

# LIQUID SCINTILLATION COUNTING OF SOME SULFUR-35 LABELED ORGANIC COMPOUNDS

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IN a study of the kinetics of the exchange reaction between elemental sulfur and substitute phenyl *isothiocyantes*, the liquid scintillation counting technique was adopted for assaying the activity of the products. This counting technique merited consideration because of the convenience and simplicity involved in sample preparation, the high efficiency of detection and the

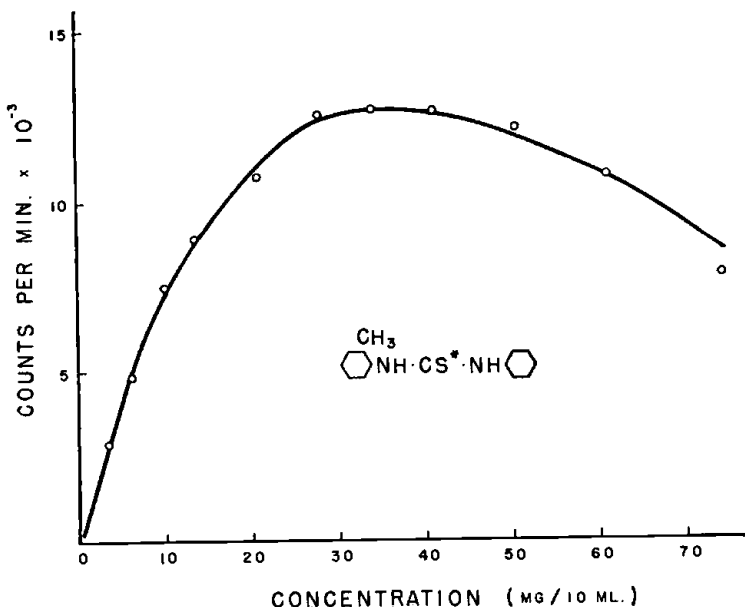


Fig. 1. Concentration-counting rate curve of a homogeneous counting system.

absence of self-absorption. The  $S^{35}$ -substitute phenyl *isothiocyantes* were isolated as thioureas. The compounds were dissolved\* in 10 ml of the liquid scintillation solution, (0.3% 2.5-diphenyloxazole (PPO) in toluene) and were counted in a Tri-Carb liquid scintillation spectrometer assembly† with

\* Heat was applied if necessary to effect solution of the sample. The counting efficiency of the liquid scintillation solution was found unaltered by this treatment.

† Manufactured by the Packard Instrument Co., La Grange, Ill.

the discriminators and voltage gain settings adjusted for optimum counting  $C^{14}$ . Preliminary study of the dependence of counting rate on the sample concentration of these thioureas indicated some apparent abnormal relationship.

Two types of curves relating counting rate versus sample concentration were obtained as shown in Figs. 1 and 2. The curve in Fig. 1, or its prototypes, were given by the more soluble thioureas which dissolved in the liquid scintillator to yield a homogeneous solution at the temperature of the

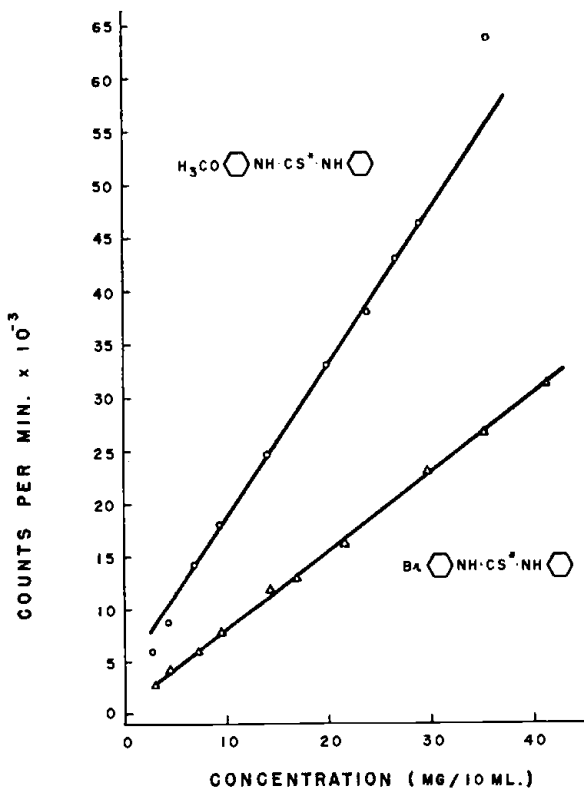


Fig. 2. Concentration-counting rate curves of inhomogeneous counting systems.

refrigerator (4°C). Other thioureas that have only a limited solubility in toluene tended to crystallize out from the solution at this temperature. It was noted that the counting rate of the samples increased and then stabilized as the process of crystallization proceeded. Consequently, these samples were counted in the suspension like condition. Surprisingly enough, an apparent linear relationship existed between the counting rate and the sample concentration as shown by the plots in Fig. 2. These lines when extrapolated to zero concentration did not pass through the origin. In some cases, when the sample concentration did not exceed the solubility of the compound in

toluene (homogeneous solution), the observed counting rate was lower than indicated by the extrapolated value. A typical example is that of 1-(*p*-methoxyphenyl)-3-phenyl thiourea, shown in Fig. 2.

Considering the case of homogeneous counting solutions, the abnormal relationship between sample concentration and counting rate is concentration dependent and may relate to the quenching property of the compound.

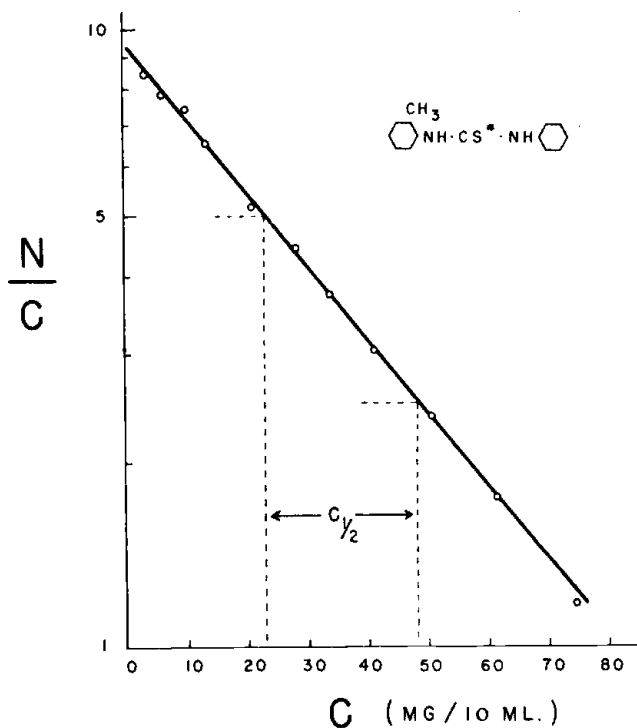


Fig. 3. Replot of the curve in Fig. 1.

According to KERR *et al.*,<sup>1</sup> the counting efficiency of a solution decreases as an exponential function of the sample concentration when quenching compounds are concerned. Therefore, the following relationship was tentatively set up:

$$N = N_0 e^{-qC} \quad (1)$$

where  $N$  = observed counts,

$N_0$  = counts that would be observed in the absence of quenching,

$e$  = base of the natural logarithm,

$q$  = quenching constant\*, and

$C$  = sample concentration.

\* The term 'quenching constant' used by KERR *et al.*<sup>1</sup> is identical with the  $C_{1/2}$  value as defined here.

The quenching constant is equal to  $0.693/C_{\frac{1}{2}}$ , where  $C_{\frac{1}{2}}$  is defined as the sample concentration that will reduce the counts to half its initial value by quenching.

Since  $N_0$  varies as the sample concentration of the radioactive compound that quenches, it follows that  $N_0$  may be replaced by the product of the specific activity of the sample and its concentration provided that the counting efficiency, counting geometry and other environmental factors remain the same. Then,

$$N = S \cdot C \cdot e^{-aC} \tag{2}$$

where  $S$  is the specific activity of the compound.

It is obvious that a semi-log plot of  $N/C$  against  $C$  will be a straight line. Figure 3 shows a replot of the data presented in Fig. 1. The slope of the line from which the quenching constant can be calculated is an intrinsic property of the compound and is independent of its specific activity (see below). The intercept on the  $N/C$  axis represents the specific activity of the compound in the absence of quenching. In this manner, it is possible to compare the activity of two quenching samples of the same compound in terms of their specific activity at 'zero' quenching without resorting to the internal-standard counting technique.

Figure 4 shows the transition from a homogeneous to a suspension-like counting system with a concomitant increase in counting rate as the samples

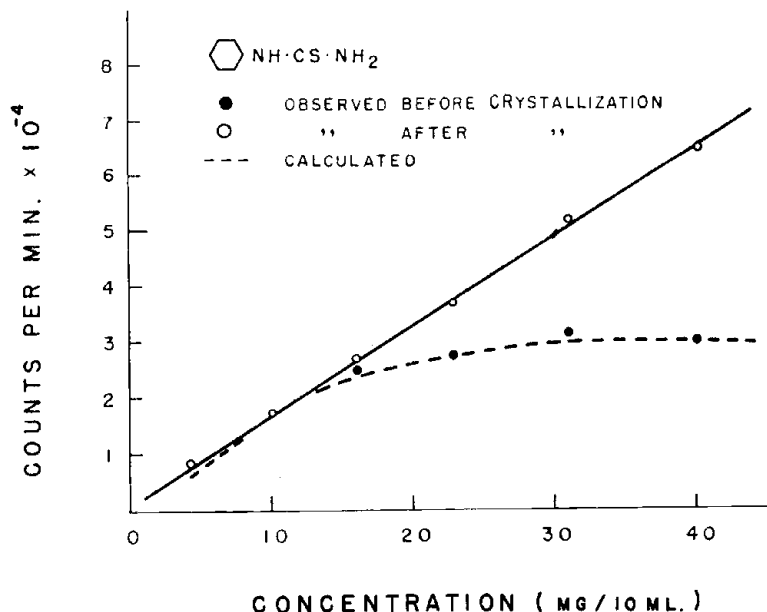


Fig. 4. Counting rate increase in the transition of homogeneous to inhomogeneous counting systems.

of phenyl thiourea were allowed to reach the concentration equilibrium. Since phenyl thiourea is relatively insoluble in toluene in the cold, homogeneous solution samples were obtained by heat. These samples were placed in the refrigerator for 20–30 min to attain a temperature equilibrium before counting; during which time there was no crystallization. In this manner, the observed values of the counting rate were found to fit the dotted curve as calculated from equation (2).

As the crystallization of the compound from the homogeneous solution proceeded, the counting rate of the sample increased accordingly. This increase in counts may be interpreted as the result of diminishing solution concentration of the quencher by crystallization and is predictable from the expression

$$S[C_1(1 - e^{-aC_1}) - C_2(1 - e^{-aC_2})] \quad (3)$$

where  $C_1 > C_2$  and  $C_2$  is a constant identical with the solubility of the compound in the liquid scintillator. It was observed that when  $C_1$  was much greater than  $C_2$  and crystallization became excessive on cooling, the increase in counts was generally less than would be given by equation (3). This appears to be due to losses caused by adverse counting geometry such as the accumulation of the crystals in the bottom of the vial, self-absorption, etc. However, the net results leads to an apparent linear relationship between the sample concentration and the counting rate as indicated by the solid line in Fig. 4 and those in Fig. 2. In addition to phenyl thiourea, this linearity was also observed among other less soluble thioureas.

It was observed that the slope of the line varied with the specific activity of the compound. Since the above samples were in part suspensions, therefore, by analogy, the suspension  $f$ -value<sup>2,3</sup> may be considered as a function of the specific activity of the suspended sample. It follows, therefore, that the  $f$ -value in such systems must also be related to the quenching property of the compound.

#### QUENCHING CONSTANT

As stated above, the quenching constant of a compound is independent of its specific activity. This is supported from the fact that identical values (within the experimental error) of the constant are obtained either by adding the inert compound to the liquid scintillator containing a known activity of  $S^{35}$ , or by adding the radioactive material to the scintillator. Both methods have been used with all the compounds studied. A typical example is given in Fig. 5 in the case of phenyl isothiocyanate, where the concentration quenching effect of the inert compound was plotted as log  $N$  vs.  $C$  and the

\* The  $f$ -value is defined as the ratio of suspension-counting efficiency to homogeneous internal-standard counting efficiency. <sup>2, 3</sup>

labeled compound as  $\log N/C$  vs.  $C$ . Both lines exhibited practically identical slopes. In Table 1 are listed some of the values obtained by these two methods.

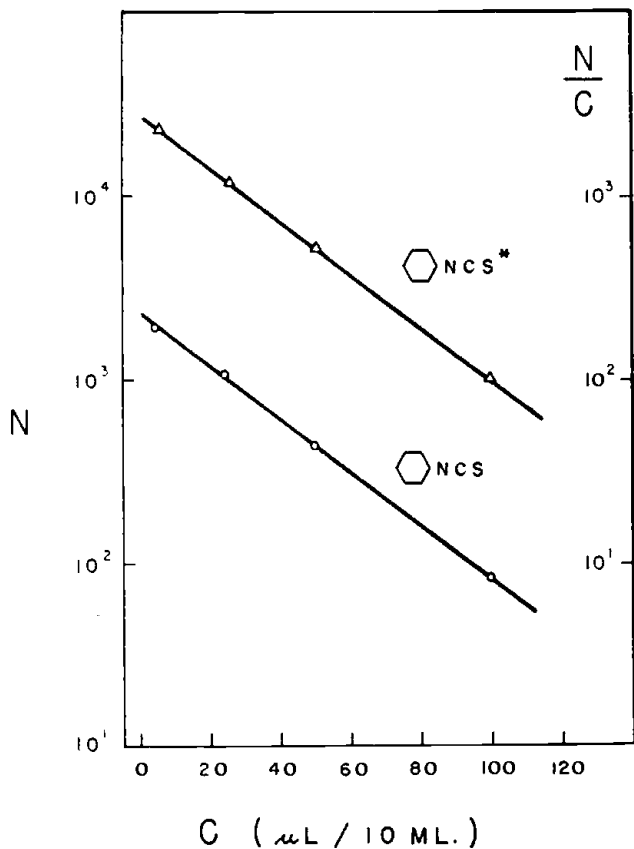


Fig. 5. See text.

#### QUENCHING PROPERTY OF THE COMPOUNDS

Using the quenching constant or  $C_{\frac{1}{2}}$  value as a criterion, the effect of substituent groups on the quenching property of the compound was also studied. In the fourth column of Table 1, the  $C_{\frac{1}{2}}$  values are given in terms of mmole/l. By comparing compounds 1, 2 and 3, it becomes apparent that the introduction of one or two phenyl groups into the thiourea molecule has an adverse effect on the quenching property of the molecule; it increases the quenching constant or decreases the  $C_{\frac{1}{2}}$  value. Introduction of other groups such as methoxy, ethoxy, etc. into one of the phenyl rings of compound 3, also affects the quenching property, but because of the limited number of compounds studied, the trend of the effect is not clear. It is possible that in

this series of compounds, the quenching property depends on the availability of the  $\pi$ -electrons in the phenyl rings since these compounds have rather close structural resemblance, i.e. two phenyl groups interposed by three atoms, as does PPO, the organic scintillator. It seems possible that the compounds could fit over the PPO in a manner so that the benzene rings of the compound lie flat against those of the PPO, thereby, facilitating the energy transfer.

TABLE I  
Half-quenching Concentration

No.	Compounds RHN-CS. NHR'*		$C_{\frac{1}{2}}$ (mg/10 ml)		$C_{\frac{1}{2}}$ (mmole/l.)
	R	R'	Labeled sample	Inert sample	
1	H	H			54.0†
2	Phenyl	H	25.5	25.5	16.8
3	Phenyl	Phenyl	13.2	12.5	5.7
4	<i>p</i> -Bromophenyl	Phenyl	16.6	16.3	5.2
5	<i>o</i> -Ethoxyphenyl	Phenyl	28.0	28.8	10.4
6	<i>p</i> -Ethoxyphenyl	Phenyl	19.0	18.0	6.8
7	<i>p</i> -Methoxyphenyl	Phenyl	20.0	18.3	7.4
8	2-Pyridyl	Phenyl	24.0	23.0	10.3
9	<i>o</i> -Tolyl	Phenyl	25.0	—	10.3
10	<i>p</i> -Tolyl	Phenyl	24.0	—	9.9
	<i>Miscellaneous</i>				
11	Phenyl <i>isothiocyanate</i>		23.2	23.5	17.3
12	Methanesulfonic acid		32.2	30.5	32.5

\* These compounds were formed by reacting the appropriate amines with labeled phenyl *isothiocyanate* obtained from exchange reaction with sulfur-35 and were purified by repeated crystallizations from solvents.

† The value is from KERR *et al.*<sup>1</sup> but expressed in mmole/l.

#### REFERENCES

- <sup>1</sup> V. N. KERR, F. N. HAYES and D. G. OTT. *Int. J. Appl. Rad. Isotopes* **1**, 284 (1957).
- <sup>2</sup> F. N. HAYES, B. S. ROGERS and W. H. LANGHAM. *Nucleonics*, **14**, No. 3, 48 (1956).
- <sup>3</sup> C. G. WHITE and S. HELF. *Nucleonics* **14**, No. 10, 46 (1956).