

Kinetic Potentiometric Determination of Creatinine in Urine with a Picrate-Ion-Selective Electrode

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INTRODUCTION

Numerous methods have been described for determining creatinine in biological fluids. Many of the currently used procedures are based on the color reaction which takes place between creatinine and sodium picrate in an alkaline medium (Jaffé reaction). These methods give erroneous results in the presence of proteins, ketone substances, and glucose (6). Thus, methods employing preliminary separation, reaction kinetic procedures, polarographic techniques, and specific enzymes in conjunction with ammonia or enzyme electrodes have been developed to minimize the effects of other Jaffé-positive materials which are found in serum and urine (1,3,5,6,8,).

Recently we reported a new picrate-selective membrane electrode and its applications to the potentiometric titration of silver and thiourea (2,4). The rapid response of the picrate-selective electrode to changes in picrate concentration makes it a valuable sensor for following the rate of picrate reactions.

In this paper, a kinetic potentiometric method is described for the determination of creatinine in urine, based on the Jaffé reaction, which is monitored with a picrate-selective electrode. Under controlled conditions, a linear relation exists between the increase in electrode potential within a fixed period of time (90 sec) and the amount of creatinine present. The method is simple, accurate, and sensitive, and no sample pretreatment is required. Recovery and comparison experiments gave satisfactory results.

MATERIALS AND METHODS

Reagents

All solutions were prepared with bidistilled deionized water from reagent grade materials.

Picric acid solution, 0.020 M. Dissolve 4.58 g of picric acid (Fluka purum) in 1 liter of water. No standardization is required.

Standard creatinine solutions. (a) Stock solution, 4.000 g/liter. Dissolve pure creatinine (Merck) dried at 100°C in water. (b) Working standards, 0.5, 1.5, 2.5, and 3.5 g/liter. Prepare from the stock solution by dilution. All creatinine standards must be stored in a refrigerator.

Ionic strength adjustment composite buffer, pH 12.6. Dissolve 257.8 g of $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ (Merck), 214.5 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (Merck), and 36.0 g of NaOH (Merck) in about 800 ml of water and heat to boiling. Cool at 37°C and dilute to 1 liter with water.

Apparatus

The picrate-selective electrode was constructed, used, and stored as previously described (4). The outer chamber of the reference electrode was filled weekly with a 10% NaCl solution.

The reaction cell was a 50-ml double-wall glass cell thermostated at $37.0 \pm 0.2^\circ\text{C}$. The temperature was checked with a thermometer in the reaction cell. The distance between the electrodes and their position in the reaction cell were rigidly maintained during all measurements. The reaction cell was emptied by suction. All measurements were taken under constant stirring with a magnetic stirring bar, at such a rate that no bubbles or vortex were formed. The electrode potential was measured and printed with an Orion Ionalyzer (Model 801 digital pH/pIon meter) coupled to an Orion digital printer (Model 751) which was adjusted to print the potential every 30 sec. The Heath-Schlumberger recording system (4) was used to monitor the electrode signal and record the reaction curve. Appropriate coupling of the two units allows synchronous printing and recording of the potential. For pH measurements a Metrohm Model E-350 B pH meter with a combination glass electrode (pH range 0–14) was used. Spectrophotometric measurements were carried out with a Bausch and Lomb Spectronic 20 spectrophotometer.

Procedure

Into the thermostated reaction cell, kept at 37.0°C, pipet 25.00 ml of the composite buffer and 1.00 ml of picric acid solution. Start the stirrer, and after the potential has stabilized and the solution temperature reached 37.0°C (1–2 min), inject 1.00 ml of the standard or sample solution into the cell and *immediately* press the “start” switch of the printer. After 120 sec (five potential measurements) stop the printer, empty the cell by suction, rinse the electrodes and the cell with water, and dry by suction. Repeat the procedure for each standard or sample analysis.

Calculations

Working curves are prepared to read grams per liter of creatinine by plotting ΔE values ($\Delta E = E_{120} - E_{30}$) against the creatinine concentrations of the standards.

RESULTS AND DISCUSSION

The effect of pH on the Jaffé reaction rate was studied. Recorded curves for the reaction at various pH levels are shown in Fig. 1. It can be seen that the reaction is very slow below pH 11, whereas above pH 11 the reaction rate increases significantly with increasing pH. A pH of 12.6 was chosen as optimum to ensure small measurement times and satisfactory

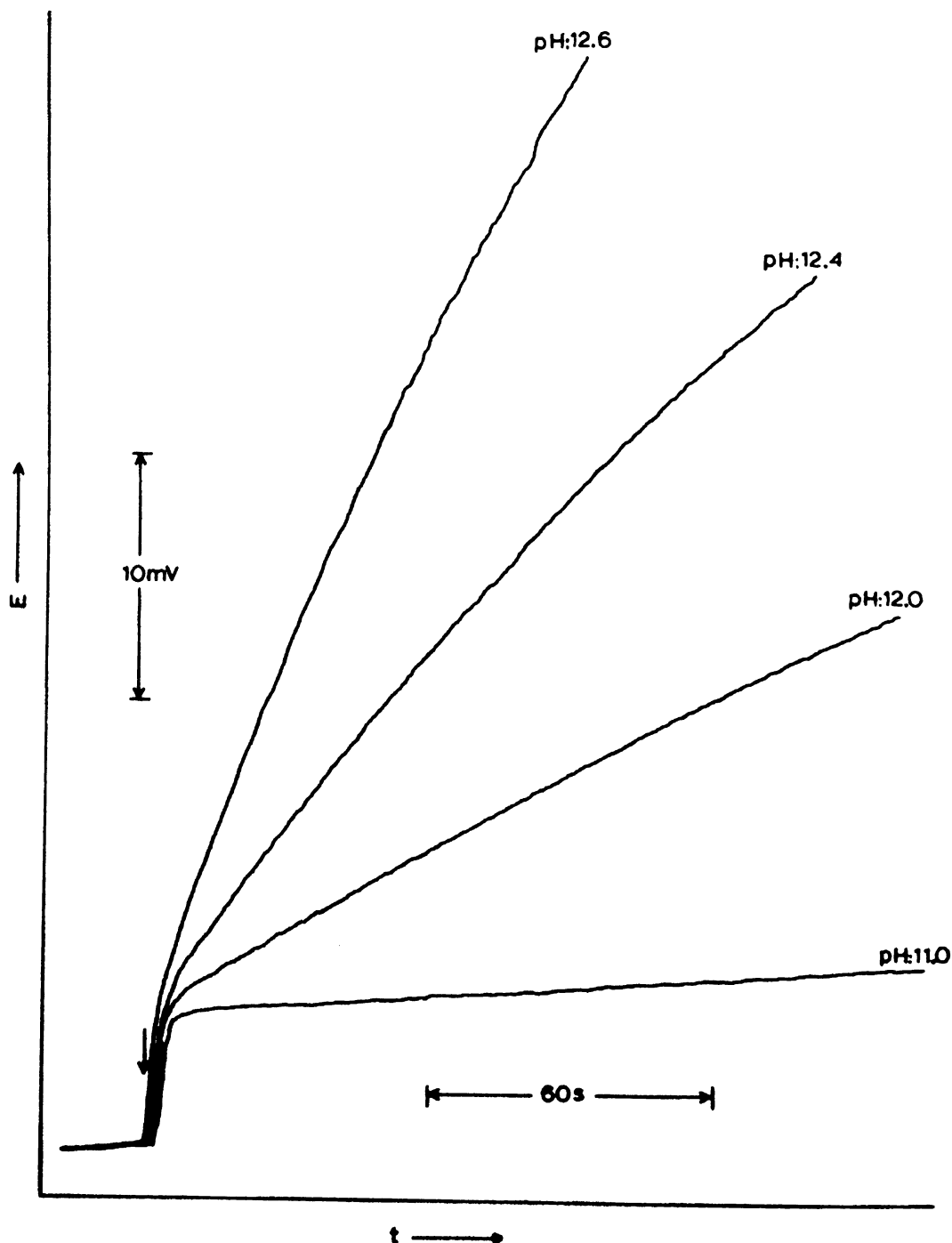


FIG. 1. Recorded curves of cell voltage vs time for the creatinine-picrate reaction at various pH levels. Creatinine concentration, 2.50 g/liter. Other conditions as under Procedure.

electrode operation in the alkaline pH range. Since the Jaffé reaction is critically pH-dependent, a buffer with sufficient buffer capacity must be used for the pH matching of standards and samples. So a final phosphate concentration of 0.74 M was chosen.

The rate of the creatinine-picrate reaction increases with increasing ionic strength.

The concentration of picrate chosen, $7.4 \times 10^{-4} M$, is a compromise to ensure a linear potential change of at least 30 mV before approaching the lower limit of the linear response of the picrate-selective electrode.

The rate of the creatinine-picrate reaction increases with increasing temperature. A temperature of 37°C was chosen as a compromise to avoid sedimentation at lower temperatures.

A premeasurement time of 30 sec was chosen to ensure that a "kinetic separation" of interfering substances present and reacting at the same time with different rates is achieved.

Figure 2 shows typical recorded curves and the corresponding working

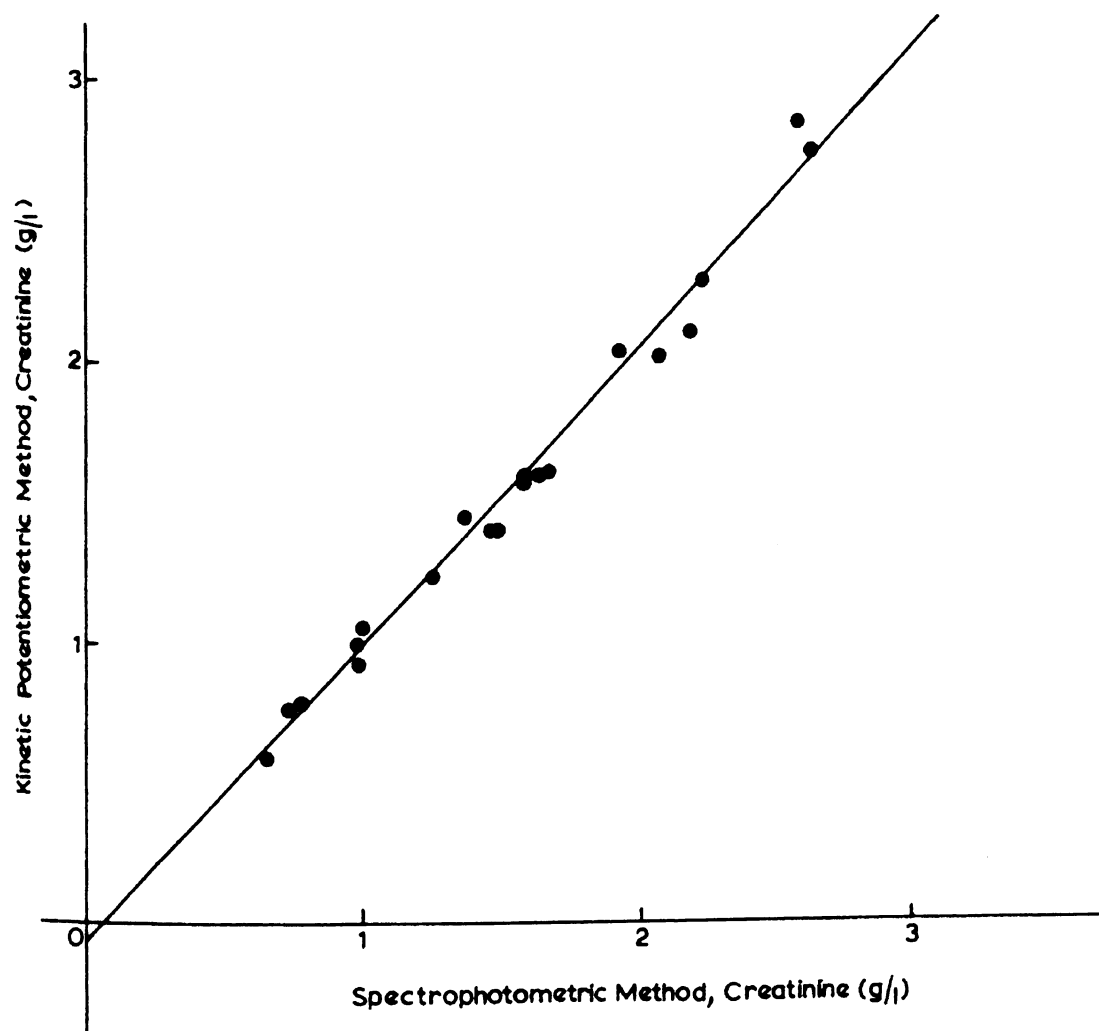


FIG. 2. Recorded curves of cell voltage vs time for the creatinine-picrate reaction and the corresponding working curve. Other conditions as under Procedure.

Erratum

Volume 22, No. 4 (1977), in the article, "Kinetic Potentiometric Determination of Creatinine with a Picrate-Ion-Selective Electrode," by E. P. Diamandis, M. A. Koupparis, and T. P. Hadjiioannou, pp. 498–504: Pages 501 and 502: Figures 2 and 3 should be reversed (the legends are correct). Page 503: The first sentence of the last paragraph of the text should read: "In conclusion, the proposed kinetic method is faster than the end point spectrophotometric method."

TABLE I
ANALYTICAL RECOVERY OF CREATININE ADDED TO EIGHT URINE SAMPLES
AND ONE URINE CONTROL SAMPLE

Creatinine (g/liter)				
Initially present ^a	Added	Total	Found ^b	Recover (%)
1.00 ^b	0.75	1.75	1.75	100
	1.50	2.50	2.46	97
1.58	1.04	2.62	2.54	92
	1.90	3.48	3.29	90
1.43	1.05	2.48	2.34	87
	1.93	3.36	3.13	88
0.68	1.12	1.80	1.77	97
	2.05	2.73	2.66	97
1.17	1.08	2.25	2.21	96
	1.97	3.14	3.03	94
0.51	1.14	1.65	1.66	101
	2.08	2.59	2.59	100
0.66	1.12	1.78	1.80	102
	2.06	2.72	2.72	100
1.44	1.05	2.49	2.47	98
	1.93	3.37	3.20	91
1.27	1.07	2.34	2.29	95
	1.95	3.22	3.11	94
Av				95.5

^a Average of two values.

^b Urine control sample.

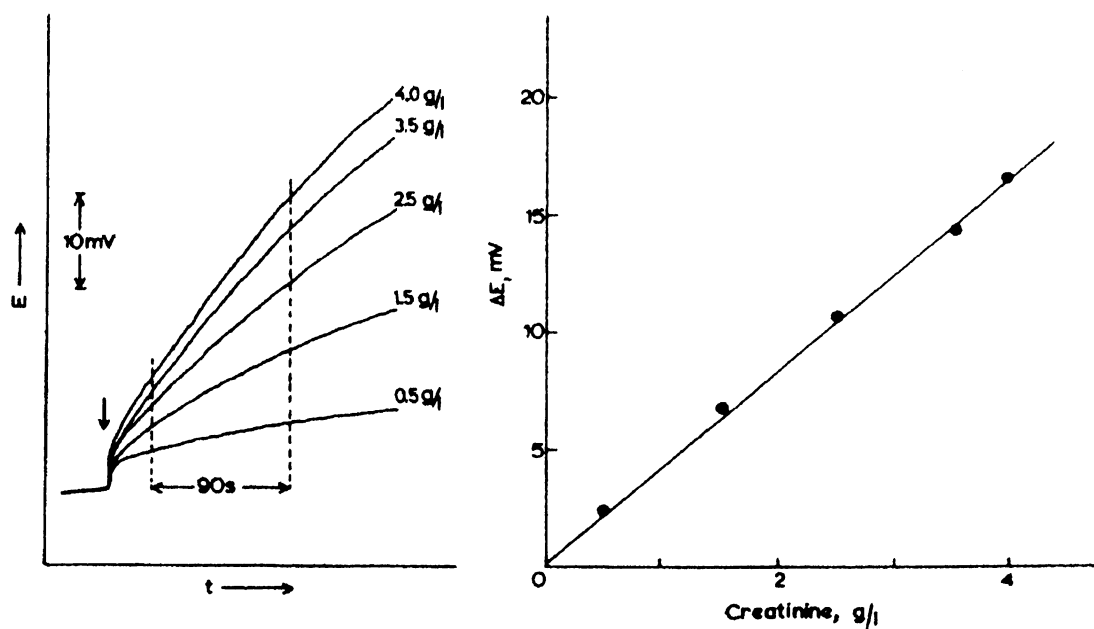


FIG. 3. Correlation between kinetic potentiometric method and spectrophotometric method for the determination of creatinine.

curve for the creatinine–picrate reaction. There is good linearity between reaction rate and creatinine concentration in the range 0.5–4 g/liter.

The response characteristics of the picrate-selective electrode were discussed elsewhere (4). However, because of the extreme conditions required for the creatinine determination (high ionic strength, pH 12.6), the electrode behavior under such conditions was reexamined. With Orion perchlorate membranes (92-81-04) consisting of polycellulose acetate the slope of the working curve was 51 mV at 37°C, the lower linear response limit was $2.4 \times 10^{-4} M$, and the operative life of the electrode was about 15 days. When Teflon membranes (Millipore 10- μ m Teflon LCWPO 1300) were used, the response characteristics of the electrode were improved. Thus, a near-Nernstian slope of 58 mV was obtained, the lower linear response limit was decreased to $1.6 \times 10^{-5} M$, and the operative life of the electrode was increased to about 1 month. Therefore, Teflon membranes were chosen for the construction of the picrate-selective electrode.

The accuracy of the method was checked with recovery experiments, in which creatinine was added to urine samples (normal human urine or urine control samples). The recovery of creatinine (amount added, 0.75–2.08 g/liter) ranged from 86 to 101%, with an average value of 95.5%. Typical results of analytical recovery studies are shown in Table 1.

The accuracy of the potentiometric kinetic method was further tested by comparing values of urine with those obtained with a spectrophotometric method (9). Results from this comparison are shown in Fig. 3. The correlation coefficient r was 0.992, and the regression line was found to be of the form $y = 1.0676 x - 0.0819$.

The relative standard deviation for a urine control sample containing 0.97 g/liter was 2.9% ($n=5$).

In conclusion, the proposed kinetic method is faster than the end point potentiometric method. The method permits analysis of about 20 samples per hr.

SUMMARY

A new kinetic method for the potentiometric determination of creatinine in urine based on the creatinine–picrate reaction in alkaline medium (Jaffé reaction) is described. The reaction is monitored with a picrate-selective electrode, and the increase in electrode potential within a fixed period of time (90 sec) is measured and related directly to the creatinine concentration. Analytical recovery of creatinine added to urine was 86–101% (average, 95.5%). Comparison with a spectrophotometric method gave a correlation coefficient of 0.992 over a concentration range of 0.6–2.6 g/liter.

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