Kinetic and Thermodynamic Aspects of *In Vitro* Interphase Transfer of Sulfonamides II: Influence of Interface Composition on Transfer of Unionized Sulfonamides

G. E. SCHUMACHER[x] and JANARDAN B. NAGWEKAR

Abstract □ The influence of interface composition on the kinetic and thermodynamic aspects of the interphase transfer of unionized sulfamethazine was studied in a two-phase *in vitro* system composed of an aqueous pH 4.3 buffer and various alcohol phases. By applying the theory of absolute reaction rates to the interphase transfer process, variations in the ΔH^* and ΔS^* values for the various two-phase systems were considered to be the results of differences in the escaping tendency of the alcohols into the interface and the influence of this parameter on the degree of hydrogen bonding and hydrophobic interaction between solute and solvate molecules in the various systems. The influence of the solubility parameter of the alcohol phase on the interphase transfer process was also examined. Based upon the observed kinetic and thermodynamic parameters, possible mechanisms for the interphase transfer of unionized sulfamethazine were proposed.

Keyphrases □ Sulfonamides (sulfamethazine)—effect of interface composition on interphase transfer, water-alcohol systems □ Transfer of unionized sulfonamides (sulfamethazine)—effect of interface composition on interphase transfer □ Thermodynamic parameters—interphase transfer of unionized sulfonamides (sulfamethazine), water-alcohol systems

In a previous paper (1), a scheme was proposed for the interphase transfer (water ↔ *n*-octanol) of three sulfonamides (sulfadiazine, sulfamerazine, and sulfamethazine) from the data obtained in terms of the thermodynamic parameters of activation. This scheme incorporated the concept of the formation, at the interface, of an "activated complex" composed of a partially desolvated solute molecule and solvate molecules. Basic to the postulation of the mechanism for formation of the activated complex is the concept of the escaping tendency of solvate molecules into the interface. Therefore, in support of this concept, the influence of interface composition on the kinetic and thermodynamic parameters for the interphase transfer of unionized sulfamethazine was examined. In addition, the influence of the concept of the solubility parameter on interphase transfer was studied.

EXPERIMENTAL

The apparatus used, the method of performing the diffusion experiments, and the procedure for determining the partition coefficients in the various two-phase systems were discussed previously (1).

Materials—Sulfamethazine[1] was specially synthesized commercially, purified, and analyzed by differential scanning calorimetry. The following reagents were also used in the highest quality obtainable: *n*-octanol, *n*-octane, *n*-heptyl alcohol, *n*-heptane, *n*-amyl alcohol, *n*-pentane, acetic acid, potassium chloride, and hydrochloric acid.

Determination of Interfacial Tension—The interfacial tension of the various aqueous-organic systems at 27° was measured with a tensiometer[2], according to the procedure recommended by the manufacturer.

Constituents of Aqueous and Alcohol Phases—The constituents of the aqueous and alcohol phases used are summarized in Tables I and II. A pH 4.3 acetate buffer[3], adjusted to an ionic strength of 0.15 to approximate the value of the fluids of the GI tract (2), was used for the aqueous phase because, as explained in the previous study (1), sulfamethazine was more than 99% in the unionized form at this pH.

RESULTS

Partition Coefficients of Sulfamethazine—The apparent partition coefficients of the unionized form of sulfamethazine in the various pH 4.3 acetate buffer-organic systems are summarized in Table III. Since the partition coefficient has long been recognized as a significant factor in membrane penetration and interphase transfer (3–8), and since it was an important parameter for interpreting previous results (1), this property was expected to play a role in the interpretation of the experimental interphase transfer data.

Interfacial Tension of Various Solvents—The interfacial tensions of the various solvents are summarized in Table IV. Since interfacial tension has been implicated as a possible factor in the transfer of drugs across an aqueous-membrane interface (10–12), this parameter of the various two-phase systems was determined as a potential aid in the interpretation of the interphase transfer data.

Kinetic and Thermodynamic Data—The kinetic scheme for the reversible interphase transfer of sulfamethazine in the various two-phase systems, as well as the methods for obtaining the kinetic and thermodynamic data, were discussed in the previous paper (1).

A summary of the kinetic and thermodynamic parameters at 36° for the interphase transfer of sulfamethazine in the various two-phase systems is given in Table V. Arrhenius plots for the interphase transfers are shown in Figs. 1 and 2.

By using the concept of the partition coefficient energy (1, 3), the difference in thermodynamic parameters of activation for forward minus back transfer (*e.g.*, ΔF_f^* minus ΔF_b^*), termed net thermodynamic parameters (*e.g.*, ΔF), was calculated (Table VI). The relationships between the partition coefficient, rate constants, and net thermodynamic parameters of interphase transfer are given in Eq. 1:

$$K_w^0 = \frac{k_f}{k_b} = e^{\Delta F/RT} = e^{(\Delta H/RT - \Delta S/R)} \qquad \text{(Eq. 1)}$$

DISCUSSION

As shown in Table V, the large positive free energy of activation (ΔF_f^* or ΔF_b^*) and the large negative entropy of activation (ΔS_f^* or ΔS_b^*) suggest that the energy barrier for interphase

[1] Matheson, Coleman and Bell, Norwood, Ohio.

[2] DuNouy model 20, Fisher Scientific Co., Pittsburgh, Pa.

[3] The acetate buffer solution contained 0.02 *M* acetic acid, 0.124 *M* potassium chloride, 0.006 *M* sodium hydroxide, and distilled water.

Table I—Constituents of the Aqueous Phases

Aqueous Phase	Percent (v/v)[a]	Mole Fraction
I. Aqueous buffer	97.80	0.996
Pentyl alcohol	1.65[b]	0.003
Pentane	0.55[b]	0.001
II. Aqueous buffer	99.88	0.99986
Heptyl alcohol	0.11[c]	0.00013
Heptane	0.01[c]	0.00001
III. Aqueous buffer	99.80	0.99975
Heptyl alcohol	0.20[d]	0.00025
IV. Aqueous buffer	99.950	0.999947
Octanol	0.045[e]	0.000047
Octane	0.005[e]	0.000006
V. Aqueous buffer	99.95	0.999947
Octanol	0.05[d]	0.000053

[a] Concentrations of the organic solvents are expressed on the basis of volumetric additions by pipet or buret. [b] The composition of a mixture composed of 3 parts pentyl alcohol and 1 part pentane, which was added to the aqueous buffer to saturate the buffer at 25° with respect to the mixture. [c] The composition of a mixture composed of 11 parts heptyl alcohol and 1 part heptane, which was added to the aqueous buffer to saturate the buffer at 25° with respect to the mixture. [d] The composition of alcohol required to saturate the aqueous buffer at 25° with respect to the alcohol. [e] The composition of a mixture composed of 9 parts octanol and 1 part octane, which was added to the aqueous buffer to saturate the buffer at 25° with respect to the mixture.

transfer is great and that a significant constraint is placed upon the solute molecule during interphase transfer. Others (3, 13) observed values of this magnitude in studying the diffusion of inorganic ions across various aqueous–organic interfaces.

In the previous paper (1), a proposal was advanced for the mechanism of interphase transfer of sulfonamides based on the thermodynamic parameters of activation of three sulfonamide homologs and the concept of escaping tendency of solvate molecules. The proposed schemes incorporated the observations of Davies (3), who suggested that an exchange of aqueous and organic solvate molecules occurs during formation of the activated complex, and of Blank (14), who suggested that some form of desolvation of the solute molecule is an important factor during interphase transfer. The schemes for the forward and back transfer of solute across the interface included the formation of an activated complex composed of a partially desolvated solute molecule and solvate molecules. Furthermore, the evidence for a twofold interaction between solute and solvate molecules, including hydrogen bonding and hydrophobic interaction, was discussed. In these schemes (Schemes I and II), as presented previously, S_{sw} and S_{sa} represent the solvated sulfonamide molecule in the aqueous and alcohol phases, respectively: S_{psw} and S_{psa} represent the partially desolvated sulfonamide molecule in the aqueous and alcohol phases, respectively, at the interface; ROH and HOH represent alcohol and water molecules in the interface, respectively; and

Table II—Constituents of the Alcohol Phases

Alcohol Phase	Percent (v/v)	Mole Fraction	Solubility Parameter of Phase at 25°[a]
I. Pentyl alcohol	72.2	0.62	10.51
Pentane	24.0	0.19	
Aqueous buffer	3.8[b]	0.19	
II. Heptyl alcohol	88.7	0.77	10.57
Heptane	9.1	0.08	
Aqueous buffer	2.2[b]	0.15	
III. Heptyl alcohol	96.2	0.77	11.07
Aqueous buffer	3.8[b]	0.23	
IV. Octanol	88.9	0.76	10.34
Octane	8.9	0.08	
Aqueous buffer	2.2[b]	0.16	
V. Octanol	98.0	0.85	10.63
Aqueous buffer	2.0[b]	0.15	

[a] Calculated using the equation of Burrell (20). [b] The concentration required to saturate the alcohol phase at 25° with respect to the aqueous buffer.

Table III—Apparent Partition Coefficients of Unionized Sulfamethazine in the Various Two-Phase Systems at 36°

Alcohol Phase	Apparent Partition Coefficient[a]
Octanol–octane	1.63
Octanol	2.20
Heptyl alcohol–heptane	2.59
Heptyl alcohol	3.00
Pentyl alcohol–pentane	5.12

[a] Expressed in terms of the ratio of solute concentration in alcohol–aqueous pH 4.3 acetate buffer phase, as obtained from interphase transfer experiments.

Table IV—Interfacial Tension of the Solvents at 27°

Alcohol Phase	Interfacial Tension[a], dynes/cm
Octanol–octane	8.7
Octanol	8.2
Heptyl alcohol–heptane	7.8
Heptyl alcohol	7.4
Pentyl alcohol–pentane	4.3

[a] Measured against an aqueous pH 4.3 acetate buffer, both phases being mutually saturated. Values are corrected for Du Nouy error.

[S_{psw}-ROH] and [S_{psa}-HOH] represent the activated complex for the forward and back transfer, respectively, at the interface.

As previously noted, basic to the postulation of the mechanism for formation of the activated complex is the concept of the escaping tendency of solvate molecules. In the case of forward transfer, as described previously, the sulfonamide molecule associates with alcohol molecules. The greater the escaping tendency of the alcohol molecules into the interface, the greater will be the opportunity for association with sulfonamide molecules due to an increase in the number of alcohol molecules per unit volume of interface. Table IV shows that the interfacial tension between the aqueous and alcohol phases decreases as the alcohol chain length decreases. Furthermore, Laiken and Nemethy (15) noted that the free energy for transfer of alcohol molecules from a hydrocarbon to an aqueous phase became more favorable as the chain length decreased. These observations suggest that the escaping tendency of alcohols increases as their hydrocarbon chain length decreases, thus resulting in the increased possibility of interaction with solute. The same general principles may apply for back transfer. The escaping tendency of water molecules into the interface increases as the alcohol chain length decreases.

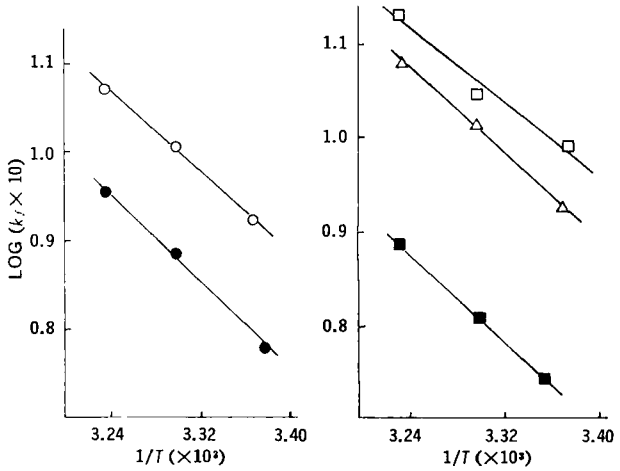

Figure 1—*Arrhenius plots of the transfer of sulfamethazine from an aqueous pH 4.3 buffer to various alcohol phases. Key:* ●, *octanol;* ○, *heptyl alcohol;* ■, *octanol–octane;* △, *heptyl alcohol–heptane; and* □, *pentyl alcohol–pentane.*

Table V—Kinetic and Thermodynamic Parameters of Activation for the Interphase Transfer of Unionized Sulfamethazine in Various Two-Phase Systems at 36°

Alcohol Phase	k, hr^{-1}		ΔH^*, cal/mole		ΔS^*, cal/mole-deg		ΔF^*, cal/mole		Ea, cal/mole	
	k_f	k_b	ΔH_f^*	ΔH_b^*	ΔS_f^*	ΔS_b^*	ΔF_f^*	ΔF_b^*	Ea_f	Ea_b
Octanol–octane	0.769	0.472	4992	3742	−43.0	−48.0	18,273	18,573	5606	4356
Heptyl alcohol–heptane	1.189	0.458	4355	2683	−44.2	−51.5	18,006	18,591	4969	3297
Pentyl alcohol–pentane	1.325	—[a]	3962	—[a]	−45.2	—[a]	17,939	—[a]	4576	—[a]
Octanol	0.896	0.408	5060	1940	−42.4	−54.1	18,174	18,662	5674	2554
Heptyl alcohol	1.187	0.396	4726	1746	−43.0	−54.8	18,007	18,680	5340	2360

[a] Not determined.

Table VI—Kinetic Parameters, Net Thermodynamic Parameters, and Apparent Partition Coefficients for the Interphase Transfer of Unionized Sulfamethazine in a Two-Phase System at 36°

Alcohol Phase	k_f/k_b[a] or K_w[a]	ΔH^b, cal/mole	ΔS^b, cal/mole-deg	$T\,\Delta S^b$, cal/mole	ΔF^b, cal/mole
Octanol–octane	1.63	1250	5.0	1545	−300
Octanol	2.20	3120	11.7	3615	−488
Heptyl alcohol–heptane	2.59	1572	7.3	2256	−585
Heptyl alcohol	3.00	2980	11.8	3646	−673

[a] As defined by Eqs. 6 and 7 of Ref. 1. [b] Net values representing the difference between activation parameters for forward transfer minus back transfer. Values are calculated from the data in Table V.

The data in Table V show that, although the magnitude of the ΔH^* and ΔS^* values for the interphase transfer of sulfamethazine varied with the composition of the alcohol phase, the values for ΔH^* and ΔS^* were always positive and negative, respectively, for both forward and back transfer. The positive ΔH_f^* suggests that the formation of the activated complex is dominated by an endothermic process. As depicted in Scheme I and as already explained, the activated complex formed for the forward transfer of partially desolvated sulfamethazine involved not only hydrogen bonding with alcohol molecules, which is an exothermic process, but also hydrophobic interaction, which is an endothermic process resulting from the release of water molecules (16, 17). Since the resultant ΔH_f^* is positive, this suggests that the positive enthalpy contribution from the hydrophobic interaction dominates the negative enthalpy contribution from hydrogen bonding. The negative ΔS_f^* values suggest that the constraint placed upon the sulfamethazine molecules during the formation of the activated complex for forward transfer, which contributes to a negative entropy, is greater than the positive entropy contribution resulting from the disorder caused by the release of water molecules.

As depicted in Scheme II, the activated complex formed for the back transfer of partially desolvated sulfamethazine involved hydrogen bonding with water molecules (which may result in displacing some alcohol molecules) and the possible formation of icebergs around the nonpolar groups, such as the methyl groups on the pyrimidine moiety of the sulfonamide. The displacement of alcohol molecules is expected to be an endothermic process, while the association of water molecules and iceberg formation is exothermic. The positive ΔH_b^* values suggest that the endothermic process is dominant. Since the alcohol molecules solvate sulfamethazine not only through hydrogen bonding, as do water molecules, but also through considerable induced dipole–induced dipole interaction, this may result in the greater energy required for the displacement of alcohol molecules as compared to the energy gained from the association of water molecules. Because of the smaller size of the water molecules, it is possible that several water molecules solvate the sulfamethazine molecule as compared to the fewer number of alcohol molecules displaced. The negative ΔS_b^* values suggest that the negative entropy of solvation dominated the positive entropy of disorder resulting from the displacement of alcohol molecules.

These considerations may also suggest an explanation for the observation that the ΔH_f^* and ΔS_f^* values recorded in Table V are greater than the corresponding ΔH_b^* and ΔS_b^* values. It is possible that the number of water molecules released in forming the activated complex for forward transfer is greater than the number of alcohol molecules displaced in forming the activated complex for back transfer. Furthermore, it is possible that the association of water molecules during the back-transfer process is greater than the association of alcohol molecules during the forward-transfer process.

Based on these considerations, the variations in the magnitude of the ΔH^* and ΔS^* values (Table V) for the interphase transfer of sulfamethazine in various aqueous–alcohol systems are the result of differences in the escaping tendency of the alcohols into the interface and the influence of this parameter on the degree of hydrogen bonding and hydrophobic interaction between solute and solvate molecules in the various systems. Thus, despite small variations in the mole fraction of alcohol in the various alcohol phases (Table II), the data in Table V suggest that the escaping tendency of alcohol is a primary factor in differentiating the thermodynamic parameters when comparing the interphase transfer of sulfamethazine in systems containing octanol–octane, heptyl alcohol–heptane, and pentyl alcohol–pentane or when comparing systems containing octanol and heptyl alcohol. Since the escaping tendency of alcohol and water molecules into the interface increases as the alcohol chain length decreases, the possibility of interaction between solute and solvate molecules in forming the activated complexes for interphase transfer improves as the chain length decreases. Therefore, it is postulated that the ΔH_f^* values decrease as the alcohol chain length decreases due to the increasingly negative enthalpy contributions arising from the increasing frequency of hydrogen bond interactions between solute and alcohol molecules for a given unit of interfacial region. Contrary to this observation is the possibility that the positive ΔH_f^* contribution might be expected to increase with the increased frequency of solute and solvate interaction due to the liberation of water molecules upon hydrophobic interaction. However, it is postulated that most of the additional alcohol molecules do not interact with the nonpolar methyl groups of the sulfamethazine molecule but rather with the aromatic moieties. This is not expected to result in the release of a significant number of

$$S_{sw} \rightleftharpoons S_{psw}$$
$$S_{psw} + \mathrm{ROH} \rightleftharpoons [S_{psw}\text{-ROH}] \longrightarrow S_{sa}$$

Scheme I—Transfer from water to alcohol

$$S_{sa} \rightleftharpoons S_{psa}$$
$$S_{psa} + \mathrm{HOH} \rightleftharpoons [S_{psa}\text{-HOH}] \longrightarrow S_{sw}$$

Scheme II—Transfer from alcohol to water

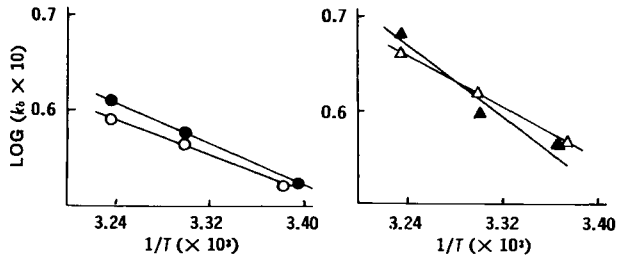

Figure 2—*Arrhenius plots of the transfer of sulfamethazine from various alcohol phases to an aqueous pH 4.3 buffer. Key: ●, octanol; ○, heptyl alcohol; ▲, octanol–octane; and △, heptyl alcohol–heptane.*

water molecules from the partially desolvated sulfonamide molecules because the aromatic moieties do not promote iceberg formation to the same extent as do alkyl groups (17, 18). Thus, this endothermic contribution is considered to be small in comparison to the exothermic interaction resulting from the hydrogen bonding of alcohol molecules. This increase in the number of interactions with decreases in alcohol chain length also results in a decrease in the ΔS_f^* values due to the increasingly negative entropy contribution arising from the constraint placed upon the interacting solute and solvate molecules. This trend is reinforced by a decrease in the opposing ΔS_f^* contribution (resulting from the release of water molecules) as the alcohol chain length decreases.

For back transfer, the escaping tendency of water molecules into the interface increases, and the possibility of interaction between solute and water molecules thus increases, as the alcohol chain length decreases. Therefore, the ΔH_b^* and ΔS_b^* values decrease as a result of the increasingly negative enthalpy and entropy contributions arising from the increasing frequency of solute–water association per unit of interface volume.

By using the data in Table V, it is possible to examine further the role of escaping tendency in the thermodynamics of the transfer process. The interphase transfer characteristics of sulfamethazine may be compared in two-phase systems in which the alcohol phase is composed of either an alcohol and its parent hydrocarbon or the alcohol alone. For this purpose, the data for the octanol–octane and the heptyl alcohol–heptane systems were compared to data obtained for the corresponding octanol and heptyl alcohol systems. In the case of forward transfer, the ΔH_f^* and ΔS_f^* values were greater for systems containing a given alcohol alone than for mixtures of the alcohol and its parent hydrocarbon. For back transfer, the pattern was reversed. The ΔH_b^* and ΔS_b^* values were greater for mixtures of a given alcohol and its parent hydrocarbon than for systems containing the alcohol phase alone.

These observations may be explained in terms of differences in the interface composition of the two types of systems. At the interface, the structural packing of heterogeneous components, especially when composed of molecules with dissimilar intermolecular bonding forces, is considered to be less rigid and coherent than a structure composed of homogeneous molecules (9). Therefore, a film of octanol molecules at the interface, which demonstrates intermolecular hydrogen bonding, is expected to be more coherent and rigid than a film composed of octanol and octane, since the latter demonstrates weaker dipole-induced dipole intermolecular forces. Therefore, based on these considerations, the escaping tendency of alcohol molecules into the interface can be expected to be greater from systems containing mixtures of a given alcohol and its parent hydrocarbon than from systems containing the alcohol alone.

In the case of forward transfer, the possibility of interaction between a partially desolvated sulfonamide molecule and alcohol molecules in forming the activated complex, as in Scheme I, is greater from systems containing the mixtures. This should result in lower ΔH_f^* and ΔS_f^* values for transfer into mixtures than into alcohol phases alone because of the increasingly negative enthalpy and entropy contributions arising from the increasing frequency of association of solute and alcohol molecules for a given unit of interface volume as discussed previously. For back transfer, the escaping tendency of water molecules into the interface is expected to be greater for systems containing a given alcohol alone than for a mixture of the alcohol and its parent hydrocarbon, as suggested by the interfacial tension data (Table IV). Furthermore, since the concentration of alcohol molecules in the interface is also greater for mixtures, the sulfamethazine molecule is expected to be less desolvated of alcohol molecules in mixtures. This should result in the displacement of more alcohol molecules upon formation of the activated complex for back transfer. Thus, the ΔH_b^* and ΔS_b^* values for the back transfer from single-component alcohol phases are expected to be less than for the corresponding phases composed of mixtures, as a result of the more negative enthalpy and entropy contributions arising from the increasing frequency of solute–water interaction in single-component systems as well as the more positive enthalpy and entropy contributions from the displacement of alcohol molecules from the solute in mixtures.

Since the solubility parameter (δ) is an index of the affinity of a solute for a solvent, Khalil and Martin (19) studied the interphase transfer of salicylic acid between water and various alkyl hydrocarbons, alkyl alcohols, and aromatic alcohols. They observed that the forward rate constants for salicylic acid increased as the difference in δ between the solute and solvent decreased. This pattern was not observed in the present study. Three alcohol phases, octanol, heptyl alcohol–heptane, and pentyl alcohol–pentane, were prepared in such a manner that the δ values for the three phases were similar at 25°: 10.63, 10.57, and 10.51, respectively (Table II). Although the range for δ values at 25° is only 1.1%, assuming that the change in δ values is negligible during the interphase transfer experiments, the range for the forward rate constants at 36° for transfer of sulfamethazine into each of the three alcohol phases was 48%, using the k_f value for octanol as a base (Table V). A comparison of the data for transfer into octanol and heptyl alcohol–heptane phases, with values of 10.63 and 10.57, respectively, shows that a 0.5% difference in δ values at 25° yields a 33% variation in k_f values at 36°, again using octanol as a base. These observations suggest that the role of the solubility parameter in interphase transfer is less important than properties of the system, such as the escaping tendency and the thermodynamic parameters associated with the interaction of solute and solvate.

An examination of Table VI corroborates the common observation that there is a relationship between interphase transfer rates and the partition coefficient. Although the net enthalpy and entropy for interphase transfer are positive, the partition coefficients and the ratio of forward to back rate constants (k_f/k_b) are greater than unity because the net entropic energy ($T/\Delta S$) exceeds the net enthalpy, resulting in a preferential transfer of unionized sulfamethazine from aqueous to alcohol phases. Thus, variations in the partition coefficient and the interphase transfer rates are related to variations in the degree of interaction of solute and solvate molecules during formation of the activated complex for forward and back transfer.

As noted previously (1), although the alcohol phases used in the simple model system employed do not simulate the complexity of biological membranes, the intermolecular forces influencing the partitioning of solutes between immiscible phases are expected to be the same forces influencing the permeation of solutes through cell membranes (8). Thus, these results are expected to provide some insight into the mechanisms for the passive transfer of solutes across aqueous–membrane interfaces.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 25, 1973, from the *College of Pharmacy, Wayne State University, Detroit, MI 48202*

Accepted for publication September 24, 1973.

Abstracted in part from a dissertation submitted by G. E. Schumacher to Wayne State University in partial fulfillment of the Doctor of Philosophy degree requirements.

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Measurement of Drug Displacement by Continuous Ultrafiltration

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Abstract □ Adaptation of the continuous ultrafiltration technique to allow precise measurement of the displacement of one ligand by another from binding sites on human serum albumin is described. The displacement of sodium urate and methyl orange by sodium salicylate is demonstrated with analysis of the number of binding sites and association constants for these reactions.

Keyphrases □ Drug displacement from human serum albumin—measurement of sodium urate and methyl orange displacement by sodium salicylate using continuous ultrafiltration □ Human serum albumin binding—displacement of sodium urate and methyl orange by sodium salicylate, measured using continuous ultrafiltration □ Binding of sodium urate and methyl orange to human serum albumin—displaced by sodium salicylate, binding measured by continuous ultrafiltration □ Ultrafiltration, continuous—measurement of sodium urate and methyl orange displacement from human serum albumin by sodium salicylate

Displacement of one substance by another from shared binding sites on the albumin molecule is increasingly being recognized as an important factor modifying expected physiological and pharmacological activity. The introduction of a continuous ultrafiltration technique (1) greatly facilitated the study of ligand–protein interactions, and the present study extends this simple ultrafiltration technique to allow a precise study of drug displacement over a wide range of ligand concentration. The displacement from human serum albumin (albumin) of the ligands, sodium urate and methyl orange, sodium *p*-[(*p'*-dimethylaminophenyl)azo]benzenesulfonic acid, by sodium salicylate was used as a model, but the technique is useful over a wide range of interactions. The only requirement is that an appropriate membrane be available to retain the macromolecule in the ultrafiltration chamber.

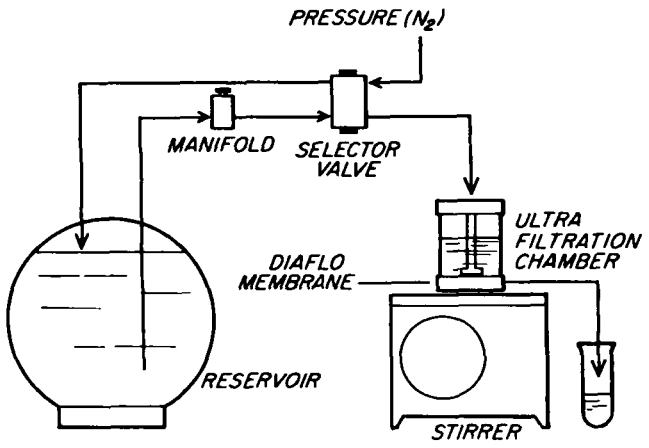

Figure 1—*Experimental setup, continuous filtration.*

EXPERIMENTAL

Crystallized human serum albumin[1] (electrophoretically pure, <0.05 m*M* of free fatty acids per m*M* of albumin) dried to constant weight was used in all studies. Methyl orange[2] was recrystallized from ethanol, dried to constant weight, and dissolved in phosphate buffer at pH 7.4. Sodium urate was made from uric acid triturated with 0.1 *N* NaOH and buffered with phosphate buffer to pH 7.4. The ionic strength of the final solutions was 0.16. Ultrafiltration was performed (Fig. 1) using an ultrafiltration chamber[3] connected through a concentration dialysis selector valve[4] to a nitrogen gas cylinder, which provided pressure for ultrafiltration, and to a 2.4-liter reservoir. A manifold in the line to the reservoir was used as a flow switch. During the experiment, since this was a closed system, the ultrafiltrate leaving the chamber was replaced by an identical volume of solution from the reservoir, with the chamber volume remaining constant. An ultrafiltration membrane[5] with a 10,000 molecular weight cut-off was used. All experiments were done at 22.5°. Methyl orange was

[1] Calbiochem.
[2] British Drug Houses.
[3] Amicon model 65.
[4] Amicon CDS-10.
[5] Diaflo PM10.