Insights on the chemistry of *a*,*c*-biladienes from a CSPT investigation

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Computer Screen Photoassisted Technique (CSPT) is a recent interesting approach for the development of optical sensor platforms using ubiquitous devices. We demonstrate here that the information obtained by CSPT measurements can be useful for other than sensing purposes and a computer set and a web camera can also be exploited as spectroscopic technique for the characterization of the compounds used as indicators. CSPT measurement data have been used to characterize novel features of the *a*,*c*-biladiene chemistry, which have been later confirmed by photophysical characterization carried out using classical instrumentation. The demonstrated analytical capabilities of a regular computer–web camera set reach a degree of complexity approaching that of fluorescence excitation-emission spectroscopy, something remarkable considering the exploitation of familiar and already well distributed devices.

Introduction

The impressive evolution and sophistication of electronic devices, together their wide distribution is one of the signatures of the last few years. Other than the implications on lifestyle, this exciting improvement offers the opportunity to take advantage of the developed devices for applications in fields for which they were not originally conceived.

Chemical sensors represent an interesting example of such opportunities, where the aim is to substitute the high-cost traditional instrumentations by commercial simple electronic devices, such as for examples scanners, *etc*.

Suslick and co-workers reported an interesting example of this approach using a flat-bed scanner to digitalize the images of spots of different chromophores before and after their exposure to different vapor analytes.[1] The color changes induced by these interactions were processed and evidenced by Photoshop® program. More recently GE HealthCare has exploited the disc drives optics and spinning capabilities of CD or DVD readers for lab-on-a-disk determinations.[2]

We have been involved in this approach by studying the possibility to use a computer monitor and a web cam to develop optical sensor platforms.[3] We called this approach Computer Screen Photoassisted Technique (CSPT). During a CSPT measurement the screen illuminates the array with a sequence of colors, while the camera captures the image of the array in synchronism with the illumination. The result is a video stream, where regions of interest (ROIs) are selected and the intensity of the recorded pixels averaged. The procedure is repeated along 50 frames in this case, corresponding to an illuminating sequence resembling the human perception of the visible spectrum. In this way it is possible to obtain a plot of the transmitted light as a function of the illuminating colors, which can be correlated with the transmittance spectrum (including emissions) of the indicator; the basic theory of CSPT has been previously reported in the literature.[4]

The method has been demonstrated for the evaluation of different bioassays and measuring strategies and optimization studies.[5] More recently we demonstrated the development of a sensor array able to discriminate mixtures of gases and volatile compounds, by using different metalloporphyrin spots deposited on a glass slide.[6]

During these studies we noted that CSPT retains the key spectral characteristics of the analyzed samples and, although based on a polychromatic light source such as a computer screen, the technique allows to generate curves that resemble those obtained by visible spectroscopy.[4a] This feature is particularly interesting, because it can open the way to use CSPT as spectrophotometric technique, which, although not as advanced or accurate as the traditional laboratory instrumentations, can be appealing in some applications for the ubiquitous diffusion of its basic constituents.

The exploitation of the 1,19-dideoxy-2,3,17,18-tetraethyl-7,8,12,13-tetramethyl-*a*,*c*-biladiene (BD, Fig. 1) as sensing material in our previous study[6] was the occasion to further investigate the spectrophotometric potentialities of this technique in a case study, and we report here the CSPT characterization of BD, compared and validated by the photophysical characterization obtained by traditional spectrophotometric techniques.

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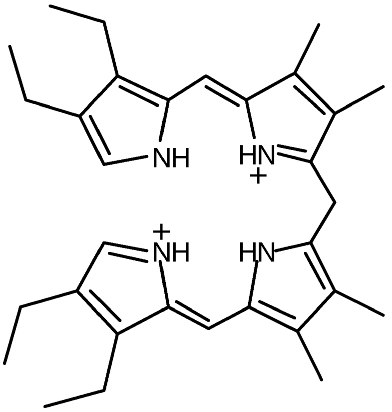


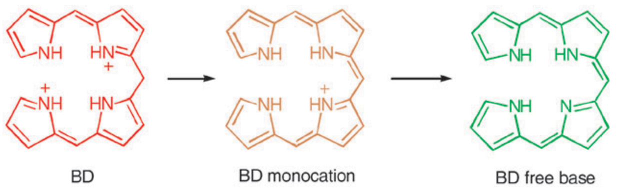
Fig. 1 Molecular structure of BD.

Results and discussion

In our previous study[6] other than metalloporphyrins, we have been interested in the exploitation of BD as indicator to test CSPT performances, because it has evident color changes induced by acid–base equilibria (Scheme 1).[7] These linear tetrapyrroles, in fact, are stable in the fully protonated form and they are usually isolated as dihydrobromide salts.[8] The formation of the monocation leads to a molecular rearrangement by tautomeric shift, with the formation of the fully conjugated form (indicated as BD monocation). This rearrangement results in a color shift from red to yellow orange. Finally a green colour is indicative of the formation of the free base 22,24-dihydro-*a*,*b*,*c*-bilatriene (indicated as BD free base).

In the fully conjugated form, both as monocation or the free base, BD is a reactive species and, depending on both the substituents present at the 1,19-positions and on the reaction conditions, different macrocycles can be obtained starting from BD.[9] For the CSPT experiments we decided to use BD, which has been previously used in our laboratories for the preparation of corrole.[10] For these measurements, focused to test of CSPT performances, we were not concerned with the low stability of BD and consequently the expected lack of reversibility of the CSPT measurements.

To preserve the solution BD optical properties also in the solid state, we dispersed the linear tetrapyrrole in a PVC membrane, a technique that has been widely used for the preparation of optical sensors.[11] In this way it is possible to obtain transparent spots having features similar to those observed in solution. The first aim was to test the performances of the CSPT set-up to follow the color changes of the BD spot after the interaction with volatile amines, such as ammonia or triethylamine. We expected that interaction with such analytes induced the formation of the free base form and consequently the red-green color change of the indicator spot.



Scheme 1 Molecular structures of the linear tetrapyrrole upon proton abstraction.

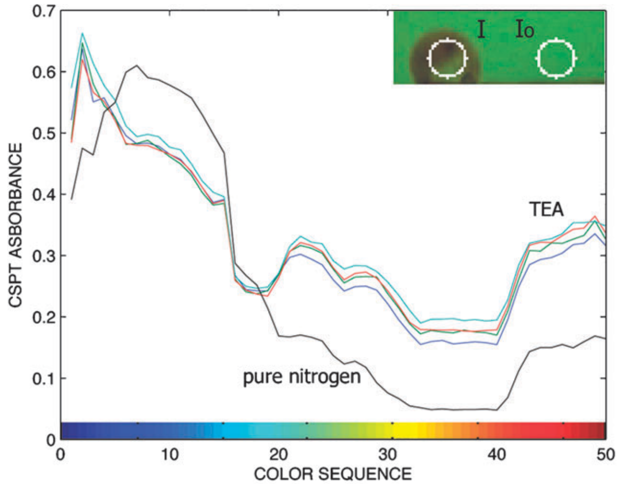


Fig. 2 The CSPT absorbance index is calculated according to eqn (1). In the inset the scene as viewed by the webcam is shown. Circles indicate the two regions of interests (ROI) centered on the biladiene spot and on the background. The average light intensity in the two ROIs defines I and I_0. Large differences are observed when the BD layer is exposed to triethylamine vapours.

The response of BD exposed to increasing concentrations of triethylamine is shown in Fig. 2. Among the manifold of possible representation of CSPT fingerprints, here the spectrophotometric mode is shown. In analogy with standard spectrophotometers, a fingerprint absorbance can be calculated from the CSPT data.[4a] It is defined by the following expression:

$$A(\text{color}) = -\log\frac{I_0(\text{color})}{I(\text{color})} \qquad (1)$$

where I and I_0 are the light intensity recorded by the webcam from the BD spot and the background, respectively. These intensities are simply calculated as the sum of the light intensity in the red, green, and blue camera regions.

Fig. 2 shows that the exposure to triethylamine results in an increase of the absorbance when the light probing the BD layer contains a significant amount of red, and a corresponding decrease of absorbance in blue-green light. It is worth noting that the first portion of the color sequence is characterized by a violet nuance containing a not-negligible amount of red; for this reason the absorbance increase produced by triethylamine appears also at the beginning of the color sequence.

The great sensitivity of BD to triethylamine is indicated by the saturation of biladiene responses. Indeed different triethylamine concentrations resulted in almost identical absorbance profiles. The concentrations used in this experiment were in the range 5–20% of the saturated pressure at standard atmospheric pressure and temperature.

An important feature of CSPT is the possibility to detect absorbance and luminescence phenomena at the same time.[12] The previously given definition of absorbance becomes inadequate in the case of luminescence, and the difference between I and I_0 allows a better representation of absorbance and emission phenomena occurring at the same time.

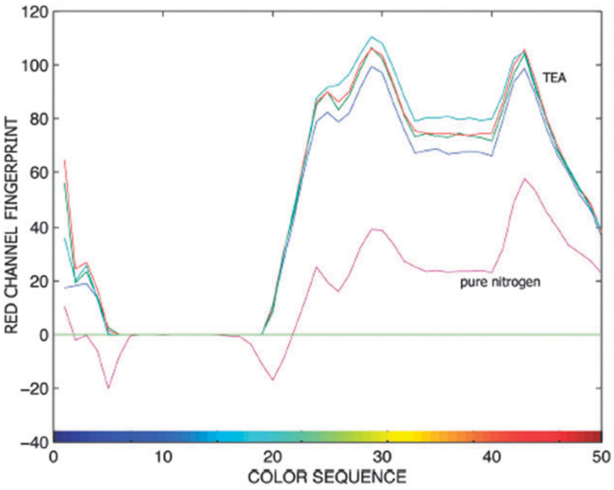


Fig. 3 The difference of intensity received in the red webcam channel between BD spot and background is plotted *versus* the illumination color sequence. Negative values of the red channel fingerprint indicate a prevalence of red light from BD exposed to pure nitrogen with respect to the background. This is interpreted as emission from BD. The addition of triethylamine vapour to pure nitrogen results in a quenching of the emission.

Emission clearly affects the intensity of the webcam red channel and the signature of emission is obtained when the red intensity from the sensing layer exceeds that from the background. In Fig. 3 for each experimental condition (nitrogen flow and triethylamine vapors) the difference between the red channel intensity from the background and the BD layer is plotted *versus* the color sequence. When exposed to pure nitrogen the BD layer curve shows two emission regions identified as negative values of red channel fingerprint. During the exposure to triethylamine the emission features disappear and, as seen in Fig. 2, there is an increase of the absorbance in the red channel. It is interesting to note that the interaction with amines led to a significant decrease of the transmitted light in the red region, supporting the quenching of BD emission in that region; it is also worth mentioning that the variation observed in this case was much more intense than those recorded for the ZnTPP-spot.[6]

A second interesting result was to observe the reversibility of BD color changes upon interaction with amines or other bases. We expected in fact that the formation of the BD free base, evidenced by the green color change, under light led to the immediate cyclization of the linear tetrapyrrole to corrole,[7] or its oxidative decomposition to smaller pyrrolic pigments. Both these events are irreversible chemical reactions and consequently they ruled out the possibility to obtain a reversible interaction: quite surprising, in our case the CSPT responses were reproducible within one day of measurements, and then a slow drift of the signal was observed. We dissolved the BD spot in dichloromethane after one measurement cycle and ran a UV-Vis spectrum to investigate the product obtained; we observed the presence of corrole bands, indicating that one of the reaction pathway of BD in the PVC membrane was effectively the cyclization to corrole. However this reaction was slow and allowed the reversible measurement under the CSPT conditions.

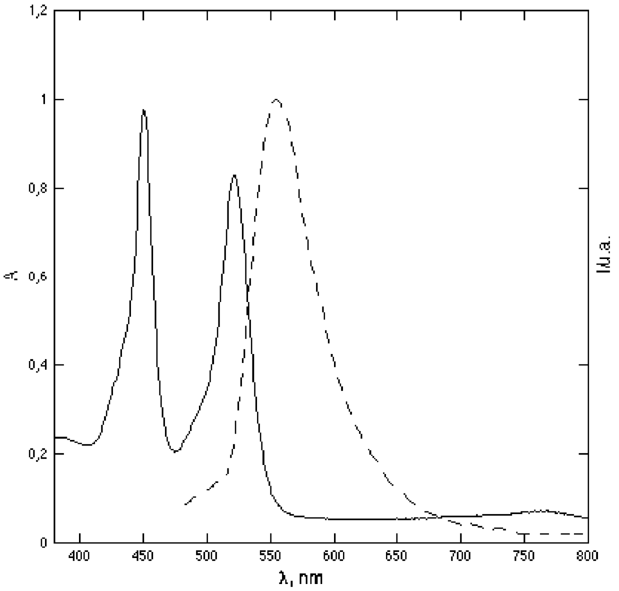


Fig. 4 UV-Vis spectrum (solid line) and fluorescence spectrum (dashed line; λ_{exc} = 450 nm) of BD in CH_2Cl_2 solution.

It is interesting to note that these results, while confirming that CSPT is useful for sensing applications, also showed that CSPT information can give a hint on the optical properties of the exploited dyes.

This is particularly significant in the case of BD, because although *a*,*c*-biladienes are well-known compounds for their synthetic usefulness, their photophysical behaviour has not been investigated in detail and, to the best of our knowledge, no reports have been published on the fluorescence emission of *a*,*c*-biladienes, probably because of their low stabilities.

To confirm this promising characteristic of CSPT, we decided to study in better detail the optical properties of BD, with the aim to fully validate the CSPT indication with the traditional spectrophotometric techniques.

The first step was the characterization of BD in CH_2Cl_2 solution. In this solvent two strong bands at 450 and 520 nm characterize the absorption spectrum (Fig. 4). The emission spectrum shows a band at 555 nm, with a quantum yield of 0.014 (Fig. 5) and an excited state lifetime shorter than 0.6 ns. The excitation spectrum is very similar to the absorption one. Addition of an excess of base leads to a dramatic change in the absorption spectrum and to a complete quenching of the fluorescence. When methanol is added to the solution an immediate change of the absorption spectrum occurs, showing the superimposition of the corrole monocation absorption bands with the characteristic absorption of the BD monocation species around 800 nm (Fig. 5), making evident both the formation and the consequent cyclization of the fully conjugated tetrapyrrole. After few minutes the spectrum is that of the corrole monocation and both absorption, emission and excitation spectra are those attributable to corrole species. These results are in good agreement with our previous studies on the reactivity of *a*,*c*-biladienes in alcoholic solvents,[9,13] but do not explain the results obtained in the CSPT measurements.

We then carried out the characterization of the photophysical properties of BD in the PVC membrane. As it can be seen

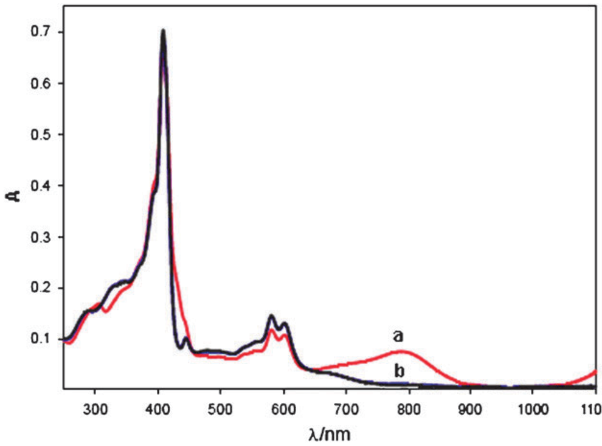


Fig. 5 UV-Vis spectra of BD in CH_2Cl_2/MeOH 1:1 mixture: (a) immediately after MeOH addition; (b) after 3 h.

from Fig. 6, a relatively large, unstructured band centered at 554 nm is present in the fluorescence spectrum. This fluorescence can be observed with the naked eyes upon excitation with the tlc lamp. The excitation spectra performed at emission wavelengths in the 540–580 nm region are all superimposable in shape showing a maximum at 453 nm (Fig. 6), indicating that only BD is present in the PVC membrane. These spectra show only the short wavelength band of BD, but this result can be attributed to the dependence of the relative intensities of these band to the BD matrix, effect attributed to the formation of hydrogen bond. The UV-Vis absorption spectrum of BD in PVC matrix confirms this hypothesis, showing a much more intense band at 450 nm (Fig. 6). The average excited state lifetime in these conditions is 3.5 ns. Assuming that the radiative rate constant of BD in the PVC matrix is similar to the one observed in CH_2Cl_2 solution, this result leads to the conclusion that the luminescence quantum yield of BD is rather high (>0.08), and that can be used for analytical applications.

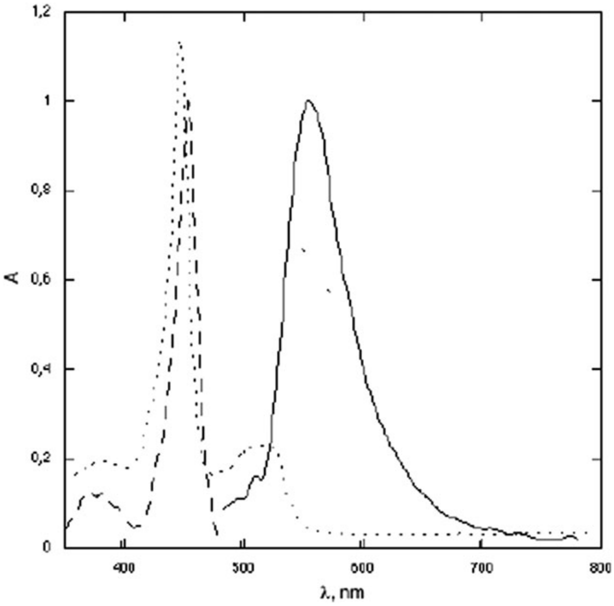


Fig. 6 Fluorescence spectrum (solid line; λ_{exc} = 450 nm), excitation (dashed line) and absorbance spectrum (dotted line) of BD in PVC membrane.

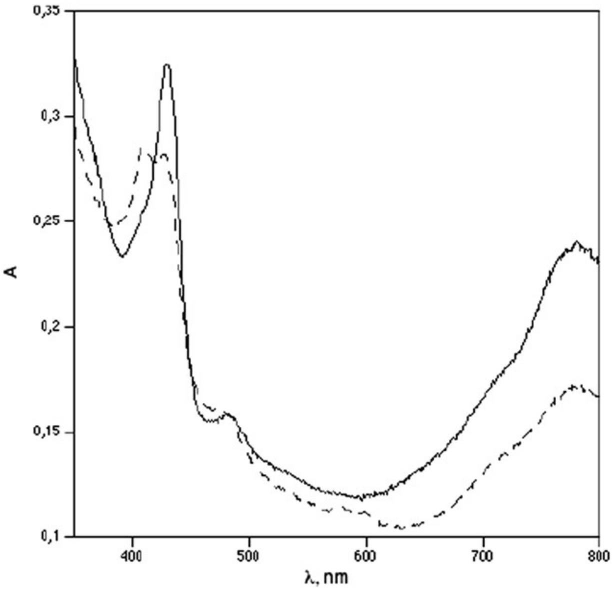


Fig. 7 UV-Vis spectra of BD in glycerol as dissolved (solid line) and after 5 hours (dashed line).

The significant decrease of the transmitted light observed in the red channel derives from the quenching of the fluorescence emission of BD, due to the formation of the BD free base, induced by the amine interaction.

We still had to explicate the unexpected reversibility of the CSPT measurements. In this case we decided to use glycerol as solvent, using this viscous solvent to try to mimic as far as possible the PVC membrane environment; the choice was successful and a completely different behavior was observed. The starting absorption spectrum indicated the formation of the fully conjugated BD monocation, as observed in the case of methanol. However in this case the cyclization to corrole was not immediate and the UV-Vis absorption spectrum was stable for hours and it took 5 hours to observe the appearance of the corrole bands in the visible spectrum (Fig. 7).

The results obtained give an explanation for the BD behavior observed during the CSPT measurements: in glycerol, the cyclization after the formation of the BD monocation is slow because the solvent viscosity makes more difficult the molecular motions necessary for the cyclization. In this way glycerol can mimic what happens in the PVC membranes; the formation of the fully conjugated form after interaction with bases does not lead to the immediate cyclization, because it is hampered by the rigidity of the polymeric matrix and this effect explains the reversibility observed in the CSPT experiments.

Conclusions

The results obtained here support another useful application of CSPT, which can be used as spectrophotometric technique, other than for sensing purposes. CSPT has been able to give information on the photophysical and photochemical behaviour of BD, which was confirmed by characterizations carried out with conventional techniques.

Of course CSPT cannot reach the information complexity and accuracy degree of conventional spectrophotometers, but it is the current ubiquitous diffusion of electronic devices having polychromatic light sources, such as computer screens or mobile phones, which makes this exploitation of CSPT particularly intriguing. In this way it is possible to open the way to a spectrophotometric technique widely distributed and at practically no-cost, using devices already available although originally conceived for other purposes.

Experimental

BD was prepared following literature methods.[9] Electronic absorption spectra were measured in CH_2Cl_2 using a Cary 1 or a Perkin Elmer λ 16 spectrophotometer. Uncorrected emission spectra and corrected excitation spectra were obtained with a Perkin Elmer LS50 spectrofluorimeter. The fluorescence lifetimes (uncertainty, ±5%) were obtained with an Edinburgh single-photon counting apparatus (D_2 filled flash lamp). In order to allow comparison of emission intensities, corrections for instrumental response, inner filter effects, and phototube sensitivity were performed.[14] PVC membranes were prepared as previously reported.[6]

The CSPT data discussed in this paper are a subset of a larger experiment aimed at identifying individual gases and to test the CSPT properties to detect the subtle changes occurring in metalloporphyrin layers exposed to gases and organic vapors. The whole experiment was published elsewhere.[6] Herewith the data related to the interaction of BD layer with triethylamine are discussed together with complementary optical studies in order to elucidate some peculiar behaviour of BD dissolved into the PVC matrix and to illustrate the potential use of CSPT as an investigation tool of the optical properties of compounds, in a spectrometer-like approach.

The basic set-up of CSPT has been thoroughly described elsewhere:[6,15] the basis of the system is a transparent tight gas measurement cell placed in the optical path between a computer screen and a web cam. For the experiments here discussed, BD was dissolved in a PVC membrane solution and deposited on a glass substrate.

Computer screens are able to display more than 16 million colours formed by different weighted combinations of red, green and blue primary spectral radiances, hence called RGB colours. During a CSPT measurement a web camera captures the image of the array under an illuminating sequence provided by the screen (a sequence of 50 colours in this work). The chosen color sequence is shown in the 3D plot of Fig. 2. It is structured in order to blend progressively the three primary colors to produce a color stream from blue to red.

From the camera images two regions of interests (ROI) are selected. One ROI is centered on the biladiene spot and the other on a background region close to the spot. RGB values of the pixel enclosed by a ROI are averaged. For each ROI a fingerprint is defined as the sequence of the red, green, and blue camera channels recorded under each illumination of the color sequence. The resulting ROI fingerprint is then a vector containing 50 (illumination colors) * 3 (camera channels) elements.

The measuring setup involved a conventional LCD screen (Philips 170S4), used as a light source, and a Logitech Quickcam pro 4000 operating at a resolution of 320 × 240 pixels used as an image detector. The measurement cell was properly shielded from ambient illumination and placed in a suitable holder keeping the geometrical arrangement small.

Software written in Matlab® controlled the illuminating sequence, the video acquisition, and extracted the information (CSPT fingerprints) from manually selected ROIs.

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