行政院國家科學委員會專題研究計畫　成果報告

離子液體應用於毛細管電泳的研究

計畫類別：個別型計畫
計畫編號：NSC94-2113-M-029-009-
執行期間：94 年 08 月 01 日至 95 年 07 月 31 日
執行單位：東海大學化學系

計畫主持人：黃承文

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一、中文摘要

離子液體（或稱為熔融鹽）是一類低熔點的非分子溶劑。由於它們特殊的物理與化學性質，近年來離子液體已成功地被用做液相−液相萃取的溶劑、氣相層析管柱的固定相、與液相層析或毛細管電泳移動相的添加劑。本研究探討利用離子液體，1-ethyl-3-methyl-imidazolium tetrafluoroborate，當作毛細管電泳移動相的添加劑，進行九種 tricyclic antidepressants 藥物的同步分離實驗。結果顯示以 50 mM 1-ethyl-3-methyl- imidazolium tetrafluoroborate 溶液當作電泳移動相，可完全分離九種化合物。

關鍵詞：離子液體；毛細管電泳；三環抗憂鬱劑

二、英文摘要

Ionic liquids (also called molten salt) are a class of non-molecular ionic solvents with low melting point. Due to their special physical and chemical properties, ionic liquids have been used as an alternative solvent in liquid-liquid extraction, stationary phases in gas chromatographic column, and as additives in HPLC mobile phase and CE eluents. In this research, we investigate the use of 1-ethyl-3-methylimidazolium tetrafluoroborate as a CE additive for the simultaneous separation of nine tricyclic antidepressants. Results indicated that the nine tricyclic antidepressants can be completely separated using a CE eluent containing 50 mM 1-ethyl-3-methyl-imidazolium tetrafluoroborate. Effects of IL concentration, buffer pH, ionic strength and types of IL on the resolution of the nine TCAs were examined in detail.

Keywords：Ionic liquid; capillary electrophoresis; tricyclic antidepressants

三、緣由與目的

Tricyclic antidepressants (TCAs) are mainly used for the treatment of depression. TCAs work by blocking serotonin and norepinephrine levels in the nervous system, essentially allowing the flow of over-firing nerve impulses, which increase anxiety, to return to normal levels. Because TCAs have many potentially serious side effects, such as increased heart rate, decreased blood pressure, drowsiness, etc., they are also frequently encountered in emergency toxicological screening, drug abuse testing, and forensic medical examinations. Analysis of TCAs in human fluids is, therefore, very important for pharmaceutical, toxicological, and forensic purposes.

Several techniques have been employed to determine the TCAs in biological samples and

pharmaceuticals, including spectrophotometry [1], spectrofluorimetry [2], radioimmunoassay (RIA) [3], gas chromatography (GC) [4], and high performance liquid chromatography (HPLC) [5,6]. Recently, capillary electrophoresis (CE) also has become a popular technique in the determination of TCAs [7-18] because of its high efficiency and versatility, very low samples and reagents consumption, low cost and minimization of the environmental pollution. However, separation of TCAs by CE is difficult due to their close mass values, chemical structure and pK_a values. Moreover, as basic drugs, TCAs are positively charged at the normal running pH of CE ($\leqq$10). Interactions between TCAs and negatively charged capillary wall often give rise to a peak broadening and lack of selectivity and efficiency. For optimal separation and efficiency, various additives, e.g. β-cyclodextrin (β-CD) [7], carboxymethyl-β-CD [8], *N*,*N*,*N*',*N*'-tetramethyl-1,3- butanediamine [9], *S*-(-)-2-hydroxymethyl-1,1'-dimethylpyrrolidinium tetrafluoroborate [10], poly-sodium 10-undecen-1-ol sulfate [11] were included in the CE background electrolyte (BGE) for careful manipulation of the electroosmotic flow (EOF) and the electrophoretic mobilities. Nonaqueous CE [12-18] also has been commonly employed to improve the resolution of TCAs.

Ionic liquids (ILs) are those compounds composed of organic cations and inorganic or organic anions which are liquids at room temperature or whose melting points are slightly higher than ambient temperature. Interests in ILs for their potential uses in different chemical processes are increasing, because they are environmentally benign, nonvolatile and nonflammable with a high thermal stability and are good solvents for a wide range of both organic and inorganic materials. Recently, ILs have been employed as additive, BGE or coating material in aqueous CE for the separation of polyphenols [19], metal ions [20,21], DNA [22], basic proteins [23], chlorophenoxy acid herbicides [24], carboxylates [25] and flavonoids [26]. ILs were also employed as electrolytes in nonaqueous CE for the separation of water-insoluble dyes [27], phenols and aromatic acids [28,29].

The purpose of this study was to investigate the use of 1-ethyl-3- methyl limidazolium-based ILs as CE additives for the simultaneous separation of TCAs. Nine TCAs, as shown in Table 1, were selected as the test analytes. Effects of IL concentration, buffer pH, ionic strength and types of IL on the resolution of the nine TCAs were examined in detail. To our knowledge, using ILs as buffer additives to enhance the CE separation of TCAs has not been reported yet.

四、結果與討論

CE separation of nine TCAs was initially attempted using a background electrolyte (BGE) of 50 mM phosphate buffer at pH 3.0 without adding ionic liquid. As shown in Fig. 1, complete separation of nine TCAs was not achieved. Attempts to enhance the resolution by just varying the pH and concentration of the phosphate buffer were also unsuccessful. The ionic liquid, 1-ethyl-3-methylimidazolium tetrafloroborate (1E-3MI-TFB), was then included in the BGE as an additive.

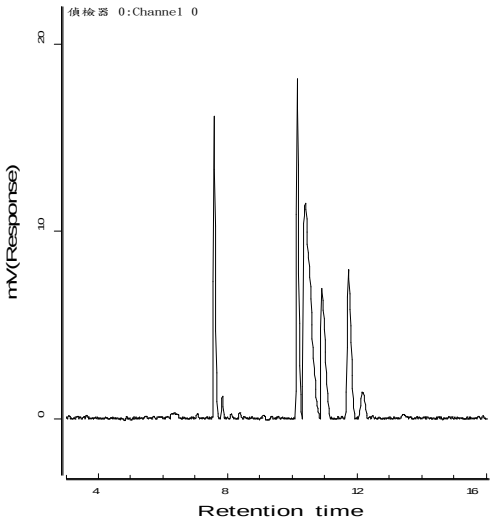

Fig. 1. Electropherogram of 9 tricyclic antidepressants using a background electrolyte without the addition of ionic liquids.

Because TCAs are basic compounds, their fraction of ionization as well as the electrophoretic mobilities will change with the pH of BGE. pH also affects the mobility of electroosmotic flow (μ_{EOF}) in the capillary. Therefore, migration time of TCAs in the capillary will be determined by the joint effects of the above factors. The effect of pH on the separation of nine TCAs was examined using electrophoretic BGEs containing 50 mM 1E-3MI-TFB and 50 mM phosphate buffer at various pH. The results are shown in Fig. 2.

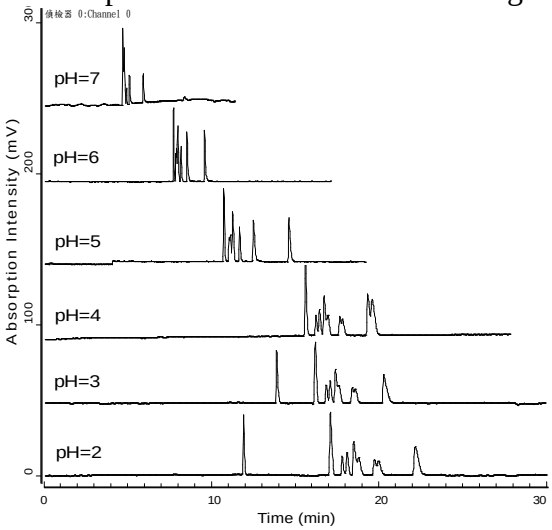

Fig. 2. Electrophoregrams of nine TCAs at different pH. BGE: 50 mM 1E-3MI-TFB and 50 mM phosphate buffer.

The analysis time (migration time of the last peak) decreased from 21 min at pH 2 to ca. 6 min at pH 7. Within the pH range studied (2 – 7), TCAs were mainly protonated because the pK_a values of all TCAs were between 7.21 and 10.2 (Table 1). In the presence of 50 mM 1E-3MI cation, μ_{EOF} was negative (toward anode). At lower pH, the mobilities of the analytes were high due to the high mole fraction of the protonated portion of TCAs, which migrated against EOF under an applied negative potential. At higher pH, the basic analytes were less protonated, and their electrophoretic mobilities were lower. Therefore, the apparent mobilities of the analytes were dominated by the anodic EOF and the analysis time was shortened. In Fig. 2, complete separation of 9 TCAs was found difficult in the presence of phosphate buffer. Further study was carried out with BGEs containing only 1E-3MI ionic liquid without phosphate buffer.

It is known that IL cations in the aqueous solution will adsorb onto the internal capillary wall, causing changes in EOF. Using dimethylsulfoxide (DMSO) as a neutral marker, the mobility of electroosmotic flow (μ_{EOF}) was found negative (toward anode) with 1E-3MI-TFB as BGE in the concentration range 25 - 120 mM. The results are illustrated in Fig. 3.

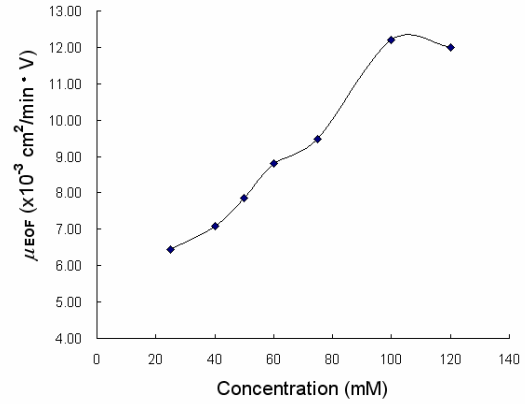

Fig. 3. Variation of electroosmotic flow mobilities with the concentration of ionic liquid.

It can be seen that μ_{EOF} increased steadily with increasing concentration of 1E-3MI-TFB from 25 to 100 mM. Above 100 mM, the μ_{EOF} became stable, which indicates saturation of the capillary wall with adsorbed 1E-3MI cations. As mentioned by Yu et al. [24], equilibrium of IL cation adsorbed onto the capillary wall was not instantaneous; the μ_{EOF} increased slowly from run to run and reached a relatively stable value after about 30 min. The μ_{EOF} in Fig. 3were measured after 30-min electrophoresis using the fresh electrophoretic BGE solution.

When using 1E-3MI-TFB as the sole electrolyte, the solution was acidic (pH ~ 3). According to the pK_a values listed in Table 1, all TCAs are protonated at pH 3 as cations. A positive high voltage was found necessary to effectively separate the nine TCAs because the electrophoretic mobilities of TCAs are larger than the μ_{EOF} at all concentrations of

1E-3MI-TFB studied. The results are illustrated in Fig. 4.

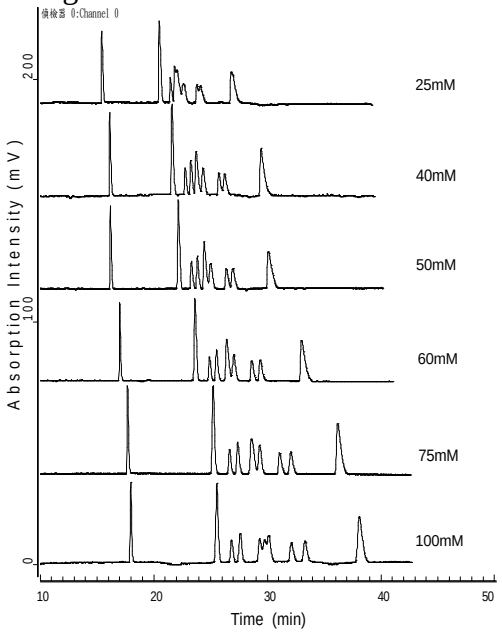

Fig. 4. Electropherograms of 9 tricyclic antidepressants using sole 1E-3MI-TFB at various concentrations.

The migration times of all TCAs increase with increasing IL concentration. The optimal resolution was observed at 50 mM 1E-3MI-TFB. Nine TCAs can be completely separated in about 30 min.

五、計畫成果自評

本研究成功地利用 50 mM 1-ethyl-3-methylimidazolium tetrafluoroborate 離子液體當作電泳移動相溶液，可完全分離九種三環抗憂鬱劑化合物。這些鹼性藥物分子的電泳分離機制主要取決於吸附在毛細管壁上的 1-ethyl-3-methylimidazolium 陽離子的濃度高低，及其所影響的負向電滲流大小。與文獻上發表的其他調整電滲流方式，本研究所發展的方法不但較簡單且效率較高。目前正在將本研究結果撰寫為學術論文形式，計畫投稿於 J. Chromatogr. A 期刊發表。

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