

Application of orthogonal functions to spectrophotometric analysis. The combined polynomial method

A. M. WAHBI AND S. EBEL

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt; Fachbereich Pharmazie und Lebensmittelchemie der Philipps-Universität Marburg, Abteilung Analytische Chemie, BRD

A general method is outlined for the linear combination of orthogonal polynomials belonging to one group of points to eliminate interferences in spectrophotometric analysis. The method is particularly useful when it is difficult to find a set of wavelengths over which the coefficient of the irrelevant absorption is negligibly small relative to the assay coefficient. The method is illustrated by the determination of salicylamide in the presence of chloroquine.

The present paper represents part of a general scheme prepared for the correction of irrelevant absorption using Glenn's method (1963, 1967) of orthogonal functions (Wahbi, 1967, 1971; Abdine, Wahbi & Korany, 1971, 1972; Wahbi & Abdine, 1973).

An absorption curve can be represented as follows:

$$f(\lambda) = p_0P_0 + p_1P_1 + p_2P_2 + p_3P_3 + \dots + p_nP_n \quad \dots \quad (1)$$

where $f(\lambda)$ is the absorbance at a wavelength λ that belongs to a set of $(n + 1)$ equally spaced wavelengths. The coefficients, p_j , of the orthogonal polynomials, P_j , are proportional to concentration only if there is no irrelevant absorption. In the presence of irrelevant absorption, each observed coefficient is the sum of two terms; thus,

$$p_j = \alpha_j c_A + p_j(z) \quad \dots \quad (2)$$

where α_j is the coefficient for the $E(1\%, 1\text{ cm})$ of the pure compound, A ; c_A is the concentration and z denotes contribution from irrelevant absorption. When $p_j(z)$ is negligibly small relative to $\alpha_j c_A$, p_j is directly proportional to c_A . This of course demands absorption curves substantially different in shape. Furthermore, in order to minimize the effect of overall shifts in the spectrophotometer's wavelength calibration, the coefficient, p_j , must be sited on a maximum or a minimum in the corresponding convoluted absorption curve (Agwu & Glenn, 1967). In certain cases, however, the irrelevant absorption may simulate in the degree of complexity the absorption curve of the analysed compound, particularly over the analytical segments. In these circumstances, it may be difficult to find a set of absorbances measured at equally spaced wavelengths over which the above-mentioned requirements are fulfilled. The combined polynomial method may offer a solution for such a problem.

In view of the orthogonality of equation (1), any coefficient, p_j , can be obtained from $n + 1$ absorbances measured at equally spaced wavelengths as follows:

$$p_j = \sum_{i=0}^n E_i P_{ji} / N_j \quad \dots \quad (3)$$

where N_j is the corresponding normalizing factor and E_i is the extinction at wavelength number i . Multiplying the values of P_j by an integer, a ,

$$ap_j = \sum_{i=0}^n E_i \left(\frac{aP_{ji}}{N_j} \right) \quad \dots \quad \dots \quad \dots \quad (4)$$

By combining multiples of more than one polynomial, e.g. aP_j and bP_k belonging to the same group of points:

$$ap_j + bp_k = \sum_{i=0}^n E_i \left(\frac{aP_{ji}}{N_j} + \frac{bP_{ki}}{N_k} \right) \quad \dots \quad \dots \quad \dots \quad (5)$$

The above equation can be rearranged as follows:

$$ap_j + bp_k = \sum_{i=0}^n E_i \left(\frac{aP_{ji}N_k/F + bP_{ki}N_j/F}{N_jN_k/F} \right) \dots \quad \dots \quad \dots \quad (6)$$

where a and b are integers and F is a common factor used to bring the numerator to the simplest integers. Equation (6) can of course be generalized for any number of polynomials belonging to the same group of points. Denoting the numerator and the denominator by P_w and D respectively, equation (6) can be written as follows:

$$p_w = \sum_{i=0}^n E_i P_w/D \quad \dots \quad \dots \quad \dots \quad (7)$$

where p_w stands for $ap_j + bp_k$. Accordingly, for a pure compound, A , $p_w = \alpha_w c_A$ where α_w is the combined coefficient for the E (1%, 1 cm) of compound A . In the presence of irrelevant absorption which contributes to p_j and p_k , each observed combined coefficient, p_w , is the sum of two terms; thus,

$$p_w = \alpha_w c_A + p_w(z) \quad \dots \quad \dots \quad \dots \quad (8)$$

where $p_w(z) = ap_j(z) + bp_k(z)$. By arranging for the numerical values and the signs of a and b to be such that $p_w(z)$ becomes negligibly small relative to $\alpha_w c_A$, the concentration can then be obtained from p_w . Moreover, p_w is selected to be optimum in the corresponding convoluted curve.

The construction of the combined polynomial, P_w , requires no more than simple arithmetic. Thus, by way of example, the computation of p_w from the quadratic polynomial, P_2 , and the cubic polynomial, P_3 , so that $p_w = p_2 - 2p_3$ and using eight-point orthogonal polynomials (Fisher & Yates, 1953) can be obtained by carrying out the following substitutions in equation (6):

$$\begin{aligned} a &= +1, b = -2, N_2 = 168 \text{ and } N_3 = 264 \\ P_w/D &= \frac{(+1)P_{2i} \times 264/24 + (-2)P_{3i} \times 168/24}{168 \times 264/24} \\ &= \frac{11P_{2i} - 14P_{3i}}{1848} \end{aligned}$$

Accordingly, P_w can be calculated for each point from $i = 0$ to $i = 7$ as shown in

Table 1. The computation of P_w so that $p_w = p_2 - 2p_3$ using eight-point orthogonal polynomials.

i	P_{2i}	P_{3i}	P_w ($11P_{2i} - 14P_{3i}$)
0	+7	-7	+175
1	+1	+5	- 59
2	-3	+7	-131
3	-5	+3	- 97
4	-5	-3	- 13
5	-3	-7	+ 65
6	+1	-5	+ 81
7	+7	+7	- 21

$N_2 = 168$ $N_3 = 264$ $D = 1848$

Table 1.

The respective p_w can be obtained by forming the product $\sum_{i=0}^n E_i P_{wi}$ and dividing by the corresponding denominator, D .

To check the correctness of the computed P_w , the following properties are strict; thus,

$$(i) \sum_{i=0}^n P_{wi} = 0 \text{ for all values of } n \quad \dots \quad (9)$$

(ii) when P_u is a polynomial belonging to the same group of points but not involved in constructing P_w , then

$$\sum_{i=0}^n P_{wi} P_{ui} = 0 \quad \dots \quad (10)$$

$$(iii) \sum_{i=0}^n P_{wi} P_{1i} / D = a \quad \dots \quad (11)$$

$$(iv) \sum_{i=0}^n P_{wi} P_{ki} / D = b \quad \dots \quad (12)$$

Choice of optimum conditions

To obtain good results using the combined polynomial method, great care must be taken in choosing (a) the polynomials and (b) the number and set of wavelengths, these choices being made with reference to the irrelevant absorption curve.

(a) *Choice of polynomials.* P_0 and P_1 , the constant and linear polynomials respectively are always ignored because irrelevant absorption—whatever its shape—contributes significantly to these polynomials. The real choice usually relates to P_2 and P_3 , the quadratic and cubic polynomials, respectively; there are few situations where higher polynomials need to be used. Furthermore, the polynomial which contributes greatly to the absorption curve of the analysed compound must be used in constructing the combined polynomial, P_w .

(b) *The number and set of wavelengths.* The number and set of wavelengths must be chosen so that (i) $p_w(z)$ is negligibly small relative to $\alpha_w c_A$ and (ii) p_w corresponds with a maximum or a minimum in the corresponding convoluted curve. In this connection, several groups of points, for example from $n = 5$ to $n = 11$, need to be tried. The coefficients p_j for compound A and p_k for the irrelevant absorption are calculated at different wavelength ranges and intervals. From the data obtained, the magnitudes and signs of a and b (eqn 6) can be varied until the above-mentioned requirements are fulfilled.

The magnitude of p_w

To obtain precise estimates of concentration, the magnitude of $p_w (=ap_j + bp_k)$ must be taken into consideration. The latter is controlled by (i) the magnitude of the individual coefficients, p_j and p_k , and (ii) the numerical values of a and b . With regard to the magnitude of the coefficients, Glenn's theory of comparative coefficients (see Wahbi, 1967) is proposed for the present case, from which it follows that both $|q_j|$ and $|q_k|$ (where $q_j = p_j \cdot N_j^{1/2}$) for compound A must exceed 0.14 for the coefficient of variation to be less than 1%. In such a case, there are no restrictions upon the numerical values of a and b . However, when these requirements could not be fulfilled and one of the comparative coefficients ($|q_k|$) was found to be far less than 0.14, it is more advisable to choose a group of points where this particular coefficient, $|q_k|$, is as small as possible. In the latter case, the integer b must be smaller than the integer a in order to minimize the effect of bp_k on the precision of p_w . If all the above-mentioned cases are available, i.e. (i) both $|q_j|$ and $|q_k|$ exceed 0.14 and (ii) $|q_j|$ exceeds 0.14 and $|q_k|$ is very small during the comparison between several groups of points, the analytical group of points for which both absolute comparative coefficients are minimum for the irrelevant absorption is to be preferred.

Determination of salicylamide in the presence of chloroquine

In 0.1N hydrochloric acid, salicylamide exhibits maxima at 235 and 299 nm and a minimum at 261 nm. Over the same wavelength range, chloroquine phosphate exhibits maxima at 235, 257, 330 and 344 nm and minima at 244, 282 and 335 nm. Fig 1 shows that the maxima and the minimum of salicylamide coincide with or are in the vicinity of a maximum, a minimum then a maximum for chloroquine, respectively. In such a case, the determination of salicylamide constitutes an example for the application of the combined polynomial method.

Application of the combined polynomial method

Choice of assay conditions. According to the above discussion, p_2 and p_3 were calculated from the absorption spectra of salicylamide (0.0011% w/v) and chloroquine phosphate (0.00064% w/v) in 0.1N hydrochloric acid using 8 to 12-point orthogonal polynomials at 2 to 6 nm intervals. The signs and values of the integers a and b were varied until the optimum conditions previously mentioned were obtained for each group of points as far as possible (Table 2).

p_w at the finally chosen sets of wavelengths using 9 to 12 points (Tables 2 & 4) were found to correspond with a maximum or a minimum in the corresponding convoluted curves. This requirement was not achieved in the case of 8 points (Fig. 1).

Table 2. Optimum conditions for the determination of salicylamide in the presence of chloroquine phosphate using the combined polynomial method.

Number of* wavelengths	Compd	$p_2 \times 10^3$	$p_3 \times 10^3$	a	b	$\frac{p_w(C)}{p_w(S)} \%$	$ q_2 \times 10^3$	$ q_3 \times 10^3$
8	S	27.390	4.019	1	-2	-0.088	355.0	65.3
	C	-6.601	-3.292					
9	S	11.270	4.752	4	1	-0.058	593.4	149.5
	C	-1.280	5.091					
10	S	-9.129	-0.189	14	-1	0.013	104.9	17.5
	C	-0.102	-1.411					
11	S	-6.581	2.815	4	-1	-0.56	192.8	184.4
	C	0.430	1.556					
12	S	-8.023	-0.257	6	1	-0.095	879.3	18.4
	C	0.135	-0.764					

* For further details see Table 4.

$$p_w = ap_2 + bp_3$$

$$|q_j| = |p_j| \cdot N_j^{1/2}$$

C and S denote chloroquine phosphate and salicylamide, respectively.

Salicylamide concentration = 1.1 mg per 100 ml 0.1N hydrochloric acid.

Chloroquine phosphate concentration = 0.64 mg per 100 ml 0.1N hydrochloric acid.

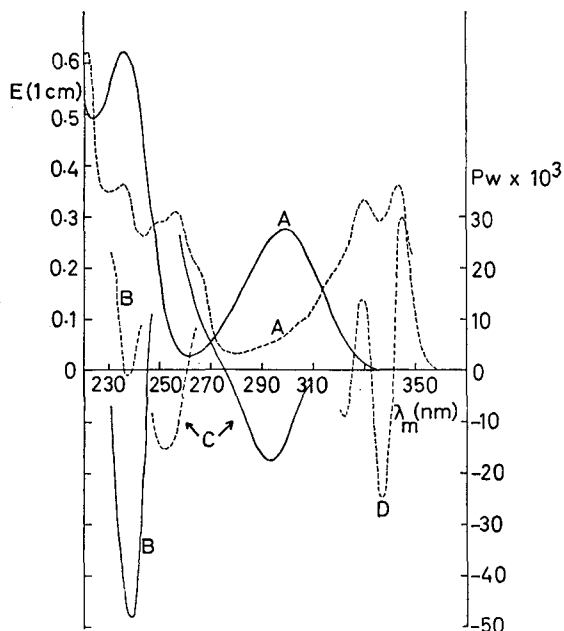


FIG. 1. The absorption curves (A) of 1.1 mg per 100 ml salicylamide (—), 0.64 mg per 100 ml chloroquine phosphate (---) in 0.1N hydrochloric acid and convoluted curves (B, C, D) derived therefrom.

B, $6p_2 + p_3$, 12 points; C, $p_2 - 2p_3$, 8 points; D, $25p_4$, 12 points.

Table 2 shows the comparative coefficients, $|q_j|$ ($j = 2 \text{ \& } 3$) for each case. For reasons previously mentioned, the use of 12 points is preferred to other numbers of points for the determination of salicylamide in the presence of chloroquine. Hence (i) $|q_2|$ greatly exceeds 0.14 whereas $|q_3|$ is very small and (ii) the two comparative coefficients are minimum for chloroquine phosphate, when compared with other groups of points. The ratio $p_w(C)/p_w(S)$ is a measure for the expected percentage error in the determination of salicylamide (S) due to the presence of chloroquine phosphate (C). In this connection, it may be concluded that using 12 point orthogonal polynomials, the calculation of $6p_2 + p_3$ which can directly be obtained by using a combined polynomial, P_w , at $\lambda_m = 239 \text{ nm}$, 2 nm intervals for a mixture of the two compounds is independent of chloroquine concentration and gives precise estimates of salicylamide. Nevertheless, the combined polynomials, P_w , were calculated for the other groups of points for the same purpose (Tables 1 & 3).

Table 3. *The computed combined polynomials P_w and the corresponding divisors for the determination of salicylamide in the presence of chloroquine phosphate.*

Points a, b	9 4, 1	10 14, -1	11 4, -1	12 6, 1
N_2	2772	132	858	12012
N_3	990	8580	4290	5148
F	396	132	858	1716
		P_w^*		
	+182	+5502	+330	+759
	+119	+1806	+114	+471
	+ 11	- 945	- 42	+165
	-107	-2761	-143	-131
	-200	-3652	-194	-389
	-233	-3628	-200	-581
	-171	-2699	-166	-679
	+ 21	- 875	- 97	-655
	+378	+1834	+ 2	-481
	<u> </u>	+5418	+126	-129
	D = 6930	<u> </u>	+270	+429
		D = 8580	<u> </u>	+1221
			D = 4290	<u> </u>
				D = 36036

* P_w fulfil equations (8) to (11).

METHODS

Instrument. A Carl-Zeiss PMQ II photoelectric spectrophotometer.

Five solutions each containing 0.5 mg salicylamide and 0.3 mg chloroquine phosphate in 100 ml 0.1N hydrochloric acid were measured over the wavelength range 220 to 360 nm at 2 nm intervals.

RESULTS AND DISCUSSION

The mean percentage recoveries for salicylamide obtained using the different combined polynomials are shown in Table 4. As expected the results obtained using 12 points were the most precise.

Table 4. Application of the combined polynomials to the determination of salicylamide in the presence of chloroquine phosphate.

Points (n + 1)	p^*_w	λ_i (nm)	λ_f (nm)	λ_m (nm)	Interval (nm)	Mean \pm s.d. (%)
8	$p_2 - 2p_3$	240	282	261	6	98.4 \pm 1.80
9	$4p_2 + p_3$	244	260	252	2	98.7 \pm 1.10
10	$14p_2 - p_3$	230	248	239	2	100.1 \pm 2.32
11	$4p_2 - p_3$	228	248	238	2	98.5 \pm 1.73
12	$6p_2 + p_3$	228	250	239	2	99.0 \pm 0.86

$$* p_w = \sum_{i=0}^n E_i P_{wi} / D \quad (\text{see Table 3}).$$

λ_i , λ_f , and λ_m are the initial, final and mean wavelengths, respectively.

Several trials have been carried out for the application of the single polynomial method (eqn 2) for the determination of salicylamide, but without success. By way of example, the mean percentage recovery was found to be $97.0 \pm 0.91\%$ using p_2 based upon 12 points, $\lambda_m = 239$, 2 nm intervals. When compared with the method of $6p_2 + p_3$ over the same set of wavelengths, a significant difference between the two means based upon the *t*-test ($P = 0.05$) was revealed. The poor results from the method using p_2 alone were due to the contribution of p_2 from chloroquine which was cancelled by the method using $6p_2 + p_3$.

The modified Vierordt's method (Glenn, 1960) has been applied to the determination of salicylamide ($\lambda_1 = 300$ nm, $\lambda_2 = 330$ nm). The mean percentage recovery was found to be 100.1 ± 0.76 . However, the presence of a linear irrelevant absorption, as may originate from differences between batches of the components of the mixture and the "reference" samples used to establish the assay coefficients, will certainly lead to erroneous results (Glenn, 1963). On the other hand, the results obtained using the combined polynomial method will not be affected by any interferences contributing to coefficients other than those involved in p_w .

Although the combined polynomial is only specific for a particular combination of two compounds, a single-step calculation only is required to find the concentration. This is in contrast to two-component spectrophotometric analysis using orthogonal functions which requires the solution of two linear equations (Glenn, 1963).

Errors in the present method are mainly attributed to (i) the general spectrophotometric sources of error, (ii) the non-zero combined coefficient, $p_w(z)$, contributed to the assay coefficient, (iii) improper magnitudes of p_w for substance A and irrelevant absorption and (iv) overall shifts in the wavelength calibration which affect coefficients sited on a slope in the convoluted curves. Neither the accuracy nor the precision of an assay result are affected by calculating the combined coefficient from a combined polynomial, P_w , rather than from the individual polynomials, P_j and P_k separately.

Chloroquine phosphate was determined in the same five mixtures by (i) calculating the quartic coefficient, p_4 , using 12 point orthogonal polynomials at $\lambda_m = 337$ nm, 2 nm intervals (Fig. 1) and (ii) measuring $E(1$ cm) at 344 nm respectively. The mean percentage recoveries were found to be 100.2 ± 2.26 and 99.3 ± 1.55 , respectively. To carry out a fair comparison between these two methods, where twelve measurements are involved in the former and only one measurement for the latter, one must take into consideration that when $E(1$ cm) is measured 12 times in succes-

sion, with the wavelength reset at 344 nm each time, the standard deviation would be reduced to *about* $1.55/(12)^{1/2} \approx 0.447$. In that case, the latter method is more precise when the variance ratio test is carried out ($P = 0.05$).

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