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ANALYSIS

SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FLUORIDES WITH THORIUM(IV) –QUERCETIN–5'–SULFONIC ACID COMPLEX

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Abstract: A spectrophotometric method for the determination of fluorides based on the thorium(IV) complex with quercetin–5'–sulfonic acid (QSA) has been developed. The principle of the method is the decrease in absorbance of Th–QSA complex, proportional to the F^- concentration. Molar absorptivity is $1.8 \cdot 10^4$ $dm^3 \cdot mole^{-1} \cdot cm^{-1}$ at λ=420 nm. At optimal conditions (pH 4.4) QSA forms complexes with Th(IV) ions in the 1:1 and 1:3 metal:ligand ratio. Stability constants of these complexes were established. The calibration graph was linear over the concentration range 0.2–1.6 $\mu g \cdot cm^{-3}F$. The method has been applied for the determination of fluorine in plant materials and in pharmaceutical preparations containing fluorine in an ionized form. Validation of the proposed method and statistical evaluation of results was carried out.

Keywords: fluorides, spectrophotometric determination, thorium(IV) complex with quercetin–5'–sulfonic acid.

In addition to the potentiometric method, the spectrophotometric determination of the fluorides is the method most widely applied for the determination of this element.

Spectrophotometric methods are mostly indirect methods (1,2) in which the content of fluorine is determined on the basis of the degree of the decolorizing dye metal – ligand complexes.

However, many of these methods are limited by the lack of sensitivity, reproducibility, simplicity, and they are often susceptibile to the presence of various interfering ions.

Hydroxyflavones are very sensitive reagents for several ion metals and some anions and find wide application in analysis. Quercetin and quercetin sulfonic acid have been used in photometric and fluorimetric determinations of several metals: zirconium (3), chromium (4) and thorium (5). These reagents are very sensitive, but they lack selectivity and require prior separation of the metal ions from the accompanying ions.

Several methods of quantitative isolation of fluoride from matrix (plant, biological material) are available (2, 7). For determination of small quantities of fluoride microdiffusion method is most advantageous (8–11).

Quercetin–5'–sulfonic acid (QSA) is a sulfonic derivative of quercetin, and is a strong acid due to the presence of the SO_3H group in the QSA molecule. Within the pH range 1–8, QSA forms water soluble complexes with some main group and d–electron metal ions, in view of favorably situated hydroxylic and carboxylic groups. Within the pH range 1.0–6.5, QSA exists as the H_5L^- ion, taking part in complexation reactions with metals.

In this work, we have investigated the possibility of using the QSA–thorium(IV) complex for the determination of fluorine in complex matrices of plant tissues. Moreover, we have studied the optimal formation conditions of the QSA complexes with thorium(IV) ion and we have determined the composition and stability constants of these complexes.

EXPERIMENTAL

Aparatus and reagents

Absorbance measurements were performed by using a spectrophotometer SPECORD M40 (Carl–Zeiss, Jena); pH measurements were performed by using a pH–meter Jenway 3030 equipped with the combination electrode OSH–10–00 (Metron, Poland).

Quercetin–5'–sulfonic acid (QSA) was obtained according to the method described by Terpiłowski *et al* (6). A stock solution of QSA ($5 \cdot 10^{-4}$ $mol \cdot dm^{-3}$) was prepared by dissolving QSA in redistilled water. A thorium (IV) stock solution

($6.25 \cdot 10^{-5}$ mol·dm^{-3}) was prepared by dissloving $Th(NO_3)_4 \cdot 5H_2O$ (Merck) in redisilled water with small addition of HNO_3, which prevents the formation of hydrolyzed species.

Sodium fluoride (POCh, Poland) was dried for 24 h at 110°C. Standard fluoride solutions (1 mg·cm^{-3} of NaF) were stored in polyethylene containers.

Sodium acetate buffer solutions (pH 4.4, 5.2) were prepared by adjusting a solution of acetic acid (0.2 mol·dm^{-3}) to pH 4.4, 5.2 with sodium acetate solution (0.2 mol·dm^{-3}) using a pH meter.

All the reagents were of analytical grade and were used without further purification. Double-distilled water was used throughout.

Isolation of fluorides (by microdiffusion) and its spectrophotometric determination in plant material

A portion of the sample (0.1–0.25 g) was placed in the middle compartment of Obrink's chamber which contained 1 cm^3 of NaOH (2 mol·dm^{-3}) used as absorbing solution in the inner compartment and 1.5 cm^3 of 80% H_2SO_4 containing 0.02% sodium dodecylsulfate in the outer compartment. About 2.5 cm^3 of 72% $HClO_4$ saturated with silicon oil DC 200 (Fluka) was then introduced into the middle compartment. The chamber was closed and maintained at 60°C for 24 h. The absorbing solution was then transferred with redistilled water to a 25 cm^3 volumetric flask and neutralized with $HClO_4$ (2 mol·dm^{-3}). Then to the above solution, 4 cm^3 Th(IV), 4 cm^3 QSA, and 5 cm^3 buffer solution (pH 4.4) were added and adjusted to 25 cm^3 with redistilled water. Absorbance was measured at 420 nm against a reagent blank (without fluoride). The fluoride concentration was calculated from the calibration graph obtained for similarly prepared standards.

Assay procedure for „Natrium Fluoratum" 0.5 mg „Polfa" tablets

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder, equivalent to about 75 μg of NaF, was transferred to the middle compartment of Orink's chamber and microdiffusion process was performed as described earlier for the isolation of fluorides (by microdiffusion) in plant material.

Potentiometric determination of fluorides

Into the solutions obtained after microdiffusion (as described above), 5 cm^3 of buffer solution (pH 5.2) was added, and the mixture diluted to 25 cm^3 with water. Then the potential of the resulting solutions was measured and fluoride concentration was calculated from the parameters of calibration curve equation, obtained with similarly treated standards. The calibration graph was linear within the range of 2–1.6 μg·cm^{-3} F^-.

An ion-selective electrode OPF-7113 and a calomel electrode (Radelkis, Hungary) with a digital pH-meter Jenway 3030 were used.

Parameters of the calibration graph for the determination of fluoride by the potentiometric method were as follows:

$E = a \cdot pF + b$ (where: a=66.9 mV·dm^3·mol^{-1} and b= –43.2 mV),

correlation coefficient r=0.9994.

RESULTS AND DISCUSSION

Spectral characteristics

According to literature data (12) UV–VIS spectra of QSA solutions are characterized by the two absorption bands λ_{max}=257 and 367 nm. As alkalization of QSA solutions proceeds, the intensity of the short wave band decreases gradually and, at the same time, a new band appears at λ_{max}=270 nm. Simultaneously, the long wave band shifts toward the long waves. In a solution of pH>8, QSA slowly decomposes and new band appears at λ_{max}=330 nm. QSA forms with Th(IV) ions intensive yellow complexes.

Figure 1 presents the spectra of the substrates and products of the QSA–thorium(IV) reaction. The wide absorption band of QSA has its maximum at λ=367 nm (curve A), thorium ions do not show any absorption in the analyzed spectrum range (280–560 nm). The QSA and QSA–thorium complex absorption bands are situated close to each other, and the introduction of Th(IV) ions in excess causes a decrease of the maximum QSA band at 367 nm, and formation of a new bathochromic shifted maximum at λ=420 nm. Because the determination of fluoride by QSA–Th(IV) complex requires the use of in QSA excess, necessary for the complete binding of thorium, its absorption band would overlap to a great measure the band of the complex formed. Thus, the Th(IV)–QSA excess, necessary for the complete binding of thorium, its absorption band would overlap to a great measure the band of the complex formed. Thus, the Th(IV)–QSA complex was characterized by absorption band at λ=420 nm, measured against the reagent blank. The optimum QSA excess was determined by the spectrophotometric titration method. Complete binding of Th(IV) ions by QSA requires a 6–8 fold excess of QSA. In further investigation the 8-fold molar excess of the ligand was used. The complex was formed immediately after the addition of the ligand to the solution of

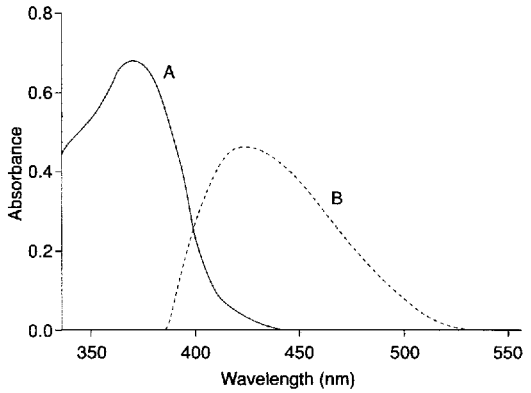

Figure 1. Absorption spectra of: (A)QSA against water, $c_{QSA}=5\cdot10^{-5}$ mol·dm^{-3}, pH=4.4; (B) Th–QSA against reagent blank, $c_{Th}=2.5\cdot10^{-5}$ mol·dm^{-3}, $c_{QSA}=2\cdot10^{-4}$ mol·dm^{-3}, pH=4.4.

thorium(IV) and it was stable for several hours.

Influence of pH on Th–QSA absorbance

The absorbance of Th–QSA complex (metal to ligand ratio 1:8) versus pH is shown in Figure 2. Within the pH range 1.0–3.2 the curve corresponds to the formation of a single complex or of several complexes, and elevation of pH above 5.2 leads to the formation of a new complex with a higher absorbance walue. Within the pH range 2.6–5.0, the absorbance of the complex investigated remained almost unchanged and pH=4.4 was used in further investigation.

Composition of complexes

The compositions of the complexes were established by the following methods: Job's method of the equimolar solutions (Figure 3); molar ratio method (spectrophotometric titration, c_{Th}=const.) (Figure 4); reversed spectrophotometric titration method, c_{QSA}=const. (Figure 4).

Absorbance measurements were made at constant pH=4.4. The ionic strength (I) was brought up to a constant value of 0.1 mol·dm^{-3} by means of $NaNO_3$ solution (1 mol·dm^{-3}).

The Job's curve (Figure 3) does not have a sharp maximum, which points out to the composition of complex. Such a shape of the curve is characteristic, when the complex formation is simultaneously accompanied by considerable dissociation or when several complexes are formed, which have similar stability (13). The point of intersection of the tangents of the left and the right branch of the Job's curve does not indicate the composition of one complex, this fact suggests that a few complexes ML, ML_2 or ML_3 may well be formed.

The reversed spectrophotometric titration curve (Figure 4) indicates that, at a thorium excess, a complex of the molar ratio M:L=1:1 has been formed. Moreover, Figure 4 demonstrates that, in the presence of excess metal ions, no multinuclear complexes were formed.

The titration curves (Figure 4) have no distinct points of inflection; therefore, in order to determine the composition of the complex the moving equilibrium method was used. This method showed

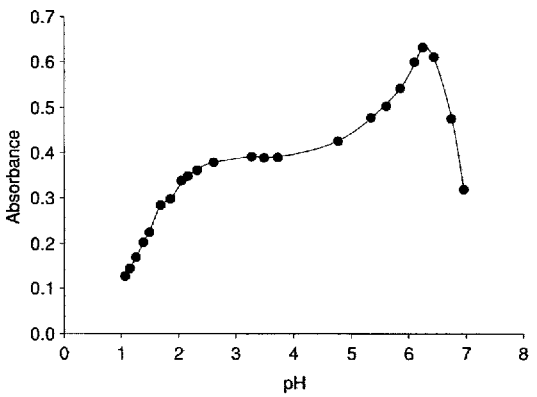

Figure 2. Influence of pH on Th–QSA complex formation, $c_{Th}=2.5\cdot10^{-5}$ mol·dm^{-3}, $c_{QSA}=2\cdot10^{-4}$ mol·dm^{-3}, λ=420 nm, I=0.1 mol·dm^{-3}.

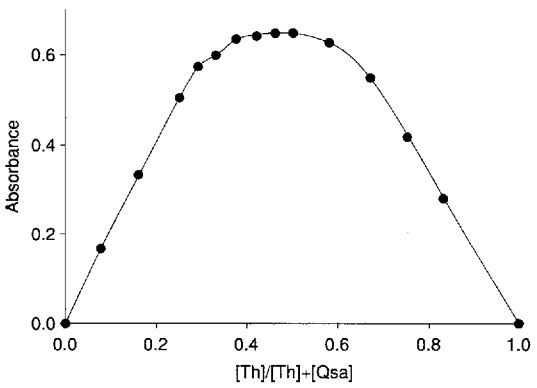

Figure 3. Determination of Th:QSA ratio by Job's method, $c_{Th}+c_{QSA}=5\cdot10^{-4}$ mol·dm^{-3}, pH=4.4, λ=420 nm, I=0.1 mol·dm^{-3}.

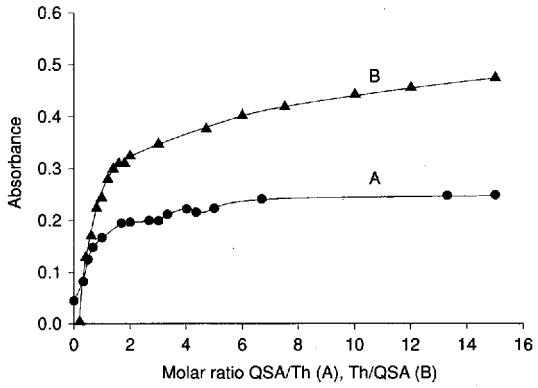

Figure 4. Determination of Th:QSA ratio by spectrophotometric titration, (A) $c_{QSA}=1.5\cdot10^{-5}$ mol·dm^{-3}, pH=4.4, λ=442 nm; (B) $c_{Th}=2\cdot10^{-5}$ mol·dm^{-3}, pH=4.4, λ=420 nm, I=0.1 mol·dm^{-3}.

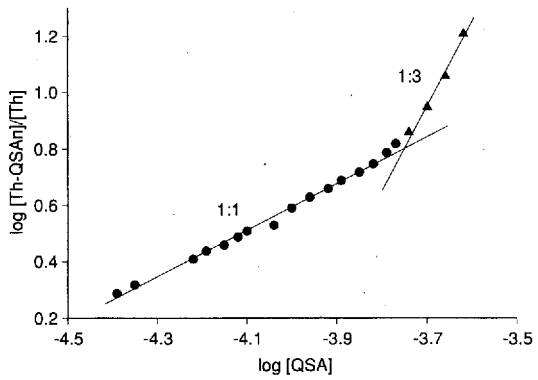

Figure 5. Determination of Th:QSA ratio by the equilibrium shift method, $c_{Th}=2\cdot10^{-5}$ mol·dm^{-3}, pH=4.4, λ=420 nm, I=0.1 mol·dm^{-3}.

(Figure 5) that, under the established conditions, thorium forms complexes with QSA in the molar ratio 1:1 and 1:3 depending on the molar excess of the ligand to the metal. The dissociation constants of the complexes Th–QSA and Th–$(QSA)_3$ were 4.14 and 11.69, respectively.

Stability constants of Th–QSA complexes

Stability constants of complexes of Th(IV) with QSA were examined by Bjerrum's method (14). They are presented in Table 1. Spectrophotometric titrations were performed by using various concentrations of thorium ($2\cdot10^{-5}$, $3\cdot10^{-5}$ and $4\cdot10^{-5}$ mol·dm^{-3}) at constant pH=4.4 and I=0.1 mol·dm^{-3}.

Influence of interfering ions

The influence of different ions (cations and anions) on the absorbance of the thorium(IV) – QSA complex was investigated at the fluoride level equal to zero. The following ions Pb^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, Zn^{2+}, PO_4^{3-} and $C_2O_4^{2-}$, were found to exert an essential influence on the absorbance of the complex already at a concentration comparable with that of thorium or even below that. The influence of other ions (Mn^{2+}, Ni^{2+}, SO_4^{2-}, and Cl^-) occur only at concentrations by exceeding thirty times or more the thorium(IV) molar concentration.

In conclusion, the reliable determination of trace amounts of fluoride content by the method based on the decrease in absorbance of the Th–QSA complex proportional to the F^- concentration is possible only after removal of or separation from the interfering ions.

As already mentioned, this method was used after fluorine had been isolated from the matrix by microdiffusion.

Validation of the method

Validation procedure of the spectrophotometric method was performed according to the guideli-

Table 1. Stability constants of complexes of Th(IV) with QSA determined by spectrophotometric method

n	[L]	K=1/[L]	$\beta_n=K_1\cdot K_2\cdot..K_n$	$\log\beta_n$
0.5	$2.10\cdot10^{-6}$	$4.76\cdot10^{5}$	$4.76\cdot10^{5}$	5.68
1.5	$2.47\cdot10^{-5}$	$4.05\cdot10^{4}$	$1.93\cdot10^{10}$	10.28
2.5	$3.73\cdot10^{-5}$	$2.68\cdot10^{4}$	$5.17\cdot10^{14}$	14.71

K_n – stability constant, β – total stability constant

Table 2. Spectrophotometric determination of fluoride by means of QSA–thorium (IV) complex

Taken ($\mu g\ cm^{-3}$)	Found x (n=6) ($\mu g\ cm^{-3}$)	Standard deviation	Coefficient of variation (%)
0.4	0.473	0.035	7.5
0.8	0.851	0.044	5.2
1.6	1.667	0.058	1.5

nes presented by Funk *et al* (15). Results were obtained in terms of the limit of detection, regression equation, linear range, correlation coefficient, and precision.

The calibration graph was prepared for six standard solutions, 6–fold replicated at each concentration within the range of 0.2–1.6 $\mu g \cdot F\ cm^{3}$.

The preliminary first and second–degree calibration functions were calculated from the measured values and the Mandell's fitting test (15) was used to verificy linearity. The second–order calibration function will not provide a significantly better fit. A graph of the data shows no apparent deviation from linearity. The correlation coefficient has a value of 0.999. The spectrophotometric calibration graph was found to obey Beer's law in the fluoride concentration range: 0.2–1.6 $\mu g \cdot cm^{-3}$. The parameters of the calibration function were $A=a \cdot c_F+b$ (where: a=–0.1820 $abs. \cdot cm^3 \cdot \mu g^{-1}$, b=0.4325 abs.)

The applicability of the linear regression equation also requires a constant imprecision (homogeneous variance of measured value) over the range. The F–test shows that there is not a significant difference between the variances. The dispersion of the measurement is independent of the analyte concentration.

The limit of detection is an important criterion when the fluoride content is very small or the amount of sample available for analysis is restricted. The limit of detection expressed in concentration units was 0.01886 $\mu g \cdot cm^{-3}$.

Within day variation of the method was verified by analyzing solutions containing known concentrations of fluoride ions (Table 2).

The presented data show the variability coefficient, obtained by determining fluorine at concentrations ranging from 0.2 to 1.6 $\mu g \cdot cm^{-3}$, to be from 7.5 to 1.5%.

Between day variation was determined and tested for significant difference by using the F–test. During the 9 consecutive days (twice each day) absorbance was measured for the solution containing a known fluoride concentration (0.8 $\mu g \cdot cm^{-3}$):

The calculated variance F–test testing value (2.52) was less than the table value, F=3.39, which means that the between day standard deviation was only negligibly larger than the within day variation, indicating high precision of the determinations.

Determination of fluorine in plant material

The proposed method has been used for determining fluoride in tea. Table 3 summarizes the results obtained by the present method and by the potentiometric method using an ion–selective electrode. Application of the statistical F–test to the ratio of variances for samples determined by both methods showed that the variance for the spectrophotometric method is not significantly different from that found by the ion–selective method. For accuracy comparison of these methods t–Student's test was used.

CONCLUSIONS

Conditions of formation of QSA with Th(IV) complexes in aqueous solutions were investigated. Th–QSA complexes were charac-

Table 3. Determination of fluorine content in plant material (tea) and NaF content in Natrium Fluoratum 0.5 mg „Polfa'' tablets by spectrophotometric method (QSA–Th(IV) complex) (x_1) and potentiometric method (x_2). Comparison of accuracy of the methods calculated using t–test (P=95%).

Plant material „Madras'' tea	x_1=268.44 mg g^{-1} d.m. x_2=264.65 mg g^{-1} d.m.	t=1.92 t_{crit}=2.15	$t<t_{crit}$
Plant material „Popularna'' tea	x_1=177.12 mg g^{-1} d.m. x_2=179.47 mg g^{-1} d.m.	t=1.61 t_{crit}=2.15	$t<t_{crit}$
Natrium Fluoratum 0.5 mg „Polfa'' tablets	x_1=0.503 mg x_2=0.502 mg	t=0.25 t_{crit}=2.23	$t<t_{crit}$

terized by absorption band at λ=420 nm (ε=1.8·10^4 dm^3·mole^{-1}·cm^{-1}). The pH of the solution exerts a significant influence on the reaction between Th(IV) and QSA. Within the pH range 2.6–5.0, almost constant absorbance was obtained for the Th–QSA (1:8) complex.

The compositions of investigated complexes were determined by two methods at constant pH 4.4. The results obtained by the equilibrium shift method prove that the formation of thorium complexes with QSA depends on the molar excess of QSA to Th(IV) ions. This method showed that, if the molar excess of QSA is less than 8–fold, the molar ratio Th:QSA is 1:1, and at higher concentrations of the ligand, 1:3 complex was formed.

The spectrophotometric procedure was critically evaluated with particular regard to linearity and range of calibration graph, precision, limit of detection, and effect of interfering ions. The potentiometric method was used as a reference method. The proposed method becomes highly selective, if the determination of fluoride is preceded by microdiffusive separation of fluorine from the matrix.

Statistical analysis of the results obtained has shown the new developed method, based on the formation QSA–Th(IV) complex, to be comparable with the potentiometric method. The method is suitable for the fluoride determination in plant material.

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