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Determination of Fluoride in Real Water, Serum And Urine Samples Using Resorcin Blue Complex By Spectrophotometry

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ABSTRACT

A simple spectrophotometric determination of fluoride in drinking and waste water has been developed using Resorcin blue-Zr yellow coloured complex. The proposed method is based on the reaction of fluoride with the coloured complex to produce colourless complex and discharging of the free ligand. The reaction reaches equilibrium at fluoride concentration of 0.062 mM. Beer-Lambert law is obeyed in the range 0.01-0.024 mM of fluoride. The molar absorptivity at 624 nm is $6.45\times10^{3} L\ mol^{-1\cdot}cm^{-1}$. Fluoride concentration higher than 1.0 $mg^{\cdot L-1}$ can be measured after proper dilution. The determination of fluoride in the range of 0.06-1.2 g m^{L-1} (SD=1.2%) was successfully performed. The detection limit, quantitation limit, and the method was successfully applied to the determination of trace amount of fluoride content of some real samples.

KEYWORDS Fluoride, real samples, Zr(IV) and Resorcin blue

Introduction

Fluorine, the 13th most abundant element of the earth's crust, represents about 0.3g / kg of earth's crust. Fluoride (F^-) occurs in almost all waters from trace to high concentration (Darma, and Daria (2011). It has been shown to cause significant effects in humans through drinking water (WHO, 2006) . Low concentration of fluoride in drinking water have been considered beneficial to prevent dental carries (Jacobson, J.S and Weinstein, L.H. (1977), but excessive exposure to fluoride can give rise to a number of adverseeffects such as causing fluorosis (Larsen, and Jensen, S.J. (1993). WHO has set alimit value of 1.5 $mg\cdot L^{-1}$ for fluoride in drinking water (ZolgharneinJ,and GhasemiJ (2009). This necessitates an accurate, simple, rapid and cost effective analytical method is of high importance. Spectrophotometric methods are widely used in the determination of fluoride because of advantages such as simplicity, convenience, accuracy and reproducibility (F. J. Green,(1990). They are based on the reaction of fluoride with coloured metal chelate complexes, producing either a mixed-ligand ternary complex or replacement of the ligand by fluoride to give a colourless metal-fluoride complex and the free ligand with a colour different to the metal-ligand complex (N. and K. R. Murty, (1979).

Resorcin blue is used in literature as pH indicator for mineral acids, strong bases and alkaloids where the visual transition interval is red at pH 4.4 to blue at pH 6.4 . It is also used as a redox indicator in the titration of Fe(II), As(III), Sb(III), U(IV), Mo(V), hydroquinone, and oxalic acid with ammonium hexa nitrato cerate (IV) in $HClO_4$ medium (H.-Z. Wu, Z.-R. Wu, J. Lin and Z.-S. Zheng, (1999). Resorcin blue is used in the determination of Cr(VI) (M. Ulrychova, and Z. Pazourkova, (1976) , and for staining cellulose in plants (S. Mustafa, M. El-Sadek, and E. A. Alla, (2002). (K. Basavaiah, and J. M. Swamy, (1999) , (M. S. Kamel and R. Sayed, (2008)employing many dyes including Resorcin blue (A.K.Mistra, P.S.Saxena and U.Gupta, (2002) and Rawa A. Zakaria, (2009).

The present study aimed to develop spectrophotometric method for determination of fluoride in drinking water using Resorcin blue – $ZrOCl_2$ yellow colored complex with fluorideions.

Experimental

Reagents and solutions

Resorcin blue and Zirconyl chloride octahydrate provided by Acros were used without any further purification. All the chemicals were of analytical reagent grade except wherestated otherwise. Solutions were prepared using doubledistilled water.

Standard fluoride stock solution was prepared by dissolving 0.1382 g of sodium fluoride provided by Merck in 250 ml water. The stock solution was further diluted as needed.Zirconyl chloride octahydrate (0.133 g) was dissolved in 25 mL of distilled water. Concentrated hydrochloric acid (LR grade) (350 mL) was added to it. The mixture was then diluted with water to make up the volume to 500 mL.

Apparatus

Elico-SL-244 double beam spectrophotometer with 1.0 cm quartz cells was used for the absorbance measurements at fixed wavelength. An Elico Li-129 model pH meter with combined glass electrode was used for all pH measurements.

Preparing of the Metal Complexes Solutions

Job's method of continuous variation was adopted for determination of the composition of the coloured complex. Zirconium to ligand ratio was also studied by making comparison between the spectra of complexes ofdifferent metal to ligand ratios such as 1:1, 1:2, 1:3, 2:1,3:1, 2:3, and 3:2. The blank was prepared by the same procedure using the solvent instead of the Zirconium ionsolution. Zirconium to ligand ratio was found to be 1:2.

The complex solutions for the spectrophotometric measurements were prepared by mixing Zirconium 1:2 Resorcin blue ratio of 5×10^{-3} M of Zirconium and 5×10^{-3} M of Resorcin blue in ethanol solution, which was then diluted to (2×10^{-4} M) that is suitable for the spectrophotometric measurements. The stability of the complex in ethanol solution was examined for two weeks, and the complex is stable.

Reaction of fluoride with the prepared complexes solutions

Various amounts of fluoride were added in the range 0 -2 mg·L–1 to 25 ml volumetric flask containing aluminium 1:2 complex solution of Resorcin blue in ethanol (2×10^{-4}M, 24.5 ml). The solution was completed to volume by water. The absorbance was measured at the wavelength of the maximum difference between the absorption spectra of the complex and the ligand which was 624 nm.

Determination of fluoride in real water samples

The method under investigation was tested using a real drinking water sample which had been collected. The sample was collected in June 2014 from a groundwater well in Kadiri , Ananthapur district . Fluoride was analysed colourimetrically using SPADNS as fluoride reagent and ELICO double beam spectrophotometer. Fluoride was measured in the sample using aluminium resorcin blue 1:2 complexes and the obtained results were compared using SPADNS method table 1 and table2. The method under investigation was tested using areal drinking water sample which had been collected and analysed by the Central Public Health Laboratory belonging to Ministry of Health and responsible for controlling water quality. The sample was collected in June 2014 from a groundwater well in Kadiri , Ananthapur district. Fluoride was analysed colourimetrically using SPADNS as fluoride reagent and ELICO double beam spectrophotometer. Nitrate, sulfate, chloride, and other characteristic data is measured. Fluoride was measured in the sample using aluminium resorcin blue 1:2 complexes and the obtained results were compared with that reported SPADNS method.

Procedure

Purified water was used for the carrier solution, and the flow rate was set to 0.8 ml/minn (P1). The flow rate of the buffer solution was set to 0.4 ml/min (P2), and the flow rate of the fluoride solution was set to 0.05 ml/min (P3). The flow rates of the three pumps were kept to within ± 0.3% of the desired values. Three solutions were mixed and passed into the cell at 1.25 ml/min of the total flow rate Fig. 1. Each serum sample (0.3 ml) was mixed with a double volume of diluting solution a(0.075 mol/l sodium acetate solution, pH 4.97). The pH value was adjusted precisely to 5.38 ± 0.02 using HCl or NaOH. Urine samples were prepared for measurement by diluting 10 times with diluting solution B (0.05 mol/l sodium acetate solution, pH 5.30). Standard or sample solutions (each 0.3 ml) were injected into the system with a 1-ml syringe. Measurement for every standard and sample solution was performed twice. The working curve of the peak heights of the potential differences to the fluoride concentrations was calculated using a curve fitness program [17].

Results and Discussion

Resorcin blue and its Zirconium Complexes

Resorcin blue is soluble in ethanol (20 mg·mL–1), methanol, acetic acid and acetone, and slightly soluble in ether.Its solubility in water is 30 mg·mL^{-1} . Resorcin blue exhibits blue colour in ethanol solution and displays twobands in the visible region at 505 and 617 nm (Fig1).The molar absorptivity at these two wavelengths is[5.98 ± 0.12] ×10^3 and [4.91 ± 0.09] ×10^3 L·mol^{-1}·cm^{-1}respectively. The obtained results from applying of Job's method of continuous variation indicated that aluminium to Resorcin blue complex ratio is 1:2. The ratio was also determined spectrophotometrically by comparing the spectra of Zr(IV)- Resorcin blue yellow complexes of different ratios witheach other's. A possible structure for the complex is given in Fig2.

The complex exhibits brown colour in ethanol solutionand has one band in the visible region at 502 nm (Figure1) where the molar absorptivity is [6.45 ± 0.22] × 10^3L·mol^{-1}·cm^{-1}. Thus, there is hypsochromic shift (decrease in the wavelength) of about 115 nm after complexation

With Zr(IV). This is resulting in a change in colour fromthat of the ligand, red to the colour of the complex of yellow.

Resorcin blue displays two bands in the visible regionat 496 and 591 nm while its zirconium complex exhibitsone band at 483 nm in water solutions. Therefore, usingof water instead of ethanol as a solvent is resulting in a hypsochromic shift in the absorption spectra of the Resorcin blue ligand and its zirconium complex of about 26and 19 nm, respectively. Thus, the solvent has a negative solvatochromism effect on the spectra of both of the ligandand it aluminium complex where increasing of the polarity of the solvent, as we move from ethanol to water, leads to a hypsochromic shift (a decrease in the wavelength. The difference in the absorption spectra betweenthe complex and the ligand is bigger in ethanol than inwater solution. This is due to the polarity of water and itsability to form hydrogen bonds in comparison with ethanol. This leads to destabilize the excited state which isexpected be less polar than the ground state .Therefore the effect of fluoride on the absorption spectra of the complex was examined in ethanol solution.

Reaction of Fluoride with the Resorcin blue Complex

Fluoride reacts with the brown zirconium Resorcin blue 1:2 complex to produce a colourless zirconium fluoridecomplex by replacement of the Resorcin blue by fluoride and liberating of the free ligand. This is resulting in achange in the colour from that of the complex, brown to the colour of the free ligand according to the equationbelow. Zirconium reacts with fluoride to give compounds .Fluoride interacts with complex under investigation tocause an increase in absorbance at 624 nm due to the releasing of the free ligand. The absorbance of the releasedfree ligand is related linearly at 624 nm to the concentration of fluoride in the range 0.0 to 0.024 mM (0.0 to 1.0mg·L^{-1}) (Fig3).

The squared correlation coefficient R^2, is 0.993, and the equation of the linear calibration curve is ($y = 0.3567x + 0.2773$) .The relationship of the reaction of fluoride with zirconium Resorcin blue1:2 complex was best described by a sixth-order polynomial function (Figure4) where the squared correlation coefficient R^2, is 0.9982.The reaction reaches equilibrium at fluoride concentrationof 0.054 mM (≈2.27 mg·L^{-1}). The equilibrium constant(Keq) was calculated from the equilibrium equation belowas 1.12 × 10^{34}. The large value for Keq indicates that the equilibrium lies far to the right.Where: [Resorcin blue] is measured using the molar absorptivity for Resorcin blue, which was found to be [5.67 ± 0.07] ×10^3 L·mol^{-1}·cm^{-1} at 624 nm. Figures 3 and 4 show that Zirconium Resorcin blue complex is suitable for determination of fluoride in therange 0.0 to 1.0 mg·L^{-1}. The sensitivity, detection limit,limit of quantification, percentage recovery of fluoride in real water sample, and the percentage recovery of 0.75,1.5, and 2.0 mg·L^{-1} fluoride of the aluminium Resorcin blue for the spectrophotometric determination offluoride at 624 nm are given in table 2.

The sensitivity was taken as the average of the slope of the calibration curve for five replicates. The detection limitand the limit of quantification were calculated as (3.3 σ/S)and (10 σ/S) respectively, where σ is the standard deviation of response and S is the slope of the calibration curve.

The recovery was measured as the average of 10 replicate. The recovery of high fluoride concentration such as1.5, and 2.0 mg·L–1 fluoride was calculated by measuringthe absorbance for the diluted solutions (1 to 3).

The interference studies were done by measuring the influence of the anions such as chloride, nitrate, and sulphate in such concentration commonly found in the natural water on the determination of 1.0 mg·L^{-1} fluoride. Chlorideand nitrate which were added in the range of 100 – 500 and 5 - 100 mg·L^{-1} respectively do not interfere with thedetermination of fluoride. Sulphate interferes with the mostvisual and photometric methods for determination of fluoride by its competition with fluoride to form a complexwith the metal and therefore it results in higher concentrations .In the present work, sulphate up to 100mg·L^{-1} does not interfere with the determination of fluoride. However, at higher concentration, sulphate interferes with determination of fluoride by causing a positive errorof about 25%. This error can be overcome by precipitating sulphate in the cold by the addition of aqueous barium chloride solution and aqueous agar-agar solution, thento separate the precipitate by filtration .

The proposed spectrophotometric method can be applied without any previous preparations, such that werenecessary to separate fluoride ions, to control fluoride incountries with low fluoride content water resources.

Fluoride concentrations in human serum and urine samples

Fluoride ion concentrations in sera of 20 Indian women aged 18–25 years were determined table 3. The subjects all lived in an area in which there is no fluoride pollution and the fluoride concentration in drinking water was <2.5 μmol/l. The fluoride ion concentrations in the serum of all women were considerably higher than the detection limit. The mean fluoride ion concentration (±S.D.) was 0.383 ±0.158 μmol/l. The concentration in about 90% of 53 samples was under 0.50 μmol/l. These values are considerably lower than previously reported values (Singer L, Ophaug R. (1982) , Cowell DC, Taylor WH. (1981) ,and Singer L, Ophaug R. (1982).Since the detection limit of this method is very low (0.016 μmol/l) , accurate measurement of fluoride ion concentrations in sera were possible. Fluoride concentrations in urine samples obtained from the same subjects were also determined table 3. A significant correlation (r=0.39, p<001) was obtained between fluoride ion concentrations in serum and adjusted urine fluoride ion concentrations. Although a significant correlation between serum and urine fluoride ion concentration in a population exposed to high fluoride concentration has been reported (Cowell DC, Taylor WH.(1981) , there has been no report on the relationship in a population not exposed to high fluoride concentration. It is thought that low fluoride ion concentrations in serum could not be determined using the current methods.

Conclusions

The relationship of the reaction of fluoride with zirconium-Resorcin bluecomplex is related linearly at 624 nmto the concentration of fluoride in the range 0.0 - 1.0 mg·L–1. However, at higher fluoride concentration, therelationship was best described by a sixth-order polynomial function. The reaction reaches equilibrium at fluoride concentration of 0.054 mM, and the equilibrium constant (Keq) was found to be 1.12 × 1034.

Zirconium Resorcin blue complex was used successfully as new spectrophotometric reagent for determination of fluoride in water in the range 0.0 to 1.0 $mg\cdot L^{-1}$.Due to its simplicity and high sensitivity, the method can be recommended as new spectrophotometric reagent for determination of fluoride in drinking water at low concentration. However, determination of fluoride at higher concentration is possible by diluting of the water sampleto fit the requirements of the proposed method.

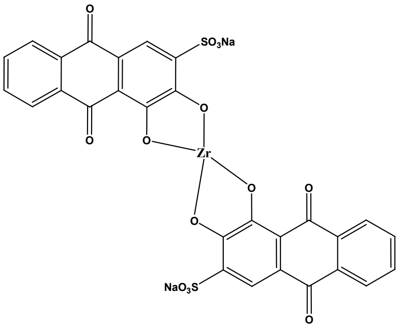

Fig 1. Possible structure for zirconiumResorcin blue1:2 complex.

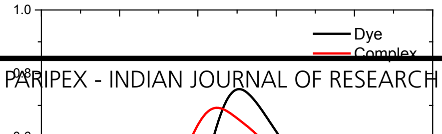

Fig 2. Electronic spectra of Resorcin blue complex and its zirconium 1:2 complex in ethanol at 2 × 10–4 M.

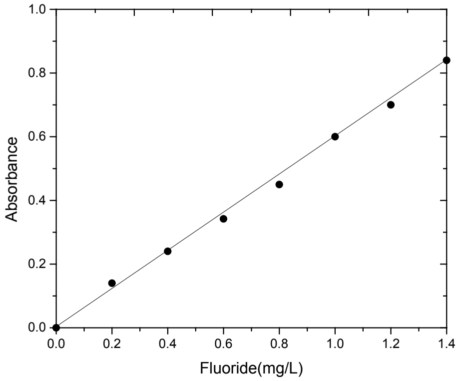

Fig 3. Calibration curve for determination of fluoride in the range (0.0 - 1.0) mg·L–1 at 624 nm by Resorcin blue zirconium complex of 2.0 × 10–4 M.

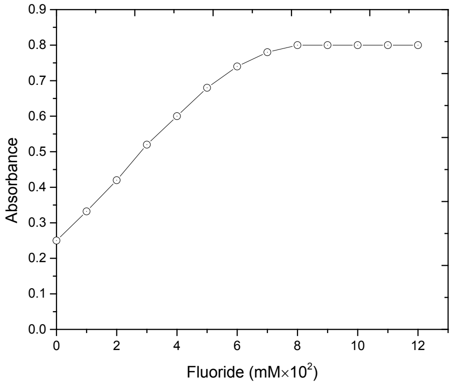

Fig 4. Absorbance of zirconium Resorcin blue 1:2 complex of 2.0 × 10–4 M versus fluoride concentration in the range 0.0 - 0.119 mM at 624 nm.

Table 1. Analytical parameters

Parameters	Values at 624 nm
Sensitivity [μg·mL–1]	0.357 ± 0.005
Detection limit [mg·L–1]	0.07
Quantification limit [mg·L–1]	0.2
Linear range/ g mL 1	0.1- 40
pH	5 5 6 2
Recovery range	99.60-99.98
RSD	

Table 2. Recovery studies of fluoride in real water samples

Source	Added ($mg\cdot L^{-1}$)	Found ($mg\cdot L^{-1}$)	Recovery (%)
Well water	10	9.98	99.80
	50	49.9	99.80
Well water 1	10	9.94	99.94
	50	48.5	97.00
Spring water1	10	9.80	98.00
	50	49.5	99.00
Spring water2	10	9.95	99.50
	50	49.8	99.60

Table 3. Fluoride concentrations in human serum and urine (N= no of determination)

sample	N	Fluoride concentrations	
		Mean ± S.D	Min-Max
Serum(women)	20	0.38 ± 0.144	0.194 ± 0.984
Urine (women)	20	19.9 ± 10.7	4.94 ± 7.3
Serum(men)	20	0.20 ± 0.10	0.026 ± 0.48
Urine (men)	20	0.44 ± 0.62	0.24 ± 0.582

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