Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1089 (2005) 101–104
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Calibration of detector responses using the shape and size of band profiles Case of a nonlinear response curve

Leonid Asnin[a,b], Georges Guiochon[a,b]*

[a] *Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA*
[b] *Division of Chemical Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6120, USA*

Received 26 April 2005; received in revised form 10 June 2005; accepted 22 June 2005

Abstract

A new method consisting of using band profiles to calibrate the response of a detector, without making frontal analysis measurements, is discussed. A model for the calibration-curve expression is proposed. It is convenient and reasonably accurate even when the detector response deviates slightly from linear behavior at low concentrations.

Keywords: Calibration curve; HPLC; UV-detector

1. Introduction

Two classes of calibration curves are used in chromatography. The curves of the first and most popular class relate the amount of a compound injected into the column and the height or area of the signal obtained as a response to this injection. This type of calibration curve is most useful in analytical chromatography. The calibration curves of the second class are often called absolute calibration curves. They directly relate the actual average concentration (C) of the compound in the detector cell at a given time to the amplitude (h) of the signal measured at that same moment. The use of such a calibration curve is necessary in the measurement of adsorption isotherms and in several other physico-chemical applications of chromatography [1,2], as well as in preparative chromatography. In all these cases, an accurate knowledge of the temporal profile of the elution bands is required [3]. A discussion of methods useful to acquire absolute calibration curves is the topic of this report.

Commonly, an absolute calibration curve is derived from the signals acquired by flushing the detector cell with a series of solutions of known concentrations and measuring the steady-state response. These signals are often the convenient by-products of the results obtained in frontal analysis (FA). This "steady-state" method, however, has its own drawbacks. It needs a significant amount of time and is highly consuming of chemicals [3]. If the absolute calibration curve is needed to account for the results of elution chromatographic experiments, this procedure cannot be applied during the experiments but before of after them, which may explain its relative lack of reproducibility.

A method of indirect absolute calibration overcoming these disadvantages was proposed earlier. It allows the drawing of a steady-state calibration curve without requiring steady-state experiments [3]. The concept of this method is based on the convolution of an appropriate calibration function, $C(h)$, and of the band profile obtained for a known amount of the compound considered (q), following the following equation

$$q = \int_{V_1}^{V_2} C(h)\,\mathrm{d}V \tag{1}$$

* Corresponding author. Tel.: +1 865 974 0733; fax: +1 865 974 2667.
E-mail address: guiochon@utk.edu (G. Guiochon).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.06.077

When an appropriate model equation is selected for the calibration curve and initial estimates of the model parameters are obtained, the detector response recorded is easily transformed into a concentration, and the concentration profile is integrated versus the volume of the mobile phase passed to give an estimate of the amount injected to generate the peak obtained. The estimates of the parameters of the model can then be adjusted and the process repeated to give the closest possible agreement between the estimated and known amounts injected.

This method was shown to give a satisfactory agreement with the results obtained by the FA calibration procedure [4]. Examples of applying this method to the determination of adsorption isotherms by the inverse method have been reported [5]. These works dealt with the derivation of the dependences of q on the peak area (S), that is curved in the range of high values of q. In practice an opposite situation can occur, as will be demonstrated later, when the plot of q versus S is nearly linear except in a narrow interval around the origin. If that region is not investigated properly, the graph appears as if it were showing a straight line, with a finite intercept. This situation can give the false impression of a linear detector response. It is worth to note that this intercept can be caused not only by a nonlinear behavior of the detector response but also by a systematic error in the operation of the injection device, by the presence of contaminants in the sample, and by sampling errors or by errors in the integration of the peak, especially for peaks exhibiting a long tailing.

The low concentration region of the calibration curve is important in most physical applications of chromatography, such as in studies of diffusion or mass transfer kinetics because this region corresponds to the initial diffuse part of the front of the peak and to its tailing. The contributions of these parts of the peak to the calculation of its moments and particularly of its second and higher order moments are sufficiently important to warrant that careful attention is paid to them. Significant calibration errors made in the low concentration region during the transformation of the detector response into a concentration profile will lead to a large error in the numerical values of these moments and of the related quantities, i.e., the HETP and the rate or diffusion coefficients. We propose a method of calibration of the detector response which is applicable when the plot of q versus S is not linear around the origin. The measurements of the injection profiles of samples provide an example of application.

2. Theory

A power expansion or a polynomial function is frequently used to calibrate a detector response [4–7]. In the cases considered here, a two-term parabolic function describes well the calibration curves determined by the steady-state method. However, preliminary experiments (not reported) showed that neither the parabolic nor any other power function is a convenient model of the absolute calibration curve for numerical applications involving the solution of Eq. (1). The use of the best two-parameter polynomial calibration curve fitted to the highest peak gives a large error in the mass balance of small samples, and vice versa. Only by using a fourth-order polynomial could Eq. (1) give a satisfactory mass balance for large and for small sample peaks in the same time (unreported data). The possibility that such a calibration curve may have two inflection points may cause serious difficulties. Hence, the problem emerged to find a suitable model for the calibration function in Eq. (1).

It is known that one can describe the nonlinear signal of a HPLC UV-detector with the following relationship [3]

$$h(C) = \epsilon_0 lC - \log\left(\frac{\sinh(2.303a_1 lC\Delta/2)}{2.303a_1 lC\Delta/2}\right) \quad (2)$$

where ϵ_0 is the zero-concentration-limit of the molar absorptivity, l is the optical path-length of the detector cell, a_1 is the derivative of the molar absorptivity with respect to the wavelength, and Δ is the spectral bandwidth. Note that $h(C)$ is the signal of the UV-detector in absorbance units, hence is dimensionless. For practical applications, the inverse function, $C(h)$, is required. Eq. (2) has no analytical solution with respect to C. Therefore, we assume that the dependence of h on C is approximately linear resulting in the following expression

$$C(h) \approx (\epsilon_0 l)^{-1} h + (\epsilon_0 l)^{-1} \cdot \log \times \left(\frac{\sinh(2.303a_1 l\Delta(\epsilon_0 l)^{-1} \cdot h/2)}{2.303a_1 l\Delta(\epsilon_0 l)^{-1} \cdot h/2}\right) \quad (3)$$

It is necessary to make some changes in Eq. (2) to take into account a nonlinear character of the $C(h)$-function. Empirically, it was found that the following relationship is a convenient working function

$$C(h) = k_0 h + k_1 k_0 \cdot \log\left(\frac{\sinh(k_2 h)}{k_2 h + (k_2 h)^2}\right) \quad (4)$$

where k_0, k_1, and k_2 are numerical parameters obtained through the fitting of the experimental data to this equation.

3. Experimental

3.1. Equipment and chemicals

The data were acquired using a HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with an auto-sampler fitted with a 100 μl loop, a column thermostat, and a variable wavelength UV-detector (model G1314A) with a high-pressure cell (volume, 14 μl; path-length, 10 mm). Because the experiments were devoted to the investigation of the broadening of an inlet profile, a correct recording of the actual injection profiles was necessary. So, the flow scheme used was the usual one in analytical chromatography, but with the column replaced with a zero-volume connector. The total volume of the system from the injection

point to the detector cell was 80 μl. The measurements were carried out at ambient temperature. The band profiles were recorded at the wavelength of 270 nm, using the data acquisition system and the HP Chemstation software.

A solution of *n*-hexane and ethyl acetate (89:11, v/v) was used as the mobile phase. The HPLC-grade solvents were from Fisher Scientific (Fair Lawn, NJ, USA) and used as supplied. The sample was (*R*)-3-chloro-1-phenylpropanol ((*R*)-3CPP), from Aldrich (Milwaukee, WI, USA). It had been purified by re-crystallization from *n*-hexane.

3.2. Procedures

The calibration of a detector using the steady-state method (or frontal analysis mode) consists in measuring the detector response after filling the detector cell with a solution of known concentration. The concentration of the solution was increased step-wise by mixing a stream of the pure mobile phase and a stream of a solution of (*R*)-3CPP in the mobile phase at a concentration of 6.02 g/l, using the binary pump. Adjusting the flow rate ratio of the two pumps allows the control of the composition of the solution filling the detector cell. The experiments were implemented at flow rates of 0.5 and 1.0 ml/min. The difference between the results at these two flow rates were less than 4% in the concentration range between 0 and 1.2 g/l and less than 0.4% between 1.2 and 6.02 g/l.

The calibration of the detector using the pulse technique was carried out at the same flow rates. The concentration of the standard solution used was also 6.02 g/l. The sample volume was increased from 1 to 100 μl, and 15 consecutive injections were made, repeated three times to evaluate the repeatability. The relative standard deviation in the peak area was less than 4.5% for sample volumes lower than 5 μl and less than 0.2% for larger volumes.

3.2.1. Fitting procedure

The coefficients of the calibration curve in Eq. (4) were determined after the following procedure. After a series of pulse experiments, the quantity q/SF_v (with F_v the flow rate) was calculated for the peak corresponding to the largest sample volume (100 μl). The function (4) was then fitted to the straight line $C = (q/SF_v)h$. The coefficients of that function were taken as the initial estimates of the parameters in the numerical calculation of the best parameters of the calibration curve, following Eq. (1). The concentration of every point of each digitized peak profile was calculated using the current calibration curve and the integral in the RHS of Eq. (1) was calculated using the trapezoid method. Since each peak consists of more than 300 data points, such an evaluation was quite precise. The integral of the peak was compared to the known injected amount. A new set of parameters was derived to reduce the sum total of the squares of the differences and the procedure repeated until the equality in Eq. (1) was satisfied with an error less than 0.1%. Then, the calibration curve was applied to the smallest peak in the series (volume, 1 μl). If the equality in Eq. (1) was not satisfied, the coefficients were corrected and then used to recalculate the area of the largest peak. Successively adjusting the parameters of the calibration curve to match the areas of the smallest and the largest peak allowed this area and the injected amount to be eventually equal within 1%. Although an automatized fitting procedure is possible [3], we employed the method of trials and errors. The fitting takes less than 5 min. All the calculations were done with the MathCad 11 software (Mathsoft Engineering & Education Inc, Cambridge, MA)

4. Results and discussions

The results of the pulse experiments are reported in Fig. 1. The graphs are nearly linear, except in a small region around the origin where they are visibly curved. Since the peak area for a concentration-sensitive detector is inversely proportional to the flow rate at constant sample size, it is expected that the product $S \cdot F_v\,|_{q=const}$ is constant. This condition was satisfied within 5%. The two absolute calibration curves, for 0.5 and for 1 ml/min, were equally well approximated by Eq. (4), with the same fitting parameters. Therefore, only the data obtained for a flow rate of 0.5 ml/min are discussed further. The best fitting parameters for Eq. (4) determined by the numerical method described earlier are $k_0 = 7.6 \times 10^{-6}$ mM/mAU, $k_1 = 230$ mAU, $k_2 = 0.005$ mAU^{-1}.

The calibration curve derived from the pulse experiments is compared with the one obtained by the "steady-state" method in Fig. 2. The agreement between these two curves is satisfactory, at least below $h = 610$ mAU (C below 0.0045 mM). Table 1 compares the results of the evaluation of the injected amounts by Eq. (1) using the two calibration curves. The frontal analysis calibration curve gives results in excellent agreement with the amounts injected at low sample sizes, and values systematically overestimated at high sample size, by 3–5%. The numerical calibration curve using sample pulses gives near coincidence of the injected and es-

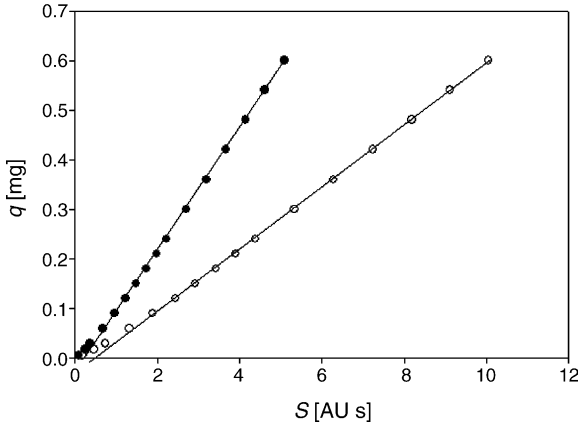

Fig. 1. Plots of the injected amount against the peak area. Flow rates 0.5 ml/min (open circles) and 1 ml/min (filled circles). Solid lines are linear fittings for straight parts of these plots continued to $q = 0$ line. Note, that the linear plots systematically deviate from experimental data around the origin.

Table 1
Material balance for peaks estimated with calibration curves measured by numerical indirect and direct (FA) methods

Sample volume (μl)	Injected amount (10^{-5} g)	Numerical method		FA method[a]	
		q (10^{-5} g)	δ (%)	q (10^{-5} g)	δ (%)
1	0.60	0.61	1.1	0.59	−1.6
3	1.81	1.91	5.5	1.79	−0.9
5	3.01	3.24	7.8	3.03	0.7
10	6.02	6.50	8.0	6.16	2.3
15	9.03	9.70	7.4	9.33	3.3
20	12.04	12.90	7.1	12.60	4.7
25	15.05	15.84	5.2	15.60	3.7
30	18.06	18.87	4.5	18.80	4.1
35	21.07	21.86	3.7	21.90	3.9
40	24.08	24.81	3.0	25.00	3.8
50	30.10	30.67	1.9	31.30	4.0
60	36.12	36.51	1.1	37.60	4.1
70	42.14	42.56	1.0	44.00	4.4
80	48.16	48.48	0.7	50.40	4.7
90	54.18	54.29	0.2	56.60	4.5
100	60.20	60.17	0.0	63.00	4.7

[a] In calculations by Eq. (1) the experimental calibration data for FA method (see Fig. 2) were approximated by function $c(h) = 3.83 \times 10^{-6}h + 6.4 \times 10^{-9}h^2$ ($[c]$ = mM, $[h]$ = mAU) with high precise ($R^2 = 0.9998$).

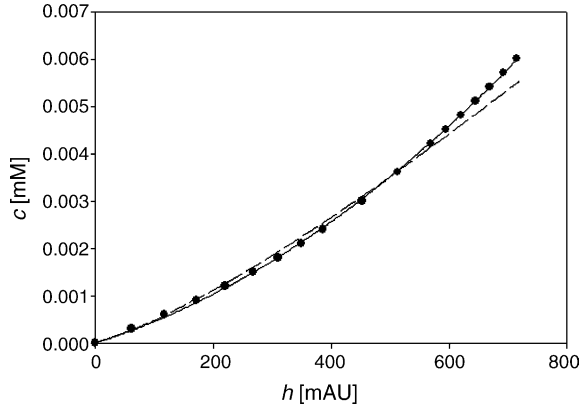

Fig. 2. Calibration curves determined by the "steady-state" method (filled circles for experimental data, solid line for a parabolic approximation) and calculated by the numerical method (dashed line). Flow rate 0.5 ml/min.

timated amounts at both ends of the range investigated and a discrepancy of up to 8% for the intermediate amounts. This result is easily understood because we used essentially the first and the last data points to fit the calibration curve.

It is worth to note that discrepancies between the amounts directly measured and calculated from the calibration curves were observed in some cases but not in other cases [4,8]. Interestingly, this discrepancy lead to a poor mass balance with the steady-state calibration curve, which is supposed to be most accurate since it is directly measured. Dose and Guiochon reported that the difference between these two types of calibration curves can amount to up to about 10% [3]. The cause of this phenomenon is not yet clear. A systematic error or the different conditions under which the detector signal forms in FA (cell filled with a homogeneous solution) and in the elution mode (cell filled with a solution the concentration of which varies widely in time and space) were suggested as possible reasons [4,8].

5. Conclusions

Linear plots of q versus S having a finite intercept are often encountered in chromatographic practice. Assuming that the detector response is linear in such case is incorrect, unless there is a strong evidence that the intercept is caused by reasons originating out of the detector itself. Calibration of detector responses is possible by mean of a numerical method based on the analysis of the chromatographic peak shapes. An appropriate model of equation for the calibration-curve must be employed in this method. Eq. (4) is convenient for this purpose. Having three fitting coefficients, it is flexible enough to approximate weak deviations of the response from linear behavior in the low concentration region and yet simple enough to allow a rapid calculation of its parameters. The new method proposed requires amounts of sample and of solvent that are about 100 times and 5 times, respectively, smaller than those consumed with the steady-state calibration method.

References

[1] S.G. Shirazi, A.M. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, Boston, MA, 1994.
[2] T. Paryjczak, Gas Chromatography in Adsorption and Catalysis, Halsted Press, New York, Ellis Horwood, Chichester, 1986.
[3] E.V. Dose, G. Guiochon, Anal. Chem. 62 (1990) 816.
[4] L. Asnin, G. Guiochon, J. Chromatogr. A 1089(2005) 105.
[5] G. Götmar, L. Asnin, G. Guiochon, J. Chromatogr. A 1059 (2004) 43.
[6] P.W. Carr, Anal. Chem. 52 (1980) 1746–1750.
[7] C.D. Pfeiffer, G.R. Larson, J.F. Ryder, Anal. Chem. 55 (1983) 1622.
[8] L. Asnin, W. Galinada, G. Götmar, G. Guiochon, J. Chromatogr. A 1076 (2005) 141.