Deconvolution of IR Spectra by Functionally-Enhanced Derivative Spectroscopy (FEDS): Why Does It Work?

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Graphical Abstract

Abstract. Functionally-enhanced derivative spectroscopy (FEDS) have been proposed as a tool for the easy spectrum deconvolution by the analysis of spectrum line function. However, given that FEDS was deduced from end-spectrum mathematical transformations using simple mathematical operations, some aspects to explain how FEDS can detect signals and differentiate them from non-signals are not completely understood. In order to increase the understanding of FEDS transform and expand its use in the analysis of HCM-systems, the residual noise remaining in the working mid-infrared spectra was studied and connected with analysis by FEDS. To exemplify the concepts developed, mid-infrared spectra of *Escherichia coli* were used as model of HCM-systems. It was demonstrated that end-spectrum noise is related with standard deviation of signal fluctuation in the spectrum permitting the easy calculation of signal-noise ratio (SNR) and the obtaining of SNR as a function of wavenumber from working spectra. In addition, our results demonstrate that spectrum obtained by FEDS transform is mainly a SNR-based spectrum, being a result of FEDS algorithm to find the data with maximum SNR and separate them by the use of simple conditional functions. It is concluded that spectral deconvolution can be performed easily by FEDS, permitting the separation of overlapped signals and the detection of hidden signals with high-SNR, with capacity to be generalized for numerous types of spectra.

Keywords: Signal-noise ratio, derivative spectroscopy, data transformation, biolayer, infrared

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1. Introduction

When an analytical measurement is performed, two basic components can be identified: A first one component is the *signal* that is understood as the fraction of information associated with the property of sample which are being investigated and, the second one is the *noise* that is undesirable information that affects the accuracy and precision of analysis, the detection limit and, in some cases, impossibilities the quantitative determination of analyte [1-2]. Particularly, into spectroscopic techniques, the effect of noise is very important when highly-complex multicomponent samples (HCMSs) are being analyzed, such as, biofilms, soils, plant extracts, tissue and biological fluids [3-4]. The main descriptor for the monitoring of effect of noise in spectroscopic analysis is the signal-to-noise ratio (SNR), which compares the amount of useful information (signal) with the noise in the analytical response. Usually, SNR can be defined as the ratio between the signal mean and the corresponding standard deviation (i.e., $SNR = \mu/\sigma$) for a set of spectral replicas [2].

The use of SNR as descriptor of quality of analytical method, in most measurements, assumes that the average power of the noise is constant and independent of the magnitude of the signal [1-2, 5]. However, Howard and Griffiths (2002) identified that, in techniques as mid-infrared or ultraviolet-visible spectroscopies, the noise in the spectrum is not constant unlike it established by noise classic derivation in spectroscopy [6]; but also, our research team during analysis of HCMSs have identified the presence of non-constant noise distribution along the spectrum which is usually neglected during the analysis. In fact, the above observation is not new, in many cases, in the most of qualitative spectroscopic analysis for pure, low-complexity or few-component samples the effect of noise contained in the end-spectrum is neglected and assumed to be a constant (here, *end-spectrum noise* is defined to be the residual noise that is not eliminated during the collection and processing of data and, therefore, it is transmitted to the working spectrum resulting of spectroscopic analysis). However, the effect of this end-spectrum noise on the spectrum analysis is significant in quantitative applications, such as, determination of thin film thickness by the analysis of penetration deep using Fourier transform infrared by attenuated total reflectance (FTIR-ATR) [7]; or when digital techniques as Fourier self-deconvolution [8-9], derivative spectroscopy [10-11], and functionally-enhanced derivative spectroscopy (FEDS) [12] are used, including convolutional neural networks for multivariate regression [13] and principal component analysis [14] among other.

On the other hand, the use of single spectrum for the analysis of end-spectrum noise has been previously proposed as a simple test for instrumental quality control in laboratories [15]. By this proposal, the extraction of noise is carried out by two steps (i) the smoothing of spectrum using the Savitzky-Golay algorithm and (ii) the smoothed spectrum is subtracted of the original spectrum. Thus, the prediction of "real" spectrum is carried out from the working spectrum and the resulting changes are related directly with the noise; in addition, the main reason for the use of Savitzky-Golay algorithm is that this is widely generalized and, in consequence, in

a strict sense, the estimation of "real" spectrum could be performed by any smoothing algorithms [16-17]. Recently, Bazar et al (2016) applied the above ideas for the evaluating of analytical signals in order to identify the spectral error identifying the presence of systematic error in the working spectra during routinely quantitative analysis [18-19].

Here, the concept of end-spectrum noise for spectroscopic techniques by the analysis of spectrum line function (SLF) from geometric and statistical arguments is developed in order to be used in the enhancing of the spectral analysis. In addition, the connection of end-spectrum noise with the SNR and its application in the spectrum digital processing by FEDS are discussed. Finally, concepts developed are exemplified in the analysis of mid-infrared spectrum of *E. coli* biolayers, however, these could be generalized for other type of spectra and samples.

2. Theory

2.1. Geometrical and statistical descriptions of end-spectrum noise

The spectrum-contained noise (R) can be defined to be the average of noise (r) in function of the wavenumber (w) and it is the fluctuation of SLF around a central value. Here, r is geometrically defined to be the average distance between two consecutive values of absorbance (b_w) throughout spectrum segment that is being analyzed. Thus, R is given by

$$R = \frac{1}{n-1} \sum r_w = \frac{1}{2(n-1)} \sum |b_{w+1} - b_w| \quad (1)$$

where n is the number of points in the analysis window. Given that $\Delta w = w_{j+1} - w_j$, n can be calculated by

$$n = \frac{(w_{max} - w_{min})}{\Delta w} \quad (2)$$

where w_{max} and w_{min} are the maximum and minimum values of w in the analysis window, respectively. Though R can be used to define a base line from of information self-contained in the spectrum, r is seen to be useful parameter for the analysis of signals in a specific spectral region.

From statistical point of view, R can be understood as the average standard deviation (s_m) calculated from mean variance between pairs of consecutive data. For this, the average between b_w and b_{w+1} (b_m) is assumed as a better estimation of the signal corresponding to $w+1$. Thus,

$$b_m = \frac{b_{w+1} + b_w}{z} \quad (3)$$

where $z = 2$ (here, z is used to obtain a generalized expression congruent with Savitzky-Golay algorithm). On the other hand, sample variance (s^2) is

$$s^2 = \frac{(b_{w+1} - b_w)^2}{z - 1} \quad (4)$$

In the equation 4, because sample average for one pair of consecutive data in the SLF is unknown, the Bessel correction was applied. Combining the equations 3 and 4 is concluded that

$$s^2 = \frac{[(z-1)b_{w+1} - b_w]^2}{z^2(z-1)} \quad (5)$$

By the above, the standard deviation (s) is given by

$$s = \sqrt{\frac{[(z-1)b_{w+1} - b_w]^2}{z^2(z-1)}} \quad (6)$$

$$s = \frac{|(z-1)b_{w+1} - b_w|}{z\sqrt{z-1}} \quad (7)$$

For pairs of consecutive data $z = 2$ along the $n-1$ consecutive data, s_m is

$$s_m = \frac{1}{n-1} \sum \frac{|b_{w+1} - b_w|}{2} \quad (8)$$

Finally, from equations 1 and 8, it is concluded that $R = s_m$, but also, r is completely congruent with geometrical description implicitly defined in the equation 1, i.e.,

$$r = \frac{|b_{w+1} - b_w|}{2} \quad (9)$$

In a generalized form, as a function of w , equation 9 can be rewritten to be

$$r_w = \frac{|b_w - b_{w-1}|}{2} \quad (10)$$

2.2. Functional-enhanced derivative spectroscopy (FEDS)

FEDS transform is an algorithm for digital analysis of spectra based on functional transformation of SLF; thus, spectral transformation is performed using mathematical and logical functions which are defined from original data [12]. The modifying of data by “functional transformation” is an approach to develop algorithms that are usually easier, faster and simpler to program than conventional algorithms (simple recursive algorithms, divide and conquer algorithms, dynamic programming algorithms, among others). By the functional transformation, a set of functions define are used to transform a set of structured data from its original form in another form of greater utility or with a specific interest. It is expected that transforming functions being “pure functions” and therefore these should be self-contained functions (i.e., data can be freely ordered and rearranged without entanglement or

interdependencies) and stateless (i.e., that executing of the same function or specific set of functions on the same input will always result in the same output data) [19-21]. By FEDS, a strategy based on “non-pure” functions are used because an infrared spectrum is a data collection with a fixed order in function of vibrational energy [19].

When FEDS transform is applied, a finite approximation method is used to compute the derivatives of the spectra. Usually, derivative algorithm utilizes the signal resolution to compute differences ($\Delta v = |v_j - v_i|$ where Δv is the separation between adjacent data). In the Equation 1 is showed the finite approximation of the first derivative for FTIR spectrum which is plotting usually in function of v :

$$y' = \frac{ds}{dv} \approx \frac{\Delta s}{\Delta v} = \frac{s(v_j) - s(v_i)}{v_j - v_i} \quad (11)$$

where s and Δs denote the signal for specific values of v and the difference between adjacent signals, respectively. For another spectrum usually used in analytical sciences, the ultraviolet-visible spectrum, the plotting of data is typically described as a function of λ [12].

The working tool in the FEDS transform is called Function P and it can be understood as a functional transformation that contracts the signals of FTIR spectrum in a function of critical points without to change the relative position of them.

The steps associated with the calculation of Function P are:

The derivative first of the inverse of normalized spectrum is easily calculated from spectrum of absorbance (a_j) as a function of v :

$$\frac{da_{N,j}^{-1}}{dv} \approx \frac{\Delta a_{N,j}^{-1}}{\Delta v} = \left(\frac{1}{v_j - v_{j-1}} \right) \left[\left(\frac{1}{a_{N,j}} \right) - \left(\frac{1}{a_{N,j-1}} \right) \right] \quad (12)$$

Assuming that $v_j - v_i$ is always a constant (this assumption is valid for almost all instrumental equipment), Equation 12 is rewritten to be

$$p = \left(\frac{1}{a_{N,j}} \right) - \left(\frac{1}{a_{N,j-1}} \right) \quad (13)$$

where p denotes an auxiliary function p in order to simplify the notation. Since Equation 13 defines positive and negative values, $|p|$ is calculated and the signals are amplified by the calculation of corresponding square root (note that absolute value operator is congruent with the definition given by equation 1, i.e., p is a segment associated with fluctuation of SLF between two consecutive wavenumbers). In this point, values of absorbance have been distorted by the transformation, however, by conditional functions the values of absorbance can be recovered exactly in the wavenumber with greater significance. Finally, Function P, here defined to be FEDS intensity (b_w) in order to avoid confusion with perturbation function, is defined to be

$$b_w = (1 + a_N) \frac{1}{\sqrt{|p|}} \quad (14)$$

where $(1 + a_N)$ is only an amplification factor for the assignation of a weight congruent with absorbance intensity. Note that, strictly speaking, equations have not limitations related with the technique, in consequence, equations should be useful for the analysis of any spectra or spectrum-like function. FEDS has been used for quantification of water contents in mixture with organic acids, determination by spectral analysis the dimerization constant and deconvolution of mid-infrared spectrum of quaternized poly(vinyl chloride) for assignation of signals [12, 22].

3. Methodology

The study of end-spectrum noise and its connecting with FEDS transform were performed in two stages: (i) a theoretical study using simulated spectra with basic SLF (straight-line spectrum and Gauss-type spectrum) and (ii) the study of HCMS, in specific, bacterial biolayers of *E. coli*.

3.1. Theoretical study by basic spectrum line functions

3.1.1. Analysis of SLF with constant noise

Two types of straight-line spectra were analyzed: $b_w = k_1$ where k_1 is a constant and $b_w = k_1 w + k_2$ where both k_1 and k_2 are constants. Simulation of straight-line spectra were performed for several synthetic noise ($r = \pm 2.0, \pm 4.0$ and ± 6.0), values of $k_1 = 4.0$ and $k_2 = 1.0$, and for two range of data (1-50 and 1-30). Later, r and SNR were calculated by linear correlation fit of data and by the use of equations 10 and 11.

3.1.2. Analysis of SLF with variable noise

In order to analyze the capacity to r to describe a non-constant noise in the straight-line spectrum a perturbation function (P) was be used. P is the function of a standing wave given by

$$P = \frac{1}{a_{wp}} \sin\left(\frac{2\pi w}{\lambda_{wp}} - t_{wp}\right) \quad (15)$$

where $1/a_{wp}$, λ_{wp} and t_{wp} are the amplitude, wavelength and displacement, respectively. Thus, P permits the modeling of controlled oscillatory noise which can be included in the spectrum by a simple sum of functions. Perturbation function was applied to straight-line spectra Gauss-type spectra. In the first case, for straight-line spectrum with $k_1 = 3.0$ and $k_2 = 0.5$, a standing wave defined by $a_{wp} = 2.5$, $\lambda_{wp} = 0.2\pi^2$ and $t_{wp} = 2.5$ was used to produce a known-synthetic noise.

On the other hand, Gauss-type spectrum is a term used to indicate that the spectrum signal or a consecutive signal collective that are being analyzed can be described by a Gaussian function (G_0). For theoretical analysis of noise and SNR, a Gauss-type analysis window can be drawn using a Gaussian function with a center (w_0) and standard deviation (s) defined to be $w_{min} < w < w_{max}$. Thus, G_0 is given by

$$G_0 = \frac{1}{s\sqrt{2\pi}} \exp\left(-\frac{(w - w_0)^2}{2s^2}\right) \quad (16)$$

G_0 was defined for $w_0 = 600$, $s = 5$ and 10, $\Delta w = 1, 2, 3, 4$ and 5 for several analysis windows and $w_0 = 650$, $s = 10$, $\Delta w = 1$ and 2 for several analysis windows. In this case, perturbation function was defined by $a_{wp} = 25$, $\lambda_{wp} = 0.2\pi^2$ and $t_{wp} = 3.4$ and added to G_0 with $w_0 = 650$ and $s = 30$.

3.2. Study of *E. coli* biolayers

3.2.1. Preparation of samples and recording of spectra

In order to exemplify the applicability of analysis of end-spectrum noise and its connecting with FEDS transform in a real system, a bacterial biolayer was deposited on ultrafiltration cellulose membrane; membrane was previously modified using a spectral marker (CISM®, Mindtech s.a.s., Colombia) which produces a characteristic mid-infrared signal at $\sim 2268 \text{ cm}^{-1}$ which is absent in the bacterial spectra and, in consequence, the presence of that signal can be directly associated with the cellulose support. In this study, this signal was used to produce a signal with a Gaussian-like shape. As bacterial model was used *E. coli* supplied by bank of bacterial strains of Research Group in Science with Technological Applications (Universidad del Valle, Colombia). Later, biolayer were dried at 40°C per 12 hours. Finally, biolayer were analyzed using an infrared spectrophotometer with attenuated transmission reflectance with a ZnSe crystal (FTIR-ATR, IR-Affinity, Shimadzu Co). Biolayer were performed in triplicate, and average spectra for each replicate were recorded by 20 scans from 500 to 4000 cm^{-1} .

Data were extracted in file format .txt in order to carry out the analysis using a spreadsheet. Two analysis windows were selected to exemplify straight-line spectra: $3700\text{--}4000 \text{ cm}^{-1}$ with $k_1 = 0$ and $2500\text{--}2700 \text{ cm}^{-1}$ with $k_1 \neq 0$, and one analysis window with Gauss-type spectrum ($2200\text{--}2400 \text{ cm}^{-1}$).

3.2.2. Pre-treatment of data

For each analysis window, data were normalized respect to the values of minimum and maximum absorbances ($a_j - a_{min}$, respectively).

$$b_j = \frac{a_j - a_{min}}{a_{max} - a_{min}} \quad (17)$$

In order to avoid calculation mistakes resulting to scaling from 0 to 1, the zero absorbance was approximated by the calculation of average value between two adjacent values of absorbance satisfying that $b_{j-1} < b_j < b_{j+1}$ where $b_j = 0$.

Since derivative spectrum is strongly sensitive to noise in the original signal, the smoothing of noise was decreased by the use of average-based spectral filter (ABSF) [12]. ABSF is given by

$$ABSF(b_i; N = 20) = \frac{1}{3} \sum_j^{j+2} (b_j) \Bigg|_{N=1}^{N=20} \quad (18)$$

ABSF is the moving average with a data window of 3 and 20 cycles ($N = 20$). As function is modified by the use of Equation 18, the

same transformation of data is performed on function domain in order to correct small displacements respect to original spectrum (i.e., maximum points in original spectrum should be the same in the Function P).

4. Results and discussion

4.1. Analysis of different types of SLF

4.1.1. Straight-line spectrum

Straight-line spectrum is the simplest situation that can be imagined, however, in this case, it is possible to identify different situations where the line shape in the analysis window could be like a straight-line; typical examples are spectrum segments in very narrow or sharp bands of FTIR spectrum. A first case is identified when the SLF is described by $b_w = k_1$ where k_1 is a constant and $r = 0$. But also, SLF can be described by $b_w = k_1 w + k_2$ or $b_w = -k_1 w + k_2$ where both k_1 and k_2 are constants and therefore $r = k_1/2$.

Therefore, for linear SLF there is an intrinsic noise or “geometric noise” (r_0) of geometrical nature associated with the slope and that can be used to characterize the noise in function of w (r_w). Because r_0 is an absolute minimum, normalized spectrum can be used to define a maximum value of SNR given that SNR is maximum when r achieves its minimum value, and SNR achieves its minimum value when $b_w = r_0$, i.e.,

$$1 < SNR \leq (1/r_0) \quad (19)$$

because $r > r_0$, “ SNR scale” for the description of end-spectrum noise as a function of w can be defined; this scale is seen to be useful for monitoring of noise effect along the spectrum and the noise changes produced by smoothing techniques. Equation 19 can be rewritten as $SNR_1 < SNR \leq SNR_0$ being $SNR_1 = b_w/r_w$ with $b_w = r_w$, $SNR_0 = 1/r_0$ and in the interval $SNR = b_w/r_w$.

In the table 1 the results of the simulation of straight-line spectrum with constant noise is shown. From input parameters used in the simulation, by the slope of ideal straight-line spectrum and equation 10 is concluded that $r_0 = 2.0$ (0.0100) and $SNR_0 = 100.5$ for range “a” whereas $r_0 = 2.0$ (0.0165) and $SNR_0 = 60.5$ for range “b” (in Table 1, the value in parentheses adjacent to r_0 corresponds to the

value of r_0 from the normalized data and a and b denote the number of data in the corresponding analysis windows). It can be seen that for the same range of data, Δw and different amounts of synthetic noise, r_0 can be calculated from linear correlation fit of $n-1$ data whereas r and SNR are easily calculated by equations 1 and 10; however, it is clear that linear correlation fit cannot be used to determine the noise amount in the spectrum.

On the other hand, from simulation shown in Table 1 can be seen that r is an adequate descriptor for the amount noise in the spectrum. Note that, if r is increased then SNR is decreased according to the expected behavior. In addition, the value of n , that is directly related with Δw and the range of data, is seen to produce a marked effect on values of r that are calculated from normalized data and on the corresponding values of SNR ; the above can be understood by the relation among SNR and r , and by the dependence of r of the following parameters: k_1 , w_{max} , w_{min} and n . Note that, for a SLF described by a straight-line function, the change in two consecutive values of absorbance is $\Delta b = b_{w+1} - b_w$ and the corresponding slope is $k_1 = \Delta b / \Delta w$. According to equation 10, $r = \Delta b / 2$ and therefore $r = k_1 \Delta w / 2$. By the use of equation 2 easily can be concluded that

$$r = \frac{k_1(w_{max} - w_{min})}{2n} \quad (20)$$

But also, it can be seen in Table 1 that R/R_n is not affected by n , Δw or the range of data suggesting that this ratio can be used for the comparison of different SLFs. In addition, values k_2 is seen to have not any effect in the description of r being only important k_1 as consequence of the previously deducted conclusion: $r_0 = k_1/2$. In the previous analysis, the correct description of noise by r can be explained since synthetic noise was constant.

It is seen from Table 2 that r_0 can be predicted by a linear correlation fit and therefore this procedure can be used not only to estimate the value of r_0 but also for the making of the noise scale using the equation 19. In addition, if k_1 is increased for the same function P , the synthetic noise is decreased respect to geometric noise, i.e., the contribution of added noise using the function P is decreased; the above behavior is satisfactorily described by R/R_n since this ratio changes from 0.13 to 0.99.

In Figure 1A the function P used has a node denoted q (q indicates

Table 1. SLF simulation of straight-line spectrum with constant noise b_n ($r = \pm 2.0$, ± 4.0 and ± 6.0) for ideal straight-line spectrum described by $b_w = k_1 w + k_2$ ($k_1 = 4.0$ and $k_2 = 1.0$), range of data 1-50 (a) and 1-30 (b) with $\Delta w = 1$ ($b_n = b_w \pm r$).

n	b_n	r_n	SNR_n	r	SNR	R/R_n
50 (a)	± 2.0	2.0 (0.010)	99.5	2.0 (0.010)	99.5	1.00
	± 4.0	2.0 (0.010)	100.5	4.0 (0.020)	50.3	0.50
	± 6.0	2.0 (0.010)	101.5	6.0 (0.030)	33.8	0.33
30 (b)	± 2.0	2.0 (0.017)	59.5	2.0 (0.017)	59.5	1.00
	± 4.0	2.0 (0.017)	60.5	4.0 (0.033)	30.3	0.50
	± 6.0	2.0 (0.016)	61.5	6.0 (0.049)	20.5	0.33

^aFor simulation, $r_0 = 2.0$ (0.010) and $SNR_0 = 100.5$ for range “a” and $r_0 = 2.0$ (0.017) and $SNR_0 = 60.5$ for range “b”. In parenthesis are shown the values of r_n and r calculated from normalized spectra. In addition, r_n and SNR_n are the values of r and SNR calculated by linear correlation fit of data in that order, r_w and SNR are values calculated from equations 10 and 11 and $R/R_n = SNR/SNR_n$

Table 2. SLF simulation of straight-line spectrum described by $b_w = k_1 w + k_2$ ($k_1 = 1.0$ to 16.0 and $k_2 = 1.0$), range of data 1-30 and $\Delta w = 1$.

k_1	r_0	r_n	SNR_n	r	SNR	R/R_n
1	0.5 (0.0161)	0.47 (0.0130)	76.8	3.67 (0.1005)	9.9	0.13
2	1.0 (0.0164)	0.97 (0.0160)	67.2	3.72 (0.0567)	17.6	0.26
4	2.0 (0.0165)	1.97 (0.0149)	62.5	3.91 (0.0317)	31.5	0.50
8	4.0 (0.0166)	3.97 (0.0166)	60.2	4.72 (0.0197)	50.7	0.84
12	6.0 (0.0166)	5.97 (0.0168)	59.5	6.09 (0.0171)	58.3	0.98
16	8.0 (0.0166)	7.97 (0.0168)	59.4	8.00 (0.0169)	59.2	0.99

* Synthetic noise was added to b_w using a standing wave with the following parameters: $a_{wp} = 0.08$, $(2\pi/\lambda_{wp}) = 1/(0.1\pi)$ and $t_{wp} = 3.8$. (in parenthesis are shown the values of r_0 , r_n and r calculated from normalized spectra).

Figure 1. (A) Function P for a standing wave defined by $a_{wp} = 2.5$, $\lambda_{wp} = 0.2\pi^2$ and $t_{wp} = 2.5$, (B) SLF resulting to add the function P to the straight-line spectrum given by $k_1 = 3$ and $k_2 = 0.5$ with corresponding r_w and SNR , (C) Function P for a standing wave defined by $a_{wp} = 2.5$, $\lambda_{wp} = 0.2\pi^2$ and $t_{wp} = 3.5$, and (D) SLF, r_w and SNR resulting to add the function P to the straight-line spectrum ($k_1 = 3$ and $k_2 = 0.5$) (scales have been normalized respect to maximum value to ease the comparison).

the zone with lower noise); thus, when the addition of synthetic noise to straight-line function is performed, the new function that is obtained is shown in Figure 1B. It can be seen that q -point corresponds to node in the SLF, but also, it can be seen that minimum value of r_w is corresponded with this point and with the zone of lower noise in the SNR function. Similar results are obtained when synthetic noise is placed at the ends of analysis window (see Figures 1C and 1D).

The above demonstrate that is possible the use of r for to detect signals with less noise and use them to amplify the signals by multiplication of original spectrum and SNR -based spectrum, in addition, it's possible to perform a spectrum transformation by the

use of SNR in function of w in order to improve the analysis of information contained in the analysis window (exemplification of previous ideas are shown for the analysis of FTIR spectrum).

For an analysis window with shape of curved-line, the segment described by two consecutive values can be assumed to be a straight-line because typically Δw is small and, in consequence, r can be easily calculated. In addition, r_0 can be determined by correlation fit, drawing the corresponding function and applying the Eq. (1). However, in many cases, the analysis of complete band and not only a segment can be useful. Thus, the study of SLF with more complex shapes (Gaussian shape) is analyzed below.

Table 3. Values of geometric noise (r_0) and maximum SNR for normalized Gaussian-type spectrum as a function of w_0 , s and Δw . Corresponding estimations by equation 15 for r_0 and SNR are shown as r_x and SNR_x , respectively.

w_0	s	Δw	Range	n	r_0	SNR	r_x	SNR _x	row
600	5	5	585 - 620	35	0.12496	8.00	0.1250	8.0	(a)
		4	584-616	32	0.1108	9.03	0.1111	9.0	(b)
		3	584-620	36	0.0769	13.01	0.0769	13.0	(c)
		2	582-618	36	0.0526	19.02	0.0526	19.0	(d)
		2	560-640	80	0.0244	41.00	0.0244	41.0	(e)
		2	550-650	100	0.0196	51.00	0.0196	51.0	(f)
		1	582-623	41	0.0238	42.02	0.0238	42.0	(g)
600	10	2	550-650	100	0.0196	51.00	0.0196	51.0	(h)
		1	550-650	100	0.0099	101.00	0.0099	101.0	(i)
650	10	2	600-700	100	0.0196	51.00	0.0196	51.0	(j)
		1	600-700	100	0.0099	101.00	0.0099	101.0	(k)

Figure 2. (a) Gauss-type function with variable synthetic noise defined by a function P ($a_{wp} = 25$, $\lambda_{wp} = 0.2\pi^2$ and $t_{wp} = 3.4$) added to G_0 ($w_0 = 650$ and $s = 30$), (b) r as a function of w for G_0+P , and (c) SNR as a function of w . In addition, the magnification of SNR to show the minimum values of r and the correct correlation with the respective maximum values of SNR (left and right boxes indicating r_1 , r_2 , r_3 and r_4 , respectively). In the top box the G_0 and P functions are shown.

4.1.2. Gauss-type spectrum

A first observation is that Gauss function has a set of values of r_0 depending on the analysis window. The above is a direct conclusion from analysis of straight-line spectrum, thus, because the slope is directly related with the value of r_0 , a change of slope is understood as a change into the r_0 , in consequence, different values of r_0 for different ranges of the Gauss function through SLF can be determined. However, like to the observed for straight-line spectrum, for Gauss-type spectrum the value of r_0 is strongly affected by the wavenumber range (i.e., w_{min} and w_{max}), Δw and n . It can be seen in the Table 3 that for different ranges and resolutions,

rows from **a** to **d**, different values of r_0 are obtained for the same Gauss function. In addition, when resolution is a constant for similar analysis windows, by instance for rows from **d** to **f**, different values of r_0 are obtained for the same function. On the other hand, it can be seen that for the same values of Δw and n , the values of r_0 are the same (see rows **f**, **h** and **j**, and rows **i** and **k**); the above illustrates the relevant of these parameters for the determination of r_0 and the careful to take during the noise behavior for different analysis windows. By the analysis of results obtained for several Gaussian functions, it can be concluded that for this type of functions an acceptable estimation of r_0 (r_x) can be calculate by

$$r_x = \frac{\Delta w}{n + \Delta w} \quad (21)$$

Results of estimation of r_0 and SNR are shown in the Table 3. It can be seen that to obtain the value of r_0 in a Gauss-type spectrum only it must be known Δw and n (rows **f**, **h** and **j** or rows **i** and **k**). As it was previously concluded, r can be calculated by equation 10.

In Figure 2 is shown a Gauss-type function with variable synthetic noise defined by a function P which was added to G_0 (P and G_0 are shown in the top box); it can be identified clearly the distribution of noise in the resulting function. Also, it is shown the description of noise by the use of definition of r as a function of w for G_0+P . The description achieved by r is completely congruent with G_0+P function ($r_1 < r_2 < r_3 < r_4$). Finally, SNR as a function of w showed a correct description of noise distribution, permitting the identification of points with lower noise.

4.1.3. Analysis of biolayers of *E. coli*

In Figure 3 is shown the average spectrum obtained for biolayer of *E. coli* (only region between 2100-4000 cm^{-1} is shown due the three analysis windows selected to exemplify the analysis can be observed in this part of spectrum). It can be seen that spectrum shows typical signals associated with vibrations associated to hydroxyl and amino groups (3000-3600 cm^{-1}), vibrations C-H of CH_3 and CH_2 (2800-3000 cm^{-1}), and it is evident that overlapping of signals. By the above, the spectrum analysis is limited by direct analysis of vibrations and it is in these situations when spectrum digital transformation is useful.

Figure 3. (A) Average IR spectrum of *E. coli* between 2100 and 4000 cm⁻¹, (B) and (C) analysis windows 1 and 2 between 3700-4000 cm⁻¹ and 2500-2700 cm⁻¹, respectively, as models of straight-line spectra, (D) analysis window 3 between 2200-2400 cm⁻¹ as model of Gauss-type spectrum and (E) illustration of bacterial biolayer.

Figure 4. (A) Analysis window between 3700-400 cm⁻¹ corresponding to normalized average spectrum ("blue plot" with 1/0.0037 as normalization factor; dash line is the corresponding correlation fit) and normalized SNR-based spectrum ("red plot" with 1/252.2 as normalization factor) and (B) Analysis window between 3700-400 cm⁻¹ corresponding to normalized-smoothed average spectrum ("blue plot" with 1/0.0027 as normalization factor) and normalized SNR-based spectrum ("red plot" with 1/19436.4 as normalization factor).

In region B, analysis window can be assumed to be a straight-line spectrum with slope zero, however, fluctuation of signal evidences the presence of residual noise in this analysis window (the maximum change is ~ 1 % of maximum signal, therefore the signal recorded in the spectrum can be considered to be noise). In region C, analysis window can be assumed to be a straight-line spectrum with slope different to zero with a subjective overlapping due to it is not possible identify a signal (a small fluctuation which could be noise being in this point lower than B, here, the maximum change is ~ 0.1 % of maximum signal). Finally, in the region D, analysis window can be assumed to be a Gauss-type window with intermediate noise in comparison with B and C.

The analysis of residual noise in the region B is shown in Figure 4. Thus, from Figure 4A, the SNR shown that two signals excel (q₁ and q₂ with SNR of 1.0 and 0.8 in the normalized spectrum with maximum SNR = 252.2 in the original spectrum; however, in this analysis window the a_{max} is only 0.0037). If spectrum is smoothed using moving average, with a cycle of twenty and a window of three, the noise is decreased and the effect of smoothing is manifested in a simplification of SNR-based spectrum (see Figure 4B). As result of smoothing, two signals q₃ and q₄ are visualized and increased respect to other signals. By near spectroscopy, the vibrations associated to overtones can be identified, thus, between 3700-400 cm⁻¹ is expected to see, for example, two second-order overtones of the CH deformation (3945 and 3757 cm⁻¹). However, these signals should be weaker by 1/10–1/50 than the bands of the first-order combination and usually they are neglected [20]. In our case, these signals also are neglected because information obtained from them is not important in the mid-infrared, but in the framework of our analysis of noise, it is evidenced that potential signals could be hidden in the noise and be revealed by analysis of SLF using end-spectrum noise concept.

In the region C, analysis window is shown in Figure 5A for original spectrum (blue plot), and SNR as a function of wavenumber (red plot). Linear correlation fit is described by $a = 0.0027w - 6.1553$ with correlation coefficient of 0.9906. In consequence, R and r_0 are estimated to be 0.00734 and 0.00135, respectively. In order to minimize the noise, a smoothing is performed (with a window of 3 and 20 cycles) and smoothed spectrum can be seen in Figure 5B. Note that, 3 new signals can be identified in the SNR-based spectrum (q₁, q₂ q₃ and q₄). These signals correspond to spectrum data in the analysis spectrum with low SNR; when smoothing is performed, segments where the distance between points is very small are smoothed producing a straight-like segment with slope near to zero, thus, by definition of r , as slope is decrease then r is decreased and SNR is increased. Therefore, points observed in SNR-based spectrum are associated with larger values of SNR and could be understood to be "hidden" signals. In the figure C is shown a comparison between FEDS intensity obtained for smoothed spectrum. The evident correlation suggests that capacity of FEDS to deconvoluted the spectrum is a result of increasing of SNR, in other words, FEDS is capable of find and increase the points on SLF with greater SNR, being the reason of capacity of FEDS transformation of increase the spectral resolution of signals.

Figure 5. (A) Analysis window between 2500-2700 cm⁻¹ corresponding to normalized average spectrum ("blue plot" with 1/0.0355 as normalization factor; dash line is the corresponding correlation fit) and normalized SNR-based spectrum ("red plot" with 1/2667 as normalization factor), (B) Analysis window between 2500-2700 cm⁻¹ corresponding to normalized-smoothed average spectrum ("blue plot" with 1/0.035 as normalization factor) and normalized-smoothed SNR-based spectrum ("red plot" with 1/593.2 as normalization factor) and (C) comparison normalized-smoothed SNR-based and FEDS spectra.

As biolayer are formed by bacterial biofilms, surface is formed by a mixture of substances with different structural features, however, this biofilm has been reported to be constituted by glycoproteins and peptides which act as adhesins and usually have units of cysteine (see figure 5E). Cysteine has two links with vibrations appearing in this analysis window. In general, S-H vibration has been published to be a weak vibration between 2550 - 2560 cm⁻¹ or 2550-2600 cm⁻¹ [21, 23]. However, a complete assignment of the vibrational spectra of L-cysteine solid state including orthorhombic and monoclinic forms by the use of a combination of computational and experimental methods show three vibrations associated with S-H bond (2543, 2545 and 2552 cm⁻¹) and two vibrations associated with N-H bond (one in the analysis window at ~2639 cm⁻¹) [24]. The analysis permits conclude that effectivity of SNR-based spectrum and FEDS transform to improve the resolution of spectra is improved by the use of smoothing techniques, but also, the use of smoothing minimize the appearing of artefacts in the modified spectrum.

The equivalence between SNR-based spectrum and FEDS transform can be understood by the incorporation of end-spectrum noise defined in this paper. It can be seen that FEDS transform can be easily written as a function of SNR by the use of equation 10, thus

$$b = f\sqrt{SNR} \quad (22)$$

By graphical analysis can be seen that $f = (1+a_n)(a_{n-1}/2)^{0.5} \approx a$.

In the region D, analysis windows for original, SNR-based and FEDS spectra (blue, red and black plots, respectively) are shown in Figure 6A. It can be seen that SNR-based and FEDS spectra are very sensitive to end-spectrum noise since both transformations are related with the changes in the SLF. Again, it is observed that smoothing improves considerably the quality of analysis (see Figure 6B).

By SNR-based and FEDS spectra four signals are identified from one single signal identified from original spectrum in the analysis window (q₀, q₁, q₂ and q₃) and two minor signals with intensity lower than 0.2 (20 % compared with maximum value q₀) and are associated with overlapping of adjacent signals, therefore, these were named overlapping' signals (see Figure 6D). On the other hand, q₀ and q₁ are main signals because they have the greater SNR. However, when SLF has slope ~ 0 for minimum and maximum points, being the maximum points associated with signals whereas minimum points are associated with intersection of overlapped signals completely defined (see Figure 6C), in consequence, q₁ is discarded.

By the above, for the biolayer analyzed q₀ at 2290 cm⁻¹ is associated with spectral marker (–NCO) [21], q₂ and q₃ at 2382 and 2340 cm⁻¹ are associated with antisymmetric C=O stretching vibration of CO₂ (g) and CO₂ (aq), respectively; which can be produced by metabolism of biofilm and re-absorbed as result of hydrogel-type behavior [25-26]. But also, q₃ is suggested that could be associated with –OH in O=P–OH [27-28], which is present on structure of phospholipids constituting the external surface of bacteria [29].

Figure 6. (A) Analysis window between 2200-2400 cm⁻¹ corresponding to normalized average spectrum ("blue plot" with 1/0.026 as normalization factor), and FEDS transformation of normalized spectrum (1/30.77 as normalization factor) and (B) Analysis window between 2200-2400 cm⁻¹ corresponding to normalized-smoothed average spectrum ("blue plot" with 1/0.025 as normalization factor), normalized-smoothed SNR-based spectrum ("red plot" with 1/2914 as normalization factor) and FEDS spectrum ("black plot" with 1/61 as normalization factor) and (C) enlargement of spectrum segment associated with point q_1 .

5. Conclusions

It is possible to carry out the study of residual noise and the corresponding SNR in the spectrum by concept of end-spectrum noise and analysis of SLF. Thus, by SNR is possible detect and differentiate the signals with greater contribution of fluctuation of SLF associated with the noise. However, the transformation of FTIR spectrum to SNR-spectrum or FEDS-spectrum is sensitive to noise and, in consequence, smoothing should be performed in order to

improve the analysis. In addition, it is demonstrated that effectivity of FEDS can be understood by end-spectrum noise concept; in this way, FEDS algorithm increases the signals with greater SNR from derivative of spectrum inverse and simple mathematical functions. From analysis of biolayers, it is concluded that when a small analysis window is selected, the effectivity of transformation is improved. Finally, it is concluded that deconvolution spectrum can be performed easily by FEDS or SNR-spectrum, permitting the separation of overlapped signals and the detection of hidden signals.

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