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Capillary zone electrophoretic separation of β-blockers using citrate buffer at low pH

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Abstract

Ten β-blockers were simultaneously separated by capillary zone electrophoresis using citrate buffer as a background electrolyte at low pH. The effects of buffer concentration and buffer pH on the migration behavior and separation of β-blockers were systematically investigated. The results indicate that the resolution of co-migrating analytes improves with increasing buffer concentration at a low pH and that the optimum pH of the buffer decreases with increasing buffer concentration. Complete separation of the β-blockers tested was achieved within a relatively short time with an appropriate concentration of citrate buffer in the range 160–400 m*M* and a proper pH in the range 3.0–1.8 using a 43 cm×50 μm I.D. fused-silica capillary at 15 kV.

Keywords: Background electrolyte composition; Pharmaceutical analysis; β-Blockers; Citrate

1. Introduction

β-Adrenergic blocking agents (β-blockers) are clinically used in the treatment of angina pectoris, cardiac arrhythmia, hypertension, anxiety attacks, thyrotoxicosis, migraine and glaucoma [1]. They are also used as doping agents in sports [2]. Various methods including gas chromatography (GC) [4], GC–mass spectrometry (MS) [3,5], supercritical fluid chromatography (SFC) [6], high-performance liquid chromatography (HPLC) [7–10] and capillary electrophoresis (CE) [11–17] have been developed to determine and identify β-blockers. Among them, HPLC is the method most utilized over the past decades. However, CE has become a popular and powerful separation technique and has been widely applied to a variety of samples [18–22]. This is due to its many advantages, such as high resolution, extremely high efficiency, rapid analysis time, small sample volume and low solvent consumption in comparison to HPLC. Thus, the application of this technique to the separation and determination of β-blockers is of great interest.

β-Blockers possess two structural features; an alkanolamine side-chain terminating in a secondary amino group and an aromatic group. The former. with typical pK_a values in the region of 9.2–9.6 [23,24], gives them their basic characteristics and the latter provides lipophilic character. Since some β-blockers are hydrophilic and others lipophilic, their polarity variation is so wide that difficulties in simultaneously determining them may occasionally be encountered.

Micellar electrokinetic chromatography (MEKC),

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which uses a background electrolyte containing surfactant as a pseudo-stationary phase, is a useful technique suitable for the separation and determination of neutral molecules and charged compounds. This technique has been used widely for drug analyses in biological matrices [14,25–30]. Separation and determination of β-blockers by MEKC was developed by Lukkari et al. [11–15], using phosphate buffer (80 m*M*) containing 10–15 m*M* cetyltrimethylammonium bromide in the pH range 6.7–7.0. However, β-blockers were poorly resolved by capillary zone electrophoresis (CZE) using 50 m*M* phosphate buffer at pH 2.5 [16]. Thus, interest in the development of new analytical methods using a CZE technique to separate β-blockers continues unabated.

In this work, we present results of a systematic investigation into the way that concentration and pH of the buffer affects migration behavior and separation of β-blockers in CZE using citric acid as a background electrolyte at low pH. Optimized separation parameters were determined.

2. Experimental

2.1. Chemicals and reagents

Ten β-blockers were used in this study. The structures of these β-blockers are shown in Fig. 1. Among them, acebutolol hydrochloride, nadolol and (±)-metoprolol(+)-tartrate were purchased from Aldrich (USA); oxprenolol hydrochloride, pindolol, propranolol hydrochloride, timolol maleate, atenolol, labetalol hydrochloride and levobunolol were supplied by National Laboratories of Foods and Drugs, Department of Health, Taiwan. Citric acid (Shimakyu, Japan) and trisodium citrate dihydrate (Showa, Japan) were obtained from the indicated suppliers. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Standard solutions of a mixture of ten β-blockers in aqueous solution containing various concentrations of each individual β-blocker ranging from 0.025 m*M* for pindolol and propranolol to 0.30 m*M* for timolol were prepared. The pH of the buffer was adjusted to the desired pH by mixing various proportions of a certain concentration (80, 120, 160, 320, 360 and 400 m*M*) of trisodium citrate solution in the range 80–400 m*M* and the same concentration of citric acid solution. All solutions were filtered through a membrane filter (0.22 μm) before use.

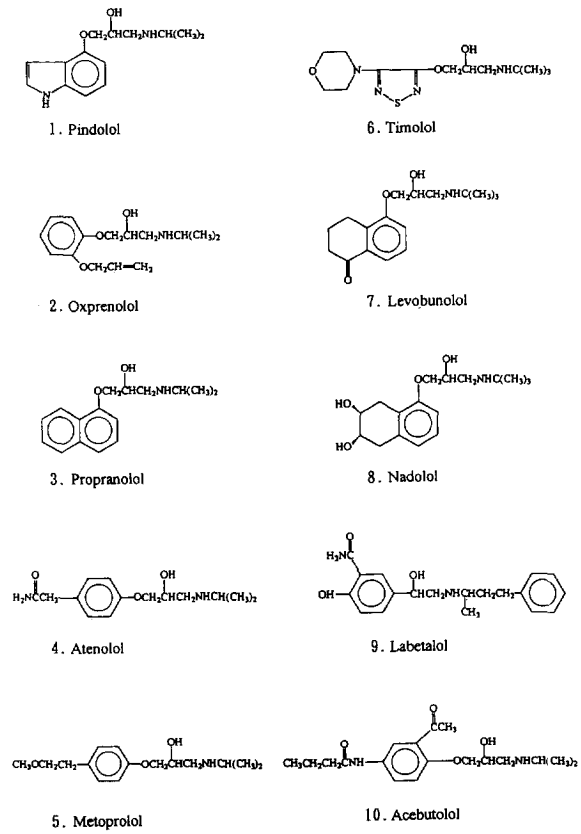

Fig. 1. Structures of the β-blockers studied.

2.2. Apparatus

Separations were made with a CE system (Spectra-Physics Model 1000, Fremont, CA, USA), equipped with a Spectra FOCUS UV–Vis detection system with a low inertia scanning (LIS) device, a fused-silica capillary cartridge thermostated with a Peltier thermoelectric device and an automatic injection system. The capillary dimensions were 43 cm×50 μm, I.D. Before installation in a capillary cartridge for on-column detection, a 0.4-cm segment of polyimide coating was burned off the tubing and the detection distance was 7.0 cm from the cathodic end. Sample injection was done in a hydrodynamic

mode over 2 s under a pressure of 1.5 p.s.i. (1 p.s.i.=6894.76 Pa). The CE system was interfaced with a microcomputer and printer with software CE 1000 1.05A. For pH measurements, a pH meter (Suntex SP-701, Taipei, Taiwan) calibrated with a precision of ±0.01 pH unit was employed.

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed for 50 min with a 1.0-*M* sodium hydroxide solution at 60°C, followed by 10 min with a sodium hydroxide solution at 60°C and 10 min with deionized and purified water at 25°C.

To ensure reproducibility, all experiments were performed at least three times and always at 25°C. The capillary was prewashed for 10 min with running buffer before each injection and was post-washed for 2 min with deionized water to maintain proper reproducibility for run-to-run injections.

For peak identification, on-column UV spectra (200–260 nm with a 2-nm wavelength increment) of β-blockers which show the characteristics of UV absorption spectra of these analytes are helpful. Thus, three dimensional spectral scans of the CE separation of a mixture of β-blockers were recorded simultaneously during the electrophoretic separation, as necessary. The suitable detection wavelength was set at 220 nm.

2.4. Mobility calculation

The β-blockers migrate before the neutral peak on the electropherogram. The electrophoretic mobility of analytes was calculated from the observed migration time, as described elsewhere [31].

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

in which μ_{ep} is the electrophoretic mobility of the solute tested, μ is 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 (water as the 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.

3. Results and discussion

Optimization of the separation of analytes in CZE is achieved by controlling either the difference in the electrophoretic mobilities (μ_{ep}) of analytes or the difference between μ_{ep} and electroosmotic flow (μ_{eo}). In practice, both μ_{ep} and μ_{eo} can be altered by varying the separation parameters, such as buffer concentration, buffer pH and applied voltage. In this work, citrate buffer was used as a background electrolyte to separate β-blockers by CZE. The effects of the concentration and pH of the buffer on the mobility and the migration selectivity of β-blockers were investigated.

3.1. Effect of buffer concentration

It is well known that, for a given type of background electrolyte, the magnitude of μ_{eo} depends mainly on the zeta potential which decreases with decreasing pH and increasing ionic strength of the buffer solution. At a given buffer pH, it is expected that an increase in the ionic strength results in a decrease in the zeta potential, thus leading to a decrease in the value of μ_{eo}. Similar arguments can be applied to account for the variation in the electrophoretic mobility of analytes. Fig. 2 shows the variation of electroosmotic flow and electrophoretic mobility of blockers as a function of buffer concentration in the range 80–400 m*M* at pH 2.0, with an applied voltage of 20 kV. As illustrated, both μ_{ep} and μ_{eo} decrease with increasing buffer concentration. These experimental observations were consistent with the prediction.

The resolution of co-migrating analytes improves with increasing buffer concentration. Fig. 3 shows the plots of the resolution of four pairs of co-migrating analytes versus the buffer concentration at 20 kV. As illustrated, nadolol (8) and labetalol (9) were effectively separated at a concentration greater than 160 m*M*; metoprolol (5) and timolol (6) or levobunolol (7) were separated at a concentration above 220 m*M*; oxprenolol (2) and propranolol (3) were separated at a concentration above 320 m*M* and timolol (6) and levobunolol (7) were separated at a concentration above 400 m*M*. Thus, complete separation of ten β-blockers was achieved within 8 min using 400 m*M* citrate buffer at pH 2.0 with an

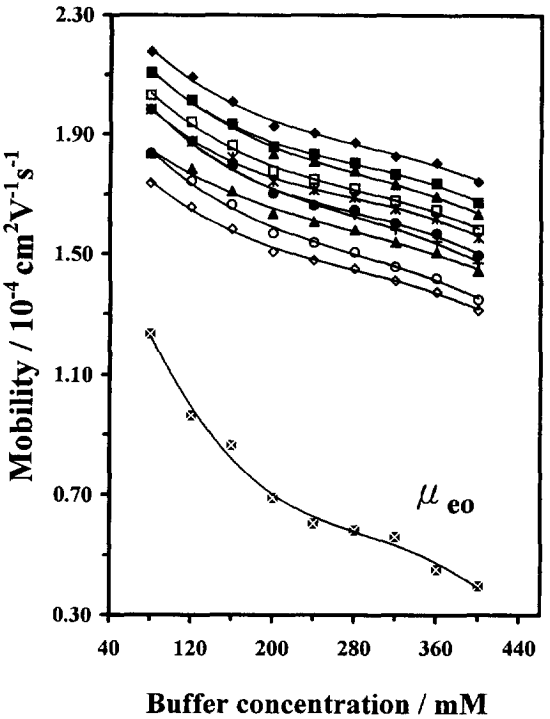

Fig. 2. Variation of the electrophoretic mobility of β-blockers as a function of buffer concentration at pH 2.0. Other operating conditions: 20 kV, 25°C. Curve identification: (◆) 1; (■) 2; (△) 3; (□) 4; (*) 5; (●) 6; (+) 7; (▲) 8; (○) 9; (◇) 10; the numbers denote the solutes shown in Fig. 1.

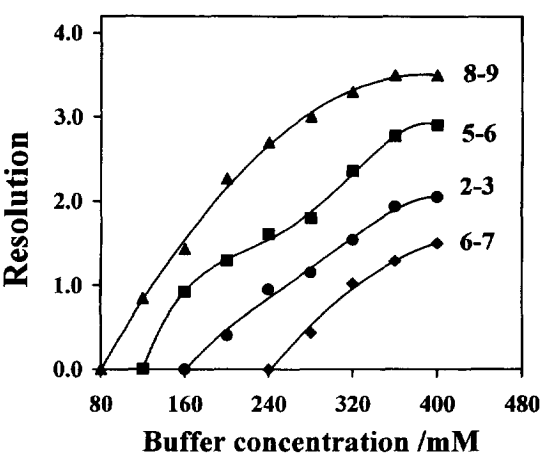

Fig. 3. Plots of the resolution of four pairs of closely migrating analytes (8–9, 5–6, 2–3 and 6–7) versus buffer concentration, with an applied voltage at 20 kV. Other operating conditions were the same as for Fig. 2. The numbers in parentheses denote the solutes shown in Fig. 1.

applied voltage of 20 kV. In addition, a greater decrease in μ_{eo} than in μ_{ep} was observed when the buffer concentration was varied from 80 to 400 m*M*. Therefore, migration times of β-blockers would increase and better resolution would be expected when the concentration of the buffer was higher.

Better resolution and baseline stability were obtained when β-blockers were separated using a lower applied voltage (i.e., 15 kV). In this case, the corresponding four pairs of co-migrating analytes were resolved at concentrations greater than 150, 210, 310 and 320 m*M*, respectively, at 15 kV. Complete separation of ten β-blockers was achieved within 11 min with the citrate buffer at a concentration of 320 ml at pH 2.0 and with a voltage of 1 kV. Fig. 4 shows the electropherogram of β-blockers obtained under these operating conditions.

3.2. Effect of buffer pH

The pH of the buffer plays an important role in the separation since it determines the extent of ionization of each analyte. Thus, manipulation of buffer pH is usually a key strategy for optimizing the separation in CZE.

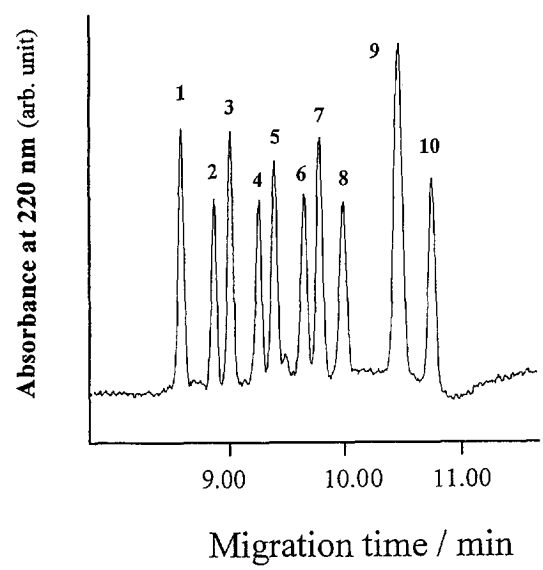

Fig. 4. Electropherogram of β-blockers obtained with citrate buffer (320 m*M*) at pH 2.0. Applied voltage: 15 kV. Other operating conditions and curve identification are the same as for Fig. 2.

Fig. 5 shows the effect of buffer pH on the electrophoretic mobility and separation of β-blockers with 80 m*M* citrate buffer at pH values varying from 2.0 to 5.0, with an applied voltage of 15 kV. As the pH of the buffer increases, the electrophoretic mobility of each analyte decreases. The resolution of co-migrating solutes improves with increasing buffer pH. However, the mobility curves for two consecutively migrating solutes may cross over when the pH of the buffer is varied. For instance, labetalol (9) and acebutolol (10) co-migrate at pH 4.3, while atenolol (4) and metoprolol (5), as well as timolol (6) and levobunolol (7), migrate together at pH 5.0. As a result, there is an optimum pH for the separation of these β-blockers with citrate buffer at each given concentration. With a buffer concentration of 80 m*M*, the optimum buffer pH determined is about 3.8.

The electroosmotic mobility of citrate buffer as a function of buffer pH at this buffer concentration is also shown in Fig. 5. Based on the fact that the migration time of the neutral marker increases with decreasing buffer pH in the range 5.0–3.5, but decreases in the range 3.5–2.0, μ_{eo} decreases with decreasing buffer pH in the range 5.0–3.5, as expected. However, μ_{eo} increases as buffer pH decreases further, down to 2.0. A reasonable explanation for this behavior is described below.

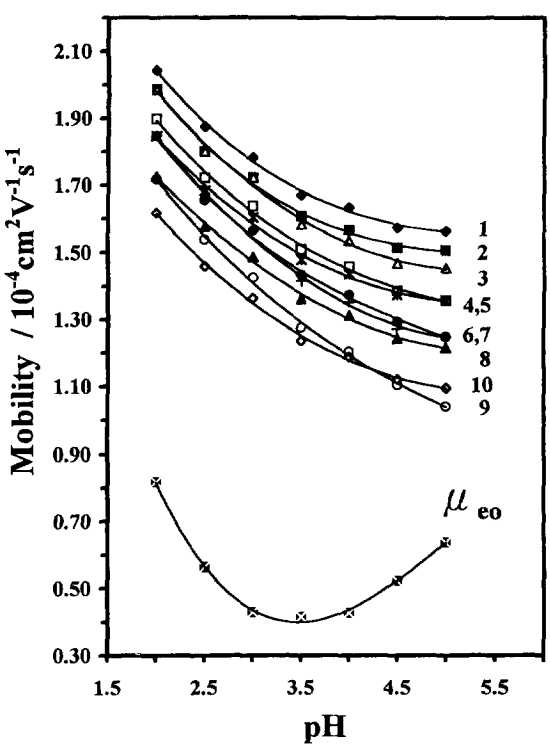

Fig. 5. Variation of electrophoretic mobility of β-blockers as a function of buffer pH with citrate buffer (80 m*M*). Applied voltage: 15 kV. Other operating conditions and curve identification are the same as for Fig. 2.

The increase in μ_{eo} with decreasing buffer pH in the pH range 3.5–2.0 may reveal that the negative charge on the capillary surface increases as the buffer pH decreases. We suspect that this is probably due to the protonation and the adsorption of anionic species of citrate buffer on the capillary surface in this pH range. In addition, current generated in the capillary was found to decrease with decreasing buffer pH. Since the ionic strength of the buffer also decreases with decreasing buffer pH, the ionic strength of the buffer correlates with the current generated. Therefore, an increase in the adsorption of anionic species on the capillary surface at a pH below 3.5 would result in a decrease in the ionic strength of the buffer and, consequently, with an increase in the, μ_{eo}. Hence, for a given buffer concentration, a minimal electroosmotic mobility observed at a certain buffer pH suggests that the resolution of β-blocker can also be affected by μ_{eo}.

Similar trends in the variation of electroosmotic mobility and electrophoretic mobility of β-blockers as a function of buffer pH were observed and lower values of μ_{ep} and μ_{eo} were obtained when a concentration of citrate buffer greater than 80 m*M* was used. It should be noted that, in order to avoid experimental complications due to Joule heating, the variation in buffer pH is confined to a smaller range as the concentration of the buffer becomes greater.

Because buffer pH and buffer concentration are two interactive separation parameters, the higher the buffer concentration employed for the separation, the lower the optimum pH of the buffer. Thus, the optimum pH of the buffer decreases from 3.8 to 1.8 when the concentration of the buffer increases from 80 to 400 m*M*. More specifically, the optimum pH values of the buffer were 3.0 and 1.8 when using 160 and 400 m*M* citrate buffer as the background electrolyte, respectively, at 15 kV. The migration patterns of β-blockers obtained under these two diifferent optimized conditions are quite similar to the one

shown in Fig. 4, except that the migration times of analytes are slightly different, as the separation conditions alter.

3.3. Reproducibility and detection limits

The variations in migration time were measured. Migration times for these β-blockers were reproducible, with relative standard deviations varying by less than 0.5% ($n=7$). The detection limits for β-blockers were found to be in the range of 1–18 μg/ml for a 2-s injection at a signal-to-noise ratio of three. Lower values of detectiion limits could be obtained by choosing the optimum wavelength for each β-blockers and/or by increasing the sample injection time.

4. Conclusion

In separating β-blockers, CZE provides an alternative and convenient method to the more common method using MEKC. Complete separation of ten β-blockers is achievable using citrate buffer as the background electrolyte at high concentrations and low pH.

Capillary electrophoresis has a distinct advantage over conventional chromatographic techniques because β-blockers in very small sample volumes can be separated nicely as cations by CZE within a relatively short time using citrate buffer. This is particularly true for samples contained in a matrix with various anionic species.

Acknowledgments

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