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Sample stacking for determination of aromatic acid impurities by microemulsion electrokinetic chromatography

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ABSTRACT

In this study, a sample stacking step coupled with microemulsion electrokinetic chromatography (MEEKC) was used to detect and analyze nine aromatic acids (benzoic acid (BA), isophthalic acid (IPA), terephthalic acid (TPA), *p*-toluic acid (*p*-TA), 4-carboxylbenzaldehyde (4-CBA), trimesic acid (TSA), trimellitic acid (TMA), *o*-phthalic acid (OPA), and hemimellitic acid (HMA)) which are common impurities produced during aromatic acid synthesis. First, the presence of both acid and water plugs at the front of the capillary improved the reproducibility in retention time and peak intensity of the tested analytes in the stacking method. Second, the pH and the electrolyte type of acidic plug and sample matrix were found to be the predominant influences on the aromatic acid stacking. The detection limits of these aromatic acids were reduced to the range of 0.00007–0.00032 $\mu g\,mL^{-1}$ by this optimal sample stacking step. This proposed on-line concentration MEEKC method was able to detect trace levels of aromatic acid impurities in commercial aromatic acid products that were not previously possible by the normal MEEKC method. Furthermore, these results in comparison with our previous studies on sample stacking MEEKC method indicated that all acidic species were concentrated by this simple stacking procedure. The sensitivity enhancement, however, was highly dependent on the types of functional groups present in the structures of analytes, and the enhancement was in the order of first the compounds carrying both carboxy and hydroxy groups (e.g. phenolic acid), followed by carboxylic acid compounds (e.g. aromatic acid), and then phenol compounds (e.g. polyphenol).

1. Introduction

Aromatic acids are useful chemical compounds and synthetic precursors widely used in various industries [1]. For example, benzoic acid (BA) has been used extensively as food additive to prevent alteration and degradation by microorganisms during food storage [2], while terephthalic acid (TPA) is an intermediate material essential for the manufacturing of polyester based plastics and fibers [3]. These aromatic acid syntheses in industry are often an oxidation process, and some by-products are also formed during preparation. For example, commercial processes for the manufacturing of TPA are based on the reaction of *p*-xylene and dioxygen in acetic acid, and some aromatic acid impurities and by-products that arise from the incomplete oxidation of *p*-xylene (for example, *p*-toluic acid (*p*-TA) and 4-carboxybenzaldehyde (4-CBA)) or the impurities present in the *p*-xylene feed stream (for example, isophthalic acid (IPA), *o*-phthalic acid (OPA), trimellitic acid (TMA), hemimellitic acid (HMA) and trimesic acid (TSA)) are also formed [4]. When TPA which contains a small amount of 4-CBA or *p*-TA reacts with ethylene glycol, it results in the production of an unacceptable yellow polyethylene terephthalate (PET) polymer or in a decrease in the polymerization degree of PET polymer [5,6]. Other impurities as mentioned above also likely cause the tubing of TPA plant to be clogged or the decrease in TPA yield [7]. Even though most impurities present in TPA may be reduced to a given concentration through time-consuming purification process such as hydrogenation, water washing, crystallizing and drying steps commonly used in TPA industry (for example, <20 $\mu g\,g^{-1}$ for 4-CBA, <10 $\mu g\,g^{-1}$ for BA and IPA, 20–40 $\mu g\,g^{-1}$ for TMA, and 80–140 $\mu g\,g^{-1}$ for *p*-TA), the trace impurities even at $\mu g\,g^{-1}$ still limit their applications [7–9]. Therefore, the detection and measurement of trace-level aromatic acid impurities in commercial or as-synthesized aromatic acid products are relatively important for quality issue reasons [5,7,8]. Since the expected levels of organic impurities in highly purified TPA range from 10 to 140 $\mu g\,g^{-1}$, and the low solubility of TPA in water, thus these impurities are often diluted by a factor of 100 during sample preparation. As a result, an analytical method has to provide the limit of detection (LOD) at or below 0.10–1.4 $\mu g\,mL^{-1}$ when it is used for the analyses of organic impurities in industrial samples, otherwise an extraction procedure for

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impurities preconcentration has to be employed prior to analysis [9,10].

Presently, capillary electrophoresis (CE) is employed as the major separation tool especially in TPA industry for the analysis of aromatic acids with reliable results [11–15]. Most reports on the usage of CE coupled with UV detection, however, had indicated poor detection sensitivities for the determination of aromatic acid (LODs were around sub-ppm [10,11]) if sample preconcentration method was not employed. Therefore, in order to improve the detection ability of aromatic acid, a suitable on-line sample concentration step prior to CE separation is necessary.

Many on-line sample concentration techniques, including sample stacking and sweeping, had been successfully developed to improve the detection ability of micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) methods [16–20]. Even though both MEKC and MEEKC have been regarded to be good separation techniques, however, inadequate separation resolutions for the aromatic acid analytes studied in this paper were achieved by MEKC method whereas MEEKC provided baseline separations for the aromatic acids according to our experimental results. For example, the best MEKC separation was obtained for the nine tested aromatic acids when a pH 2 phosphate solution (50 mM) composed of 40 mM SDS was used as running solution, but three aromatic acids, TPA, *p*-TA and 4-CBA, still had similar migration velocities (their resolutions were smaller than 0.5). So far, there had been some reports on the use of on-line sample concentration techniques in combination with MEKC or MEEKC for the analyses of acidic compounds [21–24], but no studies employing on-line concentration MEEKC analyses of the type of aromatic acid impurities mentioned in this study have been reported in the literature to date. Several studies on on-line concentration procedure coupled to MEEKC separation had been reported by our group in recent years [23–25], and these results indicated that sample stacking provided relatively high detection sensitivity for acid species such as phenolic acid. There was, however, little improvement for basic (or cation) species because they were likely to be partially adsorbed on the surface of capillary inner wall during the sample introduction (for example the sensitivity enhancement factor (SER) increased by 96 000–238 000-fold for phenolic acid compounds [23], and 180–540-fold for tobacco alkaloid cations [25]). This seemed to indicate that the sample stacking method was not suitable for basic species, but it was very efficient in improving the detection abilities for all acid species. During the development of the on-line concentration MEEKC method for polyphenol compounds which are another type of acid species, however, only a 120–715-fold increase in SER was achieved [24]. These polyphenols have five to eight hydroxy groups bonded to the benzene rings in the structure, whereas phenolic acids possess both hydroxy and carboxy moieties in the benzene rings, and this results in lower pK_a and higher aqueous solubilities obtained for the phenolic acids. The pK_a of phenolic acids and polyphenol are approximately 4.3 and 8.4, respectively. The aqueous solubilities are about 0.018–0.066 mol L^{-1} for phenolic acids, and 0.0032–0.029 mol L^{-1} for polyphenols. All of the tested polyphenols and phenolic acids have hydroxy groups, but in the case of phenolic acids, the presence of an extra carboxy moiety seemed to greatly improve the concentration effect of sample stacking [23,24], and it led us to conclude that the existence of carboxyl group in the structure of an analyte likely plays a key role in determining its sensitivity enhancement.

In this study, an on-line concentration method which consists of a sample stacking step and MEEKC separation was used to detect nine aromatic acids (BA, 4-CBA, TPA, *p*-TA, IPA, OPA, TSA, TMA and HMA), which are commonly found in the oxidation processes of aromatic acid synthesis. These aromatic acids possess carboxy moieties (one to three carboxy groups) but they do not have any hydroxy groups in their structures; and they have low pK_a (pK_{a1} and pK_{a2} are in the range of 2.1–5.4) and a wide range solubilities (0.0097–0.17 mol L^{-1}), thus they were suitable as model compounds for the examination of whether the existence and the numbers of carboxy group in analyte's structure affect the sensitivity enhancement. Effects of acid plug and sample matrix (solution pH, type and concentration of electrolyte) were examined in order to optimize the on-line concentration method of aromatic acids. Finally, the on-line concentration MEEKC was applied to analyze trace-level impurities in highly pure commercial aromatic acid products and crude terephthalic acid (CTA) products.

2. Experimental

2.1. Chemicals and reagents

The chemical structures, pK_a and solubilities of the aromatic acids studied in this paper are shown in Fig. 1. BA, IPA, TPA, *p*-TA, 4-CBA, TSA, TMA, and OPA were purchased from Merck (Darmstadt, Germany). HMA was obtained from Acros (Morris Plains, NJ, USA). Theses aromatic acid standards were individually dissolved in methanol or aqueous solution of 1N NaOH at a stock concentration of 100 μg mL^{-1}. All other chemicals were reagent-grade.

2.2. Real samples and pretreatment

Several aromatic acid chemicals of analytical-grade (≥98% in purity) and CTA samples were used as test samples in the study. The CTA samples, which are in the form of dried TPA crystals obtained by the reaction of *p*-xylene and dioxygen in acetic acid and without further purification by hydrogenation [7], were kindly provided by a PTA (purified terephthalic acid) manufacturer in Taiwan. These CTA or aromatic acid solids of 1.0 g were dissolved in 20 mL of 1N NaOH solution, and then filtered through a 0.2 μm membrane without other treatment. All filtered liquids were diluted with a borate-boric acid buffer of pH 8 (16.5 mM) in a ratio of 1:999 or 1:1 for on-line concentration or conventional MEEKC, respectively, and were then directly analyzed by the appropriate MEEKC method.

2.3. Preparation of microemulsion solution for MEEKC

All microemulsions were prepared on a w/v basis in 50 mM phosphate buffer of pH 2.0 (the buffers were measured by volume). The 50 mM phosphate solution of pH 2.0 was prepared by adding 0.49 g phosphoric acid into 100 mL of deionized water. After the addition of various ratios of surfactant (sodium dodecyl sulfate (SDS), 3.75%), cosurfactant (cyclohexanol, 5.0%), and oil (*n*-octane, 0.975%) to phosphate solution of pH 2.0 (50 mM, 90.3%), the mixture was then sonicated for 30 min until homogeneous. The microemulsion solution was not filtered prior to use.

2.4. Apparatus and operating conditions for CE

All experiments were performed with a Beckman Coulter MDQ capillary electrophoresis system equipped with a photo diode-array detector (Fullerton, CA, USA). Beckman Coulter MDQ 32 Karat software was used for instrumental control and data analysis. Separations were performed in a 50.2 or 60.2 cm total length (40 or 50 cm to detector) of 50 μm i.d. uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). The capillaries were conditioned prior to separation by washing with deionized water (5 min) then with running buffer (5 min). Sample and standard solutions were pressure-injected into the capillary column for normal MEEKC (0.5 psi, 5 s), and were electrokinetically injected into