Functionally-Enhanced Derivative Spectroscopy (FEDS): A methodological approach

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Graphical Abstract

Abstract. Functionally-Enhanced Derivative Spectroscopy (FEDS) is a simple, fast and useful method which can be used to increase mathematically the spectral resolution of infrared spectra. Though is a developing analytical technique, at the present, several applications for its use in the analysis of infrared spectra have been described. However, it is use is limited because it is a new methodological propose which requires to advance in its evaluation and interpretation. By the above, a detailed description of procedure for its application is an important alternative to increase its use. The objective of this work is to describe the methodological steps for the use of FEDS.

Keywords: Vibrational spectrum, mid-IR spectrum, deconvolution, derivative spectroscopy, spectral analysis

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Protocol

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1. Introduction

Mid-infrared spectroscopy (Mid-IR Spectroscopy) is a well-known spectroscopic technique, based on the use of infrared radiation to produce changes of vibrational states at molecular level. In addition, it is a non-destructive, fast, easy to use and highly sensitive analytical methodology characterized because to require a small sample size without complex pre-treatment procedures. Mid-IR Spectroscopy is a very useful method for the determination of the presence or absence of functional groups, the analysis of molecular interactions, the monitoring and examination of changes in the intensity of signals (Alvarez-Ordoñez et al., 2011; Ojeda and Dittrich, 2012; Prakash et al., 2013). However, for the analysis of complex systems, the main limitations of infrared spectroscopy are the obtaining of very similar spectra in very different samples but with similar components, a high overlap of signals, and the widening of adjacent signals in multicomponent systems (Alvarez-Ordoñez et al., 2011; Ojeda and Dittrich, 2012; Prakash et al., 2013). For the decrease of negative impact of these limitations, usually mathematical and computerized methods are used, being the most common the Fourier deconvolution (Friesen and Michaelian, 1991; Vazhnova et al., 2013; Palencia, 2018). However, though from a conceptual approach the Fourier deconvolution is simple, its application is limited by the 'complexity of computation', the appearing of negative intensities from calculations, the highly sensitive to the noise and the appearing of 'false' signals resulting of mathematical arguments without physical meaning (Friesen and Michaelian, 1991). In contrast, Functionally-Enhanced Derivative Spectroscopy (FEDS) is seen to be a simpler method for the deconvolution and the increase of spectral resolution of infrared signals (Palencia, 2018). Although FEDS was thought for the analysis of multicomponent systems, one-component systems also can be analyzed using the same procedures, and given that spectra for these systems can be defined as a combination of multiple vibrational patterns resulting of different energetic states, the use of analysis FEDS for one-component system maintains the same objective than that for multi-component systems. A summarize of applications of FEDS are described in the Table 1. The objective of this work is to describe the methodological steps for the use of FEDS by the analysis of ethanol as model of one-component system.

2. Protocol: mIR-FEDS 2020

Protocol described here, mIR-FEDS 2020, it contains small changes respect to procedure described in the initial publication of 2018. These are the result of review and adaptation of FEDS algorithm during the analysis of a higher sample number and types. The FEDS application is based on four stage: recording of spectra, smoothing, auto-scaling, deconvolution by algorithm FEDS and analysis.

2.1. Smoothing of spectra

The aim of this procedure is to decrease the spectral noise and variability of data. Two key steps are included:

Table 1. Summarize of applications of FEDS 2018-2020.

System	Objective	Reference
Pure liquids	Spectral characterization of water, ethanol, butanol, hexanol, formic acid, acetic acid, citric acid	(Palencia, 2018)
Binary liquid mixture	Deconvolution for the determination of dimerization constant of acetic acid in water	(Palencia, 2018)
Humified organic matter	Spectral characterization of humin from agricultural soils	(García-Quintero et al., 2018)
Microbiological surface	Characterization of artificial biofilms of nitrogen-fixing bacteria (<i>Azotobacter chroococcum</i>)	(Berrio et al., 2018)
Nanomaterials	Deconvolution of surface plasmon resonance in ultraviolet spectroscopy of silver nanoparticles	(Sanchez et al., 2018)
Biosurfaces	Mid-infrared spectral characterization of fish scales from "Bocachico" (<i>Prochilodus magdalenae</i>)	(Anaya-Tatis et al., 2019)
Polymer surface	Deconvolution of mid-IR spectra of PVC surface for biomedical materials	(Palencia et al., 2019a)
Multicomponent liquid systems	Detection of solutes in the phase of bulk liquid membranes	(Palencia et al., 2019b)
Binary liquid mixture	Deconvolution of mid-IR spectrum of triethylamine-acetone	(Otálora and Palencia, 2019)
Polymer hydrogels	Deconvolution of mid-IR spectra of polymer hydrogels	(Otálora et al., 2019)
Polymer fibers	Deconvolution of mid-IR spectra of surface of cellulose fibers modified with pyrolytic bio-oil	(Palencia et al., 2020b)
Polymer matrixes	Deconvolution of mid-IR spectra of functional polyurethanes based on cationic aminoglycosides and loaded with phytohormones	(Palencia et al., 2020a)
Polymer matrixes	Antibacterial and non-hemolytic cationic polyurethanes with N-carboxymethyl-N,N,N-triethylammonium groups	(Palencia et al., 2020d)
Microbiological surface	Characterization of outer surface of <i>Helicobacter pylori</i> biofilms	(Palencia et al., 2020c)

• **Ensuring reproducibility:** For a sample, spectra must be recorded at least in triplicate, using the same experimental parameters. This is suggested in order to decrease the noise and the random effects by the use of averaged spectrum. In addition, spectra are usually recorded in absorbance or transmittance units, they should not be modified by the equipment software, and some type of baseline correction should not be applied. The above is in order to advance in the building of comparable FEDS spectra, therefore, all information related with number of replicates, resolution, among others should be reported.

• **Decreasing the noise:** Any smoothed algorithm can be used, however, it is suggested ABSF because is simple and easy to usage (Palencia et al., 2018).

Table 2. Example of smoothing based on ABSF filter.

j	Averaged data		Smoothed data	
	ν_j (cm ⁻¹)	a_j (u.a.)	w_j (cm ⁻¹)	b_j (u.a.)
1	501.0	0.824	$w_1 = \frac{a_1 + a_2 + a_3}{3} = 501.0$	$b_1 = \frac{a_1 + a_2 + a_3}{3} = 0.827$
2	502.0	0.827	$w_1 = \frac{a_2 + a_3 + a_4}{3} = 502.0$	$b_1 = \frac{a_1 + a_2 + a_3}{3} = 0.823$
3	503.0	0.831	$w_1 = \frac{a_3 + a_4 + a_5}{3} = 503.0$	$b_1 = \frac{a_1 + a_2 + a_3}{3} = 0.817$
4	504.0	0.812	504.0	0.813
5	505.0	0.808	504.5	0.814
6	506.0	0.820	505.0	0.820

Note that equation 2 is only a different form to write the equation 1. It is important to note that, as result of smoothing using the equation 1, a number of data equal to two times the number of applied cycles is lost (i.e., if analysis window is 500 cm⁻¹ to 4000 cm⁻¹ then the new range obtained will be 520 cm⁻¹ to 3980 cm⁻¹). Smoothing by ABSF filter is exemplified step-by-step in Table 2. Corresponding illustration is shown in Figure 1.

2.2. Auto-scaling of spectra

The goal of this procedure is to delimit the absorbance data between zero and one. For that

$$q_j = \frac{b_j - b_{\min}}{b_{\max} - b_{\min}} \quad (3)$$

where q_j is the corresponding auto-scaled absorbance. In order to avoid calculation mistakes resulting to scaling from 0 to 1 during the application of FEDS algorithm, the zero absorbance, $q_j = 0$, must be approximated by the calculation of average value between two adjacent values of absorbance satisfying: $q_{j-1} < q_j < q_{j+1}$ when $q_j = 0$. Note that any other procedure could be performed in order to eliminate $b_j = 0$. Here, it is recommended the calculation of the average between adjacent values because it is easy to automatize even in a spreadsheet and, being the lowest absorbance value, the effect on the spectrum is negligible.

Note that FEDS can be applied to the entire spectrum or to a portion of it. It is desirable to perform FEDS at intervals and not the full spectrum, since a signal with a relatively high intensity can cause a strong decrease in the intensity of the adjacent data. A narrow interval provided greater ease of analysis, an aspect of great importance considering that the complexity of the FEDS spectrum is greater than that of the original spectrum. An illustration using the same data in Table 1 is shown in the Table 2.

2.3. Transformation of data by FEDS

For the obtaining of FEDS intensities, the first step is the calculation of the first-order derivative of the inverse of normalized spectrum, later, it is calculated the square root of the absolute value of the previous result, and subsequently, it is calculated the respective inverse. A factor $1+q_j$ is introduced in order to assign a weight to the intensities from the experimental data. Finally, FEDS data are

Figure 1. Illustration of smoothing based on ABSF filter for data in Table 1 (Number of cycles = 1).

$$ABSF(a_i; N = 20) = \frac{1}{3} \sum_{j=1}^{j+2} (a_j) \Bigg|_{N=1}^{N=20} \quad (1)$$

where a_j is the j -th absorbance. ABSF is the moving average with a data window of 3 and 20 cycles ($N = 20$). Thus, for each a_j , a_{j+1} and a_{j+2} , the corresponding average value is calculated, and subsequently, this procedure is repeated N times. In addition, the same transformation of data must be performed on values of wavenumbers (ν) to correct the displacements respect to original spectrum (i.e., maximum points in original spectrum should be the same in the original and smoothed spectra) (Palencia, 2018). Here, it is recommended the use of $N = 20$, but depending of quality of spectra in term of noise, values of N larger than 20 could be used. Thus, for the first cycle, new spectral data are calculated by

$$b_j = \frac{a_j + a_{j+1} + a_{j+2}}{3} \quad (2)$$

normalized respect to the maximum value. The above can be described in one single steps, but, in order to be didactic, three steps are shown below:

$$x_j = \left| \frac{1}{q_j} - \frac{1}{q_{j-1}} \right| \quad (6)$$

$$y_j = \frac{1 + q_j}{\sqrt{x_j}} \quad (7)$$

$$p_j = \frac{y_j}{y_{max}} \quad (8)$$

where x_j and y_j are auxiliary functions, p_j is the normalized result of called "function P", and y_{max} is the maximum value of auxiliary function y . Note that, all data are transformed without alteration in their relative position, and with adequate scale factor (ϕ) is possible to increase or decrease the FEDS intensities. In particular, if $\phi < 1$, then intensities are increased. Thus,

$$p_j = \frac{y_j}{\phi y_{max}} \quad (9)$$

2.3. Analysis of FEDS spectrum

To carry out the analysis of FEDS spectra is possible to define several strategies. Really, analysis depend on each researcher. However, to be ease the analysis, here it is shown a systematic procedure. Besides, a standard terminology is introduced and recommended.

Always that FEDS analysis is applied, after to obtain the corresponding FEDS spectrum (here, by spectrum should be understood the total spectrum or any range of interest). In addition, it is recommended to carry out its overlapping with the experimental spectrum, thus, it is possible identify the main signals of IR spectrum transferred to FEDS spectrum. Note that, it is expected that all signals in the IR spectrum appear in the FEDS spectrum, this signal are named as "inherited signals". The above sometimes is not true because the relative intensity with respect to the normalization signal, the overlapping of signals, and the width of the adjacent signals influences the transfer of the signals from the IR spectrum to the FEDS spectrum. However, the overlapping of spectra permits identify the inherited signals which have the same physical meaning assigned in the infrared spectroscopy. Therefore, the overlapping of the spectra eases the assignation of signals since the problem is reduced to classical analysis used in IR spectroscopy.

On the other hand, other signals are "pure signals", it is to say, signals resulting of FEDS and not necessarily they are associated with signals of IR spectrum. Two types of pure signals can be identified: "Hidden signals", and "Gauss-overlay signal". In the first case, Hidden signals are signals that were hidden and that by action of the algorithm are showed in the FEDS spectrum, these signals have a physical meaning equal to the respective signals in the IR spectrum, however, they are not clearly visible and, in consequence, their assignation should be performed very carefully, using

comparisons with IR spectra of the compounds or substances that are most similar to the samples, or by comparing with simulated spectra using some computational method well-established. Gauss-overlay signals are signals caused by the effect of adjacent bands. They are not yet fully understood and their study is currently in development. However, it is known that when two adjacent Gaussian signals overlap, depending on the distance and degree of overlap, a third signal associated with such overlap emerges. This signal impact the shape of FEDS spectrum, but has the advantage that they are relatively easy to identify, usually, they are associated with a relative minimum. These signals have been used to measure displacements and to validate the existence of two overlapping signals (Palencia et al., 2020; Palencia et al., 2021). In Figure 2 is exemplified FEDS deconvolution for mid-IR of soil sample obtained by attenuated total reflectance (ATR).

3. Conclusions

A systematic description of protocol for the application of FEDS was described. It can be concluded that FEDS is an easy tool for the mid-IR analysis of complex samples where overlap makes analysis difficult. In addition, an important factor determining the quality of analysis is the reproducibility, in consequence, at least three spectra should be recording for each sample. Finally, three types of FEDS signals can be identified throughout the spectrum: (i) Inherited signals, (ii) Hidden signals, and (iii) Gauss-overlay signals. It is necessary advance in the standardization of signals for an adequate implementation of the tool in short time.

Table 2. Example of normalization using the equation 5 ($q_{max} = 1$ and $q_{min} = 0$).

Smoothed data		Normalized data		Change of	Observations
w_j (cm^{-1})	b_j (u.a.)	w_j (cm^{-1})	q_j	$q = 0$	
502.0	0.823	502.0	1.000	1.000	q_{max}
503.0	0.817	503.0	0.367	0.367	-
504.0	0.813	504.0	0.000	0.217	$q_{min} = (0.367 + 0.067)/2$
504.5	0.84	504.5	0.067	0.067	-

Figure 2. Example of FEDS spectrum (red line) obtained by deconvolution of mid-IR spectrum (blue line) of one soil sample: **H** denotes "Inherited signals", **h** denotes "Hidden signals" and **G** denotes Gauss-overlay signals.

Conflict interest. Authors declare that there is no conflict of interest regarding the publication of this paper.

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