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Discussion

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

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In our recent contribution to this journal on the capillary electrophoretic separation of β-blockers using citrate buffer at low pH [1], the variation of electrophoretic mobility as a function of buffer pH was investigated in the pH range 2.0–5.0 with citrate buffer at a constant concentration. As the electrophoretic flow decreased with increasing pH of the buffer from 2.0–3.5 and the reverse situation occurred from pH 3.5–5.0, we suggested that the pH and ionic strength effects are simultaneously present. However, based on the fact that the pH of the buffer investigated is much less than the pK_a values of β-blockers, Reijenga and Ingelse [2] believe that the selectivity changes cannot be attributed to different extents of ionization because β-blockers must be considered fully protonated. They concluded that the selectivity effects are solely due to minor ionic strength effects. Here we would like to point out that Reijenga and Ingelse overestimated the effect of ionic strength and they should not completely ignore the effect of buffer pH.

In order to find out the influences of ionic strength and buffer pH on the mobility and the selectivity of β-blockers, the following two experiments were carried out. The first experiment was conducted by varying ionic strength of the buffer electrolyte at a fixed buffer pH to see how the ionic strength influences the mobility and selectivity of β-blockers. The second experiment was conducted by varying the pH of the buffer at a fixed value of ionic strength so that the effect of buffer pH on the mobility and selectivity of β-blockers can be examined.

In the first experiment, the pH of the buffer was fixed at 2.0 and the ionic strength of citrate buffer (80 m*M*) was varied by adding 10, 50, 100 and 150 m*M* of KCl to the buffer solution. Fig. 1 shows the variation of electrophoretic mobility of β-blockers obtained at varied ionic strength at pH 2.0. As expected, the electrophoretic mobility decreases exponentially as the ionic strength of citrate buffer increases. The electrophoretic mobility decreased by a factor of about 0.68–0.70 when the ionic strength of the buffer increased from 0.01 *M* to 0.16 *M*. In comparison with Fig. 2 which is the same as Fig. 5 of Ref. [1], except that the ionic strength instead of pH is indicated in the abscissa: it is noted that the mobility curve of propranolol merges with that of oxprenolol; the same situation also happens to timolol and *levo*-bunolol. The enhancement in the selectivity of timolol and metoprolol, and that of labetolol and nadolol as well, decreases to a considerable extent. Therefore, the selectivity of β-blockers

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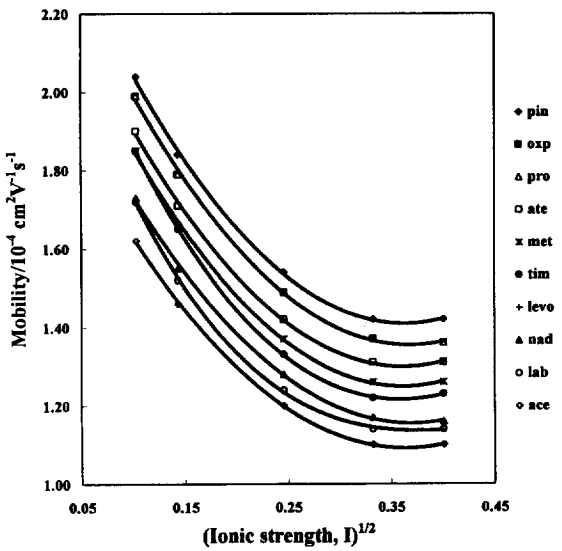

Fig. 1. Effect of ionic strength on the electrophoretic mobility of β-blockers with addition of various amount of KCl electrolyte to citrate buffer (80 m*M*) at pH 2.0. Capillary: 43 cm×50 μm, I.D., Other operating conditions: 15 kV, 25°C. Curve identification: (♦) pindolol; (■) oxprenolol; (△) propranolol; (□) atenolol; (*) metoprolol; (●) timolol; (+) *levo*-bunolol; (▲) nadolol; (○) labetolol; (◇) acebutolol.

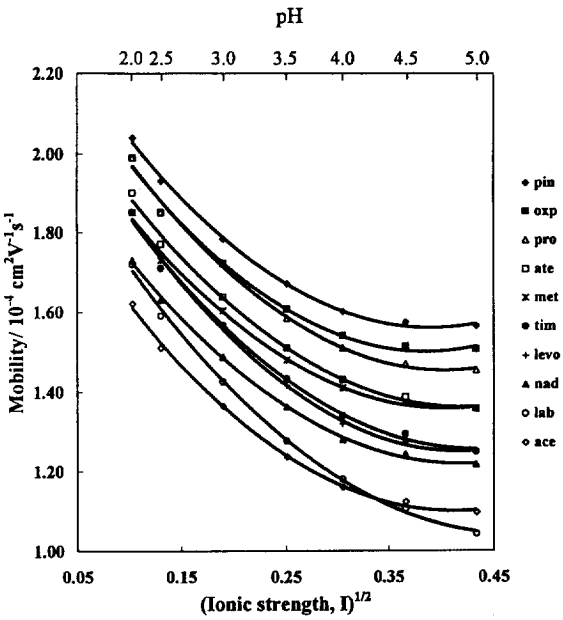

Fig. 2. Variation of electrophoretic mobility of β-blockers at varied buffer pH with citrate buffer at a constant buffer concentration (80 m*M*). The ionic strength instead of pH is indicated in the abscissa for the purpose of comparison with Fig. 1. Other operating conditions and curve identification are the same as for Fig. 1.

in Fig. 2 cannot be accounted for by the contribution of ionic strength alone. This is particularly true for labetolol and propranolol. Evidently, the contribution to the selectivity enhancement by buffer pH should not be ignored.

To add further support to the effect of buffer pH on the selectivity, the second experiment was conducted. In this experiment, the pH of the buffer was varied from 4.5 to 2.0 at fixed ionic strength (134.5 m*M*) by adding 41, 72, 98, 118 and 124 m*M* of KCl to citrate buffer (80 m*M*) at pH 4.0, 3.5, 3.0, 2.5 and 2.0, respectively. Fig. 3 shows the variation of electrophoretic mobility of β-blockers obtained. The electrophoretic mobility was found to decrease with decreasing buffer pH from 4.5–2.0. The selectivity of labetolol and nadolol and that of propranolol and oxprenolol varies most markedly. It is of great interest to note that the trends in the variation of selectivity as a function of buffer pH in these molecules are similar to those observed in Fig. 2. For instance, the selectivity of acebutolol and labetolol decreases with increasing buffer pH from 2.0 to 4.5 both in Fig. 3 and in Fig. 2, whereas the reverse

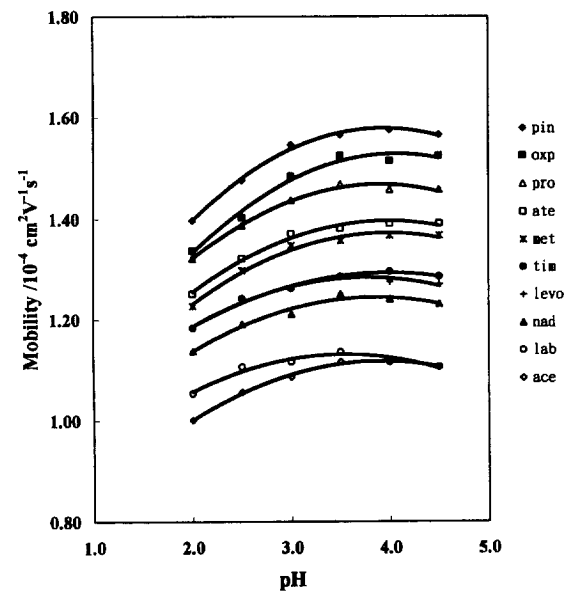

Fig. 3. Variation of electrophoretic mobility of β-blockers as a function of buffer pH with addition of KCl electrolyte to citrate buffer to keep buffer electrolyte at a constant ionic strength (134.5 m*M*). Other operating conditions and curve identification are the same as for Fig. 1.

situation occurs for propranolol and oxprenolol. Thus, the influence of buffer pH on the selectivity of β-blockers should not be ruled out.

Based on the pK_a values of β-blockers reported in the literature [3]: 7.4 for labetolol, 8.8 for pindolol and timolol and 9.2–9.7 for the rest of β-blockers studied in this work, it is unlikely that the effect of buffer pH can be attributed to the different extents of ionization of the β-blockers. What the origin of the contribution from buffer pH is, is not clearly known. The possibility of molecular interactions between β-blockers and citrate buffer is then considered. Since β-blockers possess characteristics of hydrogen bonding, we suggest that different extents of hydrogen bonding interaction may be involved between individual β-blockers and citrate buffer. As labetolol possesses the most prominent characteristics of hydrogen bonding [4], the remarkable migration behaviour of this molecule in Fig. 3 or Fig. 2 may reflect the influence of this interaction.

References

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