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Integrated calibration method (ICM) is one of the innovative approaches in the field of analytical calibration. ICM allows for only one estimation of analyte concentration in a sample. Furthermore, the signal obtained in a form of two overlapping peaks was difficult to interpret in some cases. Consequently, ICM gives a possibility to: a) verify analytical results in terms of accuracy, b) diagnose an examined analytical system in terms of interferences, and c) find an adequate way to eliminate the interference effect and to obtain results with improved accuracy. The presented work covers construction of a novel multicommutated flow manifold designed for realization of ICM, along with its operating rules. Accuracy and repeatability of the system were examined via spectrophotometric determination of chromium(III) and the obtained results were shown. Moreover, ICM realized with the use of the novel system was tested in terms of verification and elimination of systematic errors in the case of selenium determination with the use of hydride generation-atomic fluorescence spectrometry (HG-AFS), in the presence of copper as an interferent. The procedure was also investigated for three real samples containing selenium, namely: a diet supplement in a form of tablets, an energy drink and a natural thermal water.

**Introduction**

The vast majority of chemical analyses require calibration to be performed. Several calibration methods are known in analytical chemistry. They differ from each other in the way calibration solutions are prepared for measurements and the manner of interpretation of measurement signals. Definitions, terms and classifications of different calibration approaches have been presented in several papers\(^1\)\(^-\)\(^4\) and some novel calibration strategies aimed at overcoming the problem of analytical inaccuracy have been developed\(^5\)-\(^8\). However, each calibration procedure allows for only one estimation of analyte concentration in a sample.

Integrated calibration method (ICM) is one of the innovative approaches in the field of analytical calibration.\(^11\),\(^12\) Since ICM is based on combination of interpolative and extrapolative methods in a single calibration procedure, it enables to obtain more than one estimation of analyte concentration, the estimations being differently resistant to errors caused by interference effect.

Consequently, ICM gives a possibility to: a) verify analytical results in terms of accuracy, b) diagnose an examined analytical system in terms of interferences, and c) find an adequate way to eliminate the interference effect and to obtain results with improved accuracy (e.g. via utilization of gradual dilution of sample and standard solutions\(^12\),\(^13\)).

In general, ICM is based on three elements of different character: methodological – integration of different calibration methods, laboratory – addition of a standard to a sample and gradual dilution of sample and standard solutions, and instrumental – application of flow techniques. Flow analysis is recommended in order to make ICM procedure simpler and faster, as well as to improve precision of measurements.

In our laboratory several ICM flow manifolds have been hitherto developed, working in different modes.\(^11\)-\(^15\) They were tested in FAAS determination of calcium and magnesium in plant material\(^12\) and water\(^13\), as well as in spectrophotometric determination of iron in pharmaceuticals\(^11\) and chlorites in water\(^13\). The flow-injection system\(^12\),\(^13\) enabled us to perform ICM relatively fast and to obtain very precise and accurate analytical results, however it required relatively large volume of a sample. Furthermore, the signal obtained in a form of two overlapping peaks was difficult to interpret in some cases.

Calibration procedure with the use of the continuous manifold\(^14\) needed to maintain flow rates of four streams of solutions at the same value, which can be difficult in practice. The sequential system\(^15\), in turn, gave an opportunity to consume small sample and standard volumes, nevertheless the calibration procedure was very complex and time-consuming.

Also multicommutated systems have been exploited in flow injection analysis. Such systems are based on various modules fully operated by a computer and connected with each other in a properly designed network. Concepts and applications of multicommutated flow techniques for development of analytical methods have been presented in many papers\(^16\)-\(^18\). To overcome the afore-mentioned drawbacks, ICM procedure has been performed with the use of a multicommutated flow system. Such a highly developed manifold is simple and easy to operate, offering short analysis time and low consumption of reagents.
Principle of ICM

Application of ICM requires preparation of six calibration solutions according to the rule presented in Fig. 1. In the solutions, a sample and a standard are mixed with a diluent or with each other in two different degrees of P or Q, where P and Q are mutually complementary. R0 originates from a blank which is an additional solution.

Seven analytical signals (R0 + R), for all the calibration solutions, are obtained in a single calibration cycle, which allows four two-point calibration graphs to be constructed as presented in Fig. 2.

As can be easily seen, two of the apparent concentrations, c1 and c2, are obtained in an interpolative way, therefore they can be suspected to be systematically different from true analyte concentration in a sample, if an interference effect occurs. Concentrations c3 and c6 are found in an extrapolative way (initially as values c3' and c6', compare Fig. 1), and, consequently, they can be expected to be the most resistant to the interference effect. Two remaining values, c4 and c5, are also obtained extrapolatively, but in an untypical (namely semi-extrapolative) way, i.e. by extrapolation of the graph c along the graph d and the other way round.

Assuming that the calibration graphs constructed in accordance to ICM procedure are linear, analytical information obtained by ICM results can be interpreted in the following way:

a) when all apparent concentrations, c1 to c6, are statistically equal to each other, the interference effect does not occur; then the final analytical result, cST, is calculated as the arithmetic mean of concentrations c1 to c6;

b) when (c1 + c2)2 = c3 + c5, the interference effect of multiplicative character occurs and c0 is calculated as the arithmetic mean of concentrations c1 + c6;

c) when the apparent concentrations do not fulfill conditions a) and b), the interference effect of non-multiplicative character is expected; then a sample and a standard solution should be progressively diluted until either condition a) or condition b) is fulfilled;

d) when the apparent concentrations do not fulfill conditions a) and b) before and during dilution process, special reagent(s) eliminating interferences need to be added to the sample and/or to the standard solution, and the results of the repeated ICM procedure should be interpreted in accordance with points a) + c).

Experimental

Reagents and solutions

Stock solution of chromium(III) nitrate 200 mmol L⁻¹ was prepared by dissolving an appropriate amount of Cr(NO₃)₃·9H₂O (POCh, Gliwice, Poland) in water. Working solutions of a standard and a synthetic sample of chromium(III) were prepared by dilution of the stock solution with deionised water. Stock
solution of selenium 1000 mg L\(^{-1}\) was prepared by dissolving Titrisol\(^{b}\) standard (Merck, Darmstadt, Germany) in water. The working standard solution at the concentration of 100 µg L\(^{-1}\) was prepared directly before analysis by dilution of the stock solution with water. Stock solution of copper 1000 mg L\(^{-1}\) was prepared by dissolving Titrisol\(^{b}\) standard (CuCl\(_2\) in H\(_2\)O, Merck) in water. Working solution of a reducing agent, containing 2% (m/v) sodium borohydride (≥98.0%, SigmaAldrich, Germany) and 0.5% (m/v) sodium hydroxide (POCh), was prepared directly before analysis. Working solutions of 3 and 6 mol L\(^{-1}\) hydrochloric acid were prepared by dilution of 37% hydrochloric acid (Merck) with water. Thus obtained sample was subsequently diluted with 1% HCl (Merck) achieving Se concentration of 32.00 µg L\(^{-1}\). Eight portions of the energy drink á 0.500 mL were digested with 5.00 mL of concentrated HNO\(_3\) (Merck). The four digests were then transferred into a 50 mL volumetric flask and diluted to the mark with water. Thus obtained sample was subsequently diluted with 1% HCl (Merck) achieving Se concentration of 32.00 µg L\(^{-1}\). The multicommuted flow calibration manifold was coupled to an atomic fluorescence spectrometer AFS 830 (Beijing Titan Instruments Co., China) equipped with a flow hydride-generation system (HG-AFS). The hollow cathode lamp was operated at 100 mA. Argon was exploited as the shielded gas and the carrier gas at the flow rate of 800 and 300 mL/min, respectively. The readout time of AFS was set at 20 s.

In the case of the thermal water no pre-treatment was realized prior to Se determination, whereas the pills and the drink were digested with the use of Multiwave 3000 microwave system (Anton Paar, Austria) in the following conditions: 600 W of max. power, 12 min of ramp time, 20 min of hold time, 0.5 bar s\(^{-1}\) rate of pressure increase and 240 °C of max. temperature. After digestion, sample solution was cooled down in air to the temperature of 25 °C.

Four tablets were ground in a mortar and four portions á 0.4 g of thus obtained powder were digested with 5.00 mL of concentrated HNO\(_3\) (Merck). The four digests were then transferred into a 50 mL volumetric flask and diluted to the mark with water. Thus obtained sample was subsequently diluted with 1% HCl (Merck) achieving Se concentration of 32.00 µg L\(^{-1}\). Eight portions of the energy drink á 0.500 mL were digested with 5.00 mL of concentrated HNO\(_3\), merged with each other and diluted to 60 mL with 1% HCl, thus achieving Se concentration of 14.67 µg L\(^{-1}\).

**Instrumentation**

Lambda 25 UV/VIS spectrometer (PerkinElmer, USA) equipped with Hellma 178.010 flow cell (Hellma GmbH, Germany) was employed for analytical signals measurement at the wavelength of 590 nm in the case of chromium(III) determination. In selenium analysis, the multicommuted calibration manifold was coupled to an atomic fluorescence spectrometer AFS 830 (Beijing Titan Instruments Co., China) equipped with a flow hydride-generation system (HG-AFS). The hollow cathode lamp was operated at 100 mA. Argon was exploited as the shielded gas and the carrier gas at the flow rate of 800 and 300 mL/min, respectively. The readout time of AFS was set at 20 s.

The multicommuted flow calibration manifold dedicated to ICM has been schematically shown in Fig. 3. It was composed of two 3-inlet and four 2-inlet solenoid valves (01540-11 and 01540-11, ColeParmer, USA) and four peristaltic pumps Minipuls 3 (Gilson, France). The special electronic measurement system KSP-2 (SNV-212, PPC-62 and TM-232 modules) (KSP, Poland) enabled to control all units of the calibration system.
Valve controller (SNV-212) consisted of 12 outputs, output voltage 12 V, output current max. 500 mA, two work modes: normal and with reduced power (protection against heating of the valves). The device was connected with a computer by the parallel interface (LPT port), controlled by “Valve and Pump Controller” software (available at: http://debiany.pl/ksp/software.html).

The calibration manifold was coupled with both of the above-mentioned detection systems. Two-positional eight-port valve (PerkinElmer) and an additional peristaltic pump Minipuls 3 (Gilson) were utilized to connect it with the flow hydride-generation system (see Fig. 3). Tygon tubings were installed in peristaltic pumps and PTFE tubings (0.78 mm i.d.) were used for all connections, loops, coils and tubes.

**Operation of the calibration manifold**

Peristaltic pumps, \( P_1 \) and \( P_4 \), of the calibration manifold (see Fig. 3) propel streams of a sample solution, \( S \), and a standard solution, \( ST \), from reservoirs with the same flow rates, \( r \), to two injection loops of the same volume, \( \alpha \) and \( \beta \), respectively, and then to the waste. Peristaltic pumps, \( P_2 \) and \( P_3 \), propel streams of a carrier, \( C \), with two different flow rates, \( p \) and \( q \), through injection loops and then through tube \( L_1 \) or \( L_2 \) towards the detector. Two directional valves, \( V_1 \) and \( V_2 \), enable flow of a carrier through injection loops \( \alpha \) and \( \beta \) with flow rate of \( p \) or \( q \), as required. Both streams are merged with each other in tube \( L_3 \) and propelled with flow rate \( p+q \) to the detector.

Each calibration procedure consists of six steps. Each step is realized in the following sequence: 1) injection loop \( \alpha \) is filled with a sample (step: 1, 3, 5 or 6) by pump \( P_2 \), or with a carrier (step: 2 or 4) with the use of pump \( P_1 \); in the same time loop \( \beta \) is filled with a standard (step: 2, 4, 5 or 6) by pump \( P_4 \), or with a carrier (step: 1 or 3) by pump \( P_3 \); 2) segments of solutions are injected from loops \( \alpha \) and \( \beta \) with the use of pumps \( P_1 \) and \( P_2 \), with the flow rate of \( p \) or \( q \), respectively, and then mixed in coil \( L_1 \) with complementary degrees of \( P \) or \( Q \). Active positions of valves \( V_{1-6} \) and action of pumps \( P_{1-4} \) in individual cycles of the proposed calibration procedure according to the above description has been presented in Table 1.

**Table 1.** Active positions of valves \( V_{1-6} \) and action of pumps \( P_{1-4} \) in individual cycles of the proposed calibration procedure

<table>
<thead>
<tr>
<th>Cycle number</th>
<th>Sequence</th>
<th>Active valve position</th>
<th>Action of pumps</th>
<th>P signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 2 1 2 1 2 1 2</td>
<td>On On On On On On On</td>
<td>R4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 1 1 2 3 1 2</td>
<td>On On On On On On On</td>
<td>R1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 1 2 1 2 1 2</td>
<td>On On On On On On On</td>
<td>R3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 1 1 2 3 1 2</td>
<td>On On On On On On On</td>
<td>R6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2 2 1 2 2 1 2</td>
<td>On On On On On On On</td>
<td>R5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1 1 1 1 3 1 2</td>
<td>On On On On On On On</td>
<td>R2</td>
<td></td>
</tr>
</tbody>
</table>

In the consecutive six steps of the procedure, all six calibration solutions needed for ICM are prepared, as presented in Fig. 4. As a consequence, as many as six individual peaks can be registered and six analytical signals, \( R_1 \sim R_6 \), of different values can be accurately measured as peak height, as seen in Fig. 5. In the case of chromium determination, instead of HG-AFS system (Fig. 3), the spectrophotometer was directly coupled with the calibration manifold. The designed manifold, coupled with spectrophotometric detection, was used with the following instrumental parameters: \( \alpha = 500 \mu L \), \( L_1 = L_2 = 25 \text{ mm} \), \( L_3 = 1000 \text{ mm} \), \( p = 6.0 \text{ mL min}^{-1} \), \( q = 3.0 \text{ mL min}^{-1} \).

![Fig. 4. Pattern of merging standard, ST, and sample, S, segments flowing through tube L3 in order to produce signals, R1 + R6.](image)

![Fig. 5. Analytical signals, R5 + R6, registered with the use of the developed multicommitted manifold](image)

For selenium determination, the designed calibration manifold was connected with on-line hydride generation system and atomic fluorescence spectrometer, as presented in Fig. 3. In this case, each of the six steps of the procedure is followed with merging of previously mixed segments of a sample, a standard and/or a carrier with 6 M hydrochloric acid propelled with pump, \( P_5 \), and subsequent introduction into a holding loop, \( HL \), assembled on an injection valve, IV. Next, the valve is switched 45° to the right and a peristaltic pump, \( P_6 \), aspires the merged segments from the loop \( HL \). Calibration solutions are mixed with the reduction agent and the generated selenium hydrides are transported by argon to the detector. The whole peak formed by the calibration manifold is registered by atomic fluorescence spectrometer and its height is measured. The calibration manifold connected with hydride...
generation system worked with the following instrumental parameters: \( \alpha = \beta = 500 \mu L, L_1 = L_2 = 25 \text{ mm}, L_3 = 1000 \text{ mm}, L_4 = 600 \text{ mm}, H_L = 1500 \mu L, \rho = 4.0 \text{ mL min}^{-1}, q = 2.0 \text{ mL min}^{-1}, f_1 = 6.0 \text{ mL min}^{-1}, f_2 = f_3 = 4.0 \text{ mL min}^{-1} \).

## Results and discussion

Synthetic sample of chromium(III) at the concentration of 20 mmol L\(^{-1}\) was examined with the use of UV/VIS spectrophotometry. HG-AFS was employed for selenium determination in a synthetic sample containing 50 µg L\(^{-1}\) of this element. Solutions containing 40 mmol L\(^{-1}\) of chromium(III) and 100 µg L\(^{-1}\) of selenium were employed as standards. In both cases water played the role of a carrier. In a single analytical procedure performed with the proposed multicommutated flow system seven analytical signals, \( R_0 - R_6 \), were obtained allowing four calibration graphs to be constructed and the analytical result to be evaluated on the basis of six estimations of the analyte concentration, according to the formulas (1)-(6). Each sample was analyzed three times and the mean results have been presented in Table 2.

### Table 2. Results of application of the multicommutated ICM system to determination of Cr and Se in test samples with the use of UV/VIS spectrophotometry and AFS, respectively

<table>
<thead>
<tr>
<th>Analyte</th>
<th>apparent concentrations</th>
<th>( c_1 )</th>
<th>( c_2 )</th>
<th>( c_3 )</th>
<th>( c_4 )</th>
<th>( c_5 )</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr 20.00 mmol L(^{-1})</td>
<td>RE (%)</td>
<td>0.2</td>
<td>-0.3</td>
<td>-0.2</td>
<td>1.3</td>
<td>1.8</td>
<td>-0.7</td>
</tr>
<tr>
<td>Se 50.00 µg L(^{-1})</td>
<td>RE (%)</td>
<td>1.3</td>
<td>0.9</td>
<td>0.7</td>
<td>2.7</td>
<td>-0.3</td>
<td>2.5</td>
</tr>
</tbody>
</table>

In general, all the obtained results (including individual estimations and their mean values) were very close to each other \((c_1 = c_2 = c_3 = c_4 = c_5 = c = c_6)\) and to the true results \((|RE| < 2.7\%\) as the samples were free of interferents and the analyte was determined within linear analytical range. This testified the proper construction and operation of the designed flow system.

However, development of the calibration system with a manifold for hydride generation and coupling it with AFS deteriorated precision of determinations. In the case of UV-VIS detection RSD did not exceed 2.5%, whereas it was higher than 5% in the case of HG-AFS. It is fully understandable as both hydride generation and atomization in argon-hydrogen diffusion flame are dynamic processes characterized with increased random error.

In order to test analytical usefulness of ICM calibration in practice, two synthetic samples (S1 and S2) containing 50 µg L\(^{-1}\) of selenium with addition of copper as an interferent in the concentrations of 20 and 2 mg L\(^{-1}\), and three real samples: tablets containing selenium yeast and vitamin E (S3), energy drink enriched with selenium (S4) and thermal spring water (S5), were examined with the use of HG-AFS. Each sample was analyzed three times and the mean results have been presented in Table 3.

### Table 3. Results of application of the multicommutated ICM system to AFS determination of Se in synthetic samples (S1 and S2) in the presence of Cu as an interferent and in a natural samples (S3-S5)

<table>
<thead>
<tr>
<th>Expected conc., (µg L(^{-1}))</th>
<th>Dilution degree</th>
<th>Apparent Se concentrations found (µg L(^{-1}))</th>
<th>Final Se conc. (µg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 50.0</td>
<td>20000</td>
<td>1.0</td>
<td>27.28</td>
</tr>
<tr>
<td>S2 50.0</td>
<td>2000</td>
<td>1.0</td>
<td>39.55</td>
</tr>
<tr>
<td>S3 32.0</td>
<td>?</td>
<td>1.0</td>
<td>32.80</td>
</tr>
<tr>
<td>S4 14.7</td>
<td>?</td>
<td>1.0</td>
<td>10.72</td>
</tr>
<tr>
<td>S5 19.87</td>
<td>1.0</td>
<td>16.56</td>
<td>17.78</td>
</tr>
</tbody>
</table>

For the tablet sample, S3, all apparent concentrations, \( c_1 - c_6 \), were statistically equal to each other. According to ICM rules, in this case the interference effect was not present in this sample. The final analytical result, calculated as the arithmetic mean of all apparent concentrations, \( c_1 - c_6 \), was very close to the expected value.

The fact that the obtained estimations were different \((c_1 = c_2 \neq c_3 \neq c_4 \neq c_5 = c_6)\) for the synthetic samples, S1 and S2, and for the real samples, S4 and S5, enabled to diagnose the presence of systematic errors in these analytical systems. It should be noticed, however, that extrapolative estimations \( c_5, c_6 \) were close to each other and significantly different from interpolative estimations \( c_1, c_2 \), which according to the interpretation method in ICM is a sign of the presence of interference effect in the examined system. It was also observed that, as expected, the interference effect was much stronger with greater amount of copper in the synthetic sample.

As the condition \((c_1 + c_2)/2 = c_3 = c_6\) was fulfilled for both synthetic samples and the samples S4 and S5, the final analytical results were calculated as the mean values of estimations \( c_1 - c_6 \) and the multiplicative character of the interference effect was diagnosed in these cases.

The negative multiplicative effect achieved owing to the presence of Cu\(^{2+}\) in a sample took place during generation of selenium hydrides. Its mechanism is based on formation of Cu\(^{6+}\) that produces a dispersed metal colloid. Selenium hydride is then captured and decomposed in an irreversible way by the colloid.

The samples were also examined with the use of gradual dilution of a sample and a standard solution. Prior to introduction into the calibration system the sample and the standard were diluted in the same degree. Despite four-fold dilution of the sample S1, the size of the effect remained unchanged, whereas dilution of the sample containing 10-fold lower concentration of copper, S2, resulted in increased values of the interpolative estimations, proving at least partial compensation of the effect.
However, irrespective of dilution, accurate final result could be achieved via extrapolative estimations, c5 and c6, due to multiplicative character of the occurring interferences.

It may be suspected that strong negative interference effect observed in the sample of the energy drink, S4, is also caused in a similar way, since the analyzed drink contains (among many other minerals) 4 mg L\(^{-1}\) of zinc, which has very similar properties to copper. The influence of interferences on interpolative estimations, c1+c2, gets clearly weaker with gradual dilution of the sample. It should also be mentioned here that in this case the interference effect is not solely multiplicative, as the extrapolative estimations, c5+c6, for the undiluted sample are characterized with the relative error of ca. 7% and get accurate only after four-fold sample dilution.

In the case of thermal water sample, decrease in selenium signal in HG-AFS measurements may be caused by the presence of not only copper but also bromides, iodides and fluorides. Halides reduce selenium(IV) ions to elemental species and cause degradation of borohydride\(^{20}\). As seen in Table 3, the detected interferences could not be eliminated by gradual dilution of the sample. However, the analytical results calculated on the basis of estimations c5 + c6 at each dilution step were very similar to each other, which enabled to evaluate concentration with acceptable accuracy.

Conclusions

The performed experiments proved that ICM could be effectively performed with the use of the designed multicommuted flow injection system. The major advantage of the developed manifold over the previously developed systems (in CFA, FIA and SIA modules) is that it may provide single peaks for each calibration solution with simultaneous relatively low reagents consumption (4 mL for both standard and sample) and short time (ca. 6 minutes) in each calibration cycle. This gives the throughput of 10 samples per hour and the total volume of the generated waste of ca. 60 mL per calibration cycle. Moreover, in the case of detection methods which do not allow for continuous signal registration and signal registration in a form of single peaks is the only possibility to conduct measurements, the developed manifold is the best among the systems proposed so far for realization of ICM. Nevertheless, it should be noted that FIA system realizes the successive dilution procedure in an automatic mode,\(^{13}\) which is not possible in the case of the presented system.

The multicommuted calibration manifold may be easily used in connection with different chemical systems of sample preparation. Properly prepared calibration solutions may be introduced into the chemical part of the system and merged with reagents which enables to produce analytical signal in given instrumental conditions and, if necessary, the chemical system can be equipped with other modules (e.g. with on-line sample separation, preconcentration or hydride generation). Moreover, ICM worked well in terms of verification and elimination of systematic errors in case of selenium determination with the use of hydride generation-atomic fluorescence spectrometry (HG-AFS) in the presence of copper as an interferent, and in analysis of real samples. It thus allowed to perform analysis with simultaneous control and improvement of result accuracy.

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Notes and references