Errors in the evaluation of Arrhenius and van't Hoff plots

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Errors in the numerical values of activation or normal enthalpies, entropies and free enthalpies calculated from Arrhenius or van't Hoff plots, respectively, are due to the neglect of equidimensionality in equations, or to inappropriate approximations. The logarithmization of dimensioned quantities should be avoided, which demands the use of relative concentrations if a change in mole number occurs in the reaction. The application of the Arrhenius plot to enzymic reactions by using $V_{\text{max.}}/E_T$ instead of the rate constant of product formation has meaning only if the reaction follows the simplest Michaelis–Menten mechanism; however, the use of the van’t Hoff plot using $K_m$ is questionable even in the latter case.

The temperature dependence of the rate of enzymic reactions and their equilibrium constant are generally analysed by using the empirical Arrhenius equation ($\ln k = \ln A^0 - E_a/(RT)$) or the van’t Hoff equation ($\Delta G^0 = -RT \ln K_{eq}$), respectively (van’t Hoff, 1884; Arrhenius, 1889). In such an analysis mistakes can frequently be found due to the fact that authors do not adhere to the simple rules: (i) the two sides of an equation must have the same dimensions, (ii) only dimensionless pure numbers have logarithms or (iii) inappropriate approximations should not be used.

Arrhenius plots

First order reactions. These can be interpreted in terms of the absolute rate theory (Eyring, 1935). In this case the first order constant (Benson, 1960; Laidler, 1963):

$$k = \left(\frac{\kappa RT}{Nh}\right) e^{\Delta S^*/R} e^{-\Delta H^*/RT}$$  

(1)

Abbreviations and symbols used: $A^0$, pre-exponential factor; $A^0/e$, $k$, rate constant; $E_a$, activation energy; $\kappa$, transmission coefficient, usually taken as unity; $N$, Avogadro’s constant; $R$, Planck constant; $S^*$, activation entropy; $k_B$, Boltzmann constant; $P$, probability factor; $d_{AB}$, average diameter of the two reacting molecules A and B in cm; $m_A$ and $m_B$, mass of A and B, respectively; $H^o$, normal free enthalpy; $S^0$, normal entropy; $G^*$, activation free enthalpy; $c_A$, $c_B$, and $c_{AB}$, molar concentrations of A, B and the activated complex, respectively; $v_0$, initial velocity; $V_{\text{max.}}$, maximum velocity; $E_T$, total enzyme concentration; $S$, substrate concentration; $K_s$, dissociation constant; $K_m$, Michaelis constant.

i.e. the pre-exponential factor:

$$A^0 = \left(\frac{\kappa RT}{Nh}\right) e^{\Delta S^*/R} = A_1 e^{\Delta S^*/R}$$  

(2)

and $A_1$ is the ‘propre-exponential factor’. Both the first order rate constant and $A_1$ have the dimension of s$^{-1}$. Consequently, in analysing the temperature dependence of a rate constant graphically either $\log(k/A_1)$ or $\log(k \cdot s)$ should be plotted as a function of $1/T$, since the former are dimensionless quantities. Moreover, in the plot $\log(k \cdot s)$ versus $1/T$ the intercept, $A^0$, is also the function of $T$ (really of $\log T$) although its temperature dependence is very weak (Benson, 1960). Since this plot is not linear theoretically, the plot $\log(k/T)(K \cdot s)$ versus $1/T$ is recommended.

The numerical values of $\Delta H^*$ and $\Delta S^*$ are correct only if $k$ is given in s$^{-1}$. Sometimes one finds Arrhenius plots where $k$ is given in min$^{-1}$ or h$^{-1}$, which may result in erroneous numerical values if it is neglected that $A_1$ has the dimension s$^{-1}$. Consequently, in these cases $A_1$ should be multiplied by 60 or 3600, respectively, to obtain the correct value (cf. Arrhenius, 1889).

Second order reactions. Here the rate constant may be interpreted either in terms of the collision theory (Lewis, 1918) or the absolute rate theory. By using the collision theory one obtains:

$$k = PNd_{AB}^2 [8\pi k_B T (m_A + m_B)/m_A m_B]^1 e^{-E_a/RT}$$  

(3)

i.e. the pre-exponential factor:

$$A^0_2 = Nd_{AB}^2 [8\pi k_B T (m_A + m_B)/m_A m_B]^1 e^{\Delta S^*/R}$$  

$$= A_2 e^{\Delta S^*/R}$$  

(4)

where $A_2$ is the ‘propre-exponential factor’. 

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In this case the dimension of $A_2$ is cm$^3$·mol$^{-1}$·s$^{-1}$. To use dimensionless quantities either log($k/10^3A_2$) or log($k$·M·s$^{-1}$) should be plotted against $1/T$, since $k$ and $10^3 A_2 = A_2^*$ have the same dimensions, i.e. M$^{-1}$·s$^{-1}$.

If log $k$ (in M$^{-1}$·s$^{-1}$) versus $1/T$ is plotted, as occurs commonly, the intercept on the ordinate will be $A_2^* + \Delta S^*/2.303R$, which generally is not taken into account (e.g. Thusius et al., 1976; Brandsnes et al., 1982). The error in $\Delta S^*$ may reach $\pm$100% or even more if log $A_2^*$ is neglected, e.g. for a protein of 150000Da and a substrate of about 500Da. Again, if $k$ is given in M$^{-1}$·min$^{-1}$ or M$^{-1}$·h$^{-1}$ or M·min$^{-1}$·s$^{-1}$, etc., the values will be in error. If the value of $\Delta S^*$ is needed, the plot log($[k/T]$(K·M·s$^{-1}$)) versus $1/T$ is recommended, which gives also theoretically a straight line, and $d_{AB}$, $m_A$ and $m_B$ should be calculated. If the latter values are not known, $\Delta S^*$ cannot be determined.

If the second order rate constant is interpreted by the absolute rate theory:

$$k = (\kappa RT/Nh)K^*$$

where $K^*$ is the equilibrium constant of the formation of the activated complex in relative concentrations, i.e.: 

$$K^* = (c_Ac_B/c_0)/(c_A/c_0)(c_B/c_0)$$

Consequently, in this case substituting the van’t Hoff equation for $K^*$, one obtains:

$$k = (\kappa RT/Nh) e^{\Delta S^*/R} e^{-\Delta H^*/RT}$$

(5)

(7)

where the equation is formally equivalent to that of the first order rate constant (Eyring, 1935) and log($k/T$)(K·s$^{-1}$) should be plotted against $1/T$. It is supposed here that only the decomposition of the activated complex limits the rate of the overall reaction. Strictly speaking, if $k$ in M$^{-1}$·s$^{-1}$ is obtained for a bimolecular reaction, the use of the Arrhenius plot tacitly assumes the validity of the collision theory, since $A_2^*$ has this dimension (Laidler, 1963). Otherwise, if $k$ is obtained in s$^{-1}$, even in the case of a bimolecular reaction the absolute rate theory must be regarded as valid and $A_1$ should be used as proper-exponential factor in the Arrhenius plot.

If the evaluation of $\Delta S^*$ is not crucial, the use of the integrated Arrhenius equation (Arrhenius, 1889) is recommended:

$$\log(k_2/k_1) = (E_a/2.303R)\{[T_2 - T_1](T_2T_1)^{1/2}\}$$

(8)

where $k_2/k_1$ is dimensionless, both being rate constants of the same process at two temperatures. It is suggested that $k_1$ at $T_1$ should be taken as constant reference values for all $k$ values.

We must draw attention to the fact that very often one finds log $v_0$ versus $1/T$ presented as an ‘Arrhenius plot’ (e.g. Coulet et al., 1975; Terrc et al., 1978; Lucas, 1978; Bragadin et al., 1979; McMurchie & Raison, 1979; Silvius & McElhaney, 1980; Kwatra & Sourkes, 1981; Rosemeyer et al., 1982). This is a fundamental mistake caused by an inappropriate approximation. Since $v_0 = v_{max}/(K_m + S)$ and $K_m$ is a function of the temperature, one cannot use $v_0$ instead of $k$, only $v_{max}/E_T$. It is not by chance that practically in all papers where log $v_0$ was plotted against $1/T$ a break in the ‘Arrhenius plot’ was found, which was wrongly interpreted as a change in protein structure since it is due only to the temperature dependence of $K_m$. One should be aware that by using only $v_{max}$ instead of $v_{max}/E_T$ for the Arrhenius plot, the intercept on the ordinate will be affected by the value of log $E_T$, which may be equal or even higher than $\Delta S^*$ (Reiner, 1969).

One can use $v_{max}/E_T$ for the Arrhenius plot if and only if $v_{max} = k_2E_T$, i.e. the reaction follows the simple Michaelis–Menten mechanism (Keleti, 1981). If, e.g. a kinetically relevant EP complex is also formed:

$$V_{max} = k_2k_3E_T/(k_3 + k_3 + k_2),$$

and $v_{max}/E_T$ cannot be used for the Arrhenius plot, since this refers only to simple elementary steps.

van’t Hoff plots

(a) In the analysis of the temperature dependence of equilibrium constants, similar misinterpretations are common. If in the reversible reaction no change in mole number occurs

$$A + B \rightleftharpoons C$$

the equilibrium constant is a dimensionless number, i.e. the use of the van’t Hoff equation is correct. In the case of first order reactions, as:

$$K_{eq} = k_{-1}/k_1 = (A_{-1} e^ {-\Delta G_{v1}^* /RT})/$$

(9)

$$A_{1} e^ {-\Delta G_{v1}^* /RT} = e^ {-\Delta G^* /RT}$$

no problem arises. Since $A_{-1} = A_1$, $R \ln(A_{-1}/A_1^0) = \Delta S^0$ (Benson, 1960; Laidler, 1963).

Generally, by plotting log $K_{eq}$ versus $1/T$ the slope of the straight line is $-\Delta H^*/2.303R$ and the intercept on the ordinate is $\Delta S^0/2.303R$. However, this is true only if the reaction is of first order in both directions.

(b) In the case of second order reactions, if they are to be interpreted by the collision theory (i.e. the $k$ values are in M$^{-1}$·s$^{-1}$), for the elimination of the proper-exponential factors one should assume that $d_{AB} = d_{CD}^2$ and $(m_A + m_B)/m_B = (m_C + m_D)/m_D$.
In most instances this is probably true. However it is
surely not true when, e.g. A is a protein or
polysaccharide or polynucleotide and C is a small
peptide or mono- or disaccharide or mono- or
dinucleotide, etc. In these cases \( \Delta G^0 = -RT \)
\( \ln(A'C_\text{eq})/A°C \). From \( \Delta S^0 \) one obtains
\( \ln(A'_c/\Delta'_{c+}) + \Delta S^0 \), since \( \ln(A'_c/\Delta'_{c+}) \)
differs from zero.

(c) If there is a change in mole number in the
reaction at equilibrium

\[ K_{\text{eq}} = k_2/k_1 = A'_1 e^{-\Delta G^0/RT}/A_1 e^{-\Delta G^0/RT} \]

since \( k_1 \) is a first-order rate constant and \( k_2 \) a
second-order rate constant.

The dimension of \( K_{\text{eq}} \) is often (but wrongly, since
it should be dimensionless; cf. Price & Dwek, 1979)
presented as \( M^{-1} \) (e.g. Schweitz et al., 1973; Vincent &
Lazdunski, 1973; Luissi et al., 1975) or \( M \) (e.g.
Burton, 1974; Hochachka, 1974; Nowak & Lee,
1977; Kozlov et al., 1977; Schultz et al., 1977;
Hofmann et al., 1979; Takahashi et al., 1981). The
right equation to be used is:

\[ \Delta G^0 = -RT \ln(A'_1 K_{\text{eq}}/A'_1) \]

since \( A'_1 K_{\text{eq}}/A'_1 \) is a dimensionless quantity and
surely \( A'_1 \neq A'_1 \). If one analyses the temperature
dependence of the equilibrium constant of a reversible
enzyme–substrate or enzyme–inhibitor complex
formation, \( d_0 \) or \( d_i \) may be assumed to equal
\( d_e \). With a protein of about 150000 Da (\( d \approx \) 7 nm)
and a substrate or inhibitor of about 500 Da, \( A'_1 \) at
300 K is several orders of magnitude higher than
\( A'_1 \), the latter being about \( 6 \times 10^{12} \) s\(^{-1}\). The
same reasoning is valid for receptor–ligand
reactions.

By plotting \(-\log K_{\text{eq}} versus 1/T \) the intercept on the
ordinate will be \( \Delta S^0/2.303 R + \log(A'_1/A_1) \). One
should be aware that also \( \log(A'_1/A_1) \) is a function of
\( T \) [really of \( \log(1/T^4) \)] (Lewis, 1918), even if its
temperature-dependence is very weak. Therefore it is
better to plot \( \log [(K_{\text{eq}} T^4)(M \cdot \text{K}^{-i})] versus 1/T \),
which also gives a straight line theoretically. Again
the concentrations of the compounds in the expres-
sion of \( K_{\text{eq}} \) should be expressed in \( M \) and not in
\( \text{mM}, \mu \text{M}, \text{etc.}, \) if the second order rate constant is
interpreted by the collision theory.

Since in such cases \( \Delta S^0 \) generally cannot be
evaluated with the desired accuracy, the use of the
integrated van’t Hoff equation (van’t Hoff, 1884;
Arrhenius, 1889) is recommended:

\[ \text{log}(K_{\text{eq}}/K_{\text{eq}}) = (\Delta H^0/2.303 R)(T_2-T_1)/T_2 T_1 \]

\( K_{\text{eq}} \) at \( T_1 \) being taken as reference for all \( K_{\text{eq}} \) values.

(d) If one can interpret also the second-order reaction in the reversible one by the absolute rate

\[ K_{\text{eq}} = k_2/k_1 = e^{-\Delta G^0/RT} \]

since

\[ K_{\text{eq}} = (c_A/c_0)/(c_B/c_0) = (c_A c_0)/(c_B c_D) \]

is a dimensionless number, as it should be (Price &
Dwek, 1979), and the propri-exponential factors in
\( s^{-1} \) are equal.

(e) Owing to the arguments presented above, the
determination of \( \Delta G^0 \) of enzyme–substrate complex
formation using a plot of \( \log K_m \) versus \( 1/T \) has no
meaning. Either \( K_m = K_1 \) and in this case point (c)
above is valid, or \( K_m = (k_1 + k_2)/k_1 = K_1 + k_2/k_1 \),
which is not interpretable according to the van’t
Hoff equation. If the mechanism of the reaction is
more complex than the simple Michaelis–Menten
mechanism, similar plots are even more meaningless
(e.g. Tro & Keleti, 1974).

General conclusions

The above statements are independent of the
phenomenological interpretation of the pre-expo-
nential factors; the latter were involved only to help
elucidate the general misinterpretations.

The use of \( k \cdot s \) or \( (k/T)(K \cdot s) \), etc. seems to be
simply a formal elimination of the logarithmiza-
tion of dimensioned quantities. However, it is the only
way to ensure that dimensional inequalities do not
lead to erroneous numerical values for the ther-
modynamic parameters.

We suggest to re-evaluate the \( \Delta S^0, \Delta H^0, \Delta G^0, \Delta H^0, \Delta S^0 \) and \( \Delta G^0 \) values calculated without taking
into account the problems outlined above. However,
it should be considered that in second-order re-
actions the numerical values of \( \Delta S^0 \) and \( \Delta S^0 \), and
consequently those of \( \Delta G^0 \) and \( \Delta G^0 \), are question-
able, due to the difficulties of evaluating \( d_{AB}, m_A, m_B \)
and \( c_{AB} \).

The \( \Delta G^0 \) values generally cannot be used to
calculate the efficiencies of metabolic processes,
since these often involve change in mole number and
therefore relative concentrations for the van’t Hoff
plot should be applied (Cornish-Bowden, 1981).

It should be noted that there are a great number of
papers which contain similar errors. I apologize for
referring to the authors mentioned, chosen by
chance, instead of several dozen (or hundred) others
which could be selected equally.

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