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요 약. 다중파장 분광광도법 적정을 사용하여 25 ℃에서 0.1 M $NaNO_3$를 함유한 에탄올-물 이성분 혼합 용매 중에서 네 가지 수용성 비타민인 엽산(비타민 B9 또는 B10), 티아민(비타민 B1), 리보플라빈(비타민 B2) 및 피리독살(비타민 B6)의 양성자이전상수를 조사하였다. 인자분석모형으로 pH-흡광도 데이터를 적절한 질량균형방정식에 곡선맞추기(curve fitting)하여 양성자이전 평형상수, 스펙트럼, 농도 도표와 성분 수를 계산하였다. DATAN 프로그램으로 산도상수를, SPECFIT 프로그램으로 표준편차와 부분상관계수를 계산하였다. Gran 도시에 바탕을 둔 4-파라미터 식, $pH = \alpha + SpcH - J\overline{H}[H^+] - J\overline{OH}K_w/[H^+]$에 의거한 유리전극 보정과정을 사용하여 pH값을 농도 척도(pcH)로 읽었으며, 용매가 양성자이전상수에 미치는 영향을 고찰하였다.

주제어: 비타민 B군, 산도상수, DATAN, 이성분 용매, 에탄올, SPECFIT/ 32

ABSTRACT. A multiwavelength spectrophotometric titration method was applied to study protolytic constants of four water-soluble vitamins, folic acid(vitamin B_c or B_9), thiamine(vitamin B_1), riboflavin(vitamin B_2) and pyridoxal (vitamin B_6) in binary ethanol-water mixtures at 25°C and an ionic strength of 0.1M $NaNO_3$. The protolytic equilibrium constants, spectral profiles, concentration diagrams and also the number of components has been calculated from the curve fitting of the pH-absorbance data with appropriate mass balance equations by an established factor analysis model. DATAN program was used for determination of acidity constant and SPECFIT program was used for calculation of standard deviations and partial correlation coefficients. A glass electrode calibration procedure based on the four parameter equation $pH=\alpha+SpcH-J\overline{H}[H^+]-J\overline{OH}K_w/[H^+]$ based on the Gran's plots was used to obtain pH-readings in the concentration scale (p_cH). The effect of the solvent on the protolytic constants was discussed.

Keywords: Vitamins B Group, Acidity Constants, DATAN, Binary solvent, Ethanol, SPECFIT/ 32

INTRODUCTION

Protolytic constants are useful physicochemical properties by which we can describe the amount of ionization of functional groups with respect to pH. These important parameters have a lot of applications in research areas such as pharmaceutical drug development, solvent extraction, acid-base titration and ion transport. The toxicity, chromatography retention behavior and pharmaceutical properties of organic acid and bases are affected by acid-base properties. In modern organic chemistry a considerable amount of theoretical foundation is based on the observation of the effect on acid-base equilibrium of changing molecular structure.[1]

The spectroscopic instrumentation which is used

invariably has the capacity to collected data in a full spectral range. Using single wavelength most of the information in the collected spectra is discarded and it requires not only the presence but also the knowledge of such suitable wavelength. However, in many cases, the spectral responses of components overlap and analysis is no longer straightforward.[2,3] The predefined model, known as hard-modeling analysis, cannot be applied if crucial information is missing. Soft modeling or model free approaches are based on much more general prerequisites, such as positive molar absorbance, positive concentration of all species, unimodality of concentration profiles, and closure(concentration of all species are the same for all solutions). Naturally, if the strengths of hard-modeling and soft-modeling methodologies are combined, a much more powerful method of data analysis can be expected.[4-6] The principal component analysis along with different curve resolution methods[1] have been applied to spectral data to extract information utilizing a large number of absorbances measured at different wavelengths.

B vitamins form a wide organic-compound group that cannot be synthesized by humans. Since these compounds are necessary for the tropism of human beings, they need to be part of our daily intake. Vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_6 (pyridoxal) and vitamin B_9 (folic acid) occur in living cells as essential substances for growth. Any deficiency of these in human nutrition will have adverse effects. Therefore, B vitamins are often supplemented to the diet as composite vitamin B tablets.

Folic acid, also known as vitamin B_9, is also referred to as folacin or folate, while its chemical name is pteroylglutamic acid. Folic acid is required for DNA synthesis and cell growth to take place and it is important for the formation of red blood cells, for energy production, for the formation of amino acids. It is also required in protein metabolism and in treating folic acid anemia. It is also used as an antagonist in treatment of blood cancer. Thiamine, also called vitamin B_1, assists in a great many bodily functions. It plays a key metabolic role in the cellular production of energy, primarily in glucose metabolism. Thiamine is unstable at high pHs, and in food it degrades by cooking of under mildly basic conditions. Riboflavin (vitamin B_2) is the prosthetic group of flavine enzymes, which are of importance in general metabolism and particularly in metabolism of proteins. Vitamin B_2 is required for the health of the mucous membranes in the digestive tract and aids in the absorption of iron and vitamin B_6. It is needed especially during periods of rapid growth, but also when protein intake is high. It is highly beneficial to the skin, hair and nails.[7] Vitamin B_6 (pyridoxal) is a common cofactor in enzymes that support amino acids metabolism. It controls the absorption, metabolism and conversion of amino acids into neurotransmitters, antibodies, digestive enzymes, muscles and tissues in the body.[8]

About 80 to 90 percent of adults consume ethanol. 5 to 10 percent of adult females consume excessive amounts of ethanol. Ethanol is quite water soluble and is also lipid soluble. It is absorbed unaltered form the stomach and small intestine, and quickly distributed to all tissues. Less than 10 percent of the total alcohol consumed is excreted in urine, sweat, and breath. People used alcohol because it is a central nervous system (CNS) depressant. Death from CNS depression would be much more frequent except that in steady consumption, sleep usually precedes a lethal dose. In gulping consumption (chugalug) stomach irritation causes vomiting before lethal concentrations are reached.[9]

Long term effects of alcohol also include; diabetes (non insulin dependent), ulcers of the stomach and intestines, severe psychological depression, impaired immune response, central nervous system damage, malnutrition and bone deterioration and osteoporosis. Nutritional deficiencies are virtually inevitable consequences of alcoholism, not only because alcohol displaces food but also because alcohol directly interferes with the body use of nutrients, making them ineffective even if they are present.[10]

There is a question; why do physicians prescribe group B vitamins for ethanol toxicanted people? After ethanol enters body, it destroyed and deteriorate group B vitamin and immediately a toxic per-

son should be treat by group B vitamins. As it is obvious, we need to know some physicochemical behaviors of B vitamins in the partially aqueous ethanol solutions. So, in this research we measure the protolytic constants of group B vitamins, as a fundamental physical constant which determine the role of the molecules in the biochemical reactions in the living organisms, in water and in different ratios of ethanol–water mixtures spectrophotometrically at 25 ℃.

THEORY

Data Analysis DATAN

The first serious attempt to analyze spectroscopic data using multivariate methods was made in 1971 by Lawton and Silvestre.[11] They showed that it was not possible to obtain an unambiguous result when analyzing sets of regular one-dimensional spectra and provided a self-modeling curve resolution method to estimate a solution range for the components spectral responses and concentrations assuming nonnegative responses.

In last decade, Kubista et al. developed a new method, called the physical constraints 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 and four protolytic forms of fluorescine. Kubista *et al.* showed that it is possible to determine components spectral responses and their relative concentrations in a series of test sample without making assumptions about spectral shapes and without using reference specta.[12-14] A possible advantage of the Kubista *et al.* method is that is mixes a soft modeling with hard modeling approach. This might be best and more general strategy, since it can handle different situations, with only a partial knowledge of the chemistry of the system. The physical constraint approach is applicable to samples that contain components that are in chemical equilibrium. The samples must differ in a physical property, such as pH and temperature, etc., that in a predictable way affects the concentrations of the components. The physical constraints method calculates spectral profiles, concentrations and equilibrium constants by utilizing equilibrium expression that related the components.

Mathematics

Spectra of reagent at different pH values are digitized and arranged in a data matrix **A**, which is decomposed into an orthogonal basis set by NIPALS or any equivalent method [15]:

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

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 **P′** vectors or by performing χ^2-test. **E** is an error matrix. Assuming linear response the spectra in matrix **A** are linear combinations of the concentrations, **C**, and spectral responses, **V**, of the chemical components.

$$\mathbf{A} = \mathbf{CV} + \mathbf{E} \approx \mathbf{CV} \qquad (2)$$

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 believe is that the components spectral responses cannot be separated, which precludes their identification. This is due to ambiguity in determining the rotation matrix, **R**. In the following equation: from (eq.1) and (eq.2) follows that there is a square matrix **R** (r × r) that satisfies:

$$\mathbf{T} = \mathbf{CR} \qquad (3)$$

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

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

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

$$\mathbf{V} = \mathbf{RP}' \quad (6)$$

The thermodynamic expression that describes the components concentration is the main constraint used to determine **R**, from which thermodynamic parameters and components spectral responses and concentration are calculated. Therefore, the strategy for determining the rotation matrix **R** is as follows. Concentrations of the chemical species are calculated from the equilibrium expressions for various trial values of the equilibrium constants, protolytic constants in this case, and are fitted to the calculated target vectors according to eq. 3. The accuracy of the fit depends crucially on the trial values of the equilibrium constants and/or acidity constants, and best fit determines these values and the elements of matrix **R**. The details of the method can be find in references.[16,17]

EXPERIMENTAL

Thiamine, riboflavin and pyridoxal with the same concentration of 1.00×10^{-4} M, and folic acid 5×10^{-5} M were made and employed as working solutions, phosphoric acid 5×10^{-4} M was only used for buffering folic acid solution. In all experiments, the ionic strength of solutions was kept constant at 0.1 M using sodium nitrate as the supporting electrolyte. Sodium hydroxide and hydrochloric acid were used for setting of the pH of solutions. All the mentioned materials were purchased from the Merck Company. Absolute spectroscopy grade ethanol (Merck) and triply distilled water were used as the solvent mixtures. The absorption spectra were recorded on an Agilent 8453 UV-Visible Diode-Array spectrophotometer using the Agilent UV-Visible ChemStation Software for data acquisition. The pH measurements were made using a 300 HANA pH-meter model equipped with a combined glass electrode. The solutions of 0.01 M oxalate and succinate buffers were employed to precalibrate the pH meter in the various binary ethanol-water mixtures. The pH values in ethanol–water solvent mixtures were corrected using the Four-PlusTM procedure for glass electrode calibrations in both aqueous and semi-aqueous media. Nine titrations of known concentration of vitamins B group were performed in 0–80% of ethanol, using NaOH solution. The operational pH scale was established by calibrating the pH measuring circuit with mentioned buffers and assuming the Nernst slope. All data reported in this study are based on the concentration scale with respect to ionic strength 0.1 M and 25 °C. As the proton concentrations generated from strong acid–base titrations can be readily calculated, the concentration pH value ($pcH=-\log[H^+]$) is related to the operational pH reading by the equation as given below.[18]

$$pH = \alpha + SpcH + JH^{-}[H^{-}] + JOH^{-}K_w/[H^{-}] \quad (7)$$

The α term corresponds to the negative logarithm of the activity coefficient of H^- at working temperature and ionic strength. The S term denotes the ratio between the actual slope and the Nernst slope. The JH^- term corrects pH readings for the non-linear pH response due to liquid junction and asymmetry potentials in moderately acidic solution (pH 1.5–2.5), while the JOH^- term corrects for any high-pH (pH>11) nonlinear effects. These parameters are determined by a weighted nonlinear least squares procedure and the results showed a good agreement with the result of fitting of solver tool pack of Excel. For aqueous titrations, the ionization constants of water (K_w) as a function of temperature and ionic strength were taken from Sweeton *et al.*[19]. For semi-aqueous titrations, literature values of K's (the ionization constants of water in the solvent–water mixtures) were utilized from references.[40,41] In processing the titration data, contribution from carbonate was incorporated into the calculations. The acid dissociation constants of carbonic acid in solvent–water mixtures were determined iteratively in parallel with the parameters as defined in Eq. (7). Gran's plots were used to find the end point of the calibration titrations.[20]

Data preprocessing and data analysis were carried out in MATLAB version 7 and using DATAN and SPECFIT/32 computer programs[21,22] respectively.

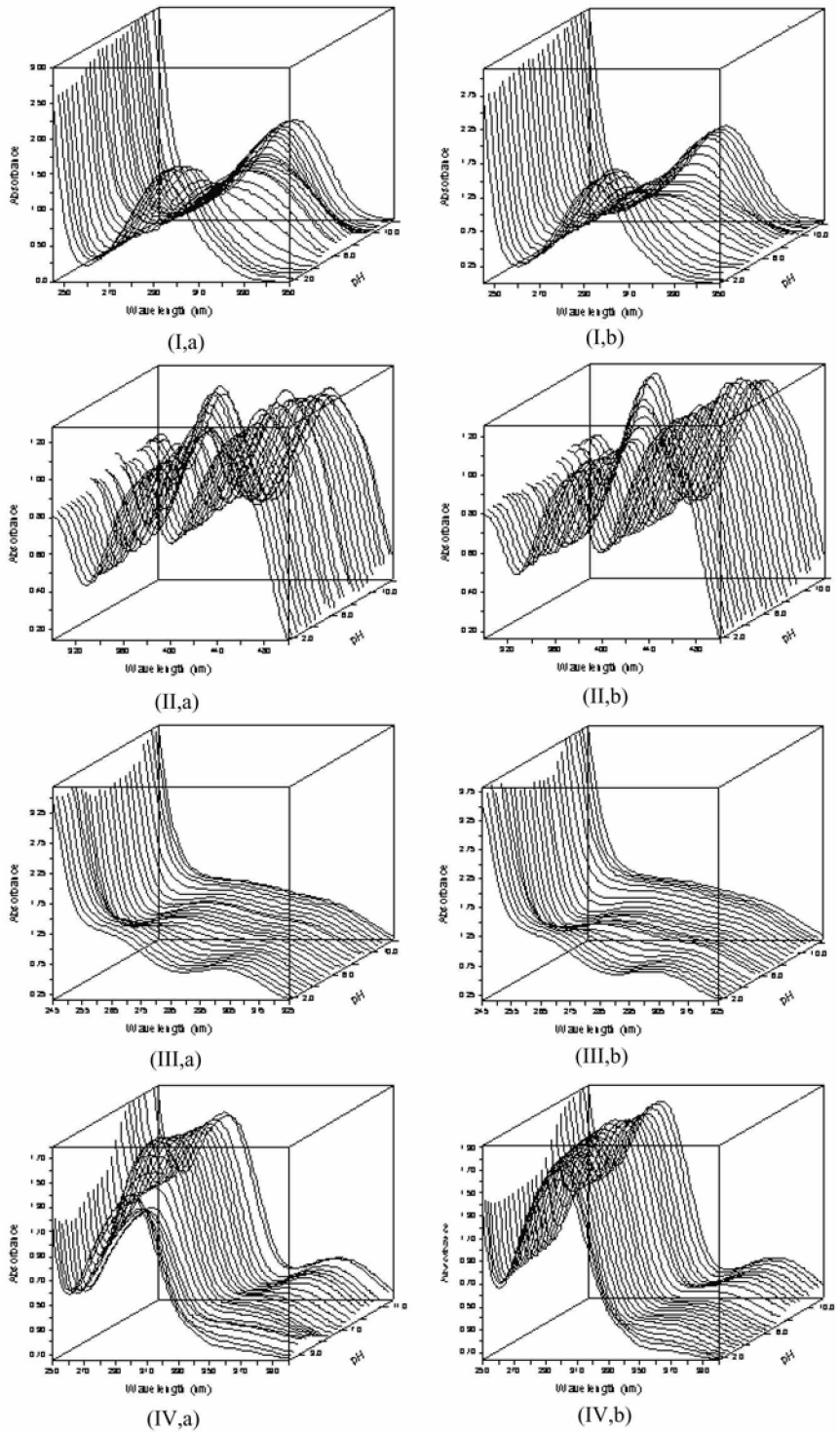

Fig. 1. Absorption spectra of vitamins in pure water (I,a) Pyridoxal. (II,a) Riboflavin. (III,a) Thiamine. (IV,a) Folic acid and 50% ethanol to water (I,b) Pyridoxal. (II,b) Riboflavin. (III,b) Thiamine, and (IV,b) Folic acid at different pH values.

RESULTS AND DISCUSSION

The electronic absorption spectra of vitamins B Group in binary solvent mixtures at various pH values were recorded. Sample spectra of each vitamin at different pH values in pure water and 50 volume percent ethanol are shown in *Fig.* 1. The principal component analysis of all absorption data matrices obtained at various pH values shows the different number of factors for each vitamin that also supported by the statistical indicators presented by Elbergali *et al.*[23]. The number of factors could be attributed to the number of dissociation equilibria of each vitamin. Protolytic constants of vitamins B group in several mixtures were evaluated using the corresponding spectral absorption–pH data and DATAN.

From inspection of the experimental spectra, it is hard to guess even the number of protolytic species involved. But the number of calculated projection vectors with clear spectral features (as compared to noise), is an evidence of the presence of four, three, two and three spectroscopically distinguishable components for folic acid, thiamine, riboflavin and pyridoxal, respectively. The outputs of the DATAN program are pK_a values, the number of principal components, projection vectors (loadings), concentration distribution diagrams, and the pure spectrum of each assumed species. In addition to the above information the standard deviation of pK_a constants and the partial correlation coefficients were obtained by SPECFIT. The pK_a values, their standard deviations and partial correlation coefficients are listed in *Table* 1. The partial correlation coefficients show the interrelations of the unknown parameters. These values are very important in nonlinear least squares methods. They simply show the multiple correlation coefficients between different fitting parameters. It means if the two parameters have high absolute correlation coefficient the changing in one of them can be compensate by other. In this situation one can't find an exact solution for the understudy system and an alternate physicochemical method should be applied. The obtained values did not show severe interrelations, so the unknown parameters are almost independent, and the unknown values can be obtained without ambiguity of the absolute values.[24]

The pK_a values corresponded to the pH dependent variation of absorption spectra in all solvent mixtures. Consider the cationic form of pyridoxal (*Fig.* 2, I) which has two dissociable protons bound to distinctly different sites, the phenolic oxygen and the ring nitrogen. Either of two protons could dissociate first as the pH is raised. However, the two microscopic dissociation constants are distinctly different. At 25 °C in the neutral (monoprotonated) form 80% of the molecules carry a proton on the N, while the remaining 20% are protonated on the less basic-O site.[25] The obtained pK_{a1} and pK_{a2} by computer fitting of spectral data are listed in *Table* 1.

Table 1. Protolytic constants of each vitamins in different ethanol + water mixture at 25 °C and constant ionic strength (0.1 M KNO3)

Ethanol	Folic acid			Pyridoxol		Riboflavin	Thiamine		PCC[b]	Folic acid		Pyridoxol	Thiamine
(wt%)	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a1}	pK_{a2}	r_{12}	r_{13}	r_{23}	r_{12}	r_{12}
0[a]	2.40	5.35	8.48	5.06	9.45	10.58	5.07	11.04					
0	2.91±0.09	4.77±0.04	8±0.05	4.95±0.03	8.94±0.05	9.96±0.14	4.79±0.03	10.32±0.04	-0.530	0.002	-0.043	-0.163	-0.125
10	2.39±0.10	4.85±0.05	7.77±0.09	4.91±0.03	9.02±0.05	9.97±0.13	4.7±0.06	10.44±0.06	-0.393	0.021	-0.116	-0.123	-0.124
20	2.4±0.26	4.63±0.11	7.99±0.07	4.88±0.03	9.09±0.06	10.08±0.2	4.71±0.04	10.26±0.04	-0.134	-0.023	0.017	-0.107	-0.108
30	2.03±0.10	4.29±0.17	8.13±0.03	4.96±0.02	9.19±0.04	10.13±0.09	4.61±0.07	10.25±0.07	0.136	-0.003	-0.010	-0.089	-0.096
40	2.08±0.36	4.63±0.39	8.14±0.06	4.98±0.03	9.24±0.04	10.23±0.09	4.57±0.05	9.84±0.04	0.347	-0.009	-0.007	-0.066	-0.120
50	1.83±0.13	4.89±0.23	8.23±0.04	5.01±0.04	9.46±0.05	10.27±0.1	4.53±0.04	9.8±0.05	0.302	0.007	0.014	-0.037	-0.114
60	1.94±0.18	4.85±0.14	8.44±0.04	5.05±0.03	9.5±0.03	10.43±0.1	4.57±0.07	9.74±0.07	0.308	0.032	0.065	-0.029	-0.144
70	1.95±0.16	4.7±0.14	8.55±0.03	5.05±0.03	9.66±0.03	10.49±0.16	4.64±0.07	9.43±0.07	.0.337	0.023	0.058	-0.015	-0.120
80	1.92±0.10	5.26±0.13	8.72±0.04	4.97±0.08	9.85±0.06	10.63±0.05	4.56±0.07	9.32±0.07	0.248	0.040	0.090	-0.024	-0.127

[a]Ref 58.

[b]Partial correlation coefficient.

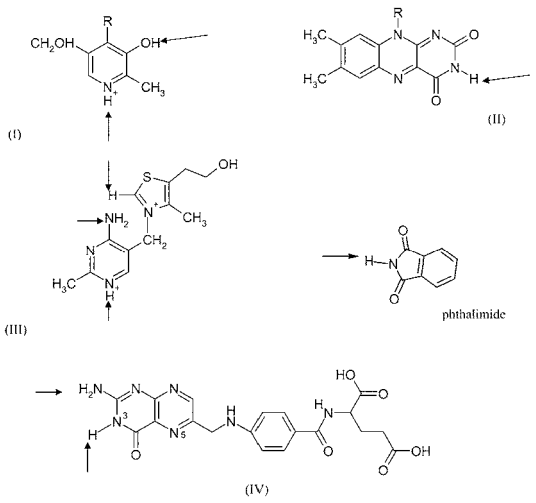

Fig. 2. The structures of (I) Pyridoxal, (II) Riboflavin, (III) Thiamine, and (IV) Folic acid.

The previously reported values of pK_{a1} and pK_{a2} at pure water are 4.64 and 8.89, respectively.[26] Riboflavin consists of a hetrocyclic isoalloxazine ring attached to the sugar alcohol, ribitol. It is stable to heat but extremely sensitive to light. One of the products of photolysis is lumichrome.[25] Freshly prepared solution of this vitamin was used as a titration solution to determine the corresponding acidity constants, avoiding thereby the photolysis of riboflavin (*Fig.* 2 II), which has a similar group to phthalimide, it has a dissociable proton bound to the ring nitrogen. The pK_a value obtained in this report, *Table* 1 for the similar functional group is between 9.5 and 11,[27] and the previously reported pK_a value for riboflavin was 10.2. The weakly basic portion of thiamine (*Fig.* 2 III) or of its coenzyme form is protonated at low pH, largely on N-1 of the pyrimidine ring.[28-30] The reported pKa value is ~4.9.[25] The hydrogen atom in the 2-position of the thiazolium ring, between the sulfur and the nitrogen atoms, dissociates as H^- during catalysis and the pK_a value of this proton has been estimated as ~18 which means that it cannot be as a acidic proton.[31] The portion that can be protonated next to the NH_2 group is the pyrimidine ring. The pK_{a2} value that we obtained in this report (*Table* 1) is comparable with previously reported values.[25] The folic acid, as shown in (*Fig.* 2 IV) has a complicated structure and allocates the obtained acidity constants to specific groups. The previously reported pK_a values are 4.82 (related to N-5 site) and 10.5 (related to N-3 site, transferring from O-position to N-position during tautomerism).[25] Three pK_a values were obtained, and they are listed in *Table* 1. As the structural information shows, folic acid possesses two carboxylic groups apart from the two acidic positions, as discussed above. It can be assumed that the obtained pK_{a1} value relates to one of the two carboxylic groups, and the other two pK_a values can be compared with reported values, 4.82 and 10.5 for pK_{a1} and pK_{a2} respectively.[26]

Since chemometrics based methods, by using the whole spectral domain, can reduce considerably the level of the noise effect (this is called the signal averaging effect), so the calculated protolytic constants are probably shows more reliability and precision in comparison to the old previous methods. One of the very important outputs of the DATAN program is the calculated spectrum of different forms of each of vitamins in each solvent mixture. The calculated spectra of all species in different solvent mixtures are shown in *Fig.* 3.

It is interesting to note that the nature of the solvent composition has a fundamental effect on each pure spectrum. As the weight percent of ethanol goes up, the absorption intensity of fully protonated species, form 1 of riboflavin at 370 nm, pyridoxal species or form 2 at 320 nm and thiamine species or form 3 at 260 nm decreased; additionally folic acid species 2 at 300 nm and folic acid species 4 at 290 nm increased. We can be described these findings by using the nonelectrostatic (Π-bonding) property on the stabilization and/or destablization of ground and excited state of $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions. The most important features of the distribution diagrams are the pH limits of the evolving and disappearing of components. The distribution diagrams are shown in *Fig.* 4.

The pK_a values of different protolytic steps of folic acid, pyridoxal and riboflavin in solvent mixtures increased by increasing the percentage of the ethanol. But the addition of the ethanol on the acid-

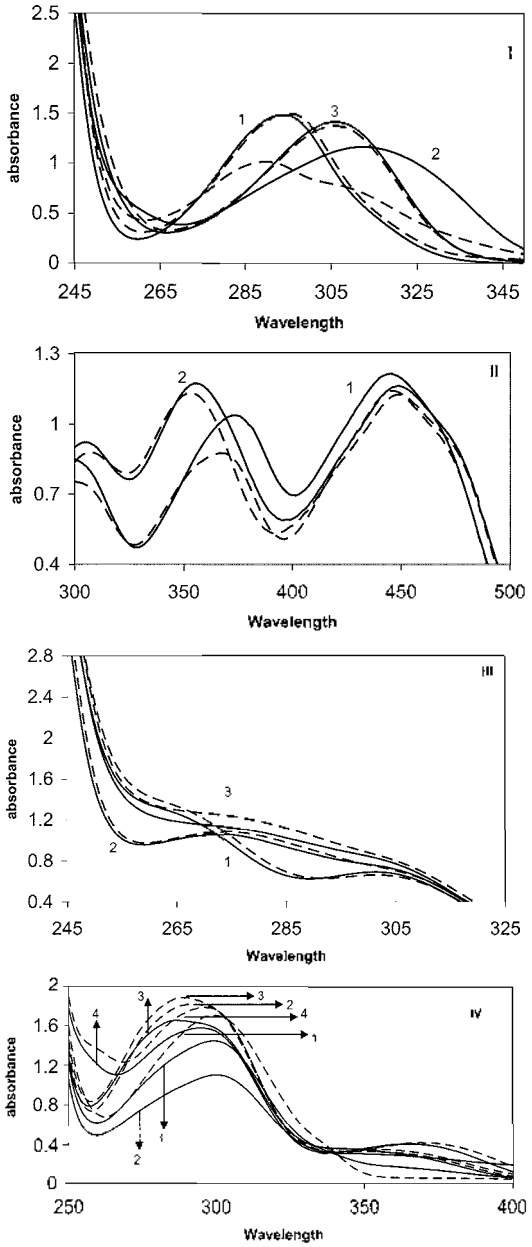

Fig. 3. Pure spectra of (I) Pyridoxal, (II) Riboflavin, (III) Thiamine, and (IV) Folic acid in pure water (—) and 80% ethanol to water (- - -).

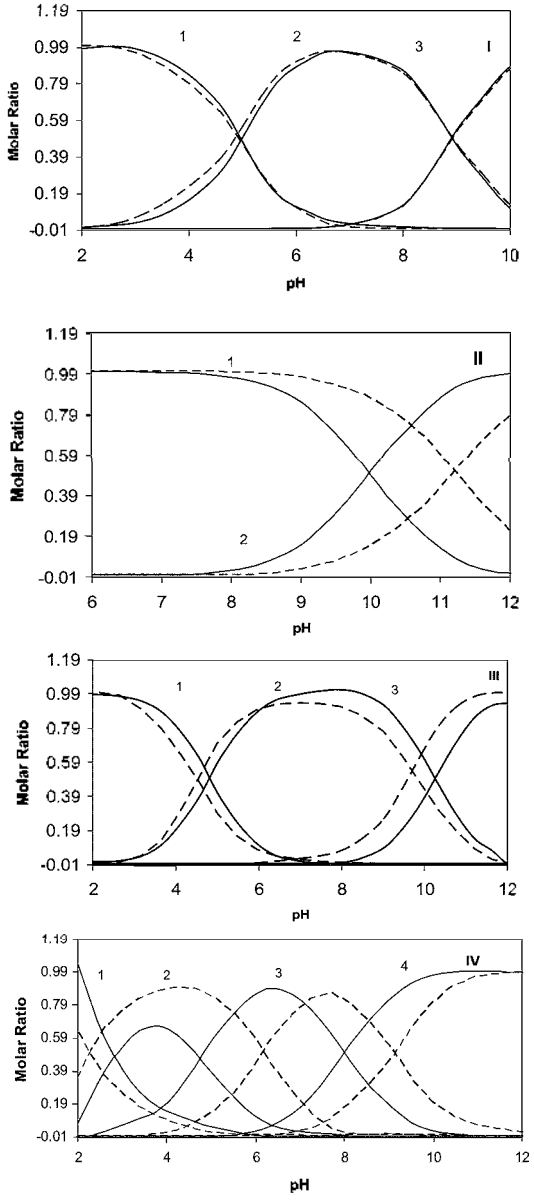

Fig. 4. Distribution diagram of different species of (I) Pyridoxal. (II) Riboflavin, (III) Thiamine, and (IV) Folic acid in pure water (—) and 80% ethanol to water (- - -).

ity constants of the thiamine has different effect; it means the increasing of the ethanol percentage in the solvent mixture decreased the acidity constants of this vitamin. These variations could be explained by the fact that there is preferential solvation in these media that is related to the structural features of these mixtures. The composition of the immediate surroundings of a solute may differ from the composition of the bulk mixture. Preferential solvation is attributable to an excess or deficiency of molecules of one of the solvents in these surround-

ings.[32] If the solute displays no preference for the solvent molecules, the solvent composition in the primary coordination shell, in the immediate neighborhood of the solute, is the same as that in the bulk. The deviation from the ideal dependence on the composition of the mixtures indicates that the solvent composition in the neighborhood of the solute may be different from that in the bulk. As discussed above, the data shown in *Table* 1 clearly illustrate the important influence of the nature of the solvent on the dissociation reactions. It has been shown that the solvating ability[33](as expressed by the Guttmann 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 DN=33.0) and dielectric constant (ε=78) which can dissociate the acid and stabilize the produced anion and hydrogen ion. Thus, it is expected that addition of ethanol with lower donor number (DN=19.0) and dielectric constant (ε=24.3) to water decreases the extent of interaction of the acid anion and the proton with solvent, and this decreases the first protolytic constant of folic acid and first step of pyridoxal and first and second steps of thiamine; and this in turn increasing the second and third steps of folic acid and only one step of riboflavin. The change in the acidity constants versus the percentages of ethanol in the binary mixtures of two solvents are shown in *Fig.* 5. The same trend has already been reported for various organic molecules in the numbers of organic solvents.[34-35] It has been reasonably assumed that preferential solvation of the charged particles by water is mainly responsible for such a monotonic dependence of the acidity constants of group B vitamins 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 the acid will be lowered. Therefore, the decrease or increase in the Ka of the any steps of each vitamin is due to increasing the mole fraction of ethanol in the binary mixed

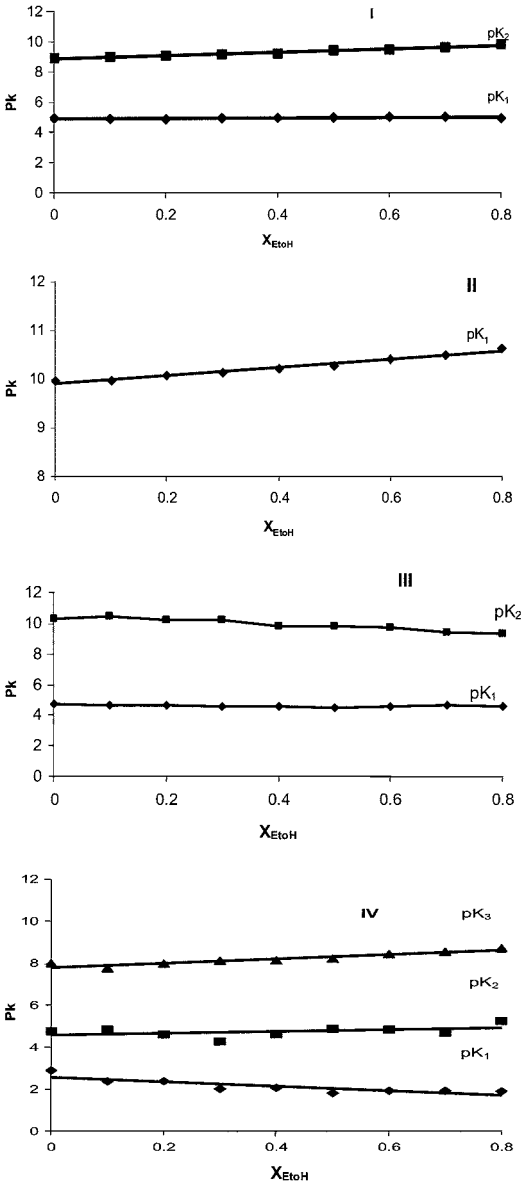

Fig. 5. pK_a values against X_{EtOH} in binary (ethanol + water) solution; (I) Pyridoxal (II) Riboflavin (III) Thiamine (IV) Folic acid.

solvent. The amounts of the variations are fully depending on the extent of the differences of physical parameters (donor number and dielectric constant) of the added organic solvents with water. Of course, as described above, the changing to solvation ability which controls the equilibrium constants are influenced by the changing to the bulk properties as such dielectric constant and donor number which these in turn has drastic effect on the solvation shell or coordination shell compositions.

CONCLUSION

The protolytic constants of the group B vitamins were calculated with spectrophotometric titrations using chemometrics based computer program DATAN. The striking advantage of the proposed method 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. The effect of binary mixture of ethanol – water on the acidity constants is investigated and it reveals that there is a complex relations between the dissociation constants and the composition of the solvent mixtures. The results show good consistency with the previous reported values. The contribution of the ethanol in the solvent composition is observed by changing in the distribution diagram, spectral profiles and the pKa values of the vitamins molecules.

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