Designing electronic eyes. Part I: 2D-COS helps in dyes choice for monocrotaline determination

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Abstract

In this work it is studied the feasibility of the use of ”D correlation spectroscopy to design an array of optical colorimetric sensors (or e-eye) to accurately determine the presence of a given compound, in this case monocrotaline. our results demonstrate that 2D correlation spectroscopy is able to characterize that compound in solution by monitoring the way absorption bands rise or falls. The second paper in this series will show the differentiation between monocrotaline and other substances by using this method.

Keywords

2D Correlation Spectroscopy, e-eyes

Introduction

Pyrrolizidine alkaloids (PAs) are natural occurring products. The main role of these secondary metabolites as defence mechanism against herbivores. The effect on the health of the predator is very diverse. For instance, these compounds can cause hepatotoxicity and cancer, both in humans and animals(1). The principal ways for how these alkaloids can be ingested by humans, is through the consumption of medicinal plants or agricultural crops, directly or indirectly. In this last case the primary uptake is made by a animal, which in turn transmit to humans (2). Honey as well as milk, could contain pyrrolizidine alkaloids (PAs), the level of PAs contamination in these foods depends on the geographical and botanical origin(3). It is important to correlate botanical and geographical origin from PAs, therefore, these alkaloids are worth studying. For the determination of these PAs in honey, usually HPLC-MS method is used(4; 5; 6). There are four types of basic structure of this family of compounds (Figure 1). With the exception of the Platynecine type of alkaloids, the other three structural types of PAs are considered as tumorigenic .

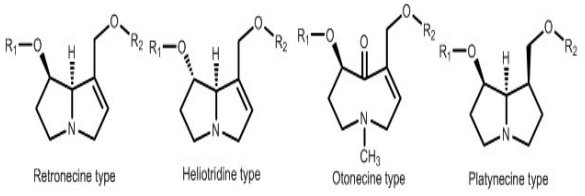

Figure 1. Basic pyrrolizidine alkaloid structures

Until few years ago, the ideal situation for an analytical chemist, was to have an selective (or better specific) probe for a given target (for selectivity and specificity we used the definition given by Vessman(7)). Therefore, an array of n different selective (or in mathematical terms: orthogonal) sensors are sufficient to discriminate n target compounds/ions. However, that situation is not common in analytical chemistry where selectivity (or specificity) is rarely encountered. Nevertheless, this can ve advantageous. The theory behind sensor arrays states that non-orthogonal sensors can give an orthogonal response to a given stimulus if appropriate pattern recognition algorithms are used. That means that a pool of sensors A, B, C will give different response when in presence of stimulus N,M,O in a way that the response of the interaction A-N is different fro A-M or B-N (8; 9; 10).

The concept of electronics eyes (e-eyes) are devices gaining popularity. The idea is to connect several fluorescent and/or colorimetric and/or Surface Plasmon Resonance sensors to suitable photo-detectors to accurately determine the analyte of interest. Some applications involving e-eyes have been developed. For instance Su et.al.(11) have used a smartphone to replace the standard ELISA test for bicinchoninic acid (BCA) protein assay and cell counting kit (CCK8). The same group have extended the method using it for detection of marine toxins(12). On the other hand, single sensor with possibilities to be updated to e-eyes were also studied. For instance, You, Park and Yeon have developed a cell-phone gadget which allows semi-quantitative quantification of thyroid stimulating hormone (TSH) with a setup using lateral flow immunochromatographic assays(13). An similarly, Gallegos et. al. have demonstrated the possibility of Label-free biodetection using a smartphone (14). Other applications are reviewed by Zhang and Liu (15)

The concept of bidimensional (2D-) correlation spectroscopy was established by Noda in 1983 see(16). From that point several developments have been made (17; 18). Basically, ”D-correlation spectroscopy seek for the sequential

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variations in thee spectral intensity of characteristic bands in vibrational, electronic or other spectroscopic techniques (19). The correlation spectra matrix is obtained first, by calculating a dynamic spectrum as:

$$\tilde{A}(\nu_i, t_k) = \begin{cases} A(\nu_i, t_k) - \bar{A}(\nu_j), & \text{for } 1 \leqslant k \leqslant m \\ 0, & \text{otherwise} \end{cases}$$

where $A(\nu_i, t_k)\ and\ \bar{A}(\nu_j)$ are individual and reference spectra, respectively. The reference spectra can be the initial (t=0), average or final ($t = \infty$) spectra.

The synchronous and asynchronous spectra are calculated as (16):

$$\Phi = \frac{1}{m-1} \sum_{j=1}^{m} \tilde{A}(v_1, t_j) \cdot \tilde{A}(v_2, t_j) \quad (1)$$

$$\Psi = \frac{1}{m-1} \sum_{i=1}^{m} \tilde{A}(v_1, t_j) \cdot \sum_{j=1}^{m} N_{ij} \tilde{A}(v_2, t_i) \quad (2)$$

In the last equation, N_{ij} is the socalled Hilbert-Noda transformation matrix, defined as:

$$N_{i,j} = \begin{cases} 0, & \text{if } i = j \\ \frac{1}{\pi(j-i)}, & \text{otherwise} \end{cases}$$

Machine learning algorithms(20; 21) are a set of mathematical techniques than allows the classification of multiple variables as well as the appropriate variable choices for this classification and statistical regression. Two of the most know of these techniques are PCA and Neural Networks. It is expected that is use can explore the small variations observed in the spectra of the e-eyes components. In this work a 2D-correlation spectroscopy study of the interaction of monocrotaline with five different solvents is made with the purpose of reveal the feasibility of construct an e-eye able to selectively determine the amount of that compound in solution.

Materials and Methods

Rhodamines 560 and 6G were obtained from Lambda-Physics, Curcumin S from Chroma-gesellscha, bromothymol Blue from BDH and Ninhydrine from May and Baker Ltd. The concentrations of the dyes were 0.2 $\mu g/\mu l$. For monocrotaline a stock solution with a concentration of 30 $\mu g/\mu l$ was prepared. Ten aliquots of that solution (50..450 μl) were added to dyes solution in order to obtain a final volume of 2 ml. UV-Vis spectra of these solutions where acquired from a Hitachi U-2010 spectrophotometer operated by Hitachi's UV Solution v2.0 software.

Data were processed in R statistical package by using the RStudio interface. The signals were not pre- or post-processed. 2D correlation spectroscopy data were calculated (and the graph were obtained) using the 2dcorr package.

Discussion

The first compound tested as marker was Rhodamine 560 (or Rhodamine 110), its structure as well as spectra and 2D correlation plots are shown in Figure 2 as can be seen, although not visible in the UV-Vis spectra, the peak at 525nm and the shoulder at 495 nm have concentration-driven intensity variations as indicate the pattern observed in that region of the synchronous plot. Moreover, as the cross peaks are positive, these changes are correlated. Therefore these peaks are candidates to be used as descriptors at least of monocrotaline presence and perhaps concentration, if a machine learning algorithm is used. The observed changes in these peaks are also related with variations in the peaks observed at 294 and 345 nm, although these peaks does not suffer noticeable variations. However they can be used as fingerprints of monocrotaline presence. The asynchronous spectrum illustrate that the changes at 525 and 495 nm are not totally synchronous, the process leading to peak decreasing is different of that promoting peak increasing.

In the case of Rhodamine 6G (Figure 3), the situation is similar Moreover the asynchronous peak at 425:525nm indicates that these peaks behave asynchronously.

Curcumin S

Ninhydrin, contrarily does not not have interactions with monocrotaline, as can be observed in Figure 4, this is not a handicap as can serve as potential "blank" assay when close figures arise from other componds.

Bromothymol blue

Differentiation with other compounds

The importance of this method for determination of the presence or absence of one contaminant is illustrated next. Figure 6 shows the comparison of the synchronous and asynchronous spectra obtained from a copy of our database of calibration curve of the interaction of crotalin with BTB and the other database obtained by incorporation of one spectrum of the interaction of BTB with ethyl-phenylamine.

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Website: http://www.sunrise-setting.co.uk

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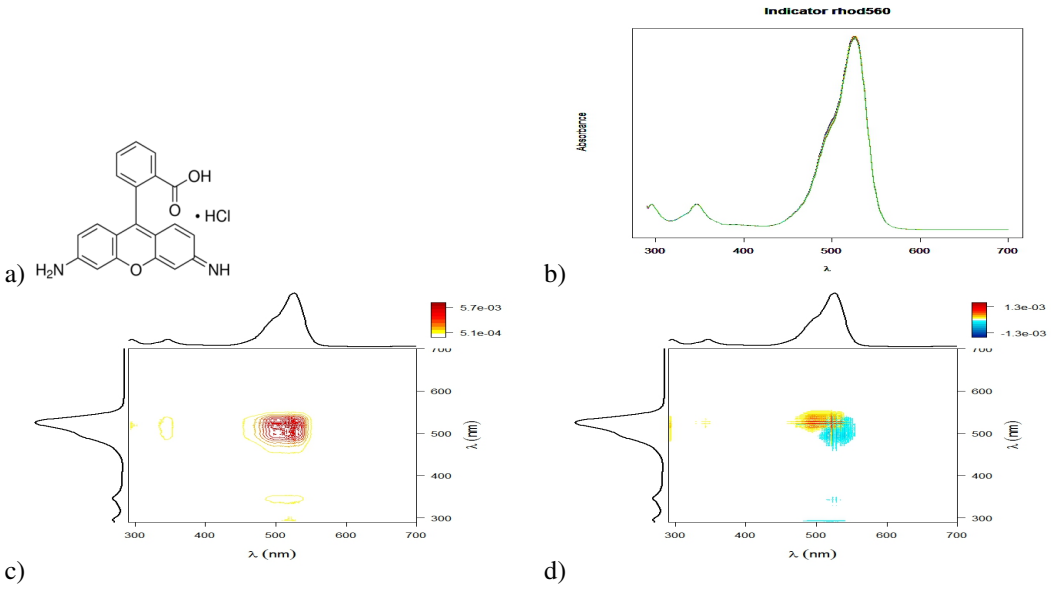

Figure 2. Structure of rhodamine 560 (a), its absorption spectra (b), synchronous (c) and asynchronos (d) correlation spectra plots

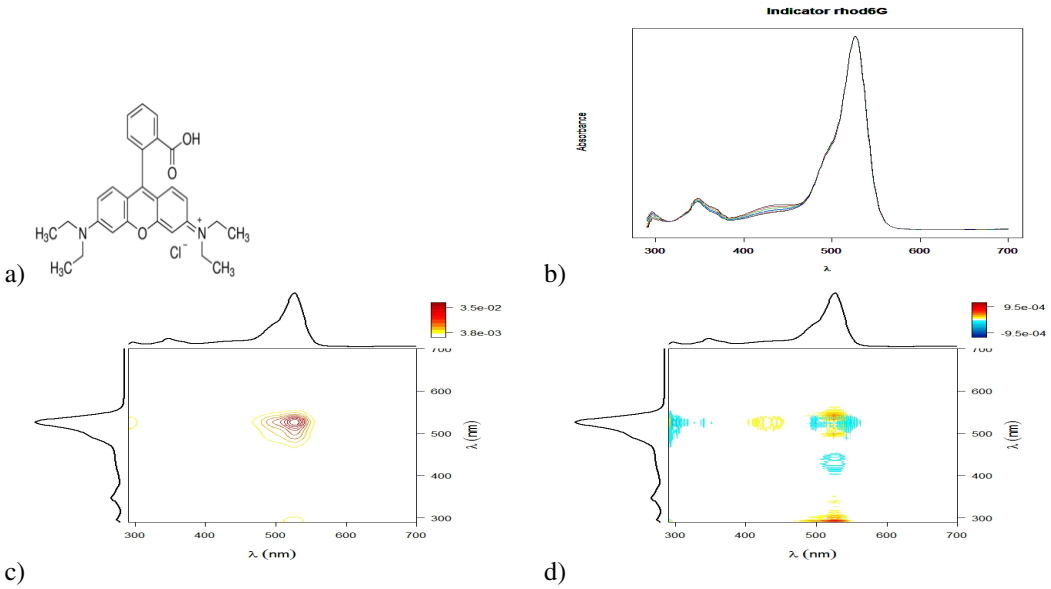

Figure 3. Structure of rhodamine 6G (a), its absorption spectra (b), synchronous (c) and asynchronos (d) correlation spectra plots

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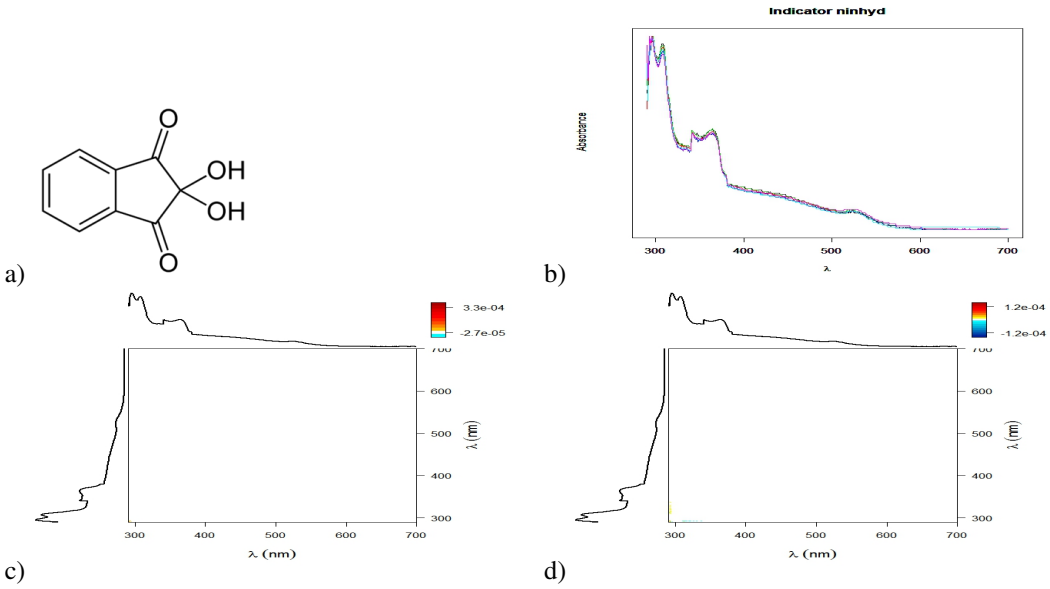

Figure 4. Structure of ninhydrin (a), its absorption spectra (b), synchronous (c) and asynchronos (d) correlation spectra plots

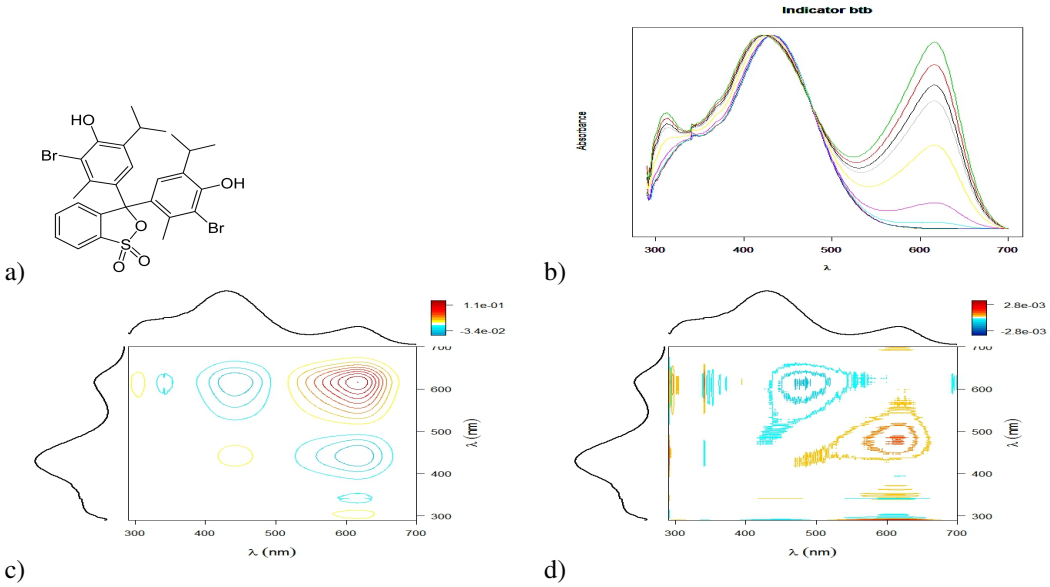

Figure 5. Structure of bromothymol blue (a), its absorption spectra (b), synchronous (c) and asynchronos (d) correlation spectra plots

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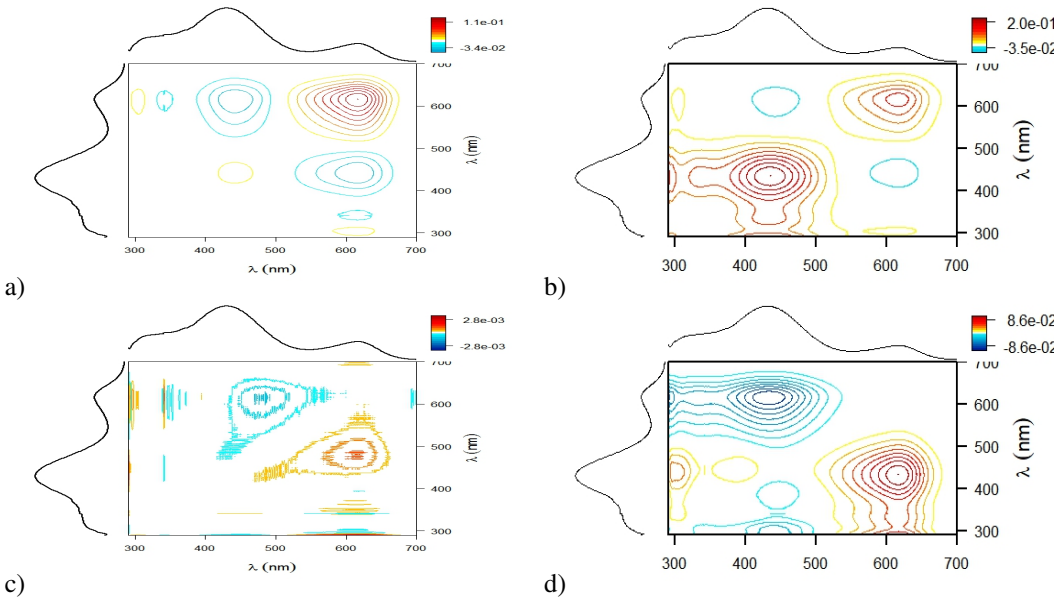

Figure 6. a) Synchronous correlation spectra plot of: the interaction of crotaline with BTB, b) Mixing these contents of the database (a) with a new spectra but the interaction of Me-Pheamine. c) and d) The corresponding asynchronos correlation spectra plots from a) and b)

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