

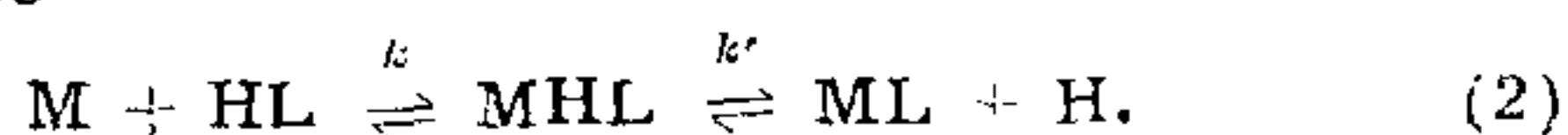
LETTERS TO THE EDITOR

ANALYSIS OF SPECTROPHOTOMETRIC DATA BY THE METHODS OF NEWTON AND ARCAND, AND McCONNELL AND DAVIDSON

SPECTROPHOTOMETRY has often been used for the determination of stability constants of 1:1 complexes formed in a reaction



The observed data can often be conveniently analysed by the methods described by Newton and Arcand¹ and McConnell and Davidson² which are fundamentally similar. The purpose of this note is to discuss these methods in a generalised way and to derive expressions for finding out stability constants in a situation in which two complex species such as ML and MHL exist in equilibrium in a reaction of the type



Recent investigation carried out by the authors on the stability constants of rare-earth complexes of 5-sulphosalicylic acid have shown that equilibria of this type are quite common at least in these complexes.³

Newton and Arcand studied complex formation between Ce^{+3} and SO_4^{-2} spectrophotometrically and derived an expression based on the fact that the ligand had no absorption at the wavelength studied. For reaction (1) it can be written as

$$-\frac{1}{K} \cdot \frac{D - D_{TM}}{a} + D_1 = D \quad (3)$$

where D is the observed optical density; D_{TM} the optical density of the solution when no complexing agent is added; D_1 , the optical density due to the complex ML when all the metal is complexed; 'a' the free ligand concentration; and K , the stability constant. The slope of the straight line obtained by plotting D against $D - D_{TM}/a$ gives the value of K . In this method the free ligand concentration must be obtained by the method of successive approximations.

If the metal, the ligand and the complex all have absorption at the wavelength under study, the expression given above must be modified as follows:

$$-\frac{1}{K} \cdot \frac{(D - D_L - D_0)}{a} + D_1 = (D - D_L - D_0) \quad (4)$$

Here D_L is the optical density due to the free ligand and D_0 that of the free metal. In practice, one generally knows the optical density of the total ligand D_{TL} (instead of D_L) and of the total metal D_{TM} (instead of D_0). It is then convenient to evaluate a quantity $\bar{D} = D - D_{TL} - D_{TM}$ which is proportional to $(D - D_L - D_0)$; the proportionality factor is $(\epsilon_{ML} - \epsilon_L - \epsilon_0)/\epsilon_{ML}$, where ϵ_{ML} , ϵ_L and ϵ_0 are the extinction coefficients of the complex, the ligand and the metal respectively. The slope of the straight line obtained by plotting \bar{D} against \bar{D}/a then gives the stability constant as before but the intercept is now $D_1 \cdot (\epsilon_{ML} - \epsilon_L - \epsilon_0)/\epsilon_{ML}$.

McConnell and Davidson's method takes into account the total metal and the total ligand concentrations. The general form of the expression is

$$\frac{l \cdot T_M \cdot T_L}{D} = \frac{T_M - T_L}{\Delta \epsilon} + \frac{1}{K \Delta \epsilon} \quad (5)$$

where T_M is the concentration of the total metal; T_L , the concentration of the total ligand; l , the path length through which the light travels and $\Delta \epsilon = \epsilon_{ML} - \epsilon_L - \epsilon_0$. The linear plot of $l \cdot T_M \cdot T_L / \bar{D}$ against $(T_M + T_L)$ has a slope of $1/\Delta \epsilon$ and the intercept of $1/K \Delta \epsilon$ from which the stability constant of 1:1 complex can be easily calculated.

Let us now consider reaction (2) in which two complex species MHL and ML coexist. If the experiment is carried out at constant pH by keeping the metal concentration constant and varying the ligand concentration, it is possible to get a mixture of the two species, the concentrations of MHL and ML being related to each other by a constant factor $[H]/k'$. For the reaction



where $[MHL]^* = [ML] + [MHL]$, at constant pH, $k_{(apparent)}$ is given by the relation

$$k_{(apparent)} = \frac{[ML] (1 + C)}{(T_M - [ML] (1 + C)) a} \quad (7)$$

where

$$C = \frac{[H]}{k'} = \frac{[MHL]}{[ML]}$$

Since

$$[ML] = \frac{(D - D_L - D_0)}{\epsilon_{ML}}$$

we get

$$\frac{1}{k_{(\text{apparent})}} \cdot \frac{(D - D_1 - D_0)}{a} = \frac{T_M \cdot \epsilon_{ML}}{(1 + C)} \quad (8)$$

when all the metal is complexed, T_M is the sum of the concentrations of ML and MHL. Therefore, $T_M = [ML]_{\text{max.}} (1 + C)$.

Defining

$$\frac{T_M \cdot \epsilon_{ML}}{(1 + C)} = D_1^*$$

where D_1^* represents the optical density when all the metal is complexed as (ML + MHL), we obtain an expression

$$(D - D_L - D_0) - D_1^* \frac{1}{k_{(\text{apparent})}} \times \frac{(D - D_L - D_0)}{a} \quad (9)$$

This expression is similar to the generalised expression (4) of Newton and Arcand. As discussed earlier, \bar{D} can be plotted against \bar{D}/a to get the value of $k_{(\text{apparent})}$. Incidentally the intercept of this straight line on \bar{D} axis is now

$$D_1^* \left\{ \frac{\epsilon_{ML}}{\epsilon_{ML}} - \frac{(1 + C)(\epsilon_L + \epsilon_0)}{\epsilon_{ML}} \right\}$$

McConnell and Davidson's expression can also be used to analyse such data. Expression (5) is still the correct expression for getting $k_{(\text{apparent})}$; however, $\Delta \epsilon$, under these circumstances, is equal to

$$\frac{\epsilon_{ML} - (1 + C)(\epsilon_L + \epsilon_0)}{(1 + C)}$$

From the value of $k_{(\text{apparent})}$, it is possible to calculate k in reaction (2) if the concentration of ML can be ascertained by a suitable method.

This type of analysis can be applied in circumstances in which one of the two species ML and MHL or both the species have absorption at some suitable wavelength.

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ANTIBIOTIC ACTIVITY OF SCLEROTIUM ROLFSII SACC.

IN an earlier communication some aspects of the study of oxalic acid production by *Sclerotium rolfsii* have been described.¹

So far, the studies on *S. rolfsii*, the causative organism of the root rot disease of many host plants, have been mainly directed towards its phytotoxicity and the considerable amount of oxalic acid produced has been incriminated as the toxic agent.² The inhibition of growth of a soil micro-organism of bacillus species by the culture filtrates of *S. rolfsii* has recently been reported.³

Since many plant pathogens are known to produce substances which besides phytotoxicity also exhibit inhibitory action on many soil micro-organisms and on human pathogens,⁴⁻⁶ antibiotic production by *S. rolfsii* has been studied and reported in this communication.

MATERIALS AND METHODS

The strain studied was isolated from diseased bean plant and maintained on potato dextrose agar. For antibiotic screening, the fungus was grown on nutrient peptone broth (Peptone 5 g., Beef extract 3 g., and distilled water 1000 ml.) at pH 6.0. Flasks containing this medium were inoculated with a single sclerotium and incubated at room temperature. Culture filtrate after 30 days incubation was used for the preliminary screening test for antibiotic action.

Test organisms.—These included pathogenic bacteria and fungi. *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella paratyphi*, *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton interdigitale*, *Aspergillus flavus*, *Erwinia carotovora*, *Pseudomonas fluorescens*, *Alternaria brassicae*, *Fusarium orthoceras*, *Helminthosporium arecae* and *Pestotia* sp. These organisms were seeded in suitable culture media in petri dishes. The standard agar cup plate method was used for the evaluation. As the rates of growth of the organisms differ, the zone of inhibition was noted when fair amount of growth of the organisms was observed in the control areas. Hence the time of recording varied from 18 hours in the case of *S. aureus* to 6 days with *T. interdigitale*.

The optimum period for maximum antibiotic activity was determined by testing the culture filtrate at varying intervals of incubation against *S. aureus*.

For the isolation of the active principles, solvent extraction procedure adopted was as follows:

1. Newton, T. W. and Arcand, G. M., *J. Am. Chem. Soc.*, 1953, **75**, 2449.
2. McConnell, H. and Davidson, N., *Ibid.*, 1950, **72**, 3164.
3. Thakur, N. V., Jogdeo, S. M. and Kanekar, C. R., Communicated for publication.