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毛細管電泳在臨床上的應用

The Application of Capillary Electrophoresis in the Clinical Settings

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一、中文摘要

臨床上經常用來診斷腎臟病病人的四種非蛋白氮化合物：尿素，尿酸，肌酸與肌酸肝可以被毛細管電泳予以鑑定分離．此四種非蛋白氮化合物在磷酸鹽溶液 PH7.4，電壓 30KV 的條件下在 30 分鐘內成功的被分離。

關鍵詞：非蛋白氮化合物、毛細管電泳、

Abstract

The capillary electrophoretic separation of the four nonprotein nitrogenous compounds (urea, uric acid, creatine and creatinine) typically employed in clinical/medical settings for monitoring renal function. Successful resolution of these compounds was achieved via use of a bile salt micelle system composed of sodium cholate at phosphate buffer (pH 7.4). The elution patterns of four NPNs can be obtained within thirty minutes with a voltage of 30 KV. The effect of varying the applied voltage, temperature and the mole ratio of phosphate buffer with bile salt surfactant on the migration behavior is also examined.

Keywords: Nonprotein Nitrogen Compounds, Capillary Electrophoresis

二、Introduction

The nonprotein nitrogen (NPN) fraction in the blood consists of about 15 compounds of clinical interest. Urea, uric acid, creatine and creatinine are four major NPN components and are routinely determined in clinical settings. They are used to monitor renal function.

Currently coupled enzymatic methods are used to measure NPNs (1-5). One of the major disadvantages is that the endogenous enzymes (e.g. latic dehydrogenase) compete for NADH in the reaction mixture, thus interfering with the indication reaction (4). Several HPLC methods have been developed to analyze uric acid (6-8) and creatinine (8-11). Because creatine assays are not readily available in most clinical laboratories, the creatine kinase levels are measured instead (12,13).

Capillary electrophoresis (CE) was developed to separate the charged molecules in a buffer-filled capillary by the application of a very high voltage (14). Micellar electrokinetic capillary chromatography (MECC) allows the resolution of uncharged molecules by adding surfactants to modify the conditions to extend the application of CE (15,16). Based on the differential binding to the micellar phase, the partition between the slow moving micelle and the fast moving aqueous phase causes differential resolution of the solutes. Sodium dodecyl sulfate (SDS) has proven very useful for separating water-soluble analytes (e.g. ascorbic acid (17)) and nonionic molecules (18,19). Cole et al. (20) successfully adopted bile salts instead of SDS to optimize the resolution of binaphthyl enatiomer separation. Hsiao et al. (21) has successfully separated steroids in phosphate buffer with sodium cholate.

CE methods for urine samples have been developed. Guzman et al. (22) and Jenkins et

al. (23) demonstrated the co-elution of urea and creatinine in borax buffer. The determination of creatinine and uric acid in phosphate buffer (pH=9.0) was developed by Mikaye et al. (24). But the simultaneous detection of these four analytes has not been reported. In this paper, the successful separation of these four analytes in phosphate buffer (pH=7.4) containing sodium cholate as well as other effects for such NPN separation is reported.

三、Result and Discussion

Figure 1 shows the CZE electropherograms of the four NPNs. Resolution of these four NPNs were not achieved in the buffer range of 0.038 M to 0.097M in the absence of sodium cholate. The creatine peak was resolved better as the buffer concentration increased (Fig. 1b-d), but urea and creatinine still co-migrated at these four chosen concentrations. The co-migration of urea and creatinine at borax buffer has been reported by Guzman et al. (22) and Jenkins et al. (23).

MECC with sodium cholate

In CE, the resolution, separation proficiency, selectivity, and elution time of the analytes can be optimized by the capacity factor, k' (e.g. assorted surfactants, surfactant concentrations, organic modifiers, temperature, voltages, etc.). However, separation of these NPNs by MECC with addition of SDS or sodium cholate in the 0.038 M phosphate or 0.10 M borate buffer solution was not achieved in the range of pH 7-9. Another approach by adding organic modifiers (e.g. methanol) in such system was also unable to separate these NPNs under the experimental conditions.

Figure 2 shows the resolution of MECC electropherograms of the four NPNs in the 0.077 M phosphate buffer of pH=7.4 with 0.075 M sodium cholate at 25^{0}C, 25 KV. The variation of the concentration of phosphate buffer with sodium cholate to adust the capacity factor, K', to separate NPNs was conducted as follows: the phosphate buffer concentrations were at different fixed sodium cholate concentrations and the data were shown in Figure 3 and Table I.

Figure 3 shows the plot of capacity factor, K', which the concentration of phosphate buffer from 0.038 M to 0.097 M with sodium cholate from 0.05 M to 0.10 M. The K' increased with an increase of phosphate buffer concentration. The difference in K' in Figure 3 could be ascribed to the difference in micellar volume. Uric acid was separated. Creatinine comigrated with either creatine or urea when the phosphate buffer concentrations were 0.038 M or 0.057 M. Four NPNs were resolved when the phosphate buffer concentrations were 0.077 M and 0.097 M.

In table I, as 0.075 M sodium cholate was added into the phosphate buffer system, the phosphate buffer concentration varied from 0.077 M to 0.097 M. The resolution factor of creatinine-creatine increased from 1.12 to 1.21, but urea-creatinine decreased from 1.18 to 0.99. On the other hand, as sodium cholate concentration increased from 0.075 to 0.10 M at 0.077 M phosphate buffer; the resolution factor of urea-creatinine increased from 1.18 to 1.69, but creatinine-creatine decreased from 1.12 to 0.93. As phosphate buffer increased from 0.077 M to 0.097 M at 0.10 M sodium cholate addition, the resolution factor of creatinine-creatine increased from 0.93 to 1.23, but urea-creatinine did not change significantly. Besides, as sodium cholate concentration increased from 0.075 M to 0.10 M at 0.097 M phosphate buffer, the resolution factor of urea-creatinine increased from 0.99 to 1.67, but creatinine-creatine did not change significantly.

We may point out that the resolution of creatine and creatinine is affected by the phosphate buffer concentrations; the resolution of urea and creatinine is affected by the sodium cholate concentrations. The results also indicate that an optimal ratio between phosphate buffer and sodium cholate concentrations for the simultaneous

separation of these three analytes is necessary (Figure 2).

Variation of voltage

Variation of voltage can have effects on migration time, resolution, peak sharpness, EOF, and joule heating. The joule heating resulting from an increase in voltage may lead to changes in EOF, ion mobility, analyte diffusion, and band broadening. Figure 4 shows the electropherograms of the four NPNs running in 0.077 M phosphate buffer with 0.075 M sodium cholate addition at different applied voltages. The migration time decreased when the applied voltage was increased (Table II), but the elution sequence did not change with different applied voltages. The joule heating effects (e.g. peak broadening) was not observed.

Temperature effect

Viscosity is a function of temperature. Therefore, as the temperature increased, the viscosity decreased; and then electrophoretic mobility and EOF increase. Some analytes may not be stable at higher temperature, and variation of temperature may lead to conformational change. Figure 5 shows the electrophoregrams of the four NPNs running in 0.077 M phosphate buffer with 0.075 M sodium cholate addition at different applied temperatures. The migration sequence did not change with the application of different temperatures. An increase in EOF and electrophoretic mobility from increased temperature leads to a shorter analysis time (Table III).

Conclusion

In conclusion, these four NPNs are separated by MECC in phosphate buffer (0.77 M) and sodium cholate (0.75 M) at 25°C and 25 KV. This MECC approach can potentially be applied in the clinical settings.

四、Acknowledgement

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