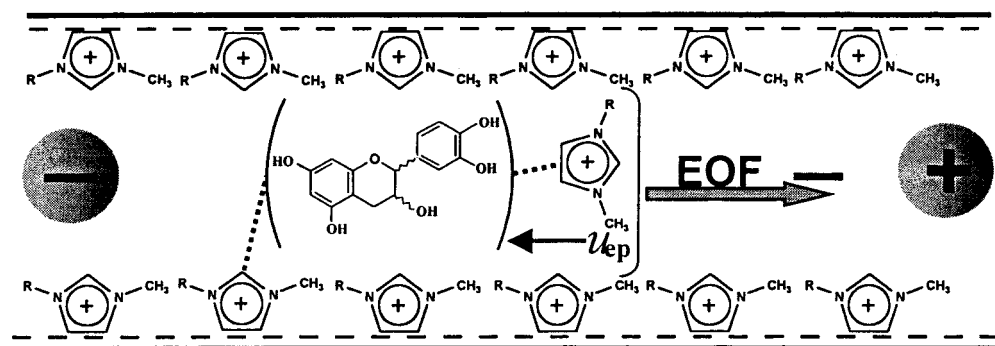
Figure 3. Mechanism of polyphenols' separation using 1-alkyl-3-methylimidazolium-based ionic liquids.
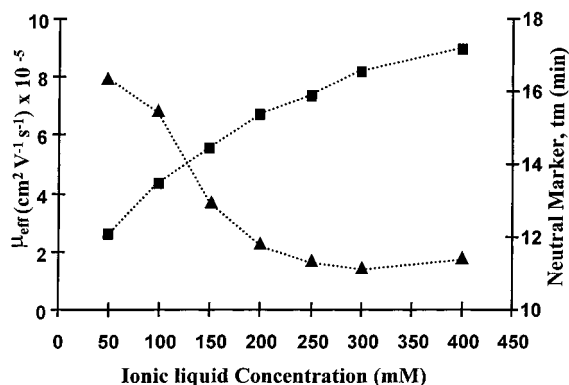
Figure 4. Effect of increasing 1-ethyl-3-methylimidazolium tetrafluoroborate concentration on the effective electrophoretic mobility of catechin (■) and on the migration time of the neutral marker (▲).

Figure 5. Separation of polyphenols using (A) 1-ethyl-3-methylimidazolium tetrafluoroborate, (B) 1-butyl-3-methylimidazolium tetrafluoroborate, and (C) 1-ethyl-3-methylimidazolium hexafluorophosphate [150 mM]; voltage, 16 kV, with anodic detection at 240 nm; asterisk (*), nitromethane.

TEA-TFB may in part be attributable to differences in the ion association constants.[38] In the case of 1E-3MI-TFB, the delocalization of the charge on the imidazolium cation should produce a lower association constant than for the TEA-TFB. Holbrey et al. noted the absence of significant hydrogen bonding between the 1E-3MI cation and the TFB anion.[14] Current theories of CE hold that the EOF emanates from the migration of the loosely held counterions (in this case, the tetrafluoroborate anion) in the outermost layer of the electrical double layer and that the magnitude of this EOF is primarily a function of the electrophoretic mobility of this ion. However, the mobility of the ions responsible for the EOF in a given system is also subject to the ion association constants with the immobilized counterions in the system.[38] Indeed, weaker association between the ionic liquid cation and its counterion is responsible for the lower melting points of this class of compounds relative to other salts.
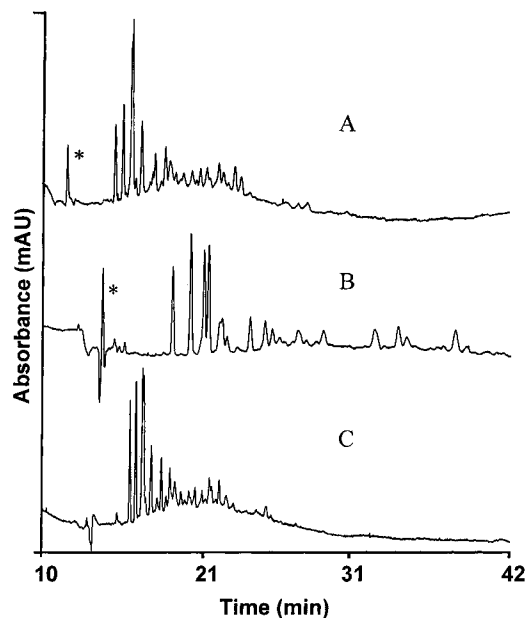
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Figure 4 also facilitates the distinction between the contribution of association of the polyphenols with either the imidazolium cations coating the capillary wall or the free cations in the bulk solution. This distinction can be observed through the relationship between the effective electrophoretic mobility ($u_{eff} = u_{meas} - u_{eof}$) of catechin and the electrolyte concentration. The mobility of catechin increased as the concentration of 1E-3MI-TFB increased. The continued increase of the effective electrophoretic mobility of catechin above ~250 mM (concentration above which EOF begins to level off) suggests that association of the polyphenols
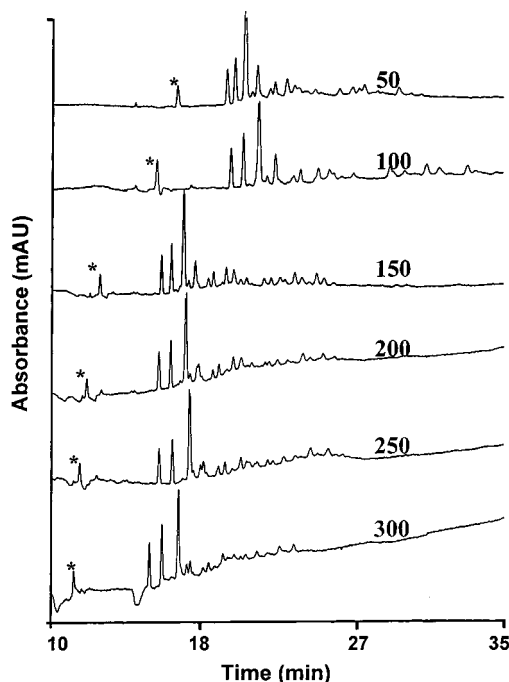
Figure 6. Effect of 1-ethyl-3-methylimidazolium tetrafluoroborate concentration on separation (50−300 mM). Voltage, 20 kV, with anodic detection at 240 nm.

Figure 7. Separation of polyphenols using 1-ethyl-3-methylimidazolium tetrafluoroborate (150 mM). Voltage, 20 kV with anodic detection at 240 nm. 7A−C, grape seed extract; 7D, polyphenol standards; asterisk (*), nitromethane.

Figure 8. Correlation between the molecular weight of the identified polyphenols and their migration times.

with the free imidazolium ion seems to dominate, although the distinction is less clear than for the TEA-TFB case.

For further investigation of the imidazolium−phenol interaction, the binding constant was calculated for 1E-3MI-TFB and catechin as a representative phenol from the CE data, using the following relationship as derived by Rundlett and Armstrong[39]

$$\frac{1}{(\mu_p - \mu_f)} = \frac{1}{(\mu_c - \mu_f)K}\frac{1}{[IL]} + \frac{1}{(\mu_c - \mu_f)}$$

where K is the binding constant, [IL] is the concentration of ionic liquid, μ_p is the corrected polyphenol mobility at the ionic liquid concentration [IL], and μ_f and μ_c are the electrophoretic mobilities of the free and complexed polyphenol. The catechin mobilities were corrected for viscosity changes by multiplying each mobility by the ratio $(\eta_0/\eta_{[IL]})$ of the measured viscosity of pure water over the viscosity of a solution containing the ionic liquid at the concentration of interest. By plotting $(\mu_p - \mu_f)^{-1}$ versus $[IL]^{-1}$ the binding constant (intercept/slope) was estimated to be $K = \sim5$ M^{-1}. The apparent Gibbs free energy $(\Delta G = -RT \ln K)$ was calculated to be ~4 kJ/mol, which seems reasonable for typical ion−dipole or ion-induced−dipole interactions.[40]

Effect of Alkyl Group on the Imidazolium Cations. To study the effect of the alkyl group on the polyphenols' separation, the 1E-3MI-TFB and the 1B-3MI-TFB ionic liquids were compared (the inorganic anion was kept the same). Figure 5 shows representative electropherograms for the separation of polyphenols using 1E-3MI-TFB (Figure 5A) and 1B-3MI-TFB (Figure 5B). Both separations were obtained at 150 mM using a 50-cm column. Other conditions are detailed in the figure legends. The electro-
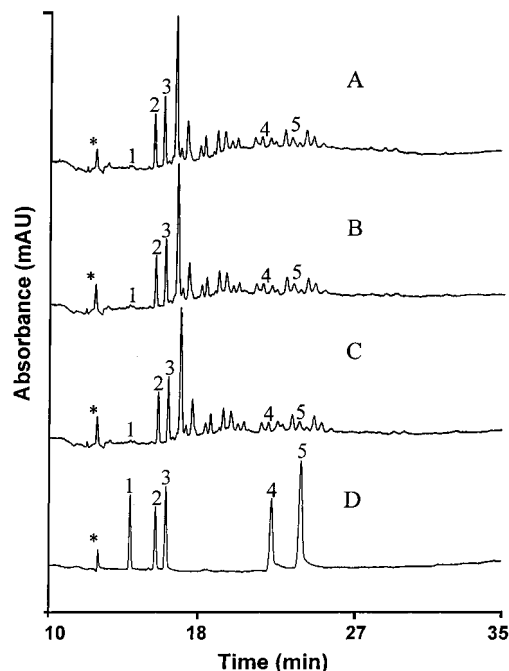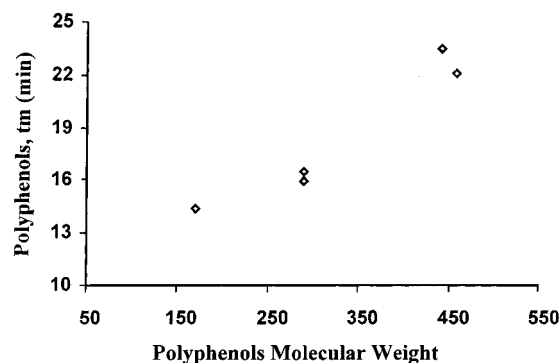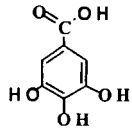
(39) Rundlett, K. L.; Armstrong, D. W. *J. Chromatogr. A* **1996**, *721*, 173−186.
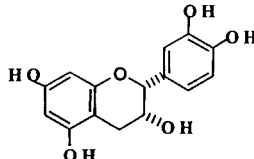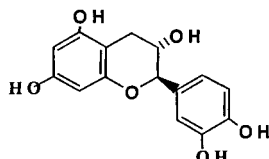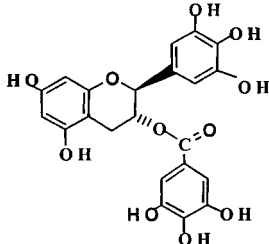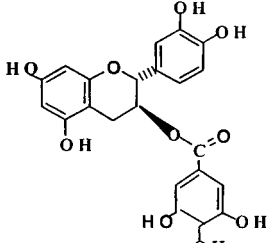(40) Atkins, P. W. *General Chemistry*; Scientific American, Inc.: 1989; p 354.

pherograms illustrate that both the separation and the magnitude of the EOF were affected by the alkyl group on the imidazolium cation. In terms of the separation, it is apparent that the butylimidazolium-based ionic liquid provided better resolution and a wider separation window; however, the resolution was improved at the expense of longer analyses time. It is also interesting to note that the 1B-3MI-TFB partially resolves the third major polyphenol peak, which to the best of our knowledge, has not been resolved in any previous studies. The improved resolution obtained with the butylimidazolium-based ionic liquid relative to its ethyl analogue could be the result of the decreased EOF, thereby affording more opportunity for the polyphenols to associate either with the cations coating the capillary wall or with those in the bulk solution migrating away from the detector. Alternatively, the affinity of the polyphenols could be higher for the butylimidazolium cation than for the ethyl analogue.

As noted by a reviewer, the decreased EOF obtained with the butylimidazolium-based ionic liquid relative to that obtained with the ethylimidazolium analogue seems to contradict EOF trends

Table 2. Polyphenols Detected

	Name	Molecular Formula	Structure
1	Gallic Acid	$C_7H_6O_5$	
2	(-)-Epicatechin	$C_{15}H_{14}O_6$	
3	(+)-Catechin	$C_{15}H_{14}O_6$	
4	(-)- Gallocatechin Gallate	$C_{22}H_{18}O_{11}$	
5	(-) -Epicatechin Gallate	$C_{22}H_{18}O_{10}$	

observed previously with tetraalkylammonium tetrafluoroborate (e.g., EOF increased with increasing alkyl chain length). Although the viscosity of the 150 mM butylimidazolium solution was determined to be slightly higher than that of an equimolar ethylimidazolium solution (data not shown), this difference in solution viscosity was not sufficient to account for the difference in EOF.

It should be noted that the magnitude of the EOF is a function of the electrophoretic mobility of the ion responsible for the EOF, but it is also dependent on the counterion charge density on the capillary walls. For instance, the relationship between EOF and pH is well documented in CE using uncoated capillaries. Quang and Khaledi[33] reported in a comparison of long- and short-chain tetraalkylammonium reagents that short-chain cationic reagents provided better capillary wall coverage and faster equilibration for the reversal of EOF than do long-chain surfactants. When using 1E-3MI-TFB, reproducible data was obtained after the first 50-min run. In contrast, 1B-3MI-TFB-mediated phenolic separations

required four or five runs of 50 min each before reproducible data was obtained, thus suggesting that equilibration of the imidazolium cations on the fused-silica capillary is reached faster with the ethylimidazolium cations than with the butylimidazolium cations.

When using quaternary ammonium EOF modifiers having long hydrocarbon tails, it has been shown that the EOF can be reduced, shut down, or even reversed, depending upon the concentration of the modifier that is used.[33,38] The anodic EOF is thought to emanate from the formation of a bilayer on the fused-silica surface.[41] It is possible that the reduced EOF observed with the butylimidazolium cation relative to the ethylimidazolium cation may be the result of either crowding of the cations on the surface of the silica or the formation of a bilayer with the butylimidazolium. In either case, the surface density of the butylimidazolium cation would be expected to be smaller than for the ethylimidazolium cation. One way to test for this might be to compare the effect of

(41) Emmer, A.; Jansson, M.; Roeraade, J. *J. Chromatogr.* **1991**, *547*, 544–550.

the concentration of these ionic liquids on the EOF or to look at a wider number of homologues. Crowding at the surface should shift the maximum EOF to a lower concentration for butylimidazolium relative to the ethyl analogue. Unfortunately, insufficient butylimidazolium was available to make this comparison.

Effect of 1E-3MI Counterion. To investigate the role of the counterion, low-melting-point ionic salts composed of the organic cation 1E-3MI with four different counterions were investigated. This included the inorganic anions tetrafluoroborate (TFB), hexafluorophosphate (HFP), nitrate (NT) and trifluoromethanesulfonate (TFMS); however, it should be noted that no separations were obtained with TFMS and NT anions. In both cases, a single peak was detected, and it was difficult to discern if the polyphenols were even eluting from the capillary column using either polarity. In addition, the presence of these anions led to an unsteady baseline. All of these issues restricted the use of such anions and were not investigated further. Although the nature and intensity of the association between ionic liquid cations and various anions is still the subject of some controversy,[12] there does seem to be some evidence that significant hydrogen bonding between the hydrogen on the C-2 carbon of the imidazolium cation and oxygen-containing anions (e.g., nitrate) may contribute to the higher melting points observed for these low-melting-point ionic salts relative to their tetrafluoroborate analogues. However, any speculation regarding correlation between the magnitude of the EOF and mutual affinity between the cation–anion pair may be more appropriate for low-melting-point ionic salts with the same anion.

The 1E-3MI with counterions TFB and HFP provided similar separation profiles (see Figure 5A,C), thus reinforcing the importance of the organic cation. Both of the electropherograms were obtained at 150 mM using a 50-cm column. Unfortunately, the results obtained with the HFP material were not very reproducible, presumably because of its low solubility in water and as a result, the EOF was not determined.

Effect of 1E-3MI-TFB Concentration. The separations shown in Figure 6, using the 1E-3MI-TFB, are representative electropherograms at concentrations from 50 to 300 mM. These results were obtained using a 60-cm capillary column. As can be seen in Figure 6, the increase of the ionic liquid concentration clearly affects the magnitude of the EOF as well as the polyphenols separation. Optimum results are obtained at 150 mM concentration.

Determination of Polyphenols Using 1E-3MI-TFB. For the characterization and identification of the phenolic compounds in the grape seed extracts, the ionic liquid 1E-3MI-TFB was used.

The optimum separation conditions included 150 mM concentration of the ionic liquid and a 60-cm capillary column with an applied voltage of 20 kV.

The capillary electrophoretic reproducibility for the separation of polyphenols found in the complex mixture is shown in Figure 7. The first three electropherograms (7A–C) illustrate the separation of the phenolics in the grape seed extract. Electropherogram 7D is the separation of standards. As can be seen from this figure, reproducible separation was achieved. The standard deviation for three consecutive injections of the grape seed extract sample for migration time of nitromethane and the polyphenols identified was <0.47 (%RSD < 2.14).

The components in the sample may be identified by referring to Table 2. The polyphenols that were detected were identified by spiking the grape seed extract sample with phenolic standards. Of the known polyphenols identified in the electropherogram, polyphenolic migration times seem to correlate with molecular weight (e.g., higher molecular weights migrate more slowly through the column). This is illustrated in Figure 8, in which the migration times of the identified polyphenols are plotted against their molecular weights.

CONCLUSIONS

Successful separation and identification of some phenolic constituents of grape seed extracts has been achieved using 1-alkyl-3-methylimidazolium-based ionic liquids as the main electrolyte solutions. The electrophoretic method proved to be simple and reliable, providing good reproducibility in terms of migration times. Analogous to previously reported polyphenolic separations using a tetraethylammonium tetrafluoroborate electrolyte, the separation mechanism relies on the association of the polyphenols with the imidazolium cations either coating the capillary wall or electrophoretically migrating in the bulk solution.