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Simple, rapid reagent-injection spectrophotometric determination of fluorides in pharmaceutical formulations

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Abstract

A new simple and rapid reagent-injection method is reported for the determination of fluorides in pharmaceutical formulations. The method is based on the inhibitory effect of fluoride ions upon the Fe(III) catalytic oxidation of 2,4-diaminophenol (DAP) by H_2O_2. Free fluoride ions form stable complexes with Fe(III), thus reducing its catalytic effect. The decrease in the absorbance of the oxidation product is monitored spectrophotometrically at 500 nm. The various chemical and physical variables of the FI system were optimized and a study of interfering ions was also carried out. A linear calibration graph was obtained from 0 to 750 mg l^{-1} for F^- ions. The precision was very good ($s_r = 0.3\%$) and the 3σ detection limit was satisfactory ($c_L = 0.471$ mg l^{-1}). The sampling rate was 90 injections h^{-1}. The method has been successfully applied to the determination of F^- ions in pharmaceutical formulations.

Keywords: Reagent-injection; Spectrophotometry; Fluoride; Pharmaceuticals

1. Introduction

At present, fluoride is widely regarded as the cornerstone of modern preventitive dentistry, as the remarkable decline in dental caries that is now occurring throughout much of the world can be largely attributed to the use of the ingested and topical forms of fluoride. The beneficial effects of fluoride may not be limited to the oral environment. Either alone or in combination with estrogen, calcium and/or vitamin D, it is used in high daily doses for the treatment of osteoporosis and other bone disorders. Fluorides are even believed to have positive effects on ischemic heart disease [1].

In addition to its established cardiostatic effect and its possible preventive or therapeutic roles in other major diseases, fluoride is a hazardous substance when large doses are taken acutely or when lower doses are taken chronically. Its effects range from dental fluorosis, reversible gastric disturbances and transient reduction in urinary concentrating ability, to skeletal fluorosis and death. The danger from fluoride poisoning is great, especially among children. For example, the 'probable toxic dose' (PTD) for a 10-kg child corresponds to only 55 ml of a mouthwash containing 0.2% NaF, or

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to 4 ml of a topical gel or solution containing 2.72% NaF [1]. For these reasons an accurate, precise, easily handled and automated method for the rapid, and with low operational cost, determination of fluoride in the pharmaceutical industry process and quality control is required. Flow injection (FI) analysis has served the above purposes [2].

The most common techniques used to determine F^- ions in pharmaceuticals are ion chromatography (IC) [3–5] and fluoride selective electrodes [6,7]. The main limitation of the IC methods is that because of the weak binding of fluoride to the ion-exchangers commonly used in IC, fluoride is generally eluted rapidly from the column and is found very close to the 'injection' peak [8], which contains the solvent and the unretained components of the sample, resulting in potential quantitative inaccuracies. On the other hand, the electrochemical methods that are based upon ion selective electrodes (ISE) [6,7] are generally not robust enough to be applied to routine analysis, since the active surface of the electrodes requires periodic regeneration because it tends to absorb impurities from the sample.

Although a large number of FI methods for the determination of fluorides in various samples have been reported, few FI procedures are applied to the determination of the analyte in pharmaceuticals [6,7]. These procedures employ a fluoride selective electrode as the detection system, either in a normal FI manifold [6], or coupled to a pervaporation unit [7]. The above-mentioned disadvantages of ISE and the fact that pervaporation is a membrane-based technique, make these methods rather unattractive for routine analysis and process control. An alternative technique that has gained most attention in FI is UV-VIS spectrophotometry, owing to its simplicity and low operational cost. Recently, a simple batch spectrophotometric method for the determination of fluoride ions has been reported [9]. The method is based on the destruction of the iron(III)-methylsalicylate complex by fluorides. However, this procedure suffers from a limited determination range (10–80 mg l^{-1}) and long reaction time (approx. 15 min).

In the present work, a simple, rapid, inexpensive and sensitive method for the determination of fluoride ions in pharmaceuticals has been developed, using a reagent-injection flow system. To the best of our knowledge this is the first spectrophotometric FIA report for the determination of F^- ions in pharmaceuticals. The method is based upon the inhibitory effect of fluoride ions [10] on the Fe(III) catalytic oxidation of 2,4-diaminophenol (DAP) by H_2O_2 [11], two procedures that have been previously developed in our laboratory. Fluoride ions form stable complexes with Fe(III), thus reducing its catalytic effect. The oxidation product is monitored spectrophotometricaly at 500 nm. The sample pretreatment for the pharmaceutical formulations was minimal, as accurately weighted amounts of the solid samples were dissolved in doubly de-ionized water and the proper dilution in doubly de-ionized water was the only pretreatment step involved for the mouthwash samples. Another significant feature of the method is that, because the proposed method combines the wide determination range and low detection limit, the samples can be further diluted thus eliminating any potential interferences — although this was not required in the present work.

2. Experimental

2.1. Instrumentation

The FI system used was a Tecator Fiastar 5010 analyzer (Tecator, Hoganas, Sweden) equipped with a FIAstar Superflow software. The detector used was a Tecator Fiastar 5023 double-beam spectrophotometer, consisting of a 5032 detector controller and a 5023-011 spectrophotometer optical unit. A printer was also included with the detection system. The absorbance of the colored oxidation product was monitored at 500 nm through a 1-cm path length flow cell with an internal volume of 18 μl. The flow system used was 0.5 mm i.d. teflon tubing throughout. For delivering the aqueous solutions Tygon pump tubes were used.

A home-made heated-refrigerated circulating water bath was used in order to adjust the solutions and the manifold temperature to the proper value.

An Orion EA940 pH-meter was employed for the pH measurements with absolute accuracy limits at the pH measurements being defined by NIST buffers.

2.2. Reagents

All chemicals were of analytical reagent grade and were provided by Merck (Darmstadt, Germany) unless stated otherwise and all the solutions were made up with doubly de-ionized water. The standard stock iron(III) chloride solution [$\gamma(Fe^{3+}) = 1000$ mg l^{-1}] also contained 0.05 mol l^{-1} HCl, in order to prevent hydrolysis of the ions. A standard stock solution containing 5 g l^{-1} F^- was prepared daily by dissolving the appropriate amount of NaF in 100 ml of doubly de-ionized water. The DAP working solution was prepared daily by dissolving the appropriate amount of doubly-recrystalized DAP in 0.2 mol l^{-1} HCl. A glycine/HCl buffer was used to adjust the pH to the proper value (pH approx. 2.75).

All solutions were de-gassed with purified nitrogen, except for hydrochloric acid which was de-gassed by ultrasonication. All stock solutions were stored in polyethylene containers. All the polyethylene containers and glassware used for aqueous solutions containing metallic cations were cleaned in (1 + 1) nitric acid, while the rest were cleaned in 3% Decon 90 and then all were rinsed with de-ionized water before use. Working solutions of Fe(III) and F^- were prepared by appropriate dilution immediately before use.

2.3. Procedure for aqueous solutions

The modified FI setup used, which was originally developed previously [12], is shown schematically in Fig. 1. It consisted of the DAP (2×10^{-3} mol l^{-1}) and buffer (glycine/HCl pH 2.64) streams which were pre-merged prior to the injection of the oxidant system/sample. A 30-μl volume containing the final oxidant system (10 mg l^{-1} Fe^{3+} and 0.1 mol l^{-1} H_2O_2) and the sample (0–750 mg l^{-1} F^-) was injected directly into the final stream. The reddish ($\lambda_{max} = 500$ nm) water-soluble product was formed on passage of the mixture through a 200-cm reaction coil. The cycle time was set to 40 s with 15-s cycle injection time. Using the cycle time of 40 s, 90 injections per hour were made.

The transient signal from the detector was recorded as a peak, the height of which was inversely proportional to fluorides concentration in the sample, and was used for all measurements. The recorded peaks were sharp and the baseline was stable. Five replicate injections per sample were made in all instances.

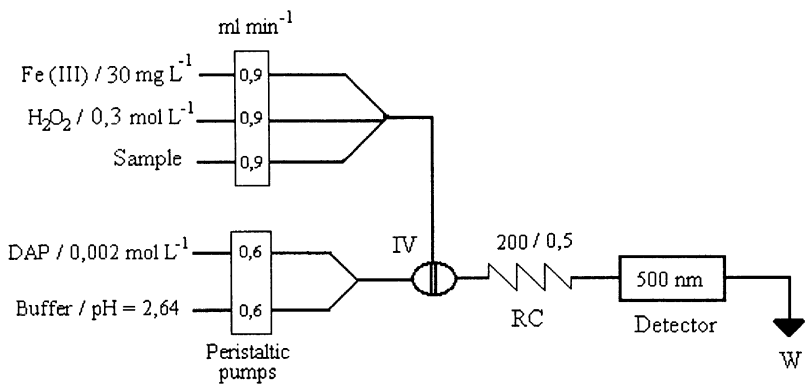

Fig. 1. Optimized flow injection manifold for the determination of fluorides. IV, injection valve (loop volume = 30 μl); RC, reaction coil (200 cm length/0.5 mm i.d.); W, waste; Buffer: glycine/HCl (pH 2.64).

2.4. Determination of fluorides in pharmaceutical formulations

For the determination of fluorides in the solid samples, five tablets of each sample were weighed, grounded by a mortar and finally homogenized. From each powdered sample, an accurately weighed amount (approx. 0.1 g) was taken and dissolved in 100 ml of doubly de-ionized water.

Regarding the mouthwashes, the only pretreatment step involved was the appropriate dilution of the samples in doubly de-ionized water.

Finally, the samples were analyzed using the above-described FI procedure for aqueous solutions.

3. Results and discussion

3.1. Preliminary studies

Based on our previous batch work [10], we propose a simple and rapid FI method determination of fluoride ions in pharmaceutical formulations, since fluorides are well known to react with Fe(III) forming stable complexes, and thus are likely to inhibit the Fe(III) catalytic oxidation of DAP by H_2O_2 in a continuous flow mode.

The preliminary studies showed that fluoride ions have an inhibitory effect upon the catalytic reaction under flow conditions, and can be determined indirectly. Due to the inhibitory effect of fluorides upon the above chemical reaction there is a non-linear relationship between the absorbance and the mass concentration of the analyte, which is shown graphically in Fig. 2. However, a linearity was achieved by using the function $1/A = f[F^-]$.

3.2. Optimization of chemical and FI variables

The effect of chemical and FI variables on the system were discussed in detail elsewhere [12]. Making the appropriate modifications, the optimized variables, the studied range and the selected optimal values are shown in Table 1.

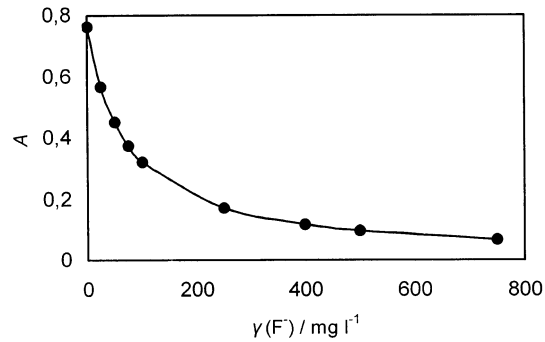

Fig. 2. Graphical depiction of the inhibition effect of fluoride ions upon the Fe(III) catalytic oxidation of DAP by H_2O_2.

3.3. Features of the proposed method

Under the above-mentioned optimal conditions and using the FI setup shown in Fig. 1, a calibration curve for citric acid was recorded. The calibration graph was linear and it was described by the regression equation

$$1/A = (i \pm s_i) + (S \pm s_S)\gamma(F^-)$$

where A is the absorbance as measured in the detector, $\gamma(F^-)$ is the mass concentration of the analyte in the aqueous solution, i is the intercept, S is the slope of the regression line and s_i and s_S the standard deviations of the intercept and the slope, respectively. The figures of merit of the proposed method are shown in Table 2.

Table 1
Optimization of chemical and FI variables

Variable	Studied range	Optimal value
Chemical variables		
Temperature (°C)	10–40	20 ± 0.2
PH	1.48–2.64	2.64
I (mol l^{-1})	0.05–1.0	0.1
c(DAP (mol l^{-1})	1×10^{-30}–8×10^{-3}	2×10^{-3}
$c(H_2O_2)$ (mol l^{-1})	0.15–4.5	0.3
γ(Fe(III)) (mg l^{-1})	2.5–40	30
FI variables		
V (μl)	30–130	30
l (cm)	30–300	200
q_v (ml min^{-1})	0.4–0.6	0.6

Table 2
Figures of merit

$S \pm s_s$	$(181.0 \pm 4.2) \times 10^{-4}$
$i \pm s_i$	1.31 ± 0.15
r ($n = 8$)	0.9992
s_r[a] (%)	0.3
Linear range (mg l^{-1})	0–750
$c_{L(k=3)}$[b] (mg l^{-1})	0.471

[a] Relative standard deviation calculated at 350 mg l^{-1} F^- ($n = 12$).
[b] Detection limit.

It should be noted that the calculated 3σ detection limit was obtained using the propagation of errors approach [13]. All the standards were run in five replicate injections ($n = 5$), while for the calculation of the 3σ detection limit the blank was run in 10 replicate injections ($n = 10$).

3.4. Interference studies

Using the FI setup shown in Fig. 1 and under the optimal conditions described above, the interference effect of several species on the determination of fluorides was examined. The criteria for interference was fixed at an e_r of less than $\pm 2\%$ of the average absorbance signal taken for a fluoride mass concentration corresponding to 50 mg l^{-1}. This criteria was established as the relative error that corresponds to $3 \times s_r$ at 50 mg l^{-1} ($n = 5$). The results are shown in Table 3. As can be seen in Table 3, there is satisfactory tolerance to the ions which are most likely to co-exist with fluorides in the pharmaceutical samples.

Table 3
Effect of diverse ions on the determination of 50 mg l^{-1} F^- under optimal conditions for aqueous solutions

Ion added[a]	Maximum tolerance level (mg l^{-1})
Na^+, Cl^-	7500
SO_4^{2-}, S^{2-}	2500
Pb^{2+}	1000
Zn^{2+}	600
Mg^{2+}	500
Ca^{2+}	250
PO_4^{3-}	100

[a] These ions in the stated level caused a relative error of $< \pm 2\%$.

Table 4
Determination of F^- ions in pharmaceutical formulations

Sample	Added (mg l^{-1})	Found[a] [mg l^{-1} ($\pm s$)]	100 R
Tablet I	–	11.2 (± 0.2)	
	100.0	112.3 (± 1.1)	101.0
	200.0	209.2 (± 1.6)	99.1
Tablet II	–	22.6 (± 0.2)	
	100.0	120.5 (± 1.1)	98.3
	200.0	226.7 (± 2.1)	101.8
Tablet III	–	33.2 (± 0.2)	
	100.0	131.9 (± 0.9)	99.0
	200.0	236.4 (± 2.3)	101.3
Mouthwash I	–	226.0 (± 1.7)	
	100.0	327.9 (± 2.5)	100.6
	200.0	423.9 (± 3.1)	99.5
Mouthwash II	–	406.0 (± 3.5)	
	100.0	496.4 (± 3.7)	98.1
	200.0	599.3 (± 4.6)	98.9

[a] Mean of five results.

3.5. Determination of fluorides in pharmaceutical samples

The proposed procedure for the determination of F^- ions was successfully applied to the analysis of commercial pharmaceutical formulations, namely three tablets each containing different amounts of NaF, and two mouthwashes. The concentration of the analyte in the samples and the percent recoveries in each case are listed in Table 4. The obtained recoveries were in the range of 98.1–101.8%, indicating that the proposed FI method is adequately accurate. Five replicate injections ($n = 5$) for each sample were made.

3.6. Accuracy of the proposed method

The accuracy of the developed method was further checked by comparison of the results obtained by the developed method with the nominal values of fluorides in the samples as claimed on their packages. The results are summarized in Table 5. The accuracy of the method as expressed by the calculated relative errors was very satisfactory, as e_r varied between 0.8 and 2.2%.

Table 5
Comparison of the results obtained by the proposed method with the nominal concentrations of the fluoride ions in the samples

Mouthwash II Sample	0.226[c] (± 0.002) FI method[a]	Normal calue Nominal value	+1.5 100 e_r
Tablet I	24.6[b] (± 0.26)	25.0[b]	−1.6
Tablet II	51.1[b] (± 0.43)	50.0[b]	+2.2
Tablet III	74.2[b] (± 0.40)	75.0[b]	−0.8
Mouthwash I	0.226[c] (± 0.002)	0.230[c]	−1.7
Mouthwash II	0.406[d] (± 0.004)	0.400[d]	+1.5

[a] Mean of five results.
[b] Found and nominal mass concentrations in mg NaF (± s)/tablet.
[c] Found and nominal fluoride ions mass fraction (± s) in sample.
[d] Found and nominal NaF mass fraction (± s) in sample.

4. Conclusions

The proposed FI method is the first spectrophotometric FIA report for the determination of fluoride ions in pharmaceutical formulations and fulfills the major demands of routine analysis. It is simple, easy to handle, it has low operational cost, and offers a high sampling rate, minimum sample pretreatment and a wide fluoride concentration range for the direct determination of the analyte without losing in sensitivity. Additionally, it shows adequate selectivity and very good precision and accuracy in all cases. These features indicate that the proposed method could be an advantageous alternative to potentiometric and ion chromatographic methods for the fast, simple and accurate determination of fluorides in pharmaceutical formulations.

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