

MULTIWAVELENGTH SPECTROPHOTOMETRIC DETERMINATION OF ACIDITY CONSTANTS OF MORIN IN METHANOL–WATER MIXTURES

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The acid-base properties of morin (2',3,4',5,7-pentahydroxyflavone) in mixtures of methanol–water at 25 °C and an ionic strength of 0.1 M are studied by a multiwavelength spectrophotometric method. To evaluate the pH–absorbance data, a resolution method based on the combination of soft- and hard-modeling is applied. The acidity constants of all related equilibria are estimated using the whole spectral fitting of the collected data to an established factor analysis model. DATAN program was used for determination of acidity constants. The corresponding pK_a values in methanol–water mixtures were determined. There is a linear relationship between acidity constants and the mole fraction of methanol in the solvent mixtures. The effect of solvent properties on acid-base behavior is discussed.

Keywords: Morin; Flavone; Flavanoids; DATAN; Acidity constants; Binary solvents; Methanol; Spectrophotometry.

Flavonoids¹ are polyphenolic natural and synthetic compounds² that possess outstanding biological and physicochemical properties^{3,4}. Flavonoids, widely distributed dietary constituents derived from plants are antioxidants and act as scavengers of oxygen radicals, such as the superoxide anion, singlet oxygen and hydroxy radicals. A wealth of evidence in literature strongly suggests the involvement of oxygen free radicals in cellular processes that underline the mechanisms of the induction of certain diseases, such as atherosclerosis, arthritis and cancer⁵. Morin, a bioactive flavonoid found in yellow Brazil wood has a number of analytical and pharmaceutical applications^{6–15}. To our knowledge, spectral properties of methanol–water morin solutions at various pH are not described in literature.

Acid dissociation constants are important parameters indicating the extent of ionization of molecules in solution at different pH values. The acidity constants of organic reagents play a fundamental role in many analytical procedures such as acid-base titration, solvent extraction, complex formation, and ion transport. It has been shown that acid-base properties affect the toxicity¹⁶, chromatographic retention behavior, and pharmaceutical properties¹⁷ of organic acids and bases. Much of the theoretical foundation of modern organic chemistry is based on the observation of the effects on acid-base equilibrium of changing molecular structure¹⁸.

Acid-base properties of methanol-water mixtures have been a constant subject of study in analytical chemistry for many years¹⁹⁻²³. The first analytical applications of methanol-water mixtures were as solvents for acid-base titration of organic compounds insoluble in water, and for the determination of acidity constants of these compounds, most of them with pharmaceutical or biological interest.

The solvation of a solute in a mixed solvent is much more complex than the solvation in a neat single solvent, and the literature offers several theories and models for this process²³. In general, they agree in that when a solute is dissolved in a mixed solvent, specific solvation effects determine that the proportion of the solvents in the solute sphere of solvation is different from that in the bulk solvent. The solute interacts more strongly with one or more solvents of the mixture and it is preferentially solvated by these solvents. Solute properties, such as pK_a value, depend on the composition and properties of this solvation sphere, and therefore they are very sensitive to preferential solvation.

The spectroscopic instrumentation used today, however, almost invariably has the capacity to collect data in a full spectral range. Using a single or a few wavelengths discards most of the information in the collected spectra and requires both the presence of knowledge of such suitable wavelengths. However, in many cases, the spectral responses of components overlap considerably and analysis is no longer straightforward^{24,25}. The determination of association constants using spectroscopic measurements is commonly accomplished by the method of Benesi-Hildebrand^{26,27}. This analysis requires that the concentration of one of the associating species be kept very much lower than the other, and it assumes that the dissociated species do not contribute significantly to the measured analytical signal. However, in many cases, the spectral responses of two and sometimes even more components overlap considerably and analysis is no longer straightforward. The single-point measurements are usually made at the edge of an absorption band, where the spectral overlap is least. However, here the

spectral responses is much lower than at the absorption maximum, the noise level may be considerable and association constants determined by the Benesi–Hildebrand method are accompanied by many systematic errors²⁸.

Using chemometric methods one can analyze whole spectra, thereby utilizing all spectral information^{29,30}. The approach is superior to any single-point measurement since several hundreds of data points per spectrum can be treated simultaneously³¹. 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 many 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^{32–35}.

Data analysis was carried out with the DATAN package, developed by Kubista group^{24,36}, using the physical constraints approach. This provides a unique solution by requiring that the calculated concentrations obey an assumed equilibrium expression and demonstrates its applicability by determining the acidity constants of two and four protolytic forms of fluorescein. A possible advantage of the Kubista method is that it mixes a soft-modeling approach with a hard-modeling one. This might be the best and more general strategy, since it can handle different situations with only a partial knowledge of the chemistry of the system. The physical constraints method calculates spectral profiles, concentrations and equilibrium constants by utilizing equilibrium expression that relates the components. The theory and application of the physical constraints method was discussed by Kubista et al. in several papers^{37–46}.

In this study, we applied the physical constraints approach for determination of the acidity constants of morin (2',3,4',5,7-pentahydroxyflavone) in water and in binary methanol–water mixtures by spectrophotometric titration at 25 °C and an ionic strength of 0.1 M. The analysis is readily performed with the DATAN program⁴⁷.

EXPERIMENTAL

Chemical and Solutions

Morin, methanol, hydrochloric acid, sodium hydroxide, and potassium nitrate were purchased from Merck. All the reagents used were of analytical reagent grade. These reagents were used without further purification. Standard stock solutions of 5×10^{-4} M morin was

prepared by dissolving appropriate amounts of morin in water. All the solutions were prepared in deionized water.

Instrumentation and Software

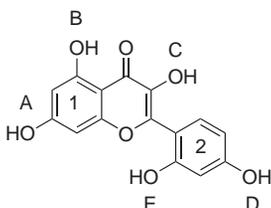
A Perkin-Elmer (Lambda 25) spectrophotometer controlled by a computer and equipped with a 1-cm path length quartz cell was used for UV-VIS spectra acquisition. Spectra between 230 and 400 nm (1 nm resolution) were obtained. The pH values were measured by a Horiba M-12 pH-meter furnished with combined calomel Ag/AgCl electrode. To calibrate the pH-meter in various methanol-water mixtures, 0.01 M solutions of oxalate and succinate buffers were employed. The data were treated in an AMD 2000 XP (256 Mb RAM) microcomputer using DATAN package.

Spectrophotometric Measurements

For 1.5×10^{-5} M solution of morin in binary mixtures, absorption spectra were measured with a titration set-up consisting of a computer interfaced to a spectrophotometer. The pH control was achieved by using the modified universal buffer solution. To account for differences in acidity, basicity, and ion activities for methanol-water solvent mixture relative to pure water, where the pH-meter is standardization using aqueous buffers at 25 °C, the pH values in methanol-water solvent mixtures were corrected using the equation $\text{pH}^* = \text{pH}(\text{R}) - \delta$, where pH^* is the corrected reading and $\text{pH}(\text{R})$ is the pH-meter reading obtained in a partially aqueous organic solvent, determined by Douheret^{48,49}. All measurements were carried out at 25 ± 0.5 °C. Ionic strength was maintained at 0.1 M by adding appropriate amounts of KNO_3 . It is noticeable that in very high acidic and basic pH, ionic strength is not adjusted in 0.1 M.

RESULTS AND DISCUSSION

The chemical structure of morin given in Scheme 1, implies a low acidity of the OH groups. Consequently, the determination of their highly alkaline dissociation constants is a difficult task. The morin studied, presents five ionizable OH groups. In this case, ring 1 and ring 2 are not conjugated, i.e., the deprotonation of OH groups of one ring system should not appreciably affect the ionization of OH groups of the other. Hence, ionization of OH



SCHEME 1
Chemical structure of morin

groups of ring 1 could be considered independent and distinguishable from those of ring 2. The previous reported values of acidity constants are mainly in water and in mixtures of ethanol with water^{50,51}. The differences observed between the pK_a values are due to probable experimental errors of old methods, against chemometric based methods which, by using the whole spectral domain, reduce considerably the level of noise. So the obtained acidity constants are more reliable and precise than previous methods. Recently, Herrero-Martinez et al.⁵² has reported the pK_a of morin and also, discussed the deprotonation site⁵³, which are listed in Table I for comparison. The obtained values of this study in water are in good agreement with this report.

The absorption spectra of morin in binary solvent mixtures at various pH values in 230–400 nm intervals were recorded. Sample spectra of morin at different pH values in water with pH ranging from 1.8 to 13.2 and 50% (w/v) methanol to water at 0.1 M KNO_3 with pH ranging from 1.9 to 13.6 are shown in Figs 1 and 2, respectively. Singular value decomposition analysis performed on all absorption data matrices obtained at various pH values for morin gives the number of components that best represent the system. The plot of eigenvalues as a function of the eigenvalue number is shown in Fig. 3, i.e., the evolution log eigenvalues is plotted as function of the prepared mixture composition. As seen in Fig. 3, six components are presented in the system. Figure 3 shows the real error^{31,54} obtained as a function of the eigenvalue number for morin system. An alternative name for the residual standard deviation (RSD) used by Malinowski³¹ is the real error. As seen in Fig. 3, there are six significant factors in the morin system. Six significant factors are also supported by the statistical indicators of Elbergali et al.⁴⁰ These factors could be attributed to the five dissociation equilibria of a pentaprotic acid such as morin. The pK_a values of morin were investigated in five different methanol–water binary mixtures spectrophotometrically at 25 °C and an ionic strength of 0.1 M.

Acidity constants of morin in several mixtures were evaluated with the DATAN program using the corresponding spectral absorption–pH data. By inspection of the experimental spectra, it is hard to guess even the number of protolytic species involved. The six calculated most significant projection vectors with clear spectral features (compared with noise) evidence the presence of six spectroscopically distinguishable components. Their shapes, however, are clearly nonphysical and cannot be directly related to the spectral response of the six protolytic forms. The output of the program are pK_a values and their standard deviations, the number of principal components,

TABLE I
Acidity constants of morin in methanol–water mixtures at 25 °C and 0.1 M ionic strength of obtained with DATAN program

Methanol wt. %	$(\epsilon)_m$	$\{1/(\epsilon)_m\} \times 10^2$	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}	pK_{a5}
0			–	6.93 (A) ^a	8.32 (E)	9.98 (D)	11.98 (B)
0	78.40	1.28	2.30 ± 0.02	6.65 ± 0.03	7.86 ± 0.04	9.58 ± 0.06	12.26 ± 0.09
10	73.82	1.35	2.56 ± 0.02	6.70 ± 0.03	8.00 ± 0.04	9.85 ± 0.07	12.54 ± 0.10
20	69.24	1.44	2.73 ± 0.03	6.92 ± 0.04	8.34 ± 0.04	10.07 ± 0.07	12.73 ± 0.10
30	64.66	1.55	3.01 ± 0.03	6.94 ± 0.04	8.52 ± 0.05	10.21 ± 0.08	12.94 ± 0.12
40	60.08	1.66	3.52 ± 0.02	7.14 ± 0.06	8.85 ± 0.06	10.46 ± 0.09	13.21 ± 0.15
50	55.50	1.80	3.79 ± 0.04	7.32 ± 0.05	9.31 ± 0.05	10.65 ± 0.09	13.32 ± 0.18

^a Main function group of Scheme 1 that contributes to the pK_a value⁵².

projection vectors (loadings), concentration distribution diagrams, and the spectrum of each assumed species.

The obtained pK_a values are listed in Table I. The pK_a values correspond to the pH-dependent variation of absorption spectra in all solvent mixtures. One of the very important outputs of the DATAN program is the calculated spectrum of different forms of morin in each solvent mixture. Sample spectra of the calculated spectra of all species in pure water and 50% methanol

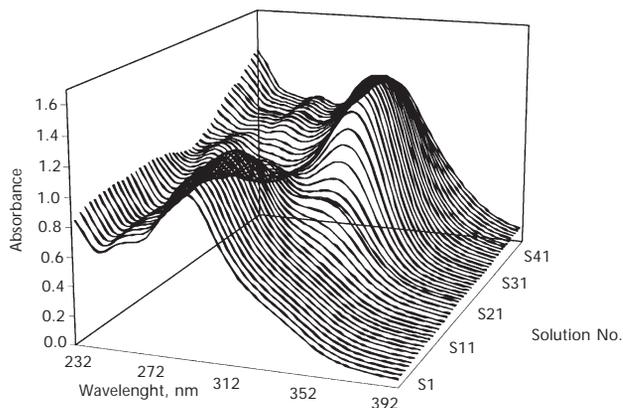


FIG. 1
Absorption spectra of 1.5×10^{-5} M morin in pure water at 0.1 M KNO_3 at different pH values

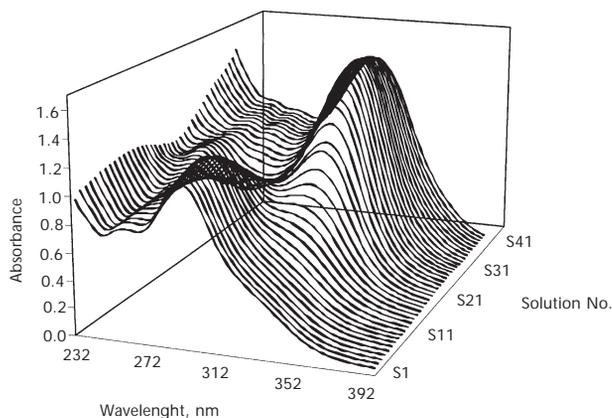


FIG. 2
Absorption spectra of 1.5×10^{-5} M morin in 50 wt.% methanol-water at 0.1 M KNO_3 at different pH values

to water are shown in Fig. 4. It is interesting to note that the nature of the solvent composition has a fundamental effect on each pure spectrum. As it is clear from Fig. 4, this effect is apparently observed in different species of morin. The solvent effect on this spectrum is very interesting. As the mole fraction of methanol increased, the absorption intensity changed for different species of morin. The most important features of the distribution diagrams are the pH limits of the evolution and disappearance of components. The sample distribution diagrams are shown in Fig. 5.

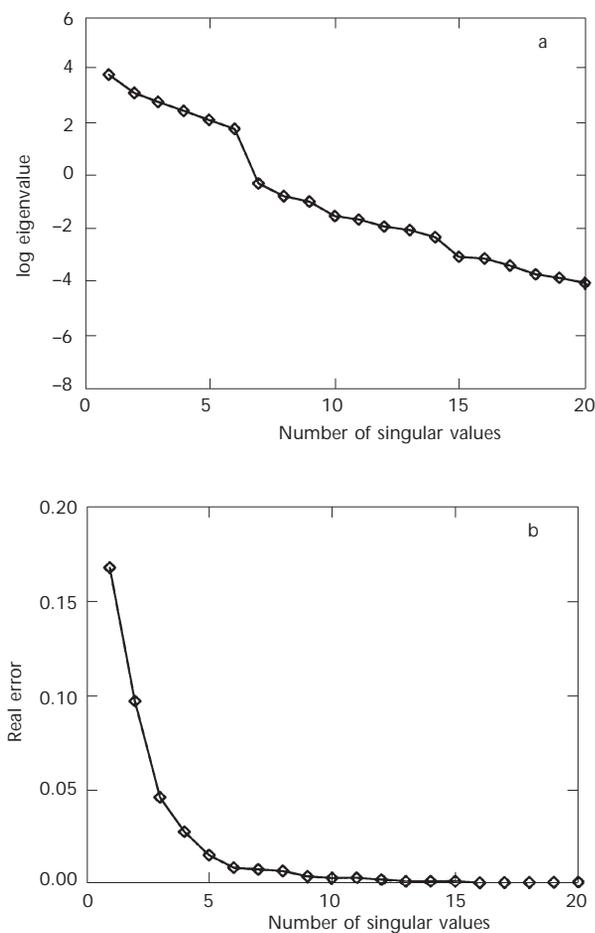


FIG. 3
Plot of logarithm of eigenvalues (a) and real error as obtained by singular value decomposition for morin in pure water (b)

Increasing the mole fraction of methanol in the medium leads to a decrease in the acid ionization constants for morin. The acid ionization constant in a pure aqueous medium ($K_{a(w)}$) is related to that in a partly aqueous medium ($K_{a(s)}$) by the relation⁵⁵:

$$K_{a(w)} = K_{a(s)} (\gamma_{A^-} \gamma_{H^+} / \gamma_{HA})$$

where γ is the activity coefficient of the respective species in a partly aqueous medium relative to that in pure water. The electrostatic effect resulting from the change in the relative permittivity of the medium influences the

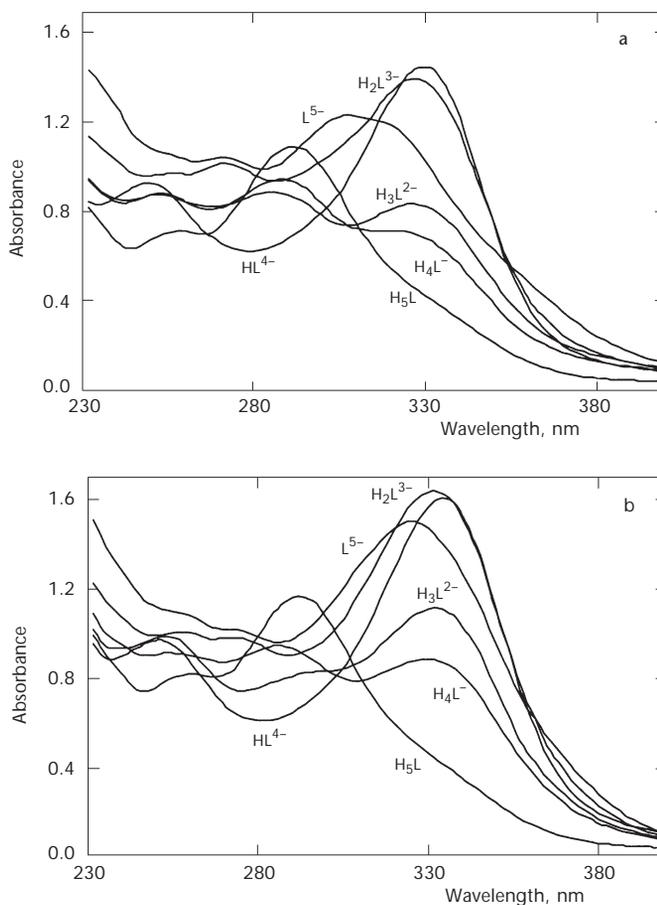


FIG. 4
Spectra of morin species in water (a) and in 50 wt.% methanol-water (b)

activity coefficient of any charged species⁵⁵. Generally, by increasing the mole percentage of methanol solvent in the aqueous medium, the relative permittivity of the medium is lowered. This will increase both the γ_{A^-} and γ_{H^+} yielding a decrease in the acid ionization constants (i.e., high pK_a values). This is consistent with the results obtained for the acid ionization constants (pK_a) of morin in various water-methanol mixtures. However, in the light of the relation⁵⁶: $pK_a = e^2/[2.303akT(\epsilon)_m]$, which describes the variation of pK_a with the relative permittivity of the medium (ϵ)_m⁵⁷, A plots of pK_a values against $\{1/(\epsilon)_m\}$ for methanol solvent, fitted by linear regression is shown in Fig. 6. The same trend has already been reported for various organic molecules in different solvent mixtures^{33,34,58}. It has been reasonably assumed that preferential solvation of the charged particles by water is mainly responsible for such a monotonic dependence of acidity constants of morin on the solvent composition.

As discussed above, this indicates that the acid ionization constants of morin obtained in various water-methanol mixtures are governed by the electrostatic effect. Acidity constants of the fifth step of dissociation of morin decrease with increasing mole fraction of methanol in the mixed solvents. It has been shown that the solvating ability⁵⁵ (as expressed by the Gutmann scale) and permeability of the solvent play a fundamental role in dissociation reactions. Water is a solvent of high solvating ability (donor number DN = 33, and permeability $\epsilon = 78.4$) which can dissociate the acid

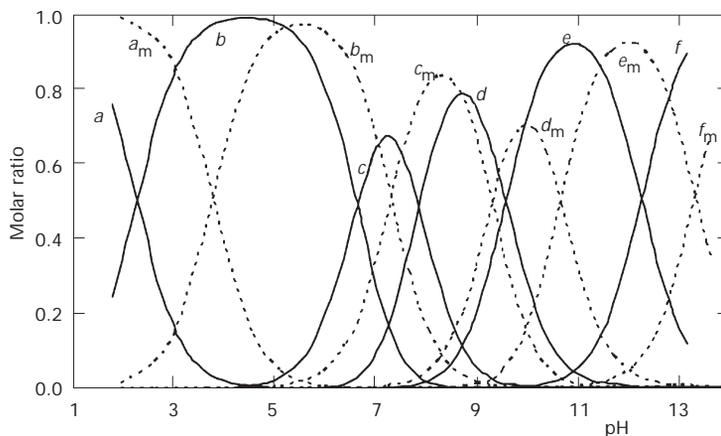


FIG. 5

Distribution diagrams of different species of morin: H_5L (a), H_4L^- (b), H_3L^{2-} (c), H_2L^{3-} (d), HL^{4-} (e) and L^{5-} (f) in water (full line) and subscript m comes for showing 50 wt.% methanol-water (dashed line)

and stabilize the produced anion and hydrogen ion. Thus, it is expected that addition of methanol with lower donor number and permeability ($DN = 19$, $\epsilon = 32.6$) to water decreases the extent of interactions between the acid anion and proton with solvent, and this decreases the acidity constants of morin.

It was recognized that solvent effects such as hydrogen bonding and solvent basicity as well as dispersion forces and proton-solvent interactions exert a profound influence on the ionization process of weak acid in the presence of organic solvent such methanol⁵⁹. The effective density of dispersion centers in the organic solvent used is higher than in pure water. Accordingly one can expect higher stabilization of the conjugate base A^- from each step of ionization by dispersion forces, which are established between the delocalized oscillator dipole of the solvent. Furthermore, the proton is expected to be highly stabilized in aqueous mixtures by its interactions with the less polar methanol and water molecules (proton-solvent interaction) compared with water molecules alone in pure water. Consequently, both A^- and H^+ are being highly stabilized upon increasing of the mole fraction of methanol in aqueous medium, i.e., γ_{A^-} and γ_{H^+} decrease. Thus, the acid ionization constants of the studied morin increase (pK_a decrease) with increasing methanol content in the medium. However, this is not the case, as is evident from the data cited in Table I. Therefore, one can conclude that both the dispersion forces and proton-solvent interaction effects do not have significant role in the ionization processes of morin.

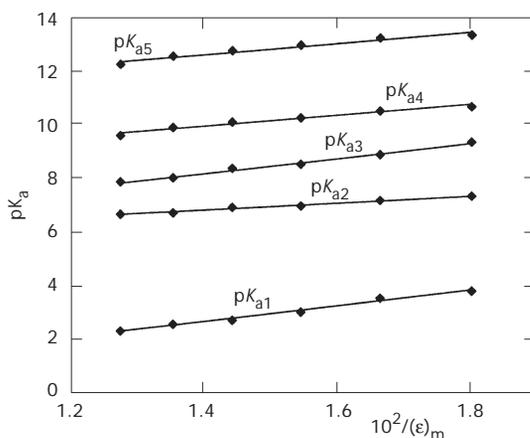


FIG. 6
Plots of pK_a values of morin against $1/(\epsilon)_m$ of the medium in various methanol-water mixtures at 25 °C and a constant ionic strength $I = 0.1$ M (KNO_3)

On the other hand, water molecules are characterized by a high tendency to act as hydrogen donors compared with other solvent molecules such as methanol. Therefore, the conjugate base A^- is expected to be less stabilized by hydrogen-bonding interaction with methanol molecule as the mole fraction of methanol is increasing (i.e., γ_{A^-} increase). This will tend to increase the pK_a values of all steps in the morin system. Accordingly, the observed increase in the pK_a values of morin upon increasing mole fraction of methanol in aqueous mixtures can be ascribed, in addition to the electrostatic effect, to the hydrogen-bonding interaction between the conjugate base A^- and methanol.

CONCLUSION

In this work, the behavior of acidity constants of morin in mixtures methanol-water at 25 °C and an ionic strength of 0.1 M is studied by a multi-wavelength spectrophotometric method. The shifts in acidity constants of morin in the mixtures were derived. There is a linear relationship between acidity constants and the mole fraction of methanol in the solvent mixtures. The effect of solvent properties on acid-base behavior is discussed. This indicates that the acid ionization constants of morin obtained in various methanol-water mixtures are governed by electrostatic effects. The results described here strongly indicate six major species in the morin system. The physical constraints method (DATAN) is a useful tool for the resolution of different species present in a multi-equilibria system.

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