Determination of stability constants of 5-(2-hydroxybenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione with copper(II) and mercury(II) ions

Morteza BAHRAM[1,*], **Nader Noroozi PESYAN**[1], **Abdolhossein NASERI**[2],
Masomeh TASBIHFOROSH[1]

[1]*Department of Chemistry, Faculty of Science, Urmia University, Urmia-IRAN*
e-mail: m.bahram@urmia.ac.ire

[2]*Department of Analytical Chemistry, Faculty of Chemistry, University of Tabriz,*
Tabriz-IRAN

In this work, a hard model chemometric method was used to determine the stability constants of copper(II) and mercury(II) complexes with the newly synthesized ligand 5-(2-hydroxybenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione in acetonitrile by UV-Vis spectrophotometry. The ligand forms stable complexes with both metal cations. The protonation constants of the ligand were also measured in an ethanol-water (1:1) mixture.

Key Words: Cu(II) and Hg(II) complexes, 5-(2-hydroxybenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione, spectrophotometry

Introduction

Complexes of divalent transition metals involving derivatives of barbiturates and salicylaldehyde are of particularly immense importance because of their similarity to the biological dioxygen carriers.[1−7]

Many experimental techniques are known for studying the formation of complexes in solution. The most common experimental techniques for the determination of stability constants are potentiometry, conductometry,

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*Corresponding author

polarography, nuclear magnetic resonance spectroscopy, UV-Vis and fluorescence spectroscopy, calorimetry, mass spectrometry, and kinetic measurements.[8−13]

Within the context of kinetic and equilibrium investigations, there are 2 fundamentally different ways of extracting information: model-based analysis and model-free analysis.[14] In model-based analysis, the goal is to extract the basic parameters of the process, i.e. calculation of the equilibrium constant in thermodynamic studies. From the experimental data in model-based analysis, and depending on the nature of the data, additional information, such as spectra of components, can also be extracted. For example, if the absorption spectra are acquired during the process, it is possible to determine the molar absorption spectra of all reacting species, even if they only exist as transitory minor species. Such spectra allow structural analysis, which is not easily achieved otherwise.[15] On the other hand, in model-free analysis, the analysis is restricted to the determination of the response curve, usually the concentration profiles of the active species, and their spectral shapes.[15] There are 3 distinct components in a model-based analysis: the data, the chemical model used to describe the chemical process, and the fitting of the parameters (e.g. equilibrium constants) that are pertinent to the model.

As part of an ongoing project on the development of chelators for bivalent metal ions (Cu(II), Hg(II), Co(II), etc.), in this work the complexation behavior of a newly synthesized ligand with some metallic cations was studied by model-based analysis. The formation constants of the complexes of the synthesized ligand, 5-(2-hydroxybenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione, with several metallic cations in acetonitrile solvent were determined by UV-Vis spectrophotometry. The protonation constants of the ligand in aqueous ethanol were also determined.

Experimental section

Reagents

The ligand 5-(2-hydroxybenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (HTDP) (Scheme 1) was synthesized according to the literature.[6,7] The reactants for the synthesis of HDTP, salicylaldehyde, and 2-thioxo-dihydro-pyrimidine-4,6-dione were purchased from Merck with the highest purity available and used without further purification. All other solvents and chemicals are commercially available (Merck) and were used without purification.
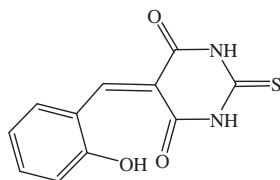
Scheme 1. Molecular structure of HTDP.

Methods

All solutions were prepared in acetonitrile. Microliter amounts of concentrated metal cation solutions (e.g. 0-150 μL from 10^{-3} M) were added to ligand solutions in increments (2 mL 10^{-4} M). The solutions were

kept constant at $25 \pm$ xx °C. The variation of the absorbances upon addition of metal ions is shown in Figure 1, indicating the formation of a complex with a Cu^{2+} ion. Scheme 2 represents the possible structure of the complex compounds of Cu^{2+} and Hg^{2+} cations with HTDP.
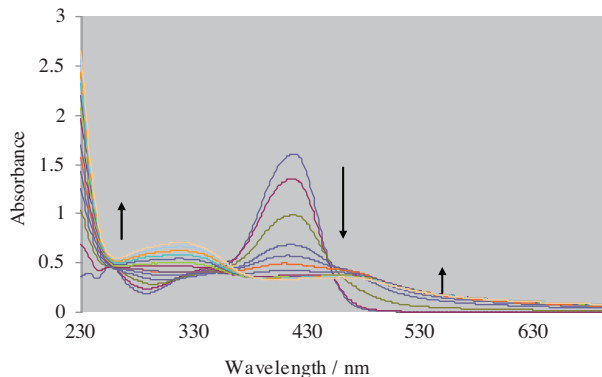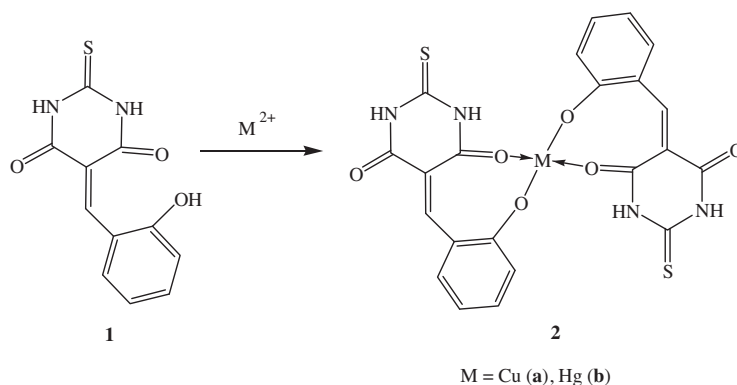

Figure 1. Changes in UV-Vis absorption spectra of 5-(2-hydroxybenzylidene)-2-thioxo-dihydro-pyrimidine-4,6-dione in acetonitrile solvent (2.5×10^{-5} mol/L); arrows indicate a change in the absorbance with increase in the concentration of Cu^{2+} ş. $[Cu^{2+}$ş] mol/L: 0, 2.5×10^{-6}, 5×10^{-6}, 7.5×10^{-6}, 1.0×10^{-5}, 1.5×10^{-5}, 1.8×10^{-5}, 2×10^{-5}, 2.3×10^{-5}, 2.5×10^{-5}, 2.8×10^{-5}, 3×10^{-5}, and 3.3×10^{-5}.

Scheme 2. Proposed structure of metal complexes with HTDP.

Results and discussion

Treatment of the experimental data

The stepwise formation constants of a 2:1 complex between a ligand L and a cation M^{n+} can be described as:

$$M^{n+} + L^{p-} \xrightarrow{k_{f1}} ML^{n-p}, \tag{1}$$

$$ML^{n-p} + L^{p-} \xrightarrow{k_{f2}} ML_2^{n-2p}. \tag{2}$$

The corresponding formation constants are defined as:

$$k_{f1} \quad = \frac{[ML^{n-p}]}{[M^{n+}][L^{p-}]} \quad , \tag{3}$$

$$k_{f2} \quad = \frac{[ML_2^{n-2p}]}{[ML^{n-p}][L^{p-}]} \quad . \tag{4}$$

The mass balances for the total ligand C_L and total salt concentration C_{salt} or total cation concentration C_M are:

$$c_L = [L^{p-}] + [ML^{n-p}] + 2[ML_2^{n-2p}] \tag{5}$$

and

$$c_M = [M^{n+}] + [ML^{n-p}] + [ML_2^{n-2p}]. \tag{6}$$

The following equation can be derived from Eqs. (1-6) with respect to ligand concentration:

$$K_{f1}K_{f2}[L]^3 + (K_{f1}(1 + K_{f2}(2C_M - C_L)))[L]^2 + (1 + K_{f1}(C_M - C_L))[L] - C_L = 0. \tag{7}$$

An ordinary method, fitting the results at a single wavelength, can often be simply used for the estimation of k_{f1} and k_{f2}. As seen from Figure 1, the ligand itself and the consecutive complexes have absorptions at the studied wavelength; thus, there is no distinct wavelength for any component. Therefore, an ordinary method cannot be used here for the accurate estimation of k_{f1} and k_{f2}. In this contribution, the model-based analysis method was employed to study the formation constants of the dicationic complexes with HTDP.

One should bear in mind that in the case of a 1:2 one step ML_2 complex formation, the following equation could be derived:

$$M + 2L \leftrightarrow ML_2, \tag{1'}$$

$$k_f = [ML_2]/[L]^2[M], \tag{2'}$$

$$C_L = [L] + 2[ML_2], \tag{3'}$$

and

$$C_M = [M^{n+}] + [ML_2]; \tag{4'}$$

$$[L]^3 + (2C_M - C_L)K_f[L]^2 + [L] - C_L = 0. \tag{5'}$$

Theory of model-based analysis

For the multivariate complexometric measurements in nt reaction concentration and $n\lambda$ wavelengths, $\mathbf{D}$ is decomposed into the product of $\mathbf{C}$, containing concentration profiles and $\mathbf{S}$ absorption spectra (absorption data are governed by the Beer-Lambert law and the measurements are well-described by a matrix equation):

$$\mathbf{D} = \mathbf{C}\mathbf{S}^T + \mathbf{E}, \tag{8}$$

where $\mathbf{D}$ is a matrix, the rows of which are formed by the absorption spectra measured as a function of the progress of the process. The columns of $\mathbf{D}$ are the absorption traces measured at different wavelengths. According to the Beer-Lambert law, this matrix can be decomposed into the product of matrix $\mathbf{C}$ containing,

columnwise, the concentration profiles of the absorbing species and a matrix $\mathbf{S}$ containing, rowwise, their molar absorptivities. Matrix $\mathbf{E}$ is a collection of the residuals, the difference between the measurement $\mathbf{D}$ and its calculated representation $\mathbf{CS}^T$.

Nonlinear least squares hard-modeling consists of finding a set of parameters for which the sum over all the squares, *ssq*, over all the elements of the error matrix, $\mathbf{E}$, is minimal.[16] This crucial sum is a function of the measurement, $\mathbf{D}$, the predefined model, and the parameters:

$$ssq = \sum E_{i,j}^2 = f(D, \mathrm{mod}el,\ parameters). \tag{9}$$

The first step in hard-modeling processes is the establishment of a correct model, e.g. the expected equilibrium species in equilibrium studies. This is generally the most difficult task and requires the knowledge and experience of the researcher.

For this case, the experimenter can use a soft-modeling approach, such as EFA and/or OPA, as a very useful method to estimate the right model.

For spectral series taken at many wavelengths, the number of parameters to be fitted can be very high, as it includes the nonlinear parameters (e.g. equilibrium constants), which define the matrix $\mathbf{C}$ and molar absorptivities that form the matrix $\mathbf{S}$.

The *roots* command of MATLAB was applied for solving the third or second order equations, Eqs. (7) or (5'), in order to establish the matrix $\mathbf{C}$ (concentration profile) by initial estimation of equilibrium constants. It is of utmost importance to recognize that the molar absorptivities are linear parameters, which can be effectively eliminated from the list of parameters that need to be fitted iteratively. At any stage in the process, the best linear parameters are explicitly calculated as $\mathbf{S}^T = \mathbf{C}^+\mathbf{D}$, where $\mathbf{C}^+$ is the pseudoinverse of the matrix $\mathbf{C}$.[17] Thus, for a given model, the sum of squares can be defined as a function of only the nonlinear parameters (e.g. the equilibrium constants), and these nonlinear parameters, which define $\mathbf{C}$, are the only ones that need to be fitted in an iterative process. This very small number of parameters is a key factor in explaining the robustness of the hard-modeling approaches.

Summarizing, given the model and the measurement $\mathbf{D}$, Eq. (8) can be written as:

$$ssq = f(\mathbf{k}) = \mathbf{D}\ -\mathbf{CS}^T = \mathbf{D}\ -\mathbf{CC}^+\mathbf{D}, \tag{10}$$

where $\mathbf{k}$ is the vector of nonlinear parameters, as defined by the chosen model. Any method that performs this minimization task can be applied. In this work, the Newton-Gauss-Levenberg-Marquardt algorithm (NGL/M)[16] was applied because it is fast and robust, and it additionally delivers basic statistical analysis of the resulting parameters.

Starting with initial guesses of equilibrium constants k_{f1} and k_{f2}, the concentration profiles based on the model proposed were constructed. These equilibrium constants were refined in an iterative approach by the NGL/M algorithm so as to minimize the *ssq*. If the improvement in *ssq* in 2 successive iterations was below a certain threshold, i.e. the shift

in the parameters resulted in no further improvement of the *ssq* value, then the process was terminated and the final values of the parameters leading to the last *ssq* were reported. It must be mentioned that in each iteration, the matrix of the linear parameters $(\mathbf{S}^T)$ was calculated explicitly as $\mathbf{S}^T = \mathbf{C}^+\mathbf{D}$.

As mentioned above, often the most difficult aspect of the model-based approach is to define the correct chemical model. A soft model process (e.g. multivariate curve resolution-alternative least squares, MCR-ALS)[18−20] can be used prior to applying any equation during any hard model approach.

The MCR-ALS method provides a set of pure theoretical spectra for each process (forced to several constraints, e.g. nonnegativity) and a set of concentration profiles, which indicate the relative changes in the concentration of every species, but, as a consequence of the lack of closure constraint, not their absolute values.

Analysis of experimental data

Figure 1. represents the complexometric spectra for the complexation of Cu^{2+} with HTDP.

Singular value decomposition (SVD) showed that there were 3 components in the investigated wavelength range of 200-800 nm (Figure 2). The results of MCR analysis for this data set, represented in Figure 3, reveal that the system may obey Eq. (6) and that the formed complex was CuL_2. Therefore, the absorbing components in this system could be assumed to be L, CuL_2, and Cu^{2+} (solvated cation).
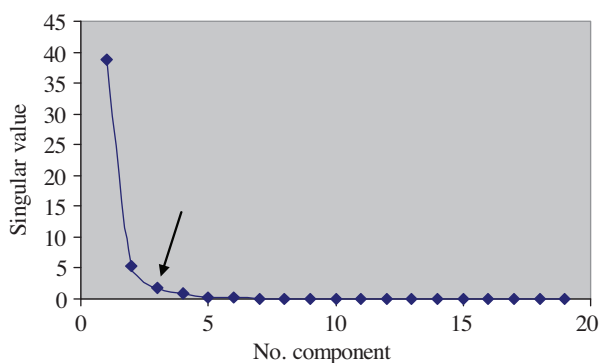

Figure 2. Consecutive singular values of singular value decomposition of Cu^{2+}-HTDP complexometric data matrix as a function of number of components.
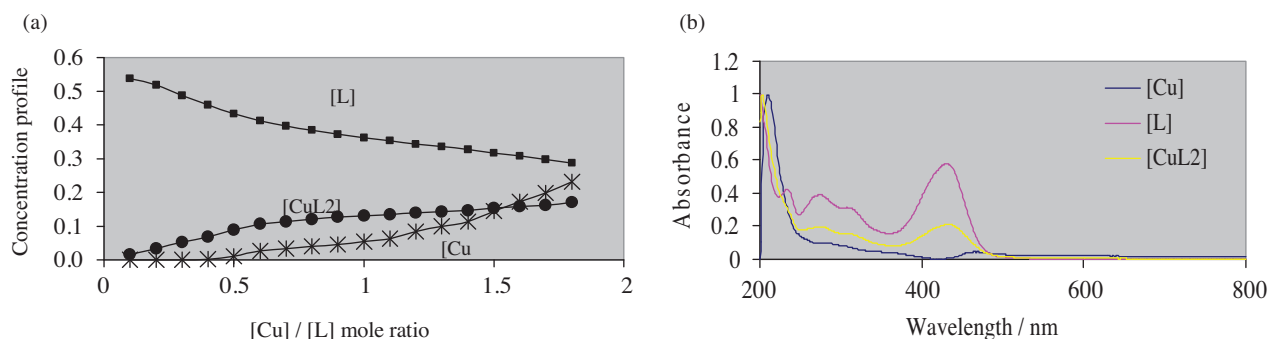

Figure 3. Results of applying MCR on the complexometric UV-Vis absorption spectra of 5-(2-hydroxybenzylidene)-2-thioxo-dihydro-pyrimidine-4,6-dione in acetonitrile with Cu^{2+}. Constraints: nonnegativity of spectra and nonnegativity and unimodality of concentration profiles.

Model-based analysis was then applied for accurate determination of K_f as well as for resolving the system and acquiring the pure spectra. The results are shown in Figure 4. The equilibrium constant of the CuL_2 complex was calculated to be $10^{6.4}$-$10^{6.5}$ using different initial estimates.
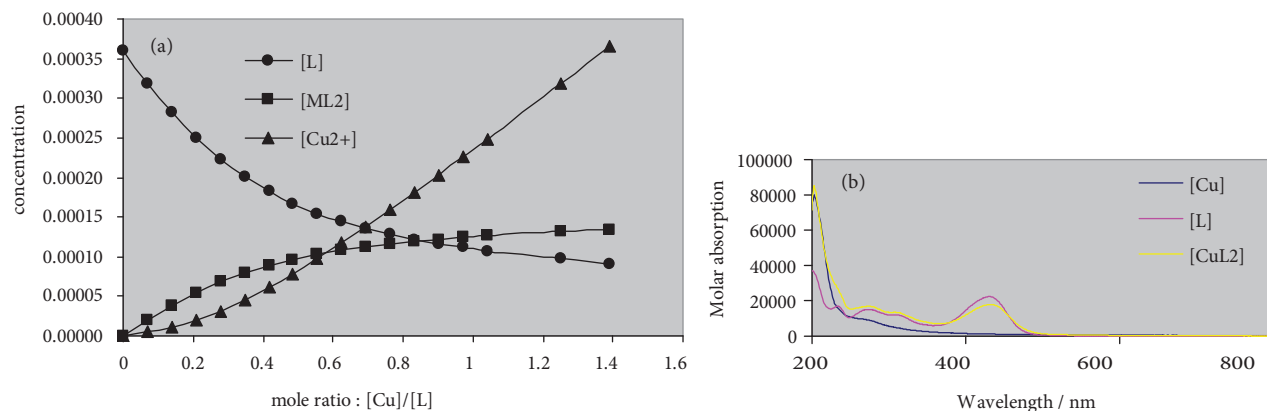

Figure 4. Results of model-based analysis of complexation of Cu^{2+} with 5-(2-hydroxybenzylidene)-2-thioxo-dihydro-pyrimidine-4,6-dione in acetonitrile.

The same study was done for the complexation of Hg^{2+} with 5-(2-hydroxybenzylidene)-2-thioxo-dihydro-pyrimidine-4,6-dione in acetonitrile. Figure 5 represents the complexometric spectra of HTDP with Hg^{2+}. The number of significant components was estimated using a singular value decomposition algorithm. Figure 6 shows that there were 2 significant components in this matrix. Because the investigated wavelength range for this data was 250-750 nm and also because the Hg^{2+} (solvated form) had no absorbance in this range, it can be concluded that the spectroscopic active components were the ligand itself and a produced complex.
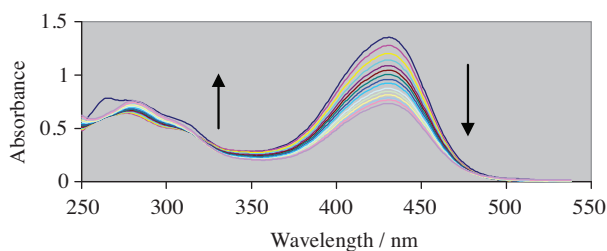

Figure 5. Changes in UV-Vis absorption spectra of 5-(2-hydroxybenzylidene)-2-thioxo-dihydro-pyrimidine-4,6-dione in acetonitrile solvent (3.0×10^{-5} mol/L); arrows indicate a change in the absorbance with increase in the concentration of Hg^{2+} ş. [Hg^{2+} ş] mol/L: 5×10^{-6}, 1×10^{-5}, 1.5×10^{-5}, 2×10^{-5}, 2.5×10^{-5}, 3.0×10^{-5}, 3.5×10^{-5}, 4×10^{-5}, 4.5×10^{-5}, 5×10^{-5}, 5.5×10^{-5}, 6×10^{-5} and 11.5×10^{-5}.

The results of MCR-ALS are also shown in Figure 7. Model-based analysis of this data set as a one step 1:2 complex is shown in Figure 8. The equilibrium constant of this complex was calculated to be $10^{7.90}$-$10^{7.96}$ using different initial estimates.
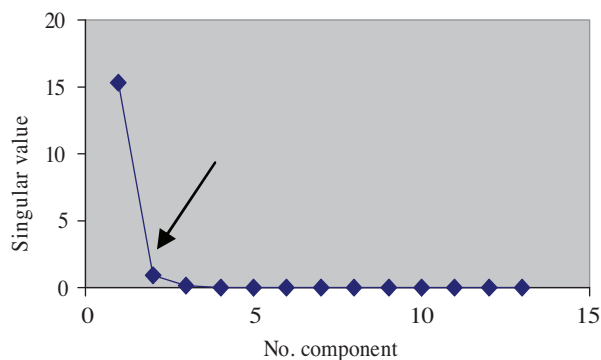
Figure 6. Consecutive singular values of singular value decomposition of Hg^{2+}-HTDP complexometric data matrix as a function of number of components.
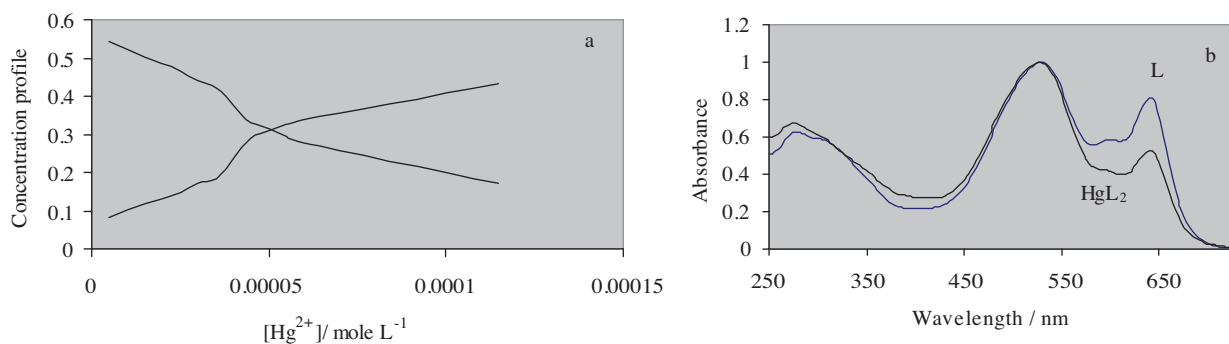

Figure 7. Results of applying MCR on the complexometric UV-Vis absorption spectra of 5-(2-hydroxybenzylidene)-2-thioxo-dihydro-pyrimidine-4,6-dione in acetonitrile with Hg^{2+}. Constraints: nonnegativity of spectra and nonnegativity and unimodality of concentration profiles.
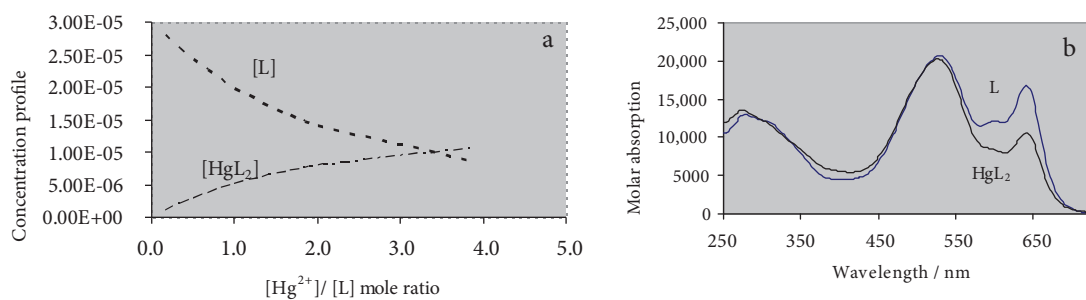

Figure 8. Results of model-based analysis of complexation of Hg^{2+} with 5-(2-hydroxybenzylidene)-2-thioxo-dihydro-pyrimidine-4,6-dione in acetonitrile.

Analysis of pH-spectral data

In order to study the pH dependency of the synthesized ligand and estimate the possible pKa value(s), the spectra of 10^{-5} mol/L of ligand at different pH values were recorded. A 50:50 ethanol-water solution was used

as a solvent and concentrated HCl, NaOH, acetic acid/acetate, and ammonium/ammonia buffers were applied for adjustment of pH values. The spectra of HTDP at different pH values are shown in Figure 9a. This data set was analyzed by MCR by applying a nonnegativity constraint for spectra and nonnegativity and unimodality constraints for the pH mode. Using the results, 3 acid/base species were obtained for this ligand, namely H_2L, HL^-, and L^{2-}, which are represented in Figures 9b and 9c. Using these results, the consecutive acidity constants (pK_{a1} and pK_{a2}) were estimated to be 3.2 and 6.3, respectively.
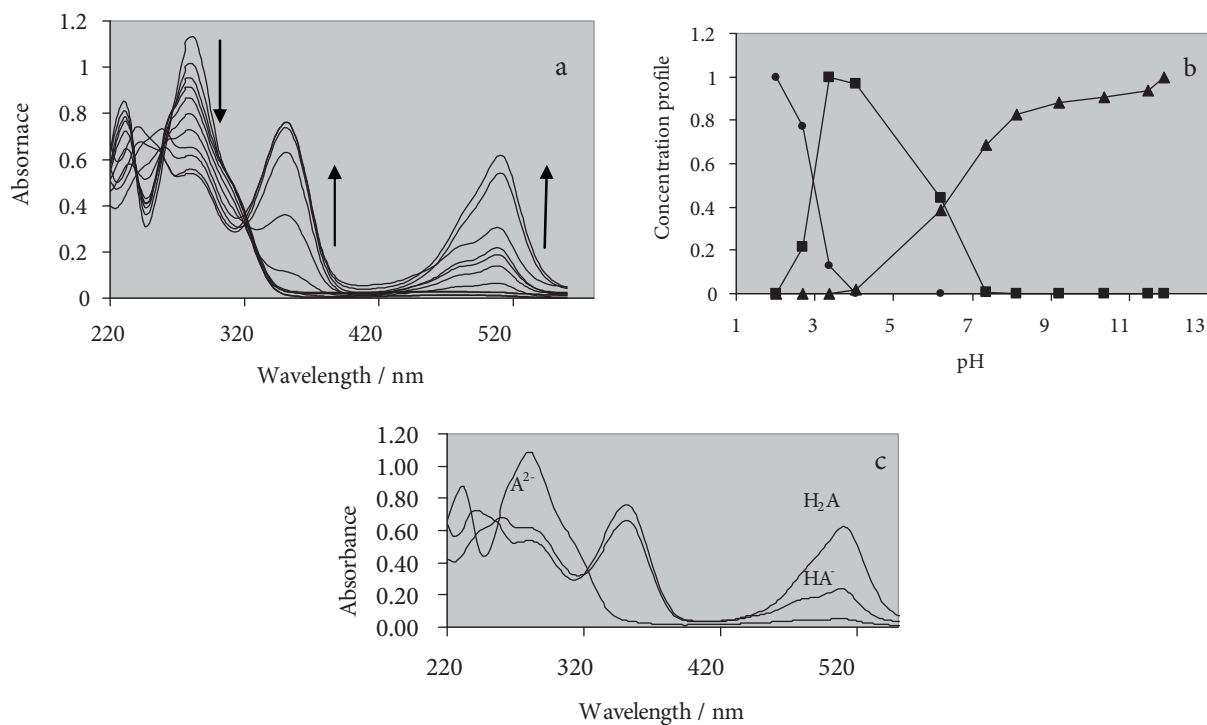

Figure 9. a) The pH-spectral titration data of 5-(2-hydroxybenzylidene)-2-thioxo-dihydro-pyrimidine-4,6-dione in aqueous solvent (pH: 2.0, 2.7, 3.4, 4.1, 6.2, 7.4, 8.1, 9.2, 10.4, 11.5, 11.9), b) the concentration profiles, and c) the spectra of H_2L, HL^-, and L^{2-} obtained using MCR-ALS. Constraints: nonnegativity of spectra and nonnegativity and unimodality of pH profiles.

Conclusion

In this work, a model-based method was used to determine the stability constants of some metal complexes of a nearly insoluble synthesized ligand, 5-(2-hydroxybenzylidene)-2-thioxodihydropyrimidine-4,6($1H$,$5H$)-dione. Among the studied metallic cations (Ni(II), Co(II), Zn(II) (not shown), Cu(II) and Hg(II)), the most stable complexes with this ligand were formed by Cu(II) and Hg(II). These results suggest that this ligand could be used as a new active emissive probe to detect Cu(II) and Hg(II) by absorption spectroscopy. This ligand could also be used for preconcentration and determination of trace amounts of these cations.

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