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**A STATISTICAL OVERVIEW OF STANDARD  
(IUPAC AND ACS) AND NEW PROCEDURES FOR  
DETERMINING THE LIMITS OF DETECTION  
AND QUANTIFICATION:  
APPLICATION TO VOLTAMMETRIC AND STRIPPING  
TECHNIQUES**

(Technical Report)

*Prepared for publication by*

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# A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: Application to voltammetric and stripping techniques

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**Abstract:** Traditional methods for determining the limit of detection (LOD) and the limit of quantification (LOQ), based on the IUPAC and ACS definitions, often are unsatisfactory. Consequently, a new, simple and statistically correct way of obtaining both the LOD and LOQ values has been derived and compared to commonly used methods. The new Upper Limit Approach, ULA, calculates the upper confidence limit of an individual blank signal using a critical value of the *t*-distribution and standard error of estimate (residual standard deviation) of regression. The uncertainty of the calibration plot position, its intercept and the mean blank signal are taken also into consideration. The proper choice of calibration model and calibration design also are discussed in detail. An improved derivation of the signal value relevant to the LOQ is based on the use of the same significance level needed for defining the LOD.

The concepts developed in this paper have been applied to the determination of cadmium by five common techniques of electrochemical trace analysis (d.c. fast polarography, differential pulse polarography, linear sweep stripping voltammetry, differential pulse stripping voltammetry, and potentiometric stripping analysis) under equivalent (as possible) conditions. The lowest LOD and LOQ values, obtained by the new reliable ULA method, were  $1.0 \times 10^{-9}$  mol/L and  $3.1 \times 10^{-9}$  mol/L Cd, resp., for differential pulse stripping voltammetry and significance level  $\alpha=0.01$ . The relevant LOD and LOQ values for  $\alpha=0.05$  are  $6.6 \times 10^{-10}$  mol/L and  $2.0 \times 10^{-9}$  mol/L Cd, resp. Potentiometric stripping analysis provided similar results.

## INTRODUCTION

One of the important goals of analytical chemistry is to report the detection and determination of the smallest concentration or, sometimes, amount of the analyte, that may be achieved with a reasonable certainty when using a given procedure. Consequently, performance of a specified trace analysis method is commonly characterised by the limit of detection (ref. 1-19) and the limit of quantification (quantitation, determination) (ref. 5-9). The following definitions of the *limit of detection*, LOD, given by the International Union of Pure and Applied Chemistry (IUPAC) and the American Chemical Society (ACS), respectively, are commonly accepted:

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(1) The limit of detection, expressed as a concentration or quantity, is derived from the smallest measure that can be detected with reasonable certainty for a given analytical procedure (ref. 1,2). (2) The limit of detection is the lowest concentration of an analyte that the analytical process can reliably detect (ref. 5). The *limit of quantification*, LOQ, is not defined in the IUPAC publications (ref. 1-4), although its numerical definition is given in an ACS document (ref. 5). The LOQ has been introduced to provide supplemental statistical separation of the blank measurement and true analyte signal distributions (ref. 8) and invented because the LOD was not considered satisfactory for quantitative analysis (ref. 9). Thus, it can be defined in words that the limit of quantification refers to the smallest concentration or the mass which can be quantitatively analysed with reasonable reliability by a given procedure.

For the sake of convenience, we will only refer in this paper to the analyte concentration (omitting the mass of the analyte), and to avoid possible confusion in terminology, the limit of quantification will be used instead of the limit of determination.

In this work statistically based definitions and methodology for correctly and unambiguously calculating LOD and LOQ will be presented. Initially, the relevant theory for calculating the LOD is summarised and a new concept of numerical defining the LOQ is given. Subsequently, procedures for the treatment of real experimental signals are described, as is the correct way of using the *t*-distribution and the appropriate transfer from the signal to the concentration domain.

Different ways of the calculation of both limits are compared in this paper using five common methods of electrochemical trace analysis. The particular example to which the methods are applied is the reduction of Cd(II) and oxidation of cadmium amalgam since the electrode process



represents a standard example of an ideally diffusion controlled reversible redox couple in electrochemistry. It is reasonable to expect similar results for other diffusion controlled systems but not of course for systems exhibiting irreversible or surface based processes. The experimental conditions in all five methods for the determination of cadmium were kept as equivalent as possible. A mercury working electrode and 0.01 mol/L HCl base electrolyte were used in all cases.

## THEORY

### Limits of detection and quantification in the signal domain using the normal distribution

The signal value,  $y_D$ , corresponding to the *limit of detection*, must reflect the value of the true signal (related to some non-zero analyte concentration) which is significantly different from the blank signal value. In contrast to the IUPAC convention (ref. 1-4), the  $y_D$  signal value is defined in the ACS document (ref. 5) and some other works (e.g. ref. 7,9,12,13) in terms of population statistics, i.e. the population mean,  $\mu_b$ , and the population standard deviation,  $\sigma_b$ , of the blank signal, as in the equation:

$$y_D = \mu_b + k_D \sigma_b \quad (2)$$

where  $k_D = 3$  (ref. 5). This definition corresponds to a  $100(1 - \alpha) = 99.865\%$  probability that the blank signal does not exceed the LOD ( $\mu_b + 3\sigma_b$ ) value (one-sided statistical test). Furthermore, this method of treating the blank signal considers only a type I error (or  $\alpha$  error, see Fig. 1), which in the above case is very low since  $100\alpha = 0.135\%$ . However, with the

definition  $y_D = \mu_b + 3\sigma_b$ , there is still a  $100\beta = 50\%$  probability that the true signal could be considered as a blank signal (type II error, or  $\beta$  error - see Fig. 1). This is a large error term so that Currie (ref. 9) and others (c.f. ref. 7) suggested the use of this limit at a higher signal value where the probabilities of the type II and the type I error are comparable. For instance, if  $100\beta = 100\alpha = 0.135\%$  then this limit is defined as  $\mu_b + 6\sigma_b$ . Such a signal value can be referred to the limit of detection by Currie and others, whilst use of the  $\mu_b + 3\sigma_b$  approach is said to give the *decision limit* (ref. 7,9). Other possible confusion can be found in the chemical literature, e.g. Kaiser (ref. 10,15) called the signal value at  $\mu_b + 6\sigma_b$  the *limit of guarantee of purity*, whereas Boumans (ref. 13) named it the *identification limit*. For the sake of clarity we will avoid the use of such a nomenclature and will use the factor  $k_D = 3$  in connection with the limit of detection  $y_D$ , and the symbol  $k_I$  will be used when we wish to refer to the factor of 6; so that in general  $y_I = \mu_b + k_I \sigma_b$ .

For the signal  $y_Q$ , which is related to the *limit of quantification*, it follows (ref. 5-9,13):

$$y_Q = \mu_b + k_Q \sigma_b \quad (3)$$

where  $k_Q = 10$ . The use of the factor of 10 as introduced in the ACS definition has no statistical significance, so we will attempt to elucidate the logical link of  $y_Q$  to  $y_D$  and  $y_I$ , and then define an alternative but statistically significant  $k_Q$  value to define LOQ.

It has been stated that signal values between  $\mu_b + k_D \sigma_b$  and  $\mu_b + k_Q \sigma_b$  represent a *region of detection* (ref. 5,7), suitable according to Currie (ref. 9) only for qualitative analysis. If a true analyte signal at the  $y_I$  level were considered, it follows that the probability  $100\gamma$  to exceed  $\mu_b + 10\sigma_b$  value is 0.00317%. It therefore seems logical to use for  $k_Q$  the value which would provide the same probability  $100\gamma = 100\beta = 100\alpha$ . This coincidence is achieved exactly for  $k_Q = 9$  when  $y_Q$  and  $y_D$  are symmetrically placed with respect to  $y_I$  (Fig. 1, part A) that defines the signal value at which the analyte *just surely can be detected*. In addition to such a selection of the upper border of the region of detection (where  $\gamma$  is used as a type I error), there exists a stronger reason for this choice based on the type II error of the signal at  $y_Q$ : At the signal level distant by  $9\sigma_b$  from the population blank mean, the significance level  $\gamma$ , expressing the risk that a single signal measured in quantitative analysis is *below* the limit where it can be surely detected (i.e.  $y_I$ ), is the same as when determining  $\alpha$  and  $\beta$  errors in the previous discussion:  $\gamma = \beta = \alpha$ .

This concept leads to the following statistically consistent definitions :

$$y_D = \mu_b + k_D \sigma_b, \quad y_I = y_D + k_D \sigma_b, \quad y_Q = y_I + k_D \sigma_b \quad (4a-c)$$

or in terms of the blank population mean  $\mu_b$ :

$$y_D = \mu_b + k_D \sigma_b, \quad y_I = \mu_b + 2 k_D \sigma_b, \quad y_Q = \mu_b + 3 k_D \sigma_b \quad (5a-c)$$

so that  $k_I = 2k_D$  and  $k_Q = 3k_D$ .

It is important to recognize that an extraordinarily high 99.865 % probability has been used in the literature for the LOD definition by assuming  $k_D = 3$  or  $k_I = 6$ . In contrast, in the actual evaluation of chemical data the probability 95% ( $\alpha = 0.05$ ) is commonly used and only

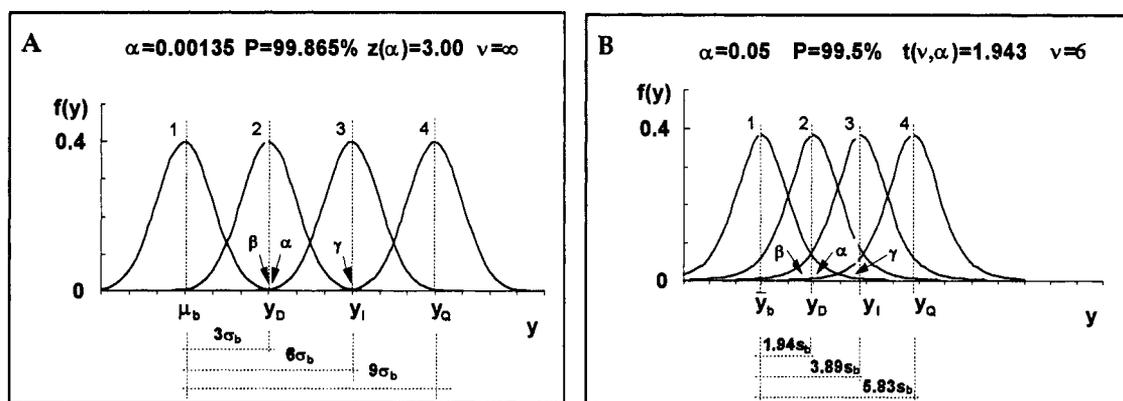


Fig. 1 Plots of the frequency of the signal values,  $f(y)$ , vs. the signal values,  $y$ , for (A) the normal distribution, and (B) the Student  $t$ -distribution with  $\nu = 6$  degrees of freedom (a chosen value). Curve 1 - blank signal, curves 2, 3 and 4 - analyte signals.

Probabilities  $\alpha$ ,  $\beta$ ,  $\gamma$  denote the risk that an individual observation is greater (curve 1) than the computed signal value  $y_D$ , or smaller (curves 3, 4) than the values  $y_D$ , and  $y_I$ , respectively. The value  $\alpha = 0.00135$  is relevant to the  $z(\alpha) = 3.000$  value, i.e. the value of the numerical factor in the IUPAC LOD definition used for multiplication of the blank standard deviation. This value determines the distance  $y_D - \mu_b$  on the signal axis as  $3\sigma_b$  if a very large number of blank observations is made. The critical value  $t(6, 0.05) = 1.943$  corresponds exactly to the quantile value  $z(0.00135) = 3.000$ , considering the different  $\alpha$  levels. The chosen value  $\alpha = 0.05$  allows to show the tails of the  $t$ -distribution more clearly.

sometimes (e.g. in the case of standardisation experiments) the 99% probability ( $\alpha = 0.01$ ) is needed. However, if 99% were used, then the previously defined  $k$ -factors have the smaller values of  $k_D = 2.326$ ,  $k_I = 4.652$ , and  $k_Q = 6.978$  (or 7.753 if  $k_Q = 10$  is used rather than our preferred value of  $k_Q = 9$ ).

### LOD and LOQ calculation in the signal domain using the Student distribution

Previous treatments of the LOD and the LOQ have been formulated in terms of the *population* characteristics  $\mu_b$  and  $\sigma_b$  related to the blank signal, which assume that numerous measurements have been made (Note a). However, in practice, only a limited number of observations of the blank signal,  $n_b$ , are made in analytical chemistry. The same is true for each of the signal observations performed in the presence of the analyte,  $n_a$ , used for construction of the calibration plot, inevitably required in the IUPAC and ACS recommended methods of the LOD and the LOQ determinations, by finding the concentration counterparts to the signals  $y_D$  and  $y_Q$ . Thus, for *real* analytical experiments, which are based on a limited data set, the following changes need to be considered:

- (1) Replacement of the population characteristics  $\mu_b$  and  $\sigma_b$  by the sample characteristics  $\bar{y}_b$  and  $s_b$ .

*Note a:* Discussion of the most recent literature is included in the section entitled *Recent Developments*

(2) Use of the appropriate Student *t*-distribution, chosen according to the number of degrees of freedom,  $\nu$ , which is related to the number of measurements,  $n$  (denoted specifically by  $n_b$  for the blank or  $n_a$  for the analyte observations):  $\nu_b = n_b - 1$  or  $\nu_a = n_a - 1$ .

(3) An appropriate choice of the probability  $P = 100(1 - \alpha)\%$  (or corresponding significance level  $\alpha$ ), which should be lower than  $P = 99.865\%$ .

After incorporation of points (1) to (3), the values of the factors  $k_D$ ,  $k_I$ , and  $k_Q$  (this time multiplying the  $s_b$  value instead of  $\sigma_b$ ) will depend on  $\nu$  and  $\alpha$  and will be related to the critical value  $t(\nu, \alpha)$  of the *t*-distribution. For a sample of  $n$  signal  $y$  observations ( $y_1, y_2, \dots, y_n$ ), the statistical *t* value may be applied generally (ref. 20,21) as

$$(y - \mu) / s \sim t, \quad \text{or} \quad (\bar{y} - \mu) / (s/\sqrt{n}) \sim t \quad (6a,b)$$

where the symbol  $\sim$  denotes that the relevant fractions in (6a) or (6b) are *t*-distributed. The more frequently used relation (6b) enables to calculate the confidence interval for  $\mu_b$  via the mean  $\bar{y}_b$ . However, for the correct calculation of  $y_D$  as the upper confidence limit of a future *individual* observation  $y_b$  (relevant to the use of  $\sigma_b$  in the ACS definition, otherwise  $\sigma_b/\sqrt{n_b}$  should be used in eq. (4a)), a *one sided t*-critical value is appropriate. In order to correctly replace the unknown  $\mu_b$  by  $\bar{y}_b$ , the *s* value for relation (6a) has to be derived from the variance of the difference ( $y_b - \bar{y}_b$ ), which will be denoted as  $s^2[y_b - \bar{y}_b]$ :

$$s^2[y_b - \bar{y}_b] = s^2[y_b] + s^2[\bar{y}_b] = s_b^2 + s_b^2/n_b = s_b^2(1 + 1/n_b) \quad (7)$$

$$s = s_b(1 + 1/n_b)^{1/2} \quad (8)$$

Then, after applying relation (6a) and eq. (8),  $y_D$  is given by

$$y_D = \bar{y}_b + t(\nu_b, \alpha)(1 + 1/n_b)^{1/2} s_b \quad (9)$$

The term  $(1 + 1/n_b)^{1/2}$ , expresses the correction for the uncertainty of the  $\bar{y}_b - \mu_b$  determination and is maximal for the smallest  $n_b$  and approaches 1 for sufficiently large  $n_b$ . This term also expresses the extent to which the  $k_D$  coefficient exceeds the  $t(\nu_b, \alpha)$  critical value:

$$k_D = t(\nu_b, \alpha)(1 + 1/n_b)^{1/2} \quad (10)$$

The same strategy of one-sided hypotheses tests can be applied to the  $y_I$  and  $k_I$ , as well as to the  $y_Q$  and  $k_Q$  calculations, assuming: (a)  $y_I$  and  $k_I$  are calculated initially and then the value of  $y_Q$  is established using the precalculated  $y_I$  value, and (b) that in this region, the signals corresponding to the *analyte* are measured and  $n_a$ ,  $\nu_a$  and  $s_a$  are used instead of  $n_b$ ,  $\nu_b$  and  $s_b$ . On this basis it follows that

$$y_I = y_D + t(\nu_a, \alpha)(1 + 1/n_a)^{1/2} s_a \quad (11)$$

$$y_Q = y_I + t(\nu_a, \alpha)(1 + 1/n_a)^{1/2} s_a \quad (12)$$

or, using substitutions for  $y_D$  and  $y_I$  from eqs. (9, 11):

$$y_l = \bar{y}_b + t(v_b, \alpha) (1 + 1/n_b)^{1/2} s_b + t(v_a, \alpha) (1 + 1/n_a)^{1/2} s_a \quad (13)$$

$$y_Q = \bar{y}_b + t(v_b, \alpha) (1 + 1/n_b)^{1/2} s_b + 2 t(v_a, \alpha) (1 + 1/n_a)^{1/2} s_a \quad (14)$$

The calculations are particularly simple if  $n_a = n_b$ , in which case:

$$y_l = \bar{y}_b + t(v_b, \alpha) (1 + 1/n_b)^{1/2} (s_b + s_a) \quad (15)$$

$$y_Q = \bar{y}_b + t(v_b, \alpha) (1 + 1/n_b)^{1/2} (s_b + 2s_a) \quad (16)$$

If the equality of variances,  $s_a^2 = s_b^2$ , were valid, the last terms in eqs. (15) and (16) would be  $2s_b$  and  $3s_b$ , respectively, as it can be seen in Fig. 1, part B. However, if the equality is not true, calculation of a pooled variance,  $s_p^2$ , or, of direct relevance, a *pooled standard deviation*,  $s_p$ , is important.

If the blank and the analyte signal measurements are assumed to be of equivalent precision (the same population standard deviation), then the pooled standard deviation can be constructed from  $s_b$  and  $s_a$ :

$$s_p = [((n_a - 1) s_a^2 + (n_b - 1) s_b^2) / (n_a + n_b - 2)]^{1/2} \quad (17)$$

A few alternatives and practical ways for obtaining a statistically efficient  $s_p$  value exist in practice. These are shown below as well as in the next part of the paper. The properly calculated  $s_p$  replaces both  $s_a$  and  $s_b$  in eqs. (9) to (16), thereby simplifying their form, e.g.

$$y_D = \bar{y}_b + t(v_b, \alpha) (1 + 1/n_p)^{1/2} s_p \quad (18)$$

$$y_Q = \bar{y}_b + 3t(v_b, \alpha) (1 + 1/n_p)^{1/2} s_p \quad (19)$$

where  $n_p$  denotes number of all observations included in the  $s_p$  calculation. By analogy, the factor of 2 is used for  $y_l$ .

An efficient way of achieving a reliable  $s_p^2$  (and  $s_p$ ) value is to average the signal variances corresponding to different calibration standards, each weighted by the appropriate number of degrees of freedom. For this purpose, the calibration needs to be performed as a series of replicative signal measurements for each concentration standard. This approach has been used for the treatment of the related problem of nonlinear calibration (ref. 22). For our particular case, the pooled variance can be re-defined in the following way:

$$s_p^2 = \left( \sum_{i=0}^{n_s} (n_i - 1) s^2[y_i] \right) / \sum_{i=0}^{n_s} (n_i - 1) \quad (20)$$

where  $n_i$  denotes number of replicative measurements for the  $i$ -th calibration standard ( $i = 0$  refers to the blank!),  $n_s$  - number of standards, and  $\bar{y}_i$  denotes the mean value of  $n_i$  replicative measurements of  $y_{ij}$ , so that

$$\bar{y}_i = \left( \sum_{j=1}^{n_i} y_{ij} \right) / n_i \quad (21)$$

and the variance  $s^2[y_i]$  is

$$s^2[y_i] = \left( \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_i)^2 \right) / (n_i - 1) \quad (22)$$

As noted in ref. 22, it is advantageous to utilise as many analyses as possible in the calculations (consistent with uniform population variance) in order to increase the total number of degrees of freedom. Consequently, calculation of  $y_D$  by use of eq. (18) is more reliable than that by use of eq. (9).

### Determination of the concentration LOD and LOQ values

Although some authors consider LOD exclusively in the signal domain (ref. 23), the real goal in trace analysis is to obtain *concentration* values of LOD and LOQ, which are denoted as  $c_D$  and  $c_Q$ , respectively. The conversion from the signal to the concentration domain is commonly made by projection of the signal, related to the LOD or LOQ and *corrected for blank*, through a *calibration plot*  $y = f(c)$  (obtained by regression), onto the concentration axis. Usually a linear calibration model  $y = q_0 + q_1 c$  is assumed and in this case the concentrations  $c_D$  and  $c_Q$ , calculated by projection, are influenced (ref. 8) by the errors of the intercept  $q_0$  and slope  $q_1$  (*analytical sensitivity*).

A non-zero intercept  $q_0$  may arise from the use of  $\bar{y}_b$  instead of  $\mu_b$ , or from the uncertainty associated with regression line position. The former problem can be overcome by using the term  $(1 + 1/n_b)^{1/2}$  in the definition of the  $y_D$  signal (eq. (9)). The second problem is associated with the fact that the true position of any regression line is given by generally unknown population regression coefficients. Irrespective of the reasons (see later) for incorrect intercept and slope values, the conversion of the signal LOD and LOQ values into concentration counterparts can lead in some circumstances to remarkable errors; e.g. in ref. 8 an approach involving correction of the errors in the intercept and slope (expressed in terms of corresponding standard deviations) revealed a difference in the LOD concentration as large as two orders of magnitude relative to the standard way of interpretation via the IUPAC definition. However, a *confidence band*, which expresses a set of confidence intervals of the signal around the regression line, can be computed by means of the *t*-statistic and used as the basis of a superior LOD or LOQ calculation. With this approach, the intersections of the appropriate signal with the upper and lower confidence limits of the calibration plot confine the relevant concentration interval which has been defined in a numerical way (ref. 22,24-26) for both possible linear models or even a non-linear calibration model. The *one-sided* upper confidence interval of the signal corresponding to the zero analyte concentration provides a statistically correct LOD value (and, subsequently, also a LOQ value) for the given  $\nu$  and  $\alpha$ , as it is shown in sections B and C.

The possibility of choosing the intercept of the calibration plot,  $q_0$ , instead of the mean blank signal,  $\bar{y}_b$ , as the zero (reference) point for the evaluation of the net signal values relevant to LOD and LOQ, as well as the possibility of selecting different forms of projection onto the concentration axis, enables the LOD and LOQ concentration values to be determined in several ways.

Prior to presenting an overview of the most important methods for the determination of concentration LOD and LOQ values a few general points need to be noted.

(a) The blank signals (for  $c = 0$ ) generally need to be *included* in the regression procedure, even though it may look strange (for some analysts) when a point having both coordinates exactly equal to zero is involved in the calculation, as occurs when the mean values of replicative *net signals* are treated in the calibration procedure.

(b) The most recommended calibration design is that consisting of the *same number of replicative signal measurements* for each concentration. The case in which the mean values of unequal number of replications are used in the calibration plot needs careful attention. The inequality might for example be a consequence of the rejection of outliers. Adequate weighing of the mean signal values can be then performed by using the number of replications in the weighted regression as described in Appendix 1. In general, a proper treatment of the regression procedure is needed regardless of whether the calibration plot consists of net or gross signal data and/or individual or averaged signal values. Surprisingly, important statistical details related to the calibration design and a relevant treatment of data in the regression analysis are rarely described in the analytical literature.

(c) For ease of presentation and because of its general importance, the mentioned *reference point*, relevant to the different methods of calculation of concentration LOD and LOQ values, generally will be denoted as  $y_R$  in the following text.

#### A. Classical approach based on IUPAC and ACS definitions

The most common application based on the IUPAC and ACS definitions employs the mean blank signal,  $\bar{y}_b$ , as the basis (reference point value) for the calculation of the signal LOD and LOQ values, *regardless of the intercept position* of the calibration plot. These values are  $y_D$  and  $y_Q$ , as expressed by eqs. (2) and (3) with  $\mu_b$  and  $\sigma_b$  replaced by  $\bar{y}_b$  and  $s_b$ , or, alternatively, by  $k_D s_b$  and  $k_Q s_b$ , for gross and net (mean blank corrected) signals, resp. On this basis, it follows that the *line parallel* to the calibration plot has to be used for the projection of the LOD and LOQ signals onto the concentration axis in order to fit geometrically to the accepted (ref. 6) numerical relationships:

$$\text{LOD} = (y_D - \bar{y}_b) / q_1 = (k_D s_b) / q_1; \quad (23)$$

$$\text{LOQ} = (y_Q - \bar{y}_b) / q_1 = (k_Q s_b) / q_1 \quad (24)$$

which are valid for  $k_D = 3$  and  $k_Q = 10$ , resp. The mentioned auxiliary parallel line passes through  $\bar{y}_b$  on the gross signal axis (or through zero on the net signal axis) and has the same slope  $q_1$  as the calibration line (Fig. 2a).

This calculation method, denoted as **SA1** (standard approach, alternative 1), only gives correct LOD and LOQ values by assuming: (a)  $q_0 = \bar{y}_b$ ; (b) all calibration points lie exactly on the calibration curve (which is equivalent to the assumption that the signals measured in the calibration procedure are without errors and therefore  $q_0$  and  $q_1$  are errorless); (c)  $\bar{y}_b = \mu_b$ , which means that the measured mean blank signal equals to the population mean,  $\mu_b$ , i.e. the true blank signal value. Such requirements are never achieved in a real experiment. If the intercept value  $q_0 > \bar{y}_b$ , then the found LOD and LOQ concentration values may be overestimated (too large); in the opposite case, if  $q_0 < \bar{y}_b$ , the found LOD and LOQ may be underestimated.

Another alternative of the standard approach, **SA2**, removes the need of any auxiliary line by using the intercept as the point of reference,  $y_R = q_0$ , as it is natural in any calibration procedure (Fig. 2b). In this case, if the condition  $q_0 > \bar{y}_b$  is valid, then the found LOD and LOQ concentration values may be underestimated; moreover, if  $q_0 > y_D$  (i.e. larger than  $\bar{y}_b + k_D s_b$ ), then the found LOD value may be even negative! On the other hand, if  $q_0 < \bar{y}_b$ , then the LOD and LOQ values may be larger than the "true" values. The conditionality of such statements follow from the fact that the incorrect position of the calibration curve (i.e. inaccurate  $q_1$  and  $q_0$ , being an error following from (b)) may either compensate for the

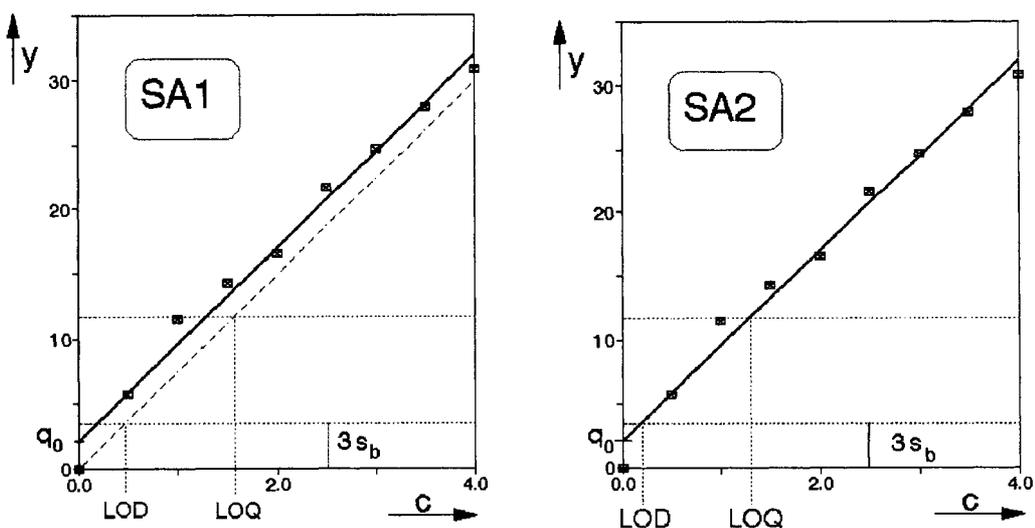


Fig. 2a,b Illustration of two alternatives of the standard approach to LOD calculation. Methods SA1 (part a on the left) and SA2 (part b on the right) are described in the text. Each calibrator point (including the blank) represents an average of 8 replicative measurements.

above-mentioned effect or may cause the error to be enhanced. The same situation prevails as far as the error described in (c) is concerned. The use of the *t*-distribution and introduction of the correction factor  $(1 + 1/n_b)^{1/2}$ , as suggested previously (eqs. (8) - (19)), eliminates the error described in (c). However, the errors relevant to (a) and (b) in the projection procedure, remain.

Several modifications of the classical IUPAC and ACS models have been published which incorporate various kinds of improvements. In this paper a few alternatives will be presented, particularly those in which regression quantities are incorporated into the LOD calculation.

Another alternative to the classical definitions, which will be denoted as the regression approach, **RA**, was developed in ref. 27. Its basic principles are:

(1) The intercept of the regression line,  $q_0$ , was used as the point of reference,  $y_R = q_0$ , instead of the mean blank signal,  $\bar{y}_b$ . (2) The blank signal standard deviation,  $s_b$ , was replaced by the regression statistic  $s_y$ , to express the variation of the signal values,  $y_i$ , around the regression values,  $\hat{y}_i$  (see Fig. 3 and eq. (26) below for details).

The  $3s_y$  value was then added to the point of reference and using this signal the corresponding concentration LOD was calculated as  $LOD = 3s_y / q_1$ . The advantages of this simple approach are: (a) The possibility of obtaining negative concentration LOD values is eliminated. (b) The number of degrees of freedom involved in the  $s_y$  calculation usually is considerably higher than that in  $s_b$ , so a more reliable  $\sigma_b$  estimation is provided in the case when  $s_y$  is correctly used for this purpose. Unfortunately, for the general straight line calibration model,  $y = q_0 + q_1c$ , this situation does not apply, as proved in section B.

However, the concept of utilising the analyte signals in addition to the blank measurements for expressing a relevant standard deviation when working in the concentration region where the

population variance remains unchanged, as well as the use of some regression output values, is the basis of the new and correct procedure for the LOD and LOQ calculation, which is presented in sections B and C for the most common calibration models.

### B. Upper limit approach related to the calibration curve with an intercept

A new approach to the LOD and LOQ determination has been developed in this paper to overcome problems that arise when the calibration curve gives a *non-zero intercept* of the *net* (blank corrected) signal vs. concentration dependence or, equivalently, when the intercept of the gross signal dependence is not equal to  $y_b$ . The most common calibration model of this kind is the general straight line (ref. 21). However, the general polynomial or any other calibration model with a non-zero intercept term (a term which is concentration independent) can be considered. The case of the calibration curve (straight line in the simplest case) passing through a fixed point (the origin) is discussed in section C.

The most important feature introduced in section B is the introduction of the *upper limit approach*, ULA, which utilises the upper boundary of the signal vs. concentration confidence band to obtain the concentration counterparts of the signal values of the detection and quantification limits. The ULA takes into consideration the uncertainty of the regression line, namely the error of the regression value,  $\hat{y}_0$ , which is expressed by its variance,  $s^2[\hat{y}_0]$  (ref. 24, 28):

$$s^2[\hat{y}_0] = s_y^2 (1/n + (c_0 - \bar{c})^2 / \sum_{i=0}^{n_s} (c_i - \bar{c})^2) \quad (25)$$

where:  $\hat{y}_0$  denotes the regression value predicted for a chosen value of the independent variable, i.e. concentration  $c=c_0$ ;  $n_s$  is the number of calibration standards (the total number of points in regression is  $n = n_s + 1$ );  $c_i$  are the concentration coordinates of the points used for constructing the calibration plot;  $\bar{c}$  is the mean value of  $c_i$ . The symbol  $s_y^2$  denotes the variance of  $y_i$  and represents an estimate of the true but unknown variance  $\sigma_y^2$ , and hence the variation of the  $y_i$  values about the unknown true values  $\psi_i$ .  $s_y^2$  is frequently called the

mean square about the regression (ref. 24). Eq. (25) is valid irrespective of the calibration model. However, the model itself is reflected in the  $s_y^2$  value, defined most commonly as

$$s_y^2 = \sum_{i=0}^{n_s} (y_i - \hat{y}_i)^2 / (n - m) \quad (26)$$

where  $m$  denotes the number of regression parameters. Thus, for example,  $m = 2$  for the general straight line (with two parameters  $q_0$  and  $q_1$ ). For another calibration model, the values  $\hat{y}_i$  and  $m$  can be different. It should be noted that the error estimates of the regression parameters, e.g. the variances  $s^2[q_0]$  and  $s^2[q_1]$  are computed by means of the  $s_y^2$  value. This procedure is shown in Appendix 1.

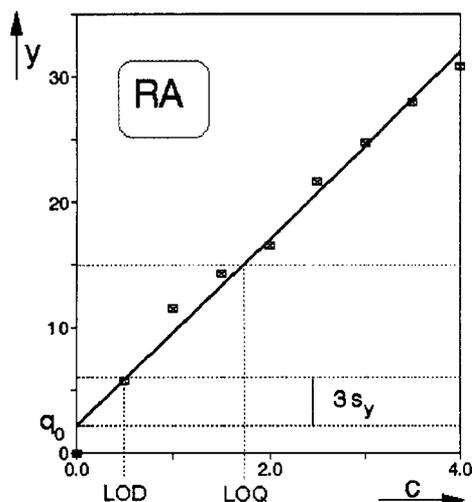


Fig. 3 Illustration of the "regression approach" to the LOD calculation which is in detail described in the text. Each point represents 8 replications.

The variance expressed by eq. (25) applies to the predicted value of  $\hat{y}_0$  (for a given  $c_0$ ), and therefore represents a mean value (ref. 24). A predicted value of an *individual* observation is still given by  $\hat{y}_0$ , but will have a variance (ref. 24,28) and a standard deviation represented by

$$s^2[y_0 - \hat{y}_0] = s^2[y_0] + s^2[\hat{y}_0] = s_y^2 \left( 1 + 1/n + (c_0 - \bar{c})^2 / \sum_{i=0}^{n_s} (c_i - \bar{c})^2 \right) \quad (27a)$$

$$s = s_y \left( 1 + 1/n + (c_0 - \bar{c})^2 / \sum_{i=0}^{n_s} (c_i - \bar{c})^2 \right)^{1/2} \quad (27b)$$

where the used variances are defined as follows:  $s^2[y_0] = s_y^2$ ,  $s^2[\hat{y}_0]$  is given by eq. (25), and  $s = (s^2[y_0 - \hat{y}_0])^{1/2}$ .

The calculation of upper and lower boundaries of the confidence band for individual observations is based on  $s^2[y_0 - \hat{y}_0]$  values multiplied by the critical t- values,  $t(\nu, \alpha/2)$ . Usually, they are placed symmetrically around the regression value  $\hat{y}_0$ . However, only the one-sided *upper* confidence limit at  $(1 - \alpha)100\%$  probability level is consistent with the IUPAC definition of LOD and LOQ:

$$\begin{aligned} y_U &= \hat{y}_0 + t(\nu, \alpha) s_y \left( 1 + 1/n + (c_0 - \bar{c})^2 / \sum_{i=0}^{n_s} (c_i - \bar{c})^2 \right)^{1/2} = \\ &= f_{\text{cal}}(c_0) + t(\nu, \alpha) s \end{aligned} \quad (28)$$

where the number of degrees of freedom  $\nu = n - m = n_s + 1 - m$ , and  $f_{\text{cal}}(c_0)$  denotes right-hand side of a given calibration model, e.g. for the general straight line calibration model it is  $q_0 + q_1 c_0$ . In the case of the general straight line, the dependence of  $y_U$  on  $c_1$ , which defines the confidence band via eq. (28), is a hyperbola (cf. Figs. 4a and 4b, for the cases ULA2 and ULA1).

Estimation of the *concentration* value  $\hat{c}_0$  corresponding to the selected value  $y = y_0$  on the signal axis represents the inverse problem encountered in the prediction of  $\hat{y}_0$  for a given  $c_0$ . Therefore, this operation is called *inverse regression* (ref. 24) or *inverse interpolation* (ref. 21). This procedure of  $\hat{c}_0$  calculation is calibration model dependent; e.g. for the general straight line the result is

$$\hat{c}_0 = (y_0 - q_0) / q_1 \quad (29)$$

or, generally, for a calibration model of the form  $y = f_{\text{cal}}(c)$ , the equation to be solved for  $\hat{c}_0$  is:

$$y_0 - f_{\text{cal}}(\hat{c}_0) = 0 \quad (30)$$

By analogy with the  $y_U$  parameter described above,  $\hat{c}_U$  is the upper limit of the  $(1 - \alpha)100\%$  confidence interval of the  $\hat{c}_0$  value found by *inverse regression* and it can be calculated using the inverse relationship  $\hat{c}_U = (y_U - q_0) / q_1$ . Of particular significance is the upper

confidence limit,  $\hat{c}_U$ , of the zero (blank) concentration,  $\hat{c}_0 = 0$ . Application of this condition in the regression equation, e.g.  $\hat{y}_0 = q_0 + q_1 c_0$ , gives  $\hat{y}_0 = q_0$ , and the substitution  $c_0 = 0$  into eq. (28) leads to a  $y_U$  value which provides  $\hat{c}_U$  by inverse regression, as the concentration LOD value:

$$\text{LOD} = \{t(\nu, \alpha) s_y / q_1\} (1 + 1/n + \bar{c}^2 / \sum_{i=0}^{n_s} (c_i - \bar{c})^2)^{1/2} \quad (31)$$

Assuming a linear calibration function, the application of the same procedure to the signal  $q_0 + 3 t(\nu, \alpha) s$  gives the LOQ concentration value as

$$\text{LOQ} = 3 \{t(\nu, \alpha) s_y / q_1\} (1 + 1/n + \bar{c}^2 / \sum_{i=0}^{n_s} (c_i - \bar{c})^2)^{1/2} \quad (32)$$

### C. Upper limit approach related to the case of a calibration curve passing through a fixed point

Section C concerns cases when calibration models are represented by a line passing through a fixed point  $[c_0, \mu_0]$ , when gross signals are plotted, or zero (i.e.  $[0, 0]$ ) when net signals are used. The common model relevant to this situation is the straight line  $y = q_1 c$  (blank signal corrected by  $\bar{y}_b$  subtraction) passing through zero. The treatment of the case when the intercept is fixed to a non-zero value is the same, except that it is necessary to shift the origin of the coordinate system to the point  $[c_0, \mu_0]$  (ref. 21). Thus, in the case relevant to C,  $c_0 = 0$ ,  $\mu_0 = \bar{y}_b$ . If the calibration dependence is forced to pass through zero or  $\bar{y}_b$  on the net/gross signal axis, then there are no problems caused by the difference between the intercept  $q_0$  and 0 or  $q_0$  and  $\bar{y}_b$ , respectively, because the position of this point of the calibration dependence is fixed. However, since it is not correct to use such a simplified calibration model arbitrarily, a testing procedure for the model validation is given at the end of this section.

The ULA1 applied to the calibration model  $y = q_1 c$  again allows for the uncertainty of the position of the calibration curve (which has only one end not rigidly fixed). By analogy with eq. (27) the variances  $s^2[y_0 - \hat{y}_0]$  of a predicted *individual* observation and the corresponding standard deviation  $s$  are:

$$s^2[y_0 - \hat{y}_0] = s^2[y_0] + s^2[\hat{y}_0] = s_y^2 (1 + c_0^2 / \sum_{i=1}^n c_i^2)$$

$$s = (s^2[y_0 - \hat{y}_0])^{1/2} = s_y (1 + c_0^2 / \sum_{i=1}^n c_i^2)^{1/2} \quad (33a,b)$$

where  $s^2[y_0] = s_y^2$ , and  $s^2[\hat{y}_0] = s_y^2 c_0^2 / \sum c_i^2 = s_{q_1}^2 c_0^2$  (ref. 21), with  $s_{q_1}^2$  being the variance of the slope. The meaning of all other symbols is the same as described in section B. The zero net signal, corresponding to the blank, is not included in the sum of  $c_i^2$ , as the zero point is the fixed point of the regression model.

The one-sided *upper* confidence limit,  $y_U$ , with the  $100(1 - \alpha)$  % probability is

$$y_U = \hat{y}_0 + t(\nu, \alpha) s_y (1 + c_0^2 / \sum_{i=1}^n c_i^2)^{1/2} = f_{\text{cal}}(c_0) + t(\nu, \alpha) s \quad (34)$$

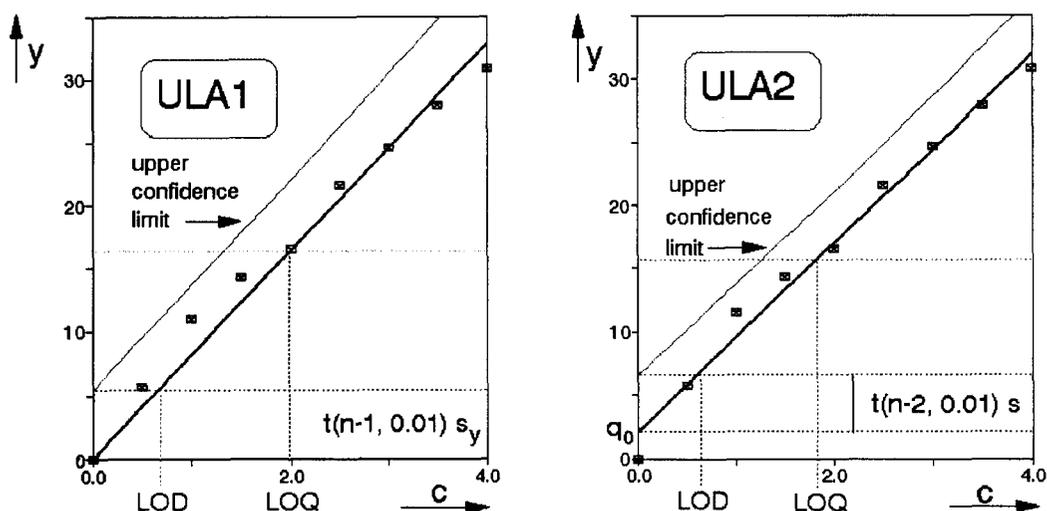


Fig. 4a,b Illustration of two alternatives of the upper limit approach to the LOD calculation. Methods ULA1 (part a on the left) and ULA2 (part b on the right) are described in the text. Each calibration point (including the blank in the ULA2 method) represents an average of 8 replicative measurements. The standard error,  $s_y$ , in the ULA1 is defined by eq. (A13) in Appendix 1, the corresponding term  $s$ , in the ULA2 is given by eq. (27b).

In order to obtain the signal value corresponding to LOD by means of eq.(34) the respective net or gross regression values  $\hat{y}_0$  must be  $0$  or  $\bar{y}_b$ , with both corresponding to  $c_0 = 0$  (analyte not present).

Finally, estimation of the concentration LOD involves the *inverse regression* of the latter signal

$$\hat{c}_U = y_U / q_1 = \{ t(\nu, \alpha) s_y / q_1 \} (1 + \hat{c}_0^2 / \sum_{i=1}^n c_i^2)^{1/2} \quad (35)$$

$$\text{LOD} = t(\nu, \alpha) s_y / q_1; \quad \text{LOQ} = 3 t(\nu, \alpha) s_y / q_1 \quad (36a,b)$$

As stated in section B, the concentration LOD value represents the upper confidence limit for the zero (blank) concentration so that  $\hat{c}_0 = 0$  is used in eq. (35) and a very simple calculation of the LOD results according to eq. (36).

The approach used in section C is based on the assumption that there is no significant intercept when the *net* signal is plotted versus concentration in the calibration procedure (the "true"  $q_0$  value is 0), or equivalently, that the difference between a possible intercept of the gross signal calibration dependence and the mean blank signal is statistically insignificant (the "true"  $q_0$  is  $\bar{y}_b$ ). A straightforward decision (ref. 28) on the validity of this assumption can be made by ascertaining whether or not the  $(1 - \alpha)100$  % confidence interval of  $q_0$  contains zero. The confidence interval  $C_I[q_0]$  is commonly defined as

$$\begin{aligned} C_I[q_0] &= q_0 \pm t(\nu, \alpha/2) (s^2[q_0])^{1/2} = \\ &= q_0 \pm t(\nu, \alpha/2) s_y \left( 1/n + \bar{c}^{-2} \sum_{i=1}^n (c_i - \bar{c})^2 \right)^{1/2} \end{aligned} \quad (37)$$

If zero is included in the interval  $C_I[q_0]$ , the null hypotheses about a non-significant intercept cannot be rejected and the intercept free calibration model is correct. This approach is applicable for confirming the fidelity of the most simple linear calibration model,  $y = q_1c$ . However, more complicated models (e.g. polynomial passing through the origin) also can be similarly tested by noting that the variance value  $s^2[q_0]$  is then directly accessible as the first diagonal element of the covariance matrix (ref. 21, 28).

## EXPERIMENTAL

### Instrumentation

Polarographic and voltammetric detection and quantification limits for Cd(II) were obtained using a Metrohm (Herissau, Switzerland) 646VA Processor with a 647VA stand and multi-mode working electrode. The following techniques were utilised with the instrument:

D.C. Tast (current sampled) Polarography (DCTP),  
Differential Pulse Polarography (DPP),  
Linear Sweep Stripping Voltammetry (LSSV),  
Differential Pulse Stripping Voltammetry (DPSV).

A three-electrode measuring system was used which consisted of either a Dropping Mercury Electrode (DME; drop time 0.5 s) or a Hanging Mercury Drop Electrode (HMDE) of surface area  $0.4 \text{ mm}^2$  as a working electrode, a Ag/AgCl/KCl (3 mol/L) reference electrode and a platinum mesh auxiliary electrode. The glassy carbon auxiliary electrode, provided by the manufacturer, was found to adsorb cadmium substantially and was therefore replaced by platinum.

Potentiometric Stripping Analysis (PSA) experiments were performed with the Trace Lab PSU20 Potentiometric Stripping Unit (Radiometer, Copenhagen), and an IPEX Computer for data manipulation. For PSA, the mercury film working electrode was prepared in the following way:

A 2 mm diameter glassy carbon electrode was polished with  $0.05 \mu\text{m}$  alumina on a Leco Inc. polishing pad, then rinsed with NANOPURE water. Prior to each experiment, mercury was plated onto the electrode from 1 mg/L  $\text{Hg}(\text{NO}_3)_2$  in 0.01 mol/L HCl to form a Hg film. A saturated calomel electrode and a platinum wire served as reference and auxiliary electrodes, respectively. The cells used in all experiments were made from high density polyethylene.

Experimental conditions for the  $\text{Cd}^{2+}$  reduction and cadmium amalgam oxidation (stripping) were kept as constant as possible for all procedures. In potential scanning experiments, a scan-rate of 4 mV/s was used in the potential range between -0.400 V and -0.800 V vs. Ag/AgCl. The staircase ramp step was 2 mV. The plating potential in all stripping techniques, including PSA, was -0.800 V vs. the relevant reference electrode, the plating time was 60 s with stirring, and the rest time was 30 s (stirrer off). For PSA, both mercury (II) and dissolved oxygen were used as chemical oxidants.

### Chemicals and reagents

The AAS (May and Baker Ltd. Dagenham, England) cadmium standard solution ( $1000 \pm 5 \text{ mg/L}$ ) was used for preparation of cadmium stock solutions. 0.01 mol/L HCl, used as the base electrolyte in all studies, was prepared from 32 % (10 mol/L) AR Grade HCl, Ajax Chemicals Ltd. (Sydney, Australia). Barnsted (Dubuque, Iowa, USA) NANOPURE deionised water,  $16 \text{ M}\Omega \text{ cm}$ , was used for preparing all solutions. Degassing of solutions was undertaken for 12 min

with high purity nitrogen except for PSA, where this step is not appropriate. When the standard addition procedure was used, degassing was repeated for 90 s after each standard addition.

## RESULTS AND DISCUSSION

### New methods for the LOD and LOQ determination

As indicated in the theory section, the standard application of the IUPAC or the ACS LOD definition, utilising projection of the corresponding  $y_D$  signal via calibration plot onto the concentration axis (denoted as SA1 and SA2), can lead to a statistically incorrect concentration LOD value (in the case of SA2 even negative values are possible!) in contrast to methods favoured by the present authors which use the intercept of the calibration plot as the reference point. In order to verify its usefulness and fidelity, the new upper limit approach, derived in detail for the two most important and simple straight line calibration models (ULA1 and ULA2), is compared to the two alternative standard ways, SA1 and SA2, as well as to the regression approach, RA, described in ref. 27, using data derived from the electrochemical determination of cadmium. The main characteristics of the five methods of the LOD calculation used in this paper are summarised in Table 1 and also are illustrated in Figs. 2-4, where the same calibration plot but the different methods of the LOD calculation are used (see Appendix 2 for details).

The concentration LOD values obtained via the five methods of calculation for each of the five electrochemical techniques for the determination of Cd(II) are shown in Table 2. In accordance with theoretical expectations, the SA1 and SA2 methods for cadmium give in some cases significantly different results relative to those obtained via use of the other three methods. Moreover, the SA2 method results in an obviously illogical negative concentration LOD value when using the DCTP technique (Table 2 as well as ref. 29). The RA values, although not theoretically rigorous, are surprisingly similar to the ULA1 and ULA2 values, but are always slightly lower (Table 2). The differences between the ULA1 and ULA2 values are in most cases very small (Table 2). The exception is the case where the ULA1 method cannot be applied properly, since the null hypothesis implying a non-significant zero intercept of the calibration plot is rejected (DCTP method, Table 2).

A relatively small difference between RA and ULA2 may be understood if the value of the bracketted term in eq. (28) (and consequently, the analogous term in eq. (31)) is separately evaluated and analysed. Such an analysis can be made in a general and quantitative way for a common case of *equidistant calibration*. For another type of calibration design, a quantitative analysis of the term also is possible, but is not general. For  $c_0 = 0$  and  $c_i$  equally spaced, the value of  $(1 + 1/n + (c_0 - \bar{c})^2 / \sum (c_i - \bar{c})^2)^{1/2}$  varies between 1.414, i.e.  $2^{1/2}$ , (for  $n = 2$ ) and 1.000 (as  $n$  approaches infinity) and depends exclusively on  $n$  and not on the concentration  $\bar{c}$  (mean value of all concentrations used in the calibration plot). The change with  $n$  is maximum for small  $n$  values, e.g. 1.414, 1.354, and 1.304 for  $n = 2, 3$ , or  $4$ , respectively. The change then becomes smaller and smaller, e.g. for  $n = 10, 20$ , and  $30$  the values of the bracketted term are 1.160, 1.089, and 1.062, respectively. The  $k_D$  factor, multiplying the  $s_y$  value is, in fact, a product of the  $t$ -critical value and the bracketted term, and is both  $n$ - and  $\alpha$ -dependent:

$$k_D(n-2, \alpha) = t(n-2, \alpha) \left( 1 + 1/n + \frac{\bar{c}^2}{\sum_{i=0}^n (c_i - \bar{c})^2} \right)^{1/2} \quad (38)$$

**TABLE 1. Basic characteristics of the investigated methods used to calculate the limit of detection <sup>a)</sup>**

Method of the LOD calculation	Ref. point $y_R$ (gross signal)	Distance $y_D - y_R$	References
SA1	$\bar{y}_b$ <sup>b)</sup>	$3 s_b$	1-4,6,8,11,16
SA2	$q_0$ <sup>c)</sup>	$3 s_b - q_0$	
RA	$q_0$	$3 s_y$	ref. 27
ULA2	$q_0$	$t(n-2, \alpha) s_y (1 + 1/n + \bar{c} / \sum_{i=0}^{n-2} (c_i - \bar{c})^2)^{1/2}$	this work
ULA1	$q_0$	$t(n-1, \alpha) s_y$	this work

<sup>a)</sup> The symbols used are explained in the text and defined in the Symbols & Abbreviations section

<sup>b)</sup> This value of  $\bar{y}_b$  refers to zero on the net signal axis; the intercept  $q_0$  is ignored

<sup>c)</sup> The intercept  $q_0$ , which is taken into account in this alternative to the standard approach, refers to regression of the uncorrected signal values

calibration design with the majority of data points being close to the origin, the  $k_D$  factor for a given  $n$  has a value equivalent to a larger  $n$  in the case of equidistant calibration. In fact, the  $\bar{c}$  values, used for the five electrochemical calibration plots relevant to the present study, varied over the range of 48% to 73% of the mean of the smallest ( $c_7 = 0$ ) and the largest ( $c_9$ ) concentrations. This is the reason, why the LOD results calculated by the ULA2 were slightly lower compared to the situation expected on the basis of an equidistant calibration design and closer to the RA results.

A possible special procedure to decrease the mean concentration  $\bar{c}$  when designing a non-equidistant calibration experiment is to double the number of the blank signal measurements and to use two points at the zero concentration on the calibration plot. In general, it is possible to design a calibration procedure so that the third expression in brackets in eq. (38) is small relative to the previous two. In the limit where this term is negligible, the expression reduces to the value  $(1 + 1/n)^{1/2}$ , consistent with eq. (18). The matrix solution used in Chapter 3.8.2 of ref. 28, applicable for a polynomial calibration model, also leads to the same value of the multiplication factor when a trivial case is considered with a unity vector describing the right-hand side of the calibration model (i.e. an exclusively zero analyte concentration is assumed).

The ULA1 method assumes a zero intercept of the calibration plot when using net signals and, in general, the calibration model is in this case described by one regression parameter less than a model using a non-zero intercept as is the case in the ULA2. In a zero intercept model, the calculated regression line is not as close to the calibration points as in the case of a non-zero intercept model, and consequently, the  $s_y$  value (expressing a variation of the calibration points around the regression line) is therefore larger in the ULA1 method of the LOD calculation than in the ULA2. It is important to emphasise that the zero net signal corresponding to the

The values of  $k_D(n-2, \alpha)$  for the equidistant calibration design, with  $\alpha = 0.01$  or  $0.05$ , and  $n = 3$  to  $30$ , are given in Table 3. Interestingly, the value close to 3 is obtained when  $n = 14$  and  $\alpha = 0.01$ . For  $n = 14$  and  $\alpha = 0.05$  the value of the multiplying  $k_D$  factor is close to 2.

From the above mathematical analysis it also follows that even though the magnitude of the third expression in the bracketed term does not depend on the value of  $\bar{c}$  in the case of equidistant calibration, it does for all other calibration designs. In the case of a non-equidistant calibration, the smaller the value of  $\bar{c}$ , the smaller the  $k_D$  factor, and, the smaller the concentration LOD value. Thus, for the general straight line calibration model and a non-equidistant

TABLE 2. Comparison of five methods for obtaining the LOD <sup>a)</sup>

Method	SA1	SA2	RA	ULA2	ULA1
DPP	$0.3 \times 10^{-8}$	$2.0 \times 10^{-8}$	$7.5 \times 10^{-8}$	$8.2 \times 10^{-8}$	<u><math>8.1 \times 10^{-8}</math></u> <sup>b)</sup>
DCTP	$0.4 \times 10^{-6}$	$-0.2 \times 10^{-6}$	$1.0 \times 10^{-6}$	<u><math>1.1 \times 10^{-6}</math></u>	$1.4 \times 10^{-6}$
LSSV	$1.8 \times 10^{-8}$	$2.3 \times 10^{-8}$	$2.8 \times 10^{-8}$	$3.0 \times 10^{-8}$	<u><math>3.0 \times 10^{-8}</math></u>
DPSV	$0.8 \times 10^{-9}$	$0.8 \times 10^{-9}$	$1.0 \times 10^{-9}$	$1.1 \times 10^{-9}$	<u><math>1.0 \times 10^{-9}</math></u>
PSA	$0.7 \times 10^{-9}$	$0.6 \times 10^{-9}$	$1.3 \times 10^{-9}$	$1.4 \times 10^{-9}$	<u><math>1.3 \times 10^{-9}</math></u>

<sup>a)</sup> All concentrations are in mol/L; 9 data points, each representing 8 replicative signal measurements were used for the construction of all calibration plots

<sup>b)</sup> The best approach is underlined. This choice follows from the testing whether the null hypothesis  $H_0 = (\mu [q_0] = 0)$  is true (intercept is not significant) or false (intercept significant); net signals are assumed

**SA1** - standard application of the IUPAC and ACS definitions neglecting the intercept  $q_0$  of the calibration plot

**SA2** - standard application of the IUPAC and ACS rules utilizing the intercept  $q_0$  of the calibration plot

**RA** - regression approach by Miller & Miller (ref. 27)

**ULA2** - upper limit approach for a calibration line with an intercept:  
 $y = q_0 + q_1 c$

**ULA1** - upper limit approach for a calibration line passing through a fixed point (the origin):  $y = 0 + q_1 c$  (net signals are assumed)

containing an intercept term, the significance of the intercept can be evaluated at the confidence level  $\alpha$  by testing whether the confidence interval of the intercept contains a zero net signal value (see Appendix 1). For this purpose a two-sided *t*-test is appropriate and  $\alpha = 0.05$  is recommended since there is no reason to use a very high probability. If the model with an intercept is rejected, the corresponding model without an intercept is correct, regardless whether it concerns the straight-line or more complicated models. On the basis of the theory of many analytical methods it follows that a calibration model without an intercept is essential and perfectly reasonable, in the absence of a systematic error. A typical analytical example is given in ref. 30 and a more general case is described in ref. 28.

It follows from the previous discussion that the **ULA1** method should be used instead of the **ULA2** if the hypothesis on the insignificant value of the intercept is accepted. Taking this into the account, the final LOD and LOQ results for Cd analysis by five electrochemical techniques are shown in Table 4. In accord with the theory presented in this paper, the signal values corresponding to the LOQ,  $y_q$ , were calculated assuming equal probabilities  $\alpha = \beta = \gamma$ , resulting in a simple use of the factor of  $3t(\nu, \alpha)$  in the LOQ calculation according to eq. (32).

### Appropriate calibration design for determination of the LOD and LOQ

The concentration range employed in designing a calibration plot for the LOD and LOQ determination has to be significantly narrower than that designed for general calibration purposes for the following several reasons:

blank, is not used as a calibration point in the **ULA1** method since this is the fixed point the calibration plot is forced to pass through. However, even if using one point less in such a case, the number of degrees of freedom in both the **ULA1** and **ULA2** methods are the same because the number of regression parameters also differs by one. No term, equivalent to the bracketted term in the **ULA2**, is used in the **ULA1** method. Thus, as a consequence of compensation effects, the calculated concentration LOD values can be very similar if the intercept of the calibration model is confirmed to be insignificant. Otherwise, the lack of the fit in the model causes that the LOD value calculated by the **ULA1** to be unacceptable, as happened in the LOD evaluation for d.c. tast polarography (Table 2).

For any calibration model

TABLE 3. Coefficients  $k_D(n-2, \alpha)$  in the LOD <sup>a)</sup> calculation by the ULA2 <sup>b)</sup> method

$n$	$k_D(n-2, .01)$	$k_D(n-2, .05)$	$n$	$k_D(n-2, .01)$	$k_D(n-2, .05)$
2	-	-	17	2.869	1.933
3	43.086	8.549	18	2.835	1.917
4	9.081	3.807	19	1.902	2.806
5	5.744	2.976	20	2.779	1.888
6	4.625	2.632	21	2.755	1.876
7	4.072	2.438	22	2.734	1.866
8	3.741	2.313	23	2.716	1.856
9	3.519	2.224	24	2.697	1.847
10	3.359	2.157	25	2.682	1.839
11	3.239	2.105	26	2.667	1.831
12	3.145	2.062	27	2.653	1.824
13	3.069	2.028	28	2.642	1.818
14	3.006	1.998	29	2.630	1.811
15	2.953	1.973	30	2.619	1.806
16	2.908	1.951	$\infty$	2.326	1.645

<sup>a)</sup> Coefficients  $k_Q(n-2, \alpha)$  in the LOQ calculation using the ULA2 are three times of the tabulated  $k_D(n-2, \alpha)$  values

<sup>b)</sup> Equidistant calibration design assumed

(1) For a sufficiently wide calibration range, the assumption of constant population variance of the signal (or constant population standard deviation  $\sigma_a$ ) is not valid and  $\sigma_a$  increases with the analyte concentration (see ref. 31 and references cited therein). However, in a narrow concentration range close to the LOD, the change of  $\sigma_a$  is unimportant and the simple theory derived in theoretical part is valid. Although it is in principle possible to include the  $\sigma_a$  increase into the calculation of both limits, the recommended method for the LOD and LOQ determination has the advantage of being both correct as well as simple. Therefore, for determination of the LOD and LOQ we recommend the use of a concentration range of only 1 to 1.5 logarithmic units above the LOD value (i.e. 10 - 30

multiple of the LOD value). This condition is fulfilled for all data in Table 2 where a 15-fold multiple of the LOD was used on average.

(2) A possible unfavourable effect of any individual data point on the estimated regression results (regression coefficients particularly) depends on the distance of the independent variable coordinate from the mean value ( $\bar{c}$  in our case) for a model with an intercept, or from zero in case of an intercept-free calibration model. This so called *leverage* effect (ref. 28) means that greater influence can be generated by a point far removed from the fulcrum of a lever than by a point closer to it. Therefore, the influence of an error in the signal value is much more pronounced if it is derived from a very remote point.

(3) The calibration model, which is valid in the region close to the LOD, may need to be modified when a large concentration range is used. For example, in a narrow range around the LOD and LOQ a simple straight-line model (and consequently simple calculations) sometimes can be used even though for a large concentration range another model is valid, e.g. a second-order polynomial.

There are many examples of LOD calculations in the analytical literature where the use of an inadequate calibration design leads to erroneously low concentration LOD values (several orders of magnitude). The main identifying feature of these inadequacies is if the concentration LOD is one or more orders of magnitude below the first analyte (i.e. non-zero) calibration concentration. Calibration design appropriate for the LOD and LOQ determination must be such that the region of calibration points is *overlapping* the determined LOD and LOQ values.

### Comparison of data obtained for different electrochemical techniques

The LOD and LOQ data, presented in Table 2 and Table 4 for the determination of Cd by five techniques were performed under conditions that are as similar as possible. However, the results in the general context, can be valid only for reversible electrochemical systems and not irreversible processes where considerably different results would be obtained (ref. 32). Furthermore, the almost constant experimental conditions used for comparison of investigated techniques are not optimal for individual techniques. For example, in case of stripping techniques we found a linear relationship between the electrochemical signal and deposition time for a time interval 20-1200 s for DPSV and 10-600 s for PSA at the concentration level close to the reported LOD. Moreover, for the PSA measurements in HCl base electrolyte,

**TABLE 4. Calculated LOD and LOQ values for the electrochemical determination of Cd(II) <sup>a)</sup>**

Method	LOD	LOD	LOQ	LOQ	Note <sup>b)</sup>
	$\alpha=0.01$	$\alpha=0.05$	$\alpha=0.01$	$\alpha=0.05$	
DCTP	$1.1 \times 10^{-6}$	$6.9 \times 10^{-7}$	$3.3 \times 10^{-6}$	$2.1 \times 10^{-6}$	ULA2
DPP	$8.1 \times 10^{-8}$	$5.1 \times 10^{-8}$	$2.4 \times 10^{-7}$	$1.5 \times 10^{-7}$	ULA1
LSSV	$3.0 \times 10^{-8}$	$1.9 \times 10^{-8}$	$8.9 \times 10^{-8}$	$5.6 \times 10^{-8}$	ULA1
DPSV	$1.0 \times 10^{-9}$	$6.6 \times 10^{-10}$	$3.1 \times 10^{-9}$	$2.0 \times 10^{-9}$	ULA1
PSA	$1.3 \times 10^{-9}$	$8.2 \times 10^{-10}$	$3.9 \times 10^{-9}$	$2.5 \times 10^{-9}$	ULA1

<sup>a)</sup> All concentrations are in mol/L; the experimental design is described in both Table 2 and the text

<sup>b)</sup> Designates the best approach

pH 4 was found to be optimal with regard to the sensitivity of the signal. Therefore, assuming a twenty times longer deposition time in DPSV and ten times longer in PSA, the LOD and LOQ data for Cd(II) by the DPSV and PSA methods would be much lower than in Table 2 and probably limited by the purity of reagents and/or possible analyte adsorption in the cell, rather than by the electrochemical technique itself. In an ideal case, the DPSV method would therefore be predicted to detect Cd below the  $10^{-10}$

mol/L concentration level on the basis of a reversible Cd(II) / Cd redox couple.

### CONCLUSIONS

In this study we have shown that traditional ways of using of the IUPAC and ACS definitions of the LOD and LOQ can cause errors. Major problems are associated with: (a) an incorrect transition from a large number of observations to a relatively small number, for which the normal distribution cannot be used, (b) the necessity to use a calibration function as the means of converting data from the signal domain to the concentration domain, (c) an incomprehension that the LOD value is based upon the signal value which an *individual* blank signal observation can exceed only at a small confidence level  $\alpha$ . A summary of the consequences and solutions to these problems is as follows:

(1) Instead of population statistics,  $\mu$  and  $\sigma_b$ , their sample equivalents  $\bar{y}_b$  and  $s_b$  are frequently used directly and without special care. This error originates straight from the IUPAC definitions (ref. 1-4), where differences in population and sample statistics are not distinguished.

(2) The use of the pooled standard deviation,  $s_p$ , gives a more reliable approximation of  $\sigma_b$  than  $s_b$  provided the assumption of a constant signal variance is valid in the calibration region. It should be noted that the use of the standard error of estimate,  $s_y$ , instead of  $s_b$  (ref. 19,27,33-35), is not a generally correct approximation for  $\sigma_b$ . However, we have proved

that it is correct if the straight line passing through the origin is a valid calibration model.

(3) Critical values  $t(v, \alpha)$  of the  $t$ -distribution, which are appropriate if a relatively small number of signal observations is made (ref. 5,7,8), have been incorrectly used in published papers since a two-sided rather than a one-sided  $t$ -test has been applied. Even though the use of critical values  $t(v, \alpha)$  has been reported as a reliable substitution of the factor  $k_D = 3$ , given in the IUPAC and ACS definitions of the LOD, the reports concerning the value of the confidence level  $\alpha$  used for  $t(v, \alpha)$  are controversial. If the confidence level  $\alpha$  is chosen, derived from the use of the factor  $k_D = 3$ , which is valid for when the normal distribution is assumed, then the consequent extraordinarily high probability level ( $P = 99.865\%$ ) leads to high values of the concentration LOD and LOQ. The same effect results when  $\alpha = 0.0005$ , which was used in ref. 8. Only in a few papers (ref. 18,19,36) has the value of  $\alpha = 0.01$ , which we consider as the optimum for the LOD and LOQ calculation via  $t(v, \alpha)$ , been suggested. The use of  $\alpha = 0.05$  (an alternative in ref. 19), which is common in analytical chemistry and has been almost exclusively used after the IUPAC - ISO Harmonization Meeting in 1993, yields lower concentration LOD and LOQ values. Lower values of the  $k_D$  factor can be unacceptable if a substantial deviation from the  $t$ -distribution were concerned and a too low probability would result from the application of Tschebyscheff's inequality (ref. 8,12).

(4) The inappropriate use of a calibration function as a convertor between the signal and concentration/mass domains is another source of error in LOD and LOQ calculations. It needs to be stressed that (a) in practice the intercept of the calibration plot is not identical with the mean blank signal value even though this is assumed in both the IUPAC and ACS definitions (ref. 1-5), (b) the calculated regression parameters are subject to errors (ref. 8). Only in the most recent official document on the calculation of LOD and LOQ (ref. 6) has the presence of a bias been reported. Long and Winefordner (ref. 8), in their Propagation of Errors Approach, analysed the errors in the LOD calculation caused by inaccurate values of the intercept and the slope of the calibration plot. Their approach, treating each source of error separately, leads to conservative, i.e. high LOD values. A more realistic approach follows from the application of regression theory, which usually (but not as a condition) takes into account the confidence intervals of the signals or the calculated concentrations (ref. 8,19,23,27,33,34,37,38). These methods use such different geometrical constructions and calculation details that it is not possible to discuss them individually. However, generally, the methods using both the lower as well as the upper confidence limits lead to higher LOD values compared to our Upper Limit Approach.

(5) In practice, appropriate calibration designs for determination of the LOD and LOQ frequently are violated. In accordance with ref. 7 "...it should be noted that, in general, it is not permissible to calculate detection limits ... at concentration levels much higher than the detection limits". A correct experimental design also encompasses the concept that the maximum possible prevention against unique interference effects for individual samples of analyte is considered (ref. 6).

## RECENT DEVELOPMENTS

Ideally, the LOD and LOQ calculation method need to be fairly simple if they are to be widely used. Some published analytical papers do not meet this requirement. In practice, the LOQ is much less frequently used than the LOD and it seems therefore impractical to recommend the reporting of three limits which are connected to the use of the factors  $k_D$ ,  $k_I$ , and  $k_Q$ . For practical reasons, we therefore recommend only the use of the LOD defined via  $k_D = 3$  and the LOQ defined via  $k_Q = 9$  for a large number of observations (where the assumption of the normal distribution is correct) and in other cases, for a limited number of observations, the use of the Upper Limit Approach which provides the necessary correction of the  $k_D$  and  $k_Q$

factors. This suggestion originates from the protocols associated with the IUPAC and ACS definitions. Obviously, approaches more remote to the IUPAC and ACS definitions of the LOD and LOQ are possible, e.g. the method based on the lower confidence limit of the analyte signal instead of the upper limit for the blank signal (ref. 37), direct use of the relative standard deviation (ref. 35,39-41), or application of a more complex statistical theory (ref. 19,33), but their inherent complexity and/or lack of compatibility with the standard IUPAC and ACS definitions would probably exclude their wide acceptance by analytical chemists.

Since 1993, when the original version of this paper was forwarded to the IUPAC, several papers (ref. 42-44) as well as the new German standard (ref. 45) and IUPAC nomenclature (ref. 46) have been published, which are relevant to the topic discussed in the previous text of this paper. Predominantly these articles, focussed on the problems of the limits of detection and quantification, are considered in this final section along with future perspectives concerning presentation of results of trace analysis.

Recently, except in ref. 42, where the use of the factor  $k_d = 3$  or, preferentially,  $k_d = 2\sqrt{2} = 2.828$  is recommended (in the statistical interpretation of the detection limit by using of signal-to-background ratio and the relative standard deviation of the background), the *t*-critical values have been commonly used. Thus, despite the differences in evaluation methods, the number of observations (measurements) generally is now being incorporated into the definition of the limit of detection. That is, *sample statistics* are gradually replacing the formerly used population statistics.

Another feature, emerging in the majority of recent reports, aimed at achieving a better approximation of the population blank standard deviation (ref. 43-46) and which matches our approach, is the use of regression parameters derived from the calibration dependence. Of course, this is not a new insight with respect to the LOD evaluation, as several variants of such an approach have been published in the past (e.g. in ref. 47-50,19,23,27) and where the inspiration for our upper limit approach originates.

A significance level of 0.05 has been recommended in the IUPAC - ISO "Detection Limit" Harmonization Meeting in 1993 as a default value. In ref. 43 and ref. 44  $\alpha = 0.05$  and  $\alpha = 0.01$  were used, respectively. Critical *t*-values for  $\alpha = 0.10, 0.05, 0.01$  and 0.003 (two-sided confidence levels) were tabulated in the nomenclature document (ref. 46), and finally, values of  $\alpha = 0.01$  or  $\alpha = 0.05$  are mentioned in the German standard (ref. 45), although only  $\alpha = 0.01$  is used in the given calculation example. For evaluation of the *expanded uncertainty U*, which provides an interval  $y \pm U$  around the estimate *y* of the measurand *Y*, the combined standard uncertainty is multiplied by a *coverage factor k* to obtain *U*. Typical values of *k* are in the range from 2 to 3 (ref. 51,52). This definition, *unchanged* in the newest document (ref. 52), corresponds to  $\alpha$  values in the range 0.0455 to 0.0027. Thus, the reasonable significance level  $\alpha = 0.01$  recommended in our previous text as well as in refs. 18,19,36,44,45,53,54 is within this interval and can be considered as optimum for the evaluation of the limit of detection. However, since the above mentioned range has not been declared officially, we have retained the limits for  $\alpha = 0.05$  in the final results which are presented in Table 4.

Three limits are defined in the German standard (ref. 45). The third limit, corresponding to the limit of quantification (Bestimmungsgrenze), is approximated as the *k*-multiple of the first limit and the suggested value  $k=3$ , used in the demonstrated example, is strikingly equivalent to our recommended approach for the LOQ calculation, i.e. to the  $9\sigma_b$  concept. Despite the calculation method differences, the similarity of our approach with that contained in the German standard also is pronounced in the stepwise calculation of the second and third limits, which enables the arbitrary choice of the coefficient used for the LOQ calculation to be omitted. The

designation of the German first limit (Nachweisgrenze) is much closer to the English term "limit of detection" than the second limit (Erfassungsgrenze).

Finally, it is necessary to consider the differences between the concept we have presented and the one originating from ref. 9 and finalized in the document in ref. 46. Importantly, the main difference is not statistical but philosophical. The reasoning behind the definition of the second limit as the limit of detection in ref. 46 is well understood. However, we consider that it is more correct to name the first limit as the limit of detection, since it is connected to the situation when a single analyte signal can be differentiated from the blank signal with a high probability. Furthermore, the concentration relative to this signal is an important performance characteristics of each measuring instrument. We believe that the second limit, declared newly (in ref. 46) as the detection limit, or minimum detectable quantity, can be also used in qualitative analysis, e.g. for identification purposes. A relatively complicated theory (employing also *non-central t-distribution*) has been fully elaborated for calculation of this limit (ref. 19,46 and citations therein). The calculated value based on this theory is approximately equal double that of the first limit (ref. 55). This result is consistent with our approach except that another designation is provided for this limit. We do not consider the second limit to be exceptionally important, since in our opinion it is just the limit at which the analyte *can be identified with a high probability* (where the  $\alpha$ - and  $\beta$ - errors are comparable) by a *single* measurement. Of course, the  $\beta$ - error value is smaller if several signal measurements are performed for qualitative analysis purposes and in this case the second limit (approximated by the equality  $\alpha = \beta$ ) shifts towards the first limit.

The "Guide to the Expression of Uncertainty in Measurement" (ref. 51) published in 1993 by ISO in collaboration with six other bodies, including IUPAC, established general rules for evaluating and expressing *uncertainty* in measurement across a broad spectrum of measurements, including measurements in Chemistry. This document is highly relevant whenever the results of a measurement are reported, since *uncertainty is a parameter associated with the result of the measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand* (ref. 51,56). It is important to note that the uncertainty of the independent variable arising from random variability in the dependent variable in linear least squares calibration is given (ref. 52) by an equation equivalent to our eq. (27b). Thus, without going into the details, the limit of detection can be defined in this paper as the concentration or amount of the analyte given by the expanded uncertainty of the analyte-free sample determined via calibration dependence and by using of the one-sided critical t-value as the coverage factor.

## REFERENCES

1. IUPAC, Analytical Chemistry Division. *Spectrochim. Acta* 33 B, 242 (1978).
2. IUPAC, Analytical Chemistry Division. *Pure Appl. Chem.* 45, 99 (1976).
3. IUPAC, Analytical Chemistry Division. *Spectrochim. Acta* 33 B, 247 (1978).
4. IUPAC, Analytical Chemistry Division. *Pure Appl. Chem.* 51, 105 (1979).
5. ACS Committee on Environmental Improvement. *Anal. Chem.* 52, 2242 (1980).
6. Analytical Methods Committee, Royal Society of Chemistry. *Analyst* 112, 199 (1987).
7. D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte and L. Kaufman. *Chemometrics: A Textbook*. 2nd Ed. Elsevier, Amsterdam (1988).
8. G.L. Long and J.D. Winefordner. *Anal. Chem.* 55, 712A, 1290A (1983).
9. L.A. Currie. *Anal. Chem.* 40, 586 (1968).
10. H. Kaiser and A.C. Menzies. *The Limit of Detection of a Complete Analytical Procedure*. A. Hilger, London (1968).
11. H. Kaiser. *Anal. Chem.* 42, 26A (1970).
12. J.D. Ingle, Jr. *J. Chem. Educ.* 51, 100 (1976).
13. P.W.J.H. Boumans. *Spectrochim. Acta* 33B, 625 (1978).
14. H. Kaiser. *Fresenius Z. Anal. Chem.* 209, 1 (1965).

15. H. Kaiser. *Fresenius Z. Anal. Chem.* 216, 80 (1966).
16. H. Kaiser. *Spectrochim. Acta* 33B, 551 (1978).
17. L.A. Currie. *Nucl. Instrum. Methods* 100, 387 (1972).
18. J.A. Glaser, D.L. Foerst, G.D. McKee, S.A. Quave and W.L. Budde. *Environ. Sci. Technol.* 15, 1426 (1981).
19. C.A. Clayton, J.W. Hines and P.D. Elkins. *Anal. Chem.* 59, 2506 (1987).
20. T. Yamane. *Statistics; An Introductory Analysis*. Harper, New York (1970).
21. J.R. Green and D. Margerison. *Statistical Treatment of Experimental Data*. Elsevier, Amsterdam (1978).
22. L.M. Schwartz. *Anal. Chem.* 49, 2062 (1977).
23. U. Bos and A. Junker. *Fresenius Z. Anal. Chem.* 316, 135 (1983).
24. N.R. Draper and H. Smith. *Applied Regression Analysis*. 2nd Ed. J. Wiley, New York (1981).
25. L.M. Schwartz. *Anal. Chem.* 48, 2287 (1976).
26. J. Mocak, S. Varga, P. Polak, S. Gergely and J. Izak. *Wiss. Zeitschr. (Merseburg)* 32, 43 (1990).
27. J.C. Miller and J.N. Miller. *Statistics for Analytical Chemistry*. Ellis Horwood, Chichester, 1st Ed. (1984); 2nd Ed. (1988).
28. A. Sen and M. Sristava. *Regression Analysis. Theory, Methods, and Applications*. Springer, New York (1990).
29. S. Mitchell. *A Comparison of Detection Limits Obtained for Various Electrochemical Techniques*. B.Sc. (Honours) Thesis. La Trobe University (1992).
30. F.C. Strong, III. *Anal. Chem.* 51, 298 (1979).
31. M. Thompson. *Analyst* 113, 1579 (1988).
32. A.M. Bond. *Modern Polarographic Methods in Analytical Chemistry*. M. Dekker, New York (1980).
33. L. Oppenheimer, T.P. Capizzi, R.M. Weppelman and H. Mehta. *Anal. Chem.* 55, 638 (1983).
34. S. Ebel and K. Kamm. *Fresenius Z. Anal. Chem.* 316, 382 (1983).
35. J. Sramkova and S. Kotrly. *Talanta* 35, 841 (1988).
36. H. Hofbauerova, E. Beinrohr and J. Mocak. *Chem. Papers* 41, 441 (1987).
37. W.R. Porter. *Anal. Chem.* 55, 1290A (1983).
38. M.A. Sharaf, D.L. Illman and B.R. Kowalski. *Chemometrics*. J. Wiley, New York (1986).
39. L.R.P. Butler. *Spectrochim. Acta* 38B, 913 (1983).
40. J. Mocak and E. Beinrohr. In: *6th Conf. Advances and Appl. Anal. Chemistry - Trace Analysis (On the Detection Limit, Determination Limit and Analytical Range)* p. 87. Slovak Tech. Univ. Press, Bratislava (1991).
41. J. Mocak and A.M. Bond. To be prepared for publication.
42. P.W.J.M. Boumans. *Anal. Chem.* 66, 459 (1994).
43. L. Sarabia and M.C. Ortiz. *Trends Anal. Chem.* 13, 1 (1994).
44. R. Ferrús and M.R. Egea. *Anal. Chim. Acta* 287, 119 (1994).
45. Deutsches Institut für Normung. *Nachweis-, Erfassungs- und Bestimmungsgrenze*. DIN 32645, Berlin (1994).
46. L.A. Currie and G. Svehla. *Pure & Appl. Chem.* 66, 595 (1994).
47. C. Liteanu and I. Rica. *Pure & Appl. Chem.* 44, 535 (1975).
48. A. Hubaux and G. Vos. *Anal. Chem.* 42, 849 (1979).
49. C. Liteanu and I. Rica. *Statistical Theory and Methodology of Trace Analysis*. E. Horwood, Chichester (1980).
50. E. Hartmann. *Fresenius Z. Anal. Chem.* 335, 954 (1989).
51. International Organization for Standardization. *Guide to the Expression of Uncertainty in Measurement*. ISO, Geneva (1993).
52. EURACHEM. *Quantifying Uncertainty in Analytical Measurement*. First Ed. Crown Copyright, LGC Information Services, Teddington, Middlesex, Great Britain (1995).
53. C. Kirchmer. In *Defection in Analytical Chemistry. Importance, Theory, and Practice* (L.A. Currie, ed.). Chap. 4. American Chemical Society, Washington, D.C. (1988).
54. J. Mocak, A.M. Bond, S. Mitchell and G. Scollary. *Abstracts of 12AC/3EC*. p. 74. Royal Australian Chemical Society, Perth, Australia (1993).
55. L.A. Currie. *Pure Appl. Chem.* 64, 455 (1992).
56. International Organization for Standardization. *International Vocabulary of Basic and General Standard Terms in Metrology*. 2nd Ed. ISO, Geneva (1993).

## APPENDIX 1

For the sake of completeness, the equations for the calculation of regression parameters, their standard deviations and confidence intervals are given in this section. They are organised according to the assumed model of the calibration function. A statistical test for the intercept and guides for the use of weighted regression are included.

**Test for the intercept - calibration function  $y = \theta_0 + \theta_1 c$** 

The regression parameters  $q_0$  and  $q_1$ , estimating the population (i.e. "true") regression parameters  $\theta_0$  and  $\theta_1$ , respectively, are:

$$q_1 = S_{cy} / S_{cc} ; \quad q_0 = \bar{y} - q_1 \bar{c} \quad (\text{A1,A2})$$

where

$$S_{cy} = \sum_{i=0}^{n_s} (c_i - \bar{c}) y_i ; \quad S_{cc} = \sum_{i=0}^{n_s} (c_i - \bar{c})^2 \quad (\text{A3,A4})$$

$\bar{y} = (\sum y_i) / n$ ,  $\bar{c} = (\sum c_i) / n$ ,  $n = n_s + 1$ , and  $\Sigma$  denotes summation.

The corresponding standard deviations of the regression parameters are:

$$s_{q_0} = s_y (1/n + \bar{c}^2 / S_{cc})^{1/2} ; \quad s_{q_1} = s_y / S_{cc}^{1/2} \quad (\text{A5,A6})$$

and the standard error of estimate,  $s_y$ , is defined as

$$s_y = \left( \sum_{i=0}^{n_s} (y_i - \hat{y}_i)^2 / (n - 2) \right)^{1/2} = \left( \sum_{i=0}^{n_s} (y_i - q_0 - q_1 c_i)^2 / (n - 2) \right)^{1/2} \quad (\text{A7})$$

The confidence intervals ( $C_i$ ) of  $q_0$  and  $q_1$  are:

$$C_i[q_0] = q_0 \pm t(n-2, \alpha/2) s_{q_0} \quad (\text{A8})$$

$$C_i[q_1] = q_1 \pm t(n-2, \alpha/2) s_{q_1} \quad (\text{A9})$$

It is necessary to test the hypotheses  $H_0$  and  $H_1$  defined as

$$H_0: \theta_0 = \theta_{00} \quad \text{against} \quad H_1: \theta_0 \neq \theta_{00} \quad (\text{A10})$$

where  $\theta_{00}$  is, in general, some preconceived value of  $\theta_0$ . In our case either  $\theta_{00} = 0$ , if net signals are assumed, or  $\theta_{00} = \bar{y}_b$  for gross signals; the statistic  $(q_0 - \theta_{00}) / s_{q_0}$  is distributed as  $t(n-2, \alpha/2)$ . The hypothesis  $H_0$  is rejected with the probability  $(1-\alpha)$  if  $(q_0 - \theta_{00}) / s_{q_0} \geq t(n-2, \alpha/2)$ , otherwise  $H_0$  is accepted if  $(q_0 - \theta_{00}) / s_{q_0} < t(n-2, \alpha/2)$ .

**Test for the intercept - calibration function  $y = \theta_1 c$** 

By analogy with the previous model, the following equations are valid:

$$q_1 = \frac{\sum_{i=1}^n c_i y_i}{\sum_{i=1}^n c_i^2} \quad (\text{A11})$$

$$s_{q_1} = s_y / \left( \sum_{i=1}^n (c_i^2) \right)^{1/2} \quad (\text{A12})$$

$$s_y = \left( \sum_{i=1}^n (y_i - \hat{y}_i)^2 / (n - 1) \right)^{1/2} = \left( \sum_{i=1}^n (y_i - q_1 c_i)^2 / (n - 1) \right)^{1/2} \quad (\text{A13})$$

**Weighted regression**

Weighted regression is usually used when the assumption concerning homogeneous variance does not hold (ref. 21,24,28). In such a case the use of the weight diagonal matrix  $\underline{W}$  is essential

$$\underline{W} = \text{diag}[w_1, w_2, \dots, w_n] \quad (\text{A14})$$

(which has non-zero elements only on the diagonal) and for *weighing* the reciprocal values of the variances are used, e.g.

$$\underline{W} = \text{diag}[1/\sigma_1^2, 1/\sigma_2^2, \dots, 1/\sigma_n^2] \quad (\text{A15})$$

where  $n$  denotes the number of points used for constructing the calibration plot. The same theory of weighted least squares is applicable if the weights  $w_1, w_2, \dots, w_n$  are expressed by means of the *number of replicative signal measurements*  $n_1, n_2, \dots, n_n$ :

$$w_i = n_i / \sum_{i=1}^n n_i \quad (\text{A16})$$

and the mean signal values  $\bar{y}_i$  are only used in the calibration plot; the  $\bar{y}_i$  being calculated by eq. (21) from the individual replicative measurements  $y_{ij}$ .

If the calibration model with the line passing through the origin is used, then summation starts from  $i = 1$ , otherwise it starts from  $i = 0$  which is related to the blank and the number of points  $n$  should be replaced by the number of calibration standards  $n_s$ .

This weighted least squares method is practical only if the number of replications is different for some of the calibration standards or the blank, i.e. if at least one equal sign in the expression  $n_1 = n_2 = \dots = n_s$  ( $= n_0$ , as noted in the previous paragraph) is not valid. If the number of replications is the same, which is strongly recommended, and population variances can be assumed constant in the region of calibration, then ordinary least squares (generally used in this paper) are applicable. In this case the mean signal values  $\bar{y}_i$  are used instead of the individual signal measurements and the number of degrees of freedom follows from the number of calibration points  $n$  instead of  $\sum n_i$ .

## APPENDIX 2

**Description of data depicted in Fig. 2**

For the reasons specified below, the data used for construction of Figs. 2-4 do not exactly correspond to any of the results presented in Table 2. Depicted data in the figures were obtained predominantly from real data associated with the determination of cadmium (II) by direct current fast polarography (DCTP). Each data point on the graphs represents the mean of 8 replications. Since all data are the *same* for the methods SA1, SA2, RA, ULA1 and ULA2, this form of presentation enables the features of the five chosen methods for the LOD evaluation to be illustrated. For the sake of clarity *net* signals are used in the figures, signal axes are drawn in nanoamperes and concentration axes in micromoles per liter. There are 8 points corresponding to the calibration standards plus the origin point [0, 0] corresponding to the blank. This point coincides with the fixed point of the model when calculating the LOD by the ULA1 method, therefore it is not used in regression in this case. Points are numbered from 0 (blank) to 8.

Of the 9 depicted points in Figs. 2-4, the points 0, 3 and 8 were unchanged from the recorded DCTP data. One half of the replications, i.e. four of eight, were simulated for the points 1, 2 and 4. All eight replications were simulated for the points 5, 6 and 7, so that the mean value in these cases are fully dependent on the simulations. The maximum relative change of the mean signal of partially or fully simulated data was 13.3 % (point 1) compared to the mean of the measured data. As a consequence of the described data design, the intercept was changed from  $q_0 = 3.47 \mu\text{mol/L}$  (real data) to  $q_0 = 2.08 \mu\text{mol/L}$  intentionally. When using the real DCTP data, the intercept was large (a negative LOD value for SA2 in Table 2 !), so it was optimal - for illustration purposes - to make changes in the data to give a lower intercept value (on the other hand, data providing a zero intercept do not show the differences in the methods of the LOD evaluation). A completely simulated experiment could have been used, however, we prefer a compromise aimed at achieving illustration power while still preserving a strong correspondence between the partially simulated data in the figures and the real data evaluated in the tables.

**SYMBOLS & ABBREVIATIONS**

- $c_i$  - concentration of the *i*-th calibration standard
- $c_0$  - arbitrary concentration used for signal prediction in regression
- $c_D$  - limit of detection (concentration domain)
- $c_Q$  - limit of quantification (concentration domain)
- $\bar{c}$  - mean concentration of the calibration standards
- $C_i[q_0]$  - confidence interval of the quantity implied as the argument in brackets, e.g.  $q_0$
- $f_{cal}$  - function defining the calibration model (calibration function)
- $k_D$  - numerical factor used for defining the limit of detection (in the signal domain) according to both the original IUPAC concept and that contained in this paper

- $k_l$  - numerical factor defining the signal limit where the type I error and the type II error ( $\alpha$ - and  $\beta$ - errors) are equal
- $k_Q$  - numerical factor used for defining the limit of quantification (in the signal domain)
- $m$  - number of regression parameters
- $n$  - number of regression points
- $n_a$  - number of the analyte signal measurements
- $n_b$  - number of the blank signal measurements
- $n_i$  - number of replicative measurements of the  $i$ -th calibration standard signal
- $n_s$  - number of calibration standards
- $q_0$  - intercept of the calibration plot
- $q_1$  - slope of the calibration plot
- $s_a$  - analyte signal standard deviation (in the region close to the LOD)
- $s_b$  - blank signal standard deviation
- $s_P$  - pooled standard deviation
- $s_y$  - standard error of estimate (residual standard deviation) in regression
- $s^2[y_i]$  - variance of the quantity implied as the argument in brackets, e.g.  $y_i$
- $w_i$  - weight of the  $i$ -th point in regression
- $y_0$  - signal value related via calibration function to the chosen concentration value  $c_0$
- $y_D$  - signal value related to the limit of detection
- $y_l$  - signal value related to the limit of detection defined by the demand of equal  $\alpha$ - and  $\beta$ - errors; it is a lower boundary of the region where the analyte is detected with a high probability (i.e.  $1 - \beta$ ) according to a single signal measurement
- $y_Q$  - signal value related to the limit of quantification
- $y_R$  - reference point on the signal axis for  $y_D$  and  $y_Q$  calculating
- $y_U$  - one-sided upper confidence limit of the signal value (a series of  $y_U$  values, corresponding to different concentrations, create a border line demarcating the confidence band)
- $\bar{y}_b$  - mean blank signal
- $\hat{y}_0$  - regression value of the signal related to the chosen concentration value  $c_0$  by the calibration function
- $\hat{y}_i$  - regression value of the signal at the  $i$ -th point of the calibration dependence
- $\alpha$  - significance level, a complementary quantity to the confidence level
- $1-\alpha$  - confidence level which expresses the probability that the expected (true) value of an estimated quantity is in the confidence interval of that quantity
- $\alpha, \beta, \gamma$  - probabilities (the overall probability is assumed to be 1); e.g.  $\alpha$  determines the risk that a true blank signal would be interpreted as the analyte ( $\alpha$ - error, e.g.  $\alpha = 0.05$ ),  $\beta$  determines the risk that a true analyte signal would be interpreted as the blank signal ( $\beta$ - error),  $\gamma$  is a form of  $\beta$ - error (type II error) which causes a single analyte signal to lie below  $y_l$
- $\mu_b$  - blank signal population mean
- $\nu$  - number of degrees of freedom
- $\sigma_a$  - population standard deviation of the analyte signal

- $\sigma_b$  - population standard deviation of the blank signal
- $\sigma_y$  - population residual standard deviation (in regression)
- $\psi_i$  - unknown population signal value ("true" value) related to the  $i$ -th calibration standard concentration
- DCTP** - Direct Current Tast (*current sampled*) Polarography
- DPP** - Differential Pulse Polarography
- DPSV** - Differential Pulse Stripping Voltammetry
- HMDE** - Hanging Mercury Drop Electrode
- LOD** - limit of detection
- LOQ** - limit of quantification
- LSSV** - Linear Sweep Stripping Voltammetry
- MFE** - Mercury Film Electrode
- PSA** - Potentiometric Stripping Analysis
- RA** - regression approach to the LOD calculation (ref. 17)
- SA1** - standard application of the IUPAC LOD definition, alternative 1
- SA2** - standard application of the IUPAC LOD definition, alternative 2
- SCE** - Saturated Calomel Electrode
- ULA1** - upper limit approach to the LOD calculation using a calibration model passing through a fixed point
- ULA2** - upper limit approach to the LOD calculation using a calibration model with an intercept

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