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Critical approach to flow injection gradient titration as a calibration method

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ABSTRACT

An attempt was made to demonstrate that flow injection gradient titration should not be considered as classical titration but rather as the indirect calibration method. This was shown experimentally by exploiting indirect spectrophotometric determination of chlorite ions in the presence of iron(II) in an acid environment with measurement of absorbance for the coloured Fe(II)/o-phenanthroline complex at 512 nm. In such an analytical system, the peaks of a cut-off profile were obtained with two characteristic points corresponding to equivalent amounts of analyte and reagent, and peak widths were used as the analytical signal. The only difference between the approach presented and the classical flow injection titration procedure was not fundamental but of a technical nature and consisted in the fact that a sample loop of relatively great volume instead of a mixing chamber was installed into the flow injection system. The analytical performance of the developed calibration procedure is also presented and discussed.

1. Introduction

Analytical calibration is a crucial stage of every analytical procedure irrespective of the instrumental technique applied. However, when reviewing the analytical literature, including academic textbooks, one notices that in fact there is no precise and clear terminology relating to this topic.

In International Vocabulary of Basic and General Terms in Metrology [1] "calibration" is defined as "...operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication". Basing on this general metrological definition the "analytical calibration" should be considered as establishing the dependence of the instrumental signal on the concentration of a substance determined (analyte) with the use of the standard solutions in order to transform the signal measured for a sample assayed into the concentration of the analyte in this sample. However, the common inappropriate approach is that the first step alone in the above definition is only perceived as being calibration. Another problem is that analytical is confused with calibration of an analytical instrument, i.e. with adjustment of a measuring system.

The great confusion exists also in nomenclature related to calibration methods: not only are different names used with reference to a given method, but they do not express the principles and the nature of the given method properly. The situation is becoming even worse with the rapid development of flow analysis, which is typified by many calibration methods with their own specific procedures and names [2,3].

Over the last decade, interest has grown in calibration itself as a fundamental domain of analytical chemistry. Metrological approaches to calibration have been presented, including such aspects as definitions, terms and classifications [4–9]. In accordance with the classification proposed by our group [6,9], each calibration method, independently of how it is conceptually and instrumentally realised, belongs to one of five groups given below. They are different from each other in terms of treatment of a sample and standard solutions and of measurement of the analytical signal:

- conventional method – the signal is measured directly for an unknown concentration of an analyte in a sample and for different concentrations of the analyte in standard solutions;
- indirect method – the signal is measured for a reactant added to both the sample and the standard solutions and remaining after reacting with the analyte in these solutions;
- internal standard method – the signal is measured for an inert substance (internal standard) added to both the sample and the standard solutions as well as for the analyte in these solutions;
- dilution method – the signal is measured for the analyte in the sample and in a single standard solution during progressive dilution of these solutions;
- titration method – the signal is measured for the analyte in the sample or for a reactant (titrant) during progressive addition of the sample to the reactant (or vice versa) evoking a reaction of well-known stoichiometry.

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It can also be shown that each of the first four calibration methods can be performed in two versions, depending on the preparation of the sample and standard solutions and, consequently, on the way of calculating the unknown analyte concentration in a sample, namely:

- interpolative version – when, in particular, the sample and standard solution(s) are prepared separately from each other, and
- extrapolative version – when the standard solution(s) are added to the sample in such a way that the analyte concentration in the sample is kept constant.

Taking the above classification into account, the most commonly used calibration method, usually called "the calibration curve method" or "the set of standards method", could be termed "the interpolative conventional method", whereas "the standard addition method" could be termed "the extrapolative conventional method". The remaining three (above-mentioned) methods are usually used in the interpolative version, but, in particular, the procedure of "the indirect extrapolative method" has been recently described and introduced into practice [10]. In such an approach all interpolative and extrapolative methods are characterised by own specific features (e.g. the "extrapolative" results are obtained with greater uncertainty than the "interpolative" ones).

According to the above classification, the titration technique is considered as a calibration method. The reason is very simple – as in the case of each of the other methods – titration needs a solution of known concentration (i.e. standard solution), which is a titrant, and the analyte concentration in a sample is found with the aid of a calibration graph, which is the titration curve. The main difference between titration and other methods is that the analytical result is calculated from a specific point (the end-point) of the calibration curve that corresponds to equivalent amounts of an analyte and titrant. As this point almost directly indicates the analytical result, titration can be exceptionally but consistently considered as a calibration method performed in the indicative version.

The calibration methods developed in flow analysis and described in the literature can easily be categorised using the above-presented classification. In almost all cases they represent either the conventional or the dilution method performed in both the interpolative and extrapolative version – some examples are presented in Table 1. However, the question is still open as to how to classify one of the oldest calibration approaches developed in flow injection analysis: gradient titration [21]. In this method, a sample is injected into a stream of titrant flowing continuously towards a detector (VIS spectrophotometer as a rule) through a well stirred mixing chamber. Due to the subsequent reaction, a peak of cut-off profile is produced, the width of which represents the analytical signal. The analyte concentration in the sample is then calculated from the calibration graph constructed with the use of a set of standard solutions.

There have been many discussions as to whether such a procedure can in fact be considered as "titration", i.e. whether it still follows the basic principles of classical titration. The basic issue was that, in contrast to the classical approach, the FIA gradient titration involves two simultaneous processes, namely chemical reaction and mass flow of the reagents, and, as a consequence, the reagents react in non-stoichiometric proportions. Moreover, it was revealed that cut-off peaks can be obtained without a reagent in the flow system, and therefore a chemical reaction is not in fact needed to obtain a calibration graph [22,23]. For these reasons, it was suggested that FIA experiments involving a gradient chamber and chemical reaction should be called "FIA pseudo-titrations" [24]. On the other hand, it was proved experimentally within only a few years after the first publication that all traditional titration techniques, i.e. acid–base, complexiometric, redox, and precipitation techniques, can be performed in the FIA mode. In such a situation even the authors of this concept admitted that "... the similarity of FIA and classical bath titrations is useful to recognise, because such recognition turns our attention to the wealth of chemistries exploited by classical titrations that are accessible to FIA adaptation" [25].

Regarding the classification of calibration methods, the crucial point is that FIA gradient titration is not of an indicative character, but it needs additional standard solutions similarly to the methods performed in interpolative or extrapolative versions. Indeed, because of the complex chemical and physical processes occurring in the injected sample zone, it is impossible in practice to mathematically model the cut-off profile and to calculate the analyte concentration in a sample using such a model directly [26]. This feature makes FIA gradient titration and classical titration quite different from each other.

Taking the above into account, in this research, the consistency between the FIA titration and another reaction-based calibration method, namely the interpolative indirect method, has been checked. For this purpose, an attempt was made to modify a typical procedure of FIA indirect method to the analyte determination in accordance with FIA titration conditions, i.e. producing cut-off peaks and using their width to construct a calibration graph. As an example, chlorite ions were spectrophotometrically determined on the basis of their reaction with iron(II) in an acid environment and of measurements of the coloured Fe(II)/o-phenanthroline complex at 512 nm.

2. Experimental

2.1. Reagents and solutions

Stock solutions of chlorites ($1.0\,g\,L^{-1}$), iron(II) ($0.5\,g\,L^{-1}$), ammonium chloride ($50\,g\,L^{-1}$), and sodium citrate (10%, w/v) were prepared by water-dissolving of an adequate amount of $NaClO_2$ (Carl Roth GmbH, Germany), $FeCl_2{\cdot}4H_2O$ (Sigma–Aldrich,

Table 1
Correspondence between flow calibration approaches and the methods termed in accordance with the proposed classification.

Flow calibration method	Ref.	New classification
Interpolative standard addition method	[11]	Interpolative conventional method
Zone sampling method	[12]	
Continuous dilution method	[13]	
Linear flow gradients method	[14]	
Merging-zones standard addition method	[15]	Extrapolative conventional method
Gradient scanning standard addition method	[16]	
Variable tube dimensions method	[17]	Interpolative dilution method
Network method	[18]	
Gradient ratio calibration method	[19]	
Gradient ratio-standard addition method	[20]	Extrapolative dilution method

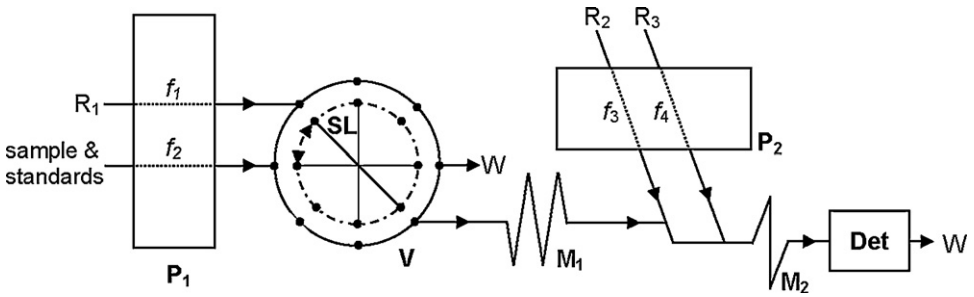

Fig. 1. Scheme of the flow-injection manifold used for the study: P_1, P_2 – peristaltic pumps; V – injection valve; SL – sample loop; M_1, M_2 – reaction coils (of volumes 0.50 and 0.25 mL, respectively); Det – UV–VIS spectrophotometer; R_1–R_3 – reagents (iron(II), o-phenanthroline, and sodium citrate, respectively); f_1–f_4 – flow rates (6.0, 6.0, 0.8, and 0.8 mL min^{-1}, respectively); W – waste.

Germany), NH_4Cl (POCh, Poland), and $C_6H_5Na_3O_7{\cdot}H_2O$ (Lach-Ner, Czech Republic), respectively. A solution of 0.25% o-phenanthroline was prepared by dissolving the required amount of solid o-phenanthroline (POCh, Poland) in 1% (v/v) HCl (Merck, Germany). Stock solutions of o-phenanthroline and sodium citrate were used without further dilution. Working solutions of chlorites, iron(II) and ammonium chloride were prepared by water-dilution of the stock solutions. All the chemicals used were of analytical grade and the solutions were prepared in deionised water.

2.2. Apparatus

The flow injection manifold used throughout the experiments consisted of two Minipuls 3 peristaltic pumps (Gilson, France), equipped with elastic Tygon tubes of various diameters, an eight-channel two-positional injection valve (PerkinElmer, USA), and a set of PTFE tubings of 0.8 mm i.d. (all connections, loops and mixing coils). The signals were measured with the use of a SPEKOL 11 UV/VIS spectrometer (C. Zeiss, Germany) equipped with a 0.018 mL flow cell of optical path length 10 mm (Zhaofa, China). The operation of the whole manifold was computer-controlled. The dedicated software, developed in our laboratory, served in the operation of instrumental modules (pumps and valve), data acquisition, signal visualisation and peak parameter measurement.

3. Results and discussion

The experiments were performed on the basis of the oxidation of Fe^{2+} ions to Fe^{3+} ions by chlorite ions in an acidic medium:

$$ClO_2^- + 4Fe^{2+} + 4H^+ = Cl^- + 4Fe^{3+} + 2H_2O \quad (1)$$

The analytical signal was measured at a wavelength of 512 nm for the coloured complex of Fe(II) ions with o-phenanthroline stabilised in a sodium citrate stream. Such a chemical system allows chlorite ions to be indirectly determined in water samples in the linear range of 0.03–1.00 mg L^{-1} [10].

The flow injection system designed to perform determinations is presented in Fig. 1. A sample of chlorites was injected from the sample loop (SL) into a stream of iron(II) solution (R_1) and directed to a reaction coil (M_1) where the reaction occurs. The remaining excess of iron(II) was merged with *o*-phenanthroline (R_2) and sodium citrate (R_3) and the coloured complex obtained in a reaction coil (M_2) was then transferred to the detector. The concentrations of solutions R_1, R_2, and R_3 were 5.0 mg L^{-1}, 0.25% (w/v) and 10% (w/v), respectively.

The designed flow injection system was used to construct calibration graphs in accordance with two slightly different procedures with the use of the same set of standard solutions (containing chlorites in concentrations from 0.0 to 1.0 mg L^{-1}). At first the standards were injected into the iron(II) solution from a loop of 0.4 mL, producing typical flow injection peaks. When they were measured in transmittance, the dependence of peak height on chlorites concentration was linear as presented in Fig. 2. Such a procedure was then consistent with calibration by the indirect method (ICM). If the standards were then injected from a loop of such great volume as 7.6 mL, cut-off peaks were produced of the same height but of different width. In this case, as expected, the signals (peak widths) were dependent non-linearly on the analyte concentration (see Fig. 2).

The latter obtained results can be easily explained (see Fig. 3). If the volume of the sample loop is relatively small, a given amount of an analyte (chlorite) is consumed totally by the reagent (iron(II)) and, consequently, a typical flow-injection peak is produced as the response of the remaining amount of the reagent. With an increasing volume of the loop, the peaks are higher and higher as a decreasing amount of the reagent has a chance to remain after the reaction with the same amount of the analyte (Fig. 3A). If the loop is sufficiently great, in the central part of the injection zone, the analyte is able to consume the reagent totally, hence the signal achieves 100% of transmittance, representing absence of the reagent in this part.

Thus, in the analytical system considered it was possible to produce a cut-off peak of two characteristic points, which correspond to equivalent amounts of the analyte and the reagent (at a given moment of the reaction). In order to do so, neither a reagent playing the role of an indicator nor a mixing chamber installed into the flow injection system was needed. When in such conditions an analyte of increasing concentration was injected into the reagent from a great loop, the amount of the analyte remaining after reaction with the reagent was greater and greater and the peak width (i.e. the distance between both points) increased (see Fig. 3B). All these features are typical for the FIA gradient titration (FIGT) procedure with a reagent playing the role of a titrant.

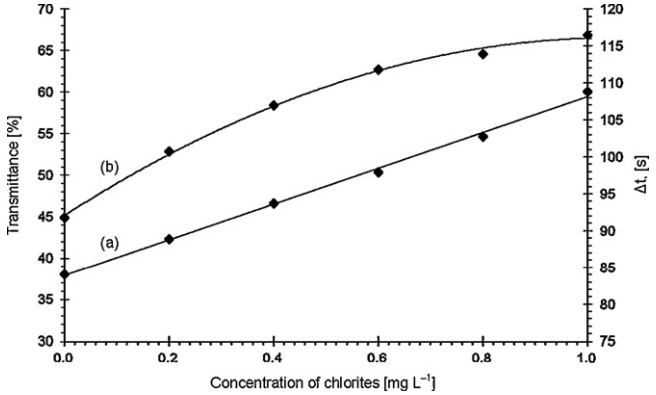

Fig. 2. Calibration graphs obtained in the analytical system considered using a sample loop of 0.4 mL (a) and 7.6 mL (b).

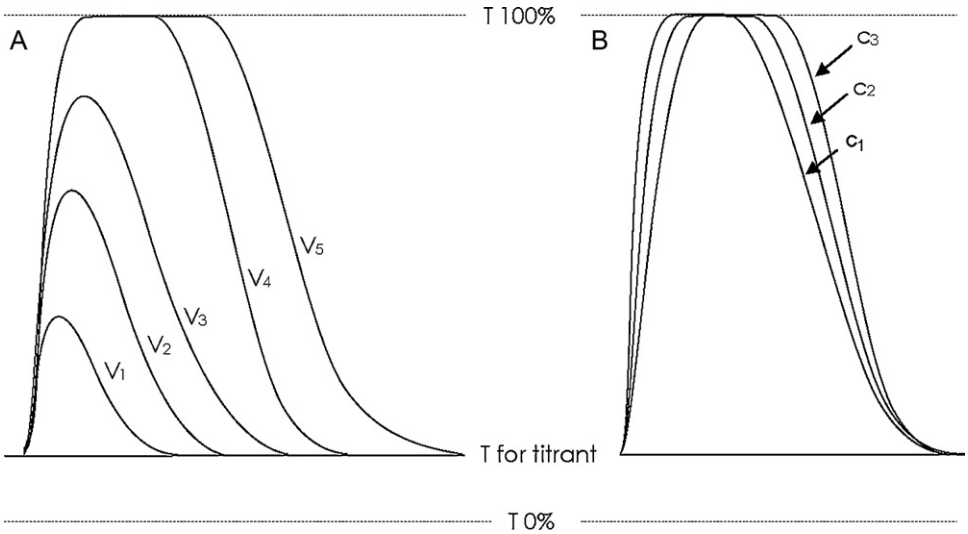

Fig. 3. The peak profiles obtained in the analytical system considered when changing: (A) the sample loop volume ($V_1 < V_2 < V_3 < V_4 < V_5$); (B) the analyte concentration ($c_1 < c_2 < c_3$) in sample injected from a loop of sufficiently great volume.

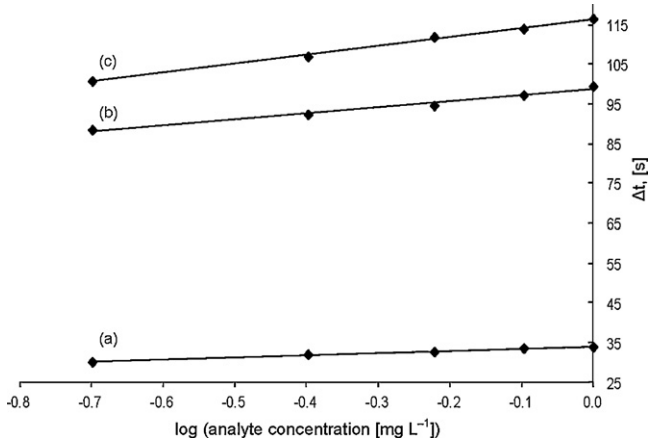

Fig. 4. Calibration graphs constructed in different instrumental conditions: (a) SL = 3.8 mL, f_1 = 6.0 mL min^{-1}; (b) SL = 7.6 mL, f_1 = 6.0 mL min^{-1}; (c) SL = 7.6 mL, f_1 = 4.0 mL min^{-1}.

As seen in Fig. 4, the calibration graphs obtained in accordance with the FIGT procedure were linear if the signals (peak widths) were presented versus logarithmic values of the analyte concentrations. The signal obtained for a given concentration could be controlled by instrumental parameters, especially by both the sample loop volume and the titrant flow rate. However, these parameters did not affect the sensitivity of the method to a great extent.

In order to compare both calibration approaches (ICM and FIGT), samples of natural spring water, named "Nałęczowianka" and "Żywiec" (both naturally free of chlorites), were analysed with chlorites added at well-known analyte concentrations. Some of them were additionally spiked with ammonium chloride, which was known to be a strong interferent in this analytical system [10]. Each sample was analysed three times using the flow injection system shown in Fig. 1 (equipped with sample loops of 0.4 and 7.6 mL, respectively). The sample compositions and the results obtained are presented in Table 2.

As revealed, the signals measured in peak-height mode (ICM) fluctuated only slightly less than those measured in peak-width mode (FIGT), but in both cases the random changes did not exceed 1% (RSD). However, the random error of the analytical results was found to be considerably grater for FIGT than for ICM. Thus, the conclusion is that FIGT is typified by much less sensitivity that ICM. On the other hand, FIGT was ascertained to be more resistant to the interference effect, giving slightly more accurate analytical results in the presence of the interferent than ICM.

Efforts were made to eliminate the interference effect by using the extrapolative version of both calibration approaches. To this end, sample Z3 (containing 0.4 mg L^{-1} of chlorites in the presence of the interferent, see Table 2) without and with known additions of ammonium chloride was measured in both the peak-height and peak-width mode and the calibration graphs obtained were extrapolated in their original forms (i.e. linear and non-linear, respectively) to blank values. The procedure applied in the latter case is presented in Fig. 5. The concentrations found (0.34 and 0.45 mg L^{-1}) were much more accurate than those obtained

Table 2
Concentrations of chlorites (c_x) and random changes of both analytical signals (RSDs) and analyte concentrations (RSD_c) obtained using indirect calibration method (ICM) and FIA gradient titration (FIGT) for analysis of Nałęczowianka (N) and Żywiec (Z) samples.

Sample	Analyte [mg L^{-1}]	Interferent [mg L^{-1}]	ICM			FIGT		
			c_x [mg L^{-1}]	RSD_s [%]	RSD_c [%]	c_x [mg L^{-1}]	RSD_s [%]	RSD_c [%]
N1	0.40	0.0	0.51	0.41	1.96	0.46	0.40	4.53
Z1	0.40	0.0	0.47	0.75	3.27	0.45	0.74	8.08
N2	0.00	500.0	0.00	0.15	–	0.08	0.92	–
Z2	0.20	500.0	0.09	0.25	5.10	0.15	0.83	8.20
Z3	0.40	500.0	0.17	0.64	5.43	0.26	0.85	8.77
Z4	0.60	500.0	0.26	0.27	2.09	0.39	0.76	8.45
Z5	0.80	500.0	0.37	0.33	1.90	0.47	0.68	7.53
Z6	1.00	500.0	0.46	0.36	1.76	0.54	0.74	8.47

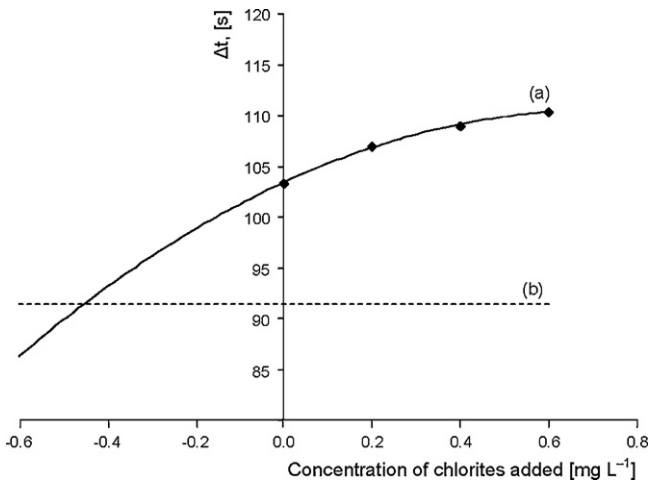

Fig. 5. Calibration graph constructed in accordance with the gradient titration procedure (a) extrapolated to analytical signal obtained for a blank (b).

using the interpolative version of both approaches (0.17 and 0.26 mg L^{-1}).

4. Conclusions

It has been proved that in UV/VIS spectrophotometry, calibration by the indirect method can be performed in a way that is typical for gradient titration, i.e. creating a cut-off peak with two characteristic points, which correspond to equivalent amounts of the analyte and the reagent, and measuring the analytical signal in the peak-width mode. The only difference between the titration procedure shown in this paper and those originally developed [21] was of a technical but not fundamental nature and consisted in the fact that a sample loop of relatively great volume but not a mixing chamber was installed into the flow injection system.

Application of the presented titration procedure seems to be very limited. The main reason is low sensitivity, which can be increased only slightly by changing instrumental parameters of the flow injection system. Nevertheless, the approach can be exploited in both the interpolative and the extrapolative version, giving results of improved accuracy in the latter version. From this point of view, it is a real calibration method and, as a consequence, it can be included into the classification mentioned above.

Perhaps each chemical system related conventionally to the indirect calibration method can be adapted in flow injection analysis to calibration by the titration approach presented here, provided that it is based on a well-known reaction producing an analytical signal in the UV/VIS range. The procedures are different from each other in terms of an instrumental parameter only, namely the volume of the sample loop. Thus, the conclusion is that there is no principle difference between calibration by the indirect method and gradient titration in flow injection analysis. Therefore – bearing in mind all essential reservations (presented previously) in relation to FIA gradient titration – it is proposed to consider this kind of titration as an indirect calibration method, but not as real titration.

The above conclusion is a contribution to the general question of logical and coherent nomenclature in analytical chemistry and, specifically, in the calibration domain. A lack of such nomenclature can become a source of numerous misunderstandings and obscurities, which, as a result, can lead to the adoption of an improper or totally fallacious analytical procedure. The situation is also unfavourable when it comes to teaching, since it is difficult to credibly convey analytical knowledge on the basis of a particular textbook or academic script when using language which is not only felt as incorrect, but which is also distinct from that which can be found in other generally available sources. Finally, one ought to remember the psychological aspect: analytical chemists are (or at least should be) particularly sensitive to 'neatness' and 'order', regardless of which field of analytical chemistry the terms refer to.

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