Determination of manganese in human teeth by flame atomic absorption spectrometry.

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Abstract

Flame atomic absorption spectrometry was applied to the determination of manganese in the whole human teeth .Combination of calcium with phosphate may cause low results in the determination of manganese by atomic absorption spectroscopy .Maximum depression of manganese absorbance is noted at mole ratio of phosphate to calcium of (3:1),correct values are obtained by incorporating (2000µg/ml) lanthanum chloride in the solution .Human teeth samples are digested with a mixture (3:1)of conc. nitric and perchloric acids. Blank absorbance value (nitric and perchloric acid mixture)should be checked from time to time .The absolute sensitivity of the method (0.126µg/ml of manganese)permits the determination of manganese in tooth material at microgram per gram concentration levels with precision of 3.8 percent. Detection limit are found to be (0.078 µg /ml of manganese).Rapid determinations of manganese at concentration down to 0.05µg/ml are possible.

الخلاصة

في البحث الحالي تم اعتماد تقنية الامتصاص الذري أللهبي لتقدير محتوى عنصر المنغنيز في الأسنان البشرية ، تم دراسة تأثير وجود فوسفات الكالسيوم(المكون الأساسي لمنشأ الأسنان) في إشارة الإمتصاص الذري للمنغنيز حيث وجد أن أقصى إنخفاض في الإمتصاصية هو عند وجود الفوسفات / الكالسيوم بنسبة (1:3) . استخدم محلول (2000مايكرو غرام/مل) من كلوريد اللانثانم لإزالة هذه التداخلات . تم إذابة و هضم نماذج الأسنان البشرية بإستخدام مزيج (1:3) من حامض النتريك / حامض البيركلوريك. تميزت الطريقة بكونها سهلة و سريعة ،كذلك تميزت بأنها ذات حساسية عالية (0.126مايكروغرام/مل) و حد كشف واطيء(0.078 مايكروغرام/مل) فضلا عن الدقة العالية في التحليل اذ بلغت نسبة الاسترجاع المئوية (97.±2.5) لكميات معلومة التركيز من عنصر المنغنيز مضافة الى محاليل من نماذج الاسنان . اجريت دراسة احصائية لنماذج من الاسنان السليمة و الاسنان المسوسة التي جمعت من اماكن مختلفة في محافظة البصرة و بمختلف الفئات العمرية لكلا الجنسين و ايجاد العلاقة بين هذه العوامل و محتوى عنصر المنغنيز فيها.

Introduction

It has been recognized that the occurrence of dental caries might be correlated with the concentration and distribution of certain trace elements in dental enamel and dentine (Awad and Abdulsahib 2004). Manganese is one of the most interesting elements ,because of its physiological role in teeth .Manganese may replace calcium in apatite (Langmyhr and Lind 1975) and increasing resistance to caries .This assumption has stimulated research into the determination of the distribution of manganese in whole human teeth .

Methods which have been applied to the determination of manganese are atomic absorption (Cook1997, Yuzefovsky etal 1997),positron emission tomography (Kim etal 1999), flame photometry (Alves etal 2000) , neutron activation (Arnold etal 2000), gas chromatography (Ombeba and Barry 1994), inductive coupled plasma (Headly etal 1996), laser techniques (Stchur and Michel 2002), anodic stripping voltammetry (O'Halloran 1982), and colorimetry (Rao etal 1993)].For field studies ,it is necessary to have a method which is rapid ,sensitive ,reliable and inexpensive .This has been accomplished by use of flame atomic absorption spectrometry .

Relatively little data are to be found regarding concentrations of manganese in the dental tissues .Increased on the outer surface with a decrease in the sub-surface layers of enamel in permanent teeth have been reported .(Brudevold etal 1960) give concentrations ranging 20ppm-5 ppm from outer to inner layer .Nixon etal (1966) report that concentrations ranging from 0.34-2.01 ppm were found again with higher concentrations of manganese on the outer enamel layer . A mean value of 0.54 ppm has been given by Soremark and Samsahl (1961) and 0.25 ppm by Battistone etal (1967).There are few figures available for concentrations in dentine . A mean concentration of 0.19 ppm ,(Soremark and Samsahl 1962) and 0.78 ppm (Langmyhr and Lind 1975)are reported .Putinam etal (1965) gives a range of 1-10 ppm manganese in dental plaque .

In this paper flame atomic absorption spectrophotometric determination of manganese in dental material was described ; the analysis was based on the digestion of teeth samples in nitric acid /perchloric acid mixtures .Lanthanum chloride was used as matrix modifier to remove the interferences effects of teeth matrix . The feasibility of this technique was demonstrated by analyzing 75 whole erupted and unerupted human teeth .

Experimental

Apparatus:

A shimadzu flame atomic absorption spectrophotometer model (AA-630-12)was used with an air-acetylene burner (10 cm) was used for air-acetylene flame (air 20 L/min, acetylene 4.5 L/min) .Instrument settings were :lamp current ,10 mA ;wave length ,279.5 nm ;slit width,0.2 nm . These conditions were maintained constant throughout the measurements.

Reagents:

All chemicals used were of analytical –reagent grade .The nitric acid was of "suprapur" quality (Merck) and (60%w/v)perchloric acid"analar" (Merck)was used .

Manganese standard solution (1000ppm) were prepared by dissolving the proper amount of high purity manganese metal in a small excess of (1:1) nitric acid .

Calcium and phosphate solutions used in the interference studies were prepared from calcium chloride and orthophosphoric acid by dissolving appropriate weights in deionized water .

Preparation of samples

Erupted ,unerupted human teeth were collected from different area in Basrah city .The teeth were cleaned carefully by scraping (with plastic equipment) and brushing ,air dried over night at 120±10C^{o} in an air oven .Weigh the dried samples accurately into a Pyrex 50 ml conical flask ,add 2 ml of concentrated nitric and 6 ml of perchloric acid. Place a reflux condenser in the flask and digest the mixture on a hot-plate until dense white fumes of perchloric acid and nitric acid evolved .Remove the reflux condenser and evaporate the contents of the flask to incipient dryness.

Procedure:

To the cooled flask in above step add 10 ml of lanthanum chloride solution to give a concentration of 2000µg/ml of lanthanum .Place the reflux condenser and boil the mixture for 5 minutes .Transfer the digest to a 50 ml graduated flask and dilute it to the mark with deionized water .Determine the manganese atomic absorption signal .

Standard addition method; aspirate the solutions containing above treated sample plus added manganese concentration of 0 ,0.25,0.50,0.75, 1.00, and 1.25 µg/ml, in the air –acetylene flame . Extrapolation of the curve of absorbance against “added” manganese concentration obtains the level of manganese in sample solutions.

Statistical analysis:

The statistical analysis was carried out using two –way analysis of variance with unbalanced repeated measurements .Statistical significance between individual time points was made by using Revised Least Significant Difference (RLSD) test. The probability level for significance was 5% less.

Results and Discussion

Interference studies*:*

The effect of teeth matrix which was consists essentially of $Ca_{10}(PO_4)_6(OH)_2$(Awad and Abdulsahib 2002) on absorbance of manganese solution containing(2.0 ppm)was investigated by adding calcium and phosphate separately ,in the amounts likely to be found in samples of teeth ,the addition of combinations of calcium and phosphate was therefore studied in more detail :by examining the effect of increasing amounts of calcium (as phosphate)on the absorbance of manganese (fig.1); and by examining the effect of increasing amounts of calcium (as chloride)in combination with increasing amounts of phosphate(as phosphoric acid) on absorbance of manganese (fig.2).Fig.1 shows that the absorbance of manganese in the flame was progressively reduced by increasing concentrations of calcium phosphate in the solution .When calcium present as the chloride, the mole ratio of phosphate to calcium at which a decrease in absorbance of manganese commenced was dependent on the phosphate concentration; maximum depression occurred when the mole ratio was(3:1) (fig. 2) .The observed effect of calcium phosphate on the absorbance of manganese example of a reduction in the vaporization rate of the solute by occlusion of the analyte in matrix , which is incompletely volatilized in the flame , it is therefore concluded that the best way of overcoming interference from alkaline earth phosphate in the acetylene-air flame is by addition of an easily volatile solute to the solution.

At the levels used in the above study, the effect of combinations of calcium phosphate on the absorbance of manganese was completely eliminated by addition(2000 ppm)of lanthanum(as chloride). Table(1) shows the effect of lanthanum on manganese atomic absorption signal which was recorded for teeth samples with and without treatment with lanthanum.

Calibration:

The representative –modified, direct calibration and standard addition calibration curves were used for solution containing up to (0.50 µg/ml) manganese. In both sets parallel calibrations appears which indicate freedom from interelement effects,either graph could be used for the determination of manganese using the absorbance measurement.Table (2) demonstrate the obtained results using both methods.

Accuracy and precision:

The reproducibility of the method was tested by carrying out 7replicates analysis on 10 ml aliquots of teeth sample; these showed an average manganese concentration of 0.66 ± 0.032µg/gm, i.e. a coefficient of variation of 1.3 % .In order to test the efficiency of recovery of manganese from teeth samples ,a 10 ml aliquots of teeth samples, from which calcium phosphate effect had been removed by addition lanthanum chloride were spiked with known amounts of manganese and then analyzed .The results of these experiments, which are summarized in table (3),indicate that the over all recovery in the analytical process is 97.0± 2.5%

Absolute sensitivity and Detection Limit:

The sensitivity (1 per cent. Absorption) and detection limit were calculated from the average slope of the calibration graph and standard deviation of the blank for 10 ml of sample solution containing0.20µg/ml of manganese, the calculated values were 0.126µg/ml and 0.078µg/ml, respectively .

Analysis of samples:

The manganese content was determined in the human teeth samples, collected from persons (men and women) living in different areas (city centre and outskirts) of Basrah city with age groups (7-60)years. The obtained results are shown in table (4) together with the literature values, which was obtained by using different analytical methods.

Fig.(3) illustrates the variation in the concentration of manganese in human teeth as a function of geographical positions. Statistically significant differences($P<0.001$) were observed between person living in city centre and others who living in outskirts for concentration of manganese .Geographical influences are thought to be the main source of variability. The observed variations are probably a reflection of the varying levels present in foods, that are generally dependent on geochemical environment in which they are living .Environmental contamination can also be a source of manganese in human teeth.

For manganese content of teeth samples as a function of sex (fig.3),however, no significant difference was shown ($P<0.05$).The means ($x\pm s.d$) were (0.727±0.135)µg/gm and (0.793±0.157) µg/gm from men and women respectively. This data indicate that there was no influence from the sexes of groups on manganese content of teeth.

Statistically significant difference between age groups were seen in the mean value of manganese concentrations in human teeth($P<0.01$) as shown in fig.4.The concentration of manganese increased with age and this concentration remained nearly constant from third(21-30years) to sixth(51-60 years) age groups of persons. The differences may be due to the exposure of manganese and other factors such as differences in diet and drinking waters.

On the other hand ,it is found out that there is a statistical relationship between the manganese content in teeth and caries (fig.5),since the concentration of manganese are found to be (0.316µg/gm) in intact teeth,(0.697µg/gm) in the mottles teeth, and (0.964µg/gm) in the caries teeth. This is a scribed to the physiological role of manganese which was replace calcium in apatite and dissociated so easily causing the prevalence of dental caries.

Conclusion

Atomic absorption employing the flame atomization technique provides a simple, sensitive and rapid method for the determination of manganese in human teeth at micro gram per gram levels. .

For teeth samples, correct values are obtained by incorporating lanthanum chloride in the solution ,since results in a greater dispersion of the involatile calcium phosphate phase and an increase in its rate of volatilization.

The method described above is quantitative and requires no prior separations .The precision of the method of about3.8 percent is satisfactory for most applications in dental caries research.

Table (1):Effect of (2000 µg/ml)lanthanum chloride on manganese atomic absorption signal in teeth samples.

Teeth samples No.	Mean of absorbance	
	With lanthanum chloride	Without lanthanum chloride
1	0.58	0.28
2	0.77	0.15
3	0.84	0.40
4	0.91	0.36
5	0.29	0.07

Table(2)Mn content (µg/gm , dry weight) of human teeth and relative standard deviation(7 replicates).

Samples	Teeth dry weight(gm)	Proposed method		Std. addition method	
		Mn content		Mn content	
		µg/gm	RSD%	µg/gm	RSD %
A	0.66	0.73	3.11	0.80	2.56
B	0.81	0.62	1.41	0.67	3.83
C	1.02	0.97	2.52	1.03	2.13
D	0.53	1.14	3.25	1.04	4.47
E	1.21	1.65	1.11	1.53	3.46

Table(3): Recovary percentage of the direct method of human teeth samples.

Sample No.	Mn content (µg/ml)	Mn added (µg)	Mn found (µg)	Recovary (%)
I	0.682	0.50	1.158	98.8
		1.00	1.63.1	97.0
		1.50	2.160	99.0
II	0.774	0.50	1.230	96.6
		1.00	1.765	99.5
		1.50	2.210	97.2
III	1.097	0.50	1.540	96.4
		1.00	1.981	94.5
		1.50	2.462	94.8

Table(4): The content (μg/gm , dry weight) of human teeth in comparison with other studies.

Mn content (ppm)	Method	Reference
10-100	Mass spectrometry	Hardwick and Martin(1967)
1-10	X-ray spectroscopy	Putinam etal(1965)
0.34-2.01	Activation analysis	Nixon etal(1966)
0.25-1.00	Activation analysis	Battistone etal(1967)
0.78	Atomic absorption spectrometry	Langmyhr and Lind(1975)
0.54±0.08	Activation analysis	Soremark and Samsahl(1961)
0.19±0.06	Activation analysis	Soremark and Samsahl(1962)
0.62-1.65	Flame AAS	Present study

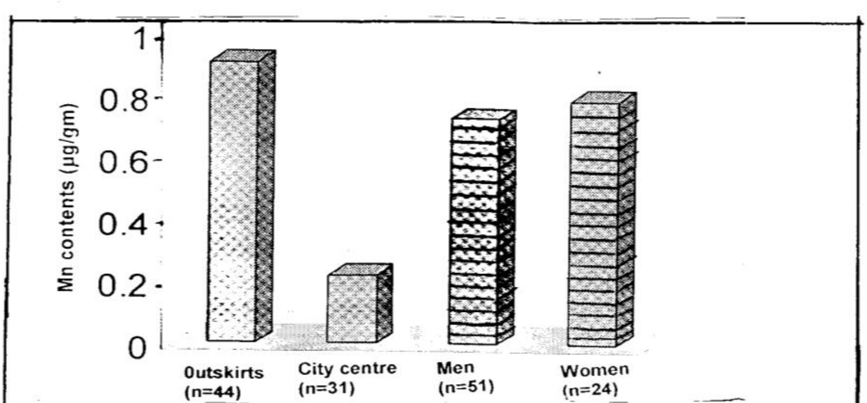

Fig.(3):Mn contents (μg/gm,dry weight) of human teeth.

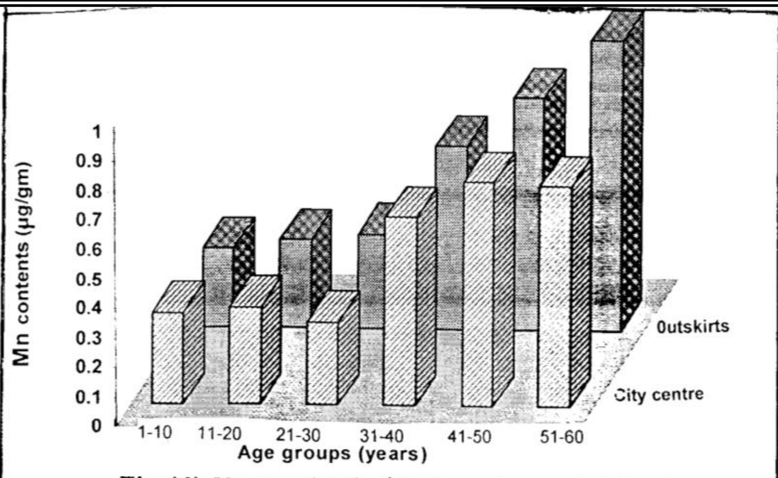

Fig.(4):Mn contents (µg/gm,dry weight) of human teeth as a function of age groups.

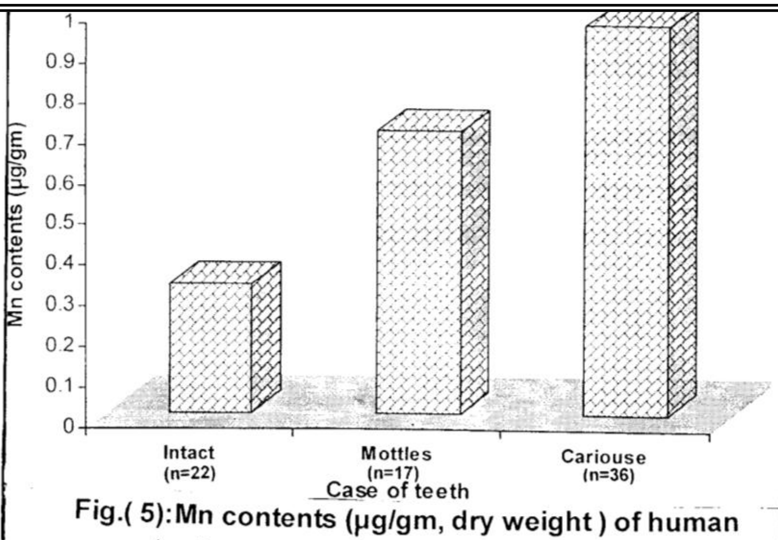

Fig.(5):Mn contents (µg/gm, dry weight) of human teeth as a function of the case of teeth .

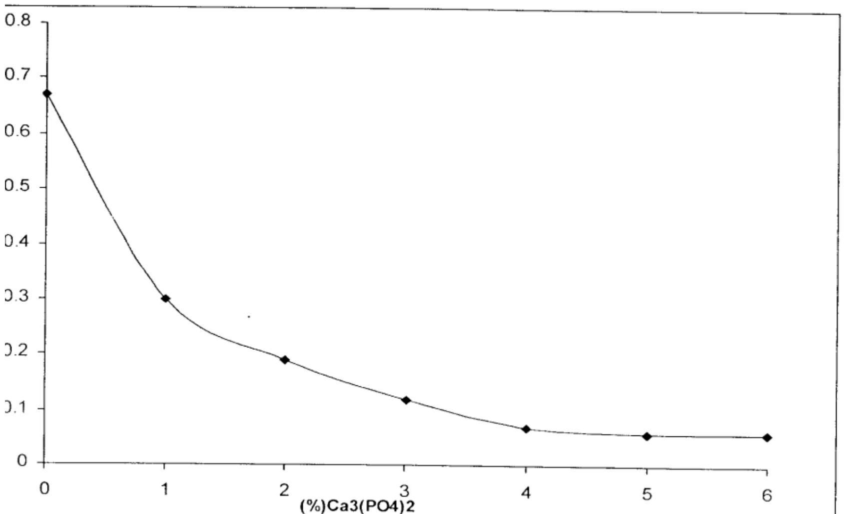

Fig.1.Effect of calcium phosphate on absorbance of(2.0ppm) manganese.

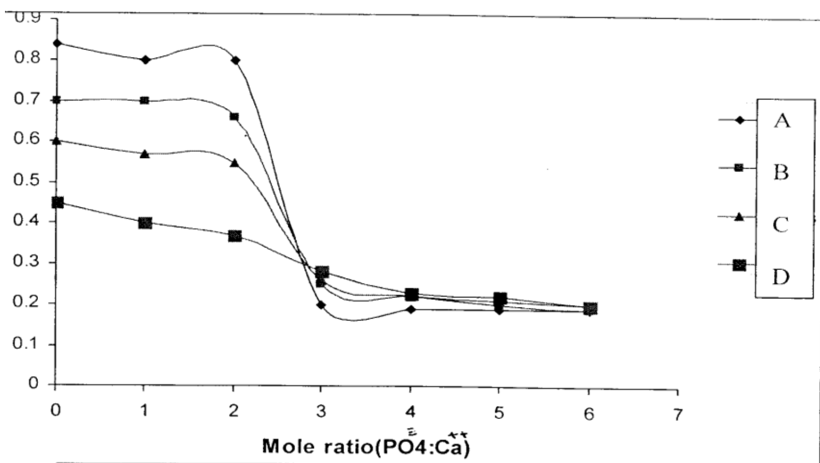

Fig.2 Effect of phosphate (as phosphoric acid):calcium(as chloride) ratio on the absorbance of(2.0ppm)manganese. calcium Concentrations (ppm):A,5000;B,7000;C,10000;D,15000.

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