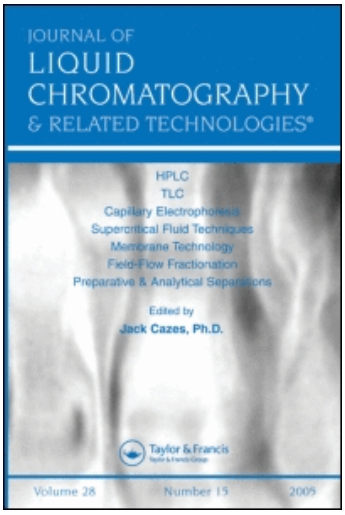

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THE DETERMINATION OF CREATININE IN HUMAN URINE BY CAPILLARY ZONE ELECTROPHORESIS WITH PHOTODIODE ARRAY DETECTION

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ABSTRACT

The application of capillary zone electrophoresis (CZE) to the separation and determination of creatinine in human urine with photodiode array detection was described. Optimum conditions for the separation were established. The effect of pH of the background buffer on the existence state of creatinine was investigated. Photodiode array detection permitted the rapid identification of creatinine in the samples analysed. By selecting 234 nm as monitoring wavelength, the interference from urea in urine was eliminated. By selecting a suitable pH, the interferences from other substances in urine were also eliminated. The linear range, detection limit, precision and recovery of the method were investigated.

INTRODUCTION

Creatinine is the main degradation product of creatine, an important compound related to muscular activity. Its level in urine is an important parameter in assessing depurating renal capacity, as well in diagnosis of muscular atrophy, or renal and thyroidal disfunctions.[1] Therefore, the determination of creatinine is very important in clinical analysis.

Measurement of creatinine in urine is routinely performed by photometric methods such as the alkaline picrate reaction according to Jaffé.[2] But various endogenous and exogenous can interfere with the analysis. Enzymatic assays, such as the creatinase-p-aminophenazon (PAP) method for creatinine,[3] are more specific, but still suffer from interference by various exogenous and endogenous substances. To avoid these interferences, high performance liquid chromatographic methods were developed. Ion-exchange,[4,5] ion-pair,[6,7] reversed-phase chromatography[8,9] and column-switching liquid chromatography[10,11] were used for the analysis of urinary creatinine. Most of these chromatographic methods suffer from some limitations, such as tedious operation, time-consuming, sample preparation requiring deproteination before analysis, and etc. For HPLC, the chromatographic column is easily contaminated and hard to clean.

High performance capillary electrophoresis (HPCE) is a powerful separation and quantitation technique that often provides high resolving power, short analysis time, low operation cost and very small sample volume. Guzman et al. have reported the determination of creatinine in human urine by CE.[12] But urea interfered with the determination of creatinine at wavelength of 210 nm, and the analysis time is quite long (needing 30 minutes). Recently, Shi et al. also developed a CE method for the determination of creatinine in urine.[13] In the method, the pH of the background buffer (phosphate solution) was adjusted to 6.4. But through our experiments, we found, at pH 6.4, creatinine suffered from interferences by other substances in urine. In this case, creatinine eluted in one peak together with other substances in urine. Therefore, the method[13] gives higher values of creatinine due to the interferences from other substances.

In this paper, we established optimum conditions for the separation of creatinine in human urine. The effect of pH of the background buffer on the existence state of creatinine was investigated. By selecting 234 nm as monitoring wavelength, the interference from urea in urine was eliminated. By selecting a suitable pH, the interferences from other substances in urine were also eliminated.

Photodiode array detection permitted the rapid identification of creatinine and other substances in the sample analysed. The linear range, detection limit, precision and recovery of the method were also investigated. The urine samples were filtered using a 0.45 μm cellulose acetate syringe filter prior to use.

EXPERIMENTAL

Instrumentation

An HP ^{3D} CE system (Hewlett-Packard, Palo Alto, CA, USA), equipped with a diode array detector was used in all the experiments. For data collection, data analysis, spectral identification, and system control, HP ^{3D} CE software was used. Polyimide-coated fused-silica capillaries with 50 cm total length, 75 μm internal diameter were obtained from YongNian Photoconductive Fibre Factory, Hebei, China. The detection window was located 8.5 cm from the end of the capillary. Pressure injection (50 mbar, 5 s) was used. The applied voltage was 15 KV. The capillaries were thermostated at 20°C. The absorbance values were recorded from 190 to 400 nm (using 234 nm as the monitoring channel). The electroosmotic velocity was measured with dimethylformamide.

Reagents

Creatinine was purchased from Fluka. Dissolve 100 mg of creatinine in a little amount of distilled water, add 1.00 mL of concentrated hydrochloric acid, then dilute to 10 mL with distilled water. A 10 mg/mL of creatinine stock solution was prepared. The solution was stored at 4°C. Less concentrated standard solutions were prepared from the stock solution by dilution using distilled water.

Urea, sodium tetraborate, sodium phosphate, sodium monohydrogen phosphate, sodium dihydrogen phosphate and dimethylformamide were purchased in China.

The carrier electrolytes were prepared as to contain 25 mM of sodium tetraborate, whose pH was adjusted to 9.6 by addition of appropriate volume of concentrated sodium hydroxide solution. Unless otherwise specified, all chemicals were of analytical reagent grade. All solutions were prepared using filtered, degassed and deionized distilled water.

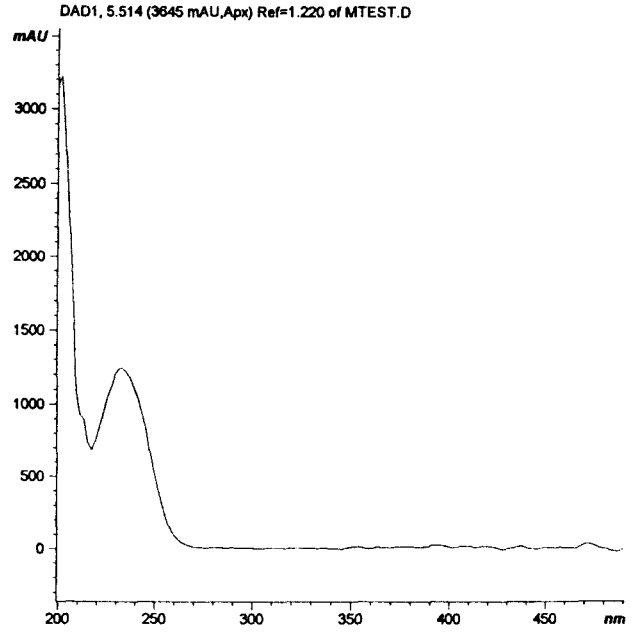

Figure 1. The UV-Vis spectrum of standard creatinine.

Analytical Procedure

Urine samples were collected from healthy volunteers who were not taking any type of drug. These samples were passed through a 0.45 μm cellulose acetate syringe filter.

The filtrates were then introduced directly into the CE system for the determination of creatinine.

After each run, the capillaries were purged under vacuum for 3 minutes with 0.1 M NaOH, followed by electrophoresis buffer for 3 minutes.

Because the urine samples were analysed directly without precleaning, it was necessary to use this rinsing procedure after each analysis to remove the impurities adsorbed on the CE column.

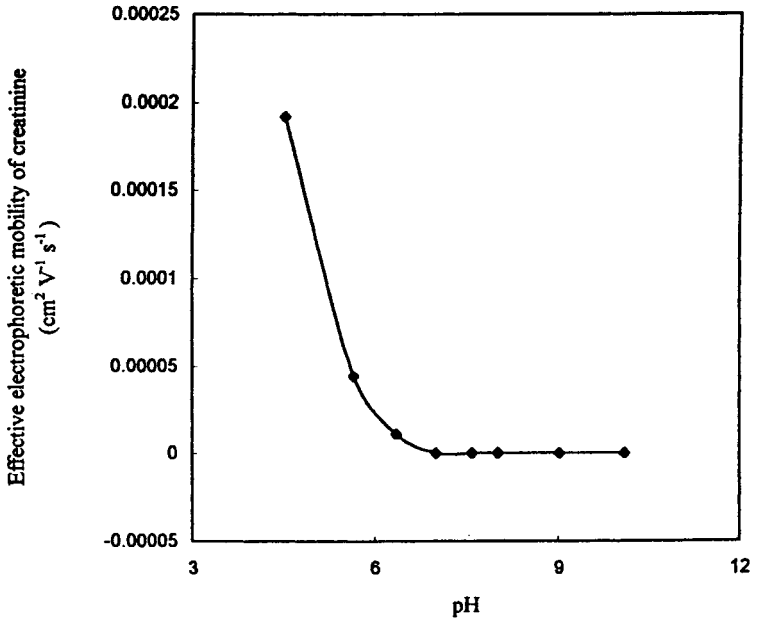

Figure 2. Effect of pH of the background buffer on the effective electrophoretic mobility of creatinine.

RESULTS AND DISCUSSION

Selection of Detection Wavelength

The UV-Vis spectrum of the standard creatinine was obtained by using the photodiode-array detector of HP ^{3D} CE instrument. Figure 1 showed the UV-Vis spectrum of the standard creatinine. Maximum absorbance was obtained at 234 nm. Therefore, 234 nm was selected as detection wavelength.

Effect of pH of the Background Buffer on the Existence State of Creatinine

In order to study the effect of pH of the background buffer on the existence state of creatinine, the pH of the buffer solution was varied by mixing appropriate portions of 25 mM of sodium phosphate, 25 mM of sodium monohydrogen phosphate and 25 mM of sodium dihydrogen phosphate solut'n.

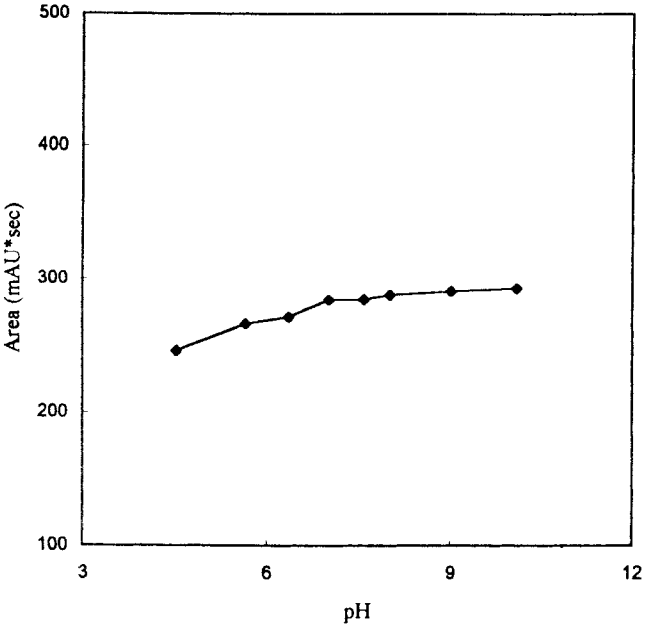

Figure 3. Effect of pH of the background buffer on the peak area of creatinine.

The solution containing 200 μg/mL of standard creatinine and 1% (v/v) of dimethylformamide as test solution. At first, we studied the effect of pH of the background buffer on the effective electrophoretic mobility of creatinine. Figure 2 depicted the relationship between pH of the background buffer and the effective electrophoretic mobility of creatinine.

When the pH of the background buffer was lower than 6.98, the effective electrophoretic mobility of creatinine was bigger than zero. In this case, creatinine eluted before neutral dimethylformamide. This is because creatinine has a positive charge due to protonation. When the pH was higher than 6.98, creatinine migrated at the same velocity of electroosmotic flow (EOF) and eluted together with dimethylformamide. Therefore, in this case, creatinine is neutral and its effective electrophoretic mobility is zero.

Secondly, we studied the effect of pH of the background buffer on the peak area of creatinine. 200 μg/mL of creatinine standard solution was used as test solution. The relationship between the pH of the background buffer and the peak area of creatinine was shown in Figure 3. When the pH of the buffer was

lower than 6.98, the area of creatinine increased slowly with increasing pH. When the pH of the buffer was higher than 6.98, the pH of the buffer almost has no influence on the peak area of creatinine.

Thirdly, when the pH of the background buffer containing 25 mM of sodium tetraborate was adjusted by addition of appropriate volume of 25 mM of phosphoric acid or concentrated sodium hydroxide solutions, a similar work was carried out and the obtained results were the same as that using 25 mM of phosphate solutions at different pH values.

Effect of pH on the Spectrum of Urea

Since urea was reported to be the major interference of creatinine in the published HPCE method,[12] interference from urea was studied. The pH of the buffer solution was varied by mixing appropriate portions of 25 mM of sodium phosphate, 25 mM of sodium monohydrogen phosphate, and 25 mM of sodium dihydrogen phosphate solutions. Twenty mg/mL of urea standard solution was used as a test solution.

We compared the UV-Vis spectrum of urea at different pH values. Our experimental results proved that the pH of the background buffer had no effect on the UV-Vis spectrum of urea. Figure 4 showed the UV-Vis spectrum of standard urea.

From Figure 4, we can see, at 234 nm, urea has no absorbance. This means that urea has no interference with the determination of creatinine in urine samples when selecting 234 nm as detection wavelength.

A similar work was performed using 25 mM of sodium tetraborate solutions at different pH values as carrier electrolytes. We obtained the same results as that using 25 mM of phosphate solutions at different pH values as carrier electrolytes.

Effect of pH of the Background Buffer on the Separation of Creatinine in Urine

Firstly, the pH of the background buffer solution was varied by mixing appropriate portions of 25 mM of sodium phosphate, 25 mM of sodium monohydrogen phosphate, and 25 mM of sodium dihydrogen phosphate solutions. A urine sample was used as test solution.

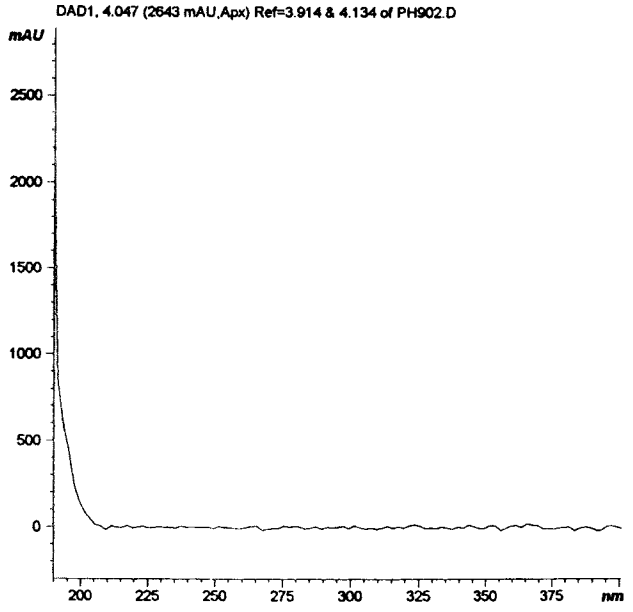

Figure 4. The UV-Vis spectrum of standard urea.

The effect of pH of the background buffer on the separation of creatinine in urine was investigated. Figure 5 showed the representative electropherograms of urine at different pH values. When the pH was higher than 6.98, creatinine was separated from other substances in urine, but other substances in urine were not separated well. With the increasing of the pH, the peak area of creatinine did not change. The identification of creatinine in urine will be discussed in the later section in detail. When the pH was 6.56, creatinine eluted together with other substances in urine and emerged in one peak. Therefore, its peak area was larger than the peak area of the separated creatinine.

According to the effect of pH on the peak area of creatinine, we can prove that creatinine suffered from interferences by other substances in urine at pH 6.56. The peak purity of creatinine in urine at pH 6.56 was further studied using photodiode array detection. The results showed that the peak was not pure. If using pH 6.56 or 6.4 as the pH of the background buffer, the determined content of creatinine in urine will be higher.

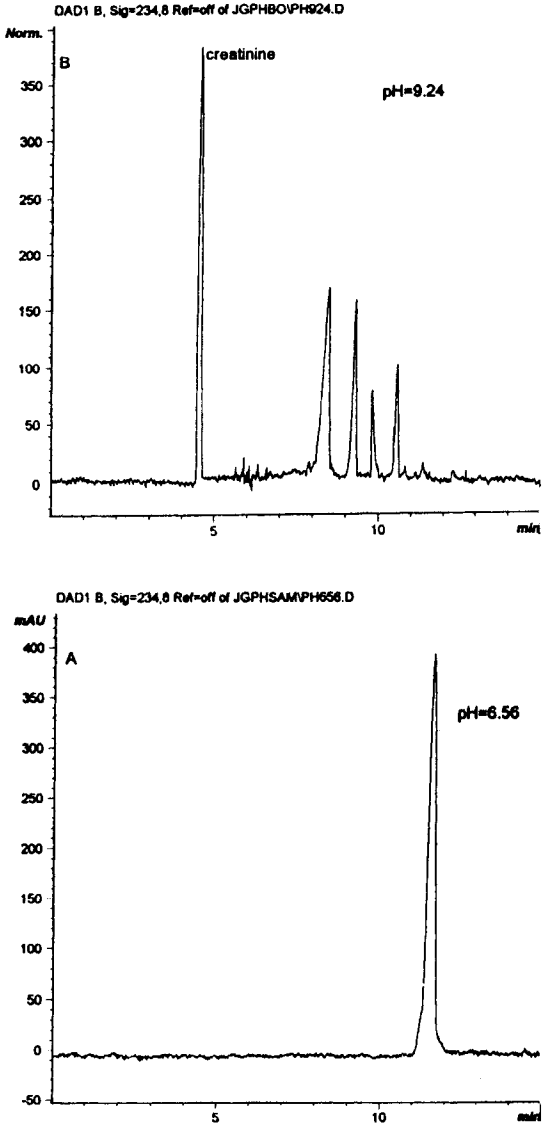

Figure 5. Effect of pH of the background buffer containing 25 mM of phosphate solutions on the separation of creatinine in urine.

Next, a similar work was carried out using 25 mM of sodium tetraborate solutions at different pH values as carrier electrolytes. Figure 6 showed the representative electropherograms of urine at different pH values. When the pH was higher than 7.0, creatinine was separated from other substances in urine well. Comparing with phosphate solution, besides creatinine, other substances in urine were also separated well when the pH of the background buffer containing 25 mM of sodium tetraborate was higher than 9.24. Using the diodearray detection, these substances in urine can be identified. Studies are currently being carried out to determine these substances in urine.

From the above experimental results, we can draw the conclusion, creatinine in urine can be separated and determined at 234 nm using 25 mM of phosphate solutions or 25 mM of sodium tetraborate solutions as a background buffer, whose pH was adjusted to 6.98-10.23. In this paper, we use 25 mM of sodium tetraborate solution (pH 9.6) as a background buffer.

Identification of Creatinine in Urine

Creatinine in urine samples was identified by comparing its migration times with that of the standard. Pure standard was also added to the samples so that the peak area of creatinine was increased in order to improve its detectability. The peak purity, with the same migration time as that of creatinine, was further studied with a photodiode array detector connected to the CE system. The UV spectrum of creatinine in urine was taken and compared with that of standard creatinine.

The peak of creatinine in the electropherogram of the urine sample has the same migration time as that of standard creatinine. The UV spectrum of the peak of creatinine in the urine sample obtained with the photodiode array detector was identical with that of the standard creatinine.

Quantitation

A correlation regression analysis was done on the data obtained from ten concentration levels of standard creatinine. The peak area is linearly related to creatinine concentration over three orders of magnitude. The regression equation of the line obtained by the least square method was, $A = 2.45C$, where A is the peak area in mAU * sec and C the concentration of creatinine in μg/mL; the correlation coefficient was 0.99991. The linear calibration range is 0-2.0 mg/mL. The detection limit of creatinine achieved at three times the signal-to-noise ratio was 10 μg/mL.

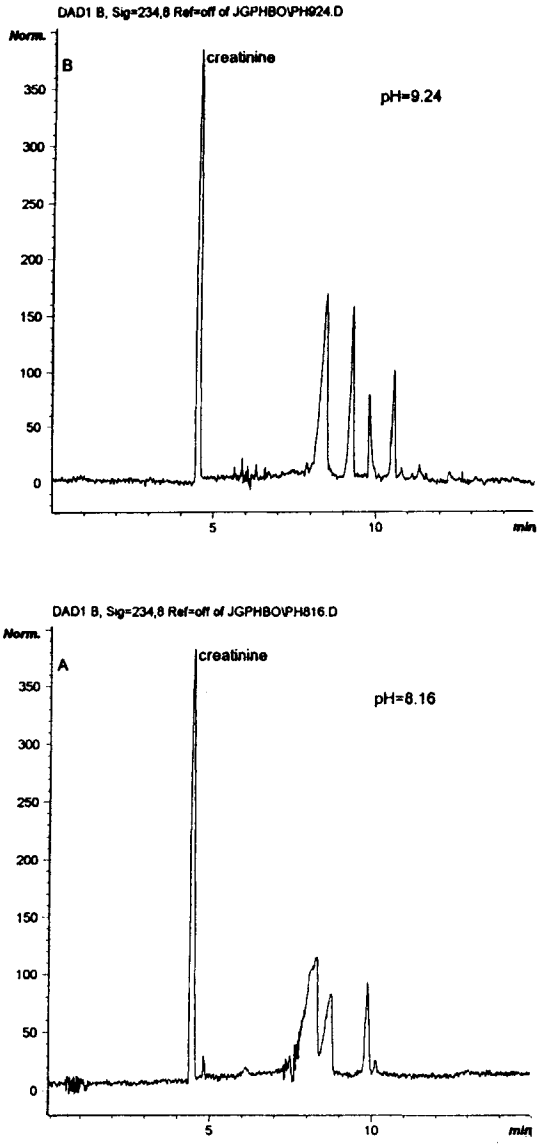

Figure 6. Effect of pH of the background buffer containing 25 mM of sodium tetraborate solutions on the separation of creatinine in urine.

Table 1

Analysis Results of Urine Samples

Sample	Detemined Content[a] (mg/mL)	RSD[b] (%)	Added (mg/mL)	Recovery[c] (%)
1	1.295	2.0	1.5	104 ± 1.2
2	0.986	1.9	1.0	98 ± 1.0
3	0.63	3.5	0.6	96 ± 4.0
4	0.597	1.6	0.6	103 ± 1.2
5	0.272	1.3	0.3	99 ± 3.0
6	0.790	0.5	0.8	103 ± 0.5

[a] Average of three determinations.
[b] Abbreviation of relative standard deviation.
[c] Mean ± relative standard deviation (n=3).

Sample Analysis

The urine samples were analysed using the CZE method developed here. The analysis results are given in Table 1. The recovery of creatinine added to urine was satisfactory as shown in Table 1. These results indicate that this CZE method is suitable for the determination of creatinine in human urine.

CONCLUSIONS

In this paper, we propose a CZE method for the rapid determination of creatinine in human urine. The method only requires filtering the sample solution and no other pretreatment. Short analysis time, simple operation and cost effectiveness of the CZE approach makes it an attractive alternative to other methods. The combination of photodiode array detection with CE permitted the rapid identification of creatinine in the urine samples analysed.

ACKNOWLEDGMENTS

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