

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

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We describe a new kinetic method for potentiometric determination of creatinine in serum, based on the creatinine-picrate reaction in alkaline medium (Jaffé reaction). The reaction is monitored with a picrate-selective electrode, and the increase in electrode potential during 270 s is measured and related directly to the creatinine concentration. Small cation-exchange columns are used to separate creatinine from interfering substances. Analytical recovery of creatinine added to serum was 100.7%. Results for a series of samples compared well with results obtained with a spectrophotometric method ($r = 0.994$).

Additional Keyphrases: *ion-selective electrodes · Jaffé reaction · kinetic methods of analysis · renal function evaluation*

Creatinine in serum is measured as a screening test in the clinical evaluation of renal function. Many methods have been described for determination of creatinine in serum (1), most of them based on the Jaffé reaction. Several compounds in addition to creatinine give the Jaffé reaction. Thus most attempts to improve the specificity depend on the separation of creatinine from the pseudocreatinine chromogens before the color reaction and have included the use of cation-exchange resins (2-4). Automated reaction-rate methods have also been described, based on the assumption that the initial rate of the Jaffé reaction is proportional to the concentration of creatinine and that the rate of reaction of the pseudocreatinine constituents is significantly different (5-7). However, these methods are also subject to interferences (8). Enzymic methods have also been used to increase specificity, involving the enzyme creatininase (EC 3.5.2.10) and enzyme electrodes (9-13).

Recently we reported a potentiometric method for determination of creatinine in urine, based on the Jaffé reaction (14). In the present paper, a kinetic method for determination of creatinine in serum is described. Creatinine is adsorbed directly from acidified serum onto a strong cation-exchanger. This removes most interfering compounds, and the Jaffé reaction can be applied to the eluted creatinine. The Jaffé reaction is monitored with a picrate electrode. Under controlled conditions, the increase in electrode potential during a fixed period of time (270 s) and the amount of creatinine present are linearly related. The method is simple, accurate, and sensitive. Recovery and comparison experiments gave satisfactory results.

Materials and Methods

Apparatus

The picrate-selective electrode was constructed, used, and stored as previously described (15). Teflon membranes were

used (14). The reference electrode was a Model K 4112 saturated calomel electrode (Radiometer A/S, Copenhagen, Denmark). The reaction cell was an 8-mL double-wall glass cell, thermostated at 37.0 ± 0.2 °C. The measurement system was the same as previously described (14).

Reagents

All solutions were prepared in doubly distilled de-ionized water from reagent-grade materials.

Sodium picrate stock solution, 10.0 mmol/L. Dissolve 2.29 g of air-dried picric acid (purum grade; Fluka AG, Buchs, Switzerland) in about 900 mL of water, neutralize to a pH of about 6 with 0.5 mol/L NaOH solution, and dilute to 1 L. No standardization is required. Prepare a 4.5×10^{-3} mol/L sodium picrate solution by dilution. Picrate solutions stored in amber-colored bottles at room temperature are stable for at least one year.

NaOH solution, 2.5 mol/L.

HCl solution, 0.10 mol/L.

Ionic strength adjustment composite buffer. Dissolve 40 g of NaOH (E. Merck, Darmstadt, F.R.G.) and 227 g of Na_2SO_4 (Merck) in about 800 mL of water, heat to boiling, cool, and dilute to 1 L with water. Store in a well-closed plastic bottle at room temperature. This buffer is stable for at least one year.

Working aqueous creatinine standards, 20, 50, 100, 150, and 200 mg/L. Prepare daily by diluting with water a stock (Merck) creatinine solution (1.000 g/L of 0.05 mol/L HCl). Stored refrigerated, this solution is stable for at least a month.

Bilirubin solution, 2 g/L. Dissolve 200 mg of bilirubin (Sigma Chemical Co., St. Louis, MO 63172) in 40 mL of 0.1 mol/L Na_2CO_3 and dilute to 100.0 mL with water. Prepare solutions containing bilirubin just before use, and protect from light.

We prepared solutions containing acetoacetate by dissolving acetoacetic acid ethyl ester (Sigma) in 0.1 mol/L NaOH. All other chemicals used in the interference studies were purchased from Sigma.

Ion-exchange columns were taken from a commercial kit intended for use in determination of thyroxine in serum (Trilute ¹²⁵I T-3 Reagent Kit; Ames Division, Miles Laboratories Inc., Elkhart, IN 46515). These plastic columns are 8 cm long, 1.1 cm in diameter, with stoppers to stop the flow. To fill the columns, the resin (Dowex resin, 50W \times 8, 200-400 mesh, Fluka) is suspended in water, then drawn into the column and allowed to settle; 500 mg of resin is used for each column. The bottom of the column is stabilized with a porous plastic disk, the top with glass wool.

During measurements all solutions were thermostated at 37.0 ± 0.2 °C.

Procedure

Place the columns in the rack and allow them to drain, then successively wash them with 2.0 mL of 2.5 mol/L NaOH and two 2-mL portions of water. Equilibrate the resin with 2.0 mL of 0.10 mol/L HCl. Put the stoppers in place and add 2.0 mL of 0.10 mol/L HCl and 500 μ L of sample or standard. Remove the stoppers and allow the columns to drain. Wash the col-

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Received July 17, 1980; accepted Dec. 30, 1980.

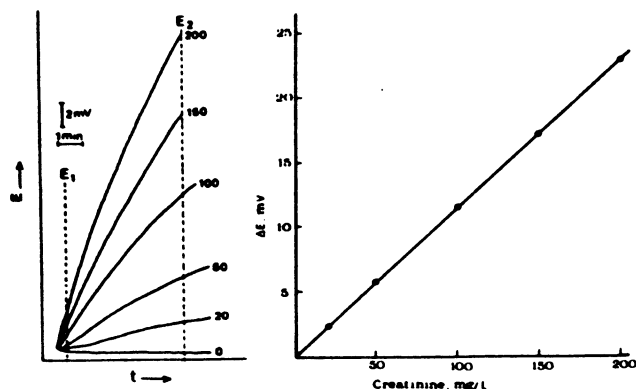


Fig. 1. Recorded curves of cell voltage vs. time for the Jaffé reaction and the corresponding calibration curve
Other conditions as under Procedure

umns with two 2-mL portions of water, remove the last drop from the tip of the column, then elute the creatinine into a test tube with two 2-mL portions of the above-described buffer. Allow the columns to drain completely after each step. The flow rate is about 1.5 mL/min. Transfer the eluate into the reaction cell thermostated at 37.0 °C, start the stirrer, and after about 1 min inject 100 μ L of 4.5×10^{-3} mol/L sodium picrate solution and immediately press the "start" switch of the printer. After 270 s (10 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, omitting the first wash with NaOH.

Calculations

Prepare working curves by plotting ΔE values ($\Delta E = E_{270} - E_{30}$) vs the creatinine concentrations of the standards, in milligrams per liter.

Results

Analytical Variables

The rate of the Jaffé reaction is first order with respect to picrate, hydroxide, and creatinine concentration (16). A 1 mol/L concentration of NaOH was chosen as optimum, to ensure short measurement times and satisfactory electrode operation. The concentration of picrate chosen, 1.1×10^{-4} mol/L, is a compromise to ensure a potential change of at least 24 mV before the lower limit of the linear response of the picrate-selective electrode (4.5×10^{-5} mol/L) is approached.

The rate of the Jaffé reaction increases with increasing temperature and ionic strength. To ensure short measurement times, we chose a temperature of 37 °C and a 1.6 mol/L final Na_2SO_4 concentration.

A premeasurement interval of 30 s ensures complete stabilization of the electrode response after the picrate solution is injected.

Response of the electrode. The response characteristics of the picrate-selective electrode were discussed elsewhere (15). However, because of the extreme conditions required for the creatinine determination (high ionic strength, high pH values) the electrode behavior under such conditions was re-examined. The electrode responded linearly over a concentration range of 4.5×10^{-5} to 1.0×10^{-2} mol of picrate per liter, with a slope of 59.0 mV per concentration decade (at 37 °C).

Linearity of the method. Figure 1 shows typical recorded curves and the corresponding calibration curve for the Jaffé reaction. There is good linearity between reaction rate and creatinine concentration in the range 0–200 mg/L.

Precision and accuracy. The accuracy of the method was checked with recovery experiments in which creatinine was added to serum samples (pathological, hemolyzed, turbid, and lipemic sera were included). Analytical recovery of added creatinine ranged from 92 to 114% (average 100.7%). Table 1 shows typical results of analytical-recovery studies.

The precision of the method was tested by analysis of commercial control sera. We analyzed by our method an assayed abnormal control serum (Hyland Division, Travenol Laboratories Inc., Costa Mesa, CA 92626) with target values given by the manufacturer ranging from 45 to 52 mg/L as measured by various methods. The mean we found was 46.6 mg/L, with a standard deviation of 3.0 mg/L ($n = 9$). An assayed normal control serum (Institut Merieux, Lyon, France), with target values given by the manufacturer ranging from 12.8 to 13.9 mg/L as measured by various methods, was analyzed by the proposed method. The mean we found was 12.0 mg/L, with a standard deviation of 2.0 mg/L ($n = 9$). The day-to-day precision of the method was assessed by repeated analysis of a pooled serum sample containing 67 mg of creatinine per liter, during a month. The method yielded a standard deviation of 5.4 mg/L (CV = 8.1%, $n = 15$).

Results by the proposed procedure (y) were compared with those by a photometric procedure (x) (17) by analyzing a series of patients' specimens by both procedures. The correlation coefficient (r) was 0.994 and the regression equation was $y = 1.051x - 0.091$ ($n = 100$).

Interference Studies

Recovery experiments (Table 1) indicated that hemolysis, lipemia, or turbidity have no effect on the accuracy of the creatinine determination by the proposed procedure.

We also examined the effect of various substances known to interfere with the Jaffé reaction, used in concentrations far in excess of those found in serum. For these experiments, we prepared creatinine/interferent mixtures and compared the results obtained with those obtained with pure 80 mg/L creatinine solutions. We found that glucose (up to 10 g/L), ascorbic acid (up to 1 g/L), acetone (up to 1 g/L), pyruvic acid (up to 250 mg/L), bilirubin (up to 200 mg/L), α -ketoglutaric acid (up to 1 g/L), and oxaloacetic acid (up to 250 mg/L) do not interfere. Pyruvic and oxaloacetic acid in concentrations

Table 1. Analytical Recovery of Creatinine Added to Serum Samples

Initially present ^a	Creatinine, mg/L			Recovery, %
	Added	Total	Found ^a	
8.8 ^b	49.5	58.3	58.7	101
	99.0	107.8	104.8	97
9.4	49.5	58.9	56.0	94
	99.0	108.4	109.6	101
19.0 ^c	49.5	68.5	69.8	103
	99.0	118.0	131.8	114
19.4	49.5	68.9	67.2	97
	99.0	118.4	127.6	109
26.4 ^d	49.5	75.9	71.7	92
	99.0	125.4	123.6	98
32.8	49.5	82.3	84.8	105
	99.0	131.8	130.6	99
63.0	49.5	112.5	114.2	103
	99.0	162.0	161.0	99
Av.				100.7

^a Average of two measurements. ^b Hemolyzed serum. ^c Turbid serum. ^d Grossly lipemic serum.

of 1 g/L caused a 10% positive error. Acetoacetic acid in concentrations of 250 mg/L and 1 g/L caused a 5 and 30% positive error, respectively. The interference of bilirubin was also examined in creatinine/bilirubin mixtures not treated on the ion-exchange columns. For these experiments, 4.00 mL of the buffer and 0.500 mL of each creatinine/bilirubin mixture were pipetted into the reaction cell and the procedure was continued from the point of starting the stirrer. Identical reaction-rate curves (E vs t) were obtained for a pure 100 mg/L creatinine standard and for creatinine/bilirubin mixtures containing, per liter, 100 mg of creatinine and up to 200 mg of bilirubin. We conclude that bilirubin does not react with picrate and does not affect the rate of the Jaffé reaction (8, 18).

Discussion

The proposed potentiometric method for creatinine in serum is an alternative to the numerous photometric methods currently used. The column procedure effectively removes the most important interfering compounds, which are not adsorbed on the ion-exchanger, and their effect is eliminated. The linearity is very useful, recovery is nearly 100%, and the precision is satisfactory.

The proposed potentiometric method has the advantage that it eliminates problems caused by optical interference—such as hemolysis, lipemia, and turbidity—because the physical quantity measured is potential, not absorbance. Only chemical interferences can affect potentiometric measurements. Although the interference from bilirubin in the kinetic Jaffé methods is well documented (8, 18), we found no interference from bilirubin on working with aqueous creatinine/bilirubin mixtures. If the explanation given by Watkins et al. (18) is correct—that bilirubin is oxidized to biliverdin by oxygen under alkaline conditions—such a change, which affects photometric kinetic procedures, leading to very low creatinine values for severely jaundiced sera, has absolutely no effect in the proposed potentiometric procedure. The column eluates can also be analyzed by continuous-flow systems that incorporate the picrate electrode as a potentiometric detector.

We thank A. Papanastasiou-Diamandi for technical assistance and H. Yatzidis for stimulating discussions.

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