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PHYSICO-CHEMICAL FACTORS GOVERNING PARTITION BEHAVIOUR OF SOLUTES AND PARTICLES IN AQUEOUS POLYMERIC BIPHASIC SYSTEMS.

II. EFFECT OF IONIC COMPOSITION ON THE HYDRATION PROPERTIES OF THE PHASES

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SUMMARY

The interfacial potential difference between the phases in Dextran 500–poly(ethylene glycol) 6000 and Ficoll 400–Dextran 70 biphasic systems was measured as a function of the ionic composition of the systems. The results obtained are compared to those on the partitioning of dinitrophenyl amino acids and human red cells obtained previously. In many cases, there is no relation between the partitioning of charged solutes and particles and the potential difference measurable in the biphasic system. It is concluded that the distribution potential measurable in a given biphasic system as well as the ionic strength of the system generally cannot be used as a feature of the system generally important for the partitioning of solutes and particles in such systems. Two main features of the phases are assumed to be important for the partition process: (i) the hydrophobic properties of the phases and (ii) the hydration properties of the phases. An approach to estimate the latter is proposed.

INTRODUCTION

In Part I[1] it was shown that the hydrophobic properties of the phases of a given aqueous polymeric biphasic system are not the only factor governing partitioning of solutes in the system. This may be attributed to the complex nature of interactions affecting the state of a solute or particle in the polar environment created by each aqueous phase of the polymeric biphasic system. It is well known[2] that the results obtained in measurements of the solid–liquid interfacial free energy depend on the liquid used —pure hydrocarbons reflect only dispersive, non-polar van der Waals interactions, while polar liquids such as water reflect both polar and non-polar interactions occurring at the interface. The partition coefficient of cells in the dextran–poly(ethylene glycol) (PEG) biphasic systems is directly related to the surface energies of the cells, as determined from the contact angles of each phase with the cell layers[2–4]. Therefore it seems possible to suggest that the partition coefficient of a solute

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molecule or particle bearing polar or charged groups is governed at least by two features of the biphasic system: (i) the hydrophobic properties of the phases[1] and (ii) the hydration properties of the phases. The latter feature represents the ability of water to solve polar and ionogenic groups of the solute molecule or particle surface.

The electrostatic potential difference between the phases of aqueous polymeric biphasic systems in the presence of salts is known[5–9] to be due to an unequal distribution of the various cations and anions between the phases. This type of interfacial electrostatic potential is called the distribution potential[10] and is derived from the difference in the hydration energies of the ions taking part in the distribution equilibrium[10–12]. Hence, this potential value in a given aqueous polymeric biphasic system seems to be a measure (in a rather limited way) of the relative hydration properties of the phases.

In this paper we report the results of a study of the effect of ionic composition on the electrostatic interfacial potential in dextran–PEG and Ficoll–dextran biphasic systems, and compare them with those obtained for the partitioning of dinitrophenyl (DNP)-amino acids in Part I[1].

MATERIALS AND METHODS

Biphasic systems

The polymer samples were the same as described previously[1]. Two aqueous polymeric biphasic systems were prepared having the following compositions: 7% (w/w) Dextran 500, 4.4% (w/w) PEG 6000 containing NaCl and sodium phosphate buffer as indicated below; 12.5% (w/w) Ficoll 400, 10.8% (w/w) Dextran 70 containing NaCl and sodium phosphate buffer as indicated below.

Measurement of interfacial potential

The difference in interfacial potential was measured with a Radelkis OP-208 pH-meter used as a mV-meter as described in ref. 6. The electrodes were Pasteur pipettes filled with 7.5% polyacrylamide gel (PAAG) containing a saturated aqueous solution of KCl. One side of the PAAG bridge was connected to a standard silver–silver chloride electrode (Radelkis, Model OP-8083), the other was exposed to one of the phases. All measurements were made at 25°C and all the biphasic systems examined were allowed to settle for 24 h. The changes in potential caused by transferring either electrode from one phase to another were determined. The measurements were performed twelve to fifteen times in each biphasic system. Two or three separately prepared systems of the same polymeric and ionic compositions were examined. The potential difference values agreed usually to within 0.2 mV and mean values were calculated.

Partition experiments

Some additional partition experiments with DNP-amino acids having aliphatic side chains were carried out as described previously[1].

RESULTS

The results shown in Fig. 1 indicate that, on replacing the sodium phosphate

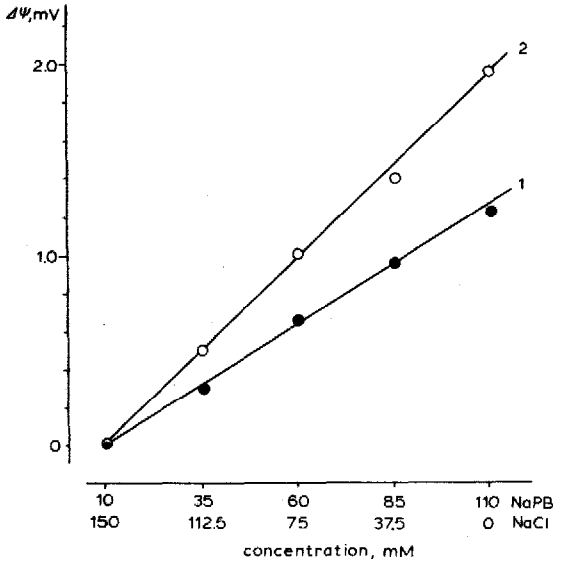

Fig. 1. Relation between the interfacial potential difference, $\Delta\Psi$, between the phases and the ionic composition of Ficoll–dextran, pH 7.4 (1), and dextran–PEG, pH 6.8 (2), biphasic systems. NaPB = sodium phosphate buffer.

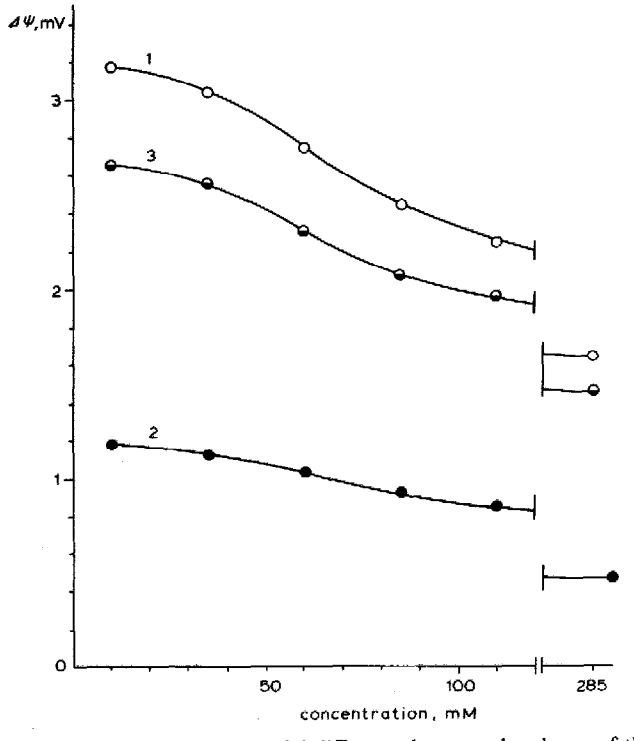

Fig. 2. The interfacial potential difference between the phases of the dextran–PEG biphasic system as a function of the concentrations of Na_2HPO_4 (1), NaH_2PO_4 (2) and sodium phosphate buffer, pH 6.8 (3).

buffer with NaCl over the concentration range 0.11 *M* buffer to 0.15 *M* NaCl in 0.01 *M* buffer, the interfacial potential difference decreases virtually to zero in both dextran–PEG and Ficoll–dextran biphasic systems. It should be noted that the Ficoll-rich and PEG-rich phases are positively charged relative to the corresponding Dextran 70-rich and Dextran-500-rich phases. Also that the interfacial potential appears to be zero in both polymeric biphasic systems containing 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer regardless of the $HPO_4^{2-}/H_2PO_4^-$ concentration ratio.

Fig. 2 indicates that the potential difference depends on the type and concentration of salt present in the biphasic system. It should be noted that the interfacial potential measured in the presence of two salts is not the sum of the corresponding values measured in the presence of each salt separately. The potential difference between the phases of the dextran–PEG system containing the following salts at the same concentration increases in the order: NaH_2PO_4 ≪ sodium phosphate buffer (pH 6.8) < Na_2HPO_4.

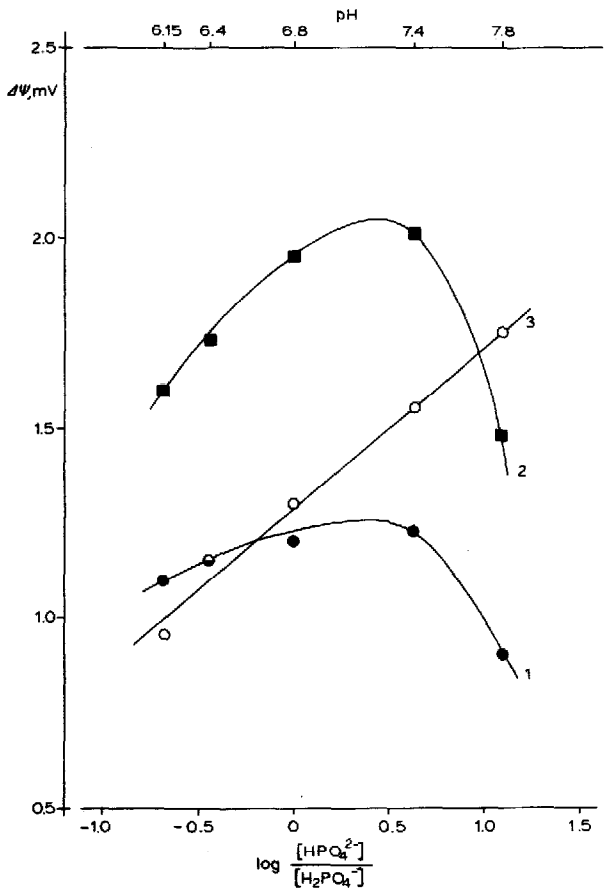

Fig. 3. The interfacial potential difference between the phases as a function of the phosphate ions' concentration ratio in the biphasic systems. 1. Ficoll–dextran (0.11 *M* sodium phosphate buffer); 2. dextran–PEG (0.11 *M* sodium phosphate buffer); 3. Ficoll–dextran (sodium phosphate buffer of various concentrations but at constant ionic strength of 0.165 *M*).

The results given in Fig. 3 show that the interfacial potential in both polymeric biphasic systems examined depends on the $HPO_4^{2-}/H_2PO_4^-$ concentration ratio in 0.11 *M* sodium phosphate buffer in the funnel-shaped manner. As the above measurements were performed under varied ionic strength of the system, the interfacial potential was then measured in the Ficoll–dextran system in the presence of different buffer concentrations but at constant ionic strength (0.165 *M*). Curve 3 in Fig. 3 shows that the potential is now linearly dependent on the $HPO_4^{2-}/H_2PO_4^-$ ratio.

From the results in Figs. 2 and 3, an increase in the concentration of sodium phosphate buffer reduces the interfacial potential difference between the phases in both polymeric biphasic systems studied. This effect appears to intensify with increasing $HPO_4^{2-}/H_2PO_4^-$ concentration ratio. Ballard *et al.*[9] reported a maximum in the potential difference in dextran–PEG biphasic systems in the presence of 0.022 *M* sodium phosphate buffer, pH 6.8. This difference in results may be due to differences in the polymer samples used.

An increase of the NaCl concentration in the dextran–PEG biphasic system containing 0.01 *M* sodium phosphate buffer reduces the potential difference as shown in Fig. 4. However, addition of 0.15 *M* NaCl to the system containing 0.11 *M* sodium phosphate buffer, pH 6.8, only reduces the potential difference from 1.95 mV to 0.79 mV. Similar observations have been made by Ballard *et al.*[9].

One of the aims of this work was to compare the interfacial potentials with the partition coefficients of solutes in aqueous polymeric biphasic systems of different ionic compositions. Hence, the corrected partition coefficients of DNP-glycine at dif-

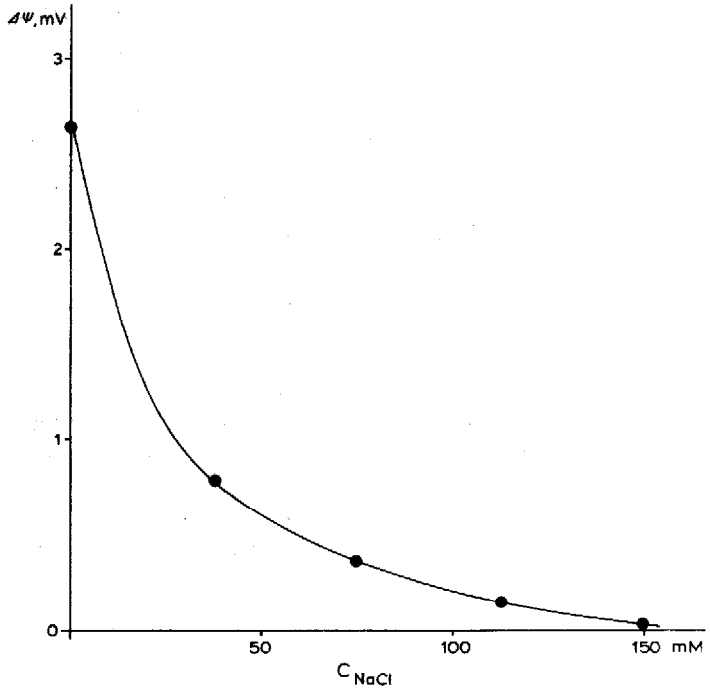

Fig. 4. The interfacial potential difference between the phases of the dextran–PEG biphasic system containing 0.01 *M* sodium phosphate buffer as a function of the concentration of NaCl.

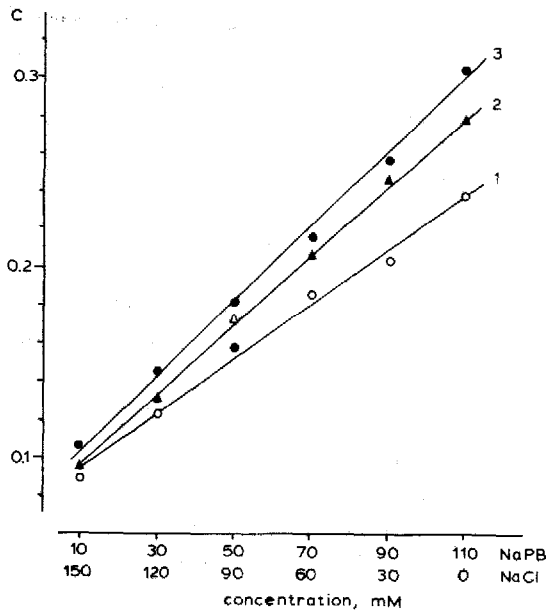

Fig. 5. Parameter C in the Ficoll–dextran biphasic system as a function of the NaCl/sodium phosphate concentration ratio, pH = 6.4 (1), 7.4 (2) and 7.8 (3).

ferent pH values in the Ficoll–dextran biphasic system obtained in Part I[1] are presented in Fig. 5 as a function of the NaCl/sodium phosphate buffer concentration ratio (the salt concentrations are related according to: $C_{buffer} = 0.11\ M - 0.67 \cdot C_{NaCl}$, where C_{buffer} and C_{NaCl} are the concentrations of the buffer and NaCl respectively). Similar relationships, parallel to those in Fig. 5, were obtained for all the DNP-amino acids examined[1].

The typical relationships between the partition coefficients of some DNP-amino acids and the concentration of NaCl in the dextran–PEG biphasic system containing 0.01 *M* sodium phosphate buffer are shown in Fig. 6. The same type of relationships has been observed by us for sodium alkyl sulphates in the absence of sodium phosphate buffer[13].

The corrected partition coefficient of DNP-glycine in the Ficoll–dextran biphasic system containing 0.11 *M* sodium phosphate buffer is presented in Fig. 7 as a function of the $HPO_4^{2-}/H_2PO_4^-$ concentration ratio. It should be noted that the partition behaviour of all the DNP-amino acids examined in this biphasic system at the same ionic strength (0.165 *M*) under various concentration of the phosphate buffer was found to be independent of the $HPO_4^{2-}/H_2PO_4^-$ concentration ratio.

The partition coefficient of DNP-glycine in both polymeric biphasic systems studied depends upon the concentration of sodium phosphate buffer as shown in Fig. 8.

DISCUSSION

The results obtained imply that the partition process in the aqueous polymeric

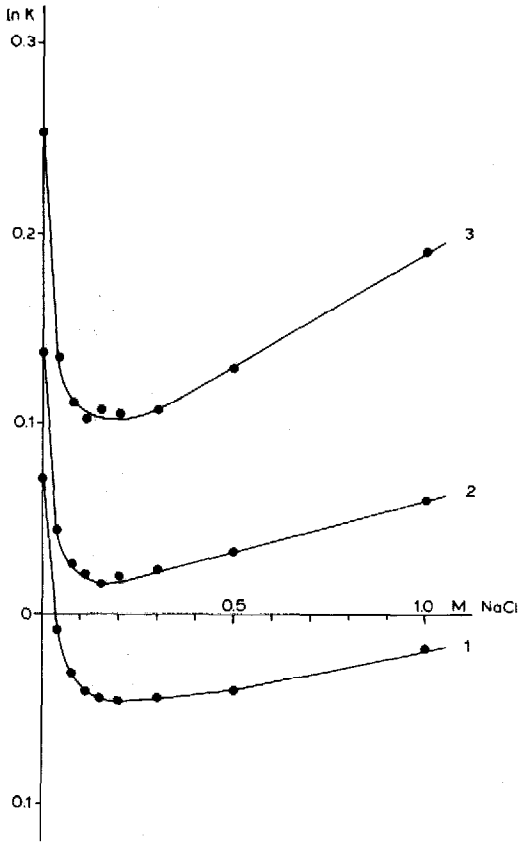

Fig. 6. Partition coefficients of DNP-amino acids as a function of concentration of NaCl in the dextran–PEG biphasic system containing 0.01 *M* sodium phosphate buffer, pH 6.8. 1, DNP-glycine; 2, DNP-norvaline; 3, DNP-2-amino-*n*-octanoic acid.

biphasic systems examined depends upon many variables. The primary independent variables capable of influencing the properties of the phases appear to be the concentrations of the sodium phosphate buffer and NaCl, and the $HPO_4^{2-}/H_2PO_4^-$ concentration ratio. Secondary variables derived from these, but presumably capable of separate effects on the properties of the phases, seem to be the NaCl/sodium phosphate buffer concentration ratio and the ionic strength of the medium.

It has been shown in Part I[1] that in order to examine the partition behaviour of a solute or particles the hydrophobic properties of the phases should be taken into consideration. The hydrophobic properties of the phases of the Ficoll–dextran biphasic system containing NaCl and sodium phosphate buffer were found to be constant over the range of salt concentrations used[1]. Therefore the relationship between the partition coefficient of a solute and the NaCl/sodium phosphate buffer concentration ratio is described by the equation[14–17]

$$\ln K = A + BI \qquad (1)$$

where I is the ionic strength of the medium, A and B are constants. It should be

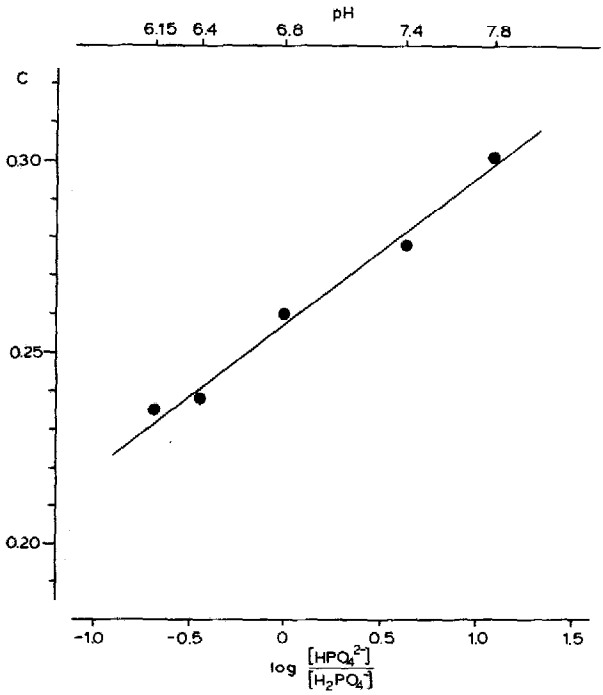

Fig. 7. The Parameter C in the Ficoll–dextran biphasic system containing 0.11 M sodium phosphate buffer as a function of the phosphate ions' concentration ratio.

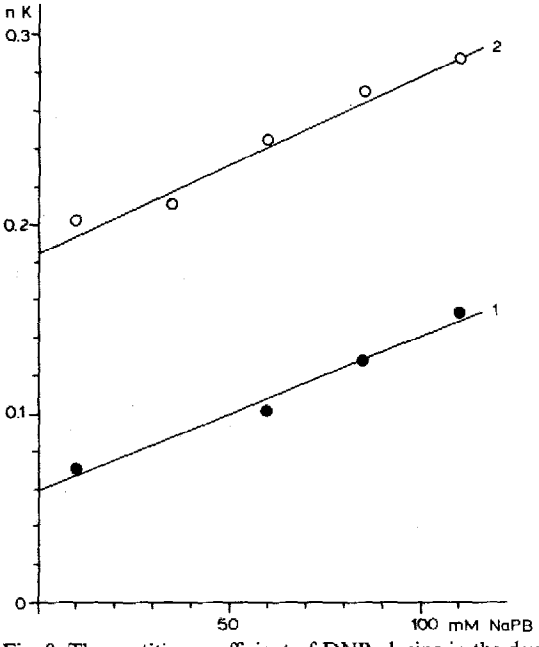

Fig. 8. The partition coefficient of DNP-glycine in the dextran–PEG, pH 6.8 (1), and Ficoll–dextran, pH 7.4 (2), biphasic systems as a function of the concentration of sodium phosphate buffer.

emphasized that I is used here as a quantitative index of the ionic composition of the biphasic system under the conditions specified.

However, conditions can be created under which the replacement of sodium-phosphate buffer with NaCl over the concentration range used is not followed by an alteration of the ionic strength of the medium, *e.g.*, in the system containing 0.11 *M* sodium phosphate buffer, pH 6.4, ionic strength 0.165 *M*. It can be seen from Fig. 5 (curve 1) that the change of the ionic composition under the above conditions is followed by an alteration in the solute partition coefficient. This implies that generally it is inadequate to use the ionic strength of the system as an index of the ionic composition. In some cases the effect of the ionic strength on solute partition behaviour is obvious. An increase of the concentration of sodium phosphate buffer accompanied by an increase of the ionic strength of the medium increases the partition coefficients of DNP-amino acids as shown in Fig. 8. The ionic strength of 0.11 *M* sodium phosphate buffer is changed when the $HPO_4^{2-}/H_2PO_4^-$ concentration ratio is varied. From Fig. 7, the partition coefficients of DNP-amino acids increase with increasing ionic strength of the Ficoll–dextran biphasic system under the conditions specified. It should be noted that the interfacial potential difference between the phases of the system decreases under the same conditions (see Fig. 3).

If the ionic strength of the buffer is kept constant at various $HPO_4^{2-}/H_2PO_4^-$ concentration ratios (by corresponding variation of the buffer concentration) the partition behaviour of a given DNP-amino acid appears to be invariable. The difference in the relative hydrophobicities of the two phases of the system under these conditions is constant, while the interfacial potential difference changes markedly (Fig. 3, curve 3). It is of particular note that most of the data obtained here indicate that there is no correlation between the variations of the solute partition coefficients and the alterations of the interfacial potential difference caused by the same variations of the ionic composition of the system. This seems to imply that the potential difference should generally not be used as an index of the effect of the ionic composition of the system on solute partition behaviour. Figs. 2 and 8 and Figs. 3 and 7 indicate that partitioning of DNP-amino acids in both dextran–PEG and Ficoll–dextran biphasic systems at various ionic compositions is unrelated to the interfacial potential difference between the phases of these systems.

It should be noted that the presence of the interfacial potential difference between the phases also cannot explain the observed variations in the partitioning of particles in the biphasic systems. From Fig. 1 at the same NaCl/sodium phosphate buffer concentration ratio, the potential difference in the dextran–PEG system (pH 6.8) exceeds that in the Ficoll–dextran system (pH 7.4). However, the quantity of human erythrocytes in the Ficoll-rich phase (96 %) is greater than (55 %) in the PEG-rich phase in the presence of 0.11 *M* sodium phosphate buffer[8,18]. Previous results[18], obtained by us on the partitioning of neuraminidase-treated human red cells particularly indicate that the partition behaviour of the cells in a Ficoll–dextran system containing 0.0452 *M* sodium phosphate buffer, pH 7.4, and 0.0972 *M* NaCl is not in agreement with the theory[5–9] of the electrostatic effect of the interfacial potential difference on the partitioning of charged particles in the biphasic systems under consideration.

The interfacial potential difference measurable in a given biphasic system in accordance with the definition of the distribution potential[10–12] represents the difference in the hydration energies of the ions taking part in the distribution equilib-

rium and, hence, seems to represent (although in a rather limited way) the difference in the relative hydration abilities of the two phases of the system. The potential difference, however, does not yield the difference in the hydration energies of a given polar or ionogenic group of the solute molecule or particle being partitioned in the biphasic system. It is possible that in the presence of an interfacial potential difference between the phases there is no difference in the hydration energies of a given ionogenic group, and the latter may be significant in a system having zero potential difference between the phases. We conclude that the difference in the hydration properties of the phases of a given biphasic system should be estimated and taken into consideration.

It seems possible to approach this problem by using the logarithm of the partition coefficient of some simple compound bearing only one polar or ionizable group as a semiquantitative measure of the above hydration difference. As the most common groups of the type required include –COOH, $-COO^-$, $-NH_2$, $-NH_3^+$ and –OH, DNP-glycine was our choice. It should be noted that we do not believe that this choice is the best, only that it seems to be equivalent to any other available and was preferred because DNP-glycine is used by us as reference for calibration of the hydrophobic properties of the systems. As the comparison of partitioning results obtained under different conditions requires an allowance for the hydrophobic properties of the phases, it seems reasonable to use the ratio ln K/E instead of the ln K value.

Thus, we suggest that the relative hydration properties of the phases of a given biphasic system be estimated in terms of the C/E values. (Parameter C corresponds to the logarithm of the partition coefficient of DNP-glycine as indicated in ref. 1). As it has been shown[1] that the C/E value in the Ficoll–dextran biphasic system containing NaCl and sodium phosphate buffer changes with the ionic composition in accordance with eqn. 1, it is clear that the type of the relationships described by eqn. 1 would not change. An application of this approach to the interpretation of partition results will be presented in Part III.

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