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Simultaneous determination of monofluorophosphate and fluoride in toothpaste by capillary electrophoresis

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Abstract

Monofluorophosphate and fluoride in various brands of toothpaste were determined by capillary electrophoresis (CE) with indirect UV detection. The electrolyte used was 10 m*M* chromate and 0.1 m*M* cetyltrimethylammonium bromide (CTAB) solution at a pH of 9.37. Analysis was completed within 8 min when the voltage was set at 15 kV (negative polarity). The CE method was compared with a reference method based on ion chromatography (IC). The results obtained by the CE method agreed with those obtained by the IC method. The CE method was found to be superior in terms of speed and simplicity.

Keywords: Toothpaste; Monofluorophosphate; Fluoride

1. Introduction

Sodium monofluorophosphate (sodium MFP) and sodium fluoride are two agents commonly added to toothpaste for caries prevention. Clinical research has proved that fluoride plays an important role in the prevention of caries, while MFP can provide new fluoride during the application of toothpaste [1]. Fluoride may react with other ingredients to form insoluble compounds, and lose its therapeutic function in storage. On the other hand, MFP will hydrolyse and release free fluoride. Consequently, quantitative analysis of MFP and fluoride in toothpaste is of great importance for quality evaluation of these dental care products.

Several techniques have been used to analyze the water-soluble fluoride species in toothpaste: fluoride ion-selective electrode electroanalysis [2–5], gas chromatography (GC) [5], isotachophoresis [6], ion chromatography (IC) [7–10] and capillary electrophoresis (CE) [5,11]. Among these methods, electroanalysis and GC have a disadvantage that MFP must be hydrolysed first before the total fluoride is measured. To our knowledge, the acid hydrolysis of MFP is difficult to control because the HF formed during hydrolysis may evaporate. The resulting fluoride has to be converted to a volatile substance for GC analysis. Ion chromatography has been demonstrated to be a powerful method for the determination of inorganic anions since its introduction in 1975 [12]. IC methods for direct determination of MFP and fluoride in toothpaste using either a dual-column (suppressed) [7] or a single-column (nonsuppressed) [8,9] version have been reported. Because of the weak binding affinity of fluoride to the ion-exchangers commonly used in IC, fluoride is generally eluted rapidly from the column under the standard separation conditions and is found very close to the so-called 'injection' peak [13], which contains the sample solvent and unretained

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components in the sample. There may be more than one species eluted within this early retention time. In the case of hydrogencarbonate-based toothpaste, sample preparation to remove hydrogencarbonate is necessary because it is an unretained component when the eluent comprises carbonate and hydrogencarbonate. If not, it will result in a very large 'injection' peak and severe early baseline disturbances [7]. If the 'injection' peak is very huge or some components coelute with fluoride, its analysis by IC may result in quantitative inaccuracies.

CE is another excellent technique for the analysis of anions. Much work has been done in the analysis of anions by CE. Its advantages for simultaneous determination of 36 anions was reported by Jones and Jandik [14]. Quantitative determination of fluorine in toothpaste by CE has been reported [5,11]. However, in one of these two CE methods, an unpublished OFM Anion BT solution (patented reagent) was used in the buffer [5]; in the other, it seemed that MFP and fluoride could not be separated completely when the ribonucleotide electrolytes were used [11].

In this paper, a new CE method with CTAB as electroosmotic flow modifier and tungstate as internal standard for the simultaneous determination of MFP and fluoride is presented and the results compared with those obtained from IC.

2. Experimental

2.1. Capillary electrophoresis

All CE experiments were carried out on a laboratory-built capillary electrophoresis system, equipped with a power supply, a UV detector (Lauerlabs, Netherlands), a fused-silica capillary and an integrator (Shimadzu C-R6A, Japan). The operating conditions for CE are listed in Table 1.

Before it was used, the capillary was pretreated with 0.2 *M* NaOH for about half an hour, then rinsed with deionized water for 2 min and the electrolyte for 10 min. To maintain reproducible migration times, the capillary was flushed with the running buffer for 2 min and a voltage of −15 kV was applied to it for 1 min before each sample was injected. The total running time for each determination was about 8 min. The capillary was filled with 0.2 *M* NaOH overnight in order to maintain the capillary wall in good condition.

The pH of the buffers was determined by a Microprocessor pH/mV/°C meter (Hanna Instruments, Model 8417).

2.2. Ion chromatography

The IC system used in this study was a Dionex

Table 1
Conditions for capillary electrophoresis and ion chromatography

Parameter	Conditions
CE	
Capillary	52 cm effective length×75 μm I.D., total length 60 cm, uncoated
Buffer	10 m*M* sodium chromate and 0.1 m*M* CTAB, pH 9.37
Voltage	−15 kV (negative polarity)
Detection	UV detection, 254 nm (indirect detection)
Injection	Hydrostatic, 10 cm for 25–30 s (ca. 32–38 nl injection volumes)
Temperature	Room temperature, 20–22°C
IC	
Column 1	Dionex HPIC-AG4A and AS4A
Column 2	Dionex HPIC-AG6 and AS6
Eluent 1	1.8 m*M* sodium carbonate and 1.7 m*M* sodium hydrogencarbonate, 2.0 ml/min
Eluent 2	20 m*M* sodium tetraborate decahydrate, 1.0 ml/min
Suppressor	Dionex AMMS (Anion Micro-Membrane Suppressor)
Regenerant	0.125 *M* H_2SO_4
Detection	Conductivity detection, 30 μS
Injection volume	50 μl
Temperature	Room temperature, 28–30°C

2010i chromatography module (Dionex, Sunnyvale, CA, USA). The operating conditions are listed in Table 1.

2.3. Reagents

Sodium MFP (95% pure) was obtained commercially from Aldrich (Milwaukee, WI, USA). Other chemicals used were analytical grade, and were purchased from either Fluka (Switzerland) or Sigma (St. Louis, MO, USA). All solutions were prepared with deionized water (18 MΩ cm) purified by a NANOpure analytical deionization system (Barnstead, Dubuque, IA, USA).

The buffer used was prepared by diluting 5 ml of 100 m*M* sodium chromate and 0.25 ml of 20 m*M* CTAB to 50 ml total volume. No further adjustment of pH was needed. The buffer had a pH assay value of 9.37. This working electrolyte was prepared fresh daily.

The standard MFP, fluoride and tungstate (internal standard) solutions (1000 μg/ml) were prepared by dissolving the appropriate amounts of the corresponding sodium salts in deionized water. These storage solutions were diluted to the proper concentrations as required.

All the above solutions were filtered through 0.45-μm membrane filters before use.

The IC eluent was prepared by diluting 6.8 ml of 0.5 *M* sodium carbonate and 7.2 ml of sodium hydrogencarbonate to 2000 ml total volume. Before use, it was filtered through a 0.45-μm membrane and degassed.

2.4. Samples

A 1.500±0.001 g amount of toothpaste was weighed into a 100-ml beaker. The tungstate internal standard solution (4 ml) and deionized water (16 ml) were then added. The solution was stirred, and, after dilution, a portion was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) in order to remove organic contaminants. The sample was filtered through a 0.45-μm membrane filter before injection into the IC columns. It was not necessary to pass CE samples through the Sep-Pak C_{18} cartridges.

2.5. CE linearity evaluation

Nine solutions of MFP, with concentrations ranging from 0.2 to 7 μg/ml, and eight solutions of fluoride, with concentrations ranging from 0.05 to 3 μg/ml, were prepared. Each solution was determined twice.

2.6. CE recovery studies

Fixed amounts of MFP and fluoride standards, ranging in concentrations from 50% to 150% of that in the samples, were used to spike four samples of toothpaste with known MFP and fluoride contents and the mixture was analyzed using the proposed procedure.

3. Results and discussion

3.1. CE analytical conditions

The principal CE parameters which affect the separation are carrier electrolyte concentration and the pH of the buffer as these influence both the electrophoretic mobilities of solutes and the electroosmotic flow (EOF) velocity [15].

An electrolyte pH range of 8–11 was evaluated. The results are shown in Fig. 1. When pH was adjusted to 8 by adding sulfuric acid, the buffer precipitated easily. Tungstate, monofluorophosphate and fluoride were separated more satisfactorily when the pH of the buffer was set at 9. As the pH of the buffer was 9.37 when the required amounts of

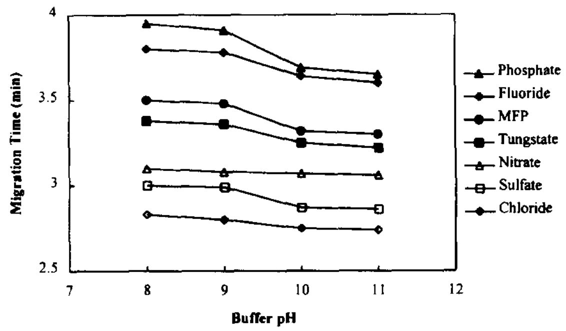

Fig. 1. Effect of buffer pH on separation. The chromate concentration was kept constant at 10 m*M* and CTAB 0.5 m*M*.

chromate and CTAB were mixed, the buffer was used without further pH adjustment.

The influence of the ionic strength of the chromate electrolyte on the selectivity of the seven anions is shown in Fig. 2. MFP and tungstate were separated poorly when the chromate concentration was 5 m*M*. In order to separate the seven anions completely over a reasonable analysis time, chromate concentration was set at 10 m*M*.

CTAB was used for reversing the natural direction of bulk flow in fused-silica capillaries. To investigate the effect of the EOF modifier (CTAB) on the separation of analytes, five solutions with different concentrations (0.1–0.5 m*M*) of CTAB were used as buffers. Variations of the concentration of CTAB within this range did not affect appreciably the separation of the analytes. Because of this, its concentration in the buffer was fixed at 0.1 m*M* for later experiments.

A solution comprising of 10 m*M* chromate and 0.1 m*M* CTAB was chosen as the final buffer. This gave excellent resolution of the anions, as shown in Fig. 3.

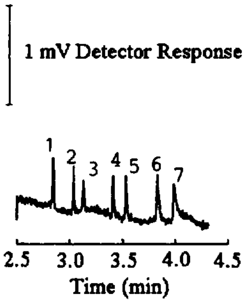

Fig. 3. Typical electropherogram of standard anions. Peaks: 1= chloride; 2=sulfate; 3=nitrate; 4=tungstate; 5=monofluorophosphate; 6=fluoride; 7=phosphate. For CE conditions see Table 1.

3.2. CE system suitability test

Tungstate was used as the internal standard as it is not a constituent in toothpaste and it was separated completely from all other anions in the electropherogram with an electrophoretic behaviour similar to that of MFP and fluoride. Fig. 4a shows a typical electropherogram of a toothpaste extract and Fig. 4b shows one of a toothpaste extract spiked with the internal standard.

3.2.1. Injection time

Sample injection times between 10 s and 40 s (hydrodynamic, 10 cm) were tested. It was found that the relative standard deviations were comparatively low when the injection time was set at 25–30 s (ca. 32–38 nl injection volumes).

3.2.2. Precision

The intra-day relative standard deviations of the CE method, on the basis of peak-height ratios for six replicate injections, were 3.6% for MFP and 4.2% for fluoride. The inter-day variations (4 days) were 4.4% for MFP and 5.8% for fluoride.

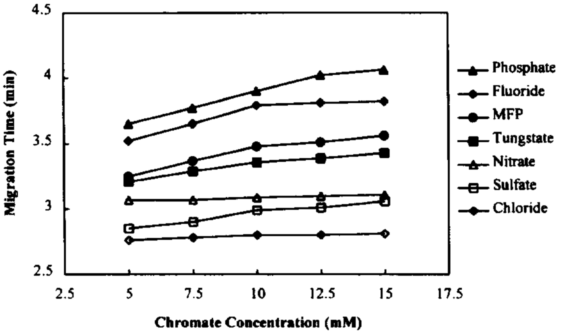

Fig. 2. Effect of chromate concentration on separation. The pH of the buffer was kept constant at 9.37 and CTAB concentration was 0.5 m*M*.

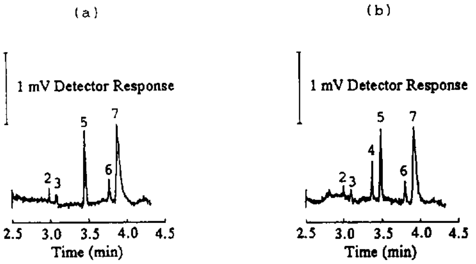

Fig. 4. (a) Electropherogram of toothpaste sample 3. Peaks: 2=sulfate; 3=nitrate; 5=monofluorophosphate; 6=fluoride; 7= phosphate. CE conditions as in Table 1. (b) Electropherogram of toothpaste sample 3 spiked with internal standard. Peaks: 2= sulfate; 3=nitrate; 4=tungstate; 5=monofluorophosphate; 6= fluoride; 7=phosphate. CE conditions as in Table 1.

Table 2
Recoveries of MFP and fluoride from four brands of toothpaste

Sample	MFP			Fluoride		
	Added (μg/ml)	Found (μg/ml)	Recovery (%)	Added (μg/ml)	Found (μg/ml)	Recovery (%)
1	200	199	99.5	10.0	8.75	87.5
	400	404	101	20.0	18.0	90.0
2	80	76.1	95.1	2.00	1.79	89.5
	200	183	91.5	6.00	5.33	88.8
3	80	78.2	97.8	10.0	8.50	85.0
	200	191	95.5	20.0	17.1	85.5
4	200	184	92.0	2.00	1.65	82.5
	400	424	106	6.00	5.26	87.7

3.2.3. Linearity

Good linearities were found in the range 0.4–4.5 μg/ml for MFP and 0.1–1.5 μg/ml for fluoride. The regression equations of these curves (peak-height ratio, y, vs. concentration, x, μg/ml) and the correlation coefficients were calculated as follows:

MFP $y=0.4328x+0.2934$ $(r^2=0.9978)$
fluoride $y=0.7177x+0.2596$ $(r^2=0.9956)$

3.2.4. Quantification limit

As described above, the limits of quantification (LOQ) were 0.4 μg/ml for MFP and 0.1 μg/ml for fluoride. The limits of detection (LOD), which represent the concentration of analyte that would result in a signal three times higher than the background noise, were not tested because the LOQs were considered more important in this work.

3.2.5. Recovery

The results of standard addition recoveries of MFP and fluoride are listed in Table 2. The recoveries were 91.5–106% for MFP and 82.5–90.0% for fluoride.

3.3. Quantification by CE and comparison with IC

To investigate the accuracy of the CE method further, the results were compared with those obtained by IC. MFP was analyzed by an AS4A column (see Table 1) and fluoride by both AS4A and AS6 columns. The Dionex AS4A is a high-speed IC column conventionally used for fast analysis of common anions. The AS6 column has a higher ion-exchange capacity than that of AS4A. As shown in Table 3, the CE analysis of MFP compares favourably with IC analysis (with the AS4A column). On the other hand, fluoride analysis by CE correlates better with that by IC with the AS6 column (Table 3). As shown in Table 4, the paired *t*-tests [16] at a 95% confidence interval indicate that there are no significant differences between the CE and IC for

Table 3
Quantitative results for MFP and fluoride in four brands of toothpaste by CE and IC

Sample	MFP (%, w/w)		Fluoride (%, w/w)		
	CE	IC (by AS4A)	CE	IC (by AS4A)	IC (by AS6)
1	0.358	0.379	0.0248	0.0163	0.0298
	0.381	0.394	0.0231	0.0166	0.0295
2	0.148	0.135	0.00388	0.00503	0.00410
	0.167	0.134	0.00373	0.00512	0.00396
3	0.265	0.274	0.0368	0.0213	0.0316
	0.272	0.274	0.0341	0.0215	0.0320
4	0.440	0.437	0.00724	0.00628	0.00690
	0.426	0.414	0.00755	0.00593	0.00658

Table 4
The paired t-test results ($|t|$ value) for the comparison of CE and IC methods

Critical value	Value for MFP between CE and IC (by AS4A)	Value for fluoride	
		Between CE and IC (by AS4A)	Between CE and IC (by AS6)
2.36	0.33	2.38	0.31

MFP results ($|t|<2.36$, the critical value) and between the CE and IC with the AS6 column for fluoride ($|t|<2.36$), but there exists a significant difference between the CE and IC with the AS4A column for fluoride ($|t|>2.36$).

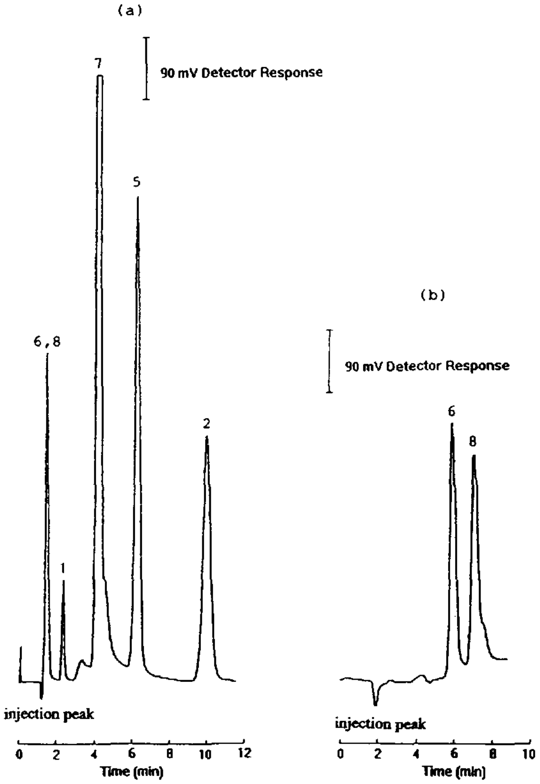

Fig. 5. (a) Ion chromatogram of toothpaste sample 2 (by AS4A column). Peaks: 6=fluoride; 8=unknown; 1=chloride; 7=phosphate; 5=monofluorophosphate; 2=sulfate. For IC conditions see Table 1. (b) Ion chromatogram of toothpaste sample 2 (by AS6 column). Peaks: 6=fluoride; 8=unknown. For IC conditions see Table 1.

In IC with the AS4A column, the fluoride (peak 6) is very close to the 'injection' peak (Fig. 5a); there is also an as yet undetermined interference (peak 8) that co-elutes with fluoride as one peak. With the AS6 column (Fig. 5b), fluoride (peak 6) and the unknown component (peak 8) are well-separated; also, in contrast to the AS4A column, there is no interference from the baseline aberration. However, gradient elution would be needed with the AS6 column for the simultaneous determination of fluoride and MFP. (It is not shown in Fig. 5b, but the retention time of MFP is off the time scale since it is too highly retained on the column under the conditions used.) Gradient elution IC with conductivity detection is not very widely used in comparison to isocratic IC, as a search of the literature reveals, and adds another level of complexity to the analysis [17] (e.g., additives have to be added to the regenerant to minimize baseline drift and additional short clean-up columns must be needed to eliminate inorganic impurities in the eluent), as well as additional expense in instrumentation. The CE method described is simpler and more advantageous than IC in general.

4. Conclusion

This paper presents the possibility of using capillary electrophoresis for the routine simultaneous determination of monofluorophosphate and fluoride in toothpaste. The method compares favourably with a reference IC method. CE has an obvious advantage over traditional methods in that it can separate many anions in a very short time. This is very useful, especially when analysis of large numbers of samples is required. It is satisfactory for the described application and can be considered for quality control in toothpaste formulating and manufacturing.

Acknowledgments

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