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Key Conditions in Capillary Electrophoresis of Amino Acids with Indirect Ultraviolet Detection

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Abstract: Key factors for capillary electrophoresis of amino acids with indirect ultraviolet detection were investigated. Buffer compositions and additives were found to be influential as expected, while buffer pH was shown to be particularly critical to the separation and detection. The effect of pH also depended on the electrophoretic mode used. Ways to obtain recurring and selective separations were suggested.

Keywords: Amino acids, capillary electrophoresis, indirect ultraviolet detection.

Indirect ultraviolet (UV) detection is becoming attractive in capillary electrophoresis (CE) of amino acids due to the avoidance of time-consuming derivatization, and ease to be developed as a universal detection mode[1-6]. However, there are still problems in practical applications. The most serious one is the irreproducible separation often resulted. We have hence started a project trying to reveal the reasons through a systematic optimization of the running conditions. In this paper, some crucial factors will be shown and discussed.

Experimental

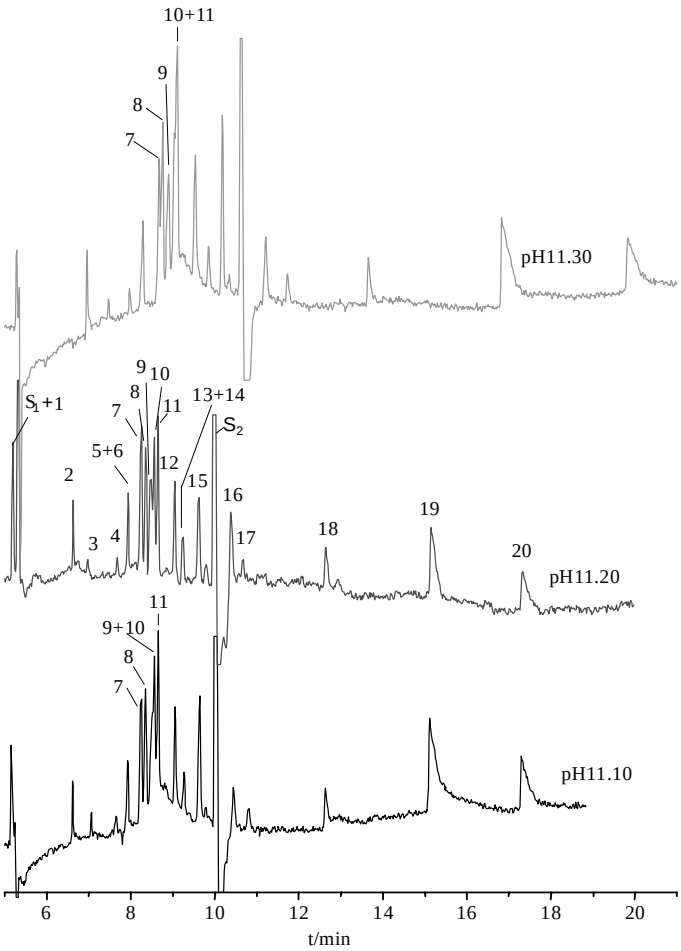
CE was performed using Beckman P/ACE model 2050 with UV detector of model 165 (Fullerton, CA, USA). A fused silica capillary of 50μmID×67cm(60cm effective) from Yongnian factory of photoconductive fibers (Hebei Province, China) was mounted. The samples used for both aqueous and nonaqueous CE were prepared by dissolving the twenty common amino acids (from Sigma, St. Louis, MO, USA) in water separately. The final concentration of the samples for injection was 0.5mmol/L for each solute. All other related reagents were of analytical reagent grade from Beijing Chemical Works (Beijing, China). Water used was doubly distilled.

Results and Discussion

Selection of background electrolyte (BGE) is commonly considered as the most important factor or the prerequisite in indirect detection. We hence examined a series of

UV absorbers, in which *p*-aminophenylsulfuric acid (APSA) and N,N-dimethylbenzylamine (DMBA) , were found to be the most effective in basic aqueous and acidic methanol media respectively. Although their selectivity and sensitivity may differ from each other, they did not show much difference in improving the reproducibility. We then turned our attention to other running conditions, including buffer electrolytes, solvents, pH and additives.

Figure 1. Effect of buffer pH on amino acids separation with indirect UV detection



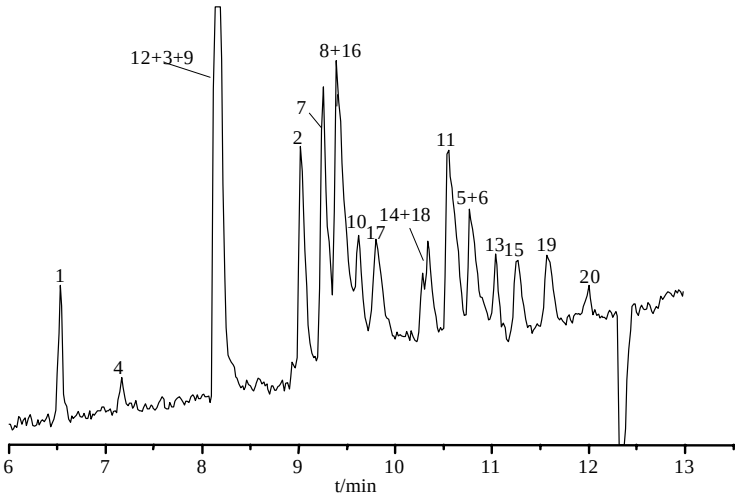
Voltage: 15kV; Indirect detection wavelength: 254nm; Temperature: 25°C; Buffer: 10mmol/L APSA, 3mmol/L borax.
1: Arg, 2: Lys, 3: Pro, 4: Try, 5: Leu, 6: Ile, 7: Phe, 8: Val, 9: His, 10: Met, 11: Gln, 12: Ala, 13: Thr, 14: Asn, 15: Ser, 16: Gly, 17: Tyr, 18: Cys, 19: Glu, 20: Asp, S: system peaks. Peaks were labeled in this way throughout the figures.

Unexpectedly, buffer pH was shown to have striking influence on the

reproducibility. When APSA and borate were used as running buffer, no discernible peaks could be detected at pH<10.80 or >11.80. The even unexpected result was obtained at near the optimal pH (11.20): A minute pH variation caused a great change of the separation as shown in **Figure 1**. At pH11.20±0.02, nearly all the amino acids were well separated except that Leu/Ile and Thr/Asn coeluted, but when pH changed to 11.30 or 11.10, the resolution between peak 7~11 greatly deteriorated. This phenomenon has also been observed in CE of amino acids with direct LIF detection mode[7]. The reasons are not yet clear, but we can conduce that the top condition in CE of amino acids is to well control the buffer pH. Borate is hence suggested to be added to the BGE solution. Otherwise, the detection may not be possible. Besides, capillary cleaning procedure and buffer renewing should be taken into consideration. We suggest renewing the running buffer before each separation. A very effective tube cleaning program is as follows: washing the columns first with 0.1mol/L HNO_3 for 5min, then with 0.1mol/L NaOH, water, and running buffer for 5min each before any injections.

As compared to aqueous CE, nonaqueous CE is not so sensitive to buffer pH. Although the separation and detection could only be achieved in buffers containing an amount of hydrochloric acid between 8mmol/L and 13mmol/L with DMBA as BGE, change of ±0.2mmol/L hydrochloric acid was allowed. This experiment also demonstrated that buffer solvents could be an important factor to the separation performance. **Figure 2** shows that the separation selectivity and migration order were different in methanol medium than in water, which was caused not only by different BGE used, but also by the changes in solvation of amino acids. Furthermore, because of the reductions of hydrophobic and electrostatic interactions between solutes and capillary wall, the peak shape could be greatly improved.

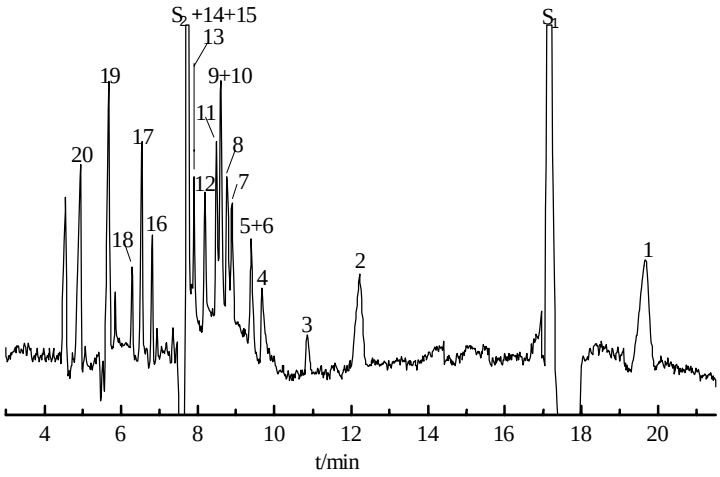
Figure 2. The nonaqueous electropherogram of amino acids with indirect UV detection



Voltage: 25kV; Indirect detection wavelength: 214nm; Temperature: 25°C; Buffer 10mmol/L DMBA, 10mmol/L HCl in methanol.

Buffer additive is another factor to regulate the peak shape and elution order. Lots of chemicals can be considered, for example, metal ions and various surfactants, of which cationic detergent like cetyltrimethylammonium bromide (CTAB) could yield symmetric peaks and reverse the elution order by changing the direction of electroosmotic flow (**Figure 3**). Nevertheless, few additives were found to improve the separation in nonaqueous CE. The reasons remain unknown at this moment.

Figure 3. Effect of CTAB on amino acids separation with indirect UV detection



Voltage: -15kV; Indirect detection wavelength: 254nm; Temperature: 25°C; Buffer: 10mmol/L APSA, 3mmol/L borax, 0.05mmol/L CTAB, pH 11.20.

Acknowledgment

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