Chemometric 방법에 의한 메탄올/물 계에서 전이 금속 이온과 소염제 Piroxicam의 산성도 및 착체 형성에 관한 분광광도법 연구

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Spectrophotometric Study of Acidity and Complex Formation of Anti-Inflammatory Drug Piroxicam with Some Transition Metal Ions in Different Methanol/Water Mixtures by Chemometric Methods

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요약. 소염제 piroxicam (PX, 4-hydroxy-2-methyl-N-2-pridyl-2H-1, 2-benzothiazine-3-carboxadiamide-1,1-dioxide)의 착물형성을 전이금속이온, Co(II), Ni(II), Cu(II), Zn(II)과 함께 메탄올(MeOH)/물 이성분 혼합물에서 25 ℃, 일정한 pH = 5.0와 I = 0.1 M에서 분광광도법으로 연구하였다. 컴퓨터 프로그램 SQUAD를 스펙트라 데이터로부터 원하는 정보를 얻는데 사용하였다. Fitting 과정의 output은 안정도 상수, 평가한 안정도 상수의 표준편차, 농도분포 다이그램, 모든 종의 스펙트럼 프로파일이다. Co(II), Ni(II), Cu(II), Zn(II)의 PX 착체의 안정도 순서는 Cu(II) > Co(II) > Ni(II) ≈ Zn(II) 순서이다. 이것은 이들 금속이온들이 기하학적 경향이 다른 이유일 것이다. PX의 산성도 상수는 다른 pH 값에서 흡수 스펙트럼으로부터 위의 조건에서 역시 결정하였다. 컴퓨터 프로그램 DATAN을 PX의 산성도 상수의 결정하는 데 사용하였다. 산성도 상수의 validity는 잘 알려진 컴퓨터 프로그램 SPECFIT/32을 사용하였다. 안정 및 산성도 상수의 용매성질, 음이온과 같은 다른 인자의 효과에 관하여 자세하게 논의하였다.

주제어: Piroxicam, 분광광도법, SQUAD, DATAN, SPECFIT/32

ABSTRACT. The complex formation of anti-inflamatory drug piroxicam (PX, 4-hydroxy-2-methyl-N-2--pridyl-2H-1,2-benzothiazine-3-carboxadiamide-1,1-dioxide) with transition metal ions Co(II), Ni(II), Cu(II) and Zn(II) in methanol(MeOH)/water binary mixtures were studied by spectrophotometric method at 25 ℃, constant pH = 5.0 and I = 0.1 M. The computer program SQUAD was used to extract the desired information from the spectral data. The outputs of the fitting processes were stability constants, standard deviations of the estimated stability constants, concentration distribution diagrams and spectral profiles of all species. The sequence of the stability constants of PX complexes with Co(II), Ni(II), Cu(II) and Zn(II) follow the Cu(II) > Co(II) > Ni(II) ≈ Zn(II) order. This may be due to different geometry tendencies of these metal ions. The acidity constants of the PX were also determined under above condition from its absorption spectra at different pH values. The computer program DATAN was used for determination of acidity constants of PX. The validity of the obtained acidity constants was checked by a well known computer program SPECFIT/32. The effects of the different parameters like solvent nature, cations characteristics on the stability and acidity constants were thoroughly discussed.

Keywords: Piroxicam, Spectrophotometric, SQUAD, DATAN, SPECFIT/32

INTRODUCTION

Ultraviolet-Visible spectroscopy or ultraviolet-visible spectrophotometry or (UV/Vis) involves the spectroscopy of photons in the UV-Visible region. UV/Vis Jahanbakhsh. Ghasemi and Alireza. Jalalvand spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds. An ultraviolet-visible spectrum is essentially a graph of light absorbance versus wavelength in a range of ultraviolet or visible regions.[1]

Synthesis and investigation of metal complexes with active pharmaceuticals in which the drug molecules play a role of ligand have been regarded as a research domain of increasing interest for inorganic, pharmaceutical, and medicinal chemistry. These studies have attracted much attention as an approach to new drug development. It has been revealed that metal complexes of anti-inflammatory drugs have lower toxicity and higher pharmaceutical effect compared to the free drug owing to the inhabitation of metal complexation with other important biological compounds.[2-5]

Piroxicam (PX, 4-hydroxy-2-methyl-N-2-pridyl-2H-1.2-benzothiazine-3-carboxadiamide-1.1-dioxide) belongs to a class of non-steroidal and an anti-arthritic drug with anti-inflammatory properties and a long biological half-life.[6] almost no side-effects and low acidity,[7,8] which acts by inhibiting enzymes involved in the biosynthesis of prostaglandins.[9-11] PX may exhibit differential anticancer effects on different cancer cell types.[12-14] It has been found to inhibit the growth of premalignant and malignant human oral cell lines, without inducing apoptosis,[15] it induces apoptosis in HL-60 cells after 48-h incubation synergistically with eicosapentaenoic acid (EPA).[6] and it is able to induce apoptosis under in vitro conditions in the fibrosarcoma (WEHI-164) cell line.[16] PX is a colorless and odorless powder with a bitter taste and belongs to the oxicam group a class of enolic acids.[17-19] The structure of PX which is shown in (*Fig.* 1), includes four different heteroatom sites that is promising for complex formation with metal ions.[17]

A stability constant (formation constant, binding constant) is an equilibrium constant for the formation of a complex in solution. It is a measure of the strength of the interaction between the reagents that come together to form the complex. There are two main kinds of complex: compounds formed by the interaction of a metal ion with a ligand and supramolecular complexes. such as host-guest complexes and complexes of anions.[20] Jannik Bjerrum developed the first general method for the determination of stability constants of metal-ammine complexes in 1941.[21] There are many areas of application in chemistry, biology and medicine. Stability constant values are exploited in a wide variety of applications. Chelation therapy is used in the treatment of various metal-related illnesses, such as iron overload in β-thalassemia sufferers who have been given blood transfusions.[22] Acid dissociation constants (acidity constants) can be a key parameter for understanding and quantifying chemical phenomena such as reaction rates, biological activity, biological uptake, biological transport, environmental fate and mechanism of action of certain pharmaceutical preparation.[23,24] The accurate determination of acidity constant values is often required in various chemical and biochemical areas. There have been several methods of the determination of acidity constants, including the use of potentiometric titration, spectrophotometry, capillary electrophoresis, and so on. Spectroscopic methods are in general highly sensitive and are suitable for studying chemical equilibria in solution. When the components involved in the chemical equilibrium have distinct spectral responses, their concentrations can be measured directly, and the determination of the equilibrium constant is trivial. In determining of acidity constants of organic reagents we are faced with several drawbacks, such as low solubility in
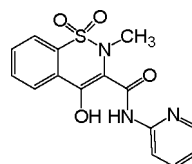

Fig. 1. The structural formula of piroxicam.

aqueous solutions and the low values of acidity constants. Therefore, in order to enhance the acidity constants on one hand and to increase the solubility on the other, we forced to choose mixed solvents. Mixed solvents are interesting, because two solvents mixed together produce a solvent with quite different properties, both, physically (dielectric, density and viscosity) and chemically (acid-base and donor-acceptor properties). In addition, the ionization degree of solute depends on the dielectric constant of solvent. Media of high dielectric constants are strongly ionizing, whereas those of low dielectric constants ionize to a lesser extent.[25] Solvent mixtures can be more convenient than individual solvents owing to enhanced solubilising efficiency, increased sharpness of color change of indicators during titration and more manageable shape of acid-base titration curves.[26,27] The stability of a transition metal complex with a bidentate chelate ligand depends on a range of factors including: number and type of the donor atoms present, the number and size of the chelate rings formed on complexation. In addition, the stability and selectivity of complexations strongly depend on the donor ability and dielectric constant of the solvent and shape and size of the solvent molecules.

PX has several possible conformational rotamers with EZE and ZZZ being the most stable ones and behaves as a bidentate chelating ligand coordinated to the metal ions such as Co(II), Ni(II), Cu(II) and Zn(II) *via* the pyridyl nitrogen and the amide oxygen (*Scheme* 1).

There is a little report on the acidity and complex formation of PX with metal ions therefore, we encouraged to investigate the acidity and complex formation of PX with Co(II), Ni(II), Cu(II) and Zn(II) by spectrophotometry based on chemometrics techniques in MeOH/water binary mixtures.

THEORY

SQUAD, SPECFIT/32 and DATAN

The Stability QUotients from Absorbance Data (SQUAD) program,[28] derived from SCOGS,[29] Leggett,[30,31] for the first time used a factor analysis
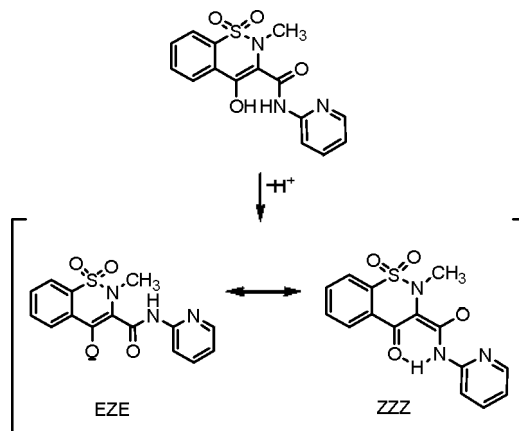
Scheme 1

method in the program for determination of stability constants. Non-linear least-squares method used is based on the minimization of the function, **E**:

$$\mathbf{E} = \sum_{j=1}^{m} (Y_{calcd} - Y_{obsd})^2{}_j w_j \qquad (1)$$

Where **m** is the number of data points and w_j is the weight of each absorbance value. The minimization approach, which was used in SQUAD is more or less similar to the SPECFIT/32, i.e. the object function is a residual matrix, which should minimized with respect to the stability constants of the selected chemical equilibrium model. Running the SQUAD has different stages that were described by Leggett in detail.[30] The calculated standard deviation of absorbance *s* (A) is used as the most important criterion for a fitness test. If, after termination of the minimization process, the condition s_{inst} (A) ≈ s (A) is met and the Hamilton R-factor is also less than 1%, the hypothesis of the chemical model is taken as being the most probable, and is accepted.

SPECFIT/32 program was developed by Gammp *et al.*[31,32] for the determination of stability constants from the spectrophotometric titration data. The mathematical features of this program have been described,[31,32] and that is similar to a general non-linear least squares program used for calculation of stability constants. The SPECFIT/32 is the latest

version of a global analysis program for equilibrium and kinetic systems with singular value decomposition and non-linear regression modeling using the Levenberg-Marquardt method.[33,34] Gammp et $al.$ used the factor analysis in this program and in continuation evolving factor analysis,[35-38] as a powerful tool for the determination of independent components in a given data matrix.

DATa ANalysis (DATAN) package developed by Kubista group,[39] called the physical constrains approach, which provides a unique solution by requiring that the calculated concentrations obey as assumed equilibrium expression and demonstrates its applicability by determining acidity constants of two, three and four protolytic forms of fluorescine.[39-41]

The spectra gathered at different pH values are digitized and arranged in a data matrix $\mathbf{A}$, which is decomposed into an orthonormal basis set by NIPALS or any equivalent method:[42]

$$\mathbf{A} = \mathbf{TP'} + \mathbf{E} \approx \mathbf{TP'} = \sum_{i=1}^{r} t_i p'_i \qquad (2)$$

Where the orthogonal target vectors t_i and orthonormal projection vectors p_i' are mathematical constructs that cannot be directly related to component spectra and concentrations, r is the number of independent spectroscopic components, which corresponds to the number of light-absorbing chemical species. It is determined by visual inspection of the t and $\mathbf{P'}$ vectors or by performing statistical methods, such as, $\chi2$-test.[43-45] $\mathbf{E}$ is an error matrix.

By assuming linear responses, the spectra in matrix $\mathbf{A}$ are linear combinations of the concentrations, $\mathbf{C}$, and spectral responses, $\mathbf{V}$, of the chemical components:

$$\mathbf{A} = \mathbf{CV} + \mathbf{E} \approx \mathbf{CV} \qquad (3)$$

If the spectral profiles of the components are known, the concentration of each component can easily be calculated, for example, by least squares minimization. If standards are not available the common belief has been that the components spectral

responses cannot be separated, which precludes their identification. This is due to ambiguity in determining the rotation matrix, $\mathbf{R}$, in the following equations: from $(Eqs.$ (2) and (3)) follows that there is a square matrix $\mathbf{R}$ ($r \times r$) that satisfies

$$\mathbf{T} = \mathbf{CR} \qquad (4a)$$

$$\mathbf{P} = \mathbf{R}^{-1}\mathbf{V} \qquad (4b)$$

Since $\mathbf{A} = \mathbf{CV} = \mathbf{C}(\mathbf{RR}^{-1})\mathbf{V} = (\mathbf{CR})(\mathbf{R}^{-1}\mathbf{V}) = \mathbf{TP'}$. If $\mathbf{R}$ can be determined, the spectral responses $\mathbf{V}$ and concentrations $\mathbf{C}$ of the components can be calculated from the target $\mathbf{T}$ and projection $\mathbf{P'}$ matrices:

$$\mathbf{C} = \mathbf{TR}^{-1} \qquad (5a)$$

$$\mathbf{V} = \mathbf{RP'} \qquad (5b)$$

The thermodynamic expression that describes the components concentration is the main constraint used to determine $\mathbf{R}$, from which thermodynamic parameters and components spectral responses and concentration are calculated. Therefore, the strategy for determining the rotation matrix $\mathbf{R}$ is as follows. Concentrations of the chemical species are calculated from the equilibrium expressions for various trial values of the equilibrium constants, and are fitted to the calculated target vectors according to $(Eq.$ (4a)). The accuracy of this fit depends crucially on the trial values of the equilibrium constants, and best fit determines their values and the elements of matrix $\mathbf{R}$.

EXPERIMENTAL

Chemical and Solutions
PX was purchased from **RAZAK** pharmaceutical Co. (Iran) with M.P (Melting Point) of 198 °C and purity of 99.8%. MeOH, hydrochloric acid, sodium hydroxide. potassium chloride and nitrate salts of metal ions were analytical grade commercial products from **Merck** Co. These reagents were used without further purification. The solutions of metal ions were prepared in a 100 mL volumetric flask by

direct weighing of the required amounts of available reagents then by dissolving in MeOH/water binary mixtures. The solutions of PX were prepared in a 25 mL volumetric flask by direct weighing of the required amounts of available PX powder then by dissolving in MeOH/water binary mixtures. The binary mixtures were consisting of 90, 80, 70, 60 and 50% V/V of MeOH to water. The doubly distilled water was used for preparation of all solutions.

Apparatus and Softwares

An **Agilent 8453** UV-visible Diode-Array spectrophotometer controlled by a computer and equipped with a 1-cm path length quartz cell was used for measurement UV-visible spectra and the Agilent UV-visible Chem Station Software was used for data acquisition.

The pH measurements were made using a **300 HANA** PH-meter model equipped with a combined glass electrode. The pH values in MeOH/water binary mixtures were corrected using the equation:

$$pH^* = pH(R) - \delta \qquad (6)$$

where **pH*** is the corrected reading and **pH(R)** is the pH-meter reading obtained in a partially aqueous organic solvent, determined by Douheret.[46,47]

Data preprocessing and data analysis were carried out in **MATLAB** software (Version 7.5, MathWorks, Inc.) environment, and the deconvolution of the obtained data matrix was performed using the software's **SQUAD, DATAN** and **SPECFIT/32**. All calculations were run on a **TOSHIBA** laptop computer with Pentium (IV) as central processing unit with Windows XP as operating system.

Procedure

For determination of the acidity constants of PX in MeOH/water binary mixtures, the absorption spectra of PX at different pH values were measured with a titration set-up consisting of a computer interfaced to a spectrophotometer. After each pH adjustment by hydrochloric acid and sodium hydroxide, solution of the PX was transferred into the quartz cell and the absorption spectra were recorded. The ionic strength of the working solution was kept constant at 0.1 M by the addition of potassium chloride. All spectrophotometric measurements were made at 25 °C. For determination of the stability constants of PX-transition metal ion complexes, 2.1 mL of a PX solution (4.76×10^{-5} M) was transferred into a quartz cell that the pH of this solution was kept constant by using hydrochloric acid and sodium hydroxide at desired value. The absorption spectra were recorded after each addition of 10 µL of metal ion solution (3.3×10^{-4} M) at the same pH into the quartz cell by calibrated micropipette. All spectrophotometric measurements were made at 25 °C.

RESULTS AND DISCUSSION

Acidity Constants

The absorption spectra of PX in MeOH/water binary mixtures at different pH values at 200 - 450 nm intervals were recorded. Typical absorption spectra of PX at different pH values are shown in (*Fig.* 2). The pK_a values of PX were investigated in
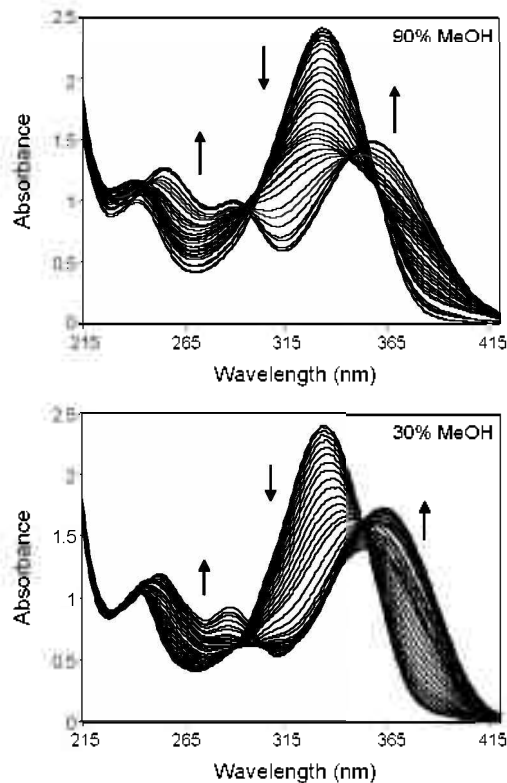

Fig. 2. The absorption spectra of PX at different pH values.

7 different MeOH/water binary mixtures spectrophotometrically at 25 °C and the constant ionic strength I = 0.1 M potassium chloride. PX exhibits weakly basic pyridyl nitrogen (pK_{a1}) and a weakly acidic 4-hydroxy proton,[48] (pK_{a2}, Scheme 2). The principal component analysis (PCA) of all absorption data matrix obtained at different pH values shows at least three significant factors. These factors could be attributed to the two dissociation equilibria of PX. Acidity constants of PX in several binary mixtures were evaluated by DATAN program using the corresponding absorption spectra pH data. Outputs of DATAN program are pK_a values, number of principal components, projection vectors (loadings), concentration distribution diagrams and pure spectrum of each assumed species. The validity of the
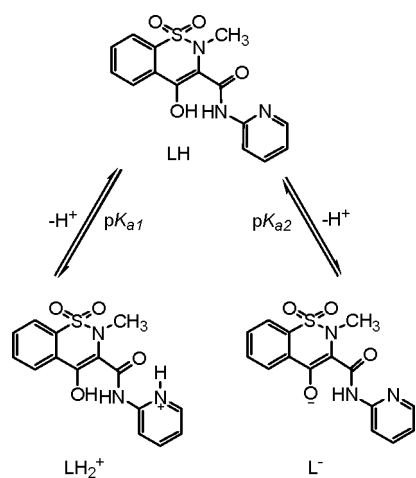
obtained acidity constants was checked by a well known computer program SPECFIT/32. The obtained pK_a values by two programs are listed in Table 1. It is not surprising to mention that the comparison of the outputs of the computer program DATAN with the computer program SPECFIT/32 reveals that there is an excellent agreement between the obtained results by two programs. The samples pure spectral profiles of two species of the PX in 30% and 90% of methanol/water are shown in (Fig. 3) The spectrum of LH shows a λ_{max} at 330 nm in 90% MeOH of binary mixture (Fig. 3) The solvent effect on this spectrum is very interesting. As the weight percent of water increased, this peak shifted to the higher wavelengths (367 nm in 30% MeOH (Fig. 3)) that we can attribute this phenomenon to a red shift phenomenon. This can be described using the polarizing forces between solvent and PX molecules on the stabilization of ground and excited states of $\pi \rightarrow \pi^*$ transition[1]. The pK_a values of PX are depended to the composition of the mixed solvent. The data are listed in Table 1. illustrates the influence of the nature of the binary solvents on the dissociation reactions. Acidity constant of first step increases and of second step decreases with increasing the weight percent of MeOH in the binary mixed solvents. It has been shown that the solvating ability.[49] (as expressed by the Gutmann donicity scale) and dielectric constant of the solvent play a fundamental role in dissociation reactions. Water is a solvent of high solvating ability, (i.e. donor number.[49] DN = 33 and dielectric constant, ε = 78) which can disso-

Scheme 2

Table 1. Data analysis using DATAN and SPECFIT/32 programs

Programs		DATAN		SPECFIT/32	
Acidity constants		pK_{a1}	pK_{a2}	pK_{a1} ± s.d.[a]	pK_{a2} ± s.d.
Solvent mixtures					
90%	MeOH	2.04	5.53	2.17 ± 0.04	5.53 ± 0.03
80%	MeOH	2.13	5.48	2.19 ± 0.01	5.49 ± 0.08
70%	MeOH	2.14	5.46	2.25 ± 0.10	5.44 ± 0.10
60%	MeOH	2.14	5.42	2.25 ± 0.10	5.42 ± 0.16
50%	MeOH	2.16	5.41	2.25 ± 0.10	5.39 ± 0.11
40%	MeOH	2.16	5.40	2.27 ± 0.09	5.38 ± 0.10
30%	MeOH	2.18	5.17	2.28 ± 0.11	5.17 ± 0.10

[a]Standard deviation of estimated acidity constants.
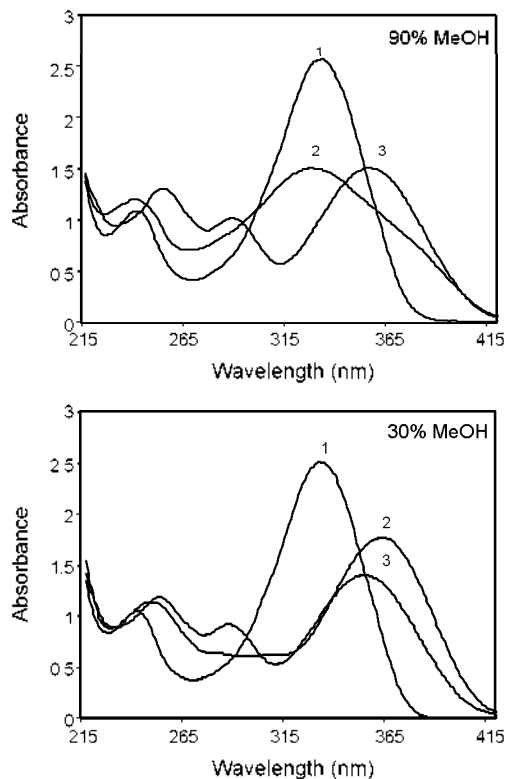
Fig. 3. Pure spectra of different species of PX, 1) LH$_2^+$, 2) LH, 3) L$^-$.

Fig. 4. Variation of acidity constants of PX with X$_{MeOH}$ in MeOH/water binary mixtures.

The L$^-$ form appeared at pH > 5.55. The same trend has already been reported for various organic molecules in different solvent mixtures.[50,51] It has been reasonably assumed that preferential salvation of the charged particles by water is mainly responsible for such a monotonic dependence of the acidity constants of the PX on the solvent composition. It is clear that, the dissociation of an uncharged acid in a solvent requires the separation of two ions of opposite charges. The work required to separate these charges is inversely proportional to the dielectric constant of the solvent. The energy required for dissociation is supplied by solvation of the ions and also the proton transfer from acid to the solvent molecule supplies an additional energy. If the dielectric constant and the solvating ability of the solvent are decreased, more energy will be required to separate the anion and cation and consequently the extent of dissociation of acid will be lowered. Therefore, the increase in first step and the decrease in second of dissociation constants are due to increasing the mole fraction of MeOH in the binary mixed solvent.

Stability Constants

The absorption spectra of complexation reaction between PX with Co(II), Ni(II), Cu(II) and Zn(II) in 5 different MeOH/water binary mixtures at 200 - 450 nm intervals were recorded. All spectrophotometric titration were carried out at constant pH =

ciate the acid and stabilize the produced anion and hydrogen ion. Thus, it is expected that addition of MeOH with lower donor number and dielectric constant (DN = 19, ε = 32.6) to water increases the extent of interaction between the acid anion and proton with solvent, and this decreases the acidity constants of acid. It is interesting to note that there is actually a linear relationship between the pK_a of two dissociation steps (first step increases and second step decreases) and the mole fraction of MeOH (X$_{MeOH}$) in the binary mixed solvents (Fig. 4). One of the very important outputs of DATAN program is calculated spectrum of different forms of PX at each binary solvent mixture. So according to distribution diagrams it is may conclude that the spectra at smaller pH than 2.1 assigned to LH$_2^+$ form because this form is dominated at this range. At pH 2.1 - 5.55 interval the LH form is dominated and hence the spectra mostly attributed to this form.
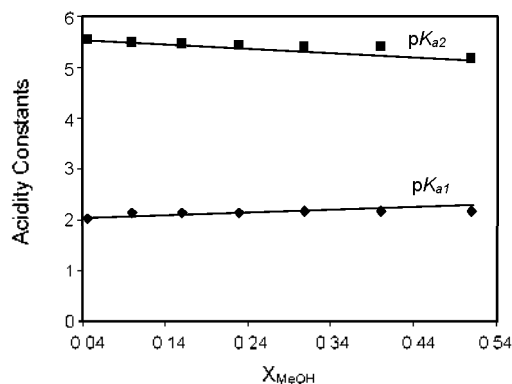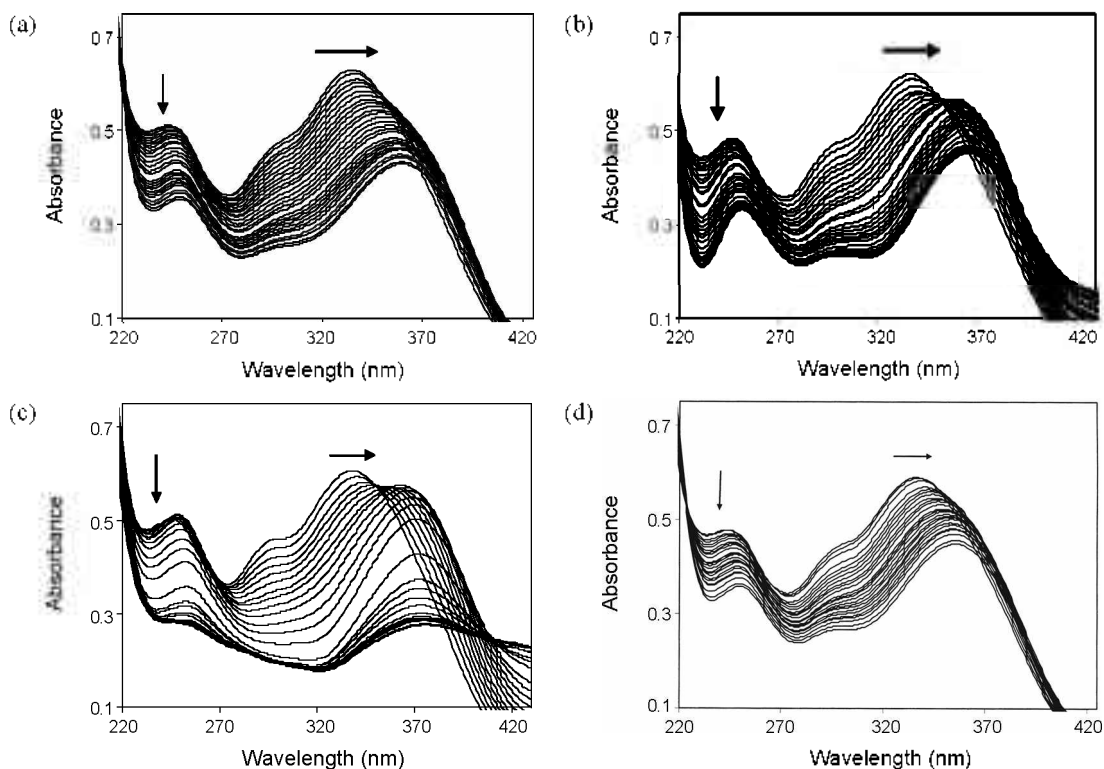
Fig. 5. Absorption spectra of complexation of PX with a) Co(II), b) Ni(II), c) Cu(II) and d) Zn(II) in 90% MeOH.

5.0 (hydrochloric acid and sodium hydroxide were used to adjust the pH), I = 0.1 M and at 25 °C. Typical absorption spectra of the spectrophotometric titration of PX with Co(II), Ni(II), Cu(II) and Zn(II) in 90% MeOH are shown in (Fig. 5). The absorption spectra of solutions containing a constant concentration of PX (4.76×10^{-5} M) at constant pH = 5.0, I = 0.1M show a marked change during the addition of varying amount of metal ions at 25 °C. The changes in absorption spectra are attributed to coordination of PX molecule to metal ions. The results show that the absorption spectrum of PX-Cu(II) and Ni(II) complexes has a hypsochromic effect and are shifted toward smaller wavelengths with increasing the polarity of solvents ($n \rightarrow \pi^*$ transition).[1] For Co(II) and Zn(II) these bands show a bathochromic shift effect and are shifted toward higher wavelengths with increasing the polarity of solvents ($\pi \rightarrow \pi^*$ transition).[1] The stoichiometry of the resulting complexes was determined from the absorbance-molar

ratio plots at maximum wavelength of the corresponding complexes for all studied systems. In the absorbance-molar ratio plots for complexation of PX with metal ions, there are two inflection points in about $C_M/C_L = 0.3$ and $C_M/C_L = 0.5$ for Cu(II) that we can attribute them to ML_3 and ML_2 complex species, respectively. For complexation of PX with Ni(II) and Zn(II) there is just one inflection point in about $C_M/C_L = 0.5$ (ML_2) and for complexation of PX with Co(II) there is also one inflection point in about $C_M/C_L = 0.3$ (ML_3).

The PCA of absorption data matrices obtained of spectrophotometric titration of PX with metal ions shows three significant factors for complexation of PX with Cu(II) and two significant factors for complexation of PX with Co(II), Ni(II) and Zn(II). The number of significant factors of the spectral data of complexation of PX with Co(II), Ni(II) and Zn(II) affected by the composition of the mixed solvents. For example in 50% MeOH for complexation of PX

with Co(II), Ni(II) and Zn(II) there is one signifi-cant factor which means at this composition there is just one detectable absorbing spices which means the complexation reaction did not take place. This phenomenon can also be seen absorbance-molar ratio plot without any inflection point (*Fig.* 6). Stability constants of complexation of PX with these metal

ions in several binary mixtures were evaluated *via* SQUAD program using the corresponding whole absorption spectral data. SQUAD. in addition to the overall stability constants. contains standard devia-tions of the estimated stability constants. concentra-tion distribution diagrams and spectral profiles of all species. Stability constants values for complexation of PX with Co(II), Ni(II), Cu(II) and Zn(II) that ob-tained with SQUAD program are shown in *Table* 2.

The data given in *Table* 2. shows the sequence of the stability constants of PX complexes with Co(II), Ni(II), Cu(II) and Zn(II) which vary as Cu(II) > Co(II) > Ni(II) $\approx$ Zn(II) in all MeOH/water binary mixtures. This may be due to different geometry ten-dencies of these metal ions. For example, the square planar complexes are most common for Ni(II) ion while the most common coordination structures for Co(II) are octahedral.[52] The stability of the resulting PX complexes increases with increasing the weight percent of MeOH in the mixed solvents. It is in-teresting to note that there is a linear relationship between logβ of PX complexes and mole fraction of MeOH in binary mixed solvents.

According the results that obtained from PCA method. absorbance-molar ratio plots and increasing of the estimated stability constants (*Table* 2) with increasing the weight percent of MeOH in mixed solvents we could rationalize that the stabilities of complexation reactions are under influence of the composition of the mixed solvents. During the com-plexation process. the ligand should be able to replace as completely as possible the solvent mole-cules in the first salvation shell of the metal ions.
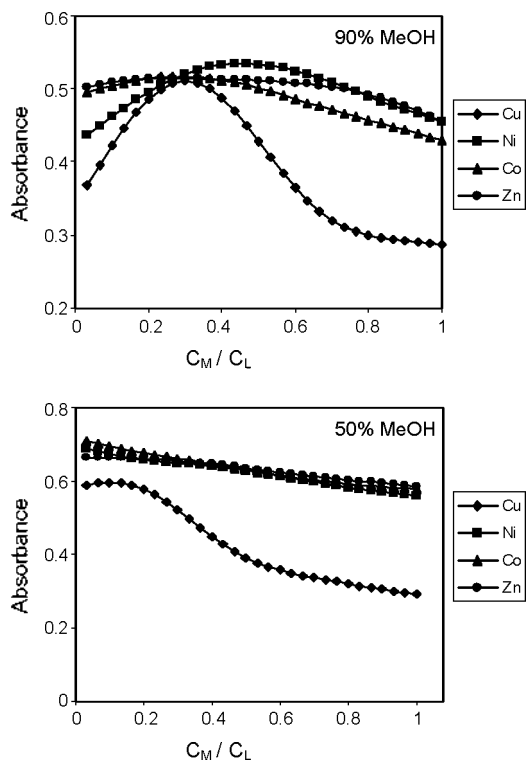

Fig. 6. Absorbance-molar ratio plot for complexation of PX with Co(II), Ni(II), Cu(II) and Zn(II).

Table 2. The stability constants of PX with metal ions in different MeOH/water mixtures

Metal Ions	Ni(II)		Cu(II)				Co(II)		Zn(II)	
Species	ML₂		ML₃		ML₂		ML₃		ML₂	
Solvent mixtures	logβ^a ± s.d.[b]	r.s.s.[c]	logβ ± s.d.	r.s.s.	logβ ± s.d.	r.s.s.	logβ ± s.d.	r.s.s.	logβ ± s.d.	r.s.s.
90% MeOH	9.39 = 0.004	0.008	18.77 ± 0.006	0.02	13.37 = 0.006	0.02	14.32 = 0.005	0.008	9.89 = 0.022	0.29
80% MeOH	9.20 = 0.50	0.08	17.86 ± 0.009	0.05	12.59 = 0.009	0.05	13.25 = 0.005	0.008	8.86 = 0.006	0.02
70% MeOH	9.09 = 0.04	0.20	13.55 = 0.01	0.06	11.26 = 0.006	0.02	12.72 = 0.004	0.006	8.12 = 0.024	0.70
60% MeOH	9.00 = 0.35	0.02	13.13 = 0.01	0.08	9.09 = 0.04	0.77	12.59 = 0.002	0.002	7.87 = 0.020	0.50
50% MeOH	....	....	13.03 = 0.02	0.19	9.20 = 0.06	0.50	....	....	....	....

[a]Stability constant. [b]Standard deviation of estimated stability constants. [c]Residual sum of squares.

Therefore, variations in the composition of the mixed solvents produce significant changes in the binding properties of the ligand, and consequently, the stability and selectivity of metal complexes. These means solvating ability and dielectric constants of the solvents play a fundamental role in complexation process. Water is a solvent of high solvating ability and dielectric constants, (i.e. donor number, DN = 33, and dielectric constant, ε = 78) which can dissociate the acid and stabilize the produced anion and hydrogen ion. Thus, with addition of MeOH (DN = 19, ε = 32.6, with lower donor number and dielectric constant) to water PX molecules could be able to replace as conveniently as possible the solvent molecules in the first salvation shell of the metal ions and increases interaction between PX molecules and metal ions, then increases the stability of metal complexes.

CONCLUSION

In this study we reported a spectrophotometric study based on chemometrics methods for evaluation of complexation of PX with some transition metal ions in different MeOH/water binary mixtures. The Stability QUotients from Absorbance Data (SQUAD) program is proposed as an efficient chemometrics technique for determination of stability constants of complexation of PX by data that obtained from spectrophotometric titration of PX with metal ions solutions. Low standard deviation values that obtained for stability constants indicate the high precision of this method. In addition we obtained concentration distribution diagrams and spectral profiles of all species by this method.

The computer program DATAN was used as an efficient chemometrics technique for determination of acidity constants of PX in different MeOH/water binary mixtures from data matrix that obtained from spectrophotometric titration of different solutions of PX at different pH values. The validity of the obtained acidity constants was checked by a well known computer program SPECFIT/32. The comparison of the outputs of the computer program DATAN with the computer program SPECFIT/32

reveals that there is an excellent agreement between the obtained results and mentioned programs. The striking advantage of the proposed methods is using of the whole spectral information in the computation process which enable us to have more precise and accurate thermodynamics constants in comparison to the classical methods such as single wavelength approach. Principle component analysis was used as a powerful technique for determination of the number of the all significant factors in spectral data that obtained for stability and acidity constants. The effect of MeOH/water binary mixtures on the stability and acidity constants is investigated and it reveals that there is a complex relation between the stability and acidity constants and the composition of the solvent mixtures.

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