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Naked-eye detection and discriminant analysis for the determination of anions based on hydroxy anthraquinone chemosensors

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ABSTRACT

Hydroxyanthraquinone derivatives are identified as synthetic receptors of anions giving the possibility of sensing, coding and doing quantitative determination in aqueous solutions of several anions. Interactions between receptor/acceptor are discussed in this paper. An anion selective receptor which is an underdeveloped field has been developed. The strategy used was colorimetric anion sensing based on attaching an appropriate chromophore to a specific anion receptor. In this work, naked eyed sensing of several anions and a multianion (acetate, bromide, carbonate, phosphate and tartrate) discriminant analysis has been developed using the signal ratios of the peaks at 450nm and 550 nm. Discriminant analysis shows that the anion groups are well separated and can be easily classified. Good sensitivity and selectivity for discrimination of these anions were obtained. And finally photometric/fluorimetric quantitative determination of sodium carbonate in aqueous solution were investigated, and the detection limits obtained were 5 μM (RSD 1.2 % (n=10), fluorimetric method) and 60 μM (RSD 0.5 % (n=10), photometric method.

KEYWORDS

Naked eyes sensing;
Hydroxyanthraquinone derivatives;
Discriminant analysis;
Carbonates;
Photometric/fluorimetric determination.

INTRODUCTION

Detection and quantification of anions play an important role due to the implication of anions in a great variety of chemical and biological processes. To understand and maintain the environment, detection and control of anions is very important because a lot of industrial and agricultural processes increase the concentration of anions in the environment. In addition, numerous biological processes involve the molecular recognition of anionic species, including DNA (a polyanion) or numerous cofactors and enzymes. Of particular interest in this regard are colorimetric anion sensors species that would allow the so called naked eye detection of anions without resort to any spectroscopic instrumentation.

The design of anion-selective receptors is an underdeveloped field in comparison to that of cations, and this lack seems to be related to the existing structural differences between anions and cations[1-3]. Several reviews concerning anion sensing are available elsewhere[4,5]. The ionic size of an anion is usually higher than that of a cation, and this implies that charge/size rate is lower and thus electrostatic in-

teractions anion/receptor are weaker. Of particular interest in this regard are "colorimetric anion sensors", species that would allow the so called naked eye detection of anions without resort to any spectroscopic instrumentation.

The strategy to prepare colorimetric anion sensing is the binding site-signalling unit approach, in which an appropriate chromophore is attached to a specific anion receptor[6]. The receptor can be positively charged or neutral but the attention of researchers in the chemistry of anion receptors is dedicated mostly to purely organic and neutral in their vast majority[7]. These chromophores may contain electron-withdrawing groups that can enhance the acidity of the anion binding subunit.

Colour changes, as signalling an event detected by the naked eye, are widely used owing to the low cost or lack of equipment required. Those chemosensors involve the binding of a specific anion substrate with receptor sites and a chromophore responsible for translating the receptor-anion association into an optical signal. This colour variation can be related to either structural or conformational changes in the receptor structure when a complex is formed or to the formation of a charge-transfer complex[3,8].

Hydrogen-bonding interactions are the key for the anion sensing[9], and the low-polarity solvent dichloromethane is the selected one to maximize these interactions. Frequently, the solvent choices are acetonitrile (ACN) or dimethylsulfoxyde (DMSO) because the analytical protocols can be easily performed in aqueous media.

In the case of hydroxyderivatives of anthraquinone, the more active positions acting as anion receptors are the 1,4,5-tri and 8-hydroxyanthraquinone (HA). The hydrogen bond functionality, preferably two or more at a time, conferred by this capacity of the –OH group, can facilitate the formation of a hydrogen bridge with the anion acceptor. The chromophoric subunit is the highly-conjugated anthraquinone (AQ) moiety.

Multicomponent arrays rely on large, moderately sensitive sensor elements that can respond to a given analyte or mixture of analytes. In this respect some indicator dyes are ideal candidates to act as optical reporter groups if they present some discrimination between analytes resulting in changes in colour. This fact can be used to signal either qualitatively or quantitatively the presence of particular analytes, in this case anions.

The signal units are arranged into a two-dimensional format for sensing and each sensor element displays relatively low sensitivity and selectivity for a given analyte, but the multicomponent array generates a pattern of responses. The interpretation of fingerprints or patterns generated from the response of every sensor element is analysed by chemometric tools and used for the analysis of complex mixtures.

Aminoanthraquinone derivatives have proven their good capacities to detect several basic anions[1,10] in dichloromethane medium and in DMSO medium[11].

Several anthraquinone reviews have been published covering both the analytical aspects (mainly related to cations detection)[12] and the photophysical behaviour of anthraquinone derivatives[13]. Kinetic[14-18], fluorimetric[19,20], photometric[21], or redox or acid-base indicators[22,23], and several other analytical applications illustrate the various analytical implications of anthraquinone derivatives in the last thirty years.

In respect the use of chemometric techniques to solve problems associated to discrimination of a set of data (photometric, fluorimetric) in order to isolate a group of data from the others, a lot of references can be obtained in the published literature[24].

In this research, it is presented the results obtained with some hydroxyanthraquinones as receptors of anions and the possibility to systematize their use for naked-eye detection and quantitative photometric and/or fluorimetric quantification. To our knowledge, there are only a few reports of a colorimetric chemosensor used to discriminate between different anions.

EXPERIMENTAL

Chemicals and reagents

Hydroxyanthraquinone (HA) derivatives were purchased from Sigma-Aldrich (Spain). Sodium salts of the anions used and acetonitrile and dimethyl

sulfoxyde were acquired from Merck (Darmstadt, Germany). Deionized water was purified by a Milli-Q SP reagent water System (Millipore, Bedford, MA, USA). All the experiments were carried out at room temperature.

Apparatus

Fluorescence measurements were performed in a Perkin-Elmer LS-50 spectrofluorometer (Concord, Canada). Spectrophotometric measurements were performed in a UV-240 Graphicord spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, USA). ELISA plate reader Microplate Reader 2001, Whittaker Bioproducts (Promega, Wisconsin, USA).

RESULTS AND DISCUSSION

In the quantitative photometric and fluorimetric determination, a multianalyte detection and coding has been used. For this, several anthraquinone receivers were placed in a spatial format to allow simultaneous detection and evaluation of multiple chemical information. The arrangement used in the data acquisition is based in plate well frame and signal acquisition was made with a transmission plate reader (ELISA plate reader Microplate Reader 2001, Whittaker Bioproducts).

Four selected coloured hydroxyanthraquinones were added to eight different plate wells and the interaction of the receptor anthraquinone and the non-specific visible anion signal was originated, hence, each well became a sensor element.

Every 5 minutes for 90 minutes the absorbance ratios at two wavelengths A450/A550 of each well were measured. The data obtained were used to make a linear discriminant analysis to determine whether a given HA can discriminate between five different anions. Discriminant analysis is a multivariate statistical procedure that mathematically defines a special discriminant function to separate study anion groups by one classification variable. A statistical program was used for discriminant analysis (Statgraphic 5.1). Thus, different groups were capable of being discriminated by different anthraquinones and finally, the combination of these mixtures made possible to determine more than one analyte at a time.

Visual sensing of anions

On the other hand, a naked-eye anion recognition by anthraquinone hydroxy derivatives was used, as well. Although hydrogen bridge interactions are fundamental to the success of the sensing of anions, and the solvent dichloromethane allows these interactions to be maximal, color changes are also observed in more polar solvents, such as DMSO or ACN, and this allows the performance of measurements in aqueous media, as well. Because of this was chosen these solvents to maximize these interactions, and it was found that a discernable colour based response was observed when more polar solvents, namely ACN and DMSO, were used. Colorimetric sensors are especially attractive if the anion can be detected by naked-eye, without the use of dedicated instrumentation and therefore having advantages over other molecular sensors.

Naked-eyed anion recognition

The colorimetric selective sensing ability of the anthraquinone receptors with anions in DMSO and ACN was monitored by UV-Vis absorption and fluorimetric measurements and by the naked eye observation. The anions were added as salts to the ACN and DMSO solutions of the receptors.

Interestingly, the colour of the solution of receptors was changed from its initial colour to different colours, visible to the naked eye (Figure 1, 2 and 3). The interaction of receptors with anions was investigated in detail through the UV-Vis spectroscopic titration, and fluorimetric spectral behaviours were observed Figure 4.

Initially we focused on 1-, 1,8-, 1,5- and 1,2,5,8-hydroxyanthraquinones as potential off-the-shelf anion sensors because they contain donor -OH groups properly positioned to bind an anion by cooperative hydrogen-bonding interaction and a chromophoric subunit in the anthraquinone skeleton, promoting bathochromic shifts associated to changes in electronic properties as a result of interactions with a bounded anionic substrate that promotes charge transfer effects.

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Chemically/biologically important anions such as carbonate, phosphate, citrate, acetate, oxalate or tartrate in aqueous/acetonitrile (ACN) or dimethylsulfoxyde (DMSO) media were the main objective of the present study.

Optical inspection of solutions of compounds ([quinone] =3.3x10^{-4} M and [anion] =1x10^{-3} M (carbonate, bicarbonate, phosphate, biphosphate, citrate, tartrate and acetate), excepting Cl$^-$, Br$^-$ and oxalate (1x10^{-4} M)) in ACN showed dramatic changes in colour (Figure 1 and 2). The changes could be detected by the naked eye. Figure 3 shows changes in colour using as solvent DMSO.

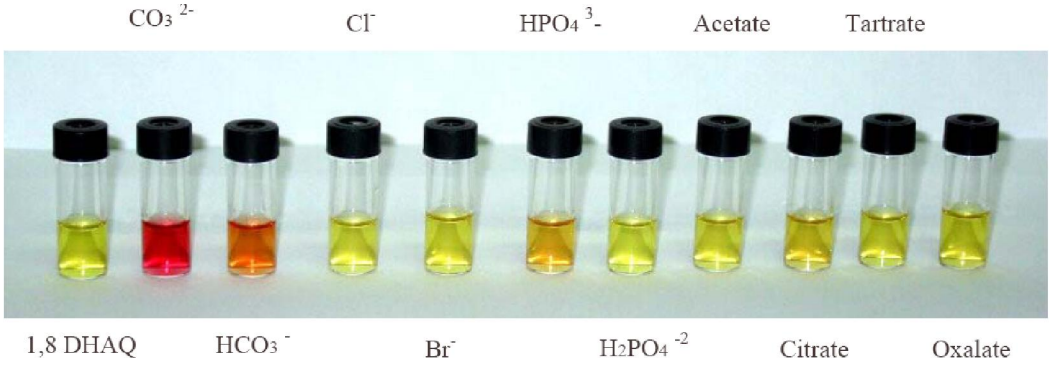

Figure 1 : Photograph showing the color of dilute solutions of 1,8-DHA in ACN [quinone]=3.3x10^{-4} M and [anion]=1x10^{-3}M, excepting Cl-, Br- and oxalate)10^{-2} M)

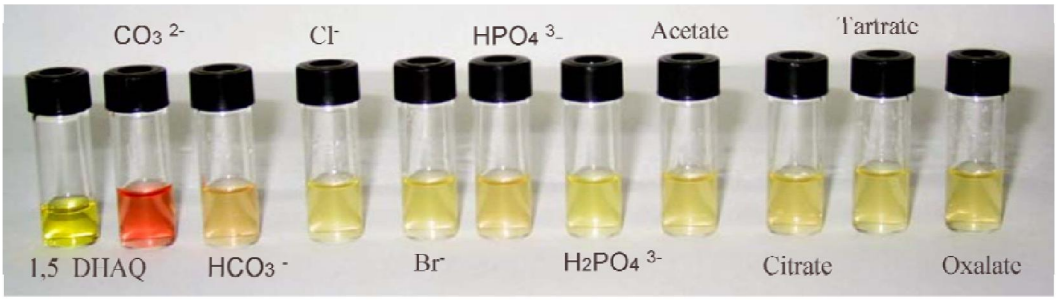

Figure 2 : Photograph showing the colour of dilute solution of 1.5 DHAQ in acetonitrile ([quinone]=3.3×10^{-4} M) and ([anion]= 1×10^{-3}M for all minus 1×10^{-2} M for Cl$^-$, Bl$^-$, oxalate.

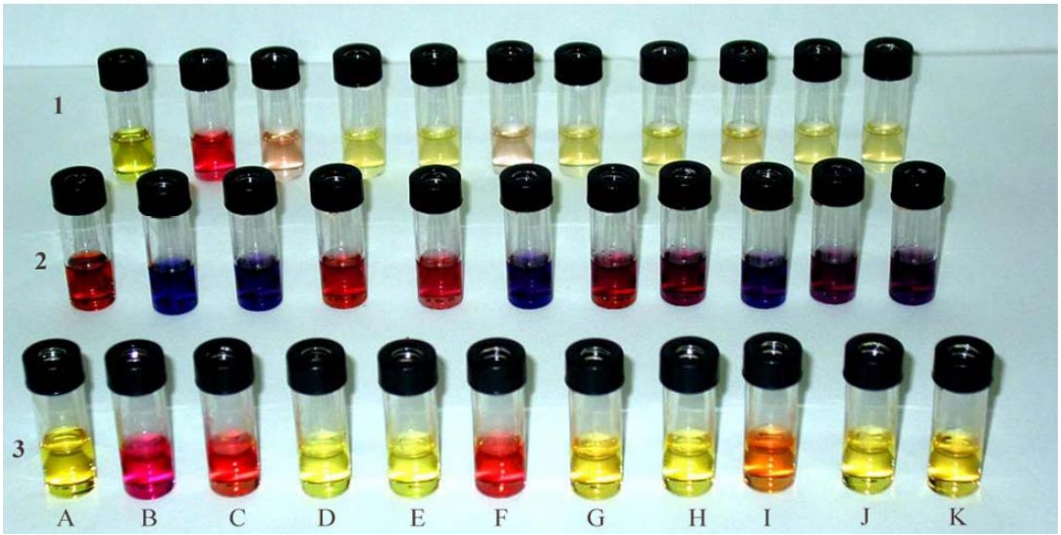

Figure 3 : Photograph showing the color of dilute solutions of 1,5-DHA (1), 1,2,5,8-THA (2) and 1,8-DHA (3) in DMS. ([quinone]=3.3x10^{-4} M) and ([anion]=1x10^{-3} M] for all excepting Cl-, Br- and oxalate that was 1.10^{-2} M). Files notation: A) AQ pure, B)CO_3^{2-}, C) HCO_3^-, D) Cl$^-$, E)Br$^-$, F)HPO_4^{3-}, G) $H_2PO_4^{2-}$, H) acetate, I)citrate, J) tartrate, K) oxalate.

Figure 1 shows the colour of dilute solutions of 1,8-DHA in ACN with the anions indicated in the figure caption; Figure 2 shows the colour of the different solutions with 1,5-DHA in ACN; and Figure 3 arranges the pictures of 1,8-, 1,5- and 1,2,5,8-hydroxyanthraquinones in DMSO with the same anions.

As it can be seen, 1,8-DHA in ACN is more selective and only carbonate, bicarbonate and bibasic phosphate show the sharpest changes because of their highest basic character. Similar reactions are present in 1,5-DHA, but the colour changes are less pronounced. As it is shown in Figure 3, 1,5-, 1,2,5,8- and 1,8- hydroxyanthraquinones in DMSO achieve a wider range of colour changes with the anions studied, especially in the case of 1,2,5,8-DHA. From the analytical point of view, this HA is a bad selective anion reagent. We can take advantage of this situation if a combination between molecular recognition and discriminant analysis is made. It allows the classification of the obtained data and their use as identification criteria.

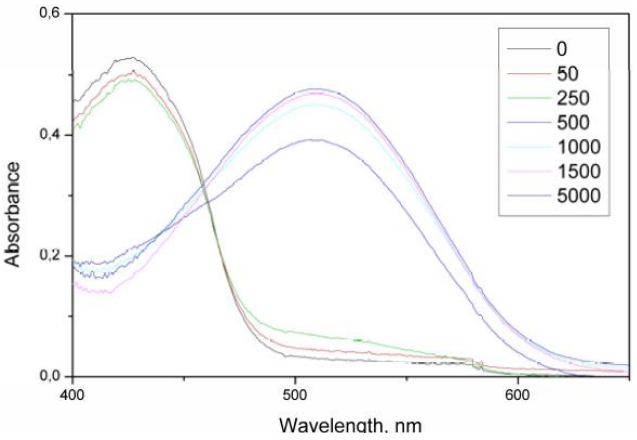

Figure 4 : Absorption spectra of 1,8-DHA 3.3 x 10^{-4} M in ACN and several concentrations of aqueous sodium carbonate from 0 to 5000 µM

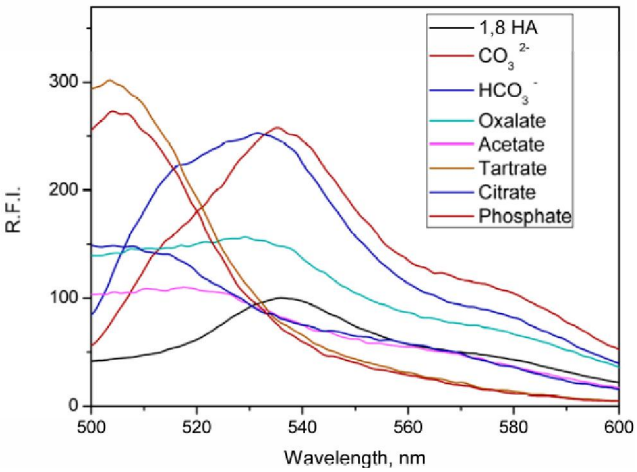

Figure 5 : Emission spectra (λ_{exc}=475 nm) of 1,8-DHA and several aqueous solutions of anions

Determination of carbonate in some water samples

The interaction of compounds with the anions was investigated through spectrophotometric and fluorimetric titrations, by adding a standard solution of each salt in ACN. Changes in UV-vis spectra of the free receptor and for the titration of anthraquinone with different concentrations of carbonate anion, from 0 to 5000 µM are shown in Figure 4.

The 1,8 DHA fluorimetric behavior against each anion studied is shown in Figure 5. Every of these experiments were carried out in ACN solutions. The anthraquinone receptor solution 3.3 10^{-4}M was treated with the representative anions carbonate, bicarbonate, oxalate, acetate, tartrate, citrate and phosphate.

As it has been written before, the 1,8-DHA/carbonate reaction gives the best conditions to be used as the basis of quantitative photometric and fluorimetric procedures. In The photometric measurements, containing concentrations of 1,8-DHA 3.3x10^{-4} M and carbonate concentrations in the range of 50-500 µM resulting in linear fits with R^2 =0.997, detection limit of 60 µM and RSD of 0.5 % (n=10).

Changes in the absorption spectrum of anthraquinone itself was found upon the addition of any of the analytes tested. These spectral changes were particularly dramatic in the case of tartatre and phosphate, being sufficiently large that these species were found to function as an effective colorimetric anion sensor.

In the case of the fluorimetric measurements, a linear range with a coefficient R^2=0.998 was obtained. This calibration curve was obtained by placing within each well, 100 µL of 1,8-DHA 10^{-4}M dissolved in 100 µL of ACN and aqueous sodium carbonate in the range of concentrations between 1-50 µM and measuring the fluorescence intensity at λ_{exc}=475 nm, λ_{em}=550 nm. The detection limit ob-

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TABLE 1 : Analytical applications. Comparison of analytical performance data

Samples	Photometric			Fluorimetric		
	Added $[CO_3^{2-}]$ (mM)	Found $[CO_3^{2-}]$ (mM)	Recovery (%)	Added $[CO_3^{2-}]$ (mM)	Found $[CO_3^{2-}]$ (mM)	Recovery (%)
Sea water	0	2.33±0.03	--	0	2.25±0.04	--
	25	25.7±0.02	93.5	25	26.7±0.05	97.8
	50	51.3±0.08	97.9	50	50.3±0.03	96.1
	150	161.89±0.02	106.4	150	--	--
River water	0	0.97±0.007	--	0	1.00±0.001	--
	25	23.1±0.02	88.5	25	25.7±0.09	98.8
	50	53.10±0.01	104.3	50	51.3±0.03	100.6
	150	163.00±0.03	108.0	150	--	--

tained was 5 μM and precision, in terms of RSD, was 1.2 % (n=10).

The applicability of the proposed method was assessed by the analysis of two environmental water samples, river and sea water. Recovery values from 88.5 to 108.0 % of the carbonate added to sea and river water samples were obtained.

From the results in TABLE, 1, it was found that, the concentration values of the carbonate added to sea water and river water samples were recovered by using both methodologies, photometric or fluorimetric measurements. Furthermore, in the sea water case, the accuracy achieved for the spiked samples demonstrates that the method is not affected by high salinity (approximately 35g l^{-1}), and consequently offers accurate determination of analytes in sea water samples. For comparison, the analytical performance data of both methods reported in TABLE, 1 are rather similar.

Multi anion discriminant analysis

Furthermore, a multi anion discriminative analysis was developed. In this, a linear discriminated analysis (LDA) was performed, where a given HA can discriminate between several different anions. For this measure the absorbance of the solutions AQ-anion was measured at two different wavelengths (450 and 550 nm) every 5 minutes for 90 minutes. At the beginning there were two classification variables (A450 and A550), but as it was independent of the concentration of HA, we calculated the ratios of absorbance at these two wavelengths (A450/A550), thus there is only one variable of classification and the corresponding anion groups to discriminate.

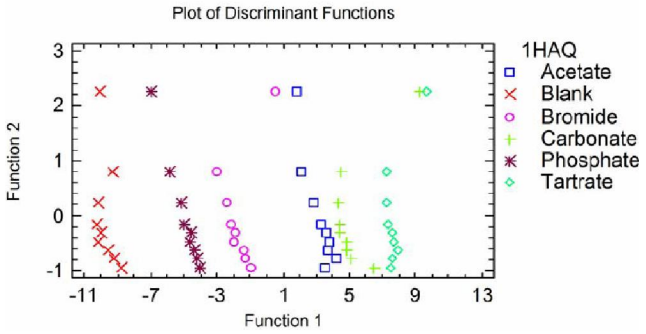

Figure 6 : Plot of discriminant functions for 1-HA, five anions and pure HA

This procedure was applied to classify different anions into different groups by using the signal ratio of the peaks at 450 and 550 nm. This chemometric technique was adopted to systematically classify different anions based on their spectra. Twelve replications of each anion were used for calibration and validations models. For each sample, nine of 12 measurements were used for the development of calibration model and rest for validation. Hence a total of 54 individual samples were used for calibration and 24 for validation. The statgraphics program defines a special discriminant function to separate study anion groups by one classification variable (ratios of signals at 450 nm and 550 nm); each of them makes an independent contribution to the overall discrimination. Taking into consideration the effect of all quantitative variables, this discriminant function produces the statistical decision for guessing to which subgroup of classification variable each subject belongs. The discrimination procedure produces quantitative variables. Figure 6 shows 2D plots to aid the visual interpretation of group differences.

The purpose of this representation is to separate the classes as much as possible. Thus, when observations are plotted, observations belonging to the same class are grouped together. The signal ratio of A450/A550 were used as *X* variables, while the *Y* variables were associated with the six different anion (one different *y* variable for each anion group)

Figure 6 shows the classification model of LDA for different anions based on the ratio of signal at 450 and 550 nm. For example, observing the tartrate group (green rhombuses) it can be seen that it is situated more to the right, in the area of the large positive values of the function, whilst the phosphate group (brown stars) always appears to the left with low negative values.

Validation tests were performed and values of R^2 were more than 0.95 for all discriminant analysis. High correlation between measured and predicted classes and low prediction errors were obtained. The defined LDA model was applied to classify the 6 anion samples (4 replicates from each class) of the external validation subset. The validation samples, a 100 % correct classification was achieved and all the anion were correctly matched to the 6 corresponding groups. These results confirmed that the predictive ability of the developed classification model was very good.

Based on the results obtained, it is believed that the use of appropriate off the shelf molecular entities could provide a generalized new approach to anion sensing. While in some occasion lack the specificity inherent in more sophisticated approaches to anion detection, based on the specific receptor design, the fact that some inherent discrimination between anionic analytes is observed, coupled with its low cost, leads to suggest that this new approach could find application in a variety of areas. This naked eye is not limited to laboratory samples but was also found to work for sea water obtained from the sea water. This observation led us to suggest that this particular species could prove useful as anion sensor.

CONCLUSIONS

To summarize, hydroxyanthraquinones derivatives show good characteristics to act as anion receptors promoting colour and fluorescence changes. The detection by the naked eye of the colour change may be a test to recognise the presence of anions in aqueous solutions. These anthraquinones compounds proved to be a colorimetric anion sensor which shows a selective coloration for each anion in ACN and DMSO. Discriminate analysis allowed the classification of different anions which react with different hydroxyanthraquinones based on colour changes. LDA method was able to discriminate accurately the different anions, with a predictive ability of 100 %, and could be used as a rapid procedure for determination of them. Two methods for determining quantitative carbonate anions have been developed in this article, a photometric and a fluorimetric method. The detection limit and precision, for both methods, are good enough, 50 μM and 1.2 % respectively, for the fluorimetric method, and 60 μM and 0.6 % for the photometric method.

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