



Why Calibration Graphs Curve in Atomic Absorption Spectrometry

Application Note

Atomic Absorption

Author

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Introduction

The application of atomic absorption spectrometry (AAS) for the determination of metal concentrations (especially at trace levels) has been very successful. The technique as envisaged by Walsh [1] of the Division of Chemical Physics, Commonwealth Scientific and Industrial Research, Australia (CSIRO) is to pass light of an appropriate wavelength through a cloud of the atoms of interest. The common light source is the hollow cathode lamp [2]. This light is preferentially absorbed by unexcited atoms. The amount of light absorbed is proportional to the number of unexcited atoms present. This has been referred to as the Beer-Lambert law [3] and can be expressed in the form of the equation below.

$$\text{Absorbance} = \log(I_0/I) \quad (1)$$

where I_0 = incident intensity

I = transmitted intensity

This expression can be re-written as shown below.

$$\text{Absorbance} = abc$$

where a = absorption coefficient (2)

b = length of the absorption path

c = concentration of absorbing atoms

The second form of the equation, (2), shows that if a and b are kept constant, then absorbance, A , is directly proportional to concentration, c . This is shown graphically in Figure 1a. In an ideal Beer-Lambert case if a concentration of atoms, c , produces an absorbance, A , then a concentration $2c$ should produce an absorbance $2A$. Also in theory, since a and b are constants, a simple measurement of absorbance should immediately give the atom concentration.



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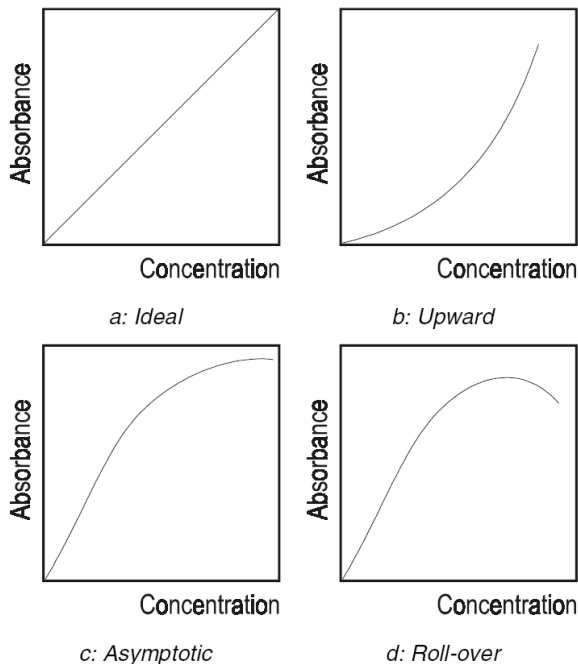


Figure 1. Possible Beer-Lambert graphs.

In practice, atomic absorption rarely shows ideal Beer-Lambert behavior. AAS is therefore a comparative technique rather than an absolute one. The spectrometer must be calibrated against a series of solutions of known concentrations ("standards") to give a calibration graph. Calibration graphs showing non-ideal behavior usually have one of three different shapes as shown in Figure 1b, c, and d. As a further complication the effects giving rise to these curved graphs may be superimposed on each other [4]. Each cause of these effects is discussed below.

Figure 1b shows "upward curvature" in which the graph curves towards the absorbance axis. This behavior is seen only for a relatively small concentration range.

Figure 1c shows the graph curving towards the concentration axis. As the concentration increases the graph tends to become parallel to the concentration axis. This "asymptotic" graph means that above a certain concentration value the absorbance does not increase at all. As will be seen in section 3.1.1, all incident light (I_0) is not necessarily absorbed equally. In this case the transmitted light (I) will reach a constant value, which gives rise to the asymptotic shape of the graph.

Figure 1d is a special variation of Figure 1c. Above a certain concentration the graph does not become parallel but curves back towards the concentration axis. This "roll-over" behavior means that two different concentrations can give the same absorbance.

Calibrating an AA Spectrometer

The operation of an AA spectrometer relies on two assumptions. The first assumption is that the path length (**b**) through the absorbing atoms is constant. The second assumption is the absorption coefficient (**c**) is also constant.

The instrument's atomizer (where the absorbing atoms are produced) is designed to have as constant a path length as possible. The operator must adjust the instrument according to the manufacturer's instructions so this assumption is true.

The absorption coefficient depends on a physical quantity, called the oscillator strength. Much work has been done in determining this as accurately as possible for each element. There is still debate on the accuracy of this constant for some elements [5].

The quickest and most practical method of calibrating an AA spectrometer is to prepare a series of calibration standard solutions. These solutions should match as far as possible the chemical and physical properties of the samples to be analyzed. The absorbances of these standards are measured and used to plot a calibration graph. Although some graphs are linear most are curved to some extent. Attempts have been made to "linearize" curved graphs [6].

A curved calibration graph is not a problem in mathematical terms. Any one of a number of curve-fit algorithms may be used to fit a line to the measured data points [7]. The difficulty is in deciding which algorithm to use.

Most commercial spectrometers allow the choice from a number of commonly used algorithms (including linear, quadratic, and cubic). The availability of all numerical data allow the user to determine which algorithm is the most suitable or to perform further analysis if required. The process of selecting the algorithm may be done during or after an analysis.

Modern computerized AA spectrometers can therefore accommodate curved calibration graphs and can interpolate to give a concentration value for a measured absorbance. However there is a potential danger in some cases where calibration graphs can give rise to false or misleading results. The operator must either take corrective action or interpret the results cautiously. The causes for curvature are discussed below.

Processes in Atomic Absorption

The causes of curvature in AA calibration graphs are as follows [1,8].

Recording

High-speed electronics have improved signal detection and processing to the extent that the recording step introduces practically no errors.

Signal integration can also be done quickly and accurately. Modern computers allow the storage and retrieval of the signal graphics for detailed analysis and further measurements.

The main benefit to the operator is the considerable quantity of information that can be captured and stored for each measurement. The recording step has no practical difficulties and is therefore not discussed further.

Atomization and Transport

The atomization and transport steps are the formation of atoms and their subsequent transport into the light path. Although extensively researched, these are still not as well understood as they could be.

Flame

Upward curvature (Figure 1b) when observed in flame AAS is almost invariably caused by chemical interference. The analytes are usually Group 1 or 2 lanthanide elements.

The most common cause of upward curvature, particularly for Group 1 metals, is a special form of chemical interference: partial ionization. The atoms are ionized at the high temperatures (2300 K to 2900 K) found in the flames used for AAS [4]. In these cases upward curvature is only seen at relatively low analyte concentration. As the concentration increases there is a decrease in the degree of ionization because ionization is a mass balance effect. At a sufficiently high concentration the graph behaves like that shown in Figure 1c although sensitivity is less than would be expected.

The solution is to add a large excess of ionization buffer, an element more easily ionized than the analyte. The ionization buffer significantly reduces upward curvature as well as increasing sensitivity. The calibration graph now resembles Figure 1a or Figure 1c over the whole concentration range.

Upward curvature can also be seen in the determination of Group 2 elements such as calcium. A species such as aluminium or phosphate ions forms a refractory compound. Low levels of these will produce upward curvature [4]. At high levels, the sensitivity is much less than expected. In this situation, the

effect is overcome using one or more of the following:

- Nitrous oxide-acetylene flame
- Careful set-up of the nebulization process
- Releasing agent

The high temperature of the nitrous oxide-acetylene flame (2800 K) is sufficient to decompose most refractory compounds. It can also ionize the Group 2 elements and an ionization buffer may be required. The impact bead of the nebulizer may be adjusted to give very fine droplets. These smaller droplets are more readily desolvated and the resultant particles more readily decomposed [3] thus minimizing chemical interference effects [9]. A releasing agent is an element (lanthanum is commonly used) that competes preferentially for the interfering species and, in effect, releases the analyte.

Furnace

In the graphite furnace chemical interferences are more common than in flame. The graphite furnace is employed for the determination of trace levels of analytes. Interfering species are almost invariably present in excess. Upward curvature is extremely rare. Most calibration graphs resemble Figure 1c. As seen in flame, chemical interferences generally affect the slope of the graph. These may be minimized with the use of chemical modifiers [10] or pyrolytic platforms [11] or both [12]. This is too large a topic to be covered here.

To summarize, chemical interferences can affect the shape of the graph. In most cases, it is mainly the slope that is affected. The presence of a chemical interference must be identified. Otherwise the chemical mismatch between standards and samples means a lower level of analyte is recorded than is actually present. At the very least a recovery study should be done to estimate the extent of chemical interferences [13].

Absorption

A considerable amount of research has been devoted to the process by which atoms absorb light. This process can be divided into two broad areas:

- Physical characteristics of the instrument
- Spectral characteristics of the absorption line

Physical Characteristics

Design of the spectrometer itself plays an important role in the shape of the calibration graph. Parameters that must be taken into account include:

- Resolution of the monochromator
- Optics around the atomizer
- Thermal distribution of the atom cloud
- Spatial distribution of the atom cloud

Resolution of the Monochromator

The function of the monochromator is to allow as much light as possible of the analytical (or resonance) emission line to reach the light detector (usually a photomultiplier tube). At the same time it must exclude light from non-resonance lines. The ability of a monochromator to discriminate among wavelengths is called "resolution". Proper design of a monochromator to achieve good resolution must consider the focal length, the number of lines on the diffraction grating and the size of the exit slits. These factors are inter-related and cannot be considered separately.

For many elements, the main resonance line is well separated from non-resonance lines and emission lines from the fill gas. Only one emission line passes through the monochromator exit slit. If more than one line falls within the slit then the calibration graph will be curved, unless all the lines have exactly the same absorption coefficient [14]. Emission lines that are too close to the resonance line to be easily resolved include:

- Unresolved multiplets
- Non-absorbed lines

Unresolved Multiplets

Many transition elements, such as vanadium and manganese have resonance lines that are part of a multiplet. Manganese has a spectroscopic triplet with lines at 279.5 nm, 279.8 nm and 280.1 nm (Figure 2).

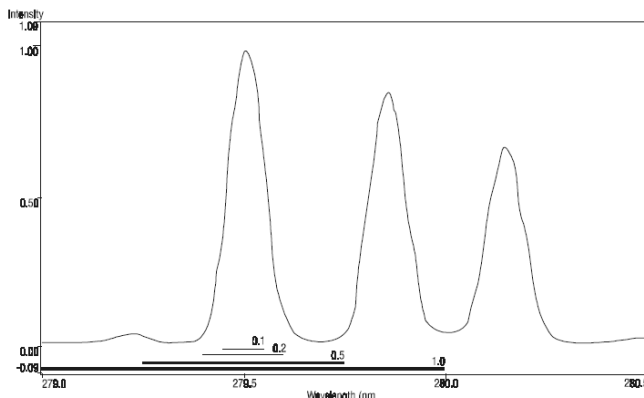


Figure 2. Manganese triplet at 279.5 nm, 279.8 nm and 280.1 nm; relative spectral bandpasses are shown centered on the 279.5 nm line.

The 279.5 nm line absorbs more strongly than the other two. A wide (1.0 nm) spectral bandpass allows all the light from the 279.8 nm and most from the 280.1 nm lines to reach the photomultiplier tube. A curved calibration graph results. A narrow spectral bandpass (0.2 nm or smaller) excludes most of the emission from the companion lines and generates relatively straight calibration graph. A family of calibration graphs can be generated as shown in Figure 3. As the spectral bandpass decreases, the amount of non-absorbed light (I) decreases and the degree of asymptotic behavior is less.

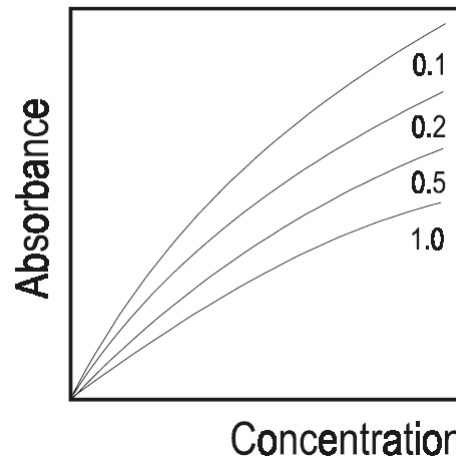


Figure 3. Family of calibration graphs that can be generated using different slit widths.

Non-Absorbed Lines

Elements such as iron and nickel have non-absorbed lines very close to the most sensitive resonance line. Clearly the amount of light absorbed is not proportional to the atom concentration. This is especially obvious at higher concentrations where the intensity of the resonance emission line is small compared with the intensity of the non-absorbed line. The effect is severe curvature of the calibration graph. Decreasing the slit width of the monochromator blocks more of the light from the non-absorbed line and the graph becomes less curved. Decreasing the slit also reduces the amount of light from the resonance line. This reduction in light usually means an increase in the baseline noise. The width of the slit used is a compromise between calibration graph curvature and the baseline noise level.

A related effect is stray light. This is caused by random reflections within the monochromator passing through the exit slit. Precautions must be taken during the design and manufacture of a monochromator to minimize the amount of stray light. The most obvious cause of stray light is incandescence from a luminous flame or a graphite furnace. The atomizer must be properly aligned to reduce this effect.

Optics Around the Atomizer

The optical path of an AA spectrometer has a focus point at the atomizer. This is shown in Figure 4a, b. One part of the beam passes through the center and the rest crosses the atomizer at varying angles [15]. The most obvious effect is the difference in path lengths. In addition the light passes through different temperatures in the flame and it is reasonable to assume the absorption coefficient will also vary. The SpectrAA monochromators have "narrow beam optics". The optical path closely matches the atomizer profile as shown in Figure 4a. There are two advantages. Variations in path length and absorption coefficient are minimized. Incandescence, especially from a furnace, entering the monochromator is reduced. The ideal situation is shown in Figure 4c. The flow-through mercury cell of the VGA-77 and the ACT-80 approach this.

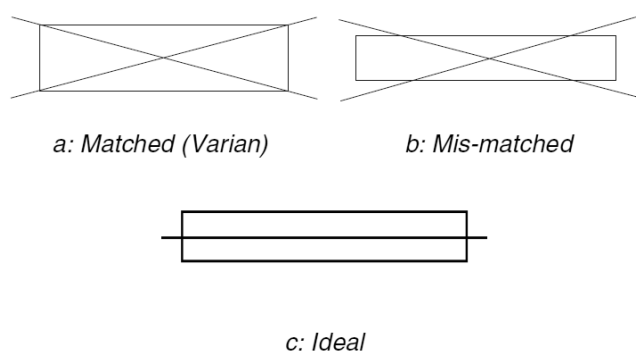


Figure 4. Representations of optical path with respect to atomizer.

Thermal Distribution of the Atom Cloud

The thermal distribution of the atom cloud must be considered. A flame has zones of different temperatures. Each zone will have its own atom concentration. Along a properly aligned system (as shown in Figure 4a) the temperature variations will be relatively small so the atom concentration is uniform over the light path. In Figure 4b, there is major temperature variation at the edges of the flame where the light path enters and leaves. The light is therefore passing through large variations in atom population.

A graphite furnace has a greater temperature variation than a flame. Careful design can give a relatively constant temperature along much of a longitudinally heated tube (Figure 5). Most of the atoms are in this region. The number of atoms in the cooler ends of the tube tends to smaller. The two effects again cancel each other. In most cases only the temperature at the center of the tube is significant [16]. The thermal distribution of the atom cloud therefore does not contribute significantly to curvature of the calibration graph. It does reduce the slope [8].

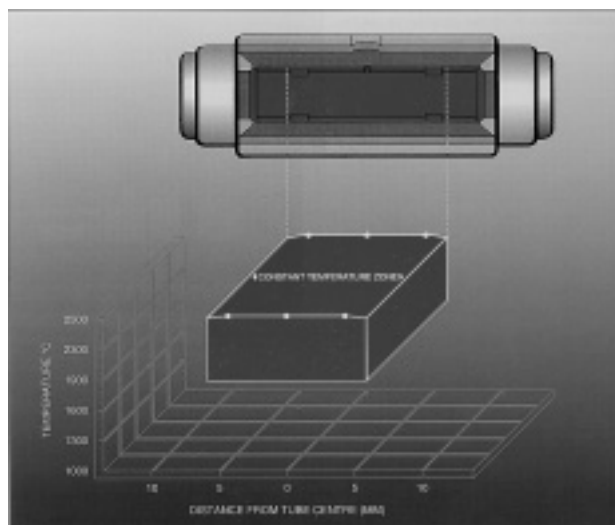


Figure 5. Constant temperature zone design of the GTA.

Spatial Distribution of the Atom Cloud

A well-designed flame atomizer should homogenize the combustion gases and the solution aerosol [1]. This contributes to uniform atom distribution. Within the flame itself there is a large amount of heat and the atoms are travelling very rapidly. The atom distribution is therefore uniform within a temperature zone. In a properly aligned burner (Figure 4a) the light path passes through a relatively homogeneous atom cloud. The width of the flame should be larger than the light path.

In a furnace a droplet of the solution is dispensed at the midpoint. The droplet tends to spread along the floor of the tube (to the partition rings) or of the platform. It is reasonable to assume that when the atoms are vaporized the transient atom cloud will diffuse upwards and then towards the ends. The atom distribution is still more or less homogeneous (depending on element). The peak atom density during atomization can contribute to non-linearity of the calibration graph [18].

The situation in which the light beam passes through a non-homogeneous atom distribution has been considered. It has been calculated that this non-homogeneous distribution causes significant curvature of the calibration graph [8].

Calibration graphs in general tend to show more curvature for furnace than for flame. Atom distribution is the biggest physical difference. High peak atom density during atomization and non-homogeneous atom distribution are the most likely contributors to curvature of the calibration graph in graphite furnace AAS.

Spectral Characteristics

The spectral lines used in AAS are better understood than the conditions of atom transport and distribution within the atomizer. One of the best conditions for atomic absorption is when the emission line is much narrower (has a smaller wavelength range) than the absorption line. In theory an infinitely narrow emission line would give rise to a linear calibration graph. In practice good results are obtained if the emission line is much narrower than the absorption line and the absorption line does not change with increasing concentration.

Absorption Profile

The absorbing atoms produced in commercial atomizers are at atmospheric pressure. The atoms and other species in the atomizer environment are continually colliding with each other. The absorption profile of the atoms is broadened by such collisions.

The atoms are also produced at relatively high temperatures (1000–3000 K). The atoms are therefore travelling at high velocities. The lines have another broadening component, Doppler, which is caused by the movement of the atoms.

The most common emission source in AAS is the hollow cathode lamp. These are designed so that the emitting atoms are at a lower temperature and pressure than the corresponding absorbing atoms. The emission lines are therefore narrower than the absorption lines. Incorrect operating conditions, such as a higher than recommended operating current, can cause broadening of the lines. More atoms are sputtered into the lamp's atom cloud. These atoms interact with each other (causing pressure broadening). The cloud tends to spread and cool down. The cooler atoms preferentially absorb light at the center of the emission line ("self-reversal"). The larger the self-reversal, the poorer the sensitivity and the more curved the calibration graph.

Hyperfine Structure

The profile of the emission line has been assumed to be a single line. Multiplets can often be resolved by the monochromator of an AA spectrometer. There is an effect that can cause splitting of the emission line itself. This splitting is caused by interactions between the nucleus and the electrons and can be due to nonzero nuclear spin, or to a mixture of isotopes [17]. In some cases both effects can occur together (the 253.7 nm Hg line and the 283.3 nm Pb line). The interactions create a number of transitions with very similar but distinct energy differences so that an emission line consists of a number of component lines very close together. This is called hyperfine structure (HFS). The difference in wavelengths is so small that an AA spectrometer monochromator cannot resolve them. The emission line is, in effect, broadened and this contributes to curvature. In addition, each HFS component does not necessarily have the same absorption coefficient and this gives rise to more curvature. This is one reason why the seemingly sharp Cd 228.8 nm line gives a curved calibration graph.

In some emission lines there are no hyperfine components. These are called "ordinary lines". The 225.9 nm Te line is an example.

Some emission lines have the HFS components so close together that they cannot be resolved. These are "quasi-ordinary lines". An example is the 213.9 nm Zn line.

The 283.3 nm Pb line is an example of a "structured line". The changes in the HFS components of the 324.8 nm Cu line have also been considered [17].

The HFS components of ordinary and quasi-ordinary lines do not usually contribute to curvature. Calibration graph curvature is caused mainly by broadening of these emission lines. The HFS of a structured line can contribute a significant amount of broadening.

Zeeman Effect

A special case of calibration graph curvature, reflex curvature, occurs in spectrometers fitted with Zeeman effect background correction systems [12,16,18]. This is shown in Figure 1d. Reflex curvature is in addition to the ones already discussed. A brief outline of Zeeman background correction is given to explain why the curvature occurs.

In a magnetic field, an atomic emission (or absorption) line is split into a number of Zeeman multiplets. The normal effect is shown in Figure 6. The single line is split into three components. The central (π) component is at the original wavelength. Two other (σ) components are shifted symmetrically away from the π component. The total intensities are the same as the intensity of the original line. The π component is polarized parallel to the applied magnetic field. The two σ components are polarized perpendicular to the applied field.

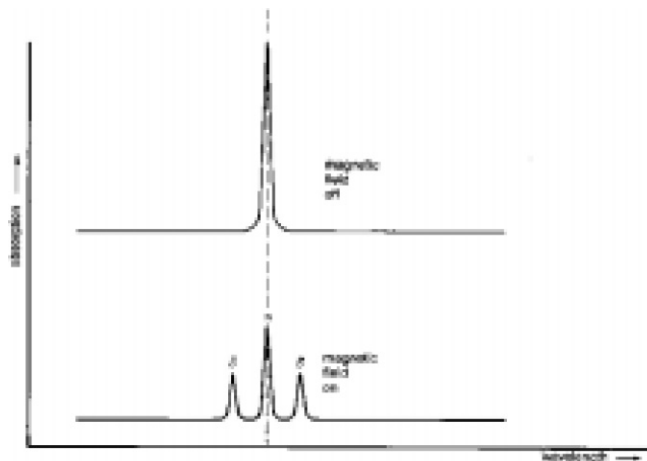


Figure 6. Example of the normal Zeeman effect (Cd at 228.8 nm).

The Zeeman splitting is more complex in the case of atoms with nuclear spin. Some examples are shown in Figure 7.

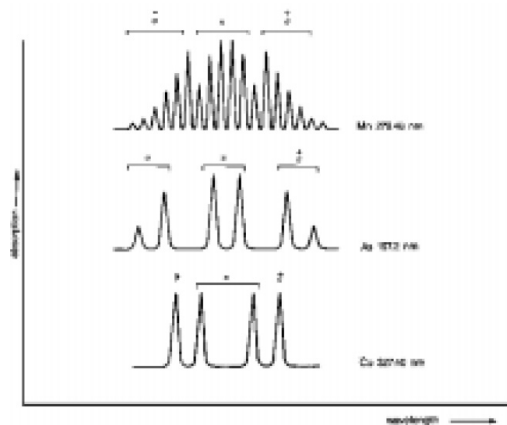


Figure 7. Examples of the anomalous Zeeman effect.

There are a number of practical configurations to use the Zeeman effect. These include applying a longitudinally-modulated magnetic field, with no polarizer; a transverse fixed (DC) magnetic field with a rotating polarizer. In the Varian Zeeman instruments, a modulated magnetic field is applied transversely to the atomizer. A fixed polarizer is used to reject the parallel polarized component of the hollow cathode lamp emission line. Only the perpendicularly polarized light is passed through the optical system.

The signal is measured with the magnetic field at maximum strength and at zero strength. At zero field strength the measured absorbance (A_{off}) is both atomic and background absorbance. When the magnetic field is applied, the absorption line is split. Only the π components can absorb parallel polarized light but the polarizer in the optical path removes the parallel polarized light. The applied magnetic field shifts the σ components sufficiently far from the lamp emission line that the atomic absorption is zero. The magnet effectively turns off the atomic absorption. The background species are magnetically insensitive and so continue to absorb (A_{on}). The difference between the two signals ($A_{off} - A_{on}$) is the corrected atomic absorbance.

The assumption that the atomic absorbance is zero, while the magnetic field is applied, is not valid for all cases. The way a structured line splits in a magnetic field may mean the σ components are not shifted far enough. In all cases the profiles broaden with increasing concentration. The wings of the σ components overlap in proportion to concentration. As this residual atomic absorption increases the calibration graph shows increasing curvature.

This reflex curvature behavior occurs with Zeeman instruments using any of the possible configurations. Both A_{off} and A_{on} show asymptotic behavior (Figure 1c) with increasing concentration. The A_{on} becomes asymptotic more slowly so that the resultant $A_{off} - A_{on}$ graph shows the reflex curvature (Figure 1d).

Summary

A range of causes contributes to the curvature of calibration graphs. Table 1 summarizes these causes and outlines strategies, if any, an operator can apply for minimizing them.

Table 1. Causes of Calibration Graph Curvature and Possible Remedial Actions

Cause	Degree	Minimizing strategies
Ionization	Large (for Group 1 and lanthanide elements)	Ionization buffer (flame)
Chemical interference	Large (for Group 2 and refractory elements)	Releasing agent (flame) Hotter $N_2O-C_2H_2$ flame (flame) Impact bead position (flame) Chemical modifiers (furnace) Platform (furnace)
Unresolved multiplets	Moderate (for transition elements)	Smallest practical slit width
Non-absorbed lines	Large (for transition elements)	Smallest practical slit width
Stray light	Large	Proper atomizer alignment
Emission line profile	Moderate	Appropriate lamp operating current Use boosted lamp
Hyperfine structure (HFS)	Moderate	Use alternative line
Atom spatial distribution	Moderate	Proper atomizer alignment (flame) Platform, in some cases (furnace)
Zeeman effect	Small to large	Use alternative line

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Printed in the USA
November 1, 2010
AA128



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