Misconceptions and Thermodynamic Untenability of Deviations from pH–Partition Hypothesis

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Abstract □ Elementary thermodynamic principles are applied to the analysis of concepts associated with pH-partitioning of weakly electrolytic solutes across proton-impermeable barriers. Several common misconceptions concerning the permeability of ionized species as well as the postulated occurrence and significance of a "virtual pH" on biomembrane surfaces are demonstrated.

Keyphrases □ pH–partition hypothesis—thermodynamic discussion of deviations and misinterpretations □ Virtual pH—theoretical aspects of relevant thermodynamic principles, misconceptions concerning the pH–partition theory discussed □ Thermodynamics—application to pH–partition theory and concept of virtual pH, misconceptions discussed, equations

The theory of nonionic diffusion, also known as the pH–partition hypothesis, was described in quantitative terms by Jacobs (1) in 1940 and was widely applied by some researchers to explain the distribution and transport of drugs across biological membranes. The principles and results were reviewed by Schanker (2) and, more recently, by Brodie (3).

The purpose of the present report is the application of elementary thermodynamic principles to the clarification of certain points concerning the pH–partitioning of weak electrolytes across biological and other barriers capable of maintaining a pH gradient. It will be demonstrated that:

1. The thermodynamic partitioning equilibrium ratio of total solute concentrations in the bulk phases is identical and independent of whether or not the barrier is permeant to the ionized species. It depends only upon the magnitude of the pH gradient and the ionization constant of the solute; it is also entirely independent of the existence of any "virtual pH" at the surfaces of the barrier which may differ from the pH of their respective bulk phases.
2. Equilibrium pH-partitioning is thermodynamically identical to the Gibbs–Donnan equilibrium.
3. It is invalid to compute estimates of the virtual pH from thermodynamic equilibrium or steady-state partitioning ratios (3) of total concentrations of solute in the bulk phases.
4. Observed deviations of total bulk phase concentration ratios from predicted equilibrium values are thermodynamically untenable, irrespective of the permeability of the barriers to ions and attributable only to the occurrence of nonthermodynamic equilibrium partitioning conditions. This applies even if the bulk phases are each simultaneously maintained in steady states in which no net transfer of total solute occurs across the barrier.
5. The occurrence of a steady-state partitioning condition is inconsistent with an assumption of an equality in the concentrations of the unionized species in the bulk phases; any computations of virtual pH and unionized–ionized species permeability coefficient ratios that at any stage involve equations utilizing this assumption are erroneous.

The ability of both uncharged and ionic solute species to permeate biological barriers is well established with the observation that ionic solutes generally permeate at slower rates. Hydrogen ion, however, is unique in that biomembranes, such as the GI barrier, are capable of maintaining large pH gradients. In this laboratory a similar capability was observed for the maintenance of pH gradients by a synthetic membrane constructed of a highly polar polysalt complex containing approximately 70% water (4, 5) and another described by Levy and Mroczak (6) in which a lipid is definitely the barrier to transport. Kavanau (7) described a mechanism that can be responsible for such observed impermeability of biological and other membranes to hydrogen ions. He considers surfaces, such as those associated with living cells and tissues, to structure water; such water exists in the immediate vicinity of the surface due to hydration of fixed ions and hydrophobic hydration of nonpolar groups. At a distance further from the surface, he postulates that considerable structure breaking of the water occurs. In this region, hydrogen bonding between water molecules is poor relative to ordinary bulk water. This unstructured region is postulated to act as an insulator with regard to proton transfer between the membrane and bulk aqueous phases. Convincing evidence that the apparent lipoidal properties of biological barriers are, in fact, also primarily a consequence of their content of structured water (proteins and lipids are primarily responsible for the structuring) was described by Smolen and Hagman (4), Horowitz and Fenichel (8), and Ling[1] (9).

The obvious pharmaceutical and general biological significance of pH–partitioning with regard to the transfer and distribution of solutes emphasizes the importance of gaining a rigorous understanding of this phenomenon. Three cases will be considered: (*a*) equilibrium pH–partitioning across a barrier permeable to the unionized species of the weakly electrolytic solute (*b*) equilibrium partitioning across a barrier permeable to both unionized and ionized species, and (*c*) steady-state partitioning across a barrier permeable to both species.

THEORETICAL

Irrespective of the manner in which the total concentrations of solute and pH in bulk phases separated by a proton-impermeable membrane are maintained, an experimentally observed or assumed net zero transfer of total solute across the barrier is indicative of either a steady-state or thermodynamic partitioning equilibrium

[1] G. N. Ling, Molecular Biology Laboratory, Pennsylvania Hospital, Philadelphia, Pa., personal communication.

(10); a basis for the distinction between these two possible conditions will be discussed here.

The assumption of a thermodynamic partitioning equilibrium requires an equality of chemical potential, μ_i (or electrochemical potential, $\bar{\mu}_i$, for charged species) for each species in all phases into which it can enter. This includes the two membrane-separated bulk phases, I and II, as well as any interphase or transition phase, T, that may exist at the surfaces or within the membranous barrier, *i.e.*, $\mu_i^{I} = \mu_i^{T} = \mu_i^{II}$. When the barrier is impermeable to the ith species, it can only be stated that $\mu_i^{I} < \mu_i^{T} > \mu_i^{II}$; *i.e.*, the chemical or electrochemical potential of the species in the barrier from which the species is excluded is greater and obviously unequal to that in the two bulk phases. In this case, it is not necessary that $\mu_i^{I} = \mu_i^{II}$. However, if the species is in equilibrium with other solute species to which the barrier is permeant, it is necessary that the chemical potential of the solute treated as a component, c, be equal in every phase into which the species can enter; *i.e.*, $\mu_c^{I} = \mu_c^{T} = \mu_c^{II}$, irrespective of the nature of the intervening barriers through which it passes.

This can readily be exemplified for the weakly acidic electrolyte, $HA \rightleftharpoons A^- + H^+$. For the first case when the barrier is permeant only to HA species, Eq. 1 must be satisfied:

$$\mu_{HA}^{I} = \mu_{HA}^{II} \qquad \text{(Eq. 1)}$$

In each bulk phase, the following ionization equilibrium relations also hold:

$$\mu_{HA}^{I} = \mu_{A^-}^{I} + \mu_{H^+}^{I} \qquad \text{(Eq. 2)}$$

$$\mu_{HA}^{II} = \mu_{A^-}^{II} + \mu_{H^+}^{II} \qquad \text{(Eq. 3)}$$

The chemical potential of the solute treated as a component is the sum of the chemical potentials of the species:

$$\mu_A^{I} = \mu_{HA}^{I} + \mu_{A^-}^{I} + \mu_{H^+}^{I} \qquad \text{(Eq. 4)}$$

$$\mu_A^{II} = \mu_{HA}^{II} + \mu_{A^-}^{II} + \mu_{H^+}^{II} \qquad \text{(Eq. 5)}$$

It follows that:

$$\mu_A^{I} = \mu_A^{II} \qquad \text{(Eq. 6)}$$

By expanding Eq. 6 in terms of the activities [A], [HA], $[H^+]$ and $[A^-]$ and the standard chemical potentials, μ_i^0, it can be rewritten as Eqs. 7 and 8:

$$(\mu_A^0)_I + RT \ln [A]_I = (\mu_A^0)_{II} + RT \ln [A]_{II} \qquad \text{(Eq. 7)}$$

$$\mu_{HA}^0 + \mu_{A^-}^0 + \mu_{H^+}^0 + RT \ln [HA]_I[A^-]_I[H^+]_I = \mu_{HA}^0 + \mu_{A^-}^0 + \mu_{H^+}^0 + RT \ln [HA]_{II}[A^-]_{II}[H^+]_{II} \qquad \text{(Eq. 8)}$$

The often justifiable assumption that the bulk aqueous phases are similar enough in composition that the partition coefficient of each species distributed between them is unity permits the removal of the μ_i^0's from Eq. 8, which can then be rewritten as Eq. 9:

$$\frac{[A^-]_{II}[H^+]_{II}}{[A^-]_I[H^+]_I} = \frac{[HA]_I}{[HA]_{II}} \qquad \text{(Eq. 9)}$$

Setting $[HA]_I = [HA]_{II}$ in effect provides one with the pH–partition hypothesis (1–3, 10); simplifying the treatment by assuming, where convenient, that activities are equivalent to concentrations yields the ratio of the total concentrations of the weak electrolyte equilibrated across a hydrogen-ion and A^--ion-impermeable barrier, as given by the familiar Eqs. 10 and 11. Equation 11 is obtained by substituting $[A^-] = [HA]\,K_a/[H^+]$ into Eq. 10:

$$\frac{C_{I,\,total}}{C_{II,\,total}} = \frac{[HA]_I + [A^-]_I}{[HA]_{II} + [A^-]_{II}} \qquad \text{(Eq. 10)}$$

$$= \frac{(1 + (K_a/[H^+]_I)}{1 + (K_a/[H^+]_{II})} \qquad \text{(Eq. 11)}$$

It will be shown that the form of Eq. 11 is identical for Case (*b*) in which the barrier is assumed permeant to the ionized species, as well as the unionized form. For this case, thermodynamic equilibrium will, in addition to Eqs. 1 and 6, require an equality of electrochemical potentials, $\bar{\mu}_{A^-}$, of A^- in the bulk phases as expressed by Eq. 12(12):

$$\bar{\mu}_{A^-}^{I} = \bar{\mu}_{A^-}^{II} \qquad \text{(Eq. 12)}$$

Expanding each side of Eq. 12 into chemical and electrical contributions yields Eq. 13, in which ψ_I and ψ_{II} are the Galvani potentials of bulk phase I and II, respectively, and F is the Faraday constant:

$$\mu_{A^-}^{0}{}_I + RT \ln [A^-]_I - F(\psi_I) = \mu_{A^-}^{0}{}_{II} + RT \ln [A^-]_{II} - F(\psi_{II}) \qquad \text{(Eq. 13)}$$

Equating standard chemical potentials and rearranging Eq. 13 yield Eq. 14, which is identical to an expression relating the equilibrium distribution ratio of ions (distributed in accordance with an ideal Donnan equilibrium, *i.e.*, when $\Delta\mu_i^0 = 0$) to the Donnan potential, E_D, given by $E_D = \psi_I - \psi_{II}$:

$$\frac{RT}{F} \ln \frac{[A^-]_I}{[A^-]_{II}} = E_D \qquad \text{(Eq. 14)}$$

Use of Eqs. 1–3 provides Eq. 15, which can be expanded and rearranged to obtain Eqs. 16–18 where r is analogous to a Donnan ratio (11):

$$\mu_{A^-}^{I} + \mu_{H^+}^{I} = \mu_{A^-}^{II} + \mu_{H^+}^{II} \qquad \text{(Eq. 15)}$$

$$RT \ln \frac{[A^-]_I}{[A^-]_{II}} = RT \ln \frac{[H^+]_{II}}{[H^+]_I} \qquad \text{(Eq. 16)}$$

$$E_D = RT \ln r \qquad \text{(Eq. 17)}$$

$$r = \frac{[A^-]_I}{[A^-]_{II}} = \frac{[H^+]_{II}}{[H^+]_I} \qquad \text{(Eq. 18)}$$

The resemblance of Eqs. 14–18 for equilibrium pH–partitioning to identical expressions appropriate to the Gibbs–Donnan membrane equilibrium (11) is not fortuitous or unexpected when it is considered that the Gibbs–Donnan equilibrium is generally applicable to describing the distribution of mobile charged species in systems containing immobile, or relatively immobile, charges. In the present case, the immobilization of charge is a consequence of the existence of a pH gradient across a relatively H^+-ion-impermeable barrier. In compliance with this analysis, existence of electrical potentials across the intestine and the movement of anions down an electrochemical potential gradient were reported previously (12, 13).

Using Eq. 18 to express $[A^-]_I = [A^-]_{II}[H^+]_{II}/[H^+]_I$ and substituting this expression into Eq. 10 for $[A^-]_I$ yield Eq. 19:

$$\frac{C_{I,\,total}}{C_{II,\,total}} = \frac{[HA]_I + ([H^+]_I/[H^+]_{II})\,[A^-]_{II}}{[HA]_{II} + [A^-]_{II}} \qquad \text{(Eq. 19)}$$

Substituting $[A^-]_{II} = [HA]_I K_a/[H^+]_{II}$ for $[A^-]_{II}$ in Eq. 19 yields Eq. 11 upon cancellation of $[H^+]_I$ from the numerator and $[HA]_I = [HA]_{II}$ between the numerator and denominator. This result is not unexpected, since the derivation of Eq. 11 in the first place requires no assumption concerning the permeability of the barrier to unionized species.

DISCUSSION

It should be quite apparent from the analysis that the equilibrium total concentration ratio of solute predicted by Eq. 11 must always be realized irrespective of whether or not the barrier is permeable to the ionized species. Conversely, the observation of the predicted ratio does not justify any assumptions concerning the permeability of the barrier to either species.

Attributing deviations from predicted equilibrium total solute distributions to the permeability of the barrier to unionized species or to the virtual pH of the absorbing surface (3, 10) is thermodynamically untenable. It is quite conceivable that a virtual pH at the mucosal surface exists which is different from that of the bulk phase in the lumen. Its origin may, as suggested (3, 10), result from the secretion of H^+ ions by the mucosal cells. In addition, a Donnan accumulation or exclusion of H^+ due to the occurrence of fixed ionic sites on the tissue surface can be operative (14). The sign and density of the surface fixed charge and, therefore, its contribution to the virtual pH may be expected to vary with the bulk phase pH.

However, the implementation of an *in vivo* bioelectrometric method (*e.g.*, 14–16) in our laboratory has revealed the variation of the fixed charge density of the lower two-thirds of the duodenal mucosal surface of the rabbit to be reversible over a pH range of approximately 3.5–10.8 and quite anomalous relative to the observed behavior of less physiologically active surfaces studied previously (14–16). The pH dependency of the fixed charge density was found to be U-shaped, with extremes at pH. 3.5 and 10.8 of −28 and −52 meq./l., respectively, and a minimum at pH 7.0 of +1.7 meq./l. The virtual pH values attributable to the fixed charge for the bulk phase pH values of 3.5, 7.0, and 10.8 were calculated as 3.46, 7.02, and 10.72, respectively. These virtual pH values, as well as a value of 5.89 corresponding to a fixed charge density of 3.0 meq./l. at a bulk phase pH of 5.9, are not significantly different, for an ionic strength of bulk solution corresponding to isotonic 0.15 *M* NaCl, from the pH of the lumen. Based on these results the existence of a postulated virtual pH of 5.3, which is insensitive to the pH of the luminal solution (3, 10), would have to arise from another source such as the secretion of H^+ ions as suggested by Brodie (3) and Hogben *et al.* (10).

That this may, however, not be the case either was suggested by the results of preliminary experiments performed to measure the pH of the *in vivo* intestinal mucosal surface relative to the bulk solution, using a flat bulb pH electrode. Placing the electrode in a pool of isotonic phosphate buffered solution in the bulk phase at a distance from the surface provided a value of pH 7.0. Lowering the electrode against the surface yielded a value of 7.4. Assuming a verity of such measurements in reflecting the surface virtual pH, the relative alkalinity of the surface is contrary to the predictions of the pH-partition hypothesis but is qualitatively consistent with our observed net positive charge density for the mucosal surface at a bulk pH of 7.0. The details of these and further experiments will be presented in a subsequent report.

The above thermodynamic analysis has demonstrated that deviations, other than those attributable to complexation and nonideal solution behavior, in the expected total solute concentration ratio, as given by Eq. 11, are impossible for a true thermodynamic equilibrium state; the observation of a C_{total} ratio predicted by Eq. 11 is a necessary condition for its occurrence. It can be further surmised that the many observed deviations from Eq. 11 (17) must be the conseqeuences of nonthermodynamic equilibrium conditions despite an observed zero transfer of total solute between the bulk phases (10). This latter condition is a special case of nonthermodynamic equilibrium which has been described as a steady state (10). For the thermodynamic equilibrium state, the equality of chemical and electrochemical potentials of all permeant species in all phases requires that the total solute flux across the barrier, J_{TOT}, as well as the flux of each species, J_{A^-} and J_{HA}, are identically zero; *i.e.*, $J_{TOT} = J_{HA} = J_{A^-} = 0$. The steady-state partitioning condition is, according to Brodie (3) and Hogben *et al.* (10), characterized by a zero net flux of total solute, which is in this manner the same as the thermodynamic equilibrium state but differs in that the flux of the individual species is nonzero, equal in magnitude but opposite in direction. The steady-state flux conditions are expressed by Eqs. 20 and 21, which can be inferred from the relations given by Hogben *et al.* (10):

$$J_{TOT} = J_{A^-} + J_{HA} = 0 \qquad \text{(Eq. 20)}$$

$$J_{A^-} = -J_{HA} \neq 0 \qquad \text{(Eq. 21)}$$

It is important to note that the postulation of a steady state in which $J_{A^-} \neq 0$ requires a permeability of the barrier to one or more cations, which must be simultaneously transferred to maintain at all times the necessary condition of electroneutrality (18); *i.e.*, the condition $\Sigma_{i=1}^{n} Z_i J_{C_i^+} - J_{A^-} = 0$, where C_{i^+} and Z_i refer to the cations and their valence, respectively, must be satisfied for a transfer of A^- to occur. If the accompanying cations are other than H^+ (*e.g.*, Na^+, K^+, or organic), a change in the pH of unbuffered bulk phases is to be expected due to shifts in the $HA \rightleftharpoons A^- + H^+$ equilibrium. This phenomenon may contribute to the observed differences in the pH of the inflowing and effluxing fluids of intestinal perfusion streams (10) and pH changes noted in everted sac experiments (19); such observations may be simply interpretable as steady-state manifestations attributable to the relative impenetrability of the barrier to protons.

Equation 11 is derived on the assumption of $[HA]_I = [HA]_{II}$, which is correct under thermodynamic equilibrium conditions; but, as shown above, it is not the case for the steady state. (When HA is the only species present, the steady state and thermodynamic equilibrium conditions of net zero flux are identical.) The use of Eq. 11 to compute a virtual pH for a system in thermodynamic equilibrium is invalid, because the pH variables appearing in Eq. 11 refer only to the bulk phases. In addition, since the postulation of a thermodynamic equilibrium requires adherence to Eq. 11 in the first place, there would be no occasion to compute a virtual pH to account for theoretically nonexistent deviations. It should, therefore, be apparent that Eq. 11 does not apply to a steady state (*i.e.*, unless the steady-state and thermodynamic equilibrium partitioning states are the same) and its use to compute a virtual pH (4) is erroneous. The subsequent use of such virtual pH values to calculate unionized–ionized permeability coefficient ratios, p_u/p_i, propagates the error. This error may be surmised to contribute to the large discrepancy between the p_u/p_i values of 4500 reported by Hogben *et al.* (10) for salicylic acid and the value of 6 more correctly obtained by Nogami and Matsuzawa (19).

SUMMARY AND CONCLUSIONS

In brief, it can be concluded that although the pH-partition hypothesis may often provide a good approximation of the solute transport and distribution behavior of biological barriers and is an excellent rule of thumb, it cannot be considered so seriously as to postulate that biological barriers are generally impermeable to ions and to attribute observed deviations from its predictions to thermodynamically untenable mechanisms which invoke a virtual pH.

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