THE USE OF ORTHOGONAL FUNCTIONS TO CORRECT FOR IRRELEVANT ABSORPTION IN TWO COMPONENT SPECTROPHOTOMETRIC ANALYSIS

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General procedures are outlined for the use of orthogonal functions to correct for irrelevant absorption in two component spectrophotometric analysis. In adapting a traditional method to the use of orthogonal functions, the essential modification occurs at the final stage of calculation, when it is necessary to substitute suitable analogues for the entities, "extinction" and "wavelength." Thus, extinction is replaced by *coefficient of an orthogonal function* and wavelength by *orthogonal function over a specified range (or set) of wavelengths.* Once these substitutions have been made, orthogonal functions may be readily incorporated into the usual methods and equations of spectrophotometric analysis.

Although the procedures are specified in terms of Legendre polynomials (as used by Ashton and Tootill, 1956), they are equally applicable to the use of trigonometric functions, which may have a major role to play in future developments. The above general procedures are illustrated by the analysis of a mixture of adrenaline and phenol.

When properly applied, Vierordt's method gives excellent results in the analysis of a two component mixture and as the result of recent work, the conditions for proper application are now much better understood than they were (Świętoslawska, 1956; Glenn, 1960; Pernarowski, 1961). For the majority of mixtures, accuracies of the order of ± 2 per cent are readily obtainable provided, (i) the absorption curves of the two components are sufficiently different, (ii) a wavelength is available at which the component in question contributes a reasonable proportion of the mixture's total absorption, and (iii) the amount of irrelevant absorption is small. In practice, the last requirement places a considerable restriction upon the application of the method, since the term, "irrelevant absorption," must also include variations of the absorbing impurity content of the components, which occur between batches. Thus, if the mixture has been prepared from batches of material that differ from the "reference" samples used to establish the assay coefficients, the overall effect is equivalent to the introduction of irrelevant absorption. The results suffer accordingly.

In cases, where the general shape of the impurity absorption curve is known, even a cursory comparison usually shows a marked difference in shape between the impurity absorption curve and the curve of the component whose concentration is sought. It is evident that if only one could extract some information from an absorption curve which was fundamentally related to its overall shape, then in most instances, there would be little difficulty in coping with irrelevant absorption. The author's

thoughts on this subject began from this point and in the first instance turned in the direction of harmonic analysis, a standard technique which is regularly used by physicists to characterise curve shapes.

The basis of harmonic analysis is that a given function can be expanded in terms of a set of orthogonal functions (of the same variable, λ). In other words, the function can be broken down into a set of fundamental shapes (orthogonal functions). Thus,

$$f(\lambda) = ag_0 + bg_1 + cg_2 + dg_3 + \ldots \quad \ldots \quad \ldots \quad (1)$$

In the present context, $f(\lambda)$ represents an absorption curve, which according to the above equation can be decomposed into the fundamental shapes, g_0, g_1, g_2, etc., which, except for g_0, are themselves functions of λ. The contribution, which a particular fundamental shape (e.g., g_2) makes to the absorption curve, is given by the appropriate coefficient (e.g., c for g_2). The calculation of such coefficients is greatly simplified by the fact that the set of functions, g_i, are mutually orthogonal, when multiplied together in an integration or matrix process over a specified range of λ. Thus, $g_i g_j = 0$ and if for simplicity, the g_i are normalised, we also have the relationship, $g_i g_i = 1$. Hence, to obtain the coefficient of a particular orthogonal function, g_3, it is only necessary to form the "product", $g_3 f(\lambda)$. Having completed this operation, the only non-zero term left on the right hand side of equation (1) is $dg_3 g_3$, which equals d, if the set, g_i, are normalised. As will be mentioned later, these coefficients are proportional to concentration and are equivalent to extinctions (or extinction coefficients where appropriate). In order to minimise errors due to irrelevant absorption, it is of course necessary to choose g_j and also the range of wavelengths so that the corresponding coefficient of the irrelevant absorption is very small relative to that of the component being determined.

In harmonic analysis, the g_i are trigonometric functions but these are slightly less convenient for calculation with a desk machine than are Legendre polynomials, as used by Ashton and Tootill (1956) when dealing with the problem of irrelevant absorption in the assay of griseofulvin in broth. However, although Legendre polynomials are used throughout this paper, there is at present no evidence that in spectrophotometric analysis they are superior to trigonometric functions. This paper is mainly concerned with preliminary thoughts on the practical application of orthogonal functions to the analysis of two component mixtures.

Calculation of Coefficients of Orthogonal Polynomials from Absorption Data

Tables of orthogonal polynomials together with the general method of application are given in standard works on numerical analysis (Milne, 1949; Fisher and Yates, 1953; Davies, 1956). In order to extract from an absorption curve the coefficient of a given polynomial, it is necessary to obtain extinctions at a number of equally spaced wavelengths. For the extraction of the coefficient of a polynomial of a given order, there is a

minimum number of wavelengths which must be used. Thus, to extract the coefficient of the "quadratic" polynomial, P_2, we need a minimum number of three extinctions, namely, E_1 at λ_1, E_2 at λ_2 and E_3 at λ_3, the wavelengths being equally spaced. The coefficient of P_2 is then obtained from the expression, $(+1)E_1 + (-2)E_2 + (+1)E_3$. The numbers in brackets are given in the standard works already mentioned and depend not only upon the order of the polynomial but also upon the number of wavelengths used. For example, the coefficient of P_2 is given by the following expression for 6 equally spaced wavelengths:

$$(+5)E_1 + (-1)E_2 + (-4)E_3 + (-4)E_4 + (-1)E_5 + (+5)E_6$$

whereas for 7 equally spaced wavelengths, it is given by

$$(+5)E_1 + (0)E_2 + (-3)E_3 + (-4)E_4 + (-3)E_5 + (0)E_6 + (+5)E_7$$

For present purposes, there is no need to normalise the coefficients so obtained. The calculation of the coefficients is very easy with a desk calculator and can be greatly simplified by the use of data sheets, so printed that after tabulating the observed extinctions in wavelength order, each extinction lies opposite the number by which it is to be multiplied.

Application of Orthogonal Polynomials to the Analysis of "A" and "B" in a Mixture

Once the coefficients of orthogonal polynomials have been calculated, they can be used in the traditional single and multi-component methods of spectrophotometric analysis just as though they were extinctions (or extinction coefficients, where relevant). The only difference relates to sign, which may be positive or negative in the case of the coefficients of orthogonal polynomials whereas with extinctions, the sign is always positive. *It is of course essential to take account of the sign of the coefficients.*

(1) *The Absorption Curves have Somewhat Different Shapes or are not too Closely Overlapped*

Let P_{1t} be a given orthogonal polynomial (e.g., P_3) which is to extend over the particular set of wavelengths, t. From the E (1 per cent, 1 cm.) of A at these wavelengths, it is possible to calculate α_{1t}, which is the coefficient of P_1 for the E (1 per cent, 1 cm.) of A at the set of wavelengths, t. If instead of E (1 per cent, 1 cm.) of A, we use E (1 cm.) of a solution of pure A at concentration, c_A, the coefficient which we obtain by the above process is referred to as μ_{1t}. The two coefficients are related by the simple expression:

$$\mu_{1t} = \alpha_{1t}c_A \qquad \text{Hence, } c_A = \frac{\mu_{1t}}{\alpha_{1t}}$$

When the solution contains B at concentration c_B, in addition to A, the equation for μ_{1t} contains an additional term, $\beta_{1t}c_B$.

Hence,
$$\mu_{1t} = \alpha_{1t}c_A + \beta_{1t}c_B \quad .. \quad .. \quad (2)$$

However, by choosing P_i and the set of wavelengths, t, with sufficient cunning, it may be possible to make the term, $\beta_{it}c_B$, negligibly small. In such a case, the assay for A can proceed as though component B were part of the irrelevant absorption, which is to be eliminated by the process of calculating μ_{it}. It may also be possible to choose another polynomial, P_j, and/or another set of wavelengths, u, so that c_B can be evaluated whilst component A is ignored.

These procedures are really limiting cases of two basic methods given in section (2). Thus, the methods suggested in this section are the same as those which receive detailed treatment in section (2), except that c_A and c_B do not have to be evaluated by means of simultaneous equations.

Before proceeding further, it is worth stressing the fact that the above equations have just the same form as the traditional equations of spectrophotometric analysis. For example:

$$E\,(1\text{ cm.})_{\lambda_1} = E\,(1\text{ per cent, }1\text{ cm.})_{A\lambda_1}c_A \quad (\text{"traditional"})$$

$$\mu_{it} = \alpha_{it}c_A \quad (\text{"orthogonal"})$$

It is evident from this pair of equations that the "μ" coefficients of the present treatment correspond to E (1 cm.) values, whereas the "α" and "β" coefficients correspond to extinction coefficients. This analogy is a general one so that any of the equations of spectrophotometric analysis can be adapted to the use of orthogonal functions by writing "coefficient of an orthogonal function" in place of "extinction". Furthermore, there is a similar general analogy between "orthogonal function over a specified range (or set) of wavelengths" and "wavelength". Hence, suffixes such as "it" that occur in the present treatment have the same significance as suffixes which denote wavelength in the traditional equations.

To sum up: orthogonal functions can be applied to any of the traditional methods of spectrophotometric analysis provided that, (i) "coefficient of an orthogonal function" be substituted for "extinction" and, (ii) "orthogonal function over a specified range (or set) of wavelengths" be substituted for "wavelength."

(2) *The Absorption Curves have Similar Shapes and Overlap Considerably*

Purely for the sake of simplicity, it is assumed throughout this section that all polynomials are of the "four point" variety. In practical applications, it may be necessary to use "higher point" polynomials (e.g., 12 point). However, the following theory would only alter in respect to the number of wavelengths specified. A given mixture may be tackled by two general methods.

(*a*) *Using Two Sets of Wavelengths, "t" and "u"*

Two polynomials, P_{it} and P_{ju} are required, each of which refers to a particular set of wavelengths. Unlike (2*b*) below, there is no need for "i" and "j" to be different. Thus, both polynomials could be P_2.

To obtain c_A and c_B, we require the experimental data listed in Table I. λ_1 to λ_4 constitute the wavelength set, "t," whilst λ_5 to λ_8 constitute the set "u".

TABLE I

NECESSARY EXPERIMENTAL DATA WHEN USING FOUR POINT POLYNOMIALS IN CONJUNCTION WITH TWO SETS OF WAVELENGTHS, "t" AND "u"

Data	Wavelengths at which data is required								Polynomial coefficients to be obtained
E (1 cm.) of mixture	λ_1	λ_2	λ_3	λ_4	—	—	—	—	μ_{it}
	—	—	—	—	λ_5	λ_6	λ_7	λ_8	μ_{ju}
E (1 per cent, 1 cm.) of A	λ_1	λ_2	λ_3	λ_4	—	—	—	—	α_{it}
	—	—	—	—	λ_5	λ_6	λ_7	λ_8	α_{ju}
E (1 per cent, 1 cm.) of B	λ_1	λ_2	λ_3	λ_4	—	—	—	—	β_{it}
	—	—	—	—	λ_5	λ_6	λ_7	λ_8	β_{ju}

From Table I, it is evident that the mixture's *E* (1 cm.) values at λ_1, λ_2, λ_3, and λ_4 are used to calculate μ_{1t}, the "mixture" coefficient of the polynomial, P_{1t}. In the same way, the mixture's *E* (1 cm.) values at λ_5, λ_6, λ_7, λ_8, are used to calculate μ_{ju}, the "mixture" coefficient of the polynomial, P_{ju}. The other coefficients are obtained similarly as indicated in Table I.

c_A and c_B are then evaluated from the following pair of simultaneous equations:

$$\mu_{1t} = \alpha_{1t}c_A + \beta_{1t}c_B$$

$$\mu_{ju} = \alpha_{ju}c_A + \beta_{ju}c_B$$

(b) Using One Set of Wavelengths, "t," Throughout

Two *different* polynomials, P_1 and P_j, are required (e.g., P_2 and P_3). To obtain c_A and c_B, we require the experimental data given in Table II.

TABLE II

NECESSARY EXPERIMENTAL DATA WHEN USING FOUR POINT POLYNOMIALS IN CONJUNCTION WITH ONE SET OF WAVELENGTHS, "t"

Data	Wavelengths at which data is required				Polynomial coefficients to be obtained
E (1 cm.) of mixture	λ_1	λ_2	λ_3	λ_4	μ_{it} and μ_{jt}
E (1 per cent, 1 cm.) of A	λ_1	λ_2	λ_3	λ_4	α_{it} and α_{jt}
E (1 per cent, 1 cm.) of B	λ_1	λ_2	λ_3	λ_4	β_{it} and β_{jt}

From Table II, it is evident that the mixture's *E* (1 cm.) values at λ_1, λ_2, λ_3, λ_4, are used to calculate, (i) μ_{1t}, the "mixture" coefficient of the polynomial, P_1, (ii) μ_{jt}, the "mixture" coefficient of the polynomial, P_j. The "α" and "β" coefficients have similar meanings, which can be seen from the same Table.

c_A and c_B are then evaluated from the following pair of simultaneous equations:

$$\mu_{1t} = \alpha_{1t}c_A + \beta_{1t}c_B$$

$$\mu_{jt} = \alpha_{jt}c_A + \beta_{jt}c_B$$

Experimental

An experimental trial of each method discussed in sections (1) and (2) was carried out using a mixture of adrenaline (0·0033 per cent w/v) and phenol (0·0060 per cent w/v). The solvent was aqueous 0·1N H_2SO_4 throughout. Extinctions were measured on a Uvispek photoelectric spectrophotometer, great care being taken with the setting of the wavelength scale. The absorption curves of adrenaline, phenol and the mixture of the two are shown in Fig. 1. The results are shown in Table III.

TABLE III

Experimental results

Assay	Wavelength range (mμ)	Intervals (mμ)	Polynomial	Percentage recoveries	
				Adrenaline	Phenol
I	264–297	3	P_2	not determined	99·2
II	241·5–296·5	5	P_3	95·8 (98·7)	not determined
III	252–285	3	P_2	101·8	97·8
	264–297	3	P_2		
IV	270–292	2	P_2 and P_3	101·0	99·4

Twelve point polynomials were used throughout, this being a compromise between (i) the need to extract as much data as possible from the (continuous) absorption curves, and, (ii) the analytical labour involved.

Discussion of Results

Assays I and II (Table III) exemplify the determination of just one component by the selection of a polynomial and set of wavelengths for which the second component makes a negligible contribution to the "mixture" coefficient (see section (1)). The poor result obtained for assay II was due to the fact that over the chosen range of wavelengths, the phenol P_3 coefficient deviates significantly from zero upon even a minute change of the starting wavelength. It is not therefore a good choice, but when allowance was made for the known small (non-zero) value of the phenol P_3 coefficient, the result improved to the recovery quoted in brackets. Assay III exemplified the method described in section (2*a*), whilst assay IV was an example of the method described in section (2*b*).

The above results are encouraging and suggest that the use of orthogonal functions warrants a careful study over a wide field of applications. The main source of error in these results is believed to reside in the setting of the wavelength scale, since above 270 mμ, the phenol absorption curve

has a particularly steep slope. In order to accumulate the extinction data that is needed to calculate the coefficients, μ, α and β, three solutions have to be measured at each of the chosen wavelengths. This can be done by measuring either, one solution at a time over the whole set of wavelengths, or, three solutions in succession at each wavelength. The second alternative eliminates wavelength setting errors entirely, for it is then possible at each wavelength to obtain the necessary extinction data from all three solutions without disturbing the wavelength scale. However, it was inconvenient to adopt such a procedure on the author's instrument.

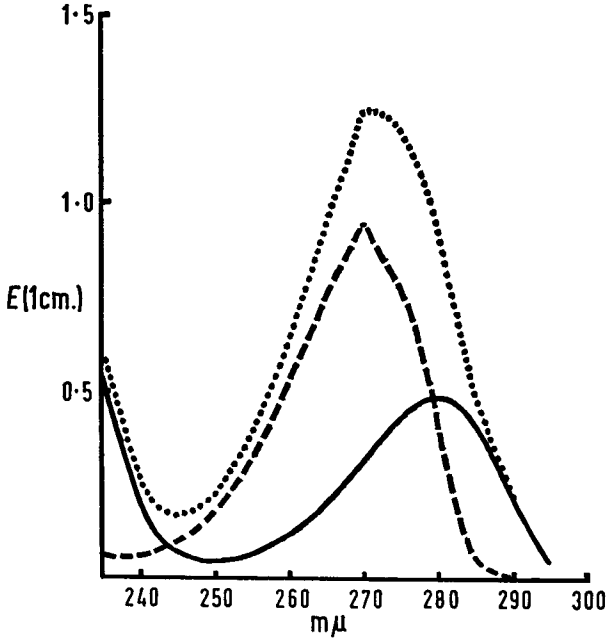

FIG. 1. Ultra-violet absorption curves of adrenaline and phenol in 0·1N aqueous H_2SO_4. —— = Adrenaline (0·0033 per cent w/v). - - - - = Phenol (0·0060 per cent w/v). ..... = Adrenaline (0·0033 per cent w/v) + Phenol (0·0060 per cent w/v).

The same mixture of adrenaline and phenol was also assayed by the modified Vierordt method (Glenn, 1960) using the wavelengths 270 mμ and 283 mμ. The recoveries were (*a*) 100·6 per cent for adrenaline and (*b*) 98·9 per cent for phenol. However, had this mixture contained a linear irrelevant absorption such that the extinction of the mixture was increased by one quarter at 270 mμ and one third at 283 mμ, the recoveries would have been, (*a*) 137·8 per cent for adrenaline and (*b*) 118·2 per cent for phenol. Nevertheless, the results obtained by the use of orthogonal functions would not have been altered in any way by the same linear irrelevant absorption, which if present would produce such catastrophic results in the modified Vierordt method.

An assay based on orthogonal functions rejects all components of the irrelevant absorption curve other than those which are used to calculate the assay coefficients. ("Component" is used in the mathematical sense throughout this paragraph.) Thus, in assays I and II, the P_3 component of the irrelevant absorption is the only one that is not eliminated, which means in particular that the constant, linear and quadratic components of the irrelevant absorption are all rejected. Assay III on the other hand rejects the constant, linear and all higher components of the irrelevant absorption but not the quadratic, P_2, component, for this was used to calculate the assay coefficients. It follows that the general procedure described in section (*2b*), which requires the use of two different polynominals over one set of wavelengths, is inherently less able to correct for irrelevant absorption than are the other methods. For example, in assay IV, the P_2 and P_3 components of the irrelevant absorption are not eliminated. Nevertheless, it is probable that on average, irrelevant absorption contributes much more to the constant and linear components of the total absorption than to any others and these are of course eliminated by any of the procedures outlined above.

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The paper was presented by the AUTHOR.