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Studies on Benzothiazole Derivatives as Chelating Agents. I. Syntheses of Benzothiazole Derivatives and Their Reactions with Metal Ions

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A number of 2-, 4- and 7-substituted benzothiazole derivatives were synthesized to inquire into the relationship between the fluorescence of metal chelates and their structures, and to investigate their applications as analytical reagents. The color and fluorescence reactions of metal ions with these benzothiazole derivatives were investigated by spot tests. On the formation of metal complexes in solution, the color change of 2-substituted derivatives was more remarkable than that of 4- and 7-substituted. As fluorometric reagents for metal ions, however, 4-aminobenzothiazole derivatives were superior to 2-amino and 7-aminobenzothiazole derivatives. 7-Substituted derivatives were inferior in the reactivity with metal ions to 2- and 4-substituted.

Non-fluorescent organic compounds capable of forming fluorescent chelates with metal ions in solution are of considerable value as analytical reagents of trace metal ions. Hitherto,

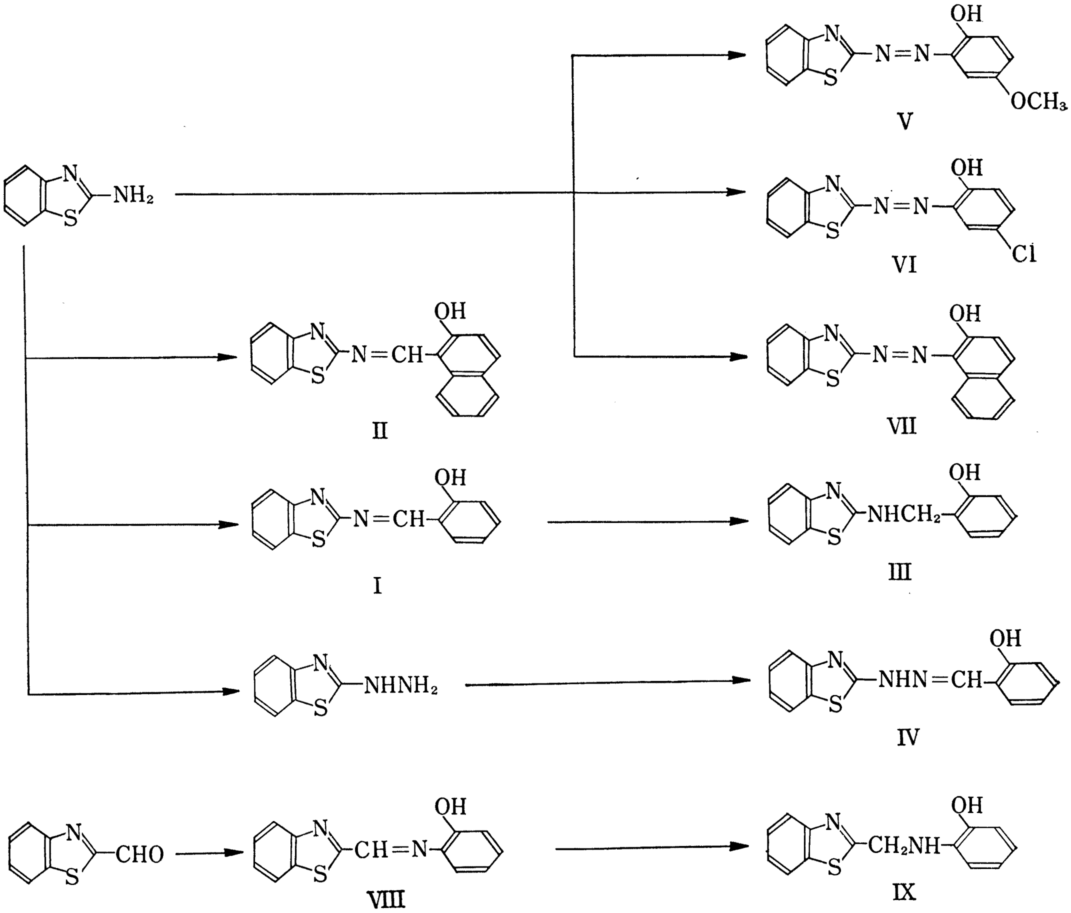

Chart 1. Syntheses of 2-Substituted Benzothiazole Derivatives

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[1] Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*

various fluorometric reagents have been developed and utilized for the microdetermination of metal ions, but the relationship between the fluorescence and the structures of reagents and their metal chelates has been scarcely clarified.

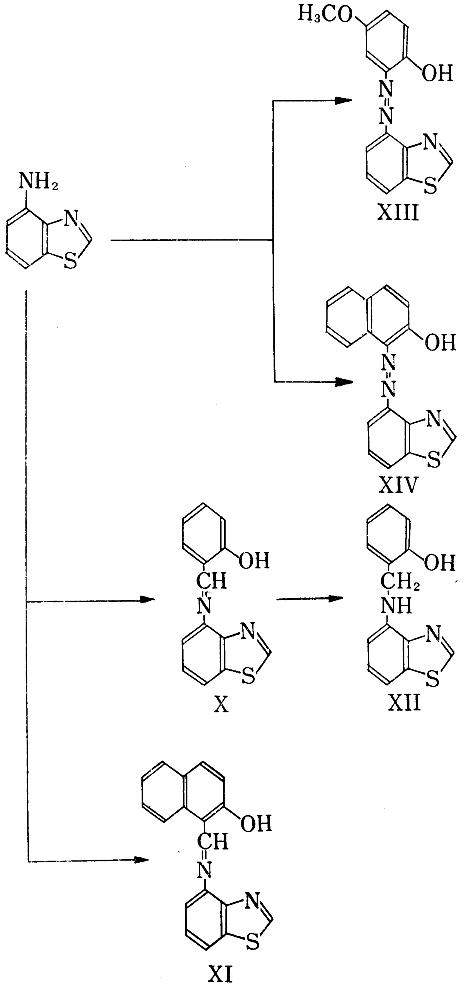

Chart 2. Syntheses of 4-Substituted Benzothiazole Derivatives

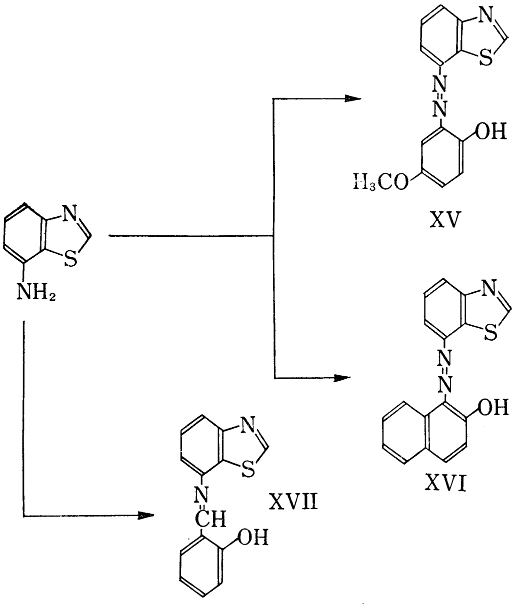

Chart 3. Syntheses of 7-Substituted Benzothiazole Derivatives

Freeman, *et al.*[2] explained the reason why non-fluorescenct *o,o'*-dihydroxyazo compounds fluoresce when they form chelates with metal ions as follows; these compounds have in common a single axis about which two major portions of the molecule can rotate to some degree. This is an ideal situation for the internal conversion, but the rotation is stopped when they form chelates with metal ions and the internal conversion of excited energy will not be allowed and fluorescence may occur. By the similar consideration, the fluorescence of some organic compounds has been accounted for.[3]

Though the above considerations may not account for all of the relationship between the fluorescence and the structure of organic compounds, it may be useful as a guiding principle for searching new chelating reagents applicable to the fluorometry. According to the scheme shown in Chart 1, 2 and 3, we synthesized new chelating reagents, having azo group, azomethine group or its reduction group as the axis of rotation, phenol or naphthol as one of the rotating portions of the molecule, and benzothiazole as the other. Benzothiazole ring has two coordinate atoms, nitrogen and sulphur, and the internal rotation of these compounds may be prohibited by the chelate formation. We intended to apply them to the determination of

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[2] D.C. Freeman, Jr. and C.E. White, *J. Am. Chem. Soc.*, **78**, 2678 (1956).

[3] E.J. Bowen and F. Wokes, "Fluorescence of Solutions," Longmans, London, 1953.

trace metal ions and to inquire into the relationship between the fluorescence and the structures of reagents and their metal chelates. The present paper deals with the syntheses of reagents and their color and fluorescence reactions with the following metal ions: Mg^{2+}, Al^{2+}, Ca^{2+}, Cr^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ga^{3+}, Sr^{2+}, Zr^{4+}, Pd^{2+}, Ag^{+}, Cd^{2+}, In^{3+}, Hg^{2+}, Pb^{2+}, Bi^{3+}, and UO_2^{2+}.

Result

2-Substituted Benzothiazole Derivatives

The intense color reactions of 2-substituted derivatives with metal ions are shown in Table I. Compound (VIII) reacts with divalent metal ions to produce red colored complexes,

TABLE I. Reactions of 2-Substituted Benzothiazole Derivatives with Metal Ions

Compound	Metal ion	Color		
		acid	neutral	alkaline
IV	Mn(II)			yellow
	Ni(II)	light green	light green	yellowish green
	Cu(II)	light green	light green	greenish brown
	Zn(II)			yellow
	Cd(II)			yellow
	Pb(II)			yellow
V	Mn(II)			greenish blue
	Co(II)	blue	blue	blue
	Ni(II)	greenish blue	greenish blue	greenish blue
	Cu(II)[a]	green	green	green
	Zn(II)	blue	blue	blue
	Ag(I)	violet	violet	violet
	Cd(II)	dark green	dark green	blue
	Hg(II)[b]	blue	blue	blue
	Pb(II)			greenish blue
	Bi(III)	dark green		
VI	Mn(II)			dark blue
	Co(II)			blue
	Ni(II)		green	blue
	Cu(II)		green	blue
	Zn(II)			dark blue
	Cd(II)			blue
	Hg(II)[c]		green	blue
	Pb(II)			violet
VIII	Mn(II)			purple red
	Co(II)	purple red	purple red	purple red
	Ni(II)	purple red	purple red	purple red
	Cu(II)[d]	purple red	purple red	purple red
	Zn(II)			purple red
	Pd(II)[e]	green	green	green
	Ag(I)			purple red
	Cd(II)			purple red
	Hg(II)	purple red	violet	violet
IX	Co(II)		pink	reddish violet
	Ni(II)		pink	reddish violet
	Cu(II)[f]	reddish violet	reddish violet	reddish violet
	Zn(II)			pink
	Pd(II)	green	green	green
	Ag(I)	green	green	green
	Cd(II)			pink
	Hg(II)	light red	violet	reddish violet

The limit of the detection: *a)* 0.1 μg/ml, *b)* 2 μg/ml, *c)* 2 μg/ml, *d)* 0.05μg/ml, *e)* 5 μg/ml, *f)* 0.5 μg/ml which can be extracted with organic solvents such as iso-amylalcohol and chloroform. This compound exhibits a green fluorescence in dioxane, but its metal complexes do not. The reactivity of the reduction product of VIII, IX, is similar to that of VIII; IX forms light red colored complexes with almost all the investigated divalent metal ions, extractable with organic solvents, but is less sensitive than VIII in the color reaction with metal ions. Generally, the aminomethyl compounds of benzothiazole derivatives show weaker color than the corresponding azomethine compounds on reacting with metal ions. IX and its metal complexes do not fluoresce.

On the other hand, the azomethine compounds derived from 2-aminobenzothiazole (I and II), are poor in the reactivity with metal ions. The reduction product of I, III, is also poor. III and its metal complexes do not fluoresce. The poor reactivity of I, II, and III may be due to the difficulty of coodination as terdentate; if they act as terdentate, their metal complexes would have 4 and 6 membered rings, whereas VIII and IX can form 5 and 5 membered rings with metal ions. However, compound (IV), which can form 5 and 6 membered rings with metal ions, has good reactivity with metal ions compared with I and II. It reacts with Al^{3+} to exhibit a blue fluorescence and itself also exhibits a weak green fluorescence in aqueous dioxane.

Table II. Reactions of 4-Substituted Benzothiazole Derivatives with Metal Ions

Compound	Metal ion	Color		
		acid	neutral	alkaline
X	Mg(II)		yellow	yellow
	Co(II)	yellow	yellow	yellow
	Ni(II)	yellow	yellow	yellow
	Cu(II)	green	green	green
	Zn(II)		yellow	yellow
	Ag(I)			yellow
	Cd(II)			yellow
	Hg(II)		yellow	yellow
	UO_2(II)			yellow
XIII	Mg(II)			purple red
	Al(III)	violet		
	Mn(II)			violet
	Fe(II)	bluish violet	bluish violet	violet
	Fe(III)		violet	violet
	Co(II)	green	green	green
	Ni(II)	violet	bluish violet	bluish violet
	Cu(II)[a]	bluish violet	bluish violet	bluish violet
	Zn(II)	violet	violet	violet
	Ga(III)	violet		
	Pd(II)	dark green		
	Ag(I)			blue
	Cd(II)			violet
	Hg(II)	blue	blue	blue
XIV	Al(III)	orange		
	Mn(II)			reddish violet
	Co(II)		red	red
	Ni(II)		red	red
	Cu(II)	reddish violet	orange	dark red
	Zn(II)			reddish violet
	Cd(II)			reddish violet
	Hg(II)			dark red

the limit of the detection: a)1 μg/ml

The 2-azo compounds (V, VI, and VII) react with divalent metal ions to produce blue — bluish violet colored complexes, which are extractable with organic solvents. The red color of these ligands in neutral region changes to blue — violet in alkaline region. Both the 2-azo compounds and their metal complexes do not fluoresce.

4-Substituted Benzothiazole Derivatives

While the 2-azo and azomethine compounds except IV do not form fluorescent complexes, some of the 4-azo and azomethine compounds form fluorescent complexes. The azomethine compound (X) reacts with Zn^{2+}, Cd^{2+}, and Mg^{2+} to emit a strong green fluorescence. Especially the fluorescence intensity of zinc complex is remarkable. These complexes are extractable with common organic solvents. X also reacts with transition metal ions to form yellow non-fluorescent complexes. Compound (XI) exhibits a weak green fluorescence, but it forms colored complexes only with Cu^{2+}, Ni^{2+}, and Co^{2+}. The reduction product of X, (XII), has poor reactivity with metal ions compared with X. This compound itself exhibits a weak green fluorescence in alkaline solution, but does not form fluorescent complexes.

The 4-azo compound (XIV) reacts with Al^{3+} to exhibit orange fluorescence. It reacts with many other metal ions to form non-fluorescent red — reddish violet colored complexes. The compound (XIII) shows a marked reactivity with metal ions; it forms violet — blue colored complexes with most metal ions, extractable with organic solvents, but both XIII and its metal complexes do not fluoresce. The significant color reactions of 4-substituted derivatives with metal ions are shown in Table II.

7-Substituted Benzothiazole Derivatives

The 7-substituted benzothiazole derivatives differ from the 4-substituted in the point that they have sulfur instead of nitrogen as the coodinate atom in the thiazole skelton. Their reactivity with metal ions is inferior to 2- and 4-substituted derivatives and both 7-substituted benzothiazole derivatives and their metal complexes do not fluoresce. The 7-azo compound (XVI) reacts with Cu^{2+}, Ni^{2+}, and Co^{2+} to form red colored complexes, but XV does not react with almost all the investigated metal ions. The behavior of 7-azomethine compound (XVIII) is similar to XV.

Conclusion

As mentioned above, the color change of 2-substituted benzothiazole derivatives on the formation of metal complexes in solution is more remarkable than that of 4- and 7-substituted; using VIII, 0.05 μg/ml of Cu^{2+} is detectable by the naked eyes. Further, 2-azo compounds in solution change from red or orange color to blue on the formation of metal complexes. As EDTA blocks most of these color formations, it is expected that these azo compounds together with 4-azo compounds can be applied to the determination of metal ions as metallochromic indicators. As fluorometric reagents for metal ions, however, 4-aminobenzothiazole derivatives are superior to 2-amino and 7-aminobenzothiazole derivatives. 7-Substituted benzothiazole derivatives are inferior to 2- and 4-substituted in the reactivity with metal ions.

Experimental

Syntheses of the Compounds——N-(2-Hydroxynaphthylidene)-2-aminobenzothiazole(II): To an ethanol solution of 2-aminobenzothiazole (1.5 g), was added an ethanol solution of an equivalent amount of β-hydroxy-α- naphthaldehyde. The mixture in ethanol was refluxed for 1 hour on a water–bath, cooled and the crystals were filtered. Recrystallization from ethanol gave 2.2 g of II as yellow needles, mp 213—214°. *Anal.* Calcd. for $C_{18}H_{12}ON_2S$: C, 71.03; H, 3.97; N, 9.20. Found: C, 71.08; H, 3.99; N, 9.14.

2-(*o*-Hydroxybenzyl)-aminobenzothiazole (III): To a suspension of sodium borohydride (0.7 g) in dioxane was added dropwise a solution of I (1.5 g) in dioxane at room temperature. After the mixture was kept standing for 1 hour, sodium borohydride was filtered off, water was added to the filtrate and the crystals were filtered. Recrystallization from aqueous ethanol gave 0.8 g of III as white needles, mp 177—178°. *Anal.* Calcd. for $C_{14}H_{12}ON_2S$: C, 65.60; H, 4.72; N, 10.93. Found: C, 65.32; H, 4.99; N, 11.09.

Salicylaldehyde-2-benzothiazolylhydrazone (IV): 2-Hydrazinobenzothiazole[4] (2.3 g) and salicylaldehyde (1.7 g) were treated with the same method as the preparation of II. Recrystallization from the mixed solvent of dioxane and ethanol gave 3.8 g of IV as white needles, mp 267—268°. *Anal.* Calcd. for $C_{14}H_{11}ON_3S$: C, 62.44; H, 4.12. Found: C, 62.18; H, 3.92.

2-(2-Benzothiazolylazo)-4-methoxyphenol (V): 2-Aminobenzothiazole, suspended in diltuted hydrochloric acid, was diazotised as usual. Coupling was effected by adding the ethanol solution of *p*-methoxyphenol to the solution of the diazotized amine. The temperature was maintained below 5°. The reaction mixture was kept standing overnight. The precipitate was filtered, dissolved in dioxane and treated with active charcoal. The product was recrystallized from aqueous dioxane. Purple red needles. mp 161—162°. *Anal.* Calcd. for $C_{14}H_{11}O_2N_3S$: C, 58.93; H, 3.89. Found: C, 58.95; H, 3.90.

2-(2-Benzothiazolylazo)-4-chlorophenol (VI): 2-Aminobenzothiazole and *p*-chlorophenol were treated with the same method as the preparation of V. Red needles. mp 218—219°. *Anal.* Calcd. for $C_{13}H_8ON_3SCl$: C, 53.89; H, 2.78; N, 14.50. Found: C, 54.00; H, 2.94; N, 14.31.

2-(*o*-Hydroxyphenylimino)-methylbenzothiazole (VIII): 2-Benzothiazolylaldehyde[5] (1.6 g) and *o*-aminophenol (1.1 g) were treated with the same method as the preparation of II. Recrystallization from ethanol gave 2.2 g of VIII as light brown needles, mp 160—161°. *Anal.* Calcd. for $C_{14}H_{10}ON_2S$: C, 66.12; H, 3.96; N, 11.02. Found: C, 65.90; H, 3.87; N, 10.87.

2-(*o*-Hydroxyanilino)-methylbenzothiazole (IX): Compound (VIII) (1.5 g) was treated with the same method as the preparation of II. Recrystallization from aqueous ethanol gave 0.9 g of IX as light pink needles, mp 187—189°. *Anal.* Calcd. for $C_{14}H_{12}ON_2S$: C, 65.60; H, 4.72; N, 10.93. Found: C, 65.81; H, 4.68; N, 10.85.

N-Salicylidene-4-aminobenzothiazole (X): 4-Aminobenzothizole[6] (1.5 g) and salicylaldehyde (1.3 g) were treated with the same method as the preparation of II. Recrystallization from ethanol gave 2.1 g of X as orange plates, mp 121—122°. *Anal.* Calcd. for $C_{14}H_{10}ON_2S$: C, 66.12; H, 3.96; N, 11.02. Found: C, 65.93; H, 4.01; N, 11.04.

N-(2-Hydroxynaphthylidene)-4-aminobenzothiazole (XI): 4-Aminobenzothiazole (0.55 g) and *β*-hydroxy-*α*-naphthaldehyde (0.86 g) were treated with the same method as the preparation of II. Recrystallization from the mixed solvent of ethanol and dioxane gave 1.5 g of XI as yellow needles, mp 177—178°. *Anal.* Calcd. for $C_{18}H_{12}ON_2S$: C, 71.03; H, 3.97. Found: C, 71.20; H, 4.15.

4-(*o*-Hydroxybenzyl)-aminobenzothiazole (XII): Compound (X) was treated with the same method as the preparation of III. Recrystallization from aqueous ethanol gave 1.4 g of XII as white needles, mp 138—139°. *Anal.* Calcd. for $C_{14}H_{12}ON_2S$: C, 65.60; H, 4.72; N, 10.93. Found: C, 65.73; H, 4.70; N, 10.82.

2-(4-Benzothiazolylazo)-4-methoxyphenol (XIII): 4-Aminobenzothiazole (1.5 g), suspended in diluted hydrochloric acid, was diazotized as usual. Coupling was effected by adding the solution of the diazotized amine to the weak alkaline solution of *p*-methoxyphenol (1.24 g) in ageous ethanol. The temperature was maintained below 5°. The precipitate was filtered, dissolved in dioxane and treated with active charcoal. Recrystallization from aqueous dioxane gave 1.2 g of XIII as reddish brown plates, mp 159—160°. *Anal.* Calcd. for $C_{14}H_{11}O_2N_3S$: C, 58.93; H, 3.89. Found: C, 58.95; H, 4.01.

1-(4-Benzothiazolylazo)-2-naphthol (XIV): 4-Aminobenzothiazole (1.5 g) and *β*-naphthol (1.44 g) were treated with the same method as the preparation of XIII. Recrystallization from aqueous dioxane gave 1.5 g of XIV as red needles, mp 244—245°. *Anal.* Calcd. for $C_{17}H_{11}ON_3S$: C, 66.87; H, 3.63; N, 13.76. Found: C, 66.70; H, 3.72; N, 13.78.

2-(7-Benzothiazolylazo)-4-methoxyphenol (XV): 7-Aminobenzothiazole[6] and *p*-methoxyphenol were treated with the same method as the preparation of XIII. The small amounts of impurity was separated by thin layer chromatography (Wakogel B-10; developing solvent: xylene). The product was recrystallized from aqueous dioxane. Orange needles. mp 155—156°. *Anal.* Calcd. for $C_{14}H_{11}ON_{32}S$: C, 58.93; H, 3.89; N, 14.73. Found: C, 58.84; H, 3.96; N, 14.60.

1-(7-Benzothiazolylazo)-2-naphthol (XVI): 7-Aminobenzothiazole and *β*-naphthol were treated with the above method. Purple red needles. mp 215—216°. *Anal.* Calcd. for $C_{17}H_{11}ON_2S$: C, 66.87; H, 3.63; N, 13.76. Found: C, 67.16; H, 3.90; N, 13.48.

N-Salicylidene-7-aminobenzothiazole (XVII): 7-Aminobenzothiazole (1.5 g) and salicylaldehyde (1.3 g) were treated with the same method as the preparation of II. Recrystallization from ethanol gave 2.2 g of XVII as yellow needles, mp 115—116°. *Anal.* Calcd. for $C_{14}H_{10}ON_2S$: C, 66.12; H, 3.96; N, 11.02. Found: C, 66.19; H, 3.79; N, 11.10.

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5) V.M. Zubarovskii, *Dokl. Akad. Nauk SSSR*, **87**, 759 (1952) [*C.A.*, **48**, 164*f* (1954)].
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Compound (I)7) and (VIII)8) have been reported.

General Procedure of the Spot Tests——One drop of a metal ion solution (10 mg of metal ion in 1 ml of water), 5 drops of buffer solution (pH=4.01, 6.86 or 9.18) and 2—3 drops of a reagent (0.1—1% solution in ethanol or dioxane) were mixed on a plate and the color development was observed. For comparison the colors of the reagents alone observed at each pH.

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