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Demonstration of a standard dilution technique for standard addition calibration

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ABSTRACT

A standard dilution approach to sequential (S-SAC) and conventional (C-SAC) standard addition calibration is introduced and described theoretically. The two calibration methods have then been demonstrated experimentally for chloride measurement in seawater samples. S-SAC showed superior results for such a sample as a function of the steeper extrapolation resulting from the calibration process. The conflicting effects on S-SAC of extrapolation precision and sensitivity to intercept correction have been discussed and recommendations concerning the optimum ratio of target analyte concentration in the calibration standard to that in the unknown sample for the use of this technique have been made.

1. Introduction

Calibration is an essential part of quantification in analytical chemistry for all but primary methods [1]. A working curve approach is usually preferred because in most cases the precision obtained by interpolation of unknown measurements between fixed reference points is better than using extrapolation techniques. Extrapolation techniques for calibration, such as standard addition, are usually required when the matrix of the sample differs from that of the calibration standards and the analytical technique employed is sensitive to the matrix [2]. Both working curve calibration and conventional (or fixed volume) standard addition calibration (C-SAC) involve the preparation of a number of solutions in separate containers. Sequential (or variable volume) standard addition calibration (S-SAC) uses only one container, and relies on adding the calibration standard into the same container as the unknown sample [3]. Because of the fixed volume nature of C-SAC (comprising a fixed quantity of unknown solution and inversely proportional, variable quantities of standard solution and blank balance solution) addition of standard solution always results in an overall increase in the mass fraction of analyte in solution. However, because S-SAC involves the use of differing solution volumes (comprising a fixed quantity of unknown solution and variable quantities of standard solution) if a standard solution of lower concentration than the unknown sample is used the mass fraction of analyte in the resulting mixture may actually decrease. This theoretical 'standard dilution' condition has been postulated in previous studies on S-SAC [3] but until now never fully described or demonstrated experimentally. In situations where the concentration of the analyte is large (with respect to the modern day expectations for 'trace' analysis) it may be impracticable to use standard solutions whose concentrations are greater than that of the sample because of the upper range of instrument response, or simply because many commercial traceable standard solutions are not available for purchase at concentrations above mass fractions of about 1 mg/g. In this work we describe for the first time the practical application of the standard dilution method, here for the measurement of the composition of seawater, and discuss its potential merits over C-SAC for this application. The standard dilution method described here is quite different from the serial dilutions method (SDM) of quantification [4] which results in a calibration relationship similar in appearance. However, in SDM, a standard solution more concentrated than the unknown is first added to the sample followed by sequential dilution using solvent with zero analyte content, whereas in standard dilution S-SAC each step involves the addition of a standard solution less concentrated than the unknown.

2. Experimental

All experiments were conducted in a temperature-controlled laboratory at 20 ± 2 °C. All solutions were prepared in fully cleaned and dried (in a nitrogen flow (oxygen free nitrogen, BOC))

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polypropylene labwear (Fisher) using deionised water (18.2 MΩ cm, Milli-Q system, Millipore). All chemicals were of high purity (+99.9%, Fisher) and were prepared according to the supplier's guidelines (drying at elevated temperatures for the inorganic salts). The eluent used for the IC analysis was an aqueous solution of 11 mM Na_2CO_3 and 2 mM $NaHCO_3$. Analysis was performed with a Dionex ICS-1500 ion chromatograph (IC), a self-regenerating suppressor unit, and conductivity detection as previously described [5]. A flow rate of 1.0 ml min^{-1} was used throughout. The volume of the sample injection loop was nominally 10 μl. Each sample was measured at least in triplicate. NPL is accredited to ISO 17025 by UKAS to perform these measurements. Filtered Atlantic Standard Seawater (OSIL, UK) was diluted to bring the chloride mass concentration within the range of the ion chromatograph's conductivity cell: in this case 11.4 ± 0.2 μg/ml. A standard solution for calibration of chloride mass concentration 0.593 ± 0.012 μg/ml was prepared gravimetrically using sodium chloride. For the C-SAC (fixed volume) quantification, 4 vessels were prepared each containing a 10 ml portion of the diluted seawater. To these vessels another 9 ml of liquid was added, comprising the following in the separate vessels: 9 ml deionised water; 6 ml deionised water and 3 ml calibration standard; 3 ml deionised water and 6 ml calibration standard; and 9 ml calibration standard. For the S-SAC (variable volume) quantification, 4 vessels were prepared each containing a 10 ml portion of the diluted seawater. To these vessels calibration standard was added in the volumes 0, 3, 6 and 9 ml. Quantification was performed on a mass concentration basis, rather than on a mass fraction basis, to avoid any physical matrix effects associated with density mismatching [6]. Extrapolation and quantification were performed with NPL's XLGenline software [7], using the peak areas provided by the proprietary software (Chromeleon software, Dionex).

3. Results and discussion

3.1. Theory of standard dilution

m_s is the mass of the unknown sample, m_a is the mass of the standard solution added at each given stage, x_s is the fractional content of target analyte in the unknown sample, x_a is the fractional content of target analyte in the standard solution and, $x_{s(meas)}$ is the fractional content of target analyte in the unknown sample determined directly by extrapolation, prior to correction [3].

We also define $r = x_a/x_s$ and $k = m_s/m_a$. Furthermore, and crucially in this special case of standard dilution, we assume that $x_s > x_a$, such that $r < 1$, throughout.

The generalised calibration condition summarised by these considerations is given in Fig. 1. Consider that a sample of known mass but unknown analyte content is placed in a vessel (together with some blank solution in the case of C-SAC). This solution is then analysed and a response is obtained, yielding P_0 in Fig. 1. (We assume that in the S-SAC case it does not matter whether the solution is consumed during analysis (like ion chromatography) or the analysis is non-destructive (like stripping voltammetry). In the latter case we may consider just one analysis vessel and in the former case we consider several vessels, similar to C-SAC. A known mass of a standard solution of known analyte content is then added to the same portion of the unknown sample, together with blank solution in the case of C-SAC to equalise volumes, and a further measurement is made. Repeating this process n times, with standard solution replacing blank solution to maintain a constant total volume in the case of C-SAC and with the total volume increasing with each addition for S-SAC, yields the points $P_{n,C}$ for C-SAC and $P_{n,S}$ for S-SAC. Previous work has demonstrated the

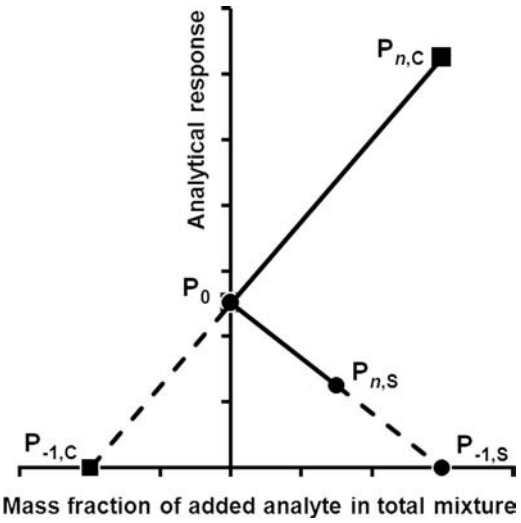

Fig. 1. Diagrammatic representation of fixed volume conventional standard addition (P_0, $P_{n,C}$ and $P_{-1,C}$) and variable volume sequential standard addition (P_0, $P_{n,S}$ and $P_{-1,S}$) calibration methods in the standard dilution scenario where the content of analyte in the sample is greater than in the standard solution ($x_s > x_a$). The solid lines represent the calibration relationship and the dashed lines the extrapolation to determine the unknown. Further explanation is given in the text.

linearity of these relationships [3]. An extrapolation of the line joining points: P_0 and $P_{n,C}$; and P_0 and $P_{n,S}$, to their intercepts with the x-axis yields $P_{-1,C}$ and $P_{-1,S}$ respectively. Note, uniquely for the standard dilution condition that the gradient of the S-SAC extrapolation is negative, as a function of the condition $x_s > x_a$, whereas the C-SAC gradient remains positive. This is because any replacement of blank solution with standard in C-SAC always increases the overall mass fraction of analyte in solution, whereas in S-SAC the addition of standard will act to reduce the overall mass fraction of analyte in solution because the volume is allowed to vary. As is shown below, this results in a shorter and more precise extrapolation for S-SAC under these conditions. We may consider for C-SAC

$$P_0 = \left(0, \frac{m_s x_s}{n m_a + m_s}\right) \tag{1}$$

$$P_{n,C} = \left(\frac{n m_a x_a}{n m_a + m_s}, \frac{n m_a x_a + m_s x_s}{n m_a + m_s}\right) \tag{2}$$

(Strictly speaking we should state that the denominator for the y-value of P_0 is $(m_s + m_b)$ since the additional material added to the original unknown sample is blank solution (of mass m_b) rather than the standard solution. However, in the n point C-SAC case we define $m_b = n m_a$; thus Eq. (1) is nevertheless correct and avoids the requirements for additional terms to be introduced). For S-SAC [3]

$$P_0 = \left(0, \frac{m_s x_s}{m_s}\right) \tag{3}$$

$$P_{n,S} = \left(\frac{n m_a x_a}{n m_a + m_s}, \frac{n m_a x_a + m_s x_s}{n m_a + m_s}\right) \tag{4}$$

Considering the generalised S-SAC strategy the real fractional content of analyte in the unknown requires a subsequent correction to the extrapolated value [3]:

$$x_s = \frac{x_a x_{s(meas)}}{x_a + x_{s(meas)}} \tag{5}$$

this accounts for the systematic bias of extrapolation associated with the gradient, V, varying as

$$V = \frac{x_a - x_s}{x_a} \tag{6}$$

The precision of extrapolation of the calibration relationship to its intercept with the x-axis, σ, relative to the precision of measurement points in the y-direction, σ_y, is given by [3]

$$\sigma/\sigma_y = \frac{1}{V}\sqrt{\frac{1}{n} + \frac{\bar{y}^2}{V^2 \sum_n (x_n - \bar{x})^2}} \tag{7}$$

where n is the number of measurement points, $\bar{y}$ and $\bar{x}$ are the average values of the y- and x-components, respectively, over all points and x_n is the value of the x-component at point P_n. The expressions for the relative extrapolation precision for these two methods have been previously obtained [8,9]. These have been used to calculate expected extrapolation precisions for the standard dilution branch of S-SAC and the equivalent precisions for C-SAC conducted with the same parameters, with the results displayed in Fig. 2. This clearly shows the theoretical extrapolation precision benefits of S-SAC over C-SAC in the standard dilution range. Precision degrades monotonically for C-SAC as r decreases. One can see from Fig. 1 that this happens because as r decreases $P_{n,C}$ moves closer to P_0 but with the same overall gradient, and so in relative terms the length of the extrapolation P_0 to $P_{-1,C}$ increases with respect to the length of $P_{n,C}$ to P_0, thereby degrading the precision of extrapolation. Conversely as r decreases in the S-SAC case the gradient steepens in a downwards direction, such that the reduction in the length of P_0 to $P_{n,S}$ is not as great as a reduction in the length of $P_{n,S}$ to $P_{-1,S}$ and so the overall precision improves tending towards high precision at very low r. The ratio of these extrapolation precisions, referred to here as ς_S/ς_C, has been calculated and is given in Fig. 3. Previous work has demonstrated the invariance of ς_S/ς_C for heteroscedastic behaviours, and for homoscedastic behaviour at high k [9]. This shows that when $r < 0.5$ we would expect S-SAC to result in improved precision over C-SAC. Compare this with all cases for $r > 0.5$ where S-SAC is never more precise than C-SAC, although at high r the differences are negligible [9].

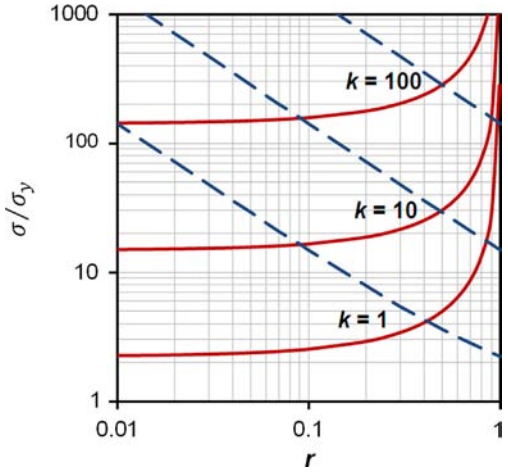

Fig. 2. The expected relative extrapolation precisions, σ/σ_y, as a function of r for the standard dilution branch of S-SAC (red, solid lines) and the equivalent precisions for C-SAC (blue, dashed lines) for three values of k, as indicated. k is defined as the ratio of the mass of the unknown sample to the mass of the standard solution added at each given stage. (For interpretation of the reference to colour in this figure, the reader is referred to the web version of this article).

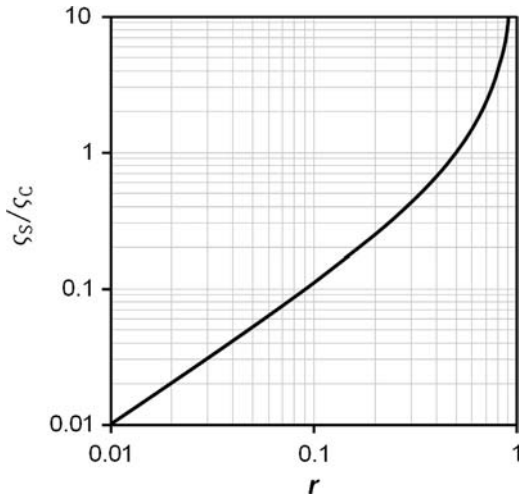

Fig. 3. The ratio of S-SAC extrapolation precision to C-SAC extrapolation precision (ς_S/ς_C) as a function of r when $k=100$ (although the relationship shows very little dependence on k [9]).

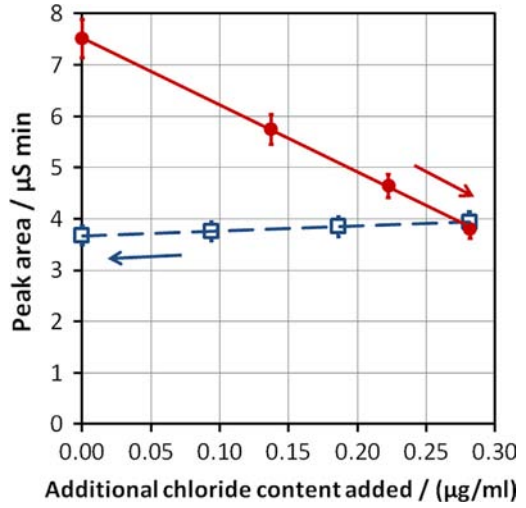

Fig. 4. Results of the exemplar measurement using the standard dilution method undertaken with the synthetic solutions described in the experimental section. The resulting calibration curves for the S-SAC (red, solid line) and C-SAC (blue, dashed line) are shown with the arrows indicating the direction of subsequent extrapolation to the x-axis. The error bars represent the expanded uncertainty ($k=2$) of the analytical measurement giving a level of confidence of approximately 95%. (For interpretation of the reference to colour in this figure, the reader is referred to the web version of this article).

3.2. Example standard dilution measurement

An exemplar measurement using the standard dilution method has been undertaken with the synthetic solutions described in the experimental section. The results, in Fig. 4, show that the standard dilution regime yields a very shallow gradient for the C-SAC extrapolation and a much steeper gradient for the S-SAC extrapolation. As a result the uncertainties in the extrapolation are significantly greater for C-SAC and the increased sensitivity of the intercept to the gradient may also result in a less accurate quantification. The mass fractions determined by the two methods were, for C-SAC: (7.3 ± 7.1) µg/ml and for S-SAC: (11.6 ± 0.8) µg/ml. Both methods provide results which agree with the known composition of the sample being measured within the uncertainty of the measurement, although the C-SAC value is inaccurate and has an uncertainty that is clearly not fit for purpose. The S-SAC value is considerably closer to the known value and has a fit for

purpose uncertainty. With $r=0.052$ for the S-SAC part of the experiment and $r=0.099$ for the C=SAC part, Eq. (7) and Fig. 2 predict a ratio of extrapolation precisions of about 10 which is close to the measured value of 8.4. Any residual deviation may result from the non-ideality of the experimental responses.

3.3. Sensitivity of intercept correction for S-SAC

As previously explained [3] the result of a S-SAC extrapolation requires correction according to Eq. (5) to account for the difference in $x_{s(meas)}$ and x_s as a result of the variation in the gradient of extrapolation as a function of r. However, under standard dilution conditions, the intercept of the extrapolation with the x-axis for S-SAC is positive, rather than negative as it would be when $r>1$, and so Eq. (5) becomes

$$x_s = \frac{x_a|x_{s(meas)}|}{x_a - |x_{s(meas)}|} \tag{8}$$

hence the measured value is particularly sensitive to the denominator, especially when $x_a \approx x_{s(meas)}$ and consequently $x_a - x_{s(meas)}$ approaches zero. This is a sensitivity which is not present when $r>1$ because the denominator comprises the addition of two positive numbers and cannot approach zero. When considering a theoretically perfect calibration relationship this is not a concern but in real life situations small measurement biases affecting the extrapolated value of $x_{s(meas)}$, and therefore also of $x_a - x_{s(meas)}$, are expected to be present. Rearranging Eq. (8) we observe

$$\frac{|x_{s(meas)}| - x_a}{|x_{s(meas)}|} = r \tag{9}$$

this demonstrates that the relative difference between $x_{s(meas)}$ and x_a is equal to r, and so this relative difference decreases as r decreases. Hence there is a need to strike a compromise when considering a suitable r for use with a standard dilution S-SAC approach. As $r \to 0$ extrapolation precision improves but the sensitivity of the intercept correction to small experimental biases is increased. Conversely as $r \to 1$ the sensitivity of intercept correction will be reduced, but the extrapolation precision will worsen dramatically. The optimum value of r will depend on the absolute value of σ_y and the coefficient of determination of the calibration, but it is likely that $0.2 < r < 0.5$ would be optimum in most situations. (If $r>0.5$ then C-SAC becomes a more precise calibration method). Of course r cannot be known *a priori*, so a quick preliminary measurement to demine its value prior to accurate work might be useful under these circumstances.

4. Conclusions

The characteristics of calibration using standard dilution S-SAC and C-SAC have been elaborated and tested experimentally. The benefits of S-SAC over C-SAC when the concentration of target analyte in the standard solution is lower than that in the unknown sample have been clearly demonstrated. However, the intercept correction for S-SAC may show significant sensitivity to small experimental biases as the ratio of target analyte in the standard solution to that in the unknown solution decreases. Because of this effect it may be necessary to aim for a value of r which balances this sensitivity against the increased theoretical extrapolation precision at low r. S-SAC has the added advantage over C-SAC in this application that it is quicker and easier to implement – because it is a variable volume technique there is no need to ensure that the different samples are prepared to the same volume as is the case for C-SAC. Furthermore, because S-SAC extrapolation takes place across shorter distances, any non-linearities in calibration, instrument sensitivity or extrapolation are mitigated somewhat.

The standard dilution S-SAC technique has shown promise for situations where the concentration of the analyte is large. In these situations it may be impracticable to use standard solutions whose concentrations are greater than that of the sample because of the limitations of instrument response, or where traceable calibration solutions with concentrations higher than the content of the unknown solution are not available.

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