ESTABLISHING PERFORMANCE PARAMETERS TO DETERMINE MANGANESE USING UV-VIS MOLECULAR ABSORPTION SPECTROMETRY

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In this study are described the development and determination of some performance parameters of an analytical method for the determination of manganese using UV-Vis molecular absorption spectrometry. The method presents a good linearity and has proved to be precise and accurate and can be used in any laboratory.

Keywords: manganese, analysis method, UV-Vis spectrophtometry, performance parameters

1. Introduction

Manganese occurs in water, sediment, biological materials and soil [1, 2]. In the environment, it does not occur as the free metal but in the form of compounds, combined with other elements, such as: silicon, chlorine, oxygen, carbon and sulphur [3]. Two types of manganese compounds are known, namely: inorganic manganese compounds and organic manganese compounds. Inorganic manganese compounds are used in the production of batteries, dietary supplements, ceramics, steel and are generated as combustion products from coal – burning industrial plants and motor vehicles. Organic manganese compounds are used in the manufacture of pesticides, fertilizers and in a gasoline additive called methlcyclopentadienyl manganese tricarbonyl (MMT) [4, 5]. These compounds are solid and can be dissolved in water or can be found in air as a suspension of small particles. There are 11 oxidation states with values between – 3 and + 7, but the most common are + 2 and + 4 [3].

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Manganese is part of highly toxic heavy metals. In excess, it gives a yellowish water, bad taste and odor, causing staining of bathroom accessories, clothes and kitchen utensils, and contributes to the water hardness, determines in pressure decrease in pipes, pumps and water heaters depositions [1, 2].

Manganese is considered an important nutrient that ensures the normal development and functioning of the body, but is toxic in high doses [4, 5]. Manganese toxicity is influenced by exposure routes [5, 6]. Routes of exposure include: exposure through soil, exposure through air and exposure through water. Exposure to manganese can be done by inhalation and ingestion, but inhalation is a primary route [5].

Excessive exposure to manganese causes central nervous system adverse effects. Another effect of manganese exposure is the „manganism", a disease whose symptoms are similar to Parkinson's and which shows tremors, muscle stiffness, difficulties with breathing or swallowing, lack of coordination, and other neuromuscular problems [4, 5]. Inhaled manganese can get in contact with the brain through ways: olfactory way(nasal airways) and is a direct path to brain tissue and to the lungs [5, 7].

In mammalian cells, manganese causes chromosome aberrations and DNA damage. In high doses, it affects fertility and it is toxic to the foetus and embryo [2, 8].

Manganese exposure can be teratogenic (cause birth defects) such as: low external ears, delayed bone hardening, short tail, cleft palate, excess toes, decreased birth weight and hydrocephaly [5, 9 13].

The determination of manganese can be done using several different methods, such as: atomic absorption spectrometry (AAS), spectrophotometry, neutron activation analysis (NAA), polarography, inductively coupled plasma - atomic emission spectrometry (ICP-AES) or mass spectrometry (ICP-MS) [2, 14], nuclear magnetic resonance method [2, 15] or a method that uses online concentration analysis [2, 16] and electrochemical methods [2].

Atomic absorption spectrometry (AAS) and atomic emission spectrometry (AES) are the most used techniques for the determination of manganese, especially in environmental and biological samples. Some of these techniques require derivatization, extraction and wet digestion before detection.

Spectrophotometric methods are the oldest methods used for the determination of manganese. Reagents used for the colorimetric determination of manganese are: oximes (formaldoxime), aromatic amines, azo dyes (PAN - (1-(2-pyridylazo)-2-naphtol) and porphyrins. Another method used for the determination of manganese is spectrophotometry with previous oxidation of Mn(II) in a strong basic medium, using a chromatogenic reagent, for example 3,3',5,5'-tertramethylbenzidine [2, 17].

Neutron activation analysis (NAA) is characterized by a high sensitivity and specificity for very low concentrations of manganese. This method has been used for the determination of manganese at very low concentrations in different samples, for the determination of manganese concentrations in plants and blood and in order to check the accuracy of results obtained by other methods [2, 18, 19].

The nuclear magnetic resonance method, known as a method that uses online concentration analysis, has been used to determine both complex and free manganese ions in aqueous media [2, 15, 16].

For the determination of traces of manganese in complex samples electrochemical methods, such as: stripping chronopotentiometry (SCP), cathodic stripping voltammetry (CSV), anodic stripping voltammetry (ASV) and adsorptive stripping voltammetry (AdSV) are used [2].

Stripping techniques are usually realized in two steps: pre-concentration/ accumulation of metal ions (electrochemical and adsorptive deposition) and quantification and re-oxidation of the accumulated metal ions. In the first step, ion metals are reduced at a constant potential for a fixed period of time and hydrodynamic conditions ensuring a steady flow of the analyte to the electrode surface [2, 20, 21, 22]. The dissociation rate constants and diffusion coefficients are used to determine the amount of metal accumulated that represents a measure of them. The last step consists in applying an anodic ramping potential for the quantification of the metal accumulated and the resulting current recorded as a function of applied potential represents re-oxidation. In voltammetric techniques the metal accumulated is stripped by a potential scan either towards more positive potentials (ASV) or towards more negative potentials (CSV) while registering the current - potential dependence. In chronopotentiometric stripping techniques the metal accumulated is stripped either electrochemically, applying a constant dissolution current (galvanostatic or constant current chronopotentiometry), or chemically, using an oxidant (potentiometric stripping analysis - PSA). In both cases the change of the potential of the working electrode is registered and evaluated [2, 23].

In the present study the development of an analytical method for the determination of manganese using UV-Vis absorption spectrometry is realized. Also the characterization of some performance parameters of this method is realized.

2. Experimental

2.1 Materials and solutions

All reagents used were analytical grade. These (manganese sulphate monohydrate, sulphuric acid, ammonium iron (II) sulphate hexahydrate, ethylenedinitrilotetraacetic acid disodium salt, hydroxylammonium chloride, formaldehyde, sodium hydroxide, ammonia) were purchased from Fluka.

The stock solution of 100 mg/L Mn (II) was prepared by dissolving manganese sulphate monohydrate ($MnSO_4*H_2O$) in water and then sulphuric acid (H_2SO_4) 3 mol/L was added. The working solution of 5 mg/l was prepared by a dilution of stock solution with distillated water.

The sulphuric acid (H_2SO_4) 3 mol/L was prepared by a dilution of the concentrated sulfuric acid.

The ammonium iron (II) sulphate hexahydrate solution [$(NH_4)_2Fe(SO_4)_2*6H_2O$] of 700 mg/L was prepared by dissolving amonium iron (II) sulphate solution in distillated water and by adding sulfuric acid 3 mol/L.

The complexon III 0, 24 mol/L was prepared by dissolving ethylenedinitrilotetraacetic acid disodium salt.

The formaldoxime solution was prepared by dissolving 10 g hydroxylammonium chloride (NH_3OHCl) in distilled water and adding 5 ml of 35 % (m/m) formaldehyde solution.

The sodium hydroxide (NaOH) solution 4 mol/L was prepared by dissolving sodium hydroxide in distilled water.

Hydroxilammonium chloride/ammonia solution is a mixture between hydroxilammonium chloride solution 6 mol/L (dissolving hydroxilammonium chloride in water) and ammonia solution 4, 7 mol/L (diluting of concentrated ammonia in distilled water) [24].

2.2. Apparatus

Volumetric flasks – capacity 50 ml, glass bottle - capacity 100 ml, cylinder – capacity 50 ml and pipettes – capacity 1ml, 5ml and 10ml were used.

The analytical control was achieved using a LAMDA UV-VIS-NIR (Perkin Elmer Life and Analytical Sciences) spectrophotometer at the wavelength 450 nm.

2.3. Procedure

In a series of 50 ml volumetric flasks were used to prepare a series of solutions of $MnSO_4$ of different concentrations and dilute to the mark with distilled water. The solutions were transferred in 100 ml glasses and add 1 ml of ammonium iron (II) sulphate hexahydrate solution and 2 ml of EDTA(ethylenedinitrilotetraacetic acid disodium salt). After mixing 1 ml of

formaldoxime solution and 2 ml of sodium hydroxide solution were added. The solutions were mixed and allowed to rest for 5 – 10 minutes, and then 3 ml of hydroxilammonium chloride/ammonia solution was added and left to stand for 1 hour for color development. The absorbance was measured at 450 nm wavelength using cells of 10 mm optical path length [24].

3. Results and discussions

The concept validation was defined from three international documents, such as: ISO 17025, ISO 9000 and International Vocabulary of Metrology (VIM). In ISO 17025 validation is definite as „confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled", in ISO 9000 as "confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled and in VIM as" verification, where the specified requirements are adequate for an intended use" [25, 26, 27, 28, 29, 30, 31].

During the validation method a set of performance characteristics are evaluated such as: linearity, limit of detection (LOD) and limit of quantification (LOQ), precision and accuracy [28].

Linearity

Linearity is the ability of an analytical method to obtain test results proportional to the concentration of the analyte in the sample. This can be evaluated in different steps of the method as calibration function (in this step the focus is on instrumental measurement) and function of the concentration of the specimen. The purpose of these steps is to determine the shape calibration function [28].

Linearity was demonstrated on 13 manganese concentrations of the standard solution prepared from manganese sulphate monohydrate using the proposed procedure. Absorbance was measured and the calibration curve was plotted, depending on the concentration absorbance.

On this basis some statistical parameters were calculated such as: the regression line equation ($y = a + bx$), the slope of the regression line (b), standard deviation of the slope (s_b), the intersection to the origin (a), standard deviation of intersection (s_a), correlation coefficient (R), determination coefficient (R^2), standard deviation (s), relative standard deviation (RSD) and the number of freedom degrees (v), regression sum of squares, standard deviation of estimate y, function F.

Table 1

Calibration data for manganese

i	Concentration [mg/L]	A_1	A_2	A_3	A_4	A_5	□
1	0.50	0.075	0.0751	0.0752	0.0753	0.0755	0.07522
2	1.00	0.1482	0.1484	0.1486	0.1485	0.1486	0.14846
3	1.50	0.2283	0.2284	0.2286	0.2287	0.2285	0.2285
4	2.00	0.2929	0.2929	0.293	0.293	0.293	0.29296
5	2.50	0.3727	0.3728	0.3727	0.3726	0.3726	0.37268
6	3.00	0.4805	0.4806	0.4806	0.4807	0.4807	0.48062
7	3.50	0.529	0.5288	0.5288	0.5288	0.529	0.52888
8	4.00	0.5978	0.598	0.6011	0.6002	0.5972	0.59886
9	4.50	0.6996	0.6998	0.6997	0.6999	0.6999	0.69978
10	5.00	0.7834	0.783	0.7829	0.7828	0.7827	0.78296
11	5.50	0.8504	0.8504	0.8509	0.8509	0.8507	0.85066
12	6.00	0.9632	0.9637	0.9638	0.9634	0.9631	0.963525
13	6.50	1.0206	1.0203	1.0199	1.0199	1.0199	1.02012

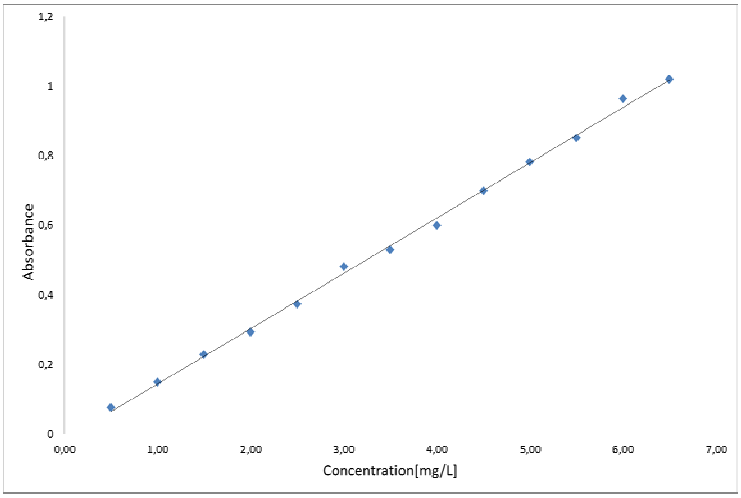

Fig. 1. Calibration curve of Manganese at 450 nm (absorbance =f(concentration))

Table 2

Calculated parameters of the linear regression analysis

Stastistic parameter	Value
Regression line equation	y = 0.1591 x – 0.015
Slope of the regression line, b	0.1591
Standard deviaton of the slope, s_b	0.00202
Intersection to the origin, a	-0.015
Standard deviation of intersection, s_a	0.0080
Correlation coefficient, R	0.9991

Determination coefficient, R^2	0.9982
Standard deviation, s	0.00005477
Relative standard deviation, RSD	0.000101
Number of freedom degrees, v	9
Residual sum of squares	0.00204
Regression sum of squares	1.1514
Standard deviation of estimate y	0.01363
Function F	6198.037

Limit of detection (LOD) and Limit of Quantification (LOQ)

The limit of detection is defined by the ICH as the lowest concentration of the analyte in a sample that can be detected but not necessarily quantifiable. This is similar to the sensitivity of the method. The sensitivity of the method is the slope of the calibration curve obtained after the graphical representation of the response, depending on concentration. ICH describes three methods to determine the limit of detection such as: visual evaluation, standard deviation of the signal based on the standard deviation of the blank and standard deviation on the signal based on the slope of the calibration curve [32, 33].

$$LOD = F*s/b,$$

where: F- constant, 3.3;

s – the blanck standard deviation;

b – the slope of the calibration curve;

Calculation of the blanck standard deviation of blanck and of the slope of the calibration curve resulted:

$$LOD = 3.3*0.00005477/0.1591$$

$$LOD = 0.001136 \text{ mg/L}$$

The limit of quantification is defined by the ICH as the lowest quantity of the analyte in a sample that can be quantitatively determined with accuracy and precision. This is used to determine the degradation products and impurities [32, 33].

$$LOQ = F*s/b$$

where: F- constant, 10;

s – the blanck standard deviation;

b – the slope of the calibration curve;

$$LOQ = 10*0.00005477/0.1591$$

$$LOQ = 0.003443 \text{ mg/L}.$$

In this study, both the limit of detection and the limit of quantification were calculated using the blank standard deviation and the slope of the calibration curve, varying only constant F.

Precision

Precision is defined by the ICH as the degree of dispersion between a set of values obtained by measuring multiple of the same homogeneous sample under specific conditions. This can be considered at three levels, such as: *repeatability, intermediate precision, and reproducibility* [32, 33].

Precision can be expressed as standard deviation (s, σ) or percentage relative standard deviation (RSD %)[34].

Repeatability

Repeatability was demonstrated by analyzing six replicates performed on the same day, the same analyst in the same working conditions, namely a concentration of 4 mg/L. In Table 3 are shown the obtained results from experiments conducted.

Table 3

Experimental results obtained from the analysis of samples performed to demonstrate the repeatability of the manganese determination method using UV-vis molecular absorption spectrometry, λ = 450 nm

Replicated measurements number	Measured signal, A (y_i)	x_i	$(x_i - \bar{x})^2, \cdot 10^4$
1	0.63482	4.084349	87,706
2	0.64966	4.177624	0,0014
3	0.65248	4.195348	3.009
4	0.65308	4.199120	4.46
5	0.6535	4.201759	5.644
6	0.65478	4.209805	10.114
		$\bar{x}$ =4.178	$\sum (x_i - \bar{x})^2 = 0.011093$
Standard deviation, s		0.007494 mg/L	
RSD		0.00179	
RSD%		0.179	

Intermediate precision

Intermediate precision was **demonstrated by using six identical samples that** were analyzed in three different days by the six different analysts, in the same laboratory and in the same working conditions, namely a concentration of 6.5 mg/L. In Table 4 are shown the obtained results from experiments conducted.

Table 4

Experimental results obtained from the analysis of samples performed to demonstrate the intermediate precision of the manganese determination method using UV-vis molecular absorption spectrometry, λ = 450 nm

Statistical parameter	Day 1	Day 2	Day 3
Average concentration [mg/L]	6.454285	6.491787	6.661890
Average absorbance	1.01188	1.017843	1.044907
Standard deviation, s	0.008302	0.004607	0.003569
RSD	0.001286	0.000709	0.000536
RSD%	0.1286	0.0709	0.0536

Accuracy

Accuracy is defined by ICH as the similarity between the accepted reference value or conventional true value and the value found [32, 33]. The accuracy can be demonstrated using different methods, such as:

- comparing the result obtained from the analysis of a sample of known concentration with the real value measurement;
- using the linearity and precision;
- by adding a known amount of active substance in a blank sample then analyzed and compared the results. This procedure is known as the recovery method [34].

In this study, we used the recovery method to demonstrate the accuracy of this method. The percentage recovery was calculated using the formula:

$$R\% = \frac{CF - CU}{CA} \times 100$$

where: CF - measured concentration of the sample fortified,

CU – substance concentration of the unfortified sample,

CA- concentration of the substance added to fortified sample [34, 16].

Table 5

Experimental results obtained from the analysis of samples performed to demonstrate the accuracy of the manganese determination method using UV-Vis molecular absorption spectrometry, λ = 450 nm

Sample	Manganese quantity in the initial sample (CU) (mg/g)	Manganese quantity added (CA) (mg)	Manganese quantity recovered in sample (CF) (mg/g)	Recovery degree (%)
5 ml	0.1840			
5 ml + 3 ml	0.1840	0.3	0.4601	92.03
5 ml + 6 ml	0.1840	0.6	0.7228	89.79
5 ml + 9 ml	0.1840	0.9	0.9718	87.53
5 ml + 12 ml	0.1840	1.2	1.3714	98.95
5 ml + 15 ml	0.1840	1.5	1.6819	99.86

4. Conclusions

In this study, emphasis was placed on establishing performance characteristics for the validation of the method for the determination of manganese through UV-Vis spectrophotometry. The developed method presents a good linearity on the selected range (0.5-6.5 mg/L). The method proved to be linear, precise and accurate and with a good LoD and LoQ.

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