

## Study of the Stoichiometry and the Kinetics of the Jaffé Reaction with a Picrate Ion-Selective Electrode

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### INTRODUCTION

The picrate-creatinine reaction (Jaffé reaction) is commonly used to determine creatinine in biological fluids. Studies on the structure and the stoichiometry of the main colored species formed and on the kinetics of the Jaffé reaction have been made by several workers (2-5, 12-15). In previous communications we described the construction of a picrate electrode (11) and its application to the kinetic potentiometric determination of creatinine in urine (7) and serum (8), based on the Jaffé reaction. In this paper, a potentiometric study of the Jaffé reaction is described and data about the stoichiometry and the kinetics of the reaction is presented. The study included the determination of the order of the reaction with respect to the chemical species involved in it. The ionic strength and temperature dependence and the activation energy for the reaction were also estimated.

### EXPERIMENTAL

#### *Apparatus*

The picrate electrode was constructed as previously described (11) but Teflon membranes were used (Millipore LCWPO 1300). The membranes were cut to appropriate sizes and put in quadruplicate to avoid leakage of the liquid ion exchanger. A single junction Ag/AgCl electrode filled with Orion's 90-00-01 solution was used as external reference electrode. All measurements were carried out in a 50-ml thermostated cell, at constant magnetic stirring. The electrode potential was measured with a Corning Model 12 Research pH/mV meter and recorded on a Sargent Model XKR potentiometric recorder.

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### Reagents

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

*Sodium picrate stock solution, 0.1000 M.* Dissolve 22.91 g of air-dried picric acid (Merck; standardized against NaOH) in about 900 ml of water, neutralize to a pH of 6 with 5 M NaOH solution and dilute to 1 liter. All solutions for the potentiometric studies were made from this solution by dilution. Picrate solutions stored in amber-colored bottles at room temperature are stable for at least 1 year.

*Creatinine solutions.* Prepare a stock solution, 0.500 M, by dissolving 2.828 g of creatinine (Merck; dried for 1 h at 100°C) in water and diluting to 50 ml. More dilute standard solutions were prepared as needed by dilution with water. All creatinine solutions must be prepared daily.

*Sodium hydroxide solutions.* Prepare a stock solution, 10.00 M, by dissolving 400 g of sodium hydroxide (Merck) in water and diluting to 1 liter. This solution was standardized by titration with standard hydrochloric acid solution. More dilute standard solutions were prepared as needed by dilution with water.

*Sodium sulfate solutions.* Prepare a 1.00 M solution by dissolving 142 g of anhydrous sodium sulfate (Merck) in water and diluting to 1 liter. Dissolution is aided by heating. A 0.30 M solution was prepared by dilution of the 1.00 M solution with water.

### Procedures

*Calibration curve.* Pipet into the thermostated reaction cell 30.00 ml of water or appropriate NaOH or Na<sub>2</sub>SO<sub>4</sub> solution, start the stirrer, and add 0.0100 ml of 0.1000 M sodium picrate solution. After the potential has stabilized, record the potential value,  $E$ , and continue with new additions of sodium picrate solution (appropriate additions are 0.0200, 0.0500, 0.100, 0.200, 0.500, 1.00, and 2.00 ml). Record the potential after stabilization in each case. Calculate the final picrate concentration,  $[P]$ ,<sup>2</sup> and plot  $E \log [P]$  to find the slope  $S$  (typically  $-58.5$  mV at 27°C).

*Stoichiometry of the picrate-creatinine reaction at various hydroxide concentrations.* Pipet into the reaction cell 10.00 ml of  $2.00 \times 10^{-2}$  M sodium picrate solution and 10.00 ml of water. Add 2.00 ml of 10.00 M sodium hydroxide solution and start the stirrer. After the potential has stabilized to  $\pm 0.1$  mV (this is shown from the recorder trace), record the

<sup>2</sup> Nonstandard abbreviations used: P, picrate; C, creatinine; PC, picrate-creatinine complex;  $[P]_0$  and  $[C]_0$ , picrate and creatinine concentrations in the cell at  $t = 0$ , respectively;  $[ ]$ , analytical concentration (moles per liter) at any time  $t$ ;  $k_{\text{obs}}$ , observed rate constant. Charges are omitted for convenience.

potential value ( $E_1$ ). Repeat as above, replacing the water with 10.00 ml of  $1.00 \times 10^{-2} M$  creatinine solution ( $E_2$ ). Calculate the stoichiometry of the reaction from the expression

$$\text{mol picrate/mol creatinine} = 2 [1 - \text{antilog} (\Delta E/S)],$$

where  $\Delta E = E_2 - E_1$ , and  $S$  is the slope of the electrode. Repeat the procedure by replacing the 10.00  $M$  sodium hydroxide solution with 7.50, 5.00, and 2.50  $M$  sodium hydroxide solutions and repeat as above.

*Stoichiometry of the picrate-creatinine reaction at various  $[P]_0/[C]_0$  ratios.* Proceed as above using 1.00 ml of 10.00  $M$  sodium hydroxide solution in all cases, and creatinine concentrations of  $1.60 \times 10^{-2}$ ,  $1.20 \times 10^{-2}$ ,  $8.00 \times 10^{-3}$ , and  $4.00 \times 10^{-3} M$ . Calculate the stoichiometry from the expression

$$\text{mol picrate/mol creatinine} = a[1 - \text{antilog} (\Delta E/S)],$$

where  $a$  is taken as 1.25, 1.67, 2.50, and 5.00, for each creatinine concentration, respectively.

*Kinetic studies.* In all cases, the final volume of the solution in the cell was 30.00 ml. The reaction was initiated with the creatinine solution (it was found the most suitable). The general procedure is as follows: pipet into the reaction cell 15.00 ml of sodium hydroxide solution (appropriate concentrations were used, to achieve the desired final hydroxide concentration), 10.00 ml of sodium sulfate solution (1.00 or 0.30  $M$ ), 4.00 ml of sodium picrate solution (appropriate concentrations were used) and start the stirrer. After the potential has been stabilized to  $\pm 0.2$  mV, inject 1.00 ml of creatinine solution (appropriate concentrations were used) and record the potential for a few minutes. A modification of this general procedure was used when working at ionic strength values of 2.00, by replacing the volumes of sodium hydroxide (5.00 ml were used instead of 15.00 ml) and sodium sulfate (20.00 ml of the 1.00  $M$  solution was used instead of 10.00 ml).

## RESULTS AND DISCUSSION

### *Electrode Response*

An evaluation of the picrate electrode response was carried out, at various background solutions and at 27 and 37°C. The slope of the electrode was calculated by regression analysis in the range  $p[P]$  from 1.94 to 4.48. Solutions tested were in sodium hydroxide from 0.20 to 1.00  $M$  and in sodium sulfate from 0.15 to 1.00  $M$ . It was found that the electrode behavior is nearly Nernstian (slope,  $S$ ,  $-58.5$  mV at 27°C or  $-60.7$  mV at 37°C) in all cases. A slight decrease in the electrode slope (1.5 mV) was noticed at 1.00  $M$  sodium sulfate solution. It is then concluded, that

the electrode response is quite satisfactory even at highly alkaline solutions. This electrode characteristic makes it a useful tool in the study of the Jaffé reaction.

It has been reported that a highly alkaline solutions some kind of interaction occurs between picrate and hydroxide (1, 6, 10). From our results it seems that the electrode response is not affected from this kind of interaction.

In the case of changing the sodium hydroxide concentration in the reaction cell, the electrode needs a few minutes to produce a stable potential (electrode memory). However, when working at the same hydroxide concentration the electrode response is very rapid.

### *Stoichiometry of the Picrate-Creatinine Reaction*

Typical recordings for the calculation of the picrate-creatinine reaction stoichiometry are shown in Fig. 1. For a constant ratio of  $[P]_0/[C]_0$ , the stoichiometry of the Jaffé reaction was calculated at various hydroxide concentrations (Table 1). The effect of the ratio  $[P]_0/[C]_0$  on the stoichiometry of the Jaffé reaction was studied at constant hydroxide concentration (Table 2). Potential values for the determination of the picrate-creatinine reaction stoichiometry were reproducible to  $\pm 0.2$  mV.

The values given in Tables 1 and 2 for the stoichiometry of the picrate-creatinine complex do not take into the account the dissociation of the complex, because of the large value of its formation constant  $K_f$  (reported values for  $K_f$  vary from 4800 (3, 4) to 18,200 (15)). When the dissociation

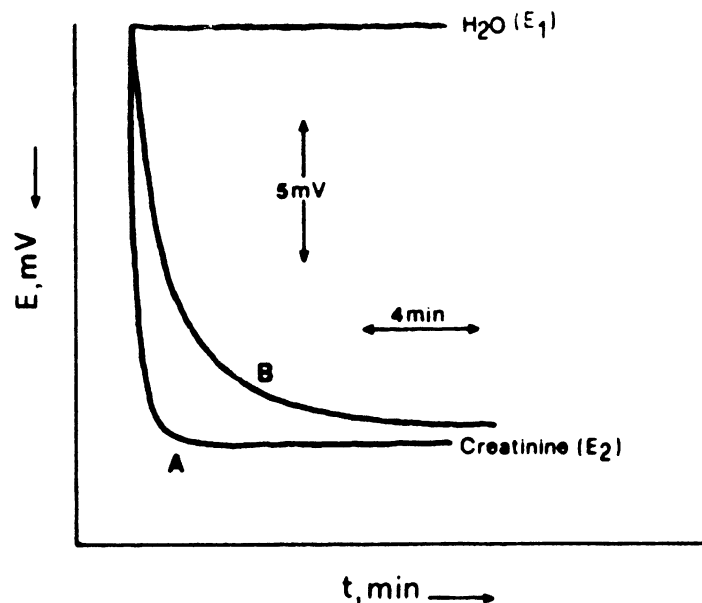


FIG. 1. Typical recordings for the calculation of the picrate-creatinine reaction stoichiometry. Conditions:  $[P]_0 = 9.09 \times 10^{-3} M$ ;  $[C]_0 = 4.54 \times 10^{-3} M$ ;  $[NaOH]$  (M) (A) 0.91; (B) 0.23. Temperature: 27°C. Other conditions as under Procedures.

TABLE 1  
EFFECT OF HYDROXIDE CONCENTRATION ON THE STOICHIOMETRY OF THE JAFFÉ REACTION AT 27°C

[NaOH] (M)	0.23	0.45	0.68	0.91
mol P/mol C	0.88	0.89	0.92	0.91

of the complex is taken into account the values for its stoichiometry are affected slightly. It is therefore concluded that the product of the Jaffé reaction is a 1:1 adduct, but a portion of the product is a 1:2 adduct between picrate and creatinine (12). The sodium hydroxide concentration does not seem to play any role in the composition of the product. It is shown (Table 2) that the ratio  $[P]_0/[C]_0$  affects the stoichiometry, and the portion of the 1:1 product increases with increasing  $[P]_0/[C]_0$  ratio.

It has been reported (9, 13) that the product of the Jaffé reaction is a 1:1 adduct between picrate and creatinine and this is in general agreement with our results. Kohashi *et al.* (12) found that a 1:2 complex between picrate and creatinine is formed even in the reaction of creatinine with excess picric acid. Blass and Thibert (2) reported in their polarographic study of the Jaffé reaction that at 1:1 ratio between picrate and creatinine the final product is 90% 1:1 and 10% 1:2 adduct.

*Picrate-Creatinine Reaction Kinetics with Creatinine in Excess*

In all the kinetic studies it is assumed that picrate and creatinine react on a 1:1 basis.

The picrate electrode exhibits near-Nernstian potential behavior for picrate at concentrations  $10^{-2}$  to  $3.3 \times 10^{-5} M$  in the pH range 3 to 14 and at constant ionic strength in accordance with

$$E = E' - \frac{RT}{F} \ln [P], \tag{1}$$

where  $E$  is the measured total potential of the system,  $E'$  is the portion of the total potential due to choice of the reference electrodes, internal solution, and ionic strength effects,  $R$  and  $F$  are the ideal gas and Faraday constants, respectively,  $T$  is the absolute temperature, and  $[P]$  is the concentration of picrate.

TABLE 2  
EFFECT OF THE RATIO  $[P]_0/[C]_0$  ON THE STOICHIOMETRY OF THE JAFFÉ REACTION AT 27°C

$[P]_0/[C]_0$	5.00	2.50	1.67	1.25
mol P/mol C <sup>a</sup>	0.96	0.92	0.89	0.85

<sup>a</sup>  $[P]_0 = 9.52 \times 10^{-3}$ . [NaOH] = 0.48 M. Other conditions as under Procedures.

When dilute picrate solutions react with an excess of creatinine (at least 10-fold) in alkaline media, and the reaction is first order with respect to picrate, the picrate concentration at any time  $t$  is given by

$$[P] = [P]_0 e^{-k_{\text{obs}} t}, \quad (2)$$

where  $k_{\text{obs}}$  is the observed pseudo-first-order rate constant. Taking the logarithms of Eq. (2) and combining with Eq. (1) give

$$E = E' + \frac{RT}{F} k_{\text{obs}} t, \quad (3)$$

where  $E' = E' - (RT/F) \ln [P]_0$ .

Thus, a plot of  $E$  versus  $t$  should be linear with a slope independent of initial picrate concentration.  $k_{\text{obs}}$  can be calculated from

$$k_{\text{obs}} = - \frac{\text{slope of the } E \text{ vs } t \text{ plot}}{\text{slope of the electrode, } S} \times 2.303 \quad (4)$$

because  $S = -2.303 RT/F$  and it was found experimentally to be  $-58.5$  mV. It can be seen from Eq. (4) that the kinetics of the Jaffé reaction can be studied by measuring the change of potential with time.

Values of  $k_{\text{obs}}$  at various initial picrate concentrations are shown in Table 3.

The  $E$  versus  $t$  plots, taken to calculate  $k_{\text{obs}}$  from Eq. (4), were linear indicating that the assumption that the reaction is first order with respect to picrate is correct. Another evidence that the reaction is first order in picrate is that  $k_{\text{obs}}$  is independent of initial picrate concentration (Table 3).

Recorded curves for the picrate-creatinine reaction ( $E$  versus  $t$  plots) at various creatinine concentrations are shown in Fig. 2. The curves are linear.

The variation of  $k_{\text{obs}}$  with creatinine concentration is given in Table 4.

If we assume that  $k_{\text{obs}} = k_1 [C]^n$  and take the logarithms, we have  $\log k_{\text{obs}} = \log k_1 + n \log [C]$ . Thus, if we plot  $\log k_{\text{obs}}$  vs  $\log [C]$ , the constants  $k_1$  and  $n$  can be determined from the intercept and the slope of the linear curve, respectively. Such a plot gave the regression equation  $y = (0.99 \pm 0.02)x - (0.310 \pm 0.041)$ , and correlation coefficient,  $r = 0.9997$ . It

TABLE 3  
VARIATION OF  $k_{\text{obs}}$  WITH PICRATE CONCENTRATION IN THE JAFFÉ REACTION AT 27°C

$[P]_0$	$5.00 \times 10^{-4}$	$1.00 \times 10^{-3}$	$2.00 \times 10^{-3}$
$k_{\text{obs}} (\text{sec}^{-1})^a$	0.00826	0.00834	0.00791

<sup>a</sup>  $[\text{NaOH}] = 0.0500 M$ ,  $[\text{C}]_0 = 1.67 \times 10^{-2} M$ ,  $\mu = 1.00$  ( $\text{Na}_2\text{SO}_4$ ).

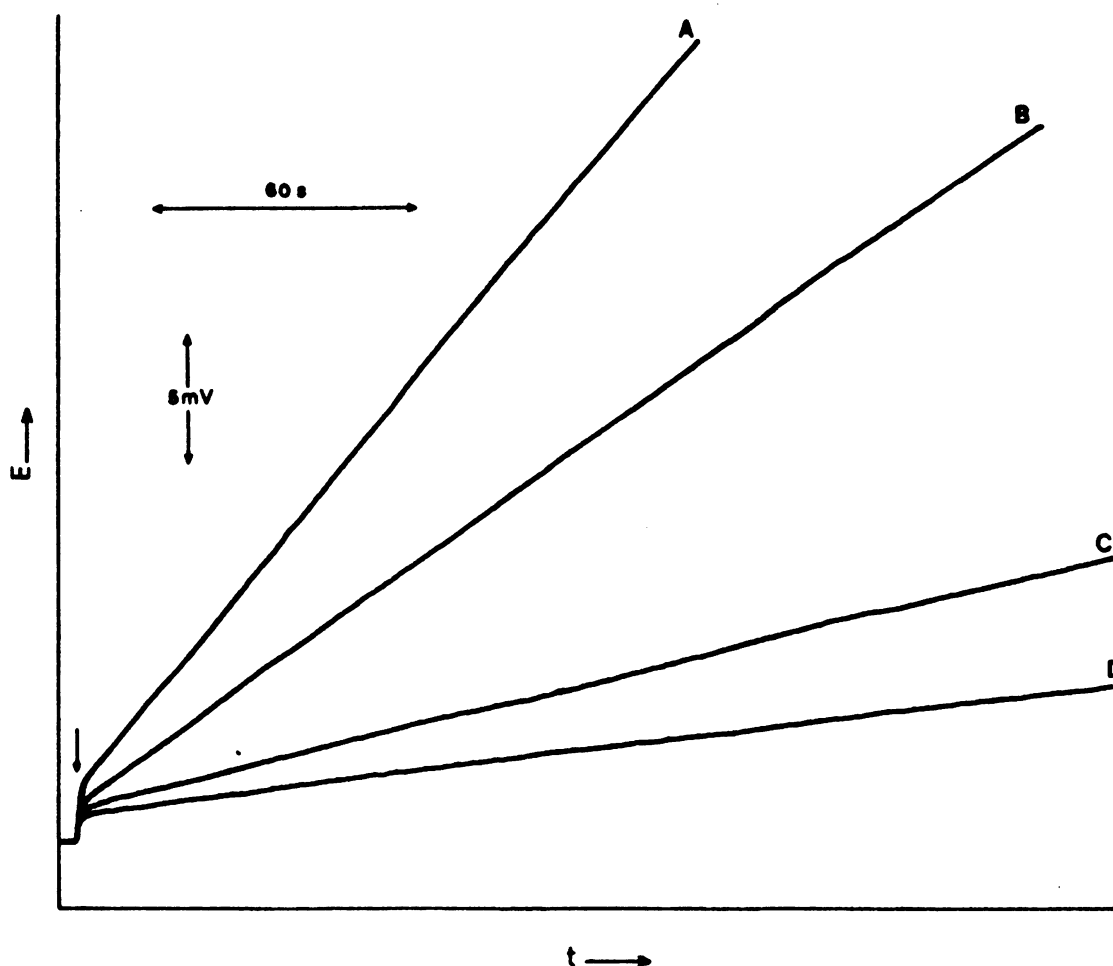


FIG. 2.  $E$  versus  $t$  plots for the picrate-creatinine reaction at  $27^{\circ}\text{C}$ , at various creatinine concentrations: ( $M$ ) (A)  $1.67 \times 10^{-2}$ ; (B)  $1.00 \times 10^{-2}$ ; (C)  $3.33 \times 10^{-3}$ ; and (D)  $1.67 \times 10^{-3} M$ . Other conditions:  $[P]_0 = 5.00 \times 10^{-4} M$ .  $[\text{NaOH}] = 0.0500$ .  $\mu = 1.00$  ( $\text{Na}_2\text{SO}_4$ ). ( $\downarrow$ ) Creatinine addition.

can be seen that the picrate-creatinine reaction is first order with respect to creatinine. A more accurate value for  $k_1$  can be obtained from a plot of  $k_{\text{obs}}$  versus  $[C]$ . When  $n = 1$ , such a plot is linear passing through the origin, and has a slope equal to  $k_1$  (in  $M^{-1} s^{-1}$ ). The plot of  $k_{\text{obs}}$  vs  $[C]$  gave the regression equation  $y = 0.516x + 4.1 \times 10^{-7}$ ,  $r = 0.9994$ .

The variation of  $k_{\text{obs}}$  with hydroxide concentration is given in Table 5.

A plot of  $\log k_{\text{obs}}$  versus  $\log [\text{OH}]$  gave the regression equation  $y = (1.06 \pm 0.07)x - (0.674 \pm 0.112)$ , and  $r = 0.998$ . It can be seen that the picrate-creatinine reaction is first order with respect to hydroxide. If we assume that  $k_{\text{obs}} = k_2 [\text{OH}]$ , a value of  $k_2$  (in  $M^{-1} \text{sec}^{-1}$ ) can be deter-

TABLE 4  
VARIATION OF  $k_{\text{obs}}$  WITH CREATININE CONCENTRATION IN THE JAFFÉ REACTION AT  $27^{\circ}\text{C}$

$[C]$ ( $M$ )	$1.67 \times 10^{-3}$	$3.33 \times 10^{-3}$	$1.00 \times 10^{-2}$	$1.67 \times 10^{-2}$
$k_{\text{obs}}$ ( $\text{sec}^{-1}$ ) <sup>a</sup>	0.000877	0.00179	0.00499	0.00871

<sup>a</sup>  $[\text{NaOH}] = 0.0500 M$ ,  $[P]_0 = 5.00 \times 10^{-4} M$ ,  $\mu = 1.00$  ( $\text{Na}_2\text{SO}_4$ ).

TABLE 5  
 VARIATION OF  $k_{\text{obs}}$  WITH HYDROXIDE CONCENTRATION IN THE JAFFÉ REACTION AT 27°C

[NaOH] (M)	0.0150	0.0300	0.0500
$k_{\text{obs}}$ (sec <sup>-1</sup> ) <sup>a</sup>	0.00238	0.00536	0.00852

<sup>a</sup> [C] =  $1.67 \times 10^{-2}$  M, [P]<sub>0</sub> =  $5.00 \times 10^{-4}$  M,  $\mu$  = 1.00 (Na<sub>2</sub>SO<sub>4</sub>).

mined from the  $k_{\text{obs}}$  vs [OH] plot. Such a plot gave the regression equation  $y = 0.174x - 1.0 \times 10^{-4}$ ,  $r = 0.998$ .

The overall rate equation is

$$\text{Rate} = k[\text{P}][\text{C}][\text{OH}], \quad (5)$$

where  $k = k_1/[\text{OH}] = 10.3 \text{ M}^{-2} \text{ sec}^{-1}$ , or  $k = k_2/[\text{C}] = 10.4 \text{ M}^{-2} \text{ sec}^{-1}$ . Butler reported a value of  $9.3 \text{ M}^{-2} \text{ sec}^{-1}$  at 25°C and creatinine in excess (4). Also, Eq. (5) is in agreement with the rate equations given by Butler (4), Vasiliades (15), and O'Keefe (13). It is mentioned that only Butler studied the Jaffé reaction with creatinine in excess over picrate.

A study of the Jaffé reaction was carried out at ionic strength values ( $\mu$ ) of 0.30, 1.00, and 2.00, at 27°C. It was found again that the reaction is first order with respect to picrate, creatinine, and hydroxide concentration, with creatinine in excess. The values of  $k$  obtained at the three ionic strength values were 7.22, 10.3, and  $16.8 \text{ M}^{-2} \text{ sec}^{-1}$ , respectively. The fact that the rate constant value increases with increasing ionic strength, indicates that the slow step in the Jaffé reaction involves ions of the same sign. This observation is in agreement with the work of Butler (4).

A study of the Jaffé reaction was carried out at 27.0, 32.5, and 37.5°C, at  $\mu = 1.00$ . It was found that the reaction always was first order with respect to picrate, creatinine, and hydroxide concentration, with creatinine in excess. The values of  $k$  obtained at the three temperature values were 10.4, 13.9, and  $18.1 \text{ M}^{-2} \text{ sec}^{-1}$ , respectively. Butler (4) reported a value of  $15.8 \text{ M}^{-2} \text{ sec}^{-1}$ , for  $k$ , with creatinine in excess, at 35°C. A plot of  $\ln k$  vs  $(1/T)$  (Arrhenius plot) gave an activation energy of 10.1 kcal/mol for the Jaffé reaction, with creatinine in excess. O'Keefe, reported a value of 9.61 kcal/mol with picrate in excess (13).

#### *Kinetic Study of the Jaffé Reaction with Picrate in Excess*

The Jaffé reaction cannot be followed with picrate in great excess, with the picrate selective electrode. So, a ratio of  $[\text{P}]_0/[\text{C}]_0 = 4.00$  was chosen as optimum for these studies. Under such conditions, potential versus time curves were recorded and from this plot the  $t_2$  was calculated ( $t_2$  is the time required for the initial potential to increase by 2.0 mV). At  $t_2$ ,  $[\text{P}] = [\text{P}]_0 10^{\Delta E/S} = 0.924 [\text{P}]_0$  and  $[\text{C}] = [\text{C}]_0 - ([\text{P}]_0 - [\text{P}]) = [\text{C}]_0$



$-0.076 [P]_0$ . At constant hydroxide concentration, if the reaction is first order with respect to picrate and creatinine,  $k_{\text{obs}}$  (in  $M^{-1} \text{sec}^{-1}$ ) can be calculated from

$$k_{\text{obs}} = \frac{1}{t_2} \left( \frac{1}{[P]_0 - [C]_0} \ln \frac{0.924[C]_0}{[C]_0 - 0.076[P]_0} \right) \quad (6)$$

There is also a first-order dependence with respect to hydroxide under such conditions. The rate equation is expressed also by Eq. (5) at 27 or 37°C and at  $\mu = 1.00$  or 2.00. The calculated value of  $k$  is  $(9.1 \pm 1.0) M^{-2} \text{sec}^{-1}$  at 27°C and  $\mu = 1.00$ ,  $(14.4 \pm 1.6) M^{-2} \text{sec}^{-1}$  at 27°C, and  $\mu = 2.00$  and  $(15.5 \pm 0.6) M^{-2} \text{sec}^{-1}$  at 37°C and  $\mu = 1.00$ . The reported value of  $k$  in the literature is 2.3 (4), 5.0 (15), and 4.6  $M^{-2} \text{sec}^{-1}$  (13). All the values are at 25°C and no ionic strength control. It was shown in our study that the value of  $k$  is affected by ionic strength which must be kept constant in kinetic studies of the Jaffé reaction. The differences with the obtained value for  $k$  in this study may be partly due to temperature and ionic strength differences and partly to the fact that the other authors used picrate in great excess (100-fold the creatinine concentration) whereas in our study only a fourfold excess is used.

## SUMMARY

Potentiometric studies with the picrate ion-selective electrode indicate that the species formed as the product of reaction between alkaline picrate and creatinine in the Jaffé reaction is a 1:1 complex. Kinetic studies indicate that the forward reaction is first order with respect to picrate, creatinine, and hydroxide concentration. The second-order rate constant  $k$  was found to be in the range 9.1–10.4  $M^{-2} \text{sec}^{-1}$  at 27°C and  $\mu = 1.00$ , with creatinine or picrate in excess.  $k$  increases with increasing  $\mu$  and temperature. An activation energy of 10.1 kcal/mol was calculated for the Jaffé reaction, with creatinine in excess.

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