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REVIEW ARTICLES

Wavelet Transforms and Applications in Drug Analysis

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SUMMARY

As it is known, the basis of the modern analytical chemistry is the instrumental analysis methods based on the evaluation of analytical signals such as spectra, chromatograms, kinetic curves and others obtained from instruments. Nowadays, the traditional evaluation of analytical signals may not always provide the desired results for the chemical and pharmaceutical analysis, where most of the analysis processes is hyper complex. Hence, combined application of conventional instrumental methods and some chemometric signal processing methods can be necessary for the analysis of complex systems. As a result, conventional analysis techniques coupled with signal processing tools enhance their ability of resolution, separation and analysis tremendously. In this context, several signal processing tools have been developed for many application areas from data analysis to data compression. One of the newest additions has been wavelets.

Wavelet transform (WT) can be classified into two categories; discrete wavelets transform and continuous wavelets transform. WT approach is a powerful signal processing tool for data reduction, denoising, baseline correction and resolution of overlapping spectra. In our previous studies, the WT signal processing tools in combination with conventional spectral analysis techniques were applied to the analysis of drugs in multicomponent samples. Very recently, fractional wavelet transform was successfully applied to increase the lower signal content and to reduce the spectral data length for the drug analysis.

In this review I will give some typical applications of the wavelet transforms to spectrophotometric, voltammetric and chromatographic signals for the analysis of drug substances.

Key Words: *Drug analysis, chemometrics, signal processing, wavelet transform, continuous wavelet transform, discrete wavelet transform.*

Dalgacık Dönüşümleri ve İlaç Analizinde Uygulamaları

ÖZET

Modern analitik kimyanın temeli spektrum, kromatogram, kinetik eğri gibi analitik sinyallerin değerlendirilmesine dayanan enstrümental analiz yöntemlerine dayanır. Günümüzde kimyasal ve farmasötik analiz süreçlerinden elde edilen ve çok karmaşık olan analitik sinyallerin geleneksel olarak değerlendirilmeleri istenen sonuçları vermeyebilir. Bu, karmaşık sistemlerin analizinde geleneksel enstrümental yöntemlerin kemometrik sinyal işleme yöntemleri ile birlikte kullanımını gerektirmektedir. Sonuç olarak, geleneksel analiz tekniklerinin sinyal işleme yöntemleri ile birlikte kullanımı bu tekniklerin çözüm, ayırım ve analiz güçlerini arttırmaktadır. Bu konu kapsamında, veri analizinden veri sıkıştırmaya kadar çeşitli uygulama alanı için pek çok sinyal işleme yöntemi geliştirilmiştir. Bunlara eklenen son yöntem dalgacıklardır.

Dalgacık dönüşümleri (DD) iki kategoriye ayrılabilir: kesikli dalgacık dönüşümü ve sürekli dalgacık dönüşümü. DD yaklaşımı, veri azaltılması, gürültü giderilmesi, taban çizgisinin düzeltilmesi, girişim yapan spektrumların ayrımı için oldukça güçlü bir sinyal işleme tekniğidir. Daha önceki çalışmalarımızda, geleneksel spektral analiz ile DD sinyal dönüşümü kullanılarak birden fazla etken madde içeren ilaç preparatlarının analizi gerçekleştirilmiştir. Yakın geçmişte ise, kesirli DD yöntemi, düşük analitik sinyallerin arttırılması ve ilaç analiznde spektral verinin azaltılması için uygulanmıştır.

Bu derlemede, dalgacık dönüşümlerinin spektrofotometrik, voltametrik ve kromatografik sinyalleri üzerine uygulanması ve ilaç maddelerinin analizi ile ilgili karakteristik uygulamalarından bahsedeceğim.

Anahtar kelimeler: *İlaç analizi, kemometri, sinyal işleme, dalgacık dönüşümü, sürekli dalgacık dönüşümü, kesikli dalgacık dönüşümü*

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Introduction

In recent years, the new advancements in computer, information science, statistics and applied mathematics with new combined analytical instrumentation devices offer new opportunities to chemists and pharmacists for their researches and analytical applications to solve complex analysis problems.

In the analytical studies, the separation techniques, LC and CE combined with various spectroscopic systems (hyphenated techniques namely LC-MS and CE-MS) have been applied to provide additional chemical information and to decrease the complexity for the analysis of multicomponent mixtures. These approaches require a preliminary separation and other tedious analytical processes during analysis for searching optimal separation and other chromatographic conditions. In addition to that, these methods using the combined devices bring high cost and time-consuming for analysis. Therefore, the separation methods based on high technology may not provide successful analytical results in all cases.

Due to the disadvantageous of the above mentioned separation techniques; analytical chemists prefer the use of the spectroscopic methods (instead of separation techniques) going to rapid analysis with low cost, if it is possible successful outcome. Particularly derivative spectrophotometry and its modified versions have been intensively used for fast quantitative resolution of multi-component mixtures without separation step. However, in all cases, these spectral methods may not lead good analytical results due to characteristics of strongly overlapping spectra of compounds, interference of main peaks with noise, baseline problems, decreasing signal intensity and worsening signal-to-noise ratio (S/N) for higher derivative orders.

In this context, to overcome the drawbacks of the above traditional separation and spectroscopic methods, analytical chemists need to develop new signal analysis techniques, approaches or methods for the efficient quantitative resolution of complex mixtures.

Recent developments in signal processing methods gave us more opportunity for the better quantitative resolution of the complex analytical problems as well as other areas of science. One of the newest additions is wavelets for the spectral quantification of compounds in mixtures. Wavelet transform (WT) has gained wide acceptance as a valuable tool for signal processing tasks, due to their wide range of applications. WT is classified as discrete wavelets transform (DWT) and continuous wavelets transform (CWT) (Daubechies, 1992). In recent times, the developments of wavelet transforms and applications in the analytical chemistry have significantly amplified the potential power of various analytical techniques (Walczak, 2000; Brereton, 2003; Chau *et al.*, 2004; Dinç and Baleanu, 2007a). Several applications of wavelets in chemistry were reported (Nie *et al.*, 2001; Shao and Zhuang, 2004; Dinç *et al.*, 2008; Süslü *et al.*, 2009).

In our previous investigations, CWT method with zero-crossing technique and ratio spectra procedure were directly applied to the spectral multicomponent determination of active compounds in mixtures in presence of the strongly overlapping absorption bands without using any priory chemical pretreatment such as derivation and extraction and successful results were obtained (Dinç and Baleanu, 2003a; Dinç and Baleanu, 2003b; Dinç *et al.*, 2003; Dinç and Baleanu, 2004a; Dinç and Baleanu, 2004b; Dinç and Baleanu, 2004c; Dinç *et al.*, 2004; Dinç *et al.* 2004; Dinç *et al.* 2005; Dinç *et al.* 2005; Dinç *et al.*, 2005; Dinç and Baleanu, 2007b; Dinç and Baleanu, 2007c; Uğurlu *et al.*, 2008; Dinç and Baleanu, 2008; Pektaş *et al.*, 2009)

Very recently, fractional wavelet transform (FWT) was successfully applied to the spectral mixture analysis to increase the lower signal content and to reduce the spectral data length for the drug analysis (Dinç and Baleanu, 2006; Dinç, *et al.* 2006; Dinç *et al.*, 2007; Dinç and Baleanu, 2010).

The aim of this study is to demonstrate the analytical applications of the CWT and FWT to the multicomponent determinations in chemical and pharmaceutical analysis.

Continuous wavelet transform

Nowadays, wavelets are popular tools as signal processing methods in chemistry. Wavelet is based on the idea of frequency-scale decomposition of signals. The CWT in signal analysis offers many advantageous over the traditional frequency decomposition. For example, the WT is localized in both time and frequency while FT does not give any information of the signal in the time domain.

In the wavelet analysis, the signal is expanded on a set of the dilatation (a, scaling parameter) and translation (b, shifting parameter) of functions;

$$\psi\left(\frac{x-a}{b}\right) \quad (1)$$

The scaling parameter is an important role to change time and frequency resolution when analyzing the signal.

The action of a given CWT on a function *f(x)* is given below;

$$CWT\{f(x);\, a,b\} = \int_{-\infty}^{\infty} f(x)\psi^{*}_{a,b}(x)dx = \langle f(x), \psi_{a,b} \rangle \quad (2)$$

where the superscript * denotes the complex conjugate and $\langle f(x), \Psi_{a,b} \rangle$ represents the inner product of function *f(x)* onto the wavelet function $\psi_{a,b}(x)$.

Fractional Wavelet Transform

Fractional wavelet transform (FWT) is a new mathematical tool for the signal and image analysis. This CWT approach is based on the signal decomposition and reconstruction. In case of the analysis of the composite signals, FWT provide functions of the spectral data compression and de-nosing by extracting the covered information from the composite signals of the components in their mixtures.

A new wavelet transform based on the fractional B-splines was initiated in early 2000s. (Unser and Blu, 2000; Blu and Unser, 2000; Blu and Unser, 2002). The mathematical idea of fractional derivatives has represented the subjected of interest for various branches of science. As it already known the splines play a significant role on the early development of the theory of wavelet transform. Brief mathematical information for FWT is explained below.

A B-spline of order n is defined as follows;

$$\beta^{n}_{+}(\mathrm{x}) = \frac{\sum_{k=0}^{n+1}\binom{n}{k}(-1)^{k}\,(x-k)^{n}}{\mathrm{n!}} \quad (3)$$

where $\binom{n}{k}$ denotes classical binomial coefficient.

Following the same idea as in the fractional calculus case, namely we replace the factorial with gamma function and n with alpha, we obtain the fractional B-spline as given below;

$$\beta^{\alpha}_{+}(\mathrm{x}) = \frac{\sum_{k=0}^{+\infty}(-1)^{k}\binom{\alpha+1}{k}\left(x-k\right)^{\alpha}_{+}}{\Gamma(\alpha+1)} \quad (4)$$

where Euler's Gamma function is given by;

$$\Gamma(\alpha+1) = \int_{0}^{+\infty} \mathrm{x}^{\alpha}\mathrm{e}^{-\mathrm{x}}\mathrm{dx} \quad (5)$$

and the generalization of the binamial coefficient is given below;

$$\binom{\alpha}{k} = \frac{\Gamma(\alpha+1)}{\Gamma(k+1)\Gamma(\alpha-k+1)} \quad (6)$$

and $n-1 \prec \acute{a} \prec \mathrm{n}$. In addition;

$$x^{\alpha}_{+} = \begin{cases} X^{\alpha}, & x \geq 0 \\ 0, & \text{otherwise} \end{cases} \quad (7)$$

denotes the one sided power funtion.

We notice that that classical forward difference of order n is replaced in (4) withe the fractional counterpart.

The fractional B-spline wavelets was defined as;

$$\psi^{\alpha}_{+}\left(\frac{x}{2}\right) = \sum_{k\in Z}\frac{(-1)^{k}}{2^{\alpha}}\sum_{l\in Z}\binom{\alpha+1}{l}\beta^{2\alpha+1}_{*}(l+k-1)\beta^{\alpha}_{+}(x-k) \quad (8)$$

We mention that the fractional splines wavelets of degree α obey the following;

$$\int_{-\infty}^{+\infty} \mathrm{x}^{n}\Psi^{\alpha}_{+}(\mathrm{x})\,\mathrm{dx} = 0, n = 0,\ldots,[\alpha] \quad (9)$$

and the Fourier transform fulfills the following relations;

$$\hat{\Psi}^{\alpha}_{+}(\varpi) = \mathrm{C}\left(\mathrm{j}\varpi\right)^{\alpha+1}, \text{as } \varpi \to 0, \quad (10)$$

We end the section claiming that the fractional spline wavelets behaves like fractional derivative operator. As a result it can be used to investigate the quantitative analysis for complex chemical mixtures.

Wavelet transforms and Applications

As it is known, the transformation of UV-VIS signals is widely used in the analytical chemistry for the quantitative resolution of two-component mixtures. This method is an approach based on the derivation of absorption spectra of components giving overlapping spectral bands in the same spectral wavelength region. However, one of the fundamental problems of this method, also known as derivative spectrophotometry, is that the signal-to-noise ratio (S/N) becomes progressively worse for higher order. In addition, this classical derivative method may not provide a good spectral resolution in some cases.

On the other hand, one of the main problems of the application of the spectrophotometric, chromatographic and voltammetric methods to the analysis of mixtures

is the overlapping peaks or overlapping signals. In addition, some of other problems of analytical chemistry are baseline correction, de-nosing and smoothing in the application of the methods based on the evaluation of analytical signals to get reliable, precise and accurate results.

To overcome the above mentioned drawbacks, the CWT and FWT approaches are used as powerful signal processing tools for chemical and pharmaceutical analysis. Typical analytical applications of the CWT and FWT in the presentation are as follows

- Application of the CWT-zero crossing technique in the binary mixture analysis

- Application of the ratio spectra-CWT approach in the binary mixture analysis

- Application of the CWT in the overlapping chromatograms

- Application of the CWT in the overlapping voltammograms

- Application of the DWT approach in the baseline correction of chromatograms

- Application of the DWT in the signal denosing

- Application of FWT-CWT-Zero crossing technique in the binary mixture analysis

Continuous Wavelet Transform-Zero Crossing Technique (CWT-ZCT)

If a binary mixture of two analytes, X and Y is considered and if the absorbance value of this binary mixture is measured at λ_i, the following equation can be written as;

$$Am_{\lambda i} = \alpha_{\lambda i} C_X + \beta_{\lambda i} C_Y \quad (11)$$

where $Am_{\lambda i}$ is the absorbance of the binary mixture at wavelength λ_i, and the coefficients $\alpha\lambda i$ and $\hat{a}\lambda i$ are absorptivities of X and Y analytes. C_X and C_Y represent the concentrations of analytes.

If CWT is applied to Eq (1), the following equation can be obtained as;

$$CWT(Am_{\lambda i}) = CWT(\alpha_{\lambda i} C_X) + CWT(\beta_{\lambda i} C_Y) \quad (12)$$

If CWT $(\alpha\lambda i\ C_X) = 0$, then we obtain the following result;

$$CWT(Am_{\lambda i}) = CWT(\beta_{\lambda i} C_Y). \quad (13)$$

Eq. (13) shows that CWT amplitudes of compound Y in binary mixture are dependent only on the concentration of C_Y and are independent on the concentration of compound X in the binary mixture. As before, the calibration graphs can be obtained by plotting $CWT(Am_{\lambda i})$ signals versus the concentration of C_Y. This procedure is repeated for X analyte in the binary mixture. Calibration functions obtained in the above steps are applied to the quantitative determination of the components in binary mixtures [10,11].

Ratio Spectra- Continuous Wavelet Transform (RS-CWT)

Let us consider a binary mixture of two analytes, X and Y and consider the absorbance value of this binary mixture measured at λ_i. The following equation can be written as;

$$Am_{\lambda i} = \alpha_{\lambda i} C_X + \beta_{\lambda i} C_Y, \quad (14)$$

where $A_{m\lambda i}$ is the absorbance of the binary mixture at wavelength λ_i, and the coefficients $\alpha\lambda i$ and $\hat{a}\lambda i$ are absorptivities of X and Y analytes. C_X and C_Y represent the concentrations of analytes. If Eq (14) is divided by the standard spectrum ($Am_{\lambda i} = \alpha_{\lambda i} C_X^o$) of one of compounds in binary mixture, the equation (6) becomes;

$$\frac{Am_{\lambda i}}{\alpha_{\lambda i} C_X^o} = \frac{\alpha_{\lambda i} C_X}{\alpha_{\lambda i} C_X^o} + \frac{\beta_{\lambda i} C_Y}{\alpha_{\lambda i} C_X^o}. \quad (15)$$

If CWT is applied to Eq (15), the following equation can be obtained as;

$$CWT\left[\frac{Am_{\lambda i}}{\alpha_{\lambda i} C_X^o}\right] = CWT\left[\frac{\alpha_{\lambda i}}{\alpha_{\lambda i}}\right]\frac{C_X}{C_X^o} + CWT\left[\frac{\beta_{\lambda i}}{\alpha_{\lambda i}}\right]\frac{C_Y}{C_X^o} \quad (16)$$

If $CWT\left[\frac{\alpha_{\lambda i}}{\alpha_{\lambda i}}\right]$ =0, then we obtain the following result;

$$CWT\left[\frac{Am_{\lambda i}}{\alpha_{\lambda i} C_X^o}\right] = CWT\left[\frac{\beta_{\lambda i}}{\alpha_{\lambda i}}\right]\frac{C_Y}{C_X^o} \quad (17)$$

The ratio-CWT amplitudes of binary mixture given in the equation (17) depend only on the concentration of C_Y and C_X^o and not on the concentration of other C_x in the binary mixture [14].

Both CWT-ZCT and RS-CWT approaches provides a short time, accurate, precision, rapid and low cost analysis of complex mixtures.

A typical application of DWT in the baseline correction of the chromatogram of B1 and B6 vitamins with lidocaine hydrochloride (LID), obtained by UPLC gradient elution is given below.

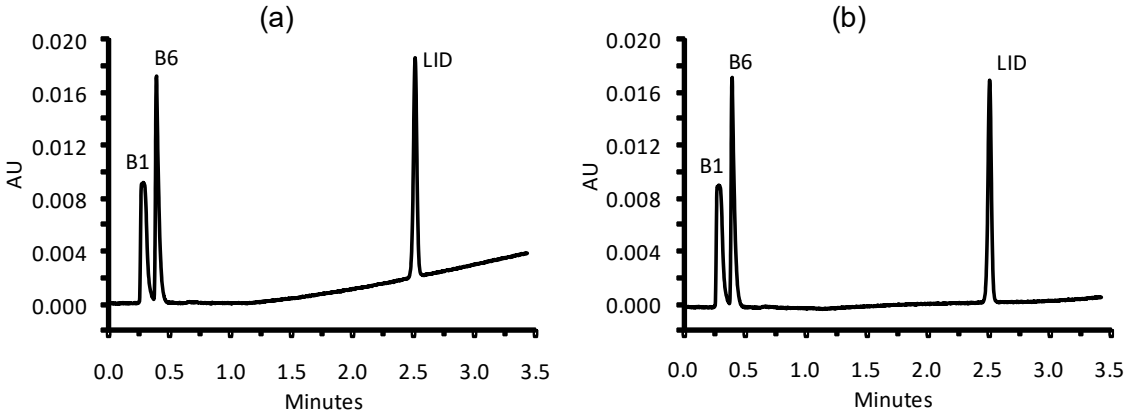

Figure 1. Original UPLC chromatogram (a) and Baseline-corrected chromatogram obtained by substraction of the nine dmeyer discrete approximation from the original chromatogram (b).

In the chromatographic analysis, gradient elution can be caused some baseline problems. In these cases, DWT is suitable for the baseline correction. Figure 1 (a) displays the original chromatogram of the ternary mixture consisting of B1 and B6 vitamins with lidocaine hydrochloride (LID) obtained by UPLC gradient elution. This chromatogram was processed by Dmeyer DWT for the baseline correction. As it can be seen from Figure 1 (b), baseline-corrected chromatogram of mixture consisting of B1 and B6 vitamins with LID was obtained.

Conclusions

Wavelet transform given in my presentation offers new possibilities and alternative ways for the resolution of mixtures of active compounds with overlapping absorption spectra, overlapping chromatograms and overlapping voltammograms. One of the main advantages of CWT approach is the simultaneous data reduction and de-noising for the signal analysis. Beside, this CWT approach provides higher peak amplitude, less noise, and sharper peaks then classical derivative spectroscopy. Wavelets transform having flexible and versatile properties gives a good resolution for the chemical and pharmaceutical analysis.

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