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FLOW INJECTION DETERMINATION OF FLUORIDE BY MORIN-ALUMINUM REAGENT WITH SPECTROFLUORIMETRIC DETECTION

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Abstract

A flow injection system is reported for the determination of fluoride based on its effects on the fluorescence intensity of Al-morin complex at pH 3.0 in ethanol-water mixture. The effect of important parameters such as excitation and emission wavelengths, pH, reagents concentrations, flow rate of the carrier stream, sample injection volume, time of measurement and interferences are reported. Cation and anion exchange resins were used to remove interferences and to preconcentrate fluoride ions prior to measurement. Calibration curve was linear over the range of 5-20000 ng ml^{-1} of fluoride. The detection limit was 3 ng ml^{-1} and the relative standard deviation for the determination of 100 ng ml^{-1} of fluoride was ±3%. The proposed method was used to determine fluoride in natural water samples. Recovery was 97.5-105% for the fluoride added to the water samples. The relative standard deviation for the analysis of these samples was less than 5%.

Introduction

Determination of fluoride is important in various samples such as soils, organic substances, biological materials, water and air. In some countries, fluoride is measured to continuously monitor and control fluoridation of municipal water supplies, because of its role in preventing dental decay and bone diseases [1,2].

A survey of the literature reveals that fluoride may be determined in different materials using potentiometry with ion selective electrodes [3-6], polarography [7], atomic absorption spectrophotometry [8], chromatography [9], inductively coupled plasma [10], spectrophotometry [11-20], kinetic spectrophotometric method [13], flow-injection spectrophotometry [16, 17] and fluorimetric methods [21-26]. Most of the spectrophotometric and fluorimetric methods that have been developed involve indirect procedures and are usually based on the formation of stable complexes between fluoride ion and complexes of multivalent metal ions, such as zirconium, aluminum, iron, thorium, and titanium. The color or fluorescence changes resulting from the reaction of fluoride ions with complexes of these metals provide indirect methods for the determination of fluoride.

Some of the reagents commonly employed for the determination of fluoride by either spectrophotometry or spectrofluorimetry include complexes of multivalent

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metal ions with a wide variety of organic reagents. Some of the most classical and widely known ones are calcein blue [21-24], 8-hydroxyquinoline-5-sulfonic acid [25], alizarines [15-17,27], xylenol orange [12,18], quinalizarin complexone [28], ferrone [20], lumogallion [26], SPADNS [29], pyrocatechol violet [30], chrome azurol S [31], and morin [32]. Many of the existing methods for determination of fluoride are subject to interferences from several ions such as iron, aluminum, nickel, zinc, arsenic, phosphate, oxalate, nitrite, etc. [15-22, 33]. Some of these interferences are usually the major or minor constituents in many samples such as soils, industrial and natural water and minerals. Because of these many interferences, in most of the reported methods, the fluoride ions are first separated by distillation [34], or ion exchange [35].

Owing to its versatility and flexibility, high sample throughput and ease of operation, flow injection analysis (FIA) is now a well-accepted analytical technique [36]. Fluorimetric methods are extremely sensitive, allowing some elements to be analyzed in the nanogram range, which is comparable to or better than what can be achieved with other analytical procedures. Therefore, a combination of FIA with a fluorimetric detection system allows very fast and sensitive methods of analysis to be performed. In the present work, a highly sensitive flow injection spectrofluorimetric method for determination of low fluoride concentrations is described. The method is based on the effect of fluoride ion on the fluorescence intensity of Al-morin complex. Cation and anion exchange resins were used to remove interferences and to preconcentrate fluoride ions prior to measurements with the FIA system.

Experimental Section

Reagents and Chemicals

All of the solutions were prepared with deionized, double-distilled water from reagent grade materials (all from Merck).

Aluminum stock solution. *(1.0 mg* ml^{-1} *as aluminum,* 3.70×10^{-2} M). Prepared by dissolving 17.5820 g of reagent grade potassium aluminum sulfate, $KAl(SO_4)_2.12H_2O$, in water, adding 1ml of 1:1 sulfuric acid and diluting the solution to 11 with water. Working solutions were freshly prepared by appropriate dilution of the stock solution.

Morin (3, 5, 7, 2', 4'-pentahydroxy flavone). 1.0×10^{-3} M. Prepared by dissolving 0.3383 g of the reagent grade material in 100 ml of 95% distilled ethanol. Working solutions of this reagent were prepared by dilution with water.

Fluoride stock solution. (1.0 mg ml^{-1}). Prepared by dissolving 2.2102 g of reagent grade sodium fluoride, previously dried at 140°C, in water and diluting to 11. This solution was stored in a polyethylene bottle. All working solutions were freshly prepared by appropriate dilution of the stock solution.

Buffer solution. (pH 3.0). Prepared by dissolving 18.9 g of monochloroacetic acid in 116 ml of 1 M sodium hydroxide solution and diluting to about 800 ml. Exact pH of the solution was adjusted with monochloroacetic acid or sodium hydroxide solutions using a pH meter. Finally, the solution was diluted to 11 with water. For potentiometric measurements, a total ionic strength adjustment buffer (TISAB) [37] was prepared by mixing (with continuous stirring) 57 ml glacial acetic acid, 5.8 g sodium chloride, and 3 g EDTA in about 500 ml of distilled water in a 1 l beaker. The resulting solution was cooled and adjusted to pH 5.0-5.5 by addition of 6 M sodium hydroxide solution. The solution was diluted to 1 l and stored in a polyethylene bottle. Samples and standards were diluted in 1:1 ratio with TISAB solution before potentiometric measurements were carried out.

Solution of interfering ions. Stock solutions of interfering ions were prepared by dissolving their suitable salts (sodium salts for the anions and nitrate salts for the cations) in water to make 1.0 mg ml^{-1} of the desired ions.

FIA carrier and reagent solution. Prepared from aluminum (1.85×10^{-5} M), morin (1.75×10^{-4} M), and buffer solution of pH 3.0 in 10% ethanol-water mixture. The use of ethanol was necessary because of the insolubility of morin in water.

Ion exchange columns. For the cation exchanger column, a strongly acidic, H^+ form exchanger (Zeo-Carb 225 from Koch-Light Lab.) and for the anion exchanger column, a weakly basic anion exchanger (Ion Exchanger-II from Merck) were packed into separate columns of about 0.5 cm i.d. and 10 cm length. Cation exchanger column was washed with 0.1 M nitric acid and anion exchanger column with 0.05 M sodium nitrate.

Apparatus

Fluorescence intensity (I_F) measurements and spectra were obtained with a Shimadzu Model RF-5000 spectrofluorimeter equipped with a data station. The excitation and emission bandwidths were selectable and usually 20 and 5 nm were used, respectively. Larger excitation and emission bandwidths were used in some experiments to obtain higher sensitivities. Standard 1.0 cm fluorescence silica cells were used.

The flow injection system was built using a DV11 pump (Mettler), Teflon and polyethylene tubing of 1 and 0.75 mm i.d., a HPLC injection system (Varian) and a Shimadzu fluorimetric flow cell. A schematic diagram of the FIA system is shown in Figure 1.

Potentiometric measurements were carried out using a fluoride ion selective electrode, Model 94-09-BN, a single junction calomel reference electrode, Model 90-01-00, both from Orion Research Inc., and a digital pH/mV meter (Model 112, Corning Scientific Instruments).

General Procedure

A mixture of aluminum ion (1.85×10^{-5} M) and morin (1.75×10^{-4} M) in 10% ethanol-water mixture buffered at pH 3.0, prepared at least 2 h before starting the measurement, is pumped at a rate of 0.35 ml min^{-1}. Standard or sample solutions are injected into the carrier stream at S (Fig. 1) and the fluorescence difference (ΔI_F) is measured. For general purposes, the following optimum conditions are suggested: length of mixing coil, 200 cm; sample injection volume, 50 µl; λ_{ex}, 420 nm; and λ_{em}, 486.4 nm.

Results and Discussion

Preliminary Investigation

In order to obtain a highly sensitive fluorimetric reagent for determination of fluoride in the FIA system, several complexes of common multivalent metal ions, capable of forming relatively stable complexes with fluoride, were examined. These systems included Al-8-hydroxyquinoline, Al-7-iodo-8-hydroxyquinoline-5-sulfonic acid, Fe(III)-phenanthroline, Al-morin, Fe(III)-bipyridyl, Fe(III)-sulfosalicylic acid and Zr-eriochrom cyanine R. A reagent (metal-ligand complex) for trace fluoride analysis by fluorimetric method ideally should be highly fluorescent. On reaction with fluoride, its fluorescence intensity should be considerably and quantitatively decreased. Further, the ligand itself should, ideally, be nonfluorescent. Among the systems studied, only Al-morin complex fulfills these requirements. Morin not only gave good analytical fluorimetric signals in the presence of aluminum, but also negligible blank fluorescence values. In addition, there was a considerable decrease in the fluorscence intensity upon addition of fluoride to the Al-morin solution. In other systems studied, either the complexes were not highly fluorescent or the fluorescence intensity hardly changed after the addition of fluoride ion to the complex solution.

Aluminum forms a highly fluorescent, 1:1 complex with morin [33]. The stability constant of this complex in 10% ethanol-water mixture was found to be 2.5×10^4 by the mole ratio method. Fluoride ion can be analyzed because of its ability to quench (decrease) the fluorescence intensity of aluminum-morin complex. Fluoride ion forms $Al(F)_n$ (n=1 to 6) complexes with aluminum ions. The stepwise formation constants of these complexes are relatively larger than the corresponding complexes with other common multivalent ions. Therefore, Al-morin complex was selected as a fluorimetric reagent for the determination of fluoride. A working curve of the fluorescence intensity of Al-morin complex versus fluoride concentration decreases as fluoride concentration increases.

In all of the measurements, the difference in fluorescence intensities of the reagent (reference) solution (Al-morin) and sample solution (Al-morin-fluoride) was considered as the analytical signal: $\Delta I_F = (I_F)_R - (I_F)_S$. The difference was taken as equal to zero when blank (distilled water) was injected.

Preliminary tests for obtaining optimum conditions were carried out using solutions that contained 100 ng ml^{-1} fluoride, 7.5×10^{-6} M aluminum and 1.5×10^{-5} M morin in 10% ethanol-water mixture. In optimization tests with the FIA system, at least five injections were made under each set of conditions. From the results, the average and standard deviations were calculated and maximum signal to noise ratio ($S/N = \Delta I_F/SD$) was used as the criterion for optimization.

Excitation and Emission Spectra

Optimum excitation and emission wavelengths (λ_{ex} and λ_{em}, respectively) for maximum ΔI_F were obtained at

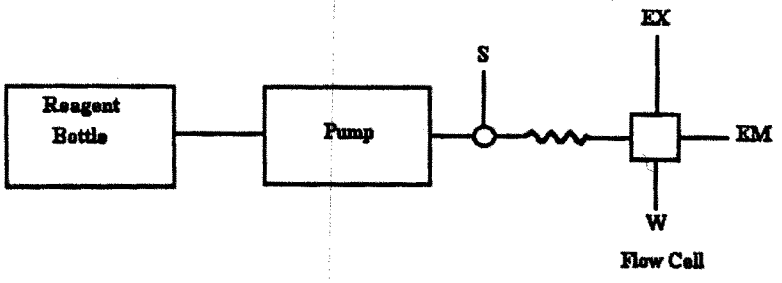

Figure 1. Schematic diagram of the FIA system for spectrofluorimetric determination of fluoride: S, sample injection; EX, exciting radiation; EM, emitted radiation; W, waste

420.0 and 486.4 nm, respectively. The fluorescence spectra hardly changed in shape and wavelength after adding fluoride to the Al-morin solution. However, the fluorescence intensity of Al-morin was suppressed remarkably after adding fluoride to the solution.

Effect of Manifold Variables

Tube length for the reaction coil in the FIA system has a considerable effect on the sensitivity of measurement. Increasing the length of the reaction coil from 50-300 cm increased the peak height. Peak broadening and tailing were also observed with long reaction coils. Satisfactory results were observed using a 200 cm mixing and reaction coil.

The sensitivity of the measurement largely depends on the flow rate of the reagent. ΔI_F values increased as the flow rate was decreased (Fig. 2), whereas peak broadening and tailing were observed with low flow rates. For practical purposes, flow rates of 0.35 ml min^{-1} were found to be satisfactory for the reagent stream.

The effect of injected sample volume was investigated. The sensitivity increased with increasing injected volume from 20-100 µl. In large sample volumes, peak broadening and tailing were observed. Therefore, 50 µl was chosen as the optimum sample volume for the present work.

Effect of pH

To determine the optimal pH for the determination of fluoride, we studied the influence of pH on the fluorescence intensity due to injection of 50 µl of 100 ng ml^{-1} fluoride over the pH range 1-10 (fixed by addition of HCl and NaOH). This study was carried out at a reagent flow rate of 0.35 ml min^{-1}. At pH values higher than 4 and lower than 2, the fluorescence intensity of Al-morin reagent and hence ΔI_F was very low. Maximum fluorescence intensity for the reference solution (Al-morin) and maximum ΔI_F in the presence of fluoride was observed at pH 3.0. At high pH, aluminum ion probably precipitates and at low pH, Al-morin complex is not stable and fluoride ion is highly protonated. The pH chosen for the subsequent experiments was 3.0±0.1, adjusted by monochloroacetic acid-sodium hydroxide buffer. The results of this study are shown in Figure 3.

Effect of Time

Time of measurement was optimized using 7.5×10^{-6} M aluminum, 1.5×10^{-5} M morin in 10% ethanol-water mixture at pH 3.0. It was observed that the

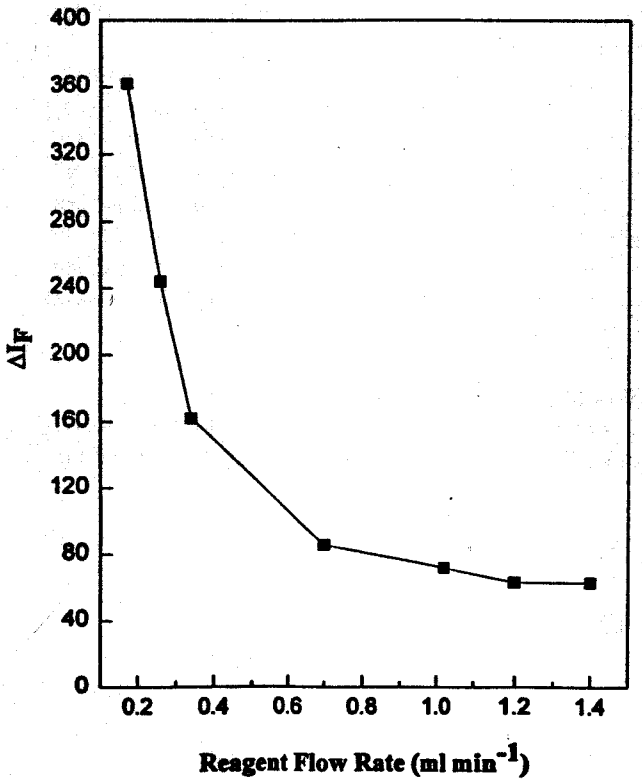

Figure 2. Effect of pumping rate (reagent flow rate) on the fluorescence intensity difference

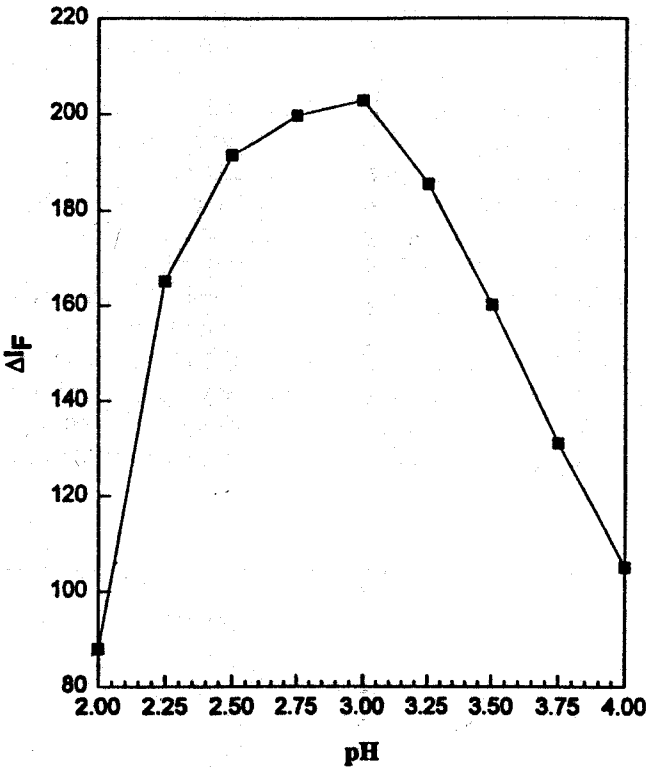

Figure 3. Effect of pH on the fluorescence intensity difference

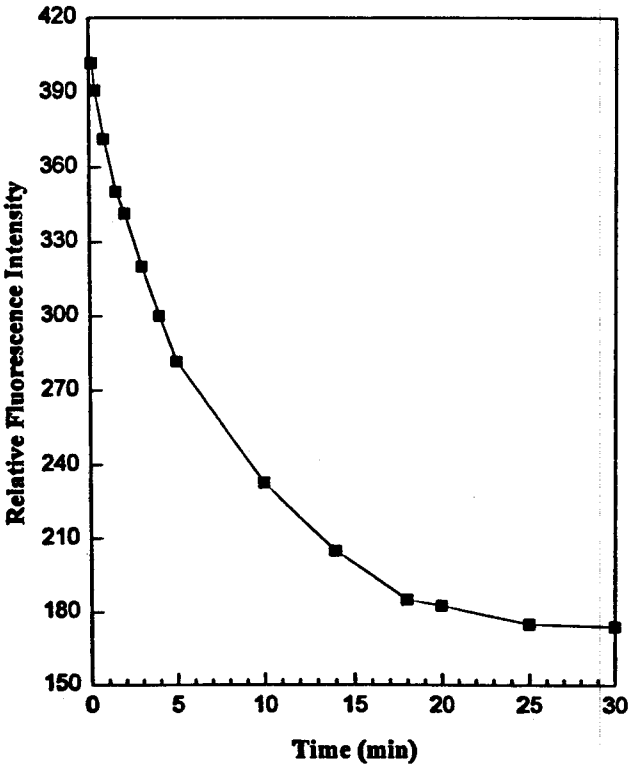

Figure 4. Effect of time on fluorescence intensity difference

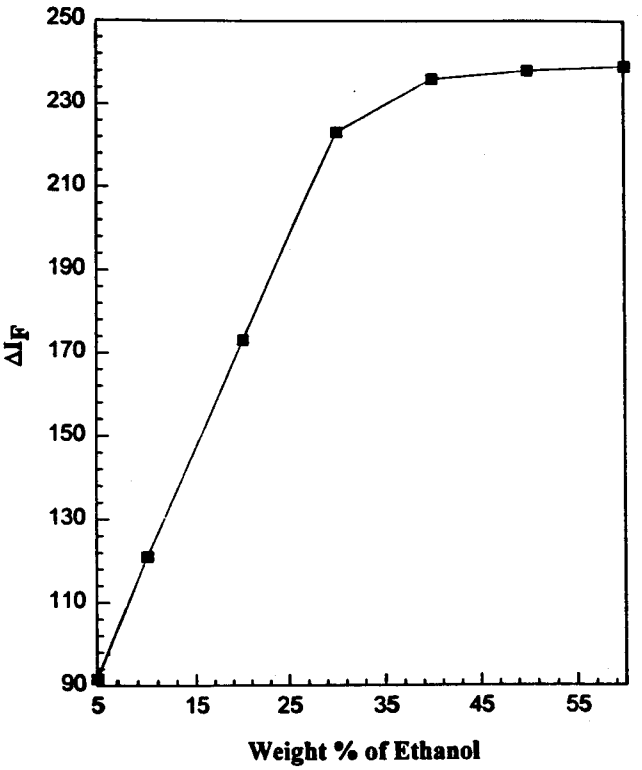

Figure 5. Effect of solvent composition on the fluorescence intensity difference

fluorescence intensity of Al-morin reagent is maximum after about 100 min. After this time the fluorescence intensity could remain stable for at least 24 h. Therefore, for subsequent measurements, the reagent was prepared at least 2 h before starting the measurement. The reaction of fluoride with Al-morin reagent was also slow and equilibrium condition with maximum ΔI_F was established after about 25 min (Fig. 4). Hence, in experiments without using the FIA system, ΔI_F was usually measured about 25 min after the addition of fluoride. In measurements with the FIA system, due to peak broadening and tailing, it was not possible to have such a delay time, because FIA peaks appeared about 120 s after injection of fluoride solution.

Solvent Composition and Reagent Concentration

It is well known that solvent affects the fluorescence characteristics and especially the quantum yield of metal chelates. In our study, ethanol-water mixture was used as the solvent, because ethanol is the common solvent for morin and a good solvent for fluorescence studies with no quenching or heavy atom effects. The composition of the ethanol-water mixture was optimized by measuring the fluorescence intensity of solutions containing 7.5×10^{-6} M aluminum, 1.5×10^{-5} M morin and 100 ng ml^{-1} fluoride in 5-60% (w/w) ethanol-water mixture at pH 3.0. The results of this study, which are shown in Figure 5, indicate that ΔI_F increases with increasing ethanol concentration up to about 40%, beyond which the fluorescence intensity levels off. Therefore, the optimum ehtanol concentration is 40%. However, in our studies 10% ethanol-water mixture was used to decrease the cost of analysis.

Tests showed that morin to aluminum mole ratio has a great effect on the fluorescence intensity of the system. In this study, a constant concentration of morin (1.75×10^{-4} M), and different aluminum concentrations, 1.0×10^{-6} - 4.0×10^{-5} M, in 10% ethanol-water mixture at pH 3.0 were used as the reagent solution. With a reagent flow rate of 0.35 ml min^{-1}, at each aluminum concentration, 50 µl of 100 ng ml^{-1} of fluoride solution was injected. Maximum sensitivity was observed at 1.85×10^{-5} M of aluminum. Tests at other morin concentrations showed that the optimum morin to aluminum mole ratio was about 10.

Selectivity

Effect of interferences. To evaluate the selectivity, the effects of several cations and anions, which generally accompany fluoride in different samples, were

Table 1. Effect of foreign ions (M) on the determination of 50 ng ml^{-1} of fluoride

Ion added	Tolerance limit, ion/fluoride Mass ratio
NO_3^-, Na^+, K^+	a
Cl^-	12000
Br^-, I^-, NH_4^+	10000
Mn^{2+}, SiO_3^{2-}	8000
Mg^{2+}	6000
Ca^{2+}, CH_3COO_2	3000
Zn^{2+}	1500
SO_4^{2-}	1200
Co^{2+}, Ni^{2+}, CO_3^{2-}	1000
Cr^{3+}	800
PO_4^{3-}	400
Pb^{2+}	200
NO_2	40
Fe^{2+}, Cu^{2+}	20
Fe^{3+}	1

[a]No interference observed even at 1 mg ml^{-1}

investigated at a fluoride concentration of 50 ng ml^{-1} and at the optimized conditions. Solutions of 1000 µg ml^{-1} of the sodium salts of phosphate, carbonate, acetate, silicate, sulfate, nitrite, bromide, iodide, chloride and nitrates of Fe(III), Fe(II), NH_4^+, Mn(II), Zn(II), Co(II), Ni(II), Cr(III), Cu(II), calcium and magnesium were prepared. Increasing volumes of these solutions were added to the fluoride solution and fluorescence intensities measured. An interference was tolerable if its effect on the fluorescence signal was less than the relative standard deviation; hence, the tolerance limit in the fluorescence measurement was the same as the relative standard deviation for this method (±3%). The limiting concentration for different ions is shown in Table 1. The maximum permissible mass ratios of other ions causing ±3% error in the fluorescence intensity were as follows: 12000-fold of Cl^-, 10000-fold of Br^-, I^-, and NH_4^+, 8000-fold of Mn^{2+} and SiO_3^{2-}, 6000-fold of Mg^{2+}, 3000-fold of Ca^{2+} and CH_3COO^-, 1500-fold of Zn^{2+}, 1200-fold of SO_4^{2-}, 1000-fold of Co^{2+}, Ni^{2+} and CO_3^{2-}, 800-fold of Cr^{3+}, 400-fold of PO_4^{3-}, 200-fold of Pb^{2+}, 40-fold of NO_2^-, 20-fold of Fe^{2+} and Cu^{2+}, and 1-fold of Fe^{3+}. The results show that Fe^{3+}, Fe^{2+}, and Cu^{2+} are potential interferences.

Removal of interfering ions. Cation and anion exchange resins were used in separate experiments to remove or decrease the effect of interferences. A series of synthetic sample solutions containing 50 ng ml^{-1} of fluoride and 0.1 mg ml^{-1} of the serious interfering species were passed through the cation exchange resin column at a flow rate of 2.5 ml min^{-1}. 50 µl of the eluate was injected into the reagent stream at a flow rate of 0.35 ml min^{-1}. The average recovery of fluoride in synthetic samples was in the range 97.0-102.5% as shown in Table 2. From this table it can be seen that the results are satisfactory. No considerable effect was observed from the cationic interfering species. The calibration curve of 2-100 ng ml^{-1} of fluoride solutions containing 0.1 mg ml^{-1} of Cu^{2+}, Fe^{2+}, and Fe^{3+}, after passing through the cation exchanger column, was linear at the optimized conditions with a correlation coefficient of 0.9998 (Fig. 6). In this investigation, the excitation bandwidth was 10 nm to obtain a higher sensitivity.

In another experiment, an anion exchange resin was used to decrease the effect of interfering species and to preconcentrate fluoride ions prior to measurements. Solutions of fluoride containing the potential interferences were passed through a column of Ion Exchanger (II) at a flow rate of 2.5 ml min^{-1}. The column was washed with about 10 ml of water and fluoride eluted with 2 ml of 0.05 M sodium nitrate. 50 µl of the eluate was injected into the carrier stream. Recoveries of 100±6% were observed with several solutions of different fluoride concentrations containing interfering species. By passing a large volume of solution through this column, a large preconcentration factor could be obtained.

Table 2. Removal of interfering ions for determination of fluoride in synthetic samples[a]

Ion added	Mean fluoride found (ng ml^{-1})	Relative standard deviation (%) (n=6)
Cu^{2+}	49.7	1.7
Fe^{2+}	50.5	2.3
Fe^{3+}	51.2	2.0
Cu^{2+}, Fe^{2+}, Fe^{3+}	48.8	2.9

[a]Conditions: interfering ions 0.1 mg ml^{-1}; fluoride ion 50 ng ml^{-1}; Al, 1.85×10^{-5} M; morin, 1.75×10^{-4} M; pH, 3.0; reagent flow rate, 0.35 ml min^{-1}; 10% ethanol-water mixture.

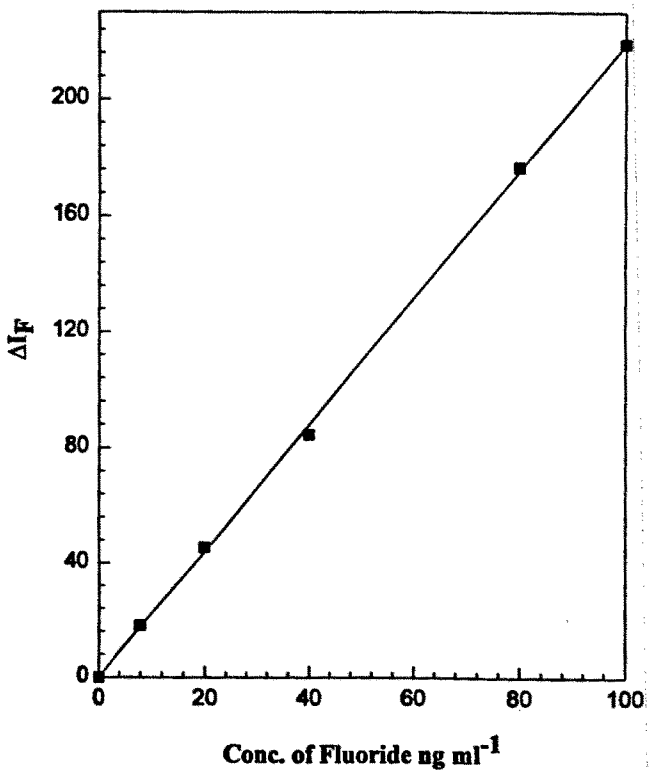

Figure 6. Calibration curve, ΔI_F vs. fluoride concentration, after elimination of interferences at the optimized conditions in the FIA system

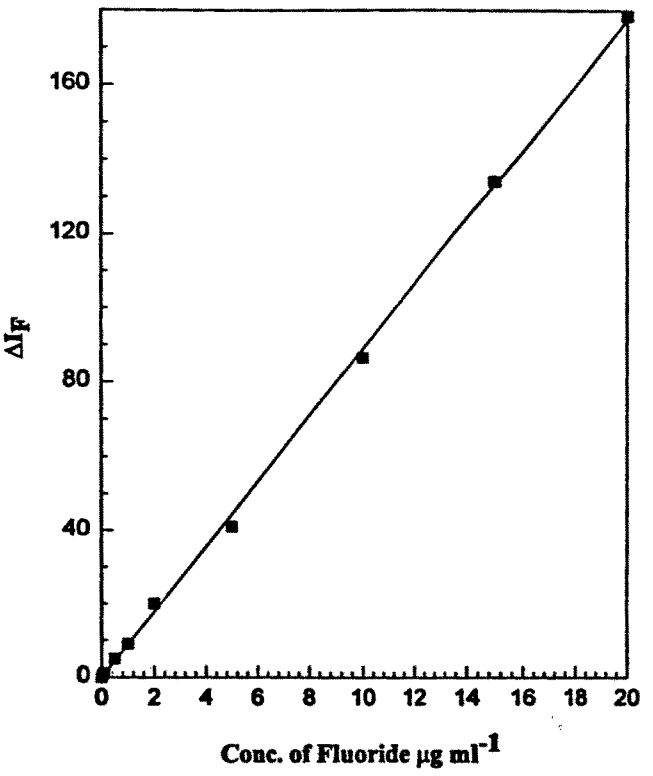

Figure 7. Calibration curve, ΔI_F vs. fluoride concentration, at the optimized conditions in the FIA system

Analytical Parameters

Working curve. Under the optimized conditions described above, i.e. 1.85×10^{-5} M aluminum, 1.75×10^{-4} M morin, 10% ethanol-water mixture, pH 3.0, reagent flow rate of 0.35 ml min^{-1}, injection volume of 50 µl, λ_{ex} 420 and λ_{em} 486.4 nm, a linear calibration graph was obtained over the concentration range of 5×10^{-3}-20 µg ml^{-1} of fluoride [ΔI_f = -0.029+8.874 × concentration of fluoride, µg ml^{-1}, n= 10] with a correlation coefficient of 0.9996 (Fig. 7).

Precision. At the optimized conditions, the relative standard deviation of 10 replicate determinations was ±3.0% for a 100 ng ml^{-1} of fluoride.

Detection limit. The theoretical limit of detection, $Y_{LD} = Y_{BL} + 3S_{BL}$, [38], for this study was 3 ng ml^{-1} of fluoride, without the preconcentration step.

Recovery Test and Determination of Fluoride in Water Samples

The standard addition method was used for recovery tests. For this purpose, the fluoride content of several well water samples from Esfahan province was determined by the above procedure (six determinations for each sample), and after addition of a known amount of fluoride the total assay was determined again with six analyses by the above method. The results thus obtained are presented in Table 3. The average recovery when natural water samples were spiked with standard fluoride solution was in the range 97.5-105% as shown in Table 3. The precision of the analysis for water samples expressed as the relative standard deviation was in the range 3-5%.

The results were compared with those obtained using the well-known potentiometric method using a fluoride ion selective electrode [37]. The results are given in Table 3. The correlation coefficient for the two sets of results using the FIA-fluorimetric and potentiometric methods was 0.9977 which shows a very good agreement between the results. The comparison of the above procedure with the potentiometric method shows that the reproducibility and accuracy of the FIA-fluorimetric method are of the same order as those of the potentiometric method. The higher sensitivity, however, expressed either by the detection limit or as the slope of the calibration curve is higher for the proposed method.

Table 3. Recovery test and comparison of the FIA and potentiometric methods

Sample no.	Fluoride added μg ml^{-1}	Fluoride found (μg ml^{-1})[b]		Recovery (%)[a]
		FIA Fluorimetry	Potentiometry	
1	–	0.22±0.01	0.22±0.01	
	0.20	0.43±0.02	0.44±0.03	105
2	–	0.32±0.01	0.33±0.02	
	0.20	0.53±0.02	0.52±0.02	105
3	–	0.10±0.005	0.10±0.01	
	0.40	0.49±0.02	0.51±0.02	97.5

[a]For the proposed method
[b]The mean results are quoted ±95% confidence limits (n= 6)

Conclusion

The proposed FIA fluorimetric method has been successfully applied to the determination of fluoride in natural water samples. The method is accurate and sensitive with a high linear dynamic range. The limit of detection was 3 ng ml^{-1} without the preconcentration step. By considering the preconcentration step, the limit of detection can be decreased at least an order of magnitude. The rate of analysis is about 30 samples per hour. Preliminary studies for very fast elimination of interfering species by incorporation of a cation exchanger column into a second line of the flow injection system was successful and further investigations are in progress.

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