no vegetation; 16 April 1966. UAIC 1943. Species: 13, 15, 20, 33, 41.

STATION 58. Smith Spring, SW ¼, SE ¼ Sec 7, T 8 S, R 3 E, Marshall Co., Ala.; el. 630 ft.; flow approx. 75 gpm.; temp. 14° C; bottom gravel; depth to 1 ft.; width to 3 ft.; watercress and mint along margin of spring run; 11 June 1966. UAIC 1947. Species: 9, 33.

STATION 59. Big Spring at Big Spring Community, SW ¼ Sec 26, T 9 S, R 2 E, Marshall Co., Ala.; el. 750 ft.; flow approx. 3,000 gpm.; temp. 16° C; bottom silty clay; depth to 3 ft.; width to 15 ft.; *Myriophyllum* abundant, some mint along margin of spring run; 15 April 1967. UAIC 2532. Species: 7, 8, 9, 13, 19, 32, 33, 37, 44.

STATION 60. Unnamed spring, SE ¼ Sec 2, T 2 S, R 7 E, Jackson Co., Ala.; el. 600 ft.; flow approx. 50 gpm.; temp. 14° C; bottom chert; depth to 1 ft.; width to 6 ft.; some algae and *Fontinalis* in spring run; 14 April 1967. UAIC 2526. Species: 33, 38, 45.

STATION 61. Unnamed spring, 3.8 mi. S. Sherwood on Tenn. Hwy. 56, Franklin Co., Tenn.; el. 600 ft.; flow approx. 50 gpm.; aqf. probably Warsaw Formation (Theis, 1936); temp. 14° C; bottom sand and chert; depth to 1 ft.; width to 12 ft.; some watercress and mint present along margin of spring run; 14 April 1967. UAIC 2527. Species: 33, 34, 44.

STATION 62. Unnamed spring, 0.9 mi. S. of Sherwood on Tenn. Hwy. 56, Franklin Co., Tenn.; el. 600 ft.; flow approx. 300 gpm.; aqf. probably Warsaw Formation (Theis, 1936); temp. 13°

C; bottom chert; depth to 1 ft.; width to 10 ft.; some watercress; 14 April 1967. UAIC 2528. Species: 12, 13, 14, 15, 33, 34, 44, 45.

STATION 63. Unnamed spring, SW ¼ Sec 23, T 2 S, R 8 E, Jackson Co., Ala.; el. 500 ft.; flow approx. 300 gpm.; temp. 13° C; bottom sand and chert; depth to 1 ft.; width to 15 ft.; mint, *Fontinalis*, and algae present in spring run; 15 April 1967. UAIC 2531. Species: 33, 37, 44.

STATION 64. Gray Spring, NE ¼ Sec 9, T 2 S, R 9 E, Jackson Co., Ala.; el. 550 ft.; flow approx. 150 gpm.; temp. 13° C; bottom sand, chert; depth to 1 ft.; width to 10 ft.; no vegetation; 15 April 1967. UAIC 2530. Species: 33.

STATION 65. Unnamed spring NE ¼ Sec 15, T 2 S, R 9 E, Jackson Co., Ala.; el. 600 ft.; flow approx. 300 gpm; temp· 13° C; bottom sand, chert; depth to 1 ft.; width to 5 ft.; *Fontinalis* on rocks; 15 April 1967. UAIC 2529. Species: 34.

STATION 66. Unnamed spring, off Old U. S. Hwy. 64-11, 3.6 mi. N. junction with U. S. Hwy. 72, Marion Co., Tenn.; el. 600 ft.; flow approx. 200 gpm.; temp. 13° C; bottom sand, chert; depth to 1 ft.; width to 10 ft.; some algae in spring run; 14 April 1967. UAIC 2525. Species: 13, 33, 34, 44.

STATION 67. Martin Spring at Martin Springs Community, Marion Co., Tenn.; el. 600 ft.; flow approx. 1,500 gpm.; temp. 12° C; bottom chert; depth to 1 ft.; width to 20 ft.; some watercress and mint around shore margin; 14 April 1967. Water issues from a cave with a low dam across its entrance making a pool approx. 40 by 50 ft. UAIC 2000. Species: 13, 33, 34.

A BEER'S LAW TEST FOR CHARGE-TRANSFER ABSORPTION SPECTRA*

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ABSTRACT

A simple procedure is presented for determining the concentration dependence of electronic absorption spectra of molecular complexes. Absorbance values for complexes of tetracyanoethylene with hexamethylbenzene and with fluorene, and 9-dicyanomethylene-2,4,7-trinitrofluorene with hexamethylbenzene are found to vary linearly with concentration.

INTRODUCTION

The vast majority of experimental investigations of molecular-complex systems have employed optical absorption measurements. Association constants and other thermodynamic properties of these systems are computed from spectral data by algebraic methods in which it is assumed that the complex species obeys Beer's law.[1] The validity of this assumption has been questioned, and it has been suggested that discrepancies reported between optical absorption results and other experimental methods may arise because of deviations from Beer's law.[2] Since a complex exists in solution only in rapid equilibrium with uncomplexed donor and acceptor, any attempt to evaluate its absorbance as a function of absolute concentration is inextricably bound to a determination of the corresponding association constant.

With a major portion of the work in this area subject to question, a rapid, sensitive procedure for deter-

mining the concentration dependence of the absorbance of molecular complexes is desirable. We present here a simple Beer's law test which should be applicable to most donor-acceptor systems.

If an electron acceptor, A, and a large excess of electron donor, D, interact in solution to form a 1:1 complex, C.

$$A + D \rightleftharpoons C$$

the association constant, K may be represented as

$$K = \frac{[C]}{([A]_0 - [C])[D]_0} \quad (1)$$

where [C] is the equilibrium concentration of complex and $[A]_0$ and $[D]_0$ are initial concentrations of acceptor and donor respectively. From eq (1)

$$[C] = \left(\frac{K[D]_0}{1 + K[D]_0}\right)[A]_0 \quad (2)$$

so that for a fixed donor concentration, [C] is directly proportional to $[A]_0$. *Assuming* Beer's law to be obeyed

$$A_c = \left(\frac{K[D]_0 b a_c}{1 + K[D]_0} \right) [A]_0 \quad (3)$$

where A_c is the measured absorbance due to the complex, b is the path length and a_c is the absorptivity of the complex at a given wavelength. In this form all terms inside the parentheses are constant. If indeed Beer's law is obeyed, a plot of A_c versus $[A]_0$ for varying acceptor concentrations should yield a straight line passing through the origin. Although by no means a requirement, the procedure will be facilitated experimentally for systems in which only the complex absorbs light in the spectral region under investigation.

EXPERIMENTAL METHODS

Tetracyanoethylene was recrystallized twice from chlorobenzene and sublimed at 1-2 torr at 100-110°C (m.p. 197°C). 9-Dicyanomethylene-2,4,7-trinitrofluorene was recrystallized twice from acetonitrile (m.p. 265-266°C). Hexamethylbenzene (m.p. 166-167°C) and fluorene (m.p. 115.7-116.3°C) were recrystallized twice from ethanol. Spectrograde 1,2-dichloroethane was purged with oxygen-free dry nitrogen (dew point less than —60°C) immediately before use. Techniques of solution preparation have been described elsewhere.[3, 4]

Spectral measurements were made with a Cary Model 14 Spectrophotometer equipped with temperature control accessories. Absorbance data were collected at 25.0 ± 0.2°C using matched sets of one and two centimeter quartz cells with pure solvent as the reference. Tests showed that absorbance measurements were easily reproducible to within 0.002 absorpance unit.

RESULTS AND DISCUSSION

The method described above has been used to test Beer's law in the systems: tetracyanoethylene-hexamethylbenzene, tetracyanoethylene-fluorene, and 9-dicyanomethylene-2,4,7-trinitrofluorene-hexamethylbenzene dissolved in 1,2-dichloroethane at concentrations comparable to those normally employed (see caption to Figure 1). In each of the mixtures only the complex absorbs light in the wavelength region near the charge-transfer band maximum. Reference to Figure 1 shows that for each system the absorbance of the complex varies linearly with the total acceptor concentration, i.e. Beer's law is obeyed. Linear least squares analyses of these lines reveal that the slopes deviate from straight lines by less than 0.05%, well within experimental error. Through the use of computer simulated spectral data it has been found that the method is extremely sensitive to even minor deviations from Beer's law. The high sensitivity of this method is in direct contrast to that of the Benesi-Hildebrand[5] and related equations. Esmlie et al.[2] have demonstrated that in the Benesi-Hildebrand approach large deviations from Beer's law may go completely undetected, while at the same time, association constants and other parameters computed from such data are seriously in error. An additional
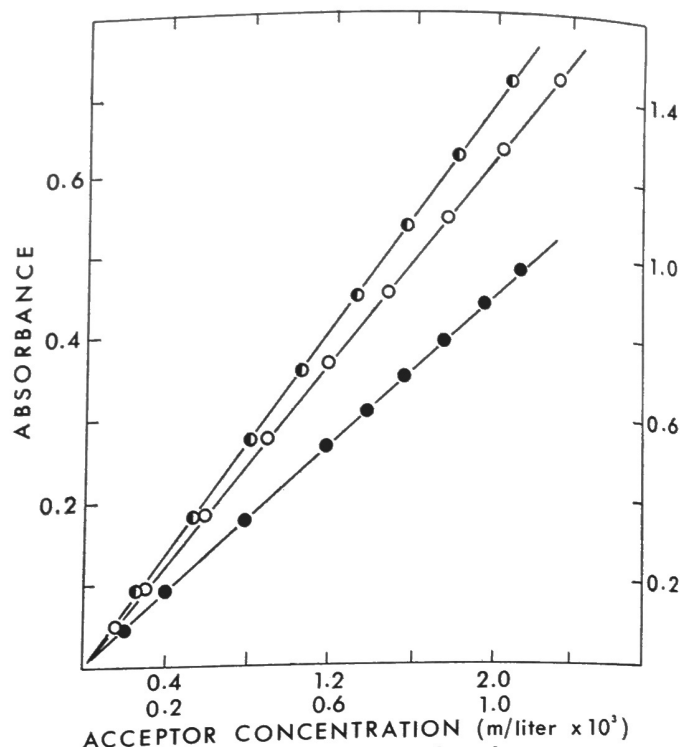

Figure 1. Molecular complex absorbance versus total acceptor concentration. ● --tetracyanoethylene (low concentration range) with hexamethylbenzene (4.8 X 10^{-2} mole/liter), 2.0 cm path length at 540 nm; ○ --9-dicyanomethylene-2,4,7-trinitrofluorene (low concentration range) with hexamethylbenzene (6.9 X 10^{-2} mole/liter), 1.0 cm path length at 530 nm; ◐ --tetracyanoethylene (high concentration range) with fluorene (12.6 X 10^{-2} mole/liter), 1.0 cm path length at 570 nm. Absorbance values for ● and ○ are indicated at the left hand side, and for ◐ at the right hand side of the figure.

advantage of the present method is the elimination of the need for a separate evaluation of K and a_c. Person[6] has shown that there is no unambiguous technique whereby the two factors can be separated.

In summary, this method offers a rapid, sensitive, and widely applicable procedure for testing Beer's law in molecular-complex systems. Since the complexes reported here represent typical donor-acceptor interactions, these results provide a strong measure of support for the various mathematical procedures in which Beer's law has been assumed.

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