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Short communication

Capillary zone electrophoretic separation of biogenic amines: influence of organic modifier

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Abstract

The influence of organic modifier on the separation of six biogenic amines in capillary zone electrophoresis was investigated using ammonium acetate as a background electrolyte. The electrophoretic mobility of each biogenic amine at a given pH decreases to a different extent on addition of organic modifier which may lead to alteration of the migration order. The results indicate that addition of acetonitrile (30%, v/v) or methanol (40%, v/v) to ammonium acetate buffer (100 m*M*) at pH 7.5 effects the resolution of co-migrating biogenic amines. Complete separation of six biogenic amines was achieved in short times using a 44 cm×50 μm I.D. fused-silica capillary at 10 kV. Methanol is relatively less effective than acetonitrile as an organic modifier to separate these biogenic amines.

Keywords: Organic modifiers; Histamine; Serotonin; Tryptamine; Tyramine; 2-Phenylethylamine; Benzylamine; Biogenic amines

1. Introduction

Biogenic amines are physiologically active organic bases in metabolic processes of animals, plants and microorganisms. They are widely present in foodstuffs and animal feed. In particular, histamine, 2-phenylethylamine, tryptamine and tyramine were reported to be responsible for diseases with symptoms similar to intoxication [1,2], whereas histamine and tyramine were considered as indicators of food poisoning or food spoilage [3]. As the concentration of biogenic amines increases as a result of spoilage of foodstuffs and animal feed, monitoring the contents of these decomposition products is commonly required.

Various methods, including gas chromatography [2], high-performance liquid chromatography (HPLC) [4–9] and mass spectrometry [10] have been developed to analyze biogenic amines in body tissues and foodstuffs. Capillary electrophoresis (CE) has become a popular and powerful separation technique that possesses many advantageous features, such as high resolution, extremely high efficiency, rapid analysis and small consumption of sample and solvent. Thus the development of a new and sensitive analytical method using capillary zone electrophoresis (CZE) to separate biogenic amines is desirable. Since reports regarding the separation of

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biogenic amines in CZE are still lacking, we present here the results of our investigation. In this work, the influence of organic modifier on the separation of these biogenic amines is emphasized.

2. Experimental

2.1. Chemicals and reagents

Six biogenic amines (Sigma, USA), as shown in Fig. 1, and ammonium acetate (Aldrich, USA) were obtained from the indicated suppliers. Methanol (MeOH) and acetonitrile (ACN) were of HPLC grade (Mallinckrodt, USA) and were used without further purification. All other chemicals were of analytical-reagent grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Standard solutions of biogenic amines were prepared at a concentration of about 20 ppm in methanolic solution. The pH of the buffer solution was adjusted with ammonia *aqua* to a desired value. All solutions were filtered through a membrane filter (0.22-μm) before use.

2.2. Apparatus and electrophoretic procedures

Separations were carried out on a capillary electrophoresis system (Spectra-Physics Model 1000, Fremont, CA, USA), as described previously [11].

All experiments were performed using a fused-silica capillary with ammonium acetate buffer systems suitable for the desired pH at 25°C and measurements were run at least in triplicate to ensure reproducibility. An applied voltage of 10 kV was selected and the total current was kept below 100 μA in order to avoid Joule heating. Sample injections were made in the hydrodynamic mode. The sample solution was typically injected for 1 s. All measurements were monitored at 215 nm.

When a new capillary was used, the capillary was washed for 2 h with 1.0 *M* NaOH at 60°C, followed by 0.5 h with deionized water at 25°C. The capillary was prewashed for 3 min with running buffer before each injection and postwashed for 2 min with deionized water, 3 min with NaOH (0.1 *M*) and 2 min with deionized water to maintain proper reproducibility of run-to-run injections.

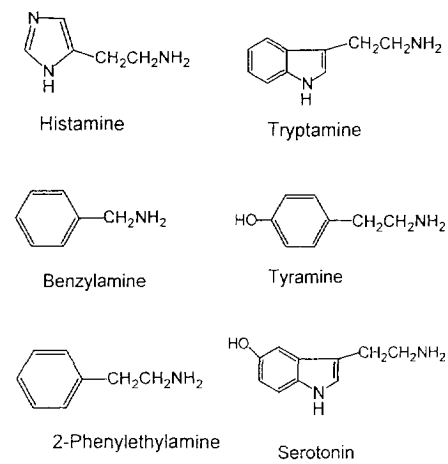

Fig. 1. Structures of biogenic amines.

For peak identification, on-column UV spectra (200–300 nm with a 2-nm wavelength increment) of biogenic amines were recorded simultaneously during the electrophoretic separation. A three dimensional spectral scan of CE separation of a mixture of biogenic amines, as shown in Fig. 2, is presented to show the characteristics of UV absorption spectra of these analytes. The optimum detection wavelength was set at 200 nm.

2.3. Mobility calculation

The electrophoretic mobility of analytes was calculated from the observed migration time as described [11].

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_t L_d}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right)$$

in which μ_{ep} is the electrophoretic mobility of the solute tested, μ the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for an uncharged solute (methanol as neutral marker), L_t is the total length of capillary, L_d is the length of capillary between injection and detection and V is the applied voltage.

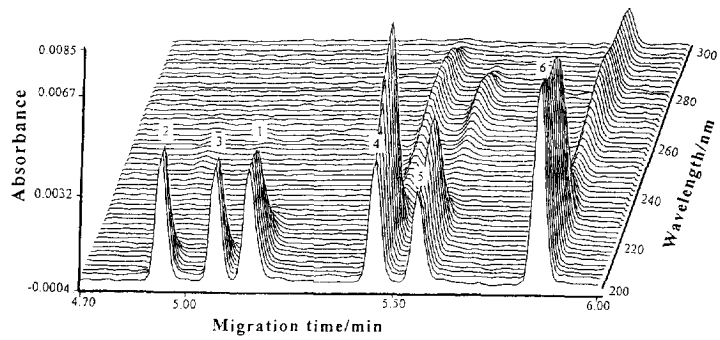

Fig. 2. Three dimensional spectral scan of CE separation of a mixture of six biogenic amines. Buffer: ammonium acetate (100 m*M*) with acetonitrile (30%, v/v) at pH 7.5. Operating conditions: 10 kV, 25°C. Capillary: 44 cm×50 μm. I.D. Peak identification: 1 = histamine, 2 = benzylamine, 3 = 2-phenylethylamine, 4 = tryptamine, 5 = tyramine and 6 = serotonin.

3. Results and discussion

3.1. Separation with buffer containing no organic modifier

With ammonium acetate (100 m*M*) as a background electrolyte and with an applied voltage of 10 kV, tryptamine and tyramine were not resolvable in the pH range 7.0–7.5; histamine and benzylamine co-migrated at pH 7.5 and were only partially resolvable at a pH in the range 7.5–8.5. Thus, complete separation of all six biogenic amines was not achievable in the pH range 7.0–8.5 with background electrolyte containing no organic modifier. At pH 7.5, the migration order follows histamine = benzylamine < 2-phenylethylamine < tryptamine = tyramine < serotonin.

3.2. Separation with buffer containing organic modifier

The addition of organic modifiers to the buffer electrolyte can serve to control electroosmotic flow [12–21]; to improve separation and resolution [13–17,22]; to enhance analyte solubility [12]; and to broaden the applicability of CZE to a wide range of non-ionic compounds [19,20,23].

As illustrated in Fig. 2, addition of acetonitrile (30%, v/v) to the buffer electrolyte effected the resolution of co-migrating analytes of biogenic amines. Complete separation of biogenic amines was achieved within 6 min. The resolution of tryptamine and tyramine was greatly enhanced, because the decrease in the electrophoretic mobility of tyramine was considerably greater than that of tryptamine. A similar situation also occurred for benzylamine and histamine. The extent of the decrease in the electrophoretic mobility of histamine was comparatively greater than those of benzylamine and 2-phenylethylamine when acetonitrile was added to the buffer. Consequently, the reversal of the migration order between histamine and benzylamine or even histamine and 2-phenylethylamine might occurred. Table 1 lists the mobility data of biogenic amines obtained with ammonium acetate buffer (100 m*M*) containing acetonitrile as an organic modifier at pH 7.5. Generally, a decreased electrophoretic mobility is accompanied by an increased proportion of acetonitrile. Depending on the nature of each individual

Table 1
Electrophoretic mobilities of biogenic amines obtained with ammonioum acetate buffer (100 m*M*) containing as organic modifier at pH 7.5

Analyte	Electrophoretic mobility				
	Acetonitrile			Methanol	
	0%	20%	30%	20%	30%
Histamine	2.95	2.63	2.52	2.21	1.87
Benzylamine	2.95	2.78	2.76	2.32	2.04
2-Phenylethylamine	2.76	2.63	2.62	2.19	1.92
Tryptamine	2.43	2.27	2.22	1.89	1.64
Tyramine	2.43	2.18	2.12	1.84	1.57
Serotonin	2.19	1.94	1.86	1.64	1.38

Mobility in unit of $10^{-4}cm^2V^{-1}s^{-1}$.

biogenic amine, the decrease in the electrophoretic mobility of each analyte was found to vary to a different extent when an organic modifier was added to the buffer electrolyte.

On addition of methanol (40%, v/v) to the buffer electrolyte at pH 7.5, effective separation of all six biogenic amines could be achieved within 11 min. Fig. 3 presents such electropherogram of biogenic amines obtained. The mobility data of biogenic amines obtained with ammonium acetate buffer (100 m*M*) containing methanol at pH 7.5 are also listed in Table 1. The electrophoretic mobility of biogenic amines decreases more substantially with the addition of methanol than with acetonitrile. However, the overall resolution of biogenic amines was better with acetonitrile than with methanol at the same concentration. Therefore, a greater proportion of methanol is necessary to be added to the buffer solution than that of acetonitrile for an effective separation.

3.3. Effect of buffer concentration

In addition to buffer pH and organic modifier, buffer concentration is also an important separation parameter. Fig. 4 presents the electropherogram of biogenic amines obtained at varied buffer concentrations from 12.5 m*M* to 100 m*M*. The results clearly indicate that, in order to effectively separate these biogenic amines at pH 7.5, the concentration of ammonium acetate buffer containing acetonitrile (30%, v/v) or methanol (40%, v/v) should be greater than 50 m*M*. Preferred buffer concentrations lie in the range 75~100 m*M*.

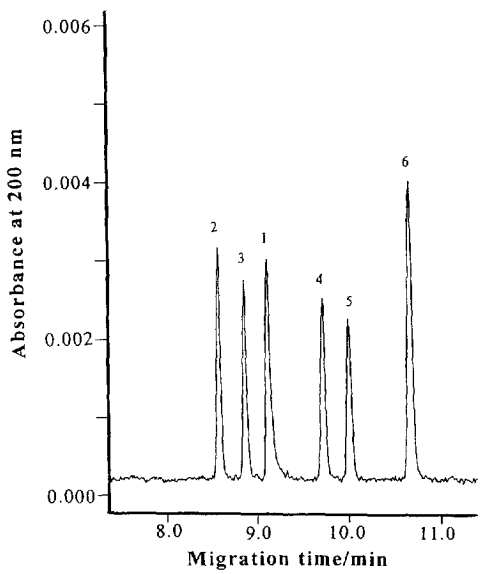

Fig. 3. Electropherograms of biogenic amines with addition of methanol (40%, v/v) in ammonium acetate (100 m*M*) at pH 7.5. Peak identification and operating conditions are the same as for Fig. 2.

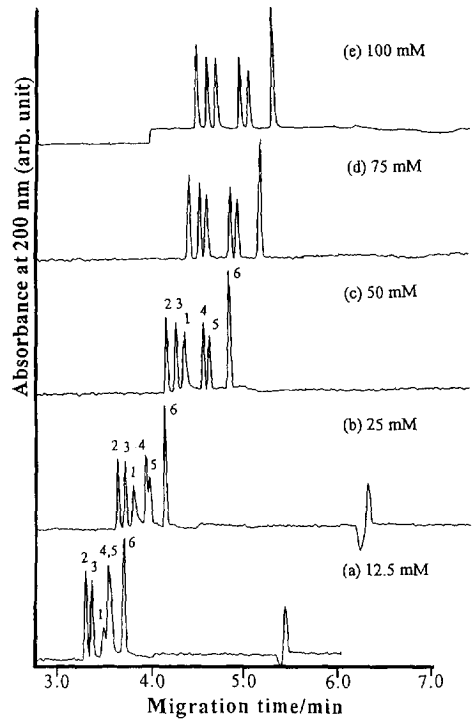

Fig. 4. Electropherogram of biogenic amines obtained at varied concentrations of ammonium acetate buffer: (a) 12.5 m*M*, (b) 25 m*M*, (c) 50 m*M*, (d) 75 m*M* and (e) 100 m*M*. Peak identification and operating conditions are the same as for Fig. 2.

4. Conclusion

Addition of acetonitrile or methanol to ammonium acetate buffer is essential to effect the resolution of biogenic amines. These amines were efficiently separated by CZE with ammonium acetate buffer (100 m*M*) containing acetonitrile (30%, v/v) or methanol (40%, v/v) at pH 7.5 with an applied voltage of 10 kV.

Acknowledgments

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