APPLICATION OF ORTHOGONAL FUNCTIONS TO PHARMACEUTICAL ANALYSIS, GENERATION OF DERIVATIVE CURVES

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يعتمد تطبيق الدوال المتعامدة في بعض طرق التحليل على توسيع المنحنى التجريبي بشكل حدود متعددة متعامدة. وسوف تتم مناقشة المبادئ النظرية للطريقة، حيث أن هذه الطريقة تتخلص من تأثير التداخلات أثناء التحليل. وهكذا، وفي المنطقة فوق البنفسجية المرئية، تم تطبيق الطريقة بنجاح لتقدير المركبات الصيدلانية في مختلف المستحضرات. كما تم استحداث طرق التحليل الذوبانية، وطرق المعايرة الدالة على الثبات، وتقدير ثابت التفكك باستخدام الدوال المتعامدة. ومن التطبيقات الأخرى المستخدمة ،الطرق الطيفية اللصفية، والطرق الطيفية الاستقطابية، وطرق الانبعاث الذري الطيفية، والتحليل الكهروكيميائي. وقد تم استخدام نسب معاملات الدالة المتعامدة لاختبار نقاء المركبات الصيدلانية وتقدير pKa. كما تم استحداث طرق الطرح الطيفي باستخدام طريقة Δpj للتغلب على قيود طريقة ΔA. فقد حلت طريقة P_w ذات الحدود العديدة المدمجة بعض الصعوبات التي تمت مواجهتها أثناء تطبيق طريقة الدالة المتعامدة المباشرة. كما تتم أيضاً مناقشة توسيع المنحنى التجريبي على هيئة دوال فورييه وتطبيقه في التحليل الصيدلاني. وتم عمل منحنيات المشتقات باستخدام الدوال المتعامدة. وهكذا فإن التفاف حزمة جاوس باستخدام الدوال الخطية والتربيعية وغيرها من الدوال المتعامدة عديدة الحدود بواسطة أحد برامج الفيجيوال بيسيك قد نتج عنه منحنيات المشتقات من الدرجة الأولى والثانية. وغيرها لهذه الحزمة، على التوالي.

Application of orthogonal functions to certain instrumental methods of analysis depends upon the expansion of an experimental curve in terms of orthogonal polynomials. The theoretical principle of the method is discussed. The method eliminates the effect of interferences during analysis. Thus, in the ultraviolet-visible region, the method has been successfully applied to the determination of many pharmaceutical compounds in different formulations. Dissolution methods of analysis, stability-indicating assays and dissociation constant determinations have been developed using orthogonal functions. Other applications to include spectrofluorometry , spectropolarimetry, atomic emission spectroscopy and electrochemical analysis have been reported. Ratios of orthogonal function coefficients have been used to test for purity of pharmaceutical compounds and also for pK_a determination. Difference spectrophotometry using the Δp_j method has been developed to overcome the restrictions of the ΔA method. A combined polynomial, P_w, method solved certain difficulties met with during the application of the direct orthogonal function method. The expansion of an experimental curve in terms of Fourier functions and its application to pharmaceutical analysis is also discussed. Derivative curves have been generated using orthogonal functions. Thus, the convolution of a Gaussian band using the linear, quadratic etc. orthogonal polynomials by means of a visual BASIC program, was found to give the first, second,etc.- order derivative curves of the band, respectively.

Key words: Orthogonal functions, derivative curves, fourier function, convolution of curves.

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1.1 Introduction:

Mathematical treatment of irrelevant absorption ranged from the simple (1) to the highly sophisticated (2) formulae, depending upon the shape of irrelevant absorption curve. The correction of linear irrelevant absorption involves measurements at three wavelengths. Wright's "base line" technique (3) is a graphical method which has been transposed later on into algebra (4). Morton and Stubbs' three-wavelength method (5) for the assay of vitamin A is an algebraic version of the base line method (3). One of the equations involved is simply the quadratic orthogonal polynomial for 3 points (+1, -2, +1).

The methods developed for the correction of non-linear irrelevant absorption are mainly depending upon the use of orthogonal polynomials. Thus, a seven-point method has been developed for the correction of a quadratic irrelevant absorption during the assay of griseofulvin in fermentation samples (6,7). The method has been further applied to the determination of cyclandelate in tablets in presence of quadratic irrelevant absorption (8). Wahbi and Abdine (9) generalized the seven-point correction method for other groups of points from n = 5 to n = 14.

1.2 Glenn's method of orthogonal functions:

Glenn outlined a general procedure for the correction of irrelevant absorption in two-component spectrophotometric analysis (10). Both methods (6, 11) depend upon the expansion of an absorption curve in terms of orthogonal polynomials (12), as shown in Fig. 1(a-d).

Fig 1a shows three curves, P_0, P_2 and P_3, when these curves are added together, the sum curve, $f(\lambda)$ in Fig 1b is obtained. Thus:

$$f(\lambda) = P_0 + P_2 + P_3 \qquad (1)$$

When P_3 is multiplied by 3, added to P_0 and P_2 (Fig 1c and 1d), another sum curve, $f(\lambda)$ is obtained.

$$f(\lambda) = P_0 + P_2 + 3P_3 \qquad (2)$$

This equation can be generalized for several multiples of P_j (j = 0, 2 and 3).

$$f(\lambda) = p_0P_0 + p_2P_2 + p_3P_3 \qquad (3)$$

Where p_j are the respective coefficients of P_j. Thus, $f(\lambda)$ can be expanded in the general form of equation 4.

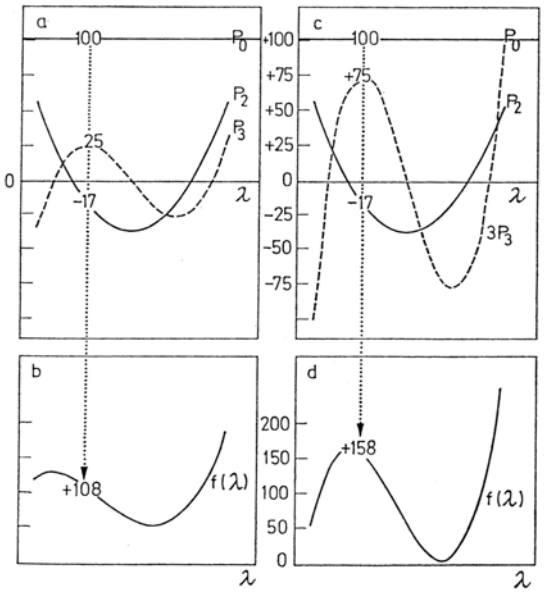

Fig. 1. Summation of curves (a-d).

$$f(\lambda) = p_0P_o + p_1P_1 + p_2P_2 + p_3P_3 + \ldots\ldots + p_nP_n \qquad (4)$$

Where $f(\lambda)$ denotes the absorption of the sample at (n+1) wavelengths, P_j are the orthogonal polynomials given in standard works on numerical analysis (12,13) and p_j, are their respective coefficients. Thus p_0P_0 is known as the constant component; p_1P_1, the linear component; p_2P_2, the quadratic component, etc. of the curve.

The coefficients, p_j are linearly related to concentration (11, 12, 14). Thus:

$$p_j = \alpha_j C_a \qquad (5)$$

Where α_j is the coefficient for the P_j for A (1%, 1 cm) of the pure compound, a, and C_a is the concentration.

In applying this method to the determination of an absorbing compound in presence of irrelevant absorption, each coefficient represents the sum of contributions from background and absorbing compound (14, 15). Thus,

$$P_j = \alpha_j C_a + p_j (Z) \qquad (6)$$

where Z denotes contribution from irrelevant absorption.

Equation (6), therefore, can be used to evaluate C_a from p_j, when there are good grounds for supposing $P_j(Z)$ to be negligible relative to $\alpha_j C_a$. To minimize $P_j(Z)$ to a negligible value, the correction of a quadratic irrelevant absorption during the assay of griseofulvin in fermentation samples (6,7). The method has been further applied great care must be taken in choosing the polynomial and range, the number of wavelengths and the mean wavelength, all these choices being made with reference to the irrelevant absorption curves.

1.2.1 Orthogonality (11):

Fig 2a shows the two standard curves P_2 and P_3 which when multiplied together give $f(\lambda)$ curve in Fig 2b. The area under the latter is equal to zero because the dotted areas cancel with the shaded areas. Thus:

$$\int_{\lambda_1}^{\lambda_2} P_2 \cdot P_3 \, d\lambda = 0 \qquad \textbf{(7)}$$

However, when P_2 (Fig 2c) is multiplied by itself, the area under the resultant $P_2 \cdot P_2$ curve is not equal to zero.

$$\int_{\lambda_1}^{\lambda_2} P_2 \cdot P_2 \, d\lambda \neq 0 \qquad \textbf{(8)}$$

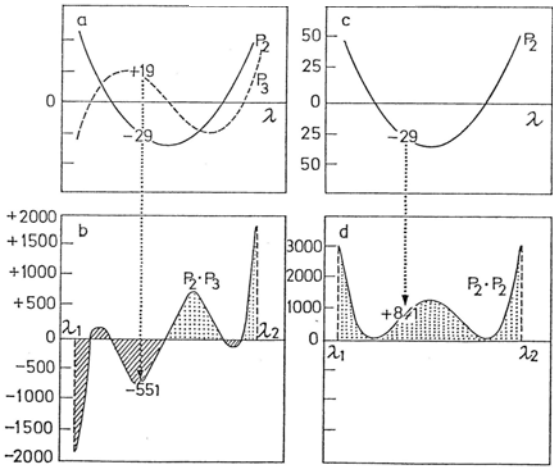

Fig. 2. Multiplication of curves (a-d).

These properties describe the meaning of orthogonality and can be generalized as follows:

$$\int_{\lambda_1}^{\lambda_2} P_j P_k \, d\lambda \begin{cases} = 0 & \text{when } j \neq k \\ \neq 0 & \text{when } j = k \end{cases} \qquad \textbf{(9)}$$

In practice, however, λ is a discrete variable. Therefore, the integration sign of equation (9) is replaced by the summation sign with the limits 0 to n for (n+1) equally-spaced points.

$$\sum_{0}^{n} P_j \cdot P_k \begin{cases} = 0 & \text{when } j \neq k \\ = N & \text{when } j = k \end{cases} \qquad \textbf{(10)}$$

Thus, $N = \sum_{0}^{n} P^2_j$ which is known as the normalizing factor.

1.2.2 Calculation of the coefficients, p_j:

Making use of the orthogonality properties (10, 11), any coefficient p_j, in the expansion equation (eq. 4) can be calculated as follows:

$$p_j = \sum_{i=0}^{n} P_{ji} \, f(\lambda)_i / N_j \qquad \textbf{(11)}$$

$$\left(N_j = \sum_{i=0}^{n} P^2_{ji}\right)$$

Where N_j is known as the normalizing factor. It is present at the foot of each column of the orthogonal polynomials listed in the standard tables (12, 13).

1.2.3 Equations of the orthogonal polynomials:

The standard equations (16) to compute, P_j, the orthogonal polynomials for the first five terms are listed as follows :

$$P_{oi} = 1 \qquad \textbf{(12)}$$

$$P_{1i} = x_i - \bar{x} \qquad \textbf{(13)}$$

$$P_{2i} = P^2_{1i} - \frac{N^2 - 1}{12} \qquad \textbf{(14)}$$

$$P_{3i} = P^3_{1i} - \frac{3N^2 - 7}{20} (P_{1i}) \qquad \textbf{(15)}$$

$$P_{4i} = P^{4}_{1i} - \frac{3N^2 - 13}{14}(P^{2}_{1i}) + \frac{3(N^2-1)(N^2-9)}{560}(P_{1i}) \quad \textbf{(16)}$$

$$P_{5i} = P^{5}_{1i} - \frac{5(N^2 - 7)}{18}(P^{3}_{1i}) + \frac{15\,N^4-230N^2+407}{1008}(P_{1i}) \quad \textbf{(17)}$$

Where N is the number of points, x is the mean of x_i and the values of P_{mi} are often conveniently scaled so as to make them integers (as small as possible) for all x_i. Tables of orthogonal polynomials are available for all numerical values from P_1 to P_5 and all points from 3-to 75-point orthogonal polynomials (13). Since $P_{oi} = 1$ for all values of N and x, the values of P_{oi} is not given in the tables.

1.2.4 Convoluted absorption curves:

The set of wavelengths associated with a given coefficient is completely defined by (i) the number of wavelengths or points, (ii) the interval, and (iii) the mean wavelength, λ_m

$$\text{where } \lambda_m = \frac{\lambda_f - \lambda_i}{2} + \lambda_i \quad \textbf{(18)}$$

Thus, having selected the number of wavelengths and interval (18, 19), it only remains to choose the optimum value of λ_m. For this purpose, p_j for the specified interval and number of wavelengths is plotted against λ_m, a process that leads to a convoluted absorption curve (17, 18, 19) . λ_m of the optimum range corresponds with a maximum or a minimum in the convoluted absorption curve. The principles for choosing λ_m are exactly those for choosing the wavelength of measurement in a normal one substance spectrophotometric analysis i.e. λ_m should correspond with that peak or minimum which is furthest removed from the abscissa scale. Furthermore, the same compromise with regard to wavelength, sensitivity also arises, so that λ_m is better sited on a broad peak or minimum than on a narrow one. Sensitivity of the coefficient to overall shifts in the spectrophotometers wavelength scale or wavelength calibration is thereby minimized. By plotting several convoluted absorption curves using different number of points, different intervals, different polynomials for both pure substance and irrelevant absorption one can maximize the coefficient p_j and find a zero-crossing or a minimum coefficient of $p_j(Z)$ to a negligible value (Eq. 6).

1.3 Computer BASIC programs:

Computer programs in BASIC have been developed to facilitate the convolution of absorption curves. These programs (20-22) have been designed to include equations (12-17) of the constant, linear, quadratic, cubic, quartic and quintic orthogonal polynomial equations. The convolution process (P_j vs λ_m) can be performed for j = 1 to 5 and any number of points up to hundred-point orthogonal polynomials at different intervals. From the convolution data obtained, one can choose the optimum wavelength range, the polynomial and the interval at which the compound to be analyzed gives maximum coefficient from the linear, quadratic, cubic, quartic and quintic components and at the same time it should give a negligibile coefficient (or zero-crossing) for the other compound or for the irrelevant absorption to be corrected for.

1.4 Applications of orthogonal functions method:

1.4.1 UV-Vis Spectrophotometry:

Glenn's method of orthogonal functions has been successfully applied to the assay of vitamin A in cod liver oil without saponification (14) using the quadratic, P_2 polynomial. In the determination of atropine sulphate injections (18), the P_4, the quadratic polynomial makes a large contribution to the absorption spectrum and hence its coefficient, p_4, afforded precise estimate of concentration. Eight-point orthogonal polynomials have been used to correct for irrelevant absorption during the analysis of tablets containing single, weakly absorbing active constituents (19). Thus, at the zero-crossing of the convoluted irrelevant absorption curve, chlorpheniramine maleate and phenyltoloxamine dihydrogen citrate have been determined using P_2, whereas diphenhydramine hydrochloride has been determined using P_3 and ephedrine hydrochloride has been determined using P_4 and P_5. The results obtained suggested that the method can be used for routine analysis of tablets without separating the active constituents from tablet fillers.

Using P_2 six-point orthogonal polynomials, naphazoline has been determined at the zero-crossing of antazoline, present in nasal drops, after precipitation of the latter using sodium carbonate solution. The results obtained were compared with those obtained by the modified Vierordt's method, and were proved to be superior to it (23).

For the determination of dihydrocodeine in presence of noscapine, P_2 14-point orthogonal

polynomials have been calculated (24) directly and after precipitation of noscapine from a solution in 0.1 M hydrochloric acid by means of a saturated sodium acetate solution. Dihydrocodeine content in the filtrate has been determined using the same polynomial, number of points and wavelengths to correct for the unprecipitated fraction of noscapine. The first method was rapid and more versatile than the second method which was time consuming and liable to error due to inevitable losses during the separation procedure.

Atropine sulphate has been assayed, in presence of phenyl mercury (II) acetate, using twelve-point P_4 & P_5 orthogonal function coefficients or using 24-point P_4 or P_5 orthogonal function coefficients under least squares (25). The shape of irrelevant absorption curve was revealed using the compensation technique and was shown to be cubic in nature. For this reason, the quartic, P_4, and quintic, P_5, polynomials have been chosen to determine atropine sulphate in injection solutions. The results obtained using orthogonal function coefficients under least squares were more precise than those obtained using P_4 & P_5 orthogonal function coefficients.

Phenytoin in pharmaceutical preparations (26) and a mixture of phenytoin and phenobarbitone (27), have been determined using the orthogonal functions method. Phenobarbitone has been determined using P_2 six-point orthogonal polynomials at 6-nm intervals, without interference from phenytoin . Phenytoin concentration has been calculated by subtracting the absorbance due to phenobarbitone from the gross curve at 240 nm. The obtained results were more accurate and precise than those obtained by ΔA and ΔA modification of Vierordt's method.

α-Tocopherol acetate has been determined in soft capsules using the orthogonal function method under least squares (28). Applying Glenn's method using P_2 at λ_m 287 nm, the results were still affected by high concentrations of ethyl oleate. Further improvement of the results was achieved by applying the orthogonal functions under least squares, using 8 coefficients over the mean wavelength range 287-294 nm, at 1-nm intervals. The results were compared with those obtained by the D_2-method.

Glenn's method of orthogonal functions has been used to determine norfloxacin in eye ointment and tablet formulations (29). The quadratic polynomial coefficient, P_2, has been calculated using six-point orthogonal polynomials at 2-nm intervals over the wavelength range 278-288 nm. The method has been found to be precise as reflected by the calculated coefficient of variation (less than 1%).

For the determination of piroxicam content in dissolution test (30), the quadratic polynomial coefficient, P_2, has been calculated using 8-point equally spaced at 3-nm intervals in the wavelength range 324-345 nm. A computer program has been developed to compute the P_2, required for the determination of piroxicam in dissolution test. The method has been proved to correct for irrelevant absorption due to gelatin capsules and other pharmaceutical excipients. For the determination of oxytetracycline hydrochloride (31), irrelevant absorption due to pharmaceutical excipients has been corrected for using p_2-eleven points, calculated over the wavelength range 244-284 nm. Phetanol in tablets (32) , papaverine hydrochloride in industrial form (33) and nifuroxime and furazolidone in pharmaceutical formulations (34) have been determined using orthogonal functions. Fluorouracil in polyphase liposome has been determined using orthogonal polynomials (35)

Glenn's method of orthogonal functions has been successfully used for the determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations (36). For the determination of the three drugs, the quadratic polynomial, P_2, has been selected, with 10-point (4-nm intervals), 6-point (6-nm intervals) and 8-point (6-nm intervals) for omeprazole, lansoprazole and pantoprazole, respectively. In each case the quadratic coefficient, P_2, at the selected λ_m (306, 293 and 295 nm, respectively) was found to be highly reproducible and proportional to the drugs concentration and showed negligible contribution for the irrelevant absorption.

The quadratic and cubic orthogonal polynomials, P_2 and P_3 using 8- and 12-point, at 2-nm intervals were used for the spectrophotometric analysis of benzyl alcohol, phenol or parabens in aqueous cyanocobalamin solutions (37). Using twelve points, proved to be more sensitive and direct and to have sufficient accuracy and precision than 8 points. The assay of ethinylestradiol by various techniques using UV spectroscopy has been reported. It was pointed that the use of orthogonal polynomials led to the most reliable results (38).

It has been reported that the determination of sulphadiamidine in presence of sulfaguanidine and

diphenhydramine in presence of ephedrine hydrochloride was performed by calculation of p_2, six points at 12-nm and 3-nm intervals for sulphadiamidine and diphenhydramine, respectively (39).

Calculation of the P_3 six points at 3-nm intervals and P_2 six-point at 4-nm intervals has been utilized for the determination of chlorpromazine and promethazine hydrochlorides, respectively, in compound tablets without prior separation (40).

Determination of certain cephalosporins (41), and total diterpene orthoesters in Yuanhua root injection (42) using the orthogonal function method has been reported.

An investigation of the mathematical bases of the method of Ashton and Tootil (6) for background correction in the spectrophotometric determination of griseofulvin in butanol extracts of fermentation liquors has shown that the results obtained were accurate and reproducible. The applicability of the method to the analysis of mixtures of griseofulvin, nystatin and mycoheptin has been shown (43).

Mixtures of acetaminophen and salicylamide (44) as well as acetaminophen, salicylamide and codeine (45) have been analyzed using orthogonal functions method.

A method based on the convolution of the ratios of absorption spectra has been proposed for resolution of binary mixtures. The ratio spectrum has been convoluted with orthogonal functions, at the optimized selected parameters. The method has been illustrated by resolving binary mixtures of ketoprofen and chlorzoxazone (46) and paracetamol and chlorzoxazone (47) in their capsules .

Mixtures of streptomycin and dihydrostrepto-mycin have been successfully determined using the orthogonal function method (48). Dihydrostrepto-mycin has been determined after acid treatment using P_2, six-point orthogonal polynomials at 8-nm intervals, whereas streptomycin has been determined, after treatment with sodium hydroxide followed by immediate neutralization, using P_2 six-point orthogonal polynomials at 10-nm intervals.

In presence of its degradation products, nystatin has been determined using P_4, eight-point orthogonal polynomials at 4-nm intervals (49). The spectrum of the degradation products has been studied using the compensation method.

The curve fitting process, using orthogonal polynomials, has been applied to the determination of sulfacetamide sodium in presence of its principal degradation product, sulfanilamide (50). Sixteen-point orthogonal polynomials have been used to determine sulfacetamide in eye drops solution. The results were compared with those obtained by the isosbestic point and the BP methods.

A stability indicating spectrophotometric method has been developed for the determination of acetaminophen in presence of its main degradation product, p-aminophenol (51). The quadratic polynomial coefficient, P_2, has been calculated using 6-point at 10-nm intervals over the wavelength range 231-281 nm. The method has been applied to the determination of acetaminophen in both fresh and expired samples of tablets and syrups. The results obtained were compared with those of the USP method, confirming that the developed method enjoys the stability indicating nature of the USP method and can be used safely in routine analysis of these preparations.

Spectrophotometric analysis of Vitamin A in presence of its degradation product using orthogonal functions has been described (52). The coefficient, p_2, has been calculated for Vitamin A and its degradation product from their absorption spectra in isopropanol, using 6-point orthogonal polynomials at 8-nm intervals.

In presence of their degradation products, nitrazepam, prazepam and dipotassium chlorazepate, have been determined using the orthogonal function method (53). Interferences by degradation products to the absorption spectra of the intact drugs have been corrected using P_2 six-points at 8-nm intervals for nitrazepam and P_4 twelve point at 6-nm and 8-nm intervals for prazepam and dipotassium clorazepate, respectively. Plots of log C% against time gave straight lines, indicating that the method is specific for the intact molecule, independent of degradation products.

A computer-assisted orthogonal function method has been applied to the determination of some phenothiazines namely, dimethothiazine mesylate, isothipendyl hydrochloride and trimeprazine tartarate, in their commerical tablets and in laboratory made mixtures with their respective sulphoxides (54).

Spectrophotometric determination of dissociation constants of some weakly acidic and basic drugs was carried out using orthogonal functions (55). By calculation of the comparative coefficient, Q_j, the pKa values of some acidic and basic drugs were obtained using the following equations :

$$pKa = pH + \log \frac{(Q_{jd} - Q_{jb})}{(Q_{jb} - Q_{ju})} \quad \textbf{(19)}$$

$$pKa = pH + \log \frac{(Q_{jb} - Q_{ju})}{(Q_{jd} - Q_{jb})} \quad \textbf{(20)}$$

Where Q_{jb}, Q_{jd} and Q_{ju} are the comparative coefficients of the buffered, dissociated and undissociated drug solutions, respectively. A graphical technique based upon plotting orthogonal function titration curves was also presented. The results obtained were in good agreement with the reported values.

The orthogonal function method has been further extended to correct for interferences in atomic emission, spectropolarimetric, fluorimetric, voltammetric and potentiometric analyses. Equation (5) applies in all cases.

1.4.2 Atomic emission spectrophotometry:

Orthogonal polynomials have been used for the elimination of spectral interferences in inductively coupled plasma atomic emission spectroscopy (56). The correction procedure described is based on the observation that emission spectra of the analyte element are usually symmetrical, with normal distribution, whereas over the same wavelength range the spectra of interfering elements are either linear or asymmetrical. Equations are presented that permit the calculation of quadratic polynomial functions to make the corrections. Emission intensities for Cr, Cu, Mn, Pd, Zn, Sn and Pb at 1-10 mg L^{-1} in 0.5% HNO_3 were measured over the appropriate wavelength ranges. Similar measurements were then made for potential interfering elements (Al, Ca, Mg and Fe). The polynomial corrections gave much improved results compared with the standard 3-point correction method. The recovery of 1-5 mg L^{-1} of analyte in presence of 250 mg L^{-1} overall of the interfering elements was also determined. Recoveries were good except for Pd (3 mg L^{-1}); the low results with small amount of Pd were attributed to its fairly low emission intensity.

1.4.3 Spectropolarimetry:

For the spectropolarimetric determination of some penicillins with 20-point orthogonal polynomials over the wavelength range 240-259 nm at 1-nm interval, the calculation of P_2 automatically rejected all other components from p_0P_0 to p_{19} inclusive present in the ORD curves of penicillin and interference as well as the error curve. The p_2 coefficient was found to be negligibly small for penicilloic acid (57).

1.4.4 Spectrofluorometry:

Orthogonal functions have been utilized to correct for the presence of interferences in spectrofluorimetric analysis. The method has been demonstrated by the determination of tetracycline and its degradation product, anhydrotetracycline, in combination (58).

Spectrofluorimetric determination of ciprofloxacin in tablets and injection has been carried out by calculating the quadratic polynomial coefficient, P_2, for 10 points of 5-nm intervals (59). Ethinyloestradiol (60) in contraceptive formulations and other drugs have been determined using orthogonal polynomials (61).

The spectrofluorimetric determination of orciprenaline in presence of oxazepam and guaifenesin in presence of sulfadiazine has been performed, using p_2 at 6-nm intervals over the wavelength range 292-332 nm for orciprenaline sulphate and 300-330 nm for guaifenesin (62).

1.4.5 Electrochemistry:

Orthogonal functions have been applied to develop a simple chemometric method to locate the half-wave potential (E ½) of DC polarograms of some metals as single or in mixtures. Upon convolution of the DC polarographic waves, using the linear orthogonal polynomial, P_1; the resultant curves were found to show maxima at the half-wave potential (E ½) of each metal. The method has been compared with the graphical DC method and the DPP method. The results obtained showed negligible differences between the three methods (63).

The resolution of partially overlapping differential pulse voltammetric (DPV) peaks and differential pulse cathodic stripping voltammetric (DPCSV) peaks, using the orthogonal function method, has been demonstrated. A binary system of Tin (II) and Pb (II), having 72 mV of DPV peaks separation in 0.04 M acetic/o-phosphoric/boric acids mixture, has been used as a model throughout the work. The method has been successfully applied to the simultaneous determination of both metals in canned soft drinks. Moreover, the applicability of the method has been demonstrated by the recovery

of lead in drinking water sample (64). Mixtures of metals with overlapping DPP have been resolved using convoluted curves (65).

Ebel and Krömmelbein (66) introduced the use of orthogonal polynomials as a curve fitting process to evaluate potentiometric titrations by Gran-plots (67). This process has been further extended (68) to potentiometric titrations of weak acids using digital computers.

Information obtained from the shape of a titration curve have been extracted in terms of orthogonal polynomial coefficients (69). In this way, it was possible to (i) distinguish a monobasic acid from a polybasic acid, having groups of closely similar strength, (ii) detect acid-base impurities in acids and bases; and (iii) study medium effects.

1.4.6 Ratios of orthogonal function coefficients:

Agwu and Glenn (17) developed the ratio p_j/p_0 for testing the purity of several pharmaceutical compounds, where j = 2,3,4,.... n, and p_0 is the coefficient of the constant component. These ratios are independent of concentration and are sensitive to the presence of interferents that contribute to p_0 . A titrimetric method has been reported, using ratios of orthogonal function coefficients, for the determination of pKa values (70). A computer program has been successfully used for calculation of these ratios. A graphical technique depending on the use of these ratios has been also presented. The method has been applied to the determination of pKa values of cholesterol, phenol, propylparaben, trimethoprim, paracetamol and phenobarbital. The results obtained were in good agreement with the reported pKa values.

1.4.7 The Δp_j method:

The peaks of compounds that undergo non-characteristic shift on changing the pH may split into subsidiary peaks without any appreciable change in intensity by changing the pH in a suitable interval. The behavior of these compounds restricts the application of the ΔA method. In these circumstances the Δp_j method (71) offered a solution for the analysis of such compounds in presence of interferences. According to the orthogonal function method (10,11), if the shape of the absorption curve changes significantly by changing the pH of the solvent from "a" to "b", the presence of a pH insensitive irrelevant absorption may be cancelled by means of

$$\Delta p_{ji} = [\alpha_{jia} C_x + p_{ji}(Z)] - [\alpha_{jib} C_x + p_{ji}(Z)] \quad \textbf{(21)}$$

$$\text{and} \quad C_x = \Delta p_{ji} / \Delta\alpha_{ji} \quad \textbf{(22)}$$

It may be possible to choose p_j and also the set of wavelengths "i" so that p_{ji} is optimum in one solvent and negligibly small in the other solvent. Accordingly, Δp_{ji} reaches the magnitude required to obtain precise and reproducible results.

Magnitude of Δp_j:

A precise estimate of concentration using the Δp_j method, is obtained using Glenn's theory of comparative coefficients (14,18,19), that is, $|\Delta p_j| . N_j^{1/2} = |\Delta q_j|$ should exceed 140 x 10^{-3} if the coefficient of variation (Δp_j) is to be less than 1. Furthermore, Δp_j should correspond to a peak or a minimum in the polynomials convoluted absorption curve.

The use of log $|\Delta p_j|$ plots against:

By analogy with the use of graphs of log $|\Delta A|$ against wavelength, graphs of log $|\Delta p_j|$ ($\Delta p_j \neq 0$) against λ_m, the mean of the set of wavelengths, for sample and pure compound may be compared to detect changes in the irrelevant absorption. The two log $|\Delta p_j|$ graphs will only superimpose when the irrelevant absorption is unaffected by the change in pH.

The Δp_j method has been used for the determination of clemizole in tablets, using 8-point orthogonal polynomials (71).

Also mepyramine maleate, aminophenazone, nialamide and chloroquine phosphate have been assayed in pharmaceutical preparations, using the Δp_j method (72). The results obtained were compared with those obtained by the p_j and ΔA methods. The relatively unsatisfactory results obtained using the p_j method are due to the contribution of the interference's coefficient to the gross coefficient, p_j. In all cases, the ΔA method gave erroneous results. The method has been used for the determination of sulphamethoxazole and trimethoprim in anti-inflammation powder for infants (73), phenytoin in pharmaceutical preparations (27) and oxazepam and dipyridamole mixtures (74).

1.4.8 The Combined polynomial method:

A modification of Glenn's method of orthogonal functions has been performed through the linear

combination of orthogonal polynomials belonging to one group of points to eliminate interferences in spectrophotometric analysis (75). 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. In view of orthogonality, any coefficient, p_j, can be obtained from n+1 absorbances measured at equally spaced wavelengths.

$$p_j = \sum_{i=0}^{n} A_i P_{ji} / N_j \qquad \textbf{(23)}$$

Multiplying the values of P_j by an integer, a,

$$a\,p_j = \sum_{i=0}^{n} A_i \frac{aP_{ji}}{N_i} \qquad \textbf{(24)}$$

By combining multiples of more than one polynomial. e.g., a P_j and b P_k belonging to the same group of points :

$$a\,p_j + b\,p_k = \sum_{i=0}^{n} A_i \frac{aP_{ji}}{N_i} + \frac{b\,P_{ji}}{N_k} \qquad \textbf{(25)}$$

The above equation can be rearranged as follows :

$$a\,p_j + b\,p_k = \sum_{i=0}^{n} A_i \frac{a\,P_{ji}\,N_k/F + b\,P_{ki}\,N_j/F}{N_j\,N_k/F} \qquad \textbf{(26)}$$

where a & b are integers and F is a common factor used to bring the numerator to the simplest integers. Equation (25) can be generalized for any number of polynomials belonging to the same group of points. Denoting the numerator and the denominator by P_{wi} and D respectively, equation (25) can be written as follows:

$$p_w = \sum_{i=0}^{n} A_i P_{wi} / D \qquad \textbf{(27)}$$

where p_w stands for a p_j + b p_k. Accordingly, for a pure compound, A, $p_w = \alpha_w C_a$ where α_w is the combined coefficient for the E (A 1%, 1 cm) of a compound A.

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

i) $\sum_{i=0}^{n} P_{wi} = 0$ for all values of n **(28)**

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

$$\sum_{i=0}^{n} P_{wi}\,P_{ui} = 0 \qquad \textbf{(29)}$$

iii) $$\sum_{i=0}^{n} P_{wi}\,P_{ji}/D = a \qquad \textbf{(30)}$$

iv) $$\sum_{i=0}^{n} P_{wi}\,P_{ki}/D = b \qquad \textbf{(31)}$$

The method has been demonstrated by the determination of salicylamide and chloroquine phosphate mixtures (75). Salicylamide has been determined using 6 $p_2 + p_3$ using 12 points whereas chloroquine phosphate has been determined in the same mixtures by calculating the quartic coefficient, p_4, using 12-point orthogonal polynomials at λ_m = 337 nm at 2-nm intervals.

The combined polynomial method has been utilized as a stability indicating method to eliminate interferences by degradation products to the absorption spectra of several drugs. A combined polynomial method has been reported for the determination of thiamine hydrochloride in presence of its degradation products (76). The coefficient $p_w = (2\,p_2 - p_3)$ of the combined polynomial P_w, calculated over the wavelength range 226-292 nm at 6-nm intervals has been found to be specific for the intact molecule, independent of degradation products. Moreover, a graph of log C% against time for a solution in borate buffer pH 10, gave a straight line with a slope of -0.0226 h^{-1}, showing that the method could be used as a stability indicating method. Similarly, procaine hydrochloride has been determined in presence of 4-aminobenzoic acid (77) without prior separation of the degradation product. The $p_w = (3p_2 + 4p_3)$ calculated over the wavelength range 272-316 nm at 4-nm intervals, using 12-point orthogonal polynomials was linearly related to concentration and independent of degradation products.

For the determination of methylphenobarbitone in presence of its degradation products, the coefficient $p_w = (4p_2 - 3p_3)$ has been calculated over the wavelength range 228-272 nm at 4-nm intervals.

The method has been also applied for assessing the stability of methylphenobarbitone (78).

The determination of benzylpenicillin and ampicillin in presence of their degradation products was performed by calculating the $p_w = (p_3 - p_4)$ over the wavelength range 247-269 nm at 2-nm intervals and $p_w = (p_2 + 2p_3)$ over the wavelength range 249-271 nm at 2-nm intervals in buffer pH 7 for benzylpenicillin and ampicillin, respectively (79). Some cephalosporins have been determined in presence of alkali induced degradation products using p_w calculated at 254-276nm for cephalexin, 248-292nm for cephalothin sodium and 232 – 276 nm for cephaloridine at 2n 4 and 4nm intervals, respectively(80).

Mixtures of chlordiazepoxide and diazepam with their degradation products, have been assayed using the combined polynomial method (81). The coefficient $p_w = (p_2 - p_3)$ calculated over the wavelength range 230-318 nm at 8-nm intervals and $p_w = (p_3 + 6p_4)$ at 272-360 nm at 8-nm intervals for chlordiazepoxide and diazepam, respectively, is reproducible and independent of the degradation products. Chloramphenicol in presence of its alkaline induced degradation products has been determined using the combined polynomial method (82).

A computer assisted combined polynomial method has been reported for the assay of acetaminophen and phenacetin in presence of their degradation products (83). The $p_w = (p_2 + 10\ p_3)$ calculated using 12-point, $(P_2 + 10\ P_3)$ orthogonal polynomials at 2-nm intervals was linearly related to concentration and independent of the degradation products. Graphs of log C% versus time were found linear for both drugs.

1.4.9 Orthogonal functions for unequal intervals:

In the application of the orthogonal polynomials for unequal intervals to spectrophotometric analysis (84), one can be faced with the problem that the polynomial integers (polynomial fundamental shapes) (10,17) especially higher order polynomials, P_3, P_4 ... etc. may not exactly fit with the fine structure of the absorption curve. This means that the optimum value of higher order coefficients (p_3, p_4, ... etc.) obtained during the convolution process does not reflect exactly the fine structure of the absorption curves (e.g. for benzenoid compounds). In this connection the unequal interval can be selected according to the shape of a specified segment on the absorption curve. The method has been illustrated by the determination of ephedrine hydrochloride and diphenhydramine hydrochloride in binary mixture. The method ended with solving a set of two linear equations similar to the conventional equal-intervals method. Determination of atropine sulphate in injections and nystatin in capsules in the presence of other interfering compounds using orthogonal polynomials for unequal intervals has been reported (85). The above mentioned two component mixture should have been assayed using the orthogonal functions equally spaced for comparison in order to appreciate the unequal intervals method and to demonstrate the reasoning of the unequal intervals. Furthermore, the convolution process led of course to loss of the assumption about fitting the fine structure of the absorption curve with the unequal interval polynomial.

1.5 Fourier functions method:

A novel method analogous to orthogonal functions method has been first developed by Wahbi *et al* (86) depending on the use of Fourier functions. Accordingly, an absorption curve, $f(\lambda)$ can be expanded in terms of the Fourier series :

$$f(\lambda) = a_0 + a_1 \cos x + a_2 \cos 2x + a_3 \cos 3x + \ldots\ldots + b_1 \sin x + b_2 \sin 2x + b_3 \sin 3x + \ldots\ldots \quad \textbf{(32)}$$

The calculation of the coefficients a_j and b_j (j = 0, 1, 2, ...) are greatly simplified by the fact that the set of the trigonometric functions, T_{jx} (where T stands for cos or sin whenever relevant) are mutually orthogonal, when multiplied together in an integration process from x = 0 to 2π (12). Thus,

$$t_j = \int_0^{2\pi} f(\lambda).\ T_{jx} d_x \Big/ \int_0^{2\pi} T_j^2 x\ d_x \quad \textbf{(33)}$$

Where t_j stands for the required coefficient. This is valid when $f(\lambda)$ is a continuous function. However, using a spectrophotometer, $f(\lambda)$ belongs to a discrete set of equally spaced wavelengths. In that case the infinite series (Eq. 31) becomes finite for (n+1) points. Thus, when (n+1) is an even number,

$$f(\lambda) = a_0 + a_1 \cos x + a_2 \cos 2x + \ldots a_{½}\ (n+1)\ \cos_{½}\ (n+1)\ x + b_1 \sin x + b_2 \sin 2x + \ldots + b_{½}\ (n-1)\ \sin_{½}\ (n-1)\ x \quad \textbf{(34)}$$

and when (n+1) is an odd number,

$$f(\lambda) = a_0 + a_1 \cos x + a_2 \cos 2x + ... + a_{½n} \cos_{(½n)} x + b_1 \sin x + b_2 \sin 2x + ... + b_{½n} \sin_{(½n)} x \quad \textbf{(35)}$$

Any coefficient, t_j, can be calculated from a set of absorbances measured at equally spaced wavelengths by expressing equation (32) in the form of summation from x = 0 to 2π-$2\pi/(n+1)$ at intervals of $2\pi/(n+1)$:

$$t_j = \sum_{i=0}^{n} f(\lambda)_i \, . \, T_j x_i \, / \sum_{i=0}^{n} T_j^2 \; x_i \quad \textbf{(36)}$$

The denominator of the above equation is the sum of the squared individual values T_j x_i. For example, the coefficient of cos x can be calculated by the following expression for 6 equally spaced wavelengths:

$$[(+1)\, A_0 + (+0.5)\, A_1 + (-0.5)\, A_2 + (-1)\, A_3 + (-0.5)\, A_4 + (+0.5)\, A_5]/3 \quad \textbf{(37)}$$

The numbers in brackets are values of cos x for x = 0 to 300° at 60° intervals.

The Fourier function coefficients, t_j are proportional to $f(\lambda)$, that is,

$$t_j = \alpha_j C_a \quad \textbf{(38)}$$

where α_j is a constant analogous to absorptivity and C_a is the concentration of the absorbing compound, A.

Similarities between equation (4) and (31) are given in table 1.

Table 1. Analogies between orthogonal functions and Fourier functions.

Component	Orthogonal function	Fourier function
Constant	p_0P_0	$a_0 \cos 0x$
Linear	p_1P_1	-
Quadratic	p_2P_2	$a_1 \cos x$
Cubic	p_3P_3	$b_1 \sin x$
Quartic	p_4P_4	$a_2 \cos 2x$
Quintic	p_5P_5	$b_2 \sin 2x$

The Fourier functions method has been applied to the determination of progesterone and testosterone propionate in oily solutions (86). Computer-assisted analysis of mixtures of acetaminophen, salicylamide with caffeine or codeine and some phenothiazines in presence of their sulphoxides have been reported. Discrete Fourier transform coefficients (combined trigonometric functions) have been utilized for analysis of a mixture of codeine phosphate, phenylephrine hydrochloride, chlorpheniramine maleate and ephedrine hydrochloride (87). The method in principle, is the multicomponent spectrophotometric method of analysis using absorbances and orthogonal function coefficients under least squares developed by Wahbi *et al* (11).

Wahbi *et al* developed ratios of Fourier to orthogonal function coefficients (88). Accordingly, the ratio a_1/p_2 should be constant for a particular compound over a set of equally spaced wavelengths, independent of concentration. Similarly, b_1/p_3, a_2/p_4 and b_2/p_5 which are independent of concentration, can be selected to be highly correlated to the general shape of the absorption curves of the two compounds investigated and to be more specific than p_j/p_0.

Ratios of Fourier to orthogonal function coefficients have been used to eliminate the personal judgment about the balance point during two-component compensation spectrophotometry (89,90). The Fourier functions method has been extended to ratio spectra of absorption curves for the determination of paracetamol and caffeine mixtures (91).

The $\Delta t_j'$ method (92) represents the application of the Fourier series method of combined trigonometric function to difference spectrophotometry. It is analogous to the Δp_j method (71) previously discussed. The $\Delta t_j'$ method, in principle, is exactly the Δp_j method.

2.0 Generation of Derivative Curves Using Orthogonal Functions:

Glenn's method of orthogonal functions is an original work for generating derivative spectra. Thus, the linear,p_1 , quadratic, p_2, cubic, p_3, and p_4 etc convoluted curves are the first D_1, second D_2, third D_3, and fourth D_4 etc order derivative curves, respectively of any experimental curve. Figs 3 and 4 shows a Gaussian band with its linear and quadratic convoluted curves. The derived curves

(93) are exactly similar in position of optima, zero crossings to the correspondingly derived derivative curves. The absorption curve of 0.02% v/v of toluene in methanol has been convoluted using P_2 for 3-points orthogonal polynomials. The resultant curve (Fig. 5) complied with the published (94) second derivative curve of the same solution in the B.P.(2003). The ratio of A/B was found to be 0.39. The Savitzky-Golay calculation (95) of derivative curves from absorption spectra depended upon using orthogonal polynomials to obtain the different derivative order after a degree of curve smoothness. The suitable program of this method is now part of the software on line with spectrophotometers. A visual BASIC program has been developed in order to facilitate the generation of derivative curves (from linear to quintic order) of any experimental curve (Fig. 6) using orthogonal polynomials equations . The program is available on CD ROM.

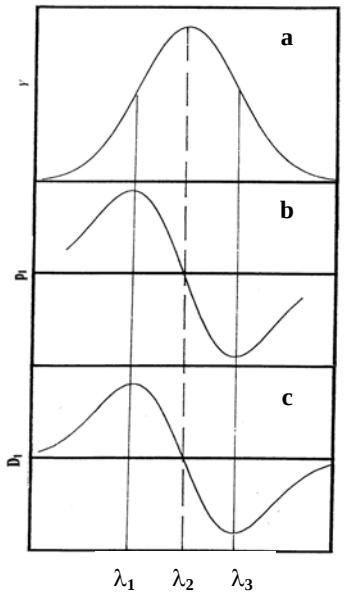

Fig. 3 Gaussian band **(a)**, its linear p_1 convoluted curve using orthogonal polynomials **(b)**, its first derivative curve, D_1 **(c)**.

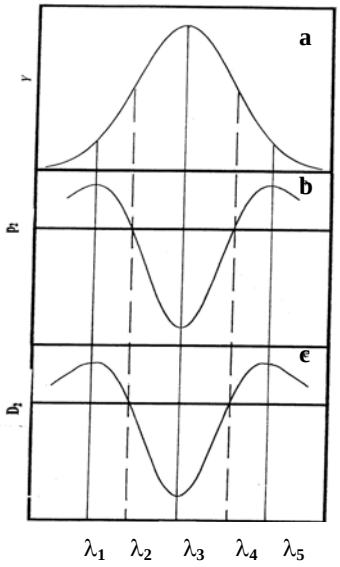

Fig. 4 Gaussian band (a), its quadratic P_2 convoluted curve using orthogonal polynomials (b), its second order derivative curve, D2 (c).

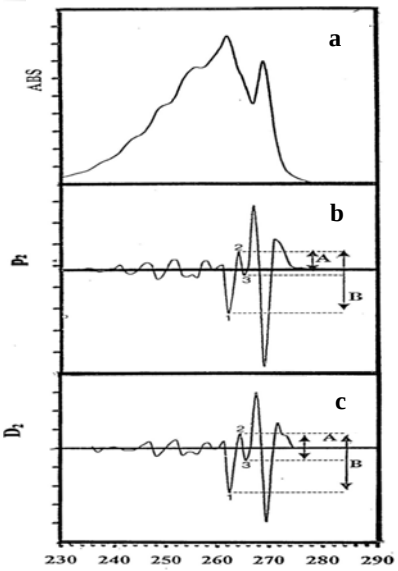

Fig. 5 Absorption curve of 0.02 v/v toluene in methanol (a), its P_2 convoluted curve using orthogonal polynomials (b), its second-order derivative curve obtained from a spectrophotometer (c). A/B is greater than 0.2, 1= 262nm, 2= 264 nm, 3= 265 nm.

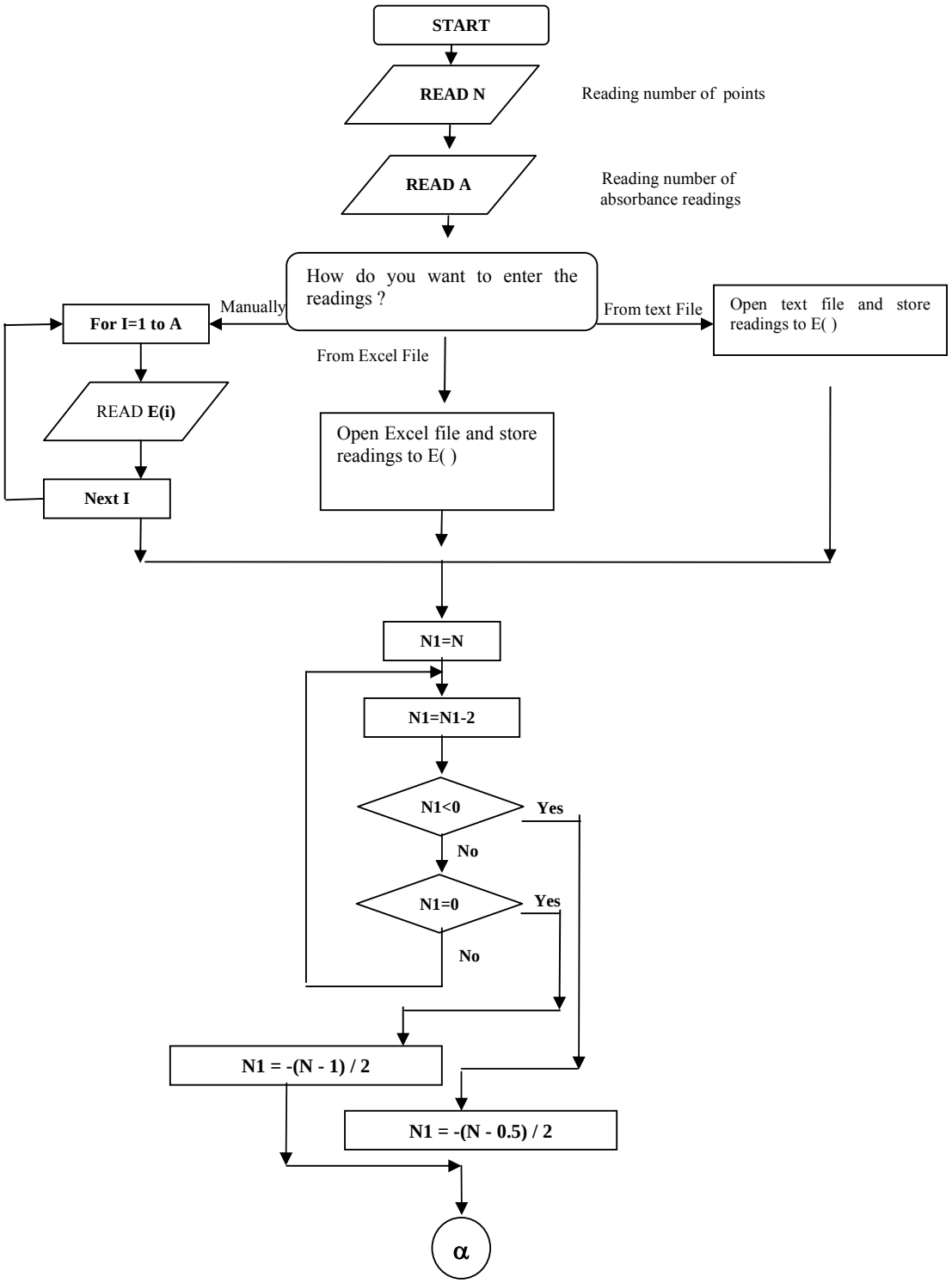

Fig. 6 Flow chart for the visual BASIC program used to obtain derivative curves.

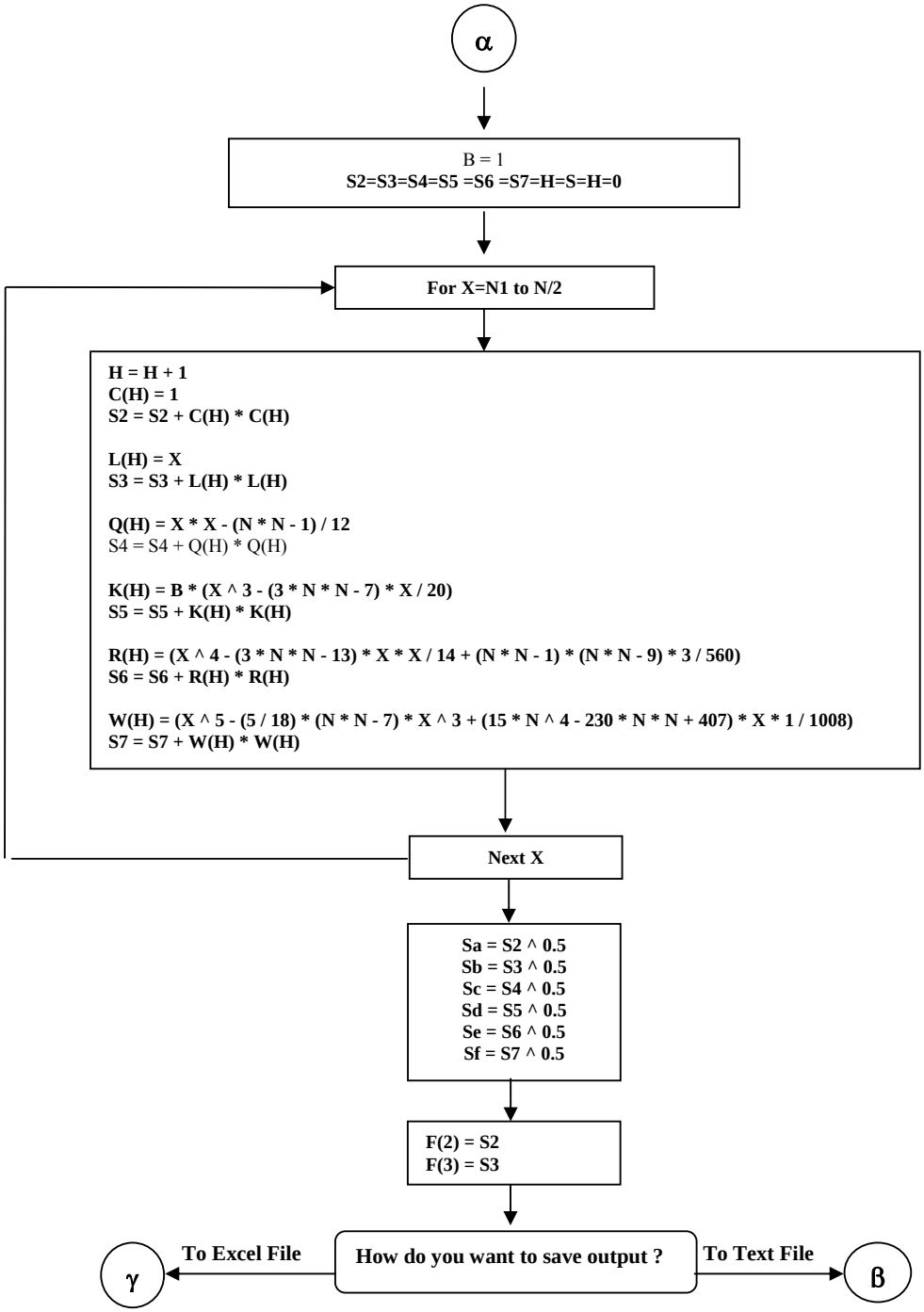

Fig. 6 Continued.

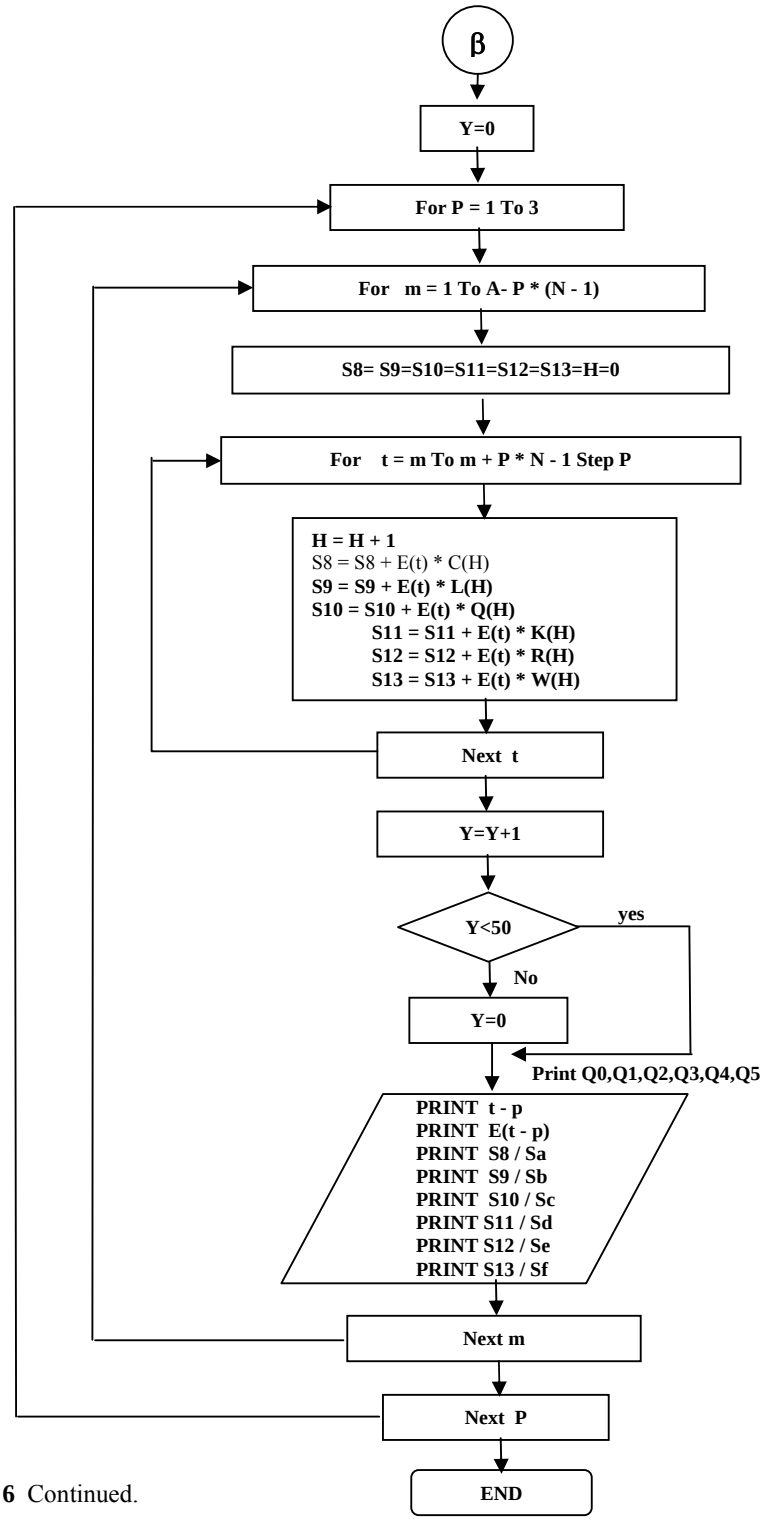

Fig. 6 Continued.

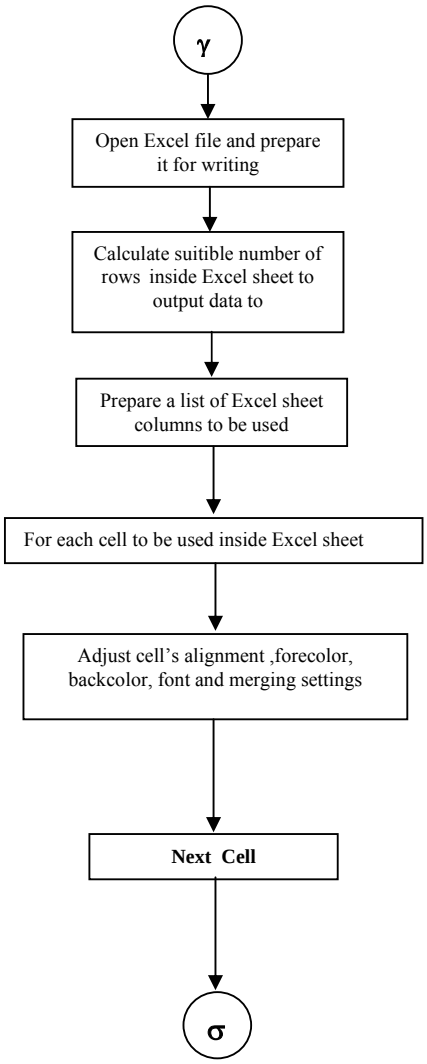

Fig. 6 Continued.

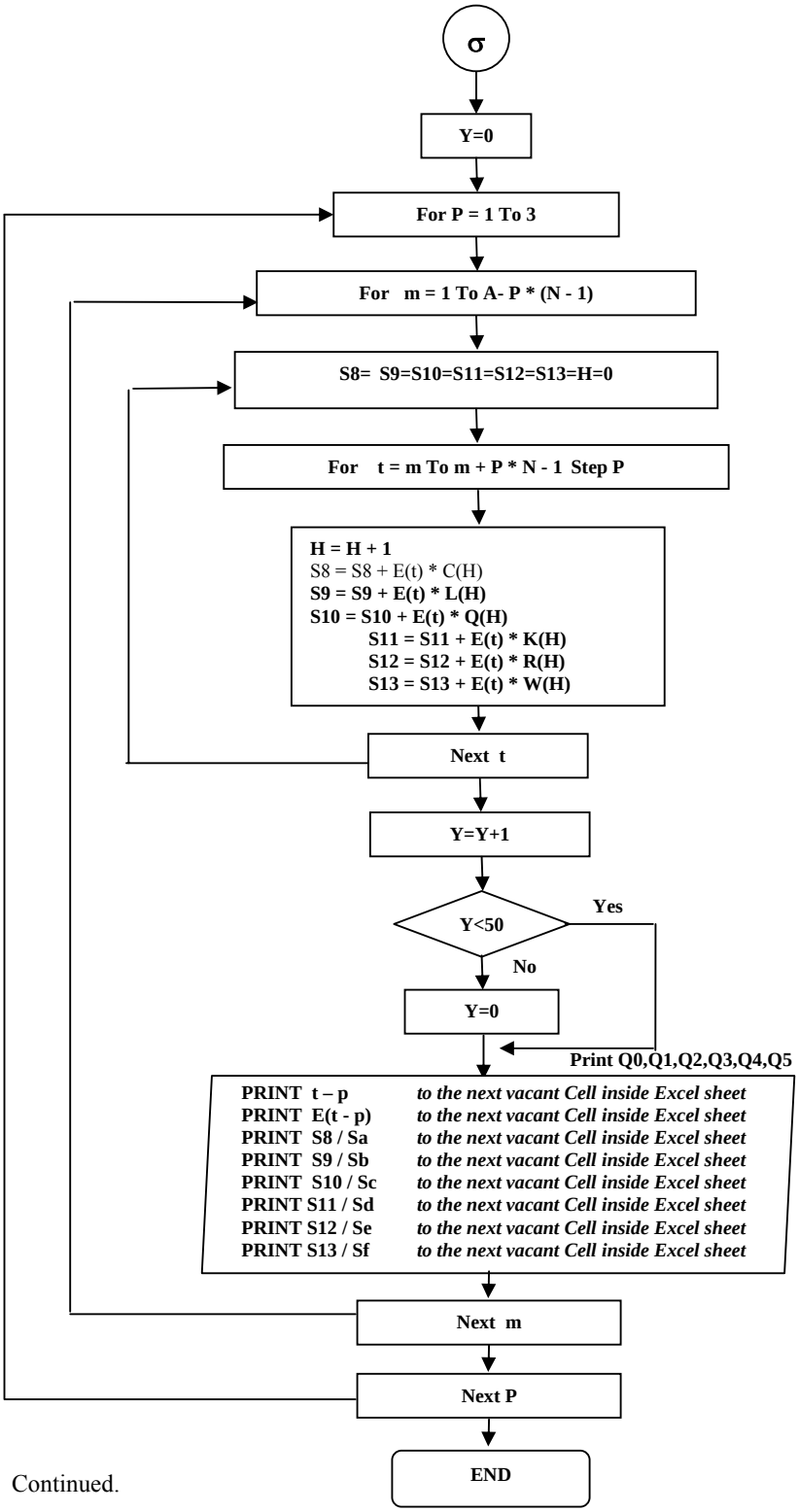

Fig. 6 Continued.

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