A highly sensitive fluorimetric method for the determination of fluoride in biological material with $\text{Al}^{3+}$-calcein complex

Abstract  A highly sensitive fluorimetric method for the determination of fluoride was established. The method was based on quenching of the fluorescence of the $\text{Al}^{3+}$-calcein complex in $\text{CCl}_3\text{COOH}-\text{CH}_3\text{COOK}$ buffer solution by fluoride. The fluorescence emission was measured at $\lambda_{\text{ex}}/\lambda_{\text{em}}$ 480/503 nm, and the experimental variables and interference in this determination were studied. The linear calibration range was 0.8 ng/mL to 150 ng/mL and the detection limit was 0.2 ng/mL. The method was applied to determine fluoride in biological materials. The recovery was in the range of 96.3% to 104.7% and the relative standard deviation was 4.6%. The results obtained from the certified reference material by the present method agreed with the certified values.

Introduction  Fluoride is known to affect various types of plants, thus both men and animals are susceptible to poisoning by fluoride-contaminated food or foodstuffs. Different procedures and methods for the isolation and determination of fluoride in biological materials have been proposed. The sample separation methods comprise mainly distillation, mineralization in alkaline medium, acid digestion, and microdiffusion, either with or without ashing of the biological material [1, 2]. The determination is mainly carried out by potentiometry with lanthanum fluoride [3] or by spectrophotometry with alizarin complexone [4]. Spectrophotometry and fluoride-ion selective electrodes have low sensitivity. For instance, the spectrophotometric detection limits for fluoride are rarely better than 20 µg/L, while it is frequently recommended that the fluoride ion-selective electrode should not be used for fluoride concentrations less than 0.1 µg/mL, because of the risk of erratic electrode response [5]. Although fluorimetry is more sensitive than potentiometry and spectrophotometry, only a few methods for the fluorimetric determination of fluoride were described in the literature [6–14]. In this paper we describe a highly sensitive fluorimetric method for the determination of fluoride. The method was applied to determine fluoride in biological materials.

Experimental  Fluorescence measurements were carried out on a Shimadzu RF-540 dual beam difference spectrofluorophotometer (Shimadzu, Kyoto, Japan), equipped with a xenon lamp and 1 cm quartz cells. The width of the excitation and emission slits was 5.0 nm. A standard solution of rhodamine B was used to adjust weekly the spectrofluorophotometer to compensate for changes in source intensity.

Reagents  All reagents were of analytical reagent grade. De-ionized water was used. The sodium fluoride stock solution of 1000 µg/mL was prepared by dissolving 0.2210 g of sodium fluoride (Sigma, USA) in 100.00 mL of water and stored in a polyethylene bottle. The aluminium (III) stock solution of 100.0 µg/mL was prepared by dissolving 0.1758 g of KAI(SO$_4$)$_2$$\cdot$$\text{12H}_2\text{O}$ (Sigma, USA) in 100.00 mL water. Calcein stock solution ($1.60 \times 10^{-4}$ mol/L) was prepared by dissolving 0.0106 g of disodium calcein (Merck, Germany) in 100.00 mL water and preserved in a refrigerator, and it was stable for two weeks. The working solutions were prepared by suitable dilution of the stock solutions with water. The CCl$_3$COOH (Merck, Germany)-CH$_3$COOK (Sigma, USA) buffer solution (pH 2.7) was prepared by addition of 0.500 mol/L CCl$_3$COOH solution and 0.500 mol/L CH$_3$COOK solution.

Procedures  A certain volume of sodium fluoride standard solution was placed in a 25 mL volumetric flask. Then, 2.00 mL of the CCl$_3$COOH-CH$_3$COOK buffer (pH 2.7), 0.70 mL of the calcein stock solution ($1.60 \times 10^{-4}$ mol/L), and 0.90 mL of the aluminium (III) solution (1.000 µg/mL) were added. The solution was diluted to the 25 mL mark with water. After heating for 15 min at 60°C, the solution was cooled to room temperature. A reagent blank was prepared in the same way but without fluoride ion. The fluorescence intensities of the solution and the reagent blank at an emission of 503 nm with reference to a blank water solution under 480 nm excitation were measured, respectively. The difference of fluorescence intensity between the reagent blank and the solution ($\Delta F$) was obtained.

Pre-treatments of samples were carried out according to the literature [1]. In short, about 0.4000 g dried mulberry leaves sample...
or certified reference material of tea leaves were placed into the distillation bottle and heated to 110 °C, and 5.00 mL of conc. H_2SO_4 and 8.00 mL of 30% H_2O_2 solution were added drop by drop. The sample was distilled at 175–180 °C for 10 min. The distillation was continued for 5 min after 2.00 mL of 30% H_2O_2 were added. The rate of the carrier gas (air) was 0.40 L/min. 15 mL of absorption solution containing 1.00 × 10^{-4} mol/L NaOH were used. After absorbing fluoride, the treated solution was diluted to 25.00 mL with water. A certain volume of the treated solution was placed in a 25 mL volumetric flask, and the other procedures were the same as for sodium fluoride standard solution as described above.

**Results and discussion**

**Choice of complexation system**

In order to obtain a better method for the fluorimetric determination of trace amounts of fluoride, the fluorescence changes that accompany the reaction of Be^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}, La^{3+}, Ce^{3+}, Zr^{4+} and Th^{4+} with calcein were examined as a function of pH and of concentrations of the reactants. A weak decrease in the fluorescence intensity of the calcein was observed in the presence of Be^{2+}, Ce^{3+}, La^{3+}, Th^{4+}, or Zr^{4+} between pH 1 and pH 14. A strong decrease in the fluorescence intensity of calcein was observed in presence of Mn^{2+} or Fe^{3+} between pH 8 and pH 10. The fluorescence intensity of Mn^{2+}-calcein or Fe^{3+}-calcein did not change after the addition of fluoride. The effect of pH on the fluorescence intensity of calcein and Al^{3+}-calcein complex is shown in Fig. 1. Between pH 8 and pH 12, the fluoride did not abstract Al^{3+} from Al^{3+}-calcein complex and released the strong fluorescent calcein. However, between pH 2 and pH 6, the fluoride could abstract aluminium from Al^{3+}-calcein complex and thus released the weak fluorescent calcein. Therefore, Al^{3+}-calcein was chosen as a new system for the determination of fluoride.

**Spectral characteristics**

The excitation and emission fluorescence spectra of the complex in the presence of fluoride ion and of the Al^{3+}-calcein alone were obtained with pH 2.7 buffer solution of CCl_3COOH-CH_3COOK, as shown in Fig. 2. A decrease in the fluorescence intensity in the maximum emission peak (503 nm) can be observed in presence of F^{-}. All results showed that the difference of fluorescence intensity (ΔF) was at the maximum at λ_{ex}/λ_{em} 480/503 nm; therefore, λ_{ex}/λ_{em} 480/503 nm was selected as operating parameter during all laboratory work. These wavelengths were not changed by the addition of fluoride, therefore, a further complexation could be excluded. It might be that fluoride reacted first with aluminium ions, the surplus aluminium ions reacted again with calcein, or aluminium ions reacted first with calcein, and fluoride ions abstracted Al^{3+} from the complex of Al^{3+}-calcein.

**Effect of temperature and heating time**

Whether fluoride ions reacted first with aluminium ions and the surplus aluminium ions reacted again with calcein, or aluminium ions reacted first with calcein, and fluoride ions abstracted Al^{3+} from the complex of Al^{3+}-calcein, it was necessary that aluminium ions reacted fully with calcein. The effects of temperature and heating time on Al^{3+} and calcein complexation were studied using a thermostat. When heating for 10 min, the fluorescence intensity of the complex increased rapidly with an increase in heating temperature from 30 °C to 60 °C; the fluorescence intensity of the complex reached the maximum at 60 °C and remained constant at 60 °C to 80 °C; finally the fluorescence intensity of the complex dropped slowly at a temperature above 80 °C. When heating at 60 °C, the fluorescence intensity of the complex increased rapidly with an increase in heating time from 0 min 10 min; it reached the maximum at the 10th min and remained constant until 20 min; finally the fluorescence intensity of the complex dropped slowly after 20 min. The decrease in the fluorescence intensity of the complex was due to the reaction of fluoride with aluminium ions.