FLUORIMETRIC DETERMINATION OF BERYLLIUM WITH *o*-PYRIDINOPHENOL

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Fluorescence properties of *o*-pyridinophenol (2-(2-hydroxyphenyl)pyridine) and its complex with a beryllium salt were studied. A selective method was developed for fluorimetric determination of beryllium, with the limit of determination 1·6 ng Be/1 ml.

o-Pyridinophenol (*o*-PP) was prepared first by Chichibabin[1] by diazotation of *o*-aminopyridine and reaction of the diazonium salt formed with phenol. Another procedure for the preparation of *o*-PP was applied by Geissman and coworkers[2] during their study of complex compounds designed for a reversible transfer of oxygen. Albert, Rees, and Tomlinson[3] determined potentiometrically the dissociation constants of *o*-PP and the stability constant of its nickel complex.

In this work we studied the fluorescence properties of *o*-PP and its complex with beryllium salt, which displayed intense blue fluorescence in an aqueous–alcoholic medium (50% v/v ethanol). The effects of pH and of the concentrations of ethanol, the reagent, and Chelaton 3 (disodium salt of (ethylenedinitrilo)tetraacetic acid) added on the fluorescence intensity were examined, the time dependence of the fluorescence intensity of the beryllium complex was established, the working curve was plotted and the precision and limit of determination of beryllium with *o*-PP were found. The effect of foreign ions on the fluorescence of the beryllium complex was investigated from the point of view of practical application of *o*-PP for the determination of small amounts of beryllium.

EXPERIMENTAL AND RESULTS

Chemicals and Apparatus

The chemicals used for the preparation of the reagent according to the Chichibabin method[1] were pure grade, the other chemicals commonly used were reagent grade purity. The melting point of the reagent prepared was 56°C. Ethanolic solutions of the reagent of accurate concentrations were prepared by weighing the substance on a microbalance Cahn M-10 (USA). For the preparation of solutions of metal salts, perchlorates of the metals were usually applied; in the case of chromium(III) salt, sulfate was used. The perchlorates were prepared by dissolving the metal or its oxide in perchloric acid, evaporation of the solution for crystallization, and recrystallization of the metal perchlorate from 0·1 M perchloric acid. The content of the metal in the approx-

0·1M stock solution was determined chelometrically or gravimetrically. Deionized water was used throughout for the preparation of solutions. As proved during the fluorimetric measurements, this water does not contain any organic substances, which would exhibit fluorescence under the conditions used and so affect the measurements. Ethanol was purified by column distillation and the fraction distilling at 77—78°C was used; its transmittance at 240 and 290 nm was 70 and 80%, respectively (with reference to redistillied water).

The pH values were measured by employing a pH-meter OP 205 (Radelkis, Hungary) with a combined electrode (Radelkis) calibrated with aqueous standard buffer solutions. As during the fluorimetric study the pH values were measured in aqueous–ethanolic solutions, where the combined electrode does not display the same function as in water[4], the measured values are denoted pH*.

The fluorimetric measurements were carried out on a spectral photometer Spekol (Zeiss, Jena GDR) with a fluorimetric attachment and an additional amplifier, in 1 cm glass cells. The source of the excitation radiation was a high pressure mercury lamp HQE 40, from whose line spectrum the radiation of the wavelength of 365 nm was selected for the excitation of fluorescence as the most suitable. The fluorescence intensities were related to that of a glass normal WG 9 with blue fluorescence, which for the amplification applied (50) was taken for 100%. The relative intensities so obtained are denoted F_r. The zero value of the fluorimeter scale was adjusted for distilled water at the maximum gain of 1 000. The fluorescence intensities were corrected for the fluorescence of the blank, which was usually represented by an aqueous–ethanolic solution of the reagent at the pH* applied, or — during the measurements of the dependence of the fluorescence intensity of the reagent on pH* and measurements of the limit of determination of beryllium — a solution ethanol–water.

Spectrophotometric measurements were performed on a recording spectrophotometer Spekord UV-VIS (Zeiss, Jena, GDR) in 1 cm quartz cells with redistilled water in reference cells.

Absorption Spectra and Fluorescence Properties of o-Pyridinophenol

o-Pyridinophenol is very low soluble in water and well soluble in common organic solvents. For the study of its properties, 50% v/v aqueous ethanolic solutions were used; they are colourless and stable enough in the acidic as well as alkaline ranges of pH. Only after 1 month's standing an observable change of colour appeared in the case of alkaline solutions — they turned slightly yellow. The absorption spectra of the aqueous–ethanolic solutions of the reagent are shown in Fig. 1, the corresponding spectral characteristics are given in Table I.

TABLE I

Absorption Maxima and Molar Absorptivities of o-Pyridinophenol in Aqueous–Ethanolic Solutions

50% ethanol		0·5M-HClO$_4$		0·5M-NaOH	
λ	ε	λ	ε	λ	ε
nm	10^3 mol^{-1} cm^2	nm	10^3 mol^{-1} cm^2	nm	10^3 mol^{-1} cm^2
254	11·7	265	7·65	254	7·60
290	6·95	298	7·45	332	6·28
316	7·78	334	7·43		

In order to test the reactivity of the reagent with metal ions and anions, the following procedure was applied: 0·5 ml of 10^{-3}M ethanolic solution of the reagent was aded to the same volume of a 10^{-3}M solution of metal perchlorate or nitrate and the pH* of the mixture was adjusted in the region of $1-13$ by additions of dilute solutions of sodium hydroxide and perchloric acid. In cases that a precipitate separated due to increased alkalinity of the solution, only such a pH* range was used where the precipitate did not appear. In order to prevent the formation of precipitates, no masking agent was added to the solutions. The fluorescence of the solutions was excited by a mercury lamp Thelta S 300 equipped with a Wood filter. Qualitative tests of reactivity of the reagent in the UV radiation were performed with the ions of the following elements: Ag, Al, Ba, Be, Ca, Cd, Co, Fe, Ge, La, Mg, Mn, Nd, Ni, Pb, Pr, Sc, Sm, Sn, UO_2, Zn, and ions of boric acid. Among the ions mentioned only beryllium ions yield a complex displaying intense blue fluorescence in alkaline medium. A weak fluorescence is exhibited by borates.

Dependence of Fluorescence Intensity of o-Pyridinophenol and its Beryllium Complex on pH*

The dependence of fluorescence intensity of the reagent itself and of its complex with beryllium ions on pH* was measured in solutions without addition of a masking agent or with addition of a solution of Chelaton 3 or of tartatic acid. In 25 ml volumetric flasks were placed 12·5 ml of 10^{-3}M ethanolic solution of the reagent, 1·25 ml of 10^{-3}M solution of beryllium perchlorate, the pH* of the mixture was adjusted by using 0·1M sodium hydroxide or 0·1M perchloric acid and brought to the volume with water. The intensity of fluorescence was measured 15 minutes
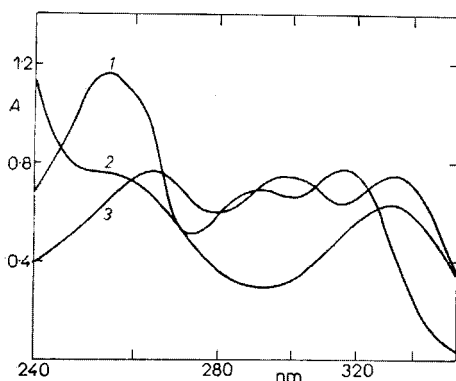

FIG. 1

Absorption Curves of o-Pyridinophenol in Aqueous–Ethanolic Solutions

$c_L = 1 . 10^{-4}$ mol/l; 1 50% C_2H_5OH, 2 0·5M-NaOH, 3 0·5M-HClO$_4$.
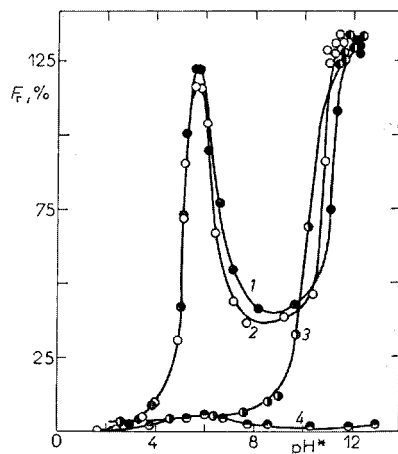
FIG. 2

Dependence of Fluorescence Intensity of o-Pyridinophenol and its Beryllium Complex on pH*

1 Be-o-PP; 2 Be-o-PP, tartaric acid; 3 Be-o-PP, Chelaton 3; 4 o-PP; $c_{Be} = 5 . 10^{-5}$ mol/l, $c_L = 5 . 10^{-4}$ mol/l.

after completing this procedure and shaking. For the investigation of the dependence with an addition of a masking agent, 2 ml of 0·05M Chelaton 3 or 0·5 ml of 1% solution of tartaric acid was added to the mixture after the addition of the beryllium salt. Likewise — just without the addition of the beryllium salt — the dependence of the fluorescence intensity of solutions of the reagent itself on pH* was measured. The plots obtained are depicted in Fig. 2.

Time Dependence and the Effect of Concentrations of Chelaton 3, Ethanol, and the Reagent on the Fluorescence Intensity of the Beryllium Complex of o-Pyridinophenol

The time dependence of the fluorescence intensity of the beryllium complex and the effect of the various concentrations were measured with solutions prepared in the same manner as those used for the investigation of the dependence on pH*. The pH* was always adjusted to 12·7—12·8.

From the measured values it follows that the fluorescence intensity of the complex of the beryllium salt with o-PP is sufficiently time stable. During long-duration measurements (12 h) the differences of the values measured did not exceed $\pm 3\%$ rel. For attaining the maximum fluorescence intensity a tenfold excess of the reagent is required under the conditions used. The maximum value is obtained immediately after adding the reagent and shaking the solution. Chelaton 3 does not affect the fluorescence intensity of the complex up to the concentration of $4 . 10^{-3}$ mol/1, but turbidity appears when higher concentrations are used. The effect of concentration of ethanol was followed in aqueous — alcoholic solutions with 45—90% v/v of ethanol. The intensity of fluorescence increases almost linearly with increasing concentration of ethanol up to 80% v/v; application of higher concentrations, however, is accompanied by a marked drop
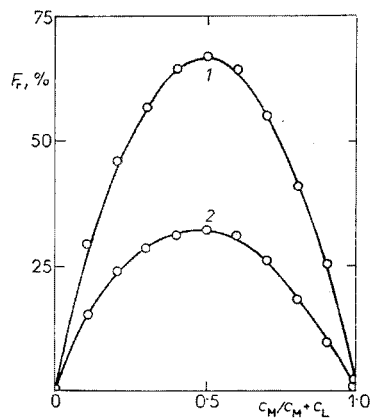

Fig. 3

Composition of the Beryllium Complex of o-Pyridinophenol

1 $c_0 = 1·6 . 10^{-4}$ mol/l; 2 $c_0 = 8 . 10^{-5}$ mol/l.
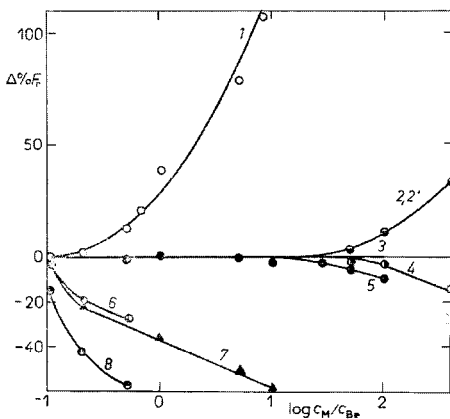
Fig. 4

Effect of Interfering Ions on the Fluorescence Intensity of the Beryllium Complex of o-Pyridinophenol

$c_{Be} = 4 . 10^{-5}$ mol/l; 1 BO_3^{3-}; 2 Al^{3+}; $2'$ Al^{3+} (without Chelaton 3); 3 Cu^{2+}, Ni^{2+}, Pb^{2+}, $Cr(VI)$, Mn^{2+}, Ag^+, $U(VI)$, Zn^{2+}, Mg^{2+}, Ba^{2+}, Ca^{2+}; 4 Ge^{4+}; 5 Co^{2+}; 6 Cr^{3+}, Zr^{4+}; 7 Fe^{3+}; 8 F^-.

of intensity. The fluorescence intensity of the reagent itself does not virtually change with increasing concentration of ethanol.

Composition of the Beryllium Complex of o-Pyridinophenol

The composition of the beryllium complex of o-PP was found by applying the Job variation method[5] for equimolar solutions and verified by employing the method of limit logarithm[6]. The measurements were carried out in solutions with pH* 12·7—12·8. The resulting Job curves for two different total concentrations of the metal and of the reagent are plotted in Fig. 3. The complex was found to contain the components in the ratio 1 : 1, this was verified by the limit logarithm method, where the ratio $\log F_r/\log c_L$ was calculated to be 0·94.

From the measured dependence of the fluorescence intensity for various total concentrations of the metal and of the ligand the conditional stability constant of the beryllium complex was calculated by the nonlinear regression method applying the program Spektfot[7] to be $\beta' = 3 \cdot 1 \cdot 10^4$.

Reproducibility of the Working Curve and Limit of Determination of Beryllium

For obtaining the plot of the working curve, 0 to 5 ml of a 10^{-3}M solution of the beryllium salt was diluted in a 25 ml volumetric flask with 2 — 3 ml of water and neutralized with 0·1M sodium hydroxide until turbidity appeared. The turbidity was dissolved with several drops of 0·1M perchloric acid, then 1·25 ml of 1M sodium hydroxide and 12·5 ml of 10^{-3}M alcoholic solution of the reagent were added and the mixture was brought to the volume with water and shaken. The pH* of such solutions lay in the range of 12·7—12·9. Fluorescence was measured 15 minutes after shaking the solution. Likewise the working plot was obtained with additions of 2 ml of 0·05M Chelaton 3; the agent was added after the beryllium salt. The working plots are linear up to the content of beryllium 0·54 μg/ml in both cases and the fluorescence intensities measured in the two cases for equal contents of beryllium did not differ from each other more than $\pm 2\%$ rel. The results obtained in the linear part of the working curve were evaluated statistically by using the regression analysis method[8]. The values of the slope K and intercept B of the working curve were calculated along with their confidence intervals with the level of significance 95%: $K = 7 \cdot 56 \pm 0 \cdot 16$ and $B = 2 \cdot 6 \pm 1 \cdot 2$. Furthermore the correlation coefficient was calculated (0·998) expressing the linearity of the dependence of fluorescence intensity on the content of beryllium. From the values obtained an equation was derived for the calculation of the content of beryllium, $viz.$

$$\mu g \ Be/ml = (\%F_r - 2 \cdot 6)/188 \cdot 8 .$$

The limit of determination of beryllium was found by measuring the fluorescence of blanks (20 measurements) and expressed numerically as triple of the standard deviation of the average obtained with the blanks[9]. For the calculated value of $3s_{\bar{x}} = 0 \cdot 3\% \ F_r$ it follows from the working curve that the minimum amount of beryllium to be determined is 0·0016 μg Be/ml.

Effect of Foreign Ions

The influence of foreign ions on the fluorescence was investigated in solutions containing 1 ml of a 10^{-3}M solution of the beryllium salt and various amounts of the ion examined. Further the procedure was the same as in the case of obtaining the working curve with Chelaton 3. The effect of aluminium ions was tested also without addition of the masking agent. The results are shown in Fig. 4 as the differences between the fluorescence intensity of the beryllium complex itself and that in the presence of the foreign ions.

DISCUSSION

o-Pyridinophenol was prepared by the procedure suggested by Chichibabin. It is complicated particularly in the point of separation of the *ortho* derivative from the *para* derivative, phenoxypyridine, and especially from small amounts of pyridylazophenol; the formation of the latter has not been reported by Chichibabin, its presence, however, was evidenced by chromatographic analysis. In fact, even a slight contamination with that substance can mislead during the study of fluorimetric and chelation properties, as it yields intensively coloured complexes with a series of metals. Pyridylazophenol was therefore separated from the mixture formed by extraction of the crystalline product with petroleum ether and repeated crystallization of the remaining crystals of o-PP from a mixture ethanol–water. In contrast to Geissman and coworkers[3], who obviously did not succeed in preparing o-PP of a sufficient purity (liquid, b.p. 135°C/2 mm Hg), we prepared by this procedure the substance in the crystalline form, with the melting point and elemental analysis in accordance with the data published[1].

Since the reagent is not soluble enough in water, the study was performed with aqueous–ethanolic solutions. The lowest content of ethanol required for maintaining the reagent in solution appeared to be 40% v/v. For practical reasons we proceeded with solutions containing 50% v/v ethanol. For the characterization of the reagent the absorption spectra of the solutions with various pH* were measured and molar absorptivities for the wavelengths of the absorption maxima were calculated. The spectral measurements performed in dependence on time showed a sufficient stability of the solutions in both the acidic and the alkaline pH* ranges. In alkaline solutions, only after 1 month a noticeable colour change appears — the solution turns yellow. A solution of the reagent in ethanol remains unchanged for a time longer than 6 months. This time stability is superior to that of morin, for instance, applied for fluorimetric determination of beryllium: in solutions of morin rather fast oxidation by atmospheric oxygen proceeds.

Qualitative tests with a series of metal ions proved that none of them enters an appreciable colour reaction with the reagent, suitable for sensitive detection or determination. Significant are the fluorescence properties of the complex of the reagent with beryllium ions: the complex displays intense blue fluorescence in the pH* range of 5–6 and in the alkaline range. Among the other ions studied only borates exhibited fluorescence, which, however, was rather weak and was not further studied.

The dependence of the fluorescence intensity of the beryllium complex on pH* shows two approximately equally high maxima, one in the relatively narrow range of pH* 5·6–6·2, the other in the alkaline region at pH* > 12·5. A similar course was obtained also in the presence of tartaric acid. The shape is, however, different when Chelaton 3 is present: only one maximum, in the alkaline region, is observed on the curve. The fluorescence maximum at pH* 5·6–6·2 can be assumed to vanish due to the

formation of the stronger complex of beryllium with Chelaton. As Chelaton 3 might be used as a masking agent during the determination of beryllium in the presence of associate ions, we carried out further investigations of the fluorescence properties of the beryllium complex in the alkaline range, pH* $12 \cdot 5 - 13$, where the intensity is not sensitive to changes of pH* and where the reagent itself does not fluoresce.

In order to find the optimum conditions of fluorescence of the complex, the effect of concentrations of ethanol, Chelaton 3 and the reagent was studied and the time dependence of fluorescence intensity followed. A tenfold excess of the reagent was found to be required for the maximum intensity of fluorescence. The intensity is sufficiently time stable (for at least 12 h) and attains its maximum immediately after the addition of the reagent. The concentration of Chelaton 3 must not exceed 4 . . 10^{-3} mol/l; in case that higher concentrations are used in the ethanol–water solution, sodium salt of Chelaton 3 precipitates and the turbid solutions are inapplicable for fluorimetric measurements. This concentration restriction of the addition of Chelaton affects infavourably the applicability of this substance as the masking agent and also determines indirectly the concentration limit of a foreign ion. Analogously is the fluorescence of the beryllium complex affected by a concentration of ethanol higher than 80% v/v, which brings about precipitation of inorganic salts from the solution even in the absence of Chelaton 3, due to their lowered solubility.

The form of the Job curves and the difference between the measured and theoretical maximum values of F_r indicate that the complex 1 : 1 formed is not very stable; this is confirmed also by the calculated value of the conditional stability constant, $\beta' = 3 \cdot 1 \cdot 10^4$.

The working curve is linear in the range of $0 - 0 \cdot 54$ µg Be/ml. For higher concentrations the curve bends to lower intensities, which can be explained by an insufficient concentration of the reagent (excess lower than tenfold) as well as by concentration quenching, which appeared for the higher beryllium contents even when the reagent was present in a tenfold excess.

The calculated value of the correlation coefficient, $0 \cdot 998$, indicates a very good linear dependence of the fluorescence intensity on the content of beryllium in the concentration range mentioned. The value of the slope of the working curve is well reproducible too. The relatively worse reproducibility of the intercept of the working curve affects infavourably the reliability of determination of lower beryllium contents. Only for the lower limit of the confidence interval, the intercept calculated by regression analysis is in a good agreement with the mean value of the blank $(1 \cdot 40\% \, F_r)$ found from 20 measurements and used for the calculation of the limit of determination of beryllium.

During the study of o-PP as a reagent for the determination of beryllium, it is of importance to examine the effect of foreign ions on the fluorescence intensity of the beryllium complex. This effect was tested in solutions with additions of Chelaton 3

as the masking agent, which in several cases prevented the formation of precipitates in the alkaline medium. The restricted concentration of Chelaton 3 in the aqueous alcoholic medium limited also the concentration of the interfering ion to $4 .10^{-3}$ mol/l, hence for a concentration of beryllium of $4 . 10^{-5}$ mol/l the associate ion may be present at most in a hundredfold excess. Under such conditions the following ions in a hundredfold excess do not interfere with the fluorescence of the beryllium complex: Ag, Ba, Ca, Cr(VI), Cu, Mg, Mn, Ni, Pb, U(VI), and Zn. The Ge(IV) ions interfere slightly, Al and Co(II) ions interfere in amounts as low as a 50fold excess. The highest interfering effect was exhibited by ions of boric acid and by chromium(III), zirconium, iron(III), and fluoride ions. From these only ions of boric acid increase the fluorescence, the remaining ions have a strongly quenching influence. The interfering effect of iron(III) ions, appreciable in $Fe^{3+} : Be^{2+}$ ratios as low as 1 : 10, could not be eliminated even by an addition of ascorbic acid or triethanolamine, often recommended in the literature[9].

From the results obtained it follows that *o*-pyridinophenol can be applied for fluorimetric determination of microgram amounts of beryllium. In comparison with morin, *o*-pyridinophenol is less sensitive, and its is advantageous by the stability of its aqueous — alcoholic solutions and that of its beryllium complex.

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