511. *Observations on Job's Method of Continuous Variations and its Extension to Two-phase Systems.*

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The principle of Job's method of continuous variations can be extended with advantage to a two-phase system provided the complex under investigation is uncharged and extractable into a water-immiscible solvent. By this new procedure it is possible to establish the composition of comparatively weak complexes in the presence of intermediate step-complexes and, if a radioactive isotope of the metal is available, the measurements can be extended to colourless complexes and to the study of a coloured complex in the presence of a similarly coloured and extractable organic reagent.

That the formulæ deduced from experimental Job curves may be in error if the total concentration of reactants is too low or not maintained constant, or if the effect of intermediate complexes cannot be neglected, is examined mathematically and illustrated for typical systems. Working with two-phase systems reduces these errors. Reference is made to the possibility of gross error in calculating stability constants from Job curves.

THE method of continuous variations, first enunciated by Job [1] and treated in great detail by Vosburgh and Cooper,[2] is one of the most widely used procedures for determining the composition of a soluble metal complex formed in solution from a metal M and ligand L

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[1] Job, *Ann. Chim.* (*France*), 1928, **9**, 113.

[2] Vosburgh and Cooper, *J. Amer. Chem. Soc.*, 1941, **63**, 437.

(charges are omitted for the sake of generality). To facilitate the subsequent discussion the basic procedure will be recapitulated.

Mixtures of M and L are prepared in various proportions subject to the condition that the total molecular concentration of the two components should remain constant. For each mixture some property characterising the complex ML_n (*e.g.* its optical absorbancy) is determined and from this is subtracted the corresponding quantity calculated on the assumption that no complex formation has taken place. A plot of the resulting value (the Job ordinate, A_j) against the mole fraction of one component will have an extremum from which the actual composition of the complex is readily calculable. Thus for the reaction:

$$\begin{array}{lcccc} & M & + & nL \rightleftharpoons & ML_n \quad . \quad . \quad . \quad . \quad . \quad (1) \\ \text{(i) if there is no reaction,} & xC & & (1-x)C & 0 \\ \text{(ii) at equilibrium} & (x-\alpha)C & & (1-x-n\alpha)C & \alpha C \end{array}$$

The value of the characteristic ordinate, A_j, is then given by

$$\begin{aligned} A_j &= \{(x-\alpha)C\varepsilon_M + (1-x-n\alpha)C\varepsilon_L + \alpha C\varepsilon_C\} - \{xC\varepsilon_M + (1-x)C\varepsilon_L\} \\ &= (\varepsilon_C - \varepsilon_M - n\varepsilon_L)\alpha C \quad . \quad (2) \end{aligned}$$

where ε_M, ε_L, and ε_C are the molecular extinction coefficients of the species M, L, and ML_n respectively, and a light-path of 1 cm. is assumed. It follows that:

$$\begin{aligned} \partial A_j/\partial x &= C(\varepsilon_C - \varepsilon_M - n\varepsilon_L)(\partial\alpha/\partial x) \\ &= \text{Constant} \times (\partial\alpha/\partial x) \end{aligned}$$

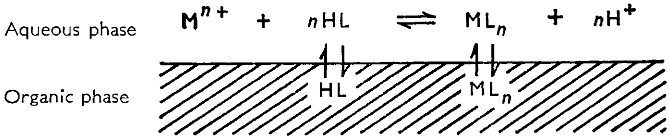
and that the position of the extremum in the Job plot will be most readily determined when ε_C is large compared with ε_M and ε_L. If the stability constant for the complex is defined by $\beta_n = [ML_n]/[M][L]^n$, logarithmic differentiation and insertion of the condition $\partial\alpha/\partial x = 0$ leads at once to the desired result:

$$x = 1/(1+n), \text{ or } n = (1-x)/x \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

at the turning point.

Extension to a Two-phase System.—One essential requirement of the procedure outlined above is that the system should be homogeneous. In many cases this is impracticable. For although the metal cation will be soluble in water but not in organic solvents, the reverse is often true of the ligand, more especially when this is a large organic molecule: many metal inner complexes are very sparingly soluble in water though soluble in organic solvents. To overcome these difficulties mixtures of solvents (*e.g.*, water–methanol–chloroform) have been used to provide a single phase, but the low solubility of the metallic inner complex has often proved the limiting factor.[3] It would clearly be of considerable general value if Job's method could be applied to a two-phase system. Although this has been tried practically and with apparent success [4] no theoretical justification for the procedure has yet been put forward.

Consider a reagent, HL (*e.g.*, oxine or dithizone), capable of partition between water and an organic solvent: allow it to react with a metal cation, M, to give an uncharged complex, ML_n, which can itself be extracted into the organic phase. At equilibrium the system can be represented diagrammatically thus:



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[3] Irving, Bell, and Williams, *J.*, 1952, 357.

[4] Pilipenko, *J. Analyt. Chem.* (*U.S.S.R.*), 1953, **8**, 317.

Partition coefficients for the reagent (p_R) and uncharged metal complex (p_C) are defined by:

$$p_R = [\mathrm{HL}]_o/[\mathrm{HL}], \text{ and } p_C = [\mathrm{ML}_n]_o/[\mathrm{ML}_n]$$

where the subscript *o* distinguishes the organic from the aqueous phase. We assume that the system *as a whole* contains xC moles of metal and $(1 - x)C$ moles of the reagent, HL, which is the acid conjugate to the ligand ion, L^-. The reagent may be added initially in the form of a solution in the organic solvent. The metal ion will, of course, be present initially in the aqueous phase which must be buffered to ensure constancy of pH and should preferably have a constant salt background to preserve constant ionic strength, in default of which the application of the law of mass action to the system may be invalidated.[5] For simplicity we postulate that the solvents are completely immiscible and that the volumes of aqueous and organic phases are equal.

Provided that there are no intermediate step-complexes [5] such as ML_j ($j \neq n$), or more complex species such as $\mathrm{H}_h\mathrm{M}_m\mathrm{L}_l$ and their hydrolysis products,[6] the concentration of the unique extractable complex ML_n in the aqueous phase after equilibration will be denoted by αC. The concentration in the organic phase will then be $[\mathrm{ML}_n]_o = \alpha C p_C$. Considerations of mass balance lead to the following equations:

$$xC = [\mathrm{M}^{n+}] + [\mathrm{ML}_n] + [\mathrm{ML}_n]_o \qquad (4)$$

and

$$(1 - x)C = [\mathrm{HL}] + [\mathrm{HL}]_o + n[\mathrm{ML}_n] + n[\mathrm{ML}_n]_o \qquad (5)$$

whence

$$[\mathrm{M}^{n+}] = xC - \alpha C(1 + p_C) \qquad (6)$$

and

$$[\mathrm{HL}] = \{(1 - x)C - n\alpha C(1 + p_C)\}/(1 + p_R) \qquad (7)$$

If the equilibrium constant for the reaction taking place in the aqueous phase is defined by:

$$K_e = [\mathrm{ML}_n][\mathrm{H}^+]^n/[\mathrm{M}^{n+}][\mathrm{HL}]^n \qquad (8)$$

then by substituting $[\mathrm{ML}_n] = \alpha C$, and values for $[\mathrm{M}^{n+}]$ and $[\mathrm{HL}]$ from equations (6) and (7), we obtain:

$$K_e = \frac{\alpha C(1 + p_R)^n\,[\mathrm{H}^+]^n}{\{xC - \alpha C(1 + p_C)\}\{(1 - x)C - n\alpha C(1 + p_C)\}^n} \qquad (9)$$

Differentiation gives:

$$\left(\frac{\partial \log K_e}{\partial x}\right)_{\mathrm{pH}} = 0 = \frac{\mathrm{d}\alpha}{\mathrm{d}x}\left\{\frac{1}{\alpha} + \frac{(1 + p_C)}{x - \alpha(1 + p_C)} + \frac{n^2(1 + p_C)}{1 - x - n\alpha(1 + p_C)}\right\}$$
$$- \frac{1}{x - \alpha(1 + p_C)} + \frac{n}{1 - x - n\alpha(1 + p_C)} \qquad (10)$$

If A_j is the absorbancy of the complex ML_n in a 1 cm. layer of the organic phase, then:

$$A_j = [\mathrm{ML}_n]_o\varepsilon_C = \alpha C p_C \varepsilon_C \qquad (11)$$

whence

$$\mathrm{d}A_j/\mathrm{d}x = C p_C \varepsilon_C(\mathrm{d}\alpha/\mathrm{d}x) \qquad (12)$$

Maximum absorbancy will occur when $\mathrm{d}A/\mathrm{d}x = 0$, whence $\mathrm{d}\alpha/\mathrm{d}x = 0$ at the extremum. Inserting this condition into equation (10) gives:

$$x = 1/(1 + n) \text{ or } n = (1 - x)/x \qquad (3)$$

as before. Hence with 1 : 1, 1 : 2, and 1 : 3 complexes the extremum will occur when $x = 0{\cdot}5$, $0{\cdot}33$, and $0{\cdot}25$ respectively, just as in the familiar method of continuous variations applied to a homogeneous system.[1,2,5] In this extension of Job's method the most sensitive wavelength at which to carry out measurements will be that for which ε_C is greatest.

[5] Woldbye, *Acta Chem. Scand.*, 1955, **9**, 299.

[6] Irving, Rossotti, and Williams, *J.*, 1955, 1906.

Measurements at High pH *and with a Coloured Reagent.*—If the pH of the aqueous phase is high enough a substantial proportion of the reagent may be transferred from the organic to the aqueous phase and it will be necessary to take into account the concentration of the free ligand ion, L^-. Equations (5) and (7) now take the form:

$$(1 - x)C = [HL] + [HL]_o + n[ML_n] + n[ML_n]_o + [L^-] \quad . \quad . \quad . \quad (5a)$$

and

$$[HL] = (1 - x)C - n\alpha C(1 + p_C)/\{1 + p_R + (K_R/[H^+])\} \quad . \quad . \quad . \quad (7a)$$

where $K_R = [H^+][L^-]/[HL]$.

The reagent itself may be coloured (with a molecular extinction coefficient of ε_r) and it is necessary to examine what effect this will have on the condition for the extremum. The actual absorbancy in a 1 cm. layer of the organic phase will be given by:

$$\begin{aligned} A &= [ML_n]_o\varepsilon_C + [HL]_o\varepsilon_R \\ &= \alpha C p_C \varepsilon_C + \left\{\frac{(1 - x)C - n\alpha C(1 + p_C)}{1 + p_R + (K_r/[H^+])}\right\} p_R\varepsilon_R \end{aligned}$$

and by subtracting the contribution

$$(1 - x)Cp_R\varepsilon_R/\{1 + p_R + (K_R/[H^+])\}$$

which would have been given by the reagent had no complex formation taken place, we arrive at:

$$A_j = \alpha C[p_C\varepsilon_C - n(1 + p_C)p_R\varepsilon_R/\{1 + p_R + (K_R/[H^+])\}] \quad . \quad . \quad (11a)$$

Thus, as before, $\partial A_j/\partial x = \text{constant} \times (\partial\alpha/\partial x)$.

If the corrected value for [HL] from equation (7*a*) is introduced into equation (8), differentiation and insertion of the condition for an extremum, $\partial\alpha/\partial x = 0$, lead at once to equation (3), showing that the theory of the simple Job method is equally applicable to these more complicated systems.

Since both p_C and $p_R \gg 1$, equation (11*a*) may be simplified to:

$$A_j = \alpha C p_C[\varepsilon_C - n\varepsilon_R/\{1 + (K_R/p_R[H^+])\}]$$

which suggests the possibility of working at wavelengths such that $\varepsilon_C \gg \varepsilon_R$ or $\varepsilon_R \gg \varepsilon_C$ for maximum sensitivity in locating the extremum.

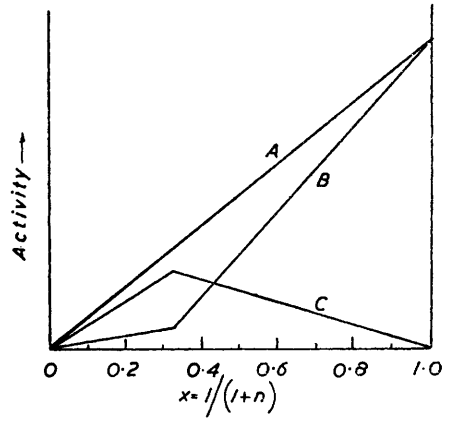
Location of the Extremum Radiometrically.—As already stated, the value of the Job ordinate, A_j, is derived from some convenient and distinctive property of the complex ML_n whose composition is under investigation. Optical absorbancy has been most often used and it is essential to choose a wavelength for which $\varepsilon_C \gg \varepsilon_M$, ε_L, ε_{HL}, and ε_{ML_j} ($j \neq n$). Even so, the contribution to the total absorbancy from intermediate complexes, ML_j ($j \neq n$) may be appreciable when the mole fraction of metal is large compared with that of the ligand. This adverse feature is absent from the two-phase modification of Job's procedure since only the formally neutral complex ML_n is present in the organic phase. Moreover, advantage can be taken of the great gain in sensitivity in locating the extremum secured by labelling the metal with a highly radioactive nuclide. Fig. 1 shows how the activity in the two phases varies with x.

Fig. 1 shows that the activity of the aqueous phase increases very slowly at first with x since most of the metal is being extracted into the organic phase as the radioactive complex, ML_n. Once the value of x exceeds $1/(1 + n)$, the activity rises rapidly, as shown in curve *B*. On the other hand the activity of the organic phase rises steeply with x until the extremum at $x = 1/(1 + n)$ and then falls steeply as shown by curve *C*. The maximum is well defined and its location can be ascertained independently of the optical absorption of the possible components of the system. Indeed, by this modification Job's method of continuous variations can be applied to systems in which all the reactants and products are optically transparent.

Errors in Concentration Measurements.—A fundamental postulate in the method of continuous variations is that the total molar concentration of metal and ligand should remain the same while x is varied. This is usually met by examining mixtures prepared from xV ml. of a stock solution of metal with $(1 - x)V$ ml. of a stock solution of ligand of exactly the same molarity. We now propose to consider what errors will be introduced if these two stock solutions are not equimolar, but of concentrations C and C' respectively.

FIG. 1. *Location of the extremum radiometrically in the application of the method of continuous variations to a two-phase system.*

A, total activity; B, *activity in the aqueous phase*; *C, activity in the organic phase.*



In a one-phase system, where the concentration of the complex at equilibrium is assumed to be given by $[ML_n] = \alpha C'$, it is easily shown that:

$$\beta_n = [ML_n]/[M][L]^n = \alpha C'/(xC - \alpha C')\{(1 - x)C' - n\alpha C'\}^n \quad . \quad . \quad (13)$$

By differentiation and insertion of the condition, $\partial\alpha/\partial x = 0$ for the extremum, we find:

$$n = (1 - x)/\{x - \alpha(C' - C)/C\}$$

$$x = \frac{1}{(1 + n)} + \frac{n}{(1 + n)} \cdot \frac{(C' - C)}{C} \cdot \alpha \quad . \quad . \quad . \quad . \quad (3a)$$

Similarly, in the two-phase system, if $[ML_n] = \alpha C'$ and $[ML_n]_o = p_C\alpha C'$, then:

$$n = (1 - x)/\{x - \alpha(1 + p_C)(C' - C)/C\}$$

or

$$x = \frac{1}{(1 + n)} + \frac{n}{(1 + n)} \cdot (1 + p_C) \cdot \frac{(C' - C)}{C} \cdot \alpha \quad . \quad . \quad . \quad (3b)$$

Equations (3*a*) and (3*b*) reduce to equation (3) when $C = C'$. In the single-phase system $\alpha \ngtr 1$. In the two-phase system the partition coefficient $p_C \gg 1$, but the product $\alpha(1 + p_C)$ cannot exceed unity. In both cases the error in the position of the extremum will therefore be determined by the product $\{n/(n + 1)\} \times \{(C' - C)/C\}$. If a 10% error in the concentration of one of the supposedly equimolecular stock solutions is assumed, the maximum range of x values for the extremum could lie between 0·55 and 0·45 for a 1 : 1 complex, between 0·40 and 0·27 for a 2 : 1 complex, and between 0·325 and 0·175 for a 3 : 1 complex. Thus, even with a systematic error of 10% in making up one of the stock solutions there is little chance of wrongly identifying a 1 : 1 complex from the position of the extremum in a Job plot. But incorrect conclusions might easily be drawn when 2 : 1 or 3 : 1 (or higher) complexes are involved, depending on which of the two stock solutions was the stronger.

The Effect of Intermediate Complexes.—Up to this point we have assumed that the complex ML_n is in direct equilibrium with M and L, *i.e.*, that intermediate step-complexes ML_j $(j = 1,2,\ldots n - 1)$ or higher complexes $(j = n + 1, n + 2,\ldots)$ are present only in negligible concentrations. .By a careful choice of wavelengths it has been possible in some cases to establish the composition of more than one step-complex in an equilibrium mixture.[2,5] An obvious restriction is that each step-complex must have a sufficiently wide range of existence; more explicitly, the concentration of metal and ligand must be such that the range of values of the free-ligand concentration covered by the Job curve (and more particularly in the neighbourhood of the extremum) should be such that the corresponding value of the degree of formation of the system, $\bar{n}$, should approximate to that of n in the formula ML_n of the complex under investigation. In brief, the relevant values of [L] should correspond with a plateau or at least a gently rising portion of the formation curve ($\bar{n}$ against pL) where ML_n is the predominant species. Failure to realise this condition has led a number of previous workers to draw erroneous conclusions from experimental Job curves.[5]

It will be obvious that errors due to ignoring the presence of intermediate complexes will be greatest when the complex under investigation is extensively dissociated and where the lower complexes are of comparable stability, *i.e.*, when the successive stability constants $K_1, K_2, \ldots$ are of comparable size and not too large. Although it is impossible to formulate explicit equations for the errors caused by neglecting the effect of lower complexes, even in the simple case where $n = 2$, some insight may be gained by considering particular cases.

Consider the application of the method of continuous variations to a homogeneous system in which the complex ML_2 coexists with ML, M, and L. If the total concentration of reactants, $C = 10^{-3}$M, and $K_1 = [ML]/[M][L] = 10^5$ and $K_2 = [ML_2]/[ML][L] = 10^3$, it is possible to calculate conjugate values of [L], $\bar{n}$, and x from the following equations:

$$\frac{\beta_1[L] + 2\beta_2[L]^2}{1 + \beta_1[L] + \beta_2[L]^2} = \bar{n} = \frac{C_L - [L]}{C_M} = \frac{(1 - x)C - [L]}{xC} \quad . \quad . \quad (14)$$

Differentiation of equation (14) yields:

$$dx/d[L] = -\{1 + xC(d\bar{n}/d[L])\}/C(\bar{n} + 1)$$

from which it is clear that as x increases, since $d\bar{n}/d[L]$ is always positive, both [L] and $\bar{n}$ will decrease monotonically (cf. Fig. 2, curve *E*).

The actual concentration of the complex ML_2 is given by:

$$\begin{aligned}[ML_2] &= \beta_2[M][L]^2 \\ &= K_1K_2[L]^2 . xC/(1 + K_1[L] + K_1K_2[L]^2)\end{aligned}$$

and, with an optimum choice of wavelength, the value of the Job ordinate, A_j, will be proportional to this. The results of such calculations are presented diagrammatically in Fig. 2 (Curve *A*). As might have been expected, even on the ligand-rich side of the graph ($x < 1/3$), the degree of formation of the system is very much below 2 and the concentration of lower complex ML and of free metal ions M cannot validly be neglected. When the metal is in excess ($x \longrightarrow 1$) the degree of formation, $\bar{n}$, falls off steeply and the Job curve is markedly distorted. But of greater significance is the striking result that the maximum of the Job plot occurs when $x = 0{\cdot}27$, from which the erroneous conclusion would be drawn that the complex had the composition ML_3 and not ML_2.

If the method of continuous variations had been applied to the same system with solutions ten times as strong ($C = 10^{-2}$M), calculations on similar lines lead to the data plotted as curve *B* in Fig. 2. Here the degree of formation is much higher (about 1·67) in the neighbourhood of the extremum; but, although this occurs at a higher mole fraction of metal ($x = 0{\cdot}301$), this fraction lies between the values expected for a 1 : 2 complex ($x = 0{\cdot}333$) and a 1 : 3 complex ($x = 0{\cdot}250$) and the choice in practice would be still harder owing to the inevitable experimental uncertainty in measuring absorbancies.

One way of raising the proportion of ML_2 in the equilibrium mixture is to increase the concentration. Curve *C* in Fig. 2 is based on data obtained by calculations for the same system as before with solutions of total concentration, $C = 10^{-1}$M. In this case the extremum is found at $x = 0{\cdot}321$, pointing unequivocably to a 1 : 2 complex. At this point the degree of formation is as high as 1·88 and about 90% of the total metal is present as the complex ML_2.

We now consider the application of the method of continuous variations to the same system of metal complexes but postulating that the uncharged species ML_2 can be solvent-extracted into an organic phase.

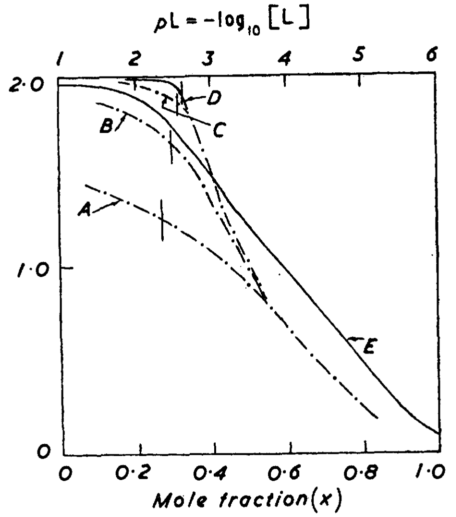


FIG. 2. *Calculated degrees of formation,* $\bar{n}$, *as a function of the mole fraction,* x, *of metal in systems for which* $\log \beta_1 = 5$ *and* $\log \beta_2 = 8$. *The total concentration of metal ligand for curves A, B, and C is* 10^{-3}, 10^{-2}, *and* 10^{-1} *respectively. Curve D presents data for a two-phase system at a total concentration of* 10^{-3}M *where the partition coefficient of the species* ML_2 *is* 999. *Curve E is the formation curve* ($\bar{n}$ versus $pL = -\log_{10}[L]$) *for the single-phase system corresponding to curve A. The vertical lines cutting curves A, B, and C show where the extrema would have occurred in a Job plot* (cf. Fig. 3).

If V, V_0 are the volumes of the aqueous and the organic phase respectively, and C, x, and p_C are defined as before, considerations of mass balance lead to the following equations:

$$(1 - x)CV = ([L] + [ML] + 2[ML_2])V + 2[ML_2]_0V_0$$

and

$$xCV = ([M] + [ML] + [ML_2])V + [ML_2]_0V_0$$

for the case where ML_2 is the extractable complex. Similar equations may be written for more complex systems. For equal phase volumes, $V = V_0$, and it follows that:

$$\bar{n}_T = \frac{\text{Total concentration of bound ligand}}{\text{Total concentration of bound metal}}$$

$$= \frac{(1 - x)C - [L]}{xC} = \frac{\beta_1[L] + 2(1 + p_C)\beta_2[L]^2}{1 + \beta_1[L] + (1 + p_C)\beta_2[L]^2} \quad . \quad . \quad . \quad (14a)$$

Equation (14*a*) is identical in form with equation (14); the only difference is that β_2 has been replaced by $\beta_2(1 + p_C)$. In other words, the degree of formation of the system *as a whole* corresponds to that which would result had the stability constant of the highest complex been increased by a factor $(1 + p_C)$. Since the percentage of any complex, ML_j, is given by the quotient $\beta_j[L]^j/(1 + \beta_1[L] + \beta_2[L]^2 + \ldots \beta_n[L]^n)$, the effect of increasing the value of β_n (relative to those of β_1, β_2, etc.) is to decrease the proportion of lower complexes ML_j $(j < n)$ relative to that of ML_n itself, so that such systems approach more and more closely to the ideal case postulated in the simplified treatment of the method of continuous variations and represented by equation (1). Since the effect of solvent extraction is equivalent to increasing the important overall constant β_n for the complex ML_n by the factor $(1 + p_C)$, the extension of Job's method to two-phase systems should have the further advantage that the position of the extremum should correspond more closely to the theoretical value, so that the possibilities of incorrect deductions should be diminished.

We illustrate this for calculations for a two-phase system with $\beta_1 = 10^5$, $\beta_2 = 10^8$, and $C = 10^{-3}$M, assuming the reasonable value of $p_C = 999$ for the partition coefficient of the complex ML_2. The results are plotted as curve D in Fig. 3. The extremum is well defined and corresponds exactly with the formation of a 1 : 2 complex. It will be recalled that the Job curve for this system at the same concentration in a single phase (Fig. 3, A) would have suggested a 1 : 3 complex. At the extremum the degree of formation of the system (as a whole) is 1·94, appreciably higher than in the case of solutions even 100 times as strong (Fig. 2, curve C) in a monophase. However, since the unique extractable species has the formula ML_2, the degree of formation in the organic phase is always two, so that the effective degree of formation in the aqueous phase is actually decreased in consequence of solvent extraction which alway removes twice as much ligand as metal.

That curves obtained by applying the method of continuous variations to a two-phase system may yet lead to the correct conclusions even though the degree of formation is very low can be illustrated by the rather extreme case presented by the solvent extraction of a metal complex (*e.g.*, a metal–dithizone complex) for which we postulate $\beta_1 = 10^6$, $\beta_2 = 10^{10}$, and $\beta_3 = 10^{12}$. The total concentration of reactants is assumed to be as low as 10^{-4}M and the partition coefficient of the complex, $p_C = 7 \times 10^5$, and that of the reagent, $p_R = 7 \times 10^4$, both strongly favour the organic phase. We further postulate that the acid, HL, conjugate to the ligand is a weak one with $K_R = 10^{-4}$, and that the aqueous phase is buffered at pH 5. The equations characterising this system will be:

$$\frac{(1 - x)C - [L]\{1 + (1 + p_R)[H^+]/K_r\}}{xC} = \bar{n} = \frac{\beta_1[L] + 2\beta_2[L]^2 + 3\beta_3[L]^3(1 + p_C)}{1 + \beta_1[L] + \beta_2[L]^2 + \beta_3[L]^3(1 + p_C)}$$

and $$[ML_n] = xC\beta_n[L]^n/\{1 + \beta_1[L] + \beta_2[L]^2 + \beta_3[L]^3(1 + p_C)\}$$

Since pH $= pK_R + 1$, 90% of the uncomplexed ligand acid in the aqueous phase will be in the form of free ions, L; but the actual concentration will be very low because p_R is so large, in consequence of which the bulk of the uncomplexed reagent remains in the organic phase. Notwithstanding, the results calculated and plotted in Fig. 4 indicate clearly that the maximum concentration of the species ML_3 in the organic phase does occur when $x = 0{\cdot}33$.

It is of interest that if one of the intermediate species had been coloured it would have been possible to apply the ordinary Job procedure to the aqueous phase at the same time in order to establish its composition.

In the two-phase systems, the decrease in free ligand concentration due to the extraction of the ligand acid into the organic phase is compensated by the apparent increase in the stability constant from its true value β_3 to its " effective " value $\beta_3(1 + p_C)$ which produces a great increase in the degree of formation of the species ML_3 (relative to that in a monophase with the same free-ligand concentration) and causes the system to approach more closely to the ideal case postulated in equation (1).

Had the equilibria discussed above been established in a monophase (so that $p_C = p_R = 0$ in the relevant equations) the degree of formation in the aqueous phase at equal values of x would be found to be higher than that in the aqueous phase of the preceding case. However, the way in which the concentrations of the species ML, ML_2, and ML_3 now vary with x is shown in Fig. 5, and it is clear that if the method of continuous variations had been used to ascertain their composition the species ML_3 could have been identified as a 5 : 1 complex and the species ML_2 as a 2 : 1 complex.

Calculation of Stability Constants from Job Curves.—The Job curve for a 1 : 1 complex has its extremum when $x = 0{\cdot}5$, and the degree of flattening in this neighbourhood is a measure of the extent of its dissociation into the components M and L. Indeed the ratio of the measured value of A_j at the extremum to the value obtained by the intersection of the tangents at $x = 0$ and $x = 1$ forms an accurate measure of the concentration term [ML], whence [M] = [L] can be calculated and thence the stability constant.[7] Although this treatment is rigorous when applied to systems in which only a 1 : 1 complex is formed it should be obvious from the foregoing discussion that it cannot validly be extended to calculations of the stability of higher complexes ML$_2$, ML$_3$, etc., unless it is possible to demonstrate that lower complexes do not contribute materially to the equilibrium mixture, *i.e.*, unless $\beta_1, \beta_2, \ldots \ll \beta_n$. The geometric construction needed to obtain the value of the Job ordinate corresponding to no dissociation (*e.g.*, by drawing tangents to the curve at $x = 0$ and $x = 1$) may be subject to considerable error, especially on the ligand-rich side of the diagram (cf. Figs. 3 and 5). Confronted with this difficulty some authors have identified the " theoretical " value of A_j, corresponding to no dissociation of the complex ML$_n$, with the point of intersection of the tangent to the Job curve at $x = 0$ (since this is usually well defined) and the ordinate through $x = 1/(1 + n)$. Unless the extremum of the Job curve also coincides with this value of x, the ratio of the experimental to the

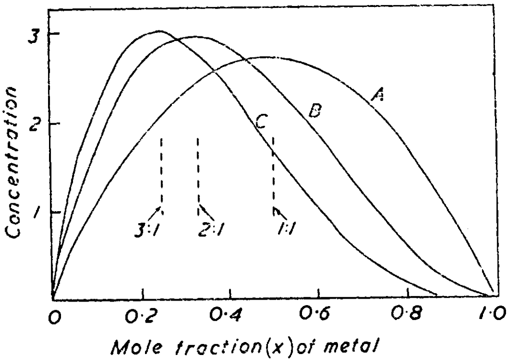


FIG. 4. *The variation in the concentrations of the species* ML, ML$_2$, *and* ML$_3$ *in the aqueous phase of the two-phase system described in the text. The ordinates of curves A, B, and C must be multiplied by* $0{\cdot}77 \times 10^7$, 10^{11}, *and* 10^{17} *respectively to give the actual concentrations of the species* ML, ML$_2$, *and* ML$_3$.

FIG. 3. *The method of continuous variations applied to the metal complexes treated in* Fig. 2. *Curves A, B, and C apply to systems with total concentrations of* 10^{-3}, 10^{-2}, *and* 10^{-1}M. *Curve D refers to a two-phase system at a total concentration of* 10^{-3}M. *The vertical lines show where the extrema should occur theoretically.*

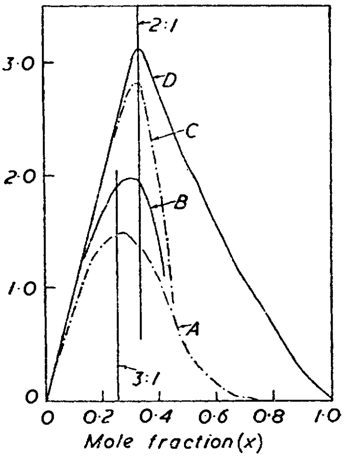
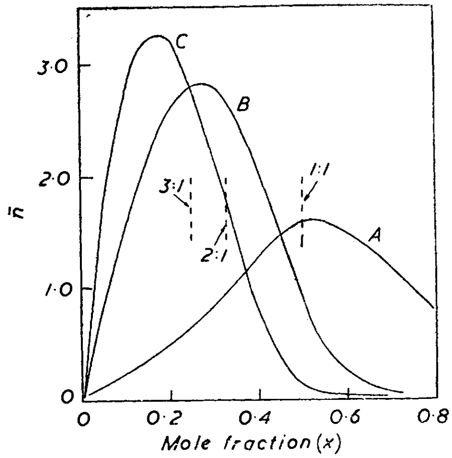


FIG. 5. *The method of continuous variations applied in a monophase to the system treated in Fig. 4. The ordinates of the curves A, B, and C must be multiplied by* $2{\cdot}5 \times 10^{-5}$, $2{\cdot}5 \times 10^{-6}$, *and* 10^{-8} *respectively to give the actual concentrations of the species* ML, ML$_2$, *and* ML$_3$.



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[7] Schwarzenbach, *Helv. Chim. Acta*, 1949, **32**, 839.

calculated value of A_j will not give a correct measure of the concentration of the species ML_n. In any case, for any value of β_n computed from this ratio alone it is tacitly assumed that all lower complexes are of negligible stability. These objections do not appear to be generally appreciated, as may be seen from a number of publications in recent years.[5]

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