Pure & Appl. Chem., Vol. 58, No. 7, pp. 1015–1022, 1986.
Printed in Great Britain.

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION

COMMISSION ON ANALYTICAL REACTIONS AND REAGENTS*

QUANTITATIVE CHARACTERIZATION OF PROCEDURES USING ULTRAVIOLET AND VISIBLE MOLECULAR ABSORPTION SPECTROPHOTOMETRY

Prepared for publication by

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Quantitative characterization of procedures using ultraviolet and visible molecular absorption spectrophotometry

Abstract - This paper gives information on the characterization of spectrophotometric methods of determination. The proposed characteristics should be helpful for practising analysts to select the most suitable photometric reagent or procedure for a particular problem from the vast number of substances and procedures recommended in the literature.

Three main groups of characteristics are proposed: 1) characterization of the reagent (purity, spectral properties, stability of reagent in solution, etc.); 2) optimal conditions for the procedure (pH, buffer used, recommended wavelength, conditional molar absorptivity, etc.); 3) characteristic parameters of the procedure (working range, equation of the calibration plot, sensitivity, precision, interferences, etc.). Particular attention is given to means of comparing different reagents and procedures and of characterising interfering species and their effects.

A further objective of the present discussion is to encourage a reduction in the number of new publications on spectrophotometric reagents with little or no practical importance which can be evaluated by means of the proposed characteristics.

INTRODUCTION

The vast number of spectrophotometric reagents and procedures recommended for inorganic analytes makes it difficult for the practising analyst to select the best reagent or procedure for a particular purpose. Thus, some old well-tested but not necessarily optimal reagents are often preferred to new and better ones, many of which have been insufficiently tested, or the procedures describing their use are not fully acceptable. Many novel reagents after critical evaluation, however, show no advantage over the Older well-tested ones. It is therefore desirable to establish criteria or recommendations for the testing of spectrophotometric procedures for various analytical purposes. Such criteria, however, can only include general considerations which are easily brought together as a whole and, if necessary, can be extended to deal with special cases of analytical practice.

The criteria recommended here characterise in a reasonably detailed way the efficiency of an individual analytical procedure and also allow the comparison of different procedures to be readily made. The problem is not a new one; it has been discussed previously by Kirkbright (refs. 1,2), Wilson (ref. 3), Röbisch (ref. 4) and Kozlicka (ref. 5). However, it was considered that these treatments of this problem were not sufficiently concise and explicit to be of value for the practising analyst and more concise recommendations for the unbiased characterization and comparison of spectrophotometric procedures and reagents are needed. The present set of criteria or recommendations is based on the earlier work of Sommer et al. (refs. 6,8) and of Ackermann et al. (ref. 7).

MAIN CHARACTERISTICS

In the development of a new and unbiased spectrophotometric procedure, a detailed examination of the equilibria for the reaction or reactions in solution should be carried out; this is necessary also to optimize available procedures.

The first requirement is the rapid and quantitative formation of a single, stable and highly absorbing species of an analyte with a particular reagent. This species should have a high molar absorptivity and a sufficient colour contrast between the desired absorbing species and the reagent alone under selected conditions. In addition the effect of various factors influencing the reaction, such as time, temperature, sequence of mixing the components, reagent concentration(s), pH and nature of buffer, inert salts, surfactants and solvents have to be examined under broad experimental conditions.

CHARACTERIZATION OF THE REAGENT

The reagent has to be unambigously described by name, preferably according to the current IUPAC nomenclature. Trivial or commercial names or synonyms should also be given if these are known. It is essential when dyes are being used to specify the Colour Index reference number.

A pure reagent of defined composition is preferred, or at least a sample of which contains no additional absorbing or interfering substances. The water content and/or amount of any inert salt and the conditions for storing the reagent should be given. The procedure for the purification of the reagent should be described, particularly with commercial or technical products, or when the reagent is first described in the literature.

The purity of the reagent and especially of a dye may usefully be checked by conventional thin-layer chromatography. The content of active substance should be determined by elemental and/or functional group analysis, acidimetric or redox titrations, etc. Some further instructions for checking the purity of organic reagents have recently been published (ref. 9).

The basic spectral properties (λ_{max}, ε_{λ}) for both acidic and basic forms of the reagent should be given together with the related pK_a values.

An indication of the stability of the reagent and of its solutions should also be mentioned.

OPTIMUM CONDITIONS OF THE PROCEDURE

Various approaches may be used to establish the optimal reaction conditions for a spectrophotometric procedure.

a) The previous evaluation of the number, stoichiometry and ranges of existence of the different species with their thermodynamic, kinetic and spectral parameters, including tests of any competitive equilibria in solution should be noted. An established pathway for the evaluation of all necessary parameters is the combined graphical and computer treatment of spectrophotometric data (refs. 10,11,12) which is useful even for involved complex equilibria. The optimum formation interval - i.e. those experimental conditions giving the greatest slope of the calibration graph and the largest value for the conditional stability constant - of the selected analyte species may be obtained from the distribution diagram or the response surfaces, calculated from appropriate, previously estimated or known, equilibrium or kinetic constants. Even preliminary or simplified calculations may be used to examine or adjust pH and component concentrations if a single or simple equilibrium is established, or if all necessary equilibrium constants are known with sufficient accuracy. Such results should, however, be verified experimentally (ref. 13).
b) Selection of the optimal formation interval of the analyte species should be carried out by means of experimental uni- or multivariant optimization procedures based on simplex or factorial design (ref. 14), which is effective even when the stoichiometry and other parameters of the analyte species are unknown. There are many spectrophotometric procedures for which the reaction mechanism is unknown, or may not be easily established, but for which optimal conditions may be obtained experimentally. Such procedures should, however, be used only under strictly controlled conditions.

The following information should be given for each spectrophotometric procedure.

i) The effect of temperature and time on the measured absorbance.
ii) The sequence of mixing the reaction components during the preparation of the solution before measurement is made.
iii) Optimal pH range, amount and composition of the buffer.
iv) Optimal concentration of the reagent; this depends on the stability of the complex formed with the analyte, the spectral properties of the reagent, the composition of the matrix as well as the recommended working concentration interval of the analyte. A 5-10 fold excess of the reagent over the analyte is normal for a stable complex, but as much as a 100-1000 fold excess may be needed for a weak complex (cf. ref. 1). The maximal usable concentration of strongly absorbing reagents is limited by their absorbance.
v) The correct wavelength for the measurement. This usually corresponds with λ_{max} of the analyte complex but may be different when it is necessary to achieve a better contrast between the analyte complex and the reagent (i.e. larger difference for λ_{max} (complex) - λ_{max} (reagent form)), or because of the larger absorptivity of the reagent at λ_{max} of the analyte complex. The absorbance of the sample solution can be measured in several ways.

1) The absorbance of the sample solution is measured against the pure solvent; the reagent blank is also measured against the pure solvent and the value for the reagent blank is subtracted.

   If matching cells with exactly the same path length are not available, the same cell should be used for measurement of both the solution and the blank to avoid error.

2) The absorbance of the sample solution is measured against the solvent but the reagent blank is not subtracted.

3) The absorbance of the sample solution is measured against the reagent blank.

   The blank may be pure solvent, the reagent solution, the solution of the sample with reagent or a synthetic solution similar to the matrix. The same kind of the blank must always be used in preparing the calibration plot and in the procedure.

There is no special preference between 1), 2) and 3). The optical properties of the reagent, the procedure for obtaining the absorbance data and the precision of the instrument as well as the type of statistical treatment to be used should be taken into account in the selection.

vi) The optical cell material and path length.
vii) The conditional molar absorptivity, ε' (in $dm^3mol^{-1}cm^{-1}$) of the measured complex, for well-defined experimental conditions.
viii) The effect of ionic strength, including the contribution from the inert salts which may be formed during the preparation of the analyte solution or sample pretreatment.
ix) The effect of other components, especially surfactants which may considerably influence the optical properties of the analyte complex and of some reagent forms.

If solvent extraction is a part of the spectrophotometric procedure, the extraction parameters such as partition coefficient, extraction constant, the solvent volume and the extraction time should also be given.

CHARACTERISTIC PARAMETERS OF THE PROCEDURE

These parameters are usually evaluated using pure analyte solutions under clearly defined conditions. It must be noted that such parameters are only valid for these conditions.

Calibration

For pratical reasons, the calibration graph is prepared from a sufficient number (normally >5) of aliquots of a standard solution following the optimal conditions for the procedure, usually for a 10 mm cell path length.

There are various ways to establish calibration graphs, but the particular one selected should be described in detail. The solutions of the substance may be (a) the pure analyte solutions, (b) synthetic solutions containing all the main components of the sample or (c) solutions prepared from standard substances. In (b) and (c) the stated composition must be similar to that of the sample. One or other of the last two ways is applied if particular sample matrices cause interference.

It is unnecessary for linear calibration graphs to be given. Instead the appropriate equation should rather be given thus:

$$A = \varepsilon' \cdot c + A'_o$$

for the corresponding concentration interval, where A is the absorbance, ε' the conditional absorptivity in $dm^3mol^{-1}cm^{-1}$, for c in mol dm^{-3} or in $cm^2\mu g^{-1}$, for c in $\mu g\ cm^{-3}$ as the concentration unit which is frequently used in analytical practice. A'_o is the absorbance of the blank at the wavelength recommended for the measurement.

Working concentration range of analyte

The number of standard solutions or of aliquots of a single solution and the necessary measurements should be prescribed with respect to the practicality of the optimized procedure and the statistical treatment to be used. The most suitable working concentration range of the analyte is given by the strictly linear section of the absorbance - concentration graph for which the Bouguer-Lambert-Beer Law is strictly obeyed. It is further limited by the need to have a sufficiently high precision for the absorbance measurements. The limits placed on absorbance measurements depend on the spectrophotometer used; the best precision is usually obtained for absorbances lying between 0.05 and 1.2 units with a single beam instrument.

A least squares calculation should be used to compute the regression equation of the linear part of the curve and for the evaluation of the conditional absorptivity and the absorbance of the blank with their associated errors, and the correlation of the straight line with the experimental points. The regression equation can be used directly to calculate unknown concentrations of the analyte. No subtraction of the blank absorbance is necessary before plotting the measured values and their treatment by linear regression.

Sensitivity

The sensitivity of the procedure is defined by the slope of the calibration plot.

dA/dc (analyte) or

$d(A - A')/dc$ (analyte.

The sensitivity corresponds to the value of the conditional molar absorptivity, ε' (in $dm^3 mol^{-1} cm^{-1}$). Instead of the above, the apparent absorptivity, k, may be used (ref. 15):

$k = \varepsilon'$/molar mass x 1000, $k' = \varepsilon'$/molar mass (in $dm^3 g^{-1} cm^{-1}$) (or) (in $cm^2 \mu g^{-1}$).

Such values are sometimes suitable because they are not dependent on the relative atomic or molecular mass of the particular substance, but the determination of ε' in $dm^3 mol^{-1} cm^{-1}$ should be obligatory for each procedure.

It should also be noted that if an excess of a high absorbing reagent is used, deviations are to be expected in the values of the molar absorptivity calculated from the slope because the reagent bound in the analyte species is not taken into consideration.

All values for the sensitivity shall be related to a path length of 10.0 mm.

Another way to characterize the sensitivity is the Sandell Index (ref. 16). Because of differing definitions [analyte concentration in $\mu g\ ml^{-1}$ for A = 0.001 or 0.01 or 0.05 (ref. 17)] its use is not recommended.

Precision

The precision may be expressed in terms of the standard deviation s_A, the probable standard deviation $s_{A(t)} = s_{A.t}$, where t is the value of the student distribution), or by the confidence interval for various selected analyte concentrations or from all points of the calibration graph because of its dependence on concentration.

For its evaluation, values of absorbance between 0.40 and 0.50 have been recommended (ref. 7) to keep the contribution of error arising from the photometric measurements to a minimum.

All the characteristic values discussed here relate to the complete procedure.

The expressions for the precision are calculated from a sufficient number ($n > 10$) of replicate measurements:

$$s_A = \sqrt{\frac{\Sigma(A_i - \bar{A})^2}{n-1}} \sim \sqrt{\frac{1}{n-1}\left[\sum_{i=1}^{n} A_i^2 - \frac{1}{n}\left(\sum_{1-n}^{n} A_i\right)^2\right]}$$

s_A = standard deviation of absorbance

A_i = replicate consecutive measurements of absorbance, carried out by the same analyst on the same instrument.

The confidence interval may be calculated by means of the equation:

$$A_i = \bar{A}_i \pm t \cdot s_{A_i} / \sqrt{n}$$

where $n \geqslant 10$, t is the value of the student distribution for the significance level $1 - \alpha = 0.95$, $\bar{A}_i$ is the arithmetic mean of the absorbance and A_i the measured absorbance value for the particular analyte concentration. The expression of the precision in terms of concentration units follows from the equation

$$s_c = s_A / \varepsilon'$$

where s_c is the standard deviation in concentration units and ε' the corresponding conditional molar absorptivity, which is either known or is determined from the slope of the calibration graph.

The absolute standard deviation, s_c, does not change much for low analyte concentrations. In contrast, the relative standard deviation

$$s_{rel} = s_c/c$$

reaches its lowest and constant value for higher ranges of concentrations. The standard deviation, s_{xy}, characterizing the spreading of points around the regression straight line, obtained from the regression analysis of data, is not usually considered as the precision of the procedure.

Upper limit of determination

Since the photometric error of measurements rapidly increases for values of absorbance above 1.20, especially for single beam spectrophotometers, this value of 1.20 is taken as the upper limiting value of measurement. An absorbance of 3.0 can, however, be measured with a sufficiently high repeatability if a highly precise double beam instrument with low stray radiation and a digital display of absorbance values is used. For the usual double beam photometers with optical, mechanical or electrical beam attenuation, or for single beam photoelectric spectrophotometers the optimal absorbance range is for values of absorbance lying between 0.1 and 1.2 units (ref. 18).

Lower limit of determination

The lower limit of a determination is calculated from the arithmetic mean of the absorbance of the blank and its standard deviation by the equation,

$$A_{lim} = \bar{A}_o + 3\, s_{A_o}$$

where $\bar{A}_o$ is the arithmetic mean of the blank absorbance, s_{A_o} is the standard deviation of the blank for $n \geqslant 15$. A_{lim} corresponds with the earlier defined detection limit (refs. 19,20,21). For practical use, the limit of a determination of higher statistical confidence may be obtained by one of the following equations:

$$A_{lim} = \bar{A}_o + b\, s_{A_o}$$

or

$$A_{lim} = s_{A_i}$$

where b = 8 or 10 (ref. 22). The value of s_A is found from replicate measurements of a number of solutions containing a low analyte concentration, c_i (for $c_i \to 0$).

In the present authors' view none of the above treatments can be preferred for the evaluation of the lower limit of the application of a spectrophotometric procedure. Nevertheless, the way used to calculate the detection or determination limit should be clearly described.

The concentration corresponding to the limit of the determination is calculated from the expression:

$$c_{lim} = (A_{lim} - \bar{A}_o)/\varepsilon'.$$

The lowest practical limit of application may also be given by

$$A = A_o + 0.05$$

for a cell path length of 10.0 mm.

Interferences

The effect of accompanying ions or substances, interferents, masking agents or various matrix components on the absorbance of the analyte solution under prescribed conditions may be expressed by a threshold concentration of the interferent (in mol l^{-1} or $\mu g\ ml^{-1}$ or another suitable unit of concentration) or as the ratio c(interferent)/c(analyte) for a selected concentration of the analyte. In all cases the given values are valid only for this concentration. An interference or a systematic error has to be considered if

$$\Delta A \geqslant b\, s_{A_i}$$

where ΔA is the systematic deviation of the absorbance from that value of absorbance obtained from a solution without interferent, and s_{A_i} is the standard deviation of the measurement estimated for the pure solution of the analyte at a particular concentration. The value of ΔA can be extrapolated graphically from the A vs. c(interferent) plots. For the commonly

used level of significance of 99.8%, a value of 3 must be set for b.

The use of a systematic deviation (bias) in absorbance, calculated according to the expression

$$A_{rel} = \frac{\Delta A}{A(true)} > \pm 0.02$$

can also be useful in practice (ref. 10), where A(true) is the absorbance of the pure solution of the analyte without interferent.

The analyte concentration corresponding to A(true) = 0.4 ... 0.5 seems to be very suitable as the basis for the determination of the threshold concentration (ref. 7) because of the low photometric error occurring at these values of the absorbance. Data should be collected in tables or arranged in groups corresponding to interference ratios of 1:1, 1:10, 1:100 etc. The effect of all likely interferents should be first investigated independently, neglecting possible non-additive influences of possible combinations of interferents. Such effects can be ascertained by testing mixtures of interferents.

Ions, compounds or agents which have been examined for possible interference should always be mentioned in the paper or report.

SURVEY OF CHARACTERISTICS

Main characteristics
Description of equilibria in solution and of the species formed.

Characteristics of the reagent
Full description by name.
Purity and purification.
Content of active substance.
Spectral properties.
Stability of the reagent solution.

Optimal conditions of the procedure
Effect of temperature and time.
Sequence of mixing the components.
pH range.
Buffer used.
Concentration of the reagent and excess to be used.
Wavelength recommended for the measurement.
Cell material and path length.
Conditional molar absorptivity of the complex.
Effect of ionic strength.
Effect of other substances, especially surfactants.

Characteristic parameters of the procedure
Equation of the calibration plot.
Working range of analyte concentration.
Sensitivity.
Precision.
Upper limit of determination.
Lower limit of determination.
Interferences.

Acknowledgement

The authors wish to express their gratitude to Dr. W.I. Stephen, University of Birmingham, U.K., for his assistance in the preparation of this report for publication.

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