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Comparison between the ion-specific electrode and SPADNS methods for analysis of fluoride concentration in the water supply

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The maintenance of adequate fluoride (F) concentration in the public water supply is fundamental for ensuring that the community use of F can reach the maximum benefit for caries control and minimum risk for dental fluorosis. Thus, surveillance systems must use accurate and valid analytical methods to determine F concentration and, according to the literature, give preference to the ion-specific electrode (F^- ISE) analysis. **Aim:** The objective of this study was to compare the accuracy of the ISE and SPADNS methods in the determination of the F concentration in the same water sample. **Methods:** Duplicate water samples were taken from 30 sampling sites in the municipality of Maringá, state of Paraná, monthly for 12 months, totaling 276 samples. An aliquot was analyzed by the FOP-UNICAMP Oral Biochemistry laboratory, using the F^- ISE method, and the other one, by the SANEPAR laboratory in Maringá/PR, using the SPADNS method. Descriptive analysis and Pearson's correlation test were applied, with a significant level of $p<0.05$. **Results:** Results were expressed as ppm F (mg F/L), and a very strong positive correlation ($r= 0.91$; $p<0.001$) was detected between the two methods of analysis. **Conclusion:** Our findings suggest that the determination of fluoride concentration in water can be made with accuracy by the SPADNS method, a standardized analysis protocol.

Keywords: Fluoridation. Water supply. Comparative study.

Introduction

Fluoridation of public water supply is a community way of using fluoride for dental caries prevention[1]. It is the best cost-benefit measure, has greater population reach, and strong impact on the control of caries development[2] and it continues to be effective even in developed countries that have shown dental caries decline such as United States[3], Australia[4], and Ireland[5]. Though, considering the high prevalence of untreated dental caries in permanent teeth (2.5 billion people in 2015) shown in Global Burden of Diseases Study[6], many efforts have been made to control the disease, and the benefits and risks of fluoridated water use have been debated worldwide[4].

However, for fluoride (F) effectiveness and to draw maximum benefits from fluoride for caries prevention and minimum risk for dental fluorosis, it is necessary, besides an adjustment in F concentration over time, the maintenance of adequate F concentration in the water supply[7-9]. In Brazil, external evaluation (heterocontrol) is one of the health surveillance actions to check the maintenance of F levels in the public water supply, regulated by Ordinance 635/BSB/1975 of the Ministry of Health[10]. For this to be implemented, F concentration in water has to be monitored by an heterocontrol. Among the several methods for the determination of the F ion by the heterocontrol, stand out the colorimetric and ion-specific electrode (ISE) methods[11].

Most studies conducted in Brazil about the quality of fluoride concentration in water heterocontrol initiatives have used the ISE analysis of F because it is considered sensitive, precise, and accurate. On the other hand, the colorimetric method, using SPADNS reagent (sodium 2-[parasulfophenylazo]-1,8-dihydroxy-3,6-naphthalene disulfonate), is the most used by the operational control (water plant treatment), because of its low maintenance cost[12]. ISE analysis, in turn, is based on the direct measurement of free F ion, present in the sample, using a specific electrode, and presenting high selectivity, better linearity, and less susceptibility to interferents[13]. Due to its higher precision and accuracy, ISE analysis is preferred over the SPADNS method[14,15].

Although both methodologies are used to analyze the concentration of F by the research and water supply companies, there are few reports in the literature, comparing the two techniques, using the same samples[14,16-18]. In addition, contradictory results have been found. While some publications suggest that the colorimetric method overestimates fluoride concentration compared to the electrode method[15-18], Motter et al.[14] (2011) reported opposite results. The reasons can be the use of non-duplicate water samples or analytical problems of using not well standardized SPADNS method[15], suggesting further studies are necessary to elucidate this controversy.

Thus, the objective of this work was to compare the accuracy in the determination of the F concentration in the same water sample, using the ISE technique by a reference research laboratory, and using the SPADNS colorimetric method by a reference laboratory of sanitary analysis.

Material and Methods

Study Design

This was a longitudinal, observational, prospective, descriptive study. To compare the methods for F determination, two analytical techniques, commonly adopted for public water supply analysis, were used; the first one, the ion-specific electrode (ISE) method, using the direct technique, and the second one, the SPADNS colorimetric method[19].

Place of study

The municipality of Maringá is located in the southern region of Brazil, and northwest of the state of Paraná. According to the Brazilian Institute of Geography and Statistics, in the study period, Maringá had a population of 357.177 inhabitants, an area of 487.7 km², and a population density of 732.1 inhabit/ km².

Selection of sites and sample collection

For the study, 30 water samples from different locations in Maringá were collected; 28 samples from the 28 Basic Health Units (BHU) of the municipality, geographically distributed according to the need of the population, and two samples from the Paraná Sanitation Company (SANEPAR), one water sample from the Pirapó River, raw, and after treatment (fluoridation and chlorination) by SANEPAR.

Duplicate water samples were collected at 30 sampling sites in the municipality of Maringá, monthly for 12 months, totaling 276 samples. One duplicate was analyzed by the FOP-UNICAMP Oral Biochemistry laboratory using the ISE method, and the other by the SANEPAR laboratory in Maringá, using the SPADNS method. Professionals of the BHUs were asked to collect the water from the faucet located near the street water entrance, preferably near the hydrometer. This faucet was left open for 30 seconds, plastic bottles were washed 3 times, and water was collected for analysis. The water sample (raw) from the Pirapó River was obtained at the SANEPAR water treatment plant before any treatment. Another sample was collected shortly after treatment, ready for distribution.

Sample Analysis

Ion-specific electrode (ISE) method

The ISE method is based on the direct measurement of free F ions with the use of an ion-selective electrode in conjunction with an ionic activity meter. The analysis was carried out at the Laboratory of Oral Biochemistry, Faculdade de Odontologia de Piracicaba - UNICAMP. Duplicate samples were buffered with the same volume of TISAB II solution (1.0 M acetate buffer pH 5.0, 1.0 M NaCl, and 0.4% CDTA) prepared in the laboratory. The electrode Orion 96-09 (Thermo Scientific Inc., Waltham, USA) coupled to an ion analyzer ORIONSTAR A214 (Thermo Scientific Inc., Waltham, USA) was calibrated with standard solutions at concentrations ranging from 0.10 to 1.00 µg F/mL in 50% (v/v) of TISAB II. Results were expressed in mg F/L (ppm F), and the coefficient of variation of the duplicate analysis was below 1%.

Colorimetric method

The colorimetric fluoride assay with SPADNS (sodium 2-[parasulfophenylazo]-1,8-dihydroxy-3,6-naphthalene disulfonate) is based on the reaction between F and the zirconium dye[20]. The intensity of the red color in water samples was measured in a spectrophotometer at a wavelength of 570 nm. Samples were analyzed in duplicate, using a Hach DR/890 photocolorimeter.

To eliminate residual chlorine and to increase the precision of the analysis, 0.2 mL sodium arsenite was added to a 10 mL water sample, and 2.0 mL SPADNS reagent was added and homogenized[21]. The concentration of fluoride was reported in parts per million (ppm).

Classification of samples

All evaluated water samples were classified according to the Technical Consensus Document on the Classification of Public Water Supply (CECOL) according to the F concentration[9].

According to the Climatological Station of the Universidade Estadual de Maringá, the maximum average temperature in the municipality of Maringá/PR, during the collection period of the water samples, was 29.9 ºC. Thus, according to CECOL, samples were classified by the temperature range, with the mean values of the maximum temperatures between 26.3 and 32.5°C. In this specific situation, the best risk/benefit combination is in the 0.55 - 0.84 ppm F range, where the anti-caries benefit is maximal and the risk of fluorosis is low.

Statistical analysis

Data processing and statistical analysis were performed in the Statistical Package for the Social Sciences (SPSS® for Windows, version 20.0, Armonk, NY, USA: IBM Corp.). Descriptive analyses were performed. The concentration values of F obtained by each method were categorized according to CECOL/USP. The paired T-Test and Pearson's Correlation test were performed to evaluate the relationship between F concentrations in the ISE and SPADNS methods. The significant level was $p<0.05$.

Results

A total of 300 samples were collected and 276 were analyzed, as 12 samples from the Pirapó River and 12 samples from the Aclimação BHU were excluded from the comparative analysis of techniques since they were water samples without fluoridation. Samples of raw water before treatment at SANEPAR (Pirapó River) showed trace concentrations of fluoride ion (<0.07 ppm F), but soon after treatment, the concentration ranged from 0.55 to 0.84 ppm F.

Concentrations of F determined by the SPADNS colorimetric analysis showed a minimum concentration of 0.54 ppm F and a maximum of 1.27 ppm F and by the ISE technique, the F concentrations varied between 0.44 and 1.20 ppm F (Table 1).

Table 1. Descriptive analysis of F concentrations (ppm) using ISE and SPADNS methods, and paired T-Test, p<0.001 (N= 276).

Month	ISE				SPADNS				
	n	Mean	Median	SD	Mean	Median	SD	p	r
1	24	1.021	1.070	0.1024	1.103	1.145	0.1149	<.001	0.949
2	23	0.665	0.660	0.0701	0.801	0.800	0.0848	<.001	0.979
3	20	0.691	0.710	0.0629	0.828	0.840	0.0744	<.001	0.961
4	26	0.755	0.760	0.0596	0.835	0.830	0.0610	<.001	0.892
5	14	0.781	0.800	0.0956	0.788	0.785	0.0817	0.659	0.789
6	26	0.738	0.740	0.0406	0.805	0.810	0.0576	<.001	0.891
7	23	0.750	0.770	0.0593	0.794	0.800	0.0660	<.001	0.868
8	24	0.789	0.810	0.0744	0.891	0.905	0.0782	<.001	0.839
9	26	0.769	0.775	0.1057	0.838	0.830	0.0957	<.001	0.889
10	25	0.784	0.800	0.0571	0.842	0.850	0.0523	<.001	0.838
11	25	0.756	0.740	0.0931	0.861	0.850	0.0965	<.001	0.952
12	20	0.669	0.640	0.0795	0.695	0.665	0.0857	0.015	0.863
TOTAL	**276**	**0.77**	**0.75**	**±0.12**	**0.84**	**0.83**	**±0.12**		

Source: Prepared by the authors, 2023.

Table 2 lists that 86% (n=237) water samples analyzed by the ISE were classified with the best risk/benefit ratio (0.55 to 0.84 ppm F) for the municipality of Maringá. By the SPADNS method, the value was lower (56%; n= 155).

Table 2. Number of water samples from the Maringá BHUs classified according to CECOL/USP in fluoride concentration intervals (ppm F), according to the chemical analysis method (ISE or SPADNS).

Classification CECOL/USP	ISE n (%)	SPADNS n (%)
0.00 to 0.44 ppm F	1 (0.4)	0 (0.0)
0.45 to 0.54 ppm F	3 (1.1)	1 (0.4)
0.55 to 0.84 ppm F	237 (85.8)	155 (56.1)
0.85 to 1.14 ppm F	34 (12.3)	106 (38.4)
1.15 to 1.44 ppm F	1 (0.4)	14 (5.1)
> 1.45 ppm F	0 (0.0)	0 (0.0)
Total	**276 (100.0)**	**276 (100.0)**

Source: Prepared by the authors, 2023.

Pearson's correlation coefficient between the water samples analyzed by the ISE and SPADNS techniques was 0.91 (r=0.91; p<0.001), evidencing a very strong positive, significant correlation between the two variables (Figure 1).

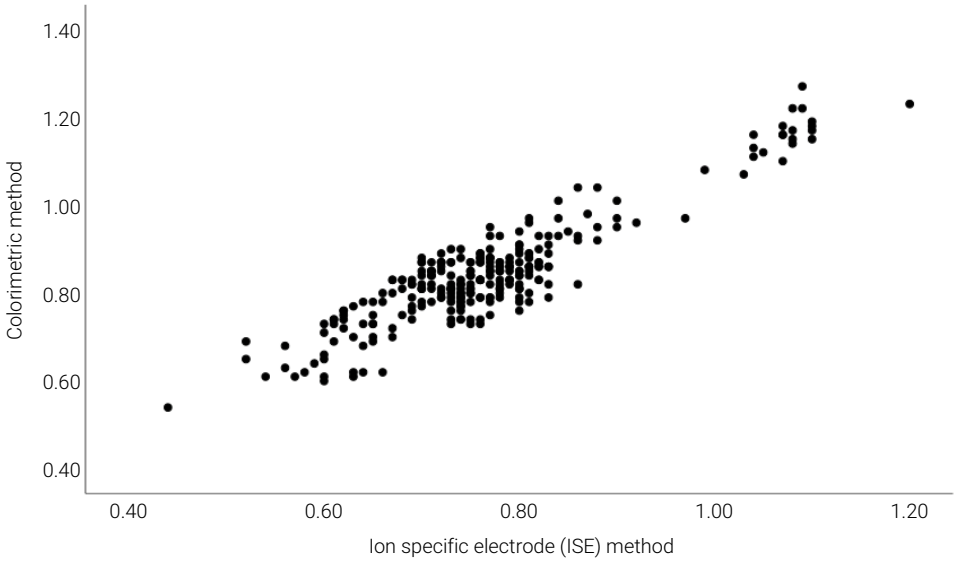

Figure 1. Correlation between fluoride concentrations determined by SPADNS colorimetric and ion-specific electrode (ISE) methods (N=276).

Discussion

Monitoring and evaluating F concentration in the public water supply is an effective way to keep the concentration of F within a range considered ideal to ensure its anticaries benefit for the population. Among different methods to determine the F concentration, the most used are the SPADNS colorimetric and ISE techniques[14].

Comparing these techniques for the determination of F concentration, there was a high, strong positive correlation (r=0.91) (Figure 1), showing that the determination of F concentration in water can be made with accuracy by both methods. Although 56% of the samples analyzed by the SPADNS colorimetric technique and 86% of the samples analyzed by the ISE electrometric technique presented concentrations of F ranging from 0.55 to 0.84 ppm F (the best risk/benefit ratio range according to CECOL[9]), minimal differences (0.01 ppm F) could alter the classification of the samples into different categories. In addition, these differences seem to have little meaning considering that the purpose of the assessment is to identify inadequate concentrations; allowing the water company to make the necessary adjustments, pursue the best risk/benefit F concentration and not to punish or condemn them. Thus, the SPADNS colorimetric method can provide them with valid and coherent results, compared to the ISE electrometric technique.

Following the guidelines presented in the Technical Consensus by CECOL[9], small variations in F concentration in the water below or above the range of the best combination of risks and benefits are acceptable over the exposure time, i.e., concentrations of F with insignificant benefit or very high risk could be tolerated only if occurring sporadically for a day over the months. Likewise, minimum benefit or high-risk concentrations could be acceptable only if not constant for more than seven days over the course of months, and moderate benefit or risk concentrations could be tolerable

only if not constant for more than 21 days in a year. However, from the identification of the need to adjust the concentration, it must be corrected immediately to guarantee quality water to the population.

In Brazil, the standards for public water fluoridation supply were established by Ordinance 635/BSB/1975[10]. When comparing this ordinance with the classification of F concentration in supply water proposed by CECOL[9], the latter takes into account the development of caries and fluorosis as chronic diseases and stratifies the concentration ranges of F according to different degrees of risk. Different from the 1975 ordinance, which sets strict limits for F concentration (minimum 0.6 ppm F and maximum 0.8 ppm F). In this way, the Technical Consensus proposed by CECOL[9] is the most coherent and flexible way to control the concentration of F by supply companies, not interfering with the safety of water consumption by the population[22-24].

Comparing these two techniques for F analysis, a very strong and significant correlation (r= 0.91) was detected between the two methods of analysis. Previous studies found a high positive correlation between ISE x SPADNS, r= 0.95 [Eldestein et al.[18] (1992)]; ISE x Gold Standard Government Laboratory in Ontario, r= 0.99; SPADNS x Gold Standard Government Laboratory in Ontario, r= 0.93 [Weinberger et al.[17] (1989)]. In our study, the two techniques had a strongly positive correlation (r= 0.91), which may demonstrate the potential for using the colorimetric technique, when calibration is carried out with the electrometric technique. Importantly, in our study, the analyses were carried out by different institutions in a blind manner, in contrast to other studies conducted in the same laboratory[15-17]. In addition, one of these studies used a SPADNS kit of analysis, which can present limited results, compared to a standardized methodology of analysis. Possibly, analytical problems may account for the differences found between both techniques used, suggesting more studies to calibrate the SPADNS technique.

Nevertheless, according to Brossok et al.[16] (1987), Weinberger et al.[17] (1989), Edelstein et al.[18] (1992), and Lins-Candeiro et al.[15] (2020), the colorimetric technique tends to give a higher value than the electrometric one, overestimating F concentration compared to the ISE technique. On the other hand, in Motter's study[14], the colorimetric method resulted in a lower value than the electrometric technique. In our study, although the colorimetric method showed numerically higher values, they did not show relevant differences according to the classification proposed by CECOL[9], which points to an adequate range of F concentration (0.55 to 0.84 ppm F), considering the best risk/benefit ratio for fluorosis, and prevention of dental caries. Thus, the colorimetric method seems to be a valid technique to be adopted by water treatment companies. In addition, they should be guided by the "CECOL/USP Technical consensus about the classification of public water supplies according to the fluoride content" to conduct daily operational control of F concentration in water and guide their teams to keep F concentration in the 0.55 to 0.84 ppm F range (in regions with average maximum temperatures between 26.3 and 32.5°C).

The colorimetric method is simple and easy to perform, due to the speed with which the reaction occurs. The cost of this methodology is considered low, which justifies its implementation as a routine analysis[25,26], provided periodic calibration is carried out using a more accurate method such as electrometric.

Thus, it is essential to monitor the F concentration in public water supply, to be routinely carried out in the water treatment plants, as well as to control the F content through heterocontrol[27]. More studies should be conducted to check if the methods for F determination in the public water supply made by the water treatment companies are presenting adequate F concentrations in the water.

In conclusion, our findings suggest that the determination of fluoride concentration in water can be accurately made using the SPADNS method, using a well-standardized protocol of analysis.

Conflict of interest

The authors have no conflict of interest to declare.

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Author Contribution

Tânia Harumi Uchida: Data curation, investigation, methodology, project administra-tion, validation, original draft, writing, review and editing.

Jaime Aparecido Cury: Data curation, investigation, methodology, writing, review and editing.

Cinthia Pereira Machado Tabchoury: Writing, review and editing.

Cacilda Castelo Branco Lima: Data curation, writing, review and editing.

Mitsue Fujimaki: Conceptualization, data curation, investigation, methodology, project administration, validation, original draft, writing, review and editing.

All authors actively participated in the discussion of the manuscript's findings, and have revised and approved the final version of the manuscript.

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