The concept of the real stability constant for complexes formed through hydrogen bonding

Józef Magoński

Department of Marine Chemistry and Marine Environmental Protection, Institute of Oceanography, University of Gdańsk, 81-378 Gdynia, Poland. E-mail: ocejm@univ.gda.pl

Received (in Cambridge, UK) 17th August 2001, Accepted 30th January 2002
First published as an Advance Article on the web 14th March 2002

On the basis of previously published results it is explained why the formation constants of complexes formed through hydrogen bonding, contrary to accepted opinion (*e.g.* Shan *et al.*, *Science*, 1996, **272**, 97), are not the general measure of their stability. Instead, a newly introduced quantity is recommended—the *real stability constant*—which takes into account all possible ways of decomposition of such complexes. The practical expression for the real stability constant of AHA_1^- type complexes (formed in reactions between HA and A_1^- with comparable steric contributions) exhibits a distinct maximum at ΔpK_a^S (defined as $pK_{HA_1}^S - pK_{HA}^S$) equal to or slightly different from zero depending on whether the hydrogen bridges join moieties belonging to compounds of the same or different families. The two major factors governing the value of this constant are the comparable proton donating properties of HA and HA_1 and the tendency towards homoconjugation in the parent systems $HA + A^-$ and $HA_1 + A_1^-$. Thus, a problem which for years seemed intuitively understandable, has finally found both theoretical explanation and experimental confirmation. The general solution found in the paper creates a base for much more constructive discussion on the proposal of Cleland and Kreevoy (*Science*, 1994, **264**, 1887) dealing with enzymatic catalysis.

1 Introduction

The problem of the stability of complexes formed through hydrogen bonding has for years been recognized by chemists as a very exciting one. Hydrogen bonds, although in general considered to be relatively weak, strongly determine the structures and properties of the key substances present in living matter *e.g.* proteins[1] or nucleic acids.[2] The reason for the enormous significance of these bonds is that the overwhelming majority of species contain proton accepting and/or proton donating centers. The interactions between these centers occur with unusual ease because of the great density of charges with opposite signs. When the stability of a given complex formed through hydrogen bonding is under consideration, the quantity that almost instantly springs to mind is its formation constant.[3] If the initially introduced species (HA and B) are the only ones which coexist with the given AHB complex in an equilibrium

$$HA + B \rightleftharpoons AHB \tag{1}$$

then its formation constant defined as

$$\vec{K}_{AHB} = \frac{[AHB]}{[HA][B]} \tag{2}$$

does reflect its stability. If, however, HA and B interact very strongly to form (besides AHB) predominantly A^- and BH^+, then the formation constant defined by eqn. (2) does not reflect the stability of the complex AHB. Since strong interactions are generally much more important than those which are extremely weak, the terms stability constant and formation constant should not be regarded as exchangeable.

An example illustrating the significance of the problem may be the heated discussion about the role of strong hydrogen bonds in enzymatic catalysis.[4–13] The proposal given by Cleland and Kreevoy,[7] which assumes that the energy released during the formation of sufficiently strong hydrogen bonds is a factor probably facilitating effective catalytic action of several enzymes, has caused serious controversy among scientists. The fundamental condition for existence of such bonds was that ΔpK_a (equal to the difference between pK_a of the protonated base and pK_a of the acid) should be close to zero. When the opponents, *e.g.* Shan *et al.*,[3] argued that systems of ΔpK_a close to zero show no particular stability (the linear dependence between the formation constant and ΔpK_a continuing when passing through this particular point), the defence[9] of the proposal remained unconvincing to the average reader such as myself. The objection of Shan *et al.*[3] was, however, based on the assumption that the formation constant of a given hydrogen bonded complex is the best measure of its stability and reflects the strength of its key bond.

To show that in the general case the formation constant does not reflect its stability it is sufficient to notice that the same hydrogen bonded complex may be formed from two different pairs of reactants. For instance, the complex anion AHA_1^- that is formed in the reaction between 2,4-dinitrophenol (HA) and the phenolate ion (A_1^-) in acetonitrile (AN):

$$HA + A_1^- \rightleftharpoons AHA_1^- \tag{3}$$

is also formed in the reaction between phenol (HA_1) and the 2,4-dinitrophenolate ion (A^-) in the same solvent.

$$HA_1 + A^- \rightleftharpoons AHA_1^- \tag{4}$$

For the same complex we have two formation constants: $\vec{K}_{AHA_1^-} = [AHA_1^-]/([HA][A_1^-])$ and $\overleftarrow{K}_{AHA_1^-} = [AHA_1^-]/([HA_1][A^-])$. The difference $\log \vec{K}_{AHA_1^-} - \log \overleftarrow{K}_{AHA_1^-}$ is exactly equal to 10.3_5, which results from the fact that it must be equal to $pK_{HA}^{AN} - pK_{HA}^{AN} = 26.6_5 - 16.3$ (AN = acetonitrile).[14] It is evident that the stability of the given AHA_1^- ion in the same solvent cannot depend on which of the two reactions (3) or (4) is being considered. If in reactions (3) and (4) the same equimolar amounts

 DOI: 10.1039/b107482m

of reagents (and the same counterion) are used, the composition of the final mixture, and hence its spectral, electrochemical *etc.* properties, are, within the limits of experimental error, exactly the same. In both cases, the conjugated equilibrium

$$HA + A_1^- \rightleftharpoons AHA_1^- \rightleftharpoons A^- + HA_1 \qquad (5)$$

would be shifted to the right to the same extent, reflecting the fact that HA is a much stronger proton donor than HA_1. The complex ion AHA_1^- could generally be regarded as stable if its equilibrium concentration significantly exceeded the equilibrium concentrations of its major decomposition products (which in this case are A^- and HA_1) and not only when it exceeds the equilibrium concentrations of HA and A_1^- which appear to be negligible. An analogous situation exists in the cases involving complexes formed between molecular species. For example, the formation constant $\vec{K}_{\mathbf{AHB}}$ [eqn. (2)] of the complex formed between picric acid (HA) and tributylamine (B) calculated from the available dissociation constant of tributylammonium picrate AHB

$$K_d = \frac{[BH^+][A^-]}{[AHB]} \qquad (6)$$

($K_d = 4.6 \times 10^{-3}$)[15] and dissociation constants of picric acid ($pK_{HA}^{AN} = 11.0$)[16] and the tributylammonium ion ($pK_{BH^+}^{AN} = 18.09$)[17] reaches an astronomical figure of the order of 10^9. This figure only tells us that the AHB complex hardly decomposes into HA and B, but it does not indicate, at all, to what degree it decomposes into BH^+ and A^-. The decomposition of tributylammonium picrate into BH^+ and A^- in diluted solutions is almost complete, and this fact is absolutely sufficient to state that the AHB complex, despite its impressive formation constant, appears to be very unstable. Another striking example may be taken from the paper of Chantooni and Kolthoff,[18] who studied the complexes formed between benzoic acids and tetramethylguanidine in acetonitrile. For the 2,4-dinitrobenzoic acid–tetramethylguanidine system, ΔpK_a^{AN} and $\log \vec{K}_{\mathbf{AHB}}$ are 7.1 and 9.57 respectively. Assuming that the analytical concentrations of HA and B, or of the complex itself, are equal to 10^{-3} M, these data allow the following approximate concentrations of the species coexisting in the equilibrium

$$HA + B \rightleftharpoons AHB \rightleftharpoons A^- + BH^+ \qquad (7)$$

to be evaluated: $[HA] \approx [B] = 10^{-7}$ M, $[AHB] = 10^{-4.5}$ M, $[A^-] \approx [BH^+] = 10^{-3.5}$ M.

These numbers illustrate very clearly that, despite the unusually high formation constant, the AHB complex constitutes only *ca.* 10% of all the species coexisting in equilibrium (6). Considering that the primary decomposition products HA, B, A^- and BH^+ and solvent molecules S participate in further equilibria (*e.g.* formation of AHA^-, BHB^+, AHS, BHS^+), one has to state that the equilibrium concentration of the AHB complex must constitute an even smaller part (<10%) of its analytical concentration.

To sum up, it must be stated that the formation constant of a given complex formed through hydrogen bonding is not a measure of its stability. The aim of this paper is, however, not only to give a reasoning for this statement: this should have been done half a century ago. The main goal of this paper is to introduce a quantity which reflects the resistance of the hydrogen bonded complex to all possible ways of its decomposition, and give the method of its determination based on simple and reliable measurements.

2 The real stability constant (RSC) concept

The stable heteroconjugate AHB should exist in the solvent (S) at a reasonably high equilibrium concentration relative to all its decomposition products. After the equilibrium (7) is reached, the complex coexists with its primary decomposition products (HA, B, A^- and BH^+) and their derivatives (*e.g.* AHA^-, BHB^+, AHS, BHS^+, SA^- and BS) formed by appropriate conjugation. In some cases, formation of other species *e.g.* acid dimers, may also be significant despite a relatively high electric permittivity.[19]

It is proposed that the quantity reflecting stability of the AHB complex (also called after Kolthoff[20] the AHB heteroconjugate) be defined as

$$\$_{AHB} = \frac{[AHB]}{[\Sigma A][\Sigma B]} \qquad (8)$$

and named the *real stability constant* (RSC) of the AHB complex. ΣA denotes the total equilibrium concentration of A moieties contained in the primary decomposition products of AHB and their derivatives:

$$[\Sigma A] = [HA] + [A^-] + 2[AHA^-] + [AHS] + [SA^-] + \ldots \qquad (9)$$

and analogously, ΣB denotes the total equilibrium concentration of B moieties contained in the primary decomposition products of AHB and their derivatives:

$$[\Sigma B] = [BH^+] + [B] + 2[BHB^+] + [BHS^+] + [BS] + \ldots \qquad (10)$$

The stoichiometric coefficient at an equilibrium concentration of AHA^- equal to 2 reflects the fact that the formation of one such species requires the prior decomposition of two AHB molecules—one into HA and B, the other into A^- and BH^+. This way, in eqn. (8) each elementary decomposition of AHB is reflected by the relevant concentration.

To express RSC with experimentally accessible values, the use of the relation between the equilibrium concentrations of hetero- and homoconjugates derived previously[21]

$$\frac{[AHA_1^-]^2}{[AHA^-][A_1HA_1^-]} = 1 \qquad (11)$$

appears to be useful. The relation applies to ionic complexes of the AHA_1^- or BHB_1^+ type, and particularly when the homoconjugation phenomena in the two parent systems, *e.g.* HA + A^- and $HA_1 + A_1^-$ systems, are comparable. Its applicability to molecular complexes seems to require some additional factor to be taken into account.[22] In the case of AHA_1^- type ionic complexes, the expression for RSC takes the form

$$\$_{AHA_1^-} = \frac{[AHA_1^-]}{[\Sigma A][\Sigma A_1]} \qquad (12)$$

If we take into account the definitions of the dissociation constants K_{HA} and K_{HA_1}, homoconjugation constants K_{AHA^-} and $K_{A_1HA_1^-}$ and formation constants for the corresponding solvent complexes K_{AHS}, K_{SA^-}, K_{A_1HS} and $K_{SA_1^-}$ we can present every term of the expression for ΣA as a function of HA:

$$[\Sigma A] = [HA] + [HA]\frac{K_{HA}}{a_{H^+}y_{A^-}} + 2K_{AHA^-}[HA]^2\frac{K_{HA}}{a_{H^+}y_{A^-}} + K_{AHS}[HA][S] + K_{AS^-}\frac{K_{HA}}{a_{H^+}y_{A^-}}[S][HA] + \ldots \qquad (13)$$

and every term of the expression for ΣA_1 as a function of HA_1:

$$[\Sigma A_1] = [HA_1] + [HA_1]\frac{K_{HA_1}}{a_{H^+}y_{A_1^-}} + 2K_{A_1HA_1^-}[HA_1]^2\frac{K_{HA_1}}{a_{H^+}y_{A_1^-}} + K_{A_1HS}[HA_1][S] + K_{A_1S^-}\frac{K_{HA_1}}{a_{H^+}y_{A_1^-}}[S][HA_1] + \ldots \quad (14)$$

A more detailed analysis of the above expressions leads to the conclusion that in each of them, the first two terms are strongly dominant. Assuming that

$$[\Sigma A] = [HA] + [HA]\frac{K_{HA}}{a_{H^+}y_{A^-}} \quad (15)$$

and

$$[\Sigma A_1] = [HA_1] + [HA_1]\frac{K_{HA_1}}{a_{H^+}y_{A_1^-}} \quad (16)$$

as well as substituting in the numerator of eqn. (12) $\sqrt{[AHA^-][A_1HA_1^-]}$ for $[AHA_1^-]$ on the basis of eqn. (11), $K_{AHA^-}[HA][A^-]$ for $[AHA^-]$ and $K_{A_1HA_1^-}[HA_1][A_1^-]$ for $[A_1HA_1^-]$ one obtains the expression

$$\$_{AHA_1^-} = \frac{\sqrt{K_{AHA^-}[HA][A^-]K_{A_1HA_1^-}[HA_1][A_1^-]}}{\left([HA] + [HA]\frac{K_{HA}}{a_{H^+}y_{A^-}}\right)\left([HA_1] + [HA_1]\frac{K_{HA_1}}{a_{H^+}y_{A_1^-}}\right)} \quad (17)$$

Dividing the numerator and denominator of this expression by the product $[HA][HA_1]$ one obtains

$$\$_{AHA_1^-} \approx \frac{\sqrt{K_{AHA^-}K_{A_1HA_1^-}\frac{[A^-][A_1^-]}{[HA][HA_1]}}}{\left(1 + \frac{K_{HA}}{a_{H^+}y_{A^-}}\right)\left(1 + \frac{K_{HA_1}}{a_{H^+}y_{A_1^-}}\right)} \quad (18)$$

Under experimental conditions (polar solvent), the quotient $[A^-][A_1^-]/([HA][HA_1])$ is very close to unity, since we are dealing with a mixture where the analytical concentrations of the reactants used (C_{HA} and $C_{A_1^-}$) are equal, while the homo- and heteroconjugation equilibria hardly affect its value. The final expression for the approximate RSC of anionic complexes takes the form

$$\$_{AHA_1^-} \approx \frac{\sqrt{K_{AHA^-}K_{A_1HA_1^-}}}{\left(1 + \frac{K_{HA}}{a_{H^+}y_{A^-}}\right)\left(1 + \frac{K_{HA_1}}{a_{H^+}y_{A_1^-}}\right)} \quad (19)$$

In the above expression two factors are noteworthy: the factor $\sqrt{K_{AHA^-}K_{A_1HA_1^-}}$ reflecting the tendency of the two key proton donors (HA and HA_1) towards homoconjugation, and the factor $[1 + K_{HA}/(a_{H^+}y_{A^-})][1 + K_{HA_1}/(a_{H^+}y_{A_1^-})]$ reflecting their relative strength. As could be expected, the stronger tendency towards homoconjugation in systems $HA + A^-$ and $HA_1 + A_1^-$ leads to a greater value of the numerator in eqn. (19) and hence, to a larger value of $\$_{AHA_1^-}$. The effect of the relative strength of HA and HA_1 may best be understood by considering that $a_{H^+}y_{\pm} \approx \sqrt{K_{HA}K_{HA_1}}$. This, in turn, has a relevance in the experimental observation [22–24] that the emf of a cell containing equimolar amounts of HA and A_1^-, denoted by $E_{AHA_1^-}$, is approximately equal to the arithmetic average of E_{AHA^-} and $E_{A_1HA_1^-}$. Here, E_{AHA^-} denotes the emf of the cell containing an equimolar mixture of HA and A^-, while $E_{A_1HA_1^-}$ denotes the emf of the cell containing equimolar amounts of HA_1 and A_1^-. The minimum value of the denominator (equal to 4) is therefore obtained when $K_{HA} = K_{HA_1}$, as in this case, $a_{H^+}y_{\pm} = K_{HA} = K_{HA_1}$ also holds. When $K_{HA} > K_{HA_1}$ and HA becomes stronger relative to HA_1, the factor $(1 + K_{HA}/a_{H^+}y_{A^-})$ increases, whereas the factor $1 + K_{HA_1}/(a_{H^+}y_{A_1^-})$ decreases, the former change being more significant than the latter. As a result, when $K_{HA} > K_{HA_1}$, and if the strength of HA increases relative to HA_1, the value of the product $[1 + K_{HA}/(a_{H^+}y_{A^-})][1 + K_{HA_1}/(a_{H^+}y_{A_1^-})]$ increases, thus leading to a lower value of $\$_{AHA_1^-}$. Similarly, $\$_{AHA_1^-}$ decreases when $K_{HA_1} > K_{HA}$ and the strength of HA_1 increases relative to HA. Thus RSC attains its maximum value (at a fixed value of the numerator) when the denominator is equal to 4. This condition requires that $K_{HA} = K_{HA_1}$ which is equivalent to $\Delta pK_a^S = 0$.

In practice, it is more convenient to determine the quotients $K_{HA}/(a_{H^+}y_{A^-})$ and $K_{HA_1}/(a_{H^+}y_{A_1^-})$ directly from potentiometric measurements, since E_{AHA^-} is a measure of K_{HA}, $E_{A_1HA_1^-}$ is a measure of K_{HA_1}, and $E_{AHA_1^-}$ is a measure of the activity of the solvated proton a_{H^+} in the equimolar mixture of HA and A_1^- (or HA_1 and A^-). The final practical expression for RSC then takes the form

$$\$_{AHA_1^-} \approx \frac{\sqrt{K_{AHA^-}K_{A_1HA_1^-}}}{\left(1 + 10^{\frac{E_{AHA^-} - E_{AHA_1^-}}{s}}\right)\left(1 + 10^{\frac{E_{A_1HA_1^-} - E_{AHA_1^-}}{s}}\right)} \quad (20)$$

where s is the slope of the glass electrode calibration curve. The effect of the two major factors on the value of RSC may be illustrated by the numerical data originating from previous work [24] for three selected 4-nitrobenzoic acid–substituted phenolate systems in acetonitrile.

For the 4-nitrobenzoic acid–2,6-dibromo-4-nitrophenolate system ($\Delta pK_a^{AN} = -3.95$) [24]

$$\$_{AHA_1^-}^{AN} = \frac{8.91 \times 10^2}{(1 + 0.00433)(1 + 34.17)} = 25.2 \quad (21)$$

For the 4-nitrobenzoic acid–2,5-dinitrophenolate system ($\Delta pK_a^{AN} = -0.55$) [24]

$$\$_{AHA_1}^{AN} = \frac{2.24 \times 10^3}{(1 + 0.4944)(1 + 1.909)} = 515 \quad (22)$$

and for the 4-nitrobenzoic acid–2,6-dichlorophenolate system ($\Delta pK_a^{AN} = 2.85$) [24]

$$\$_{AHA_1^-}^{AN} = \frac{2.37 \times 10^3}{(1 + 77.61)(1 + 0.1087)} = 27.2 \quad (23)$$

Therefore, it does not matter which of the two possible key proton donors (HA or HA_1) is stronger, but how strongly their relative proton donating properties are differentiated. When this differentiation is large, one obtains a high value for the denominator of eqn. (19) [or (20)], which correspondingly renders a low RSC value. The symmetry of eqns. (19) and (20) implies that RSC attains the same value independently of whether equimolar amounts HA and A_1^- or equimolar amounts of HA_1 and A^- were mixed, which is in full agreement with the chemical equilibrium law. The RSC values calculated as indicated above for twelve 4-nitrobenzoic acid–substituted phenolate systems in acetonitrile and for ten 2,4,6-trichlorophenol–substituted phenolate systems in the same solvent are plotted against ΔpK_a^{AN} in Figs. 1 and 2, respectively. To enable

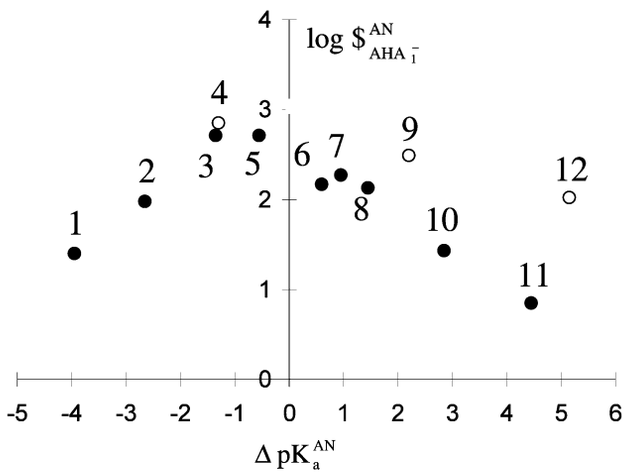

Fig. 1 The relationship between logarithm of the real stability constant (log $\$^{AN}_{AHA_1^-}$) and ΔpK_a^{AN} for 4-nitrobenzoic acid (HA)–substituted phenolate (A_1^-) systems in acetonitrile. The dark points denote systems where substituted phenolates have comparable steric hindrances. The numbering and log $K_{A_1HA_1^-}$ values[14] for particular phenolates involved are: **1**) 2,6-dibromo-4-nitrophenolate (1.9), **2**) 2,4-dinitrophenolate (2.05), **3**) pentachlorophenolate (2.6), **4**) 3,4-dinitrophenolate (3.35), **5**) 2,5-dinitrophenolate (2.7), **6**) 2,4,6-triiodophenolate (2.3), **7**) 2,4,6-tribromophenolate (2.4), **8**) 2,4,6-trichlorophenolate (2.65), **9**) 4-nitrophenolate (3.75), **10**) 2,6-dichlorophenolate (2.75), **11**) 2-nitrophenolate (2.25), **12**) 3-nitrophenolate (4.8).

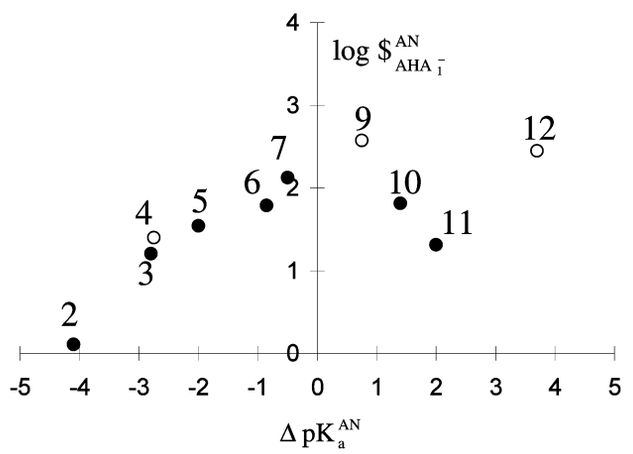

Fig. 2 The relationship between logarithm of the real stability constant (log $\$^{AN}_{AHA_1^-}$) and ΔpK_a^{AN} for 2,4,6-trichlorophenol-substituted phenolate systems in acetonitrile. The dark points denote systems where substituted phenolates have comparable steric hindrances. The numbering for particular phenolates involved is the same as in Fig. 1.

any reasonable comparison, a sufficient number of experimental points have been retained by assuming that the effect of one nitro group in the *ortho*-position on the homoconjugation constant is comparable to that caused by the two halogen substituents in those positions. Such an assumption seems to be justified in view of the comparable homoconjugation constants $K^{AN}_{AHA^-}$ of the corresponding phenol–phenolate systems.[14]

Within each of the two figures, systems with comparable average steric effects and hence with comparable values of the numerator in eqns. (19) and (20) are then represented by the dark points. In both cases the distinct maximum of the relationship under study may be observed, although in a somewhat different position. In 2,4,6-trichlorophenol–substituted phenolate systems it occurs at $\Delta pK_a^{AN} = 0$ while in 4-nitrobenzoic acid–substituted phenolate systems it occurs at ΔpK_a^{AN} close to -1. Undoubtedly, one of the possible reasons for this difference could be the neglect of those components in the sums ΣA and ΣA_1 which contain one of the two homoconjugation constants (K_{AHA^-} or $K_{A_1HA_1^-}$). It is then unfortunate that the terms $2K_{AHA^-}[HA]^2K_{HA}/(a_{H^+}y_{A^-})$ and $2K_{A_1HA_1^-}[HA_1]^2K_{HA_1}/(a_{H^+}y_{A_1^-})$ are dependent on $[HA]^2$ and $[HA_1]^2$ respectively, thus creating a problem in obtaining the exact expression for RSC as a function of measurable values only. The evaluation of the relative contributions of the terms that have been neglected in the approximate form of the expression for RSC is therefore important. One cannot rule out the possibility that taking into account the different values of the formation constants K_{AHS} and K_{A_1HS} would cause a maximum value of RSC to appear (in Fig. 1) closer to $\Delta pK_a^{AN} = 0$. Thus, the need, recognized earlier[23] for the correct quantitative approach to the formation of solvent heteroconjugates returns, as it is of fundamental importance for solving the problem of the stability of hydrogen bonded complexes. It is also possible that the general structural asymmetry of hydrogen bridges in systems with 4-nitrobenzoic acid forces the most stable system to occur at a ΔpK_a^{AN} value slightly different from zero.

The greater stability of complexes formed by phenolates unsubstituted in *ortho* positions (3-nitrophenolate, 4-nitrophenolate, and 3,4-dinitrophenolate) is most likely to be caused by the fact that these species are free of the steric substituents. The stronger delocalization of the negative charge in the 3,4-dinitrophenolate ion than in 4-nitrophenolate or 3-nitrophenolate ions seems to be the reason for the somewhat reduced stability of the complex containing the former species. This delocalization lowers the density of the negative charge at the key oxygen atom and consequently weakens the hydrogen bond formed.

The formation constant and the real stability constant are equivalent only if the interactions are extremely weak (ΔpK_a^S highly negative) because equilibrium concentrations of the products of the proton exchange reaction are, in such a case, negligible. These two quantities respond differently to an increasing value of ΔpK_a^S, particularly after passing the region close to $\Delta pK_a^S = 0$. While the formation constant continues its linear increase,[22–24] the real stability constant, after reaching it maximum value around ΔpK_a^S close to zero, systematically decreases. At extremely high ΔpK_a^S, when the products of the proton exchange reaction strongly dominate, the formation constant, being very large, becomes a very good measure of instability of hydrogen bonded complexes. On the contrary, since it is in such a case very low, the real stability constant retains its meaning it accordance with its name. From the energetic point of view the real stability constant is a measure of the free energy difference between the complex formed and all species which coexist at equilibrium and which result from its decomposition. This fact raises a very fundamental question, whether the energy of a given hydrogen bonded complex may, at all, be attributed to only one strictly defined reaction.

A separate problem deals with the possibility of the coexistence of the two tautomeric forms of the same heteroconjugate AHB in the equilibrium

$$AH\cdots B \rightleftharpoons A^-\cdots {}^+HB \qquad (24)$$

called the proton transfer equilibrium.[25] As stated originally[21] and confirmed recently,[26] this equilibrium, though so commonly assumed by many investigators, has not, so far, been proven experimentally. The proof requires that the absorbance ratio of the two bands, each attributed to the corresponding form, remains constant regardless of the analytical concentration ratio of the proton donor (HA) and the proton acceptor (B). In the chemical literature, no spectrum can be found from which these two bands can successfully be extracted. This fundamental condition is not fulfilled even approximately, thus making the concept of the double minimum proton potential speculative.

The most general conclusion that may be drawn from the above discussion deals with the most favourable conditions for the formation of strong hydrogen bonds. As indicated by eqn.

(18) and confirmed by experimentally-based Figs. 1 and 2, for a given type of hydrogen bridge and at comparable steric constraints, the factor reflecting relative strength of both key proton donors coexisting in equilibrium plays a decisive role. The strongest hydrogen bonds are then generally formed when the proton donating properties of HA and HA_1 are comparable. Only the differentiated tendencies to form homoconjugates (AHA^- and $A_1HA_1^-$) and solvent heteroconjugates (*e.g.* AHS and A_1HS) seem to cause in this respect some, however not very significant, deviations. Thus, a problem which for years has seemed only intuitively understandable, has finally found a quantitative explanation and simple experimental confirmation. Since the optimum ΔpK_a^S providing the most stable hydrogen bonds is indeed close to zero, the objection given by Shan *et al.*[3] and dealing with the proposal of Cleland and Kreevoy[7] appear to be groundless. This proposal may then be now discussed much more constructively than before.

Conclusions

1. The real stability constant is the only known quantity which takes into account all possible ways of decomposition of complexes formed through hydrogen bonding and therefore is recommended as the best measure of their stability.
2. The most stable hydrogen bridges formed between species of comparable steric contributions are those for which ΔpK_a^S is close to zero as reflected both by theoretically predicted and experimentally determined maximum values of the real stability constant.

Acknowledgements

The author wishes to thank Professor C. Perrin (Department of Chemistry, University of California) and Professor J. P. Guthrie (Department of Chemistry, University of Western Ontario) for a valuable discussion (during the 15th IUPAC Conference on Physical Organic Chemistry in Göteborg) on the problem presented in this paper.

References

1 L. Pauling and R. B. Corey, *Nature*, 1951, **168**, 550.
2 J. D. Watson and F. H. C. Crick, *Nature*, 1953, **171**, 737.
3 One of the recent assumptions that the formation constant of a given hydrogen-bonded complex is still considered as a measure of its stability is made *e.g.* in the paper: S. Shan, S. Loh and D. Herschlag, *Science*, 1996, **272**, 97.
4 W. W. Cleland, *Biochemistry*, 1992, **31**, 317.
5 J. A. Gerlt and P. G. Gassman, *J. Am. Chem. Soc.*, 1993, **115**, 11552.
6 J. P. Guthrie and R. Kluger, *J. Am. Chem. Soc.*, 1993, **115**, 11569.
7 W. W. Cleland and M. M. Kreevoy, *Science*, 1994, **264**, 1887.
8 P. A. Frey, S. A. Whitt and J. B. Tobin, *Science*, 1994, **264**, 1927.
9 A. Warshel, A. Papazyan, P. A. Kollman, W. W. Cleland, M. M. Kreevoy and P. A. Frey, *Science*, 1995, **269**, 102.
10 S. Scheiner and T. Kar, *J. Am. Chem. Soc.*, 1995, **117**, 6970.
11 J. P. Guthrie, *Chem. Biol.*, 1996, **3**, 163.
12 J. A. Gerlt, M. M. Kreevoy, W. W. Cleland and P. A. Frey, *Chem. Biol.*, 1997, **4**, 259.
13 C. L. Perrin and J. B. Nielson, *Annu. Rev. Phys. Chem.*, 1997, **48**, 511.
14 J. Magoński, Z. Pawlak and T. Jasiński, *J. Chem. Soc., Faraday Trans. 1*, 1993, **89**, 119.
15 J. F. Coetzee and G. P. Cunningham, *J. Am. Chem. Soc.*, 1965, **87**, 2534.
16 I. M. Kolthoff and M. K. Chantooni, Jr., *J. Am. Chem. Soc.*, 1965, **87**, 4428.
17 J. F. Coetzee and G. R. Padmanabhan, *J. Am. Chem. Soc.*, 1965, **87**, 5005.
18 M. K. Chantooni, Jr. and I. M. Kolthoff, *J. Phys. Chem.*, 1976, **80**, 1306.
19 Z. Pawlak, J. Magoński and T. Jasiński, *J. Mol. Struct.*, 1978, **47**, 329.
20 I. M. Kolthoff, *Anal. Chem.*, 1974, **46**, 1992.
21 J. Magoński, *J. Solution Chem.*, 1990, **19**, 597.
22 J. Magoński and B. Rajzer, *J. Chem. Soc., Perkin Trans. 2*, 2000, 1181.
23 J. Magoński, *Anal. Chim. Acta*, 1999, **384**, 27.
24 J. Magoński, *Phys. Chem. Chem. Phys.*, 2000, **2**, 2743.
25 T. Zeegers-Huyskens and P. Huyskens, in *Molecular Interactions*, W. J. Orville-Thomas and H. Ratajczak (eds), John Wiley & Sons, New York, 1980, pp. 1–106.
26 J. Magoński, *J. Phys. Org. Chem.*, 2002, **15**, 204.