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A thermodynamic analysis of the Collander equation and establishment of a reference solvent for use in drug partitioning studies

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A thermodynamic analysis of the Collander equation, $\ln P_I = a + b \ln P_{II}$ (I and II refer to two different partitioning systems with partition coefficients P_I and P_{II}, respectively), is given and applied to three forms of correlation. The intercept, a, is shown to have no general fundamental significance whereas the slope, b is shown to reflect differences in non-aqueous solvent properties; b is also shown to be of use in scaling solvent behaviour to select solvents which closely represent biological membrane properties for use in partitioning studies. Laboratory and literature data are subjected to the analysis.

There is much interest in the relationship between biological response (BR), drug structure and partition coefficients. The partition of a drug substance between water and some non-aqueous solvents has been used extensively for establishing correlations between BR and drug structure (for reviews see Leo et al 1971; Kubinyi 1979; Tomlinson 1983). From the site of administration to the site of action a drug must traverse a variety of hydrophilic and lipophilic barriers. Partition between aqueous and non-aqueous solvents appears to mimic this process insofar as homologous series of compounds behave in a predictable way as solutes and as drugs.

Selection of the appropriate non-aqueous solvent to represent the properties of a biological system is, however, a serious problem (Leo & Hansch 1971; Leo et al 1971; Smith et al 1975; Tomlinson 1983) and it seems unlikely that the most commonly used solvent, octan-1-ol, could be the optimum for the wide variety of systems to which octan-1-ol/water partition coefficients are applied (Pomona College Medicinal Chemistry Project). Indeed Taft et al (1985) have recently examined the physicochemical properties that influence these partition coefficients (solvatochromic parameters that measure solute dipolarity/polarizability, hydrogen bond acceptor basicity and molar volume).

There are at present no criteria, although there are *methods*, apart from complete quantitative structure-activity relationship (QSAR) studies, by which the correlation between a solvent system and a biological system (e.g. micro-organisms, tissues, isolated cells, whole organs or animals) can be judged.

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The successful use of octan-1-ol has been ascribed to its hydrophobic and hydrophilic properties (Kubinyi 1979), to its hydrogen bonding ability (Smith et al 1975) and to its solvating and associating properties (Smith et al 1975). Other solvent systems, such as cyclohexane and iso-octane, have been proposed on thermodynamic grounds (Rytting et al 1972), and Kubinyi (1979) has discussed the application of the Collander equation (Kubinyi 1979) to biological correlations. This equation

$$\ln P_I = a + b \ln P_{II} \quad (1)$$

has been applied to partitioning systems in three ways (Kubinyi 1979). In the first of these it was found to correlate the water/solvent partition coefficient of a solute in one system (P_I) with the value for the same solute in a second system (P_{II}).

The values found by linear regression for a and b are usually applicable only to homologous series of solutes and when correlating between fairly similar types of solvent, polar, non-polar, hydrocarbon, etc. Indeed the solutes too have been classified as H-bond donors or acceptors, different values of a and b being applied to each. Kubinyi (1979) also, in an attempt to set $b = 1$ (because some data implied that this was an appropriate value for b and because some correlations were discovered to be poor unless the solute systems were sorted into, for example, H-bonding or non-H-bonding systems), discusses the inclusion of extra terms in the Collander equation which, it is suggested, take account of judgements about the H-bonding ability of homologous series or substituent groups.

The Collander equation has also been extended (whilst maintaining the same form) to cover correla-

tion between partition coefficients for unrelated solutes distributed between two solvent systems (Kubinyi 1979) and to the distribution of a single solute between varied solvent systems (Korenman et al 1985).

A statistical analysis of this equation has not included any attempt to define a and b as other than regression coefficients (Dunn & Wold 1978).

It is important therefore to establish whether the various forms of the Collander equation have any theoretical basis and, if so, what conclusions may be drawn from the results of the theoretical analysis as to the nature of the transfer process.

In particular, the significance of a and b should be exposed in such an analysis. To date, a and b have been interpreted in several different ways. It has been suggested that b, is necessarily equal to one (Kubinyi 1979). It is also said to reflect the similarity of the solvent environment with respect to the solute (Katz & Diamond 1974a, b). Again, b is described as being a measure of the sensitivity of the system to perturbation by hydrophobic effects (Leo & Hansch 1971; Smith et al 1975). The intercept, a, is said to reflect "the sensitivity of the biochemical system and the intrinsic activity of a set of congeners" (Leo & Hansch 1971; Leo et al 1971; Smith et al 1975). It has also been described as a selectivity constant (Katz & Diamond 1974a, b). Leo & Hansch (1971) have also indicated that values of a are correlated with solvent lipophilicity and with water content at saturation. Rekker (1977) has likewise discussed the Collander equation and the values of a and b, coming to rather similar conclusions and has proposed an index of wetness based on factors other than these correlation parameters.

Case 1. Partition of a homologous series of compounds between two solvent systems

We have recently shown (Beezer et al 1986a) that the following equation, which is developed from Cratin's analysis (Cratin 1968; the basic details of the approach are also found in Langmuir 1925), can be used to describe the thermodynamic basis of the partitioning process for members of a homologous series.

$$\ln P = \frac{-n\Delta\mu^{\theta}_{L}}{RT} - \frac{\Delta\mu^{\theta}_{H}}{RT} + \ln\frac{V_W}{V_o} \qquad (2)$$

where P is the partition coefficient, expressed in molar concentration terms for the transfer process

solute in water → solute in non-aqueous solvent;

n is the number of similar lipophilic groups, e.g. CH_2, present in the molecule, each of which makes a contribution $\Delta\mu^{\theta}_{L}$ to the overall value of the Gibbs function of transfer; $\Delta\mu^{\theta}_{H}$ is the contribution to the overall Gibbs function of transfer of the hydrophilic, or parent structure; V_W and V_o are the molar volumes of the aqueous phase and non-aqueous phase, respectively. This equation of course simply formalizes the additivity postulates common to all linear Gibbs energy relationships.

Application of equation (2) to the Collander equation (i.e. to transfer between water and solvent system I and between water and solvent system II) yields

$$\frac{-n\Delta\mu^{\theta}_{L}(I)}{RT} - \frac{\Delta\mu^{\theta}_{H}(I)}{RT} + \ln\frac{V_W}{V_o(I)} =$$

$$a - \frac{nb\Delta\mu^{\theta}_{L}(II)}{RT} - \frac{b\Delta\mu^{\theta}_{H}(II)}{RT} + b\ln\frac{V_W}{V_o(II)} \qquad (3)$$

The value of a, the value of the intercept when $\ln P_{II} = 0$, is hence given by

$$a = \ln\frac{V_W}{V_o(I)} - \frac{n\Delta\mu^{\theta}_{L}(I)}{RT} - \frac{\Delta\mu^{\theta}_{H}(I)}{Rt} \qquad (4)$$

From the data given in a previous paper (Beezer et al 1986a) and reproduced here as Table 1 (transfer of *m*-alkoxyphenols from water to (i) heptane, (ii)

Table 1. Values of $-\Delta\mu^{\theta}_{L}$, $-\Delta\mu^{\theta}_{H}$ derived from plots described by equation (2). Values in kJ mol^{-1}. Values relate to solute transfer from water to described non-aqueous solvent*.

Temp. K	$-\Delta\mu^{\theta}_{L}$	$-\Delta\mu^{\theta}_{H}$	Solvent
283·15	3·09	−4·08	h
	0·81	11·28	pc
288·15	3·15	−3·95	h
	1·01	11·06	pc
	2·83	9·78	o
293·15	3·09	−3·25	h
	1·12	10·72	pc
	2·90	9·76	o
298·15	3·07	−2·92	h
	1·11	10·69	pc
	2·96	9·75	o
303·15	3·16	−2·68	h
	1·11	10·57	pc
	3·03	9·78	o
308·15	3·32	−2·88	h
	1·17	10·26	pc
	3·09	9·82	o
313·15	3·10	−1·79	h
	1·13	10·07	pc

* pc, Water-saturated propylene carbonate; o, water-saturated octan-1-ol; h, water-saturated heptane.

propylene carbonate and (iii) octan-1-ol), it is possible to calculate the value of n such that $\ln P_{II} = 0$ and hence the value of a in this circumstance. Calculation of n at 298·15 K such that equation (4) is satisfied produces, for solvent I (heptane) and solvent II (propylene carbonate), a value of n = −6·15. Insertion of this value of n into equation (3) and again setting T = 298·15K yields excellent agreement between calculated values of a and experimentally derived values. That n = −6·15 clearly has no physical significance.

The value of b, the slope of the Collander plot, may be evaluated by consideration of two sequential points in the plot e.g. for n = 1 and n = 2 and can be shown to be $b = \Delta\mu^{\theta}_{L}(I)/\Delta\mu^{\theta}_{L}(II)$. Inspection of the data in Table 1 shows the $\Delta\mu^{\theta}_{L}$ values are essentially independent of temperature. As $\Delta\mu^{\theta}_{L}$ values are insensitive to temperature this also means that Collander plots at a series of temperatures should be parallel. This is indeed observed and the values of b derived from the equation shown above and from plots of $\ln P_I$ vs $\ln P_{II}$ are shown in Table 2.

Case 2. Partition in two solvent systems: no homologues present

Commencing from equation (2) and without factoring the Gibbs function into group contributions then the following equation results

$$\frac{-\Delta G(I)}{RT} + \ln\frac{V_W}{V_o(I)} = a + b\left[\frac{-\Delta G(II)}{RT} + \ln\frac{V_W}{V_o(II)}\right] \quad (5)$$

from which it can be deduced that the value of b is given by

$$b = \frac{\Delta G(I) - \Delta G'(I)}{\Delta G(II) - \Delta G'(II)} \quad (6)$$

where the primed functions refer to the values for another substituted compound in the family.

If ΔG is set equal to $\Delta G_{parent} + \Delta G_{substituent}$ then b is given by

$$b = \frac{\Delta G_{subs}(I) - \Delta G'_{subs}(I)}{\Delta G_{subs}(II) - \Delta G'_{subs}(II)} \quad (7)$$

Thus straight line plots of the Collander form of equation are to be expected for such systems.

The value of a may also be derived and is equal to the value of the left-hand side of equation (5) when the term in square brackets is set to zero, i.e. when $\Delta G(II) = RT \ln V_W/V_o(II)$.

Case 3. Partition correlation for some acids and their esters in various solvent systems

In this case the substituent remains constant but the solvent systems change. Therefore we consider the form of the Collander equation given below as

$$\frac{-\Delta G(I)}{RT} + \ln\frac{V_W}{V_o(I)} = a + b\left[\frac{-\Delta G'(I)}{RT} + \ln\frac{V_W}{V_o(I)}\right] \quad (8)$$

for one point on the graph and

$$\frac{-\Delta G(II)}{RT} + \ln\frac{V_W}{V_o(II)} = a + b\left[\frac{-G'(II)}{RT} + \ln\frac{V_W}{V_o(II)}\right] \quad (9)$$

for another point on the graph. The primed symbols refer to the parent acid compound partitioned between the same solvent system. Algebraic manipulation shows that b is given by

$$b = \frac{\Delta G(I) - \Delta G(II)}{\Delta G'(I) - \Delta G'(II)}$$

and a by the left-hand side of equation (8) when the term in square brackets is set to zero, i.e. when $\Delta G(I) = RT \ln V_W/V_o(I)$.

DISCUSSION

Cases 2 and 3 are of interest as they rationalize, theoretically, the experimental observations of linear plots; they contain information on differences in group and overall Gibbs function values. Such values are more accessible and useful if derived through studies described by Case 1. The values of a in both these cases are shown to be of little fundamental interest. Indeed the forms of the Collander equation make it plain that the intercept value a is that value of $\ln P_I$ which results from setting $P_{II} = 1$ (i.e. $\ln P_{II} = 0$)—a purely arithmetic statement.

Furthermore the equations defining b given above for the three cases make it clear that b = 1 is not a necessary result; it may arise from other features of the process. Consideration of Case 1 and the resultant definition of b does yield interesting results. The definition of b includes terms that relate to the interaction of the lipophilic groups with solvents I and II (both referred to water). This

parameter may serve as an index of solvent character that would allow identification of a bulk solvent which represents more satisfactorily the properties of the biological membrane. From the formulation of b it is apparent that if some solvent system (water/lipid-like solvent) is selected as a *reference* system and used in this equation as system II then it will be possible to scale (at least for consideration of CH_2 groups) all other solvent systems relative to the chosen system. An obvious candidate for the reference system is water/octan-1-ol. This choice of reference solvent is based mainly upon the large volume of data for water/octan-1-ol systems and dooes not imply that octan-1-ol is the best or even a good mimic of the properties of biological membranes. Values of b, relative to octan-1-ol taken as b = 1 for the partition of *m*-alkoxyphenols between water and octan-1-ol, heptane and propylene carbonate (Beezer et al 1986a) are shown in Table 2. The corresponding values of b for the 'partitioning' of some esters of cortisone and hydrocortisone into liposomes, both above and below the lipid phase transition temperature, and also their partition into isopropyl myristate as bulk solvent, are also shown in Table 2. As a first approximation liposomes may be closer to biological membranes than are bulk solvents and, of the several partitioning studies reported with liposomes, we have recalculated the data given for esters of cortisone and hydrocortisone to yield values of b. Values of b for the same esters partitioning into ether are also given in Table 2. Table 3 lists values of b calculated from other data available in the literature for a variety of systems.

Table 2. Values of b calculated for partitioning of homologous series between water and various organic solvents and liposomes. The water/octan-1-ol system is designated system II (see text).

Solvent system I		
Water	Solute	b
Propylene carbonate	*m*-Alkoxy phenols	0·36
Liposome ($T < T_c$)*	21-Alkyl esters of hydrocortisone	0·546
IPM†	21-Alkyl esters of cortisone	0·582
Liposome ($T > T_c$)*	21-Alkyl esters of hydrocortisone	0·616
IPM†	21-Alkyl esters of hydrocortisone	0·625
Liposome ($T < T_c$)*	21-Alkyl esters of cortisone	0·672
Octan-1-ol	*m*-Alkoxy phenols	1
	Both ester series	1
Heptane	*m*-Alkoxy phenols	1·04
Diethyl ether	21-Alkyl esters of cortisone	1·30
Diethyl ether	21-Alkyl ester of hydrocortisone	1·53

* Above or below the lipid phase transition temperature, T_c.
† Isopropyl myristate.

It is clear from the data discussed by, for example, Kubinyi (1979) and by Leo & Hansch (1971), that values of b derived from Collander plots depend on the nature of the solutes studied and their relationship to the solvents used in the partitioning experiments. Thus the values of b shown in Table 3 show wide variation. As noted in the introduction to this paper it is unlikely that any one solvent will adequately represent all the physicochemical properties of a biological membrane. However, the values of b determined for a given series of solutes may well indicate the relationship between bulk solvent properties and membrane properties. In order to test this hypothesis a reliable data set is required. There are, as has been noted before, few data available for the same homologous series of compounds studied in a range of solvent systems with strict control of environmental conditions, e.g. temperature etc. We cannot therefore be totally confident yet about the utility of b values in selecting bulk solvents appropriate to mimic membranes with respect to specific solutes. The surprising feature of the data for the esters of cortisone and hydrocortisone is that their b values for partition into liposomes and into isopropyl

Table 3. Values of a, b and regression coefficients calculated from literature data for transfer of the named solutes from water to the named solvents compared to transfer to octan-1-ol (see text). Varied conditions were used to study the partitioning processes. Solutes studied; benzene, toluene, ethyl benzene, propyl benzene.

Solvent	a	b	r	Ref.
Cyclohexane	1·17	0·72	0·99	Korenman et al 1985
n-Pentane	−1·13	1·20	1·00	"
n-Hexane	−1·09	1·14	0·98	Korenman et al 1978
n-Decane	−0·64	1·11	0·99	"
n-Hexadecane	−0·83	1·25	0·98	"
n-Butanol	−2·30	1·66	0·97	"
n-Pentanol	−3·41	1·83	0·98	"
n-Hexanol	−4·83	2·04	0·99	"
n-Heptanol	−4·58	1·95	1·00	"
n-Nonanol	−5·22	2·03	1·00	"

Solutes studied: limited numbers of alcohols in the series methanol to decanol. No attempt made in calculation of b to recalculate to common concentration scale because of lack of detail in original paper.

Solvent	a	b	r	Ref.
Dipalmitoyl lecithin bilayer	1·85	0·93	0·99	Jain & Wray 1978
Egg lecithin bilayer	0·16	1·12	1·00	"
Erythrocyte membranes	2·18	0·95	1·00	"
SDS micelles	−2·99	1·18	1·00	Hoiland et al 1984
Heptane	1·76	0·90	1·00	Lissi 1981
Octane	1·13	0·90	1·00	Goffredi & Liveri 1981; Aveyard & Mitchell 1969
Dodecane	1·16	1·05	2 points:	Manabe et al 1975

myristate lie in the region in which, in comparison with *m*-alkoxyphenols, more water is present in the non-aqueous solvent system than is present at saturation in octan-1-ol. (These conclusions are at variance with those of Leo & Hansch (1971) who found intercept values to be correlated with wetness of solvent system.) Thus liposomes appear, according to these data, to be quite 'wet'. They certainly do not behave like the low dielectric solvents heptane and diethyl ether (which are relatively anhydrous). Furthermore, if liposomes can be regarded as good models of real biological cells then these data imply too that membranes are quite 'wet'. There are, however, no data available to test this conclusion. The ordered bilayers of the biological cell are not entirely mimicked by the multi-lamellar vesicles (MLVs) which were studied in the reports whose results have been used to establish the data in Table 2. MLVs will contain quite large amounts of water (between the lamellae) and hence there may be a series of 'microscopic' partitioning equilibria established. A more sensitive and controlled test would be to investigate the behaviour of bilayer liposomes of uniform size distribution.

The data shown in Table 3 broadly support these conclusions though of course the absolute values of b do indeed reflect the dependence of this parameter upon the nature of both the solute itself and upon the solvent systems chosen for study. These data have been drawn from different publications by different authors who, whilst studying the same solutes, have not controlled all other experimental conditions, so these b values must be regarded with some caution.

The conclusion of the present work however is that it is possible to demonstrate the linearity of the various forms of the Collander equation, that the parameter a in this equation has no fundamental physical significance, but that the b parameter does have physical significance. We therefore await the appearance of reliable data sets to enable testing of the proposal that the b parameter can be used to sort bulk solvent systems into those which, relative to given series of compounds, will adequately represent the particular and dominant membrane solvent properties appropriate for the particular class of solute under study.

There is in addition to the approach outlined above a complementary thermodynamic criterion which may allow selection of solvent systems to represent lipoidal phases. Preliminary results (Beezer et al 1986b) from the microcalorimetric measurement of $\Delta_{trans}H$ (the enthalpy of transfer of a solute from water to some non-aqueous solvent system) for transfer of *m*-alkoxyphenols from water to *Escherichia coli* cells have yielded the results shown in Table 4. Comparison of these data with those for transfer to bulk solvent systems (Table 4) reveals that none of the bulk solvent systems behaves as do the cells. Indeed the negative values for $\Delta_{trans}H$ found for transfer to cells confirms the view that cells behave as if more 'wet' than octan-1-ol but more dry than propylene carbonate; liposomes, too, fall into that range of values.

Table 4. Values of $\Delta_{trans}H$ (kJ mol^{-1}) for transfer of *m*-alkoxyphenols from water to cells, octan-1-ol, heptane and propylene carbonate.

Solute	Cells	Octan-1-ol	Heptane	Propylene carbonate
m-Methoxy	−0·22	−8·03 ± 0·19	20·9 ± 0·6	23·2 ± 0·3
m-Ethoxy	−1·1	−6·95 ± 0·15	19·3 ± 0·9	23·4 ± 0·4
m-Propoxy	−2·02	−6·94 ± 0·14	16·0 ± 0·5	23·9 ± 0·4
m-Butoxy	−4·06	—	13·9 ± 0·5	23·4 ± 0·4
m-Pentoxy	−5·14	—	12·0 ± 0·4	23·2 ± 0·3

As $\Delta_{trans}G$ (the Gibbs function for the transfer process) for transfer to cells is difficult to measure (because of the problem of the definition of equilibrium in the partition-only process), then perhaps the easier-to-measure quantity $\Delta_{trans}H$ will be an alternative parameter to use to evaluate bulk solvents with properties most akin to those of biological membranes (we do not here underestimate the role of $\Delta_{trans}S$ in determining values of $\Delta_{trans}G$).

This analysis has simply assumed that the relative 'wetness' of the non-aqueous solvent is the sole determinant of behaviour; the analysis of Taft et al (1985) makes it clear that this cannot be so. In the absence of further data with which to explore the relationships described in this paper and their bearing upon the Taft analysis, 'wetness' is highlighted as an important feature of the transfer process.

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