

A Study of the Inosine–Sodium Hydroxide–Water and Inosine–Potassium Hydroxide–Water Systems

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Solubility data have been obtained for the inosine–NaOH–H₂O and inosine–KOH–H₂O systems at 30 °C. Two different solid phases of the potassium salt of inosine (C₁₀H₁₁N₄O₅·K·H₂O and C₁₀H_{11.5}N₄O₅·0.5 K·0.5 H₂O) were found in the latter system, while only one solid phase of the sodium salt of inosine (C₁₀H₁₁N₄O₅·Na·2.5 H₂O) was found in the former system. The hemi-potassium salt of inosine is incongruent and is decomposed by pure water, whereas the mono-alkaline-metal salts are stable. The solubilities of the two mono-alkaline-metal salts of inosine in water were determined at 0–50 °C and formulated as follows:

$$\text{Na-salt, } \log S = 0.01359 \cdot t + 0.3997$$

$$\text{K-salt, } \log S = 0.00733 \cdot t + 1.6513$$

where S represents the gram weight of the anhydrous salt per 100 g of water. The X-ray powder diffraction data of the above three salts are given.

The equilibrium for the inosine–alkaline-earth metal hydroxide–water system was reported in the preceding paper.¹⁾ This paper will describe the results for the inosine–alkaline metal hydroxide–water system. The solubilities of the alkaline metal salts of inosine in water were also determined.²⁾

Results and Discussion

Table 1 shows the composition data in the solution phase for the inosine–sodium hydroxide–water system at 30 °C. The solubility of inosine increases linearly with an increase in the amount of sodium hydroxide until its mono-sodium salt is precipitated, and then it decreases with a steep slope until the mole ratio of inosine and sodium ion in the solution phase reaches 1 : 1. Thereafter, it increases again.

Table 2 shows the composition data in the solution phase and in the solid phase for the inosine–potassium

hydroxide–water system at 30 °C. The solubility of inosine also increases with an increase in the quantity of potassium hydroxide with a slope nearly equal to that of sodium hydroxide until its mono-potassium salt is precipitated; thereafter it decreases a little until the mole ratio of inosine and potassium ion in the solution phase reaches about 2 : 3. Then, it increases again. However, on the way to the point where the mono-potassium salt begins to be precipitated, there is a clear region where the hemi-potassium salt of inosine is precipitated. Such a region is not found in the sodium hydroxide system.

The composition of the hemi-potassium salt of inosine can be explained as a molecular compound between one mole of the mono-potassium salt of inosine and one mole of free inosine. This salt is incongruent and is decomposed by pure water, because the region where this salt is stably present does not include the line on which the mole ratio of the inosine and the

TABLE 1. COMPOSITION DATA FOR THE INOSINE–SODIUM HYDROXIDE–WATER SYSTEM AT 30 °C

Run No.	Solution Phase (Weight %)		Solid Phase ^{a)}
	Inosine	NaOH	
1	3.18	0	I(α)
2	5.81	0.243	I(α)
3	7.88	0.465	I(α)
4	11.72	0.849	I(α)
5	13.70	1.076	I(α) (metastable)
6	14.55	1.137	I(α) (metastable)
7	12.67	1.000	I(α) + S
8	12.87	1.040	I(α) + S
9	12.24	0.988	S
10	9.58	0.908	S
11	7.47	0.891	S
12	5.61	0.839	S
13	5.57	1.148	S
14	6.94	1.967	S
15	9.52	3.548	S

a) I(α): Inosine in the α-form, C₁₀H₁₂N₄O₅; S: Mono-sodium salt of inosine, C₁₀H₁₁N₄O₅·Na·2.5H₂O

TABLE 2. COMPOSITION DATA FOR THE INOSINE–POTASSIUM HYDROXIDE–WATER SYSTEM AT 30 °C

Run No.	Solution (Weight %)		Wet residue (Weight %)		Solid phase ^{a)}
	Inosine	KOH	Inosine	KOH	
1	3.18	0	—	—	I(α)
2	19.04	2.44	69.90	0.905	I(α)
3	30.88	4.16	—	—	I(α) (m)
4	26.57	3.66	68.98	5.77	I(α) + K/2
5	27.22	4.36	70.53	7.98	K/2
6	33.24	6.48	69.95	8.37	K/2
7	38.70	7.89	72.70	11.69	K/2 + K
8	36.04	7.76	77.52	16.14	K
9	34.36	8.81	73.98	15.94	K
10	33.92	11.89	76.13	16.83	K
11	36.26	14.82	71.65	16.73	K
12	40.19	17.31	76.99	17.62	K
13	42.94	20.14	75.22	18.08	K

a) I(α): Inosine in the α-form, C₁₀H₁₂N₄O₅; K/2: Hemi-potassium salt of inosine, C₁₀H_{11.5}N₄O₅·0.5K·0.5H₂O; K: Mono-potassium salt of inosine, C₁₀H₁₁N₄O₅·K·H₂O; m: metastable

TABLE 3. SOLUBILITIES OF MONO-ALKALINE-METAL SALTS OF INOSINE IN WATER

Temp. (°C)	S (g, anhydrous salt/100 g water)	
	C ₁₀ H ₁₁ N ₄ O ₅ ·Na·2.5H ₂ O	C ₁₀ H ₁₁ N ₄ O ₅ ·K·H ₂ O
0	2.57	43.2
5	—	50.8
10	3.35	55.4
30	6.47	74.6
50	12.22	100.5

TABLE 4. X-RAY POWDER DIFFRACTION PATTERNS OF ALKALINE METAL SALTS OF INOSINE

S ^{a)}		K ^{b)}		K/2 ^{c)}	
<i>d</i> (Å)	<i>I</i> / <i>I</i> ₀ ^{d)}	<i>d</i> (Å)	<i>I</i> / <i>I</i> ₀ ^{d)}	<i>d</i> (Å)	<i>I</i> / <i>I</i> ₀ ^{d)}
7.31	10	7.11	20	10.10	20
6.92	40	6.28	20	8.35	50
5.72	10	5.37	15	6.76	30
5.50	50	5.20	10	6.13	100
5.16	50	4.75	10	4.77	40
5.01	90	4.65	30	3.88	60
4.48	90	4.27	100	3.68	30
4.21	40	4.05	20	3.56	25
3.82	50	3.87	15	3.52	30
3.75	100	3.58	30	3.10	40
3.41	40	3.41	50		
3.34	80	3.36	15		

a) Mono-sodium salt of inosine, C₁₀H₁₁N₄O₅·Na·2.5H₂O. b) Mono-potassium salt of inosine, C₁₀H₁₁N₄O₅·K·H₂O. c) Hemi-potassium salt of inosine, C₁₀H_{11.5}N₄O₅·0.5K·0.5H₂O. d) The scale is so chosen as to make the most intense line have the value 100.

potassium ion is 1 : (1/2).

On the other hand, the two mono-alkali-metal salts are stable in water. Table 3 shows the solubilities of these salts in water at 0—50 °C. Each logarithm of the solubility is linear to the temperature and is expressed as follows:

$$\text{sodium-salt: } \log S = 0.01359 \cdot t + 0.3997$$

$$\text{potassium-salt: } \log S = 0.00733 \cdot t + 1.6513$$

where *S* represents the gram weight of the salts per 100 g of water in terms of anhydride. No change in the solid phase was observed in the temperature range examined.

It is of interest that the alkaline metal salts of inosine examined in the present study show no similarity among each other with respect to solubility behavior and crystal structure, while the alkaline-earth metal salts of inosine do.¹⁾ The solubility of the mono-potassium salt and that of the mono-sodium salt are quite different from each other. The former is ten times more soluble in water than the latter. On the other hand, the hemicalcium salt and the hemi-strontium salt give solubilities nearly equal below a decimal point.¹⁾ The hemi-alkaline-earth metal salts are both monohydrates, and their X-ray diffraction patterns are similar.¹⁾ On the other hand, the mono-sodium salt possesses two and a half moles of water of crystallization, while mono-

potassium salt possesses one mol of that; moreover, the X-ray diffraction patterns shown in Table 4 also indicate no resemblance.

There is another large difference between the two phase diagrams plotted with the data in Tables 1 and 2 in the mole ratios of inosine and alkaline metal ion at the point where mono-alkaline-metal salt begins to be precipitated. The ratios are 1 : 0.54 for the sodium hydroxide system and 1 : 0.98 for the potassium hydroxide system respectively. Because the latter value is very close to 1 : 1, it sometimes seems as if the mono-potassium salt of inosine were incongruent and were decomposed by pure water, especially when the salt possesses an impure composition.

Experimental

Materials. The inosine (α -form)³⁾ used was of a commercial A-grade of the Ajinomoto Co., Inc.; the other materials were of a reagent grade.

Analysis. The inosine was determined by means of the UV absorbance at 250 nm in 0.1 M HCl using the molar extinction coefficient of 11,800 under the above conditions. The alkaline metal hydroxide was determined by titration with standard sulfuric acid, using a methyl red-bromocresol green indicator. The X-ray powder diffraction patterns (CuK α) were used for the identification of the solid phase in equilibrium. The degree of hydration of the crystals was determined by Karl Fischer titration.

Preparation of the Crystals. *Mono-sodium Salt of Inosine:* Inosine (26.8 g) was dissolved in 1/2 M NaOH (200 ml) at 60 °C; then the solution was cooled at 10 °C. Precipitated thick prism crystals were filtered out and dried in air. Yield, 13 g; mp 198—202 °C. Found: Na, 6.90; H₂O, 13.5%; $\epsilon_{250\text{ nm}}$ in 0.1 M HCl, 9,450. Calcd for C₁₀H₁₁N₄O₅·Na·2(1/2)H₂O: Na, 6.87; H₂O, 13.4%; ϵ , 9,440. *Mono-potassium Salt of Inosine:* Inosine (145 g) was dissolved in 22% aqueous KOH (180 g), and the solution was allowed to stand at 50 °C overnight with the addition of seed crystals. The seed crystals had previously been obtained by the rapid cooling of a little portion of that solution. The precipitated long prism crystals were filtered out, washed with 80% ethanol, and dried at 100 °C for 5 hr. Yield, 14 g; mp 163—6 °C. Found: K, 12.18; H₂O, 5.66%; $\epsilon_{250\text{ nm}}$ in 0.1 M HCl, 9,760. Calcd for C₁₀H₁₁N₄O₅·K·H₂O: K, 12.06; H₂O, 5.55%; ϵ , 9,810. *Hemi-potassium Salt of Inosine:* Inosine (200 g) was dissolved at 80 °C in 12% aqueous KOH (240 g), after which the mixture was filtered with a glass filter in order to obtain a clear solution. When it was cooled to 50 °C, a very small amount of seed crystals was added; the mixture was then left to stand for two days at 50 °C. The seed crystals had previously been obtained by the rapid cooling of a little portion of that solution. The precipitated needle crystals were quickly filtered out, sandwiched between filter paper with pressure in order to remove the mother liquor completely, and then dried for 5 hr at 100 °C. Yield, 30 g; mp 172—5 °C. Found: K, 6.86; H₂O, 3.41%; $\epsilon_{250\text{ nm}}$ in 0.1 M HCl, 10,430. Calcd for C₁₀H₁₂N₄O₅·C₁₀H₁₁N₄O₅·K·H₂O: K, 6.60; H₂O, 3.04%; ϵ , 10,660.

Determination of Equilibria. The equilibria were usually approached by dissolving the crystals in water or aqueous alkaline hydroxide at a constant temperature. In a few cases they were approached by cooling the supersaturated solution. The details in Table 1 show that the equilibria at 30 °C were approached by dissolving inosine in the α -form (Runs No. 1,3,7 and 11), by dissolving sodium salt of inosine

(No. 9, 10 and 12—15), by dissolving the above two materials (No. 8), and by cooling the supersaturated solutions (No. 2, 4, 6 and 7). The supersaturated solutions were prepared by dissolving inosine in aqueous NaOH at 60 °C. Those in Table 2 show the results obtained by dissolving inosine in the α -form (Runs No. 1—3 and 5), by dissolving the hemi-potassium salt of inosine (No. 6), by dissolving the above two materials (No. 4), by dissolving the mono-potassium salt of inosine (No. 8—13), and by dissolving both hemi- and mono-potassium salts (No. 7). Those in Table 3 show the results obtained by dissolving the alkaline metal salts of inosine in water at a constant temperature (0—50 °C). It took 16 hr for the equilibrium to be reached by tumbling the bottles holding the mixture on a water bath at a constant temperature. The mixture in the bottles was

quickly filtered with a glass filter, and the solutions and the residues were submitted to analysis.

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References

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