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Monitoring glucose, calcium, and magnesium levels in saliva as a non-invasive analysis by sequential injection multi-parametric determination

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ABSTRACT

The use of saliva for diagnose and surveillance of systemic illnesses, and general health has been arousing great interest worldwide, emerging as a highly desirable goal in healthcare. The collection is non-invasive, stress-free, inexpensive, and simple representing a major asset. Glucose, calcium, and magnesium concentration are three major parameters evaluated in clinical context due to their essential role in a wide range of biochemical reactions, and consequently many health disorders. In this work, a spectrophotometric sequential injection method is described for the fast screening of glucose, calcium, and magnesium in saliva samples. The glucose determination reaction involves the oxidation of the aldehyde functional group present in glucose with simultaneous reduction of 3,5-dinitrosalicylic acid (DNS) to 3-amino, 5-nitrosalicylic acid under alkaline conditions, followed by the development of colour. The determination of both metals is based on their reaction with cresolphtalein complexone (CPC), and the interference of calcium in the magnesium determination minimized by ethylene glycol-bis[β-aminoethyl ether]-N,N,N′,N′-tetraacetic acid (EGTA). The developed multi-parametric method enabled dynamic ranges of 50 – 300 mg/dL for glucose, 0.1 – 2 mg/dL for calcium, and 0.1 – 0.5 mg/dL for magnesium. Determination rates of 28, 60, 52 h^{-1} were achieved for glucose, calcium, and magnesium, respectively. Less than 300 µL of saliva is required for the multi-parametric determination due to saliva viscosity and inherent necessity of dilution prior to analysis. RSDs lower than 5% were obtained, and the results agreed with those obtained by reference methods, while recovery tests confirmed its accuracy.

1. Introduction

Saliva as a matrix to diagnose and surveillance of systemic illnesses and general health, has been arousing great interest worldwide being a highly desirable goal in healthcare [1]. The main advantage of saliva sampling is the non-invasive, stress-free, inexpensive, simple collection method. Moreover, sampling and handling of an oral fluid is safer for operator and the patient; no special training or equipment is needed, and it can be self-collect, if required. In that way, multiple samples can be collected from the same individual at the optimum analyse times taking into account fluctuations (diurnal or monthly) [2]. This mean of diagnose can be especially relevant and useful in the case of the very young and the old, and in large-scale screening or epidemiological studies [3]. In fact, molecules, like several drugs, hormones and steroids were already routinely analysed in saliva samples despite the low concentrations present [2,4–6], and for many cases a close relationship between saliva and serum concentration has been found. There are, however, disadvantages in the use of saliva for diagnose. The most significant relates to the variation of the analytes concentration with the saliva flow rate, the low concentration of the analytes in saliva, the need for more sensitive methods of quantification, and the difficulty in the interpretation of the tests results due to the complex matrix [2].

Glucose, calcium, and magnesium are three major parameters routinely screened in clinical context and epidemiological research due to its importance to the human body homeostasis. Diabetes is a serious, chronic disease of public health concern, one of four priority non-communicable diseases targeted for world action [7]. Overall, 422 million adults were estimated to live with diabetes with a prevalence of 8.5% in the adult population. WHO projects that diabetes will be the 7th leading cause of death in 2030 [8]. Both, diagnosis and monitoring are commonly accomplished through testing of blood sugar (glucose) levels, however a significant correlation between salivary and blood glucose concentration was already been establish in previous studies [3,9–11]. Several methods are available for glucose measurement in blood and saliva, including spectroscopy (Infrared, fluorescence, Raman) [12–14], liquid chromatography–mass spectrometry (LC-MS)

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and gas chromatography–mass spectrometry (GC-MS) [15,16], photoacoustic probes [17], micro electromechanical sensors (MEMS) [18], and electrochemical methods [19,20].

Calcium and magnesium play essential roles in a wide range of biochemical reactions being critical to the overall human body nutrition and health. Magnesium is the second most abundant cation in the cell, and a physiological antagonist of calcium. These metals are associated with neuromuscular transmission, cardiac muscle activity, control of hormones and neurotransmitters, blood coagulation, immunity and metabolism of osseous tissues [21,22]. Moreover, due to the function in saliva buffering capacity, both elements are also closely related with oral health [23,24].

In that way, it is not surprising that many clinical diseases, such as genetic disorders or dental health, are associated with calcium and magnesium unbalance [23–26]. The routine based monitorization and screening, measure calcium and magnesium concentration in serum or plasma [21]. Nevertheless, some studies have already been performed with the saliva matrix, and a correlation between the plasma and saliva levels was found [21,27]. Also, it is important to note that in the same individual, the concentration of magnesium seems to be very stable within the day [28]. In that way, this analyte can be used for the normalisation of most variable parameters analysed, like glucose. Most of the available studies took advantage of the atomic absorption spectrophotometry, potentiometric, or colorimetric methods [29] for the determination of both metals.

The aim of this work was to develop a simple, fast method for glucose, calcium, and magnesium screening in saliva, without sample pre-treatment, as an alternative non-invasive analysis that can easily be use in clinical/analytical context. The colorimetric sequential injection multi-parametric method proposed is based in the use of 3,5-dinitrosalicylic acid (DNS) reagent for determination of reducing sugar described by Miller [30] in the glucose determination and an adaptation of a previous work by Mesquita and Rangel [31] applied to the calcium and magnesium determination. The reaction for glucose determination involves the oxidation of the aldehyde functional group present in glucose, incorporated in the manifold as in-line approach. Simultaneously, DNS is reduced to 3-amino, 5-nitrosalicylic acid under alkaline conditions, and the colour development was measured at 575 nm. The calcium and magnesium determinations are based on the reaction with cresolphtalein complexone (CPC) and calcium interference in the magnesium determination is minimized by using ethylene glycol-bis(β-aminoethyl ether)-N,N,N,N-tetraacetic acid (EGTA) as masking agent.

2. Materials and methods

2.1. Reagents and solutions

All solutions were prepared with analytical grade chemicals and Milli-Q water (MQW; specific conductance less than 0.1 μS cm^{-1}).

The 1% DNS solution was prepared by dissolving 1 g of 3,5-dinitrosalicylic acid 98% (Acros Organics, New Jersey, USA), 0.05 g sodium sulfite (Sigma, Germany) and 1 g sodium hydroxide (Merck, Germany) to a final volume of 100 mL, resulting in final concentrations of 43.8 mM of DNS, 4 mM of Na_2SO_3, and 0.25 M of NaOH.

Glucose stock solution of 139 mM was prepared by weighing 2.5 g of the solid, $C_6H_{12}O_6$ (Merck, Germany) in 100 mL of MQW. Glucose working standards in the range of 0.5–6 g/L (50–600 mg/dL) were weekly prepared from the stock solution.

A 14 g/L 2-amino-2-methylpropan-1-ol (AMP) buffer solution was prepared by dissolving 1.4 g of AMP (Merck, Germany) in 100 mL of water. The cresolphtalein complexone solution was daily obtained by dissolving 20 mg of the solid (Sigma, Germany) in 100 mL of AMP buffer. The ethylene glycol-bis[β-aminoethyl ether]-N,N,N′,N′-tetraacetic acid solution was obtained by dissolving 48 mg of the solid (Sigma, Germany) in 20 mL of the AMP buffer.

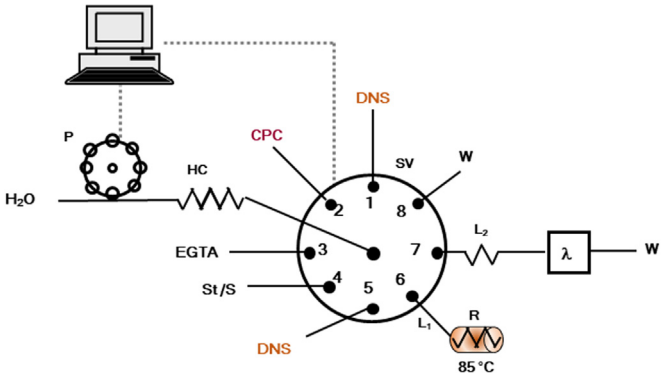

Fig. 1. Manifold for the spectrophotometric determination of glucose, calcium, and magnesium in saliva samples: CPC, cresolphtalein complexone 0.2 g/L; EGTA, ethylene glycol-bis[β-aminoethyl ether]-N, N,N′,N′-tetraacetic acid 4.8 g/L; DNS, Dinitrosalicylic acid 10 g/L and sodium sulfite 0.5 g/L in 0.25 M NaOH; St, standards; S saliva sample; W, waste; L_i, connection tubes of 17, 100 cm respectively; R, reaction coil of 1 m length; P, peristaltic pump; HC, holding coil 3 m long; SV, eight port selection valve; λ, spectrophotometer at 575 nm.

Calcium and magnesium stock solution of 50 mg/L were obtained by dilution of the 1000 mg/L atomic absorption standard (Spectrasol, USA). Calcium working standards in the range of 1–20 mg/L, and magnesium working standards in the range of 0.5–5 mg/L, were weekly prepared in 0.03 M HNO_3, obtained from a proper dilution of the concentrated nitric acid (d = 1.39, 65%), from the 50 mg/L stock solution.

Synthetic saliva [32] was prepared by dissolving 2.24 mg of potassium chloride (VWR-Prolabo, USA), 544.36 mg of monopotassium phosphate (Merck, Germany), 77.69 mg of calcium chloride dihydrate (Sigma-Aldrich, Germany), 19.04 mg of magnesium chloride (Sigma-Aldrich, Germany), 4766.20 mg of $C_8H_{18}N_2O_4S$ HEPES (Sigma-Aldrich, Germany) in 1 L of water (pH ≈ 7.0).

2.2. Sequential injection manifold and procedure

The described sequential injection (SI) method for the multi-parametric determination of glucose, calcium and magnesium with a single manifold with in-line oxidation of glucose is depicted in Fig. 1.

The solutions were propelled by using a Gilson Minipuls 3 (Villiers-le-Bel, France) peristaltic pump (P), equipped with 1.02 mm i.d. Tygon pumping tubes connected to the central channel of an eight port selection valve (Valco VICI51652-E8). All tubing connecting the different components of the system was made of PTFE from Omnifit (Cambridge, UK), with a 0.8 mm i.d. A heated UV digester (GlobalFia, USA) was used for the in-line oxidation of glucose with an internal volume of 500 μL (1 m of tube 0.8 mm id).

The detection system consisted in an Ocean Optics USB 4000 charge coupled device (CCD) spectrophotometer, equipped with a pair of 400 mm fiber optic cables and a Mikropack DH-2000 deuterium halogen light source. A flow cell (Hellma 178.710-QS), with a 10 mm light path and 80 μL inner volume, placed on an Ocean Optics cuvette holder was used.

Data acquisition of the signal obtained at a wavelength of 575 nm for glucose, calcium, and magnesium, was performed through the Ocean Optics—Spectrasuite software running on a personal computer (Tsunami – Intel(R) Atom(TM) 2 GB/149 GB) with Windows XP Home Edition. The selected wavelength was set as corresponding to the maximum absorbance observed when mixing the prepared reagents with a standard.

A personal computer (Samsung SD 700) equipped with a PCL818L interface card, running with a homemade software written in QuickBasic 4.5, controlled the selection valve (SV) position and the