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A comparative study on the ionization constants of a Schiff's base in different environments by multiwavelength UV-Vis spectroscopy

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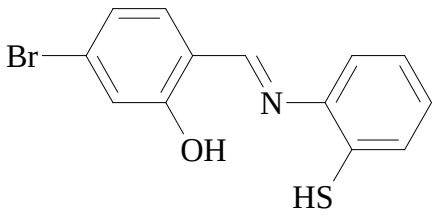
In the present study, the Schiff's base 5-bromo-2-([(2-mercaptophenyl)imino] methyl)phenol was covalently immobilized on agarose, cellulose acetate and sol–gel membranes. A multiwavelength spectrophotometric method was applied to study the acidity constants of immobilized and dissolved forms of the mentioned compound in universal buffer solutions of various pH,recorded over the wavelength range 200- 800 nm. The protolytic equilibrium constants, spectral profiles, concentration diagrams and number of components were calculated. Not unexpectedly, different apparent pK_a values were obtained. The values of the acidity constants of 5-bromo-2-([(2-mercaptophenyl)imino] methyl)phenol were different in various environments. The reason was that solute properties, such as ionization constants, depend on the composition and properties of its surrounding sphere, therefore they are very sensitive to membrane components.

Keywords: Schiff's base; Acid dissociation constants; Spectrophotometry; Principal components analysis; Environments.

INTRODUCTION

The accurate determination of pK_a values is often required in various chemical and biochemical areas and its vital importance is in understanding the distribution, transport behavior, binding to receptors and mechanism of action of certain pharmaceutical preparations. The acidity constants of organic reagents play a fundamental role in many analytical procedures such as acid-base titration, complex formation, different extraction procedures like SPE, ISEs,in optodes and pH sensors.

Different methodologies have been proposed for the experimental determination of acid dissociation constants including ^{1}H-NMR spectroscopy [1], capillary electrophoresis [2], FT-IR spectrometry [3], UV-VIS absorption and fluorescence spectrophotometry [4] and potentiometry [5]. Among these techniques, UV-Vis spectrophotometry has been utilized for the determination of pK_a values of acidic substances due to its high sensitivity (10^{-6} M) [6]. Here, the compound under investigation must possess chromophore(s) in proximity to the ionization center(s) so that the protonated and deprotonated species exhibit sufficient spectral dissimilarity [7]. If the molar absorption coefficients of the ionizing species are known, the pK_a values can be computed by fitting the spectral data obtained at a particular wavelength channel to established equations [6]. However, this method suffers from some limitations. For instance, the absorption data of the fully deprotonated and protonated forms cannot be obtained if these species are not stable within 1-pH units of the pK_a values. Furthermore, the calculation may be difficult to apply for multi-protic substances with overlapping pK_a values. Recently, different chemometric methods based on factor analysis have been developed for multi-wavelength spectrophotometric determination of overlapping acidity constants. Programs and algorithms used for the determination of acidity constants from absorbance data are SQUAD [8], SPECFIT [9] and DATAN 2.1 [10-12]. Fortunately, these problems can be solved very satisfactorily by combining the data obtained in the spectrometric determinations with multivariate analysis and non-linear regression [13].



LH_2

Fig. 1. Molecular structure of the compound used in this work.

In continuation of our research in this field [14, 15], this work was undertaken to determine the acidity constants, concentration distribution diagrams and pure spectra of the species involved inthe Schiff's base, 5-bromo-2-([(2-mercaptophenyl)imino] methyl)phenol **LH_2**, (Fig. 1)**,** covalently immobilized on agarose, triacetyl cellulose (photographic film tape) and sol–gel glass film membranes at 25 °C in aqueous solutions, using a spectrophotometric method.

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EXPERIMENTAL

Materials and instruments

The Schiff's base **LH_2** with the chemical name 5-bromo-2-([(2-mercaptophenyl)imino] methyl)phenol, was synthesized and purified as reported [16, 17]. Other chemicals were of reagent grade,purchased from Fluka or Merck.

A Jenway (USA) model 3020 pH meter with a combined glass electrode was used for pH determinations after calibration against standard Merck buffers. A Shimadzu (Japan) model 1650PC double-beam spectrophotometer was used forrecording the electronic absorption spectra.

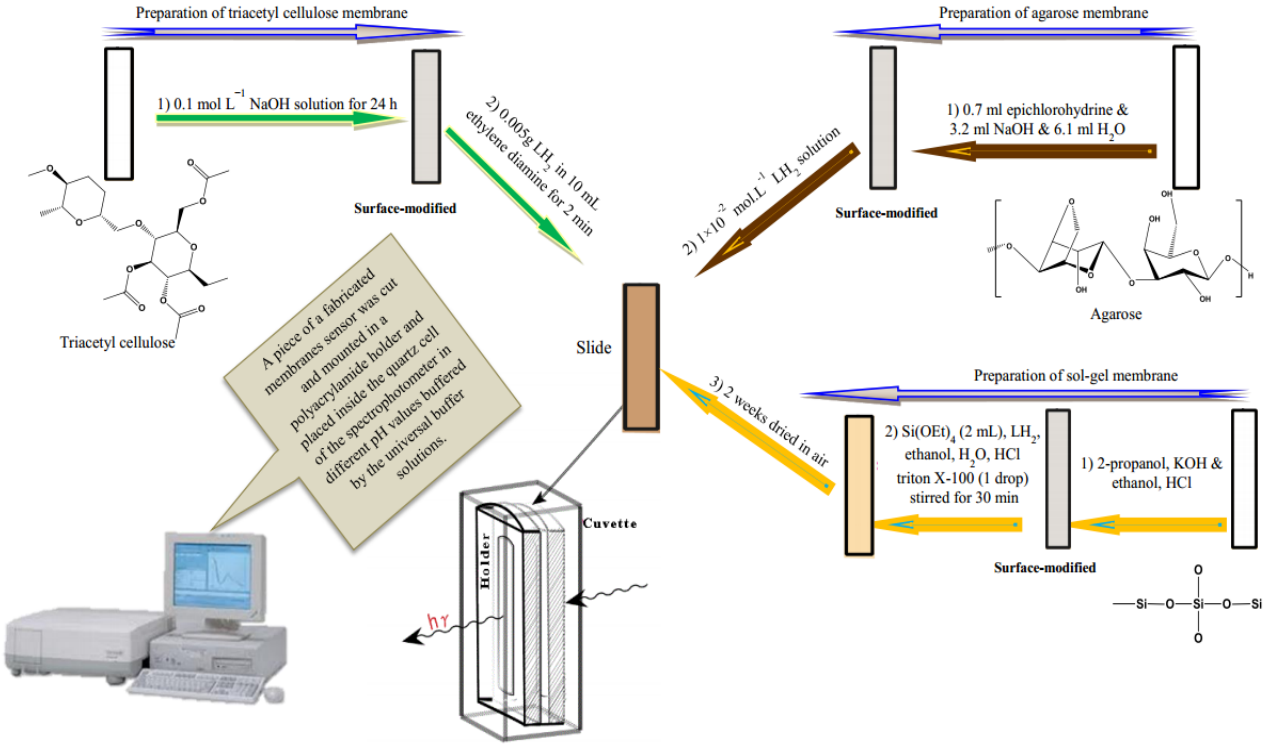
The multivariate analysis Add-in for excel was downloaded for PCA analysis [13] from the Centre for chemometrics website at http://www.chm.bris.ac.uk/org/chemometrics/addins, and the other data treatments were performed in MATLAB for Windows (Mathworks, Version 7.10) [18].

Procedures

A method described elsewhere was used for constructing transparent triacetyl cellulose, agarose and sol–gel membranes with a sensing layer of LH_2, as presented in Scheme 1 [19-24]. For analysis, about 2 ml of a sample buffered by anuniversal buffer was transferred into a 1 cm quartz cell equipped with a membrane sensor. A home-made polyacrylamide holder was used for holding the membranes inside the quartz cells of the spectrophotometer. To obtain the UV-Vis spectra of the dissolved forms of LH_2, 2.5 mL of 5.0×10^{-5} M LH_2 in universal buffer solutions of various pH were measured over the wavelength range from 200 to 800 nm. The concentration values of the immobilized LH_2 on membranes with different composition were calculated by absorbance measurements at the maximum wavelength and same pH in comparison to those of their standard soluble forms.

RESULTS AND DISCUSSION

In the present study, the Schiff's base 5-bromo-2-([(2-mercaptophenyl)imino]methyl)phenol, **LH_2,** is considered as a di-protic solute. The mentioned structure presents two ionizable OH and SH groups. In this case, ring 1 and ring 2 are conjugated*via* an imine linkage, thus deprotonation of the OH group of one ring system should appreciably affect the ionization of the SH group of the other. To determine the acid dissociation constants of this molecule, its UV–Vis spectra were recorded atvariouspH. Using chemometric methods one can analyse the whole spectra, thereby utilizing all spectral information, which considerably reduces the level of error and the result couldbe even



Scheme 1. Experiment setup for absorbance measurements.

further improved. Therefore,multiwavelength spectrometry can be a powerful alternative for systems where a single wavelength is appliedfor every component. The approach is superior to any single-point measurement since several hundreds of data points per spectrum can be treated simultaneously. So the obtained acidity constants are more reliable and precise than those obtained by previous methods [14, 25, 26].

The resulting absorbance spectra were recorded for the protonated form of LH_2at different pH values of the universal buffer. Sample plots in Fig. 2 show the corresponding spectra of LH_2 immobilized in agarose and photographic film.

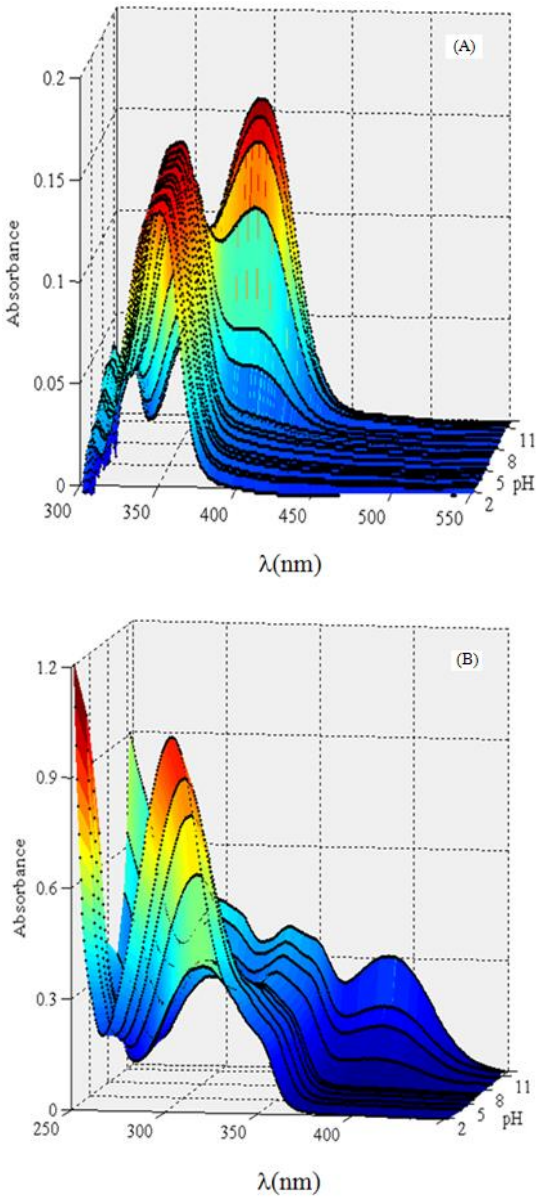


Fig. 2. UV-Vis absorption spectra of LH_2 immobilized in photographic film (A)and agarose (B)membranes as a function of pH.

The most suitable wavelengths in the UV region in the low and in the high pH range with their corresponding molar absorption coefficients (L $mol^{-1}cm^{-1}$) for the compound **LH_2** in various environments are summarized in Table 1. Absorption maxima (λ_{max}) in the UV region observed for **LH_2** in the different systems are assigned to $\pi \rightarrow \pi^*$ transition of the benzenoid system towards the other ring present in its structure.

Table 1. Absorption maxima (λ_{max}) and molar absorption coefficients of 5-bromo-2-([(2-mercaptophenyl)imino] methyl)phenol in various environments. The shorter and longer wavelength bands are observed in the low and in the high pH range, respectively.

Entry	System	λ_{max}(nm)	Molar absorption coefficient
1	Triacetyl cellulose membrane(photographic film)	345, 394.5	32000, 30000
2	Agarose membrane	303.5, 392.5	30400, 10100
3	Sol–gel glass	304.5, 370	27400, 10000
4	Aqueous solution	395.5, 452	18100, 17880

A fundamental question is how many spectroscopically active species are present in this experiment? To answer this question principal components analysis (PCA) was performed on the data matrix. The observed rank of the data matrix was three or two depending on the composition and properties of its surrounding sphere like aqueous solution, agarose, cellulose acetate and sol–gel membranes. Although the rank of a matrix is a mathematical concept, in the ideal case it corresponds to the number of present species. Cross-validation is also a complementary method to determine the number of PCs. A simple graphical approach is to take the number of PCs, where the graphs of PRESS or RSS are level off, see Figure 3. As seen in Fig. 3, there are two or three significant factors in the corresponding plots.

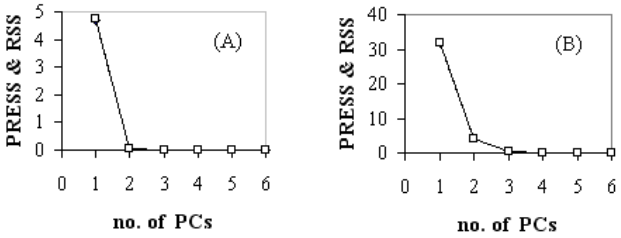


Fig. 3. Plots of PRESS & RSS *vs.* principal components for LH_2 immobilized in photographic film (A) and agarose (B)membranes.

These factors could be attributed to the protogenic species available in the dissociation equilibria of the mentioned compound [25, 26].

The two successive acid–base equilibria of the compound used in this workin different environmentsare represented by the following equations:

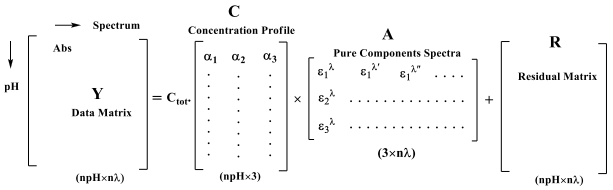
$$LH_2 + H_2O \rightarrow LH^- + H_3O^+ \quad (1)$$
$$LH^- + H_2O \rightarrow L^{2-} + H_3O^+ \quad (2)$$

where LH_2 is the neutral form of the molecule, LH^- (deprotonated form of LH_2) is the monoanion species and L^{2-} (deprotonated form of LH^-) is the dianion solute. The equilibrium constants that characterize the ionization reactions described in equations (1) and (2) are indicated as K_1 and K_2, respectively. The reaction described in Eq. (1) predominates in the low pH range, while the second ionization reaction occurs in the high pH range [26, 27].

The monoprotic and diprotic acid-base equilibrium models were developed by combination of Beer law and law of mass action which can be summarized in a matrix equation as Y=CA+R. A Matlab-based program for nonlinear regression with Newton-Gauss-Levenberg/Marquardt (NGL/M) algorithm was used [18]. This model was used by minimization of the sum of squares to fit experimental data and to calculate the pure spectra, distribution diagrams and acid dissociation constants of the mentioned molecule in various environments.

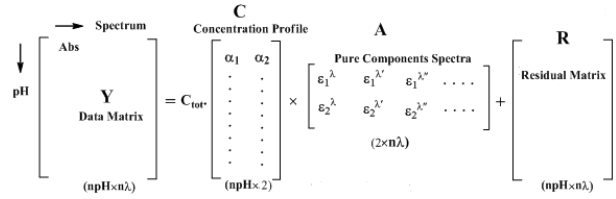
If two chemical equilibria (three species, i.e., LH_2, LH^- and L^{2-}) are apparent at various pH values the following model has been used. It is shown here in corresponding final matrix notation.

$$Y = C \times A + R$$



Another model for a sole chemical equilibrium is represented here:

$$Y = C \times A + R$$



Three principal components were observed in aqueous solution, agarose and sol–gel membranes using multiwavelength spectrometryat various pH, which are equal to three chemical species in the spectrum. In the other membrane (photographic film) two chemical species were observed in the corresponding spectrum (Figs. 4 and 5). In order to show the compatibility of the proposed model with the experimental data, in the best case utilizing the sum of squares and the dimensions of the corresponding experimental data matrix, i.e. a & b was calculated $[ssq/(a\times b)]^{1/2}=0.004$ which is a good indication for the compatibility of experimental data with model.

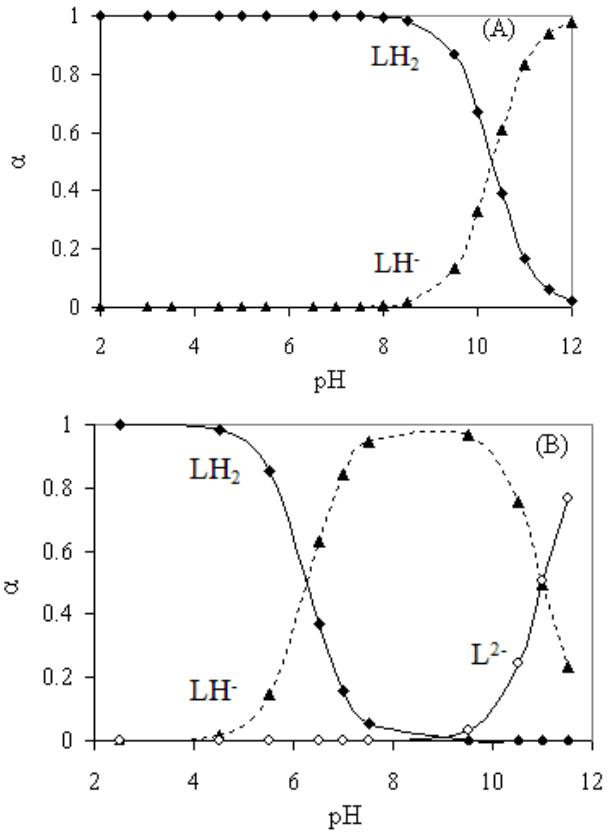


Fig. 4. Distribution diagrams of LH_2 immobilized in photographic film (A)and agarose (B)membranes at different pH values.

As shown in Table 2, different pK_a values were obtained for the immobilized forms of LH_2 on agarose and on sol–gel membranes. The same results were found for the dissolved form. This can be related to the relatively high hydrophilicity of agarose and sol–gel permitting faster diffusion ofprotons in the membrane. Therefore, the response is fast and hydrogen release is easier,leading to a decrease in pK_a values during the deprotonation process.

Also, the authors believe that the environment of the photographic film may well change the pore size and result in a prolonged response time of the sensor to hydrophilic species, then a sole pK_a will be obtained at various pH values.

Table 2. Determination of experimental pK_1, pK_2 values of the title compound in various environments.

Entry	Environment	Number of present species	pK_1	pK_2
1	Triacetyl cellulose membrane[a]	2	10.312±0.004	----
2	Agarose membrane	3	6.265±0.010	11.000±0.013
3	Sol–gel glass	3	7.040±0.020	11.211±0.015
4	Aqueous solution	3	6.764±0.010	11.115±0.021

[a]Photographic film

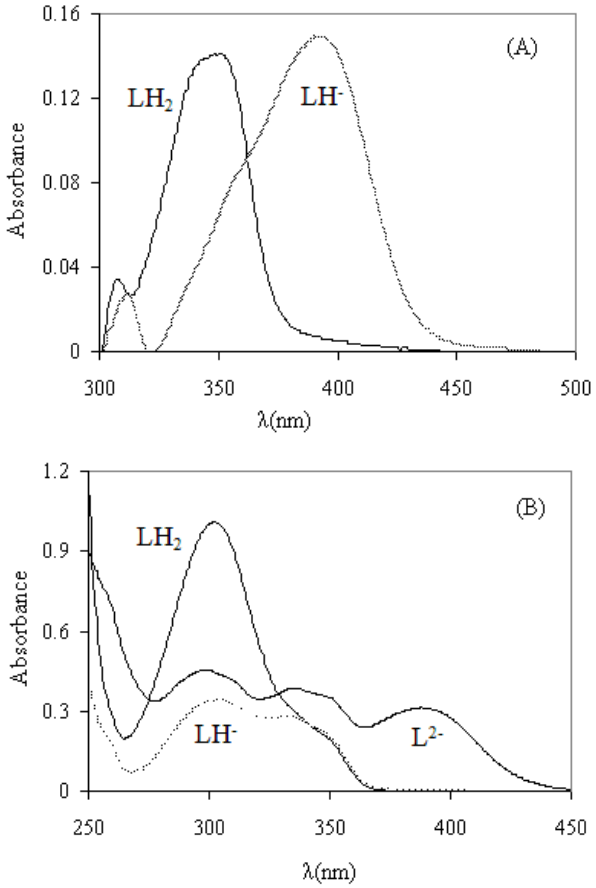


Fig. 5. Pure spectra of each assumed species of LH_2 immobilized in photographic film (A) and agarose (B)membranes.

CONCLUSIONS

In this work the acidity constants of 5-bromo-2-([(2-mercaptophenyl)imino]methyl)phenol covalently immobilized on agarose, triacetyl cellulose and sol–gel membranes at different pH values were studied by a multiwavelength spectrophotometric method. A model based on theNewton-Gauss-Levenberg/Marquardt (NGL/M) algorithm was used to find the minimum of the sum of square function values to fit experimental data and to calculate the ionization constants, the pure spectrum profiles of each chemical species and the concentration profiles of the mentioned molecule in several environments. The influence of composition and properties of environmentson the obtained values of the studied molecular structure was investigated.

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СРАВНИТЕЛНО ИЗСЛЕДВАНЕ НА ЙОНИЗАЦИОННИТЕ КОНСТАНТИ НА ШИФОВА БАЗА В РАЗЛИЧНИ СРЕДИ ЧРЕЗ UV-Vis СПЕКТРОСКОПИЯ С ПОЛИХРОМАТИЧНА ДЪЛЖИНА НА ВЪЛНАТА

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(Резюме)

В настоящото изследване Шифовата база 5-бромо-2-([(2-меркаптофенил)имино]метил)фенол е ковалентно имобилизирана върху агароза, целулозен ацетат и зол-гел мембрани. Приложен е полихроматичен спектрофотометричен метод за изследване на константите на киселинност на имобилизираните и разтворените форми на споменатото съединение в универсални буферни разтвори с различно pH в областта на дължини на вълните 200-800 нм. Протолитните равновесни константи, спектралните профили, диаграмите на концентрациите, както и броят на компонентите са изчислени. Не неочаквано бяха получени забележимо различни pKa стойности. Константите на киселинност на 5-бромо-2-([(2-меркаптофенил)имино]метил)фенол се различават една от друга в различни среди. Причината е, че свойствата на разтвореното вещество, катостойност на йонизационните константи, зависят от състава и свойствата на заобикалящата го сфера, поради което са много чувствителни към мембранните компоненти.