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Getting More out of a Job Plot: Determination of Reactant to Product Stoichiometry in Cases of Displacement Reactions and *n*:*n* Complex Formation

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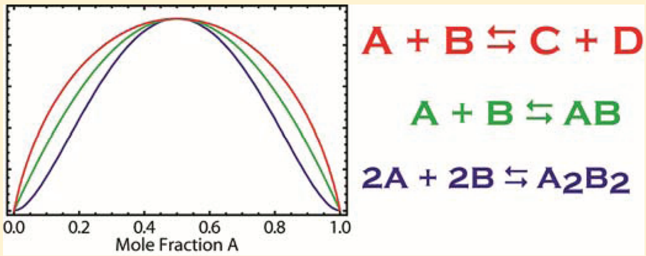
Supporting Information



ABSTRACT: The method of continuous variation (often referred to as Job's method) is an easy and common method for the determination of the reactant stoichiometry of chemical equilibria. The traditional interpretation of Job plots has been limited to complex association equilibria of the type $n\mathrm{A} + m\mathrm{B} \rightleftharpoons \mathrm{A}_n\mathrm{B}_m$, while little focus has been placed upon displacement type reactions (e.g., $\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{C} + \mathrm{D}$), which can give Job plots that look quite similar. We developed a novel method that allows the user to accurately distinguish between 1:1 complex association, 2:2 complex association, and displacement reactions using nothing more than a pocket calculator. This method involves preparing a Job plot of the system under investigation (using regularly spaced mole fractions), normalizing the measured quantities (such as the concentration of $\mathrm{A}_n\mathrm{B}_m$ or C for the above reactions) to their maximum value (i.e., at mole fraction 0.5), and determining the sum of the normalized values. This sum is then compared with theoretically predicted normalized sum values that depend on the nature of the equilibrium. The relationship between, on the one hand, the sum of the normalized values and, on the other hand, the reaction equilibrium constant and the concentration of the stock solutions used for the preparation of the Job plot is also explored. The use of this new technique for the interpretation of Job plots permits users to readily determine information that can be obtained otherwise only with laborious additional experiments, as illustrated by the analysis of four Job plots taken from the literature.

INTRODUCTION

Several techniques have been developed for the determination of the stoichiometry of chemical equilibrium reactions, including the method of continuous variation,[1−4] slope ratio method,[5] and mole ratio method,[6] to name a few. Due to its simplicity, the method of continuous variation (often referred to as Job's method), first described by Ostromisslensky,[1] Dension,[2] and Job,[3] is widely regarded as the most popular of these methods.[7,8] A common use of Job's method is the determination of the ratio of the reaction coefficients n and m in an association equilibrium of the form

$$n\mathrm{A} + m\mathrm{B} \rightleftharpoons \mathrm{A}_n\mathrm{B}_m \tag{1}$$

where A and B represent the free species at equilibrium (i.e., a ligand and a substrate), and $\mathrm{A}_n\mathrm{B}_m$ represents a molecular complex of A and B.[3,7,9−13] In this method, the measured concentration of the complex $\mathrm{A}_n\mathrm{B}_m$ (or a parameter that is proportional to its concentration, such as its UV/vis or infrared absorbance[14] or the integrated NMR signal intensity[15]) is plotted against the mole fraction of one reactant while the sum of the reactant concentrations, c^0_{SUM}, is kept constant. This plot is referred to as a Job plot. It follows from eq 1 that $c^0_{\mathrm{A}} = c_{\mathrm{A}} + nc_{\mathrm{A}_n\mathrm{B}_m}$ and $c^0_{\mathrm{B}} = c_{\mathrm{B}} + mc_{\mathrm{A}_n\mathrm{B}_m}$, where c_{A}, c_{B}, and $c_{\mathrm{A}_n\mathrm{B}_m}$ refer to the species present in solution, while c^0_{A} and c^0_{B} refer to the total concentrations of the reactants A and B, respectively. The latter are related to c^0_{SUM} at any given mole fraction f of species A by

$$c^0_{\mathrm{A}} = f \times c^0_{\mathrm{SUM}} \tag{2}$$

$$c^0_{\mathrm{B}} = (1 - f) \times c^0_{\mathrm{SUM}} \tag{3}$$

For association equilibria of this type, the highest concentration of the complex $\mathrm{A}_n\mathrm{B}_m$ and, thereby, the maximum in the Job plot is given by

$$x = \frac{n}{n + m} \tag{4}$$

where x is the mole fraction of A at the maximum point of the curve.[8] Clearly, this relationship makes Job's method an extremely powerful tool to determine the stoichiometries of equilibrium reactions, which is the cause for the popularity of the method.

Because of its utility and simplicity, Job's method has been utilized to interrogate a wide variety of systems, including ligand/metal complexes,[16] complexes of inorganic anions and organometallic compounds,[17,18] the binding of DNA to fluorescent dyes,[19] and the interaction of organic hosts and guests,[20,21] to name just a few. Because of the widespread use of Job's method,

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several reports highlighting the technical aspects of the method have been published.[12−14,22−25] In particular, a number of techniques have been developed to directly determine the formation constant of the complexes that are investigated by fitting of Job plot curves.[10,12,13,16,22]

However, while the conventional interpretation of a Job plot allows for the immediate determination of the empirical ratio of the reactants in a chemical equilibrium by the location of the maximum in the plot, distinguishing between 1:1, 2:2, and higher *n*:*n* stoichiometries on the basis of a Job plot is more difficult, because they all exhibit a maximum at the same mole fraction (i.e., $f = 0.5$). Recently, a method developed by Sayago and co-workers utilized Job's method with spectrophotometry to distinguish between *n*:*m* stoichiometries of the types 1:1 and 2:2.[9] While the use of this method accurately confirmed the presence of a weakly bound 2:2 complex, it relied upon the prediction of a maximum absorbance and replotting of the Job data in a linearized form derived from a method developed by Heller and Schwarzenbach.[26] Even more difficult is the differentiation between an association equilibrium as described by eq 1 and a displacement reaction of the type

$$\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{C} + \mathrm{D} \tag{5a}$$

where the concentration of either species C or D is detected and plotted as a function of the mole fraction of either A or B. The method described herein considerably simplifies this problem, as it can be used to quickly and accurately determine the stoichiometry of chemical equilibria from Job plots. Specifically, this contribution focuses on the three most common reaction types, which are 1:1 complex association (i.e, eq 1 where $n = m = 1$), 2:2 complex association ($n = m = 2$), and the 1:1 displacement reaction (eq 5a). Note that any treatment of the latter also applies to the special case of

$$\mathrm{AB} + \mathrm{C} \rightleftharpoons \mathrm{A} + \mathrm{BC} \tag{5b}$$

where B is transferred from A to C. A typical example for such a reaction is a H^+ transfer. Examples of other reactions that involve two reactants and give a Job plot with a maximum at the mole fraction of 0.5 seem to be very rare.

Distinguishing between the formation of complexes of the type $\mathrm{A}_n\mathrm{B}_m$ and displacement reactions using the method introduced in this contribution is achieved by normalization and summation of the measured concentration of a product of the reaction in question. As with other methods for interpreting Job plots, this method is limited to the presence of a single equilibrium.[7] To simplify the discussion herein, we refer to the species written on the left-hand side of equilibria 1, 5a, and 5b as reactants, while those written on the right-hand side are referred to as products.

DESCRIPTION OF DIFFERENT EQUILIBRIA

1:1 Complex Association. In the case of 1:1 complex association, as described by eq 1 for n = m = 1, the system is described by:

$$K = \frac{c_{\mathrm{AB}}}{c_{\mathrm{A}} \times c_{\mathrm{B}}} \tag{6}$$

$$c_{\mathrm{A}}^0 = c_{\mathrm{AB}} + c_{\mathrm{A}} \tag{7}$$

$$c_{\mathrm{B}}^0 = c_{\mathrm{AB}} + c_{\mathrm{B}} \tag{8}$$

where K is the equilibrium constant describing the complex formation, and c_{i} is the equilibrium concentration of species i. Solving the set of eqs 2, 3, and 6−8 for c_{AB} in terms of c^0_{SUM}, f, and K results in

$$c_{\mathrm{AB}} = 1 + c^0_{\mathrm{SUM}}K - \sqrt{1 + 2c^0_{\mathrm{SUM}}K + [c^0_{\mathrm{SUM}}K(1-2f)]^2} \tag{9}$$

2:2 Complex Association. For a 2:2 complex association, as described by eq 1 for $n = m = 2$, the system is described as

$$K = \frac{c_{\mathrm{A_2B_2}}}{(c_{\mathrm{A}} \times c_{\mathrm{B}})^2} \tag{10}$$

$$c_{\mathrm{A}}^0 = 2 \times c_{\mathrm{A_2B_2}} + c_{\mathrm{A}} \tag{11}$$

$$c_{\mathrm{B}}^0 = 2 \times c_{\mathrm{A_2B_2}} + c_{\mathrm{B}} \tag{12}$$

Unlike in the case of 1:1 complex association, combining eqs 2, 3, and 10−12 results in a (fourth-order) polynomial that cannot be solved algebraically to give a single discrete equation describing the system.[18] As for any fourth-order polynomial, there are four algebraic solutions to this problem, but the Job plots cannot be calculated from one of these algebraic solutions alone. Instead, it depends on the actual numerical values of K, c^0_{A}, and c^0_{B}, whose algebraic solution has to be used to calculate the concentration of $\mathrm{A_2B_2}$. Consequently, this system of equations was solved for the following discussion numerically, using (as for all other graphs and calculations) the software Mathematica 7 (Wolfram Research, Champaign, IL).

1:1 Displacement Reaction. Finally, for a 1:1 displacement reaction as described by eq 5a, the equilibrium is described by

$$K = \frac{c_{\mathrm{C}} \times c_{\mathrm{D}}}{c_{\mathrm{A}} \times c_{\mathrm{B}}} \tag{13}$$

$$c_{\mathrm{A}}^0 = c_{\mathrm{A}} + c_{\mathrm{C}} \tag{14}$$

$$c_{\mathrm{B}}^0 = c_{\mathrm{B}} + c_{\mathrm{C}} \tag{15}$$

$$c_{\mathrm{C}} = c_{\mathrm{D}} \tag{16}$$

Solving the set of eqs 2, 3, and 13−16 for c_{C} or c_{D} in terms of c^0_{SUM}, f, and K results in

$$c_{\mathrm{C}} = c_{\mathrm{D}} = \frac{c^0_{\mathrm{SUM}}K - c^0_{\mathrm{SUM}}\sqrt{K^2(1-2f^2-4Kf^2+4Kf)}}{2(K-1)} \tag{17}$$

THEORETICAL PREDICTION OF JOB PLOTS

Figure 1 shows on the left-hand side representative plots of the three systems described above for several equilibrium constants and a c^0_{SUM} value of 10 mM. For simplicity, the concentration of the measured species is plotted, rather than a measurable parameter that is proportional to its concentration, such as absorbance or integrated NMR signal intensity. To facilitate the direct comparison of the curve shapes and to emphasize the difference between the three different systems, the same plots are also shown on the right-hand side, but this time normalized to their maximum value, c_{MAX}. Because the type of equilibria

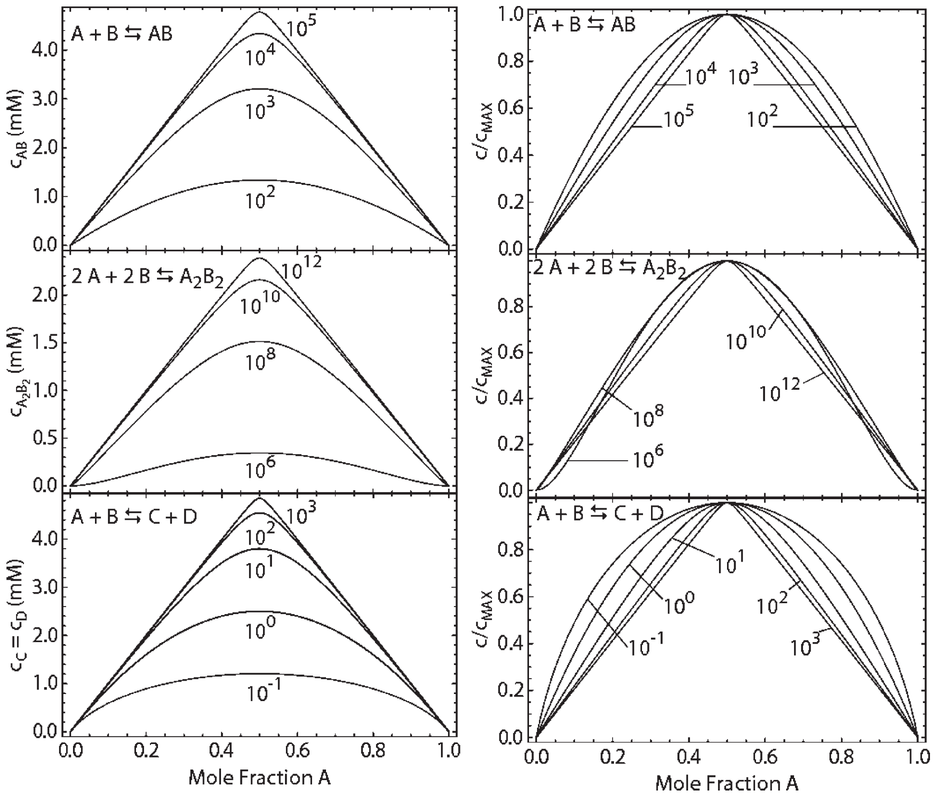


Figure 1. Theoretical (left) and normalized (right) Job plots for 10 mM stock solutions (i.e., c^0_{SUM} = 10 mM) and several equilibrium constants for 1:1 association (top), 2:2 association (middle), and 1:1 displacement (bottom) equilibria as described by eq 5a. The different curves are labeled with their respective equilibrium constants, K.

described here have an equimolar reactant stoichiometry, c_{MAX} is the concentration of the measured species at $f = 0.5$. Therefore, the plotted variable c/c_{MAX} is the ratio of the concentration of the measured species at point f and the concentration of that species at $f = 0.5$. In other words, c/c_{MAX} is the concentration of the measured species normalized with respect to its concentration at $f = 0.5$.

As pointed out in the literature, the shape of the Job plot curves shown in Figure 1 distinctly varies with the equilibrium constant.[8,25] For all three equilibrium stoichiometries described herein, the predicted curves approach a limiting triangular shape for reactions characterized by very large equilibrium constants: i.e., reactions resulting in the formation of very stable associates or displacement reactions favoring very strongly the products C and D. Because these triangular shapes are identical for all three systems, the determination of the stoichiometry from Job's method alone is not possible in these cases. Note, however, that use of a more dilute stock solution can eliminate this problem (see below).

It is also clear from the normalized plots that, for small equilibrium constants, the Job plot curves for all three types of equilibria take on distinctly different shapes. As can be clearly observed in Figure 1, the integrated areas under the normalized Job plot differ greatly for the three equilibrium stoichiometries. The distinctly different shapes for the Job plot allow for determination of the equilibrium stoichiometry for systems with small equilibrium constants. Indeed, previous methods to determine reaction stoichiometry and/or binding constant are applicable only for rather small equilibrium constants.[9,10,12,16,27]

NUMERICAL EVALUATION OF EXPERIMENTAL JOB PLOTS

By quantitatively comparing the area under the normalized theoretical Job plots, a method may be developed to determine the reaction stoichiometry. However, experimental Job plots are not continuous functions of the mole fraction on the x axis, making it impossible to determine the area from a discrete and limited number of measured data points without making some approximations. Indeed, experimental Job plots are prepared using measured concentrations of reaction products only for a limited number of distinct mole fractions. Therefore, rather than calculating the area under an approximated continuous curve, it is much more convenient to consider the sum of the individual data points after normalization to c_{MAX}, as described in Theoretical Prediction of Job Plots. Consequently, the method developed herein can be summarized as follows.

(1) Prepare a Job plot as outlined in the literature,[8,14] varying the mole fraction of A (and consequently B) at constant intervals of Δf.

(2) Verify that the maximum of the resulting Job plot curve is at $f = 0.5$ and that the curve is symmetric around $f = 0.5$. This is to confirm that the equilibrium under investigation involves the reaction of equal amounts of reactants A and B and that the system being interrogated does not exhibit multiple equilibria. If the curve either is not centered at $f = 0.5$ or is asymmetric, the approach developed herein is not applicable.

(3) Determine the c/c_{MAX} value for each data point by dividing the measured quantity, c (i.e., either the concentration of the product or a measured observable that is

directly proportional to its concentration), at each point of *f* with the measured quantity at $f = 0.5$ (c_{MAX}).

(4) Calculate the sum of c/c_{MAX} over the entire range of *mole fractions* from 0 to 1.

The calculated sum, as described by step 4, is directly related to the area under the continuous curve and will be represented in the following as $\Sigma c/c_{MAX}$. Therefore, the experimentally determined values of $\Sigma c/c_{MAX}$ can be used to determine the equilibrium stoichiometry by comparison to theoretically predicted values for $\Sigma c/c_{MAX}$.

Note that even if in step 3 a measured quantity other than concentration is used for *c* and c_{MAX}, special pretreatment of the data is not required, provided that the measured quantity is directly proportional to the concentration of the reaction product. This is a direct result of the elimination of the proportionality constant between the measured quantity and the species concentration (e.g., molar absorptivity) using the normalization described in step 3.

RESULTS AND DISCUSSION

Effect of Equilibrium Constant on $\Sigma c/c_{MAX}$. As mentioned above, the area under the Job plot curve for any fixed c^0_{SUM} is highly dependent on the equilibrium constant, *K*. Because this area is directly related to $\Sigma c/c_{MAX}$, the value of $\Sigma c/c_{MAX}$ is also highly dependent upon the equilibrium constant. The relationship between $\Sigma c/c_{MAX}$ and the equilibrium constant of the system is shown in Figure 2. For this plot, a mole fraction interval, Δf, of 0.1 was chosen.

The curves for $\Sigma c/c_{MAX}$ versus *K* shown in Figure 2 illustrate two important features. First, for very large equilibrium constants, the value of $\Sigma c/c_{MAX}$ reaches a constant value. For the example of $\Delta f = 0.1$ used for Figure 2, this value is 5. This phenomenon is expected since, in the limit of large equilibrium constants, the Job plots approach an isosceles triangle: i.e., a triangle with two equal sides (as shown in Figure 1). More importantly, in the limit of extremely large formation constants, $\Sigma c/c_{MAX}$ approaches a numerical value that is the same regardless of stoichiometry. This value only depends on the number of experimental points in the Job plot: i.e., the number of mole fractions for which a measurement was performed. In the general case of *n* data points distributed equally between 0 and 1 on the mole fraction scale, the limiting value of $\Sigma c/c_{MAX}$ is $(n - 1)/2$ for all three types of reactions in question.

Second, in the limit of *small* equilibrium constants, a constant value of $\Sigma c/c_{MAX}$ is also achieved, but the value of $\Sigma c/c_{MAX}$ depends on the type of reaction. Thus, calculating $\Sigma c/c_{MAX}$ for systems in which the lower limit is reached allows for the direct determination of the type of equilibrium by comparison with the theoretically predicted values of $\Sigma c/c_{MAX}$ for the three different equilibria. Table 1 contains an abridged list of the numerical values for $\Sigma c/c_{MAX}$ for the three systems in the limit of small equilibrium constants. A more extensive list may be found in the Supporting Information for this article.

As in the limiting case of *large* equilibrium constants, the values of $\Sigma c/c_{MAX}$ in the limiting case of *small* equilibrium constants can also be readily predicted from the number of data points, *n*, of the Job plot. As plots of $\Sigma c/c_{MAX}$ values versus *n* show (see Table 1 and the corresponding table in the Supporting Information), $\Sigma c/c_{MAX}$ in the limiting case of small equilibrium constants can be the calculated as $0.77 \times (n - 1)$ for the displacement reaction, as $0.66 \times (n - 1)$ for the formation of the product AB, and as $0.53 \times (n - 1)$ for the formation of A_2B_2. These relationships are empirical but predict for all Job plots with at least nine data points the $\Sigma c/c_{MAX}$ values with an error of less than 1.0%. The few exceptions with errors in the 1.0−3.4% error range are all for Job plots characterizing displacement equilibria investigated with 13 data points or fewer, where $\Sigma c/c_{MAX}$ is a slightly less accurate estimate of the area under the Job plot curve.

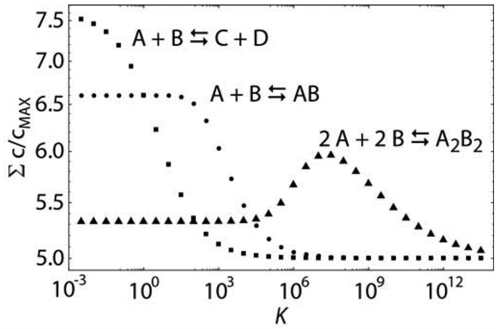


Figure 2. Plot of $\Sigma c/c_{MAX}$ versus *K* for 1:1 displacement (squares), 1:1 association (circles), and 2:2 association (diamonds) equilibria for 10 mM stock solutions and $\Delta f = 0.1$ (i.e., for Job plots with 11 data points equally distributed on the mole fraction axis).

Table 1. Theoretical Values of $\Sigma c/c_{MAX}$ in the Limit of Small Equilibrium Constants as a Function of the Number of Data Points, *n*, of the Job Plot

		$\Sigma c/c_{MAX}$		
no. of data points[a]	Δf	1:1 association	1:1 displacement	2:2 association
9	0.1250	5.25	5.96	4.27
11	0.1000	6.60	7.55	5.34
16	0.0667	9.96	11.49	8.00
21	0.0500	13.30	15.41	10.67
26	0.0400	16.64	19.33	13.33
41	0.0250	26.65	31.04	21.34

[a] Including $f = 0$ and $f = 1$.

Interestingly, the maximum in the curve for A_2B_2 in Figure 2 is always observed at $0.60 \times (n - 1)$, which shows that, except for very minor deviations for very small values of *n*, the shapes of the curves depicted in Figure 2 do not depend on the number of data points. Figures that are equivalent to Figure 2, but that are prepared for different numbers of data points (*n*) in the Job plots, differ from Figure 2 only by a scaling factor for the *y* axis.

It is also clear from Figure 2 that experimentally determined, intermediate values of $\Sigma c/c_{MAX}$ immediately exclude the occurrence of certain equilibrium stoichiometries. For instance, if a Job plot has been prepared for $\Delta f = 0.1$ and passes the symmetry test, and $\Sigma c/c_{MAX}$ lies between 6.60 and 7.55, formation of AB or A_2B_2 can be excluded. Consequently, there is strong evidence for a displacement reaction. Similarly, if for $\Delta f = 0.1$ the experimentally determined value of $\Sigma c/c_{MAX}$ lies above 6.00, the possibility of 2:2 association may be eliminated. Also, if for a Job plot with $\Delta f = 0.1$ a value of 5.34 for $\Sigma c/c_{MAX}$ is calculated, and the Job plot exhibits inflections near the mole fractions of 0 and 1 (giving it the characteristic bell shape shown in Figure 1), this is consistent with A_2B_2 formation. Consequently, AB formation and displacement can be excluded. In the event that the system being

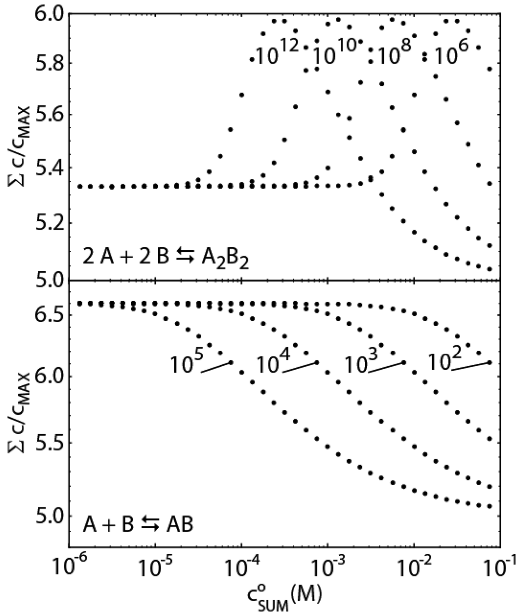


Figure 3. Relationship of $\Sigma c/c_{MAX}$ on c^0_{SUM} for $\Delta f = 0.1$ and several formation constants for the 1:1 and 2:2 association reactions. All curves are labeled with the respective formation constant.

interrogated cannot be determined using one of these checks, it can be concluded that either the limit of weak binding for an A_nB_m formation is not reached or a displacement reaction is occurring. In such a case, more information may be attained by varying the value of c^0_{SUM} used to determine the Job plot.

Note that the $\Sigma c/c_{MAX}$ values for the displacement and the 2:2 association case are 17% larger and 20% smaller, respectively, than in the case of 1:1 stoichiometry. These are large differences and will typically permit a distinction between different types of equilibria within experimental error, even where a casual look at the Job plots might suggest that the differences between the Job plots for the different types of equilibria are not very pronounced.

Effect of Stock Concentration on $\Sigma c/c_{MAX}$. Figure 2 may appear somewhat discouraging if *K* lies outside the lower limiting range. However, the use of a stock solution of lower concentration can often easily shift the Job plot toward the low binding limit. In other words, for the purpose of the method of Job plot analysis presented here, a binding constant is only large or small in comparison to the c^0_{SUM} value employed. Dilution of the stock solution to shift the Job plot toward the low binding limit is limited only by the lower detection limit of the instrumental method used to measure the concentration of the observed species (e.g., UV/vis, infrared, or NMR spectrometry).

Figure 3 shows the relationship between $\Sigma c/c_{MAX}$ and c^0_{SUM} for 1:1 and 2:2 association systems. The 1:1 displacement reaction has been omitted from this plot, as it can be shown easily from eq 17 that for this system $\Sigma c/c_{MAX}$ is independent of c^0_{SUM}.

As it can be seen from Figure 3, $\Sigma c/c_{MAX}$ exhibits a strong dependence upon the c^0_{SUM} value chosen when preparing the Job plot. As such, the limiting value of $\Sigma c/c_{MAX}$ observed for small equilibrium constants may be determined experimentally by varying the c^0_{SUM} value chosen for the Job plot, thus allowing the determination of the reaction stoichiometry over a wide range of equilibrium constants.

While in an intermediate case the most obvious option for the determination of the correct type of equilibrium is to lower c^0_{SUM} to a value sufficiently low such that $\Sigma c/c_{MAX}$ reaches its limiting value, it is not the only option. Even in the case of an experimental technique that has a detection limit that is insufficiently low to permit working with c^0_{SUM} low enough to reach the lower limiting case, there are still prospects to determine the type of equilibrium from Job plots. If the measured $\Sigma c/c_{MAX}$ is intermediate to the limiting values determined above and shows no dependence on c^0_{SUM}, the reaction may immediately be concluded to be a 1:1 displacement reaction. Alternatively, if $\Sigma c/c_{MAX}$ shows a positive slope with respect to c^0_{SUM}, the equilibrium is a 2:2 association reaction.

Table 2. Analysis of Job Plots from Refs 18, 21, 28, and 29

ref	figure[a]	published reacn type[b]	$\Sigma c/c_{MAX}$	error (%)[c]
18	2	2:2 association	5.49	2.72
21	11	1:1 association	6.55	−0.83
28	2	proton transfer	7.54[d]	−0.17
29	4	1:1 association	7.26[e]	10.0

[a] Figure number in original publication. [b] Published conclusion. [c] Percent error in measured $\Sigma c/c_{MAX}$ versus theoretical Job plot curve for the reaction type indicated. [d] Average result from four Job plots. [e] The data point at 100% host was omitted from this reanalysis.

Application to Published Data. To demonstrate the utility of the method described in this article, four Job plots published previously[18,21,28,29] were analyzed, and the conclusions gained by this new method of Job plot analysis were compared with the conclusions reported in the original literature. The plots were chosen as examples for all types of equilibria discussed in this paper. A summary of these results is shown in Table 2 and is discussed in the following.

Using the analysis method introduced in this paper, two examples of association reactions from the literature were analyzed and the original authors' conclusions confirmed. First, a Job plot describing the complexation of iron(III) with chrome azurol S, a known 2:2 association reaction, was reanalyzed.[18] As expected, reanalysis of the Job plot presented in the original article indicates that, with an error of only 3%, this system is a 2:2 association reaction. Another association system that we reanalyzed is the complexation of citrate by a fluorescent tricationic guanidiniocarbonyl pyrrole.[21] By monitoring the decrease in the fluorescence of this receptor upon addition of citrate, the authors nicely showed a 1:1 receptor:citrate stoichiometry and confirmed complex association by ^{1}H NMR spectroscopy. This conclusion is strongly supported by reanalysis using the method described above, the error being only −0.83%.

While distinguishing between 1:1 and 2:2 association reactions may be achieved using established analysis techniques, 1:1 displacement reactions are not as readily identified and can be quite easy to miss. This is particularly the case when the species observed for the Job plot is the minority species, as we reported in a recent publication.[28] In this article, we characterized the interaction of 2,4-dinitrotoluene (DNT) with nucleophilic bases in DMSO. It has long been assumed that polynitroaromatic compounds such as DNT form colored Meisenheimer complexes with nucleophiles,[30−35] and the addition of an aliphatic amine to a solution of DNT in DMSO indeed produces an intense blue color. However, the presence of a Meisenheimer complex could not be confirmed by NMR spectroscopy. This led us to utilize a variety of techniques to investigate this system, leading to the conclusion that the interaction between DNT with a base in DMSO results in the deprotonation of an extremely small fraction of the DNT and not the formation of a Meisenheimer

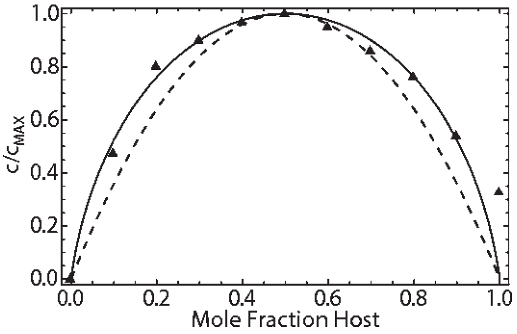
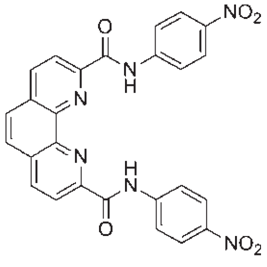


Figure 4. Data from Figure 4 in ref 29 normalized to the maximum at $f = 0.5$, along with theoretically expected Job plots for 1:1 displacement (solid line) and 1:1 association (dashed line) in the limiting case of small equilibrium constants.

complex. As expected, reanalysis of Job plots for the interaction of DNT with diethylamine shows very clearly that this interaction is a 1:1 displacement reaction, with an error of only −0.17% (Table 2, third row).

Another common system in which there can be difficulty in distinguishing between 1:1 association and 1:1 displacement is the case of hydrogen-bonding receptors for basic anions.[36] Such receptors often use guanidinium, amide, or urea functional groups (or their thio derivatives) as hydrogen bond donors to bind the anion of interest.[21,36] To enhance the hydrogen bond donor strength of these groups by increasing the partial positive charge on their hydrogens, these receptors are often substituted with electron-withdrawing groups that also make these receptors more acidic. This leads to the possibility of deprotonation rather than complex formation.[37,38] As shown previously in the literature, the straightforward identification of deprotonation in such systems is somewhat complicated because the titration plot of a deprotonation reaction can be satisfactorily fitted using a 1:1 association model,[36] which can easily lead to erroneous conclusions. Therefore, one has to be careful and may have to rely upon a second, complementary technique to identify the occurrence of deprotonation. As an example, Table 2 includes the reanalysis of the interaction between the diamide receptor **1** and acetate, which has been previously interpreted as a 1:1 complex association (fourth row of Table 2).[29] Our reanalysis of the published Job plot shows that this interpretation exhibits a 10.0% divergence between the theoretically expected value of $\Sigma c/c_{\mathrm{MAX}}$ and the value determined from the experimental data. This is a large deviation considering that, in the limit of small equilibrium constants, erroneously fitting a perfect 1:1 displacement reaction with a 1:1 complexation model would result in a difference in $\Sigma c/c_{\mathrm{MAX}}$ of only 14.4% (see Table 1 for 11 data points).



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Indeed, this system can be fitted much more accurately based on a 1:1 displacement reaction, giving a deviation of only −3.8% from the theoretically expected value. The visual difference between the two fits is striking. When the experimental data is normalized to c_{MAX} and plotted along with the theoretically expected plots for 1:1 association and the displacement reaction (see Figure 4), the 1:1 association fit clearly show that the 1:1 association gives a much worse fit. The systematically positive residuals for this fit are a characteristic feature of an inappropriate model. Clearly, a reevaluation of the reaction of acetate with receptor **1** is warranted.

CONCLUSIONS

Job's method is a powerful tool for the determination of the stoichiometry of the reactants in a chemical equilibrium. However, previously developed methods for interpreting Job plots have been limited to complex association reactions.[7] Only recently, a method was developed by Sayago and co-workers to accurately discern between 1:1 and 2:2 association reactions.[9] This approach required linearization of the data, followed by least-squares analysis of a linear fit of the data.

The new technique described in this paper allows rapid determination of the reactant:product stoichiometry of chemical equilibria with equimolar reactant stoichiometry by simple normalization and summation of equally spaced data points in a Job plot. We have shown that the resulting $\Sigma c/c_{\mathrm{MAX}}$ reaches upper and lower limiting values, the lower limiting value being distinct for the different reaction stoichiometries. Careful selection of the stock concentrations allows the user to access these regions for the case of 1:1 and 2:2 association reactions, while $\Sigma c/c_{\mathrm{MAX}}$ is shown to be independent of c^0_{SUM} for 1:1 displacement reactions. This independence is an extremely useful diagnostic when intermediate values of $\Sigma c/c_{\mathrm{MAX}}$ are observed for the system being studied.

The method proposed here is not intended to replace numerical fits of Job plots entirely. The latter can still be very useful to determine equilibrium constants. However, fitting of Job plots with several types of reaction models to determine which equilibrium model fits the data best is a very time-consuming process, especially if multiple Job plots are fitted simultaneously and/or if Job plots and titration curves are fitted simultaneously with one set of parameters (for an example, see ref 28). Moreover, Job plot fitting is even more complicated when the extinction coefficient of the reaction product (or alternatively any other proportionality constant between the measured quantity on the reaction product) cannot be determined readily.[28] Under such circumstances, the remarkably simple method introduced here considerably simplifies the study of the equilibrium and the ultimate determination of equilibrium constants. We tested this method by reanalyzing Job plots that were previously presented in the literature, demonstrating the simplicity and utility of the method not only to confirm known complex stoichiometries but also to readily detect errors in the interpretation of systems investigated less thoroughly in the past. Given the regular use of Job plots across different fields of chemistry and in related disciplines, this new method of analysis should prove to be a useful tool in studies of a wide range of chemical equilibria.

ASSOCIATED CONTENT

S Supporting Information. A table giving theoretical values of $\Sigma c/c_{\mathrm{MAX}}$ based upon the number of data points, n, for n ranging from 7 to 41. This material is available free of charge via the Internet at http://pubs.acs.org.

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