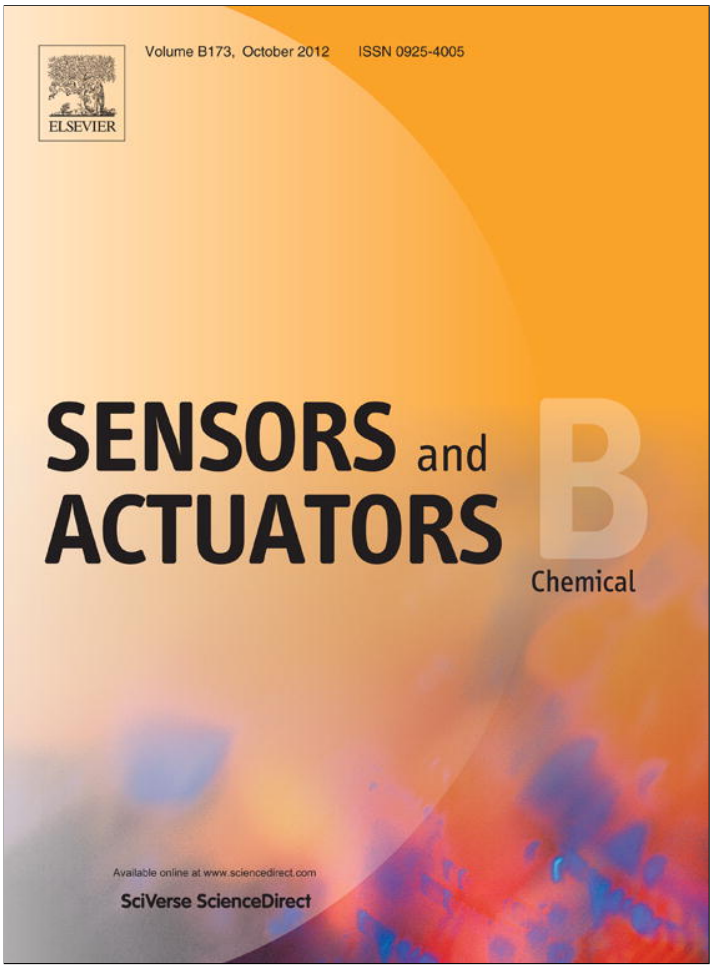

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Detection limits of chemical sensors: Applications and misapplications

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ABSTRACT

The limit of detection (LOD) and the sensitivity of a chemical sensor are defined using IUPAC guidelines. The LOD from simulated and experimental data is calculated from a calibration curve using a simple statistical model that was implemented into a spreadsheet program. This definition of the LOD is compared with the commonly used definition of the LOD, which is based on the product of sensitivity and the theoretical instrument resolution.

1. Introduction

The following instructions for "Xerox Enhanced Atomic Microscopy (XEAM)"were once posted near a departmental photocopier

- Take a photocopy of a sheet of paper at 4-fold magnification.
- Now take another photocopy of the enlarged copy – again at 4-fold magnification.
- Repeat the process 15 times for a total magnification of $1{:}4^{15}$ or 1:1.07 billion.
- At this point a 0.27 nm feature of the paper has been enlarged to fill the entire paper copy. The black smudge you see is an individual carbon atom (diameter about 0.22 nm)!

The resolving power in this XEAM experiment was calculated by assuming that one can obtain the ultimate spatial resolution (the detection limit) by extrapolation from experiments at low magnification assuming an error-free linear calibration with constant magnification (sensitivity). Many authors of articles that describe spectroscopic detection systems and chemical sensors use a similar assumption. It is quite common to find in the literature a measured sensitivity, which was obtained at high concentrations, and a calculated detection limit that may be orders of magnitude below the lowest measured value. Frequently, these detection limits are incorrectly calculated by dividing the resolution of, typically, only one of the instrumental components of the system with the sensitivity, i.e. the slope of the calibration curve. This is akin to assuming that all measurements fall almost exactly on the calibration curve and the standard deviation of the signal from the sensor is much less than the discretization-limited instrumental resolution.

Some authors have justified their approach by referring to an article on refractive index sensors by White and Fan [1], who stated – correctly, of course – that the detection limit can be obtained by dividing the sensor resolution with its sensitivity

$$x_{\mathrm{LOD}} = \frac{R}{r} \tag{1}$$

However, the resolution, R, is a quantity that needs to be obtained either by repeated measurements near the suspected LOD or by statistical analysis of a calibration curve. One cannot simply assume that the sensor resolution is identical to the discretization limit of one of the components of the system. While White and Fan defined the "sensor resolution" of their particular refractive index sensor as being related to the "smallest possible spectral shift that can be accurately measured", they also stated that this is not a quantity related solely to, e.g. the spectrometer's spectral resolution, but an experimentally determined quantity that also includes, e.g. amplitude noise and temperature-induced noise. Unfortunately, the "resolution of the measurement" is a term that is used in widely different ways in optical engineering (e.g. "spectral resolution of a

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spectrometer") in physics (e.g. "vertical resolution of an oscilloscope") and in analytical chemistry, where it stands for "$t{\cdot}s_y$", i.e. the value of the Student t-function multiplied with the standard error of the signal, which is obtained by averaging the signal near the LOD. In this article we provide a rigorously derived but user-friendly set of equations that permit the calculation of a detection limit from a linear calibration curve. A spreadsheet program is provided in the electronic database of the journal that permits the entry of data pairs forming a calibration curve and that then calculates the LOD. It is *not* the goal of this article to provide a survey of the considerable research effort that went into statistical data analysis (see e.g. Refs. [2–5] for reviews), nor is it claimed that the analysis below will be correct for any type of data set. Some limitations of the model are given below.

Two different methods will be reviewed that may be used to calculate a limit of detection. The more commonly used method involves the repeated measurement of the sensor's response when exposed to a blank solution, i.e. one without analyte, and a solution containing the analyte at a concentration close to the LOD. The second method describes how the LOD may be obtained from a linear calibration curve. This requires the determination of confidence intervals and measurements of the sensor's response at different concentrations including some near the LOD. The second method is particularly useful when literature data need to be re-evaluated.

To illustrate the applications and limitations of both methods, the limits of detection are calculated using both simulated and experimentally obtained calibration curves.

2. Method I: determination of the limit of detection from standard deviations at low concentration

The following paragraphs provide a sketch of the accepted procedures by analytical labs and are consistent with guidelines of the *American Chemical Society* [6]. The reader is referred to analytical chemistry text books for more information on the derivations and for details on chemical quantitation. Here we follow Harris in his description of the LOD determination [7].

- We first require repeated measurements of a blank sample, i.e. one that does not contain analyte. The sensor response is repeatedly measured. The *American Chemical Society* recommends $k = 10$ of such measurements [6], but many agencies only require 7 measurements of the blank's response. The mean value, $\bar{y}_{\text{blank}}$ is obtained by averaging.
- We then prepare samples that contain analyte at a concentration about 1–5 times higher than the expected LOD and again perform k measurements at this concentration. The mean value and the standard deviation of the measurement, s_y, can be determined. Ideally the samples should be prepared independently from each other and using different stock solutions.
- The signal at the detection limit is then calculated from [8].

$$y_{\text{LOD}} = \bar{y}_{\text{blank}} + t_{\alpha,k-1}s_y \tag{2}$$

Here $\bar{y}_{\text{blank}}$ is the average signal of the k measurements of the blank samples and $t_{\alpha,k-1}$ is the α-quantile of Student's t-function with $k-1$ degrees of freedom where $(1-\alpha)$ designates the required confidence level. For example, if it is required that the measurement at the LOD has a 99% probability of being larger than the blank, then $\alpha = 0.02$, or $(1-\alpha) = 0.98$, owing to the two-sided nature of the t-distribution. When 10 samples are analyzed ($k = 10$) one obtains $t_{0.02,9} = 2.821$, whereas $t_{0.02,7} = 2.998$ for $k = 8$. A critique of Eq. (2) is given by Mocak et al. [9]. Frequently, a less stringent threshold of $(1-\alpha) = 0.95$ may be appropriate [4].

- The concentration at the detection limit can then be calculated from the sensitivity, r, i.e. the slope of the calibration curve. Assuming a linear calibration curve near the LOD, we calculate the minimum detectable concentration as

$$x_{\text{LOD}} = \frac{ts_y}{r} \tag{3}$$

Eq. (3) is identical to Eq. (1), since the sensor resolution at the LOD, is simply $t_{\alpha,k-1}$-fold larger than the measurement uncertainty, $R = ts_y$. For convenience the value of the student t-function is frequently assumed to be $t = 3$, but this implies that a minimum of about 16 samples (8 blanks and 8 low-concentration samples) have been analyzed. In the sensor literature it is frequently overlooked that with a single measurement near the estimated LOD it is not possible to determine the LOD, since one cannot determine the measurements' standard deviation s_y from such a single measurement.

It is important to note that the calculations above assume that the errors are normally (Gaussian) distributed, and that the error distributions of the blanks and the low-concentration measurements have an identical width.

3. Method II: determination of the limit of detection using a calibration curve

Frequently it is necessary to compare the performance of one's own sensor system to a system that has been reported previously and may not have been characterized using the above method. Assuming that the authors of the previous study provided a calibration curve, how can one estimate the LOD of their measurements?

If we were provided with n data pairs forming a linear calibration curve $\{x, y\}$, we can calculate the sensitivity as the slope of a linear fit

$$r = \frac{\Delta y}{\Delta x} = \frac{n\sum(x_i y_i) - \sum x_i \sum y_i}{D} \tag{4}$$

The signal offset, i.e. the intercept of the calibration curve, is similarly calculated as

$$b = \frac{n\sum x_i^2 \sum y_i - \sum(x_i y_i)\sum x_i}{D} \tag{5}$$

Here, the determinant in the denominator is given by

$$D = \begin{vmatrix} \sum x_i^2 & \sum x_i \\ \sum x_i & n \end{vmatrix} = n\sum x_i^2 - \left(\sum x_i\right)^2 \tag{6}$$

The standard deviations of the sensitivity and offset and their corresponding covariance (s_{rb}) are calculated from

$$s_r = s_y\sqrt{\frac{n}{D}};\quad s_b = s_y\sqrt{\frac{\sum x_i^2}{D}};\quad s_{rb} = -s_y^2\frac{n\bar{x}}{D} \tag{7}$$

where the average standard deviation of the sensor response is estimated from the standard error of the fit [10]

$$s_y = \sqrt{\frac{\sum(y_i - rx_i - b)^2}{n-2}} \tag{8}$$

These equations are commonly found in textbooks on analytical quantitation and are readily incorporated into a spreadsheet program. An example is provided as an electronic supplement.

Of importance in re-analysis of the previously published calibration curves is the uncertainty of the concentration measurement, s_x. Following Harris [7] we can calculate the uncertainty, s_x, at the concentration x by propagating uncertainties

$$s_x = \frac{s_y}{|r|}\sqrt{\frac{1}{k} + \frac{x^2 n}{D} + \frac{\sum x_i^2}{D} - \frac{2x\sum x_i}{D}} \tag{9}$$

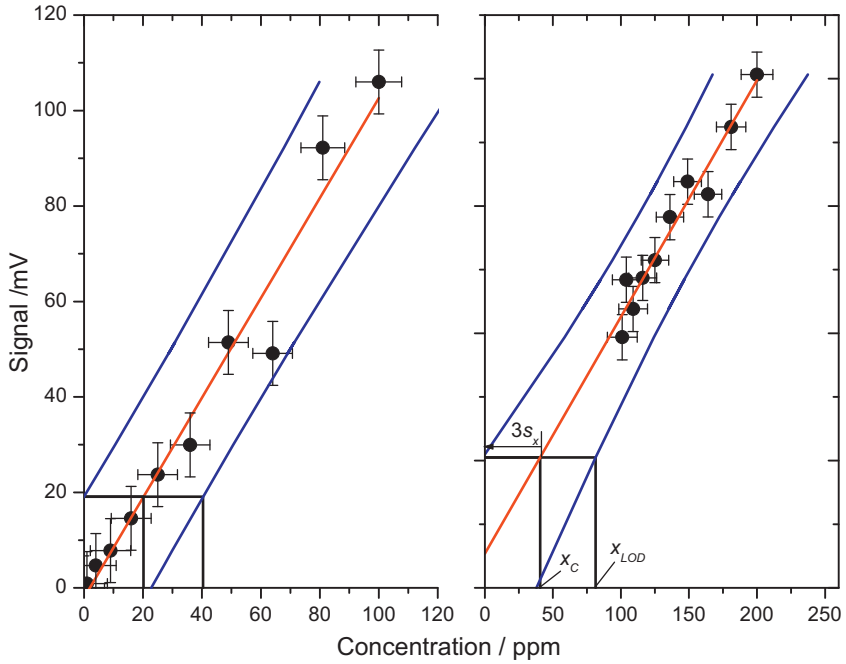

Fig. 1. Two different simulated linear calibration curves showing the curves for a linear fit (red) and the 99% confidence intervals (blue) calculated as $\pm 3s_x$. For each datum the uncertainties of the signal ($\pm s_y$) and of the concentration ($\pm s_x$) were calculated using Eqs. (8) and (9) and are shown as error bars. The error bars are therefore not the result of multiple measurements at a single concentration. The values for x_{LOD} and y_{LOD} are shown with black lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The last term in the root considers the covariance of the errors of the slope and intercept [11]. Here, k designates the number of repeat measurements that are averaged to obtain a single data pair for the calibration curve. If the literature curve does not provide error bars one may assume that only a single measurement was performed at a given concentration x and that $k = 1$. We define x_C as the concentration which is larger than the uncertainty at this concentration by a factor given by the student-t function, i.e.

$$x_C = ts_x = \frac{ts_y}{|r|}\sqrt{\frac{1}{k} + \frac{x_C^2 n}{D} + \frac{\sum x_i^2}{D} - \frac{2x_C \sum x_i}{D}} \tag{10}$$

After squaring both sides of Eq. (10) one finds the detection limit from the root of the resulting quadratic equation as

$$x_{LOD} = 2x_C = \frac{2ts_y}{nt^2 s_y^2 - Dr^2} \times \left(ts_y \sum x_i - \sqrt{\frac{D^2 r^2}{k} + Dr^2 \sum x_i^2 - n\frac{D}{k} t^2 s_y^2 - Dt^2 s_y^2} \right) \tag{11}$$

Please consult Supplementary material in the journal's data base for details on the derivation. The relation between x_C, x_{LOD}, and s_x is shown in Figs. 1 and 6. When we, again, set $t = 3$, the signal, $y_{LOD} = y_{blank} + rx_{LOD}/2$ at this detection limit corresponds to concentration, x_{LOD}, at which an average of k measurements has a 99% chance of being larger than that of a blank sample. The practical meaning of the above calculation is apparent from a linear calibration curve (Fig. 1a) which was generated using simulated and slightly scattered data. The linear fit is quite good ($R^2 = 0.97$), but the lines designating the confidence intervals nevertheless indicate that the lowest concentrations which are outside the confidence interval of a blank sample are higher than 40 ppm. This means that the lowest measured concentrations lie much below the detection limit.

An equation similar to Eq. (11) was already presented as part of the *IUPAC Recommendations for the Nomenclature for the Presentation of Results of Chemical Analysis* [4]. The factor of two in Eq. (4.19) of Ref. [4] and in Eqs. (10) and (11) arises from the requirement to span the entire confidence interval of concentrations at the y_{LOD}, i.e. it takes into consideration that there are uncertainties in the slope and intercept of the regression curve. We note that for poorly correlated data sets ($R^2 < 0.7$) the IUPAC expression gives a detection limit that may differ from that given by Eq. (11) and may even be negative. A comparison of Eq. (11) with Eq. (4.19) of Ref. [4] is provided in Appendix.

A closely related method of obtaining the LOD by statistical analysis of the calibration curve was presented by Hubaux and Vos [12], and is considered superior to the above statistical analysis if the data set is clustered in particular ways. The authors of both articles [4,12] pointed out that the concentration at the limit of detection is no more than an estimate and may differ by more than a factor of two – even for data sets producing very similar calibration curves with identical strong correlation, $R^2 > 0.98$. This may be verified by generating numerous random data sets using the spreadsheet that is available in Supplementary information. Since the limit of detection is so strongly dependent on the actual distribution of the sampled data, there really is no point in giving the LOD with a large number of significant figures.

Finally, quoting Harris "it is not reliable to extrapolate any calibration curve, linear or non-linear, beyond the measured range of standards." On the other hand with the above method there are some scenarios in which one may be able to justify an extrapolation of the calibration curve to a detection limit that may be lower than the lowest measured concentration. Fig. 1b shows a hypothetical linear calibration curve with $R^2 = 0.95$. Here, the LOD that was estimated using Eq. (11) is somewhat lower (80 ppm) than the lowest measured concentration (100 ppm). In this case one may be excused for violating Harris' rule and give a detection below the lowest measured concentration.

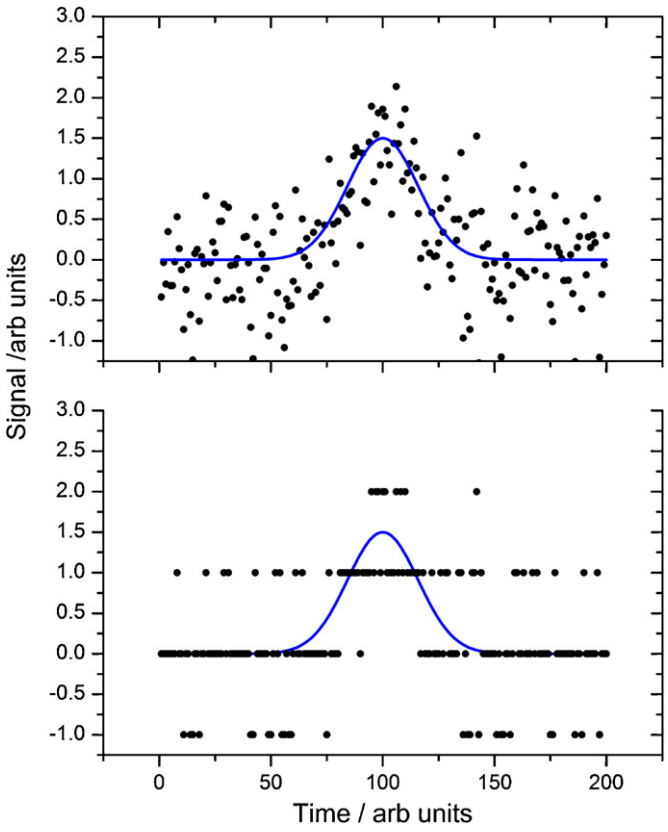

Fig. 2. Simulated data set with the signal sampled at two different resolutions. The upper trace shows a peak (solid line) and simulated data with a standard deviation of 0.5 and sampled at 10 bit resolution. The data points in the lower panel assume that the same data was sampled at 4 bit resolution.

In the above analysis it is assumed that (a) the errors are normally distributed, (b) the concentrations, x, of the $\{x, y\}$ data pairs are known exactly and (c) that the standard deviation of the signal is the same for all concentration measurements. As was pointed out by Lavagnini and Magno [2] the last assumption is, in fact, not correct for many calibration curves and their article describes cases in which the measurement uncertainty is dependent on the concentration.

Of course, it is also assumed that the calibration curve is linear and, in particular, that the sensitivity near the LOD is identical to that of the entire calibration curve. This condition is also not always fulfilled, since sensor measurements may saturate at high concentrations and there may be an induction range or hysteresis at low concentrations.

4. Method III: determination of the limit of detection from the instrumental resolution limit

Is the ratio of instrument resolution and sensitivity then a meaningless quantity? In cases of low-resolution measurements near the LOD, the frequently quoted product of the inverse of sensitivity and the smallest measurable signal increment can indeed be a quantity that is useful for comparison. For example, if the detection limit has been determined using the required number of blanks and samples one may realize that the standard deviation of the measurement, s_y, is lower than the increment at which the signal can be sampled (Δy). In this case the signal at the LOD, y_{LOD}, is, indeed, limited by the resolution at which the signal can be sampled, $y_{LOD} = 3\Delta y$, and discretization determines the sensors' limit of detection.

For example, in Fig. 2 the same simulated signal peak was sampled at 4-bit and at 10-bit resolution. At high sensor resolution

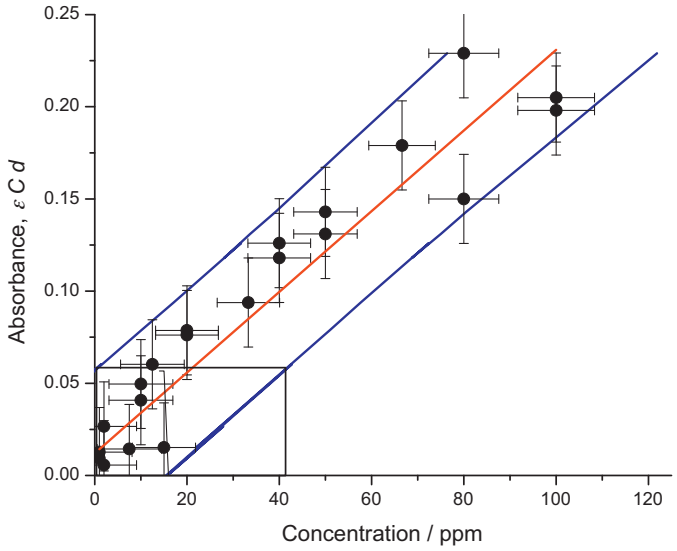

Fig. 3. A calibration curve that was obtained by measuring the ring-down time of a fiber loop cavity that contained a liquid absorption cell (see Andachi et al. for details [13]). A detection limit $x_{LOD} = 42$ ppm is calculated using Eq. (11). When the measurements at 100 ppm and 80 ppm are omitted one obtains $x_{LOD} = 22$ ppm. The error bars are defined as in Fig. 1 and differ from those in Ref. [13].

the upper trace of Fig. 2 shows a signal with a peak that is $3s_y$ higher than the baseline noise ($s_y = 0.5$), i.e. the signal at the LOD is $y_{LOD} = 1.5$. For the lower curve, recorded at low resolution the signal LOD, $y_{LOD} = 3$, is reasonably calculated from the signal increment ($\Delta y = 1$) and not from the lower standard deviation of the baseline signal, $s_y = 0.5$. While one can correctly identify discretization of the sensor signal as providing a lower limit of detection, it is not correct to assume that the LOD will improve by a factor of 256 when sampling at 10-bit resolution instead of 4-bit resolution. In fact the LOD improves only by a factor of two. This illustrates that even in the case of a discretization-limited measurement the LOD is rarely linearly correlated to the instrumental resolution of the acquisition system – in contrast to how Eq. (1) is frequently interpreted.

5. Application to experimental data

In the following we use previously published calibration curves of three different chemical sensors to demonstrate practical applications as well as the limitations of Method I and Method II in determining the detection limit.

Fig. 3 shows a calibration curve obtained by Andachi et al. for the determination of the dye methylene blue in a fiber-loop cavity ring-down experiment [13]. Light from a gain-switched 660 nm laser diode was coupled into a 4.69 m multimode fiber loop (125/50 μm) using a 99:1 fiber:fiber coupler. A custom-made absorption cell with a length of 100 μm was inserted into the loop and contained methylene blue dye in methanol at different concentrations. The ring-down times of 4096 light pulses were averaged and, together with the ring-down time of the used fiber cavity containing the blank solution, t_0, used to determine the absorption term εCd shown in Fig. 3. Details of the experiment are given in Ref. [13]. Using the calibration curve and Eq. (11) a limit of detection $x_{LOD} = 42$ ppm is obtained. When the values at the highest concentrations are omitted, as was done in Ref. [13], we calculate $x_{LOD} = 22$ ppm. The latter value is slightly higher than the LOD given by Andachi et al. ($x_{LOD} = 20$ ppm).

This calculation may be contrasted to one that is sometimes found in the cavity ring-down literature. Cavity ring-down times are related to optical absorption of a sample in the cavity through

$$\alpha(\nu) = \frac{nL}{c_0 d}\frac{\tau_0 - \tau}{\tau_0 \tau} \tag{12}$$

and the minimal detectable absorption loss – or the detection limit – is calculated as [14,15]

$$\alpha_{\min}(\nu) = \frac{nL}{c_0 d}\frac{\Delta\tau_{\min}}{\tau_0^2} \tag{13}$$

The parameters in Andachi's experiment were $L = 4.69$ m, $d = 100\,\mu$m, $n = 1.46$, and the empty cavity ring-down time $t_0 = 45$ ns. With these parameters we obtain $\alpha_{\min}(\nu) = 1.13 \times 10^{11}\ \mathrm{m^{-1}\,s^{-1}}\ \Delta\tau_{\min}$. Generally, $\Delta\tau_{\min}$ is not given by the bandwidths of the data acquisition card, the photodetector and the oscilloscope, but has to be experimentally determined. In this case the minimum measurable change of ring-down time, $\Delta\tau_{\min}$, is determined from $x_{\mathrm{LOD}} = 22$ ppm (50 μM) and the absorption coefficient of methylene blue, $\varepsilon = 1.5 \times 10^5\ \mathrm{L\,mol^{-1}\,cm^{-1}}$. Their product is $\alpha_{\min}(\nu) = 7.9\ \mathrm{cm^{-1}}$ and consequently $\Delta\tau_{\min} = 7$ ns. If one assumes, incorrectly, that the minimal resolvable change in ring-down time $\Delta\tau_{\min}$ is solely determined by the bandwidth of the detection system – here about 30 GHz from the bandwidth of the oscilloscope and the rise time of the photodetector – the minimum detectable absorption loss would be calculated as $\alpha_{\min}(\nu) = 0.03\ \mathrm{cm^{-1}}$ and $x_{\mathrm{LOD}} = 90$ ppb. This latter calculation ignores fluctuations in the sample concentrations, the shape of the ring-down transient, intensity noise arising from electronics or optical alignment, and ultimately laser shot noise. Of course, the minimum detectable change in ring-down time is also not simply given by the fitting error of an experimental intensity decay curve to an exponential function. This fitting error only indicates how well each of the experimental decay waveforms can be described by an exponential function.

As a second example we present a very different chemical sensor for volatile organic compounds was recently described by one of the authors and his collaborators [16]. A silicon-on-insulator (SOI) micro-ring resonator was coated with polydimethyl/polydiphenylsiloxane (PDMS/PDPS) co-polymer and the response to m-xylene vapor at various concentrations was determined from the shift of the SOI ring cavity resonance wavelengths. As m-xylene partitions into the polymer coating, the refractive index of the polymer increases and the effective index of the propagated mode consequently also increases. From the calibration curve shown in Fig. 4 and using Eq. (11) the detection limit is determined as $x_{\mathrm{LOD}} = 325$ ppm.

The actual detection limit may be somewhat higher since the calibration curve does not appear to be linear at very low concentrations. Close inspection of Fig. 4 reveals that at low concentrations of m-xylene the refractive index of the polymer does not appear to increase. This may be attributed to the combined effect of swelling of the polymer, which decreases the refractive index, and increase of the xylene volume fraction, which increases the refractive index. This "induction range" leads to an underestimate of the detection limit.

As a third example Fig. 5 shows the calibration curve presented by Yang et al. [17] for the determination of coumarin dye concentrations using photoacoustic measurements with a fiber Bragg grating as a transducer. The data are displayed both on a regular and on a logarithmic scale. The respective uncertainties, which were calculated using Eqs. (8) and (9) were scaled accordingly. It is clearly inappropriate to assume that the standard deviation of the photoacoustic signal is scaled logarithmically throughout the entire concentration range as this would imply that the uncertainty is zero for the blank solution. On the other hand the measurement error is

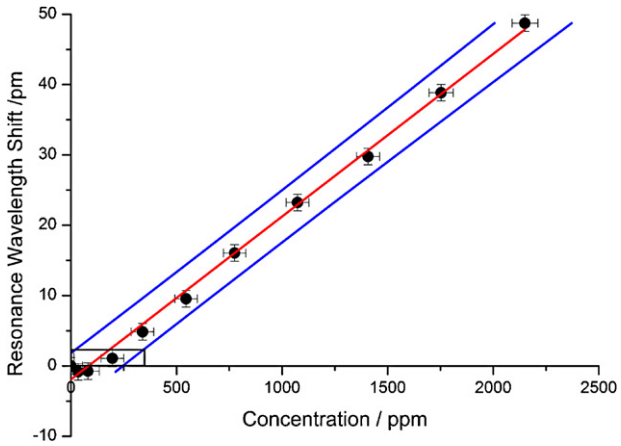

Fig. 4. Response of the resonant wavelength of a functionalized silicon-on-insulator microring resonator to m-xylene vapor (adapted from Loock et al. [16]). The micro-ring resonator was coated with a film of a PDMS/PDPS co-polymer which increased its refractive index upon exposure to m-xylene and other aromatic volatile organic compounds. The refractive index change increases the effective index of the propagated mode and causes a wavelength shift. The detection limit is determined as $x_{\mathrm{LOD}} = 325$ ppm. The error bars are defined as in Fig. 1.

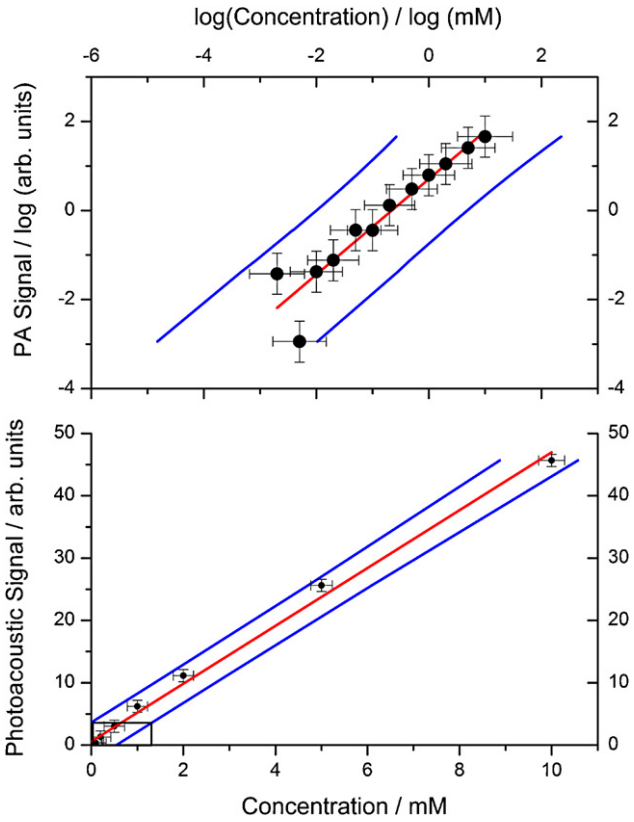

Fig. 5. Photoacoustic signal as a function of coumarin 440 dye concentration recorded using a fiber Bragg grating as a transducer. The top figure shows the data displayed in Fig. 7 of Yang et al. [17] on a logarithmic scale and assumes that the errors of the both concentration and photoacoustic signal are scaled logarithmically. While x_{LOD} cannot be determined with this assumption, the width of the confidence interval spans 2.5 orders of magnitude in concentration. The lower panel shows the same data but assumes that the photoacoustic signal measurement errors are constant over the entire measurement range. The detection limit is then determined as $x_{\mathrm{LOD}} = 1.3$ mM. The error bars are defined as in Fig. 1.

not constant, either, since x_{LOD} calculated from the linearly scaled calibration curve gives x_{LOD} = 1.3 mM, i.e. a concentration that was shown by Yang et al. to be 500-fold higher than what could be determined by multiple measurements near the LOD, i.e. using Method I presented above. The standard deviation of a series of measurements at 1 μM, 2 μM, 5 μM, and 10 μM showed that a 5 μM solution produced a signal that was $3s_y$ higher than the background noise [17] (see Eq. (3)). With this example we intend to illustrate that the method of determining a limit of detection using a calibration curve cannot be relied upon when the measurement uncertainties are not constant over the range of measurements. A comprehensive treatment of these cases has been presented by Lavagnini and Magno [2].

6. Summary

In conclusion, detection limits of chemical sensors may be obtained in two different ways:

(1) As recommended by IUPAC and most analytical labs one should determine the average signal level when repeatedly measuring a blank sample, as well as the average signal and its standard deviation of samples at one, or ideally more, concentrations near the LOD.
(2) When re-analyzing previously published and incompletely characterized data, the concentration at the LOD may also be estimated from the calibration curve using Eq. (11) or the equations presented in Ref. [4]. This can only be accurate when a large number of calibration measurements have been performed in the concentration regime near the LOD. Of course, Eq. (11) assumes a calibration that is linear over the entire measurement range and also assumes that the systematic measurement error is negligible or has been corrected. It also needs to be assumed that the uncertainties of the concentrations and of the signal are similar for all measurements, that they are normally (Gaussian) distributed, and that the calibration curve shows no hysteresis, induction range, or saturation. In any case, the LOD calculated from the calibration curve cannot be more than an estimate and another calibration with similarly distributed data can produce an LOD that may be different by a factor of two or more. Given the many assumptions that go into Method II it is better to obtain the LOD by repeated measurements near the suspected LOD, i.e. using by Method I. [18]

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Appendix I. Relation of Eq. (11) to Eq. (4.19) by Currie and Svehla (Ref. [4])

In Eq. (4.19) of Ref. [4] Currie and Svehla give the detection limit ("the estimate for the minimum detectable quantity") as

$$x_{LOD} = \frac{2t\sqrt{s_b^2 + s_y^2}}{r} \frac{1 - \left(\sum x_i / \sqrt{n\sum x_i^2}\right)\left(s_b / \sqrt{s_b^2 + s_y^2}\right) t(s_r/r)}{1 - t^2(s_r^2/r^2)} \quad (14)$$

This can be rearranged using Eqs. (6) and (7) to give

$$x_{LOD} = \frac{2ts_y}{nt^2 s_y^2 - Dr^2}\left(ts_y \sum x_i - \sqrt{r^2 D^2 + Dr^2 \sum x_i^2}\right) \quad (15)$$

Supplementary material deposited in the journal's database gives a detailed derivation of Eqs. (11) and (14). When comparing to the detection limit defined by Eq. (11)

$$x_{LOD,1,2} = \frac{2ts_y}{nt^2 s_y^2 - Dr^2} \times \left(ts_y \sum x_i - \sqrt{\frac{D^2 r^2}{k} + Dr^2 \sum x_i^2 - n\frac{D}{k} t^2 s_y^2 - Dt^2 s_y^2}\right) \quad (11)$$

it is found that the expressions differ by the last two terms in the root. Also, in the derivation of Eq. (11) k is retained as a parameter, whereas Currie and Svehla implied it to be $k = 1$. Eqs. (15) and (11) give the same result when

$$\frac{Dr^2}{k} + r^2 \sum x_i^2 \gg t^2 s_y^2 \left(\frac{n}{k+1}\right) \quad (16)$$

This is very commonly the case, but differences are found when the data are poorly correlated, or, more specifically, when the product of t with the standard deviation of the sensor response s_y is large compared to the sensitivity of the measurement, r. Currie and Svehla also remarked that the LOD can no longer be defined by the IUPAC definition if the uncertainty of the slope (=sensitivity) s_r is t-fold larger than the slope r. In any case it is important to remember that the LOD is "the maximum null-signal upper limit for a *particular realization* of the calibration curve" [4], and that another calibration curve of the same sensor with similarly scattered data can provide a very different estimate.

From a qualitative perspective, the differences between the two approaches are subtle, but can be traced to two sources. First, Eq. (15) (Currie and Svehla) incorporates the uncertainty in the estimated blank into the signal detection limit, whereas Eq. (11) does not. This results in different critical concentration limits ($x_C = (y_{LOD} - b)/r$) for the two methods Both methods use the calibration data to determine the LOD as the concentration where the probability that ($x < x_C$) is less than α. The second difference is that Eq. (11) assumes that the uncertainty in the estimated x does not change from that at x_C, whereas Eq. (15) does not make this assumption. In virtually all practical situations, these differences are inconsequential, especially in view of other assumptions that are likely to be violated.

We emphasize that no claim is made to the superiority of Eq. (11) over existing definitions. It is introduced only because the derivation from Eq. (10) is straightforward, there is an obvious graphical interpretation (Figs. 1 and 6), and its derivation does not require the introduction of more parameters. The spreadsheet program associated with this article provides Eqs. (11) and (15).

Appendix II. Differences between Methods I and II

While there are only small differences between the LOD calculated from Eq. (11) and Eq. (15) there is an approximately 2-fold difference to an LOD calculated using Method I, i.e. when the standard deviation of the signal, s_y, is measured near the LOD. Fig. 6 is an illustration that shows measurements of 8 replicate blanks ($x = 0$) and 8 replicate standards of the same concentration ($x =$ "10" in arbitrary units). The simulation assumed a slope of $r = 10$, an intercept of 10, and a measurement error standard deviation of $s_y = 13$. For simplicity, t was taken to be 3.

The black line shows the LOD_1 as calculated from Method I, i.e. using Eq. (3). It considers the regression line to be error-free and sets the signal limit of detection based on the estimated uncertainty of the signal, s_y, which in this case is the same whether one uses the pooled replicates or regression standard error.

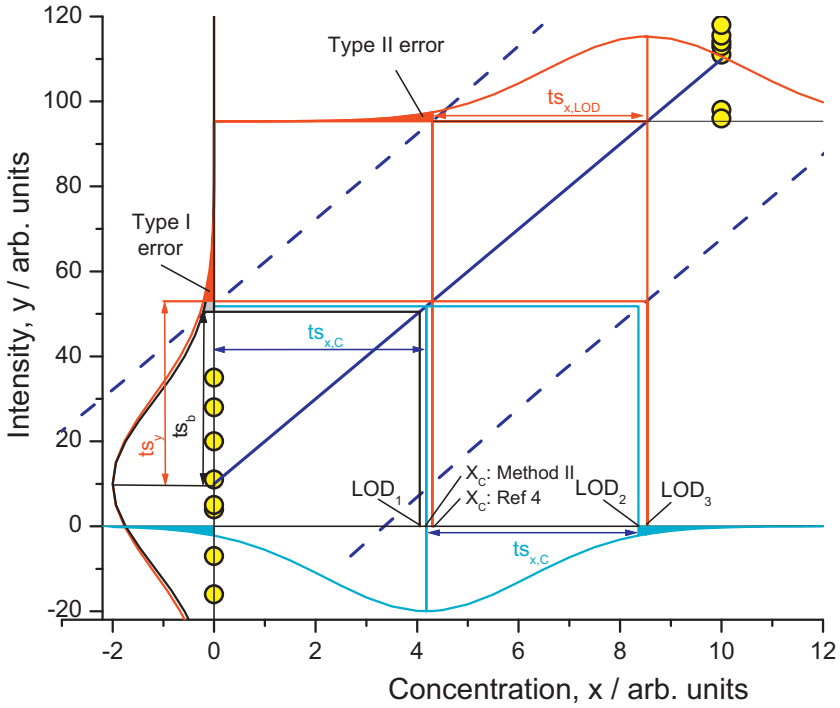

Fig. 6. Simulated data to illustrate the difference between limits of detection (LOD) obtained using Method I (LOD_1) and Method II (LOD_2). The LOD calculated according to Ref. [4] is given as LOD_3.

The $LOD_1 = 4.05$ is then determined directly from the regression line.

In deriving Eq. (11) it was assumed that LOD_2 is the concentration at 2 ts_x (here, $LOD_2 = 8.36$ and $t = 3$) where s_x is the estimated uncertainty of x at the concentration x_{LOD}. The relation is shown by the solid blue line. This procedure implicitly incorporates the uncertainty of the linear regression parameters. Note that the critical concentration (x_C) for Method II is close to LOD_1, but it is not identical since the latter uses the estimated uncertainty in y and the former uses the confidence bounds for estimated x. The value $LOD_3 = 8.53$ was calculated according to Ref. [4] and is only slightly higher than (red line) LOD_2.

Appendix C. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2012.06.071.

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Biographies

Hans-Peter Loock is a Professor of Chemistry with an interest in the development of fiber-optic and micro-photonic sensors for absorption, refractive index, strain, and fluorescence. His group specializes on the chemical detection in very small sample volumes. Together with his group and with collaborators he has developed optical detection methods that allow for chemical quantitation but do not require accurate intensity measurements. He has obtained an Engineering Chemistry degree from the Technical University of Darmstadt (Germany) in 1991 and a Ph.D. in physical chemistry from the University of Victoria, Canada. After two years of postdoctoral work at the Steacie Institute for Molecular Sciences (NRC, Ottawa) he joined Queen's University in 1999.

Peter Wentzell is a Professor of Chemistry at Dalhousie University, where he has been since 1989. He obtained his PhD from Michigan State University and held an NSERC Post-doctoral Fellowship at the University of British Columbia. His principal research interests are in the area of chemometrics, with a focus on the role of analytical measurement errors in multivariate measurements and on the development of data analysis strategies for high-throughput biological methods.