Theory of the Measurement of Weak Molecular Complexes. II. Consequences of Multiple Equilibria

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Abstract: The consequences of multiple equilibria on the calculation of formation constants and other molecular constants for weak complexes are considered from a theoretical standpoint. A microscopic model is used to illustrate the averaging process involved in the assignment of formation constants for higher order complexes, and the slopes, and intercepts of various plotting forms usually applied to the evaluation of weak complexes are derived for a PX, PX_2 system. It is shown that, except in the special case where all the microscopic constants of the system are closely similar, curved lines are to be expected on all of the usual plots. At very low saturation fractions, however, curvature is small, and in the limit as the concentration of the excess component approaches zero, the plotted curves are indistinguishable from straight lines. The slopes and intercepts of such lines have widely differing experimental meaning and cannot be interpreted simply as "K" or "$K\epsilon$" except in cases of very little practical interest. From the shape of the theoretical curves, it appears that it is necessary to measure of the order of 75% of the entire saturation curve before a given stoichiometric model can be assigned to the fitted data.

In the preceding paper of this series, we have attempted to point out where errors may arise in the evaluation of formation constants of weak complexes by the usual methods of analysis.[1] In particular, we have tried to show the necessity for collection of roughly 75% of the data comprising the entire saturation curve in order to prove the model, or range of models, fitting the phenomenological equation "straight line." We would like now to enquire into specific conditions under which the entire binding curve cannot be represented as a straight line, *i.e.*, to consider the consequences of multiple equilibria on the slopes and intercepts of the Benesi–Hildebrand[2] and Scott[3] plots, from which virtually all formation constants for weak complexes have been evaluated to date. The reason for so doing is to examine the possible origin of observed or suspected anomalies[4–11] in the reported values of "K" or "ϵ" other than those generated by insufficient data to obtain an accurate slope or intercept from the appropriate plot.[1,4]

The problem of higher order complexes has been more or less exhaustively treated[12] in the literature and the concept has been applied by Ross and Labes[9] to

weak molecular complexes of the "charge-transfer" type. Carter, *et al.*,[7] proposed a solvent binding theory to account for anomalies in the values of "K" and "ϵ"; however, it was later found necessary to include a higher order complex in the theory in order to fully explain the anomalies in the calculated constants.[8] Johnson and Bowen[13] concluded from numerical calculations that straight lines are obtained from the Benesi–Hildebrand plot in spite of the presence of higher order complexes with widely differing step formation constants. However, this conclusion can be shown to be generally false except under certain conditions (data obtained over a limited range of the saturation curve), and little can be said concerning the stoichiometry for these cases. In addition, Johnson and Bowen made no attempt to predict theoretical values for the slope and intercept of the plot, which can have widely different experimental meaning for different multiple equilibria systems. Equations of multiple equilibria specifically derived for charge-transfer complexes have also been presented by Briegleb,[14] but again no attempt was made to evaluate the slopes or intercepts of the various plots.

The Microscopic and Macroscopic Binding Models. For purposes of clarity, we shall rederive the normal equilibrium equations of a simple model of multiple binding (complexes of the type PX and PX_2) from the standpoint of a microscopic model. Although no additional information concerning the formation constants of the over-all macroscopic equilibrium reaction results from this treatment, the microscopic model is directly applicable to kinetic methods and has the advantage of clearly demonstrating the averaging processes involved in the application of the usual macroscopic model to the binding process, and the possible mechanisms of such binding processes.

Consider a dilute component P, at constant total concentration [P_0], to which two molecules of a second component X can be bound according to the scheme in (1).

(1) Part I: D. Deranleau, *J. Am. Chem. Soc.*, 91, 4044 (1969). Note that in the present paper, the notation K is used for the macroscopic binding constant, whereas in the preceding paper, k was used for the same constant. The purpose is to emphasize the difference between simple 1:1 binding, for which the microscopic constant k and the macroscopic constant K are identical, and multiple binding, where in general K is a function of several k's.
(2) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 71, 2703 (1949).
(3) R. L. Scott, *Rec. Trav. Chim.*, 75, 787 (1956).
(4) W. B. Person, *J. Am. Chem. Soc.*, 87, 167 (1965).
(5) P. H. Emslie, R. Foster, C. A. Fyfe, and I. Horman, *Tetrahedron*, 21, 2843 (1965).
(6) P. H. Emslie and R. Foster, *ibid.*, 21, 2851 (1965).
(7) S. Carter, J. N. Murrell, and E. J. Rosch, *J. Chem. Soc.*, 2048 (1965).
(8) S. Carter, *ibid.*, A, 404 (1968).
(9) S. D. Ross and M. M. Labes, *J. Am. Chem. Soc.*, 79, 76 (1957).
(10) N. J. Rose and R. S. Drago, *ibid.*, 81, 6138 (1957).
(11) L. J. Andrews and R. M. Keefer, "Molecular Complexes in Organic Chemistry," Holden-Day Inc., San Francisco, Calif., 1964.
(12) See, for example, F. J. C. Rossotti and H. Rossotti, "The Determination of Stability Constants," McGraw-Hill Book Co., Inc., New York, N. Y., 1961; J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, New York, N. Y., 1958; G. Weber in "Molecular Biophysics," B. Pullman and M. Weissbluth, Ed., Academic Press, New York, N. Y., 1965.
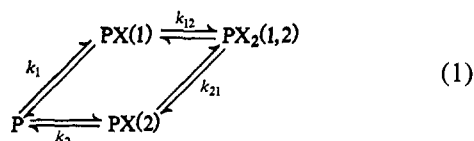(13) G. D. Johnson and R. E. Bowen, *J. Am. Chem. Soc.*, 87, 1655 (1965).
(14) G. Briegleb, "Elektronen-Donator-Acceptor-Komplexe," Springer-Verlag, Berlin, 1961.

$$PX(1) \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} PX_2(1,2)$$

$$P \underset{k_2}{\rightleftarrows} PX(2) \qquad\qquad (1)$$

The k's are the microscopic association constant for the reactions shown, and the model implies that there are two microscopically distinct species PX which have the possibility of binding a molecule of X on either site 1 or site 2; that is, the sites are distinguishable. All possibilities are allowed by the model, for example, $k_1 = k_2 = k_{12} = k_{21}$ (equivalent and independent sites); $k_{12} > k_1$ and/or $k_{21} > k_2$ (normal or mixed cooperative binding); $k_2 = k_{21} = 0$ (cooperative binding in which the second site is created by the addition of the first molecule of X); $k_1 \neq k_2 \neq k_{12} \neq k_{21}$ (competitive equilibria with interactions between sites); etc. If the absorbance of the solution of P and X is due entirely to the formation of complexes with microscopic extinction coefficients $\epsilon(1)$, $\epsilon(2)$, $\epsilon(1,2)$, not necessarily the same, we can write

$$A = \epsilon(1)[PX(1)] + \epsilon(2)[PX(2)] + \epsilon(1,2)[PX_2(1,2)] \quad (2)$$

presuming that Beer's law holds for each species. The total concentration of the dilute species P is

$$[P_0] = [P] + [PX(1)] + [PX(2)] + [PX_2(1,2)] \quad (3)$$

and introducing the association constants defined by

$$[PX(1)] = k_1[P][X]$$

$$[PX(2)] = k_2[P][X]$$

$$[PX_2(1,2)] = k_{12}[PX(1)][X] = k_1 k_{12}[P][X]^2 \quad (4)$$

$$= k_{21}[PX(2)][X] = k_2 k_{21}[P][X]^2$$

and eliminating [P] from eq 2 and 3

$$\frac{A}{[P_0]} = \frac{\{k_1\epsilon(1) + k_2\epsilon(2)\}[X] + k_1 k_{12}\epsilon(1,2)[X]^2}{1 + (k_1 + k_2)[X] + k_1 k_{12}[X]^2} \quad (5)$$

This is the microscopic form of the equation given by Briegleb,[14] and with $k_2 = k_{21} = k_{12} = 0$ reduces to the simple Benesi–Hildebrand or Scott relationships, or to that due to Scatchard[15] and used for charge-transfer calculations by Foster, et al.[16] Note that the term in [X] involves a summation over the two possibilities for the binding of the first mole of X. The appropriate macroscopic form of the equation is found by introducing the macroscopic association constants

$$K_1 = k_1 + k_2 \qquad K_1 K_2 = k_1 k_{12} = k_2 k_{21} \quad (6)$$

and the weighted-average (macroscopic) extinction coefficients[17]

$$\epsilon_1 = [k_1\epsilon(1) + k_2\epsilon(2)]/(k_1 + k_2) \qquad \epsilon_2 = \epsilon(1,2) \quad (7)$$

obtaining[14] for the over-all equilibrium reaction P $\rightleftarrows$ PX $\rightleftarrows$ PX$_2$

$$\frac{A}{[P_0]} = \frac{K_1\epsilon_1[X] + K_1 K_2\epsilon_2[X]^2}{1 + K_1[X] + K_1 K_1[X]^2} \quad (8)$$

Similar equations can be derived with the chemical

(15) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).

(16) R. Foster, D. L. Hammick, and A. A. Wardley, *J. Chem. Soc.*, 3817 (1953).

(17) From the definition $\epsilon_i = \Sigma_i k_i \epsilon(i)/\Sigma_i k_i$; L. E. Orgel and R. S. Mulliken, *J. Am. Chem. Soc.*, **79**, 4839 (1957).

line shift (nmr spectroscopy) as the measure of complexing. When $\epsilon_2 = 2\epsilon_1 = 2\epsilon$, eq 8 reduces to a form of Adair's equation[18] for two sites

$$\frac{A}{2\epsilon[P_0]} = \frac{1}{2}\left[\frac{[K_1[X] + 2K_1 K_2[X]^2]}{1 + K_1[X] + K_1 K_2[X]^2}\right] \equiv s \quad (9)$$

where the factor 1/2 has been introduced to normalize the values of the saturation fraction s to the condition[1] $0 \leq s \leq 1$. Note that when the approximation [X] $\simeq$ [X$_0$] cannot be made, eq 8 or 9 is fourth order in [X].

Form of the Graphs of Eq 8. A plot of [P$_0$]/A vs. [X]$^{-1}$ (Benesi–Hildebrand or reciprocal plot), or of [P$_0$][X]/A vs. [X] (Scott or half-reciprocal plot), or of A/[P$_0$][X] vs. A/[P$_0$] (Scatchard plot) will in general give a curved line except in the special case where all the microscopic association constants are identical ($K_1 = 4K_2 = 2k$) and all the microscopic extinction coefficients are identical ($\epsilon_2 = 2\epsilon_1 = 2\epsilon$). Under these conditions, eq 8 is necessarily a straight line on any of these three plots according to the relationship

$$\frac{A}{2\epsilon[P_0]} = \frac{k[X]}{1 + k[X]} = s \quad (10)$$

Except for the factor 2 in the denominator on the left-hand side, eq 10 is identical with the equation obtained for the formation of only a 1:1 complex.[1] If the microscopic constants are merely of the same order of magnitude for each microscopic species, the plots may be *experimentally* indistinguishable from a straight line over the entire saturation curve, depending on how accurately the data can be obtained. In any case, there will be portions of the saturation curve which can always be represented as straight lines on any of the three plots mentioned; these will occur as [X] becomes very small or very large, and correspond to small and large saturation fractions, respectively. Since in the case of observable curvature the slope is constantly changing *except* in these regions, the observed slope or intercept calculated on the assumption of a straight line for the entire curve (simple Benesi–Hildebrand or Scott treatment) will depend on the region in which the data are collected.

The importance of these considerations has apparently not been widely recognized in the application of equilibrium methods to weak molecular complexes, although a large amount is known about the fundamental principles.[12] Thus Ross and Labes[9] interpreted several different sets of multiple equilibria data, ranging from 40 down to 5% of total saturation, all as straight lines, and Johnson and Bowen[13] were led to conclude that a second-degree equation would yield straight lines when plotted according to the simple Benesi–Hildebrand treatment. The problem in both these cases is again one of insufficient data, as curvature is small over the ranges of saturation fraction employed. By symmetry, maximum deviation from the straight line case occurs at a saturation fraction of 0.5, and this figure was not obtained in either of the two works mentioned above. In the experiment of Ross and Labes,[9] it appears that the observed formation constant decreases with increasing saturation fraction as more and more of the curved line is least squared as a straight line; the experiment which gave the "best" value was

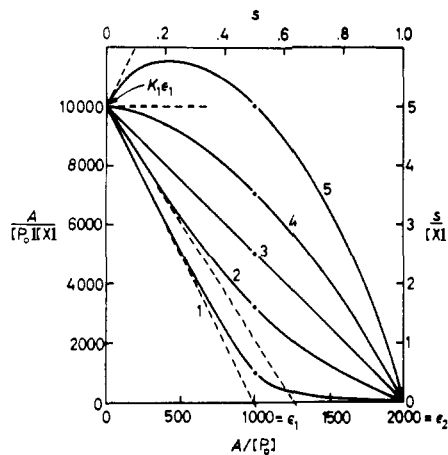(18) G. S. Adair, *J. Biol. Chem.*, **63**, 529 (1925).

Figure 1. Scatchard plots of various types of multiple equilibria. Curves 1 and 2 are examples of ordinary, noncooperative equilibria, curves 4 and 5 are examples of equilibria with cooperative effects, and curve 3 is the straight line obtained when all the microscopic constants are identical. The equivalent saturation fraction scales are shown at the top and right of the graph, and the crosses mark the point of half-saturation; see Table I for constants used to compute the curves. Dashed lines represent the limiting slopes at low saturation.
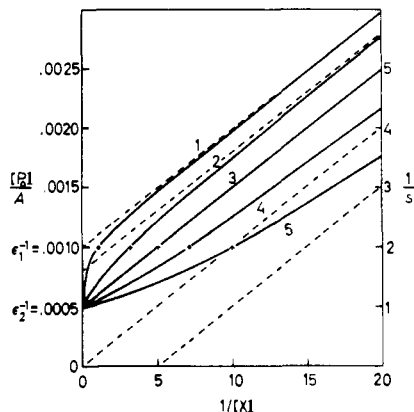

Figure 2. Benesi–Hildebrand, or reciprocal plots of various types of multiple equilibria (conditions same as for Figure 1).

one in which the saturation fraction did not exceed 5% and represents a limiting case (see below). Although Johnson and Bowen[13] concluded that curvature was negligible, it is apparent even in their data, which were all obtained at low saturation fractions and are thus not representative of the entire experiment. As pointed out previously,[1] the Benesi–Hildebrand plot is a particularly bad plot on which to see curvature because of the reciprocal doubling effect. Johnson and Bowen[13] also concluded that the plots were linear when complexes of the type P_2X were present; this finding is correct, as shown theoretically by Deranleau[19] for the general case of complexes of the type P_iX_j under the same conditions ([P_0] = constant, [X_0] is variable).

An examination of the actual graphs for various microscopic models comprising the over-all reaction $P \rightleftharpoons PX \rightleftharpoons PX_2$ helps to clarify the situation. Examples of these graphs are shown in Figures 1–3, and the numerical values of the various parameters used to obtain the curves are given in Table I, along with the concentrations of X required to reach half-saturation,

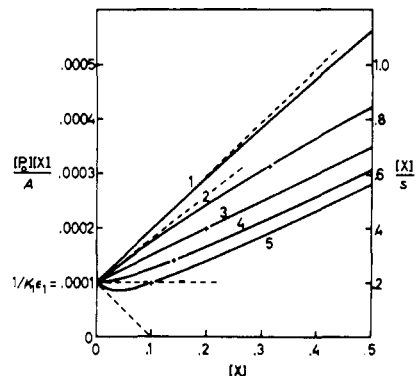(19) D. Deranleau, J. Chem. Phys., 40, 2134 (1964).

Figure 3. Scott or half-reciprocal plots of various types of multiple equilibria (conditions same as for Figure 1, but note that the s scale cannot be uniquely given, as it is different for each curve).

[X]$_{1/2}$. Although a particular set of values was chosen for representation, the situation is not basically different for other sets. In the case of the Benesi–Hildebrand (Figure 2) and Scott (Figure 3) plots, representation of

Table I. Microscopic and Macroscopic Formation Constants and Extinction Coefficients Used for Data in Figures 1–3[a]

Curve no.	K_1	K_2	$k_1 = k_2$	$k_{12} = k_{21}$	[X]$_{1/2}$
1	10	0.1	5	0.2	1.2
2	10	1	5	2	0.32
3	10	2.5	5	5	0.20
4	10	5	5	10	0.14
5	10	10	5	20	0.10

[a] Units are l. mole^{-1} for K, k; mole l.$^{-1}$ for [X]$_{1/2}$. The additional constants $\epsilon_1 = \epsilon_2/2 = \epsilon = 1000$ l. mole^{-1} cm^{-1} ($\epsilon(1) = \epsilon(2) = \epsilon(1,2)/2$) and [$P_0$] = 10^{-3} mole l.$^{-1}$ were used in the calculations. Under these conditions [X]$_{1/2} = 1/(K_1K_2)^{1/2}$.

the entire saturation curve is not feasible because of the open upper limit for the abscissa.[1] It is obvious from the figures that curvature, if present, will be most easily seen on the Scatchard plot (Figure 1), and of the three plots shown curvature is least obvious on the Benesi–Hildebrand plot, especially at low saturation ratios. This is the graphical explanation of why Johnson and Bowen[13] were unable to obtain significant curvature; in only two of the four cases studied for PX_2 formation did the saturation fraction reach $\sim$0.4, and, similar to the case of Ross and Labes,[9] the theoretically generated curves were subjected to straight-line fitting procedures.

Slopes and Intercepts of the Plots. Since the values of the slopes and intercepts of the various plots are obviously composite values, it is pertinent to examine at this point the actual values of the *limiting* slopes and intercepts at small saturation ratios, under which conditions much if not most weak-complex formation data have been obtained. The slopes of the various plots are easily obtained by differentiation of the relevant form of eq 8 and are

$$\frac{d([P_0]/A)}{d(1/[X])} = \frac{\epsilon_1 + 2K_2\epsilon_2[X] + K_1K_2(\epsilon_2 - \epsilon_1)[X]^2}{K_1(\epsilon_1{}^2 + 2K_2\epsilon_1\epsilon_2[X] + K_2{}^2\epsilon_2{}^2[X]^2)} \quad (11)$$

$$\frac{d([P_0][X]/A)}{d[X]} =$$

$$\frac{K_1\epsilon_1 - K_2\epsilon_2 + 2K_1K_2\epsilon_1[X] + K_1K_2{}^2\epsilon_2[X]^2}{K_1(\epsilon_1{}^2 + 2K_2\epsilon_1\epsilon_2[X] + K_2{}^2\epsilon_2{}^2[X]^2)} \quad (12)$$

Table II. Limiting Slopes and Intercepts of Various Plots for the Model $P \rightleftharpoons PX \rightleftharpoons PX_2$

Plot	Low saturation limit $s, [X_0] \to 0$		High saturation limit $s \to 1, [X_0] \to \infty$	
	Slope	Intercept	Slope	Intercept
Scatchard (Figure 1)	$-K_1\left(1 - \dfrac{K_2\epsilon_2}{K_1\epsilon_1}\right)$	$K_1\epsilon_1$	$-K_2\left(1 - \dfrac{\epsilon_1}{\epsilon_2}\right)^{-1}$	ϵ_2
Benesi–Hildebrand (Figure 2)	$\dfrac{1}{K_1\epsilon_1}$	$\dfrac{1}{\epsilon_1}\left(1 - \dfrac{K_2\epsilon_2}{K_1\epsilon_1}\right)$	$\dfrac{1}{K_1\epsilon_2}\left(1 - \dfrac{\epsilon_1}{\epsilon_2}\right)$	$\dfrac{1}{\epsilon_2}$
Scott (Figure 3)	$\dfrac{1}{\epsilon_1}\left(1 - \dfrac{K_2\epsilon_2}{K_1\epsilon_1}\right)$	$\dfrac{1}{K_1\epsilon_1}$	$\dfrac{1}{\epsilon_2}$	$\dfrac{1}{K_1\epsilon_2}\left(1 - \dfrac{\epsilon_1}{\epsilon_2}\right)$

$$\frac{d(A/[P_0][X])}{d(A/[P_0])} = \frac{d(A/[P_0][X])}{d[X]} \bigg/ \frac{d(A/[P_0])}{d[X]} =$$

$$-\frac{K_1^2\epsilon_1 - K_2\epsilon_2 + 2K_1K_2\epsilon_1[X] + K_2^2\epsilon_2[X]^2}{K_1\epsilon_1 + 2K_2\epsilon_2[X] + K_1K_2(\epsilon_2 - \epsilon_1)[X]^2} \quad (13)$$

for the Benesi–Hildebrand, Scott, and Scatchard forms, respectively. The limiting slopes and intercepts, at low and high saturation ratios, are given in Tables II and III for the various cases. It should be strongly emphasized that *in virtually no cases of interest will division of the limiting slope by the intercept (or vice versa) yield unique values for "K" or for "ε."* Unique values for both constants will be obtained only for the case in which $K_1\epsilon_1 \gg K_2\epsilon_2$, which is of very little practical interest. In view of this restriction, we propose to introduce an apparent formation constant and an apparent extinction coefficient defined, for the PX, PX_2 system, for example, by the relations

$$K_{app} = K_1[1 - (K_2\epsilon_2/K_1\epsilon_1)]$$
$$\epsilon_{app} = \epsilon_1[1 - (K_2\epsilon_2/K_1\epsilon_1)]^{-1} \quad (14)$$
$$K_{app}\epsilon_{app} = (K\epsilon)_{app} = K_1\epsilon_1$$

These apparent constants would apply to the straight-line fitting of data at low saturation fractions, and constants so obtained should be clearly labeled in those cases where proof of the actual stoichiometry is lacking.[20] It is worthwhile to mention that such proof is

Table III. Slope and Intercept of the Line of Identical Microscopic Formation Constants and Extinction Coefficients[a]

Plot	Slope	Intercept
Scatchard (Figure 1, curve 3)	$-k = -K_1/2$	$2k\epsilon = K_1\epsilon$
Benesi–Hildebrand (Figure 2, curve 3)	$1/2k\epsilon = 1/K_1\epsilon$	$1/2\epsilon$
Scott (Figure 3, curve 3)	$1/2\epsilon$	$1/2k\epsilon = 1/K_1\epsilon$

[a] $K_1 = 2k = 4k_2, k_1 = k_2 = k_{12} = k_{21} = k, \epsilon_2 = 2\epsilon_1 = 2\epsilon.$

not obtainable by application of a second method such as nmr, as similar definitions of apparent constants will result there as well because of the presence of multiple equilibria. On the other hand, if the entire curves are measured, all four relevant constants can be obtained as long as the curves are experimentally distinguishable from a straight line. In case they are not, a decision must be made from other evidence that the straight

line is one corresponding to a simple 1:1 binding model, or one corresponding to a PX, PX_2, ..., PX_n system with identical microscopic association constants and microscopic extinction coefficients. In any case, it appears from Figures 1–3 that of the order of 75% or more of the total saturation range is necessary to be sure that curvature exists, in agreement with previous considerations.[1]

A brief consideration of the magnitude of the correction terms in eq 14 is illuminating. In the case of identical microscopic association constants and extinction coefficients, the actual step constant k (see eq 10) will always be correctly obtained, since $K_{app} = K_1/2 = k$, but both K_1 and the extinction coefficient will be in error by a factor of 2. Note that the macroscopic constant K_1 is in this case also an apparent constant, being twice as large as the actual constant for the first step. This can have rather interesting consequences on the thermodynamic functions $\Delta G°$ and $\Delta S°$ but will not affect $\Delta H°$ as obtained from a van't Hoff plot.[14] Note also that the product, $K_1\epsilon_1$, is always correct according to eq 14.

If the extinction coefficient of the complex PX_2 is well behaved, that is, no large hyper- or hypochromic effects are present, $\epsilon_2 \simeq 2\epsilon_1$, and for ordinary multiple binding we can set an upper limit of about two to the error in K_1 from the condition $K_1 \geqslant 4K_2$ (for complexes of the type PX_j, the upper limit of error will be of the order $j!$). For cooperative binding, however, $K_1 < 4K_2$, and the correction term when $K_1 < 2K_2$ and $\epsilon_2 = 2\epsilon_2$ becomes negative and potentially very large. Although an instance of a negative limiting intercept has been reported,[21] the observation is probably due to insufficient data to determine a valid slope or intercept[1,5] rather than to cooperative binding, as positive values were obtained on a different concentration scale. An assumption sometimes made in the handling of eq 8 for real systems[11,12] is that when the term containing $[X]^2$ is very small, eq 8 reduces to the simple 1:1 binding case

$$\frac{A}{[P_0]} = \frac{K_1\epsilon_1[X]}{1 + K_1[X]} \quad (15)$$

From Table II, it is seen that this assumption is independent of $[X]$ itself in the limit of low saturation fraction, depending only on the ratio $K_2\epsilon_2/K_1\epsilon_1$. When this ratio is larger than 0.01 or so (1% error or more), it is false to conclude[9] that the binding of the first molecule of X can be studied in the absence of complications

(20) By such an assignment, for example, the limiting slope of the Scatchard plot would be $-K_{app}$, and the intercept (at $s = 0$), would be $(K\epsilon)_{app}$.

(21) M. W. Hanna and A. L. Ashbaugh, *J. Phys. Chem.*, **68**, 811 (1964).

produced by attachment of a second molecule of X, *regardless of the ratio* [P$_0$]/[X$_0$]. By the definition of the equilibrium constants, the amount of complex formation varies with the absolute concentrations [P$_0$] and [X$_0$], not with their ratio, which is concentration independent. Thus for systems in which $K_2\epsilon_2/K_1\epsilon_1$ is appreciably larger than 0.01, eq 15 is inapplicable at *any* concentration of X. We might also reemphasize that when [X] becomes very small, the saturation fraction (depending on the values of the formation constants) approaches zero, and we enter a region where it may be difficult or impossible to determine accurate values of the limiting slope or intercepts of the plots.[1]

Finally, we note that methods commonly in use[22,23]

(22) M. J. S. Dewar and C. C. Thompson, *Tetrahedron Suppl.*, 7, 97 (1966).
(23) P. R. Hammond, *J. Phys. Chem.*, 72, 2272 (1968).

for "proving" the existence of 1:1 complexes, such as Job's method,[24] have been shown to be inappropriate for systems in which more than one molecule of X is bound, leading to erroneous values for the formation constants.[25] It can be concluded from a careful examination of the literature that there are few if any weak complexes determined by spectroscopic means which have been rigorously proven to consist only of 1:1 complexes, other than those which are isolable as crystalline complexes amenable to chemical analysis.

(24) P. Job, *Ann. Chim.* (Paris), 9, 113 (1928).
(25) *Cf.* discussions by Rossotti and Rossotti.[12]

Local Orbital Guide to Allowed Interconversions of C$_4$H$_7^+$ Ions

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Abstract: A guide to adiabatic reaction pathways, parallel to the method of Hoffmann and Woodward but convenient for three-dimensional molecules of low symmetry, is developed in terms of localized molecular orbitals. Calculations on the C$_4$H$_7^+$ system illustrate the technique; several predictions are made. The *syn*-allylcarbinyl ion closes to cyclobutyl ion by a disrotatory motion. The cyclopropylcarbinyl cation rearranges to a nonplanar cyclobutyl ion by bending the *exo*-methylene toward a ring carbon. The CH bond at the formally charged carbon naturally assumes the axial position essential to a stable cyclobutyl ion. The protonated planar methylene cyclopropane may undergo a pseudorotation by conrotatory motion of the *exo*- and a ring methylene. A detailed description of delocalization in these ions is given by a newly developed semiempirical local orbital analysis.

I. Introduction

The Hoffmann–Woodward rules provide an invaluable guide to low-energy paths in chemical reactions and are widely verified by experiment.[2] The arguments leading to these rules rest upon the correlation between orbitals of an original geometry A and a final geometry B. At first decisions were based on the fate of the highest occupied orbital in A; if this orbital passes into an occupied orbital of B, the nuclear motion probably requires little energy. It is "thermally allowed." If the orbital correlates instead with an excited orbital of B, A goes to B only by free-radical, multistep, or photochemical processes. Since these pathways are either very demanding of energy or require special experimental conditions, they are easily distinguished from the thermally allowed reactions. At the suggestion of Longuet-Higgins and Abrahamson,[3]

the arguments were put on a firmer basis by consideration of the correspondence of each member of the set of occupied orbitals in A with its counterpart in B.

Application of the Hoffmann–Woodward approach and arguments analogous to it becomes more difficult in large, three-dimensional systems of low symmetry. In this report, we describe a reformulation of the Hoffmann–Woodward approach, in the language of localized molecular orbitals, which avoids this difficulty. We rely on the fact that, if a set of molecular orbitals ΦA correlates with another set ΦB, one may form arbitrary linear combinations within the sets without destroying their correspondence. The energy localized orbitals[4] are particularly suitable linear combinations in this context because of the high degree of transferability of many local orbitals. The transferability makes the correspondence of individual orbitals very easy to discern: a CH bond orbital of A usually corresponds to a similar CH bond orbital in B, for example. The localized orbitals (LO's) we refer to in

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