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Determination of Fluoridesand Exposure of Children to Intake from Toothpaste and Mouthwash

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Abstract: Fluorides are common ingredients in toothpaste and mouthwash, due to their effect in the prevention of dental caries.However, use too much of fluoride products for oral hygiene is one of factors that may be a potentially toxic for children. The aim of this study was to determine a content of fluoride in five different types of toothpaste and five different types of mouthwashes commercially available on the Bosnian and Herzegovinian markets and based on a certain content of fluoride, to show the exposure of children to fluorides from the toothpaste and mouthwash. The fluoridecontent was determined by a simple, fast and economical potentiometric method using fluoride ion-selective electrode. The content of fluorides in the toothpaste was in the range from 0.05 to 49.60 ppm, and in the mouthwash was in the range from 0.10 to 1286 ppm. The obtained content of fluorides in samples of toothpaste and mouthwash appears to be different in comparison with the certified values. From the analyzed toothpastes, two are intended to be used by children and three by adults, the same applies to samples of mouthwash. The calculated statistical data showed that the content of fluoride in the analyzed toothpastes and mouthwashes did not have statistically significant differences in products intended for children and adults.

Keywords: Toothpaste, Mouthwash, Fluoride, Fluoride ion-selective electrode, Potentiometry.

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I. INTRODUCTION

Fluorides are micronutrients that have different effects on the human organism. From the chemical point of view, fluorine is the most electronegative and reactive element commonly found in the form of fluorides [1]. Exposure of the organism to high concentrations of fluoride over a long period can lead to negative effects on the human health, such as musculoskeletal disorders, neurological diseases, inhibition of enzyme activity [2, 3]. At low concentrations, fluorides play an important role in the prevention of dental caries, and deficiency of fluorides can lead to dental problems, especially in children [4]. Due to this fact, they are added to numerous oral hygiene products such as toothpastes and mouthwashes. Fluorides are in oral hygiene products preferably added in the form of sodium fluoride (NaF), sodium mono-fluorophosphate (Na_2FPO_3), tin fluoride (SnF_2) or in the form of different amines. Toothpastes and mouthwashes frequently contain NaF and Na_2FPO_3, where NaF is more effective in the prevention of dental caries than Na_2FPO_3. The difference in efficiency of NaF and Na_2FPO_3 can be explained by the different dissociation of these salts and the different quantities of the liberated fluoride ions. Sodium fluoride, in an aqueous solution, is completely dissociated, while Na_2FPO_3 by dissociation releases about 6% fluoride ions [5]:

$$NaF \rightarrow Na^{+} + F^{-}$$
$$Na_2FPO_3 \rightarrow 2Na^{+} + FPO_3^{2-}$$
$$FPO_3^{2-} + OH^{-} \rightarrow F^{-} + HPO_4^{2-}$$

The toothpaste and mouthwashes are not the only sources of fluoride, children intake fluorides also through food, water, and other products that contain the fluorides [6]. Babies and young children are approximately three to four times more exposed to fluorides than adults. This occurs mostly due to the use of toothpaste and mouthwash since control of swallowing in children is not sufficiently developed in comparison to adults [7]. Considering that the differences between toxic and therapeutic doses of fluorides are the small, fast and precise quantification of fluorides in products for oral hygiene is important in quality control and for

assessing the daily fluoride intake. Several analytical methods are permanently in use: electro analysis with a fluoride ion selective electrode [8], gas chromatography [9], capillary electrophoresis [10], and atomic absorption spectrometry [11]. Potentiometric determination of fluoride with a fluoride ion selective electrode is a quick and simple method, which is most commonly used. This method detects only free fluoride in the solution.

The aim of this study was to determinate fluoride in toothpastes and mouthwashes commercially available on the Bosnian and Herzegovinian market with the potentiometric method by using fluoride ion selective electrode, and to evaluate the exposure of children to fluoride from these oral hygiene products.

II. MATERIALS AND METHODS

Instrumentation

Potentiometric determination fluoride in products for oral hygiene was performed using Fluoride Ion Selective Electrode (mod. 6.0502.150, Metrohm, Switzerland) connected to reference electrode Ag/AgCl (3 M KCl, mod. 6.0750.100, Metrohm, Switzerland) and the electrodes were attached to a read-out device HANNA potentiometer (USA). Wherever it was possible, glassware has been replaced by polyethylene labware to decrease influence fluorides ion. For stirring the solution during the measurements, a magnetic stirrer hotplate was used (IKA, ika-3622001, Ontario, U.S.). The pH values were measured using pH-meter METTLER TOLEDO (FE20 FiveEasy, Switzerland).

Chemicals and reagents

Sodium fluoride was purchased from Kemica (Zagreb, Croatia) all other reagents and chemicals were obtained from Sigma-Aldrich Co. LLC. All chemicals were p.a. grade. For the preparation of all solutions, and measurements triply distilled water was used. The NaF used to prepare the stock solution (100 mg/L) was previously dried at 110^{o} C. The stock solution was used to prepare diluted solutions (1-100 mg/L). Total Ionic Strength Adjustment Buffer (TISAB) solutions are used for potentiometric measurements. The TISAB solution was prepared by mixing 0.15 g sodium citrate, 29 g NaCl and 28.5 mL acetic acid with 250 mL of triple distilled water. Then NaOH (5 M) was added until pH was 5.17 and the volume was adjusted to 0.5 L with triply distilled water. The prepared buffer solutions were stored in a plastic bottle until use. Potentiometric measurement is performed by a mixing: 15 mL of TISAB solution with 15 mL of sample.

Samples

Different toothpaste and mouthwash, commercially obtainable from Bosnianand Herzegovinianmarkets, produced by different manufacturers such as Germany, Italy, Poland, Russia, Switzerland, France, and the UK were used in this study. Fluorides were determined in five samples of tubes of toothpaste and five samples of mouthwash: $\mathbf{T_1}$ – mouthwash for children (UK); $\mathbf{T_2}$ – mouthwash for children (UK); $\mathbf{T_3}$- mouthwash (Italy); $\mathbf{T_4}$-mouthwash (Switzerland) and $\mathbf{T_5}$ – mouthwash (Germany).$\mathbf{P_1}$- toothpaste for children (Germany); $\mathbf{P_2}$- toothpaste for children (France); $\mathbf{P_3}$- toothpaste (UK); $\mathbf{P_4}$- toothpaste (Poland); $\mathbf{P_5}$ – toothpaste (Russia). The samples were collected randomly, and selected samples including the most readily available and common ones.

Sample preparation

The sample of the toothpaste (0.50 g) is dissolved in triply distilled water using a magnetic stirrer, then quantitatively transferred to a 50 mL volumetric flask and filled with triply distilled to the mark. To speed up the dissolution of the sample, before adding triply distilled water to the mark, we heat it up for 10 minutes using a water bath at 40^{o}C. After cooling, 5 drops of propanol are added to reduce the formation of the foam. The mouthwash sample is transferred (5 mL) to a 50 mL volumetric flask, 5 drops of propanol were added, and then filled with triple distilled water to the mark [12].

Potentiometric measurements

The standard solution or sample solution (15 mL) was transferred into a plastic process vessels and 15 ml of TISAB buffer solution then added. During the measurement, the solution is constantly mixed with the magnet and magnetic stirrer. After each change of the solution, the electrode is washed with triply distilled water. Solutions are mixed for 2 minutes, after which the voltage is measured. Each sample was analyzed at a minimum in triplicate. The concentration of the analyte (sample) is determined using a calibration curve, which is constructed on the basis of standard solutions (1-100 mg/L).

Mathematical-statistical data processing

The measurement results are displayed as the values of ppm fluorides in five paste samples and five mouthwash samples, with standard deviation (*SD*), the standard mean error (*SEM*) as a variation indicator within

the group. For the evaluation of statistical significance (*p*) difference in the measured mean values in two groups the unpaired *t*-test was used. SigmaPlot 11 computer program was used for data processing.

III. RESULTS AND DISCUSSION

The contentof fluoride determined by the potentiometric method with ion-selective electrode is shown in **Table 1**. The results of the measurements are presented as ppm fluoride in five toothpaste samples (P_1-P_5) and five mouthwash samples (T_1-T_5). The content of fluoridewas obtained on the basis ofthreemeasurements for each sample.

Table 1: Content of fluoride in toothpaste and mouthwash determined by potentiometric method using fluoride ion selective electrode.

Sample	Content of fluoride /0.5 g	E (mV)	Content of fluoride (ppm)
P_1	32.15	62.33	1286
P_2	9.70	91.90	388
P_3	35.39	59.97	1416
P_4	30.28	63.80	1211
P_5	0.05	222.63	2.00
T_1	46.35	53.30	185.4
T_2	50.68	51.10	203
T_3	0.014	254.47	0.10
T_4	49.60	51.63	198.4
T_5	34.90	60.30	241.2

The content of fluoride in samples of toothpaste was in the range from 2.00 to 1416 ppm, and in mouthwash was in the range from 0.10 to 241.2 ppm. The highest content of fluoride was detectedin the toothpaste P_3 produced in England (UK), and in mouthwash T_5 produced in Germany. The lowest content of fluoride was detected in the toothpaste P_5 produced in Russia, which according to the declaration should not have fluoride at all, and in mouthwash T_3 produced in Italy.

Taking into account fluoride content in samples specified by the manufacturer, a standard linear calibration curve was constructed with a concentration range of fluoridefrom 1 to 100 ppm (**Fig. 1**). The calibration was repeated three times, yielding a linear dependence (R^2= 0.9998). In order to assess the sensitivity of the method, the limit of detection (LOD) and the limit of quantification (LOQ) were determined. The LOQ and LOD were calculated from equations [13]:

$$LOQ = \frac{10 \cdot s}{m}; \quad LOD = \frac{3 \cdot s}{m}$$

where*s* is the standard deviation of electrode response (n=3) at 1 ppmfluoridesand *m* is the slope of the calibration curve (**Fig. 1**).The value for LOD is 0.037 mol/L, and LOQ is 0.12 mol/L.

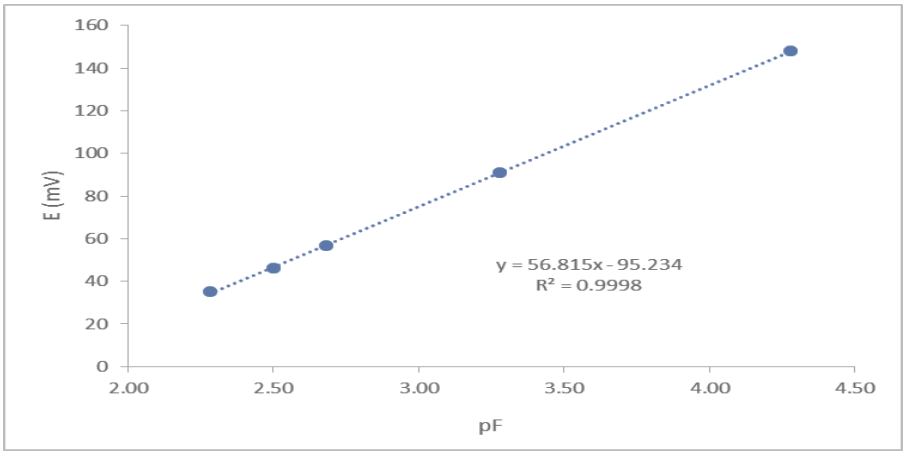

Fig.1: Calibration curves of potentiometric method (with Fluoride Ion Selective Electrode) for the determination of fluoride.

The content of fluoride in toothpaste and mouthwash determined in this research is in agreement with the previous study reported by Švarc-Gajić et al. [12]. Studies have shown that deviations occur between the experimentally determined content of fluoride and the content of fluoride specified on the declaration. Similarly, our results showed that there are deviations between experimentally determined content fluoride and content specified on the declaration as shown in **Table 2**.

Table 2: Fluoride content certified and calculated in toothpaste and mouthwash.

Sample	Certified contentfluoride (ppm)	Calculated content fluoride (ppm)	Recovery (%)
P_1	1450	1286	88.70
P_2	500	388	77.60
P_3	1400	1416	101.14
P_4	1450	1211	83.52
P_5	0	2.00	-
T_1	220	185.4	84.27
T_2	225	203	90.22
T_3	45	0.1	-
T_4	225	198.4	88.18
T_5	225	241.2	107.2

Based on the values shown in **Table 2**, it can be concluded that the highest deviation is shown by the toothpaste P_2 and the mouthwash T_3.

In their research, Ellwood et al. establish that there are middle doses of toothpaste and mouthwash for children [14]. Based on the indicated middle dosing of the toothpaste and mouthwashes, in **Table 3** and **Table 4** is shown the daily fluoride exposure of children.

Table 3: Daily exposure of childrento fluoride through different commercial available toothpastes

Doses of toothpaste - mass of toothpaste (g)	Exposure of fluoride for one brushing per day (mg)	Exposure of fluoride for two brushing per day (mg)
Thin smudge of toothpaste (**0.125 g)**	P_1 0.161 P_2 0.049 P_3 0.177 P_4 0.151 P_5 $2.5 *10^{-5}$	P_1 0.322 P_2 0.098 P_3 0.354 P_4 0.302 P_5 $5*10^{-5}$
Small pea of toothpaste (**0.25 g)**	P_1 0.322 P_2 0.098 P_3 0.354 P_4 0.302 P_5 $5 *10^{-5}$	P_1 0.644 P_2 0.196 P_3 0.708 P_4 0.604 P_5 $10*10^{-5}$
Half of the brush head (**0.5 g)**	P_1 0.644 P_2 0.196 P_3 0.708 P_4 0.604 P_5 $1 *10^{-4}$	P_1 1.288 P_2 0.392 P_3 1.416 P_4 1.208 P_5 $2 * 10^{-4}$
Full brush head (**1.0 g)**	P_1 1.288 P_2 0.392 P_3 1.416 P_4 1.208 P_5 $2 * 10^{-4}$	P_1 2.576 P_2 0.784 P_3 2.832 P_4 2.416 P_5 $4* 10^{-4}$
Total full brush head (**1.50 g)**	P_1 1.932 P_2 0.588 P_3 2.124 P_4 1.812 P_5 $3 *10^{-4}$	P_1 3.864 P_2 1.176 P_3 4.248 P_4 3.624 P_5 $6*10^{-4}$

Table 4: Daily exposure of children to fluoride through different commercial available mouthwash

Doses of mouthwash - volume of mouthwash (mL)	For one aplication per day (mg)	For two aplication per day (mg)
10	T_1 1.85	T_1 3.71
	T_2 2.03	T_2 4.06
	T_3 0.001	T_3 0.002
	T_4 1.98	T_4 3.97
	T_5 2.41	T_5 4.82
20	T_1 3.71	T_1 7.42
	T_2 4.06	T_2 8.12
	T_3 0.002	T_3 0.004
	T_4 3.97	T_4 7.94
	T_5 4.82	T_5 9.64
30	T_1 5.56	T_1 11.12
	T_2 6.09	T_2 12.18
	T_3 0.003	T_3 0.006
	T_4 5.94	T_4 11.88
	T_5 7.23	T_5 14.46

Based on the contents of fluoride shown in **Tables 3** and **4**, the exposure of children to fluoride from the toothpaste can be estimated. The mentioned exposure can be estimated based on the dose applicated and the content of fluorides in the toothpastes and mouthwashes.

The obtained values of fluoride in five toothpaste and five mouthwashes were grouped on: **1. group** with two toothpastes for children, **2. group** with three toothpastes for adults, **3. group** with two mouthwashes for children and **4. group** with three mouthwashes for adults.

The average content of the fluoride of **1.group** was 836.84 ± 183.57 ppm and **2. group** was 876.06 ± 220.50 ppm, ($p>0.05$) "non-significant".

The average content of the fluoride of **3.group** was 194.06 ± 3.92 ppm and **4. group** was 146.35 ± 37.08 ppm, ($p>0.05$) "non-significant".

The results of statistical study were shown that content of fluoride into toothpaste and mouthwash do not have an excessive deviation of fluoride in products intended for children and adults.However, amount of fluoride from the toothpaste in systemic circulation depends on the amount of swallowed paste, and therefore the eventual phenomenon of toxicity is dependent to proper oral hygiene.Considering that fluoride from the toothpastes can be intake in the body up to 80% of the total daily intake of fluorides it can conclude that the only risky group of consumers are children, because they do not have developed reflex of swallowing. In this manner can occur toxic effects in children [15].The optimal daily intake of fluoride is 0.05 - 0.07 mg fluoride per kilogram of body weight [16]. Therefore were children are 3-4 times more exposed to fluorides than adults, because children can swallow up to 40% of the toothpaste. So, oral hygiene should necessarily be supervised up until the seventh year of the children [15].

IV. CONCLUSION

The content of fluoride in toothpaste and mouthwashes commercially available on the Bosnian and Herzegovinian market was determined with the potentiometric method using a fluoride ion selective electrode. From the results obtained from this study, it was concluded that there are deviations in the declared and experimentally determined content of fluoride. The largest deviation in the content fluoride was determined in the mouthwash produced in Italy and the toothpaste produced in France. Since children have a poorly developed swallowing reflex, they are three to four times more exposed to fluoride than adults. Therefore control of oral hygiene from parents is necessarily recommended until the age of seven. Statistical data shows that analyzed toothpaste and analyzed mouthwash do not have a significant difference in the content of fluoride in products intended for children and adults. In the analyzed samples of toothpaste and mouthwash, both intended for children and those intended for adults, a content of fluoride that have been obtained are not potentially toxic at the recommended daily doses.

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