

A spectrophotometric investigation of the complex between titanyl oxalate and 3-hydroxyflavone in water ethanolic mixtures

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It has been established, by the application of suitable spectrophotometric methods and pH-metric measurements, that titanyl oxalate anion and 3-hydroxyflavone (3HF) form a $[\text{TiO}(\text{C}_2\text{O}_4)_2(\text{C}_{15}\text{H}_9\text{O}_3)_2]^{4-}$ complex. The investigation of the composition and the concentration stability constant of the complex were carried out in a 50 % aqueous ethanol solution at room temperature (20 °C), in the pH range from 1.9 to 9.0. The concentration stability constant of the complex, $\log \beta_2$, ranged from 16.65 at pH 5.0 to 13.96 at pH 7.0. The conditions for the spectrophotometric determination of 3HF by means of the complex formation were investigated in the concentration range from 2.5×10^{-5} to 3.0×10^{-4} mol dm⁻³ 3HF.

Keywords: complex, 3-hydroxyflavone (3HF), titanyl oxalate, spectrophotometric methods.

INTRODUCTION

Flavones are a very important group of plant phenolics that occur in various kinds of fruits, vegetables and plant beverages.¹ They show some significant physiological and pharmacological effects, such as antiinflammatory, antimicrobial, anticancer. They are also used for the prevention of cardiovascular diseases. All this makes them constituents of many pharmaceutical preparations.

3HF, C₁₅H₉O₃H, a flavonoid derivative, which possesses one hydroxyl group in position 3 and one carbonyl group in position 4 of the molecule,² what allows it to form complex compounds with many different metals. 3HF has been investigated spectrophotometrically using its interaction with metal ions.³⁻⁹ However, there are no data in the literature about the titanyl oxalate-3HF interaction. The aim of the present work was to investigate this complex and to optimize this complexation reaction in order to develop a new and simple spectrophotometric method for the determination of 3HF using titanyl oxalate as the analytical reagent.

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EXPERIMENTAL

Apparatus

The spectrophotometric measurements were performed using a Beckman DU-650 spectrophotometer in a 1 cm quartz cell. For the pH-metric determinations, a Radiometer pH M 28 - pH meter with a glass - saturated calomel electrode was used.

An U1 Ultra Thermostat Medigen (Dresden) was used for maintaining a constant temperature (with an accuracy of ± 0.1 °C) during the experiments.

Reagents

The reagents used were: $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$, abs. ethanol, NaOH, HNO_3 , NaNO_3 (all Merck), 3HF (Aldrich-Chemie) all p.a. grade.

General procedure

Since 3HF is not soluble in pure water, and $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$ is not soluble in pure ethanol, 50 % aqueous ethanol was selected as the most suitable solvent in which the solubility of both these compounds is optimal. All measurements were carried out at room temperature (20 ± 0.1)°C.

The pH of all solutions were adjusted by the addition of HNO_3 or NaOH, and the ionic strength of the final solutions was kept constant at 0.01 mol dm^{-3} by the addition of 1.0 mol dm^{-3} NaNO_3 .

For the application of the Job's method, $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$ and 3HF stock solutions of the same concentration ($c_{\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2} = c_{3\text{HF}} = 5 \times 10^{-3} \text{ mol dm}^{-3}$) were mixed in different ratios 0.0 - 1.0). In the mole ratios method, solutions containing a constant $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$ concentration ($2 \times 10^{-4} \text{ mol dm}^{-3}$) with varying 3HF concentrations $1 \times 10^{-4} - 6 \times 10^{-4} \text{ mol dm}^{-3}$, were used.

RESULTS AND DISCUSSION

Absorption spectra

The reaction of 3HF with titanyl oxalate anion was investigated at pH 5.0. This pH was chosen because this was the value established when these components were mixed with a large excess of 3HF (7 : 1). The spectra were recorded from 350

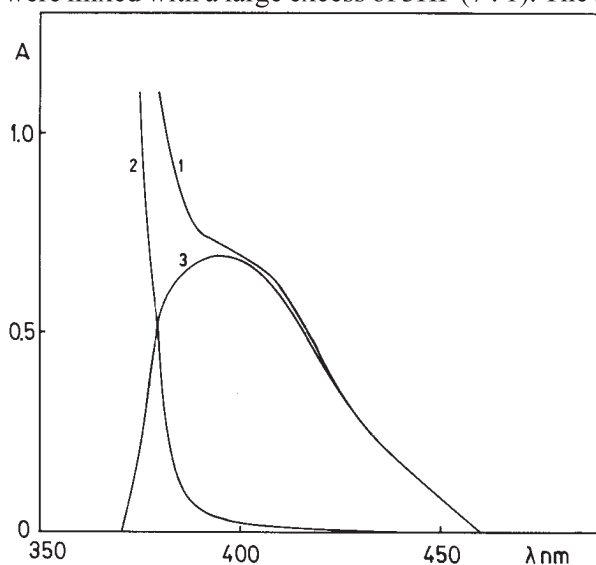


Fig. 1. Absorption spectra: 1) $3.5 \times 10^{-4} \text{ mol dm}^{-3}$ 3HF and $5.0 \times 10^{-5} \text{ mol dm}^{-3}$ $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$ in 50 % aqueous ethanol, blank: 50 % aqueous ethanol; 2) $3.5 \times 10^{-4} \text{ mol dm}^{-3}$ 3HF in 50 % aqueous ethanol, blank: 50 % aqueous ethanol; 3) curve 1 - curve 2.

to 500 nm. In this region titanyl oxalate has no significant absorbance in the applied concentrations, while 3HF shows strong absorption below 380 nm (Fig. 1, curve 2). the absorption curve representing the formation of the complex between 3HF and titanyl oxalate (Fig. 1, curve 3) was obtained by subtraction from the absorption curves of mixed solutions of 3HF with titanyl oxalate (curve 1) of the absorption curve of 3HF in the same concentration as in the mixture (Fig. 1, curve 2). The complex has a yellow-green color with an absorption maximum at 395.0 nm.

Composition of the complex

The composition of the complex was determined by the application of the Job's method¹⁰ and by the mole ratios method.¹¹ The first method is based on the continual variation of equimolar $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$ and 3HF solutions so that the sum of the metal and ligand concentration in all solutions always remains the same. At pH 6.65, the obtained curve $A = f(x_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}})$ (Fig. 2) has a maximum at $x_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}} = 0.33$ [$x_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}}$ denotes the mole fraction of $\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}$], showing the formation of a $\text{TiO}(\text{C}_2\text{O}_4)_2^{2-} : 3\text{HF} = 1 : 2$ complex. When the mole fraction of $\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}$ is increased, the complex absorbance decreases sharply until $x_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}} = 0.5$. The absorbance first remains almost constant when the mole fraction of the metal is further increased but slightly decreases towards zero, as the metal mole fraction approaches 1. The distortion of the curve at $x_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}} = 0.5$ instead of a linearly drop of A towards $x_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}} = 1$ indicates the formation of a slight amount of a 1 : 1 complex. The Job's method was performed at $\lambda = 440$ nm, away from the absorption maximum, to avoid the influence of ligand absorption.

In the second method, when the absorption at pH 5.0 is plotted against $c_{3\text{HF}} / c_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}}$, three straight lines are obtained which intercept at $c_{3\text{HF}} / c_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}} = 1$ and at $c_{3\text{HF}} / c_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}} = 2$ (Fig. 3). These two changes in slope indicate the existence of a 1 : 1 complex, as well as a 1 : 2 complex.

When the mole ratios method was applied for the determination of the composition of the complex, it is obvious that two complex species were present in the solution. The investigations could be performed at $\lambda = 390$ nm, near to the

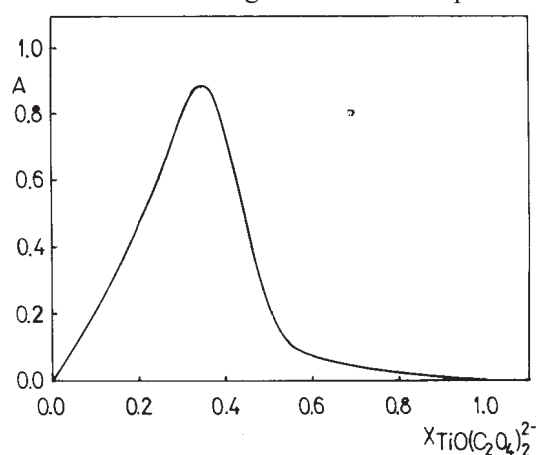


Fig. 2. Method of continuous variations of equimolar solutions of $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$ and 3HF: $c = 5 \times 10^{-3} \text{ mol dm}^{-3}$, blank is 3HF at the same concentration as in the mixtures, $\lambda = 440$ nm, pH 6.65.

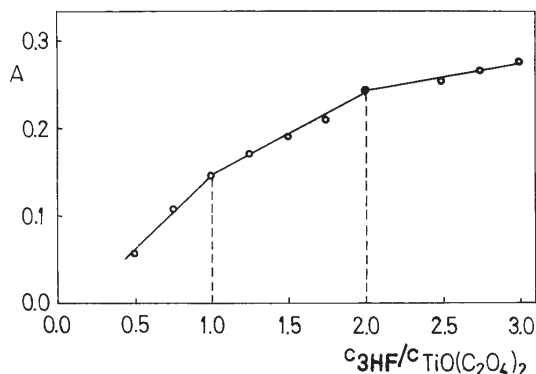


Fig. 3. Method of molar ratios: mixture $2.0 \times 10^{-4} \text{ mol dm}^{-3} \text{ K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$ and 3HF (from 1×10^{-4} to $5 \times 10^{-4} \text{ mol dm}^{-3}$), blank is 3HF at the same concentration as in the mixtures; pH 5.0; $\lambda = 490 \text{ nm}$.

absorbance maximum, because the influence of the ligand absorption at this low pH value was negligible.

Our attempt was to find the experimental conditions when the amount of the 1 : 1 complex present in the solution would be the smallest, that is when the 1 : 2 complex would be predominantly present in the solution. For this reason the Job's method was applied at pH 6.65. At $x_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}} = 0.33$, the very sharp and intensive maximum indicates a large amount of the 1 : 2 complex, but from the sudden change of A at $x_{\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}} = 0.5$ it can be assumed that these conditions do not favor the formation of the 1 : 1 complex.

According to the results obtained using these two methods, it is obvious that in more acid solutions, besides the 1 : 2 complex, some amount of the 1 : 1 complex is also formed. With the increasing pH, 3HF, which is a weak acid, is predominantly present as the anion, making the formation of the 1 : 2 complex a favorable process. Hence, at higher pH values, it can be assumed that only the 1 : 2 complex is present in solution.

The reaction of complex formation

Three solutions in 50 % aqueous ethanol were prepared to investigate the reaction of complex formation. The first solution contained $2 \times 10^{-4} \text{ mol dm}^{-3}$ 3HF, the second one contained $2 \times 10^{-4} \text{ mol dm}^{-3} \text{ K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$, whereas the third solution was mixed solution containing $c_{3\text{HF}} = c_{\text{TiO}(\text{C}_2\text{O}_4)_2} = 2 \times 10^{-4} \text{ mol dm}^{-3}$. The results obtained by measuring the pH of these solutions are presented in Table I.

TABLE I. pH-metric investigation of complex

Solution	3HF	$\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$	3HF + $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$
pH	6.05	4.96	4.85
c_{H^+}	8.91×10^{-7}	1.10×10^{-5}	1.41×10^{-5}
Σc_{H^+}		1.19×10^{-5}	1.41×10^{-5}

The results of these pH-metric measurements showed that the pH of the mixed solution containing both components was the lowest. That is the H^+ concentration in the solution containing both components was higher than the sum of the H^+ ion concentration in the other two solutions ($\Delta \text{H}^+ = 2.2 \times 10^{-6} \text{ mol dm}^{-3}$). (This result was ob-

tained simply by the adding the results in the table). This effect can be explained by assuming that metal ions are linked to the anions form of 3HF, whereby H^+ is liberated. Hence, the formation of the complex can be represented by the following reaction:



where n can be either 1 or 2, depending on the pH applied.

Effect of pH

The investigation of the complex between titanyl oxalate and 3HF was carried out in the pH range from 1.9 to 9.0. The shapes of the absorption curves and the positions of the absorption maxima are pH dependent, indicating the formation of different types of complexes at different pH values. In order to clarify the pH effect, the absorbance of a mixture containing $2.5 \times 10^{-5} \text{ mol dm}^{-3} K_2TiO(C_2O_4)_2$ and $5 \times 10^{-4} \text{ mol dm}^{-3}$ 3HF, and of a solution containing $5 \times 10^{-4} \text{ mol dm}^{-3}$ 3HF in this pH range was measured. From the two curves obtained the curve of the complex absorption $\Delta A = f(pH)$ was calculated (Fig. 4).

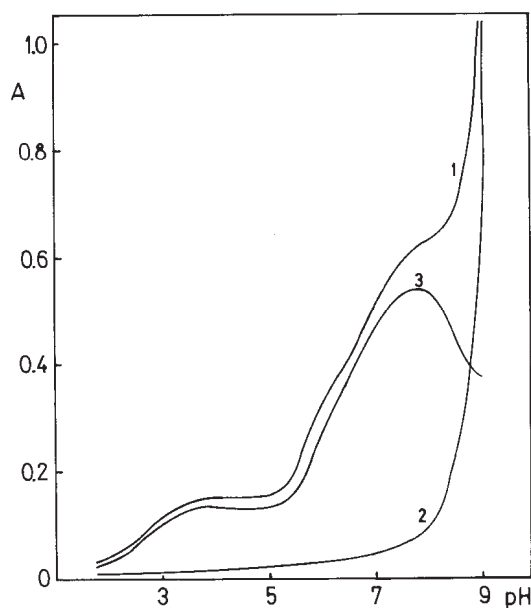


Fig. 4. Dependence of the absorbance on pH: 1) mixture $2.5 \times 10^{-5} \text{ mol dm}^{-3} K_2TiO(C_2O_4)_2$ and $5 \times 10^{-4} \text{ mol dm}^{-3}$ 3HF; 2) $5 \times 10^{-4} \text{ mol dm}^{-3}$ 3HF; 3) curve 1—curve 2; blank is 50 % aqueous ethanol: $\lambda = 425 \text{ nm}$.

The investigation of the spectra of the complex at different pH values, clearly showed that the absorption maxima does not change its position ($\lambda_{\text{max}} \approx 395 \text{ nm}$) until pH 8. When the pH is further increased, the maximum of the complex absorbance is shifted towards higher wavelengths ($\lambda_{\text{max}} \approx 410 \text{ nm}$). From Fig. 4, it can be clearly seen that the intensity of the absorbance is almost constant in the interval $3 < \text{pH} < 5$, but on further increasing the pH it starts suddenly to increase, and at pH 7.75 reaches its maximum, after which it decreases.

On the basis of the results regarding the complex composition and the influence of pH, it can be concluded that the most suitable conditions for the formation of

the $\text{TiO}(\text{C}_2\text{O}_4)_2^{2-} : 3\text{HF} = 1 : 2$ complex, are neutral solutions, $\text{pH} \approx 7$. In more acid solutions, $\text{pH} < 5$, the content of this complex is smaller, and some amount of the $1 : 1$ complex is also formed. However, because of the rather small values of the complex absorbance, it can be said that the acid solutions are generally unfavorable for the formation of complex species between titanyl oxalate and 3HF. In basic solutions, $\text{pH} > 8$, there is a bathochromic shift of the absorption maxima and a sharp decrease of the complex absorbance which indicates that there is a change in the structure of the formed complex, and a decrease of the complex concentration. This is probably due to the formation of metal hydroxy complexes.

Stability constant of the complex

According to the dependence of the complex absorbance upon the pH, presented in Fig. 4, and everything discussed above, it was possible to define the most convenient conditions where the $\text{TiO}(\text{C}_2\text{O}_4)_2^{2-} : 3\text{HF} = 1 : 2$ complex is predominantly present. It can be assumed that in the pH range from 5 to 7, the concentrations of the $\text{TiO}(\text{C}_2\text{O}_4)_2^{2-} : 3\text{HF} = 1 : 1$ complex, as well as of the metal hydroxy complexes, are negligible, and can be omitted from the calculations.

The concentration stability constant of the $\text{TiO}(\text{C}_2\text{O}_4)_2^{2-} : 3\text{HF} = 1 : 2$ complex, $\log \beta_2$, was calculated using the Bjerrum's method,¹² the dissociation constant of 3HF in mixed water–ethanolic solution ($K_d = 5.62 \times 10^{-11}$),⁶ and the equations related to the equilibrium reactions in the solutions. The highest concentration of the complex was found at pH 7.75, and it may be assumed that at this pH value the concentration of the $\text{TiO}(\text{C}_2\text{O}_4)_2^{2-} : 3\text{HF} = 1 : 2$ complex is approximately equal to the total $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$ concentration ($[\text{Complex}] \approx [\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}]_0$) in view of the fact that the concentration of 3HF in the solution is twenty times higher than that of the $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$.

Therefore, the molar absorptivity of the complex was calculated from the expression:

$$a = A_{\max} / [\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}]_0 \quad (1)$$

The concentrations of the $[\text{Complex}]$, $[\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}]$, and $[\text{C}_{15}\text{H}_9\text{O}_3^-]$ were calculated from the following equations:

$$[\text{Complex}] = A/a \quad (2)$$

$$[\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}]_0 = [\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}] + [\text{Complex}] \quad (3)$$

$$[\text{C}_{15}\text{H}_9\text{O}_3\text{H}]_0 = [\text{C}_{15}\text{H}_9\text{O}_3\text{H}] + [\text{C}_{15}\text{H}_9\text{O}_3^-] + 2[\text{Complex}] \quad (4)$$

$$K_d = [\text{H}^+] [\text{C}_{15}\text{H}_9\text{O}_3^-] / [\text{C}_{15}\text{H}_9\text{O}_3\text{H}] \quad (5)$$

The concentration stability constant:

$$\beta_2 = [\text{Complex}] / [\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}] [\text{C}_{15}\text{H}_9\text{O}_3^-]^2 \quad (6)$$

was calculated at three different pH values, and the obtained results are presented in Table II.

TABLE II. Concentration stability constants of the $[\text{TiO}(\text{C}_2\text{O}_4)_2(\text{C}_{15}\text{H}_9\text{O}_3)_2]^{4-}$ complex

pH	$\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}$	$[\text{C}_{15}\text{H}_9\text{O}_3^-]$	[Complex]	β_2	$\log \beta_2$
5.0	1.87×10^{-5}	2.74×10^{-9}	6.25×10^{-6}	4.44×10^{16}	16.65
6.0	1.24×10^{-5}	2.67×10^{-8}	1.26×10^{-5}	1.43×10^{15}	15.15
7.0	3.56×10^{-6}	2.57×10^{-7}	2.14×10^{-5}	9.12×10^{13}	13.96

$$a_{425} = 21600 \text{ cm}^{-1} \text{ mol}^{-1} \text{ dm}^3$$

In the acid media (pH < 3), the complex absorbance decreased abruptly (Fig. 4) which can probably be explained by decomposition of the complex due to protonation of the ligand. In the pH region between 3 and 5, the complex absorbance is almost constant but rather small, indicating that these are unfavorable conditions for complex formation. This is one more reason why concentration stability constant, β_2 , were calculated only for the segment of the curve from pH 5 to pH 7.

With increasing of pH, from pH 5 to pH 7, the concentration stability constant, β_2 , decreases (Table II). This can be explained by the more intensive increase in the concentration of the anionic form of the ligand than by an increase in the complex concentration.

The results obtained at higher pH values (pH > 7.75) again shows a decrease in the complex concentration, which may be explained by decomposition of the complex caused by the action of hydroxyl ions, or by the formation of metal hydroxy-complexes.

Determination of 3HF

The high stability of the complex allows the quantitative determination of microamounts of 3HF. Solutions containing a constant $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2$ concentration ($1 \times 10^{-3} \text{ mol dm}^{-3}$) and various 3HF concentrations were prepared. All the solutions were prepared with 50 % aqueous ethanol which was found to be the optimum solvent for complete dissolution of both components. The investigations were performed at pH 4.8, which was the native value obtained after complex formation, and did not need any further adjustment. Complex absorbance was redorded at $\lambda = 430 \text{ nm}$ where the absorbance of 3HF is negligible, so 50 % aqueous ethanol was used as the blank.

TABLE III. Spectrophotometric determination of 3HF ($n = 8$)

Taken (mol dm^{-3})	Found (mol dm^{-3})	SD	CV/%
5.0×10^{-5}	5.2×10^{-5}	1.58×10^{-6}	3.04
2.0×10^{-4}	1.9×10^{-4}	5.34×10^{-6}	2.81
3.5×10^{-4}	3.4×10^{-4}	7.06×10^{-6}	2.09

A linear dependence of the absorbance on the concentration was obtained for 3HF concentrations in the range from 2.5×10^{-5} to $3.5 \times 10^{-4} \text{ mol dm}^{-3}$. By the application of the least squares method, the regression equation: $y = 0.00198 + 0.12723x$ ($n = 8$) was calculated with a high correlation coefficient of 0.9998. The accuracy of the method was determined for three different 3HF concentrations (Table III).

3HF form complexes with a great number of metals and, therefore, its quantitative determination is not possible in the presence of more than one complexing species.

ИЗВОД

СПЕКТРОФОТОМЕТРИЈСКО ИСПИТИВАЊЕ КОМПЛЕКСА ИЗМЕЂУ ТИТАНИЛ
ОКСАЛАТА И 3-ХИДРОКСИФЛАВОНА У ВОДЕНО-ЕТАНОЛНОЈ СМЕШИ

МАРА АЛЕКСИЋ, СЛАВИЦА БЛАГОЈЕВИЋ, ДУШАН МАЛЕШЕВ и ЗОРИЦА РАДОВИЋ

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Коришћењем погодних спектрофотометријских и рН-метријских метода утврђено је да титанил оксалат и 3-хидроксифлавон (3HF) граде $[\text{TiO}(\text{C}_2\text{O}_4)_2(\text{C}_{15}\text{H}_9\text{O}_3)_2]^{4-}$ комплекс. Испитивање састава и одређивање концентрационих константи стабилности овог комплекса вршено је у 50 % етанолном раствору, на собној температури (20 °C), у области рН од 1,9 до 9,0. Израчунате вредности концентрационе константе стабилности комплекса, $\log \beta_2$, су у интервалу од 16,65 при рН 5,0 до 13,96 на рН 7,0. Одређени су и услови за спектрофотометријско одређивање 3HF коришћењем награвеног комплекса у концентрационом интервалу од $2,5 \times 10^{-5}$ до $3,0 \times 10^{-4} \text{ mol dm}^{-3}$.

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