# Flow injection on-line dilution for zinc determination in human saliva with electrothermal atomic absorption spectrometry detection

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## ARTICLE INFO

## ABSTRACT

An automated method is described for the determination of zinc in human saliva by electrothermal atomic absorption spectrometry (ET AAS) after on-line dilution of samples with a significant reduction of sample consumption per analysis (<0.4 mL including the dead volume of the system). In order to fulfill this aim without changing the sample transport conduits during the experiments, a flow injection (FI) dilution system was constructed. Its principal parts are: one propulsion device (peristaltic pump, PP) for either samples, standards or washing solution all located in an autosampler tray and for the surfactant solution (Triton X-100) used as diluent, and a two-position time based solenoid injector ($TBSI_1$) which allowed the introduction of 10 μL of either solution in the diluent stream. To avoid unnecessary waste of samples, the $TBSI_1$ also permitted the recirculation of the solutions to their respective autosampler cups. The downstream diluted solution fills a home made sampling arm assembly. The sequential deposition of 20 μL aliquots of samples or standards on the graphite tube platform was carried out by air displacement with a similar time based solenoid injector ($TBSI_2$). The dilution procedure and the injection of solutions into the atomizer are computer controlled and synchronized with the operation of the temperature program. Samples or standards solutions were submitted to two drying steps (at 90 and 130 °C), followed by pyrolysis and atomization at 700 and 1700 °C, respectively. The aqueous calibration was linear up to 120.0 μg L$^{-1}$ for diluted standard solutions/samples and its slope was similar ($p > 0.05$) to the standard addition curve, indicating lack of matrix effect. The precision tested by repeated analysis of real saliva samples was less than 3% and the detection limit ($3\sigma$) was of 0.35 μg L$^{-1}$. To test the accuracy of the proposed procedure, recovery tests were performed, obtaining mean recovery of added zinc of $97.8 \pm 1.3\%$. Furthermore, Zn values estimated by the procedure developed in this work were compared with those obtained by a standard addition flame-AAS method applied to 20 randomly selected saliva samples. No significant differences ($p > 0.05$) were obtained between the two methods. Zinc levels in saliva samples from 44 healthy volunteers, 15 male and 29 female, with ages between 20 and 51 years (mean $30.50 \pm 9.14$ years) were in the range 22–98 μg L$^{-1}$ (mean of $55 \pm 17$ μg L$^{-1}$), similar to some and different from others reported in the literature. It was found that zinc values for male were statistically higher ($p = 0.006$) than for female.

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# 1. Introduction

Whole saliva is a unique and complex secretion, containing a mixture of oral fluids and constituents of non-salivary origin, with numerous functions in the oral cavity [1]. Water represents 99% of its composition and the remaining 1% consist of organic molecules, electrolytes and oligoelements. It is well known that the composition of saliva depends on a number of factors related to physiological, pathological and environmental changes. The fluid composition may differ on account of varying contribution rate and quality from the individual glands, the presence or absence of a stimulatory source, the type of food ingested, the circadian cycles and the presence of infections [1,2]. Monitoring one or various salivary parameters, one can lead to the cause of any baseline levels impairment.

Zinc is an essential trace element which is present in small amounts in all tissues and body fluids, including saliva. Several diseases that affect the oral cavity have been shown to be related to changes in salivary zinc concentration: incidence of dental caries [3], periodontal diseases [4], abnormal test acuity [5], etc. Therefore, knowledge about concentrations of zinc in saliva has become increasingly important. Estimation of baseline salivary zinc concentrations is sensitively carried out by inductively coupled plasma-mass spectrometry (ICP-MS) [5,6] or atomic absorption spectrometry (AAS) with electrothermal atomization (ET AAS) [7,8], as conventional flame-AAS proves impractical: (1) because zinc values are below or near the detection limit of the instrument, which makes necessary the use of the standard addition method, thus increasing the time of analysis and sample handling, and (2) due to a high sample consumption (see below).

Given its high selectivity and sensitivity, its wide availability in analytical and clinical laboratories, as well as its relatively low cost and sample economy, ET AAS is the method of choice for the routine analysis of biological samples. However, there are two major problems for its application to the determination of zinc: (i) the achievement of a too high sensitivity (less than 0.3 pg of zinc for 1% absorption), and (ii) the great risk of contamination due to excessive sample handling and/or addition of reagents, as zinc is an over-abundant element in the environment.

There are different means of decreasing the sensitivity in ET AAS: the use of an alternative atomic line or the dilution of samples with water or any appropriate reagent. The alternative zinc line at 307.6 nm has about 1000-fold less sensitivity than its resonant line at 213.9 nm, which makes it too insensitive for the purpose of this work [7,8]. An adequate dilution scheme with water or any reagent looks suitable to bring samples concentrations within the instrumental working range and also to reduce matrix interferences which might occur with concentrated samples. The use of mineral acids and oxidizing agents to digest the samples, and also of chemical modifiers to correct the background should be avoided as it proved inappropriate for zinc analysis due to impaired blank signals which lead to inaccurate results [9]. The dilution procedure with pure reagents or water fulfills the requirements mentioned above and it is advantageously facilitated by coupling the detection system with a flow injection (FI) manifold

which reduces: the analysis time, the number of preparatory steps and the sample volume needed for each analysis.

Up to date, only spectrometric techniques with continuous detecting systems like flame-AAS [10–18], ICP-MS [19–21], spectrophotometry [22–25] and fluorimetry [26] have been coupled with FI manifolds for on-line dilution. Generally, the purpose was to extend the analytical dynamic range [10,12,14,17,26], to increase sample throughput and precision and to reduce the sample pretreatment steps, thus minimizing the risk of contamination [11,13,16,18,19,22,23].

In such systems, the on-line dilution was achieved in manifolds based on different principles, like zone penetration [11], dispersion of micro-volumes [12,14,18] and zone sampling [26], implementation of the cascade method [16,22] or using different devises, like recirculating loops [10,19], dialysis units [24], dilution chambers [16,17,25], binary inlet static mixers [23], etc. Each of these approaches have their drawbacks: some manifolds are too complex to be implemented in other laboratories, the precision is impaired by the peristaltic pumps fluctuations at large flow rates and by the generation of air bubbles in the flow streams, the dialysis membranes or the manifold tubing are fragile for long-term usage, etc.

Coupling a continuous flowing system with the discontinuous ET AAS detection is guaranteed by the characteristic precise timing of FI and by the development of an adequate interface to perfectly synchronize the injection of the desired flowing segment into the atomizer [27–30]. Our experience in the development of such systems allows us to develop a FI on-line dilution manifold with ET AAS detection for the contamination-free determination of zinc in whole, unstimulated saliva samples. Such a system will allow us to achieve the main purposes of this study: (i) bring zinc concentrations of samples within the instrumental working range, (ii) there is a significant reduction of sample and reagent consumption, (iii) reduce sampling handling, (iv) avoid samples digestion, (v) avoid the use of chemical modification, and (vi) demonstrate the robustness of the experimental arrangement and make complete automation of the analysis possible.

# 2. Experimental

## 2.1. Reagents

All the chemicals used in this study were of analytical-reagent grade or higher. Only high-purity de-ionized and distilled water (DDW) with specific conductance <0.1 μS cm$^{-1}$, obtained in a Milli-Q system (Millipore, Bedford, Massachusetts, USA) and frequently tested to be zinc-free, was used in the experiments. A zinc stock solution of 1000 mg L$^{-1}$ (Titrisol, Merck) was used to prepare daily standards. The carrier (diluent) solution was prepared by diluting Triton X-100 (Merck) in water to obtain a concentration of 0.02% (v/v). At this surfactant concentration no traces of zinc were detected. All standards were stored in appropriate polystyrene screw-cup containers soaked overnight in 2% (w/v) EDTA and then thoroughly washed with several portions of zinc-free water to remove any traces of zinc. Argon gas of 99.99% purity served as purge gas for the graphite furnace. The only glassware employed in this