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Quantitative Analysis by Derivative Spectrophotometry (II).

Derivative spectrophotometry and methods for the reduction of high frequency noises

Man Ki Park and Jung Hwan Cho

College of Pharmacy, Seoul National University, Seoul 151, Korea

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Abstract □ One of the problems of derivative spectrophotometry, the decrease of signal-to-noise ratio by derivative operations, was solved by three concepts of digital filtering, ensemble averaging, least squares polynomial smoothing and Fourier smoothing. The authors made several computer programs written in APPLE SOFT BASIC language for the actual applications of the concepts of these digital filters on UV spectrophotometer system. As a result, ensemble averaging could not be used as a routine operation for the spectrophotometer used. The maximum S/N ratio enhancement factors achieved by least squares polynomial smoothing were 6.17 and 7.47 for the spectra of Gaussian and Lorentzian distribution models, and by Fourier smoothing, 16.42 and 11.78 for the spectra of two models, respectively.

Keywords □ Derivative Spectrophotometry, High Frequency Noise, Signal-to-Noise Ratio (S/N Ratio), Synthetic Spectrum, Digital Filters, Computer Program.

On the application of derivative spectrophotometry which involves the utilizations of derivative spectra, one of the most serious problems is that noises, particularly high frequency noises, are amplified by derivative operations. So, the Signal-to-Noise ratio (S/N ratio) becomes greatly lowered along the orders of derivative operations. For this reason, there is a great restriction on using the derivative spectra for the purposes of quantitative analysis as well as qualitative analysis. Therefore, the methods for reduction of high frequency noises should be established.

Various methods have been evaluated for the noise reduction. There are several reports about the utilizations of the concepts of convolution[1-19], ensemble averaging[20], Fourier smoothing[21-28], Kalman filter[29,30], factor analysis[31], cross correlation[32,33] and Chebyshev filter[34] in the fields of electrochemistry, FT-NMR, IR, CD spectroscopy and chromatography, etc. Authors made computer programs written in APPLE SOFT BASIC language for the applications of ensemble averaging, least squares polynomial smoothing, and Fourier smoothing which empolys nine different filter functions. We made use of these programs to reduce the high frequency noises in the real or synthetic UV spectra.

EXPERIMENTAL METHODS

Instruments

UV spectrophotometer was model UV-4050 of LKB Biochrome, England, and computer was APPLE II plus personal computer of APPLE Co., USA. The communication between two systems was accomplished via RS-232C interface card. All computer programs were written in APPLE SOFT BASIC language and then compiled to machine language codes using APPLE SOFT BASIC Compiler®. The data transfer between programs was done via files on the floppy diskettes.

Computer Programs and their Theoretical Backgrounds

Synthetic UV spectrum Generation

To evaluate the efficiency of digital filtering, we made a computer program which generates synthetic UV spectra as a reference according to Gaussian (1)[36] or Lorentzian (2)[26] distribution model.

$$A = G(\lambda) = H \cdot \exp\left(\frac{-(\lambda_{max}-\lambda)^2}{2 \cdot \sigma^2}\right), \sigma = \frac{\text{fwhm}}{\sqrt{8 \ln 2}} \quad (1)$$

$$A = L(\lambda) = \frac{H}{1+4\frac{(\lambda_{max}-\lambda)}{\text{fwhm}}} \quad (2)$$

where, H, peak hight; A, absorbance value; λ wavelength; σ, the variance of peak; fwhm, the full width at the half maximum of peak; λ_{max}, absorbance maximum wavelength.

With this program, synthetic UV spectra can be generated, which have several peaks as many as needed in a selected spectral range. In addition, this program has a routine to generate the high frequency noises in random fashion with a certain level, using RND function of BASIC language. Using this routine, noisy spectra were generated and compared with noise-free reference spectra before and after digital filtering operations. Fig.1 shows the synthetic UV spectra generated by this program.

Calculation of S/N ratio

For the calculation of S/N ratio, noise was defined as root mean of squares (RMS)[37] of differences between the derivative values of noisy spectra and noise-free reference spectra at every spectral point, and signal as the peak height (Si) of the highest peak of reference spectrum. Using these definitions, S/N ratio was calculated by following equations[37].

$$S/N = Si/RMS \qquad (3)$$

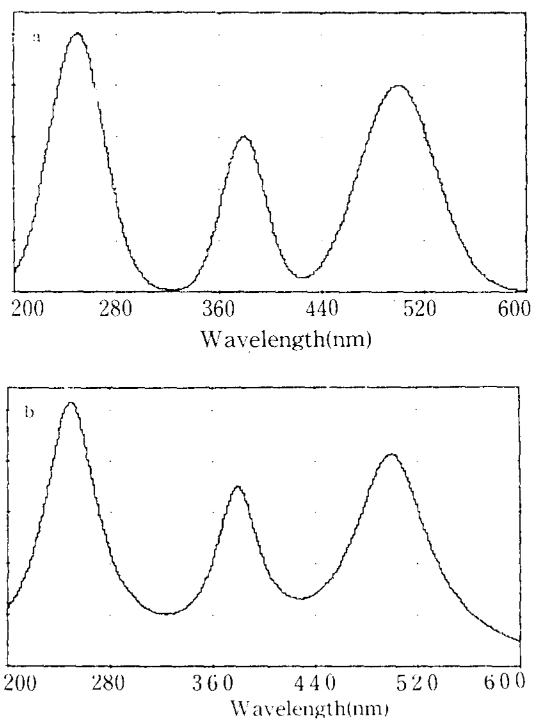


Fig. 1. The examples of synthetic UV spectra.
a. Gaussian distribution model
b. Lorentzian distribution model

$$RMS = \frac{\sqrt{\sum_{i=1}^{N} (x_i^o - x_i)^2}}{N} \qquad (4)$$

where, X_i^o, derivative value of i-th sampling point of reference spectrum; X_i, derivative value of i-th sampling point of noisy spectrum; N, the number of sampling points.

Ensemble averaging

Ensemble averaging, calculation of averages of signals acquired by the successive scans is based on the fact that the noises are reduced because of their randomness. The S/N ratio of n sacns can be estimated by following equation[20].

$$(S/N)_n = \sqrt{n} \cdot (S/N)_1 \qquad (5)$$

where, n is the number of scans, $(S/N)_1$ is the S/N ratio of 1 scan and $(S/N)_n$ is the S/N ratio of n scans. To apply this concept, the computer program for wavelength scan, supplied with spectrophotometer from LKB Biochrome, was revised.

Least squares polynomial smoothing and digital differentiation

In the following equation (6)[2] of convolution operation, convoluting integers, C_i, and normalizing factor, N, determine whether the result of convolution is noise reduction (*i.e.*, digital filtering) or digital differentiation.

$$Y_j^* = \frac{\sum_{i=-m}^{i=m} C_i \cdot Y_{j+i}}{N} \qquad (6)$$

where, Y_j^*, convoluted value; Y_{j+i}, the raw value; 2m+1, the number of data points in convoluting block. Convoluting integers and normalizing factors are determined by the types of transfer functions, the degree of polynomials and the number of data points in the convoluting block (*i.e.*, convoluting block size, 2m+1). Several reports[2-6] already presented with these values on the well-defined mathmetical backgrounds. A program was made which can utilize these values presented in such reports. The transfer functions used are cubic, quartic, and modified cubic functions[5].

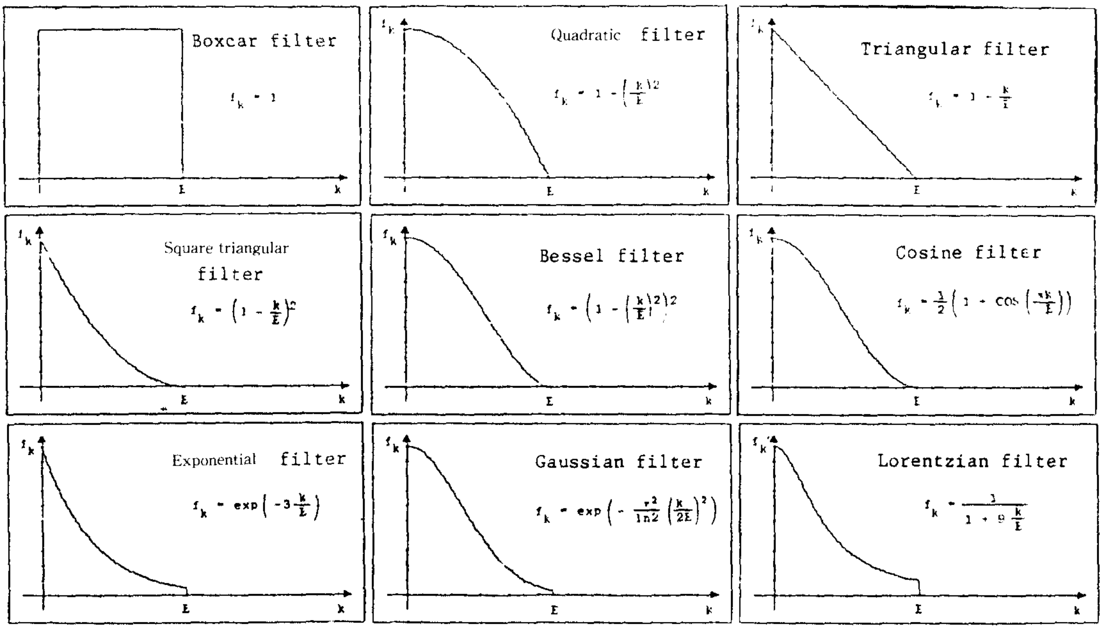
Fourier smoothing

There are three steps in accomplishing Fourier smoothing operation. The first step is the conversion of UV spectral data to frequency domain data by forward Fourier transform, which generates two sets of real valued transform data, R_k and I_k[27]

$$R_k = \frac{1}{N} \sum_{j=0}^{N-1} x_j \cdot COS\left(\frac{2\pi jk}{N}\right), \quad k=0, 1, \cdots, N-1 \qquad (7$$

$$I_k = \frac{-1}{N} \sum_{j=0}^{N-1} x_j \cdot SIN\left(\frac{2\pi jk}{N}\right), \quad k=0, 1, \cdots, N-1 \qquad (8$$

where, X_j, the raw noisy UV spectral data; N, the



ig.2. The diagrams and equations of filter functions which are used in the Fourier smoothing.

umber of sampling points of UV spectral data. The econd step is the determination of cut-off freuency and weighting of the data by appropriate ilter function. Nine different filter functions, boxar, quadratic, triangular, square triangular, besel, cosine, exponential, Gaussian, and Lorentzian ilter functions were used. Fig.2 shows the diarams and eqùations of these filter functions. The hird step is the inverse Fourier transform of weighed data to normal spectral data. The correspondng discrete inverse Fourier transform can be exressed as the following equations[27].

$$_0 = R_0 + 2 \sum_{k=1}^{E-1} f_k R_k \quad (9)$$

$$_j = R_0 + 2 \sum_{k=1}^{E-1} f_k R_k \cdot \mathrm{COS}\left(\frac{2\pi jk}{N}\right) - f_k I_k \cdot \mathrm{SIN}\left(\frac{2\pi jk}{N}\right), \; j = 1, 2, \cdots, N-1 \quad (10)$$

here, E, the index number of cut-off frequency; $_j$, smoothed spectral data; f_k, the weight value etermined by filter function.

RESULTS AND DISCUSSION

he Decrease of S/N ratio by the derivative perations

Fig.3 shows that there is significant decrease of /N ratio when the order of the derivative operaons become higher. This trend acts as a great restriction on applying the concepts of derivative spectrophotometry unless the proper solutions can be presented. So, several programs were made for widely-distributed computer system, APPLE II plus personal computer, to achieve noise reduction by using three concepts of digital filters discribed above. Although at this time, these programs are made for the LKB UV spectrophotometer system, they can be adapted for the other spectrophotometer or chromatography systems with ease.

Ensemble averaging

The spectra shown in Fig.4 are those that are acquired by repetitive scans of 1 mg% pyridoxine · HCl dissolved in phosphate buffer (pH 7.4). The more scans were repeated, the less noises were involved. The S/N ratio was gradually increased along the repetition of scanning, when the S/N ratio was calculated using the spectrum of 8 scans as a reference spectrum. However, it took 150 seconds to acquire a spectrum of 1 scan, so it took 20 minutes for 8 scans in the range of wavelength, 220-380 nm at 1 nm interval. In other words, unless a new technique in scanning of UV spectrophotometer is adapted, ensemble averaging cannot be used as a routine operation. Moreover, theoretically, S/N ratio of only 10 times as great as that of one scan can be achieved by 100 scans. Therefore, this is very time-consuming.

Least squares polynomial smoothing

Fig.5-a and 5-b represent the S/N ratio enhan-

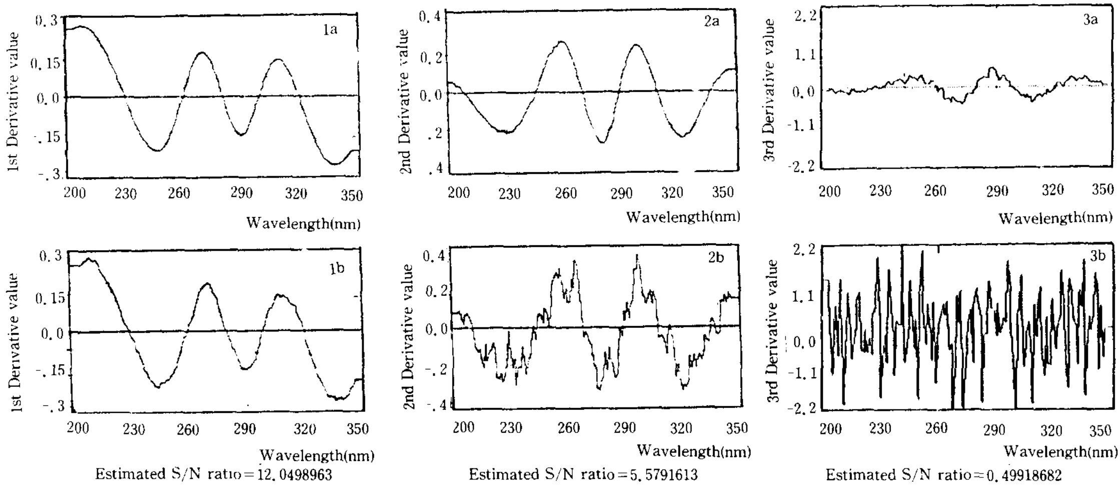


Fig.3. S/N ratio lowering by the derivative operations.

1. 1st derivative 2. 2nd derivative 3. 3rd derivative
a. noise-free reference spectrum
b. noisy spectrum
* All spectra are generated by UV spectra synthesis routine,

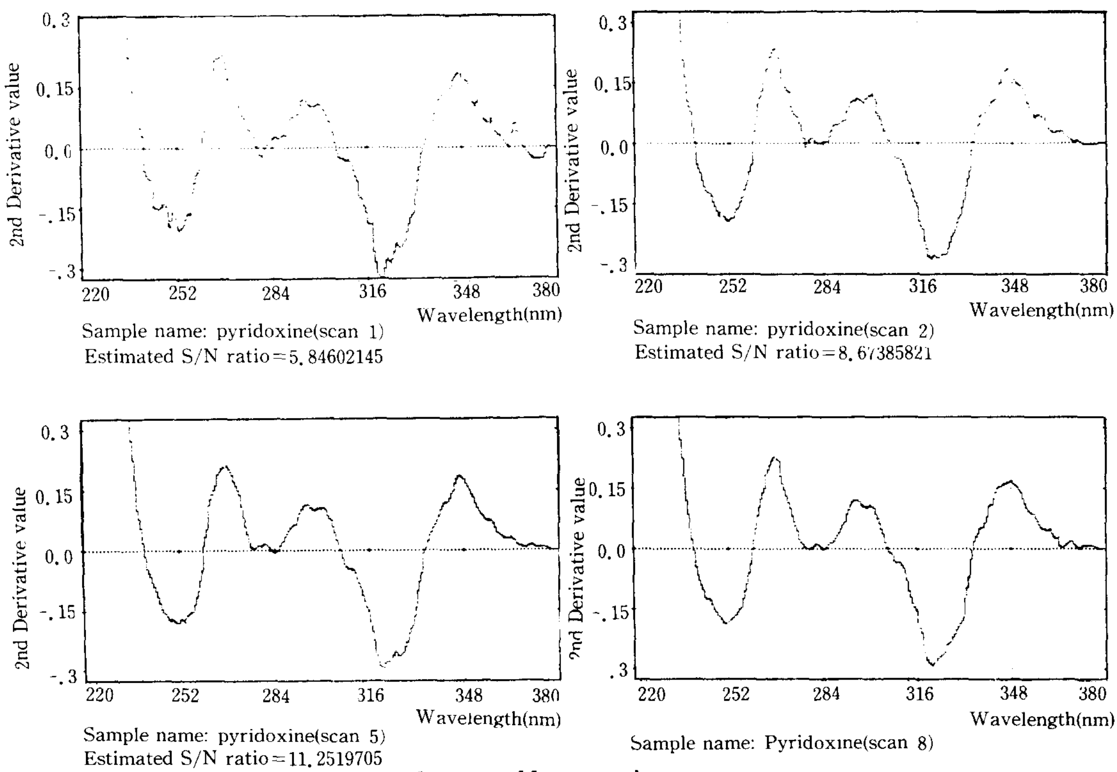


Fig.4. The S/N ratio enhancement by ensemble averaging.

* S/N ratios were calculated using the spectrum of 8 scans as the reference spectrum.

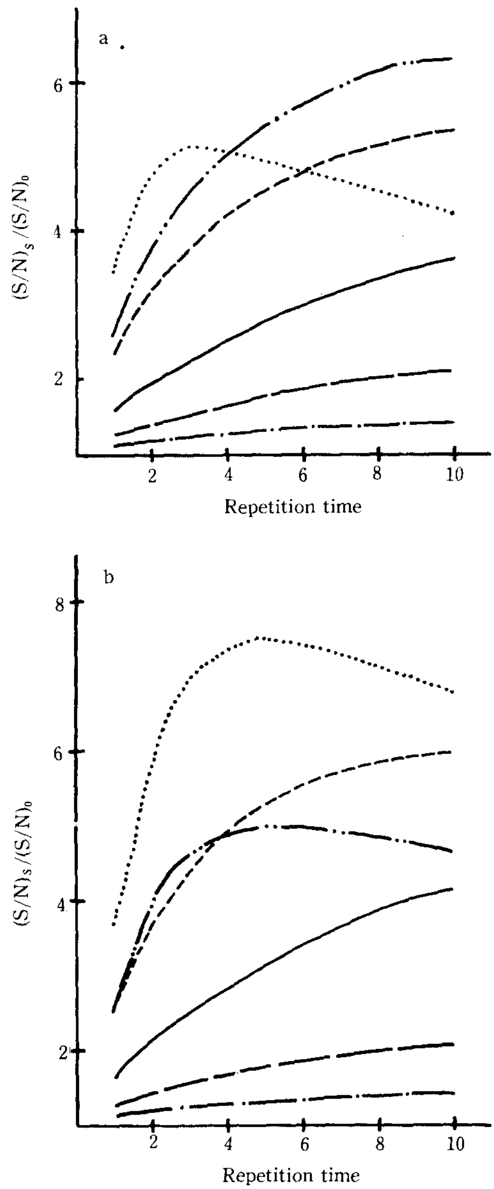


Fig. 5. S/N ratio enhancement by least square polynomial smoothing.

a. Gaussian distribution model
b. Lorentzian distribution model
—— Cubic 2m+1=7
—·— Quartic 2m+1=7
—··— Modified cubic 2m+1=7
----- Cubic 2m+1=9
—— Quartic 2m+1=9
······ Modified cubic 2m+1=9
$(S/N)_0$: S/N ratio of original noisy spectra
$(S/N)_S$: S/N ratio after smoothing operation

cement by using least squares polynomial smoothing to synthetic UV spectra of Gaussian and Lorentzian distribution models, respectively. S/N ratio enhancemtnt factors were calculated as the ratio of S/N ratios of spectra before and after noise reduction. The S/N ratio enhancements were examined by varying the transfer functions, convoluting block size, and the number of repetition of convolution operation. The more convolution operations were repeated, the greater S/N ratio were obtained. That is, S/N ratio enhancement factor was gradually increased. However, there was the maximum S/N ratio enhancement factor obtainable. In other words, there was the optimal number of repetition for each of transfer functions and convoluting block sizes. It can be deduced that there may be signal distortions by too many repetitions of convolution operations, resulting in the increase of RMS differences. It took 10 seconds in average to process least squares polynomial smoothing a time for the data of 151 sampling points (200-350 nm, interval 1 nm).

Fourier smoothing

Fig.6-a and 6-b represent the S/N ratio enhancement by using Fourier smoothing to synthetic UV spectra of Gaussian and Lorentzian distribution models, respectively. S/N ratio enhancement was examined by varying filter functions and cut-off frequencies. Each of filter function has optimal cut-off frequency as shown in the figures. Even for the same filter functions, the optimal cut-off frequencies are different for two models of synthetic UV spectra. Therefore, it is very important that the proper cut-off frequency should be determined in considerations of shape and fwhm of peak in spectrum of a certain system and the origins of noises and their frequencies. As shown in figures, there was the maximum value of S/N ratio enhancement factor for a given filter function. It can be deduced that there may be signal distortions in the left of the optimal cut-off frequency and insufficient noise reductions in the right. Using quadratic, bessel, cosine and Gaussian filter functions,the maximum S/N ratio enhancement factors were obtained at relatively low cut-off frequencies. Therefore, these filter functions are more useful for the purpose of noise reduction because with low cut-off frequency, the inverse Fourier transform can be processed more rapidly. It took *c.a.* 100 seconds in average, to process the data of 151 sampling points (200-350 nm, interval 1 nm), although this can be varied according to the size of data, the cut-off frequency, and the type of filter function.

Comparison of three methods

Ensemble averaging could not be used as a rou-

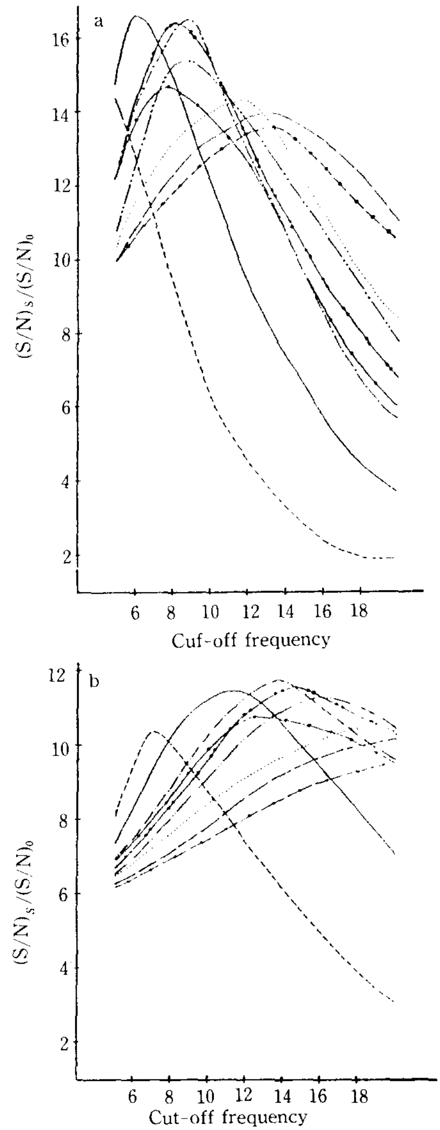


Fig. 6. S/N ratio enhancement by Fourier Somoothing.

a. Gaussian distribution model
b. Lorentzian distribution model

———— Quadratic filter
——•—— Triangular filter
——••—— COSINE filter
——··—— Gaussian filter
——·—— Bessel filter
------ Boxcar filter
—— —— Square Triangular filter
—•— —•— Exponential filter
·········· Lorentzian filter

$(S/N)_0$: S/N ratio of original noisy spectra
$(S/N)_S$: S/N ratio after smoothing operation

tine operation for the purpose of noise reduction of UV spectral data. In consideration of processing time only, least squares polynomial smoothing method is the preferred one. But using this algorithm, relatively small S/N ratio enhancement factors were obtained, which are 6.17 and 7.47 at maximum for the synthetic spectra of Gaussian and Lorentzian distribution models, respectively. In contrast with this, although Fourier smoothing took relatively long processing time, reasonably good S/N ratio enhancement factors were obtained, which are 16.42 and 11.78 at maximum for two models, respectively. Furthermore, processing time can be greatly reduced by some improvement of computing ability of computer system, for examples, the utilization of math coprocessor or acceleration of instruction fetch cycles using improved clock circuit of computer system concerned. Therefore, Fourier smoothing can be used with great efficiency for the purpose of noise reduction in the derivative values of UV spectral data.

CONCLUSION

The problems of noises, particularly high frequency noises, in the derivative spectrophotometry were solved by computer programs, without any improvements or changes of optical system mechanical structure, or electronic system oi spectrophotometer. In addition, since the origins oi noises are of very wide range, only the computei program utilizing various filter functions can afforc optimal S/N ratio.

When the less noises are involved in the spectra data and derivative spectra of lower order (first oi second order) are to be used, least square: polynomial smoothing can be used in advantage o its short processing time. But when the high level o noises are involved in the spectral data or derivative spectra of higher orders (above third order) ar(needed, Fourier smoothing is preferred one in spit(of its relatively long processing time, because witl this algorithm, reasonably good S/N ratio can b(achieved. Among the filter functions which are use(in Fourier smoothing, quadratic, bessel, cosin and Gaussian filter functions were more useful fo the purpose of reduction of high frequency noises i the synthetic UV spectral data.

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