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

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INTRODUCTION

Ion-selective electrodes and gas-sensing probes have already been employed for continuous-flow measurements in industrial process control, environmental or pollution research, and biomedical analysis. In earlier communications we described the picrate electrode (*4*) and its application to the manual potentiometric determination of creatinine in urine (*1*) and serum (*2*). In this paper, a continuous-flow kinetic potentiometric method is described for the determination of creatinine in urine, based on the Jaffé reaction. The reaction is monitored with a picrate-selective electrode, and the increase in electrode potential within a fixed period of time is related directly to the creatinine content of the sample. Forty samples of untreated urine can be analyzed per hour by this method. Sample consumption is 0.15 ml. The method is simple, specific, accurate, and sensitive. 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, 3.00×10^{-3} M. Dissolve 0.687 g of air-dried picric acid (Merck) in water and dilute to 1 liter. No standardization is required. Store in an amber bottle. The solution is stable for at least 1 year.

Ionic strength adjustment composite buffer, pH 12.0. Dissolve 134 g of Na_2HPO_4 $7H_2O$ (Merck) and 142 g of Na_2SO_4 (Merck) in about 800 ml of water (dissolution is aided by heating). Cool, adjust the pH to 12.0 by adding saturated NaOH solution, and dilute to 1 liter. The solution is stable at room temperature. If sedimentation is observed, dissolve it before use by heating.

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Standard creatinine solutions. (a) A stock solution of 10.00 g/liter was prepared by dissolving 1.000 g of pure creatinine (Merck; dried for 1 h at 100°C) in 100 ml of 0.100 *N* HCl. (b) Working standards of 0.500, 1.00, 2.00, and 3.00 g/liter were prepared from the stock solution by dilution with 0.1 *N* HCl. All creatinine standards were stored in a refrigerator when not in use. Standards are stable for at least 1 month.

Apparatus

The body of an Orion 92 electrode equipped with Teflon membranes (Millipore LCWPO 1300) was used. The Teflon membranes were cut to the appropriate sizes and placed in quadruplicate to avoid any leakage of the liquid ion exchanger. The flow-through channel was constructed by embedding the lower cup of the electrode in a plastic material (Serifix resin, H. Struers, Scientific Instruments, Copenhagen) and drilling holes of appropriate diameters, as shown in Fig. 1. Commercial flow-through cups for liquid membrane electrodes can also be used for this application. The picrate electrode was constructed as previously described (*4*). The reference electrode used was an Orion Model 90-01 single-junction Ag/AgCl electrode; it was filled with Orion's 90-00-01 solution. All potentiometric measurements were carried out with a Corning Model 12 Research pH/mV meter and recorded on a Sargent–Welch Model XKR potentiometric recorder. Sampler II and proportioning pump III were from the Technicon Auto-Analyser II system. The timing functions of the sampler

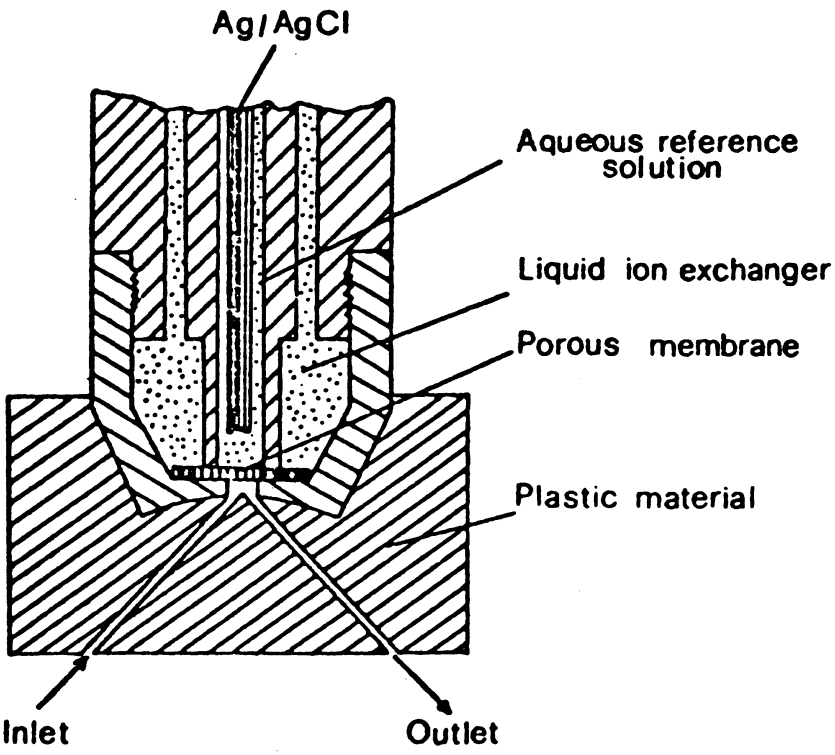

FIG. 1. The Orion 92 series liquid membrane electrode modified to be used in continuous-flow analysis systems.

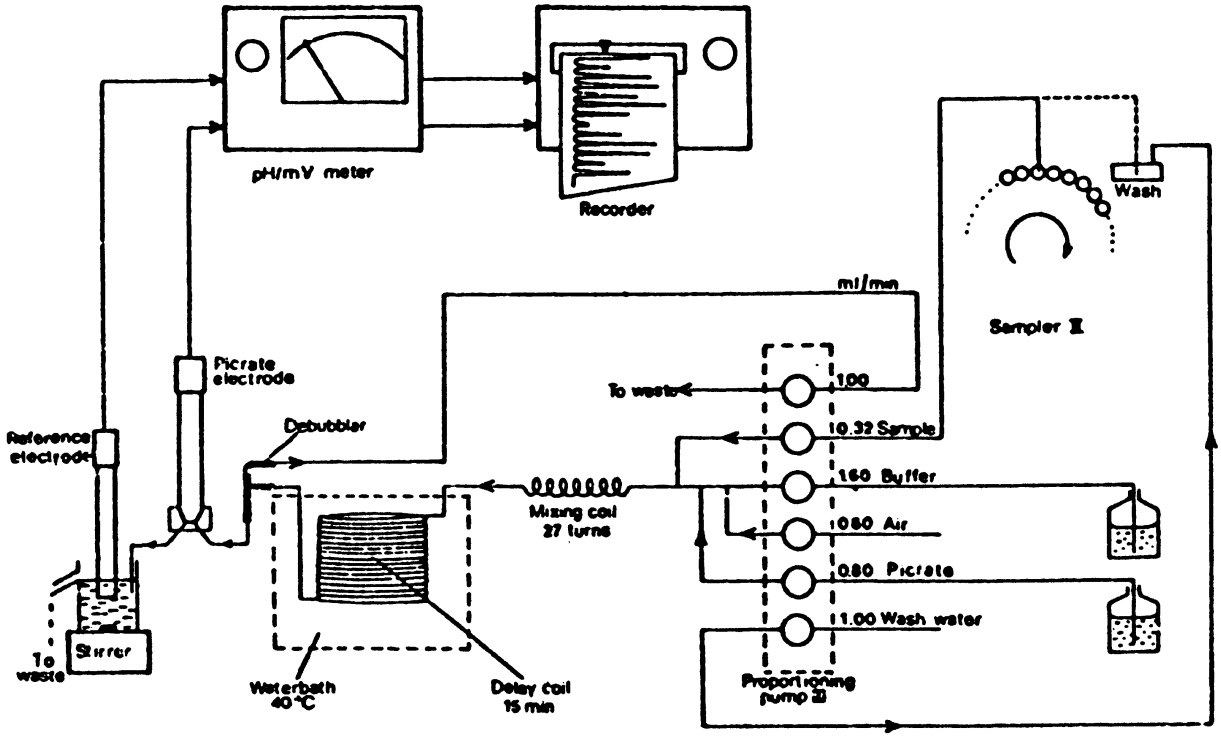

FIG. 2. Flow diagram of the automated analysis system.

II were controlled by a specially designed "sample-wash timer" connected to sampler II as described elsewhere (*3*). A schematic diagram of the automated analysis system is shown in Fig. 2.

Procedure

The optimum conditions for the analysis are indicated in Fig. 2. With urine samples loaded in the 2-ml plastic sample cups, the system is set to operate at 40 samples per hour with a 1:2 sample:wash ratio. The delay coil in the heated water bath provides an incubation period of ~15 min at 40°C, during which the Jaffé reaction proceeds. After debubbling, the stream reaches the sensing picrate electrode.

RESULTS AND DISCUSSION

The response characteristics of the picrate electrode were discussed elsewhere (*4*) and were found to be the same in the flow-through configuration.

Figure 3 shows a typical recording of a series of creatinine determinations carried out by the above procedure using calibration aqueous creatinine solutions. From the potential difference between the reagent baseline and the peak maxima (peak values in millivolts) and the known creatinine concentration (expressed in grams per liter) of the calibrating solutions, a calibration curve can be constructed (Fig. 4). The actual peak maximum of a 2 g/liter creatinine standard solution is about 75% of the

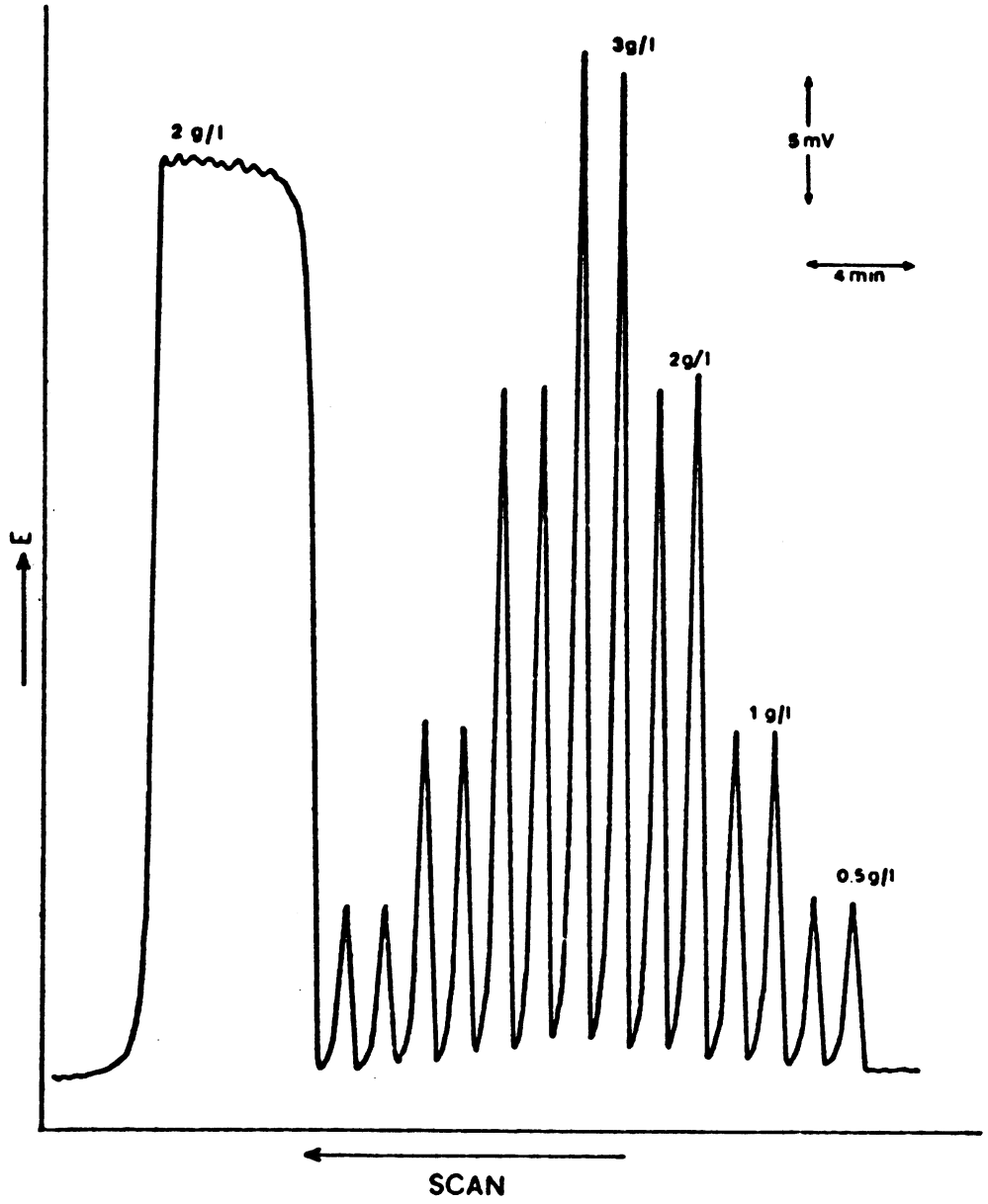

FIG. 3. Typical calibration sequence with aqueous creatinine standards.

peak maximum obtained under sample continuous-flow conditions (steady-state response) as is shown in Fig. 3.

The relative standard deviations for an aqueous 1 g/liter creatinine standard solution and for a urine control sample containing 0.97 g/liter creatinine (Lederle Diagnostics) were 0.84% (n = 9) and 1.3% (n = 14), respectively.

The accuracy of the method was checked with recovery experiments in which creatinine was added to urine samples (amount added: 0.45–0.91 g/liter). The recovery ranged from 90 to 111% with an average of 96.7%. The results of analytical recovery studies are shown in Table 1.

The accuracy of the proposed method was further tested by comparing values for urine with those obtained by a spectrophotometric method (5).

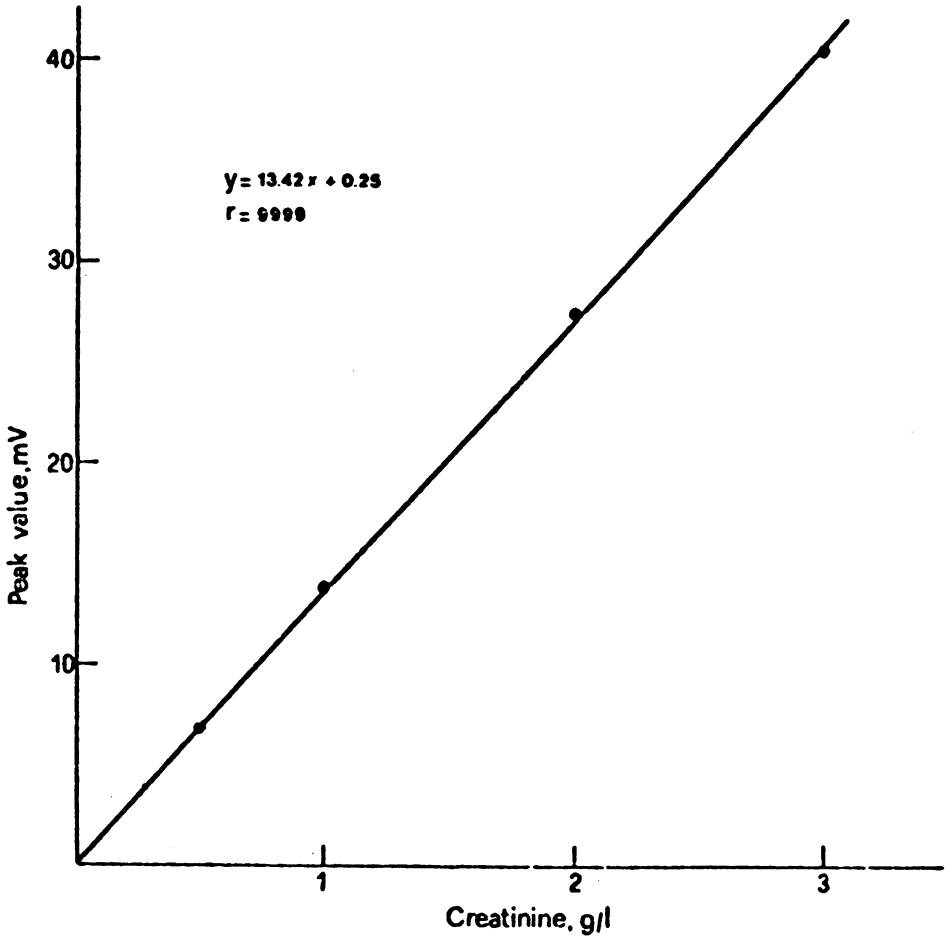

FIG. 4. Calibration curve for the determination of creatinine in urine.

Results from this comparison are shown in Fig. 5. The correlation coefficient r was 0.994 and the regression line was found to be of the form $y = 0.864x + 0.165$ ($n = 50$).

The specificity of the proposed method was tested by analyzing mix-

TABLE 1
ANALYTICAL RECOVERY OF CREATININE ADDED TO URINE SAMPLES

Creatinine, (g/liter)				
Initially present	Added	Total	Found	Recovery (%)
0.90	0.45	1:35	1.34	97.8
	0.91	1.81	1.78	96.7
1.25	0.45	1.70	1.68	95.6
	0.91	2.16	2.07	90.1
1.72	0.45	2.17	2.22	111.1
	0.91	2.63	2.59	95.6
0.90	0.45	1.35	1.32	93.3
	0.91	1.81	1.75	93.4
Average				96.7

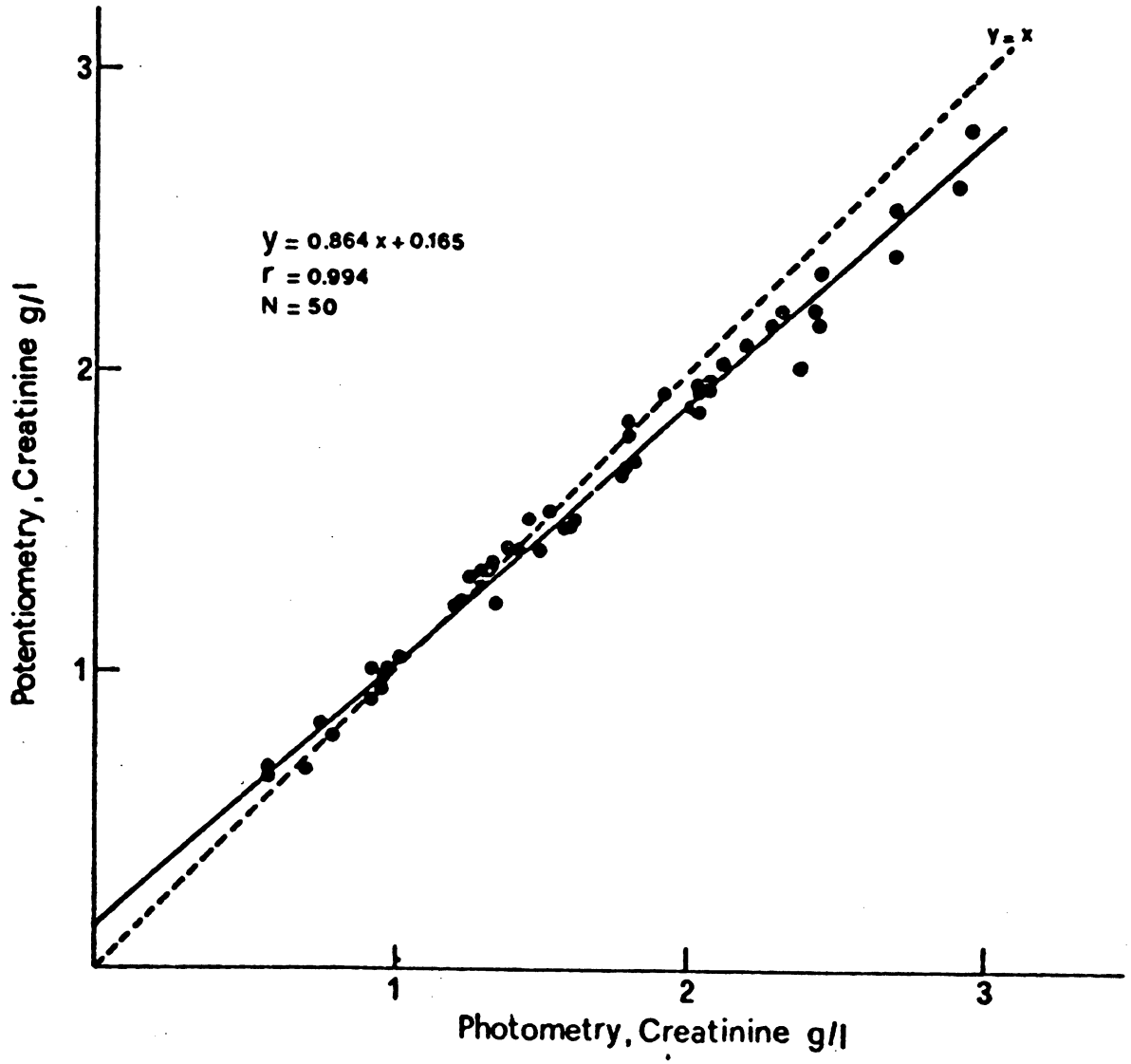

FIG. 5. Comparison of results by the automated method with results by the photometric method.

tures of creatinine (1.5 g/liter) and interferent. It was found that albumin up to 0.5 g/liter, glucose up to 3 g/liter, and ascorbic acid up to 0.8 g/liter cause errors smaller than 4% in all cases. However, it is known that only negligible amounts of the Jaffé-positive noncreatinine chromogens are found in urine.

In conclusion, the proposed kinetic potentiometric method is suitable for the continuous determination of creatinine in urine. It offers high sampling rate, accuracy, precision, and specificity. Urine samples do not need special dilutions or pretreatment before analysis. As for all potentiometric methods it is not affected by optical interference such as turbidity, colored samples, etc.

SUMMARY

A completely automated potentiometric method for the determination of creatinine in urine is described. Creatinine reacts with picrate in alkaline media (Jaffé reaction) in a flow

system, and the decrease in picrate activity is continuously monitored with a picrate-selective flow-through electrode. Creatinine in urine, in the range 0.5–3 g/liter, was determined in a sample volume of 0.15 ml, with a relative standard deviation of about 1%. Forty samples per hour can be analyzed without previous dilution or pretreatment. Recovery of creatinine added to urine samples ranged from 90 to 111% with an average of 96.7%. The method compares favorably with a photometric method. The proposed automated method is suitable for routine clinical measurements and screening tests.

REFERENCES

1. Diamandis, E. P., Koupparis, M. A., and Hadjiioannou, T. P., Kinetic potentiometric determination of creatinine in urine with a picrate ion-selective electrode. *Microchem. J.* 22, 498–504 (1977).
2. Diamandis, E. P., and Hadjiioannou, T. P., Kinetic potentiometric determination of creatinine in serum with a picrate ion selective membrane electrode. *Clin. Chem.* 27, 455–457 (1981).
3. Efstathiou, C. E., Papastathopoulos, D. S., and Hadjiioannou, T. P., A digital "sample-wash timer" for the Technicon sampler II. *Chem. Biomed. Environ. Instrum.* 11(1), 49–56 (1981).
4. Hadjiioannou, T. P., and Diamandis, E. P., Analytical study of a new picrate selective membrane electrode. *Anal. Chim. Acta;* 94, 443–447 (1977).
5. Henry, R. S., Cannon, D. C., and Winkelman, J. W., "Clinical Chemistry, Principles and Technics," 2nd ed., p. 545. Harper & Row, New York, 1974.