

Fig. 1. The formation of HRP from apoHRP and Lba (O), Lbc (●) and MbI (▲) in 0.02 M sodium phosphate buffer, pH 7.0. Ordinate: HRP formed as calculated from the activity measurements; abscissa: concentration of hemoglobin in incubation solution.

peroxidatic activity of Lba, Lbc, Mb I, and apoHRP. The activity obtained is therefore a consequence of the formation of holoHRP.

Banerjee¹⁰ has defined the equilibrium constant for the dissociation of a hemo-protein to yield dimeric hematin and protein and derived the value $\log K = 15.24$ at 25°C for metmyoglobin at pH 7.0. A comparison of the affinities of the apoproteins of Lba, Lbc, and Mb I allows a relative value of 12.81 for Lba and 12.91 for Lbc to be estimated for the hematin-protein association constant, assuming the constant for Mb I to be equal to the above-mentioned 15.24.

These findings present a new case of hematin transfer between proteins. Under the conditions studied (pH 7.0) heme is about 370 times more firmly bound to the apoprotein in horse Mb I than in Lba, and 250 times more firmly than in Lbc. A comparative study on urea denaturation of Lba, Lbc and sperm whale myoglobin indicates that sperm whale myoglobin is more stable to urea denaturation than the

two leghemoglobin components.¹¹ It appears therefore, as through the "heme pocket" of Lba and Lbc is more open than that of myoglobin, and allows an easier migration of the heme.

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On the Nonsymmetry of Reaction Parameter (ρ) and the Substituent Parameter (σ) in the Hammett Equation and Similar Extrathermodynamic Relationships

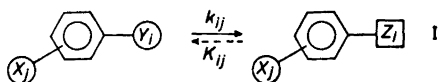
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The Hammett equation (1) has empirically been found to well describe aromatic

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reactivity data (m and p substitution) of reaction series of type I (for recent reviews, see Refs. 1 and 2):



Often, the Hammett equation is written in the reduced form as

$$\log k_{ij} - \log k_{i0} = \rho_i \sigma_j \quad (1a)$$

However, as will be argued below, for mathematical and statistical reasons, the following, full formulation, should be preferred

$$\log k_{ij} = \alpha_i + \rho_i \sigma + e_{ij} \quad (1b)$$

In eqn. 1b, the parameters α_i and ρ_i are specific for the i :th reaction (defined by the reaction center Y_i in scheme I). The former (α_i) is closely, but not exactly, corresponding to $\log k_{i0}$ in eqn. 1a. The substituent parameter σ_j is specific for the j :th substituent (X_j in scheme I). The residuals (e_{ij}) denote the part of the observed data ($\log k_{ij}$) which is not described by the systematic part in eqn. 1b. They contain contributions of two, principally different, types, namely (a) errors of measurement and (b) model errors, due to simplifications inherent in eqn. 1.*

Hine,³ making a theoretical interpretation of the Hammett equation in terms of free energy differences between reactant and products (or transition states) in scheme I in conjunction with a complementary scheme describing the change of the substituent X_j to the substituent W_j , arrived at the following formulation of the Hammett equation, which is formally symmetrical in the reaction and substituent variable

$$\log k_{ij} - \log k_{i0} = \tau_{ij}(\sigma_{Y_i} - \sigma_{Z_i})\sigma_{X_j} \quad (2)$$

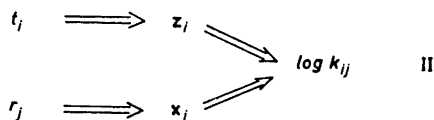
Wepster⁴ recently extended this formalism also to the extended Hammett equation of Yukawa and Tsuno,⁵ obtaining the following equation

$$\log k_{ij} - \log k_{i0} = \tau_{ij}[(\sigma_{Y_i} - \sigma_{Z_i})\sigma_{X_j} + (\sigma_{Y_i}^R - \sigma_{Z_i}^R)\sigma_{X_j}^R] \quad (3)$$

* Eqn. 1 is empirically valid both for rate (k) and equilibrium (K) constants. For brevity, however, the notation is restricted to the former.

Though the derivations of Hine and Wepster are formally correct, and consequently also eqns. (2) and (3), these formulations imply, incorrectly, a symmetry between the reaction variable(s) and substituent variable(s) in the Hammett and Yukawa-Tsuno equations. However, in their full formulations (eqn. 1b and analogously for the Yukawa-Tsuno equation), these equations are not symmetrical. In the Hammett equation, the variation of the reaction center is described by *two* parameters (α_i and ρ_i), while the variation of the substituent can be described by a single parameter (σ_j). The nature of this asymmetry can, moreover, be formulated in stringent mathematical terms as follows (a full account of this treatment is given in Ref. 6):

Consider the variation in reaction center in scheme I as being described (formally) by the macroscopic variable t , varying among the states t_i with each state corresponding to a particular reaction $Y_i \rightarrow Z_i$. Similarly, the variation of the substituent (X_j in scheme I) is described by the variable r with the states r_j . In addition, the influence of these macroscopic variables (t and r) on the observed variable ($\log k_{ij}$) is symbolized to occur *via* the microscopic (hypothetical) vector variables \mathbf{z} and \mathbf{x} , respectively.



Hence, $\log k_{ij}$ is a function of two vector variables, say $F(\mathbf{z}, \mathbf{x})$. The behaviour of this function can be studied by means of Taylor expansions; different cases can be seen:

1. The vector variables \mathbf{z} and \mathbf{x} contain only one independent variable each, denoted by z and x , respectively. The Taylor expansion is, in the usual symbols:

$$\begin{aligned} \log k_{ij} = F(z, x) &= F(z_0, x_0) + F'_z(z - z_0) + \\ &F'_x(x - x_0) + \frac{1}{2}F''_{zz}(z - z_0)^2 + F''_{xx}(x - x_0)^2 + \frac{1}{2}F''_{zx}(x - x_0)^2 + R_3(3) = F(z_0, x_0) - \\ &F'_z F'_x / F''_{zx} + (F'_z + F''_{zx}(z - z_0) + \\ &F''_{zz} F''_{xx}(z - z_0)^2 / 2F'_z)(F'_z / F''_{zx} + \\ &(x - x_0) + F''_{xx} / 2F'_x(x - x_0)^2) + R_2(3) \\ &= C + h(z)g(x) + R_2(3) \quad (4) \end{aligned}$$

Here F_z' denotes the value of $\delta F/\delta x$ at the point (z_0, x_0) and so on. The remainders, $R(3)$ and $R_2(3)$, which are different, contain terms of third and higher orders, such as $F_{xxx}'''(z-z_0)(x-x_0)^2$.

Since now the macroscopic variables t and r vary discretely, so do the microscopic variables z and x . Rewriting $h(z_i)$ as β_i and $g(x_j)$ as θ_j , we see that, provided that the variation of the microscopic variables z and x over the experimental range of t and r is sufficiently small to make the remainder $R_2(3)$ small compared to the errors of measurement of $\log k_{ij}$, the data will in this first case conform well to the simple one-component model:

$$\log k_{ij} = c + \beta_i \theta_j + e_{ij} \quad (5)$$

2. Analogously, it can be shown that for the case that the vector variable \mathbf{z} contains several independent variables while the vector variable \mathbf{x} still contains only one, a second order approximation is⁶

$$F(\mathbf{z}, x) = f(\mathbf{z}) + h(\mathbf{z})g(x) + R(3) \quad (6)$$

which, translated to the discrete case in the same way as above, gives:

$$\log k_{ij} = \alpha_i + \beta_i \theta_j + e_{ij} \quad (7)$$

3. Finally, higher order approximations of functions in both single variables and vector variables (\mathbf{z} and/or \mathbf{x}) all give the same discrete model:

$$\log k_{ij} = \alpha_i + \sum_{a=1}^M \beta_{ia} \theta_{aj} + e_{ij} \quad (8)$$

We are now in the position to make a rigorous interpretation of the simple Hammett equation (1b) which is seen to be equivalent to eqn. (7). This implies that several microscopic variables (denoted by the vector \mathbf{z}) vary with the variation of the reaction center (the macroscopic variable t), while only one microscopic variable (x) varies with the variation of the substituent (the macroscopic variable r). In addition, the variation of these microscopic variables over the experimental range where the Hammett equation is valid, is so small that the second order approximation holds good ($R(3)$ is small).

The formalism of Hine and Wepster is relevant only for cases when data are well fitted by the simpler model (5), indicating that both the reaction variable and the substituent variable influence the reaction

rate (or equilibrium constant) via one single microscopic variable each.

From the present derivation it also follows that it is of less importance which transformation of the observed data which is used in the models. The function $F(\mathbf{z}, x)$ can as well correspond to $\sqrt{k_{ij}}$ or $\log \log k_{ij}$ as to $\log k_{ij}$. The parameter scales will naturally be different, but there is presently no indication that $\log k_{ij}$ will always be the best transformation for the use in an empirical model of the ETR type.

Finally, the present treatment shows that, since the influence of any number of macroscopic variables can be incorporated in the variable \mathbf{z} , it is indeed possible to formulate the Hammett equation and other similar ETR's with a single substituent scale which is independent of temperature, pressure, solvent, and so on. The influence of the latter variables can in principle be incorporated in the vector variable \mathbf{z} and hence be described by the two parameters α_i and θ_j in eqn. (1b). Whether this is possible for a particular class of chemical phenomena, however, is a question that only can be settled by the analysis of corresponding empirical data. The present derivation only shows the possible existence of such a single substituent scale, not the experimental domain where it is applicable.

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