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Spectrophotometric Determination of Fluoride in Groundwater Using Complexes of Flavonoid Chrysin

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Abstract: A simple spectrophotometric method is developed for determination of fluoride in drinking water by using complexes of chrysin. The method is based on the reaction of the coloured complexes with fluoride where its colour changes, due to the formation of the colourless fluoride complex and liberating of the free ligand, is dependent on the concentration of fluoride in water samples. The molar absorptivity for the complexes at the wavelength of maximum absorption in the visible region, 371 nm is 2.75 × 103 L mol^{-1} cm^{-1}. The method allows a reliable determination of fluoride in the range 0.3–3.0 mg L^{-1} which is compatible to WHO limit value of 1.5 mg L^{-1}. The sensitivity, detection limit, quantitation limit, and correlation coefficient for the method were found to be 0.211 µg mL^{-1}, 0.1 mg L^{-1}, 0.3 mg L^{-1}, and 0.9914 respectively. The percentage recovery of 1.5 mg L^{-1} fluoride is 101.3.

***Keywords*:** *Drinking water, Fluoride determination, Spectrophotometric method, Aluminium chrysin complex*

INTRODUCTION

Fluoride is a natural element found at varying concentrations in all drinking water (Winston and Bhaskar 1998). WHO has considered fluoride as one of the very few chemicals that have been shown to cause significant effects in people (WHO 2006). Low concentration of fluoride in drinking water have been considered beneficial to prevent dental carries (Maliyekkal *et al*. 2008), but excessive exposure to fluoride can give rise to a number of adverse effects such as causing fluorosis (WHO 2006; Wang *et al*. 2000; Armienta and Segovia 2008). Approximately 62 million people including 6 million children suffer from fluorosis (UNICEF 1999; Meenakshi 2004; Singh 2007). There is a narrow margin between the desired and harmful doses of fluoride (Czarnowski *et al*. 1996). WHO has set a limit value of 1.5 mg L-1 for fluoride in drinking water (WHO 2004). Therefore, an accurate, simple, rapid and cost effective analytical method is of high importance (Barghouthi and Amereih 2012).

Spectrophotometric methods, which are widely used in the determination of fluoride, 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 of the metal – ligand complex (Einaga and Iwasaki 1981). These methods have largely replaced titrimetric methods because they are more easily adapted to instrumental and automated procedures (Jacobson and Weinstein 1977).

Flavonoids, 2-phenylbenzo-γ-pyrone derivatives, are a broadly distributed class of naturally occurring pigments present in many types of plants, occurring mainly as glycosides in fruits, vegetables, nuts, seeds, flowers, and bark (Walle et al 1999; Lau *et al.* 2002; Zeng *et al.* 2003; Castro and Blanco 2004). Flavonoids show biological activity, which was greatly responsible for the interest in this group of compounds (Zeng *et al.* 2003; Lemanska *et al.* 2001; Ognibene *et al.* 2008). The best known and mostly widely used flavonoids are quercetin, morin, rutin, and their sulfo derivatives. Less studied is chrysin (5,7- dihydroxyflavone), which, similar the above – mentioned flavonoids, is insoluble in water; this hinders its uses, e.g., in analytical chemistry (Zeng *et al.* 2003; Pusz and Nitka 1997; Pusz *et al.* 2003).

The present study aimed to develop spectrophotometric method for determination of fluoride in drinking water in the range of 0.0 to 2.0 mg l^{-1}, compatible to WHO limit value of 1.5 mg l^{-1}, using complexes of chrysin as fluoride reagent for spectrophotometric determination.

MATERIAL AND METHODS

Materials

Chrysin (5,7-dihydroxyflavone) provided by Acros (99%, 11032 0050), aluminium chloride hexahydrate provided by Fluka (Purum p. a., 06232), and aluminium nitrate provided by Merck (p. a., 433 A846663) were used without any further purification. All the chemicals were of analytical reagent grade except where stated otherwise. Solutions were prepared using double distilled water. Chrysin ligand solutions and its aluminium complexes solutions were prepared using ethanol from Merck (reagent 96%, 159010). Standard fluoride stock solution was prepared by dissolving 0.1382 g of sodium fluoride provided by Merck (ACS reagent, 106449) in 250 ml water. The stock solution was further diluted as needed.

Apparatus

Beckman DU-7500 single beam spectrophotometer with 1.0 cm quartz was used for wavelength scanning and for spectral studies. Hitachi U- 1500 UV/Vis single beam spectrophotometer with 1.0 cm quartz cells was used for the absorbance measurements at fixed wavelength.

Preparing of chrysin complexes solutions

Job's method of continuous variation was adopted for determination of the composition of the coloured complex (Werner and Boltz 1971a; Werner and Boltz 1971b). Aluminium to ligand ratio was also studied by making comparison between the spectra of complexes of different 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 aluminium ion solution.
The complex solutions for the spectrophotometric measurements were prepared by mixing the proper aluminium to chrysin ligand ratio of 1×10^{-3} M of aluminium and 1×10^{-3} M of Chrysin ligand in ethanol solution, which was then diluted to ($\approx5\times10^{-4}$ M) which was suitable for the spectrophotometric measurements.

Reaction of fluoride with the prepared complexes solutions

Various amounts of fluoride were added in the range 0 – 3 mg L^{-1} to 25 ml volumetric flask containing aluminium chrysin 1:3 complex in ethanol (5×10^{-4} M, 24.5 mL). Water was added to reach the required volume (25 ml). The absorbance was measured at the wavelengths of the maximum difference (393 nm) in the electronic spectra between the ligand and the complex. The spectra of the reaction of various amounts of fluoride with the complex were compared.

Determination of fluoride in a real groundwater samples

The method under investigation was tested using a real 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 2011 from a groundwater well in Tubas District (Aqaba well). Fluoride was analysed colourimetrically using SPADNS as fluoride reagent and Hack – DR/2010 as spectrophotometer. Nitrate, sulfate, chloride, and other characteristic data of the sample are given in Table 1. Fluoride was measured in the sample using the proposed spectrophotometric method and the obtained results were compared with that reported by the Central Public Health Laboratory using SPADNS method (Table 1 and Table 2).

RESULTS AND DISCUSSION

Chrysin ligand

5,7-dihydroxyflavone which is commonly known as chrysin is one of the lesser known flavonoids that except for being synthesized and characterized as solid complexes of several metal ions such as Co(II), Ni(II), Cu(II), Cd(II), Pb(II), Ga (III), In (III), and Fe(III) etc., has not been paid great attention (Zeng *et al.* 2003; Pusz *et al.* 2000; Alluis and Dangles 1999). Chrysin is a mild antioxidant presents at high levels in honey and propolis, and it is a dietary supplement for bodybuilding (Walle *et al.* 1999; Lau *et al.* 2002). It has also been shown recently to be a potent inhibitor of drug-metabolizing enzymes, and of human immunodeficiency virus activation in models of latent infection (Walle *et al.* 1999).

The electronic spectrum of chrysin in ethanol has two intensive bands at 209 and 267 nm; and a less intensive band at 313 nm. It follows from literature that the band at 313 nm is related to the absorbance of cinnamonyl system, whereas that at 267 nm is related to the absorbance of (benzoyl system). The spectra are related to the π to π * transitions in the ligand molecule (Castro and Blanco 2004; Alluis and Dangles 1999). The molar absorptivity at 313 nm is $[8.93 \pm 0.15] \times10^{3}$ L mol^{-1} cm^{-1}.

Table 1. Analytical data of Aqaba groundwater sample analysed by Ministry of Health laboratories

pH	Conductivity Micro.S cm^{-1}	Fluoride mg L^{-1}	Nitrate mg L^{-1}	Chloride mg L^{-1}	Sulfate mg L^{-1}	TDS Mg L^{-1}
7.17	826.00	0.68	0.33	90.33	87.00	413.00

Table 2. Sensitivity, detection limit, quantification limit, and recovery of the method.

Parameters	393 nm
Sensitivity [µg mL^{-1}]	0.211 ± 0.006
Detection limit [mg L^{-1}]	0.1
Quantification limit [mg L^{-1}]	0.3
Recovery of 1.0 mg L^{-1} %	104.1 ± 4.6
Recovery of 1.5 mg L^{-1} %	101.3 ± 4.1
Recovery of 2.0 mg L^{-1} %	99.6 ± 3.9
Recovery of real water sample	107.1 ± 4.9

Aluminium chrysin complexes

The obtained results from applying of Job's method of continuous variation indicated that aluminium to chrysin ratio can be 1:1 or 1:3. This is in agreement with that reported by Pucz *et al.* (2000) and Alluis and Dangles (1999) respectively. The reaction of aluminium chrysin 1:1 and 1:3 complexes with fluoride showed that the maximum difference in the absorption spectra was occurred with the complex of 1:3 ratio. Therefore, the further discussion will be focused on this complex.

The absorption spectra of the aluminium chrysin 1:3 complex in ethanol exhibit two bands in the ultraviolet region and one band in the visible region. The bands in the ultraviolet region are one intensive band at 280 nm and a less intensive one at 313 nm. The molar absorptivity at 280 and 313 nm is [1.51 ± 0.04] $\times 10^4$ and [9.32 ± 0.11] $\times 10^3$ L mol^{-1} cm^{-1} respectively. The new band that appeared in the visible region is a characteristic feature of the electronic spectra of the complexes of polyhydroxy flavones with metal ions, and it is assigned to ligand to metal charge-transfer and it is responsible about the colour of the complex. For the complex under investigation, the new band, which is centered at 371 nm, is characteristic for 4C=O and 5C-OH chelation of the ligand. The molar absorptivity at 371 nm is [2.75 ± 0.09] $\times 10^3$ L mol^{-1} cm^{-1}. There is a bathochromic shift of about 53 nm after complexation with aluminium due to ligand to metal charge transfer, this leads to a change in the colour from the colour of chrysin, colourless to the colour of the complex, yellow. The stability of the complex in ethanol solution was examined by measuring the absorbance of the solution at different time of intervals during 15 days and the complex is stable.

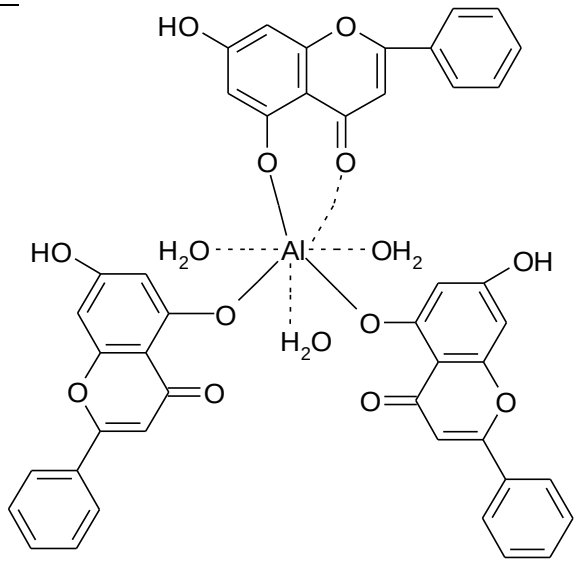

Figure 1. Possible structure for aluminium chrysin 1:3 complex.

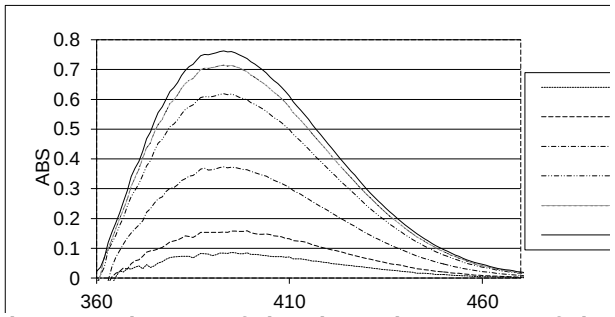

Figure 2. Changes of the absorption spectra of the complex in ethanol at 5×10^{-4} M due to the addition of various amounts of fluoride.

Determination of fluoride

Fluoride reacts with the yellow aluminium chrysin 1:3 complex to produce a colourless aluminium fluoride complex by replacement of the chrysin by fluoride and liberating of the free ligand. This is resulting in a change in the colour from that of the complex, yellow to the colour of the free ligand according to the next equation.

$$Al\ [chrysin]_{3yellow} + 6F^{-1} \rightarrow AlF_6^{-3}{}_{colourless} + 3[chrysin]_{colourless} \quad \text{(Eq. 1)}$$

Aluminium reacts with fluoride to give compounds of the nature of (AlF_6^{-3}) or ($AlF_y(OH)_{6-y})^{-3}$ (Macnulty *et al.* 1956). The absorption spectra of the reaction of fluoride with the complex showed that fluoride interacts to cause a decrease in the absorbance of the complex at 393 nm. The absorbance of the aluminium complex is related linearly at 393 nm to the concentration of fluoride in the range of 0.0 to 3.0 mg L^{-1}, the squared correlation coefficient R^2, is 0.9914, and the equation of the linear calibration curve is (y = -0.2069x + 1.1126). Figure 2 showed the effect of various amounts of fluoride on the absorption spectra of 5×10^{-4} M of the complex where each spectrum in Figure 2 represents the absorption spectra of the complex minus the absorption spectra of the complex after adding a given amount of fluoride.

The sensitivity, detection limit, limit of quantification, and the percentage recovery of 1.0, 1.5, and 2.0 mg L^{-1} fluoride using aluminium chrysin complex for the spectrophotometric determination of fluoride at 393 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 limit and the limit of quantification were calculated as (3.3σ/S) and (10σ/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 proposed spectrophotometric method was tested by comparing fluoride contents with that analyzed by the Central Public Health Laboratory. The recovery of fluoride in the real water sample (Table 2) is in good agreement with that reported by the Central Public Health Laboratory using SPADNS colourimetric method (Table 1).

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 and 1.5 mg L^{-1} fluoride. Chloride and nitrate which were added in the range of 100 – 500 and 5 – 100 mg L^{-1} respectively do not interfere with the determination of fluoride. Sulphate interferes with the most visual and photometric methods for determination of fluoride by its competition with fluoride to form a complex with the metal and therefore it results in higher concentrations (Ruzicka *et al.* 1966; Dixon 1970). In the present work, sulphate up to 100 mg L^{-1} does not interfere with the determination of fluoride. However, at higher concentration, sulphate interferes with determination of fluoride by causing a positive error of about 40%. This error can be overcome by precipitating sulphate in the cold by the addition of aqueous barium chloride solution and aqueous agar-agar solution, then to separate the precipitate by filtration (Dixon 1970).

CONCLUSION

Aluminium chrysin 1:3 complex was successfully used as new spectrophotometric reagent for determination of fluoride in drinking water. The proposed method is cheap, reproducible, rapid, and it allows a reliable determination of fluoride in the range of 0.3–3.0 mg L^{-1} which is compatible to WHO limit value of 1.5 mg L^{-1}. Due to its simplicity and high sensitivity, it can be recommended as new fluoride reagent for controlling the amount of fluoride in the countries suffers from fluoride health problem.

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