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The Extraction of Inosine with Liquid Anion Exchangers

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Inosine can be extracted with a liquid anion exchanger. The most useful pH values are about 10 and over 10 for extraction with *n*-octylamine and with methyltrioctyl ammonium chloride respectively; this suggests that the extraction is principally due to electrostatic attraction. The extraction processes may, on the basis of the experimental results, be represented as follows:

$$\mathrm{HxR} + 2\mathrm{OA} = \mathrm{HxR}\cdot 2\mathrm{OA}$$

$$\mathrm{HxR}^- + (\mathrm{Q}^+\cdot\mathrm{Cl}^-) = (\mathrm{Q}^+\cdot\mathrm{HxR}^-) + \mathrm{Cl}^-$$

The isolation of inosine is of fundamental importance not only for biochemical study, but also for the industrial production of seasoning.[1,2] Although ion-exchanging resins have often been used for the separation of nucleic bases or nucleosides in the hydrolysate of nucleic acids,[3] and also for the isolation of inosine from the fermentation broth,[4] there are some defects in this process from the commercial point of view. The extraction of inosine with liquid anion exchangers, a method which has been studied as offering an improvement of the discontinuity of the treatment with the resins, will be described in this paper.

Experimental

Materials. The inosine was of the best commercial grade produced by the Ajinomoto Co. and was used without further purification.

Methyltrioctyl ammonium chloride (MTA), a product of the Kao Soap Co., mixed with the same volume of benzene (benz.) or kerosene (ker.), was washed with the same volume of a 2 N sodium hydroxide solution and, if necessary, twice with the same volume of 2 N hydrochloric acid, and finally with a proper amount of water.

The *n*-octylamine (OA) and *n*-octanol (OcOH) were of a commercial grade, which had been prepared by the Tokyo Kasei Co., and were used without further purification.

Procedure. The required liquid anion exchanger thus prepared was diluted to a proper degree with benzene, kerosene, or *n*-octanol. Twenty mililiters of this solution were mixed and vigorously shaken at room temperature for some 5 min with the same volume of an inosine solution, during which time the concentration and pH were adequately adjusted.

The mixture was then separated into two phases by letting it stand or by centrifugation. No change in the volumes of the two phases was observed during the extraction unless an emulsion formed.

The concentrations of inosine in both phases were, by the method which has been described by Wyatt,[5] determined spectrophotometrically after a paper-chromatography run in a solvent system composed of *n*-butanol, acetic acid, and water (volume ratio 4 : 1 : 1).

The concentration of *n*-octylamine was calculated from the total nitrogen value by the micro-Kjeldahl method. Methyltrioctyl ammonium chloride was analyzed by Metcalfe's method.[6]

Results

The Effect of the pH. The effect of the pH on the destribution ratio (D) is shown in Fig. 1. The distribution ratio is the ratio of the concentrations in the two phases. Thus:

$$D = \frac{(\mathrm{HxR})_\mathrm{O}}{(\mathrm{HxR})_\mathrm{W}}$$

where o and w represent the organic phase and the water phase respectively.

The higher the pH, the faster the phases separated in all the systems; *n*-octylamine in octanol formed a stable emulsion with water at pH values lower than 9.

The change in the net charges of the reactants with the pH is shown in Fig. 2.

The Concentration Effect of the Ion Exchanger. In order to determine the species of inosine extracted, the distribution ratio (D) was measured as a function of the ion-exchanger concentration. The results are shown in Tables 1 and 2 and in Fig. 3, where C_1=the concentration of *n*-octylamine, methyltrioctyl ammonium chloride,

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1) Japanese Pat. 14550 (1961).
2) Japanese Pat. 18319 (1965).
3) S. P. Colowick and N. O. Kaplan, editors "Methods in Enzymology," Vol. 3, Academic Press, New York (1957), Section 5.
4) Japanese Pat. 2886 (1964).
5) G. R. Wyatt, "The Nucleic Acid," Vol. I, Academic Press, New York (1955), p. 243.
6) L. D. Metcalfe, *Anal. Chem.*, **32**, 70 (1960).

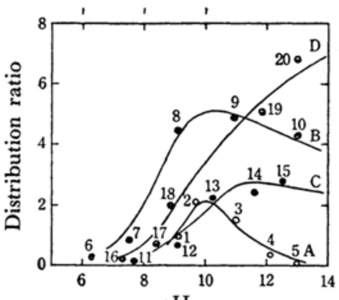

Fig. 1. Effect of pH.

Initial inosine concentration in water was about 2 g/d*l*. Organic phase was composed of the same volume of anion exchanger and diluent.

	A	B	C	D
Anion exchanger	MTA	OA	MTA	MTA
Diluent	OcOH	ker.	OcOH	benz.
Inosine concn. in water phase at equil. (g/d*l*)	^{1}0.56	^{6}0.65	^{11}0.95	^{16}0.85
	^{2}0.36	^{7}0.59	^{12}0.71	^{17}0.71
	^{3}0.56	^{8}0.15	^{13}0.30	^{18}0.42
	^{4}0.74	^{9}0.15	^{14}0.31	^{19}0.21
	^{5}0.78	^{10}0.15	^{15}0.25	^{20}0.15

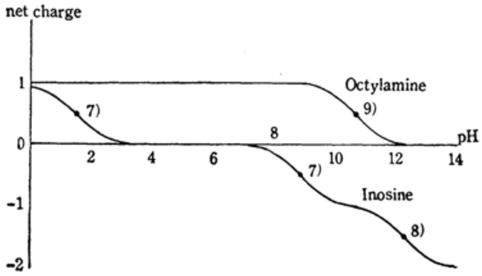

Fig. 2. Plot of net charge *vs.* pH.

pK_a values are cited from following literatures.
7) A. Albert, *Biochem. J.* (*London*), **54** 646 (1953).
8) P. A. Levene, H. S. Simms and L. W. Bass, *J. Biol. Chem.*, **70** 243 (1926).
9) C. W. Hoerr, M. R. McCorkle and A. W. Ralston, *J. Am. Chem. Soc.*, **65**, 328 (1943).

or methyltrioctyl ammonium hydroxide, and C_2= the concentration of the extracted inosine in the organic phase.

The Concentration Effect of Inosine. The effect of the inosine concentration in the water phase is shown in Table 3 and Fig. 4, where is

TABLE 1. EFFECT OF *n*-OCTYLAMINE (OA) CONCENTRATION IN ORGANIC PHASE

n-Octylamine mol/*l* (C_1)*1	Final pH in water phase*2	Inosine in org. phase mol/*l* (C_2)	Distribution ratio (D)	C_1-C_2
4.0	10.0	0.047	3.23	3.95
3.0	10.0	0.048	2.75	2.95
2.4	9.6	0.038	2.25	2.36
2.0	9.6	0.023	1.18	1.98
1.7	10.2	0.017	0.83	1.68
1.2	10.2	0.009	0.40	1.19
0.6	10.0	0.003	0.14	0.60

*1 Diluted with *n*-octanol
*2 Adjusted with 4 N HCl

TABLE 2. EFFECT OF THE CONCENTRATION OF METHYLTRIOCTYL AMMONIUM CHLORIDE (MTAC) OR METHYLTRIOCTYL AMMONIUM HYDROXIDE (MTAH)

Anion exchanger	Concn. of MTAC or MTAH*1 mol/*l* (C_1)	Ionsine in org. phase mol/*l* (C_2)	Distribution ratio (D)	C_1-C_2
MTAC	0.48	0.032	10.1	0.45
	0.40	0.030	8.3	0.37
	0.30	0.029	5.5	0.27
	0.20	0.027	3.6	0.17
	0.10	0.022	1.7	0.08
	0.05	0.016	0.8	0.03
MTAH	0.43	0.033	7.8	0.40
	0.27	0.033	7.8	0.24
	0.18	0.032	5.7	0.15
	0.09	0.027	2.7	0.06
	0.04_5	0.021	1.3	0.02

Final pH was adjusted between 11 and 12 with 4 N HCl.
*1 Diluted with kerosene

TABLE 3. EFFECT OF INOSINE CONCENTRATION

Inosine concn., mol/*l*		Chlorine concn. in water phase mol/*l*	Distribution ratio (*D*)	K_{Cl}^{HxR}
Org. phase	Water phase			
0.0072	0.00025	0.028	28.8	1.7
0.0140	0.00100	0.036	14.0	1.1
0.0205	0.00186	0.041	11.0	1.0
0.0272	0.00253	0.050	10.7	1.2
0.0329	0.00436	0.054	7.5	0.9
0.0486	0.00730	0.072	6.7	1.1
0.0631	0.0114	0.091	5.5	1.2
0.0902	0.0216	0.128	4.2	1.4
0.115	0.0342	0.173	3.4	1.7
0.140	0.0465	0.212	3.0	2.2

Final pH was adjusted between 10 and 11 in water phase.

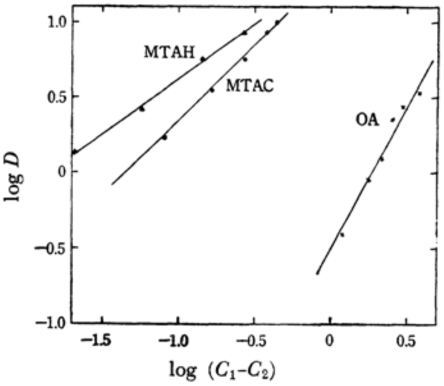

Fig. 3. The dependance of *D* on the concentration of residual anion exchanger.

also shown the apparent selectivity constant of inosine to the chlorine ion, which is calculated from the following equation:

$$K_{Cl}^{HxR} = \frac{(HxR)_O \cdot (Cl)_W}{(Cl)_O \cdot (HxR)_W}$$

The chlorine concentration in water was measured at equilibrium by Mohr's method.

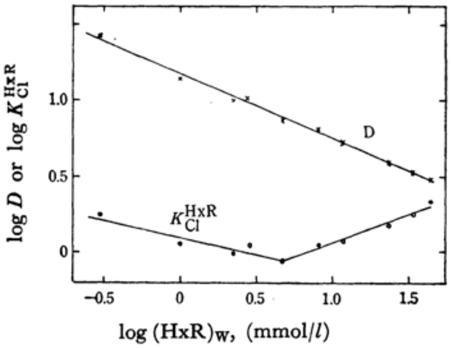

Fig. 4. Effect of inosine concentration.

Discussion

It is clear from Figs. 1 and 2 that, in the octylamine system, the distribution ratio depends closely on the difference in the net charges of the reactants.

Because of the ionic dissociation, the quarternary ammonium group keeps a positive charge at higher pH values than in the case of octylamine; this may account for the difference between Curve A and the others in Fig. 1.

Thus, the effect of pH suggests that the extraction is principally due to a electrostatic attraction similar to the adsorption by anion-exchanging resins.

If the extraction process of inosine (HxR) with *n*-octylamine (OA) is written as follows:

$$HxR + 2OA = HxR \cdot 2OA \qquad (1)$$

then the equilibrium constant is expressed as:

$$K = \frac{(HxR \cdot 2OA)}{(HxR)(OA)^2}$$

and the distribution ratio is given by:

$$D = \frac{(HxR \cdot 2OA)}{(HxR)} = K(OA)^2$$

Hence:

$$\log D = 2 \log (OA) + \text{const.} \qquad (2)$$

where OA is nearly equal to $(C_1 - C_2)$ in Fig. 3.

Moreover, if the process of the extraction with the quarternary ammonium chloride (Q^+–Cl^-) is written as follows:

$$\begin{aligned} HxR^- &+ (Q^+\text{-}Cl^-) \\ &= (Q^+\text{-}HxR^-) + Cl^- \qquad (3) \end{aligned}$$

the equilibrium constant and the distribution ratio are expressed as:

$$K = \frac{(HxR)_O(Cl)_W}{(Cl)_O(HxR)_W} \qquad \begin{aligned} &\text{o: organic phase} \\ &\text{w: water phase} \end{aligned}$$

$$D = \frac{(\mathrm{HxR})_\mathrm{O}}{(\mathrm{HxR})_\mathrm{W}} = K\frac{(\mathrm{Cl})_\mathrm{O}}{(\mathrm{Cl})_\mathrm{W}}$$

$$= K\frac{(\mathrm{Cl})_\mathrm{O}}{(\mathrm{HxR})_\mathrm{O}} = \frac{K(\mathrm{Cl})_\mathrm{O}}{(\mathrm{HxR})_\mathrm{O}} \qquad (4)$$

Therefore,

$$\log D = \log\,(\mathrm{Cl})_\mathrm{O} - \log\,(\mathrm{Cl})_\mathrm{W} + \text{const.} \qquad (5)$$

where $(\mathrm{Cl})_\mathrm{O}$ is equal to (C_1-C_2) in Fig. 3.

The same discussion holds in the case of the extraction with the quarternary ammonium hydroxide.

As Eq. (2) coincides with the experimental results, the species of inosine extracted with *n*-octylamine may be considered to be a complex composed of one mole of inosine and two moles of the amine.

In Fig. 3, the inclination of (MTAC) is steeper than that of (MTAH), but they are both about unity. This fact agrees well with Eq. (5), because the effect of the chlorine ion concentration in the water phase on the distriubtion ratio can not be neglected; on the other hand, the quantity of hydroxide ions produced in the ion-exchanging reaction is negligibly small at pH values so high as in this experiment.

Therefore, the process of the extraction with the quarternary ammonium chloride may be expressed as Eq. (3).

In Table 3, the chlorine concentration in the water phase is more than the inosine concentration in the organic phase; this means that the quantity of chlorine ions released from the anion exchanger is more than that of the inosine extracted.

This may be supposed to be due to the partial hydrolysis of the quarternary ammonium chloride, which may follow the inosine extraction, because, as has been discussed above, it is not considered that one mole of inosine replaces two moles of the chlorine ion.

The discussion in this paper may also apply to the extraction of nucleic bases and other nucleosides with liquid anion-exchangers.

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