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EFFICACY OF THE CORRECTIONS APPLIED IN THE RESOLUTION OF OVERLAPPING CHROMATOGRAPHIC PEAKS BY THE PERPENDICULAR DROP METHOD

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SUMMARY

Systematic errors associated with the use of the perpendicular drop method for the determination of the areas of incompletely resolved chromatographic peaks were evaluated experimentally for various peak size ratios and degrees of overlap. The experimentally determined errors were compared with those estimated theoretically using the Gaussian peak shape model. A theoretical analysis was carried out on the applicability of the model of a triangular peak to the mathematical resolution of overlapped chromatographic peaks.

INTRODUCTION

Detection and resolution of incompletely separated peaks is an important step in quantitative analysis by gas chromatography (GC). The commonly used empirical resolution techniques of perpendicular drop and triangulation¹ give satisfactory results only in favourable cases, characterised by Gaussian or near Gaussian peaks, moderate overlaps, and not very high ratios of the sizes of the larger and smaller peak. In more complex cases, where it may be impossible even to detect visually the true number of the component peaks in the incompletely resolved portion of a chromatogram, curve-fitting techniques²⁻⁷ may be effective. Owing to the iterative character of the above techniques, a practical approach to this problem is possible only with the aid of computers. Besides digital procedures the application of analogue techniques to the resolution of overlapping peaks has been described⁸.

A common feature of all curve-fitting techniques is the necessity of defining an adequate peak shape model; procedures involving models of Gaussian, bi-Gaussian as well as non-Gaussian peaks have been published. In practice, however, it happens frequently that even the computer-based procedures of mathematical peak resolution are inapplicable. Such cases occur when the unresolved segment of the

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chromatogram is composed of peaks of generally different shapes or when peaks of so different sizes are present in the segment that the dimensions of the smaller peak and of the asymptotic parts of the larger one, on which the smaller peak is superimposed, become comparable. In such situations, the most reliable solution of the resolution problem is the decision made by a skilled chromatographer, consisting in drawing adequate images of the component peaks. At this point, however, the matter becomes more or less an art.

The methods of mathematical resolution were also employed to determine the systematic errors incidental to the component peak area determination by the methods of perpendicular drop and triangulation⁹. In virtue of the concepts similar to those employed in the above cited work, PROKSCH *et al.*¹⁰ have calculated a system of factors for correcting the component peak areas determined by the perpendicular drop method to obtain the respective true peak areas. In both cases mentioned above^{9,10}, a Gaussian curve was adopted as a peak shape model. KAISER AND KLIER¹¹ have recommended a procedure based on a model of triangular peak for the mathematical resolution of overlapped peaks.

The procedure involving the use of the above correction factors combines the simplicity of the perpendicular drop method with the exactness of the methods of mathematical peak resolution. This rational approach undoubtedly may provide for quick and accurate determination of the true component peak areas, however, the accuracy is again determined by the extent to which the model peak shape chosen in calculating the correction factors approximates the real chromatographic peaks. The present paper is an experimental check-up of the applicability of the correction factors of PROKSCH *et al.* in the above sense. Attention is also given to KAISER AND KLIER's triangular model.

THEORETICAL

Determination of the empirical correction factors

The choice of a Gaussian peak shape model makes it possible to express the correction factors as functions of merely two parameters, A_2'/A_1' and R_s , where A_2' and A_1' are the apparent areas of the larger and of the smaller segment of the composite peak, respectively, as determined by the perpendicular drop method, and R_s stands for the degree of resolution defined by $\Delta/\bar{\sigma}$ where Δ is the distance between the maxima of the two component peaks and $\bar{\sigma}$ denotes the average standard deviation of the latter. Hence, the appropriate correction factors may be readily found for any particular case of two overlapping peaks, assuming the latter are Gaussian. However, the correction factors may also be determined directly from two chromatograms of a given binary mixture if one of the chromatograms consists of overlapping peaks and is evaluated by the perpendicular drop method while the other is run under conditions permitting complete separation of the components, thus rendering directly the true peak areas. These empirical correction factors are obviously independent of the peak shape and, therefore, the comparison of the factors obtained by both procedures, for the given mixture and degree of overlap, may give a measure of the applicability of the theoretical correction factors.

Let us consider a chromatogram of a pair of incompletely resolved peaks where A_2 and A_1 designate the true areas of the larger and of the smaller peak, A_2' and A_1'

stand for the areas found for the larger and for the smaller segment of the composite peak area divided by the separation perpendicular, and S is the total composite peak area, respectively. The true area fractions, a_2 and a_1 , are given by

$$a_2 = A_2/(A_2 + A_1) = A_2/S \quad (1)$$

$$a_1 = A_1/(A_2 + A_1) = A_1/S \quad (2)$$

Eqns. 1 and 2 imply the calculation of a_2 and a_1 from the images of true peaks 2 and 1 in the composite chromatogram. However, these peak area fractions may be determined from the corresponding chromatogram where peaks 2 and 1 have been completely resolved, which is facilitated by the fact that area fractions are independent of the absolute sizes of the respective areas.

The correction factors have been defined¹⁰ by the equations

$$f_2 = A_2/A_2' \quad (3)$$

$$f_1 = A_1/A_1' \quad (4)$$

which may be rewritten, on combining them with eqns. 1 and 2, to read

$$f_2 = a_2S/A_2' \quad (5)$$

$$f_1 = a_1S/A_1' \quad (6)$$

The above equations provide for calculating the correction factors from directly measured experimental data. In order to distinguish between the theoretical factors obtained from PROKSCH's tables and the empirical factors calculated by eqns. 5 and 6, we shall use the designation f_t and f_e for the theoretical and for the empirical factors, respectively.

Systematic errors of the peak area determination with the use of the theoretical factors

The relative systematic error of the determination of the true peak areas by applying the perpendicular drop method without any corrections at all, δ , may be expressed by

$$\delta_2 = (A_2' - A_2)/A_2 \quad (7)$$

$$\delta_1 = (A_1' - A_1)/A_1 \quad (8)$$

Upon substituting for A_2 and A_1 from eqns. 3-6, eqns. 7 and 8 may be rewritten to read

$$\delta_2 = (1/f_{e2}) - 1 = (A_2'/a_2S) - 1 \quad (9)$$

$$\delta_1 = (1/f_{e1}) - 1 = (A_1'/a_1S) - 1 \quad (10)$$

where the subscript e indicates that the empirical factors have been employed.

Assuming that the products $A_2'f_{e2}$ and $A_1'f_{e1}$ represent the true areas A_2 and A_1 , it is possible to express also the errors that will affect the results when using PROKSCH's theoretical factors. Thus, denoting the above errors by δ_G (Gaussian peak shape model), we can write

$$\delta_{G2} = (A_2'f_{t2} - A_2'f_{e2})/A_2f_{e2} = (f_{t2}/f_{e2}) - 1 \quad (11)$$

$$\delta_{G1} = (A_1'f_{t1} - A_1'f_{e1})/A_1'f_{e1} = (f_{t1}/f_{e1}) - 1 \quad (12)$$

The applicability of KAISER AND KLIER's triangular model may be checked by carrying out an appropriate comparison of the latter with the more realistic Gaussian

model. With this end in view, a pair of overlapping triangles specified by the parameters of KAISER AND KLIER's model was analysed in terms of a Gaussian curve. The analysis was based on the following concept. Let us consider two triangles with the heights h_2 and h_1 (subscript 2 again denotes the larger component), equal base widths, d , and with the apexes separated from each other by a horizontal distance Δ . It can be shown that the horizontal distance (x) between the perpendicular separating the true areas of the triangles and the apex of the smaller triangle is given by

$$x = \frac{d + \Delta(h_1/h_2)^{\frac{1}{2}}}{1 + (h_1/h_2)^{\frac{1}{2}}} - \frac{d}{2} \quad (13)$$

Now, let us suppose that the pair of the triangles is substituted by a pair of Gaussian curves with the same height ratio and the same horizontal distance between the peak maxima, the standard deviations of the curves amounting to one fourth of the triangle base width. Provided the smaller peak is considered as that with the lower retention time, the individual Gaussian curves may be described by the equations

$$\varphi_1(t) = h_1 \exp[-8(t - t_1)^2/d^2] \quad (14)$$

$$\varphi_2(t) = h_2 \exp\{-8[t - (t_1 + \Delta)]^2/d^2\} \quad (15)$$

the corresponding composite peak being defined by

$$\varphi_3(t) = \varphi_1(t) + \varphi_2(t) \quad (16)$$

The symbol t in eqns. 14-16 stands for time, t_1 denoting the time corresponding to the maximum of the smaller peak. In this context, the abscissa x is related to the time corresponding to the separation perpendicular, t_s , by

$$t_s = t_1 + x \quad (17)$$

provided x is expressed in time units. It can be shown that the combination of eqns. 13 and 17 is an equivalent of KAISER AND KLIER's formula for determining the separation line between the two triangles¹¹. The true areas of peaks 1 and 2 are obviously given by

$$A_1 = \int_0^{\infty} \varphi_1(t) dt \quad (18)$$

$$A_2 = \int_0^{\infty} \varphi_2(t) dt \quad (19)$$

while the areas that will be obtained according to the procedure suggested by KAISER AND KLIER are

$$A_1' = \int_0^{t_s} [\varphi_1(t) + \varphi_2(t)] dt \quad (20)$$

$$A_2' = \int_{t_s}^{\infty} [\varphi_1(t) + \varphi_2(t)] dt \quad (21)$$

Thus, eqns. 18-21 afford the estimation of the relative systematic error of the peak

areas determined in virtue of the triangular model, the estimate being of course referred to the Gaussian model. The above error is designated by $\delta_{K(G)}$ (cf. Table III).

EXPERIMENTAL

The experimental material was obtained by chromatographing on columns of different lengths a series of binary mixtures of various proportions of the components, thus obtaining a system of chromatograms with the peak area ratios and degrees of peak overlap ranging within appreciably wide limits. One of the chromatograms of each mixture presented completely resolved peaks.

The model substances were *m*-xylene and *o*-xylene of analytical grade purity (Koch-Light Laboratories Ltd., Great Britain). The mixtures were chromatographed on stainless-steel columns filled with a packing of 10 wt. % of Apiezon L on Chromosorb W 60/80 mesh and kept at 100°. The internal diameter of the columns was 3 mm and their lengths varied within 25–120 cm, as required for the particular degree of resolution. The above system rendered fairly symmetrical peaks for both components.

All measurements were carried out on a Hewlett-Packard high efficiency gas chromatograph, Model 402 (U.S.A.), employing flame ionisation detection. The flow rates of the carrier gas (N₂), hydrogen, and air were set to about 30, 40, and 400 ml/min, as measured at the detector outlet, respectively. The injection port and the detector were kept at a temperature of 150°. The sample charges amounted to some tenths of a microliter and were injected by a Hamilton 7001 N (1 μ l) syringe (Hamilton Co., Whittier, Calif., U.S.A.), at appropriate sensitivity attenuations. The recorder chart speed was varied so as to obtain peaks of comparable widths with the columns of different lengths. The peak areas were determined from the records provided by a Disc chart integrator, Model 229-A (Disc Instruments, Inc., Santa Anna, U.S.A.). Three chromatograms were recorded and processed in all cases.

RESULTS AND DISCUSSION

When processing the results obtained by evaluating the chromatograms it became necessary to take into consideration also the sequence of the peaks in the chromatogram, which is obviously at variance with the theory based on the Gaussian peak model. Therefore, the results have been assorted accordingly.

Table I contains the data concerning the cases in which the smaller peak precedes the larger one. In these cases, the experimentally determined systematic errors due to neglecting the corrections are negative for the smaller peak and positive for the larger one. The above finding as well as the trends displayed by the bias upon varying the peak area ratio and the degree of overlap are in compliance with the above-mentioned theory. Therefore, the application of the factors of PROKSCH *et al.* appreciably lessens the systematic error in this case. The I_2 and I_1 in Tables I and II are the percentage coefficients of variation of the experimental correction factors f_{e2} and f_{e1} , respectively, and have been quoted to characterise the reliability of the respective data. The values of the factors represent the averages of three independent determinations, and the coefficients of variation have been expressed for the average values by the method of DEAN AND DIXON¹².

The results concerning the cases with the smaller peak located after the larger

TABLE I

DATA CONCERNING THE CASES WITH THE SMALLER PEAK PRECEDING THE LARGER ONE

A_2'/A_1'	$\Delta/\bar{\sigma}$	f_{e2}	f_{e1}	δ_2 (%)	δ_1 (%)	I_2 (%)	I_1 (%)	f_{t2}	f_{t1}	δ_{G2} (%)	δ_{G1} (%)
1.5	5.4	1.002	0.997	-0.2	0.3	0.137	0.525	I	I	-0.2	0.3
1.6	4.8	0.979	1.035	2.1	-3.4	0.525	1.074	0.999	1.002	2.0	-3.2
1.7	3.4	0.957	1.075	4.5	-7.0	0.382	0.568	0.986	1.025	3.0	-4.7
1.7	2.9	0.958	1.064	4.4	-6.0	0.329	0.327	0.969	1.054	1.1	-0.9
2.6	5.4	0.997	1.009	0.3	0	0.335	0.750	I	I	0.3	-0.9
2.7	4.8	0.984	1.040	1.6	-3.8	0.227	0.309	0.998	1.003	1.4	-3.6
2.8	3.8	0.980	1.055	2.0	-5.2	0.330	0.472	0.989	1.031	0.9	-2.3
3.5	2.5	0.926	1.260	8.0	-20.6	0.160	0.562	0.917	1.290	-1.0	2.4
5.5	5.4	1.004	0.977	0.4	-2.4	0.285	0.268	0.999	1.003	-0.5	2.7
6.0	4.3	0.992	1.050	0.8	-4.8	0.145	0.289	0.996	1.031	0.4	-1.9
6.3	3.8	0.985	1.096	1.5	-8.8	0.048	0.609	0.988	1.080	0.3	-1.5
6.1	3.6	0.990	1.077	1.0	-7.1	0.242	0.592	0.983	1.103	-0.7	2.4
12.8	5.6	0.999	1.008	0.1	-0.8	0.133	0.568	I	1.006	0.1	-0.2
14.5	4.0	0.991	1.127	0.9	-11.3	0.044	0.630	0.992	1.115	0.1	-1.1
16.0	3.7	0.987	1.240	1.3	-19.4	0	0.693	0.988	1.199	0.1	-3.3
16.6	3.3	0.982	1.287	1.8	-22.3	0.217	1.443	0.977	1.389	-0.5	7.9
29.6	3.7	0.995	1.222	0.5	-18.2	0.043	5.132	—	—	—	—
34.2	3.5	0.991	1.410	0.9	-29.1	0.042	1.443	—	—	—	—
39.3	3.4	0.988	1.614	1.2	-38.0	0.210	0	—	—	—	—
44.5	2.5	0.984	1.817	1.6	-45.0	0.339	1.924	—	—	—	—

TABLE II

DATA CONCERNING THE CASES WITH THE SMALLER PEAK FOLLOWING THE LARGER ONE

A_2'/A_1'	$\Delta/\bar{\sigma}$	f_{e2}	f_{e1}	δ_2 (%)	δ_1 (%)	I_2 (%)	I_1 (%)	f_{t2}	f_{t1}	δ_{G2} (%)	δ_{G1} (%)
1.2	5.2	1.039	0.956	-3.8	4.6	1.032	1.122	I	I	-3.8	4.6
1.2	4.8	1.027	0.977	-2.6	2.4	0.464	0.548	0.999	1.001	-2.7	2.5
1.2	4.1	1.041	0.948	-3.9	5.5	0.467	0.650	0.998	1.003	-4.1	5.8
1.1	3.1	1.007	0.920	-7.1	8.7	1.056	1.001	0.995	1.006	-7.6	9.3
2.1	5.6	1.013	0.955	-1.3	4.7	0.202	0.713	I	I	-1.3	4.7
2.2	5.4	1.004	0.991	-0.4	0.9	0.267	0.297	I	I	-0.4	0.9
2.2	3.6	1.010	0.974	-1.0	2.7	0.589	0.291	0.987	1.029	-2.3	5.6
1.8	2.7	1.085	0.845	-7.8	18.3	0.419	0.380	0.955	1.081	-12.0	29.5
4.2	5.3	1.018	0.922	-1.8	8.5	1.001	0.453	0.999	1.003	-1.9	8.8
4.0	3.9	1.029	0.882	-2.8	13.4	0.537	1.076	0.990	1.041	-3.8	18.0
4.3	3.5	1.018	0.928	-1.8	7.8	0.159	0.679	0.980	1.086	-3.7	17.0
7.5	5.3	1.016	0.882	-1.6	13.4	0.198	1.117	0.999	1.006	-1.7	14.1
6.8	5.0	1.028	0.812	-2.7	23.2	0.700	2.001	0.999	1.010	-2.8	24.4
6.7	4.9	1.032	0.788	-3.1	26.9	1.345	2.276	0.998	1.012	-3.3	28.4
7.7	4.5	1.013	0.902	-1.3	10.9	0.829	2.196	0.996	1.028	-1.7	14.0
12.8	5.0	1.012	0.831	-1.2	20.3	0.277	1.215	0.999	1.018	-1.3	22.5
15.9	4.9	1.003	0.953	-0.3	4.9	0.233	2.211	0.998	1.026	-0.5	7.7
13.7	3.4	1.016	0.781	-1.6	28.0	0.187	1.920	0.979	1.291	-3.6	65.3

one are summarised in Table II. It is evident from the values of f_{e2} and f_{e1} as well as δ_2 and δ_1 that the actual situation is just contrary to that predictable by virtue of the properties of Gaussian curves. In all these cases the systematic error of the uncorrected area of the smaller peak is positive whereas the area of the larger peak has a negative error. The consequence of the above situation is the introduction of an additional error by applying the correction factors of PROKSCH *et al.* which is apparent from the comparison of the values of δ_2 and δ_1 with those of δ_{G2} and δ_{G1} .

The above situation may be explained as follows. A chromatographic peak, even when it represents a concentration region falling into a linear part of the respective sorption isotherm, is always modified by a convolution component, irrespective of the other sources of peak skew. The above convolution component leaves a major part of the peak practically unskewed, except the tail occurring just at the foot of the trailing part, which becomes enlarged by the exponential decay component. As this enlargement is proportional to the peak size, it is insignificant with the smaller peak and, therefore, plays a small role if this peak is before the larger one. However, when a small peak follows closely after a large one, the former, being actually superimposed upon the convolution tail of the large peak, renders excessive values rather than deficient ones when evaluated by the perpendicular drop method. The above enlargement of the smaller segment is obviously to the detriment of the segment of the larger peak.

Under the above conditions, the true correction factors will vary significantly with even slight variations in the extent of the exponential decay component, especially as concerns the smaller peak. Therefore, minor experimental irregularities, particularly those incidental to injecting the sample, may be critical in the above respect. This is probably why it was not possible to find any correlation between the experimental correction factors and the respective values of A_2'/A_1' and $\Delta/\bar{\sigma}$, though the former have been determined with fair precision.

TABLE III
DATA CONCERNING THE MODEL OF A TRIANGULAR PEAK

A_2'/A_1'	$\Delta/\bar{\sigma}$	$\delta_{K(G)2}$ (%)	$\delta_{K(G)1}$ (%)	f_{t2}	f_{t1}	δ_{G2} (%)	δ_{G1} (%)
3	5.40	-0.54	1.63	I	I	0	0
	4.20	-1.31	4.06	0.994	1.017	0.60	-1.67
	3.00	-2.16	6.77	0.959	1.123	4.28	-10.95
	1.80	-2.39	7.54	—	—	—	—
6	5.40	-0.77	4.72	0.999	1.004	0.10	-0.40
	4.20	-1.66	10.34	0.994	1.037	0.60	-3.57
	3.00	-2.60	16.46	0.956	1.263	4.60	-20.82
	1.80	-3.00	19.14	—	—	—	—
9	5.40	-0.92	8.43	0.999	1.006	0.10	-0.60
	4.20	-1.79	16.70	0.994	1.054	0.60	-5.12
	3.00	-2.70	25.75	0.959	1.371	4.28	-27.06
	1.80	-3.14	30.18	—	—	—	—
15	5.40	-1.09	16.70	0.999	1.009	0.10	-0.89
	4.20	-1.89	29.51	0.995	1.082	0.43	-7.58
	3.00	-2.70	42.87	0.963	1.531	3.84	-34.68
	1.80	-4.08	66.60	—	—	—	—

Table III presents some selected data illustrating the errors associated with the use of the corrections according to KAISER AND KLIER's model. The values of $\delta_{K(G)_2}$ and $\delta_{K(G)_1}$ were calculated by eqns. 7 and 8, A_2' and A_1' being expressed by means of eqns. 20 and 21, respectively. The errors designated δ_{G_2} and δ_{G_1} are the theoretical errors of the uncorrected area segments, expressed under the presumption that the composite peak consists of Gaussian components; the δ_G values were calculated by $\delta_G = (A' - A'f_t)/A'f_t = (1/f_t) - 1$. Hence, the whole case has been referred to the Gaussian model. It is apparent from the data in Table III that the separation perpendicular determined by the formula derived in virtue of the triangular model is generally false. The application of this method leads to errors of the absolute values roughly comparable with the errors brought about by neglecting the corrections, but having opposite signs. Owing to the above-mentioned findings, this situation actually applies in cases where the smaller peak precedes the larger one. In the converse cases, the error associated with the application of KAISER AND KLIER's procedure will be about twice as large as that for the uncorrected data. These great discrepancies appear to be due to the fact that it is not possible to allow for the asymptotic parts of the peak by means of the triangular model, while it is just these parts that represent one of the main sources of the correction problems, particularly in cases of moderate overlaps.

CONCLUSIONS

The applicability of the factors defined in virtue of the Gaussian peak shape model to the correction of the areas determined by the perpendicular drop method is limited to the cases where the larger peak is preceded by the smaller one. If the smaller peak follows the larger one, excessive values of the area of the smaller peak and deficient values of that of the larger peak are obtained by the above method even when the composite peak consists of fairly symmetrical components. As this situation is just contrary to that expected by the theory based on the Gaussian peak model, additional systematic errors would be introduced by applying the correction factors in the above case. The discrepancies between the theory and reality appear to stem from the tailing effects, the significance of which rises with increasing peak size.

The application of the triangular model in locating the separation perpendicular leads generally to false results; the areas obtained by the plain perpendicular drop method are mostly more accurate than those determined by virtue of the above model.

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