COMPARISON OF SOME METHODS FOR AXIAL SPREADING CORRECTION IN GEL PERMEATION CHROMATOGRAPHY

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Artificial chromatograms were used in testing five published methods of data correction in gel permeation chromatography. The methods were compared with respect to correction efficiency (expressed by an agreement between the calculated and the known courses of the chromatogram being corrected and by an agreement between the calculated and the given number, weight and *z*-average molecular weights), to the sensitivity to experimental errors in the read-off heights of real chromatograms, and also with respect to the computer time requirements.

In contrast to other types of column chromatography a rather low separation efficiency is characteristic of gel permeation chromatography (GPC) of polymers; a chromatogram obtained in the separation of a polydisperse polymer usually has the shape of a single band, which is a superposition of a large number of unresolved overlapping peaks of the individual components. A number of procedures have been described in the literature, which allow to calculate the corrected molecular weight distribution from the chromatographic record, while taking into consideration the imperfect resolving power of real GPC columns. The significance of such axial spreading correction is the higher, the lower the efficiency of the given column system and the narrower the distribution of the sample under investigation; the correction is of great importance especially in such cases when one must, in solving problems of the mechanism of polymerization using the GPC data, decide about the uni- or multimodal character of distribution, and when any further rise in column efficiency is neither feasible nor practical.

From the mathematical point of view, correction of the GPC data requires solving the integral Tung equation[1]

$$f(v) = \int_{-\infty}^{\infty} G(v, y)\, w(y)\, \mathrm{d}y\,, \qquad (1)$$

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in which $f(v)$ is the original chromatogram (normalized so that $\int f(v)\,dv = 1$), w is the sought function – chromatogram corrected for axial spreading, and the function $G(v, y)$ (the so-called spreading function) is the response of the respective chromatograph to a narrow injected pulse of a monodisperse fraction having the retention volume y.

In this paper we shall confine ourselves to the case in which the spreading function in Eq. (*1*) has the Gaussian form so that the Tung equation will read

$$f(v) = \int_{-\infty}^{\infty} \sqrt{(h/\pi)} \exp\left[-h(v - y)^2\right] w(y)\,dy\,; \qquad (2)$$

h is the spreading factor, which is either constant ($h = h_0$) or is a function of the elution volume ($h = h(y)$).

Many methods described so far[1-12] and used in solving the integral Eq. (*1*) differ in their respective mathematical complexity and in their requirements on the storage space and operational speed of the computer employed, and also presumably in the efficiency of correction and versatility. Some of the earlier correction procedures have been compared in two papers of Duerksen and Hamielec[13,14]. Since that time, a number of new methods have been suggested; this paper offers a critical evaluation of some of them.

METHODS

To compare and evaluate all procedures offered so far for solving Eq. (*2*) would be a very extensive task. We therefore ruled out such procedures[2,3] which are too demanding as to the operational speed and the computer storage space, because in the case of a routine treatment of the GPC data the expenses connected with the computer time are by no means insignificant. We also ruled out in advance such methods which are known to lead to a numerically unstable solution[1,4], or which are not sufficiently universal in that they do not allow an easy algorithmization of some decisions[5-7,10] (these include, in the first place, methods of the numerical Fourier transformation[6,7,10], in which the required limits of integration in the inverse transformation depend on the type of the chromatogram or on the accuracy of the height readings[10]).

Methods Compared

1) Method of Pierce and Armonas[8] (P. A.): This approximative procedure is attractive because of its simplicity. We used the algorithm published by the authors of the method with a single modification, which allowed the local value of the resolution factor h to be substituted into the correction formula in such cases where h was a function of the elution volume.

2) Method of Chang and Huang[9] (CH. H.): A rapidly converging procedure, in which instead of the integral Eq. (*2*) an equivalent problem of the calculus of variations is solved. We used an original algorithm obtained by courtesy of Professor Huang and proceeded exclusively according to the iteration scheme II (*cf.*[9]). The iteration was stopped when an agreement better than 1% between the areas of the original and recalculated normalized chromatogram was attained.

3) Second polynomial method of Tung[10] (T.): A procedure in which sections of the function $f(v)$ corresponding to nine consecutive abscissae are approximated by least-squares fourth-order polynomials with coefficients calculated from the given nine consecutive data points. (A number of misprints appeared in the description of the algorithm in the original paper[10], which had to be corrected before implementing the algorithm on the computer.) In the evaluation of chromatograms with a variable $h(v)$, different values of h corresponding to the central of the nine points under consideration were successively substituted.

4) Method of Vozka and Kubín[11] (V. K.): A procedure published recently, in which an equivalent boundary-value problem is solved instead of the integral equation, Eq. (*2*). The algorithm is very simple and the experience acquired so far suggests that its efficiency is good even in the evaluation of very complicated chromatograms. In the present paper the original procedure was used (third-order method with the number of steps $k = 3$ and with the increment $\Delta v_c = 1{\cdot}5$ count — *cf.*[11]) and also a modified procedure derived with the aim to improve the correction efficiency in a non-iterative, once-through application[11]. The modification consists in that the necessary derivatives were calculated not by using an interpolation polynomial, as in the original work[11], but a smoothing least-squares sixth-order polynomial based on 11 symmetrically distributed points[15]. Here, the increment of the elution volume employed in the calculation could be reduced to Δv_{calc} 0·5 without the inaccuracies involved in the height readings of real chromatograms being reflected in artificial oscillations in the calculated course of $w(v)$.

5) Method II according to Ishige, Lee and Hamielec[12] (I. L. H.): Although the procedure is rather exacting as to the computer time and storage space, our experience shows that it is both universal and highly efficient even in the most complicated cases. The criterion of completion of the iteration was the same as that used in the CH. H. method.

All methods were programmed in the BASIC language and implemented on a programmable WANG 2200 calculator with 12 kB memory.

Testing Chromatograms

In order to compare corrected chromatograms calculated by the above methods with the known course, the testing was carried out with five artificial chromatograms constructed as follows.

The test chromatograms were always based on a combination of one or several Schulz–Zimm molecular weight distributions with known values of the number, weight and z-average molecular weight; these distributions were superimposed in a known ratio to form the resulting distribution curve. The correct (theoretical) average $\overline{M}_n$, $\overline{M}_w$ and $\overline{M}_z$ values of this distribution were then calculated by means of the equations

$$\overline{M}_n = \left[\sum_i \alpha_i / M_n^{(i)}\right]^{-1}; \quad \overline{M}_w = \sum_i \alpha_i M_w^{(i)}; \quad \overline{M}_z = \sum_i \alpha_i M_w^{(i)} M_z^{(i)} / \overline{M}_w, \qquad (3)$$

in which $M_n^{(i)}$, $M_w^{(i)}$, $M_z^{(i)}$ respectively are the number, weight and z-average molecular weights of constituent Schulz–Zimm molecular weight distributions and α_i are their weight fractions in the mixture ($\sum \alpha_i = 1$).

The distribution thus obtained was then used in calculating the corrected — $w(v)$ — and uncorrected — $f(v)$ — chromatograms by employing a procedure described earlier[11]. In the calculation of $f(v)$ by the numerical integration of Eq. (2), either a constant $h_0 = 0{\cdot}25$ was substituted, or the resolution factor was regarded as a function of the elution volume described by the relationship[11]

$$h(v) = -0{\cdot}93102 + 0{\cdot}02541v - 0{\cdot}00007v^2 . \qquad (4)$$

Table I summarizes characteristics of the individual testing chromatograms. From the first distribution, which was a superposition of four Schulz–Zimm functions, two chromatograms (1A, 1B) were calculated, one with a constant (1A) and the other with a varying (1B) resolution factor; especially in the latter case in the region of lower elution volumes, where the resolving power was already rather low, the small peak corresponding to the third fraction appeared only as a barely discernible tail on the peak of the fourth fraction. The second distribution was given by a single Schulz–Zimm curve; the respective chromatogram (2) had to prove the aptness of the individual methods to correct without artificial oscillations very narrow ($M_w/M_n = 1{\cdot}1$) peaks for which the contribution of axial spreading is a very important one, and in particular to recover correct values of the molecular weight averages. Finally, the third distribution was a superposition of two equally high, narrow and very close distribution curves, whose parameters were chosen so that at a constant resolution factor (chromatogram 3A) the two individual peaks have just merged into a band with a single maximum; at a varying resolution factor (chromatogram 3B) the resulting chromatogram consisted of one peak with a barely perceptible shoulder.

In addition to the correction efficiency expressed through an agreement between the true and the recovered courses of $w(v)$, and through an agreement between the true and the recovered average molecular weights, all tested methods were also examined as to the effect of inaccuracy of the input, "experimental" data (heights of chromatograms). The effect was simulated in such a way that the calculated heights of normalized, "uncorrected" chromatograms were first multiplied by 10^3 and then rounded-off to one decimal place (precision code 1), to the even first decimal

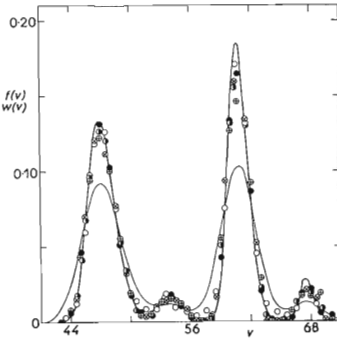

FIG. 1

Results of Correction of Chromatogram 1 A by Various Methods at Precision Code 2

– – – Uncorrected chromatogram; ——— original, known function $w(v)$. Methods: ○ P.A., ⊗ CH.H., ⊕ T., ◑ original V.K., ● method I.L.H.

TABLE I

Characteristics of Testing Chromatograms and Starting Molecular Weight Distributions
α_i — weight fractions

Chromato-gram	Parameters of constituent Schulz–Zimm distributions			
	α_i	$M_n^{(i)}$	$M_w^{(i)}$	$M_z^{(i)}$
1A	0·05	$5 . 10^3$	$5{\cdot}25 . 10^3$	$5{\cdot}5 . 10^3$
	0·45	$4 . 10^4$	$4{\cdot}4 . 10^4$	$4{\cdot}8 . 10^4$
1B	0·05	$3{\cdot}2 . 10^5$	$3{\cdot}36 . 10^5$	$3{\cdot}52 . 10^5$
	0·45	$2{\cdot}56 . 10^6$	$3{\cdot}07 . 10^6$	$3{\cdot}58 . 10^6$
2	1	$1 . 10^5$	$1{\cdot}1 . 10^5$	$1{\cdot}2 . 10^5$
3A	0·5	$4{\cdot}5 . 10^5$	$4{\cdot}95 . 10^5$	$5{\cdot}40 . 10^5$
3B	05	$1{\cdot}35 . 10^6$	$1{\cdot}49 . 10^6$	$1{\cdot}62 . 10^6$

place (precision code 2), to five at the first decimal place (precision code 5), and eventually to an integer (precision code 10). According to our experience precision code 1 corresponds to the limiting attainable precision of the GPC data with negligible noise and a perfect baseline, while precision code 10 represents data with the lowest still acceptable precision (at the maximum height of chromatogram 10 cm the ordinates are read off with a precision of 1 mm).

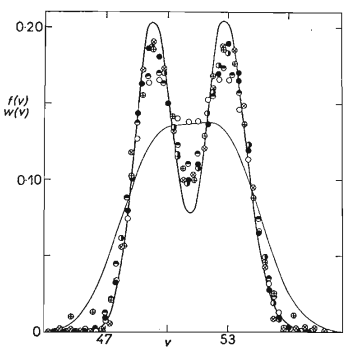

FIG. 2

Results of Correction of Chromatogram 3 A at Precision Code 2

Description as in Fig. 1, ⊖ modified, once-through procedure V.K.

TABLE I
(*continued*)

Parameters of composite MWD's			Spreading factor
$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_z$	
			0·25, constant
$4{\cdot}63 \cdot 10^4$	$1{\cdot}421 \cdot 10^6$	$3{\cdot}493 \cdot 10^6$	
			variable, Eq. (*4*)
$1{\cdot}0 \cdot 10^5$	$1{\cdot}1 \cdot 10^5$	$1{\cdot}2 \cdot 10^5$	variable, Eq. (*4*)
$6{\cdot}75 \cdot 10^5$	$9{\cdot}9 \cdot 10^5$	$1{\cdot}35 \cdot 10^6$	0·25, constant
			variable, Eq. (*4*)

RESULTS AND DISCUSSION

Fig. 1 shows the uncorrected and corrected chromatogram 1A (Table I) and the results of correction obtained by the tested methods for precision code 2. In such simple case with a constant resolution factor the results of all methods agree reasonably well with the known course of w (thick curve), although a closer examination reveals that the best agreement is achieved by employing the I.L.H. method, while the results of the approximative method P.A., and in some regions of the chromatogram also of the Tung method somewhat depart from the correct course. This is also corroborated by the corrected molecular weight averages given in Table II (Δ_n, Δ_w and Δ_z are deviations in per cent of the calculated values from the theoretical ones, *e.g.* $\Delta_n = (M_n/M_n^{true} - 1) \cdot 100$); the last column in Table II gives the sum of the absolute values of these deviations as a criterion of the overall goodness of fit. The results obtained by the correction with the non-iterative modified V.K. method are not presented in Fig. 1, because they could not be discerned from points calculated by employing the original V.K. method used in three steps.

The respective comparison of the individual methods for the chromatogram 3A (precision code 2) is shown in Fig. 2. Here too the fit with the real course of w is satisfactory with the exception of the method P.A., where the correction is clearly insufficient. The Tung polynomial method provides good correction in the main chromatogram region, but at its edges the calculated course shows oscillations which are also reflected in the considerable deviations of the corrected molecular weight averages calculated by employing this method – (Table II). (The perfect agreement between the corrected molecular weight averages calculated by using the P.A. method

with the given values is probably due to a fortuitous compensation of effects in the correction of a bimodal, strictly symmetrical chromatogram.) The once-through modified V.K. method gives satisfactory results of the course of w and of the corrected molecular weight averages, even though they are demonstrably poorer than those obtained by the I.L.H. and CH.H. methods and by the original V.K. procedure.

The correction of chromatograms calculated with a variable resolution factor $h(v)$ according to Eq. (4) represented a more exacting test of all methods; in the case of the methods V.K., P.A. and T., successive substitution of varying h-values into the correction formulae is at variance with the assumptions used in deriving them, and is thus of empirical character. The method CH.H. strictly requires a constant $h = h_0$; consequently, the chromatograms 1B, 2 and 3B were evaluated by substituting the integral average value $\bar{h}$ calculated in advance from the known course of $h(v)$

TABLE II

Corrected Molecular Weight Averages Obtained by the Individual Methods for Chromatograms with Constant Resolution Factor at Precision Code 2

Δ_n, Δ_w, Δ_z — per cent deviations from true values M_n, M_w, and M_z, respectively.

Method	$M_n \cdot 10^4$	Δ_n	$M_w \cdot 10^5$	Δ_w	$M_z \cdot 10^5$	Δ_z	$\sum\lvert\Delta\rvert$
			Chromatogram 1 A				
True	4·630	—	14·21	—	34·93	—	—
Uncor.	4·197	−9·4	15·70	+10·5	46·85	+34·1	54·0
I.L.H.	4·562	−1·5	14·34	+ 0·9	35·75	+ 2·4	4·8
CH.H.	4·447	−4·0	14·28	+ 0·5	36·89	+ 5·6	10·1
V.K.o.[a]	4·492	−3·0	14·24	+ 0·2	35·70	+ 2·2	5·4
V.K.m.[b]	4·417	−4·6	14·25	+ 0·3	35·65	+ 2·1	7·0
P.A.	4·296	−7·2	13·62	− 4·2	35·10	+ 0·5	11·9
T.	4·408	−4·8	14·49	+ 2·0	38·24	+ 9·5	16·3
			Chromatogram 3 A				
True	67·50	—	9·90	—	13·50	—	—
Uncor.	61·48	−8·9	10·89	+10·0	17·82	+32·0	50·9
I.L.H.	67·81	+0·5	10·02	+ 1·2	13·82	+ 2·4	4·1
CH.H.	67·61	+0·2	10·03	+ 1·3	14·02	+ 3·9	5·4
V.K.o.[a]	67·78	+0·4	10·09	+ 1·9	13·85	+ 2·6	4·9
V.K.m.[b]	67·62	+0·2	10·08	+ 1·8	14·00	+ 3·7	5·7
P.A.	67·73	+0·3	9·81	− 0·9	13·45	− 0·4	1·6
T.	66·62	−1·3	10·20	+ 3·0	15·04	+11·4	15·7

[a] Original Vozka, Kubín method operated with numerical volume count increment $\Delta v_c = 1·5$ count and in three steps. [b] Modified, once-through method with $\Delta v_c = 0·5$.

for the range of elution volumes corresponding to the uncorrected chromatogram. Only the I.L.H. correction procedure is able to cope without any further assumptions with a situation when the resolution factor is not constant.

Fig. 3 shows the courses of the uncorrected chromatogram and of the known function $w(v)$ together with the results of the individual tested methods for the chromatogram 1B (precision code 2). As expected, the best fit with the known course of $w(v)$ is offered by the method I.L.H.; also the procedures P.A. and the original V.K. adequately reproduce the course of the initial function within the whole range of the elution volumes. The Tung method gives unsatisfactory results especially at the beginning of the chromatogram, where the resolution factor is low, and consequently the separation efficiency is poor. The method CH.H. employs the average value $\bar{h}$ and accordingly exhibits a low correction efficiency at the beginning and a too high efficiency in the final region of the chromatogram. The modified V.K. procedure gives, considering its simplicity, a satisfactory fit between the calculated and the given courses, but was accompanied by a rather pronounced artificial oscillation in the region of low elution volumes, which particularly affected M_z (Table III).

A corresponding comparison for chromatogram 2 (precision code 2) of a polymer with a narrow distribution curve is shown in Fig. 4. In this case all tested methods give good fit with the reality.

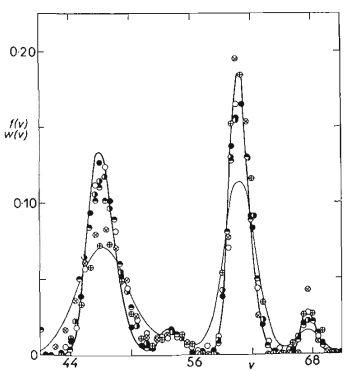

FIG. 3

Results of Correction of Chromatogram 1 B at Precision Code 2

Description as in Figs 1, 2.

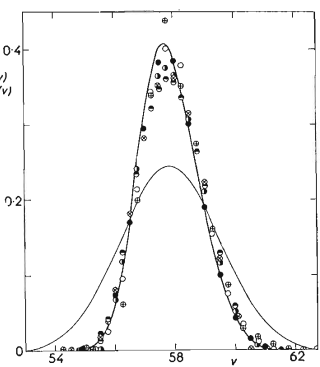

FIG. 4

Results of Correction of Chromatogram 2 at Precision Code 2

Description as in Figs 1 and 2.

Lastly, Fig. 5 shows a comparison of results obtained by the individual procedures for the chromatogram 3B (precision code 2). The decrease in the resolution factor in the region of low elution volumes had as a result that the uncorrected chromatogram (thin curve, Fig. 5) was only asymmetrical and did not suggest the presence

TABLE III

Corrected M Averages Computed by Using the Individual Methods for Chromatograms with Varying Resolution Factor at Precision Code 2

The meaning of symbols Δ and modifications of the V.K. method are explained in Table II.

Method	$M_n \cdot 10^4$	Δ_n	$M_w \cdot 10^5$	Δ_w	$M_z \cdot 10^5$	Δ_z	$\sum\lvert\Delta\rvert$
			Chromatogram 1 B				
True	4·63	—	14·21	—	34·93	—	—
Uncor.	4·35	− 6·1	17·48	+23·0	65·71	+88·1	117·2
I.L.H.	4·62	− 0·2	14·62	+ 2·9	37·00	+ 5·9	9·0
CH.H.	4·23	− 8·6	15·86	+11·6	52·12	+49·2	69·4
V.K.o.	4·60	− 0·7	14·61	+ 2·8	40·97	+17·3	20·8
V.K.m.	4·65	+ 0·4	16·74	+17·8	64·58	+84·9	103·1
P.A.	4·53	− 2·2	13·42	− 5·6	35·08	+ 0·4	8·2
T.	3·47	−25·1	11·58	−18·5	64·05	+83·4	127·0
			Chromatogram 2				
True	10·00	—	1·100	—	1·200	—	—
Uncor.	9·24	− 7·6	1·196	+ 8·7	1·542	+28·5	44·8
I.L.H.	10·05	+ 0·5	1·111	+ 1·0	1·221	+ 1·8	3·3
CH.H.	9·96	− 0·4	1·107	+ 0·6	1·241	+ 3·4	4·4
V.K.o.	9·86	− 1·4	1·095	− 0·5	1·208	+ 0·7	2·6
V.K.m.	9·97	− 2·1	1·090	− 0·9	1·211	+ 0·9	3·9
P.A.	9·71	− 2·9	1·071	− 2·6	1·175	− 2·1	7·6
T.	9·56	− 4·4	1·059	− 3·7	1·161	− 3·3	11·4
			Chromatogram 3 B				
True	67·50	—	9·90	—	13·50	—	—
Uncor.	59·72	−11·5	11·43	+15·5	21·29	+57·7	84·7
I.L.H.	68·05	+ 0·8	10·05	+ 1·5	13·85	+ 2·6	4·9
CH.H.	67·05	− 0·7	10·33	+ 4·3	16·26	+20·4	25·4
V.K.o.	64·65	− 4·2	9·79	− 1·1	14·12	+ 4·6	9·9
V.K.m.	64·00	− 5·2	9·75	− 1·5	14·80	+ 9·6	16·3
P.A.	62·77	− 7·0	8·98	− 9·3	12·64	− 6·4	22·7
T.	58·70	−13·0	9·97	+ 0·7	21·57	+59·8	73·5

TABLE IV

Effect of Accuracy of Input Data on Resulting Corrected Molecular Weight Averages for Chromatogram 1 A

V.K.o. is the original Vozka, Kubín method operated with $\Delta v_c = 1{\cdot}5$ count in three steps.

Precision code	Method				
	I.L.H.	CH.H.	V.K.o.	P.A.	T.
	Number average, $M_n \cdot 10^4$				
1	4·59	4·52	4·53	4·53	4·50
2	4·56	4·45	4·49	4·30	4·41
5	4·61	4·57	4·58	4·55	4·57
10	4·56	4·35	4·35	4·30	4·46
	Weight average, $M_w \cdot 10^5$				
1	14·3	14·3	14·2	13·7	13·5
2	14·3	14·3	14·2	13·6	14·5
5	14·3	14·3	14·3	13·8	14·5
10	14·3	14·2	14·3	13·7	14·5
	z-average, $M_z \cdot 10^5$				
1	35·7	36·8	35·8	34·9	38·1
2	35·8	36·9	35·7	35·1	38·2
5	35·8	36·7	35·8	35·3	37·2
10	35·7	35·3	36·5	34·9	37·1

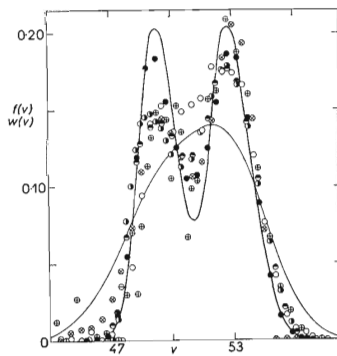

FIG. 5

Results of Correction of Chromatogram 3B at Precision Code 2

Description as in Figs 1 and 2.

of another peak – this case has therefore become the strictest test of the individual methods. The Figure shows that none of the correction procedures could represent the starting curve adequately. The best result was again obtained by using the I.L.H. method, which yielded two peaks of approximately the same height; two distinctly separated peaks, though of different height, were also obtained by the methods CH.H., V.K. and by the modified method V.K., even though the first method did not adequately represent the marginal regions of the chromatogram owing to the enforced constant value of $\bar{h}$. The method P.A. only suggests the existence of a second peak; the Tung method fails completely, as it leads to wild oscillations in the corrected curve.

The corrected molecular weight averages calculated by employing the individual procedures in the case of chromatograms with a variable resolution factor are summarized in Table III and confirm in principle the conclusions formulated on the basis of the courses shown in Figs 3–5. It is worth mentioning that the method P.A. compared with the other methods gives distinctly lower M_w and particularly M_z values.

The effect of precision of the input data was examined using the chromatogram 1A, which was evaluated by all methods for precision codes 1, 2, 5 and 10. With the exception of the P.A. method and of the original V.K. method, all testing procedures involve some kind of smoothing algorithm; it could be expected, therefore, that an impaired accuracy of the input data would not affect the results to any marked degree. This assumption proved to be true – points obtained in correction with various degree of accuracy differed from each other so little that the differences

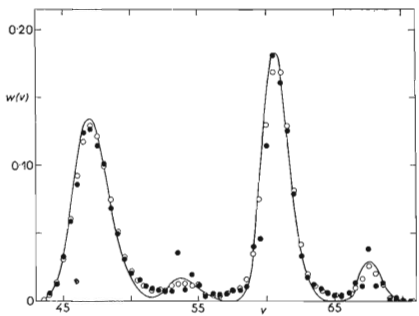

FIG. 6
Correction of Chromatogram 1 A by the Method P.A. at Various Accuracy of Input Data
——— Given course of $w(v)$. Precision code: ○ 1, ● 10.

were barely preceptible in the usual graphic representation. The situation was somewhat worse in the case of the P.A. method for precision code 10; this is documented by Fig. 6, in which the results of correction by the P.A. method are plotted for precision codes 1 and 10. In the latter case artificial oscillation appeared in the region of both lower peaks. Neither here, however, was the reduced accuracy of data reflected too markedly in the corrected molecular weight averages (Table IV).

The demands of the individual testing methods on the operational speed of the computer are documented by Table V, which gives the net computation time in minutes needed for the evaluation of the chromatograms with a Wang 2200 minicomputer. The approximative P.A. procedure is the fastest; both V. K. methods are only somewhat slower, while the two iteration procedures, CH.H. and particularly I.L.H., require ten to hundred times more computer time.

CONCLUSION

In the correction of simple chromatograms (unimodal or nearly baseline-separated peaks, constant resolution factor and good accuracy of the input data), all methods tested here give satisfactory results. The P.A. method is very simple and rapid and yields very good recovery in the correction of narrow chromatograms of unimodal fractions; in the case of more complex chromatograms its small correction efficiency is a drawback, and at a lower accuracy of experimental data it has a tendency towards artificial oscillations. The Tung method cannot be recommended except perhaps in the simplest cases, because it tends to the formation of spurious peaks and is not

TABLE V

Time in Minutes Needed for Calculating Corrected Chromatograms with a Wang 2200 Minicomputer

V.K.o. — original Vozka, Kubín method operated with numerical volume count increment $\Delta v_c = 1{\cdot}5$ count and in three steps, V.K.m. — modified, once-through method with $\Delta v_c = 0{\cdot}5$.

Chromatogram	Method					
	I.L.H.	CH.H.	V.K.o.	V.K.m.	P.A.	T.
1 A	121	28	2	1	0·5	5
1 B	160	165[a]	2	1	0·5	5
2	125	12	1·3	0·8	0·4	4
3 A	165	32	1·5	0·9	0·5	5
3 B	508	122	1·5	0·9	0·5	5

[a] Tolerance 0·01 not satisfied.

able to cope with a variable resolution factor at the real accuracy of experimental data. The CH.H. method gives outstanding recovery even for complicated chromatograms, if the resolution factor is constant or little dependent on the elution volume; in an opposite case, however, it has a tendency to undercorrect at the beginning and to overcorrect in the last section of the chromatogram. In the majority of cases occurring in practice the original V.K. method is satisfactory being very rapid and sufficiently efficient; at lower requirements on accuracy a once-through modification of the V.K. method may prove satisfactory; in this case the whole calculation of the corrected chromatogram can be carried out with a manual calculator. Only in the most demanding cases, when extreme accuracy and high resolution are required, the I.L.H. method must be used, but the price in the necessary computer time to be paid is rather high.

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