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Derivative Ultra-Violet/Visible Absorption Spectrophotometry and Its Areas of Application

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ABSTRACT

Derivative spectroscopy is one of the important technique for as resolution of multi component systems. Many of the modern pharmaceutical formulations are so complex because they contains number of excipients such as diluents, disintegrating agents, stabilizers, coloring agents or dyes, flavors etc. along with the active ingredient. Derivative technique is becoming increasingly popular in analytical spectrophotometry as a background correction and as a resolution enhancement technique. Derivative spectroscopy is a technique which offers an alternative approach to the enhancement of sensitivity and specificity in mixture analysis. Derivative spectrophotometry is a technique which is based on derivative spectra of a basic, zero-order spectrum. Derivative spectroscopy is widely used in different fields of the analysis.

Key words: UV-Spectrophotometer, Derivative spectroscopy (DS), Multi-component analysis, Clinical Chemistry.

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INTRODUCTION

Ultraviolet (UV) – visible spectroscopic method of analysis is widely used in the analysis of drugs in different pharmaceutical formulations due to its good sensitivity and cost effectiveness. A conventional absorption spectrum is a plot of absorbance A against wavelength λ but as we have seen electronic spectra usually show broad band with little structure and much overlapping of adjacent bands. For the purpose of spectral analysis in order to relate chemical structure to electronic transition, and for analytical situation in which mixture contribute interfering absorption, a method of manipulating the spectral data called derivative spectroscopy.[1] It offers a convenient solution to a number of well defined analytical problems, such as resolution of multicornponent systems, sample turbidity or matrix background and enhancement of spectral details[2-3, 10]. Spectrophotometers are basic equipment of every laboratory because Uv Spectrophotometry is one of the cheapest technique, but there is disadvantage and limitation of the spectrophotometry that is its low selectivity. Derivatization of spectra is one of the simplest methods for an increasing selectivity.

Derivative Spectrophotometric has been extensively used in the determination of drugs in multi components having overlapping spectra, which eliminates interference from formulation matrix by using the zero-crossing techniques[4]. It has found application in many fields of analysis, especially in pharmaceutical, biochemical, clinical and as well as in inorganic or organic analysis. It has been recognized that derivative spectrophotometry can eliminate the effect of background signals and hence does not require optically clear sample solutions[5, 37]. Derivative spectrophotometry is now a reasonably priced standard feature of modern micro-computerized UV/Vis spectrophotometry.[5]

Techniques for generation of derivative spectra: [5, 6, 7,8,10]

Derivative spectra may be generated by any of fallowing three techniques

By modification of the optical system:

The earliest derivative spectra were obtained by modification of the optical system. Spectrophotometers with dual monochromator set a small wavelength interval (Δ λ, typically 1-3nm) apart, or with the facility to oscillate the wavelength over a small range, are required (Figure 1). In either case the photo detector generates a signal with amplitude proportional to the slope of the spectrum over the wavelength interval. Instruments of this type are expensive and are essentially restricted to the recording of first derivative spectra only.

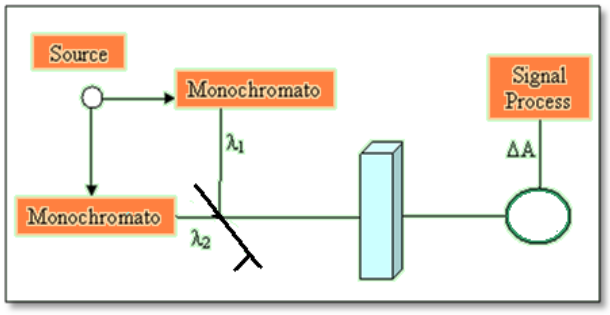

Figure 1: Representation of spectrophotometers with dual monochromator.

By Resistance capacitance (RC) modules

The second technique to generate derivative spectra is electronic differentiation of the spectrophotometer analog signal. Resistance capacitance (RC) modules may be incorporated in series between the spectrophotometer and recorder to provide differentiation of the absorbance, not with respect to wavelength, but with respect to time, thereby producing the signal dA /dt. If the wavelength scan rate is constant (dλ /dt = C), the derivative with respect to wavelength is given by

$$dA/d\lambda = (dA/dt) / (d\lambda/dt) = (dA/dt)(1/C)$$

Derivative spectra obtained by RC modules are highly dependent on instrumental parameters, in particular the scan speed and the time constant. It is essential, therefore, to employ a standard solution of the analyte to calibrate the measured value the instrumental conditions selected.

Microcomputer differentiation:

The third technique of generation of derivative spectra is based upon microcomputer differentiation. Microcomputers incorporated into or interfaced with the spectrophotometer may be programmed to provide derivative spectra during or after the scan, to measure derivative amplitudes between specified wavelengths and to calculate concentrations and associated statistics from the measured amplitude.

Advantages of Derivative spectroscopy:

An effective enhancement of resolution:

Derivative spectroscopy can be useful to separate two or more components or the mixture of compounds with overlapping spectra; Spectral resolution of multi component systems by measurement at two wavelengths; where the interferent has identical molar absorptivity while the analyte does not, can result in good exclusion of interferences.

Accurate determination of λ_{max}:

It is possible to determine the λmax of any drug or any compound which can be absorbed in the

Uv range. With the help of derivative spectroscopy Not only the drugs but also the other compounds without separation in biological fluids like blood, saliva etc. can be determined.[3]Generally, the second derivative is more useful than the first ones [9]

Used for sample verification[10]

Identification of compounds and to interpret drug excipients in drug formulation:

The derivative spectra methods allow us to use the wavelength of highest value of maxima or minima signals. Moreover, the presence of a more number of maxima and minima wavelengths gives an opportunity to select a particular wavelength for determination of active compounds without the interference from other compounds or formulation excipients.

Different orders of derivative spectroscopy:

First order derivative spectrum:

The first order derivative spectrum is a plot of the rate of change of absorbance with wavelength against wavelength i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ vs. λ. A first order derivative starts and finishes at zero. The maximum positive and maximum negative slope respectively in the D spectrum corresponds with a maximum and a minimum respectively in the D^1 spectrum. This bipolar function is characteristic of all odd-order derivatives. The λ max in first order derivative spectrum is a wavelength of zero slope and gives, $dA/d\lambda = 0$

Though it is useful, First-derivative spectra are difficult to interpret. Explanation of the first-derivative (D1) spectrum and its relationship to the absorption spectrum (Abs) is explained in figure 2.

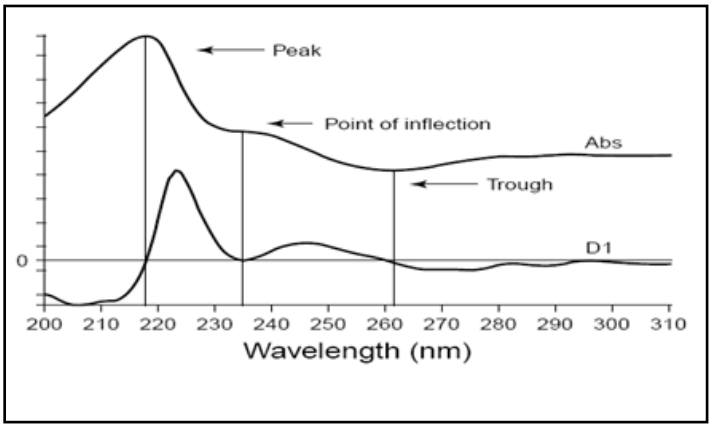

Figure 2: Explanation of the first-derivative (D1) spectrum and its relationship to the absorption spectrum (Abs). [11]

Second order derivative spectrum:

The second order derivative spectrum is a plot of the curvature of the D spectrum against

wavelength or a plot of $d^2A/ d\lambda^2$ vs. λ. The maximum negative curvature in the D spectrum gives a minimum in the D^2 spectrum, and the maximum positive curvature in the D spectrum gives two small maxima called satellite bands in the D^2 spectrum. The wavelength of maximum slope and zero curvature in the D spectrum correspond with cross-over points in the D^2 spectrum.

The second-derivative spectrum can be thought of as an inverted spectrum. In the second-derivative spectrum sharp peaks will be made even sharper. Broad peaks will become flattened and because of this reason this technique can be used both as a peak-enhancement and background-rejection tool[13]. The second-derivative spectrum, like its absorbance counterpart, still contains quantitative information. If we take the second derivative spectrum (by derivatizing the absorbance spectrum twice), we obtain a plot such as that in Figure 4.

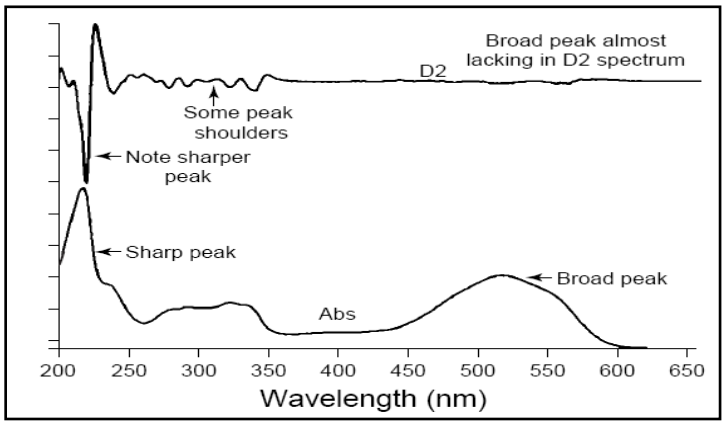

Figure- 3: Second order derivative (D2) spectrum and its relationship to the absorption spectrum (Abs) [11]

Third order derivative spectrum:

This is higher order of second order derivative spectra and is characteristic of all odd-order derivatives

Fourth order derivative spectrum:

The fourth derivative shows a positive band. The presence of a strong negative or positive band, with the minimum or maximum at the same wavelength as λmax of the absorbance band, is characteristic of the even-order derivatives.[5] Note that the number of bands observed is equal to the derivative order plus one.

Disadvantages of derivative spectrophotometry[6]

Though the derivative spectroscopy is very useful, it has some disadvantages. The main disadvantage of this technique is its low reproducibility. This is caused by the following reasons:

Dependence on instrumental parameters:

The main disadvantage of this technique is its dependence on instrumental parameters like speed

of scan and the slit width [12]. The instrumental conditions of recording parent zero-order spectrum have strong influence on the shape and intensity of its derivative generations. The spectrum which we obtain is more or less distorted by instrumental noises and as the consequence the derivative spectrum is distorted too. The Derivatization can amplify the noise signals in the resulted curves.

Non-robust properties of the Derivatization parameters:

Another disadvantage of derivative spectrophotometry is non-robust character of the selected parameters of elaborated methods. They can be used only for the system for which they were chosen. As the analytical use of derivative spectrophotometry is based on the analysis of the derivative spectra, the introduction of additional compound into the studied object changes the shape of Derivatization results. The selected parameters of derivative spectrophotometric method are applicable only for the studied system and every change in its composition require the re optimization and selection of new parameters of Derivatization.

lack of homogeneous protocol of optimization the parameters of the method and presentation of results.

APPLICATION OF DERIVATIVE SPECTROSCOPY:

Pharmaceutical analysis:

Multicomponent analysis:

Derivative spectroscopy is an analytical technique of a great importance for resolving the mixtures of drugs with overlapping spectra. DS has been applied successfully in determination of individual drug or drug mixtures. Interference of spectra of drug additives and drug decompositions is a disadvantage of normal spectrophotometery which is overcome by DS which allows the elimination of undesirable interference. The different orders of the derivative spectroscopy are used in the analysis of single drugs and for the simultaneous determination of multicomponent mixture of the pharmaceutical compounds

Table 1: Following drugs have been reported to be estimated simultaneously by the Derivative spectroscopy method

Drugs	Order of DS	Ref.
Atenolol	Zero(276 nm), First, Second And Third Order Derivatives	13
Diazepam	First-Order Derivative	14
Ezetimibe	First(259.5 nm) second(269 nm) and third-Order (248 nm) Derivative	15
Fluoxetine	First (235 nm) And second order derivatives 229-238.5 nm)	16

Gliclazide	Zero order (226 nm) and First Order Derivatives (217nm)	17
Glipizide	First order derivative. λmax 286 nm λmin63nm.	18
Irbesartan	Third(224 nm) and fourth order derivative(230 nm)	19
Mosapride	First order derivative (300 nm)	20
Nabumetone	First order derivative	21
Olanzapine	First and second order Derivative(222 nm and 230 nm)	22
Amlodipine Besylate And Hydrochlorothiazide	First order derivative (238.5 nm for HCT and 271 nm for AMLO).	23
Amoxicillin And Cephalexin	-	24
Furosemide And Spironolactone	Ratio derivative spectrophotometry	25
Atorvastatin Calcium and Amlodipine Besylate	Zero and first order derivative(241nm and 250 nm)	26
Ceftriaxone Sodium and Tazobactum Sodium	First order derivative(247 nm and 260 nm)	27
Linezolid (An Antibacterial Drug)	Zero (250 nm)first order and second derivative(251 nm)	28
Metformin, Pioglitazone And Glimepiride	First order Derivative(287 nm and 317.9 nm)	29
Moxifloxacin and Cefixime	First and ratio order first derivative	30
Naphazoline And Antazoline	Ratio derivative spectrophotometry	31
Olmesartan Medoxomil And Hydrochlorothiazide	Ratio derivative at zero-crossing	32
Ondansetron and Metoclopramide	First order derivative (266 nm and 253nm)	33
Paracetamol,Aceclofenac And Rabeprazole	First order derivative	34
Atenolol, Nifedipine, Aspirin And Dipyridamole	Second order derivative	35

For determination of partition coefficient:

Partition of drugs between an aqueous phase and biomembranes is really important factor which affects on absorption of drug in body To determine the partition coefficients spectrophotometrically , drug and vesicle mixture solutions should be separated into aqueous and lipid phases by centrifugation or filtration (hygroscopic desorption), since lipid vesicles cause intense background signals by light scattering. However, the separation procedures are troublesome and may disturb the equilibrium states of the sample solutions. From literature It has been known that derivative spectrophotometry can eliminate the effect of back-ground signals and improve resolutions of overlapped signals[36-38].Therefore, if derivative spectrophotometry can be applied to the determination of partition coefficients of drugs between lipid vesicles and water, partition coefficients can be obtained more accurately and more easily without disturbing

the equilibrium state by the separation procedures.

The partition coefficients between lipid bilayers of dimyristoyl-L-phosphatidylglycerol unilamellar liposomes and water were determined using derivative spectrophotometry for Chlordiazepoxide Benzodiazepine, Isoniazid and Rifampicin tuberculostatic drugs and dibucaine local anaesthetic.[41-39]

Determination of partition coefficients of chlorpromazine and promazine were done by using second derivative spectrophotometric. Absorption spectra of chlorpromazine and promazine in the presence of lecithin (egg yolk) bilayer vesicles were measured against the reference solutions containing the same amount of lecithin vesicles alone and their second derivative spectra were obtained by the Savitzky-Golay method.[36]

By using the second derivatives of ultraviolet absorption spectra, the partition coefficients between lipid bilayer of phosphatidylcholine (PC) vesicles and buffer for five new Phenothiazines were determined. The λmax of absorption band for each of the investigated Phenothiazine derivatives (PDs) was shifted to the longer wavelengths in the presence of PC vesicles with increasing of lipid concentration.[40]

The absorption spectra of six Phenothiazine derivatives, Chlorpromazine, Triflupromazine, Promazine, Promethazine, Trifluoperazine and Prochlorperazine, measured in the solutions containing various amounts of human erythrocyte ghosts (HEG) showed bathocromic shifts according to the amount of HEG. The partition coefficients of these drugs were determined by using second order derivative spectroscopy.[41]

For determination of Dissociation Coefficient:

For developing new APIs, the pKa has become of great importance because the transport of drugs into cells and across other membranes is a function of physico-chemical properties, and of the pKa of the drugs .When a drug has ionizable group(s) in its structure, the affinity of the drug for biomembranes depends on its dissociation constant (pK), since the lipophilicity of the drug in the neutral state is usually far higher than that in the ionic state. Thus, the pK is a very important physicochemical property of drugs[42]. The largest change in absorbance occurs at the pH corresponding to a pKa value. These changes are usually identified from the first derivative of the absorbance against time plot.

Recently, it has been recognized that derivative spectrophotometry, particularly of second or higher order, can eliminate the effect of the background scattering signal caused by turbidity of sample solutions whose pka has to be determined. Therefore, if second-derivative spectrophotometry can be employed to measure the concentration of dissolved compounds in a

turbid sample solution, the dissociation constants of the sparingly soluble compounds could be obtained very easily.[43]

In Clinical Chemistry:

Clinical samples are characterized by a very complicated matrix and low level of analyte. The sensitivity and selectivity of spectrophotometric measurements usually is too low for the direct use for clinical purposes. The assays of clinical interest with spectrophotometric determination require intensive pretreatment steps involving extraction, enrichment and cleaning operations, using solvent or solid phase extraction. Derivative spectroscopy offers a simple alternative approach for the enhancement of sensitivity and specificity in clinical chemistry. Main advantage of first-derivative spectra is that they diminish or eliminate contributions from background (blank) absorbance that do not change with wavelength. An additional advantage of second-derivative spectra is its ability to diminish or eliminate effects of spectra that change with wavelength but with rates of curvature that are small relative to rates of curvature of spectra for components of interest; in other words, with second-derivative spectra, a component with one or more sharp peaks is easily differentiated from a component with a broad peak. [44]

The second-order derivative method was proposed for the direct determination of Pefloxacin [45] in serum. The detection limit of determination was 15 ng of analyte in 1 ml of serum. The same group of authors [46] has proposed derivative Spectrophotometric method for determination of Fleroxacin in human serum. The first derivative method was proposed for determination of Triamterene and Leucovorin in biological fluids: urine and serum samples[47] the first and the second derivative method have applied for assay of Guanoxan sulfate in pharmaceutical formulations as well as in spiked human urine and serum by Author Gazy[48]. The method elaborated for determination of some Cephalosporin antibiotics [49] based on the first derivative spectra was used for their determination in physiological serum and glucosed physiological serum. The mentioned previously derivative method concerned on determination of Acrivastine [50]was applied for its assay in urine samples

Analysis of food, cosmetics colorants and dyes:

Derivative spectrophotometric methods have found applications in analysis of food or cosmetics

Color is the first sensory quality by which foods are judged, and food quality and flavor are closely associated with color. Mixtures of two or three colorants were commonly used as additive in food and soft drinks to achieve suitable and ideal colors. In addition, colorants often used commercially as alimentary additive in pharmaceutical and cosmetic. Visible spectrophotometric methods can be used for their quantitative analysis. However, the absorption

spectra of some colorants are much overlapped and direct measurements of the absorbance are not suitable for resolving mixtures of these colorants without a separation step. This problem can be overcome by using DS.DS is an analytical technique of great utility for resolving mixture of substances with overlapping spectra by the simultaneous use of the zero crossing method and derivative of ratio spectra method .Other graphical and numerical measurement methods such as that of derivative quotient spectra are used in literature[50-53], these methods have permits the determination of different mixtures of food colorants.

The recent applications of derivative spectrophotometry in food or cosmetics analysis are gathered in Table 3.

Table 3.Applications of derivative spectrophotometry in food or cosmetics analysis

Substance	Type of Substance	Remark	Ref.
colorants E-123 (Amaranth), E-124 (Ponceau 4R) and E-120 (Carminic acid)	Colorants	1st derivative ratio spectra	54
cola, coffee, tea	Some beverages	Second derivative third derivative	55
Tartrazine, Amaranth & Curcumin	Colorants	first derivative spectra at	56
Erythrosine and Sunset yellow,	Colorants	first derivative	57
Oxytetracycline	medicated food	second derivative synchronous spectrofluorimetry	58
Oryzanol	Food	second derivative	59

CONCLUSION

Derivative spectrophotometry is the well established analytical technique with a number of possible applications in organic as well as in inorganic field of analysis. DS is a relatively modern technique which has proved to be very advantageous in solving particular analytical problems which normal spectroscopy is not able to solve. The advantages of the derivative UV-Vis spectroscopy in the quantitative analysis and the interpretation of overlapping bands are well known. The other advantages are minimization or elimination of background absorption, quantitative determination of trace components, characterization of individual pure compounds, the analysis of trace components in complex absorbing matrices, measurements on turbid, scattering solutions and suspensions, Derivative spectrophotometry as a technique which allows to non-invasive extraction of information included in basic spectrum appears to be a very valuable tool in physico-chemical studies. It permits to investigate the reaction equilibria or kinetics without disturbing their run studies. It permits to investigate the reaction equilibria or kinetics without disturbing their run. Nowadays, derivative spectrophotometry is fully available with software's controlling modern spectrophotometers. Analysts receive an elegant tool which

allows extraction of analytically useful information from spectra. An understanding of specific features of this technique and its proper utilization leads to simplification of procedure and to increase a selectivity of assay.

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