

THE SOLUBILITY OF GLUTAMIC ACID IN WATER AND CERTAIN ORGANIC SOLVENTS*

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The solubility of glutamic acid in water was determined by Bayliss (1), Pfeiffer and Wurgler (2), Pfeiffer and Angern (3), and others. In the course of an investigation of certain properties of *d*-glutamic acid there became available samples of this acid of considerable purity. It was of interest, therefore, to extend these measurements with a view to determining the effect of the temperature on the solubility of glutamic acid in water and also to provide data for the solubility of this acid in ethanol, methanol, and acetone, about which very little is known.

EXPERIMENTAL

Glutamic Acid—Our investigation was carried out with three commercial *d*-glutamic acid preparations. They were all subjected to repeated crystallization from water. The nitrogen content of Samples I and II did not differ beyond the experimental error from the amount required by the theory. Sample III yielded, however, a larger amount of nitrogen. The number of carboxyl groups was determined by titration in ethanol and acetone. The agreement between the theory and experimental values was satisfactory. Table I shows such a titration of Sample II. These measurements will be discussed at greater length in one of the following sections.

Solvents. Water—Boiled, distilled water, kept from contact with the CO₂ of the air, was used in the investigation.

Ethanol—Commercial absolute ethyl alcohol was used as a starting material. It was then subjected to the following purification. First, a small amount of H₂SO₄ was added and the alcohol distilled. Following this distillation 3 gm. of AgNO₃ in 3 cc. of

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water per liter and then 3 gm. of KOH previously dissolved in 30 cc. of the alcohol were added. This procedure frees the alcohol from aldehydes. The solution was left standing overnight. After distillation, the alcohol was shaken with CaO and again left standing overnight and then distilled. This last procedure was repeated

TABLE I

Estimation of Glutamic Acid by Titration in Presence of Ethanol or Acetone

Indicator, 2.0 cc. of 0.08 per cent thymolphthalein; 0.0359 gm. of glutamic acid in 5.0 cc. of H₂O for every titration.

Substance added (1)	Amount added (2)	0.1 N Na alcoholate required (3)	Blank (5 cc. H ₂ O) 0.1 N Na alcoholate (4)	Equivalent of base bound $\frac{(3) - (4)}{10^{-4}} \times$ (5)	Equivalent weight, $\frac{0.0359}{(5)}$ (6)
C ₂ H ₅ OH	cc.	cc.	cc.		
	5.00	4.90	0.03	4.87	73.7
		4.88	0.03	4.85	74.0
	10.00	4.94	0.06	4.88	73.6
		4.94	0.06	4.88	73.6
		4.93	0.06	4.87	73.7
	15.00	(5.08)	0.095	(4.98)	(72.1)
		5.01	0.095	4.91	73.1
		4.96	0.095	4.86	73.8
	20.00	5.01	0.11	4.90	73.2
	25.00	5.06	0.17	4.89	73.4
	35.00	5.13	0.23	4.90	73.2
(CH ₃) ₂ CO	None	4.82	0.02	4.80	76.8
		4.87	0.02	4.85	76.0
	5.00	5.02	0.05	4.87	75.6
	10.00	5.08	0.07	5.01	73.6
	15.00	5.10	0.09	5.01	73.6
	20.00	5.13	0.12	5.01	73.6
		5.17	0.12	5.05	73.0
	25.00	5.15	0.13	5.02	73.4

Parentheses indicate an error of unknown origin.

twice. During the last two distillations only the middle fraction of the distillate was collected.

Methanol, c.P.—The alcohol was purified in the same way as ethanol.

Acetone, c.P.—The acetone was purified by distilling it once from calcium oxide.

Method of Analysis—Three methods may be conveniently used for determining the amount of glutamic acid dissolved in a solvent. It can be determined by evaporating the solution and weighing the amount of acid dissolved, if the substance is pure, by analyses for nitrogen, and by analyses for carboxyl groups. In our investigation we used the last two methods. Nitrogen was determined by the micromethod suggested by Pregl (4) (with CuSO_4 as the oxidizing agent). The amount of carboxyl groups was determined by titration of the acid with 0.1 N sodium alcoholate in the presence of ethanol or acetone, thymolphthalein being used as an indicator. This method is quite well known. It came into existence largely through the contributions of Foreman (5), Willstätter and Waldschmidt-Leitz (6), and Martens (7). This method is quite reliable if two precautions are taken: first, if the amount of alcohol or acetone necessary to produce the largest titer is determined and secondly if the formation of a precipitate is avoided. In the latter case the end-point becomes unstable and color fades quickly.

A comparative titration of a sample of glutamic acid in the presence of varied amounts of ethanol and acetone is reported in Table I. It is evident that when 10 cc. of ethanol or acetone are added a maximum value is reached, which is not affected (within experimental error) by further addition of either of these substances. This value is in close agreement with the theoretical value for the equivalent weight, the latter being 73.54 gm.

In our determination of the solubility of glutamic acid in water we therefore used 10 cc. of acetone. In these titrations the total concentration of acetone and ethanol (as a solvent for thymolphthalein and as sodium alcoholate) was thus never less than 76 per cent. Occasionally these determinations were checked by nitrogen analysis.

The method becomes less accurate when only very small amounts of glutamic acid are present. Therefore, the determination of the solubility of the acid in ethanol, methanol, and acetone was made by microanalysis for nitrogen.

Determination of Solubility—The required solvent and glutamic acid were placed in Pyrex flasks and shaken for a given period in a shaking machine in an air thermostat. The shaking was vigorous enough to keep a part of the precipitate always in suspension.

TABLE II

Solubility of Glutamic Acid in Water and Certain Non-Aqueous Solvents at 25.0° ± 0.5°

Solvent	Sample No.	Saturation	Time for equilibrium	Glutamic acid in 50 cc.	Solubility, glutamic acid dissolved in 50 cc.	
(1)	(2)	(3)	(4)	(5)	(6)	
H ₂ O	I	1st	6½ days	5.00	0.442	
				2.50	0.438	
			8½ "	5.00	0.435	
				2.50	0.432	
			2nd	10 hrs.	4.10	0.431
					2.50	0.440
		3rd	43 "		1.60	0.442
				3.20	0.446	
				2.00	0.438	
		1st	65 "		3.20	0.434
					2.00	0.443
						0.447
		II	1st	7 days	4.00	0.441
					2.00	0.440
				75 hrs.	1.00	0.441
				50 "	2.00	0.437
		III	1st	50 "		0.430
				30 "		0.437 by N
					2.00	0.431
					0.426	
					0.429	
					0.435	
CH ₃ OH	II	2nd	10 days	1.00	0.0038	
				2.00	0.0029	
		3rd	12 "	0.50	0.0028	
				1.00	0.0030	
	4th	20 "		2.00	0.0030	
				0.50	0.0026	
				1.00	0.0028	
			2.00	0.0026		
					0.0029	

TABLE II—*Concluded*

Solvent	Sample No.	Saturation	Time for equilibrium	Glutamic acid in 50 cc.	Solubility, glutamic acid dissolved in 50 cc.
(1)	(2)	(3)	(4)	(5)	(6)
C ₂ H ₅ OH	II	2nd	10 days	<i>gm.</i>	<i>gm.</i>
				0.50	0.0029
				1.00	0.0030
		5th	30 "	2.00	0.0024
				0.50	0.0029
				1.00	0.0024
		2.00	0.0026		
				0.0027	
(CH ₃) ₂ CO			Not more than		0.00003

When needed, a part of the content of the flask was quickly filtered (at room temperature) through No. 42 Whatman filter papers and the filtrates analyzed as described. All solubility experiments were carried out at $25.0^{\circ} \pm 0.5^{\circ}$ and all data refer to volumes at 20° .

Results

The data obtained are reported in Table II. As may be noted, we have varied in these measurements the number of saturations, the time, and the starting amount of glutamic acid in a given volume of solvent. Though the existence of a large amount of impurity in our preparation is doubtful, due to good analytical agreement, there is a slight possibility that an optical isomer of *d*-glutamic acid may have a different solubility. Washing a preparation, or in other words saturating it several times with fresh lots of solvent, would necessarily change the ratio of the two isomers (if their solubility were different) as well as change the solubility itself. This would be equally true if the amount of glutamic acid per given volume of solvent were varied.

Our experiments show that this is not the case. The solubility does not vary as a function of the number of saturations, or the amount of glutamic acid involved. An equilibrium is probably established in less than 10 hours. The experiments indicate that

we are dealing with a single chemical individual. They do not exclude the possibility of the presence of *l*-glutamic acid, but exclude the possibility that this optical isomer, if present, has a different solubility in water from *d*-glutamic acid; all certainly being true within our experimental error.

The solubility of glutamic acid in methanol and ethanol is much lower than in water. The value for ethanol is lower than the value usually accepted (8) (0.07 gm. in 100 cc.). The solubility

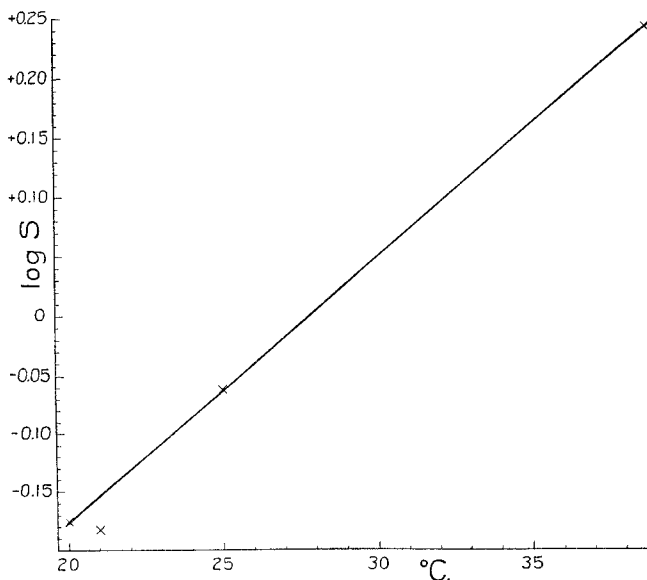


FIG. 1. The effect of temperature on the solubility (*S*) of glutamic acid in water.

in both of these alcohols differs little, but glutamic acid seems to be slightly less soluble in ethanol than in methanol. The solubility in acetone was below our experimental grasp, but is certainly not more than 6×10^{-5} gm. per 100 cc.

Pfeiffer and Wurgler (2) and Pfeiffer and Angern (3) measured the solubility of glutamic acid in water at 20° and 21° respectively. Bayliss (1) carried out similar measurements at 38.5°. Combining these observations with our own, we can calculate the effect of temperature upon the solubility of this acid.

We know that the solubility must be related to the temperature by the equation

$$\ln \frac{N_1}{N_2} = \frac{-\Delta H}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)$$

where N_1 and N_2 are mol fractions of glutamic acid in solution at the absolute temperatures T_1 and T_2 , respectively; R has the value of 1.986; and ΔH is the heat of solution. In dilute solutions the logarithm of solubility plotted against the temperature should yield a straight line.

Such a plot is found in Fig. 1, in which $\log S$ is the logarithm to the base 10 of the solubility of glutamic acid in gm. per 100 cc. of solution. The straight line passes nicely through the value obtained by Pfeiffer and Wurgler (2) (0.669 gm.), but the value obtained by Pfeiffer and Angern (3) (0.658 gm.) appears to be too low.

Using the equation and the value indicated in Fig. 1, we can calculate the heat of solution, which is close to 9600 calories. If glutamic acid is a "normal" substance, then this value according to Gehloff (9) must be near to the heat of fusion. It should also be the same for all solvents.

SUMMARY

1. The solubility of *d*-glutamic acid was determined in the following solvents at 25°.

Solvent	Solubility, mols per 1000 gm. solvent
Water.....	0.0595
Methanol.....	4.8×10^{-4}
Ethanol.....	4.6×10^{-4}
Acetone.....	Not more than 2.5×10^{-6}

2. The heat of solution, in water, of *d*-glutamic acid was calculated to be 9600 calories.

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