Multivariate optimisation of a rapid and simple automated method for bismuth determination in well water samples exploiting long path length spectrophotometry

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An automated spectrophotometric system is proposed for the determination of bismuth in well water samples, using multi-syringe flow injection analysis (MSFIA) and exploiting a liquid waveguide capillary cell (LWCC). This method is based on the colorimetric reaction of bismuth and methylthymol blue (MTB) in the presence of polyvinylpyrrolidone (PVP) in acid medium (0.1 mol L^{-1} HNO_3). The Bi(III)–MTB complex was measured at 600 nm. The method was optimised by multivariate techniques. Some figures of merit of the proposed system are worth being highlighted, such as its wide linear working range (between 4.9 and 600 μg L^{-1}), its low detection limit (1.5 μg L^{-1} of bismuth) and its high intra-day precision and inter-day precision (0.7% (n = 12) and 1.4% (n = 5), respectively, both expressed as RSD). Moreover, a high injection frequency of 30 h^{-1} is achieved, as the proposed analyser is a powerful tool for fast Bi(III) determination. The method developed was successfully validated by analysing reference samples (pharmaceutical samples) by comparing the results with those obtained by ICP-OES and it was satisfactorily applied to well water samples. Besides, the present system is miniaturised allowing in situ measurements in control processes and field analysis.

1. Introduction

Bismuth is a trace element present in the environment as bismuthinite (Bi_2S_3), bismite (Bi_2O_3) and bismuth sulphide-telluric (Bi_2Te_2S), associated with minerals of lead, copper and tin [1]. The natural abundance of bismuth is 0.2 mg L^{-1} [2]. Bismuth levels in environmental matrices are usually in the parts per million (mg L^{-1}) or parts per billion (µg L^{-1}). In soil samples it is at 1 µg g^{-1}, in sediments in the range of 0.07–49.6 mg g^{-1} [3]. Bismuth compounds are used in cosmetics, nanomaterials, medical treatments and its radioisotopes for tumours treatment [4]. Bismuth is widely used as active ingredient in pharmaceutical preparations, e.g. in treatments for gastrointestinal diseases [5], given its low toxicity and the high therapeutic efficacy and few side effects of its compounds. Thus, given its widespread use, it is necessary to have fast and robust methods for bismuth determination, which can be used as control tools for bismuth monitoring.

Different methods for bismuth determination can be found in the literature, exploiting various detection techniques, such as thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS) [6], inductively coupled plasma optical emission spectrometry (ICP-OES) [7], inductively coupled plasma mass spectrometry (ICP-MS) [8], luminescence [1] and UV-vis spectrophotometry [9]. UV-vis spectrophotometric detection is an affordable technique that provides good precision, and the possibility to obtain miniaturised portable systems. Furthermore, long path length capillary cells permit us to increase sensitivity and improve detection limits. Liquid waveguide capillary cells (LWCCs) have been widely used to determine environmental contaminants at trace levels [13]. LWCCs are based on the use of a capillary with a lower refractive index than the liquid core contained in it, so the light introduced into the liquid core of the capillary is totally internally reflected down the capillary towards the detector, detecting as much of the optical signal as possible while minimising background noise [16].

A number of colour reagents have been employed for bismuth determination, e.g. methylthymol blue (MTB) [9], xylenol orange [10], *N*-(2-acetamide)iminodiacetic acid [17] and lead tetramethylenedithiocarbamate [18]. MTB has higher affinity for ions with higher charge, having greater affinity for ions with oxidation state (III) [19]. For instance, MTB can form complexes with aluminium (III) [20], zirconium, hafnium and bismuth [21] in acid medium. MTB can be used selectively for bismuth determination since the complex has different maximum absorbance’s wavelengths for the above-cited metals and also different reaction media are required, e.g. in the case of iron (III), the maximum of absorbance of the 1:2 Fe(III)–MTB complex is at the same wavelength as the bismuth (III) complex but it reacts at a different pH, i.e. pH 6 [22]. However, if samples with high iron content are analysed, further masking agents will be required to avoid the interference of the 1:1 Fe(III)–MTB complex.

Modern analytical strategies tend towards automation and miniaturisation. In this context, the use of flow analysis techniques for automation has provided a number of enhanced analytical methods affording high throughput, higher precision, lower reagents consumption and waste generation [23]. Spectrophotometric determination of bismuth has been previously automated using flow injection analysis (FIA) [25], sequential injection analysis (SIA) [9] and multi-commuted flow injection analysis (MCFIA) [10]. Multi-syringe flow injection analysis (MSFIA) combines the benefits of the aforementioned techniques, such as the multi-channel operation and high injection throughput of FIA, together with the robustness and versatility of SIA and MCFIA [23]. Therefore, we implemented a MSFIA-LWCC system for bismuth determination.

Multivariate experimental design combined with the concepts of multiple response treatments and desirability function permit simultaneous, quick and efficient optimisation. By exploiting multivariate optimisation techniques not only single factors affecting the response signal are studied but also interactions between them [27]. Furthermore, a global knowledge of the system is achieved involving a small number of experiments. As a general rule, when the experimental variables and the responses have been defined, the experiments can be planned and performed in such a way that a maximum of information is gained from a minimum of experiments. Moreover, a useful tool for multi-response evaluation, i.e. minimise the blank signal while maximising as much as possible the response signal, is the desirability function, which allows the simultaneous optimisation of more than one response, finding the best compromise for all of them [28].

Therefore, we present a simple and rapid method for bismuth determination in well water samples. The combining of multi-syringe flow injection analysis and LWCC makes of the present system a powerful tool for in situ bismuth determination, aiming to develop an environmentally friendly and low-cost bismuth analyser.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical grade reagents and Millipore quality water. Bismuth standards were prepared from a standard solution of 1000 µg L^{-1} of bismuth (Scharlau, Barcelona, Spain) by dilution in 0.1 mol L^{-1} HNO_3. Other reagents used were: HNO_3 (65% Scharlau, Barcelona, Spain), methylthymol blue (MTB) – sodium salt (Panreac, Madrid, Spain), polyvinylpyrrolidone (Fluka, Switzerland), aluminium nitrate nonahydrate (Merck, Darmstadt, Germany), EDTA, ascorbic acid, sodium fluoride (96%), hydrofluoric acid (48%), sodium chloride and iron nitrate nonahydrate (Scharlau).

The chromogenic reagent solution was prepared by dissolving the required amount of MTB in an aqueous solution containing 6 g L^{-1} of polyvinylpyrrolidone (PVP).

2.2. Samples

All samples were prepared in a final solution of 0.1 mol L^{-1} HNO_3.

The proposed method was applied to well water samples from Palma de Mallorca (Spain). In order to validate the proposed method, pharmaceutical samples were analysed. These contained different bismuth compounds such as bismuth subgallate (cream ‘synalar rectal’), bismuth gluconate (liquid-injectable solution ‘Labcatal’) and bismuth subcitrate (tablet ‘gastrodenol’).

Well water samples and the liquid-injectable solution did not require digestion these were just acidified prior being processed. Solid samples (tablet and cream) were weighed, tablets were ground by a mortar to homogenise, prior microwave digestion in a microwave oven (MLS-1200 Mega- Milestone). Thus, 0.2 mg of sample were mixed with 10 mL of nitric acid (65%) and the following microwave digestion programme was used: 6 min at 250 W, 6 min at 400 W, 6 min at 650 W, 6 min at 250 W and 10 min without power supply. The clear digests were heated again to near-dryness and diluted in 0.1 mol L^{-1} HNO_3 to the required volume for analysis.

2.3. Manifold and software

The MSFIA-LWCC spectrophotometric system for Bi determination is shown in Figure 1. The developed analyser is based on the use of a multi-syringe burette (BU4S; Crison Instruments, Barcelona, Spain), which can simultaneously handle up to four syringes. Each syringe has a three-way solenoid valve at the head (N-Research, Caldwell, NJ, USA), allowing the injection of the reagents and sample to the system only when required and their return to their reservoirs when not. This system used three glass

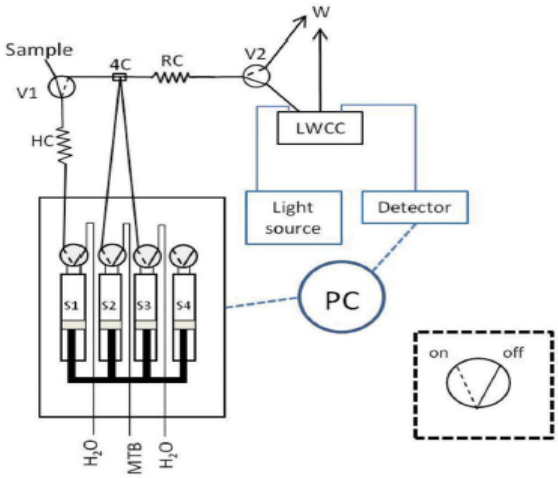


Figure 1. Schematic depiction of the developed MSFIA-LWCC bismuth analyser. 4C, four-way connector; HC, holding coil; LWCC, liquid waveguide capillary cell; PC, personal computer; RC, reaction coil; S, syringe; V, external solenoid valve; W, waste.

syringes of 10 (S1), 5 (S2) and 10 (S3) mL (Hamilton, Bonaduz, Switzerland). Syringe S1 is used for sample injection containing the carrier (H_2O), S2 dispenses the colorimetric reagent and S3 drives the reaction product to the detector. The system has two external three-way solenoid valves (V1, V2) (Takasago Electric Inc., Nagoya, Japan), which direct the flow in the desired way, i.e. on: to the detector; off: to waste.

Figure 1. Schematic depiction of the developed MSFIA-LWCC bismuth analyser. 4C, four-way connector; HC, holding coil; LWCC, liquid waveguide capillary cell; PC, personal computer; RC, reaction coil; S, syringe; V, external solenoid valve; W, waste.

The flow network was constructed with PTFE (polytetrafluoroethylene) tubing of 1.5 mm i.d. for reagents and sample load position, and 0.8 mm i.d. for the rest of the system. A holding coil (HC) with a volume of 1 mL was connected to S1 and used for sample loading. A reaction coil (RC) was used to mix the reaction plug. A four-way connector made of PMMA (polymethylmethacrylate) was also implemented.

The detection system was constituted by a miniaturised spectrometer USB 2000 (Ocean Optics, USA), connected to a computer via an USB interface coupled to a liquid waveguide capillary cell-LWCC (World Precision Instruments, Inc. FL USA) made from a 1 m type II Teflon AF (550 μm i.d., 100.0 ± 0.5 cm effective path length and 240 μL internal volume). Two optical fibres of 400 and 600 μm i.d. (Ocean Optics) were used to connect the deuterium–halogen light source (DH-2000, Ocean Optics) used, the LWCC and the detector.

Samples were also analysed by an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 5300DV, Perkin Elmer Inc.).

Instrumental control, data acquisition and processing were done with the software AutoAnalysis 5.0 (Sciware Systems, Bunyola, Spain). The distinctive feature of this software based on dynamic link libraries (DLLs) at 32 bits is the possibility of using a single and versatile application without further modification for whatever instrumentation and detection system needed.

Optimisation was made in a multivariate approach using the software Statistica 6 (Statsoft, USA).

2.4. Analytical procedure

The analytical protocol for bismuth determination is described in Table 1 and summarised as follows. First the tube of the liquid located between reservoir of the sample and V1 is conditioned and filled with the new sample to avoid crosscontamination. Then the sample is loaded into the holding coil (HC), with V1 in ‘off’. Afterwards, syringes S1 and S2 are activated (dispense in ‘on’ position) to simultaneously dispense and mix the sample and the colorimetric reagent solution in the reaction coil (RC). Finally, the mixture is pushed towards the detector with water (syringe S3 dispense in ‘on’) to be quantified.

2.5. Optimisation of experimental conditions

The experimental operating conditions of the developed method for bismuth determination were optimised through experimental design exploiting the computer statistic package Statistica. First, a screening method was applied to find the parameters affecting significantly the analytical response and to discard those with negligible effects upon it. Five variables were included in this first study, namely: the flow rate, the length of the reaction coil, concentration of MTB, volume of MTB and sample volume. The screening was carried out applying a 2^k full factorial design taking into account the effects of individual variables and their second-order interactions. The Pareto chart is commonly used to represent in a graphical way the standardised effect of each variable and their interactions. A red line sets the limit to consider if a variable has a significant effect upon the analytical response or not. Variables and interactions are ordered from the highest to the lowest significant, also showing the standardised effect in labels.

Once the variables with a significant effect upon the response signal were selected, a face centred central composite design including quadratic terms was performed.

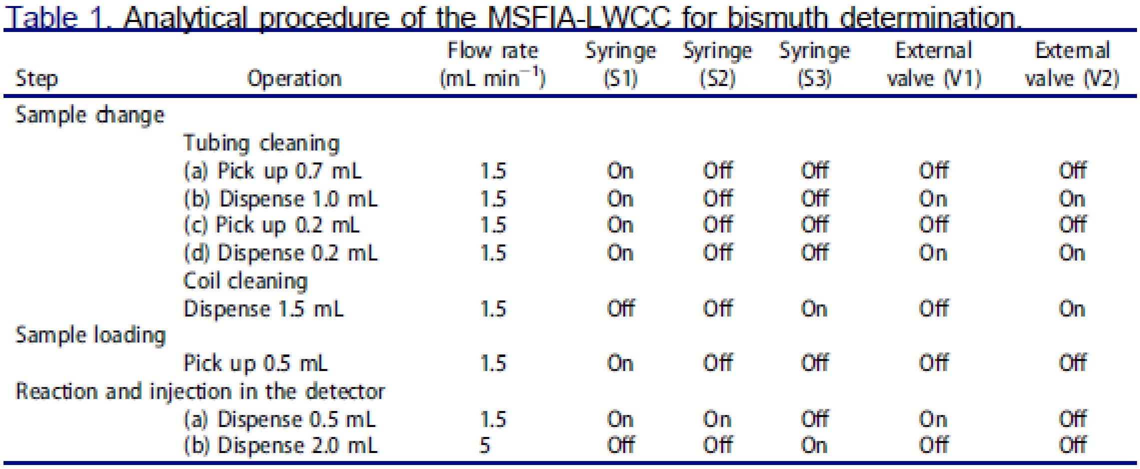


Table 1. Analytical procedure of the MSFIA-LWCC for bismuth determination.

Step	Operation	Flow rate (mL min^{-1})	Syringe (S1)	Syringe (S2)	Syringe (S3)	External valve (V1)	External valve (V2)
Sample change							
	Tubing cleaning						
	(a) Pick up 0.7 mL	1.5	On	Off	Off	Off	Off
	(b) Dispense 1.0 mL	1.5	On	Off	Off	On	On
	(c) Pick up 0.2 mL	1.5	On	Off	Off	Off	Off
	(d) Dispense 0.2 mL	1.5	On	Off	Off	On	On
	Coil cleaning						
	Dispense 1.5 mL	1.5	Off	Off	On	Off	On
Sample loading							
	Pick up 0.5 mL	1.5	On	Off	Off	Off	Off
Reaction and injection in the detector							
	(a) Dispense 0.5 mL	1.5	On	On	Off	On	Off
	(b) Dispense 2.0 mL	5	Off	Off	On	Off	Off

The desirability function was used in order to find a compromise between the lowest blank signal and the highest Bi(III)–MTB complex signal. The desirability function is a useful tool to treat more than one response and to find a compromise between them. This function, proposed by Derringer and Suich [28], transforms predicted values, i.e. calculated with the mathematical model equation, from each response into a dimensionless scale (0–1). The overall desirability is calculated by determining the geometric mean of individual desirabilities. Then goals are set for each individual response, i.e. 1 is given to the wanted response and 0 to the undesirable response.

All optimisation studies included three centre points. The ranges of the variables used in the experimental design are listed in Table 2.

3. Results and discussion

3.1. Chemical conditions and system set-up

MSFIA is a very versatile flow technique allowing parallel operations. Moreover, its coupling to LWCC provides lower detection limits improving the sensitivity of the developed method for bismuth determination. Furthermore, the developed MSFIA-LWCC system is compact and miniaturised, being portable for in situ measurements.

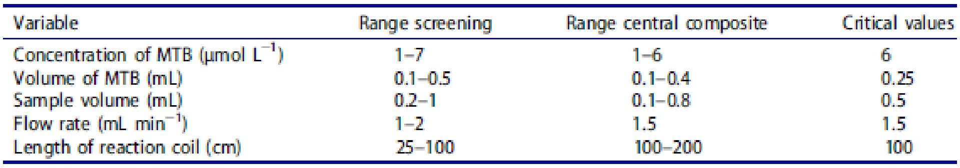
The absorbance of the coloured Bi(III)–MTB complex was measured at 600 nm. The Bi (III)–MTB complex can be measured at 548 and 600 nm; we measured at 600 nm because there was a greater difference between the blank and the complex signal. The correction wavelength used was 760 nm.

In previous works PVP was used to increase the solubility of the chromogenic reagent [9,29]. Thus, the effect of PVP was evaluated at two different wavelengths, i.e. 548 and 600 nm. As can be seen in Table 3, in the presence of PVP the colorimetric reaction showed increased sensitivity and bathochromic effect, showing a higher net absorbance at 600 nm and allowing the elimination of possible interferences. Thus, 6 g L−1 of PVP [9] were added in further studies. In previous works it has been reported that the formation of Bi(III)–MTB complex takes 5 s [9]; in this method the reaction time is studied depending on the length of the reaction coil.

3.2. Optimisation of the MSFIA-LWCC method for bismuth determination

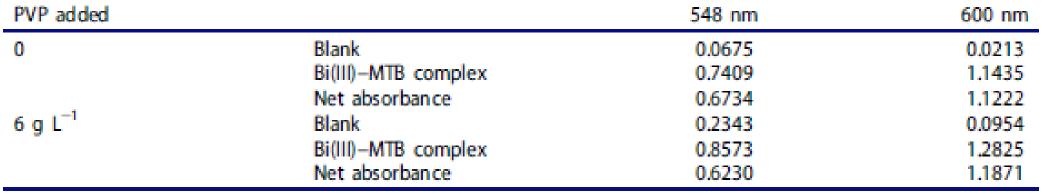
The experimental design was built in a dimensionless coordinate system using factor coding, wherein the highest and lowest levels are given as +1 and −1, respectively. For the screening, a full factorial 25 design was carried out with a total of 35 experiments

Table 2. Experimental domains and optimum values obtained by multivariate optimisation of the proposed method.



Variable	Range screening	Range central composite	Critical values
Concentration of MTB ($\mu mol\ L^{-1}$)	1–7	1–6	6
Volume of MTB (mL)	0.1–0.5	0.1–0.4	0.25
Sample volume (mL)	0.2–1	0.1–0.8	0.5
Flow rate ($mL\ min^{-1}$)	1–2	1.5	1.5
Length of reaction coil (cm)	25–100	100–200	100

Table 3. Study of the effect of polyvinylpyrrolidone (PVP) at two different wavelengths.



PVP added		548 nm	600 nm
0	Blank	0.0675	0.0213
	Bi(III)–MTB complex	0.7409	1.1435
	Net absorbance	0.6734	1.1222
6 $g\ L^{-1}$	Blank	0.2343	0.0954
	Bi(III)–MTB complex	0.8573	1.2825
	Net absorbance	0.6230	1.1871

including 3 centre points. Results showed that the five variables, their interactions except sample volume – flow rate, and the curvature were significant in the studied experimental domain using a three-way interaction model, as can be seen in the Pareto chart (Figure 2). However, the desirability function (Figure 3) was studied to observe the global effect of the variables upon the analytical signal and the flow rate showed a minimal effect on the signal. Thus, it was set at 1.5 mL min−1 to prevent overpressure in the system guaranteeing the maximum sample throughput. Ranges of the other four variables were modified according to results observed in the screening, i.e. the MTB concentration and volume were lowered in order to minimise the blank signal, the sample volume was also slightly modified because its increase affected the MTB-complex response more, and the RC length range was increased as a higher ratio (complex signal divided by blank signal) was attained at the highest length studied. Optimum values were obtained by a central composite design including quadratic terms (Table 2), performing a total of 27 experiments. The desirability function (d) was studied for the blank, the Bi(III)–MTB product and

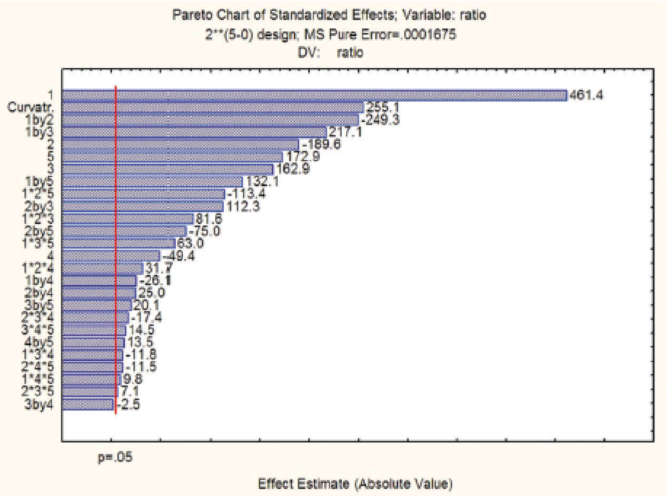


Figure 2. Pareto chart of the 2^5 screening for five variables analysing the ratio between the analytical response and the blank. The red line sets the limit to consider if a variable has a significant effect upon the analytical response or not (p = 0.05). 1, MTB concentration; 2, MTB volume; 3, sample volume; 4, flow rate; 5, RC length; X by Y, two-way interaction (representing X and Y, the interacting variables); X × Y × Z, three-way interaction (representing X, Y and Z, the interacting variables).

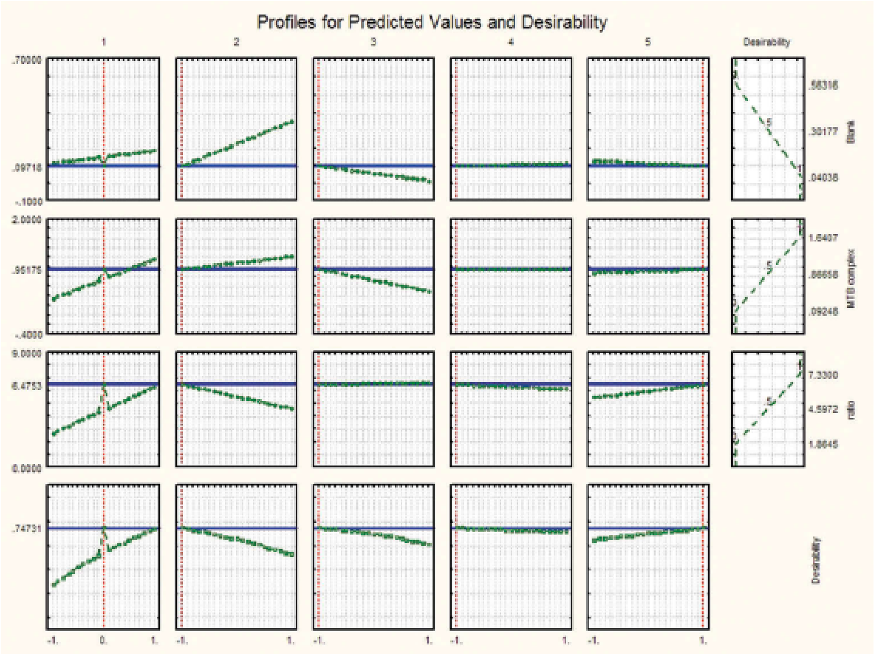


Figure 3. Desirability chart of the screening for the blank, the Bi–MTB product and the ratio between them. Plotting from left to right: the MTB concentration, the MTB volume, the sample volume, the flow rate and the RC length; and from up to down: the response signal for the blank, the Bi(III)–MTB complex, the ratio between the complex signal and the blank signal and finally the desirability function aiming to find a compromise between the lowest blank signal and the maximum complex signal. The red line shows the critical value for each variable according to the desirability. The green line shows the experimental data and the blue line the best value obtained according to the goals established. 1, MTB concentration; 2, MTB volume; 3, sample volume; 4, flow rate; 5, RC length.

the ratio between them (Figure 4). As stated before, we aimed to reach the highest net absorbance, so d = 1 was set to the highest complex signal, the ratio and the lowest blank signal. As can be seen in Figure 4, the sample volume showed a maximum for the ratio and the Bi(III)–MTB signal and a minimum for the blank at 0.15, codified value, corresponding to 0.5 mL. The MTB concentration showed a maximum positive effect at the highest level studied for the Bi(III)–MTB complex and for the ratio whereas the blank showed no significant differences in the studied range. The MTB volume and the reaction coil length had no significant effect upon the response signal in the studied range. However, the Bi (III)–MTB complex and the blank did increase with the chromogenic reagent volume. Thus, the ratio was studied and the central studied level was selected as the optimum value, since the blank does not significantly increase up to this volume. The optimum value for the reaction coil length was the minimum length studied, since the signal decreases when increasing its length due to dispersion of the reaction plug. Therefore, a 100-cm-long reaction coil was selected for further assays. Critical values of the studied variables are shown in Table 2.

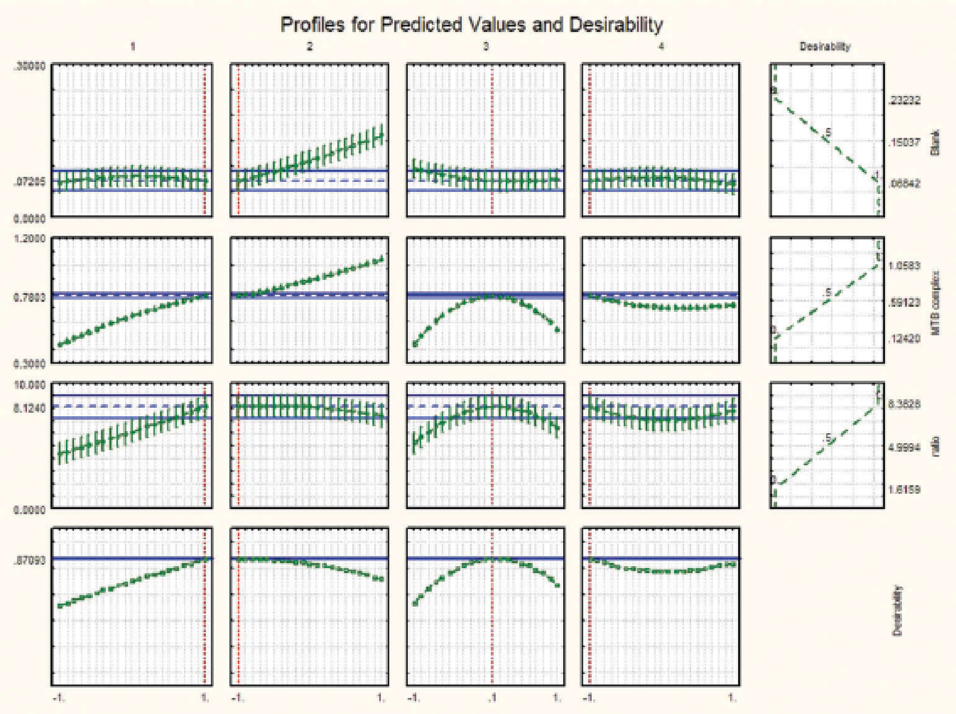


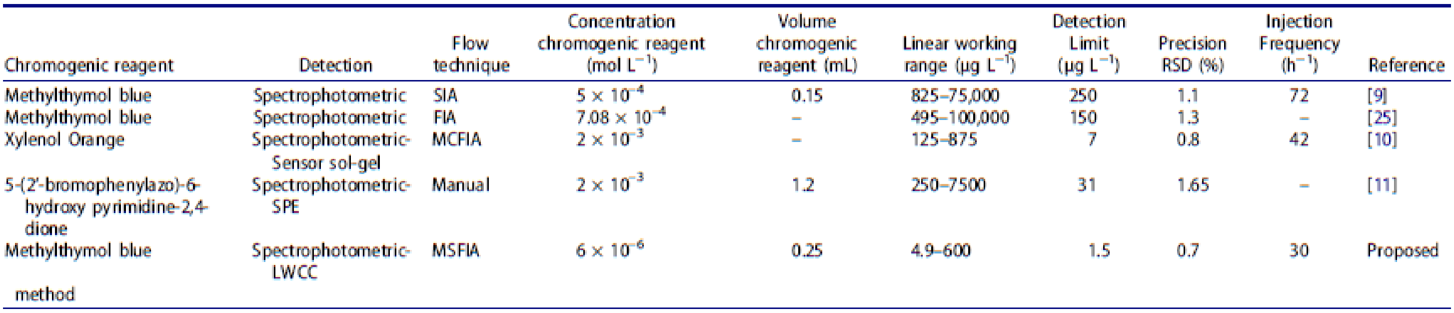
Figure 4. Desirability chart of the face centred central composite design for the blank, the Bi–MTB product and the ratio between them. Plotting from left to right: the MTB concentration, the MTB volume, the sample volume and the RC length; and from up to down: the response signal for the blank, the Bi(III)–MTB complex, the ratio between the complex signal and the blank signal, and finally the desirability function aiming to find a compromise between the lowest blank signal and the maximum complex signal. The red line shows the critical value for each variable according to the desirability. The green line shows the experimental data and the blue line the best value obtained according to the goals established. 1, MTB concentration; 2, MTB volume; 3, sample volume; 4, RC length.

3.3. Analytical parameters

In order to evaluate the performance of the proposed MSFIA-LWCC method, its figures of merit were studied.

The calibration curve for bismuth determination (net absorbance versus bismuth concentration in μgL−1) was obtained with a statistically satisfactory fit (y = 0.0018x + 0.0249, R2 = 0.9982, n = 8). Awidelinearworking rangewas accomplished, i.e. 4.9–600 μg L−1 and the limit of detection (LOD) obtained was 1.5 μg L−1 (Table 4). This LOD was calculated as three times the standard deviation of 10 replicates of the blank divided by the slopeofthe calibrationcurve,accordingto the IUPAC [30,31]. The intra-day precision was evaluated as the relative standard deviation (RSD) from 12 successive injections of 250 μg L−1 of bismuth and the inter-day precision was calculated from results obtained on different working days (n =5). Results were 0.7% and 1.4%, respectively. An injection frequency of 30 h−1 was attained with the proposed analyser.

Table 4. Comparison of the present method with previous method for Bi determination by spectrophotometric detection.



Chromogenic reagent	Detection	Flow technique	Concentration chromogenic reagent (mol L^{-1})	Volume chromogenic reagent (mL)	Linear working range (μg L^{-1})	Detection Limit (μg L^{-1})	Precision RSD (%)	Injection Frequency (h^{-1})	Reference
Methylthymol blue	Spectrophotometric	SIA	5×10^{-4}	0.15	825–75,000	250	1.1	72	[9]
Methylthymol blue	Spectrophotometric	FIA	7.08×10^{-4}	–	495–100,000	150	1.3	–	[25]
Xylenol Orange	Spectrophotometric-Sensor sol-gel	MCFIA	2×10^{-3}	–	125–875	7	0.8	42	[10]
5-(2′-bromophenylazo)-6-hydroxy pyrimidine-2,4-dione	Spectrophotometric-SPE	Manual	2×10^{-3}	1.2	250–7500	31	1.65	–	[11]
Methylthymol blue method	Spectrophotometric-LWCC	MSFIA	6×10^{-6}	0.25	4.9–600	1.5	0.7	30	Proposed

As can be seen in Table 4, the detection limit obtained is lower than that obtained by Amin et al.[11], exploiting solid phase extraction prior spectrophotometric detection reaching an LOD 20 times higher (31 μg L−1) than the one achieved with the proposed method. Also, the use of solid phase extraction (SPE) decreases the injection frequency.

Regarding other automated methods exploiting flow techniques and spectrophoto-metric detection, the consumption of the chromogenic reagent in the present method is lower or similar to that observed for other automated methods, as can be seen in Table 4. The LOD is 4.5 times lower than the one reported using an MCFIA method [10], 167 times lower than in the SIA method [9] and 100 times lower than in the FIA method [25].

Furthermore, the proposed method has a higher precision and is more miniaturised, given the small size of the spectrophotometer used. However, the injection throughput is lower than in previously reported methods because of the washing of the system to prevent contamination between samples and the lower flow rate used due to the LWCC. Nonetheless, the sensitivity increase is remarkable and the injection throughput is still high.

3.4. Interferences

As cited above, the MTB has higher affinity for ions with higher charge, having greater affinity for ions with oxidation state (III) [19]. MTB can form complexes with several metals but taking into account the medium and the absorption wavelength, only Al3+ and Fe3+ were selected as potential interfering ions since these could give a positive interference. Also, it has been reported that the ion chloride can produce a negative interference by masking the bismuth [9]. Thus, the effect of these interfering ions for the determination of bismuth was studied. The criterion for interference was established in±10% of the analytical response.

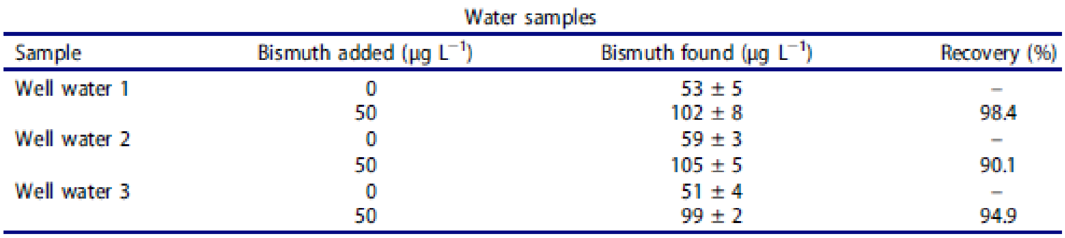
Aluminium concentration has been reported to be at 100 μg L−1 in well water [32]. Solutions containing 250 μg L−1 of bismuth were prepared, adding increasing amounts of the potential interfering ions. Al3+ and Cl– showed no significant interference in the concentration ranges of 0–1 and 0–60 mg L−1, respectively.

The concentration of iron in well water is between 1 and 2 mg L−1; this data was used as reference value to evaluate its interfering degree [32]. The ion Fe3+ presented interference at 200 μg L−1, requiring the use of ascorbic acid as masking agent. Thus, by adding 1 × 10−5 mol L−1 of ascorbic acid, the level of tolerance was raised up to 2mgL−1 of Fe3+.

3.5. Application of the developed MSFIA-LWCC method for Bi determination in well water samples and validation

The applicability of the proposed automated method was evaluated by analysing three well water samples from different locations in Palma de Mallorca (Spain). As can be seen in Table 5, a concentration of 50 μg L−1 was found in these samples and satisfactory recoveries were obtained in the add-recovery test with the proposed method.

Table 5. Analysis of well water samples with the proposed method.



Water samples			
Sample	Bismuth added (μg L^{-1})	Bismuth found (μg L^{-1})	Recovery (%)
Well water 1	0	53 ± 5	–
	50	102 ± 8	98.4
Well water 2	0	59 ± 3	–
	50	105 ± 5	90.1
Well water 3	0	51 ± 4	–
	50	99 ± 2	94.9

Moreover, in order to validate the present method, different reference samples (pharmaceutical samples) were analysed. These samples were analysed with the pro-posed system and by ICP-OES performing three replicates. Results are shown in Table 6. As can be seen, the t-values obtained were all below the critical value. Thus, no significant differences at the 95% confidence level were obtained between the values found by the proposed method and by ICP OES. Thus, these results together with the good recoveries achieved in well water samples validate the present method for bismuth determination.

4. Conclusions

A new low-cost and miniaturised analyser for bismuth determination exploiting multi-syringe flow injection analysis and long path length spectrophotometry has been developed. The use of multivariate optimisation combined with the desirability function allowed the effective optimisation of the proposed bismuth analyser. The automation of the proposed system by MSFIA provides a decrease of reagents consumption and makes of this method a potential, simple, fast and low-cost control tool for bismuth determination in well water samples.

One of the main advantages of the developed automated method is the miniaturisation achieved, which allows its in situ utilisation for monitoring purposes and in field measurements. Moreover, the methodology accomplished is more economical and environmentally friendly in comparison with previous automated methods for Bi(III) determination.

The applicability of the developed system as bismuth analyser in well water samples has been validated by its application to well water samples from Mallorca and the good results obtained in the add-recovery test. Furthermore, the method was successfully validated using reference samples.

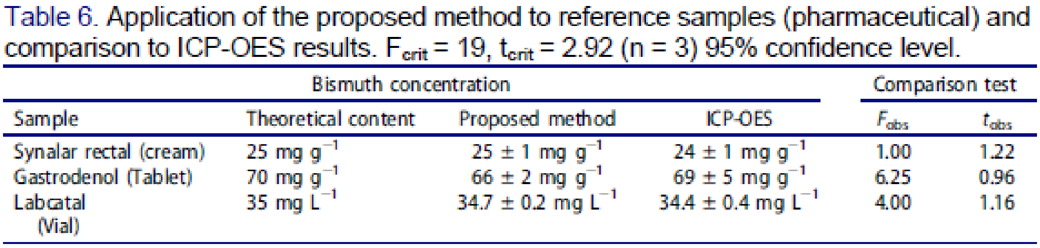


Table 6. Application of the proposed method to reference samples (pharmaceutical) and comparison to ICP-OES results. $F_{crit} = 19$, $t_{crit} = 2.92$ ($n = 3$) 95% confidence level.

	Bismuth concentration			Comparison test	
Sample	Theoretical content	Proposed method	ICP-OES	F_{obs}	t_{obs}
Synalar rectal (cream)	25 mg g^{-1}	25 ± 1 mg g^{-1}	24 ± 1 mg g^{-1}	1.00	1.22
Gastrodenol (Tablet)	70 mg g^{-1}	66 ± 2 mg g^{-1}	69 ± 5 mg g^{-1}	6.25	0.96
Labcatal (Vial)	35 mg L^{-1}	34.7 ± 0.2 mg L^{-1}	34.4 ± 0.4 mg L^{-1}	4.00	1.16

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] M.A. Taher, M. Rahimi and H. Fazelirada, J. Lum. 145, 976 (2014). DOI:10.1016/j. jlumin.2013.09.025.

[2] N. Yang and H. Sun, Bismuth: Environmental Pollution and Health Effects. Encyclopedia of Environmental Health (Elsevier, Burlington, 2011), p. 414.

[3] A.K. Das, R. Chakraborty, M.L. Cervera and M. De La Guardia, Trends Anal. Chem. 25, 599 (2006). DOI:10.1016/j.trac.2006.01.006.

[4] B.A. Fowler, D.W. Sullivan Jr and M.J. Sexton, Volumen II Specific Metals, Handbook on the Toxicology of Metals (Academic Press, Ámsterdam, 2015), p. 656.

[5] F. Cui, L. Wang and Y. Cui, J. Pharm. Biomed. Anal. 43, 1033 (2007). DOI:10.1016/j. jpba.2006.10.003.

[6] C. Zeng, L. Ji, C. Zhou, F. Zhang, M. Liu and Q. Xie, Microchem.J.119,1 (2015). DOI:10.1016/j. microc.2014.10.003.

[7] M. Sun and Q. Wu, J. Haz. Mat. 192, 935 (2011). DOI:10.1016/j.jhazmat.2010.11.044.

[8] K. Norisuye and Y. Sohrin, Anal. Chim. Acta. 727,71(2012). DOI:10.1016/j.aca.2012.03.042.

[9] P.D. Tzanavaras, D.G. Themelis and A. Economou, Anal. Chim. Acta. 505, 167 (2004). DOI:10.1016/S0003-2670(03)00017-5.

[10] P.C. Jerónimo, A.N. Araújo, M.B. Montenegro, D. Satinský and P. Solich, Anal. Chim. Acta. 504, 235 (2004). DOI:10.1016/j.aca.2003.10.049.

[11] A.S. Amin and I.A. Zaafarany, J. Taibah Univ. Sci. 9, 490 (2015). DOI:10.1016/j. jtusci.2015.01.003.

[12] G. Gumus, H. Filik and B. Demirata, Anal. Chim. Acta. 547, 138 (2005). DOI:10.1016/j. aca.2005.03.028.

[13] L.J. Gimbert and P.J. Worsfold, Trends Anal. Chem. 26, 914 (2007). DOI:10.1007/s00216-009-2745-5.

[14] F. Maya, J.M. Estela and V. Cerda, Anal. Bioanal. Chem. 394, 1577 (2009). DOI:10.1007/s00216-

009- 2745-5.

[15] J. Avivar, L. Ferrer, M. Casas and V. Cerda, Anal. Bioanal. Chem. 397, 871 (2010). DOI:10.1007/s00216-010-3600-4.

[16] R.N. Páscoa, I.V. Tóth and A.O. Rangel, Anal. Chim. Acta. 73,1(2012). DOI:10.1016/j. aca.2012.05.058.

[17] A. González-Portal, F. Bermejo-Martínez, C. Baluja-Santos and M.C. Diez-Rodríguez, Microchem. J. 31, 368 (1985). DOI:10.1016/0026-265X(85)90128-6.

[18] J. Szpunar, Anal. Chim. Acta. 251, 275 (1991). DOI:10.1016/0003-2670(91)87147-Y.

[19] E. Sandell, Colorimetric Determination of Traces of Metals (Interscience Publishers, New York, 1959).

[20] M. Sadaaki and Y. Takashi, Polyhedron 1, 405 (1982). DOI:10.1016/S0277-5387(00)80828-1.

[21] K. Cheng, Anal. Chim. Acta. 28,41(1963). DOI:10.1016/S0003-2670(00)87194-9.

[22] B. Karadakov, D. Kantcheva and P. Nenova, Talanta 15, 525 (1968). DOI:10.1016/0039-9140(68) 80126-2.

[23] V. Cerdá, L. Ferrer, J. Avivar and A. Cerdá, Flow Analysis; A Practical Guide (Elsevier Science, Ámsterdam, 2014).

[24] V. Cerdà, J. Avivar and A. Cerdà, Pure Appl. Chem. 84, 1983 (2012). DOI:10.1351/PAC-CON-

11- 11-14.

[25] D.G. Themelis, P.D. Tzanavaras and J.K. Papadimitriou, Analyst. 126, 247 (2001). DOI:10.1039/b008661o.

[26] V. Cerdà, J.M. Estela, R. Forteza, A. Cladera, E. Becerra, P. Altimira and P. Sitjar, Talanta 50, 695 (1999). DOI:10.1016/S0039-9140(99)00196-4.

[27] R. Leardi, Anal. Acta Chim. 652, 161 (2009).

DOI:10.1016/j.aca.2009.06.015.

[28] G. Derringer and R. Suich, J. Quality Technol. 12, 214 (1980).

[29] J. Hernandéz, B. Moreno, R. Carabias and L. Gutierrez, Microchem. J. 35, 288 (1987). DOI:10.1016/0026-265X(87)90112-3.

[30] G.L. Long and J. Winefordner, Anal. Chem. 55, 712 (1983). DOI:10.1021/ac00259a060.

[31] J.C. Miller and J.N. Miller. Statistics for Analytical Chemistry (Ellis Horwood, Englewood Cliffs, NJ, 1992).

[32] S. Pozdniakovaa, A. Padarauskasa and G. Schwedt, Anal. Chim. Acta. 351,41(1997). DOI:10.1016/S0003-2670(97)00331-0.