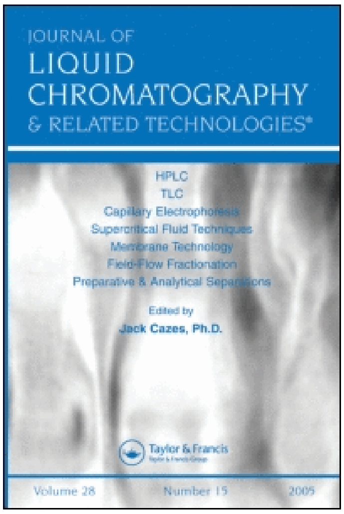

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Errors in the Analysis of Minor Components within Environmental Samples Caused by the Physical Matrix Effect: Implications for Automated Analysis and Standardised Methods

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Errors in the Analysis of Minor Components within Environmental Samples Caused by the Physical Matrix Effect: Implications for Automated Analysis and Standardised Methods

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Abstract: It is generally agreed that for the purposes of performing analytical determinations, the highest accuracy calibration standards are those prepared by mass (gravimetrically) as opposed to by volume (volumetrically). However, the use of gravimetrically prepared standards to calibrate analytical techniques that rely on fixed volume injections can cause systematic errors. These errors can occur even when the analytical technique does not suffer from a chemical matrix interference. The physical matrix of the sample has been shown to be the cause of these errors. This effect is demonstrated experimentally for the analysis of minor components within two environmental samples. Proposals are offered for methods to overcome this 'physical matrix effect'.

Keywords: Physical matrix, Automated analysis, Gravimetrically, Volumetrically

INTRODUCTION

Calibration is an essential part of analytical measurement.[1,2] It relates the amount of analyte to the analyser response by the measurement of a set of standards of known composition.[3] This calibration relationship is then used to determine the amount of analyte in a sample of unknown composition, given the response level of the analyser for the unknown.

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It is generally accepted in chemical metrology that more accurate calibration standards may be prepared using gravimetric, rather than volumetric means,[4,5] especially at the trace level.[6] The reasons for this are manifold:

- Individual gravimetric measurements can be routinely made with a smaller uncertainty than volumetric measurements (for example: relative uncertainties of $<1 \times 10^{-5}$ compared to 1×10^{-4} for measurement of 100 g or 100 mL of water respectively). This is especially important when the preparation of a standard involves multiple steps.
- For dilute preparations, gravimetric methods are simpler and involve fewer vessels and usually fewer preparative steps than volumetric preparation – each step introducing increased uncertainty in the final composition.
- Gravimetry is more flexible, allowing bespoke compositions to be easily created. Volumetric preparation relies to some extent on the graduations available on the volumetric flasks used.
- The composition of a gravimetrically prepared solution when expressed as a mass fraction remains constant (assuming buoyancy effects are always corrected for when weighing), whereas the composition of a volumetrically prepared solution expressed as a mass concentration will vary with temperature.
- Volumetry is ultimately traceable to standards of mass via gravimetry and, therefore, lies further down the traceability chain and necessarily cannot be more accurate than gravimetric preparation.

For these reasons it has become usual for high accuracy analytical procedures to use gravimetry for the production of calibration standards, and for sample preparation and handling procedures. Since analytical results are usually expressed in the same units as those used to described the composition of the calibration standards used for the measurement, an analytical result produced using gravimetrically prepared standards is expressed in mass fraction terms, with units of kg/kg, whereas an analysis result produced using volumetric standards is expressed in mass concentration terms, with units of kg/m^3.

When the analyte in the unknown solution is part of a complex matrix of other components, analysis may be significantly more complex. The response of the analyser to an analyte in a complex matrix may not be the same as for the same amount of analyte in a simple solution – ‘a chemical matrix effect’. This is usually overcome by ‘standard addition’ calibration, which involves the addition of known amounts of a simple matrix standard to the unknown complex matrix. After mixing, the matrix, which is assumed to be invariant, then has an equal effect on the analyser response from the analyte in both the unknown sample and the standard addition solution. Standard addition is a more complex calibration technique than a normal working curve method, involving at least three extra weighing steps per measurements (required to prepare a mixture of sample, calibration standard and balance solution of known of mass composition). Hence, for the analysis of large numbers of samples, and for automated high-throughput analysis, the use of a standard addition methodology is not practical; measurement techniques that show no chemical matrix effects for the required analysis are far preferable.

Notwithstanding this, the use of mass fraction based calibration with analytical instrumentation, that injects fixed sample volumes, can impose a physical matrix dependent error on the measurement results, despite the analysis method being independent of the chemical matrix. This paper describes the origin of this effect, the implications for high accuracy, and high-throughput analysis, and the calibration methodology that can help overcome it. A practical demonstration of the effect during ion chromatographic measurement of two different types of environmental sample is presented, and the likely analysis error introduced by a variety of different environmental samples is presented. This work has implications for a variety of published standards relating to the measurement of minor components in complex environmental matrices by fixed volume injection methods.

EXPERIMENTAL

All solutions were prepared in fully cleaned (deionised water) and dried (nitrogen flow (oxygen free nitrogen, BOC)) polypropylene labware (Fisher) using deionised water (18.2 $\mathrm{M\Omega \cdot cm}$, MilliQ 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).

A series of solutions were prepared gravimetrically. Five synthetic seawater samples of approximate equivalent salinity $s = 35$[7] were obtained by preparing aqueous solutions of nominal sulphate mass fraction 503×10^{-6}, and nominal chloride mass fraction of 17×10^{-3}, using the respective sodium salts. Calibration standards of appropriate mass fraction containing only sulphate were prepared to bracket the expected sulphate mass fraction of the synthetic seawater.

Additionally, five sampled ambient air filters were taken (48 mm diameter cut-outs from 5, 24 hour samples collected using a Graseby-Andersen High Volume sampler (PM_{10} fraction, 68 $\mathrm{m^3 \cdot h^{-1}}$)). The anionic content of these filters was extracted by sonication (5 mins) in approximately 20 mL of deionised water. Each extract was then split into two approximately equal portions, whose exact masses were noted. To one of the two portions was then added approximately 25% of its mass again in deionised water, and to the other was added approximately 25% of its mass again in ethanol. Exact masses were noted at each stage. The mass fractions of chloride and nitrate in final samples were approximately 2.0×10^{-7} and 1.5×10^{-6}, respectively. The addition of ethanol to these extracts simulates measurement of the anionic content of particulate matter following extraction with water and wetting agents (such as described in VDI 3497–3 : 1988).[8] Calibration standards of appropriate mass fraction containing only chloride and nitrate were prepared to bracket the expected analyte mass fraction in the ethanolic extracts.

Measurements were performed with a Dionex DX-120 ion chromatograph, with a G14 guard column, AS14 anion separation column, ASRS – Ultra II self-regenerating suppressor unit, and a DS4 conductivity detector. Samples were introduced via a 0.45 μm polypropylene filter (Whatman). The eluent used in all cases was an aqueous solution of 11 mM Na_2CO_3 and 2 mM $NaHCO_3$. A flow rate of $0.9\,\text{mL}\cdot\text{min}^{-1}$ was used throughout. The volume of the sample injection loop was nominally: 25 μL for the measurement of the synthetic seawater sample; and 125 μL for the measurement of the ethanolic extracts. The measurement procedure was fully automated using a Dionex AS40 automatic sampler.

Each measurement consisted of five repeat runs on the ion chromatograph. Quantification was performed with NPL's Xgenline software,[9] using the peak areas provided (Peak Net software, Dionex). All measurement results and mass fractions of prepared solutions had associated uncertainty statements, calculated according to the GUM approach[10] and quoted at the 95% confidence interval.

The experiments were performed within a controlled environment where the temperature did not deviate from the average by any more than 2°C during the entire measurement process and, therefore, changes in ambient conditions did not have any significant effect on the measurements reported here.

THEORETICAL ASPECTS

The mass fraction w_i of component i in a mixture is given by[11,12]

$$w_i = \frac{m_i}{\sum_j m_j} \tag{1}$$

where m_i is the mass of component i and $\sum m_j$ is the sum of the masses over all component parts of the mixture, including component i. The mass concentration γ_i of component i in the same mixture is given by:

$$\gamma_i = \frac{m_i}{V} \tag{2}$$

where V is the volume of the total mixture, so therefore, at a given temperature t,

$$\gamma_i = \rho_t \cdot w_i \tag{3}$$

where ρ_t is the density of the mixture at temperature t.

Several analytical techniques based on chromatographic or mass spectrometry principles may show no chemical matrix effect; for instance: ion chromatography (IC) when no overlapping peaks are present, or inductively coupled-mass spectrometry (ICP-MS) where the matrix does not affect the ionisation of the sample element and no isobaric interferences are present. These rely on detecting the amount of substance, n, introduced into the instrument via a fixed volume sampling method. In IC, this is in the form of a fixed volume sample loop, and in ICP-MS this takes the form of introducing a nebulised sample at a fixed volume flow rate for a fixed time period. Consider that the sensitivity of the measurement instrument is k, such that:

$$I_i = k \cdot n_i \tag{4}$$

where I_i is the analyser response on introducing an amount n of component$_i$. Therefore, for the measurement of the amount of component i in an unknown sample, $n_{i,sam}$, using a calibration standard of content, $n_{i,cal}$:

$$\frac{k \cdot n_{i,sam}}{I_{i,sam}} = \frac{k \cdot n_{i,cal}}{I_{i,cal}} \tag{5}$$

If a fixed volume of sample, v, is introduced into the analytical instrument during each measurement then, for a mass fraction based analysis:

$$\frac{k \cdot v \cdot \rho_{sam,t} \cdot w_{i,sam}}{I_{i,sam} \cdot M_{i,sam}} = \frac{k \cdot v \cdot \rho_{cal,t} \cdot w_{i,cal}}{I_{i,cal} \cdot M_{i,cal}} \tag{6}$$

where $\rho_{sam,t}$ and $\rho_{cal,t}$ are the density of the sample and calibration standard, respectively, at temperature t. $M_{i,sam} = M_{i,cal}$, and are equal to the molar mass of the target analyte. This yields the unknown sample mass fraction as:

$$w_{i,sam} = \frac{I_{i,sam} \cdot \rho_{cal,t} \cdot w_{i,cal}}{I_{i,cal} \cdot \rho_{sam,t}} \tag{7}$$

since $\rho_{sam,t}$ is generally unknown, it is usually assumed that $\rho_{sam,t} = \rho_{\ cal,t}$, and, therefore, that measured sample mass fraction is $w'_{i,sam} = I_{i,sam} \cdot w_{i,cal}/I_{i,cal}$. This may not be a robust assumption for environmental samples, in particular, which may have complex matrices, and high ionic contents. If $\rho_{sam,t} \neq \rho_{cal,t}$ then the relative error of the measured mass fraction value is given by:

$$\frac{w'_{i,sam} - w_{i,sam}}{w_{i,sam}} \equiv \delta_r = \left(\frac{\rho_{sam,t}}{\rho_{cal,t}} - 1\right) \tag{8}$$

Hence, the measured mass fraction value will be overestimated for samples that are denser than the calibration solutions, and underestimated for samples that are less dense than the calibration solution.

RESULTS AND DISCUSSION

The Analysis of Environmental Samples by Standard Methods

The origin of the systematic error for mass fraction based analysis occurs not because of the analyser's response to the different matrices of the environmental sample and calibration standard (a chemical matrix effect), but because of the physical properties of the solutions themselves – in this case, variation in the solution density between the sample matrix and the calibration solution (a physical matrix effect). As will be demonstrated, the error occurs because the measurement is performed on a mass fraction basis rather than a mass concentration basis. Two experimental examples are illustrative of this phenomenon; the results of these investigations are presented in the following sections.

The Analysis of Minor Anions in High Ionic Content Environmental Samples

The measurement of minor anions in high ionic content waste waters, such as described in standard method ISO 10304–2 : 1997,[13] or in sea water[14,15] has been illustrated by the analysis of a synthetic seawater solution. The results of analysis by ion chromatography, using gravimetrically prepared standards with compositions described by mass fraction, are displayed in Figure 1. This shows a clear systematic measurement biases in the measurement

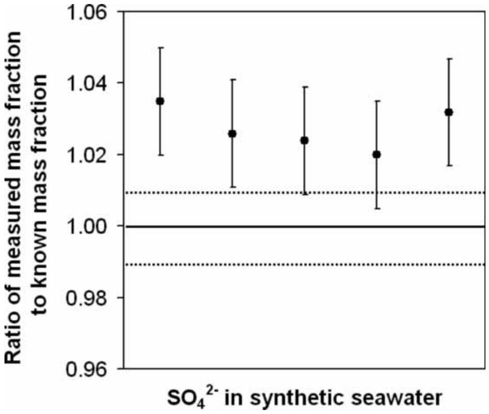

Figure 1. The ratio of the measured mass fraction to the known mass fraction for the measurement of sulphate in 5 synthetic seawater solutions of approximate salinity $s = 35$. The error bars represent the uncertainty of the measurements at the 95% confidence interval. The dotted lines represent the uncertainty in the known mass fraction at the 95% confidence interval.

process. In the case of these synthetic seawater measurements, the error is positive, as the unknown sample is more dense than the calibration standards. The observed average error for measurements of seawater was +2.7%, whilst the expected error calculated from Equation (8) and reference [7] is +2.5%.

The Analysis of the Anionic Content of Particulate Matter on Ambient Air Filters Following Extraction with Water and Wetting Agents

The measurement of the anionic content of particulate matter following extraction with water and wetting agents, such as described in standard method VDI 3497–3 : 1988[8] has been illustrated by the analysis of chloride and nitrate in five sampled ambient air filters extracted with a simulated 4:1 water to ethanol solution. The results of the analysis by ion chromatography, using gravimetrically prepared standards with compositions described by mass fraction, are displayed in Figure 2. Again, this shows clear systematic measurement biases in the measurement process. In the case of these ambient air ethanolic extracts the error is negative since this sample is less dense than the calibration solutions. The observed average error for measurement of the chloride and nitrate in ethanolic extract was −3.6%, whilst the expected error calculated from Equation (8) and reference [7] is −3.2%.

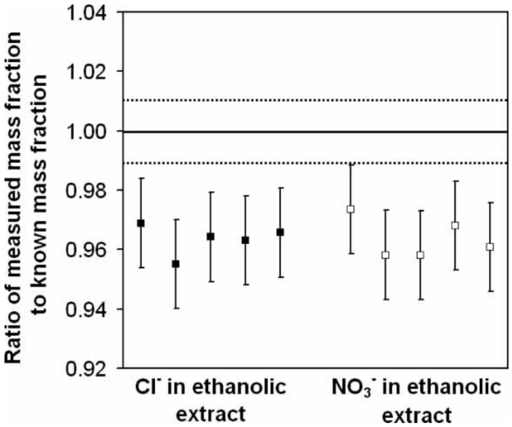

Figure 2. The ratio of the measured mass fraction to the known mass fraction for the measurement of chloride and nitrate in an ethanolic extract of five ambient air particulate samples. The error bars represent the uncertainty of the measurements at the 95% confidence interval. The dotted lines represent the uncertainty in the known mass fraction at the 95% confidence interval.

Correcting the Observed Measurement Error

The calculation of the magnitude of these errors is possible in these exemplar situations, since the compositions of the samples were known and their density could be calculated. Cleary, this is not normally the case for ‘unknown samples’. These errors can be significant and need to be overcome in order to achieve accurate measurement results for these and similar situations. There are several methods that can be considered in order to correct for this error. These are described and evaluated in Table 1. It can be seen that Methods 4 and 5 are the only realistic courses of action to eliminate any error from the measurement. It is clear that Method 4, standard addition calibration, would be effective in overcoming the physical matrix effect. However, this technique is time consuming and requires relatively large quantities of unknown sample. It is also not suitable for use with large numbers of samples in routine analytical measurement, or in automated high-throughput techniques. Therefore, Method 5 is the most suitable universal method for eliminating any error in these measurements. It is also applicable to automated high-throughput analysis regimes. The application of Method 5 is explained in more detail below.

Table 1. Possible methods to overcome the measurement error caused by the “physical matrix” effect

Method to overcome observed error	Advantages	Disadvantages
Use of volumetrically prepared standards	Eliminates any error	Would increase the uncertainty of the measurement dramatically (see Introduction)
Expanded measurement uncertainty	Produces an uncertainty statement that allows for any error	Would require prior investigation to determine size of error. Doesn’t correct error-is intrinsically unsatisfactory
Performing a density correction	Eliminates any error	Requires knowledge of the composition of the ‘unknown sample’ and is therefore unworkable in most cases
Density matching via standard addition calibration	Eliminates any error	This methodology is very time consuming and not practical for automated, high-throughput analysis methodologies
The use of mass concentration based, instead of mass fraction based, units	Eliminates any error	Requires knowledge of the density of calibration solutions. In most cases this may be easily calculated

Consider if Equation (6) was rewritten in terms of standards and samples whose composition was described on a mass concentration basis; this would yield:

$$\frac{k \cdot v \cdot \gamma_{i,sam}}{I_{i,sam} \cdot M_{i,sam}} = \frac{k \cdot v \cdot \gamma_{i,cal}}{I_{i,cal} \cdot M_{i,cal}} \tag{9}$$

Therefore, this yields the unknown mass concentration as:

$$\gamma_{i,sam} = \frac{\gamma_{i,cal} \cdot I_{i,sam}}{I_{i,cal}} \tag{10}$$

with no systematic error in the measurement result relating to the matrix of the sample.

For a routine analysis application, the use of mass concentration units for the measurement procedures is, therefore, the best method for overcoming the observed error. It is still essential that standard solutions are prepared gravimetrically rather than volumetrically in order to ensure accuracy. However, these standards should then be labelled with mass concentration units. This should be possible as long as preparation and analysis is done under temperature controlled conditions (although the density of aqueous solutions is not particularly sensitive to small temperature changes – approximately 1 part in 10^3 for a 5°C change). The components of the calibration standard will be present at known levels and, therefore, the density of the final solution may be readily calculated.[7] In most applications, the analyte component of the calibration standard will be present in such low concentrations that the density of the

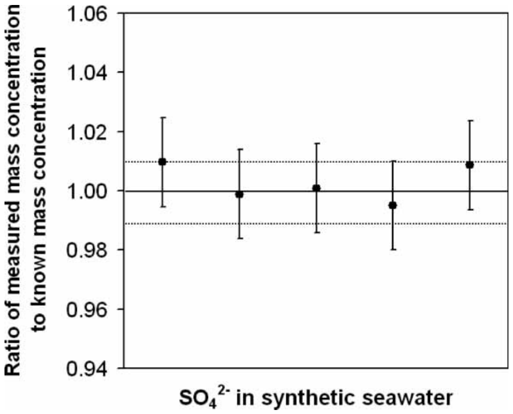

Figure 3. The ratio of the measured mass concentration (converted from the data in Figure 1) to the known mass concentration for the measurement of sulphate in 5 synthetic seawater solutions of approximate salinity $s = 35$. The error bars represent the uncertainty of the measurements at the 95% confidence interval. The dotted lines represent the uncertainty in the known mass concentration at the 95% confidence interval.

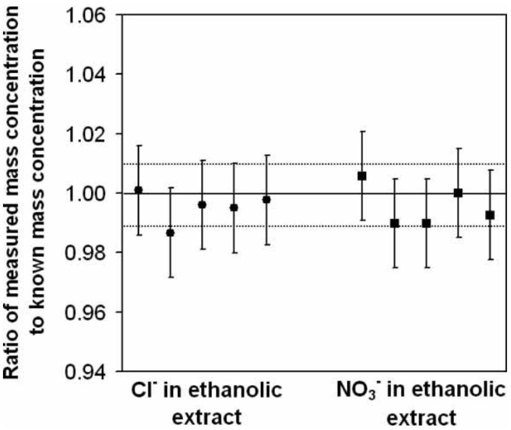

Figure 4. The ratio of the measured mass concentration (converted from the data in Figure 2) to the known mass concentration for the measurement of chloride and nitrate in an ethanolic extract from five ambient air particulate samples. The error bars represent the uncertainty of the measurements at the 95% confidence interval. The dotted lines represent the uncertainty in the known mass concentration at the 95% confidence interval.

mixture will be approximately that of the solvent. Since in the examples presented here the density of the measured solutions were known, the effect of making a mass concentration based measurement, rather than a mass fraction based measurement may be observed. In effect, this is the equivalent of performing a density correction. Recalculation of the data in Figures 1 and 2, on a mass concentration basis, according to Equation (3), is shown in Figures 3 and 4.

Performance of a mass concentration measurement has removed the systematic error in the measurement, as predicted by Equation (10). It should be remembered that the magnitude of quantities containing volume terms, such as mass concentration might be considered less useful since their magnitude is dependent on pressure (for gaseous mixtures) and temperature. If mass fraction units are to be replaced by mass concentration units, a statement of the temperature at which the measurement is valid must also be made.

CONCLUSIONS

It is widely acknowledged that the gravimetric preparation of standard solutions is more accurate and reproducible than volumetric preparation. These solution standards are most usually used to determine the sensitivity of a measuring instrument via the process of calibration. For a large number of analytes and measurement methods, no significant chemical matrix effect exists which can interfere with the measurement process. In these situations a normal working curve approach to calibration is undertaken with standard solutions made up in simple matrices.

It has been shown that a 'physical matrix effect', can exist, where although the response of the measuring instrument to a given amount of analyte is not affected, a measurement procedure using mass fraction based units can produce a biased result. This occurs principally because the density of the analyte can vary from that of the calibration solution. In analytical techniques that rely on a fixed volume injection to introduce the sample it has been shown that this can lead to a mass fraction analysis that is biased by an amount related to the difference in density between the sample and the calibration solution. This has been demonstrated experimentally for the analysis of sulphate in synthetic seawater samples and the measurement of the anionic content of particulate matter following extraction with water and wetting agents where average errors of +2.7% and −3.2%, respectively, were observed. The approximate error caused by the physical matrix effect for analytical methodologies using fixed volume injection techniques for the measurement of a minor component in a range of other sample matrices is given in Table 2 for comparison.

Several methods have been considered to overcome this error. Standard addition calibration, the method commonly used to overcome chemical matrix effects, would eliminate this error. However, this technique is time consuming and requires relatively large quantities of unknown sample.

Table 2. The approximate error caused by the physical matrix effect for analytical methodologies using fixed volume injection techniques for the measurement of minor components in a range of environmental sample matrices. These predictions assume that calibration standards are prepared with ultra-pure deionised water. Values with asterisks (*) indicate that the error depends on the exact composition of the sample

Sample matrix	Indicative predicted measurement error (%)
Sweetened soft drink	up to +4.0*
Seawater (salinity, $s = 40$)	+3.0
Blood plasma	+2.6*
Seawater (salinity $s = 35$)	+2.5
Seawater (salinity $s = 30$)	+2.0
Normal medical saline	+0.5
Waster water	up to +0.4*
Mineral water	up to +0.2*
Pure water	0
Ground water contaminated with light oils, organic compounds	up to −0.5*
Particulate matter extracted by 9:1 water to ethanol solution	−1.9
Particulate matter extracted by 4:1 water to ethanol solution	−3.2

Furthermore, it is also not suitable for use with large numbers of samples, or in automated high-throughput techniques.

It is proposed that the method employed to eliminate this error is the gravimetric preparation of simple matrix solution standards as before, but to follow this with conversion of the analyte mass fractions (kg/kg) therein to mass concentrations (kg/m^3) via Equation (3). The measurement should then be performed of a mass concentration basis. For the majority of cases this conversion, which requires the density of the calibration standard to be known, should be simple. Complex calibration mixtures may be more troublesome.[16]

Although the performance of a mass concentration based measurement has removed the systematic error in the measurement, it should be remembered that the magnitude of quantities containing volume terms, such as mass concentration might be considered less useful since their magnitude is dependent on temperature. If mass fraction units are to be replaced by mass concentration units, it is important that a statement of the temperature at which the measurement is valid must also be made.

This work has implications for a variety of published standards relating to the measurement of minor ionic components in complex environmental matrices[8,13] by fixed volume injection methods. These procedures may suffer from the physical matrix effect described in this paper in measurements performed and expressed using mass fraction based units.

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