

Short Communication

New approach for area determination of an overlapped pair of chromatographic peaks

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ABSTRACT

Based on an exponentially modified Gaussian model, a new approach for the area determination of an overlapped pair of peaks was developed. Empirical equations for the accurate calculation of the area ratio are presented. Individual peak areas can be calculated from the area ratio and the total area of an overlapped pair. This method can be applied over a wide range provided that there is a clear and precise valley except for shoulder peaks. The relative error of the peak area determination is less than $\pm 5\%$.

INTRODUCTION

The occurrence of unresolved or overlapped chromatographic peaks is a commonly encountered practical problem. The perpendicular-drop method is generally employed for the measurement of overlapped peaks. Foley [1] reported that the error due to a combination of peak tailing and differences in peak size can be exceed 200% for peak area measurement by this method. Jeansonne and Foley [2] reported a method with an accuracy of $\pm 4\%$ for the first peak area of an overlapped pair when the relative valley is less than 45%. Lin and Lu [3] reported a table of correction factors for the quantitative area determination of overlapped peaks. In previous papers [4,5] we reported two methods for the measurement of the areas of an overlapped pair of peaks. These methods were all based on the

calculation of the first peak area of an overlapped pair, the second peak area being obtained by subtraction of the first peak area from the total area measured by an electronic integrator. In this paper, we report an approach for area determination based on the area ratio of an overlapped pair of peaks.

THEORY

The following exponentially modified Gaussian (EMG) peak-shape model is used:

$$h(t) = \left(\frac{A}{2\tau}\right) \exp\left(\frac{\sigma^2}{2\tau^2} - \frac{t-t_g}{\tau}\right) [1 + \operatorname{erf}(Z/\sqrt{2})] \quad (1)$$

where

$$Z = \frac{t-t_g}{\sigma} - \frac{\sigma}{\tau} \quad (2)$$

$$\operatorname{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-y^2} dy \quad (3)$$

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t_g and σ are the retention time and standard deviation of the parent Gaussian peak, respectively, τ is the time constant of the exponential decay, $h(t)$ is the height at time t and the peak asymmetry is characterized by $S = \sigma/\tau$.

According to McWilliam and Bolton [6], assuming that the asymmetry and peak widths of two peaks are equal, the profile of an overlapped pair can be simulated by the following equation:

$$H(t) = h(t) + rh(t - d) \tag{4}$$

which represents an EMG function $h(t)$ followed after time d by another, $h(t - d)$, of equal width, where r is the area ratio of the second to the first peak ($r = A_2/A_1$, where A_1 and A_2 are the areas of the first and second of an overlapping pair of peaks) and $H(t)$ is the superposed height at time t .

From previous work [5] and ref. 2, the height of the first peak of an overlapped pair is less affected by the overlap (the increment of the height is usually less than 5%), as shown in Fig. 1. Therefore, it is reasonable to assume that the height of the first peak remains almost unchanged after overlap, i.e., $h_1 \approx h_{10}$, where h_1 and h_{10} are the heights of the first peak after and before overlap, respectively.

The height of the second peak of an overlapped pair can be expressed as

$$h_2 = h_{20} + h_{12} \tag{5}$$

where h_{20} and h_2 are the heights of the second peak before and after overlap, respectively, and h_{12} is the increment of the second peak height resulting from the overlap with the first peak; h_{12} depends on the asymmetry of the first peak and the degree of overlap and, from eqn. 1, it can be expressed as

$$h_{12} = \left(\frac{A_1}{2\tau}\right) \exp\left(\frac{\sigma^2}{2\tau^2} - \frac{t_{r2} - t_g}{\tau}\right) [1 + \text{erf}(Z_{12}/\sqrt{2})] \tag{6}$$

where

$$Z_{12} = \frac{t_{r2} - t_g - \sigma}{\sigma} \tag{7}$$

$$t_{r2} = t_{r1} + d \tag{8}$$

and t_{r1} and t_{r2} are the retention times of the first and the second peak, respectively, before overlap.

Dividing each term of eqn. 5 by h_1 , we obtain

$$\frac{h_2}{h_1} = \frac{h_{20}}{h_1} + \frac{h_{12}}{h_1} \approx \frac{h_{20}}{h_{10}} + \frac{h_{12}}{h_1} \tag{9}$$

From eqns. 1, 6 and 9, the following form can be obtained:

$$\frac{h_2}{h_1} = \frac{h_{20}}{h_{10}} \approx \gamma - \exp\left(-\frac{d}{\tau}\right) \frac{1 + \text{erf}(Z_{12}/\sqrt{2})}{1 + \text{erf}(Z_{11}/\sqrt{2})} \tag{10}$$

where

$$Z_{11} = \frac{t_{r1} - t_g - \sigma}{\sigma} \tag{11}$$

and γ is the apparent height ratio of an overlapped pair of peaks.

The unknown in the last term of eqn. 10 are unknown in practice and eqn. 10 is inconvenient to use. In order to make eqn. 10 practicable, we arbitrarily substituted $\exp[-c\Delta t/W_{0.1(r)}]$ for the last term in eqn. 10. Thus, eqn. 10 becomes

$$r \approx \gamma - \exp\left[-\frac{c\Delta t}{W_{0.1(r)}}\right] \tag{10a}$$

where $W_{0.1(r)}$ is the rear half-width at 10% height, Δt is the distance of an overlapped pair and c is a coefficient. The general expression can be obtained as follows. First, by using linear regression of the theoretical value of the last term in eqn. 10 and the calculated value of $\Delta t/W_{0.1(r)}$, the regression coefficient was taken as the initial value of c in eqn. 10a.

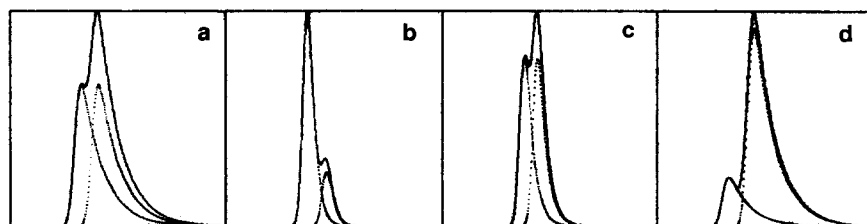


Fig. 1. Effect of the second peak of an overlapped pair on the height of the first peak. (a) $S = 0.2$, $r = 1:1$; (b) $S = 1$, $r = 1:5$; (c) $S = 1$, $r = 1:1$; (d) $S = 0.2$, $r = 5:1$.

Then, an iteration multiple regression program, *i.e.*, simplex algorithm, was used to adjust the residual sum of squares about the empirical regression equation (eqn. 20 or 21) to a minimum. Finally, eqns. 20 and 21 were obtained. The error resulting from the approximation we made can be reduced by this regression treatment.

The area of the first and second peak can be calculated by eqn. 12 or 13:

$$A_1 = \frac{A_t}{1+r} \quad (12)$$

and

$$A_2 = \frac{r A_t}{1+r} \quad (13)$$

where A_t is total area of overlapping peaks.

The expressions for the relative error of the first and second peak area ($\%RE_{A_1}$, $\%RE_{A_2}$) can be deduced as below. In general, the relative error ($\%RE$) is

$$\%RE = \frac{O-T}{T} \cdot 100 \quad (14)$$

where O and T are the calculated and real values, respectively. For the first peak area,

$$O = \frac{A_t}{1+r_{cal}} \quad (15)$$

$$T = \frac{A_t}{1+r} \quad (16)$$

where r and r_{cal} are the real area ratio and the area ratio calculated from eqn. 20 or 21, respectively.

Substituting eqns. 15 and 16 into eqn. 14, we obtain

$$\begin{aligned} \%RE_{A_1} &= \left(\frac{1+r}{1+r_{cal}} - 1 \right) \cdot 100 \\ &= \frac{r-r_{cal}}{1+r_{cal}} \cdot 100 \end{aligned} \quad (17)$$

Similarly,

$$\%RE_{A_2} = \frac{r_{cal}-r}{r(1+r_{cal})} \cdot 100 \quad (18)$$

Obviously, the relative error of area ratio ($\%RE_r$) is

$$\%RE_r = \frac{r_{cal}-r}{r} \cdot 100 \quad (19)$$

EXPERIMENTAL

All calculations were run on an IBM-PC computer using a BASIC program. In this paper, the area ratio is restricted to 4:1 to 1:4, and the asymmetry (σ/τ) varied from 0.2 to 2, corresponding to $f_{0.1}$ from 1.003 to 4.147 (the definition of $f_{0.1}$ is shown below).

Gas chromatographic experiments were performed on a Model 102 G gas chromatograph (Shanghai Analytical Instrument Factory), connected with a Shimadzu C-R1B data processor. The column used was 2 m \times 3 mm I.D. and contained 15% dinonyl phthalate as the stationary phase coated on 60-80-mesh white diatomite support (Shanghai Reagent Plant). The chromatograms were recorded at a chart speed of 120 mm/min. Overlapped peak pairs were generated according to the literature [2]. By consecutive injections of test solutions consisting of toluene-benzene or toluene-ethylbenzene at short time intervals, the overlapped profiles of two toluene peaks were obtained. Benzene and ethylbenzene were used as internal standards. From the principle of the internal standard method, the real r value of overlapped toluene peaks can be accurately determined.

High-performance liquid chromatographic experiments were performed on a Model 5060 instrument (Varian, Palo Alto, CA, USA) equipped with a UV-VIS detector. The mobile phase was 75% methanol-deionized water (75:25). The flow-rate was 0.8 ml/min and the UV detector was set at 275 nm. A 150 \times 4.6 mm I.D. MCH-5 (ODS) column was used isothermally at 30°C. *p*-Chlorotoluene and *m*-chlorotoluene were taken as test materials. Under the conditions described above, *p*-chlorotoluene was eluted first. The chromatograms were recorded at a chart speed of 30 cm/min. Working solutions of *p*- and *m*-chlorotoluene of 0.1% and 0.2% in methanol, respectively, were used. The different mixing solutions were made by mixing different volumes of the two working solutions and diluting to 1 ml. The degree of overlap and the area ratio of an overlapped pair of peaks are dependent on the mixed solution. Dilute solutions of the two test compounds were prepared by taking the same volume of each working solution as that in the preparing mixed solution separately and diluting to 1 ml with methanol. The true area of either peak of

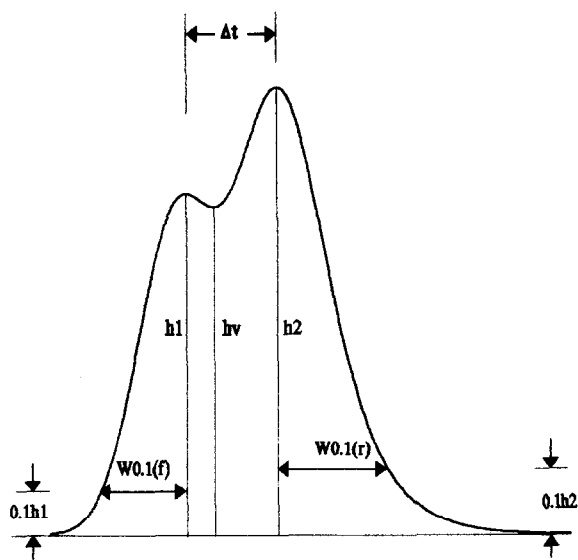


Fig. 2. Schematic diagram of parameters. $\gamma = h_2/h_1$; $f_{0.1} = W_{0.1(r)}/W_{0.1(f)}$.

an overlapped pair can be determined by injecting each diluted solution three times and taking the mean. The overlapping chromatograms of phenanthrene and *n*-butylbenzene obtained in previous work [5] were also used.

The data needed were measured manually except that the total area and the area of the internal standard were provided by a data processor.

RESULTS AND DISCUSSION

The following two equations were obtained from 200 and 160 data sets calculated from eqn. 4, respectively:

$$r = 0.0052 + 1.0007 \gamma - 1.1473 \exp\left[-\frac{2.62 \Delta t}{W_{0.1(r)}}\right]$$

$$f_{0.1} = 1.399 - 4.147 \quad (20)$$

$$r = -0.0152 + 1.0150 \gamma - 0.3300 \exp\left[-\frac{2.45 \Delta t}{W_{0.1(r)}}\right]$$

$$f_{0.1} = 1.003 - 1.445 \quad (21)$$

where $f_{0.1} = W_{0.1(r)}/W_{0.1(f)}$ and $W_{0.1(r)}$ and $W_{0.1(f)}$

TABLE I
THEORETICAL RELATIVE ERROR OF AREA RATIO
CALCULATED FROM EQN. 20 OR 21

S	$A_2:A_1$					
	4:1		1:1		1:4	
	%V ^a	%RE _r	%V ^a	%RE _r	%V ^a	%RE _r
0.2	99.77	-2.29	90.59	0.63	93.83	0.44
0.4	99.72	4.02	99.16	0.21	97.08	0.82
0.6	95.77	-2.82	97.26	0.01	98.12	-0.50
0.8	97.47	2.69	95.17	0.98	96.44	-0.96
1.0	93.08	2.03	94.50	-2.73	95.91	-1.50
1.2	92.69	4.65	99.04	3.24	98.70	-0.40
1.4	97.36	2.16	93.78	2.70	94.11	-0.38
1.6	97.13	0.22	97.84	-0.25	92.02	-0.48
1.8	99.86	0.60	96.61	-0.74	90.20	-0.41
2.0	99.33	-0.42	95.50	-1.13	98.30	-2.44

^a %V = $(h_v/h_{\min}) \cdot 100$, where h_v and h_{\min} are the height of the valley and the smallest height between two peaks of an overlapped pair, respectively; see also Fig. 1.

are the rear and front half-widths at 10% height, as shown in Fig. 2. The correlation coefficients of eqns. 20 and 21 are 0.9999 and 0.9998, respectively. The precisions of eqns. 20 and 21 are 2.13×10^{-2} and 2.68×10^{-2} , respectively.

Table I and II list the theoretical relative errors of the area ratio and the relative error of the area ratio for the experimental overlapped peak pair calculated from eqn. 20 or 21, respectively. Overall, the value of %RE_r listed in Table II is greater than that in Table I. This mainly results from the error in measurement. The greater is RE_r, the greater are RE_{A1} and RE_{A2}. The sign of RE_{A1} is opposite to that of RE_r and RE_{A2}, which can be explained as follows:

$$r = \frac{A_2}{A_1}$$

$$dr = -\frac{A_2}{(A_1)^2} \cdot dA_1 + \frac{1}{A_1} \cdot dA_2$$

$$\frac{\Delta r}{r} \approx -\frac{\Delta A_1}{A_1} + \frac{\Delta A_2}{A_2}$$

Therefore,

$$\%RE_r \approx -\%RE_{A1} + \%RE_{A2} \quad (22)$$

TABLE II

RELATIVE ERRORS OF AREA RATIO OF THE EXPERIMENTAL OVERLAPPED PAIR CALCULATED FROM EQNS. 20 AND 21

Experimental conditions: see text unless stated otherwise.

$r = A_2:A_1$	%V	$f_{0.1}$	% RE_{A1}	% RE_{A2}	% RE_r	
0.3237:1	98.93	1.590	-0.24	0.73	0.97	GC
0.5938:1	93.69	1.898	1.64	-2.76	-4.33	GC
0.6384:1	89.36	1.919	-1.70	2.70	4.50	GC
0.6955:1	90.79	1.878	0.54	-0.78	-1.31	GC
1.1984:1	86.63	1.413	-1.66	1.39	3.10	GC
1.5434:1	91.14	1.362	2.79	-1.81	-4.47	GC
1.6958:1	93.70	1.380	0.54	-0.31	-0.85	GC
2.1000:1	74.67	1.420	0.82	-0.39	-1.21	GC
0.4241:1	93.95	1.940	0.33	-0.77	-1.10	LC
0.7234:1	97.31	1.964	2.21	-3.05	-5.15	LC
1.060 :1	76.96	1.634	-0.65	0.62	1.28	LC
1.6964:1	84.02	1.757	0.26	-0.15	-0.41	LC
1.852 :1	91.18	1.432	2.10	-1.13	-3.16	LC ^a
1.869 :1	81.94	1.376	-0.18	0.09	0.28	LC ^a
0.9346:1	84.58	1.279	0.68	-0.73	-1.40	LC ^a
2.804 :1	86.05	1.354	1.34	-0.48	-1.80	LC ^a
2.778 :1	88.85	1.337	3.84	-1.38	-5.03	LC ^a
0.4673:1	89.49	1.498	1.56	-3.34	-4.83	LC ^a
0.9259:1	77.93	1.486	-1.27	1.38	2.68	LC ^a

^a Experimental conditions: see ref. 5.

This new approach for the determination of peak area based on area ratios is more accurate and simple than methods reported in the literature [2–5]. Eqns. 20 and 21 can be used for calculating the area ratio of two overlapping EMG peaks with an accuracy of $\pm 5\%$ provided that there is a clear and precise valley except for shoulder peaks. Further, it can be easily programmed. If a data acquisition system is used, the accuracy will be improved.

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