

combined and rechromatographed on a second, freshly prepared column of Sephadex G-75 which, in turn, was subsequently calibrated with natural material as described above. After combination of the appropriate fractions, amino acid analysis indicated the presence of about 75 μg of the desired tetraheptapeptide. This protein fraction was allowed to react with 142 μg of $\text{Hg}(\text{OAc})_2$ in aqueous acetic acid at 25° for 6 hr to remove the cysteinyl blocking groups. After the addition of 0.1 ml of a 50% aqueous solution of mercaptoethanol, the mixture was kept at 25° for 17 hr and the solution was then desalted by passage through Sephadex G-25. Fractions containing excluded protein were combined (2.6 ml).

An aliquot (2.0 ml) was converted into enzymatically active material as described below, and a 0.6-ml aliquot was retained as a control. The former was treated with 0.07 ml of a 1% solution of aqueous mercaptoethanol and then with 26 μg of S-peptide. The pH was adjusted to 6.5 and the phosphate ion concentration to 0.2 M, two drops of CHCl_3 was added, and the mixture was held at 25° for 5 days. In an assay⁴ using RNA as substrate, Mr. I. Putter found this solution to contain about 2 μg of RNase-S activity. By contrast, the 0.6-ml aliquot, which had been processed in a completely analogous manner, but to which no S-peptide had been added, was devoid of enzymatic activity (less than 0.02 μg). Similarly, Dr. M. Zimmerman, using an assay⁵ involving polycytidilic acid as the substrate, found the 2.0-ml solution to contain 1.2 μg of RNase-S activity and the 0.6-ml aliquot to be inactive (less than 0.01 μg). As expected, none of the reagents employed, including S-peptide, showed any enzymatic activity.

Simultaneously with the above experiments parallel experiments were carried out on 130 μg of natural acetamidomethylated S-protein.³ The aliquot (2.0 ml) to which S-peptide had been added yielded about 10 μg of enzymatic activity. Oxidation without added S-peptide (0.6-ml aliquot) again served as a negative control.

It may be concluded therefore that, under conditions where a 100- μg aliquot of natural acetamidomethylated S-protein gave 8–10 μg of RNase-S activity, an aliquot of about 60 μg of our synthetic protected protein gave 1.2–2 μg of RNase-S activity. It is also apparent that enzymatic activity is obtainable from our synthetic protein only when S-peptide is added.

The data presented herein represent a repeat of our first synthesis of the protected tetraheptapeptide. This earlier experiment had also led to enzymatically active material after deblocking and oxidation in the presence of S-peptide.

We are now attempting to prepare sufficient quantities of protein to permit us to carry out the oxidation step at more favorable concentrations and to permit a more complete purification and characterization of the enzymatically active material.

Acknowledgment. The authors are deeply grateful to Mr. I. Putter and Dr. M. Zimmerman of these

(4) This assay was carried out essentially according to the method of P. Cuatrecasas, S. Fuchs, and C. B. Anfinsen, *J. Biol. Chem.*, **242**, 1541 (1967).

(5) S. B. Zimmerman and G. Sandeen, *Anal. Biochem.*, **10**, 444 (1965).

laboratories for carrying out the assays reported herein. We are indebted to them as well as to Professor S. Beychok of Columbia University and to Messrs. F. Kahan and P. Cassidy of these laboratories for numerous discussions concerning assay problems.

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Conductances of Potassium Perchlorate in the Plastic Phase of Sulfolane

Sir:

For the last few years we have been studying the physicochemical properties of sulfolane.¹ This solvent belongs to a family of substances (so-called "globulaire"²) which have one or more transitions below the freezing point.

Thus, in the temperature range 28.45–15.45°, the sulfolane molecules are able to freely rotate on their own axes, and, in some respects, they resemble a liquid.³ Evidence for this is given by several studies on this solvent.^{1,4}

We have measured the resistance of a potassium perchlorate solution in the plastic phase of sulfolane in order to obtain information on the transport properties of electrolytes in this new phase of matter.

During the solidification the potassium perchlorate solution was maintained at reduced pressure (10^{-5} torr) in order to avoid fractures between the electrode surfaces. Resistances were measured by a Jones and Josephs bridge; 30,000 ohms of the bridge resistance was shunted in parallel with the cell. A cell with a constant of 0.2706 cm^{-1} was used. The cell was calibrated by comparison with another cell, which in turn was calibrated with potassium chloride solutions at 25° using the Jones and Bradshaw⁵ standards. In the calculations of the conductivities of the solution, no allowance was made for the variation of the cell constant with the temperature. The concentration of the solution was deduced *a posteriori* from the known conductivity values of potassium perchlorate in sulfolane solutions at 30°. The temperature of the oil bath was controlled within $\pm 0.01^\circ$ by a series of calibrated thermometers. With the same solution the measurements were performed several times, and each time the entire cycle, as depicted in Figure 1, was explored. In the plastic phase the conductance values from several runs were reproducible within $\pm 2\%$; this is probably due to small fractures in the solid invisible with the naked eye.

(1) L. Jannelli, M. Della Monica, and A. Della Monica, *Gazz. Chim. Ital.*, **94**, 552 (1964); M. Della Monica, L. Jannelli, and U. Lamanna, *J. Phys. Chem.*, **72**, 1068 (1968).

(2) B. Timmermans, *J. Phys. Chem. Solids*, **18**, 1 (1961).

(3) C. P. Smyth, *ibid.*, **18**, 40 (1961); L. A. K. Staveley, *ibid.*, **18**, 46 (1961); R. B. Guthrie and J. P. McCullough, *ibid.*, **18**, 53 (1961).

(4) R. L. Burwell and C. Langford, *J. Am. Chem. Soc.*, **81**, 3799 (1959); R. Garnsey and J. E. Prue, *Trans. Faraday Soc.*, **64**, 1206 (1968).

(5) G. Jones and B. C. Bradshaw, *J. Am. Chem. Soc.*, **55**, 1780 (1933).

(6) M. Della Monica, U. Lamanna, and L. Jannelli, *Gazz. Chim. Ital.*, **97**, 367 (1967).

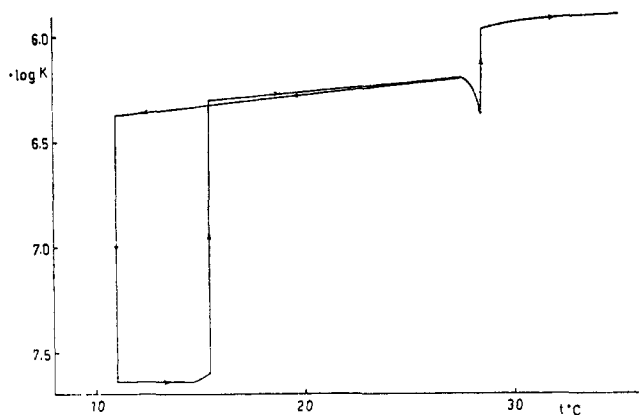


Figure 1. Plot of $-\log K$ vs. temperature for potassium perchlorate in sulfolane.

In Table I the conductance values at various temperatures for one run are given. From Table I the graph of Figure 1 has been constructed; in this diagram the arrows indicate the succession of temperature used.

Table I. Temperature Dependence of Log of the Conductivity for a $KClO_4$ in Sulfolane Solution^a

Data obtained decreasing the temperature		Data obtained increasing the temperature	
Temp, °C	-Log K	Temp, °C	-Log K
26.00	6.210	11.00	7.638
23.00	6.245	12.00	7.638
21.00	6.269	13.00	7.638
17.00	6.306	14.00	7.638
16.00	6.319	15.00	7.620
15.90	6.319	15.10	7.615
15.70	6.321	15.30	7.602
15.50	6.323	15.42	7.595
15.40	6.324	15.45	6.305
15.30	6.325	15.53	6.303
15.25	6.326	15.73	6.301
15.00	6.329	16.35	6.294
14.50	6.333	24.95	6.213
14.00	6.339	27.50	6.192
13.50	6.344	27.70	6.211
13.00	6.350	27.81	6.214
12.00	6.361	28.00	6.238
11.00	6.371	28.10	6.256
		28.33	6.333
		28.40	6.338
		28.45	6.282
		28.50	5.961
		29.02	5.955
		30.00	5.930
		31.00	5.921
		33.00	5.900
		35.00	5.891

^a $c = 1.2 \times 10^{-4} M$ at 30°.

Some interesting results can be deduced. Firstly, the sharp change of the conductivity at the transition point allows a precise determination of this temperature. The value is reproducible if obtained raising the temperature; when the temperature is decreased the system exceeds the transition point, and it may stay in a metastable state for several days. In this way a temperature of 15.45° was obtained which agrees very well with the value obtained from cryoscopic¹ and dielectric constant⁷ measurements. Furthermore, the melting point agrees

(7) U. Lamanna, O. Sciacovelli, and L. Jannelli, *Gazz. Chim. Ital.*, **96**, 114 (1966).

with the value obtained from cryoscopic determinations on pure sulfolane; therefore, owing to the high cryoscopic constant of this solvent (64), this accord is possible only when very dilute solutions are analyzed.

Figure 1 shows that in the temperature range 27.5–28.5° the conductivity of the solution at first decreases with increasing temperature and then rapidly increases near the melting point.

Figure 1 also shows that the mobility of the ions in the plastic phase of sulfolane is too high if compared with the mobility of the same ions in liquid sulfolane (the viscosity of the plastic sulfolane has not been measured, but it is probably very high).

To explain these facts we formulate the hypothesis that the ions are immersed in small liquid zones dispersed within the bulk of the solid solvent. Consequently, even if the measured resistance of the conductor between the two electrodes increases, the resistance of each liquid zone decreases as an effect of the increased electrolyte concentration. A similar hypothesis has been formulated to explain how certain chemical reactions are markedly accelerated when the solution is frozen.⁸ The formation of liquid zones dispersed in the solid phase also seems consistent with the anomalous conductivity near the melting point; the broadening of the liquid zones can decrease the local concentration of the salt so that the conductivity decreases.

More definite conclusions on this problem will be made when conductivity data of several electrolytes at various concentration are obtained. Therefore, we are trying to obtain conductivity data in this phase of sulfolane which are reproducible within a few parts in a thousand.

(8) T. C. Bruice and A. R. Butler, *J. Am. Chem. Soc.*, **86**, 4104 (1964); R. E. Pincock and T. E. Kiovsky, *ibid.*, **87**, 2072 (1965); R. E. Pincock, *ibid.*, **87**, 1274 (1965); R. E. Pincock and T. E. Kiovsky, *ibid.*, **87**, 4100 (1965).

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Received November 5, 1968

The Stability of Ferrocenylcarbonium Ions¹

Sir:

The high degree of stabilization of α -ferrocenylcarbonium ions is manifested in the unusual reactivity of ferrocene derivatives in a variety of reactions believed to proceed through such ions as intermediates.² This great stability has invited speculations about the structure of these carbonium ions and the nature of their stabilization. It was proposed some years ago on the basis of solvolysis results that a portion of the stabilization may arise from direct overlap of iron orbitals with the p orbital of the formally cationic carbon.³ Recently, this interpretation has been questioned, and an alternative model involving "carbon-iron hypercon-

(1) Presented in part at the Great Lakes Regional Meeting of the American Chemical Society, Milwaukee, Wis., June 1968.

(2) For reviews, see M. Cais, *Record Chem. Progr.*, **27**, 177 (1966); *Organometal. Chem. Rev.*, **1**, 435 (1966).

(3) (a) J. H. Richards and E. A. Hill, *J. Am. Chem. Soc.*, **81**, 3484 (1959); (b) E. A. Hill and J. H. Richards, *ibid.*, **83**, 3840, 4216 (1961); (c) D. S. Trifan and R. Bacskai, *Tetrahedron Letters*, No. 13, 1 (1960).