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Fluorometric Method for Determination of Mercury(II) with Rhodamine B

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Mercury(II) is able to quench the fluorescence of Rhodamine B in the presence of potassium iodide. The quenching was affected by pH of the solution and the concentration of potassium iodide, but not markedly by several anions. The degree of quenching was closely correlated with mercury(II) concentrations at the fixed concentrations of the dye and potassium iodide, so that mercury(II) can be fluorometrically determined in this method. Cystline and egg albumin protected the quenching by mercury(II) in the presence of potassium iodide. The fluorescence of Rhodamine B was not quenched by organic mercury(II) compounds in the presence of potassium iodide. Fluorescence of the dye was also quenched by some metal ions, such as bismuth(III), thallium(III), palladium(II) and platinum(IV) in the presence of potassium iodide.

Mercury is a violently poisonous heavy metal and comes frequently into the problem of public. Numerous methods for the determination of mercury(II) have been reported. Among them, colorimetric methods for mercury(II) were commonly used in various fields, but it was often interfered by some metals which were difficult to separate from it. New specific methods for mercury(II) determination in the presence of other metals are desirable.

It is known that Rhodamine B has a very intensive, orange fluorescence in a dilute solution and combines with mercury (II) at the concentration of less than 3M hydrochloric acid.[2]

It was found that the fluorescence of Rhodamine B is quenched with mercury(II) in the presence of potassium iodide, and the present report is on a method for fluorometric determination of mercury(II).

Experimental

Materials——Egg albumin was a product (Lot 5901210) of E. Merck Ag. Darmstadt. All of other reagent used were commercial analytical grade and were used without further purification. A 1.0% egg albumin solution was prepared by dissolving in deionized water and adjusting to pH 8.5 with 1N NaOH. Stock solutions (10^{-2}M) of $CaCl_2$, $MgCl_2$, $CdCl_2$, $FeCl_3$, $MnCl_2$, $TlCl_3$, $PtCl_4$, $SnCl_2$, $CuSO_4$, $SrSO_4$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Be(NO_3)_2$, $NaAsO_2$ and lead acetate were made by dissolving each compound in deionized water and 10^{-2}M $SbCl_3$ solution in ethanol. Solutions (10^{-2}M) of $ZnCl_2$, $AlCl_3$ and $PdCl_2$ were made by the addition of a minimum volume of 1N HCl until disappear of white turbidity. A solution of 10^{-2}M $Bi(NO_3)_3$ was prepared by the addition of a minimum volume of conc. HNO_3 to give a clear solution. Phenylmercuric acetate and ethylmercuric chloride were dissolved in deionized water and ethanol, respectively to form a 10^{-2}M solution. *p*-Chloromercuribenzoic acid was dissolved in a minimum volume of 0.1N NaOH and adjusted to 10^{-2}M with deionized water. Freshly prepared 10^{-3}M cysteine solution was used. The solutions of 10^{-3}M $HgCl_2$ and 10^{-2}M KI were mainly used in the experiments. The buffer solutions were prepared by the method of Britton and Robinson.[3] Solution of 1.0M sodium acetate was also adjusted to pH 4.5 with 1.0M acetic acid.

Methods——Visible absorption spectra were taken with a Hitachi recording spectrometer, model EPS-3, by the use of cells with a 10 mm light path. Fluorescence emission spectra and their intensity were measured with an Aminco-Bowman Spectrofluorometer by the use of a quartz cell with 10 mm light path, slit width of 2 mm, and activation at 486 mμ. pH was measured with a Hitachi-Horiba pH meter, M-5.

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[1] Location: *5-9-1, Shirogane, Minato-ku, Tokyo.*

[2] E.B. Sandell, "Chemical Analysis" Vol. 3, Interscience Publishers, Inc., New York, 1959, pp. 210—213.

[3] Britton and Robinson, *J. Chem. Soc.*, **1931**, 458, 1456.

Results

The dilute solution of Rhodamine B shows a bluish-red color with orange fluorescence. As can be seen in Fig. 1, visible absorption spectra of the dye did not change in the presence of equimolar concentration of mercury(II) or 10 times one of potassium iodide, and only slightly in the presence of both. It seems, therefore, that Rhodamine B is not a good indicator for colorimetric determination of mercury(II) under the conditions used. Its fluorescence emission spectra are shown in Fig. 2, in which the intensity at 586 mμ was approximately 2-fold higher in 90% ethanol than in aqueous solution. The intensity did not change by the addition of equimolar concentration of mercury(II) in both solvents. The fluorescence of the dye was almost quenched in the presence of equimolar concentration of mercury(II) by the addition of 10 molar excess of potassium iodide in aqueous solution, but only slightly in 90% ethanol. Fluorescence peak of the dye at 487 mμ in the presence of both mercury(II) and potassium iodide may be due to random dispersion of excitation light with the colloid formed from the reactants. An examination was made on the effect of the concentration of potassium iodide on the fluorescence quenching of the dye with mercury(II). As shown in Fig. 3, the intensity did not decrease in the concentration below 7×10^{-5}M KI, but did completely of 2×10^{-4}M KI to 4×10^{-5}M Rhodamine B and mercury(II).

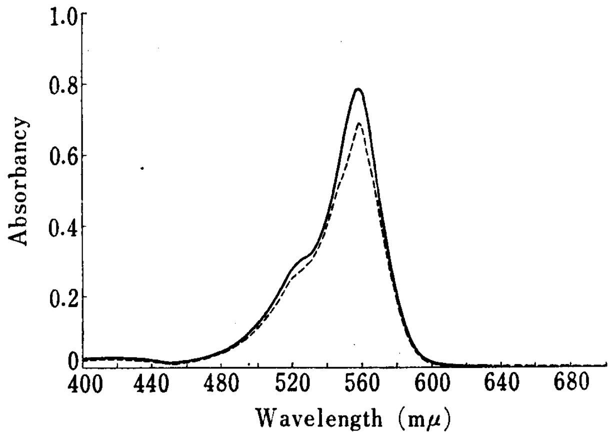

Fig. 1. Absorption Spectra of Rhodamine B in the Presence of Mercuric Chloride and Potassium Iodide

——: 8×10^{-6}M Rhodamine B, 8×10^{-6}M Rhodamine B and $HgCl_2$, or 8×10^{-6}M Rhodamine B and 8×10^{-5}M KI
------: 8×10^{-6}M Rhodamine B and $HgCl_2$, and 8×10^{-5}M KI

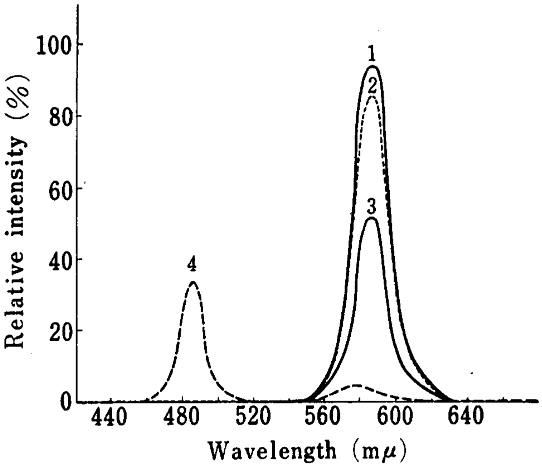

Fig. 2. Fluorescence Emission Spectra of Rhodamine B in the Presence of Mercuric Chloride and Potassium Iodide

1. 4×10^{-5}M Rhodamine B, 4×10^{-5}M Rhodamine B and $HgCl_2$, or 4×10^{-5}M Rhodamine B and 4×10^{-4}M KI in 90% ethanol
2. 4×10^{-5}M Rhodamine B and $HgCl_2$, and 4×10^{-4}M KI in 90% ethanol
3. 4×10^{-5}M Rhodamine B, 4×10^{-5}M Rhodamine B and $HgCl_2$, or 4×10^{-5}M Rhodamine B and 4×10^{-4}M KI in aqueous solution
4. 4×10^{-5}M Rhodamine B and $HgCl_2$ and 4×10^{-4}M KI in aqueous solution

Bouchard4) reported that the fluorescence of Rhodamine B was quenched by some anions including KI. It might, thus, be thought that fluorescence quenching by mercury(II) was markedly accelerated by some other anions than KI. As shown in Table I, however, the fluorescence intensity was not affected in the 1/125 concentrations of half-quenching ones of $NaNO_2$, $Na_2S_2O_3$, KBr and KCl in the presence of 4×10^{-5}M $HgCl_2$, but did slightly in that of KCNS. It is, thus, probable that the fluorescence quenching of Rhodamine B is specific to both mercury(II) and potassium iodide. The above facts suggest that mercury(II) can

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4) J. Bouchard, *J. Chim. Phys.*, 33, 325 (1936).

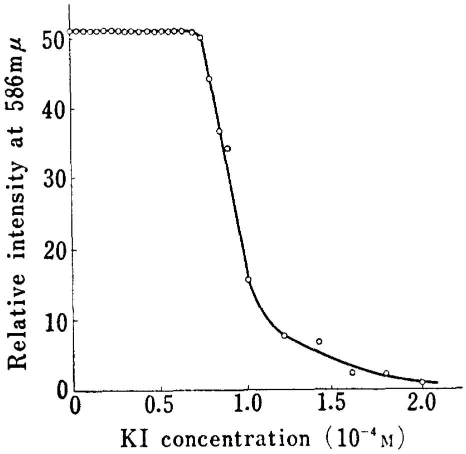

Fig. 3. Effect of the Concentration of Potassium Iodide on the Quenching of Rhodamine B with Mercury (II)

Reaction mixture consisted of 4×10^{-5}M Rhodamine B and $HgCl_2$, and indicated amount of KI in a total volume of 5.0 ml.

Table I. Effect of Some Quenching Reagents on the Fluorescence of Rhodamine B in the Presence of Mercury (II)

Reagent	Half-quenching concentration[a] (M)	Relative intensity
None	—	49.8
KCl	0.5	49.8
KBr	0.15	49.8
KI	0.05	2.8
KCNS	0.16	46.3
$Na_2S_2O_3$	0.12	49.8
$NaNO_2$	0.7	49.8

a) Values presented by Bouchard[4]-Reaction mixture consisted of 4×10^{-5}M Rhodamine B and $HgCl_2$, and 1/125 concentration of half-quenching of each salt in a total volume of 5.0 ml.

be fluorometrically determined with Rhodamine B in the presence of potassium iodide in aqueous solution.

Effect of pH on the fluorescence quenching is shown in Fig. 4, in which the fluorescence was not quenched at pH higher than 7.0, but completely at pH lower than 4.0 with Britton-Robinson's buffer. Standard curve for mercury(II) determination is presented in Fig. 5. The fluorescence intensity decreased linearly in concentrations lower than 1×10^{-5}M $HgCl_2$.

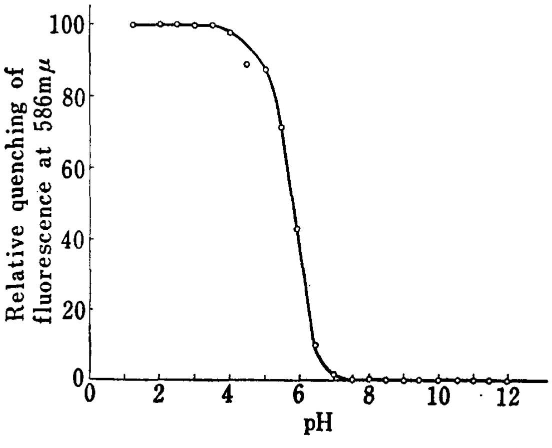

Fig. 4. Effect of pH on the Quenching of Rhodamine B with Mercury (II) and Potassium Iodide

Reaction mixture consisted of 4×10^{-5}M Rhodamine B and $HgCl_2$, 4×10^{-4}M KI and Britton-Robinson's buffer of indicated pH value in a total volume of 5.0 ml.

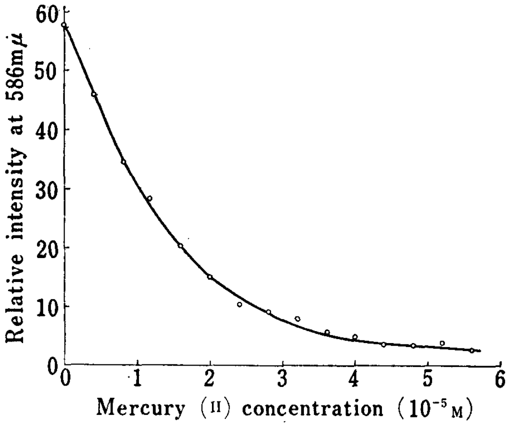

Fig. 5. Standard Curve for Mercury (II) Determination by Fluorometry

Reaction mixture consisted of 4×10^{-5}M Rhodamine B, 4×10^{-4}M KI, and indicated amount of $HgCl_2$ in a total volume of 5.0 ml.

Effect of various anions on mercury(II) determination was investigated (Table II). The determination gave slightly low results in the presence of 0.1M phosphate, borate, malonate, nitrate or glycine and 10^{-2}M tartrate, but not of 0.1M citrate, lactate, sulfate or phthalate in 0.8M acetate buffer (pH 4.5).

The acetate buffer also showed little effect on the mercury(II) determination.

Table II. Effect of Several Anions on the Fluorescence Quenching of Rhodamine B with Mercury (II)

Anion	Quenching (%)	Anion	Quenching (%)
None	100.0	Tartrate	97.5
Phosphatde	91.5	Citrate	100.0
Borate	94.1	Lactate	100.9
Malonate	94.1	Sulfate	101.7
Nitrate	95.8	Phthalate	102.5
Glycine	96.6		

Reaction mixture consisted of 4×10^{-5}M $HgCl_2$ and Rhodamine B, 4×10^{-4}M KI, 0.8M acetate buffer (pH 4.5) and 0.1M of each anion except tartrate which was 10^{-2}M, in a total volume of 5.0 ml.

Examination was made on whether fluorescence quenching of the dye was specific to inorganic mercury(II) or not. As can be seen in Table III, 4×10^{-5}M ethylmercuric chloride or *p*-chloromercuribenzoate did not affect the fluorescence intensity of Rhodamine B in the presence of 4×10^{-4}M KI, while 4×10^{-5}M phenylmercuric acetate slightly decreased its intensity.

Table III. Failure of the Reaction of Organic Mercury (II) Compounds with Rhodamine B

Material		Decrease of relative intensity at 587 mμ
Ethylmercuric chloride	− KI[a]	0
	+ KI[b]	0
p-Chloromercuribenzoate	− KI[a]	0
	+ KI[b]	0
Phenylmercuric acetate	− KI[a]	0
	+ KI[b]	6.7

a) Reaction mixture consisted of 4×10^{-5}M Rhodamine B and organic mercury (II) compound in a total volume of 5.0 ml.
b) Reaction mixture consisted of 4×10^{-5}M Rhodamine B and organic mercury (II) compound, and 4×10^{-4}M KI in a total volume of 5.0 ml.

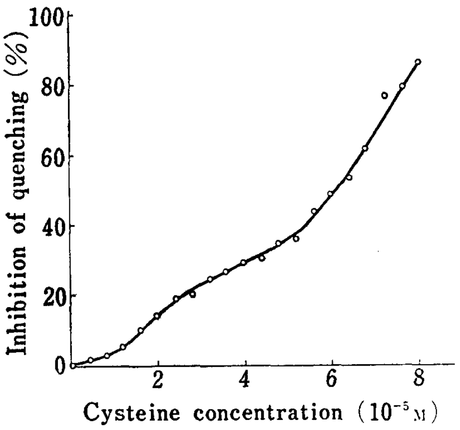

Fig. 6. Effect of Cysteine on the Quenching of Rhodamine B by Mercury (II) and Potassium Iodide

Reaction mixture consisted of 4×10^{-5}M Rhodamine B and $HgCl_2$, 4×10^{-4}M KI, and indicated amount of cysteine in a total volume of 5.0 ml.

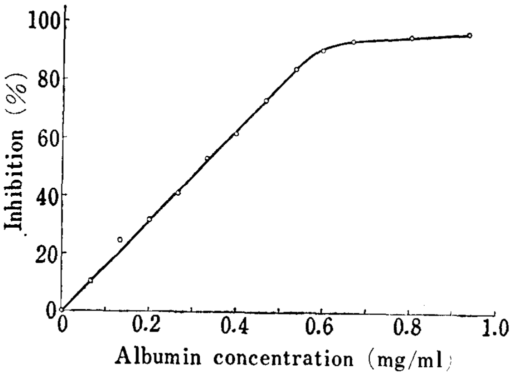

Fig. 7. Effect of Egg Albumin on the Quenching of Rhodamine B by Mercury (II)

Reaction mixture consisted of 4×10^{-5}M Rhodamine B and $HgCl_2$, 4×10^{-4}M KI, 0.4M acetate buffer (pH 4.5), and indicated amount of egg albumin in a total volume of 5.0 ml.

The same concentration of latter compound became turbid with the addition of only 4×10^{-4}M KI. It seems that the fluorescence quenching by phenylmercuric acetate is due to the formation of such precipitate.

Effect of cysteine on fluorescence quenching of the dye with mercury(II) and potassium iodide was examined, since some sulfhydryl compounds could form insoluble mercaptide with mercury(II) (Fig. 6). Equimolar and 2-fold excess concentrations of cysteine to mercury(II) could protect 30 and 86% of the quenching, respectively.

Mercury(II) can also combine with and precipitate proteins. When egg albumin was used as a protein, the quenching with mercury(II) was inhibited by it, which protected 90% of the quenching in a concentration of 0.6 mg/ml(Fig. 7). This fact suggests that mercury(II) determination in biological materials must take the presence of sulfhydryl groups or protein-like substances into consideration.

The specificity of this method to other various metal ions is shown in Table IV. The fluorescence quenching of Rhodamine B was observed by cadmium(II), iron(III), antimony(III), bismuth(III), thallium(III), palladium(II) and platinum(IV), especially the latter four metal ions, but not metal ions other than these, in the presence of 4×10^{-4}M KI. It is necessary to remove these interfering metal ions, if present, before mercury(II) determination by this method.

Table IV. Interaction of Various Metal Ions with Rhodamine B

Metal salt	Decrease of relative intensity at 586 mμ		Metal salt	Decrease of relative intensity at 586 mμ	
	−KI[a]	+KI[b]		−KI[a]	+KI[b]
$CaCl_2$	0	0	$Co(NO_3)_2$	0	0
$MgCl_2$	0	0	Lead acetate	0	0
$ZnCl_2$	0	0	$NaAsO_2$	0	0
$SnCl_2$	0	0	$Bi(NO_3)_3$	9.2	39.6
$MnCl_2$	0	0	$CdCl_2$	11.5	11.5
$AlCl_3$	0	0	$FeCl_3$	1.9	1.9
$CuSO_4$	0	0	$SbCl_3$	7.1	7.9
$SrSO_4$	0	0	$TlCl_3$	3.8	45.4
$Be(NO_3)_2$	0	0	$PdCl_2$	5.0	45.0
$Ni(NO_3)_2$	0	0	$PtCl_4$	0.1	45.8

a) Reaction mixture consisted of 4×10^{-5}M Rhodamine B and the metal salt in a total volume of 5.0 ml.

b) Reaction mixture consisted of 4×10^{-5}M Rhodamine B and the metal salt, and 4×10^{-4}M KI in a total volume of 5.0 ml.

Discussion

Rhodamine B is able to combine with certain metals forming chloro acids to give red-violet salts, which are extractable with various organic solvents such as benzene. Gallium (III),[5] gold(III),[6] thallium(III)[7] and antimony(V)[8] can be determined sensitively in this way. It is also known that iron(III) forms its salt extractable with organic solvents and tungsten(IV) forms insoluble Rhodamine B tungstate, not extracted by benzene.[2]

Rhodamine B reacts, but not very sensitively, with a number of other elements such as molybdenum(IV), tantalum(V) and mercury(II) at acid concentration lower than 3M hydrochloric acid, below which some amounts of colorless lactone form of the dye are extracted with

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[5] H. Onishi and E.B. Sandell, *Anal. Chim. Acta*, **13**, 159 (1955).

[6] E.B. Sandell, *Anal. Chem.*, **20**, 253 (1948).

[7] H. Onishi, *Bull. Chem. Soc. Japan*, **29**, 945 (1956); H. Onishi, *ibid.*, **30**, 567 (1957).

[8] W.G. Fredrick, *Ind. Eng. Chem., Anal. Ed.*, **13**, 922 (1941); S.H. Webster and L.T. Fairhall, *J. Ind. Hyg. Toxicol.*, **27**, 184 (1945).

organic solvent and the solution becomes violet on standing as a result of slow hydrolysis of this lactone. Nonsensitive metal ions cannot be colorimetrically determined in this way, unless the excess dye was decolorized by oxidation with bromine, *etc.*

The fluorescence of Rhodamine B, on the other hand, could be quenched in the presence of $4\times10^{-5}\text{M}$ $HgCl_2$ and $4\times10^{-4}\text{M}$ KI, but not with each of them alone. The quenching occurred completely at pH lower than 4.0, but not higher than 7.0, while the fluorescence intensity of Rhodamine B itself is not affected by pH. Quenching of the dye with mercury(II) is dependent on the concentration of potassium iodide, and maximum quenching with $4\times10^{-5}\text{M}$ $HgCl_2$ is attained by $2\times10^{-4}\text{M}$ KI, of which concentration corresponds to 1/250 concentration of the half-quenching by potassium iodide itself. The fluorescence intensity was unaffected by 1/125 concentrations of other reagents, KCl, KBr, $NaNO_2$, $Na_2S_2O_3$ and KCNS in the presence or absence of $4\times10^{-5}\text{M}$ $HgCl_2$. The quenching was specific to both mercury(II) and potassium iodide, so that mercury(II) can be determined fluorometrically in this method. Mercury(II) determination was not affected by various anions, but by cysteine and egg albumin. Since organic mercury(II) compounds did not quench the fluorescence of Rhodamine B in the presence of potassium iodide, the quenching is specific to inorganic mercury(II).

This method has a limited use for mercury determination because of the interference by several metal ions other than mercury(II).