Infrared Spectroscopy of Aqueous Carboxylic Acids: Malic Acid

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Quantitative infrared (IR) titration of 1.80 M malic acid is presented where factor analysis (FA) was used to obtain the principal species' spectra and their abundances. Three malic species and three water species were obtained. The distribution of the species as a function of pH was made from which their pK_a values were determined. The experimental points of the distribution curves correspond to the values calculated from the thermodynamic equilibrium equations. A precise determination of aqueous malic IR bands was obtained from the real spectra of the malic species. The hydrates' hydration numbers were determined to be 2.0 ± 1.0, 3.0 ± 2.0, and 4.0 ± 0.5 for malic acid, mono-, and disodium malate, respectively. The hydrates are stable throughout the pH range where the species are present. The double and single CO stretch bands of malic acid are situated at 1719 and 1272 cm^{-1}, respectively. The antisymmetric and symmetric CO stretch bands of mono- and disodium malate are situated at 1580, 1400 cm^{-1} and 1563, 1395 cm^{-1}, respectively. Malic acid shows four very broad bands in the 3800−1800 cm^{-1} region as a continuous absorption assigned to the OH stretch of hydrogen bonded water and alcoholic groups to carboxylic groups. The 2930 and 2580 cm^{-1} bands, which are far from the 3500 cm^{-1} band, indicate strong hydrogen bonds. Disodium malate shows one large band at 3320 cm^{-1} assigned to the OH stretch of solvated water and alcoholic groups. Monosodium malate has the bands of both malic and disodium malate slightly displaced, but with half their intensities.

1. Introduction

Malic acid ($HO_2CCH_2CH(OH)CO_2H$) is an important dicarboxylic acid present in fruits such as apples, plums, berries, etc. It is also present in small amounts in living cells, being one of the intermediates of the Krebs cycle responsible for the breakdown of food to release energy. In these systems, malic acid exists in aqueous environments at different pH values; this is a challenge for chemists and biochemists wishing to develop efficient quantitative analytical methods for its study. The molecular organization of such a system is also of some interest because it is a double carboxylic acid with which water could form a complex. Previous infrared (IR) spectroscopic studies on malic acid are not numerous. One utilized a HPLC−FTIR tandem method to quantify carbohydrates and organic acids, malic acid among them, in wines.[1] Another study dealt with the simultaneous determination by IR of citric, malic, and tartaric acids in soft drinks.[2] A third involved an IR titration of succinic and malic acids where a two-dimensional FTIR correlation spectroscopy allowed the carboxylate groups to be identified.[3] The spectra in this last study were limited to the 1900−1000 cm^{-1} range for solutions of a pH between 2 and 12. The water and malic principal species and their abundances were not sorted.

In a previous study intended to evaluate the usefulness of IR spectroscopy in quantifying acidic and basic water, we used malic acid and disodium malate as probes to evaluate our methods in dealing with the different kinds of water organizations encountered in acidic and basic aqueous solutions.[4] Being a strong IR absorber, water makes quantitative measurement difficult but not altogether impossible, as several of our papers have shown. For example, titration of glycine, *N*, *N*-((butyloxy)propyl)amino diacetic acid (BOPA), NaOH, H_3PO_4, and H_2SO_4 proved the feasibility of the technique.[5−10] More than a simple quantitative measurement, these IR titrations yielded some other useful information, such as the spectra of the different species in the solution, the determination of several complexes, some not previously established, and the determination in solution of the number of water molecules solvated to the different species.

Many, if not all, carboxylic acids show low intensity absorption in the 3000−1800 cm^{-1} region that many authors refer to as a "continuum of absorbance"—an ambiguous expression. In a previous study with basic and acidic water as well as with several aqueous salt solutions that showed some absorption in that region, we assigned that absorption to combination bands and OH stretch bands.[5,7,11,12] Because malic acid, which has two carboxylic groups giving three species and two values of p*K*a, shows such an absorption, it is a good molecule to either verify this assignment or help propose another one. Furthermore, malic acid has an alcoholic OH group in the center of the molecule that increases the difficulty of water subtraction but renders the substance soluble in the whole 0−14 pH range. Because of its importance to the food industry and of its interest to biochemistry, it is worthwhile to look at the IR titration of malic acid, especially in view of the rapidity and nondestructiveness of this analytical technique.

IR spectroscopy is a technique that allows the different species present in mixtures to be identified and their abundances to be

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determined.[4−19] Because the OH stretch vibrations are strong IR absorbers, transmission measurements of aqueous solutions are difficult to obtain.[20] Raman spectroscopy was used to overcome this difficulty because Raman signals of the OH stretch vibrations are far less intense than their IR absorption.[21] On the other hand, the attenuated total reflectance (ATR) configuration allows the complete mid-IR (MIR) spectra of aqueous systems to be obtained with high reproducibility.[4−19,22,23] We have developed an adequate quantitative method with ATR that provides consistent and reliable results as long as the following basic requirements are met: (1) the use of a proper crystal whose refractive index is far from that of the solution,[14] (2) an adequate angle of incidence of the IR beam, (3) an adequate length of the ATR crystal. When these conditions are met, the IR-ATR spectra reflect the system's chemical changes: that is, the ATR(ν) spectra closely follow the imaginary part of the refractive index spectra $k(\nu)$.[14,18] Using this technique to study alkali halide aqueous solutions as well as several acid−base titrations, we found that water forms stable clusters with the ions when a binary salt (NaCl, KCl), a strong acid (HCl), or a strong base (NaOH) is dissolved in it.[4−12,15,16,18] We also detected the formation of complexes in aqueous sulfuric and phosphoric acids,[8−10] some of which had previously been deduced only from thermodynamic measurements.

We applied principal factor analysis (FA) in previous studies to a series of IR spectra in order to determine the number of species, their abundances, and their spectra. This type of analysis is suited to cases where some of the principal spectra can easily be obtained from the set of experimental spectra. Other methods of applying FA can be used[24] that usually give abstract spectra. These can then be transformed into real spectra using more demanding methods.[17,25]

The IR titration of 2.66 M glycine was reported in ref 5. In that analysis, the water bands were removed from the experimental spectra by subtracting only a pure water spectrum. Since some low intensity absorption persisted, a second-order polynomial function was applied in the 1800−900 cm^{-1} region to remove it. Factor analysis was then applied to obtain the real spectra and the MFs of the glycine species.[5] Such a procedure effectively titrated aqueous glycine by giving the spectra of the glycine species and their abundances, but it did not permit a quantitative analysis of the water content of the aqueous solutions. Such information would be useful in determining the composition of the species present in the solutions. Furthermore, it did not yield the complete mid-IR spectra (4800−700 cm^{-1}) of the pure species.

We demonstrated in subsequent papers,[4,7,11] that four distinct types of water may be present in aqueous solutions: pure water, salt-solvated water, acidic water, and alkaline water. We further performed the quantitative subtraction of the water spectrum using these four types of water.[5,8,9] On the bases of these results, it was possible to subtract quantitatively the water absorption bands from a series of IR titration spectra of BOPA obtained in the 0−14 pH range[6] without the need of an arbitrary baseline correction. This allowed us to determine the complete mid-IR spectra of the four BOPA ionic species. In a study of sulfuric acid, we reported its IR titration and gave a detailed quantitative analysis of the amount of water in strong interaction with the ionic species.[9] In an another study of saline solutions, it was found that the salt solutions and pure water spectra are not orthogonal even at the solubility limit−the solution spectrum contains some of the pure water spectrum.[15] To surmount this difficulty, we developed the equations permitting the extrapolation of a saline solution spectrum to that of "pure" salt-solvated spectrum, a spectrum devoid of absorption of pure (or ordinary) water.[15,18] From the "pure" salt-solvated spectrum, the molar ratio of water to solute in the solvated species could be determined.

The purpose of the present study is to apply the above-mentioned technique to analyze the complete MIR spectra obtained from the titration of malic acid with aqueous HCl and NaOH. We want to determine (i) the number of species present in the mixtures, (ii) their abundances as a function of pH, (iii) their composition, (iv) their pK_a values, (v) the species hydration numbers, (vi) the origin of the "continuum of absorption".

2. Theoretical Considerations

2.1. Volumetric Titration Curves. The dissociation equilibrium equations for malic acid are

$$\underset{AH_2}{HOOCCH(OH)CH_2COOHH} \overset{K_1}{\rightleftharpoons} H^+ + {}^-OOCCH(OH)CH_2COOH \overset{K_2}{\rightleftharpoons} \underset{A^{2-}+2H^+}{{}^-OOCCH(OH)CH_2COO^-} + 2H^+ \quad (1)$$

where the bottom line is the symbolic representation of the equilibrium equation. The dissociation constants are

$$K_1 = \frac{[AH^-]\,[H^+]}{[AH_2]} \quad (2)$$

$$K_2 = \frac{[A^{2-}]\,[H^+]}{[AH^-]} \quad (3)$$

In eq 1, H^+ stands for H_3O^+ and the anionic form AH^- represents the two isomers of monosodium malate ${}^-OOCCH(OH)CH_2COOH$ and $HOOCCH(OH)CH_2COO^-$.

We have developed the titration equations for aqueous glycine,[5] BOPA,[6] sulfuric,[9] and phosphoric[8] acids on the bases of the dissociation equilibrium equations, the equations of the conservation of species, and the equation of electroneutrality. In ref 6 were developed the equations for the titration of an aqueous solution of BOPA starting from any ionic state of the molecule. BOPA is a glycinate containing two carboxylic groups that can be ionized to their cationic forms. Since the cationic form does not exist in malic acid, we modified eq 28 of ref 6 by putting K_1 equal to ∞, resulting in $y_1 = 0$ (amount of cationic form). In the present case, the other constants K_2 and K_3 become K_1 and K_2, respectively. For malic acid titration, the relation between titrant mass m_δ and proton concentration becomes

$$m_\delta = V\frac{\rho_0\frac{\epsilon_2}{M_2}\left[\frac{\left(\frac{K_1}{[H^+]}+2\frac{K_1K_2}{[H^+]^2}\right)}{1+\frac{K_1}{[H^+]}+\frac{K_1K_2}{[H^+]^2}}\right]-[H^+]+\frac{K_0}{[H^+]}}{\delta\frac{\epsilon_\delta}{M_\delta}+(1-\rho_\delta)\frac{\epsilon_2}{M_2}\left[\frac{\left(\frac{K_1}{[H^+]}+2\frac{K_1K_2}{[H^+]^2}\right)}{1+\frac{K_1}{[H^+]}+\frac{K_1K_2}{[H^+]^2}}\right]} \quad (4)$$

where V is the total solution volume, δ selects the titrant used (base, $\delta = +1$; acid, $\delta = -1$), ϵ_2 is the relative concentration

of the stock solution (w/w), ϵ_δ is the titrant solution relative concentration, ρ_0 is the stock solution density (g/L), ρ_δ is the variation of the total sample density divided by the titrant partial density, M_2 is the malic acid molar mass, and M_δ is the titrant molar mass.

Equations 2, 3, and 4 can be transformed to give the relation between pH and titrant mass. The amount of the ionic species of malic acid is obtained by

$$[AH_2] = \frac{A}{1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2}} \quad (5)$$

$$[AH^-] = \frac{A}{\frac{[H^+]}{K_1} + 1 + \frac{K_2}{[H^+]}} \quad (6)$$

$$[A^{2-}] = \frac{A}{\frac{[H^+]^2}{K_1K_2} + \frac{[H^+]}{K_2} + 1} \quad (7)$$

where A represents the solute total concentration.

2.2. Factor Analysis. Factor analysis of a spectral data set is a process by which the principal factors of an evolving system can be identified and their concentrations obtained from derived multiplying factors (MFs). An evolving system is a system that is modified when a parameter such as temperature, pressure, concentration, or, in this case, pH is varied.

Once identified, the principal spectra are multiplied by estimated multiplying factors (MFs) that are varied until the difference between calculated and experimental spectra is minimized using a least-squares fit procedure. The residues that are not at the zero level, and that show a regular pattern indicate the presence of another factor. The largest residue serves to determine the spectrum of the supplementary species, which is then introduced into the FA procedure. The procedure is stopped when the residues have reached a minimum that cannot be efficiently reduced. We then obtain the number of principal factors, their abundances, and their spectra. The MFs multiplied by the principal spectra concentrations give the concentrations of the species.

The principal factors represent the separated species when all the species' concentrations evolve differently. However, when two or more species evolve in concert with the varying parameter, FA cannot separate them because the relative concentrations of the species remain constant. The principal factors in such situations contain more than one molecular species, as was observed for aqueous propanol and sucrose.[19,23]

3. Experimental and Data Treatment

3.1. Chemicals and Solutions. D−L-Malic acid (Aldrich Chemical Co., purity >99%) was used without further purification. Deionized water was used to prepare the aqueous solutions. Aqueous NaOH, 50.8% (w/w) and concentrated HCl 37.0% (w/w, density 1,19 g/mL, Fisher Scientific) were used for the titration. The high titrant concentrations were used to maintain the sample concentrations approximately constant. Both acid and base were calibrated by standard methods.

Pure water, 1.54 M HCl, and 2.23 M NaOH solutions were used to obtain the reference spectra (or principal spectra) of neutral, acidic, and basic water.[6,7] A 1.849 M malic acid stock solution was made up. Each sample was prepared by weighing the titrant in an empty 10 mL volumetric flask and completing the volume with the stock solution. The resulting total mass was measured. A series of 24 samples in the pH range 0 to 14 were prepared. Samples resulting in homogeneous solutions were divided into two parts, one for the IR measurements and the other for the pH measurements.

3.2. pH Measurements. The pH was measured at ambient temperature (24 ± 2 °C) with a pH meter (Omega model PHH-253) equipped with a combination electrode (Analytical Sensors, Inc., model PH10107B-03-B). A two-point calibration was made at pHs 2.00 and 7.00 as well as at pHs 7.00 and 10.00 prior to any series of measurements.

3.3. IR Measurements. The IR measurements were obtained using a model 510P Nicolet FT-IR spectrometer with a TGS detector. Two KBr windows isolated the measurement chamber from the outside. The liquid samples were contained in a Circle cell (SpectraTech, Inc.) equipped with a ZnSe crystal rod (8 cm long) in an ATR configuration; with the incident beam at an angle of 45° to rod's axis, there are 6.6 effective internal reflections. The solid sample was placed as a powder on a ZnSe ATR horizontal crystal (SpectraTech, Inc.). The spectral range of the ZnSe crystals in the spectrophotometer is 4800−650 cm^{-1}. The spectra were taken under nitrogen flow to ensure low residues of CO_2 and water vapor. Twenty scans of 4 cm^{-1} resolution were accumulated for each spectrum. All measurements were taken at 26.5 ± 0.3 °C. The cell was carefully dried before each measurement. Model 510P is a single-beam spectrometer and a background was taken with the cell empty before the measurement of each sample.

The IR measurements consisted in obtaining the ATR background intensity R_0 and the ATR sample intensity R. The ratio of R/R_0 produced intensity I for the spectral range studied. Thereafter, the 2153 data points $\{I(\tilde{\nu})$ vs $\tilde{\nu}$ (in cm^{-1})$\}$ of each spectrum were transferred to a spreadsheet program on a personal computer where the numerical treatments were performed. Next, intensities I were transformed into absorbances $\log(1/I)$ (abbreviated in some cases as au). A small baseline shift (<0.005 au) was made to obtain a null mean absorbance in the 4600−4450 cm^{-1} region, where water absorbs very little.[20]

3.4. Factor Analysis. FA was performed using a personal computer[7,8] in a two-step procedure that consisted in determining the multiplying factors (MFs) first of the water spectra and second of the malic species spectra.

4. Results and Discussion

4.1. Experimental ATR Spectra. The composition of the 24 samples of aqueous malic acid is given in Table 1 and the experimental spectra are presented in Figure 1. The spectra show the broad intense $\nu_{1,3}$ band of water from 3700 to 2800 cm^{-1} and its ν_2 band at 1638 cm^{-1}. A vertical line drawn at this position helps emphasize the changes that occur with variations in pH. The presence of the libration band of water below 650 cm^{-1} produces the strong absorption that starts near 1000 cm^{-1}. Water absorption band features are not constant throughout the entire series, but evolve slightly in passing from an acidic environment to a basic one. The spectral features not due to water are those of the solutes that evolve with pH.

4.2. Water Multiplying Factors (MFs). The water spectral subtraction, from which the water MFs are obtained, was made with acidic, alkaline, and "pure" water spectra on the bases of the following criteria: (i) no negative bands, with special attention paid to a small water band near 3660 cm^{-1}; (ii) the intensity levels in the 2620−2580 and 1850−1800 cm^{-1} regions are minimized. Observed in both acidic and alkaline water, the

TABLE 1: IR Titration of Malic Acid: Composition of the Solutions and Results of Factor Analysis

	gravimetry (MW 134.09)				factor analysis MFs					
			malic acid 24.8% w/w		water species			malic species		
pH	HCl 37% w/w g/10 mL	NaOH 50% w/w g/10 mL	g/10 mL	mol/L	HCl 1.54 M	H_2O pure	NaOH 2.23 M	COOH COOH	COOH COO^-	COO^- COO^-
0.28	0.312		10.596	1.792	0.430	0.410	0.000	1.790	0.000	0.000
0.39	0.239		10.650	1.801	0.390	0.450	0.000	1.801	0.000	0.000
0.69	0.179		10.774	1.822	0.350	0.490	0.000	1.810	0.000	0.000
1.04	0.072		10.854	1.835	0.280	0.560	0.000	1.825	0.009	0.001
1.35			10.937	1.849	0.250	0.588	0.000	1.818	0.028	0.002
2.25		0.199	10.822	1.830	0.230	0.611	0.000	1.585	0.254	−0.009
2.59		0.358	10.684	1.807	0.220	0.631	0.000	1.389	0.423	−0.005
2.82		0.512	10.583	1.790	0.210	0.647	0.000	1.209	0.592	−0.011
3.05		0.693	10.464	1.770	0.190	0.667	0.000	0.996	0.771	0.003
3.27		0.874	10.314	1.744	0.180	0.688	0.000	0.777	0.959	0.009
3.45		1.033	10.221	1.729	0.170	0.700	0.000	0.599	1.109	0.021
3.64		1.193	10.083	1.705	0.150	0.725	0.000	0.464	1.137	0.103
3.86		1.378	9.949	1.683	0.130	0.741	0.000	0.322	1.156	0.205
4.08		1.535	9.859	1.667	0.120	0.750	0.000	0.230	1.097	0.340
4.30		1.706	9.747	1.648	0.100	0.768	0.000	0.121	1.034	0.482
4.53		1.902	9.642	1.630	0.090	0.768	0.000	0.074	0.855	0.700
4.72		2.041	9.545	1.614	0.070	0.780	0.000	0.027	0.728	0.860
5.06		2.235	9.468	1.601	0.050	0.787	0.000	−0.009	0.518	1.093
5.51		2.380	9.292	1.571	0.030	0.790	0.000	−0.004	0.207	1.377
12.90		2.576	9.218	1.559	0.000	0.775	0.040	0.000	0.000	1.560
13.32		2.702	9.137	1.545	0.000	0.689	0.120	0.000	0.000	1.550
13.53		2.881	8.990	1.520	0.000	0.550	0.250	0.000	0.000	1.520
13.79		3.106	8.854	1.497	0.000	0.406	0.390	0.000	0.000	1.500
13.90		3.264	8.712	1.473	0.000	0.297	0.491	0.000	0.000	1.470

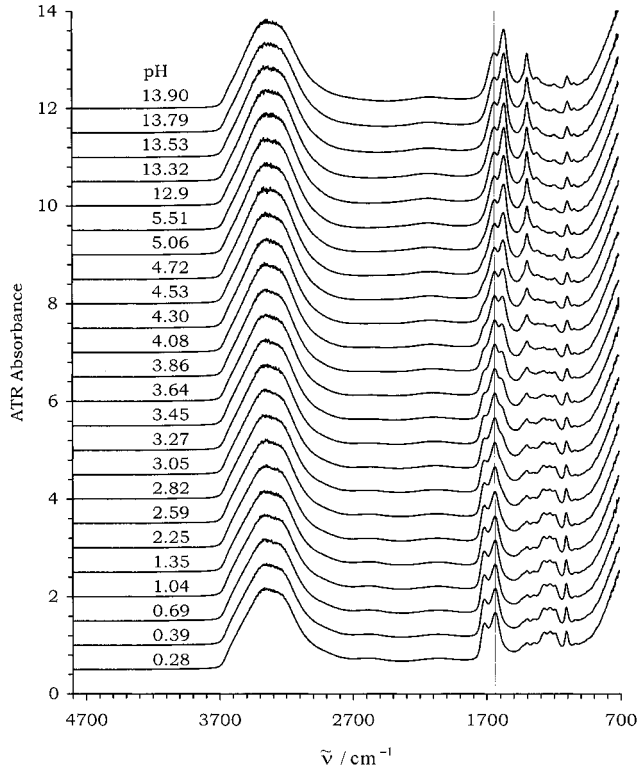


Figure 1. IR titration of 1.80 M malic acid by HCl and NaOH: 24 ATR−IR spectra in the 0.28−13.90 pH range (Table 1 gives the composition). The spectra are shifted by 0.5 au from each other. The vertical line at 1638 cm^{-1} indicates the $\delta(H_2O)$ band.

absorption in these regions is principally due to the so-called "continuum of absorbance". The water spectra are not orthogonal spectra, but this does not prevent FA from being performed.[7,14−16]

The principal water spectra used for the subtraction of the aqueous malic spectra are shown in Figure 2A: (a) 2.23 M NaOH, (b) 1.54 M HCl, and (c) pure water. Table 2 lists the

TABLE 2: Mean Intensity (in au) of the Water Principal Spectra (Figure 2A) in Critical Regions

spectral region	pure H_2O	2.23 M NaOH	1.54 M HCl
2620−2580 cm^{-1}	0.03	0.28	0.17
1850−1800 cm^{-1}	0.10	0.31	0.35

mean ATR intensity of the water principal spectra in the 2620−2580 and 1850−1800 cm^{-1} regions, which are critical regions for the subtraction. Subtraction of the water spectra produced a low intensity level near 3200 cm^{-1}; when overdone, this subtraction produced a negative band near 3660 cm^{-1}. The subtraction was stopped before this occurred (criterion i). The results led to a maximum water spectral subtraction. The subtraction of the acidic and basic water spectra produced the spectra in the low and high pH regions of Figure 3. Part of the broad band observed near 3450 cm^{-1} comes from the alcoholic OH. The remaining absorption at 1638 cm^{-1} (vertical line) is discussed in section 4.5.

Figure 2B shows the water MFs of Figure 1 spectra. These MFs vary linearly with equivalent titrant (titrant quantity necessary to produce one neutralization of the sample). The transformation of these values into concentrations is made in section 4.4.

4.3. Malic Species Multiplying Factors (MFs). Given that malic acid's two pK_a values are situated at pH 3.40 and 5.11,[26] the solute is completely ionized (>99.9%) at pH = 13.53 into the dianionic form (A^{2-}), while at pH = 0.39 it is completely nonionized (>99.5%). Consequently, two malic acid principal spectra were obtained at high and low pH once the water spectra were subtracted from the experimental spectra. These spectra were selected as the first two principal spectra for the FA procedure. After subtracting these spectra from the Figure 3 spectra, some intense residues remained. A third principal spectrum selected at pH = 4.08 was introduced in FA that gave minimal residues (Figure 4C) which are at the noise level. The three malic principal spectra normalized to 1 M malic acid are shown in Figure 4B.

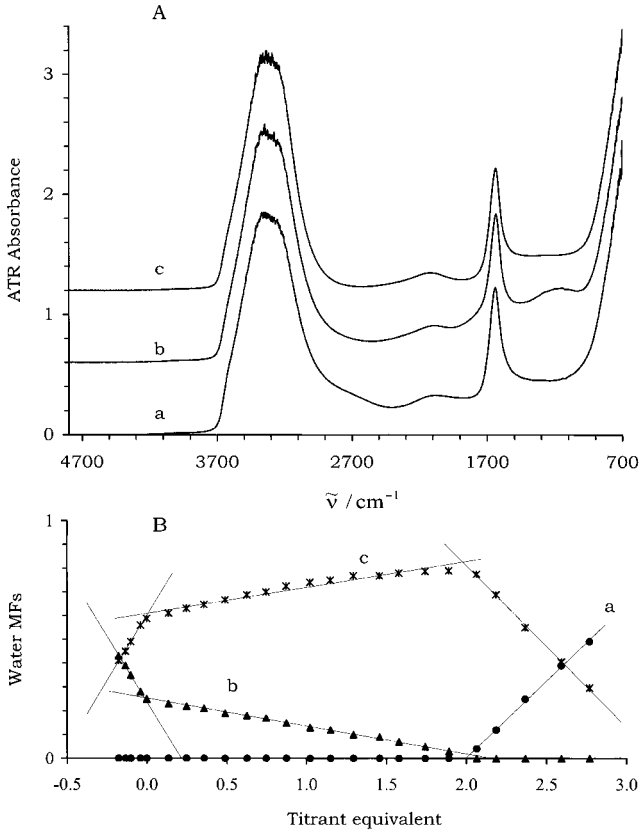


Figure 2. (A) ATR−IR water principal spectra. (B) Water MFs of Figure 1 spectra. (a) 2.23 M NaOH for basic water (●), (b) 1.54 M HCl for acidic water (▲), and (c) pure water (*).

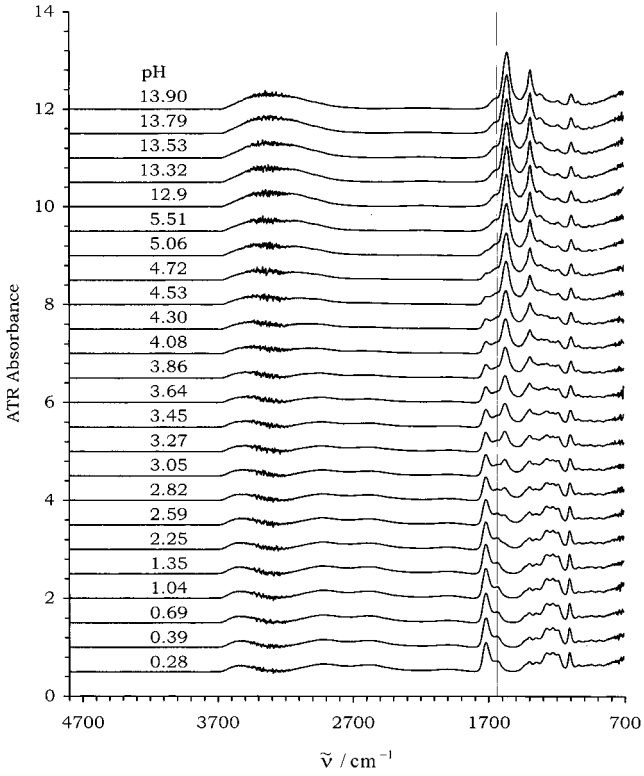


Figure 3. Figure 1 spectra after the water spectra subtractions (same presentation as that in Figure 1). The vertical line at 1638 cm^{-1} indicates the $\delta(H_2O)$ band.

Malic species relative concentrations obtained from the MFs are plotted as a function of pH in Figure 4A (symbols). The

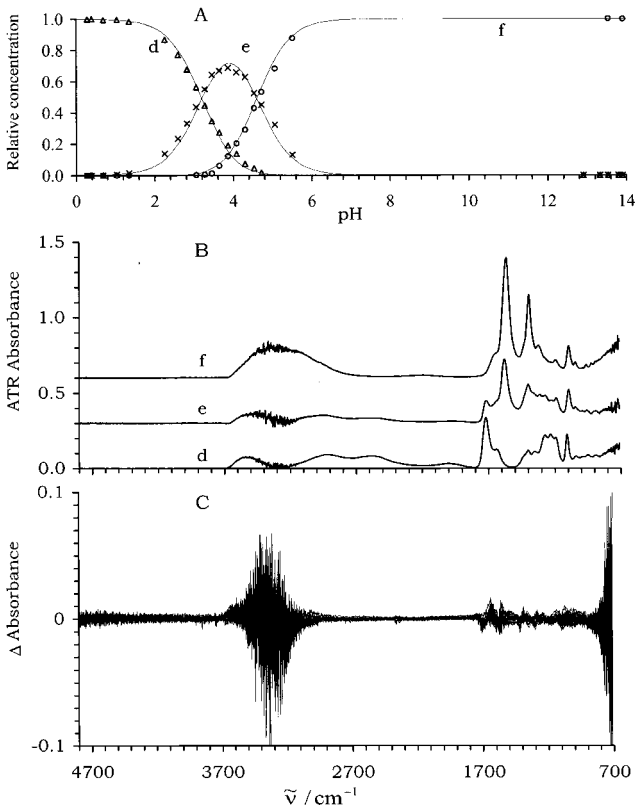


Figure 4. FA results of the 1.80 M malic acid titration: (d) malic acid (△), (e) monosodium malate (×), and (f) disodium malate (○). **(**A) relative distributions of malic principal species against pH. Symbols represent the experimental data; the full lines come from the theoretical calculations. **(**B) Molar principal species spectra (spectrum e shifted 0.3 au; spectrum f shifted 0.6 au). (C) Residues: difference between experimental (Figure 1) and calculated (∑MF × principal spectra) spectra. (Note scale expansion.)

full lines represent the species abundance calculated using eqs 5−7, the constants pK_1 = 3.2 and pK_2 = 4.6 obtained with eqs 2 and 3, and the MFs. Theoretical calculations and FA results are in good agreement. Both values of pK_a are close to the reported values of 3.40 and 5.11, respectively.[23] The small difference between the two sets of values is attributable to ignoring the activity coefficients and to experimental errors. The residues from the difference between calculated and experimental spectra (Figure 4C) are very small and are near the noise level limit (note the expanded scale compared to Figures 1 and 3). This indicates that the results are reliable.

4.4. Species Abundance as a Function of Titrant Equivalent. Due to the logarithm relation between pH and the related concentration, it is difficult to assess the direct relation between the species abundance and titrant equivalent. We present these relations in Figure 5 using the same malic symbols as in Figure 4A. The acidic and alkaline water MFs (Figure 2B), once transformed to molar equivalents, are also set in Figure 5 and the linear curve characteristics are given in Table 3. Below 0 titrant equivalent (HCl added is negative), the HCl curve is linear with a slope -0.99_3 (± 0.01). Similarly, above 2 titrant equivalent, the NaOH curve is linear with a slope of 0.98_1 (± 0.02). Both curves are straight lines with slopes of one, indicating that the water subtraction procedure is accurate in these titrant equivalent regions. The situation between 0 and 2 titrant equivalent is discussed below.

4.5. Water in Strong Interaction with Solute Species. Some absorption is observed at 1638 cm^{-1} (vertical line in Figure 3) even after the acidic, alkaline, and pure water spectra subtraction.

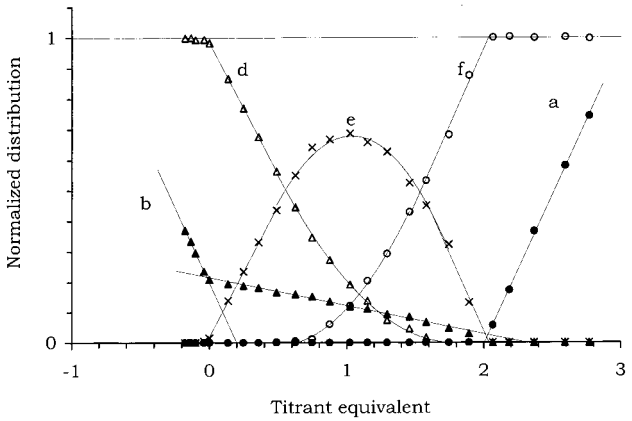


Figure 5. Normalized distribution against titrant equivalent of 1.80 M malic acid species: (a) 2.23 M NaOH for basic water (●), (b) 1.54 M HCl for acidic water (▲), (d) malic acid (△), (e) monosodium malate (×), and (f) disodium malate (○). Pure water is not shown.

TABLE 3: Linear Curves Characteristics of Acidic and Alkaline Water (Figure 5)

titrant equivalent[a]	$-1 < X < 0$	$0 < X < 1$	$1 < X < 2$	$X > 2$
titrant added	HCl	NaOH	NaOH	NaOH
acidic or alkaline water	acidic	acidic	acidic	alkaline
slope	−0.993	−0.093	−0.093	0.981
origin at the ordinate	0.197	0.215	0.215	−1.966

[a] Titrant equivalent: One titrant equivalent is the titrant quantity necessary to produce one neutralization of the sample.

TABLE 4: Characteristics of the Malic Acid Stock Solution (1.849 M, pH = 1.35)

species	HCl 1.54M	H_2O pure	NaOH 2.23M	COOH COOH	COOH COO^-	COO^- COO^-
MFs (Table 1)	0.250	0.588	0.000	1.818	0.028	0.002
concentration/M	0.385	32.55	0.00	1.818	0.028	0.002
molar equivalent (Figure 5)	0.208	17.60	0.00	0.983	0.015	0.001
H^+/M	0.000	0.000	0.000	0.000	0.028	0.004

Since the carbonyl groups do not absorb at this position, we assign this absorption to water in strong interaction with the malic species. We now look at the particular situation of the species.

4.5.1. Water in Strong Interaction with Unionized Malic Acid. The MFs of the 1.85 M malic acid stock solution (0 titrant equivalent, pH = 1.35) are reported in Table 4. The molar concentrations are obtained from these MFs, as are the equivalent titrant molar concentrations. The total amount of H^+ in this solution is evaluated by summing the elements in the fifth line of Table 4 obtained from eq 1 (0.032 mol/L, or a pH of 1.49). The small difference between this value and that from the pH meter reading (1.35) is attributed to the solute and H^+ activity coefficients.

However, the amount of acidic water retrieved by FA is 0.385 mol/L (Table 4). This amount is more than 1 order of magnitude greater than the H^+ concentration obtained from both the pH meter and equilibrium eq 1. Since no HCl was added to the solution, the excess of acidic water cannot come from "free" protons (H^+ or H_3O^+) but must be due to water in strong interaction with nondissociated malic acid (>98%, Table 4). Therefore, the over subtraction of acidic water made in section 4.2 must be reversed by adding the estimated amount of "acidic water" to the spectrum of the solute (Figure 4Bd; see ref 47). The corrected spectrum is shown in Figure 6d. The H^+ discrepancies observed between MF and pH values (calculated and measured) is not a new phenomena, having previously been observed in the phosphoric acid IR titration.[8]

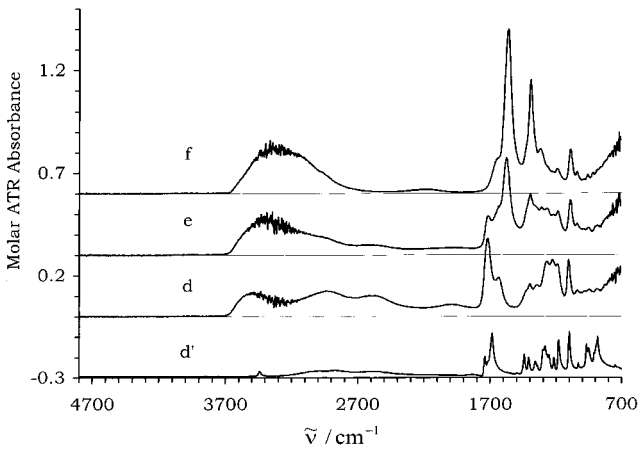


Figure 6. Molar IR spectra of (d′) solid malic acid, (d) malic acid, (e) monosodium malate, and (f) disodium malate (spectrum d′ shifted −0.3 au, spectrum e shifted 0.3 au, and spectrum f shifted 0.6 au). Light horizontal lines indicate the baseline.

4.5.2. Water in Strong Interaction with Monosodium Malate. The monosodium malate spectrum retrieved by FA was obtained from the sample at pH = 4.08 where the malic acid concentration is 1.667 mol/L (Table 1). The H^+ concentration at this pH is negligible ($\sim 10^{-4.08}$ M) when compared to that of malic acid species (1.667 M). The acidic water MF retrieved is 0.120 (Table 1), which corresponds to $[0.120(1.54/1.667)] = 0.111$ HCl molar equivalent (Figure 5). At this concentration, the relative composition of the sample is 0.138, 0.662 and 0.200 for malic acid, monosodium malate and disodium malate, respectively (Figure 5). Nonionized malic acid was found to be associated to acidic water (0.208 mol/L equivalent HCl for each mol/L malic acid, section 4.5.1). Disodium malate was found free from acidic and alkaline water (section 4.5.3). Therefore, one mole of aqueous monosodium malate is associated to $\{[0.111-(0.208 \times 0.138)]/0.662\} = 0.124$ mol/L of acidic water (equivalent HCl). This amount of acidic water, subtracted to obtain Figure 4Be**,** must be replaced. After doing this (see ref 48), spectrum e in Figure 6 was obtained.

4.5.3. Water in Strong Interaction with Disodium Malate. Figure 5 indicates that malic acid is completely ionized at 2 titrant equivalent to form disodium malate. Acidic and alkaline water are not associated with this species. Hence, the spectrum obtained (Figure 4Bf) needs no correction and is shown in Figure 6f. However, the shoulder observed near 1650 cm^{-1}, which is attributed to the deformation band of water, indicates that some water is still present in this species as solvated water. The absorption in the 3700−3000 cm^{-1} region comes in part from the OH stretch of the solvated water and in part from the alcoholic OH of disodium malate.

4.6. Hydration Numbers of Solute Species from IR Spectra. Once the acidic, basic, and pure water spectra have been subtracted from the aqueous solution spectra, the resulting spectra indicate that water is still present. These spectra can be used to evaluate the number of water molecules bound to the solute species giving the hydration numbers. FA applied to the IR malic acid titrations (spectra in Figure 1) produced six principal species, three types of water species and three malic species. The 1.54 M HCl and 2.23 M NaOH spectra used in the FA procedure are not orthogonal spectra because they contain some of the pure water spectra.

We have to calculate the exact composition of the species retrieved by FA in order to evaluate the malic acid species

TABLE 5: MFs of the Experimental Spectra Used to Obtain the Principal Spectra (Giving Matrix P)

exptl solutions	principal spectra					
	acidic water	neutral water	alkaline water	malic acid	monosodium malate	disodium malate
1.54 M HCl	1	0	0	0	0	0
pure water	0	1	0	0	0	0
2.23 M NaOH	0	0	1	0	0	0
pH = 0.39	0.390	0.450	0	1.801	0	0
pH = 4.08	0.120	0.750	0	0.230	1.097	0.340
pH = 13.53	0	0.550	0.250	0	0	1.520

hydration numbers. The principal water spectra used for FA are not orthogonal because both 1.54 M HCl and 2.23 M NaOH spectra contain some of the pure water spectra, but their compositions are known.[26] We extract the MFs related to the six principal species spectra, $\mathbf{S}^{\mathrm{P}}$ (Figures 2A and 4B). Note that the Figure 4B spectra were normalized to 1.0 M. The relation between the experimental spectra $\mathbf{S}_{\mathrm{P}}^{\mathrm{exp}}$ and principal spectra $\mathbf{S}^{\mathrm{P}}$ is

$$\mathbf{S}_{\mathrm{P}}^{\mathrm{exp}} = \mathbf{P} \times \mathbf{S}^{\mathrm{P}} \tag{8}$$

The MFs that are presented in Table 5 form matrix **P**. The inverse of matrix **P** with the experimental spectra ($\mathbf{S}_{\mathrm{P}}^{\mathrm{exp}}$) gives the principal spectra $\mathbf{S}^{\mathrm{P}}$:

$$\mathbf{S}^{\mathrm{P}} = \mathbf{P}^{-1} \times \mathbf{S}_{\mathrm{P}}^{\mathrm{exp}} \tag{9}$$

Matrix **P** and its inverse, matrix $\mathbf{P}^{-1}$, are listed in Tables 5 and 6, respectively. The water content of the solute's principal species ($\mathbf{S}_i^{\mathrm{P}}$) was obtained by multiplying each coefficient of $\mathbf{P}^{-1}$ by the water content of the corresponding experimental solution. The sums obtained by adding the results were corrected by the method described in section 4.5. The resulting water concentrations (mol/L) divided by the concentration of the solute species (1 mol/L) gave the hydration number: 2.0 ± 1.0, 3.0 ± 2.0, and 4.0 ± 0.5 for malic acid, mono-, and disodium malate, respectively. The error is greater on the hydration number of malic acid and monosodium malate than of disodium malate because it is not possible to use the small water band near 3660 cm^{-1} for the water subtraction (criterion i in section 4.2) that would permit a more precise subtraction.

4.7. Spectral Features. The spectra of aqueous malic acid and of mono- and disodium malate separated from the water spectra are presented in Figure 6. To this figure we have added the spectrum of solid malic acid that contains no water (Figure 6d′) in order to compare it with that of aqueous malic acid (Figure 6d). The assignment of the bands is given in Table 7. Because they are basic to our understanding of the malic system and to carboxylic acids, we discuss in the following sections the solid malic acid spectrum, the CO bands of aqueous malic species, and their absorption in the 3700−1800 cm^{-1} region where low intensity continuous massive absorptions are observed.

4.7.1. Solid Malic Acid Spectrum. To obtain a spectrum comparable to that of aqueous malic acid, we normalized the spectrum of the solid malic by using the CO stretch band of the alcoholic CO−H group; this vibration is not greatly affected by the different environmental situations. The solid malic acid spectrum (Figure 6d′) shows a small band situated at 3442 cm^{-1} that we assign to free OH stretch absorption of the alcoholic group because it is sharp and situated at a fairly high wavenumber. Being very small, this band cannot represent all the alcoholic groups. Indeed, some of these groups H-bonded to the carboxylic C═O would shift the absorption toward the 2580 cm^{-1} small broad band. Beside the small 3442 cm^{-1} band, there is no absorption in the 3700−3300 cm^{-1} region, which indicates that the OH carboxylic groups do not absorb there. The only possibility for the absorption of these groups resides in the 3000 and 2880 cm^{-1} bands, the most intense bands in this spectral region. Consequently, we assign these two bands to the two OH carboxylic groups hydrogen bonded in a dimer organization. Compared to the 3400 cm^{-1} band of liquid water, the 3000 and 2880 cm^{-1} band positions suggest stronger hydrogen bonds than in liquid water. Because these bands are broad (near 200 cm^{-1} each), they indicate a wide range of hydrogen bonds.

In the carbonyl region, the very small band at 1840 cm^{-1} is assigned to a combination band. The band absorbing between 1760 and 1600 cm^{-1} is assigned to carboxylic C═O carbonyls. This band has four components. Its high frequency indicates that the 1740 cm^{-1} component are not H-bonded. From its intensity, approximately 25 % of the C═O groups are in this category. The 1688 cm^{-1} component, which is the most intense one, is at the lowest frequency range of the carbonyl band. This indicates that these groups are strongly hydrogen bonded, which could only occur with the carboxylic OH group of a partner molecule. We are left with two small bands situated at 1718 and 1700 cm^{-1}. Being red shifted compared to the free C═O component, these components indicate that some carbonyl groups are hydrogen bonded, but less so than with the 1688 cm^{-1} one. For these reasons we assign the 1718 and 1700 cm^{-1} to the carbonyl associated to the alcoholic OH groups.

We obtain from these considerations on solid malic the molecular picture illustrated in Scheme 1A. This shows the dimer part of a greater organization where two OH carboxylic groups of two malic acid molecules are hydrogen bonded to two C═O carbonyl groups in a face-to-face organization. Some alcoholic OH groups are hydrogen bonded while others are not. Some carbonyl groups are not bonded. The carboxyl-to-carboxyl H-bonds are favored because they form stronger bonds than what alcoholic OH groups can. Because of the hopping nature of the proton, two configurations are possible.

4.7.2. CO Bands in Aqueous Malic Solutions Spectra. Five types of CO bonds (single and double) are encountered in the malic spectra to which correspond the same number of bands (Figure 6 and Table 7). The sharp band situated near 1110 cm^{-1} in all three malic species, not much affected by the ionic situation of malic acid, is assigned to the alcoholic C−O groups.

In the absorption situated between 1800 and 1550 cm^{-1}, the component at 1631 cm^{-1} is very near the ν_2 deformation band of pure liquid water situated at 1638 cm^{-1} (Figures 1 and 2A). Since this band occurs at a frequency too low to assign it to the carbonyl band, we assign it to ν_2 of associated H_2O. Compared to pure water, the bathochromic shift of this band indicates that the H-bonds in these solutions are stronger than those found in pure water, a situation requiring that these H-bonds be formed with the carbonyl groups. The 1721 cm^{-1} band is assigned to the carbonyl groups. This position is situated between that of the free and associate carbonyl bands of solid malic acid (respectively at 1740 and 1688 cm^{-1}, Figure 6d′). This indicates that the carbonyl groups of aqueous malic solutions are hydrogen bonded, with weaker H-bonds than those occurring in solid malic. This is only possible if they are bonded to water. Compared to the malate carbonyls (Table 7 and below), the relatively high position of the C═O carbonyl groups typical of carboxylic acids indicates that they are not ionized.

The band situated at 1280 cm^{-1} with an intensity of approximately 0.18 au is assigned to the single bond C−O

TABLE 6: P^{-1}, Transformation Matrix Used to Obtain Principal Spectra from Experimental Spectra

	experimental solutions					
principal species	1.54 M HCl	pure water	2.23 M NaOH	pH = 0.39	pH = 4.08	pH = 13.53
acidic water	1	0	0	0	0	0
neutral water	0	1	0	0	0	0
alkaline water	0	0	1	0	0	0
1 M malic acid	−0.217	−0.250	0	0.555	0	0
1 M monosodium malate	−0.064	−0.519	0.51	−0.116	0.912	−0.204
1 M disodium malate	0	−0.362	−0.164	0	0	0.658

TABLE 7: IR Band Positions (in cm^{-1}) and Intensities (in au) of Aqueous Malic Species (1 M)[a]

	malic acid $HO_2CCH_2CH(OH)CO_2H$			monosodium malate $HO_2CCH_2CH(OH)CO_2^-$ Na^+		disodium malate Na^+ $^-O_2CCH_2CH(OH)CO_2^-$ Na^+	
	solid Figure 6d′	Figure 6d		Figure 6e		Figure 6f	
approximate assignment	ν/cm^{-1}	ν/cm^{-1}	I/au	ν/cm^{-1}	I/au	ν/cm^{-1}	I/au
νO−H (alcohol)	3442 w						
νO−H (H_2O, $-CO_2H$, and R•O−H•R)		3500	0.12, lar	3400	0.17, lar	3320	0.22, lar
C−H_2 asy st		2940[b]	vw	-		2970[b]	vw
C−H_2 symm st		2895[b]	vw	-		2925[b]	vw
CH st		2840?[b]	vvw	-		2889?[b]	vvw
νO−H (carboxylic, H-bonded to water)	3000, w, lar 2880, w, lar	2930	0.12, lar	2940	0.6, lar		
νO−H (water, H-bonded to carboxylic)	2580, vw, lar	2580	0.1, lar	∼2540	0.05		
H_2O comb						2190	0.02, lar
H_2O comb		1995	0.05, lar	1960	0.03		
comb (1184 + 666 = 1850)	1840 vvw						
ν(C═O)	1740, m; 1718, sh; 1703, sh; 1688 s	1721	0.38	1720	0.19		
δH_2O		1631	∼0.10	∼1645	∼0.05, sh	∼1652	∼0.05, sh
$\nu(CO_2^-$ asy)				1575	0.47	1563	0.80
	1443, m	∼1440	sh	∼1430	∼0.05, sh	∼1440	∼0.03, sh
δ(COH)	1410, m	1406	0.16				
$\nu(CO_2^-$ sym)				1402	0.30	1398	0.55
	1360 w, 1343 sh	1350	0.12	1360	∼0.05		
				1315	∼0.06	1320	0.18
ν(C−O)	1307, vw; 1287, m; 1270, sh; 1255, vw	1280	∼0.18	1275	∼0.09	1270	0.02, sh
	1218 w	1230	∼0.18	1230	∼0.05	1230	0.02
	1184 s	1188	∼0.18	1195	∼0.18	1195	∼0.10
ν(C−OH)	1108 s	1110	0.27	1093	0.27	1095	0.21
	1033 w	1040	0.05	1041	∼0.05	1043	∼0.08
	971 m	985	0.02	995	∼0.04		
	953 w	948	0.05	980	∼0.05	960	∼0.03
						925	∼0.02
	910 sh; 900, sh; 884 m	890	0.05	900	∼0.05	895	∼0.01
	750 vw						
	723 vvw						
	666 s						

[a] Abbreviations: asy, asymmetric; symm, symmetric; st, stretch; comb, combination; w, weak; lar, large; v, very; sh, shoulder; m, medium; s, strong. [b] Obtained with second derivatives.

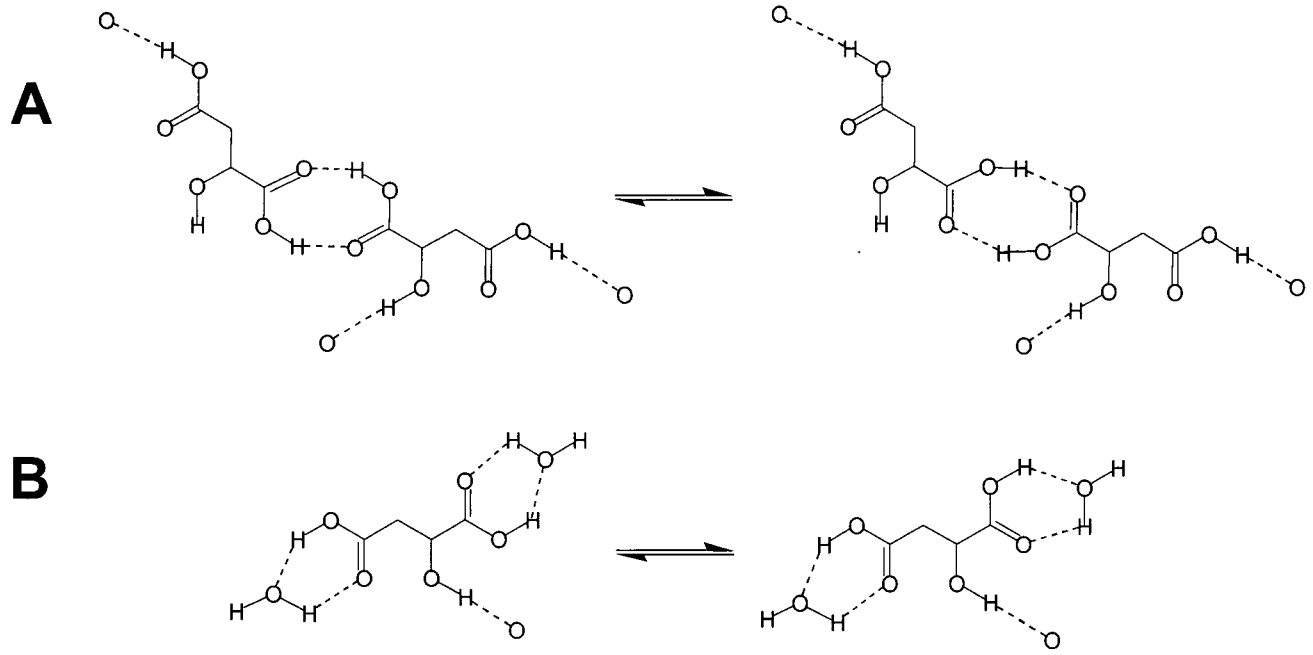
groups. This assignment is made because this band is absent in the disodium malate (Figure 6f) and because a similar band at around the same position is observed in several carboxylic acid spectra.

Two strong and sharp bands dominate the spectrum in disodium malate (Figure 6f): one is situated at 1563 cm^{-1} with an intensity of 0.80 au and the other is at 1398 cm^{-1} with an intensity of 0.55 au. Because the 1563 cm^{-1} band is situated lower than the 1721 cm^{-1} band of malic acid, and because the 1398 cm^{-1} band is situated higher than the 1280 cm^{-1} malic acid band, the 1563 and 1398 cm^{-1} malate bands are assigned to the asymmetric and symmetric carbonate vibrations, $-CO_2^-$, where the charge resonates between the two oxygen atoms.

Monosodium malate has two sets of CO bands (single and double bonds): those of malic acid and those of disodium malate. These occur at half the intensities of the parent molecules and with the band positions slightly shifted (Figure 6e, Table 7). Although small, the differences between monosodium malate spectrum and malic acid and disodium malate are sufficient for factor analysis to make the separation. This gave its abundance throughout the titration pH range (Figure 5). On the other hand, the separation of the two monosodium malate isomers was not possible because they evolve together during the titration.

4.7.3. Aqueous Malic Absorption in the 3600−3800 cm^{-1} Region. The position of the bands in this region is listed in Table 7 and the spectra are presented in Figure 6. Disodium malate is solvated with four water molecules (Section 4.6). Its spectrum shows a broad massive band situated between 3700 and 2700 cm^{-1}, with a maximum near 3320 cm^{-1}. Compared to the OH stretch band of water situated between 3700 and 2800 cm^{-1} (with its maximum near 3320 cm^{-1}), the OH stretch band of disodium malate is a little broader, but its maximum occurs at the same place. We infer from this observation that the 3500 cm^{-1} band is due principally to water solvated to disodium

SCHEME 1: Hydrogen Bonding in Malic Acid[a]



[a] A, solid malic acid as a dimer with external hydrogen bonding; B, aqueous malic acid with water molecules hydrogen bonded. [a]Abbreviations: asy, asymmetric; symm, symmetric; st, stretch; comb, combination; w, weak; lar, large; v, very; sh, shoulder; m, medium; s, strong. [b]Obtained with second derivatives.

malate in a complex formation. The H-bonded alcoholic OH absorption is also situated in that spectral region. We observe some small features near 3000 cm^{-1} that can be enhanced by the second derivative technique to yield three very weak features situated at 2970, 2925, and possibly one at 2889 cm^{-1}. These are assigned to CH_2 asymmetric and symmetric stretch and CH stretch bands of malic acid, respectively. In the region between 2700 and 1800 cm^{-1}, only the very weak but broad band situated at 2190 cm^{-1} is observed. This band is assigned to the combination band of water, $\nu_2 + \nu_{lib}$. Its position is slightly displaced compared to the pure water position (2115 cm^{-1}) because it is solvated, not "free", water. The "continuum of absorbance" is not observed on this species spectrum.

Malic acid is solvated with two water molecules (Section 4.6). The 3700−1800 cm^{-1} region is dominated by four weak broad bands situated at 3500, 2930, 2580, and 1995 cm^{-1} (Figure 6). Because of its continuous formation, this diffuse absorption is sometimes referred to as the "continuum of absorbance".[27−45] However, this absorption is associated with the presence of water for two reasons: no H_3O^+ is present in the solution, and the absorption in the 3700−1800 cm^{-1} region is much more intense than in solid malic acid where there is no water (Figure 6d,d′). The second derivative technique allowed us to see three very weak bands situated at 2940, 2895, and possibly one at 2840 cm^{-1}, which we assign to CH_2 antisymmetric and symmetric and CH stretch bands. The integrated intensity between 3700 and 1800 cm^{-1} is around 75 cm^{-1} au, which is a value comparable to that of the disodium malate (∼68 cm^{-1} au). For disodium malate, 9.0 ± 0.5 OH groups are available (four H_2O and one alcoholic OH) and for malic acid, 7 ± 2 OH groups are available (two H_2O, two carboxylic OH, and one alcoholic OH). Although crude, this comparison does indicate that the absorption in this region can be assigned to the OH groups of solvated malic acid. While the broad band situated between 2200 and 1800 cm^{-1} (maximum at 1995 cm^{-1}) is near that observed in pure water at 2115 cm^{-1} and that of malate water at 2190 cm^{-1}, the intensity in all three cases is weak. We can therefore assign the 1995 cm^{-1} aqueous malic acid band to the combination band $\nu_2 + \nu_{lib}$ of the associated water. The 3500 cm^{-1} band (3700−3200 cm^{-1}) is assigned to the OH stretch of the alcoholic group and to that of associated water because the absorption of these groups are situated in that region.

We are left with two broad bands situated at 2930 and 2580 cm^{-1}. The 2930 cm^{-1} band and its broadness are almost coincident with the 3000 and 2880 cm^{-1} bands observed in solid malic acid (Figure 6d′), to which species we have assigned the two bands to the H-bonded of the carboxylic OH groups. In the case of aqueous malic acid, we also assign the 2930 cm^{-1} band to the H-bonded of the carboxylic OH groups. However, the situation in aqueous malic acid is different from that in solid malic. The OH groups are bonded to the water oxygen. This association makes for the spectral difference observed in the two cases: in aqueous malic acid there is one band situated at 2930 cm^{-1} whereas in solid malic, two components are observed at 3000 and 2880 cm^{-1} (Figure 5d,d′). We are left with the OH stretch of the solvated water to which we assign the remaining band situated at 2580 cm^{-1}. The association of water to malic acid in the aqueous solutions is illustrated in Scheme 1B. Using the relation between the positions of the bands and the internuclear distances,[46] we estimate the distance between the malic OH and water O at 1.7 A and that between the water OH and carboxylic C═O carbonyl at 1.6 A. Theses distances are shorter that that encountered in liquid water, which is around 1.9 A. Since water in such a situation is quite mobile, these distances vary considerably, probably around 20%. This in turn explains the broadness of these OH stretch bands.

5. Conclusion

The IR titration of malic acid was carried out in the 0−14 pH range and principal factor analysis was performed on the derived MIR-ATR spectra. Three types of water were used in the analysis: pure water, 1.54 M HCl to retrieve the acidic water, and 2.23 M NaOH to retrieve basic water. After subtracting

these types of water from the experimental spectra, factor analysis on the resulting spectra yielded three principal malic species: malic acid, which is not ionized; monosodium malate, composed of half an acid and half a salt; and disodium malate, a double ionized salt. We obtained the spectra of these species and determined their abundances. The malic species abundance obtained from the IR spectra agrees in all cases with the thermodynamic theoretical calculations. From the distribution of the species the pK_as determined agree with literature values.[26] These results indicate that the method used is reliable. Furthermore, the complete MIR malic spectra were obtained in an aqueous environment normal for this natural product, but the spectra were subtracted from the cumbersome water spectra that render the localization of the malic bands and their subsequent assignment difficult.

Beside the three types of water that were subtracted from the solution spectra, some water spectrum remained. This indicates that some other type of water is also present in the solution. This remaining water can only be water strongly bonded to the malic species. Because our measurements are quantitative, we were able to determine the hydration numbers of the solvated species: 2.0 ± 1.0, 3 ± 2, and 4.0 ± 0.5 for malic acid, mono-, and disodium malate, respectively. This is the first time that hydrate numbers could be determined on aqueous malic species in the 0−14 pH range, or on any aqueous carboxylic acids for that matter.

A comparison between the malic species IR spectra allowed the assignment of the principal bands of malic acid, mono-, and disodium malate in an aqueous environment to be made. A more thorough normal coordinate analysis could make the assignment of all the small bands that could not be determined in the present study.

As for many carboxylic acids, a low intensity, broad, continuous absorption that some authors refer to as a "continuum of absorbance" was observed in the 3700−1700 cm^{-1} region with malic acid, but not with disodium malate. The intensity with monosodium malate is about half that of malic acid. This is in agreement with the fact that monosodium malate is half acid and half salt. This absorption is dominated by four broad peaks situated at 3500, 2930, 2580, and 1995 cm^{-1} that are assigned, respectively, to malic acid solvated water and alcoholic OH, hydrogen bonded carboxylic OH, hydrogen bonded water OH, and a water combination band.

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(47) The spectrum used for the subtraction of acidic water (section 4.2) was that of 1.54 M HCl (Figure 2Ab). This spectrum, multiplied by 0.208/1.54 = 0.135, was added to the molar spectrum of unionized malic acid (Figure 4Bd). Since the spectrum of 1.54 M HCl (Figure 2Ab) contains part of the pure liquid water spectrum,[12,14,15,18] the pure water spectrum was thus subtracted, and this subtraction was maximized according to the criteria given in section 4.2 without any consideration to the apparent "continuum" of absorbance in the 2800−1800 cm^{-1} region. As a result, the pure liquid water spectrum multiplied by 0.097 was therefore subtracted.
(48) The spectrum used for subtraction of acidic water (section 4.2) was that of 1.54 M HCl (Figure 2Ab). This spectrum, multiplied by 0.124/1.54 = 0.081, was added to the molar spectrum of monosodium malate (Figure 4Be). Since the spectrum of 1.54 M HCl contains part of the spectrum of pure liquid water,[12,14,15,18] the pure water spectrum was subtracted from the above resulting one, and this subtraction was maximized according to the criteria given in section 3.4.1 without any consideration to the apparent "continuum" of absorbance in the 2800−1800 cm^{-1} region. As a result, the pure liquid water spectrum multiplied by 0.013 was therefore subtracted.