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A CONTINUOUS-DILUTION CALIBRATION TECHNIQUE FOR FLAME ATOMIC-ABSORPTION SPECTROPHOTOMETRY

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Summary.—Calibration in atomic-absorption spectrometry is achieved by means of a concentration-gradient chamber using a single concentrated standard solution. Calibration is rapid and extends over the entire working concentration range of the analyte, irrespective of the shape of the conventionally obtained calibration curve. No curve-fitting approximations are involved. With well-designed apparatus, deviations are less than 1%.

In most instrumental analytical techniques no absolute mathematical relationship exists between the magnitude of the quantity observed and the quantity of determinand. The relationship is generally a complex function of instrumental parameters which rarely have long-term stability, and the chemical environment of the analyte. The latter often has a profound effect on instrument response, so it is then not possible to ignore the chemical matrix when relating signal to analyte. Accordingly, most instruments require calibration immediately before analytical determinations are made. This is certainly true of atomic-absorption spectrometry. Variation in cleanliness, optical alignment, and performance of the line-source, causes day-to-day changes in instrument response. During operation, baseline drift may arise from the gradual stabilization of electrical circuits and variations in source intensity, whilst overall sensitivity may be influenced by changes in nebulizer efficiency, sample flow-rate, and flame chemistry (resulting from changes in fuel-to-oxidant ratio and sample matrix). Certainly, whenever such operational parameters are changed, recalibration is necessary.

Calibration in atomic-absorption spectrometry is usually done by stepwise dilution of a concentrated stock solution to produce a range of standard solutions for analysis. The resulting set of points on the plot of absorbance *vs.* concentration is used as the basis of the calibration curve. In many modern instruments, a microcomputer fits an equation to the calibration data. Thus the development of micro-processor technology has allowed the calibration data-processing step to be incorporated within the instrument itself. The preparation of standards, however, remains a tedious and time-consuming task which greatly reduces sample throughput. Automated calibration is therefore an attractive prospect for atomic absorption.

In considering prospects for automated calibration procedures, the so-called "flow-injection" techniques¹ developed over the last eight years or so, look

promising. The basis of flow injection is the controlled, reproducible, flow-induced dispersion obtainable when a plug of sample is injected into a reagent carrier stream as it flows through narrow-bore tubing. The changing concentration profile of the sample solution may be monitored by downstream detectors, though usually it is the concentration of reaction product that is monitored as the basis of the analysis. The modification, by stages, of the sample concentration profile, from rectangular, through skew peak, to Gaussian peak, has been described by several authors.¹⁻³ Růžička and Hansen⁴ defined the dispersion, D , by the equation:

$$D = C_m/C_p \quad (1)$$

where C_m is the concentration of the injected sample and C_p is the concentration at the peak maximum.

These authors have described the dependence of dispersion on flow-rate and tube dimensions. The degree of dispersion may thus be preselected to match the requirements of a particular analytical procedure. The versatility and reproducibility of the dispersion process were such that Růžička and Hansen⁴ were able to state, "We can replace beakers, pipettes and volumetric flasks with small open-ended tubes through which we pump the solutions." This is just the basis required for the automation of calibration procedures and it should be possible to produce a range of standards from a single stock solution by varying the dispersion to which it is subjected. Systems based on this concept are presently being studied.⁵

Methods of producing concentration gradients by using mixing-chambers and differential flow-rates have been described by Block and Ling.⁶ Early work with continuous-flow titration techniques employed magnetically-stirred mixing-chambers.⁷ Whilst the predictable, reproducible performance of mixing-chambers is well-known and understood, certain associated properties, notably sample hold-up and excessive sample dispersion, render them unsuitable

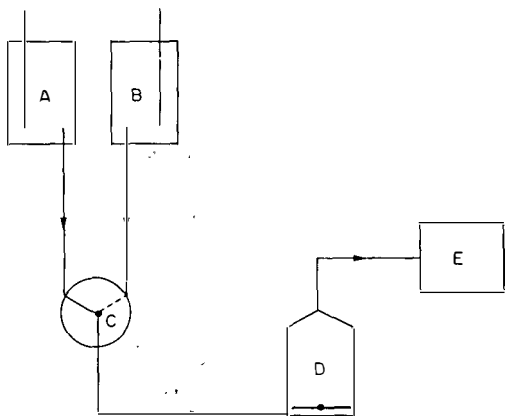


Fig. 1. Schematic diagram of basic apparatus for generating an exponential concentration gradient. A and B are constant-head reservoirs (Marriott bottles) containing water and concentrated stock solution, respectively. C is a rotary valve allowing rapid switching between one solution and the other. D is the mixing-chamber. E represents the detector and recording system.

for general use in flow-injection systems.² Their elimination was brought about by a recent return to a true flow-injection technique⁸ wherein the mixing-chamber function was achieved by means of a carefully designed flow manifold equivalent to one theoretical mixing stage. The removal of the mixing-chamber enabled the realization of high-speed continuous-flow titrations.

Electrochemists studying continuous-flow titrations have employed concentration-gradient programming, achieved by the merging of a constant-flow carrier stream with either a constant-flow reagent stream of increasing concentration⁹ or a reagent stream of constant concentration and increasing flow-rate. The practice of making measurements on alternately increasing and decreasing concentration profiles has been called the "Triangle-Programmed Titration" technique.¹⁰

Mixing-chambers have also long found favour amongst electrochemists,¹¹ who continue to defend the merits of the device.¹² Similar arguments may be advanced for the application of the mixing-chamber gradient technique to atomic-absorption calibration, namely, precise, reproducible performance described by exact mathematical equations readily related to the system parameters, and a smooth concentration profile compatible with the relatively slow response characteristics of the detection system, together with the fact that all this is achieved with simple, readily available apparatus.

The basic system, shown in Fig. 1, consists of a water-filled ideally-stirred tank into which a stream of stock solution is switched to produce an effluent concentration which is an exponential function of the time elapsed after switching. The use of this system for continuous-flow atomic-absorption calibration has previously been suggested.^{13,14} In this paper, the

results of a preliminary study of the system represented in Fig. 1 are reported. The objectives were to assess the viability of such a calibration technique and to identify the experimental conditions necessary for its successful application.

PRINCIPLE OF THE METHOD

An arrangement of the type illustrated in Fig. 1 is used to generate an exponential concentration-time profile of the type described by the equation

$$C = C_m [1 - \exp(-ut/V)] \quad (2)$$

where C is the effluent concentration at time t after the start of the concentration gradient, C_m is the concentration of stock calibrant solution, u is the flow-rate, and V is the volume of the mixing-chamber.

This equation may be derived from a consideration of the mass balance in the mixing-chamber (see *e.g.*, Danckwerts¹⁵ or Levenspiel¹⁶). The effluent from the mixing-chamber is led directly to the nebulizer of an atomic-absorption spectrometer, and the resultant absorbance-time profile is recorded with a rapid-response chart-recorder. Since in atomic-absorption work absorbance is generally not a linear function of concentration, this curve soon deviates from its initial simple exponential form, as shown in Fig. 2.

Used in conjunction with equation (2), Fig. 2 is a continuous calibration plot relating absorbance to concentration. In practice, instead of relabelling of the time axis, the absorbance-time profile is generated and then the sample is admitted directly to the nebulizer at the same flow-rate as the calibrant and its absorbance is noted. By interpolation (see Fig. 3), a corresponding value of t is obtained which is substituted into equation (2), giving the concentration of the sample. For fixed instrument settings, in the absence of interferences, absorbance is a function of only the concentration and flow-rate. Thus, if the absorbances of sample and calibrant (measured at

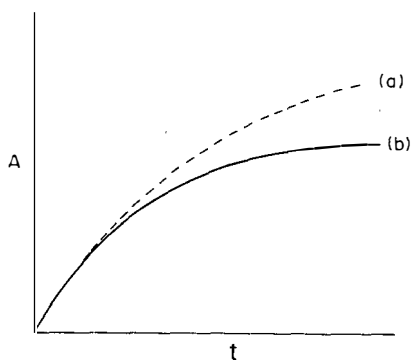


Fig. 2. Absorbance-time profile. Curve (a) corresponds to a hypothetical profile $A = A_m [1 - \exp(-ut/V)]$ where A is the absorbance at time t , and A_m the absorbance corresponding to C_m if the sensitivity were independent of concentration. Curve (b) is the profile generally observed, *i.e.*, curve (a) modified by instrument response.

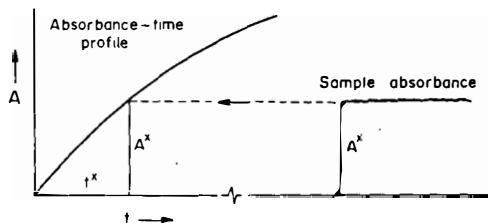


Fig. 3. Interpolation of sample absorbance on absorbance-time profile to find the characteristic time value for the sample, t^x .

the same flow-rate) are equal, their concentrations are equal and

$$C_x = C_m[1 - \exp(-ut^x/V)] \quad (3)$$

where C_x is the concentration of the sample and t^x is the time found by interpolation from the absorbance-time profile.

However, in common with other flow techniques, the creation of practical conditions under which equation (3) is valid requires careful attention to methodology. The minimum requirements are that the assumptions made in the derivation of equation (3) are valid, namely,

- (a) that mixing-chamber performance is ideal, *i.e.*, it conforms with equation (2);
- (b) that the flow-rate may be accurately determined and remains constant during the recording of both calibration and sample absorbances;
- (c) that no instrumental drift occurs between the recording of the calibration and sample absorbances;
- (d) the starting time of the absorbance-time calibration profile may be accurately located.

In addition, minimizing the length of connecting tubing, ensuring that fittings are well-matched and polished, minimizing pump noise by operating at maximum speed and minimum roller pressure, and avoiding air-bubbles, are reported¹⁷ to have a beneficial effect on precision.

EXPERIMENTAL

Apparatus

Mixing-chamber. This was constructed from borosilicate glass, as a sealed unit in the form of a vertical cylinder (diameter 2 cm, maximum height 3 cm) with a slight dome to allow the escape of air bubbles. The volume (determined by filling with water and weighing) was 8.40 ml. The inlet and outlet guides were slightly tapered to allow a push-fit of the 0.58 mm bore PTFE tubing inside a sleeve of 1.14 mm bore PTFE tubing (Corning Ltd., Laboratory Division, Stone, Staffs.). A soft-iron magnet-follower was constructed from a pin (13 × 0.5 mm) sealed inside a short piece of the 0.58 mm bore PTFE tubing.

Investigation of mixing-chamber performance. The apparatus is represented in Fig. 1, with the carrier and stock calibrant solutions gravity-fed from two constant-head vessels. The valve was a Rheodyne type 5011 six-position rotary valve and the instrument used was a Varian 634 S spectrophotometer fitted with a home-made flow-cell measuring 50 × 2 × 1 mm, mounted vertically with the largest

faces normal to the light-beam. An identical cell, filled with distilled water, was mounted in the blank cell compartment of the instrument. The instrument response was monitored by a J. J. 100 chart-recorder (J. J. Lloyd Instruments Ltd.).

Solutions

Copper(II) sulphate pentahydrate (16 g/l.) in distilled water. Magnesium, nickel and chromium, 1000-ppm stock solutions (B.D.H. Chemicals Ltd.).

Procedures

Mixing-chamber performance. With conventional cuvettes it was established that solutions of copper sulphate pentahydrate exhibited an absorption maximum at 808 nm and obeyed Beer's law up to concentrations of at least 16 g/l. A range of flow-rates was obtained by varying the head of liquid between sample reservoir and waste outlet. Flow-rate was measured by averaging the volumes of effluent collected during six 1-min intervals. Seven absorbance-time growth profiles were recorded at different flow-rates over the range 3.6–8.2 ml/min.

Atomic-absorption calibration. The apparatus is shown schematically in Fig. 4. Solutions of magnesium, nickel and chromium were used. Their concentrations and the respective instrument settings employed are shown in Table 1. Magnesium was chosen for its high sensitivity and for the limited curvature of its conventional calibration plot, nickel as an element exhibiting pronounced curvature of the calibration plot, and chromium because of its reported calibration irregularities.¹⁸

The absorbance-time growth profiles were generated by using the mixing-chamber, and the time taken for 10 ml of calibrant to be consumed from the reservoir was recorded simultaneously. The mixing-chamber was then "short-circuited" by making the direct connection Z (Fig. 4), and the samples were introduced through S without changing the flow-rate. Data from the chart were analysed with the aid of a microcomputer.

RESULTS AND DISCUSSION

Mixing-chamber performance

Assuming ideal mixing-chamber performance, the growth of concentration within the chamber is ex-

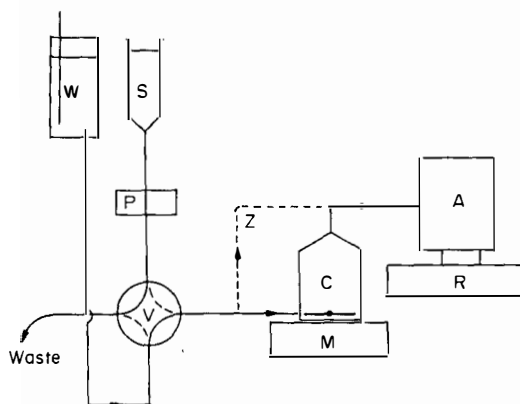


Fig. 4. Apparatus for instrument calibration. A is a Shandon Southern A3300 atomic-absorption spectrometer, C the mixing-chamber, M a Gallenkamp model SS615 combined magnetic stirrer and hot-plate, P a Gilson Minipuls 2 peristaltic pump, R a Tekmann TE200 chart-recorder, S a 10-ml Teflon non-wetting syringe barrel as sample reservoir, V an Altex model 201-25 eight-port injection valve, W a constant-head reservoir of distilled water, and C and A were connected by 7.5 cm of 1.14 mm bore tubing, and C and A by 5.5 cm of 0.58 mm bore tubing.

Table 1. Solutions and instrument settings employed for atomic-absorption calibration

Setting	Magnesium	Nickel	Chromium
Monochromator, nm	285.2	232.0	357.9
Band-pass, nm	0.18	0.1	0.1
Lamp current, mA	3.5	5.0	3.0
Air flow, arbitrary units	8.0	8.2	8.65
Acetylene flow, l/min	2.8	2.8	5.0
Sample concentration, ppm	0.25-2.0	10-75	2-20
Calibrant concentrations, ppm	2, 5, 10	100, 200	25, 50, 75
Flow-rate, ml/min	8.4	8.5	8.4

Table 2. Investigation of mixing-chamber performance by solution spectrometry; data from plots of $\ln[A_m/(A_m - A)]$ against t

Flow-rate, ml/min	Gradient, min ⁻¹	Correlation coefficient	V (calculated), ml
3.56	0.403	0.99994	8.8
4.48	0.505	0.99998	8.9
5.07	0.599	0.99999	8.5
5.68	0.630	0.99996	9.0
6.18	0.709	0.99998	8.7
6.72	0.764	0.99997	8.8
7.50	0.886	0.99999	8.5

pressed by equation (1). Applying Beer's law, we have $C/C_m = A/A_m$, and hence

$$A = A_m [1 - \exp(-ut/V)] \quad (4)$$

and

$$\ln\left(\frac{A_m}{A_m - A}\right) = ut/V \quad (5)$$

Thus a plot of $\ln[A_m/(A_m - A)]$ against t should be a straight line with slope u/V , from which V can be calculated for a known flow-rate. The results are summarized in Table 2, and show good agreement with the predicted exponential response. The values of the flow-rates were determined with a mean relative standard deviation of 1.4% and the mean and 95% confidence interval for V were 8.74 ± 0.41 ml.

Observation of a theoretical chamber-volume which probably exceeds the true volume confirms Pungor's findings, which suggested that the effect

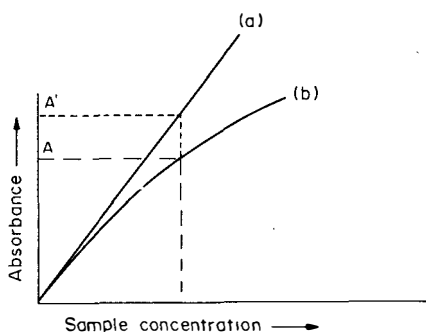


Fig. 5. "Correction" of absorbance for instrument response. (a) is a hypothetical linear calibration plot, assuming constant sensitivity, (b) is the real atomic-absorption calibration curve.

might be due to a mixing contribution within the tubes connected to the chamber. Owing to non-linear instrument response, the corresponding atomic-absorption absorbance-time profiles are not simple exponential-growth functions. Therefore they cannot normally be used directly to evaluate mixing-chamber performance. Two methods of circumventing the difficulty have been employed for test purposes. First, truly exponential absorbance-time curves can be obtained for an element of high sensitivity by use of a solution sufficiently dilute for the conventional calibration curve to be linear over the concentration range from zero to C_m (for example 0.5-ppm magnesium solution). Secondly the generally non-exponential absorbance-time curve may be "corrected" to remove the modification due to instrument response. This is accomplished by reference to the conventional calibration curve, obtained under the same experimental conditions (see Fig. 5). The exponential profile is again observed. In correcting the atomic-absorption absorbance-time profile, any absorbance A observed at time t , and corresponding to a concentration C , is replaced by the corresponding absorbance A' .

The results for a 2-ppm magnesium solution are given in Table 3. The times are measured with respect to an arbitrary zero.

A plot of $\ln[A_m/(A_m - A)]$ against t [equation (5)] is a straight line (correlation coefficient 0.9997). It should be noted that the use of the conventional calibration curve was confined to this preliminary investigation of mixing-chamber characteristics, which is not part of the main calibration procedure.

Ideal mixing requires that a small element of calibrant entering the chamber is instantly and uniformly dispersed throughout the chamber, and is thus recognised at the exit at the same instant. Errors are introduced if this process takes an appreciable time. Inadequate stirring causes the apparent theoretical volume of the chamber to fall,¹⁴ whilst dispersion in other parts of the apparatus causes it to rise. With such a large mixing-chamber, stirring must be vigor-

Table 3. The "correction" of absorbance for instrument response by using the conventional calibration curve

t, sec	0	5	10	20	30	50	70	∞
A	1.5	11.1	19.8	34.6	45.9	61.2	70.5	83.0
A'	1.5	11.1	20.3	36.3	49.2	68.2	83.6	115.0

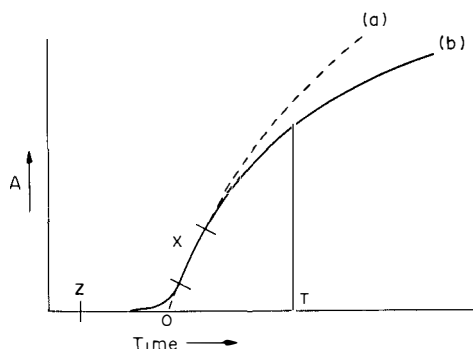


Fig. 6. Time-axis zero of the absorbance-time profile: Z = arbitrary time-zero; O = true zero; ZT = measured time, t_m ; ZO = time error, t_e ; OT = true time, t .

ous if ideal mixing is to be approached, but it must also be smooth. At very high stirring rates the magnetic-follower motion became erratic, resulting in excessive noise in the recorded calibration signal.

Atomic-absorption calibration

Flow-rate. The technique requires the flow-rates of calibrant and sample to be equal. Moreover, the flow-rate must be accurately measured, since it is required in the calibration equation. Ten replicate measurements of the time for the passage of 10 ml through the system gave a mean flow-rate of 8.37 ml/min with a standard deviation of 0.03 ml/min.

Instrumental drift. Any drift in instrument sensitivity between calibration and sample analysis causes an error. Such errors will be minimized in a fully-automated sequence in which calibration and measurement cycles can be run in close succession. The problem also arises, of course, in conventional calibration procedures.

Time-axis zero. In practice, the time-axis zero is

not sharply defined on the chart. The interconnection of the various components by tubing, no matter how short, causes some dispersion of the water-calibrant boundary. This smooths the sharp intersection of the curve with the time-axis, see Fig. 6.

The problem of locating the time-zero on the horizontal scale has been overcome by computer-assisted analysis of a small interval (X) of the curve after the point of inflection but within the linear range of the instrument response, so that the portion X has truly exponential form. An arbitrary time-zero Z is chosen, from which all times, t_m , are measured. These measured times contain a constant time error t_e , given by

$$t_e = t_m - t$$

where t_m is the time measured from the arbitrary zero and t is the time measured from the true zero.

Over the interval X, the curve is given by equation (4). Substituting for t gives

$$\begin{aligned} A &= A_m \{1 - \exp[-u(t_m - t_e)/V]\} \\ &= A_m [1 - \exp(-ut_m/V) \exp(ut_e/V)] \\ &= A_m [1 - k \exp(-ut_m/V)] \end{aligned}$$

where $k = \exp(ut_e/V)$

With time measured from the arbitrary zero, equation (2) becomes, similarly,

$$C = C_m [1 - k \exp(-ut_m/V)] \quad (6)$$

The value of k is computed by least-squares analysis of a plot of A vs. corresponding values of $\exp(-ut_m/V)$, using data from the arc X of the calibration curve; k is then the negative ratio of the slope to the absorbance intercept of this plot. An Apple II BASIC program has been written for doing the calculation. It also calculates concentrations from the time-values for the samples. Copies may be obtained from the authors by request. The results for a number of determinations are given in Table 4.

Table 4. Results obtained by continuous-dilution calibration method

Element	True concentration, ppm	Concentration found, ppm		
		C_m^*1	$C_m^{\dagger}2$	$C_m^{\S}3$
Mg	0.25 ($A = 0.19$)	0.27	0.25	0.26
	0.50	0.52	0.48	0.50
	1.00	1.03	1.00	1.01
	1.50	1.59	1.50	1.52
	2.00 ($A = 1.02$)	—	2.02	2.04
Cr	2.0 ($A = 0.16$)	1.99	2.06	2.09
	4.0	3.99	4.07	3.95
	6.0	5.94	6.10	5.86
	10.0	10.1	10.1	9.8
	15.0	15.3	15.2	15.1
	20.0	20.6	20.4	20.0
Ni	10 ($A = 0.47$)	10.3	10.3	—
	20	20.6	20.7	—
	30	31.0	30.6	—
	40	41.2	40.7	—
	50	51.5	50.4	—
	75 ($A = 1.19$)	77.7	75.8	—

*Mg 2 ppm, Cr 25 ppm, Ni 100 ppm.

†Mg 5 ppm, Cr 75 ppm, Ni 200 ppm.

§Mg 10 ppm, Cr 75 ppm.

The concentrations found rarely deviated by more than 4% from the sample concentrations, and in most of the analyses trends in the errors in the results were evident, suggesting that the deviations contained a contribution from systematic errors (e.g., an incorrect flow-rate or deviation from ideal performance of the mixing chamber), rather than being entirely due to random errors. It is thought that systematic errors could be reduced and the results improved by use of a carefully-designed permanent assembly of the apparatus, incorporating precise measurement techniques. A particular improvement would be to extend the role of the microcomputer to include storage and processing of the absorption-time profile and thus dispense with the chart-recorder. Ultimately, a microcomputer-controlled system is envisaged wherein a sample-input device would be switched into a "calibration" mode to record this profile, before returning to the "analysis" mode to determine sample concentrations.

CONCLUSIONS

The viability of a continuous-dilution calibration technique for atomic-absorption work has been demonstrated. The technique uses readily available or easily constructed apparatus. The results obtained suggest that less than 1% uncertainty should be attainable with a carefully-designed assembly. This is confirmed by recent results obtained by using a microcomputer interfaced with the atomic-absorption spectrometer.¹⁹

Of fundamental importance in this system is an efficiently stirred mixing-chamber having the shortest possible connecting tubes and zero dead-volume. The technique depends upon the predetermined and reproducible performance of this chamber.

In addition to eliminating the need for preparation of standards, continuous dilution calibration has

other advantages over conventional calibration. The technique is simple, easily automated, and operable with existing spectrometers. Instead of use of a limited number of data for calibration purposes, the entire calibration curve is recorded. This provides a vast amount of data suitable for computer smoothing and processing. No curve-fitting procedure is involved, and thus the technique may be applied regardless of any curvature or irregularities in the calibration plot.

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