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Evaluation of the direct and diffusion methods for the determination of fluoride content in table salt

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Objective: The aim of this study was to assess methods currently used for analyzing fluoridated salt in order to identify the most useful method for this type of analysis. ***Basic research design:*** Seventy-five fluoridated salt samples were obtained. Samples were analyzed for fluoride content, with and without pretreatment, using direct and diffusion methods. Element analysis was also conducted in selected samples. Fluoride was added to ultra pure NaCl and non-fluoridated commercial salt samples and Ca and Mg were added to fluoride samples in order to assess fluoride recoveries using modifications to the methods. ***Results:*** Larger amounts of fluoride were found and recovered using diffusion than direct methods (96%-100% for diffusion vs. 67%-90% for direct). Statistically significant differences were obtained between direct and diffusion methods using different ion strength adjusters. Pretreatment methods reduced the amount of recovered fluoride. Determination of fluoride content was influenced both by the presence of NaCl and other ions in the salt. ***Conclusion:*** Direct and diffusion techniques for analysis of fluoridated salt are suitable methods for fluoride analysis. The choice of method should depend on the purpose of the analysis.

Key Words: Fluoride, fluoride analysis, salt

Introduction

Fluoride (F) determination in salt became relevant since salt fluoridation was proposed as an adequate vehicle for public fluoridation. This alternative to water fluoridation started to be successfully used in several countries throughout the world (Bergmann and Bergmann, 1995; Estupiñán-Day, 2000; Marthaler and Sener-Zanola, 1985; Secretaría de Salud, 1995). Because of safety and quality control issues, constant monitoring of salt F concentration has been recommended (Estupiñán-Day, 2000; Maupomé-Carvantes *et al*., 1995). It has been suggested that a lack of quality control in the manufacturing process, as well as problems in the methodologies used, may be responsible for the wide range of F concentrations reported for salt (Maupomé-Carvantes *et al*., 1995). Reports have shown that fluoridated salt has varying amounts of F, not always compliant with governmental regulations. Different F concentrations have been reported within a single bag (Estupiñán-Day, 2000; Franco Cortés *et al*., 2003; Maupomé-Carvantes *et al*., 1995), as well as changes in F concentration over time (Galindo and Galindo, 1992). Other investigators have further hypothesized that the varying ranges of F in salt are the result of changes in the availability of ionic F in salt. They have attributed this phenomenon to F complex formation with other elements present in salt, such as stabilizers, bleaching agents and anti-compactants (Alanen and Pohto, 1977).

The analytical techniques that are currently used to conduct salt fluoridation monitoring have not been standardized and universal procedures for the determination of F in this type of sample have not been established. The need for standardized methods was highlighted by the significant differences found among results of eight laboratories that participated in an international collaboration where the same sample set, included salt samples. Results of this collaboration clearly showed that the choice of technique made for specific types of samples played a significant role in the differences found and that certain techniques appeared to provide more precise and true results for certain types of samples (Martínez-Mier, *et al*., 2003).

There are few reports in which methods to analyze fluoridated salt have been assessed. Results of previous studies have specifically suggested that F determination in salt samples may be affected by the method used to conduct the analysis (Venkateswarlu, 1990). Differences have been reported for several of the steps involved in the determination of F in salt samples. These include pretreatment of samples, separation and concentration of F and actual measurement of F ions (Venkateswarlu, 1977 and 1990). Therefore, the aim of this study was to assess the most commonly used methods for analyzing fluoridated salt samples in order to identify which one yielded more precise and true results.

Materials and Methods

Salt samples were obtained from five large producers, eight packers and five supermarket chains that commercialize their own brand of fluoridated salt (private labels) in Mexico. Eighteen different brands of fluoridated salt were obtained. Six duplicates of commercial brands (three from manufacturers and three from packers) and one du-

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plicate from a private label brand were obtained resulting in 25 one-kg packages of fluoridated salt. Since three samples were collected from each one, 75 samples of salt were analyzed in total. Five of the samples were ground salt, while 70 of the samples were refined. The theoretical value of the samples, according to the manufacturers reported concentrations, was 250 ± 50 µg/g F. These salt samples were obtained from the evaporation of seawater by open air or vacuum. Fluoride was later added to salt using the wet method, which uses potassium fluoride (KF) or sodium fluoride (NaF) in a solution that is continuously sprayed onto salt. In addition to the fluoridated samples, non-fluoridated samples (salt samples that had not undergone the fluoridation process) were obtained from two commercial packers. Ultra pure grade NaCl samples (Ultra pure NaCl, J.T. Baker, The Netherlands) and aliquots from the two commercial non-fluoridated salt samples were additionally prepared to contain known amounts of F (5, 10, 25 , 50, 100 and 250 µg/g,) by spraying a solution of either KF or NaF.

Approximately 90 g were taken from each one-kg package; 30 g from the top, 30 g from the middle and 30 g from the bottom of the package. Each top, middle, and bottom sample was stored in 60 ml Nalgene sterile containers. All samples were numbered and packed according to international regulations and transported to our laboratory.

Pretreatment of samples

Two previously reported pretreatment methods recommended for salt analysis were tested (Venkateswarlu, 1990). In the first pretreatment method, 10 g of salt were ashed at a temperature of 315.55° C for two, four or six hours. Samples were placed in an acid-washed, pre weighed, silica dish. Muffle furnaces were preheated at approximately 315.55° C for about 2 hours. Total ash weight was then determined and recorded. As controls, ultra pure 10 g NaF samples were ashed at this same temperature and at varying times alone or in combination with 10 g ultra pure NaCl. One g of ashed salt was then mixed with 1 ml of deionized water. In the second pretreatment method, acid dissolution, 1 mg of the sample was dissolved adding 20 µL of 5M perchloric acid ($HClO_4$: Sigma Chemical Co., USA); 40 µL of deionized water and 40 µL of citrate/EDTA (294g $Na_3C_6H_6O_7$, 74.5g Na_2EDTA in 900 ml deionized water + 8 g of NaOH pellets [to adjust pH to a range of 7.9 to 8.1.]) was added to buffer the solution. Samples were capped and analyzed immediately.

Fluoride Analysis Methods

The two basic methods used for F determination were: direct analysis, which is used for F analysis of samples that contain free, ionic fluoride. The second method was diffusion analysis, which releases both free and bound fluoride, subsequently concentrates it, and is used for samples in which fluoride may be in a covalent or complexed form. One research technician conducted analyses of all samples, while a second research technician conducted duplicate analysis in order to determine inter-technician reproducibility. Duplicate analyses were conducted every tenth samples by the main research technician to determine repeatability.

Analyses were conducted in two phases; initially comparisons were made between direct and diffusion analysis, with and without pretreatment of commercially fluoridated salts, and ultra pure fluoridated salt. Direct analysis was conducted following recommendations for monitoring fluoridated salt as published by the Pan American Health Organization (PAHO) (Estupiñán-Day, 2000) and for diffusion analysis we used modifications (Soto-Rojas *et al*., 2004) to the hexamethyldisiloxane (HMDS: Sigma Chemical Co., USA) micro-diffusion method of Taves (1968) and Whitford (1996). A second phase of experiments was designed to obtain data to explain differences found among the results initially obtained using different modifications to the methods.

Direct analyses were performed using a combination F ion-selective electrode (Orion #96-909-00) and an Orion 720A pH/ion meter (Fisher Scientific Co., USA). In the initial phase, salts were diluted with deionized water (1:10 w/v) in a beaker and were pipetted out of the original container into vials with Total Ionic Strength Adjustment Buffer (TISAB) and then analyzed. Samples were mixed 1:1 (v/v) with TISAB II. Samples were placed directly under the electrode with continual stirring. Fluoride content values were obtained by comparison of the millivolt reading of each sample to a standard curve prepared from the analysis of F standard solutions conducted at the time of sample analysis. In the case of the acid pretreated samples, no further dilutions were performed. Standard solutions used to construct this curve had similar amounts of percholric acid, and the citrate/EDTA NaOH solution.

For diffusion analysis, 1 ml of sample from a salt dilution (1:10 v/v) was pipetted into a plastic petri dish (Falcon 5 cm-plastic petri dishes, Fisher Scientific Co., USA). A 0.05N sodium hydroxide (NaOH, A.R.: Sigma Chemical Co., USA) trap solution (50 µl) was placed, and 1 ml 3N sulfuric acid (H_2SO_4: Sigma Chemical Co. USA) saturated with HMDS, was added. Samples were diffused overnight, the trap was then recovered and buffered to pH 5.2 with 25 µl of acetic acid 0.10 N (CH_3COOH_9: Sigma Chemical Co., USA), and 25 µl of TISAB II (Fisher Scientific Co., USA) as recommended by Soto-Rojas *et al.* (2004). The recovered solution was adjusted to a final volume of 100 µl with deionized water. Fluoride content of each sample was also obtained by comparison of the millivolt reading of the sample to a standard curve prepared from the data for diffused F standard solutions. The range of standard concentrations used for the direct and the diffusion methods was from 0.05 to 50.0 µg/g.

Additional analyses were conducted in an attempt to explain differences observed in the initial results. In one modification, approximately 0.1 g of salt was poured undiluted, into vials containing deionized water (1 ml), those same vials were placed under the electrode for reading. Other experiments included the use of different types of TISAB. TISAB II. 1:1 (v/v), or 10:1 (v/v) TISAB III (Fisher Scientific Co., USA) were tested. A subset of samples (25) was analyzed using a 3:1 (v:v) TISAB II dilution. In order to test if the effect of adding NaCl to F standards used to construct the calibration curve produced better results, fluoridated salt was measured using F standards with ultra pure NaCl added at a similar proportion. A set of F standards was prepared using high purity NaF and deionized water. The solutions were prepared by dissolving the appropriate amounts of NaF in water. A second corresponding set of standards was prepared using, 0.2M aqueous solution of ultrapure NaCl instead of water. A third set of standards was prepared at constant F concentration (5 µg/g), in variable concentrations of ultrapure NaCl (0, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0M). Each of these modified samples or standards were mixed in a scintillation vial 1:1 (v/v) with TISAB II. A final method, standard addition, which has been recommended for use when the linear calibration plots of mV reading vs. log (F concentration) slope made with deionized water is different from the slope from the samples being analyzed (Liberti and Mascini, 1969) was utilized. In this method known amounts of standard fluoride solutions were added to dilutions of salt samples. The concentration of the samples was obtained using Gran's plot.

Element analysis of fluoridated salt.

Because it has been proposed that F complex formation with other elements present in salt could explain some of the significant differences in the results, we assessed the composition of salt samples in our study. It was our objective to identify possible interferences that could explain differences among different methods and pretreatment of samples by performing an elemental content analysis. Element analysis was performed in ten fluoridated salt samples for which results using the analysis techniques showed the highest or lowest recovery of fluoride samples from the commercially available fluoridated salts, plus the two non-fluoridated commercial salts, for a total of 12 samples. Almost all alkaline, earth-alkaline and metals were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES). This technique is based in the atomic spectra emitted by a sample, which is used for the determination of its qualitative or quantitative elemental composition. Samples were prepared dissolving a known weight of each salt sample. All samples were filtered to eliminate impurities commonly found in commercial salt using acid-resistant syringe filters (Acrodisk product number 4497, Gelman Scientific, Ann Arbor, MI, USA). To measure sodium ions, samples were diluted 1:100 (v/v) in deionized water. Only in one sample, a 1:10 (v/v) dilution was necessary to measure calcium (Ca) and potassium. All the standards were prepared from commercial stocks of 1000 µg/g. A multi-elemental standard (MERCK IV for ICP, MERCK, Germany) was used for Ag, Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr and Zn. The standards for As, Cl, Hg, Mo, Sb, Se, Si, Sn, Ti and V were prepared from individual stocks (Monoelemental Standards of 1000 µg/g for ICP, J.T. Baker, The Netherlands). Standards were prepared by dilution with deionized water. Interferences and limits of detection were consulted in the Atlas of Spectral Information of Inductively Coupled Plasma-Atomic Emission Spectroscopy (Winge, 1985). After element analysis was concluded, in order to test the possible effect of ions other than Na, Cl and F present at the time of analysis, the effect of Ca and Mg on direct fluoride measurements under the electrode was studied. Calcium chloride and magnesium chloride were added to pure salt samples at levels similar to those found in the commercially available samples (0.87 g Ca/kg salt and 0.85 g Mg/kg salt).

Statistical Analyses.

The concordance of results among techniques was assessed within each type of sample using a variety of statistical measures. Initially we compared the mean values for each technique using a mixed model analysis of variance (ANOVA) with sample number as the random effect and technique as the fixed effect. Differences among techniques were an indication of problems in the methods that had resulted in a systematic bias. Pair-wise comparison analyses were run to assess dyads of methods. The agreement among the techniques was expressed quantitatively using intraclass correlation coefficients (ICC). For our study design, the Shrout and Fleiss (1979) reliability coefficient was the most appropriate form of the ICC. Intra- and inter- technician variation (the reproducibility and repeatability) of duplicate laboratory fluoride analyses in this study were measured by determining ICCs. Statistical significance was set at $p<0.05$.

Descriptive analyses were performed to assess concordance. Samples containing known amounts were analyzed to determine the percent error for each method for each of the standard samples and also averaged across the standards. Finally, percent recovery was calculated for each known sample and the values were examined within and across techniques.

Results

The reproducibility and repeatability for the laboratory analyses in this study was excellent. ICC values for repeatability were 0.98, and the ICC values for reproducibility were 0.99. ANOVA repeated measurements analysis showed statistically significant differences among different pretreatments of samples (Table 1). Ashing of samples reduced the amount of recovered F. Seventy-six to 82% of F loss was found when compared to results of unashed samples measured by diffusion or directly , respectively. Results of ultra pure NaF ashing at 315.55º C (for two, four or six hours) demonstrated that F was not lost when ashed alone a or in combination with salt, while results of the ashing of ultra pure NaF with ultra pure NaCl demonstrated an average F recovery of 27% for all ashing times. Dissolution in acid also resulted in lower amounts of F recovery, with a 25% difference when compared to results obtained using the diffusion method, the average F obtained using the dilution in acid was of 170 ± 60 µg/g, which was below the average obtained with the direct method and TISAB II which was of 197 ±44. The observed difference was not statistically significant.

ANOVA repeated measurements also showed statistically significant differences among the methods initially tested. Pair-wise analysis showed statistically significant differences between results obtained using direct measurements (adding TISAB II) and measurements using diffusion ($p=0.002$). Initial results of direct vs. diffusion

Table 1. Mean fluoride concentration (SD) of salt samples

Sample type	*N=*	*Direct TISAB II * Mean ± SD (F μg/g)*		*Direct TISAB III * Mean ± SD (F μg/g)*		*Diffusion TISAB II * Mean ± SD (F μg/g)*	
		*Unashed***	*Ashed*	*Unashed***	*Ashed*	*Unashed***	*Ashed*
All Commercially Fluoridated Samples	75	197 ± 44	35 ± 22	161 ± 35	28 ± 2	266 ± 67	5 ± 43

* p= <0.05 between direct TISAB II and direct TISAB III, between direct TISAB II and diffusion, and between direct TISAB III and diffusion

** Samples not ashed, nor acid pretreated

Table 2. Percent recovery of fluoride.

Pretreatment	*Method*	*Percent recovery*
Ashed*	Direct and Diffusion	18-24%
Acid dissolution**	Direct	75%
Diluted in deionized water (1:10 w/v)	Direct	67%
No pretreatment	Direct	84%-93%
No pretreatment	Diffusion	96%-100%

* Mean for all ashing times

** Samples were pretreated with perchloric acid/citrate/EDTA and NaOH

Statistically significantly different (p< 0.05)

Table 3. Elemental Analysis of 12 salt samples by Inductively Coupled Plasma-Optic Emission Spectroscopy

Element	*Na*	*Cl*	*K*	*Li*	*Ca*	*Sr*	*Mg*	*Si*	*Mn*	*Hg*
Mean ± SD (g/kg)	436 ± 34	560 /± 140	0.86 ± 1.90	0.023 ± 0.004	0.87 ± 1.66	0.071 ± 0.091	0.041 ± 0.033	0.04 ± 0.049	0.03 ± 0.012	0.02 ± 0.005
N *	12	12	12	12	9	8	5	4	3	2

* Samples containing element

analyses showed that for commercially fluoridated salt the average F concentration was 77 ± 22 μg/g when analyzed directly and with diffusion the average was 226 ± 67 μg/g. Results of the analysis of non-fluoridated commercial salt samples showed that the average concentration for these samples was 11 ± 4 μg/g when measured directly, and with the diffusion method the F concentration was 13 ± 7 μg/g. No differences were found between non-fluoridated commercial salt samples to which KF was added versus the non-fluoridated commercial salt samples to which NaF was added. Mean F concentrations of commercially available fluoridated salt samples using different techniques are shown in Table 1.

A comparison between the results obtained by direct measurement of salts or by previously diluting them showed statistically significant differences. For commercially fluoridated salt the average F concentration was 197 ± 44 μg/g if measured undiluted with TISAB II using the direct method, 77 ± 22 μg/g if they were diluted and an aliquot was pipetted from the original solution. When the result of the direct analysis of commercial salts that were fluoridated in our laboratory were compared to the values obtained when the salts were diluted (1:10 v/v deionized water) in a beaker, pipetted and measured, a statistically significant difference was found ($p < 0.05$). On the other hand, for commercial salt fluoridated in our laboratory the recovery when poured into deionized water was 84%; however, it was 67% when these salts were diluted. For diffusion analysis 96% was recovered.

ANOVA repeated measurements also showed statistically significant differences among results obtained using different TISAB solutions. Pair-wise comparison analysis showed statistically significant differences between results obtained using direct measurements with TISAB II and with TISAB III ($p<0.001$); and between the direct method with TISAB III and diffusion ($p=0.03$). No significant differences were observed between the subset of samples that were analyzed using a different dissolution with TISAB II (3:1). The percentage difference between pairs was on average 22.40%, 11.20% and 27.43%, respectively. ICCs were 0.99 between the direct method using TISAB II and the direct method using TISAB III and 0.94 between the direct method using TISAB II and diffusion. Diffusion analysis showed on average larger F recoveries.

Fluoride recovery was 90% when both ultra pure NaCl and NaF were poured into deionized water prior to the addition of TISAB II. A statistically significant difference was found between results obtained using direct readings of ultra pure NaCl and NaF and those obtained using the diffusion method to analyze these same ultra pure samples (100% recovery). There was a 6% difference in recovery of fluoride between results obtained using undiluted ultra pure NaCl and NaF and those of undiluted commercial salt fluoridated in our laboratory when direct values were compared. Results of percent recoveries obtained using all techniques to analyze commercially fluoridated and non-fluoridated samples are presented in Table 2.

Results obtained using F standards with NaCl added produced an overall 10% increase in values. A set of standards prepared using high purity NaF using TISAB II buffered deionized water and a second set of standards using 0.2M of ultrapure NaCl instead of water produced linear calibration plots of mV reading vs. log (F concentration) of similar slopes. A third set of standards with constant F concentration (5 μg/g) but variable concentrations of ultrapure NaCl produced a curved plot of mV reading vs. log (NaCl concentration), which tended to a constant mV value with decreasing NaCl concentration. Results obtained using the method of incremental constant addition, resulted in a recovery of 93% while parallel direct analysis of the same samples using direct analysis gave recoveries of 86%.

Results of element analysis are shown in Table 3. This methodology demonstrated the presence of Li in all samples, Ca, Sr in most analyzed samples, and Mg in less than 50% of the samples. Hg, Mn and Si were detected in some samples, traces of Ba (0.004), B (0.005), V (0.02) and Al (0.003) were found in one different sample each. Other elements were not detected. When results of element analysis of these 12 salt samples were correlated to results obtained from methodologies used to measure F concentration, it was found that the samples that contained the highest concentration of all combined elements, were the ones that showed the highest difference between direct and diffusion methods. A negative correlation was found between the content of Sr and smaller differences found in the results obtained using ashed or unashed samples. Specifically for direct analysis, K, Li, Ca and Sr were negatively correlated, which could indicate that their presence had an effect on direct F measurements.

Results of our tests showed that when we added Ca to ultra pure salt, the recoveries of F were of 76%. When these salts with added Ca were diluted in deionized water (1:10 v/v) and then 24 hours later pipetted from the original beakers and measured, values fell to 69%. The addition of Mg resulted in same day/same beaker recoveries of F of 86% and samples that were pipetted and read 24 hours later had a recovery of 71%.

Several comparisons were conducted among results obtained for different types of samples. No significant differences were found among samples fluoridated by the packer or producer or for salt distributed directly by the packer, producer or by a supermarket chain as a private label, regardless of technique. No significant differences were also found for salt obtained from the top, middle or bottom portion of the packages using any of the techniques. There were also no differences when the type of packaging (bag, bottle, plastic, or carton) was compared. Finally, the analysis of F content differences between ground and refined salt also showed no statistically significant difference.

Discussion

Results of a thorough assessment of analytical methods commonly used to analyze fluoridated salt which included several different combinations of methods indicated that the use of the diffusion method with no prior treatment of the samples showed higher recoveries of F for both diluted and undiluted samples. Increasing the proportion of TISAB or using different types of TISAB did not yield percentage recoveries as large as those obtained with diffusion. The analysis of ultra pure NaCl with known amounts of F showed that diffusion analysis had the best percentage of recovery. In addition, results of the analysis of pre- and post-fluoridation samples that were sent to us directly from the packers demonstrated better recoveries with diffusion analysis. The trend observed in these results seems to support the use of diffusion analysis as the method for determination of total F in samples.

Results of certain experiments warrant careful consideration and point to a possible explanation for the differences observed. Five experiments were particularly considered when hypothesizing: 1) when using the direct method with TISAB II in commercially non-fluoridated samples the percentage of F recovered was lower than the percentage of F recovered with the same method using ultra pure NaCl; 2) the values obtained using diluted ultra pure NaCl using the direct method were lower than the values obtained using diffusion. Some precipitation may have taken place when samples were diluted and then aliquots taken from the initial solution; and 3) the results for commercially fluoridated samples consistently showed larger amounts of F found with diffusion analysis. 4) the fluoride ion specific electrode measures activity not concentration as supported by the different mV readings for a given F amount in a buffered solution of ultrapure NaCl than for the same amount in NaCl-free buffer. The F concentrations were the same but the F activities were different because of the difference in ionic strength. 5) Results of incremental analysis calculated using Gran's plot resulted in higher recoveries that those obtained by direct analysis using a linear calibrate, but lower recoveries than those obtained with diffusion analysis. Interpretation of these results allows us to hypothesize that differences observed could be partially due to both the presence NaCl in all of the samples and to the presence of other ions (only present in commercially available salts), which interfered with F analysis even in buffered samples.

As previously stated, our results seem to support a hypothesis which proposes that the presence of other ions interferes with the direct measurement of F, either by binding with it or by directly interfering with electrode readings even after buffering. Specifically for salt obtained from the sea, Riva (1966) proposed that it contained SO_4 and Cl, which interfered with the analysis of F and suggested adding these ions, plus NaCl, to the standard solutions at the same ratio by which they are present in seawater. In our study, preparation of standards by adding ultra pure NaCl to measure fluoridated salts increased the recovery of F. The addition of Ca to standards, which is a common practice for the analysis of some types of samples such as enamel biopsies, could also be a practice useful for salt samples. However, determination of the average Ca content for each sample set may prove difficult, as demonstrated by the large variation in Ca content observed in our samples. The analysis of samples using the constant addition method calculating the concentration using Gran's plot instead of a linear calibrate resulted in the highest recoveries of any direct measurement technique. Although this technique has been employed in other disciplines, its use in dentistry has been limited and according to our results, it is potentially useful when analyzing salt samples.

Riva (1966) also reported that seawater samples contained small quantities of F, which is in agreement with our findings. This is in disagreement, however, with the results of another study by Fuge (1988) who reported concentrations above 1.2 – 1.4 µg/g in seawater, which are higher than the ones we found. These differences could be attributed to the different sources of seawater studied in our study and Fuge's study.

The results of Alanen and Pohto (1977), who studied the effect of tricalcium orthophosphate added to salt as a stabilizer, seem to also support this hypothesis, and are in agreement with our findings. These investigators found that only 17% of F added was recovered when directly analyzed, while the diffusion method liberated 50 to 60% of the F. They highlighted that the bioavalilability of F in salt may be hindered by the stabilizer, anticaking or free flowing agents used. The salt samples analyzed in the present study used different stabilizers and anti-humectants; these were sodium silicoaluminate (contained in 13 brands), sodium ferrocyanide (contained in seven brands) while the following were contained in three salt brands each, lactose maltrodextrin, potassium chloride, and silicon dioxide. None of our samples had tricalcium orthophosphate, so valid comparisons cannot be made. The amounts of these compounds were not stated in labels.

On the other hand, our results are in disagreement with those of Marthaler and Sener-Zanola (1985). These researchers added different F compounds to salt obtained from Latin America, including some from Mexico, and studied the availability of F added to salt. Using the direct method, recoveries ranged from 231 to 269 F µg/g, when 300 µg/g F was added as a powder. When F was added in a humid state, 80 to 100% was recovered. They hypothesized that aluminum or other elements did not seem to form complexes with F. They used TISAB in their experiments and the high levels of CDTA used in TISAB directly intend to compensate for aluminum presence. However, these investigators did not conduct element analysis to determine which other ions were present in the samples they analyzed.

The presence of certain elements in our samples seems to further support the complex-formation hypothesis, since we found some elements that could potentially interfere with the F analyses. These were Ca, Mg, Sr, and Mn. One sample had more Ca than the amount allowed by Mexican regulations (1993). Magnesium and manganese were within permissible limits. Concentration limits of lithium and strontium were not included in the regulations. The presence of these elements could partially explain differences found between direct and diffusion methods since diffusion would free F bound to other ions, concentrating F in the trap and preventing the interference of those ions with the electrode even when not bound to F. The amount of these ions could have larger variations from sample to sample and not all of our samples were assessed for element analysis. There were only two previous investigations to which the results of element analysis could be compared. Using ICP, Herrador *et al*., (1998) analyzed 20 samples of salt reporting averages (expressed as g/kg) of 0.47 Ca, 0.48 K, 0.58 Mg, 0.016 Sr, and 0.003 Mn. Except for Mn our results were higher and this could be explained by the different origin of the sea water used to produce salt.

Finally, the results we observed when ions were added to ultra pure salt further demonstrate the effect other ions have on F analysis even when samples are buffered, as they showed that the addition of Ca to ultra pure NaCl resulted in decreased recoveries of F. Calcium, Mg and strontium could theoretically interfere with F analysis because they can form compounds with F if the sample is not sufficiently diluted. Specifically, at the 1:10 v/v dilution we used in most of our experiments and at the Ca concentration we found through element analysis, our samples would be well above the saturation of CaF_2. However, the CDTA in TISAB, or the EDTA and citrate used in the acidified samples should have bound the Ca and other metal ions and released all the F. A possible explanation for the higher values found in undiluted samples could be found by evaluating the results obtained when aliquots were taken from diluted samples and transferred to TISAB II; in these cases un-dissolved CaF_2 could have remained behind in the container and may account for the lower values found in the direct samples.

Other investigators have suggested that the different ranges of F values found in samples relate to a non-homogeneous distribution of F within packages (Estupiñán-Day, 2000; Giron-Amaya, 1999; Maupomé-Carvantes *et al*., 1995). Our results do not support this hypothesis. Samples analyzed in this investigation were collected from three different areas of the package. When concentrations were analyzed according to sampled area, this was not statistically significant. Although we observed variations in concentrations of F within the same sample, these differences were not statistically significant.

Our results seem to indicate that ashing was not appropriate for salt samples since most of the F was lost during the procedure. We included this step as part of our tested methodologies because descriptions of ashing procedures have been pointed out as an appropriate step when analyzing soil and salt samples by other researchers (Venkateswarlu, 1990). Further study is needed to determine why F is lost during ashing in the presence of salt. The negative correlation between the presence of certain elements and differences between ashed and unashed samples seems to indicate that the formation of F complexes may reduce F volatilization. Acid dissolution of samples was also suggested as a pretreatment by Venkasteswarlu (1990). This method was used to assess recoveries and it did not result in better recoveries of F than the ones reported with diffusion or the direct and TISAB II measurements. Therefore our results do not support Venkateswarlu's (1990) recommendation to ash soil and salt samples or to acid dissolve them in order to improve detection of F content when using a direct technique.

Based on our results, we conclude that the diffusion method was most effective when determining the total F content of salt. The results of these analyses should inform legislation regarding the constant monitoring of salt fluoridation programs. When deciding upon an ideal method for analysis of fluoridated salt, all aspects of the method, including cost-effectiveness and complexity of technique should be considered. It is recommended that the diffusion method should be used for the determination of total F in salt samples. The less technique sensitive constant addition direct method using TISAB II at a 1:1 dilution, where samples have not been previously diluted, and where concentrations are calculated using Gran's plot can be employed when the goal is the determination of available F, using standards prepared with the addition of ultra pure NaCl. The standardization of techniques and the understanding of the differences among techniques would facilitate the correct monitoring of salt fluoridation programs by public health policy makers.

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