PERFORMANCE DIFFERENCES OF TWO POTENTIOMETRIC FLUORIDE DETERMINATION METHODS IN HARD DENTAL TISSUE

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SUMMARY: A comparison between two ion selective electrode (ISE) potentiometric methods is reported for determining the amount of fluoride in hard dental tissue after placement of fluoride-releasing dental restorations. The two methods are: (1) the direct method involving linear calibration (LC), and (2) a spiking method involving multiple standard additions (MA). Results showed that measurements performed by the LC method underestimate the amount of fluoride released by up to 30%. Recovery tests demonstrated that the use of MA and blank correction procedures is useful for an accurate and sensitive ISE determination of fluoride in hard dental tissues.

Keywords: Dental materials; Fluoride ISE potentiometry; Linear calibration; Multiple spiking addition.

INTRODUCTION

In dental research, the potentiometric ion selective electrode (ISE) method[1-4] is commonly used for fluoride determinations. The method is fast, simple, and specific.[5] A direct quantification method, using a linear calibration (LC) plot, is often preferred, but it is unable to detect and remove bias due to matrix effects.[6] More powerful methods of quantification, based on spiking of analytical samples (e.g., the standard addition method),[7,8] are available, although they are rarely used for this purpose.[1,9] The aim of this study was to compare ISE determinations of the amount of fluoride in hard dental tissue after use of fluoride releasing restorative materials by the direct LC method versus a constant volume multiple addition (MA) method.

MATERIALS AND METHODS

Specimen preparation: Twenty-five 3-mm thick root disks from the coronal third of the roots, measuring approximately 6 mm in width and length were obtained from extracted bovine incisor teeth. The discs were embedded in a methlyl methacrylate resin (Leocryl, Leone Italia) and abraded through cementum with 600-grit silicone carbide paper. In the area of the exposed dentin surface near the root-crown junction, a cavity was prepared, 1.0 mm in depth and 1.5 mm in diameter, using a diamond burr on a high speed drill (SUPERtorque 655 Kavo, Germany) under water-spray. Next, the specimens were randomly divided into five groups and treated respectively with: 1) a 3rd generation self-etching adhesive (Xeno III, Dentsply, Germany)[10]; 2) an ionomeric cement (Ketac-Cem Radiopaque, 3M ESPE AG, Germany) plus a thin layer of bonding agent (Clerafil

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SE bond); 3) a light-cured bonding system consisting of a self-etching primer and a bonding agent (Clearfil SE bond, Kuraray, Europe GmbH, Germany); 4) an ionomeric cement (Ketac-Cem Radiopaque, 3M ESPE AG, Germany); 5) a control group with no restorative material on dentinal surface. The specimen were then submitted to a demineralizing-remineralization procedure under four 24-hr cycles: 1 hr in a demineralizing solution[11] and 23 hr in a remineralizing solution.[12]

Analytical procedure: A) SAMPLE MINERALIZATION AND PRE-TREATMENT: Six grams of the dentinal sample powder, obtained from each specimen by abrasion with 600-grit silicone carbide paper, was transferred to a 50-mL volumetric flask and dissolved in 40 mL of 37% HCl solution and then diluted with water to the mark. A 5.0-mL aliquot of the above solution was transferred to a 25-mL volumetric flask, diluted 1:1 with water, neutralized with a 6 M NaOH solution to pH 4.5, and made up to the mark with water. B) LC FLUORIDE EVALUATION: Six solutions (each containing 2.39 mol/L of HCl neutralized with NaOH) were prepared containing 10.0, 5.0, 1.0, 0.5 and 0.1 mg F^-/L, respectively. The last one was kept as the analytical blank. In a beaker, 25.0 mL of each solution was mixed 1:1 with TISAB solution, and the potential was measured beginning with the blank solution. Each sample was determined in quintuplicate. C) MA FLUORIDE DETERMINATION: In a beaker, 25.0 mL of each sample was mixed 1:1 with TISAB solution and the potential was measured, beginning with the most diluted one. Later, three consecutive additions of fluoride standard solution were made, collecting the potential value after each addition. Table 1 reports the concentration and the volume of each addition. The Gran's-like linearization procedure[7,13] provided the analytical concentration. Each sample was determined in quintuplicate. D) BLANK EVALUATION: To increase the accuracy and precision in the measurement of very low fluoride concentrations (up to few μg/L), the Villa procedure[15] was always used.

Table 1. Operating parameters of MA method

Material	Concentration of standard spiking fluoride solution (mg F^-/L)	Standard volume of each addition (μL)
1 (Xeno)	50	100
2 (CVI+ClearS-bond)	100	100
3 (ClearS-bond)	50	50
4 (Cement VI)	500	50
5 (Dentine, control)	50	50
Blank	50	50

Equipment: A Model 96.09 F^- ISE, a Model 900200 Ag/AgCl reference electrode, and a Model 290 mV digital meter, all by Orion, were used. Temperature was 20±0.5ºC. Appropriate fixed volume Eppendorf Research Series 2100 pipettes were used. Everywhere possible, glassware was replaced with polyethylene labware.

Reagents: Analytical grade reagents were used throughout. NaF used for standard was 99.99%. Water was ultra pure grade. The TISAB solution was purchased from Thermo-Orion (code 94-09-11).

Statistical analysis: The data and the robustness of the analytical methods used were statistically analyzed using Student's t-test ($p<0.05$).

RESULTS

Table 2 reports the total fluoride concentration by means of an LC plot. The equation of the calibration line obtained is $y = (-59.1\pm0.2)x + (159.2\pm0.3)$, with a correlation coefficient $R= 0.9999$. Table 2 shows the analytical data obtained using the MA method.

Table 2. Fluoride amounts in the bulk material evaluated by means of LC and MA methods

Group Sample[a]	LC method C F^-±SD[b] (mg/kg)	MA method C F^-±SD (mg/kg)	Matrix index
1	9.380±0.394	9.853±0.453	0.98
2	17.81±0.69	21.27±0.53	0.78
3	2.632±0.126	2.667±0.139	1.03
4	45.33±0.50	53.72±0.64	0.71
5	2.094±0.107	2.938±0.147	0.77
Blank	-		1

[a]Each sample has been measured in quintuplicate. [b]SD = estimated standard deviation.

Analytical values for samples 2, 4, and 5 are quite different from those obtained using the LC plot (Student's *t*-test). An analytical interference is evidenced by the substantial differences of their slope values, respect to the blank one. The matrix interference in each situation was evaluated by a normalization of the slopes of the different experimental MA lines. A dimensionless parameter, matrix index (MI), has hence been defined and evaluated (Table 2):

$$MI = \frac{a_{sample}C_{blank}}{a_{blank}C_{sample}}$$

where a_{sample} and a_{blank} are the slopes of both linear regressions considered, C_{sample} and C_{blank} are the concentrations of the relevant fluoride spiking solutions (Table 1). Values of MI close to 1 mean that data relative to the matrix of the sample are affected by the same bias as the analytical blank, *i.e.* a situation of "no interference." On the other hand, MI lower (or higher) than 1 implies an analyte underestimation (or overestimation) by matrix interference. Group 1 and 3 samples gave a matrix index close to 1 (the blank value), whereas in groups 2 and 4 a significant matrix effect was observed (0.78–0.71 MI). Samples from group 5 (dentin, control group) showed an MI value of 0.77.

DISCUSSION

Nowadays, dentinal root caries is an increasing problem[15] and to prevent them, it is essential to consider their cause and how to protect against them. Fluoride is considered an important tool[16-17] in arresting the processes of secondary caries; otherwise, in relation to water fluoridation, debate is still open,[18-19] and a precautionary approach to the use of fluoride would consider all the available evidence on efficacy, safety, and alternatives.

The need to obtain high reproducibility and reliability in the measurement of fluoride in solutions or in dental tissues is therefore essential.[1] Comparison of data sets in Table 2 shows significant differences. Linear calibration always tends to underestimate the fluoride amount. The MI value reveals that groups 2, 4, and 5 (i.e., the "inorganic-based" materials) evidence a strong matrix interference that, if not corrected, leads to substantial under-evaluation of the amount of fluoride released (up to ca. 30% for sample 4 ionomeric cement). It is interesting to note that, in both methods the dentinal samples (i.e., the control group), show MI values not too far from the value observed for ionomeric cements. In the same conditions polymer-based materials (samples 1 and 3) show low or negligible matrix interference.

Recovery tests, performed in triplicate with samples 3 and 4 (i.e., a pure organic and an inorganic-based material, respectively) show good accuracy: the recoveries were between 96 and 102% (sample 3) and between 98 and 101% (sample 4). These results show that the proposed method is bias-free. Another advantage to combine the MA ISE potentiometric determination of fluorides with the blank evaluation/subtraction is represented by a significant lowering and "customization" of the limit of detection. In this way, the limit of detection is related to the nature of the sample, and not to a data set of standard solutions, as usually happens in the LC method.

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