JOURNAL OF APPLIED POLYMER SCIENCE VOL. 16, PP. 2123–2130 (1972)

The Dialysis of Non-Electrolytes Through Regenerated Cellulose (Cuprophan). II. The Dialysis of Binary Mixtures

S. BOTTOMLEY, F. M. PARSONS, and P. M. G. BROUGHTON,
Biochemistry Department, Coventry and Warwickshire Hospital, Coventry, Renal Research Unit, The General Infirmary at Leeds, and Department of Chemical Pathology, University of Leeds, England

Synopsis

Solutions containing two organic compounds of different concentrations were dialyzed through Cuprophan using a modified Muir-Ross cell. The dialysis rate of the solute present in a low concentration (hypoconcentrate) was altered by the presence of a large concentration of a second solute (hyperconcentrate). The effect was unrelated to the molecular radius of the hyperconcentrate or, for acids and bases, to the dissociation constant. It was related, though not proportionately, to the solubility (expressed in mole/l.) of the hyperconcentrate. It is postulated that the effect is due to changes in the hydrogen-bonded structure of the water induced by the dissolution of solutes in this solvent.

INTRODUCTION

Most studies of dialysis rate have been made with solutions of single components. However, knowledge of the dialysis rates of the compounds in blood is of fundamental importance with the artificial kidney. Dialysis rates of components of complex mixtures may differ from those in simple aqueous solutions owing to chemical[1,2] or physical[3–5] interactions between the various solutes present. It is difficult to adapt the artificial kidney for in vitro work, whereas the Muir-Ross cell has been shown to be a suitable apparatus for investigating the dialysis of single-component solutions.[6] A study of the dialysis rates of mixtures of two organic compounds was therefore carried out using the Muir-Ross cell.

EXPERIMENTAL

A modified Muir-Ross dialysis cell[7] was employed using the experimental arrangement and procedure described elsewhere.[6,8] Dialysis rate was expressed as the "half-clearance time" ($t_{1/2}$), and its 95% confidence limits were estimated.[6]

The membrane used, Cuprophan (Bemberg A.G., Wuppertal, West Germany), was a regenerated cellulose membrane of dry thickness 10.5–11.5 μm.

The solutes were used in their purest commercially available form. Urea, creatinine, and glucose were determined by standard Technicon Auto-Analyzer procedures (Technicon Instruments Co. Ltd., Basingstoke, Hants). Raffinose was determined by the HCl/alcoholic resorcinol reaction of Kulka.[9]

RESULTS

Dialysis of Raffinose in the Presence of Other Organic Solutes

Mixed solutions of urea and raffinose of varying concentrations were dialyzed against water, and their $t_{1/2}$ values were calculated. The results are given in Table I. In all the experiments, the half-clearance time of urea, $(t_{1/2})_U$, was not altered by the presence of raffinose. However, the raffinose half-clearance time $(t_{1/2})_R$, was decreased, i.e., the sugar dialyzed faster in the presence of urea. The magnitude of the effect, shown by the percentage decrease in $(t_{1/2})_R$ in Table I, depended on the urea concentration. The same decrease in $(t_{1/2})_R$ was obtained with solutions of 2 g/l. raffinose + 4 g/l. urea and with 1 g/l. raffinose + 2 g/l. urea. Similarly, equal decreases in $(t_{1/2})_R$ were obtained in the experiments where ratios of the urea-to-raffinose concentration of 8:2 and 4:1 were used. Thus, equal ratios of the urea-to-raffinose concentration produced the same decrease in $(t_{1/2})_R$.

TABLE I
Dialysis of Mixtures of Urea and Raffinose of Varying Concentrations[a]

Raffinose concn., g/l.	Urea concn., g/l.	Ratio of urea concn. to raffinose concn.	$(t_{1/2})_U$, min	$(t_{1/2})_R$, min	$\Delta(t_{1/2})_R$, %
0	2	—	76.0 ± 5	—	—
2	0	0	—	406 ± 24.5	0
2	2	1	75.2 ± 5	381.5 ± 23	−6.0
2	4	2	78.7 ± 6	368 ± 22	−9.4
2	6	3	74.9 ± 5	351 ± 21	−13.5
2	8	4	77.3 ± 6	350 ± 21	−13.8
2	10	5	75.9 ± 5	334.5 ± 20	−17.6
0	2	—	75.8 ± 5	—	—
1	0	0	—	420 ± 25	0
1	2	2	76.2 ± 5	383.5 ± 23	−8.7
1	4	4	76.5 ± 5	357.8 ± 21.5	−14.8
0	1.25	—	75.9 ± 5	—	—
0.25	0	0	—	425 ± 25.5	0
0.25	1.25	5	76.7 ± 5	350.5 ± 21	−17.5

[a] Experimental conditions were as described in ref. 6. The 95% confidence limits of $(t_{1/2})_R$ and $(t_{1/2})_U$, shown as ± values, were 6% and 6.6%, respectively, and were estimated as outlined in ref. 6.

TABLE II
Dialysis of Mixtures of Creatinine and Raffinose of Varying Concentrations[a]

Raffinose concn., g/l.	Creatinine concn., g/l.	Ratio of creatinine concn. to raffinose concn.	$(t_{1/2})_C$, min	$(t_{1/2})_R$, min	$\Delta(t_{1/2})_R$, %
0	1.76	—	112.3 ± 7		
1	0	0	—	423 ± 25.5	0
1	1.76	1.76	114.0 ± 7	432 ± 26	+2.2
1	5.28	5.28	111.8 ± 7	430 ± 26	+1.6
1	9.42	9.42	116.5 ± 7	453.5 ± 27	+7.2
2.0	0	0	—	423 ± 25.5	0
2.0	8.84	4.42	117.2 ± 7	423 ± 25.5	0
2.0	18.84	9.42	113.4 ± 7	461 ± 28	+9.0

[a] Experimental conditions were as described in ref. 6. The 95% confidence limits of $(t_{1/2})_R$ and $(t_{1/2})_C$, shown as ± values, were both 6% and were estimated as outlined in ref. 6.

TABLE III
Combined Effects of Urea and Creatinine on the Dialysis Rate of Raffinose[a]

Solution dialyzed	Raffinose concn., g/l.	Urea concn., g/l.	Creatinine concn., g/l.	$\Delta(t_{1/2})_R$, %
Raffinose + urea	2.0	10.0	—	−17.6
Raffinose + creatinine	2.0	—	18.84	+9.0
Raffinose + urea + creatinine	2.0	10.0	18.84	−10.0

[a] Experimental conditions were as described in ref. 6.

Similar experiments were carried out with solutions of creatinine and raffinose of varying concentrations. The results, given in Table II, show that the half-clearance time of creatinine $(t_{1/2})_C$ was not altered by the presence of raffinose, whereas $(t_{1/2})_R$ was significantly increased at the highest creatinine concentrations used. The same percentage increases in $(t_{1/2})_R$ were obtained with solutions of 1 g/l. raffinose + 9.42 g/l. creatinine and with 2 g/l. raffinose + 18.84 g/l. creatinine.

Thus, opposite effects were produced by urea and creatinine on $(t_{1/2})_R$. When both compounds were mixed with raffinose in the same concentrations used in mixtures of raffinose + urea and raffinose + creatinine, a decrease of 10% in $(t_{1/2})_R$ was obtained, as shown in Table III. Thus, the opposing effects of the two compounds tended to cancel each other. When mixtures of urea and creatinine of varying concentrations were dialyzed, no changes in $(t_{1/2})_U$ or $(t_{1/2})_C$ were observed.[8] Thus, any chemical interactions between urea and creatinine in a mixture of the two were excluded. It was concluded that the effects of these two solutes on $(t_{1/2})_R$ were due to opposite and reversible interactions between each solute and raffinose.

TABLE IV
Effect of pK, Molecular Radius, and Solubility of Hyperconcentrate on Change Produced in $(t_{1/2})_R$[a]

Hyperconcentrate	pK of hyperconcentrate	Molecular radius hyperconcentrate, nm	Mass solubility of hyperconcentrate at 37.5°C, g/l.	Molar solubility of hyperconcentrate at 37.5°C, mole/l.	$\Delta(t_{1/2})_R$, %
Thiourea	14.96 (pK_b)	0.294	276.0	3.45	−7.8
Acetamide	14.51 (pK_b)	0.274	1333.4	22.6	−16.7
Urea	13.82 (pK_b)	0.258	1575.0	26.25	−17.6
Phenylacetic acid	4.28 (pK_a)	0.352	16.32 (25°C)	0.12 (25°C)	+11.1
Creatinine	4.80 (pK_a)	0.303	87.01 (16°C)	0.77 (16°C)	+7.0
Glutaric acid (1) (2)	4.34 / 5.41 (pK_a)	0.340	990.0	7.5	−25.8

[a] Experimental conditions were as described in ref. 6. Raffinose concentration was 2 g/l. Moles of hyperconcentrate per mole of raffinose was 50:1. Solubility of raffinose at 37.5°C is 445.5 g/l., or 0.75 mole/l. Molecular radii of the hyperconcentrates were obtained from ref. 8, pK values from ref. 10, and solubilities from ref. 11.

Since urea and creatinine have different dissociation constants and solubilities, the effects of these properties on the dialysis rate of raffinose were investigated with other acidic and basic solutes. The stronger solution (e.g., urea, creatinine) was termed the hyperconcentrate, and the weaker one (raffinose), the hypoconcentrate; and in each case, the ratio of their molar concentrations was 50:1. The results, given in Table IV, show that every hyperconcentrate tested either increased or decreased $(t_{1/2})_R$, but the magnitude and direction of the effect were not related to pK. Phenylacetic acid, creatinine, and glutaric acid have similar pK_a values, but whereas the first two solutes produced increases in $(t_{1/2})_R$, glutaric acid gave a decrease in $(t_{1/2})_R$. Thiourea, urea, and acetamide have similar pK_b values and produced decreases in $(t_{1/2})_R$, but of different magnitudes. The changes in $(t_{1/2})_R$ produced by different hyperconcentrates did not appear to be related to their molecular sizes (expressed as radius r). The effect of the hyperconcentrate on $(t_{1/2})_R$ was not related to its mass solubility (expressed in g/l.), but was related, though not proportionately, to its molar solubility (expressed in mole/l.). Those hyperconcentrates with molar solubilities greater than that of raffinose decreased $(t_{1/2})_R$, while those with molar solubilities less than that of the sugar increased $(t_{1/2})_R$.

Dialysis of Glucose in the Presence of Various Hyperconcentrates

Similar investigations were made of the effect of various hyperconcentrates on the half-clearance time of glucose, $(t_{1/2})_G$, using a concentration ratio of 50 moles hyperconcentrate to 1 mole glucose. The results are given in Table V. There was no correlation between the molecular radius

TABLE V
Effect of Various Hyperconcentrates on the Half-Clearance Time of Glucose[a]

Hyperconcentrate	Molecular radius of hyperconcentrate, nm	Mass solubility of hyperconcentrate at 37.5°C, g/l.	Molar solubility of hyperconcentrate at 37.5°C, mole/l.	$\Delta(t_{1/2})_G$, %
Urea	0.258	1575.0	26.25	−8.3
Creatinine	0.303	87.01 (16°C)	0.77 (16°C)	+9.8
Fructose	0.355	549.0	3.05	+9.6
Sucrose	0.449	2308.5	6.75	+10.6
Raffinose	0.563	445.5	0.75	+1.7

[a] Experimental conditions were as described in ref. 6. Glucose concentration was 2 g/l. Moles of hyperconcentrate per mole of glucose was 50:1. The 95% confidence limits of $(t_{1/2})_G$ were ±5.9%. Solubility of glucose at 37.5°C is 1512.0 g/l., or 8.4 mole/l. Molecular radii of the hyperconcentrates were obtained from ref. 8 and solubilities, from ref. 11.

TABLE VI
Effect of Urea on $(t_{1/2})_R$, at Two Different Temperatures[a]

Temp., °C	Molar solubility of urea, mole/l.	Molar solubility of raffinose, mole/l.	Ratio of solubility of urea to solubility of raffinose	$\Delta(t_{1/2})_R$, %
37.5	26.25	0.75	35.0	−17.6
50.0	33.43	1.45	23.0	−17.9

[a] Experimental conditions were as described in ref. 6. Raffinose concentration was 2 g/l., and urea concentration was 10 g/l. Solubilities of urea and raffinose were obtained from ref. 11.

or the mass solubility of the hyperconcentrate and the change produced in $(t_{1/2})_G$. There was no direct correlation between the molar solubility of the hyperconcentrate and the magnitude of the change in $(t_{1/2})_G$. Urea, with a greater molar solubility than glucose, was the only solute which decreased $(t_{1/2})_G$. Raffinose had no significant effect on $(t_{1/2})_G$, while creatinine, fructose, and sucrose produced equal increases in $(t_{1/2})_G$, despite a ninefold difference in their molar solubilities.

A mixture of urea and raffinose was dialyzed at 37.5°C and at 50°C, the solubilities of each solute being different at these temperatures. The results given in Table VI show that the change induced in $(t_{1/2})_R$ by the presence of urea was the same at both temperatures. This confirmed the lack of correlation between the molar solubility of the hyperconcentrate and the magnitude of the change induced in the $t_{1/2}$ of the hypoconcentrate.

DISCUSSION

Franz, Galey, and Van Bruggen[4,5] studied dialysis rates of sugar mixtures. They showed that a hyperconcentrate sugar dialyzing through a

membrane caused a hypoconcentrate sugar, placed in equal concentrations on both sides of the membrane, to dialyze in the same direction. This effect was achieved at zero net osmotic flow of the solvent and was proportional to the concentration and molecular radius of the hyperconcentrate. This phenomenon was ascribed to a physical interaction between the two sugars: as the hyperconcentrate diffused down its concentration gradient, it collided with, and pushed molecules of, the hypoconcentrate through the membrane pores.

This hypothesis could explain the present results where decreases in $(t_{1/2})_R$ and $(t_{1/2})_G$ were obtained in the presence of a hyperconcentrate. It does not explain the increases in $(t_{1/2})_R$ and $(t_{1/2})_G$ produced in some cases (Tables II, IV, and V) or the lack of correlation between the magnitude of the effect and the molecular radius of the hyperconcentrate (Tables IV and V). Thus, another explanation must be sought.

In every experiment it was found that the graphs of log concentration against time, expressing dialysis rate, for both the hypoconcentrate and hyperconcentrate, were single, straight lines. This indicated that the two solutes remained in the same form throughout the experiment and that an irreversible chemical reaction between them did not occur.

It is possible that changes in the structure of the water, both bound and unbound to the hypoconcentrate, are brought about by the presence of the hyperconcentrate. From studies of the changes in the thermodynamic properties of water which occur on the dissolution of solutes in the solvent, a model for water has been proposed.[12] Water is said to consist of at least two forms, one of which is hydrogen-bonded (bulky) water and the other, monomeric (dense) water. The two forms exist in an equilibrium which can be changed by dissolving a second component in the water, the changes being characterized by excess enthalpies and entropies of mixing.[13]

From experiments with carbohydrates, it has been suggested that these solutes can be accommodated in water without much modification of the hydrogen-bonded solvent structure[14] and may even extend the hydrogen-bonded system. However, urea is known to cause a breakdown of hydrogen bonds, possibily by a dilution effect, changing the bulky/dense equilibrium, rather than by direct action on the hydrogen bonds.[12,15]

Thus, it is possible that the addition of urea to solutions of raffinose or glucose will cause a breakdown of the structure of the water, both bound and unbound to the sugar. Since the sugar and urea will probably be closest together for a longer time while in the pores of the membrane, the effect of urea on the water bound to the sugar is likely to be greater within the membrane pores than in bulk solution. Thus, this enhanced interaction between the urea and raffinose molecules appears to be caused by the greater resistance to solute diffusion offered by the water in the membrane pores than by that in bulk solution. This postulate is supported by the finding of Galey and Van Bruggen[5] that the effect of a hyperconcentrate on the dialysis rate of a hypoconcentrate is greater with a smaller membrane pore size. Other effects which may enhance the interaction between the

two solutes while in the membrane pores, such as absorption of either solute onto the membrane, appear to be unlikely. As stated previously, in all the experiments the dialysis plot of each solute in the mixture was a single, straight line, indicating that the solutes did not absorb onto the membrane. Since friction between solute and solvent molecules is greater in the membrane pores than in bulk solution,[16] the effect of urea on the structure of the water not bound to the sugar is also likely to be greatest within the membrane pores. Hence, the hydrated sizes of raffinose and glucose will be smaller in the presence of urea, and the water system in the membrane pores through which the sugars must diffuse will become less "rigid." This will result in an increase in the dialysis rates of the sugars, i.e., a decrease in $(t_{1/2})_R$ and $(t_{1/2})_G$. The effectiveness of urea as a disrupter of water structure increases with concentration[12] and may account for its greater effect on $(t_{1/2})_R$ at higher concentrations. The constancy of $(t_{1/2})_U$ in the presence of raffinose may be due to urea having a greater influence on water structure than raffinose has.

The decreases in $(t_{1/2})_R$ produced by thiourea, acetamide, and glutaric acid can be explained in a similar way to that for urea. These solutes are among the class of compounds listed by Franks[12] as effective breakers of the hydrogen-bonded system present in water.

The effect of adding fructose, sucrose, or raffinose to a glucose solution may be to extend the water structure already enhanced by the presence of glucose. Thus, the water barrier to glucose diffusion in the membrane pores will become more "rigid," and the hydrated size of glucose will increase. Consequently, the dialysis rate of glucose will decrease, i.e. $(t_{1/2})_G$ will increase.

The decrease in the dialysis rate of raffinose brought about by creatinine and phenylacetic acid may be related to their low solubilities in water and their nonpolar character. Dissolution of such substances in water is accompanied by a large decrease in entropy,[12] implying structure formation in the water system.

This hypothesis explains the effects observed in the present experiments. A relationship between the molar solubility of the hyperconcentrate and its effect on the $t_{1/2}$ of the hypoconcentrate was found. This may imply that the forces exerted by a solute on the structure of water may be a factor in determining the solubility of that solute in water.

These results show that the physical interactions between two solutes when dialyzed together, i.e., diffusion of the hypoconcentrate down the concentration gradient of the hyperconcentrate,[8] may not be the only effect taking place. Reversible chemical interactions between the solutes and the solvent may also have to be taken into account. The importance of the interactions proposed here can be further determined by altering the capacity for hydrogen bond formation in the dialysis system. This could be achieved by using substituted derivatives of the solutes already employed, by pH changes, or by varying the composition of the solution with other solvents of different hydrogen-bonding abilities.

References

1. L. C. Craig, *Meth. Enzym.*, **11**, 870 (1967).
2. L. C. Craig, H. C. Chen, and E. J. Harfenist, *Progr. Separ. Purif.*, **2**, 219 (1969).
3. D. F. Grossman, K. F. Kopp, and J. Frey, *Proc. Eur. Dial. Transpl. Assoc.*, **4**, 250 (1967).
4. T. J. Franz, W. R. Galey, and J. T. Van Bruggen, *J. Gen. Physiol.*, **51**, 1 (1968).
5. W. R. Galey and J. T. Van Bruggen, *J. Gen. Physiol.*, **55**, 220 (1970).
6. S. Bottomley, F. M. Parsons, and P. M. G. Broughton, *J. Appl. Polym. Sci.* **16**, 2115 (1972).
7. W. M. Muir and D. S. Ross, *Int. Confer. Med. Biol. Eng.*, **7**, 499 (1967).
8. S. Bottomley, M. Phil. Thesis, University of Leeds, 1971.
9. R. G. Kulka, *Biochem. J.*, **63**, 542 (1956).
10. *Handbook of Chemistry and Physics*, 42nd ed. The Chemical Rubber Publishing Co., Cleveland, 1960–1961.
11. H. Stephen and T. Stephen, Eds., *Solubilities of Inorganic and Organic Compounds*, Vol. 1, Parts 1 and 2, Pergamon Press, Oxford, 1963.
12. F. Franks, in *Proceedings of the Symposium on Equilibria and Reaction Kinetics in Hydrogen-Bonded Solvent Systems*, A. K. Covington and P. Jones, Eds., Taylor and Francis Ltd., London, 1968, pp. 31–47.
13. J. S. Rowlinson, *Liquids and Liquid Mixtures*, Butterworth, London, 1959.
14. D. T. Warner, *Ann. N.Y. Acad. Sci.*, **125**, 605 (1965).
15. J. A. Gordon and W. P. Jencks, *Biochemistry* (N.Y.), **1**, 47 (1963).
16. B. Z. Ginzburg and A. Katchalsky, *J. Gen. Physiol.*, **47**, 403 (1963).

Received October 12, 1971
Revised February 7, 1972