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DETERMINATION OF IONIC CHARGE BY LIQUID–LIQUID PARTITION

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SUMMARY

The possibility of determining the net charge of ionic substances by studying their partition between two liquid aqueous phases in equilibrium has been investigated. The partition has been influenced by including three different salts in the systems; *viz.*, sodium sulphate, sodium chloride or sodium perchlorate. In aqueous dextran–polyethylene glycol systems, the difference between the logarithms of partition coefficients, determined for systems containing two different salts, is proportional to the net charge of the ionic molecule present at low concentration. This offers a simple way to determine the net charge of the water-solvated ion by liquid–liquid partition. Simple ions and organic acids and bases as well as dyes and proteins have been investigated. Deviations in the net charges, determined by partition, from the expected values are discussed in the light of the known properties of solvated ions.

INTRODUCTION

The partition of molecules between two phases is the basis of all chromatography. For ionic solutes, the two-phase system is either solid–liquid or liquid–liquid. The former type includes ion exchangers or other adsorbents together with water as the liquid phase, and the latter type usually consists of water and an organic solvent. The partition of an ionic compound between the two phases depends not only on the net charge of the molecules, but also on several other factors, *e.g.*, size of the ion and its polarizability. In water-organic solvent systems the ions are only present as free solvated molecular species in the water phase while they form non-charged ion pairs in the organic phase. It is, however, possible to obtain systems where both phases are aqueous[1] and contain 75–98% of water. These two-phase systems are obtained by dissolving two polymers, usually dextran and polyethylene glycol, in water. The partition of ionic compounds in these systems has been shown to depend on the kind of neutral salt present (in excess). The partition coefficient (K) is defined as the ratio between the concentrations of the solute in the upper and lower phases. From both experimental considerations[1] and from direct partitioning experiments[2,3], with proteins at various pH values, it was found that the partition coefficient of an

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ionic compound, in the presence of a neutral salt in excess, can be described by the equation:

$$\log K = \log K_0 + \gamma \cdot Z \tag{1}$$

where Z is the net charge of the partitioned molecules, γ is a factor that depends on the neutral salt used and is directly related to the interfacial potential[1], and K_0 is the partition coefficient obtained with a salt that gives $\gamma = 0$, *i.e.*, a stable zero interfacial potential. As has been shown for a number of proteins[4–6], the K_0 value depends on the kind of protein and the concentration of phase-forming polymers, but is nearly independent of the salt used to influence the partition.

Equation 1 offers a unique possibility for determining the net charge of a molecule directly and without knowledge of its molecular weight. If the partition coefficients of the ion are K_1 and K_2 in two two-phase systems with the same composition with respect to dextran and polyethyleneglycol, but containing different neutral salts, yielding the γ values γ_1 and γ_2 respectively, and assuming that the K_0 values are the same in both cases, eqn. 1 gives

$$Z = \frac{\log K_1 - \log K_2}{\gamma_1 - \gamma_2} \tag{2}$$

Consequently, the net charge should be proportional to the difference between the logarithms of the two partition coefficients of the ion.

MATERIALS AND METHODS

Two-phase systems

The two-phase systems were prepared from 20% (w/w) aqueous solutions of dextran with MW = 500,000 (Pharmacia, Uppsala, Sweden) and polyethylene glycol, with MW = 3000–3700 (Union Carbide, New York, U.S.A.). The systems (5, 10 or 20 g) contained 8% (w/w) dextran, 8% (w/w) polyethylene glycol, 0.5–5 mmol/kg of the substance to be tested, and 125 mmol/kg sodium sulphate, 250 mmol/kg sodium chloride or 250 mmol/kg sodium perchlorate. They were kept in closed test tubes and equilibrated at 25 ± 0.2°C by inverting the tubes 80 times (mixing time 45 sec). The systems were then centrifuged to speed up the settling. Equal volumes were withdrawn from the phases with 2.0- or 0.5-ml constriction pipettes and analysed. Ribonuclease I (ribonucleate 3′-pyrimidinooligonucleotido-hydrolase, EC 3.1.4.22, from bovine pancreas, type I-A, Sigma, St. Louis, MO, U.S.A.) and ovalbumin (grade V, Sigma) were partitioned at various pH values by using a titration method described earlier[7]. The salts used were of analytical grade.

Analytical methods

The H^+ and OH^- were determined by titration with 5 m*M* sodium hydroxide and 10 m*M* hydrochloric acid, respectively, to pH 7.0 by using a Radiometer titrigraph fitted with a glass electrode. In the case of OH^-, where the analytical concentration changed with time, samples of the phases were withdrawn every 10 min and titrated. The concentrations at the time for separation of the phases were found by

extrapolation. Cl^-, Br^-, I^- and SCN^- were titrated potentiometrically with 5 m*M* silver nitrate by using silver and Hg/Hg_2SO_4 electgrodes; Cu^{2+}, Ag^+ and Sn^{2+} were titrated photometrically with 2 m*M* sodium sulphide at 550 nm by using a Zeiss PMQ II spectrophotometer with a cuvette of our own construction, with automatic stirring.

Photometric titration was also used for Fe^{3+}, determined by titration with 1 m*M* potassium ferrocyanide at 620 nm, for $Fe(CN)_6^{4-}$ (titrant: 1 m*M* ferric chloride plus 1 m*M* hydrochloric acidc; 620 nm), and for $Fe(CN)_6^{3-}$ (titrant: 1 m*M* ferrous sulphate plus 1 m*M* sulphuric acid; 620 nm). Pyridine was determined by absorbance measurement at 253 nm after dilution (20×) with 10 m*M* sulphuric acid, chromate and dichromate at 370 nm, and phthalic acid and its salts at 268 nm all after dilution (10×) with 0.2 *M* sodium hydroxide, indigodisulphonate diluted with water (20×) at 610 nm, methyl red diluted with 0.1 *M* hydrochloric acid (100×) at 513 nm, and ribonuclease I and ovalbumin diluted with water (7.7×) at 280 nm. The absorbance was measured against a correspondingly diluted phase from a blank system.

RESULTS

The logarithms of the partition coefficients (log *K*) of the ions studied (together with a few neutral molecules) in two-phase systems containing 0.125 mmol/kg sodium sulphate are reported in Table I together with the changes in the log *K* values observed when this salt is replaced by sodium chloride or sodium perchlorate. The apparent net charges have been calculated via eqn. 2 by setting the charge of H^+ equal to +1.0, which gives $\gamma_1 - \gamma_2 = 0.10$ (1 = $NaClO_4$; 2 = Na_2SO_4) or 0.05 (1 = $NaClO_4$; 2 = NaCl as well as 1 = NaCl; 2 = Na_2SO_4). Halide ions, OH^-, Sn^{2+}, $Fe(CN)_6^{3-}$, pyridinium ion, pyridine, phthalic acid, hydrogen phthalate ion and methyl red (as uncharged zwitterion) give experimental values that differ by only 0.3 charge unit or less from the stoichiometric values. In contrast, the calculated charges of SCN^-, Cu^{2+}, Ag^+ and the charged dyes are larger (0.7–3.0 units) than assumed, while the values are lower than expected for Fe^{3+}, $Fe(CN)_6^{4-}$, $Cr_2O_7^{2-}$, CrO_4^{2-} and phthalate ion (1.2 units or less, with the exception of the hexacyanoferrate ion).

The difference in log *K* values of a protein, comparing systems with different salts, $\Delta \log K$ (Fig. 1), are proportional to the net charge of the protein (*Z*) calculated from their titration curves[8,9] (for lowest salt concentration) and using pI 4.6 for ovalbumin[10]. By using the same charge scale as above (based on partition of solvated protons) the charges on the ribonuclease molecule at various pH values are found to be 80% (sodium chloride–sodium sulphate) and 65% (sodium perchlorate–sodium sulphate), respectively, of the stoichiometric values. For ovalbumin, the corresponding values are 100 and 67%, respectively. For these calculations, the isoelectric points are adjusted so that the straight lines in Fig. 1 go through the origin. This means a shift of 1 unit for ovalbumin and 0.3 unit for ribonuclease.

DISCUSSION

In contrast to most other two-phase systems, the aqueous systems used here allow ionic compounds to exist in both phases as water-solvated ions. This is possible since both phases have high water contents. The system used in this work have phases

TABLE I

PARTITION OF IONS AND NEUTRAL MOLECULES

The two-phase systems contained excess of neutral salt (125 mmol/kg Na_2SO_4, 250 mmol/kg NaCl or 250 mmol/kg $NaClO_4$). Temp. = 25°C. K = partition coefficient.

Partitioned ion or molecule	*Concentration mmol/kg*	*Source*	*Adjusted pH*	*log K with Na_2SO_4*	*Δ log K = log K_1 − log K_2*			*Ionic charge calculated from Δ log K*		
					A: 1 = $NaClO_4$ 2 = NaCl	*B: 1 = NaCl 2 = Na_2SO_4*	*C: 1 = $NaClO_4$ 2 = Na_2SO_4*	*A*	*B*	*C*
H^+	5	HCl	–	0.04	0.05	0.05	0.10	+1.0	+1.0	+1.0
OH^-	5	NaOH	–	−0.84	−0.05	−0.05	−0.10	−1.0	−1.0	−1.0
Cl^-	5	NaCl	7.0	0.04	–	–	−0.10	–	–	−1.0
Br^-	5	KBr	7.0	0.09	–	–	−0.13	–	–	−1.3
I^-	5	KI	7.0	0.15	–	–	−0.13	–	–	−1.3
SCN^-	5	KSCN	7.0	0.18	–	–	−0.17	–	–	−1.7
Cu^{2+}	1	$CuCl_2$	3.0	−0.11	0.15	0.13	0.28	+3.0	+2.6	+2.8
Fe^{3+}	2.5	$FeCl_3$	3.0	−0.02	0.10	0.09	0.19	+2.0	+1.8	+1.9
Sn^{2+}	1	$SnCl_2$	3.0	0.02	0.13	0.06	0.19	+2.6	+1.2	+1.9
$Fe(CN)_6^{3-}$	2.5	$K_3Fe(CN)_6$	6.0	0.30	−0.15	−0.13	−0.28	−3.0	−2.6	−2.8
$Fe(CN)_6^{4-}$	2.5	$K_4Fe(CN)_6$	6.0	−0.09	−0.13	−0.09	−0.22	−2.6	−1.8	−2.2
Pyridinium ion	1	Pyridine	3.5	−0.01	0.05	0.06	0.11	+1.0	+1.2	+1.1
Pyridine	1	Pyridine	7.0	0.10	0.00	0.00	0.00	0	0	0
Ag^+	2	$AgNO_3$	3.0	−0.15	–	–	0.22	–	–	+2.2
$Ag(NH_3)_2^+$	2	$AgNO_3$ NH_3(50 m*M*)	–	−0.05	–	–	0.14	–	–	+1.4
$Cr_2O_7^{2-}$	1	K_2CrO_4	4.0	−0.01	–	–	−0.16	–	–	−1.6
CrO_4^{2-}	1	K_2CrO_4	8.0	−0.16	–	–	−0.14	–	–	−1.4
Phthalic acid	1	Acid	2.0	0.26	–	–	0.00	–	–	0
Hydrogen phthalate ion	1	Potassium salt	4.0	0.11	–	–	−0.08	–	–	−0.8
Phthalate ion	1	Potassium salt	7.0	−0.04	–	–	−0.14	–	–	−1.4
Indigo di-sulphonate ion	0.5	Sodium salt	7.0	0.48	−0.15	−0.25	−0.40	−3.0	−5.0	−4.0
Methyl red ion (−1)	1	Methyl red	7.0	0.25	–	–	−0.17	–	–	−1.7
Methyl red (uncharged)	1	Methyl red	4.0	0.27	–	–	−0.02	–	–	−0.2

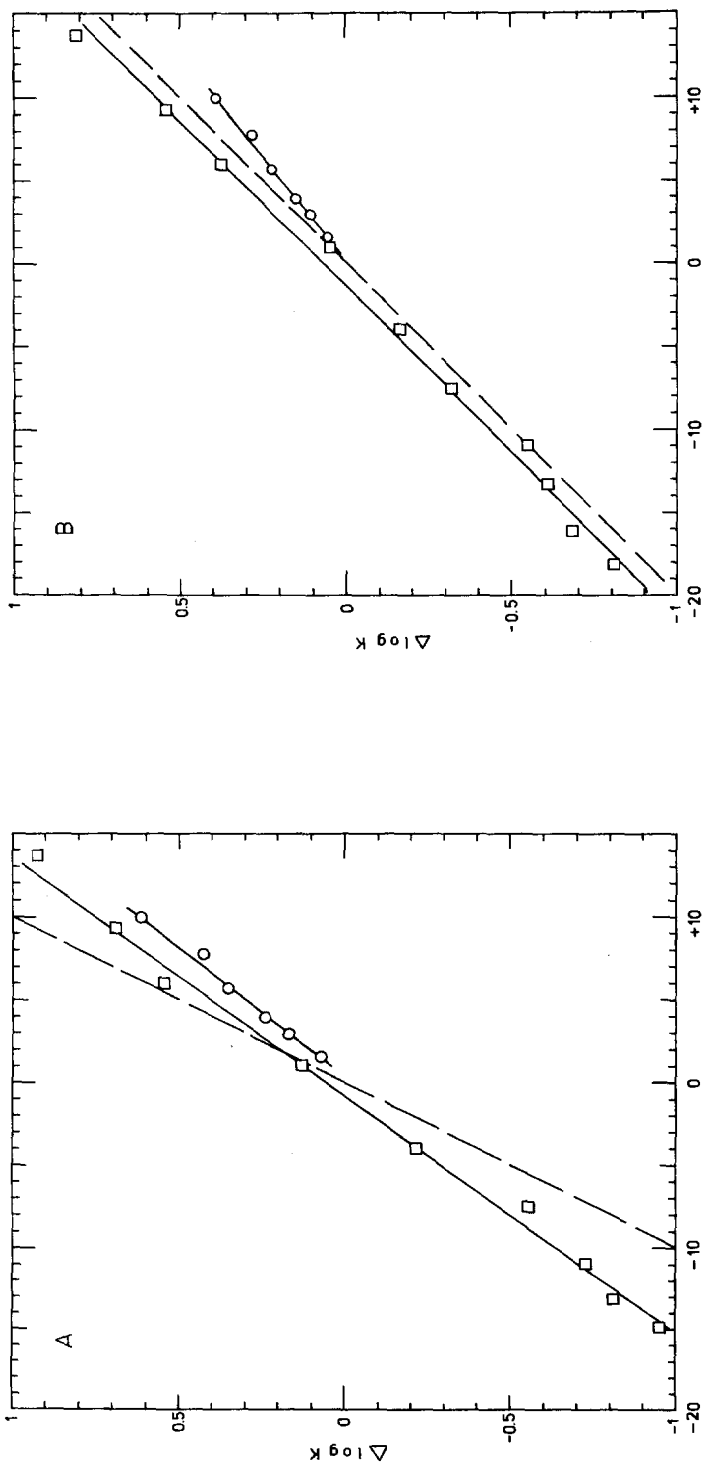

Fig. 1. Difference in the logarithmic partition coefficients, $\Delta \log K$, of protein-comparing systems containing (A) $NaClO_4$, respectively, Na_2SO_4; and (B) NaCl, respectively, Na_2SO_4, as function of the protein net charge (Z) for ribonuclease A (○) and ovalbumin (□). The broken lines indicate the values predicted from the calibration with hydrogen ions as described in the text.

where 88% of the upper phase and 77% of the lower phase is water[1]. The effect of neutral salts on the partition of proteins has been correlated with small differences in the affinity of the cation and anion of the salt for the two phases[2], causing an electric interfacial potential. This potential difference, which was been determined with electrochemical methods[2,11], influences in turn the partition of other ionic components in the system present in low concentrations. The suggested method for determining the net charge of a (water-solvated) ion is based on the fact that the interfacial potential can be changed by substituting one neutral salt for another one. The simple relationship between net charge and partition coefficients, as expressed in eqn. 2, is a consequence of the absence of any solid phase, and thus all kinds of steric restrictions, as occurs with solid ion exchangers, are avoided. With such exchangers, direct binding depends on the availability of the charged groups on the ion exchanger for the solute ions. In the case of large charged ions, *e.g.*, proteins, the availability is limited and depends on the molecular volume. In recent work, Rounds and Regnier[12] showed that indeed the so-called Z term in their model for ion-exchange chromatography of proteins gives the number of charges on the molecule interacting with the ionic adsorbent and is not to be mistaken for the net charge of the molecule. Also, unequal distribution of charges over the surface of the binding to such a degree that even proteins with a net charge opposite to the charge of the ion exchanger will not be bound. Such phenomena are neither expected nor seen when ions are partitioned in aqueous two-phase systems.

Half the number of the molecules/ions investigated here were found to have the charge values denoted by their formulae, while the rest diverge from the nominal values. Generally, the magnitude of these deviations is less than one charge unit, with a few exceptions. It is notable that, in all cases, the signs of the charges determined are the same as that assumed. Since both the two phases have high concentrations of water, *viz.*, 88 and 77% (w/w), it may be assumed that the ions have the same properties (charge, solvation, etc.) as in aqueous solution.

The deviations from the nominal values of the ionic charge are probably due to the presence, in solution, in two or more ionic species in which the tested ion can be a part. If these solute species differ in charge, the Δ log K value will be a measure of the mean of their charges.

The ion may also form complexes with other ionic components present. Such complex formation may occur with ions of one of the neutral salts and not the other ones. In this case, eqn. 2 does not hold since the ions actually partitioned are not the same when the salt is changed. This explains the abnormal value obtained for Ag^+. This ion forms complexes with sulphate while it probably is not as much affected by perchlorate. In the presence of ammonia (50 mmol/kg) in the system the partitioned ion is $[Ag(NH_3)_2]^+$, which is stronger than the sulphato complex[14,15]. Further interactions may be involved in that the apparent charge is found to be $+1.4$.

The negative divalent ions, as well as the tetravalent hexacyanoferrate(II) ion may interact with the sodium ions present in relatively high concentration (0.25 M), which would explain the low charge values obtained. Interaction with counter ions also explains diminution of protein net charge.

The solvated ions can, in some cases, exchange protons with the surrounding and thereby change their effective charge. Ferric ions and Cu^{2+} are known to have this ampholytic character[16]. These ions can further be connected via oxygen bridges to larger units.

Association between dye molecules in water solutions have been reported[17,18]. The high charge values of indigo disulphonate and methyl red may be due to a partial dimerization. Besides the possible interactions mentioned above, formation of ion pairs may result in experimental partition coefficients, which differ from those of the free ion.

The presented method provides a rapid way to determine the charge value (with sign) of ionic species in aqueous solution. It may be used for studies on variation of charge with pH, as has been demonstrated with proteins, or on the influence of other substances on the charge of an ion. It is significant that the charge of an ion can be determined by this method, without knowledge of its molecular weight being required.

Any suitable pair of neutral salts can be used and pH can be chosen within the interval 2–10. Other concentrations of polymers can also be used, as can be seen from the phase diagrams of dextran–polyethylene glycol–water[1]. The main limitation of this method is that the concentration of the ion studied has to be at most 1/50 of the concentration of neutral salt present in the system[2].

Partition of ions between the water inside and outside a hydrophilic gel shows sometimes similar charge dependence as described here. Data from gel chromatography of small ions on strongly cross-linked gels[19,20] show good correlation between the charges and differences in log K_D (K_D = distribution coefficient) when eluents consisting of aqueous solutions of various salts (in excess) are compared. This relationship can be deduced from the thermodynamic model for gel chromatography presented by Hjertén[21].

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