

used to convert viscosities at 24.05° to other temperatures in the vicinity of room temperature, the estimated additional error being about 0.05% for each degree removed from 24.05°.

### Results

Table I lists efflux times, densities, viscosities and  $d \ln \eta / dT$  of several mixtures of DMA and water. Each viscosity is the median of several different measurements. The average magnitude of the percentage deviation for all measurements from their respective medians was 0.067%.

TABLE I

Water (mole fract.)	Density (g./cc.)	Viscosity (cp.)	$-\frac{d \ln \eta}{dT} \times 100$ (%/°C.)
0.0298	0.9408	1.005	1.58
.0519	.9420	1.037	1.61
.0694	.9429	1.062	1.64
.0926	.9444	1.105	1.68
.2763	.9565	1.564	2.11
.4360	.9694	2.300	2.58
.5550	.9804	3.112	3.06
.6259	.9867	3.615	3.38
.6897	.9925	3.957	3.58
.7347	.9955	4.029	3.64
.7737	.9981	3.926	3.61
.8015	.9990	3.741	3.55
.8783	.9994	2.782	3.32
.9515	.9969	1.599	2.97
1.0000	.9973	0.910	2.24

The solutions were stored in screw-cap bottles and, as a test of stability, efflux times were re-measured for some of the solutions after storage periods ranging from 41 to 52 days. In each case, the time increased slightly, the increases ranging from 0.05% in the best case to 0.46% in the worst. In light of the very great dependence of viscosity on composition, these changes must be considered negligible.

The density of pure DMA, obtained by extrapolation, is 0.9392 g./cc.<sup>5</sup> while its viscosity is 0.961 centipoise.<sup>6</sup>

### Discussion

The theory of viscosity of binary liquid mixtures is not well developed. Various treatments have been developed for ideal mixtures,<sup>7</sup> but in general no agreement is to be expected in cases where one component is associated or where association exists between the two components.

In the case of the DMA-water system, water is associated through hydrogen bonding, while DMA is generally considered to be unassociated. The viscosity maximum must be attributed either to compound formation or to a higher degree of as-

sociation than exists in either water or DMA alone. Association can, of course, be considered to be a specific type of compound formation.

Geddes,<sup>1</sup> in discussing the dioxane-water system, states that when fluidity ( $1/\eta$ ) is plotted *versus* weight per cent., the composition at which the fluidity deviates most from a straight line connecting the fluidities of the pure components is the composition of a compound formed between these components. This is justified only in cases where some very simple considerations are applicable. Some conditions under which this may be true are discussed by Bingham and Brown.<sup>8</sup> In Geddes' case the fact is emphasized that this point of maximum deviation occurs at a molar ratio (water:dioxane) of exactly 5:1, although no measurements were made between ratios of 3.9:1 and 6.4:1. In the DMA-water system this point of greatest deviation occurs at a water:DMA molar ratio of about 2.7:1, but no conclusions are to be drawn from this fact.

The qualitative explanation for a greater degree of association in mixtures of water-DMA than in water alone is straightforward. The carbonyl bond is highly polar. In resonance terminology, it has a high percentage of ionic character,<sup>9</sup> the oxygen being strongly negative, and stronger hydrogen bonds are formed between water and carbonyl oxygens than between water molecules. In the case of DMA, this effect is strengthened by the nitrogen atom, the resonance form II being perhaps fully as significant as form I.<sup>9</sup>



The importance of structures similar to II in the case of mono-N-substituted amides has been discussed by Gutowsky and Holm<sup>10</sup> among others.

Certain implications of this explanation are clear. It predicts, for example, that similar, but less pronounced, behavior will be found in mixtures of simple ketones with water. In the case of mono-N-substituted amides mixed with water, the maximum in the viscosity-composition curve will be shifted toward the amide end and may become only a fairly broad, nearly flat region, due to the very strong hydrogen bonding in the amide itself.

(8) E. C. Bingham and D. F. Brown, *J. Rheol.*, **3**, 95 (1932).

(9) L. Pauling, "The Nature of the Chemical Bond," 2nd ed., Cornell Univ. Press, Ithaca, N. Y., 1948, pp. 75, 131, 133, 207.

(10) H. S. Gutowsky and C. H. Holm, *J. Chem. Phys.*, **25**, 1228 (1956).

## INTERACTIONS OF SERUM ALBUMIN AND SYNTHETIC POLYELECTROLYTES IN VARIOUS BUFFER SYSTEMS

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In the previous paper,<sup>1</sup> we reported experimental results on the interaction of albumin with polymers

(1) H. Noguchi, *Biochim. et Biophys. Acta*, **22**, 459 (1956).

(5) Using the temperature dependence of density of DMA obtained by J. R. Ruhoff and E. E. Reid, *J. Am. Chem. Soc.*, **59**, 401 (1937), this value is equivalent to 0.9430 g./cc. at 20°. Ruhoff and Reid report values at 20° (their own and two earlier ones) of 0.9413, 0.9405 and 0.9434 g./cc. Considering the possible great effect of certain impurities, this agreement is highly satisfactory.

(6) G. R. Lester, T. A. Grover and P. G. Sears, *THIS JOURNAL*, **60**, 1076 (1956), report the value 0.919 cp. for DMA at 25°. This is equivalent to about 0.932 cp. at 24.05°. Better agreement would be desirable, but again the strong dependence of viscosity on purity can explain the discrepancy.

(7) For example see S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," 1st ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1941, pp. 514-516.

TABLE I  
COMPLEX FORMATION BETWEEN ALBUMIN AND POLYANION AT pH 5.2 AND 3°

Buffer	0.2 M buff.	0.1 M buff.—0.1 M salt			0.1 M buff.—0.1 M salt		
Salt	None	NaCl	KCl	KBr	NaCl	KCl	KBr
SCMC	Not formed	Formed	Formed	Formed	Not formed	Not formed	Not formed
(Complex $u \times 10^6$ )		(-7.0)	(-6.9)	(-6.9)			
DS-1	Formed	Formed	Formed	Formed	Formed		
(Complex $u \times 10^6$ )	(-6.1)	(-7.0)	(-5.3)	(-5.9)	(-6.3)		

TABLE II

MOBILITIES ( $u \times 10^5$ ) OF ALBUMIN OF THE CONCENTRATION OF 0.53% AND POLYANION OF 0.25% IN VARIOUS ACETATE BUFFERS AT 0°

Buffer	0.1 M buff.—0.1 M salt				0.1 M buff.—0.1 M salt			0.2 M buff.
Salt	None	NaCl	KCl	KBr	NaCl	KCl	KBr	None
pH 5.6 Albumin	-2.7	-3.3	-3.6	-3.7	-3.0	-3.1		
pH 5.2 Albumin	-1.9	-2.2	-2.3	-2.5	-2.0	-2.0	-2.2	-1.6
pH 5.2 SCMC	-11.0	-10.6	-11.6	-9.6	-10.0	-11.5	-11.4	-10.9
pH 5.2 DS-1	-16.8	-14.8			-15.0	-15.2	-15.7	

in the alkaline side of the isoelectric point of albumin. According to our findings, electric charges are necessary for polymers to form a soluble complex with albumin. In that work<sup>1</sup> we had paid no attention to the effect of buffer composition on the interaction, but in the course of successive experiments we found that the composition of buffer solutions had an influence on the interaction. In order to clarify the mechanism of the interaction of albumin with polyelectrolytes, we have investigated the soluble complex formation between them in various buffer systems.

### Materials and Method

Human serum albumin was prepared by Cohn's method and found to be pure electrophoretically. Its isoelectric point was 4.7 in 0.1 M acetate buffer.

Sodium carboxymethyl cellulose (SCMC) was obtained from Hercules Powder Co. The molecular weight was 70,000 and the degree of substitution was 0.77.

Purified sodium dextran sulfate (DS) was kindly supplied by Dr. Sasaki of Nagoya University School of Medicine.<sup>2</sup> Two kinds were used for the present experiment: DS-1: molecular weight 32,000; degree of esterification 1.45 and DS-2: molecular weight 270,000; degree of esterification 2.04.

Solutions of albumin, polyelectrolytes and their mixtures were examined by Tiselius electrophoretic apparatus and the formation of the soluble complex was confirmed from the electrophoretic pattern.<sup>1</sup>

### Results

(1) **Interaction in Acetate Buffer Containing Neutral Salts.**—In Table I are shown the results of electrophoresis on the mixture of albumin and polyelectrolytes, whose concentrations are 0.68 and 0.25%, respectively, in 0.1 M acetate buffer containing various concentrations of sodium chloride, potassium chloride and potassium bromide, and in 0.2 M acetate buffer. The results at pH 5.6 are similar to those at pH 5.2. From this table we can conclude that the soluble complex formation between albumin and SCMC is disturbed by increase in concentration of salts or buffer. On the other hand, DS has a stronger affinity to albumin than SCMC and added salts have no influence on the soluble complex formation between DS and albumin.

As given in Table II, the mobility of polyelectrolytes is hardly changed by addition of salts

or increase in buffer concentration, while the mobility of albumin is slightly increased (negatively) with increasing concentration of salts and decreased with increasing buffer concentration. There is found a difference in the effect on the mobility among various kind of salts. The increase of the mobility by salts may be due to the adsorption of anions on albumin.<sup>3,4</sup> When DS is added to the buffer containing 0.1 M KCl and KBr, there appear flocculent white precipitates.<sup>5</sup>

By comparing Table I with Table II, however, we can see that independently of the degree and the direction of the change in mobility, the increase in the concentrations of salts and buffers always has the effect of breaking the soluble complex between albumin and SCMC. It was also confirmed by viscosity measurements that the molecular shape of SCMC and DS is not changed by addition of salts or acetate buffer to 0.1 M acetate buffer. At such high concentrations of small ions, the long-range electrostatic interaction between ionized groups is sufficiently suppressed. From these results it is allowable to say that the complex formation between albumin and SCMC is brought about by the short-range electrostatic interaction between ionized groups on them, as suggested in the previous paper,<sup>1</sup> and that small ions break the complex by screening this interaction. On the other hand, DS probably has another specific interaction with albumin molecule besides the simple electrostatic interaction. Although the SCMC has a lower degree of substitution than DS, this difference is not the cause of the difference in affinities to albumin because it was found that the degree of esterification of DS had no influence on the ability of complex formation in the range from 0.6 to 2.0.

(2) **Interaction in Other Buffer Systems.**—In addition to the acetate buffer, these three buffers having pH 5.6 were employed: (a) 0.1 M succinic acid–0.1 M sodium borate buffer of Kolt-hoff–Vleeschhouwer, (b) 0.1 M monopotassium

(3) L. G. Longworth and C. F. Jacobsen, *THIS JOURNAL*, **53**, 126 (1949).

(4) B. S. Schlessinger, *ibid.*, **62**, 916 (1958).

(5) S. Sasaki (personal communication) has observed qualitatively in the process on the preparation of DS that potassium salt of DS is less soluble compared with the sodium salt.

(2) S. Sasaki and H. Noguchi, *J. Gen. Physiol.*, **43**, 1 (1959).

citrate-0.1 *N* sodium hydroxide buffer of Kolthoff-Vleeschhouwer and (c) 0.1 *M* potassium biphthalate-0.1 *N* sodium hydroxide buffer of Clark-Lubs. According to our results, SCMC can form a soluble complex with albumin only in the acetate buffer, while DS can form the complex in all buffer systems except in potassium biphthalate-sodium hydroxide buffer, the ionic strength of which is 0.18. The mobilities of albumin, DS, and the complex of them measured in solutions mixed at various concentrations are given in Table III. When the concentration of DS is between 0.125 and 0.25%, we could observe only one pattern in all buffer systems; the mobility of this single peak was intermediate between those characteristic of albumin and DS, corresponding to that of the soluble complex. As seen in the table, the mobility of the soluble complex in solutions containing free albumin and no free DS differs considerably from the mobility in solutions containing free DS and no free albumin. This result may arise from the differences in the viscosity of solutions and in the proportion of albumin and DS in the complex.

TABLE III

COMPLEX OF SERUM ALBUMIN AND DS-2 AT pH 5.6 AND 0°

Buffer	Ionic str.	Albumin concn. (%)	DS-2 concn. (%)	Albumin $u \times 10^6$	Complex $u \times 10^6$	DS-2 $u \times 10^6$
Acetic acid-sodium acetate	0.10	0.83	0.40		-10.4	-16.8
		.83	.0625	-2.8	-4.2	
Succinic acid-sodium borate	.23	.83	.375		-9.9	-15.6
		.83	.0625	-3.8	-5.1	
Monopotassium citrate-NaOH	.24	.83	.40		-9.4	-15.1
		.83	.0625	-4.3	-5.9	

At present, we cannot separate the specific effect of buffer anions due to binding on albumin from their non-specific effect due to screening. At least apparently, however, we can arrange various anions in the order of the affinity to albumin: acetic ion, SCMC, succinic ion and citrate ion, DS, biphthalate ion.

### Discussion

Thus, we found that the composition of buffer has a considerable influence on the formation of soluble complex between polyanions and albumin, particularly through the specific interaction between small anions and albumin. The order of affinity of various small anions and polyanions is determined. Special attention must be paid to the difference between DS and SCMC. This means that the  $-\text{SO}_3$  groups on polyanions have a stronger affinity to albumin than  $-\text{COO}^-$  groups. A similar phenomenon was found in the interaction between fibrinogen and these polyanions as reported in the previous paper.<sup>2</sup> There we assumed that  $-\text{SO}_3^-$  groups have a stronger tendency to make hydrogen bonds with amino groups on protein than  $-\text{COO}^-$  groups. A similar situation is expected in the present case.

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## THE ENRICHMENT OF LITHIUM ISOTOPES BY ION-EXCHANGE CHROMATOGRAPHY.<sup>1</sup> II. THE INFLUENCE OF TEMPERATURE ON THE SEPARATION FACTOR

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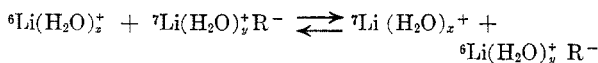
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Equilibrium constants for isotopic exchange reactions generally decrease with increasing temperature.<sup>2</sup> The manner in which the equilibrium constant of a reaction depends on temperature is readily deducible from thermodynamics. The van't Hoff reaction isochor defines the temperature coefficient of  $\ln K_e$ .

$$\left[ \frac{\partial \ln K_e}{\partial T} \right] = \frac{\Delta H^0}{RT^2}$$

Assuming that  $\Delta H^0$  is independent of the temperature or that it varies only very slowly with the temperature, the equation may be integrated between two temperatures. This integration is valid if the temperature range is not large.  $\Delta H^0$  therefore can be determined from the slope of the line obtained by plotting  $\log K_e$  against  $1/T$ . The heat of isotopic exchange is naturally quite small. Glueckauf,<sup>3</sup> using the data of Betts, Harris and Stevenson<sup>4</sup> for  $\text{Na}^{24}$  and  $\text{Na}^{22}$  separation by ion exchange, has calculated the heat of isotopic exchange to be 0.4 cal./mole.

The effect of temperature on the equilibrium constant ( $\alpha$ ) for the reaction



where  $x > y$ , and  $\text{R}^-$  are the exchange sites on the resin, was measured in a series of isotopic separation experiments performed at several temperatures between 3 and 59°. The method used in these experiments was described in the previous paper.<sup>5</sup>

TABLE I

SUMMARY OF EXPERIMENTS TO DETERMINE THE EFFECT OF TEMPERATURE ON  $\alpha$ 

Temp., °C.	Flow rate $10^{-3}$ , cm./sec.	Plates	$\frac{(\text{Li}^6/\text{Li}^7)_{\text{res.}}}{(\text{Li}^6/\text{Li}^7)_{\text{aq.}} (\alpha)}$
3	1.13	1400	1.0033
20	1.42	2000	1.0031
40.5	1.92	1900	1.0028
59	2.02	1700	1.0026

### Experimental

One column was used in these experiments. Constant temperature water ( $\pm 0.3^\circ$ ) was circulated in a jacket around the resin column. The resin was analytical grade sulfonated polystyrene-divinylbenzene Dowex X-16 ground to 200-400 mesh. The resin column dimensions were 19 mm. i.d. and 1080 mm. long. The resin was washed thoroughly with 0.25 *N* HCl before adding 1 ml. of 8.75 *N* LiCl as the lithium band to the top of the column. The eluent in each experiment was 0.25 *N* HCl. The temperatures at which the experiments were made were 3, 20, 40.5 and 59°. The analyses were made as described previously.<sup>5</sup>

(1) This paper is based on work performed for the U. S. Atomic Energy Commission by Union Carbide Corporation.

(2) H. C. Urey, *J. Chem. Soc.*, 562 (1947).

(3) E. Glueckauf, *Trans. Faraday Soc.*, **54**, 1203 (1958).

(4) R. H. Betts, W. E. Harris and M. D. Stevenson, *Can. J. Chem.*, **34**, 65 (1956).

(5) D. A. Lee and G. M. Begun, *J. Am. Chem. Soc.*, **81**, 2332 (1959).