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2.2.21. FLUORIMETRY

Fluorimetry is a procedure which uses the measurement of the intensity of the fluorescent light emitted by the substance to be examined in relation to that emitted by a given standard.

Method. Dissolve the substance to be examined in the solvent or mixture of solvents prescribed in the monograph, transfer the solution to the cell or the tube of the fluorimeter and illuminate it with an excitant light beam of the wavelength prescribed in the monograph and as near as possible monochromatic.

Measure the intensity of the emitted light at an angle of 90° to the excitant beam, after passing it through a filter which transmits predominantly light of the wavelength of the fluorescence. Other types of apparatus may be used provided that the results obtained are identical.

For quantitative determinations, first introduce into the apparatus the solvent or mixture of solvents used to dissolve the substance to be examined and set the instrument to zero. Introduce the standard solution and adjust the sensitivity of the instrument so that the reading is greater than 50. If the second adjustment is made by altering the width of the slits, a new zero setting must be made and the intensity of the standard must be measured again. Finally introduce the solution of unknown concentration and read the result on the instrument. Calculate the concentration c_x of the substance in the solution to be examined, using the formula:

$$c_x = \frac{I_x c_s}{I_s}$$

c_x = concentration of the solution to be examined,

c_s = concentration of the standard solution,

I_x = intensity of the light emitted by the solution to be examined,

I_s = intensity of the light emitted by the standard solution.

If the intensity of the fluorescence is not strictly proportional to the concentration, the measurement may be effected using a calibration curve.

In some cases, measurement can be made with reference to a fixed standard (for example a fluorescent glass or a solution of another fluorescent substance). In such cases, the concentration of the substance to be examined must be determined using a previously drawn calibration curve under the same conditions.

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2.2.22. ATOMIC EMISSION SPECTROMETRY

GENERAL PRINCIPLE

Atomic emission is a process that occurs when electromagnetic radiation is emitted by excited atoms or ions. In atomic emission spectrometry the sample is subjected to temperatures high enough to cause not only dissociation into atoms, but also to cause significant amounts of collisional excitation and ionisation of the sample atoms to take place. Once the atoms and ions are in the excited states, they can decay to lower states through thermal or radiative (emission) energy transitions and electromagnetic radiation is emitted. An emission spectrum of an element contains several more lines than the corresponding absorption spectrum.

Atomic emission spectrometry is a technique for determining the concentration of an element in a sample by measuring the intensity of one of the emission lines of the atomic vapour of the element generated from the sample. The determination is carried out at the wavelength corresponding to this emission line.

In this chapter only atomisation in flame is dealt with. The method of inductively coupled plasma-atomic emission spectrometry (ICP-AES) is described in a different general chapter.

APPARATUS

This consists essentially of:

- a sample introduction and nebulisation system;
- a flame to generate the atoms to be determined;
- a monochromator;
- a detector;
- a data-acquisition unit.

Oxygen, air and a combustible gas such as hydrogen, acetylene, propane or butane may be used in flames. The atomisation source is critical, since it must provide sufficient energy to excite and atomise the atoms. The atomic spectra emitted from flames have the advantage of being simpler than those emitted from other sources, the main limitation being that the flames are not powerful enough to cause emission for many elements allowing their determination. Acidified water is the solvent of choice for preparing test and reference solutions, although organic solvents may also be used if precautions are taken to ensure that the solvent does not interfere with the stability of the flame.

INTERFERENCES

Spectral interference is reduced or eliminated by choosing an appropriate emission line for measurement or by adjusting the slit for spectral band-width. Physical interference is corrected by diluting the sample solution, by matching the matrix or by using the method of standard additions. Chemical interference is reduced by using chemical modifiers or ionisation buffers.

MEMORY EFFECT

The memory effect caused by deposit of analyte in the apparatus may be limited by thoroughly rinsing between runs, diluting the solutions to be measured if possible and thus reducing their salt content, and by aspirating the solutions through as swiftly as possible.

METHOD

Use of plastic labware is recommended wherever possible.

Operate an atomic emission spectrometer in accordance with the manufacturer's instructions at the prescribed wavelength. Optimise the experimental conditions (flame temperature, burner adjustment, use of an ionic buffer, concentration of solutions) for the specific element to be analysed and in respect of the sample matrix. Introduce a blank solution into the atomic generator and adjust the instrument reading to zero or to its blank value. Introduce the most concentrated reference solution and adjust the sensitivity to obtain a suitable reading.

It is preferable to use concentrations which fall within the linear part of the calibration curve. If this is not possible, the calibration plots may also be curved and are then to be applied with appropriate calibration software.

Determinations are made by comparison with reference solutions with known concentrations of the element to be determined either by the method of direct calibration (Method I) or the method of standard additions (Method II).

METHOD I - DIRECT CALIBRATION

For routine measurements 3 reference solutions of the element to be determined and a blank are prepared and examined.

Prepare the solution of the substance to be examined (test solution) as prescribed in the monograph. Prepare not fewer than 3 reference solutions of the element to be determined, the concentrations of which span the expected value in the test solution. For assay purposes, optimal calibration levels are between 0.7 and 1.3 times the expected content of the element to be determined or the limit prescribed in the monograph. For purity determination, calibration levels are between the limit of detection and 1.2 times the limit specified for the element to be determined. Any reagents used in the preparation of the test solution are added to the reference solutions and to the blank solution at the same concentration.

Introduce each of the solutions into the instrument using the same number of replicates for each solution, to obtain a steady reading.

Calculation. Prepare a calibration curve from the mean of the readings obtained with the reference solutions by plotting the means as a function of concentration. Determine the concentration of the element in the test solution from the curve obtained.

METHOD II - STANDARD ADDITIONS

Add to at least 3 similar volumetric flasks equal volumes of the solution of the substance to be examined (test solution) prepared as prescribed. Add to all but 1 of the flasks progressively larger volumes of a reference solution containing a known concentration of the element to be determined to produce a series of solutions containing steadily increasing concentrations of that element known to give responses in the linear part of the curve, if at all possible. Dilute the contents of each flask to volume with solvent.

Introduce each of the solutions into the instrument using the same number of replicates for each solution, to obtain a steady reading.

Calculation. Calculate the linear equation of the graph using a least-squares fit, and derive from it the concentration of the element to be determined in the test solution.

VALIDATION OF THE METHOD

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

LINEARITY

Prepare and analyse not fewer than 4 reference solutions over the calibration range and a blank solution. Perform not fewer than 5 replicates.

The calibration curve is calculated by least-square regression from all measured data. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when:

- the correlation coefficient is at least 0.99,
- the residuals of each calibration level are randomly distributed around the calibration curve.

Calculate the mean and relative standard deviation for the lowest and highest calibration level.

When the ratio of the estimated standard deviation of the lowest and the highest calibration level is less than 0.5 or greater than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression.

Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

ACCURACY

Verify the accuracy preferably by using a certified reference material (CRM). Where this is not possible, perform a test for recovery.

Recovery. For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. For other determinations, for example for trace element determination, the test is not valid if recovery is outside of the range 80 per cent to 120 per cent at the theoretical value. Recovery may be determined on a suitable reference solution (matrix solution) which is spiked with a known quantity of analyte (middle concentration of the calibration range).

REPEATABILITY

The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

LIMIT OF QUANTIFICATION

Verify that the limit of quantification (for example, determined using the 10 σ approach) is below the value to be measured.

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2.2.23. ATOMIC ABSORPTION SPECTROMETRY

GENERAL PRINCIPLE

Atomic absorption is a process that occurs when a ground state-atom absorbs electromagnetic radiation of a specific wavelength and is elevated to an excited state. The atoms in the ground state absorb energy at their resonant frequency and the electromagnetic radiation is attenuated due to resonance absorption. The energy absorption is virtually a direct function of the number of atoms present.

This chapter provides general information and defines the procedures used in element determinations by atomic absorption spectrometry, either atomisation by flame, by electrothermal vaporisation in a graphite furnace, by hydride generation or by cold vapour technique for mercury.

Atomic absorption spectrometry is a technique for determining the concentration of an element in a sample by measuring the absorption of electromagnetic radiation by the atomic vapour of the element generated from the sample. The determination is carried out at the wavelength of one of the absorption (resonance) lines of the element concerned. The amount of radiation absorbed is, according to the Lambert-Beer law, proportional to the element concentration.

APPARATUS

This consists essentially of:

- a source of radiation;
- a sample introduction device;
- a sample atomiser;
- a monochromator or polychromator;
- a detector;
- a data-acquisition unit.

The apparatus is usually equipped with a background correction system. Hollow-cathode lamps and electrodeless discharge lamps (EDL) are used as radiation source. The