A Spectrophotometric Study of Xylenol Orange as a Color Reagent for Beryllium

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Xylenol orange reacts with many metal cations in a weakly or a slightly acid medium to form red or reddish-violet complexes, and it has widely been used as a metal indicator in complexometric titrations. This dye also reacts with beryllium ions to give a red coloration. The reaction is applied to the spectrophotometric determination of trace amounts of beryllium. The purpose of this study is to find the optimum conditions under which up to 6 μg. of beryllium can be determined, to ascertain the effect of diverse ions on the determination, and to determine the composition of the complexes formed. It will also be found that xylenol orange is a better color reagent for beryllium than eriochrome cyanine R1) and 2-phenoxyquinizarin3, 4-disulfonic acid2).

Experimental

Standard Beryllium Solution. — A solution of beryllium perchlorate was prepared by dissolving the beryllium hydroxide, freshly precipitated from a beryllium sulfate solution in the presence of ethylenediaminetetraacetic acid (EDTA), which served as a masking agent for contaminating metal ions, in perchlorc acid (0.2 N in excess). The beryllium content was determined gravimetrically as oxide.

Buffer Solution.—A hexamethylenetetramine - perchloric acid mixture was prepared by dissolving about 4 g. of hexamethylenetetramine in 100 ml. of water and by adding a perchloric acid solution in order to bring the pH to a desired value.

The other reagents and apparatus used were the same as those reported on previously.3) All experiments were carried out at 25 ± 0.5°C.

Standard Procedure for the Beryllium Determination.—An aliquot of the standard beryllium solution was introduced into a 25 ml. volumetric flask. Ten milliliters of a pH 5.8 buffer, 2.5 ml. of a 1×10^{-3} M xylenol orange solution and then a small amount of water were added to adjust the volume. After having been mixed, the solution was allowed to stand for about 30 min. The absorbance of the solution was measured against a reagent blank treated in a similar manner.

Results

Absorption Curves.—When a buffered, aqueous solution of beryllium is mixed with a xylenol orange solution, a red complex is immediately formed. Figure 1 shows the absorption curves of xylenol orange and its beryllium complex. The absorption curve of the beryllium complex obtained with a reagent blank as reference has an absorption maximum at about 495 mμ.

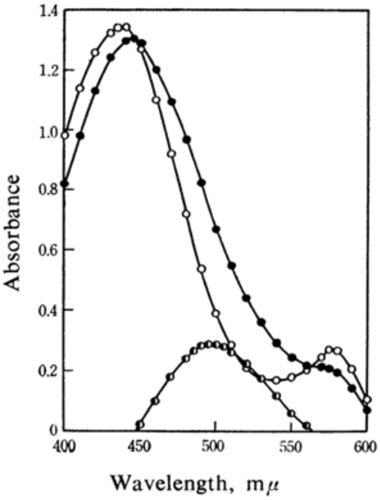

Fig. 1. Absorption curves of xylenol orange and its beryllium complex at pH 5.8.

Be : 4.5 μg., XO : 1.003×10^{-4} M
—○— XO vs. water
—●— Be-XO + XO vs. water
—◐— Be-XO + XO vs. a reagent blank

The Effect of pH and Choice of Buffer. — The pH is very important because the reagent functions as an acid-base indicator. The effect of pH on the color development of the complex was investigated by measuring the absorbance of mixtures containing 4.5 μg. (0.5 μmol.) of beryllium and 2.5 μmol. of the reagent at different pH values; the results are shown in Fig. 2, from which it can be seen that the optimum pH range for analytical purposes lies between 5.6 and 6.2. The buffer system, hexamethylenetetramine - perchloric acid, has a great

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1) S. Umemoto, This Bulletin, 29, 845 (1956).

2) E. G. Owens, II, and J. H. Yoe, *Anal. Chem.*, 32, 1345 (1960).

3) M. Otomo, This Bulletin, 36, 137 (1963).

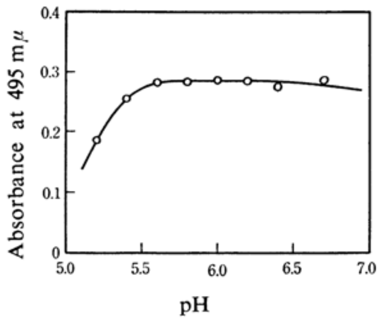

Fig. 2. Effect of pH on color development.
Be: 4.5 μg., XO: 1.003×10^{-4} M

buffering capacity in the pH range from 4 to 7. The amount of the buffer had no effect on the color intensity of the complex provided that more than 3 ml. of the said solution was used. For the sake of comparison, an acetate buffer was also used, but the observed absorbance was always lower than that obtained with the hexamethylenetetramine-perchloric acid buffer.

The Effect of the Amount of Xylenol Orange.—Two series of colored solutions were prepared; one contained 4.5 μg. of beryllium, and the other, 9.0 μg. of beryllium. The results are shown in Fig. 3. For both series, about a 4-fold excess of the reagent over the beryllium concentration is necessary in order to obtain the maximum color intensity. As a compromise between sensitivity and absorbance by the blank solution, 2.5 ml. of a 1×10^{-3} M reagent solution was employed.

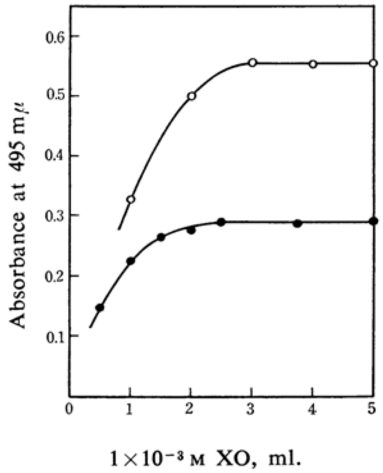

Fig. 3. Effect of amount of xylenol orange.
pH: 5.8, Reference: a reagent blank
—●— Be 4.5 μg.
—○— Be 9.0 μg.

The Color Stability.—The red color of the complex develops immediately at room temperature, and its intensity is independent of the temperature, at least up to 80°C. The complex is very stable, and there was no change in absorbance over a period of 24 hr.

Adherence to Beer's Law.—The color system obeys Beer's law over the concentration range up to 0.63 μmol. (about 6 μg.) of beryllium.

Sensitivity.—The sensitivity of the reaction as expressed by Sandell's notation[4] is 0.0006 μg. of beryllium per cm^2.

The Effect of Anions and Complexing Agents.—The influence of various anions and some complexing agents was examined. These materials were added individually to solution containing 4.5 μg. of beryllium. The results are summarized in Table I. Chloride, nitrate and sulfate ions do not interfere, even at a high concentration. Phosphate and oxalate ions at lower concentration have no effect, either; however, these ions give negative errors at higher concentrations. Fluoride and citrate ions bleach the color of the complex, even when less than 1 μmol. is present. Nitrilotriacetic acid (NTA) and EDTA, which form stable complexes with many other metal ions to decompose the corresponding xylenol orange complexes, have almost no influence on the complex formation between beryllium and xylenol orange unless large amounts are added.

TABLE I. EFFECT OF ANIONS AND COMPLEXING AGENTS

Be taken: 4.5 μg.

Anion or complexing agent added, μmol.		Be found μg.	Deviation μg.
Cl^-	200	4.5	±0.0
	500	4.6	+0.1
F^-	1	4.3	−0.2
	5	2.8	−1.7
NO_3^-	100	4.5	±0.0
	200	4.5	±0.0
SO_4^{2-}	100	4.6	+0.1
	250	4.5	±0.0
$C_2O_4^{2-}$	5	4.2	−0.3
	20	3.8	−0.7
$C_4H_4O_6^{2-}$	10	4.5	±0.0
	50	4.5	±0.0
PO_4^{3-}	5	3.9	−0.6
	20	3.8	−0.7
$C_6H_5O_7^{3-}$	1	4.0	−0.5
	10	1.8	−2.7
NTA	1	4.5	±0.0
	10	4.2	−0.3
EDTA	0.1	4.5	±0.0
	2	4.2	−0.3

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[4] E. B. Sandell, "The Colorimetric Determination of Traces of Metals," Interscience Publishers, New York (1950), p. 49.

Therefore, NTA and EDTA can serve as excellent masking agents to prevent the interference of other heavy metals. The latter has been used as a masking agent in several methods of beryllium determination,[5-8] and recently Owens and Yoe[2] used the Calcium-EDTA complex as a sequestering agent in the spectrophotometric determination of beryllium.

The Effect of Cations.—Xylenol orange is a selective reagent for certain cations when it is used in a relatively strong acid medium. However, in a slightly acid or a neutral medium it reacts with many metal cations to give red or reddish-violet complexes, indicating that a suitable masking agent must be used to increase the selectivity. Disodium salt of EDTA was chosen for this reason. Most common bivalent metal cations except cobalt can be effectively masked by EDTA, as Table II shows. Many ter- and quadrivalent cations, including aluminum, bismuth, thorium and zirconium, however, disturb the estimation.

TABLE II. MASKING EFFECT OF EDTA ON BERYLLIUM DETERMINATION

Beryllium taken: 4.5 μg.
Cations added: each 2.0 μmol.
EDTA added: 2.5 μmol.

Cation	Be found, μg.	Deviation, μg.
Bi^{3+}	4.8	+0.3
Cd^{2+}	4.5	±0.0
Co^{2+}	5.4	+0.9
Cu^{2+}	4.4	−0.1
Fe^{3+}	4.5	±0.0
Hg^{2+}	4.4	−0.1
La^{3+}	4.7	+0.2
Mg^{2+}	4.4	−0.1
Mn^{2+}	4.4	−0.1
Pb^{2+}	4.3	−0.2
Pd^{2+}	4.4	−0.1
Zn^{2+}	4.4	−0.1

Complex Formation

First, attempts were made to characterize the beryllium - xylenol orange complex by two methods, the method of continuous variations and the mole ratio method. These attempts were performed at various pH values between 5.0 and 7.0, while the absorbance measurements were made at seven wavelengths between 440 and 520 mμ. Both methods revealed that beryllium forms chiefly a 1 to 1 complex with xylenol orange at a pH below 5.0; it gives, however, a mixture of 1 to 1 and 1 to 2 complexes at higher pH values. The results were further supported by the following experiments.

The Formation of the 1 to 1 Complex.—A method which had been described by Newman and Hume[9] was applied to the complex formed at relatively lower pH values. If $Be_n(XO)$ is the only beryllium complex in a detectable concentration in solutions containing beryllium and xylenol orange, the apparent formation constant at a given pH value, $(K_1')_H$, may be defined as:

$$(K_1')_H = [Be_n(XO)]/[Be]^n[XO] \tag{1}$$

where [XO] represents the molar concentration of the free xylenol orange at equilibrium. The absorbance of a solution containing the complex and the free xylenol orange is:

$$D = [Be_n(XO)]\varepsilon_{C_1} + ([XO]_t - [Be_n(XO)])(\varepsilon_{XO})_H \tag{2}$$

where ε_{C_1} is the molar extinction coefficient of the complex; $(\varepsilon_{XO})_H$ is the apparent molar extiction coefficient of xylenol orange at a given pH value, and $[XO]_t$ is the total molar concentration of xylenol orange. The concentration of the complex, beryllium and xylenol orange at equilibrium are expressed, respectively, as:

$$[Be_n(XO)] = (D - [XO]_t(\varepsilon_{XO})_H)/(\varepsilon_{C_1} - (\varepsilon_{XO})_H) \tag{3}$$

$$[Be] = [Be]_t - n(D - [XO]_t(\varepsilon_{XO})_H)/(\varepsilon_{C_1} - (\varepsilon_{XO})_H) \tag{4}$$

and

$$[XO] = [XO]_t - (D - [XO]_t(\varepsilon_{XO})_H)/(\varepsilon_{C_1} - (\varepsilon_{XO})_H) \tag{5}$$

By introducing Eqs. 3, 4 and 5 into Eq. 1 and by taking logarithms, Eq. 6 is derived:

$$\log\{(D - D_{XO})/(D_C - D)\} = \log(K_1')_H + n\log\{[Be]_t - n(D - D_{XO})/(\varepsilon_{C_1} - (\varepsilon_{XO})_H)\} \tag{6}$$

where D_{XO} is the absorbance of the total xylenol orange and D_C is the absorbance of a solution containing a large excess of beryllium ions and the given amount of xylenol orange.

The experimental values of $\log\{(D - D_{XO})/(D_C - D)\}$ are plotted against the values of $\log\{[Be]_t - n(D - D_{XO})/(\varepsilon_{C_1} - (\varepsilon_{XO})_H)\}$ at pH 4.5 for five wavelengths between 480 and 510 mμ. When the value of n was assumed to be unity, the plots gave straight lines with a slope of 1.08 (on the average), as may be seen from Fig. 4. When other values, e. g., one-half, two,

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[5] H. V. Meek and C. V. Banks, *Anal. Chem.*, **22**, 1512 (1950).

[6] C. L. Luke and M. E. Campbell, ibid., **24**, 1056 (1952).

[7] C. W. Sill and C. P. Willis, ibid., **31**, 598 (1959).

[8] P. W. West and P. R. Mohilner, ibid., **34**, 558 (1962).

[9] L. Newman and D. N. Hume, *J. Am. Chem. Soc.*, **79**, 4571 (1957).

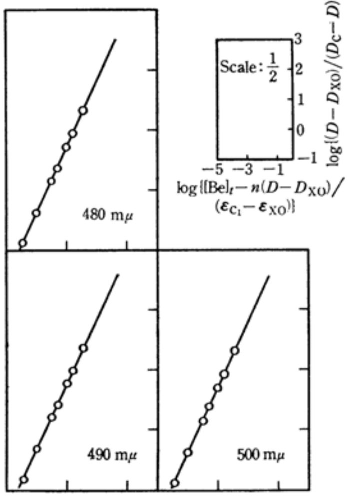

Fig. 4. Plots of $\log\{(D-D_{XO})/(D_C-D)\}$ vs. $\log\{[Be]_t-n(D-D_{XO})/(\varepsilon_{C_1}-\varepsilon_{XO})\}$.
$[XO]_t$: 4.0×10^{-5} M, pH: 4.5
μ: 0.10($NaClO_4$), Reference: water

etc., were assumed for n, however, no straight lines with slopes of the assumed values were obtained. Therefore, it may be concluded that the beryllium-xylenol orange complex formed at the investigated pH value has a molar ratio of 1 to 1. As is shown by Eq. 6, the intercept of the straight line should give the value of $\log(K_1')_H$ at pH 4.5. The values of ε_{C_1}, n and $\log(K_1')_H$ obtained are summarized in Table III.

TABLE III. VALUES OF ε_{C_1}, n AND $\log(K_1')_H$ AT pH 4.5

	Wavelength, mμ		
	480	490	500
ε_{C_1}	12550	12050	11130
n	1.10	1.06	1.08
$\log(K_1')_H$	4.00	3.91	3.92

The Formation of a Mixture of 1 to 1 and 1 to 2 Complexes.—To determine the composition of the complex formed in a higher pH region, the presence of a mixture of 1 to 1 and 1 to 2 complexes was assumed and a method described by McConnell and Davidson[10] was applied. If the total concentration of xylenol orange is high enough compared with that of beryllium and if the pH value of the solution is kept constant, the successive apparent formation constants at given pH values, $(K_1')_H$ and $(K_2')_H$, may be written, respectively, as:

$$(K_1')_H=[Be(XO)]/([Be]_t-[Be(XO)_n])[XO]_t \tag{7}$$

$$(K_2')_H=[Be(XO)_n]/[Be(XO)][XO]_t^{n-1} \tag{8}$$

If the absorbance of the solution is measured against a solution containing the same amount of free xylenol orange as that added to beryllium, it may be given by:

$$D=[Be(XO)](\varepsilon_{C_1}-(\varepsilon_{XO})_H)+[Be(XO)_n](\varepsilon_{C_2}-n(\varepsilon_{XO})_H) \tag{9}$$

where ε_{C_1} and ε_{C_2} are the molar extinction coefficient of Be(XO) and $Be(XO)_n$ respectively, and where $(\varepsilon_{XO})_H$ is the apparent molar extinction coefficient of xylenol orange at a given pH value. From Eqs. 7, 8 and 9, the following relationship may be derived:

$$[Be]_t/D=1/(\varepsilon_{C_2}-n(\varepsilon_{XO})_H)+F/(\varepsilon_{C_2}-n(\varepsilon_{XO})_H)(K_1')_H(K_2')_H[XO]_t^n \tag{10}$$

$$F=1+(K_1')_H[XO]_t-(K_1')_H[Be]_t[XO]_t(\varepsilon_{C_1}-(\varepsilon_{XO})_H)/D$$

The experiments were carried out in the concentration range, $[XO]_t=0.89\times10^{-4}$—3.57×10^{-4} M, the pH value being held constant at 5.30 and the ionic strength being held constant at 0.1. Although the total concentration of xylenol orange was not sufficiently large compared with that of beryllium (1.996×10^{-5} M), it is assumed, for the first approximation, that the equilibrium concentration of xylenol orange nearly equals the total concentration. The graphical analysis was done at three wavelengths, 480, 490 and 500 mμ, using the value of $(K_1')_H$ at pH 5.30 (1.60×10^6), a value which was obtained according to the method described by Buděšinský,[11] and the calculated values of $(\varepsilon_{C_1}-(\varepsilon_{XO})_H)$. Although good straight lines were not obtained, the values of $(K_2')_H$ at pH 5.30 at the three wavelengths were estimated from the slopes as 7.3×10^3, 5.6×10^3 and 5.5×10^3 respectively. Therefore, for the second approximation to the solution of Eq. 10, the deviation between the total concentration of xylenol orange was calculated using the first approximation for $(K_2')_H$. The correction is small at relatively higher concentrations of xylenol orange, but a large correction (about 30%) was required at the lowest concentration of xylenol orange. The corrected values of the total concentration of xylenol orange were then used, and the linear character of the plots of $[Be]_t/D$ vs. $F/[XO]_t^n$ was examined. Figure 5 shows the results obtained when the value of n equals two, indicating that the plots are good straight lines within the range of experimental error. The third approximation was further applied. However, it was

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[10] H. McConnell and N. Davidson, ibid., 72, 3164 (1950).

[11] B. Buděšinský, *Collection Czechoslov. Chem. Commun.*, 27, 226 (1962).

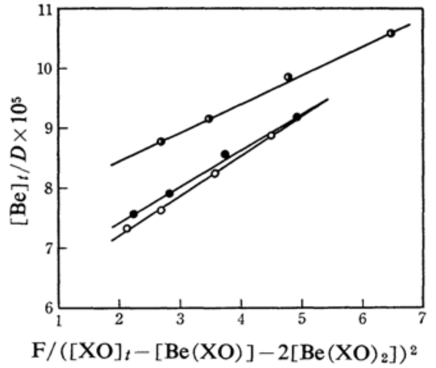

Fig. 5. Plots of $[\mathrm{Be}]_t/D$ vs. $\mathrm{F}/([\mathrm{XO}]_t-[\mathrm{Be(XO)}]-2[\mathrm{Be(XO)_2}])^2$.

$[\mathrm{Be}]_t$: 1.966×10^{-5} M, pH : 5.3
μ : 0.10 ($NaClO_4$), Reference : water
Wavelength : —◑— 480 mμ, —●— 490 mμ
—○— 500 mμ

found that there is little difference between the second and third approximations.

Finally, the concentrations of the 1 to 1 and the 1 to 2 complexes were calculated at pH 5.8 and at varying concentrations of xylenol orange, using the following relationships, which can easily be obtained by solving Eqs. 7 and 8:

$$[\mathrm{Be(XO)}]=(K_1')_\mathrm{H}[\mathrm{Be}]_t[\mathrm{XO}]_t/(1+(K_1')_\mathrm{H}[\mathrm{XO}]_t+(K_1')_\mathrm{H}(K_2')_\mathrm{H}[\mathrm{XO}]_t^2) \qquad (11)$$

$$[\mathrm{Be(XO)_2}]=(K_1')_\mathrm{H}(K_2')_\mathrm{H}[\mathrm{Be}]_t[\mathrm{XO}]_t^2/(1+(K_1')_\mathrm{H}[\mathrm{XO}]_t^2+(K_1')_\mathrm{H}(K_2')_\mathrm{H}[\mathrm{XO}]_t^2) \qquad (12)$$

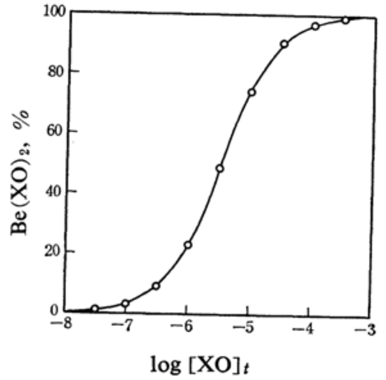

Fig. 6. Percentage occupied by $Be(XO)_2$ in a mixture of Be(XO) and $Be(XO)_2$ at pH 5.8.

Be : 2.0×10^{-5} M

The percentage occupied by the 1 to 2 complex in a mixture of the 1 to 1 and 1 to 2 complexes was calculated as a function of the concentration of xylenol orange. The curve in Fig. 6 was obtained when the total molar concentration of beryllium was constant as 2.0×10^{-5} M. The reciprocal of the value of log $[\mathrm{XO}]_t$ at the intercept of the 50% line with the curve should give the value of log $(K_2')_\mathrm{H}$ at pH 5.8. The pH value of 5.8 was chosen because the determination of beryllium was performed at this pH value.

Summary

The red color produced by adding a xylenol orange solution in excess to a beryllium solution has been studied spectrophotometrically in order to ascertain the optimum conditions for the determination of beryllium. The absorption curve of the colored system shows an absorption maximum at 495 mμ when measured against a reagent solution of pH 5.8. Beer's law is obeyed over the range up to 6 μg. of beryllium. The composition and the apparent formation constant of the two different complexes have then been obtained by applying some spectrophotometric techniques. Beryllium forms a 1 to 1 complex with xylenol orange below pH 5; it gives, however, mixture of 1 to 1 and 1 to 2 complexes at higher pH values.

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