Molecular Complexes.[1,2] Nonparallel and Nonlinear Slopes of NMR Scatchard Plots Caused by Additional Unspecific Shielding

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Abstract: If NMR shift data of molecular complex equilibria A + D ⇄ AD, where D is aromatic, are used to determine the equilibrium quotient K from an A signal under conditions of excess of D, it is necessary to take into account the additional unspecific shielding (AUS) by unsuccessful collisions between A and D molecules. This AUS influences the chemical shifts of both free A and complexed A (i.e., AD), both shifts being functions of D concentration. In all methods of K evaluation one has to consider that the chemical shifts of both species are not constant for solutions of different D concentrations. Therefore, one of the best methods, the Scatchard–Foster–Fyfe treatment, may yield curved and nonparallel (i.e., nucleus dependent) slopes which should be equal to $-K$ according to theory. By assuming a linear dependence of AUS, $a_1[D_0]_i$ for A and $a_2[D_0]_i$ for AD, a modified Scatchard equation is developed and discussed. On the basis of this equation a proposal is made to overcome the methodical problem of AUS.

There has been much discussion and controversy about the correct performance and evaluation of NMR shift experiments designed for the determination of molecular complex parameters, especially of the equilibrium quotient for 1:1 complexes. The main problems are (1) the choice of the reference signal (internal or external) and the choice of the concentration range[3] and of the concentration units,[2] and (2) results, which do not coincide with results derived from other methods or which are inconsistent in themselves. As far as the last problem is concerned, sometimes the Scatchard plots are not parallel for different nuclei of one partner of the investigated complex equilibrium, or the Scatchard plots are not linear. Explanations for such irregular results have been put forward among others on the basis of activity effects or by assuming the presence of termolecular (or even higher molecular) complexes.[3,4] We now wish to show a new aspect of these problems, which to our knowledge has been overlooked up to now.

We may consider an equilibrium

$$A + D \rightleftarrows AD \tag{1}$$

investigated by measuring the shift δ_i of acceptor A as a function of total donor concentration $[D_0]_i$ in a number of different A- and D-containing solutions. The index i refers to the solution i. Usually the total acceptor concentration $[A_0]$ is held constant.

We can analyze and interpret the observed chemical shifts according to different known methods.[2a,5] One of the most reliable and most often used methods is based on the equation of Scatchard or Foster and Fyfe,[2a,5] respectively:

$$\Delta_i/[D_0]_i = -K\Delta_i + K\Delta_{AD} \tag{2}$$

Here Δ_i is the observed shift (weighted mean of the shifts of complexed and free acceptor) relative to the resonance of the free acceptor (measured in a solution containing no D):

$$\Delta_i = \delta_A - \delta_i \tag{3a}$$

and Δ_{AD} is defined correspondingly as the relative shift of the pure complex:

$$\Delta_{AD} = \delta_A - \delta_{AD} \tag{3b}$$

K is the equilibrium quotient.

We shall confine our considerations to the frequent case of a planar acceptor molecule A which complexes with an aromatic donor D in a fast equilibrium forming a face-to-face complex; i.e., the molecular planes of A and D will be (at least approximately) parallel in the complex AD. Now, for the sake of simplicity, let us suppose we have an ideal reference signal, which will not suffer from additional shielding when the donor D is added (in practice, e.g., an external reference with corrections for the susceptibility variations). Here, as well as in other cases, the Foster–Fyfe or Scatchard equation (2) requires the values of δ_A and δ_{AD} to remain constant in the presence of donor, even in the presence of a large excess of donor, which is the usual experimental condition. However, the acceptor A will not only undergo face-to-face collisions with the aromatic donor which will yield the stack-formed complex, but also such collisions which cannot yield the complex on account of unfavorable topographic arrangement of the colliding molecules. In other words, there are successful collisions and unsuccessful ones, if one considers the complex-yielding collisions as successful. This means that the acceptor in the free state A as well as in the complexed form AD will suffer additional unspecific shielding (AUS) as a net result of these unsuccessful collisions.[6] This AUS is related to aromatic solvent induced shift (ASIS). The size of this AUS is a function of the donor concentration $[D]_i$. At least in the case of a weak complex we may substitute the total donor concentration $[D_0]_i$ for the concentration $[D]_i$ of the free donor (probably the mean between $[D]_i$ and $[D_0]_i$ would be correct) so that the shifts of free acceptor and complex varying under the influence of AUS can be expressed (see Figure 1) by

$$\delta_{A,i} = \delta_{A,0} - f_1([D_0]_i) \tag{4a}$$

$$\delta_{AD,i} = \delta_{AD,0} - f_2([D_0]_i) \tag{4b}$$

where the index 0 in connection with a shift quantity refers to the condition $[D]_i = [D_0]_i = 0$ (i.e., no AUS). The following definitions then are trivial (see Figure 2):

$$\Delta_{AD,00} = \delta_{A,0} - \delta_{AD,0} \tag{5}$$

$$\Delta_{AD,ii} = \delta_{A,i} - \delta_{AD,i} = \Delta_{AD,00} - f_1([D_0]_i) + f_2([D_0]_i) \tag{6}$$

According to the concept of AUS, each Δ_i determined in the usual manner is an apparent one, and we have to introduce instead of it

$$\Delta_{ii} = \delta_{A,i} - \delta_i \tag{7}$$

and

$$\Delta_{0i} = \delta_{A,0} - \delta_i = f_1([D_0]_i) + \Delta_{ii} \tag{8}$$

The variable Δ_{0i} (the former Δ_i) is in fact that one which is used for the construction of the Scatchard plot or the corre-

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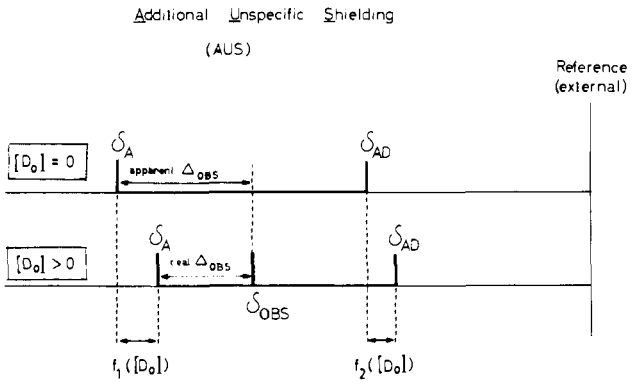

Figure 1. Effects of AUS. Real and apparent mean shift Δ_i of a solution containing A, AD, and D.

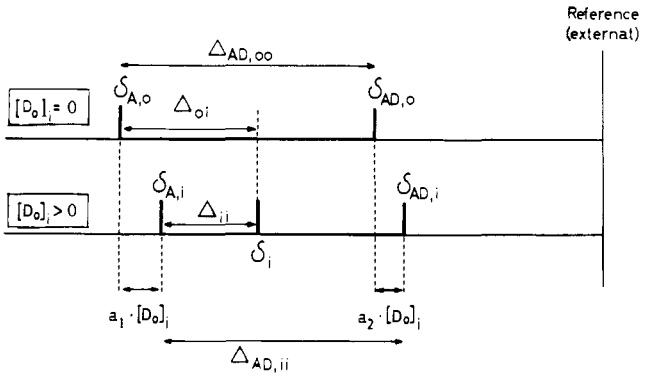

Figure 2. Linear dependence of AUS and representation of Δ_{ii}, Δ_{0i}, $\Delta_{AD,00}$, and $\Delta_{AD,ii}$.

sponding plots needed for the other methods of evaluation of K from the shift data. But, like Δ_i in the known Scatchard–Foster–Fyfe treatment, only Δ_{ii} is related directly to the relative complex concentration (or saturation fraction[3]) by

$$\Delta_{ii} = \frac{[AD]_i}{[A_0]} \Delta_{AD,ii} \tag{9}$$

so that we may transform the usual Scatchard–Foster–Fyfe equation (2) into

$$\frac{\Delta_{ii}}{[D_0]_i} = -K\Delta_{ii} + K\Delta_{AD,ii} \tag{10}$$

$$\frac{\Delta_{0i} - f_1([D_0]_i)}{[D_0]_i} = -K\{\Delta_{0i} - f_1([D_0]_i)\} + K\{\Delta_{AD,00} - f_1([D_0]_i) + f_2([D_0]_i)\} = -K\{\Delta_{0i} - f_2([D_0]_i)\} + K\Delta_{AD,00}$$

$$\frac{\Delta_{0i}}{[D_0]_i} = -K\{\Delta_{0i} - f_2([D_0]_i)\} + K\left\{\Delta_{AD,00} + \frac{f_1([D_0]_i)}{K[D_0]_i}\right\} \tag{11}$$

Here the usual approximation $[D]_i \approx [D_0]_i$ is included.

As a first approximation we may expect a linear dependence on $[D_0]_i$ of AUS (see Figure 2):

$$f_1([D_0]_i) \approx a_1[D_0]_i \tag{12a}$$

$$f_2([D_0]_i) \approx a_2[D_0]_i \tag{12b}$$

With this approximation the modified Scatchard equation (11) changes to

$$\Delta_{0i}/[D_0]_i = -K(\Delta_{0i} - a_2[D_0]_i) + K(\Delta_{AD,00} + (a_1/K)) \tag{13}$$

This modified Scatchard equation, which takes into account the AUS, reveals some interesting consequences and the need for a reinvestigation of possibly a number of published complex parameters (compare Figure 3):

(1) A linear Scatchard plot and a correct value for K can be expected only if $a_2 = 0$, i.e., if for the acceptor in the complexed form no AUS is at work.

(2) If $a_2 > 0$, the usual Scatchard plot must be curved upwards and any direct Scatchard (or other treatment) of the experimental data must yield a K value which is too small. If the original shift data have been referred to the signal of an internal reference substance and if the AUS effect on this reference substance is stronger than the AUS effect on the acceptor A (more precisely, on the particular nucleus the shift of which is observed), a_2 will be negative and the direct Scatchard plot will be curved downwards with the opposite influence on K. In this case, the Scatchard plot may show even a maximum in Δ_{0i}, and the corresponding plot of the experimentally determined shifts Δ_{0i} vs. $[D_0]_i$ will show a maximum in Δ_{0i} simultaneously (see Figure 4). Obviously, the best reference substance would be an internal reference with the same a_2 as that of the investigated acceptor nucleus.

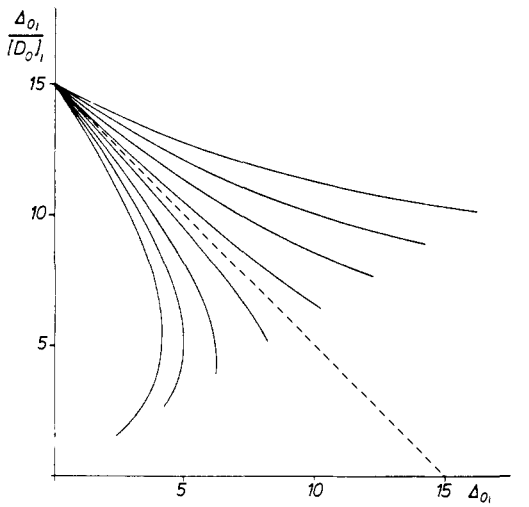

Figure 3. Theoretical Scatchard plots for different values of a_2, constructed from the following parameters: K = 1 L/mol; $\Delta_{AD,00}$ = 8 Hz; a_1 = 7 Hz·L/mol; a_2 from top to bottom +7, +5, +3, +1, 0, −1, −3, −5, −7 Hz·L/mol.

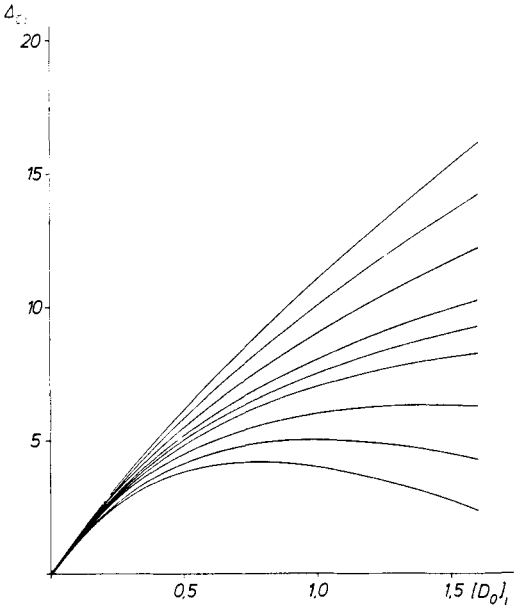

Figure 4. Theoretical plots Δ_{0i} vs. $[D_0]_i$, each one constructed from the corresponding Scatchard plot of Figure 3.

(3) It ought to be possible to obtain the correct K value in the case of a curved Scatchard plot simply by adding a linear term $a_x[D_0]_i$ to the experimental Δ_{0i} and varying a_x until a linear (or the most linear) Scatchard plot is obtained (then $a_x = a_2$).

(4) If $a_1 > 0$, the complex shift Δ_{AD} will be found too large: $\Delta_{AD} + a_1/K$ instead of Δ_{AD}. Independent of this statement, the influence of an increased or decreased value of K on the evaluation of Δ_{AD} is obvious.

According to the concept of AUS, $a_1 \geq a_2$ always should hold. The size of the errors in K and complex shift Δ_{AD} depends on the relative size of AUS. For a given coefficient a_1 the error in Δ_{AD} will be large for small values of K and small for large values of K (compare dashed plots (3) and (4) in Figure 5). The equilibrium quotient K will be found markedly too small if a_2 is relatively large in comparison to the shifts Δ_{0i} (compare plots (1), (2), and (3) in Figure 5). As a logical consequence of the fundamental concept of AUS, the values of a_1 and a_2 must depend on the intramolecular geometric position of the observed nucleus in the acceptor molecule, at least as far as only geometrical factors determine the relevant features of the unsuccessful collisions, i.e., frequency of collisions, duration of contact, nearest intermolecular distance, and intermolecular orientation. If the nucleus is not much influenced by the additional anisotropic shielding during the "unsuccessful" collisions, i.e., if the nucleus is situated near the center of the acceptor molecule, then the error in K will be small and even negligible, because a_1 and a_2 will be small. If the observed nuclei are situated in the very periphery of the acceptor molecule or if they even are jutting out from the molecular contour, as will be the case with a methyl group, a_1 and a_2 will be large and K may be found markedly too small. On the other hand, a_2 as the AUS coefficient of the complexed acceptor must decrease when the size of the molecular plane of the donor becomes larger, thereby preventing more and more the access of donor molecules in unsuccessful collisions, at least the access near enough to exert a marked influence on the complexed acceptor. In other words, the error in K will be smaller for the same acceptor if a donor with a bigger molecular size is used.

It seems necessary to emphasize that the error in K and the degree of curvature of the Scatchard plot (if referred to the above defined ideal reference) depend on the relative size of the product $a_2[D_0]_i$ compared with Δ_{0i}. For a given a_2 this error will be greater the smaller K is. Further, one ought to keep in mind that a slight curvature may be hidden in the plot by scattering of the experimental points and by a too short range of saturation fraction.[3] The high sensitivity to scattering of experimental data requires high precision and a sufficient number of experimental data when the proposed procedure for linearizing the Scatchard plot is applied.

Usually, the errors which are introduced by using $[D_0]_i$ in place of $[D]_i$ (i.e., the usual approximation) in the modified Scatchard equation will be smaller than the errors caused by AUS. They may be eliminated by an iterative procedure similar to that described earlier.[2a]

Literature examples for a nuclear dependence of K are found easily. To our knowledge, all published methyl-nonmethyl cases show a smaller figure for K derived from the methyl signal (the largest deviations differ by a factor of about $1/2$ from the respective nonmethyl K). On the other hand, it is not so easy to find curved Scatchard plots in the literature, since normally the original experimental data (concentrations and shifts) are not published (which we recommend strongly to do)

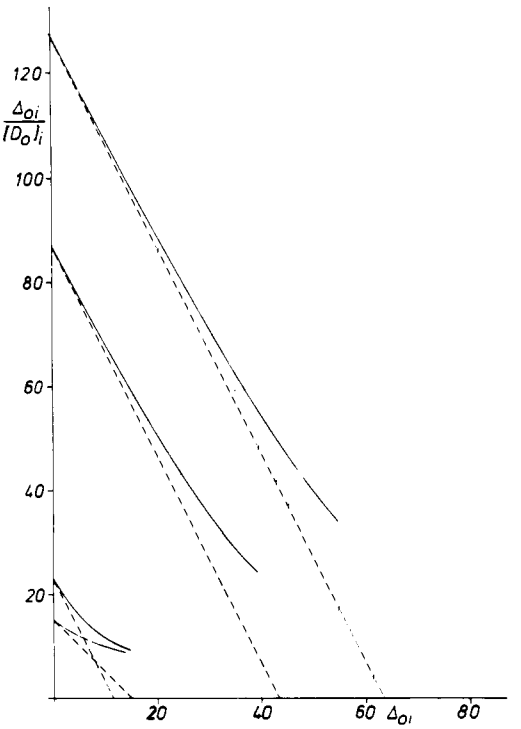

Figure 5. Theoretical Scatchard plots showing the influence of different values of K and Δ_{AD}, respectively. Parameters: $a_1 = 7$ Hz·L/mol; $a_2 = 5$ Hz·L/mol ($a_2 = 0$ for the dashed plots). From top to bottom: (1) $K = 2$ L/mol, $\Delta_{AD,00} = 60$ Hz; (2) $K = 2$ L/mol, $\Delta_{AD,00} = 40$ Hz; (3) $K = 2$ L/mol, $\Delta_{AD,00} = 8$ Hz; (4) $K = 1$ L/mol; $\Delta_{AD,00} = 8$ Hz. Plot (4) is identical with plot (2) of Figure 3.

and most researchers hesitate to consider a curved Scatchard plot as a correct experimental result. But first tests of the theory with our own experimental results show indeed that linearizing curved Scatchard plots can lead to much better consistency within the investigated system.

We assume that similar considerations ought to be applied to nonaromatic complexing agents, although the actual effect on the evaluated parameters may be much smaller.

The terms acceptor and donor have been used for convenience. Of course, we may exchange A and D, if the actual experiments require this.

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References and Notes

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(6) Compare the discussion of contact effects in the Benesi-Hildebrand treatment of light absorption data: L. E. Orgel and R. S. Mulliken, *J. Am. Chem. Soc.*, **79**, 4839 (1957).