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Spectrophotometric Determination of Aluminum by Reaction with Ethyl Pyridoxyliminopyruvate; An Attempt at Analytical Application of an Enzyme Model

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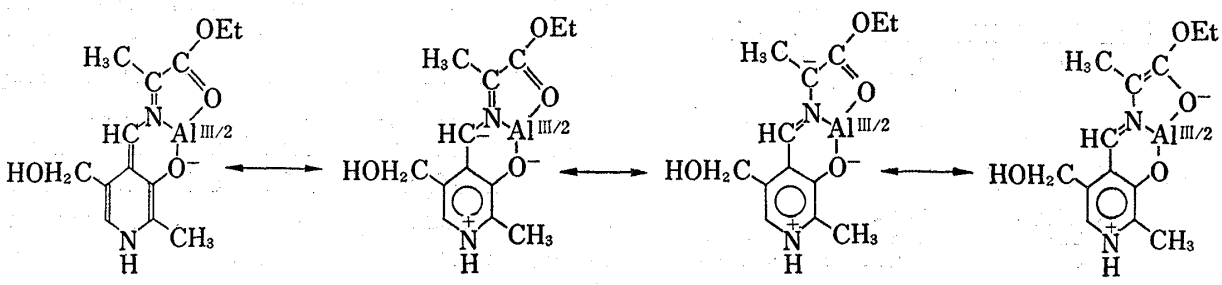
Aluminum can be determined by reaction with ethyl pyridoxyliminopyruvate in methanol. The neutral methanolic solution of pyridoxamine and a methanolic solution of ethyl pyruvate are mixed and heated at 70° for 15 min. This Schiff base solution is added to the methanolic solution of a sample containing aluminum nitrate. The time-absorbance curves have to be recorded for each sample, because the coloration of species absorbing at 488 nm is unstable. Calibration curve is obtained by plotting the maximum values in the time-absorbance curve against aluminum concentration. This spectrophotometric method can be used in the concentration range of 0.89 to 2.11 μg/ml of aluminum.

Keywords——aluminum; ketimine Schiff base; aluminum chelate; metal ion-catalyzed transamination reaction; colorimetry; pyridoxamine; ethyl pyruvate

Enzymes have been employed in analytical chemistry, because of their high sensitivity and selectivity. However, the enzymic methods have some defects[2] and the preparation of enzymes is often troublesome and tedious. Therefore, in order to find an analytical method which would be as specific as an enzymic analysis, the authors directed their attention to nonenzymic model reactions, and possibility of the use of these reactions was examined.

Matsumoto and Matsushima[3] reported that the ketimine Shiff base derived from pyridoxamine and ethyl pyruvate reacts specifically with aluminum and forms a species which exhibits an intense absorption band at 488 nm. This absorption band was very similar to that observed in an enzymic reaction.[4] The absorption intensity reached its maximum 15 min after the addition of aluminum, then decreased gradually, and disappeared in several hours. The final spectrum had the absorption characteristics of aldimine-metal chelate derived from pyridoxal, an amino acid derivative, and a metal ion. Thus, it has become apparent that the species absorbing at 488 nm is an intermediate in the transamination reaction. They gave the following resonance structures to the intermediate.



1) Location: *Gofuku, Toyama.*
2) I. Fridovich, *J. Biol. Chem.*, **239**, 3519 (1964).
3) S. Matsumoto and Y. Matsushima, *J. Am. Chem. Soc.*, **94**, 7211 (1972); *idem, ibid.*, **96**: 16, 5228 (1974).
4) Y. Morino and E.E. Snell, *J. Biol. Chem.*, **242**, 2800 (1967).

Based on these findings, We elaborated a specific assay method for aluminum.

Experimental conditions for the Schiff base formation were examined first. Because the Schiff base[5] exhibits maximum absorption at 333 nm, the effect of temperature at this wavelength was examined and its results are given in Fig. 1. The optimal temperature for the Schiff base formation was 70°. When the solution was cooled in ice after completion of the reaction, the Schiff base was stable for 10 hr.

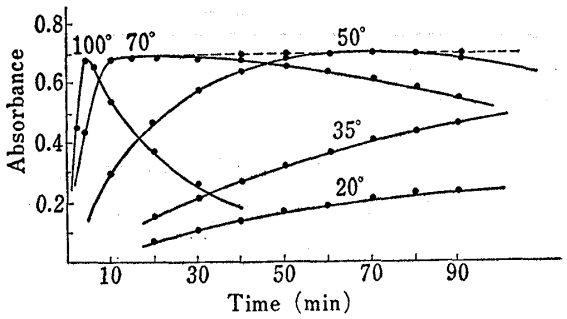


Fig. 1. Effect of Temperature on Time-Absorbance Curves for Schiff Base Formation at 333 nm

The Schiff base formation was incomplete in methanol containing water. The yield in methanol containing 10% water was 65.1% of that in anhydrous methanol. When a colored substance formed, the maximum absorbance was obtained in anhydrous methanol and the absorbance was markedly decreased in the presence of water. These results are given in Table I.

Table I. Effect of Water

H_2O content in MeOH (%, v/v)	Schiff base absorbance	Absorbance of colored substance	
		Expt. 1	Expt. 2
0	0.7	0.84	0.84
0.5	0.7	0.43	0.62
1	0.68	0.24	0.45
10	0.46	0.04	0.13

Expt. 1 indicates the effect of water content on absorbance at 488 nm, when aluminum nitrate in anhydrous methanol was added to the Schiff base formed in methanol containing water. When aluminum nitrate dissolved in methanol containing water was added to the Schiff base formed in anhydrous methanol, the effect of water content is presented in Expt. 2.

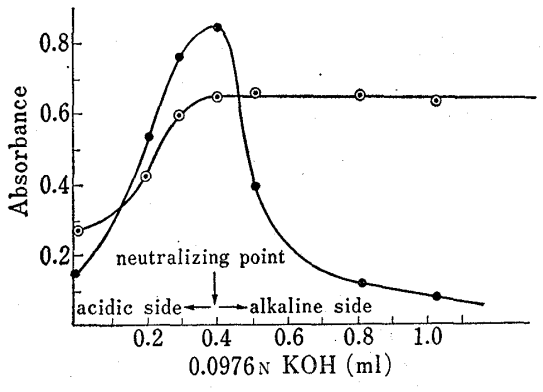


Fig. 2. Effect of Alkaline Methanol

—⊙—⊙—, Schiff base formation curve at 333 nm.
pyridoxamine-2HCl: 1×10^{-3} M
ethyl pyruvate: 1×10^{-1} M
The reaction solution was diluted with 5 volumes of methanol for spectrum measurement.

—●—●—, colored substance formation curve at 488 nm.
pyridoxamine-2HCl: 1×10^{-4} M
ethyl pyruvate: 1×10^{-2} M
aluminum nitrate: 0.5×10^{-4} M

In order to study the effect of alkali concentration on the formation of the Schiff base and the colored substance, a given volume of an alkaline methanol solution with known normality was added to pyridoxamine-2HCl. Its results are given in Fig. 2. Formation of both the Schiff base and the colored substance reached maximum at the neutralizing point (neutrality). On the alkaline side, however, the Schiff base formation did not vary so much, while formation of the colored substance decreased markedly. Furthermore, both formations decreased markedly in acidic condition.

In order to study the effect of anions, the reaction was allowed to proceed with alumi-

5) Y. Matsuo, *J. Am. Chem. Soc.*, 79, 2016 (1957).

num sulfate or aluminum chloride as aluminum nitrate. Absorbances at the maximum were 44% and 1% with sulfate and chloride, respectively. The reason for such a large dependence of absorbance on anions is not clear.

Besides Al(III), Ga(III) ion developed a similar absorption spectrum, and In(III) ion formed a broad absorption band at 500 nm. No visible absorption was produced in 480—500 nm region by Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Zn(II), Cd(II), Pd(II), Ba(II), Hg(II), Sn(II), Mg(II), and Ca(II) ions. Matsushima[3] reported that Cu(II) ion did not exhibit any absorption under these conditions, but it was found in the present study that although Cu(II) ion in a high concentration (31.8 μg/ml) did not exhibit the absorption, it did so in a lower concentration (3.18 μg/ml). An absorption band appeared at 500 nm reached its maximum in 3 min and disappeared rapidly.

Effect of temperature on the formation of the colored substance was examined. The time-absorbance curves at different temperatures were recorded at 488 nm. In the temperature range of 15—30°, only the time for the maximum yield of the colored substance varied, but the absorbance at maximum remained unchanged.

Table II. Effect of Coexisting Metal Ions

No.	Amount of aluminum (μg/ml)	Metal ion present (μg/ml)		Recovery of aluminum (%)
1	1.35	Nickel	0.58	100
2	1.35	Nickel	1.77	98.8
3	1.35	Nickel	2.36	102.4
4	1.35	Nickel	2.95	102.4
5	1.35	Magnesium	0.24	102.0
6	1.35	Magnesium	0.96	101.2
7	1.35	Magnesium	1.20	102.4
8	1.35	Zinc	0.65	98.8
9	1.35	Zinc	1.95	100
10	1.35	Zinc	2.60	98.8
11	1.35	Zinc	3.25	100

The calibration curve showed a linear relationship in the concentration range of 0.89—2.11 μg/ml of aluminum. In the assay of 2.11, 1.81, and 1.51 μg/ml of aluminum in four separate experiments, the standard deviations were 1.07, 0.82, and 1.22, respectively. Aluminum was determined in the presence of other metal ions by these assay procedures and its results, given in Table II, show no interference from Ni, Mg, or Zn.

Experimental

Chemicals——Pyridoxamine dihydrochloride and ethyl pyruvate were obtained from commercial sources. They were purified by recrystallization or by distillation before use. Inorganic substances were certified reagent grade chemicals and were used without further purification. Dotite Spectrosol "methanol" was used as a solvent.

Standard Aluminum Solution——To 0.0135 g of aluminum metal in a beaker, 20 ml of 10% nitric acid was added, the beaker was covered with a watch glass, and warmed gently in a water bath until aluminum dissolved completely. This solution was concentrated until a white solid appeared and then evaporated to dryness in an evacuated chamber. The semi-crystalline $Al(NO_3)_3$ thus obtained was dissolved in 50 ml of MeOH and 1 ml of this solution was diluted to 10 ml with MeOH. Each milliliter of this final MeOH solution (1×10^{-3} M) contained 27 μg of Al. A working solution was prepared by diluting this stock solution with MeOH.

Spectrophotometry——Absorption spectra were taken with a Hitachi Model 124 recording spectrophotometer (Hitachi, Ltd. Tokyo) with a 10 mm cell-path.

Assay Procedure——The standard procedure finally developed was as follows: A solution of pyridoxamine was prepared by dissolving its dihydrochloride in MeOH with an equimolar amount of KOH. The

neutral MeOH solution (10 ml) of pyridoxamine (2×10^{-3} M) and a MeOH solution (10 ml) of ethyl pyruvate (2×10^{-1} M) were mixed and sealed in an ampule. The ampule was allowed to stand at 70° for 15 min and then cooled in ice water. To the MeOH sample solution (9 ml) containing a given quantity of $Al(NO_3)_3$, this Schiff base solution (1 ml) was added with stirring. The moment of the addition of Al^{3+} was taken as the initiation of the reaction. An aliquot was placed in a glass-stoppered 10 mm glass cell and submitted to absorption measurement. Time-absorbance curves were recorded at 488 nm with pure MeOH as a blank. Calibration curves were obtained by plotting the maximum values in the time-absorbance curve against aluminum concentration. Mixing of the solutions and the spectral measurement were carried out at room temperature.

Conclusion

The present work may be characterized by the fact that the nonenzymic transamination reaction can be applied to the determination of aluminum, although a number of analytical methods for aluminum have already been presented. This method is appreciably high in specificity, compared with other colorimetric method[6] in which the presence of Fe(III), Cr(III), Sn(II), Hg(II), Ca(II), and Ba(II) ions interfere with the determination and the assay conditions of which is troublesome. The chief inhibitor in the present method will be only copper in practical analysis of metals, although Cu(II), Ga(III), and In(III) ions are inhibitory to the reaction. It is the defect of this analytical method that water cannot be used as a solvent, and because the coloration is unstable, the time-absorbance curves have to be recorded for each sample. Further, sulfate and chloride anions affect the reaction, so that it requires the use of the nitrate, and for this reason, metals are dissolved in diluted nitric acid before use; this method is therefore appropriate for the assay of aluminum alloys.

6) O.B. Winter, W.E. Thrum, and O.D. Bird, *J. Am. Chem. Soc.*, **51**, 2721 (1929); L.P. Hammett and C.T. Sottery, *ibid.*, **47**, 142 (1925).