A PARTIAL KINETIC ANALYSIS OF THE CHEMILUMINESCENCE (CL) OF PHENAZINE METHOSULFATE (PMS)*

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In an accompanying paper Chayet et al. (1963) reported some preliminary observations on a chemiluminescence (CL) of phenazine methosulfate (PMS) in the presence of hydrogen peroxide (HOOH) induced by the reductants reduced nicotinamide adenine dinucleotide (NADH) and ascorbic acid (AA). In this paper we present the kinetic analyses for the overall CL "flashes" induced by NADH and AA.

We have found that these CL "flashes" obey the kinetics for a series first order reaction, i.e.,

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C .$$

Of several methods available for solving the "flash" data for the equations of the curves, notably the analytical methods of Schoepfle (1940, 1941), Frost and Pearson (1961), and Johnson (1952), we have found the analytical and/or graphical methods of Johnson to be the more useful.

The data fit parabolic equations of the following combined exponential form:

$$\text{CL-signal} = 10^{bt + a} - 10^{dt + c} . \qquad 1)$$

By plotting the CL-signals (which we have normalized with the maxima at 100) on semi-logarithmic paper, as shown in Fig. 1, it may be seen that the decay becomes linear, i.e., first order, and the equation for the line may be written as

$$\log y' = bt + a \qquad 2)$$

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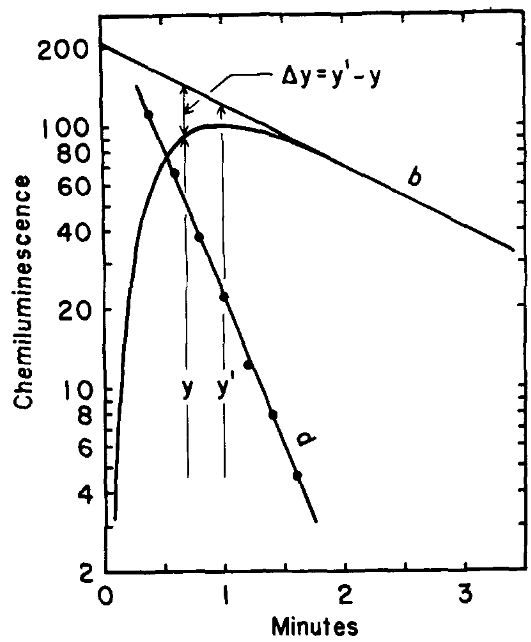

Fig. 1. Diagramatic presentation of the graphic kinetic analysis used for determining the first order rate constants k_1 and k_2. The chemiluminescent curve was obtained using a PMS-HOOH-AA system (Chayet et al., 1963). b = slope for the line log y' = bt + a, and d = slope for the line log Δy = dt + c. k_1 and k_2 may be determined from these slopes as indicated in the text.

From the expression for the first order rate constant,

$$k_2 = \frac{2.3}{t} \log \frac{100}{y'}$$, combined with equation-2 , k_2, in

reciprocal seconds (where the abscissa is expressed in minutes) may be obtained by multiplying the slope "b" of the line by 2.3/60. By extending the decay, first order, line to zero time, and taking the differences between the extended curve, y', and the signal curve, y (graphically or analytically), values of Δy may be obtained, which, when plotted semilogarithmically, permit the equation of the line, log Δy = dt + c, to be obtained. From the slope, d, of this line the first order rate constant, k_1, may be calculated in the manner as for k_2 above.

We have obtained the following equations and first order rate constants for the CL emissions for the PMS-HOOH systems described in this paper:

NADH-PMS-HOOH system:

$$\text{CL-signal} = 10^{-1.855t + 2.311} - 10^{-13.823t + 3.013};$$

$$k_2 = 0.071 \text{ sec.}^{-1},\ k_1 = 0.532 \text{ sec.}^{-1}.$$

AA-PMS-HOOH system:

$$\text{CL-signal} = 10^{-0.235t + 2.318} - 10^{-1.146t + 2.489};$$

$$k_2 = 0.009 \text{ sec.}^{-1},\ k_1 = 0.044 \text{ sec.}^{-1}.$$

The agreement between the experimental and the theoretically calculated curves may be seen in the results presented in Figs. 2 and 3.

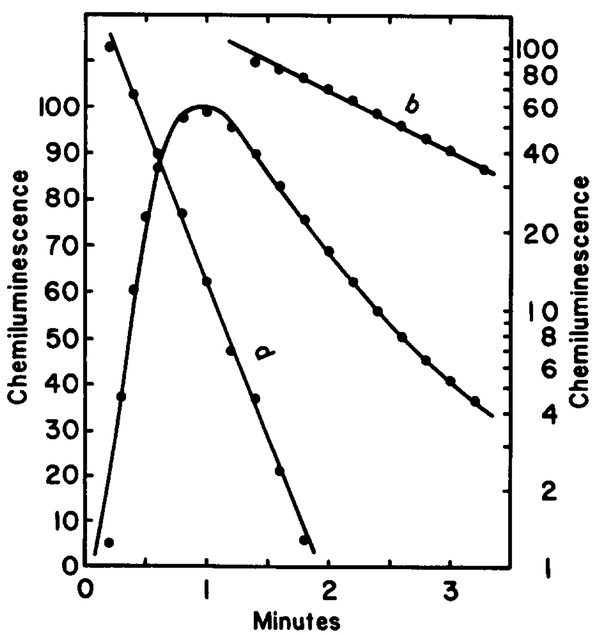

Fig. 2. Chemiluminescence vs time for a PMS-HOOH-AA system showing the agreement of the theoretically calculated curve (......) with the experimentally obtained curve (———). The curve is described by a series first order equation (see text). Components for this system are given in Fig. 3 (Chayet et al., 1963). Shown also are the first order decay plots, from the slopes, b and d, of which k_1 and k_2 may be calculated.

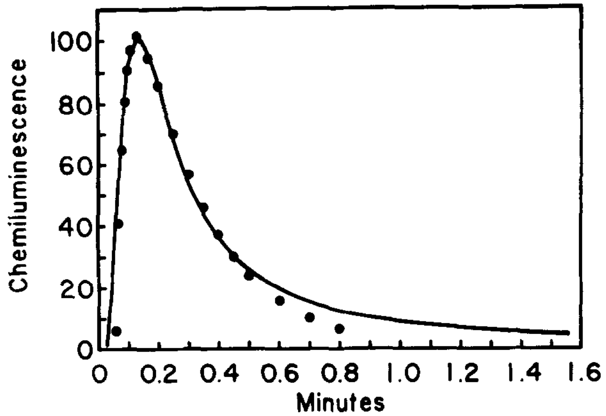

Fig. 3. Chemiluminescence vs time for a PMS-HOOH-NADH system showing the agreement of the theoretically calculated curve (......) with the experimentally determined curve (———). Components for this system are given in Fig.1 (Chayet et al., 1963).

We are not able at this time to identify the molecular species participating in the reactions for which the rate constants k_1 and k_2 have been determined. We presented evidence in the previous paper (Chayet et al., 1963) that k_2 for the PMS-HOOH-NADH system is a pseudo first order constant and expressed the opinion that the same may be true for the other constants.

In Fig. 4 we present an empirical finding which we have observed for the PMS-HOOH-AA system, the PMS-HOOH-NADH system, the photo-induced riboflavin-HOOH system, and the CL-flash from Cypridina material (Chance et al., 1940), the mechanistic significance of which is not yet apparent. The CL-signal gives a linear relation, both up and down, when plotted against the square-root of time. When the apparent pseudo first order reaction reverts to its true second order character, as evidenced by the downward phase for the PMS-HOOH-NADH system (see Fig. 1, Chayet et al., 1963), the square root correlation ceases.

We are struck by the uniformity in the kinetic behavior exhibited by the photo-induced riboflavin-HOOH system, the PMS-HOOH-NADH system and the PMS-HOOH-AA system. The similarity between these "artificial" systems and bioluminescent chemiluminescences (see, e.g., Chance et al., 1940 and Schoepfle, 1940, 1941) suggests that a general mechanism may be common to all.

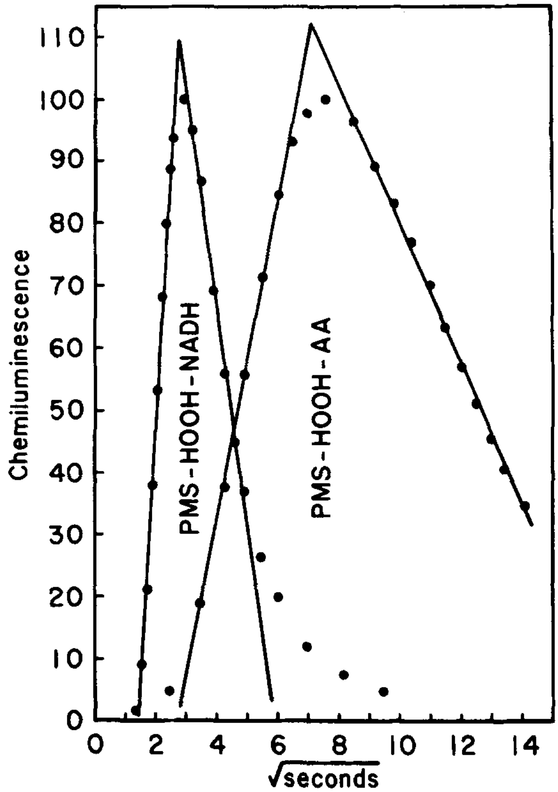

Fig. 4. Chemiluminescence vs square-root of time for the PMS-HOOH-AA, and PMS-HOOH-NADH systems described in Figs. 2 and 3. Note the linearity of the flash responses when expressed in this fashion.

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