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A Critical Look at Calibration Procedures for Flame Atomic-absorption Spectrometry

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The present treatment of calibration procedures in the analytical literature is described and the reasons for the poor coverage are discussed. The computational bases of a number of currently available atomic-absorption instrument calibration computer programs are described and discussed. Two computer-based methods are compared using the same calibration data. Additional features of these programs are also discussed. Some desirable features of such programs are proposed. The use of the standard additions method is examined in terms of the shape of the plot of absorbance *versus* concentration of interferent. The success of the method depends on using only the plateau region of this plot.

Keywords: Calibration; atomic-absorption spectrometry; standard additions

The relationship between the concentration of analyte and the response of an analytical instrument is usually a complex function of a variety of instrument parameters and interference effects from other components of the sample. Thus all instrumental methods must include a calibration step as an integral part of the over-all procedure. The calibration may simply be to establish the instrument response *versus* concentration relationship or the procedure may be designed to compensate for interference effects arising from other sample components, as in the matching of standards to samples or in the standard additions method. All of these procedures are regularly used in flame atomic-absorption spectrometry as each instrument has its own highly individual response characteristics, heavily dependent on the operating conditions selected by the user and, in many instances, on other components in the sample. The response is individualised to such an extent that manufacturers tend to quote the slope of the calibration function near the origin as, literally, a "figure of merit" for a particular model. Analogous claims for molecular absorbances are never made by manufacturers of solution spectrometers as there are good reasons for expecting the absorbance value measured to be independent of the instrument used. It is unlikely, though, that this situation will ever be achieved with atomic-absorption spectrometers because the complex sequence of physical and chemical steps of atom production is very much a function of an individual instrument's design. In practice, other effects such as the age of the light source and choice of operating parameters will also have a marked influence on the absorbance - concentration relationship.

As calibration procedures are vital for achieving accuracy in any analytical procedure that uses an instrumental finish, it is disappointing that the topic is given such poor coverage in general text books on analytical chemistry, although some of the more recent texts^{1,2} do give a small amount of space to the topic. Even in specialist texts on analytical atomic spectrometry,^{3,4} the topic is only given minimal treatment. The topic does not appear to be covered adequately in the original literature either, with one or two notable exceptions.^{5,6} There is therefore a danger that, with the advent of computer-based calibration procedures, the importance of an accurate calibration procedure will be increasingly overlooked. In addition to fitting a curve to the calibration points, calibration software packages normally include other features such as identification of the linear portion and extrapolation for the standard additions method, resloping of a stored calibration graph based on one standard and indication of when the "curvature" has exceeded a certain value, etc. Currently there appears to be a reluctance on the part of manufacturers to reveal the details of what their various programs actually do.⁷ This is exemplified by a recent paper in which the validity of using

stored working curves was discussed but no information was provided on how the program "resloped" the calibration.⁸ This can only further the blind acceptance of the accuracy of the calibration program.

In this paper, the bases of the computerised calibration procedure of a number of different atomic-absorption instruments are described and the performances of two different instruments evaluated. A problem with the standard additions method that has not been discussed previously in analytical atomic-absorption text books is outlined and discussed in terms of the shape of the plot of percentage interference against interferent to analyte concentration ratio.

Curve Fitting Procedures

Until the recent arrival of inexpensive, readily available calculating devices, either as separate units or built into instruments, calibration has consisted of obtaining the instrument response for a series of analyte concentrations covering the range to be encountered in the analysis in question, plotting the points on graph paper and drawing the calibration graph following visual inspection of the pattern produced by the points using a pencil and ruler or "Flexicurve." Such a procedure is time consuming and naturally there has been a move towards using the readily available computing power to fit equations to the set of points, and then calculate analyte concentrations by substitution of measured absorbance values into the equation.

Manufacturers' literature indicates that a variety of ways are actually used to fit the curve to the calibration points.

Experimental

Information was requested from five manufacturers of atomic-absorption spectrometers currently available in the UK on the method used to fit the curve to the calibration points. To compare different methods, calibration data generated by one instrument (a Shandon Southern A3300) was used for the calibration of two instruments with different curve fitting programs (a Pye Unicam SP9 computer and a Baird Atomic Datacomp Model A5195 connected to a Baird Atomic A3400 spectrometer). In addition, data for chromium taken from Thompson's paper⁹ for a situation in which a region of negative slope was observed were also used for calibrating the two instruments.

Procedure

Any convenient hollow-cathode lamp was used. The usual resonance line was selected. The flame was not lit. The integration period was set at 4 s and three replicate readings

were taken. The appropriate calibration points were obtained by slightly changing the monochromator wavelength (neither instrument has a continuously variable slit width) so as to measure absorbance on the wings of the instrument-broadened emission profile from the lamp, thus changing the absorbance reading while leaving the gain setting unchanged. With the Pye Unicam SP9 instrument a chart recorder was used to monitor the absorbance reading as this is not displayed during a calibration run.

Results

The way in which the various programs deal with the calibration data are set out below.

Baird Atomic

The Datacomp accessory¹⁰ used in the experiments reported here uses the equation

$$C = rA^2 + pA + q \quad \dots \quad (1)$$

where C is concentration, A is absorbance and p , q and r are coefficients calculated for a least-squares fit of the curve to the points. Any number of standards up to eight can be accommodated. The Alphastar system¹¹ (used with the current range of Baird Alpha instruments) uses an equation similar to that of Varian, namely

$$A/C = rA^2 + pA + q \quad \dots \quad (2)$$

The coefficients are calculated by an iterative least-squares procedure and up to 20 standards can be handled. The program includes a "reslope" facility based on correcting subsequent measurements by a sensitivity correction factor calculated from a single new standard result.

Instrumentation Laboratory

A different procedure is used, depending on the number of standards.^{8,12} With two standards a quadratic equation is solved so that the curve is forced through the calibration points. If three standards are used, a similar procedure is used for a cubic equation. For four or five standards (the maximum number that can be handled) a cubic equation is used but the best fit curve is computed by a least-squares procedure. The program includes a reslope facility based on a single standard that corrects the absorbances of other standards by a factor calculated from a comparison between the single standard's new absorbance and its original absorbance.¹³ If stored calibration data are lost from memory, they may be re-entered via the instrument key-board.

Perkin-Elmer

The program uses the equation

$$C = \frac{pA^2 - rA}{qA - 1} \quad \dots \quad (3)$$

For three standards, the values of p , q and r are evaluated by solving the simultaneous equations so that the curve passes through the calibration points.¹⁴ For more than three standards (up to eight), the equation is calculated by a least-squares fit procedure.¹⁵

Pye Unicam

The program does not use a single equation but calculates a different equation between each calibration point.¹⁶ A straight line is drawn between the blank and the lowest standard. The mean of the slope of this line and the slope of the line between the first and second points is used to define a parabola between these points, which is used as the calibration relationship for establishing sample results. The process is repeated for up to five standards, so that a different parabola is calculated for the region between each pair of adjacent calibration points. Single point recalibration is possible and the program will detect the onset of curvature for use in the standard additions mode.¹⁷ This is done by comparing the slope of the straight line between adjacent calibration points with the slope between the two previous points. Curvature is detected when a 5% difference in slopes is observed.

Varian

The program uses the following equation¹⁸:

$$A/C = rA^2 + pA + q \quad \dots \quad (4)$$

When three standards are used the simultaneous equations are solved so that the curve passes through the calibration points. If one or two standards are used then r and p or r are set equal to zero, respectively. If four or five standards are used then the values of r , p and q are obtained by solving the simultaneous equations corresponding to the two standards below and the one standard above the unknown concentration. This, in effect, generates a family of overlapping parabolas. A reslope facility based on a single standard and a standard additions mode calibration are also available.

Comparison of Curve Fitting Methods

The calibration values generated from the Baird Atomic A3300 for the elements magnesium and nickel are given in Table 1, which also contains data calculated from Fig. 3 of reference 9. The table shows the values calculated by each program when the absorbances of the data points were input as unknowns. The calibration data and calculated concentrations are illustrated in Fig. 1 for nickel and in Fig. 2 for chromium.

Discussion

Although the method of linear regression analysis using the least-squares method is the only "curve" fitting method that is dealt with to any extent in analytical text books, it is obvious that this procedure is of little use in dealing with atomic-

Table 1. Comparison of Pye Unicam (PU) SP9 and Baird Atomic (BA) Datacomp curve fitting programs

Calibration data						Concentrations found, p.p.m.					
Mg		Ni		Cr		Mg		Ni		Cr	
C	A	C	A	C	A	PU	BA	PU	BA	PU	BA
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.011	0.00	1.66	0.00	0.12
0.25	0.18	10.0	0.32	5.0	0.36	0.25	0.23	10	6.12	5.0	5.30
0.50	0.34	25.0	0.58	7.5	0.52	0.50	0.47	25	24.3	7.5	7.73
1.00	0.61	50.0	0.82	10.0	0.74	1.00	1.02	50	54.0	11.5	11.11
1.50	0.81	75.0	0.96	12.5	0.72	1.50	1.53	76	76.4	9.9	10.81
2.00	0.96	100	1.07	15.0	1.00	2.00	1.97	100	96.9	14.9	15.2

absorption calibrations that are well known to be, in general, initially straight lines becoming progressively more curved at higher concentrations. Effects such as ionisation and flame reaction kinetics may cause the curve to have a more complex shape with points of inflection and even regions of negative slope. The computational methods of the linear least-squares method are probably used in the programs that fit the "best curve" to the points by calculating coefficients by a least-squares method so that the computation is carried out with a linearised function. Such procedures probably account for the persistence of the term "curve correction," whereas "curve fitting" would be much more appropriate. As is sometimes mentioned in text books,² the slope and intercept by the least-squares method have uncertainties associated with them that can be expressed as an interval at the appropriate level of confidence. This means that with a known uncertainty in a measured value, a confidence interval can be calculated for a predicted value. Although this is well known in statistical circles (see, for example, reference 19), the use of such confidence intervals has not been practised in analytical chemistry circles, probably because the equations involved are cumbersome and thus the computations were tedious and time consuming. However, with the computing power that is now readily available in the present generation of instruments, such calculations could be readily performed. The inclusion of a confidence interval on the computer printout would go a considerable way to removing the blind acceptance of the accuracy of results on computer printout that was mentioned earlier.

It is also known that the uncertainty in the measured values of absorbance increases as the concentration increases and, although the absolute standard deviation increases, the relative standard deviation passes through a minimum. As pointed out by van Dalen and de Galan,⁶ it is relatively simple to choose a calibration concentration range within which the results have less than a desired relative standard deviation from the Ringbom plot. From the point of view of curve fitting procedures, the increase in uncertainty of the measured points means that a weighted regression analysis should be used, *i.e.*, the curve should be forced to pass closer to some points than others. An exactly similar argument applies to why the use of such weighted procedures has not become common practice as was discussed for the use of confidence intervals on predicted results and exactly the same counter argument applies; with the present generation of instruments the calculations can be readily performed.

Confidence intervals and weighting factors will be discussed at greater length in another paper.²⁰

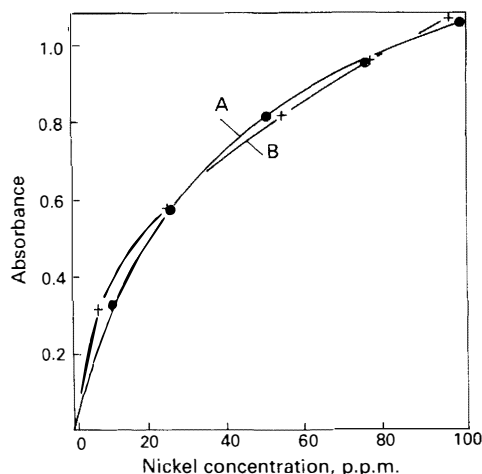


Fig. 1. Calibration graphs for nickel. A, Data produced by a Baird Atomic A3300 instrument; B, best-fit relation from a Baird Atomic Model A5195 Datacomp accessory

The present position with regard to curve fitting procedures is unsatisfactory in that the current instrumentation uses at least four different equations and one "composite" method. In some instances the curve is a best fit to the points calculated by some "minimising of the squares of the absorbance residuals" procedure and in some instances the curve is forced to pass through the calibration points. The problems associated with trying to fit all calibration graphs to a single type of mathematical function can be readily seen from the poor fit between the calculated curve and the actual curve in the case of nickel (see Fig. 1). Neither method of calibration could cope with the region of negative slope of the chromium calibration (see Fig. 2). Both methods though coped adequately with the much "straighter" and smooth curve of the magnesium calibration.

Conclusions

Attractive as computerised curve fitting procedures are in principle, there are a number of drawbacks with the present generation of atomic-absorption spectrometers. Unless a top of the range instrument with a video display unit is used, it is not possible visually to inspect the data points and various unusual or unexpected features may be overlooked. There could be the presence of outliers due to errors in preparing calibration solutions or the occurrence of inflection points or regions of negative slope.

Although it is important to ensure appropriate selection of optimum spectral band pass and lamp current to minimise the curvature due to stray light while maintaining an adequate signal to noise ratio, it is important that curve fitting procedures can deal with situations exemplified by Fig. 1. The concentration range for which the relative standard deviation is less than a desired value (selected from the Ringbom plot⁶) may permit the use of upper absorbance values well into the curved region of the calibration plot.

Until such time as such display units are available on all instruments and appropriate weighting procedures are used with printout of confidence intervals for predicted results, the accuracy of a given calibration procedure can only be assessed by visual inspection of a manually plotted graph.

Standard Additions Method

Statements such as "the method of standard additions is a well known and useful technique that often makes it possible to obtain accurate determinations in the presence of matrix interferences"¹⁵ are fairly common in manufacturers' literature. The method is usually mentioned briefly in analytical

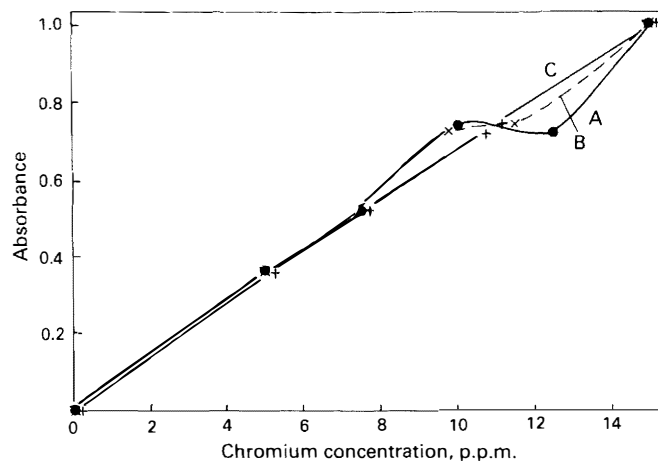


Fig. 2. Calibration graphs for chromium. A, Data from reference 9; B, best-fit relation from a Pye Unicam SP9 computer; C, best-fit from a Baird Atomic A5195 Datacomp accessory

atomic spectrometry texts and the limitations that (1) it is necessary to work with the linear portion of the calibration graph and (2) the method will not correct for errors arising from non-specific background absorption are usually explained.²¹ The principle of the method is illustrated in Fig. 3, from which it is clear that for the method to be successful, the interference effect encountered must affect the slope of the calibration (the sensitivity), *i.e.*, each point must be subjected to the same percentage depression.

There are many situations in which this constant percentage depression is not obtained.

Experimental

A Pye Unicam SP 90 Series 2 atomic-absorption spectrometer was used with an air - acetylene flame throughout. Stock 1000 p.p.m. solutions of calcium and aluminium were obtained from BDH Chemicals and a 1000 p.p.m. phosphate solution was prepared by diluting AnalaR-grade orthophosphoric acid.

Procedure

The instrumental conditions were selected to give maximum sensitivity for calcium in the absence of interferences.

The effects of increasing the concentration of phosphate and the concentration of aluminium on a fixed concentration of calcium were investigated and a number of solutions containing various amounts of calcium and phosphate or aluminium were analysed by the standard additions procedure.

Results

The results of the study of the phosphate and aluminium interferences are shown in Fig. 4 and the results of the analysis of the various solutions are given in Table 2. It was found that with solutions containing the same concentration of calcium and phosphate ranging from 1 to 50 p.p.m. errors of up to -30% were observed. When the phosphate to calcium ratio was greater than 2, satisfactory results were obtained but no aluminium to calcium ratio could be found for which accurate results were obtained.

Discussion

The shapes of the interference plots (Fig. 4) are similar to those reported by other workers,^{22,23} and are typical of the effects of a range of interferences due to stable compound formation of incomplete volatilisation.

The effects observed here may be more severe than observed with other instruments or would be observed for different initial operating conditions. The initial conditions

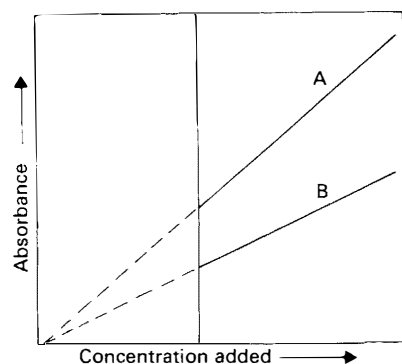


Fig. 3. Principle of the standard additions method. Extrapolation of curve A (no interferences) gives the analyte concentration from the intercept on the concentration axis; extrapolation of B (interferences present) is only accurate if all points on A suffer the same percentage depression

were selected on the basis of maximising sensitivity for calcium in the absence of any interference effects, as it is felt that this is the most commonly used instrument optimising strategy.

It is thus obvious that the standard additions method will be inaccurate in situations that are typified by the calcium - aluminium effect as there is no constant percentage depression plateau, and will only give satisfactory results when situations typified by the calcium - phosphate effect are encountered. In theory, the "calcium - aluminium" type of effect should be detected from an upwards curvature of the calibration, but in practice the magnitude of this effect may be small and it may easily be overlooked. In the work reported here, very good correlation coefficients were achieved for a linear regression analysis of the measured region of the calibration under conditions in which curvature would have been theoretically expected. The use of releasing agents, such as lanthanum, does not provide a simple solution to the problem as the released signal never falls on the horizontal zero interference line and may, in some instances, actually be greater than the signal obtained in the absence of interferent. This is probably due to additional ionisation-suppression effects. The same argument has then to be applied to the enhanced signal, all the measurements for the analysis must be made under conditions at which a constant percentage enhancement is obtained. The extent to which interference effects are observed is often highly dependent on instrumental operating conditions such as fuel to oxidant ratio and observation height. Hence it may be that the operating conditions necessary for successful application of the standard additions method (achieving a linear calibration over the required range with constant percentage depression or enhancement) are different from those selected for maximum sensitivity of the analyte element alone. Problems in the determination of calcium in cement associated with the effects discussed above have been reported by Hosking *et al.*²⁴

Table 2. Standard additions method of analysis for calcium

Concentration in test solutions, p.p.m.			Calcium found, p.p.m.
Ca ²⁺	PO ₄ ³⁻	Al ³⁺	
5	200	—	5.0
20	200	—	20.2
50	200	—	50.0
7	—	2	6.3
10	—	5	7.9

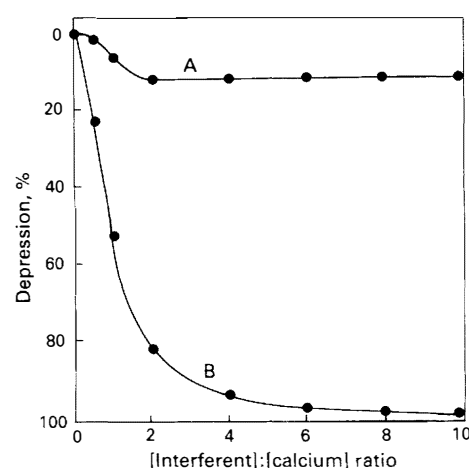


Fig. 4. Graph of percentage depression in calcium absorbance against ratio of interferent concentration to calcium concentration. A. Phosphate interference; B. aluminium interference

Conclusions

The various constraints that apply to the standard additions method mean that great care is needed if accurate results are to be obtained. Method development should, if possible, include investigation of the linearity of the calibration and the shape of the plot of percentage depression or enhancement *versus* ratio of interferent concentration to analyte concentration. In order to be certain of achieving such a constant level of interference it may be necessary, as part of the sample preparation procedure, to add a certain amount of the interferent.

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