Kinetic Studies with Ion Selective Electrodes: Determination of Creatinine in Urine with a Picrate Ion Selective Electrode

A Laboratory Experiment

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Ion selective potentiometry has become a well established analytical method in the last few years. Several examples of its use in analytical applications and theoretical studies have been reported in the literature (*1–3*). The advantages of ion selective electrode (ISE) methodology over conventional methods of analysis are mainly the simplicity and the low cost of the equipment required. It is very easy to set up experiments which can be used effectively to demonstrate the basic characteristics of ISE (*4*).

Kinetic methods of analysis often have advantages over equilibrium (end point) techniques, especially when mixtures of closely related compounds (e.g., isomers) or compounds that react slowly or catalytically acting compounds (e.g., enzymes) are to be analyzed (*5*). The selectivity and the sensitivity of kinetic methods of analysis combined with the selectivity and sensitivity of ISE provide an excellent and versatile combination, which may lead to totally new analytical schemes.

Creatine is a physiological component in blood, brain, and muscles and is important in the flow of energy in muscle tissue. Its final catabolic product is creatinine which is excreted in urine via the kidneys. Creatinine level in serum and urine is a very sensitive index of renal function since increase in serum and decrease in urine demonstrate renal failure. The ratio of urine to serum creatinine level in conjunction with urine flow rate is a significant clinical test for renal examination (Creatinine Clearance Test, C_{cr}).

Various methods have been described for the determination of creatinine in serum and urine. Most of them are based on the reaction of creatinine(C) and picrate(P) in alkaline medium to form a 1:1 red complex (Jaffé reaction) (*6, 7*). Recently, we reported the kinetic determination of creatinine in urine with a picrate ISE (*8*).

In this experiment, the kinetic study of the Jaffé reaction with the picrate ISE and a kinetic method for the determination of creatinine in urine is presented. The exercise could be used to familiarize the students with the application of ISE in kinetic studies and clinical analysis.

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The picrate ISE response is expressed by the Nernst equation

$$E = E' - \frac{RT}{F} \ln a_P \qquad (1)$$

where E is the measured total potential of the electrochemical system, E' is the portion of the total potential due to reference electrode and internal filling solution, R and F are the ideal gas and Faraday constants, respectively, T is the absolute temperature, and a_P is the picrate activity. Using picrate solutions of high constant ionic-strength the picrate concentration [P] can be used in eqn. (1) instead of the activity.

When dilute picrate solutions react with an excess (at least tenfold) of creatinine in alkaline media, if the reaction is first

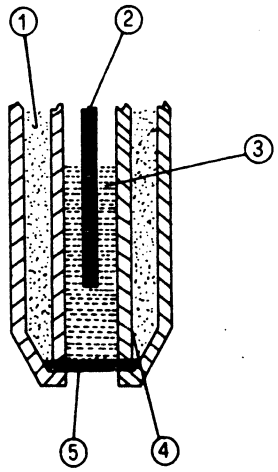

Figure 1. Details of construction of the picrate electrode.
1. Tetrapentylammonium picrate dissolved in 2-nitrotoluene (liquid ion exchanger).
2. Internal reference electrode, Ag/AgCl.
3. Aqueous internal reference solution.
4. Electrode body.
5. Porous hydrophobic membrane (Millipore, Teflon®, LCWPO 1300).

order with respect to picrate, the picrate concentation $[P]_t$ at any time t is given by

$$[P]_t = [P]_0 \cdot e^{-K_{obs} \cdot t} \quad (2)$$

where $[P]_0$ is the picrate concentration at $t = 0$ and K_{obs} is the observed pseudo-first order rate constant. By taking the logarithm of eqn. (2) we have

$$\ln [P]_t = \ln [P]_0 - K_{obs} \cdot t \quad (3)$$

Combining eqns. (1) and (3) we have

$$E_t = E' - \frac{RT}{F} \ln [P]_0 + \frac{RT}{F} \cdot K_{obs} \cdot t = Constant + \frac{RT}{F} \cdot K_{obs} \cdot t \quad (4)$$

It can be seen from eqn. (4) that if the reaction is first order with respect to picrate while the other reactants (C and OH^-) are in great excess, the E versus t plots are linear with a slope equal to $K_{obs} \cdot RT/F$.

The electrode response is usually expressed by eqn. (5) instead of eqn. (1).

$$E = E' - \frac{2.303\,RT}{F} \log [P] = E' - S \log [P] \quad (5)$$

Factor S is called the slope of the electrode and is calculated experimentally from the calibration curve of the electrode, i.e., the E versus log [P] plot. The constant K_{obs} can be calculated from the linear E versus t reaction curve because the slope of such a curve is $K_{obs}(RT/F) = K_{obs} \cdot S/2.303$. Therefore,

$$K_{obs} = \frac{Slope\ of\ E\ versus\ t\ reaction\ curve\ (mV/s)}{Slope\ of\ the\ electrode\ S(mV)} \times 2.303 \quad (6)$$

Experimental

The Picrate Ion Selective Electrode

The picrate electrode was constructed and stored as previously described (9). A single junction Ag/AgCl reference electrode was used in conjunction with the picrate electrode. Details of the electrode construction are shown in Figure 1. The measuring electrochemical cell is

Ag, AgCl	Cl^-(0.1 M), P(0.01 M)	Membrane	Sample	Ag, AgCl
Internal reference electrode	Internal reference solution	Containing $(C_5H_{11})_4NC_6H_2N_3O_7$ Liquid ion-exchanger	solution electrode	External reference

A block diagram of the recording and measurement system is shown in Figure 2.

The electrode potential was measured with an Orion Model 801 digital pH/pIon meter and recorded either by an Orion digital printer (Model 751) or a Heath-Shlumberger recording system. Instead of the printer or the recording system, a stopwatch can be used to read the potential from the digital electrometer at fixed time intervals. From the printed or recorded values of E versus time the slope of the reaction curve in mV/s can be calculated easily.

All measurements were carried out at 25°C in a thermostated cell under constant magnetic stirring.

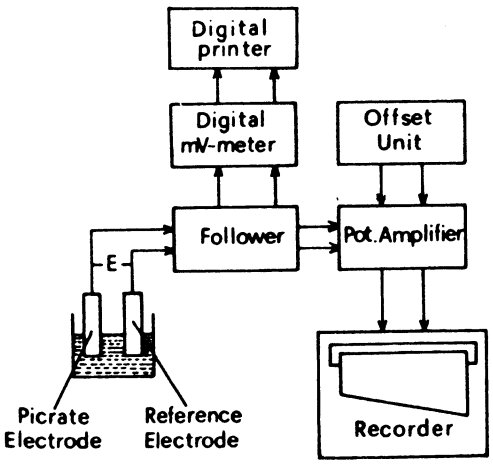

Figure 2. Block diagram of the recording and measurement system.

Reagents

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

Creatinine stock solution, 10.00 g/l, in 0.005 M H_2SO_4. More dilute working solutions of 0.500, 1.000, 2.00, and 3.00 g/l are prepared by dilution with 0.005 M H_2SO_4 solution. All creatinine solutions are stored in a refrigerator when not in use.

Sodium picrate stock solution 0.100 M, is prepared by dissolving 22.9 g of air-dried picric acid (**CAUTION! Do not heat solid picric acid; it is explosive**) in about 900 ml of water, neutralizing to a pH of about 6 with 5 M NaOH solution and diluting to 1 l. More dilute standard solutions, 3.00×10^{-2}, 1.00×10^{-2}, 3.00×10^{-3}, 1.00×10^{-3}, and 3.00×10^{-4} M (50 ml of each), are prepared by serial dilution.

Composite solutions of Na_2SO_4-NaOH, of ionic strength $\mu = 1.00$, are prepared by appropriate mixing of 1.000 M NaOH(Titrisol) and 1.00 M Na_2SO_4 solutions, as follows: S_1 (0.2 M NaOH):100.0 ml NaOH + 133.3 ml Na_2SO_4, dilution with water to 500 ml. S_2 (0.1 M NaOH): 10.00 ml NaOH + 30.0 ml Na_2SO_4, dilution to 100 ml. S_3(0.05 M NaOH):5.00 ml NaOH +31.7 ml Na_2SO_4, dilution to 100 ml. S_4(0.02 M NaOH):2.00 ml NaOH +32.7 ml Na_2SO_4, dilution to 100 ml.

Measurements and Calculations

Step 1: Calibration Curve of the Picrate Electrode

Pipet into the thermostated reaction cell 25.00 ml of solution S_1 and 1.00 ml of 3.00×10^{-4} M sodium picrate solution. Start the stirrer, and after 1–2 min record the potential to ±0.01 mV. Repeat the procedure with all standard picrate solutions ($1.00 \times 10^{-3} - 1.00 \times 10^{-1}$ M). Calculate the final picrate concentration, [P], in each case, and construct the plot E versus log [P] (calibration curve). Estimate the slope of the electrode, S (it is nearly Nernstian ~ 59 mV).

Step 2: Effect of Creatinine Concentration on the Reaction Rate

Pipet into the reaction cell 25.00 ml of solution S_1 and 0.500 ml of 1.00×10^{-2} M sodium picrate solution. Start the stirrer and after the potential has been stabilized (1–2 min) add rapidly 2.00 ml of each of the standard creatinine solutions (0.5–3 g/l). Record the reaction curves or print the electrode potential every 0.5 min or take three potential values at fixed time intervals (e.g., every 1 min). From these data estimate the reaction curve slope (mV/s) at any creatinine concentration. Draw a plot of reaction curve slope ($\Delta E/\Delta t$) versus creatinine initial concentration (g/l) and use it to analyze a number of urine samples following exactly the above procedure. From the estimated reaction curve slopes and the slope of the electrode S calculate K_{obs} by eqn. (6) and draw a plot of K_{obs} versus final creatinine concentration (the molecular weight of creatinine is 113.11).

Step 3: Effect of Hydroxide Concentration on the Reaction Rate

Pipet into the reaction cell 25.00 ml of solution S_1 and 0.500 ml of 1.00×10^{-2} M sodium picrate solution. Start the stirrer and after the potential has been stabilized add rapidly 2.00 ml of 3.00 g/l creatinine solution and record, print, or take three potential values as above (step 2). Repeat the procedure with solutions S_2, S_3, and S_4. From the collected data calculate the reaction curve slope and, by eqn. (6), K_{obs} at any hydroxide final concentration. Draw a plot of K_{obs} versus final hydroxide concentration.

Results and Discussion

Typical data used to construct the calibration curve of the picrate electrode and estimate the slope of the electrode are shown in Table 1. The slope was found, 58.6 mV, and the calibration curve shows a very good linearity ($r = 0.99997$) over the concentration range used.

Recorded curves for the picrate-creatinine reaction at various creatinine concentrations are shown in Figure 3.

The calculated reaction curve slopes ($\Delta E/\Delta t$) used to construct a typical calibration curve for urine creatinine analysis are shown in Table 2.

Using the procedure described in step 2, a Lederle Urine

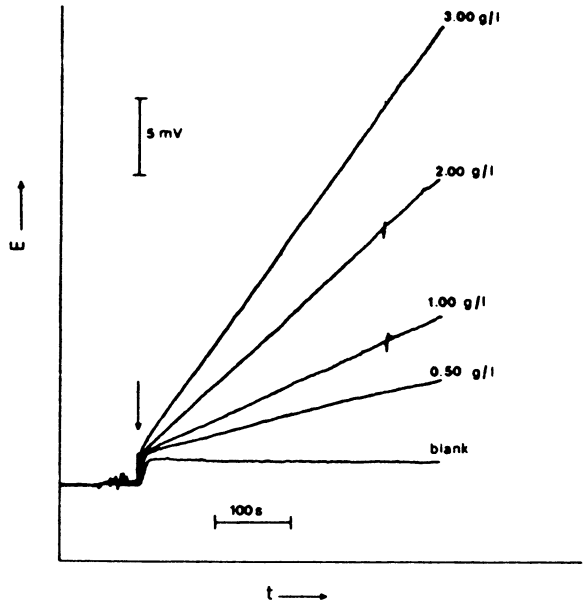

Figure 3. Recorded curves of cell voltage versus time for the picrate-creatinine reaction at 25°C, at various creatinine concentrations (indicated). Other conditions as under procedure, step 2.

Table 1. Typical Measurements Used for the Calibration Curve of Picrate Electrode

Picrate Final concentration, M^a	− log [P]	E, mV[b]
1.154×10^{-5}	4.938	213.4
3.846×10^{-5}	4.415	183.6
1.154×10^{-4}	3.938	155.4
3.846×10^{-4}	3.415	124.1
1.154×10^{-3}	2.938	96.2
3.846×10^{-3}	2.415	66.2

Slope = 58.6 mV, r = 0.99997
[a] In 0.2 *M* NaOH, μ = 1.00
[b] Single measurement

Table 2. Calibration Curve Used for Creatinine Determination [a]

Creatinine, g/l	Reaction Curve Slope (mV/s)[b]
0.500	0.0118
1.00	0.0227
2.00	0.0458
3.00	0.0690

[a] Calibration curve: slope = 0.02294, intercept = 0.000049, r = 0.99995
[b] Single measurement

Control of 0.97 g/l creatinine concentration (Autoanalyzer, Jaffé, expected range of 0.83–1.11) was analyzed and was found to contain 1.00 g/l creatinine. The RSD was 0.8% for five measurements. Students will grow enthusiastic determining their own urine creatinine.

From the linear reaction curves shown in Figure 3 it is clear that the assumption that the reaction is first order in picrate is correct. So at this time the rate is

$$Rate = K_{obs}[P] \quad (7)$$

A plot of K_{obs} versus [C] obtained as described in step 2 is shown in Figure 4. The plot is linear and passes through the origin, indicating that the reaction is first order in creatinine. (This will be clearer by plotting ln K_{obs} versus ln [C]. The slope of this plot will be 1, the creatinine reaction order.) The rate at this time is given by

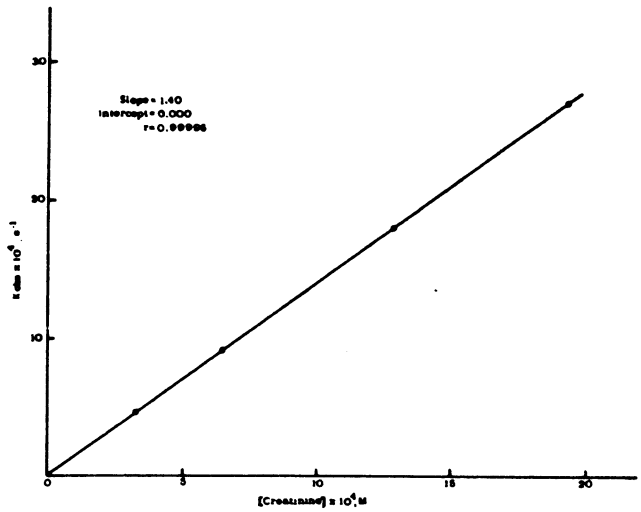

Figure 4. K_{obs} versus creatinine concentration plot at 25°C to calculate K_1.

$$Rate = K_1[P][C] \quad (8)$$

where K_1 is the second order rate constant and its value is calculated from the K_{obs} versus [C] plot (the slope in Figure 4). A value of 1.40 M^{-1} s^{-1} was found.

From the reaction curves obtained from the measurements in step 3 a similar plot of K_{obs} versus hydroxide concentration is drawn. Such a plot is linear and passes through the origin indicating that the reaction is first order in hydroxide. Therefore, the rate is given by

$$Rate = K_2[P][OH^-] \quad (9)$$

where K_2 is the second order rate constant and its value is equal to the slope of the K_{obs} versus [OH$^-$] plot. A value of 0.0150 M^{-1} s^{-1} was obtained (r = 0.998).

By combining eqns. (8) and (9) we have the reaction rate law of the Jaffé reaction

$$Rate = K[P][C][OH^-] \quad (10)$$

where K is the rate constant and is calculated from eqns. (11) or (12), at constant hydroxide or creatinine concentration (μ = 1.00 and temperature 25° C at all cases), respectively:

$$K = \frac{K_1}{[OH^-]} = 7.7\ M^{-2}\ s^{-1} \quad (11)$$

$$K = \frac{K_2}{[C]} = 7.8\ M^{-2}\ s^{-1} \quad (12)$$

In conclusion, it should be pointed out that this experiment could significantly help students to understand how ion selective electrode technology can be used in kinetic studies and clinical analysis.

Acknowledgment

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