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On-line electrochemical preconcentration and flame atomic absorption spectrometric determination of manganese in urine samples

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Abstract

A sensitive and selective method was developed for the determination of traces of manganese in urine using on-line electrochemical preconcentration followed by flame atomic absorption spectrometry detection. A home made flow-through polypropylene cell (4.5 cm long × 0.8 cm diameter filled with glass marbles) with an effective inner volume of 0.5 ml containing a working and a counter electrode, both of glassy carbon and a Pt pseudo reference electrode was located in a flow injection manifold specially designed for the purpose of this work. The manganese was deposited from buffer solution of NH_3/NH_4Cl at pH 9.00 through an oxidizing process at a current of 400 mA during 7 min. A flow of HCl 0.1 mol l^{-1} at 4 ml min^{-1} through the cell, chemically dissolved the deposit. A small portion (15 μl) of the concentrate was introduced in a continuously flowing system by means of a timing device and was then carried to the detector for the manganese quantification. All electrochemical and spectroscopic variables as well as possible interferences in both systems were systematically studied. The relative standard deviations for ten consecutive measurements of manganese solutions of 2.0 and 20 μg l^{-1} were of 2.3 and 1.5%, respectively, while for a sample processed five times was less then 5%. The accuracy of the developed procedure was evaluated by adding known amounts of manganese standard to urine samples and following the whole procedure. Recoveries within the range 97.2–102.8% were obtained. To further prove the accuracy, a Seronorm Trace Elements in Urine, Batch 403125 sample with a reported concentration of 13 μg Mn l^{-1} was also analyzed. The experimental value obtained was of 12.7 ± 0.1 μg l^{-1}, which does not differ significantly from the reported amount ($p < 0.05$). A preconcentration factor of 40, a linear range between 0.015 and 60 μg l^{-1} and a limit of detection of 15 ng l^{-1} permitted the determination of manganese in real urine samples from non-exposed subjects in the range 0.5–2.8 μg l^{-1}.

Keywords: Manganese; Electrochemical preconcentration; Flame atomic absorption spectrometry detection; Flow injection manifold; Urine samples

1. Introduction

Manganese (Mn) is recognized as both, an essential and a neurotoxic trace element. As an essential trace element, Mn plays an important role in: bone and tissue formation, reproductive functions and the activation of many enzymes, which are involved in vital metabolic processes [1]. Element deficiency is not a common occurrence since dietary sources provide an adequate supply of 2–8 mg of Mn per day. However, toxic levels may be reached in workers or individuals leaving near mines, ore-processing plants or manufactures of varnish, pharmaceutical products, ceramics and pottery. There is still little information on the biochemical mechanism, which could explain the Pasrkinson-like symptoms, caused by chronic inhalation of excess levels of Mn [1,2]. Additionally, overall exposure to Mn in urban environment may increase due to the use of a manganese-containing com-

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pound in gasoline as antiknock agent. Monitoring its levels in environmental samples [3–6] as well as in whole blood and urine [7–14] would be advisable in order to establish Mn baseline values in such samples. Biomonitoring Mn in body fluids, especially blood and urine is also essential for the control of possible nutritional deficiencies and perhaps for preventing environmental exposure. Therefore, it is mandatory the availability of simple, economic, sensitive and selective analytical methods for the determination of traces of Mn in different kinds of samples.

Several methods have been described for the determination of Mn at low concentrations in biological specimens, although electrochemical (EQ) [3–7] and spectroscopic [8–14] techniques are the most frequently employed. Among them, only inductively coupled plasma mass spectrometry (ICP-MS) [8,9] and atomic absorption spectrometry with electrothermal atomization (ETAAS) [10–13] have been used for the determination of Mn in urine samples from non-exposed subjects. The MS detector is rather complex and expensive, fact that has limited the widespread use of ICP-MS for routine work in laboratories and hospitals. ETAAS has been the method of choice for biomonitoring of trace elements and its selectivity and sensitivity was further improved by separation/preconcentration techniques like for instance chelate formation of Mn with cupferron followed by solvent extraction [12] or coprecipitation with samarium hydroxide [13]. Flame atomic absorption spectrometry (FAAS), although is the simplest and the cheapest of them all, has the poorest sensitivity and therefore it is not applicable to the direct determination of Mn concentrations at $\mu g\,l^{-1}$ levels. However, its association with preconcentration techniques would make it useful over the range 0.05–10 $\mu g\,l^{-1}$, generally found in biological fluids [15,16].

EQ techniques, like cathodic stripping voltammetry [3–5] with solid [3,4] or mercury [5] working electrodes are the most popular for the simultaneous determination of trace metals including Mn, in environmental samples. Their application to body fluids is limited, probably because of the complexity of the biological matrix which damage the electrode surface or to the inherent mutual interferences due mainly to transition elements peak potentials overlapping [17]. Additionally, the rigid control of all EQ parameters during the redox reactions necessary in such techniques make their application to the analysis of biological samples troublesome.

Instead, Beinrohr [18,19] utilized electrolysis as a separation and preconcentration principle by incorporating a microcell of 30 μl inner volume in a flow injection (FI) system. The deposited analyte can be chemically stripped by flushing the electrolytic cell with a diluted acid and finally determine the analyte on-line by FAAS [18] or ETAAS [19]. No applications to real samples were reported whatsoever.

The aim of this work was to design and evaluate an on-line system for the EQ preconcentration followed by FAAS quantification of Mn in urine samples.

2. Experimental

2.1. Apparatus

The absorbance measurements were carried out on a Varian, Model 1475-A atomic absorption (AA) spectrometer equipped with: a Mn hollow cathode lamp (operated at 5 mA); a wavelength set at 279.5 nm and a slit-width of 0.2 nm; a 10 cm air/acetylene burner positioned 5 mm below the optical path of the instrument and a deuterium lamp background corrector. A FI system described below was linked to the AA spectrometer with a 15 cm long and 0.8 mm inner diameter poly (tetra fluoroethylene) PTFE tubing.

For comparison purposes, a Perkin-Elmer (PE) Model 4100 ZL atomic absorption spectrometer, equipped with a Zeeman effect background corrector, a transversally heated graphite tube atomizer (THGA) and a PE Model AS-71 autosampler was used. A PE Mn hollow cathode lamp, PE pyrolytically coated graphite tubes with integrated L'vov platforms and an Epson Model LX-810 printer were employed. The graphite furnace heating program and the procedure earlier optimized by Burguera et al. [20] were used.

For the preliminary evaluation of the EQ parameters, a BAS Epsilon potentiostat/galvanostat controlled by a PC with Epsilon software, coupled to a three electrodes H type cell. The working electrode was a glassy carbon disk and the counter electrode was a Pt coil facing the working electrode. All measurements were made against an Ag/AgCl reference electrode. The working compartment was connected to the reference electrode by a Luggin capillary.

The flow through EQ cell (Fig. 1) used in the combined system was made from a polypropylene vial tightly closed with its screwed cup of the same material. Two parallel glassy carbon rods with a total surface of 1.5 cm^2 and of 2.0 cm^2 were used as working and counter electrode, respectively. Between them, a 1.3 cm long Pt wire was inserted as a pseudo-reference electrode. To further reduce the inner volume of the cell to 0.5 ml, its body was filled with glass marbles of 2.0 mm of diameter. A Lambda, Model LR 616 FM power supply feed the cell when connected on-line with the rest of the system.

The EQ and AAS systems were coupled in a FIA configuration, which comprises two multiple-way Cole-Palmer solenoid valves of 24 V and 0.6 A, controlled by a homemade power supply. The components of the power supply were: two transformers of 24 A and 6 V, two Weaston bridges of rectified diodes, one of 24 A and 1.5 V and the other of 10 V and 0.5 A; two condensers, one of 24 V and 1.2 mF and other of 10 V and 0.5 mF; one contact loading coil of 12 V and two switches. The doubly stopping shutter of the solenoid valves either close or open their sets of tubing (Fig. 2) at a fixed time, thus allowing the sequential introduction of samples or washing solution in the system as described in the procedure. The solenoid valve (ISV) called injection valve, has a set of tubing indicated in Fig. 3 with 1, 2, 3 and 4, while the set

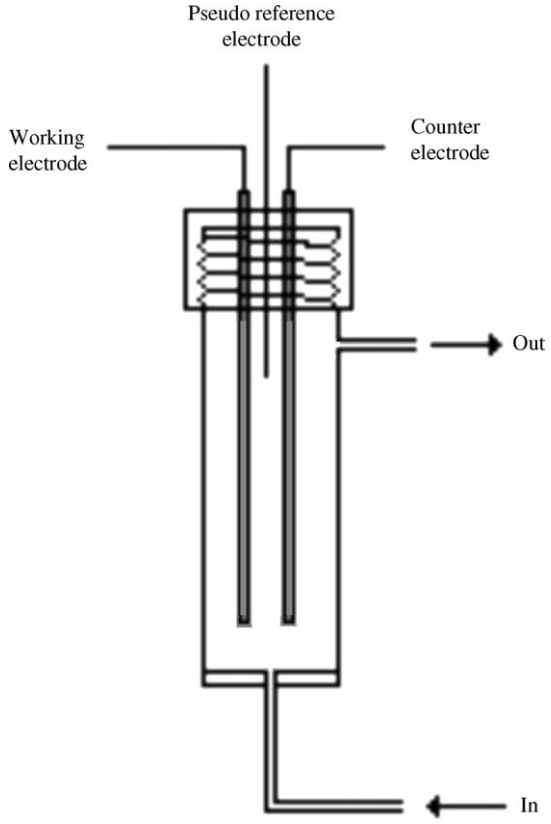

Fig. 1. Electrochemical flow-through cell (4.5 cm height × 0.8 cm of diameter): glassy carbon (working and counter electrode); Pt pseudo reference electrode. Electrodes characteristics are given in the text.

of tubing named X, Y and W belong to the solenoid valve (WSV) used for washing the system. All tubing used was of silicon rubber with an internal diameter of 0.8 mm. The operation time of the solenoid valves was controlled by a Gralab programmable temporizer, Model 900, while for the propulsion of the solutions, a digital ISMATEC peristaltic pump, Model IPC with 12 channels and eight rollers provided with Tygon tubing was used.

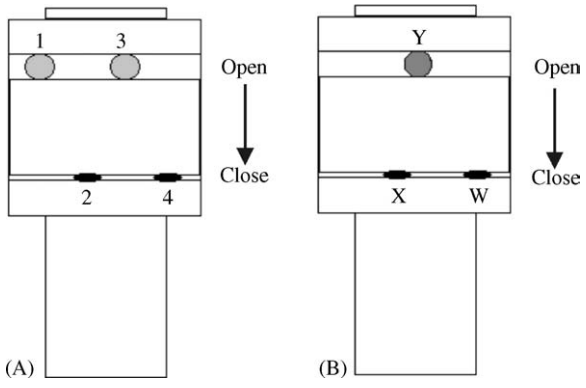

Fig. 2. Functioning of the solenoid valves incorporated to the FIA system. A and B indicate IVS and WVS, respectively, in step 1 position (see Fig. 3).

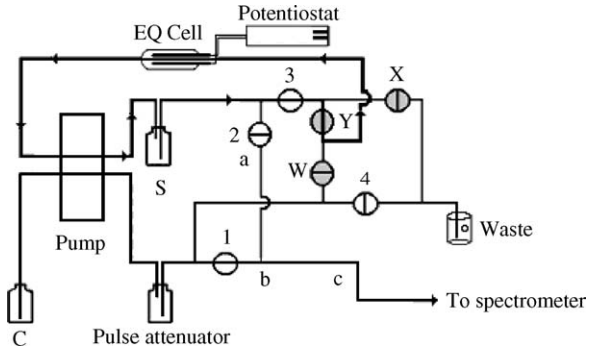

Fig. 3. Flow injection-electrochemical preconcentration system for the determination of manganese in urine samples by FAAS. The system is shown in its preconcentration and dissolution step. Arrows show the direction of the recirculated solution through the EQ flow cell. S contains Mn standard or urine sample during the preconcentration, acid solution during the stripping step and water during the washing step; C always contains water.

2.2. Reagents and samples

All chemicals used in this study were of the highest available analytical grade. Milli-Q ultrapure water with a specific resistivity of 18 MΩ cm^{-1} was used for standards preparation, reagents dilution and rinsing of the cleaned laboratory material.

An ammonia/ammonium chloride (NH_3/NH_4Cl) buffer solution of pH 9.0 was prepared by mixing 50 ml of 1 mol l^{-1} hydrochloric acid (HCl) with 72.4 ml of 1 mol l^{-1} ammonia solution [5].

Daily working standards used for the EQ deposition of Mn were prepared by diluting appropriate volumes of 1.000 g Mn l^{-1} solution prepared from $MnSO_4 \times H_2O$ (99% from Merck) in a buffer solution of NH_3/NH_4Cl at pH 9.00, while HCl (37% from Merck) 0.1 mol l^{-1} was used for the chemical dissolution of Mn.

To confirm the accuracy of the technique developed in this work, a standard reference material (Seronorm^{TM} Trace Elements in urine, batch 403125 from Nicomed, Oslo, Norway) with a recommended content of 13 μg Mn l^{-1} was used.

Urine samples obtained from 10 healthy laboratory workers and students were void by each volunteer directly in sterile acid washed polypropylene containers provided with tight cups of the same material. After collection, the samples were kept under refrigeration (4 °C) if the analysis was not carried out immediately and were 1:1 diluted in pH 9.0 buffer electrolyte immediately before analysis.

It is sufficiently documented that Mn is difficult to be reliably determined in biological samples, due to uncontrolled contamination during sample collection and/or preparation procedures [21,22]. To avoid hazardous contamination, all glassware and plastic containers were new and kept separate from other laboratory material. It was also soaked for at least 4 h in 2 mol l^{-1} HNO_3, rinsed several times with ultrapure water and then inverted to drain dry. The washings were tested to be free of Mn by the alternative ETAAS techniques used in this work [20].

2.3. Procedure

The general procedure consists on three fundamental steps:

- Step 1. Preconcentration and dissolution: a portion of 20 ml of sample or standard diluted 1:1with pH 9.0 buffer or prepared in the buffer, respectively, is located in a 50 ml bottle in position S (Fig. 3). At this stage: (1) the power supply applies a constant current of 400 μA to the working electrode; (2) valve ISV is activated so that its conduits 1 and 3 are opened while 2 and 4 are closed; and (3) valve WSV has the conduits X and W closed and Y opened. In this way, either a sample or standard solution is automatically recirculated through the EQ cell during 7 min. Meanwhile, the carrier flow (water located in position C) is directed towards the AA spectrophotometer. Finally, the container in position S is substituted by another one containing 2 ml of 0.1 mol HCl l^{-1}, which chemically dissolves the Mn deposited on the working electrode.
- Step 2. Charging, injection and detection: valve ISV is activated to open conduits 2 and 4 and to close the conduits 1 and 3. The timer sends a pulse of 4 s to this valve, which allows 15 μl of the acidified solution to fill the segment ac of conduit 2, while the carrier is directed through conduit 4 to waste in order to avoid an overpressure within the system. Then, the valve ISV is activated again to open conduit 1 and 3 and to carry the manganese preconcentrated acidic solution which filled the segment bc to the detector. During the last operation, conduits 2 and 4 are obviously closed.
- Step 3. Washing: for this operation, both valves are activated in such a way that conduits X, W, 1 and 3 are opened, while Y, 2 and 4 are closed. The carrier solution located in positions C and S is recirculated through the whole system for about 2 min to remove traces of HCl from the EQ cell and from all the conduits. In this way, the system is ready for another cycle.

3. Results and discussion

3.1. System optimization

For the optimization of the experimental EQ (deposition potential, scan rate, pH, etc.) and spectroscopic (slit width, lamp current, burner position, air: acetylene ratio, etc.) parameters, one factor-at-a-time was changed while holding the others constant, using aqueous Mn standards of 0.7 and 3.0 mg l^{-1}, respectively. The optimum conditions shown in Table 1, where considered those in which the maximum sensitivity, the highest sample throughput as well as the best precision are reached. As there is enough knowledge about the significant factors affecting the EQ deposition of Mn on solid electrodes [3,4] and its behavior in an air/acetylene flame [23], the optimization process was fast and reliable.

Table 1
Optimal parameters for the EQ preconcentration and AAS detection of traces of Mn in urine

System	Parameter	Units
EQ	Electrolyte (NH_3/NH_4Cl)	50 ml HCl 1 mol l^{-1} + 72.4 ml NH_3 1 mol l^{-1}
	pH	9.0
	Deposition current	400 μA
	Deposition potential	400 mV
	HCl concentration for dissolution	0.1 mol l^{-1}
AAS	Wavelength	279.5 nm
	Lamp current	5 mA
	Burner height with respect to optical axis	5 mm
	Air flow rate	6.0 l min^{-1}
	Acetylene flow rate	2.0 l min^{-1}
	Air/acetylene ratio	3
	Slit width	0.2 nm
	Nebulizer flow rate	7 ml min^{-1}
Coupled	Carrier flow rate	7 ml min^{-1}
	Time of preconcentration (for 20 ml of sample	7 min
	Sample volume to detector	15 μl
	Dispersion tubing length	23 cm

In preliminary EQ experiments, the H-type EQ cell was used in batch mode. Deposition potentials between −200 and 700 mV versus Ag/AgCl with a scan rate ranging from 0 to 300 mV s^{-1} were varied in turn for Mn concentrations from 0.5 to 1.0 mg l^{-1} in a buffer solution of NH_3/NH_4Cl at pH 9 until obtaining the combination that gave the highest oxidation peak. The optima parameters are given in Table 1.

Several authors who used voltammetric methods to determine traces of Mn have found that the redox reaction of Mn(II) at a solid electrode is pH dependant: the potentials of the cathodic and anodic peaks are shifted towards more negative values with increasing pH, indicating a protonation reaction during the oxidation process [3,4,6]. This protonation reaction, which takes place after the electrons loss, is verified in the graph $I_{pc}/\nu^{1/2}$ (Fig. 4), where I_{pc} is the current of the cathodic peak and ν the scan rate. This is the typical response of an electrochemical mechanism involving a diffusion controlled electron transference followed by a chemical reaction and this represents an experimental evidence of the

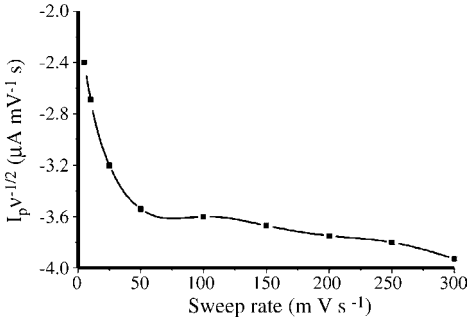

Fig. 4. Variation of Ipc/$\nu^{1/2}$ vs. ν for the system Mn(IV)/Mn(II), characteristic of a chemical reaction coupled to an electronic transference.

pH effect. The slope values of the oxidation potential versus pH graphs of 118 mV/pH [4] and 114 mV/pH [6] reported in the literature, suggested a four-proton and two-electron process, as shown in the following equation [4,6]:

$$Mn^{2+}(H_2O)_x \rightleftarrows MnO_2(H_2O)_{x-y}(s) + 4H^+ + (y-2)H_2O + 2e^-$$

The highest oxidation peaks were obtained around pH 9.0, at lower pH values, the formation of MnO_2 is probably incomplete and at higher values, competitive production of $Mn(OH)_2$ may occur, which eventually is oxidized to $Mn(OH)_4$ and subsequently precipitated in the solution. In view of these considerations, an NH_3/NH_4Cl buffer of pH 9.0 was used in all EQ experiments carried out in this work.

The representative cyclic voltammogram indicates an oxidation peak at 190 mV attributed to the oxidation of the dissolved Mn(II) to hydrated Mn(IV) oxide, while a reduction peak was located at 40 mV, which corresponds to the reduction of Mn(IV) back to Mn(II). As expected, the peak current values increases linearly as the concentration of Mn is increased.

The material obtained at the glassy carbon electrode is chemically stripped with diluted HCl. This process is practically instantaneous since the acid is completely dissociated and rapidly disaggregates the deposit. Mn concentrated in this acidic solution is then determined by FAAS.

The spectroscopic parameters were also optimized first in the batch mode by aspirating a solution of 3.0 mg l^{-1} in the air/acetylene flame, following the criteria of high sensitivity and reproducibility. The best results were obtained under the conditions listed in Table 1.

In the combined system, all these parameters were checked again with special emphasis on the carrier and sample or standards flow rates, preconcentration time, sample volume and dimensions of the tubing connecting the FI assembly with the AA nebulizer. In such arrangements, the nebulizer uptake limits the solutions flow rates in the FI system: a higher flow would produce an overpressure and a lower flow would form vacuum bubbles. Therefore, the carrier and sample flow rates were kept equal to the aspiration rate at 7 ml min^{-1}. The preconcentration time will depend on the volume of sample available and on its flow rate through the EQ cell. For instance, a sample volume of 20 ml will pass through the flow cell in approximately 3 min. The Mn deposition efficiency in the EQ cell is strongly dependent on the recirculating time; it increases from 42 to 75% and closed to 100% after 3, 5 and 7 min, respectively. As indicated in Fig. 5, the sample should be therefore recirculated for at least 7 min in order to assure the complete deposition of the Mn(II). As indicated in the procedure, for the intercalation of a sample plug in the FI system, a temporized system will activate the valve ISV for a controlled time. The duration of these electronic pulses are proportional to the amount of sample incorporated in the system. Thus, varying the pulse between 1 and 10 s, the volume was varied between 4 and 35 μl, respectively. Fig. 6 shows

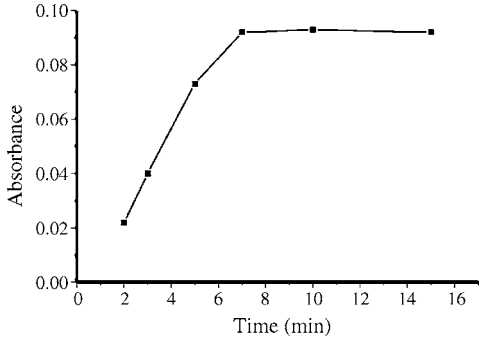

Fig. 5. Effect of the EQ preconcentration time on the absorbance (20 ml of a Mn standard of 20 μg l^{-1}).

that for volumes higher than 20 μl, the instrumental response is independent of the volume, while for lower volumes the reproducibility was poor (higher than 8%). Considering the criteria of obtaining best sensitivity and reproducibility, a volume of 15 μl was considered as optimum. To avoid high dispersion of the sample plug before reaching the nebulizer, the tubing interfacing the FI manifold with the AA spectrometer was kept as short as the physical distribution of the components permitted (23 cm long × 0.8 cm i.d.).

3.2. Interferences of foreign ions

Under the conditions established above, various amounts of some major components of urine [29] were added to a Mn(II) solution of 0.2 μg l^{-1} and the whole procedure described in Section 2.3 was followed. As the analyte reaches the FAAS detector free of other sample components, this study was undertaken in the EQ system only. When a change in the absorbance obtained from the analyte solution containing the interfering components was within 5% of that from the analyte alone, the co-existing species was regarded as tolerant. The tolerated concentrations are well above the physiological levels in urine [24], proving that none of these components affect the EQ deposition of Mn at the glassy carbon electrode and that selective determination of Mn(II) is achieved by using the procedure developed in this work.

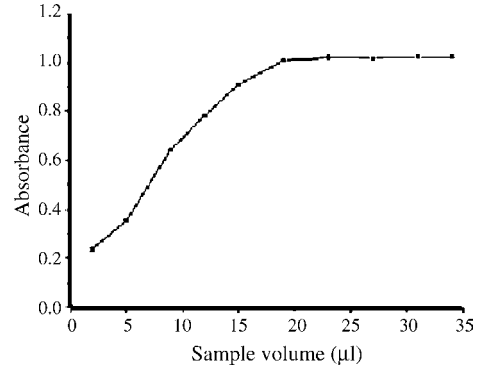

Fig. 6. Variation of preconcentrated sample volume incorporated in the combined system.

3.3. Analytical performance and method validation

The combined system's analytical parameters like linear range, sensitivity and detection limit were verified by using aqueous Mn(II) solutions prepared in NH_3/NH_4Cl buffer of pH 9.0.

The calibration curve, linear from 0.015 to 60 $\mu g\,l^{-1}$, obeyed the equation: Absorbance = intercept + slope [Mn], where intercept = 0.005 ± 0.001, slope = 0.029 ± 0.010 and [Mn] = Mn concentration in the linear range. The standard addition calibration curves constructed for three urine samples at two analyte concentration levels (0.20 and 20.0 $\mu g\,l^{-1}$) had similar slopes to the analytical functions of the aqueous solutions (not significant differences at 5% error level were obtained), revealing negligible matrix effects. The correlation coefficient was in all cases >0.9990 and the detection limit defined as the Mn concentration equivalent to three times the standard deviation of the reagents blank ($n = 6$) was of 15 $ng\,l^{-1}$. The relative standard deviations (R.S.D.) calculated for ten consecutive measurements of manganese solutions of 2.0 and 20 $\mu g\,l^{-1}$ were of 2.3 and 1.5% respectively, while for a sample processed five times was less then 5%. The preconcentration factor calculated as the ratio of slopes corresponding to the calibration curve obtained with and without preconcentration × 1000, was of 40.

For method validation, three approaches were studied: (i) the recovery of the analyte spiked into urine samples; (ii) analysis of a standard reference material; and (iii) the determination of Mn in all urine samples by ETAAS [20]. For recovery studies, increasing amounts of Mn were added to five urine samples randomly chosen, in such a way that the total Mn concentration remained within the linear range of the calibration curve. The average recovery was of 99.6 ± 1.2 (range 97.2–102.8%), indicating the adequacy of the procedure for the determination of Mn. Furthermore, as the nature of the Mn in the samples might be different to that added as a standard, the proposed procedure was also applied to the determination of Mn in Seronorm™ Trace Elements in urine, batch 403125. The amount of 12.7 ± 0.1 $\mu g\,Mn\,l^{-1}$ found, is in good agreement with the recommended content of 13 $\mu g\,Mn\,l^{-1}$. The Student's *t*-test [25] shows an experimental value of 1.84 while its theoretical value for 4° of freedom was of 2.78, indicating that the two means are not statistically different for a significance level of 0.05. However, the concentration of Mn reported for the Seronorm urine standard is a lot higher than its values in real samples and it will be useful to have access to standard reference materials with lower Mn content, which resembles real urine samples. Therefore, to further check the accuracy of the combined EQ-FAAS procedure developed in this work, the ten urine samples from non-exposed subjects were also analyzed by the well-established ETAAS procedure [20]. The linear regression equation $Y = 0.030 + 1.006X$ ($r = 0.9978$), where X and Y are the Mn concentrations obtained by ETAAS and by our EQ-FAAS procedure respectively, shows statistically indistinguishable results ($p < 0.05$) between both methods.

3.4. Analysis of real urine samples

Once set and optimized for aqueous standards and for the reference material, the combined procedure developed in this work was applied to the determination of Mn in real urine samples. The manganese concentrations found in the urine samples of the non-exposed volunteers were in the range 0.5–2.8 $\mu g\,l^{-1}$ with a mean average of 1.80 ± 0.51 $\mu g\,l^{-1}$, showing certain concordance with values reported in the literature of 0.50–1.60 $\mu g\,l^{-1}$ [20]; 0.08–2.67 $\mu g\,l^{-1}$ [26]; 0.12–1.9 $\mu g\,l^{-1}$ [27,28] or 0.0–7.9 $\mu g\,l^{-1}$ [29]. However, other authors reported that most of the real samples have Mn concentrations below their instrumental limit of detection [9,11]. The use of ultrapure water and reagents and clean-room conditions are essential in order to assure the accurate determination of Mn at low levels.

4. Conclusion

The results obtained in this work show that traces of manganese can be accurately and selectively determined in complex matrix samples like urine by cheap and robust analytical arrangements. The flow injection manifold assembled here, permitted reliable automatic handling of standards and samples, reducing the risk of contamination and allowing the 40-fold preconcentration of Mn in the EQ system. The sampling frequency was of at least six samples per hour with minimum of sample preparation.

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