trons on a heteroatom in a three-membered ring is employed to explain the electronegative character of that atom.

Experimental

Heats of Mixing Determinations.—The apparatus and method have been described previously.[13] The ethers were introduced in sealed ampoules in the manner described for volatile ethers in a previous paper.[1] A slightly different value (461 cal./mole) was reported previously for the heat of mixing of propylene oxide with chloroform, but the agreement is within experimental error.

(13) G. G. Zellhoefer and M. J. Copley, THIS JOURNAL, **60**, 1343 (1938).

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Tracer-diffusion in Liquids. V. Self-diffusion Isoelectric Glycine in Aqueous Glycine Solutions[1]

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The ordinary chemical concentration gradient diffusion of isoelectric glycine in its aqueous solutions has been measured by many workers.[2-4] The dependence of these chemical concentration gradient diffusion coefficients upon the concentration of solution have been compared[2] with those calculated from Gordon's formula[5] where D and D_0

$$D = D_0\left(1 + c\frac{\partial \ln y}{\partial c}\right)\frac{\eta}{\eta_0} \qquad (1)$$

are the diffusion coefficients of the solute in its solutions at molal concentration c and at infinite dilution, respectively, y the activity coefficient of the solute at concentration c, and η and η_0 are the macroscopic viscosities of the solution and pure solvent, respectively. Lyons and Thomas showed that for the case of aqueous glycine solutions, the factor (η/η_0) overcorrects the retardation of diffusion due to change in mobility of the glycine molecule. These authors inferred from their measured temperature dependence of the diffusion coefficient of glycine that the above overcorrection is due to the breaking down of the structure of water in the immediate vicinity of glycine molecules.

In self-diffusion, however, since the composition of solution is chemically uniform along the diffusion path, the activity coefficient of the diffusing tracer-molecules is constant, the term $(1 + c \times (\partial \ln y/\partial c))$ reduces to unity. Furthermore, since in self-diffusion there is no net transport of solvent in any direction, the "hydrodynamic effect" of Onsager and Fuoss[6] (which is often referred to as the "electrophoretic effect" in the diffusion of electrolytes) vanishes. We may thus expect the self-diffusion coefficient of isoelectric glycine to be theoretically simpler and hence possibly even more interesting. In the present work the self-diffusion coefficients of isoelectric glycine in its aqueous solutions at 25° are measured and discussed.

Experimental

Tracer Solution.—The radioactive glycine ($^+H_3NCH_2COO^-$) was supplied by Tracerlab, Inc., Boston, Mass., and obtained on allocation from the Isotopes Division, U.S. Atomic Energy Commission, Oak Ridge, Tennessee. The specific activity of this radioactive glycine was about 0.02 mc./mg. A dilute solution of this radioactive glycine in conductivity water was prepared and kept in a sterilized bottle at 0° as stock solution. In making radioactive solution for diffusion measurement, a small measured volume of this stock solution was evaporated to dryness in a platinum crucible under a heat lamp. The radioactive residue (practically invisible) was dissolved in inactive glycine solution of accurately known concentration and with pH at 5.98. The increase in total glycine concentration due to the dissolution of the above mentioned radioactive residue is negligible.

Diffusion Measurements.—All the measurements were carried out at 25.00 ± 0.01°. The experimental procedure is the same as that described in Paper I of this series.

Analysis of Samples.—The counting of the radioactive diffusion samples was carried out by means of a windowless flow counter: In order to eliminate errors due to self-absorption of the weak β-particles from C^{14}, the volumes of the c_0-samples were so adjusted that each of them contained approximately the same amount of solid material as the corresponding c_{av}-sample. All samples were dried in desiccators for more than 24 hours before counting. Consequently, the measured values of (c_{av}/c_0), which determine the values of Dt/l^2, are practically free from self-absorption errors.

Results and Discussion

The self-diffusion coefficients of isoelectric glycine in its aqueous solutions at 25° as determined in the present work are listed in Table I.

TABLE I

SELF-DIFFUSION COEFFICIENTS OF ISOELECTRIC GLYCINE IN AQUEOUS GLYCINE SOLUTIONS AT 25°

Concn., mole/l.	$D \times 10^5$, cm.²/sec.
0.01	1.06 ± 0.02
.10	1.05 ± .02
.25	1.03 ± .02
.50	0.990 ± .026
1.00	.929 ± .018
1.50	.871 ± .018
2.00	.830 ± .011

Each value listed in Table I is the average result of 3 to 12 measurements. These self-diffusion coefficients are plotted *vs.* volume molal concentration of glycine in Fig. 1. Diffusion coefficients of glycine for ordinary chemical concentration gradient diffusion as reported by Lyons and Thomas[2] are also included in Fig. 1 for comparison. From the definition of the diffusion coefficients, it is easy for us to see that at infinite dilution the self-diffusion coefficient of isoelectric glycine should be identical to that for chemical concentration gradient diffusion. Figure 1 indicates that as the concentration approaches zero, the two sets of diffusion coefficients tend toward the same limiting value. Since the optical method used by Lyons and Thomas has been developed to a high degree of precision, this agreement can be considered as a further confirmation on the general reliability of the present capillary method for measuring tracer-diffusion in liquids. The dotted curve in Fig. 1 represents values of $D/(1 + c(\partial \ln y/\partial c))$ in equation (1) calculated

(1) Contribution No. 1135 from the Department of Chemistry of Yale University. Previous papers of this series: THIS JOURNAL, **74**, 1182, 6317 (1952); *ibid.*, 1611 (1952); *ibid.*, 1612 (1952); *ibid.*, **75**, 1769 (1953).

(2) M. Lyons and J. V. Thomas, *ibid.*, **72**, 4506 (1950).

(3) L. G. Longsworth, *ibid.*, **74**, 4155 (1952).

(4) See E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 412 for earlier references.

(5) A. R. Gordon, *J. Chem. Phys.*, **5**, 522 (1937).

(6) L. Onsager and R. M. Fuoss, *J. Phys. Chem.*, **36**, 2689 (1932).

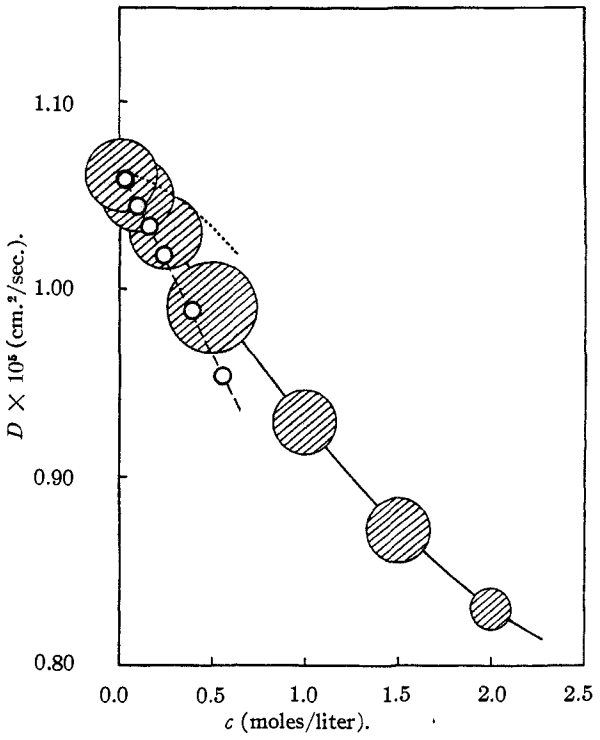

Fig. 1.—Diffusion coefficients of isoelectric glycine in its aqueous solution at 25°: ●, self-diffusion coefficients determined in the present work; O, chemical concentration gradient diffusion coefficients determined by Lyons and Thomas.

by Lyons and Thomas from their data. It may be seen from Fig. 1 that these values, which when corrected for "hydrodynamical effect" would represent the mobilities for chemical concentration gradient diffusion, are higher than the self-diffusion coefficients of isoelectric glycine at the corresponding concentrations. From this we may infer that the actual mobilities for the chemical concentration gradient diffusion of isoelectric glycine are still higher than the corresponding self-diffusion coefficients.

A possible cause of this decrease in self-diffusion coefficient of isoelectric glycine with increasing concentration in dilute solutions is that the actual driving force for self-diffusion (equal to the absolute temperature times the entropy gradient) is opposed by a smaller but finite force due to the time of relaxation effect. This time of relaxation effect is due to the interaction between dipolar ions and therefore should presumably increase with concentration of the solution. The effect of interaction between dipolar ions on the equilibrium properties of these solutions in the dilute concentration range have been discussed by Fuoss.[7] But the quantitative formulation of a theory for the relaxation effect in the self-diffusion of dipolar ions analogous to that for the diffusion of simple spherical ions[8] appears to be difficult because of the absence of spherical symmetry of charge distribution of both the diffusing dipolar ion and its surrounding atmosphere of dipolar ions. Thus, the equilibrium charge distribution surrounding a diffusing dipolar ion can be disturbed both by the translational motion of the center of mass of the dipolar ion (as in the case of self-diffusion of simple spherical ions), or by a pure rotation of the latter. Since these two mechanisms are probably linked together in the actual process of diffusion, it is difficult at this time to make quantitative calculations of the relaxation effect without introducing further simplifying assumptions of doubtful validity. It appears advisable, therefore, to postpone detailed discussion of the present subject until more data on the self-diffusion of dipolar ions have become available.

(7) R. M. Fuoss, THIS JOURNAL, **56**, 1024 (1934); *ibid.*, **58**, 982 (1936).

(8) L. Onsager, *Ann. N. Y. Acad. Sci.*, **46**, 241 (1945).

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The Uronic Acid Component of Chondroitinsulfuric Acid[1]

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Hexosamine glycosides hydrolyze with difficulty and when hexosamines are glycosidically linked to a hexuronic acid, as in the cartilage heteropolysaccharide chondroitinsulfuric acid,[2,3] the acid conditions requisite to break this bond lead largely to the destruction of the uronic acid moiety. In the case of the related polysaccharides heparin and "mucoitinsulfuric acid,"[4] this difficulty was overcome by the employment of an oxidative hydrolysis with bromine and concentrated sulfuric acid at 0°, whereby the D-glucuronic acid entity present was recovered as D-glucaric (D-glucosaccharic) acid. We report herein the extension of this procedure to cartilage chondroitinsulfuric acid whereby its hexuronic acid component is adequately identified as D-glucuronic acid, in confirmation of the work of Bray, Gregory and Stacey,[5] who isolated a crystalline methyl ether of this substance on acid hydrolysis of the methylated, degraded polysaccharide. In early experiments, Levene and Jacobs[6] obtained a silver salt on treatment of chondroitinsulfuric acid, designated glycothionic acid by them, with hydrobromic acid and bromine under unspecified conditions. The silver content of this salt was in agreement with that required by a hexuronic acid but the substance was not further characterized.

Experimental

An amount of 500 mg. of purified barium chondroitinsulfate (from cartilage)[3] was dissolved at 0° in a mixture of 10 ml. of concentrated sulfuric acid (sp. gr. at 15.56°, 1.84), 4 ml. of water and 10 drops of bromine and maintained at 3° for 6 days. Additional quantities of bromine were added

(1) Supported by fellowship funds granted by The Ohio State University Research Foundation to the university for aid in fundamental research (Project R-11670-P369).

(2) P. A. Levene, *J. Biol. Chem.*, **140**, 267 (1941).

(3) M. L. Wolfrom, R. K. Madison and M. J. Cron, THIS JOURNAL, **74**, 1491 (1952).

(4) M. L. Wolfrom and F. A. H. Rice, *ibid.*, **68**, 532 (1946); **69**, 1833 (1947).

(5) H. G. Bray, J. E. Gregory and M. Stacey, *Biochem. J.*, **38**, 142 (1944).

(6) P. A. Levene and W. A. Jacobs, *J. Exptl. Med.*, **10**, 557 (1908).