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Thermodynamic modeling of naringenin protonation equilibria in $NaClO_4$ aqueous solutions by specific ion interaction theory and Pitzer equations

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Abstract. The protonation equilibria for the flavonoid naringenin were studied at 25°C using combined multi-wavelength spectroscopic and pH-potentiometric methods as a function of the ionic strength. Over a wide range of ionic strengths, 0.10–3.00 mol dm^{-3}, the investigation was performed in different aqueous solutions of $NaClO_4$ as the background electrolyte. The dependence on ionic strength of protonation constants was modeled by the Brönsted–Guggenheim–Scatchard Specific Ion Interaction Theory (SIT) and Pitzer approaches. Apart from the values of SIT interaction coefficients and Pitzer parameters, the protonation constants at infinite dilution (zero ionic strength) were obtained. On the basis of these results, it was found that Pitzer mode l gives more satisfactory results rather than the SIT method.

Keywords. Protonation constant; Naringenin; Ionic strength dependence; SIT model; Pitzer parameters.

1. Introduction

Flavonoids are natural products that are present in most plants, in particular, in fruits and vegetables, and are thus important constituents of the human diet. They are categorized into flavonols, flavones, flavanones, isoflavones, flavonols, and anthocyanidins. The flavonoid nucleus consists of benzo-γ-pyrone (ring A and ring C) and benzene (ring B) moieties with hydroxyl, carbonyl, sugar, or methyl groups attached to this base structure.

During the past decade, flavonoids have generated growing interest because of their broad biological and pharmacological effects such as vasoprotective, anti-inflammatory, anti-viral, and anti-fungal actions.[1–3] Many of these effects are thought to result from their antioxidant properties, which may be due to their ability to scavenge free radicals and chelate metal ions.[4] Stoichiometric studies in reducing the capabilities of flavonoids indicate that antioxidant ability is markedly influenced by the position of hydroxyl groups on the B or A rings and the extent of conjugation between the B and C rings.[5] Radical scavenging by flavonoids occurs by electron donation from the free hydroxyls on the flavonoid nucleus with the formation of a less reactive flavonoid aroxyl radical, which is stabilized by resonance and therefore plays a moderate role in the propagation of radical-induced damage in biological systems. Free radicals like some reactive oxygen species are believed to be involved and are responsible for the development of different cancers and cardiovascular diseases. The use of antioxidants for the prevention of such effects induced by these compounds has great input on advice for human nutrition and health.[6]

Naringenin (4′,5,7-trihydroxyflavanone) is one of the polyphenolic compounds that is mostly found in grapefruit and, in lower concentration, also in tomato.[7] This flavonoid has been shown to inhibit in vitro the growth of cancer cells in human and can exhibit estrogenic,[8] anticarcinogenic,[9] and antioxidative properties.[10,11] Naringenin has antioxidant and antitumor activity and may play a role in cancer, heart disease, hypertension, circulation, Alzheimer's disease, etc.[12,13] Naringenin has also been shown to reduce hepatitis C virus production by infected hepatocytes (liver cells) in cell culture. This seems to be secondary to naringenin ability to inhibit the secretion of very-low-density lipoprotein by the cells.[14]

The acidic dissociation constants (pK_a values) have played an important part in understanding the ionic composition of many biologically active molecules. The chemical and biological activity of these substances would be expected to vary with the degree of ionization. For this reason, accurate knowledge of the ionization

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constants for different substances is a prerequisite to an understanding of their mechanism of action in both chemical and biological processes. Furthermore, these parameters describe the amount of various species as a function of pH.[15,16]

In the present work, the protonation constants of the flavonoid naringenin have been determined in different aqueous solutions of $NaClO_4$ as a background electrolyte to examine the dependence of acid–base equilibria on ionic strength. The parameters that define this dependency were analyzed by the specific ion interaction theory (SIT) and Pitzer equation, with the aim of obtaining further information with regard to their variation as a function of charges involved in the dissociation process.

2. Experimental

2.1 *Reagents and chemicals*

Naringenin, figure 1, was obtained from Fluka as analytical reagent grade material and was used without further purification. The NaOH and perchloric acid solutions were prepared from chemicals supplied by Merck. Sodium perchlorate was supplied by Merck and it was kept at room temperature in vacuum at least 72 h before use. All dilute solutions were prepared by mixing double distilled water; the conductivity of which did not exceed 0.05 ± 0.01 μS cm^{-1}.

2.2 *Apparatus and procedure*

The protonation constants were evaluated at 25°C from the measurements of absorbance versus *emf* by titration of a 50 mL naringenin (30 μmol dm^{-3}) solution with 0.10 mol dm^{-3} sodium hydroxide solution in different ionic strengths (0.10–3.00 mol dm^{-3} of $NaClO_4$). The electromotive force was measured using a Jenway (model 3520) research potentiometer equipped with a combined pH electrode. The combined pH electrode was modified by replacing its aqueous KCl solution with 0.01 mol dm^{-3} NaCl + 0.09 mol dm^{-3} $NaClO_4$ saturated with AgCl.

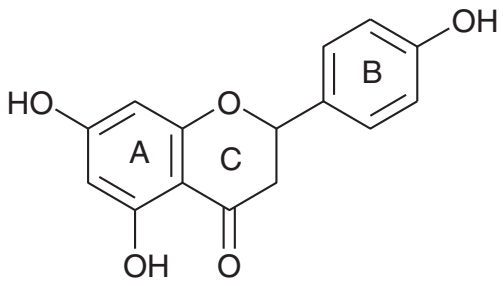

Figure 1. Chemical structure of flavonoid naringenin.

Spectrophotometric measurements were performed on a UV–Vis Shimadzu 2100 diode-array spectrometer equipped with 10 mm quartz cells and a Julabo F12 circulating water bath to keep the temperature constant within ±0.1°C. In the UV–Vis spectrophotometric titrations, the test solution was pumped to a spectrometric flow cell by means of a peristaltic pump. After each addition of titrant, and after waiting for the potential reading to be stable, a spectrum was recorded, all relevant data were stored, and a new volume of titrant was added to restart the cycle. During the course of titrations, a stream of nitrogen was passed through the reaction vessel to eliminate the adverse effect of the atmospheric carbon dioxide and oxygen.

The protonation constants of naringenin were calculated by processing the UV–Vis spectrophotometric titration data using the computer program STAR (STability constants by Absorbance Reading), which calculate stability constants and molar absorbances of the pure species by multilinear regression.[17] In this program, the refinement of equilibrium constants is performed based on the minimization of the nonlinear least-squares sum defined by the difference between the calculated and the experimental data of the spectrophotometric titration spectra.

3. Results and Discussion

3.1 *Glass electrode calibration*

Before each spectrophotometric titration, the potentiometric cell was calibrated to obtain formal electrode potential E°_{cell} at each of ionic strength so that the hydrogen ion concentration was measured rather than the activity. The glass electrode was calibrated based on the Gran method.[18,19] Approximately 20 mL $HClO_4$ solution of a known concentration was titrated by stepwise addition of standardized NaOH solution at constant ionic strength of $NaClO_4$ and 25°C, in nitrogen atmosphere. The equilibrium *emf* values of the cell were recorded after allowing potential stabilization. Titration was carried out in triplicate to ensure reproducibility. The E°_{cell} values were computed from the Nernst equation knowing the exact concentration of H^+ in each titration points. According to the Nernst equation, the potential of a potentiometric cell equipped by a glass electrode (Ag|AgCl|NaCl-$NaClO_4$‖$NaClO_4$, $H^+(c_{H+})$‖ glass electrode) can be presented as

$$E_{cell} = E^{\circ}_{cell} + k\log[H^+] + k\log\ \gamma_{H^+} + E_{LJ} \qquad (1)$$

where E_{LJ} is the liquid junction potential, $k = 2.303RT/F$ in which R, T, and F have the usual meanings and γ_{H+} is the activity coefficient of hydrogen ion. Because the ionic strength of the solution is kept

Table 1. Calibration constants obtained for the combined glass pH electrode in different ionic strengths of $NaClO_4$ at 25°C.

k	E_a	I/mol dm^{-3}
60.24	527.67	0.10
59.70	430.97	0.25
59.86	412.97	0.50
59.68	446.00	0.75
59.36	413.81	1.00
59.45	445.14	1.50
59.85	450.11	2.00
59.57	460.68	3.00

constant for each experiment, the activity coefficient of hydrogen ion is constant too. The nonideality of solutions is then included in E_a (the specific constant of the potentiometric cell in acidic region), and thus

$$E_{cell} = E_a + k \log[H^+] \quad (2)$$

where E_a is the $E^\circ_{cell} + k \log \gamma_{H+} + E_{LJ}$. The hydrogen ion concentration can be easily calculated by

$$[H^+] = \left(M_{HClO_4} V_0 - M_{NaOH} V_1\right) / (V_0 + V_1) \quad (3)$$

where M_{HClO4} and M_{NaOH} are the molarities of acid and base, V_0 and V_1 are the initial volume of acid and the added volume of sodium hydroxide solution, respectively. The E_a values were calculated from measured *emf* and known concentration of solvated proton in every titration point by linear regression analysis. For each experiment at constant ionic strength, the slopes obtained from least squares analysis were close to the theoretical Nernst value (59.167 mV at 25°C) with correlation coefficients of $r^2 = 0.99$. The calibration constants (E_a and k) obtained for the combined glass pH electrode at working ionic strengths are given in table 1.

Accordingly, the pH values can be properly measured at each of constant ionic strength by

$$pH = (E_a - E_{cell}) / k \quad (4)$$

3.2 *Determination of protonation constants*

Typically, figure 2 shows the electronic spectral changes of flavonoid naringenin in the pH range of 2–12.8 at constant ionic strength of 1.00 mol dm^{-3} $NaClO_4$.

For mathematical analysis of deprotonation equilibria, absorbance data gathered in different pH values were used to construct a matrix $\boldsymbol{R}$ of size $m \times n$, where m is the number of different pH values in which absorbance determined in n wavelengths at 1 nm intervals.

According to Beer's law, a least squares analysis was performed in STAR program environment to decompose the data matrix $\boldsymbol{R}$ into a matrix of pure concentration,

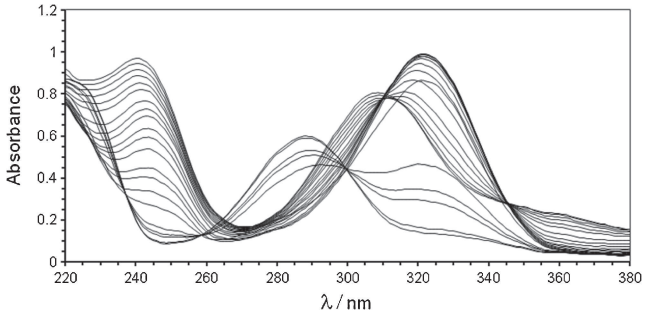

Figure 2. The electronic absorption spectral changes of naringenin in the pH range of 2–12.8 at constant ionic strength of 1.50 mol dm^{-3} $NaClO_4$ and 25°C.

$\boldsymbol{C}$, and a matrix of pure spectral profiles, $\boldsymbol{S}$, with the optimal residual error matrix $\boldsymbol{E}$.

$$\boldsymbol{R} = \boldsymbol{C}\boldsymbol{S} + \boldsymbol{E} \quad (5)$$

In iterative cycles, those matrices $\boldsymbol{C}$ and $\boldsymbol{S}$ were determined that best represent the original matrix $\boldsymbol{R}$. The matrix of pure concentration profiles is related to protonation constant and total concentration of compounds. Under suitable constraints, the nonlinear least-squares fitting goes on until one finds the best set of parameters (pK_a and molar absorptivities of the species) that result in a minimum of $\boldsymbol{E}$.

The optimal fitting between theoretical and experimental absorbance data was achieved, when all of three possible deprotonation equilibria of naringenin were considered in calculation. The pK_a values of naringenin calculated by STAR program in different ionic strengths are reported in table 2, in the molal concentration scale. In figure 3, the equilibrium distribution of various species of the naringenin in ionic strength of 1.50 mol dm^{-1} $NaClO_4$ is shown as a function of pH.

Experimental protonation constant values (table 2), determined in $NaClO_4$ aqueous solutions at different ionic strengths, are shown as a function of the square root of ionic strength (in mol kg^{-1}) in figure 4, which shows that the pK_a values of naringenin are nearly always at their minimum at an ionic strength range 0.30–0.90 mol kg^{-1}, which is a characteristic of $pK_a = f(I)$. According to the electrolyte solutions at low ionic strength, the dependence accounts for the Coulomb interaction between the ions that are screened by the ion atmosphere, while at higher concentrations the dependence accounts for the disturbances in ion–solvent interactions.[23,24] At low ionic strength (less than 0.10 mol kg^{-1}), the Coulomb interactions are of primary importance. However, as the ionic strength increases, the ionic atmosphere becomes more compressed and screens the ionic charges more effectively, so the intermolecular interactions (dipole–dipole or even multipole–multipole)

Table 2. The pK_a values of naringenin in different ionic strengths of $NaClO_4$ at 25°C, where K_1 (mol kg^{-1}), K_2 (mol kg^{-1}), and K_3 (mol kg^{-1}) refer to the thermodynamic protonation equilibria of H_2L^-, HL^{2-}, and L^{3-}, respectively (where L^{3-} represents the fully dissociated naringenin).

I		pK_1	pK_2	pK_3	
mol dm^{-3}	mol kg^{-1}	mol kg^{-1}	mol kg^{-1}	mol kg^{-1}	Ref.
0.10	0.10	6.84 ± 0.02	9.63 ± 0.08	11.64 ± 0.12	present work
0.25	0.25	6.80 ± 0.01	9.52 ± 0.10	11.44 ± 0.12	〃
0.50	0.51	6.73 ± 0.03	9.39 ± 0.09	11.36 ± 0.14	〃
0.75	0.78	6.76 ± 0.02	9.31 ± 0.05	11.32 ± 0.11	〃
1.00	1.05	6.78 ± 0.01	9.40 ± 0.01	11.35 ± 0.01	〃
1.50	1.62	6.91 ± 0.03	9.52 ± 0.02	11.56 ± 0.04	〃
2.00	2.21	7.00 ± 0.01	9.64 ± 0.01	11.66 ± 0.05	〃
3.00	3.50	7.22 ± 0.05	9.90 ± 0.11	11.77 ± 0.10	〃
≤ 0.001		6.70	9.10	-	20
0.05 (NaCl)		6.80	10.40	<11.50	21
1.00 (mol dm^{-3} TBAC)[a] in 50% (v/v) H_2O-DMSO		8.01	10.24	11.38	22

[a]Tetra *n*-butylammonium chloride

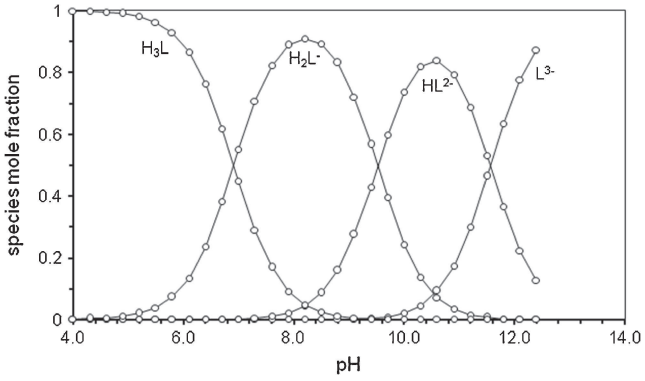

Figure 3. Distribution diagram of the different species of naringenin as a function of pH at 25°C and ionic strength 1.50 mol dm^{-3} $NaClO_4$.

become more important. These forces at higher ionic strength possibly have a primary role between the ions.

3.3 *Ionic strength effect*

The acidic dissociation equilibria can be expressed by Eq. (6)

$$H_{4-n}L^{1-n} \rightleftharpoons H^+ + H_{3-n}L^{-n} \quad (6)$$

Where, for example, L^{-3} represents the full deprotonated species of naringenin. A stepwise thermodynamic acid dissociation constants K_n for these equilibria is given by

$$K_n = \frac{[H^+][H_{3-n}L^{-n}]}{[H_{4-n}L^{1-n}]}\frac{\gamma_{H^+}\gamma_{H_{3-n}L^{-n}}}{\gamma_{H_{4-n}L^{1-n}}} = K_n^0\frac{\gamma_{H^+}\gamma_{H_{3-n}L^{-n}}}{\gamma_{H_{4-n}L^{1-n}}} \quad (7)$$

where γ_i is the activity coefficient of species i, and K_n^0 refers to the protonation constant, expressed as a concentration quotient, at infinite dilution in a particular medium. By taking negative logarithms on Eq. (7)

$$pK_n = pK_n^0 - \log\gamma_{H_{3-n}L^{-n}} - \log\gamma_{H^+} + \log\gamma_{H_{4-n}L^{1-n}} \quad (8)$$

The determination of pK_n^0 value requires that the activity coefficient values areknown. The activity coefficient of species can be expressed by the specific ion interaction theory (SIT) and Pitzer models.

3.4 *SIT model*

SIT model was first outlined by Brönsted and later elaborated by Guggenheim and Scatchard.[25–30] Owing to its simplicity (i.e., there is only one linear parameter for ionic strength dependence of formation constants), it can still be considered as one of the most popular prediction strategies in solution chemistry. In the original SIT model, activity coefficient of ion i with charge z_i can be expressed by Eq. (9) in the solution of ionic strength I_m(in molal scale) at 25°C.

$$\log\gamma_i = -z_i^2\frac{A\sqrt{I_m}}{1+B\sqrt{I_m}} + \sum_j \varepsilon_{ij}m_j \quad (9)$$

where A is the limiting Debye–Hückel law slope (0.509 kg$^{0.5}$ mol$^{-0.5}$) and B is the empirical constant (1.5 kg$^{0.5}$ mol$^{-0.5}$). The ion interaction coefficient ε_{ij}, usually called SIT parameter, interprets the specific short range interactions of ion ith with ion jth in its molal concentration m_j. The SIT model assumes that interaction coefficient is zero for two electrically like sign ions or neutral species (this is the Brönsted principle of specific ionic interaction). Although SIT parameters are approximately considered to be constant for some ionic media in $0.50 \le I_m \le 3.50$ mol kg^{-1}, literature indicate that they are functions of I at lower and higher ionic

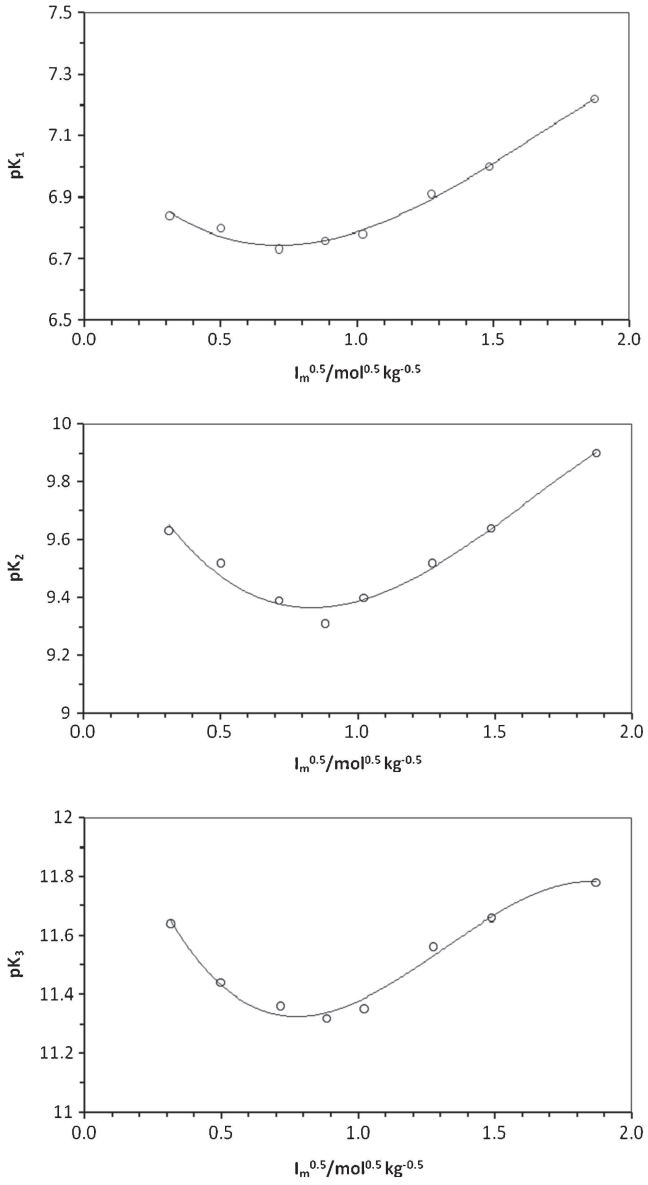

Figure 4. Plots of pK_1, pK_2, and pK_3 (in molal scale) of naringenin versus the square root of ionic strength (in mol kg^{-1}) of the supporting electrolyte at 25°C.

strengths.[31] An expertise group in Italy modified the SIT model and introduced concentration dependency of εas the following term.[32]

$$\varepsilon = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1 + I} \qquad (10)$$

where ε_0 and ε_∞ parameters take into account the dependence of SIT parameters on medium ionic strength and are the values of ε for $I \to 0$ and $I \to \infty$, respectively.

By considering the modified SIT model for calculation of activity coefficients of species in $NaClO_4$ as supporting electrolyte, Eq. (8) can be reformulated as follows

$$pK_n = pK_n^0 + z^* \frac{A\sqrt{I_m}}{1 + B\sqrt{I_m}} - \Delta\varepsilon I_m \qquad (11)$$

Where,

$$z^* = \sum (\text{charges})^2_{\text{products}} - \sum (\text{charges})^2_{\text{reactants}} \qquad (12)$$

$$\Delta\varepsilon = \varepsilon_{H_{3-n}L^{-n},Na^+} + \varepsilon_{H^+,ClO_4^-} - \varepsilon_{H_{4-n}L^{1-n},Na^+} \qquad (13)$$

It should be noted that pK_a and I_m values are in molal concentration scale; for $n = 1$ in $\varepsilon_{H_{4-n}L^{1-n},Na^+}$, ε for neutral fully protonated naringenin was considered zero; also, all SIT interaction coefficients were defined to be concentration dependent by using Eq. (10).

Experimental pK_a values (table 2) were fitted into Eq. (11) as a function of ionic strength by least-squares regression analysis. The results of the SIT calculations of our data are given in table 3. The goodness of fit of the SIT model was judged by the square correlation coefficient, associated with values of standard deviation obtained for each of the regression coefficients. Ion interaction coefficients of $\varepsilon_0^{Na^+,H_{3-n}L^{-n}}$ and $\varepsilon_\infty^{Na^+,H_{3-n}L^{-n}}$ were calculated according to those values of $HClO_4$($\varepsilon_0^{H^+,ClO_4^-} = 0.062$ and $\varepsilon_\infty^{H^+,ClO_4^-} = 0.167$) reported in literature.[32]

3.5 *Pitzer Model*

Pitzer equation for activity coefficient of a cation (M) in mixed solution of anions (a) and cations (c) has the following form.[33–35]

$$\ln\gamma_M = z_M^2\left(\sum_a\sum_c m_a m_c B'_{a,c} + f_\gamma\right) + \sum_a m_a\left(2B_{M,a} + ZC_{M,a} + \sum_{a'} m_{a'}\psi_{M,a,a'}\right)$$

Table 3. Results of SIT analysis on pK_a values of naringenin in different ionic strengths of $NaClO_4$ (0.10–3.00 mol dm^{-3}) at 25°C.

n	pK_n^0	$\Delta\varepsilon_0$	$\Delta\varepsilon_\infty$	$\varepsilon_0^{Na^+,H_{3-n}L^{-n}}$	$\varepsilon_\infty^{Na^+,H_{3-n}L^{-n}}$	r^2
1	7.04 ± 0.03	0.08 ± 0.08	0.23 ± 0.02	0.02	0.06	0.97
2	10.06 ± 0.05	−0.03 ± 0.15	0.32 ± 0.04	−0.07	0.22	0.94
3	12.18 ± 0.07	0.64 ± 0.24	0.24 ± 0.06	0.50	0.29	0.81

$$+2\sum_{c} m_c\theta_{M,c} + \sum_{a}\sum_{c} m_a m_c \left(\psi_{M,c,a} + |z_M| C_{a,c}\right) \quad (14)$$

In a single 1:1 electrolyte, the molality of ions of electrolyte (m_c & m_a) is the same as ionic strength and the contribution of terms containing molality of other solutes becomes negligible. Therefore, Pitzer equation can be rewritten as

$$\ln\gamma_M = z_M^2\left(I_m^2 B'_{a,c} + f_\gamma\right) + I_m\left(2B_{M,a} + ZC_{M,a}\right) + 2I_m\theta_{M,c} + I_m^2\left(\psi_{M,c,a} + |z_M| C_{a,c}\right) \quad (15)$$

The expression for an anion (X^{z-}) can be derived by changing M, c and a to X, a and c, respectively. And, for neutral species:

$$\ln\gamma_N = 2I_m\lambda_N \quad (16)$$

Terms in Eq. (15) are defined by

$$f_\gamma = -A_\phi\left[\frac{I_m^{0.5}}{1+1.2I_m^{0.5}} + \frac{2}{1.2}\ln(1+1.2I_m^{0.5})\right] \quad (17)$$

$$Z = \sum_i m_i |z_i| \quad (18)$$

$$C_{ij} = \frac{C_{ij}^\phi}{2\left|z_i z_j\right|^{0.5}} \quad (19)$$

$$B_{ij} = \beta_{ij}^{(0)} + \beta_{ij}^{(1)}\left(\frac{1-(1+2I_m^{0.5})\exp(-2I_m^{0.5})}{2I_m}\right) = \beta_{ij}^{(0)} + \beta_{ij}^{(1)}F \quad (20)$$

and B' is the derivative of B respective to the ionic strength:

$$B'_{ij} = \beta_{ij}^{(1)}\left(\frac{-[1-(1+2I_m^{0.5}+2I_m)\exp(-2I_m^{0.5})]}{2I_m^2}\right) = \beta_{ij}^{(1)}F' \quad (21)$$

The parameter A_ϕ is the Debye–Huckel constant for the osmotic coefficient with value of 0.3915 (kg mol^{-1})$^{0.5}$ at 25°C; $\beta^{(0)}$, $\beta^{(1)}$ and C^ϕ are Pitzer parameters describing the interactions between two ions with the opposite signs (−+interactions); the θ parameter describes the interaction between two ions with the same signs (++ or −−); ψ and λ are triple interaction parameter (−+− or +−+) and interaction parameter for neutral species, respectively.

Pitzer equation for the activity coefficient of the proton and different protogenic species of naringenin can be obtained in $NaClO_4$ as follows:

$$\ln\gamma_{H^+} = (f_\gamma + \beta^{(1)}_{NaClO_4} I_m^2 F') + 2I_m(\beta^{(1)}_{HClO_4}F) + 2I_m(\beta^{(0)}_{HClO_4} + \theta_{HNa}) + I_m^2\left(\psi_{HNaClO_4} + C^\phi_{HClO_4} + \frac{C^\phi_{NaClO_4}}{2}\right) \quad (22)$$

$$\ln\gamma_{H_3L} = 2I_m\lambda_{H_3L} \quad (23)$$

$$\ln\gamma_{H_2L^-} = (f_\gamma + \beta^{(1)}_{NaClO_4} I_m^2 F') + 2I_m(\beta^{(1)}_{H_2L^-,Na^+}F) + 2I_m(\beta^{(0)}_{H_2L^-,Na^+} + \theta_{H_2L^-,ClO_4^-}) + I_m^2\left(\psi_{H_2L^-,NaClO_4} + C^\phi_{H_2L^-,Na^+} + \frac{C^\phi_{NaClO_4}}{2}\right) \quad (24)$$

$$\ln\gamma_{HL^{2-}} = (f_\gamma + \beta^{(1)}_{NaClO_4} I_m^2 F') + 2I_m(\beta^{(1)}_{HL^{2-},Na^+}F) + 2I_m(\beta^{(0)}_{HL^{2-},Na^+} + \theta_{HL^{2-},ClO_4^-}) + I_m^2\left(\psi_{HL^{2-},NaClO_4} + \frac{C^\phi_{HL^{2-},Na^+}}{\sqrt{2}} + C^\phi_{NaClO_4}\right) \quad (25)$$

$$\ln\gamma_{L^{3-}} = (f_\gamma + \beta^{(1)}_{NaClO_4} I_m^2 F') + 2I_m(\beta^{(1)}_{L^{3-},Na^+}F) + 2I_m(\beta^{(0)}_{L^{3-},Na^+} + \theta_{L^{3-},ClO_4^-}) + I_m^2\left(\psi_{L^{3-},NaClO_4} + \frac{C^\phi_{L^{3-},Na^+}}{\sqrt{3}} + \frac{3}{2}C^\phi_{NaClO_4}\right) \quad (26)$$

Introducing Eqs. (22)–(26) in Eq. (8), a general expression was found for ionic strength dependency of protonation constant in a single 1:1 electrolyte as follows:

$$pK_n = pK_n^0 + \frac{z^*(f_\gamma + \beta^{(1)}_{NaClO_4} I_m^2 F') + 2I_m A_{n1}F + 2I_m A_{n2} + I_m^2 A_{n3}}{\ln(10)} \quad (27)$$

where A_{n1}, A_{n2}, and A_{n3} are constants defined by

$$A_{n1} = \beta^{(1)}_{HClO_4} + \beta^{(1)}_{H_{3-n}L^{-n},Na^+} - \beta^{(1)}_{H_{4-n}L^{1-n},Na^+} \quad (28)$$

Table 4. Results of Pitzer analysis on pK_a values of naringenin in different ionic strengths of $NaClO_4$ (0.10–3.00 mol dm^{-3}) at 25°C.

n	pK_n^0	A_{n1}	A_{n2}	A_{n3}	r^2
1	6.73 ± 0.02	-5.61 ± 0.39	-1.94 ± 0.11	6.03 ± 0.40	0.99
2	9.42 ± 0.03	-11.05 ± 0.69	-4.03 ± 0.20	11.91 ± 0.72	0.98
3	11.26 ± 0.04	-15.30 ± 0.99	-5.48 ± 0.28	16.36 ± 1.04	0.96

$$A_{n2} = \beta^{(0)}_{HClO_4} + \beta^{(0)}_{H_{3-n}L^{-n},Na^+} + \theta_{H_{3-n}L^{-n},ClO_4^-} + \theta_{H^+,Na^+} - \beta^{(0)}_{H_{4-n}L^{1-n},Na^+} - \theta_{H_{4-n}L^{1-n},ClO_4^-} \quad (29)$$

$$A_{n3} = \psi_{H^+,Na^+,ClO_4^-} + \psi_{H_{3-n}L^{-n},Na^+,ClO_4^-} + \frac{C^{\phi}_{H_{3-n}L^{-n},Na^+}}{\sqrt{n}} + C^{\phi}_{HClO_4} + C^{\phi}_{NaClO_4} - \frac{C^{\phi}_{H_{4-n}L^{1-n},Na^+}}{\sqrt{n-1}} - \psi_{H_{4-n}L^{1-n},Na^+,ClO_4^-} \quad (30)$$

If $n = 1$, for neutral species (H_3L), the terms $\beta^{(1)}$, θ and ψ are zero and term $\beta^{(0)}$ should be replaced by λ in Eqs. (28)–(30).

Experimental pK_a values were fitted into Eq. (27) and results are given in table 4 and then are compared with the SIT model results. Although the SIT model is simpler than Pitzer, the Pitzer approach gives a significantly better fit in the ionic strength range investigated. Moreover, it was found that from $n = 1$ to 3, the coefficient A_{ni} increases. This indicates that the ionic strength dependency of protonation constants increases for higher deprotonated species of naringenin. This effect is mainly attributed to higher electrostatic interactions between species of naringenin and electrolyte due to the increase in the charge on the species during acid dissociation equilibria.

4. Conclusion

Deprotonation of the flavonoid naringenin was investigated by a combination of spectroscopic and potentiometric methods in a wide range of ionic strength supplied by sodium perchlorate at 25°C. The pK_a values were calculated by multivariate curve fitting implemented in STAR program. The SIT and Pitzer equations were applied successfully to describe the ionic strength dependency of pK_a values. The thermodynamic deporotonation constant at infinite ionic strength was calculated together with overall specific ion interaction coefficients. A comparison between pK_n^0(protonation constant at infinite dilution) results obtained from the SIT and Pitzer models demonstrates that the values from Pitzer equation give more accurate fitting results.

Table of symbols

Symbols	Dimension	Representation
A , B	$(kg\ mol^{-1})^{0.5}$	Debye–Hückel parameters
A_ϕ	$(kg\ mol^{-1})^{0.5}$	Debye–Huckel constant for the osmotic coefficient
C^ϕ	-	Third virial coefficient in the Pitzer equation
E_a	mV	The pseudo-Nernstian standard potential
I_m	mol kg^{-1}	Ionic strength in molal units
k	mV	The Nernstian slope
m	mol kg^{-1}	Molality of ions
z	-	Charge of ions
$\beta^{(0)}$, $\beta^{(1)}$	-	Second virial coefficient in Pitzer equation
γ	-	Activity coefficient of species
ε	kg mol^{-1}	Specific interaction coefficient of ions
$\Delta\varepsilon$	kg mol^{-1}	The difference of specific coefficients
θ, ψ, λ	-	Pitzer interaction coefficients

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