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ORIGINAL PAPER

Lactonization and Protonation of Gluconic Acid: A Thermodynamic and Kinetic Study by Potentiometry, NMR and ESI-MS

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Abstract In acidic aqueous solutions, the protonation of gluconate is coupled with the lactonization of gluconic acid. With a decrease of pC_H, two lactones (δ- and γ-) are sequentially formed. The δ-lactone forms more readily than the γ-lactone. In 0.1 mol·L^{-1} gluconate solutions, if $pC_H > 2.5$ then only the δ-lactone is generated. When the pC_H is decreased below 2.0, formation of the γ-lactone is observed although the δ-lactone still predominates. In solutions with $I = 0.1$ mol·L^{-1} $NaClO_4$ and room temperature, the deprotonation constant of the carboxylic group was determined to be $\log_{10} K_a = 3.30 \pm 0.02$ using the NMR technique, and the δ-lactonization constant obtained by batch potentiometric titrations was $\log_{10} K_L = -(0.54 \pm 0.04)$. Using ESI-MS, the rate constants for the δ-lactonization and the reverse hydrolysis reaction at $pC_H \approx 5.0$ were estimated to be $k_1 = 3.2 \times 10^{-5}$ s^{-1} and $k_{-1} = 1.1 \times 10^{-4}$ s^{-1}, respectively.

Keywords Gluconic acid · Protonation · Lactonization · NMR · ESI-MS

1 Introduction

Gluconic acid, a polyhydroxyl carboxylic ligand, has been investigated for many years, mainly due to its importance in a variety of industrial, pharmaceutical and biological processes [1, 2]. Many studies dealt with solutions of high pH because gluconate forms strong complexes with metal cations in neutral to basic solutions [3–7]. Fewer studies have

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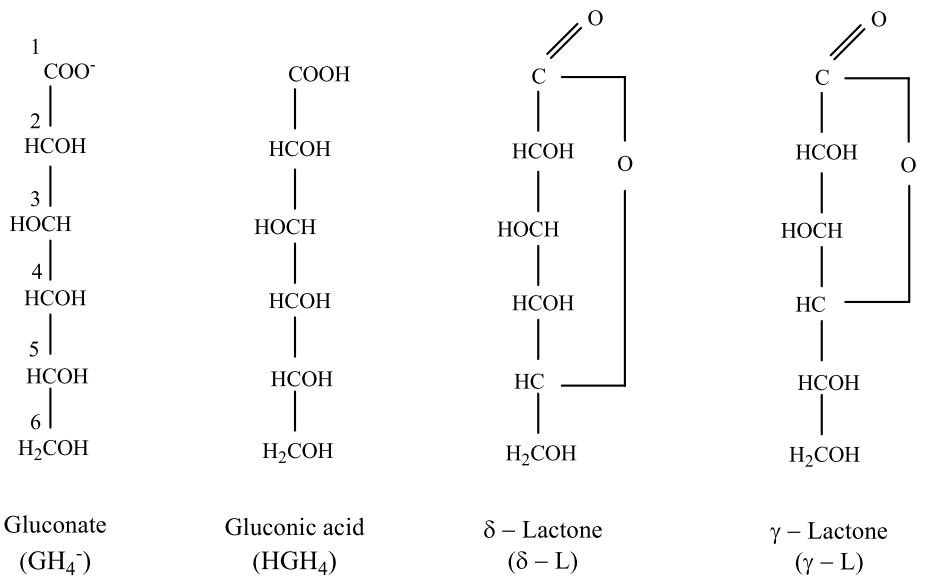
been conducted in solutions of low pH, and thermodynamic data that describe solution behavior under acidic conditions are scarce and in disagreement. Two reasons likely contribute to this situation: (1) under acidic conditions the ability of gluconic acid to bind metal ions is weak or moderate [8, 9], making the studies less attractive for applications; and (2) the studies at low pH are complicated by the lactonization of gluconic acid [10, 11], a slow reaction that is coupled to fast chemical processes such as protonation/deprotonation and complexation. The published literature [12] shows that such lactonization also occurs with a similar polyhydroxycarboxylic ligand, isosaccharinic acid. Although the formation of a lactone is catalyzed by the hydrogen ion, lactonization as well as its reverse reaction (lactone hydrolysis) does not alter the acidity of the solution. These complicating factors make a direct determination of the protonation and complexation constants difficult by conventional techniques such as acid/base potentiometry under acidic conditions.

Recently, the complexation of gluconate with lanthanides and actinides has been a subject of study because gluconate is present in some high-level radioactive wastes and its presence affects the f-electron element speciation in the waste processing solutions. The development of strategies for the treatment of these waste streams requires thermodynamic data concerning the f-electron element complexation under a wide range of proton concentrations ranging from acidic to basic. For this purpose, a description of protonation and lactonization of gluconic acid in acidic solutions is necessary.

Gluconic acid refers to *D*-gluconic acid derived from natural *D*-glucose. To be consistent with the notations in the literature, gluconate, gluconic acid, and δ- and γ-lactone, as shown in Scheme 1, are denoted here as GH_4^-, HGH_4, and δ- and γ-L, respectively, where the first H of HGH_4 refers to the proton on the carboxylate group and H_4 refers to the four hydrogen atoms on the secondary alcohols [3, 13]. The six carbon atoms are numbered starting from the top in the order as C1 to C6.

In this paper, we report thermodynamic and kinetic data on the deprotonation and lactonization of gluconic acid. Our results were obtained by multiple techniques including ^{13}C



Scheme 1

NMR spectroscopy, specially-designed potentiometric titrations, and electrospray ionization coupled with mass spectrometry (ESI-MS). This work aids in defining the conditions under which lactonization is important, the form of the lactones that are produced, their rate of production, and the corrected protonation constant of the gluconate ligand under acidic conditions.

2 Experimental

Unless specifically described differently, all the experiments were conducted in $I = 0.1$ mol·L^{-1} $NaClO_4$ solutions and at room temperature. Deionized and boiled water was used in the preparation of all solutions except those used for the NMR experiments. All chemicals were reagent grade or higher. Sodium gluconate (Acros) was used as received without further purification.

All experiments, except the NMR data collection, were conducted under an inert atmosphere, involving either bubbling argon through the solutions or by using a glove box filled with argon.

2.1 NMR

NMR experiments were conducted to understand the deprotonation and lactonization of gluconic acid, and further to determine the deprotonation constants. The stock solution of gluconate was prepared by dissolving sodium gluconate ($NaGH_4$) in 99.96% D_2O (Cambridge Isotope Laboratories). Working solutions of gluconic acid were prepared by mixing appropriate amounts of the stock solution, DNO_3 (70% w/w in D_2O, Cambridge Isotope Laboratories) or NaOD (40% w/w in D_2O, Cambridge Isotope Laboratories), and D_2O. The final concentration of gluconate in the working solutions was 0.1 mol·L^{-1} and the acidity (pC_D or $-\log_{10}[D^+]$) ranged from 1.0 to 13.4. The prepared solutions were stored for three days before the ^{13}C NMR spectra were recorded on a Varian 300 Spectrometer at the Center of NMR Spectroscopy at Washington State University. Separate experiments had demonstrated that this time was sufficient for the lactonization reaction to achieve equilibrium.

In the NMR measurements, the magnetic field was stabilized by locking it to the D signal of the solvent. The sample temperatures were regulated to be 22.0 °C during all acquisitions. For individual samples, 5,000 to 10,000 scans were taken (with the proton decoupler turned on) and were averaged to obtain the ^{13}C spectra. Sodium 2,2-dimethyl-2-silapentane-sulfonate (DSS) was used to reference all ^{13}C NMR spectra, following the procedures reported elsewhere [14, 15]. The pC_D of the solutions, except for the two "end" solutions, was measured by a potentiometric procedure similar to that described in the next section. In this case, the electrode was calibrated by a standard acid/base titration in D_2O. The values of pC_D in D_2O were subsequently converted to pC_H in H_2O by the relationship: $pC_D = pC_H + 0.40$ [16]. The acidities of the two "end" solutions ($pC_D = 1.0$ and 13.4) were directly calculated from the amounts of D^+ and OD^- added during preparation of the working solutions because these values are outside the working range of the pH electrode.

2.2 "Batch" Potentiometric Titrations

Because the gluconate protonation reaction is coupled with the formation of lactone, and the latter is a much slower reaction, a batch titration approach was designed to determine the lactonization constant in conjunction with the protonation constant obtained from the NMR experiments.

In a batch titration experiment, a number of individual solutions containing the same quantity of gluconate were prepared. Different amounts of acid or base were added into each solution to simulate one step in a titration, in which each solution represented a point in a typical titration. After the solutions were stored for at least three days, the concentration of the hydrogen ion in each solution was determined using a Metrohm pH meter (Model 713) equipped with a Ross combination pH electrode (Orion Model 8102). The electrode had previously been calibrated by a standard acid/base titration so that the Emf reading of the electrode could be converted to pC_H. A detailed description of the electrode calibration is provided elsewhere [17, 18]. Multiple batch titrations were conducted with solutions of different concentrations of gluconate. Given the protonation constants obtained from NMR, the equilibrium constant for lactonization of gluconic acid was calculated with the program HYPERQUAD [19].

2.3 ESI-MS

ESI-MS measurements were explored as a method to examine the formation of a lactone and were subsequently applied to estimate the rate constants of lactonization/hydrolysis. All ESI-MS measurements were performed on an Agilent 1100 series instrument, using an electrospray ionization method coupled with a quadrupole mass analyzer at Washington State University. A sample was injected into the electrospray by pressure infusion (50 mbar), and was run with the following instrumental conditions: (1) 3500 V negative tip voltage; (2) 110 fragmentor factor; (3) 4.0 mL per minute flow rate of the sheath liquid; (4) 25 PSI spray gas; and (5) a 150 °C drying gas. The mass spectrum of each run was averaged from the recorded Total Ion Chromatogram (TIC) data.

A gluconate stock solution was prepared by dissolving sodium gluconate in water. A sodium acetate solution, used as the sheath liquid for the electrospray measurements, was prepared by dissolving an appropriate amount of sodium acetate (Aldrich) in water and then diluting it with an equal volume of methanol (Aldrich). The final concentration of sodium acetate was controlled at 5 mmol·L^{-1}. The solutions were degassed with an ultrasonic water bath (Branson) before being loaded into the instrument.

In the experiments, gluconic acid samples with varying values of pC_H were prepared by diluting the gluconate stock solution with water, and then acidifying the samples with perchloric acid. These samples, after being allowed to equilibrate for three days, were run under the instrumental conditions mentioned above to determine the specific mass signals for the species present in these solutions.

For the kinetic experiments, a 50 mmol·L^{-1} $NaGH_4$ sample was prepared by diluting the gluconate stock solution with water, and then used to measure a mass spectrum to define a starting point of the lactonization reaction. After this sample was acidified by 10%, a series of mass spectra were recorded as a function of time under the same instrumental conditions as described above.

In order to estimate the lactonization/hydrolysis rate constants, the measured mass spectra versus time data were treated by the following procedures. We started by taking the intensities of two ionic signals, where one is for a gluconate anion ($m/z = 195$) and the other is for a lactone ($m/z = 337$), from the mass spectrum recorded at time t. The intensities of those two signals (I_{195} and I_{373}) are assumed to be correlated linearly to [GH_4^-] and [L], respectively. With consideration of the limitations associated with the sample injection (pressure infusion) and the electrospray ionization for quantitative analysis [20], the following correction was made to the lactone signal. Because under our experimental conditions the gluconate anion was present in large excess over gluconic acid or lactone, we assumed

that any change in $[GH_4^-]$ by lactonization was negligible throughout the kinetic course. As a result, an averaged intensity of the gluconate signal (I_{ave}) could be referred to as an expected value for the gluconate anion, and therefore the intensity of the lactone signal at the time t was corrected to I_t by the following equation:

$$I_t = I_{373}(I_{195}/I_{ave}) \quad (1)$$

This corrected intensity (I_t) was used in the correlation with the lactone concentration.

3 Results and Discussion

3.1 Assignment of ^{13}C NMR Peaks

Within the pC_H range studied, we observed six primary peaks for the six carbons of the gluconate molecule, and each of these six primary peaks sometimes had two smaller peaks associated with them depended on chemical conditions. This is shown in Fig. 1. Due to the rapid exchange of the proton ion involved in protonation reactions, chemical shifts of

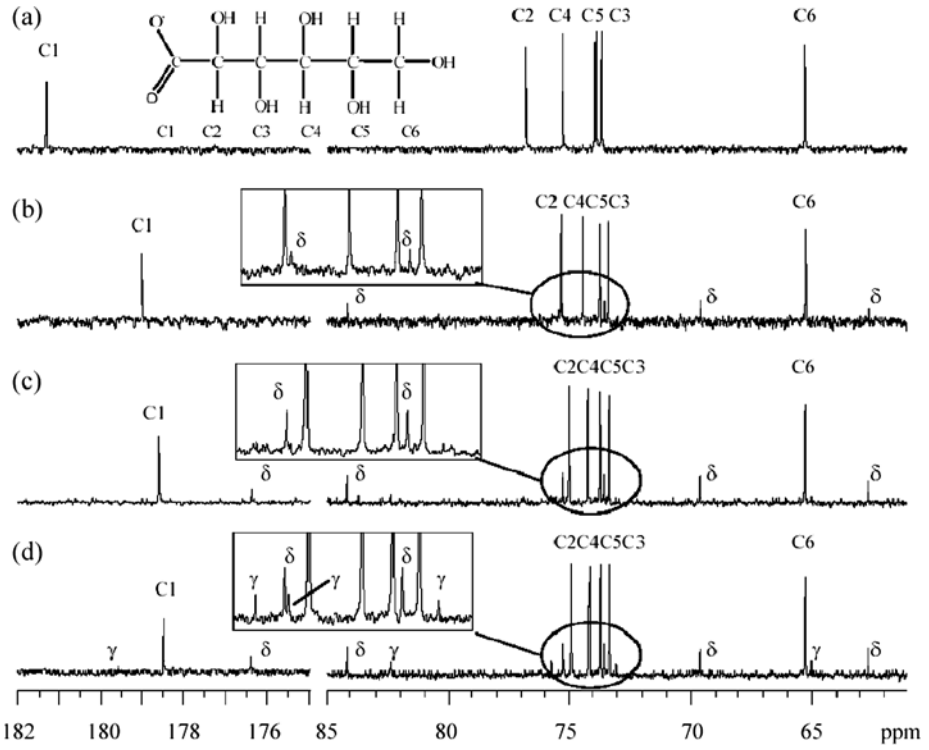


Fig. 1 ^{13}C NMR spectra of gluconic acid and its related lactones. A Varian 300 Spectrometer operated at 75.5 MHz was used for recording the spectra: 5000–10000 scans for individual spectra, 0.6 Hz line broadening, $C_{NaGH4}{}^0 = 0.1$ mol·L^{-1} in D_2O, and $t = 22$ °C (room temperature). Acid/base concentrations used for pC_H adjustment are: $C_{DNO3} = 70\%$ w/w in D_2O and $C_{NaOD} = 40\%$ w/w in D_2O. The peak identification is relative to the standard ^{13}C NMR spectrum of gluconate [Aldrich 18,633-3] and δ-lactone [Aldrich G2000-1]. (**a**) $pC_H = 6.66$; (**b**) $pC_H = 2.54$; (**c**) $pC_H = 1.99$; (**d**) $pC_H = 1.42$

individual carbons in the conjugate acid and base cannot be separated [21]. As a result, one group with six sharp intensive peaks, existing over the entire pC_H range and shifting with the pC_H, is attributed to the conjugate species (HGH_4 and GH_4^-). These peaks are assigned to C1 (C=O), C2, C4, C5, C3 and C6, in the order of increasing field (Fig. 1), as previously published [11].

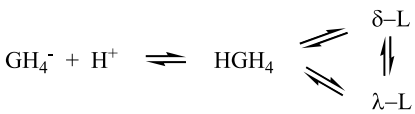
As the pC_H value approaches the acidic range, two additional groups appeared sequentially in the spectra with slight intensities (Fig. 1). The first group to appear represents the δ-lactone, and thus the second peak is assigned to the γ-lactone. The intensities of the lactone peaks increase with decrease of the pC_H, but the chemical shifts of these peaks remain constant, implying that lactonization and hydrolysis have no effect on the NMR measurement for these two lactones. This observation is in agreement with the earlier work by Sawyer et al. [10] and Combes et al. [11], where they have confirmed the slow kinetics of lactonization/hydrolysis. For example, in the work by Combes et al., the rate constants for lactonization and hydrolysis were determined to be around 10^{-5} s^{-1} at $pC_H = 2.4$ [11]. These reaction rates are quite low in terms of the ^{13}C NMR time scale. Therefore, the recorded ^{13}C NMR spectra for the lactones, as indicated in Fig. 1, are independent of those reactions [21].

The sequential appearance of the two groups of lactone peaks also suggests that formation of the two lactones occurs differently. The δ-lactone is formed more readily than the γ-lactone. As indicated from Fig. 1a–b, if the pC_H is >2.5, then only the δ-lactone is formed. When the pC_H is decreased to <2.0, the γ-lactone starts to form in measurable amounts (Fig. 1c–d) although the δ-lactone form still predominates.

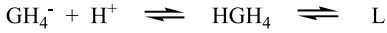
3.2 Protonation constants by NMR

In acidic aqueous solutions, gluconic acid (e.g., protonated gluconate) undergoes lactonization into δ- and γ-lactone, and the two lactones interconvert with each other as shown in Scheme 2 [10]:

Scheme 2



Scheme 3



As discussed in the previous section, only the δ-lactone is formed when $pC_H > 2.5$. Furthermore, the pC_H is always > 2.5 under the experimental conditions for the batch potentiometric titrations and ESI-MS that were used for evaluating the thermodynamic and kinetic properties of lactonization. We, therefore, made the following simplifications in this study, i.e., we ignored the interconversion of the two lactones and considered only the δ-lactone (designated as L) formation (Scheme 3). The equilibrium constants of protonation (K_a) and δ-lactonization (K_L) were defined as Eqs. 2 and 3, respectively:

$$K_a = \frac{[HGH_4]}{[GH_4^-][H^+]} \tag{2}$$

$$K_L = \frac{[L]}{[HGH_4]} \tag{3}$$

where $[HGH_4]$, $[GH_4^-]$, $[H^+]$ and [L] denote the molar concentrations of HGH_4, GH_4^-, H^+ and L, respectively.

Owing to the slow kinetics of the lactonization/hydrolysis reactions [10, 11], the gluconate protonation reaction can be monitored separately from the lactonization/hydrolysis reaction by observing the NMR chemical shifts as a function of pC_H [21] so that a protonation constant can be independently determined. Taking a simple protonation reaction (Eq. 4) as an example, we can describe the principle behind this determination method. The protonation constant (K_H) is defined by Eq. 5:

$$A + H \leftrightharpoons HA \tag{4}$$

$$K_H = \frac{[HA]}{[A][H]} \tag{5}$$

where [A], [HA], and [H] are the molar concentrations of A, HA, and H, respectively.

Because the two conjugate species (A and AH) undergo interconversion through the rapid exchange of a proton, an observed chemical shift (δ_{obs}) for each nucleus is a mole-fraction weighted average of the two resonances δ_A and δ_{HA}, as described by Eq. 6 [21]:

$$\delta_{obs} = \frac{[A]\delta_A + [HA]\delta_{HA}}{[A] + [HA]} \tag{6}$$

Manipulation of Eq. 5 yields $[HA] = K_H[A][H]$, and substitution of this relation into Eq. 6 gives the relationship between the observed chemical shift (δ_{obs}) and the proton ion concentration ([H]), as described by Eq. 7:

$$\delta_{obs} = \frac{K_H[H]\delta_A + \delta_{HA}}{1 + K_H[H]} \tag{7}$$

where K_H, δ_A, and δ_{HA} are constant. The program HYPNMR2000, having the approach described above, was used to fit the experimental data of δ_{obs} versus pC_H to obtain the value of K_H.

The results of fitting the gluconate chemical shifts as a function of pC_H using the program HYPNMR2000 [22] are depicted in Fig. 2, and the calculated protonation constants are summarized in Table 2. As the pC_H changes from 2.0 to 6.0, the largest displacement of the chemical shift occurs for the C1 (C=O) atom (Fig. 2), which suggests that the carboxylic group is deprotonated. The displacement magnitude (≈3.0 ppm) and direction (e.g., towards higher frequency with increasing values of pC_H) are consistent with previous work [23], confirming that the carboxylic group is the most acidic site of the HGH_4 molecule. Figure 2 also reveals that the C2 and C4 atoms exhibit the next largest displacements. In view of the pC_H range under consideration and the expected dissociation constants of aliphatic alcohols [26], it is unlikely that these displacements are attributable to the deprotonation of a gluconate hydroxyl group. A more plausible explanation is that deprotonation of the carboxylic group initiates a modification of the conformation of the gluconate molecule, resulting in alterations in the hydrogen-bonding environment of the C2 and C4 hydroxyl groups, but not of the C3, C5 and C6 groups. Similar behavior has been described by Cho et al. [24] for isosaccharinic acid (ISA).

The calculated protonation constant of the carboxylic group is $\log_{10} K_a = 3.30 \pm 0.02$ (Table 2). This value indicates that gluconic acid is a slightly stronger acid than the simple monocarboxylic acids (e.g., $\log_{10} K_a \approx 4.6$ for acetic, butanoic and hexanoic acids [25]), but it is similar to that of other α-hydroxycarboxylic acids (e.g., $\log_{10} K_a \approx 3.6$ for hydroxyacetic, 2-hydroxybutanoic and 2-hydroxyhexanoic acids [25]) and isosaccharinic acid

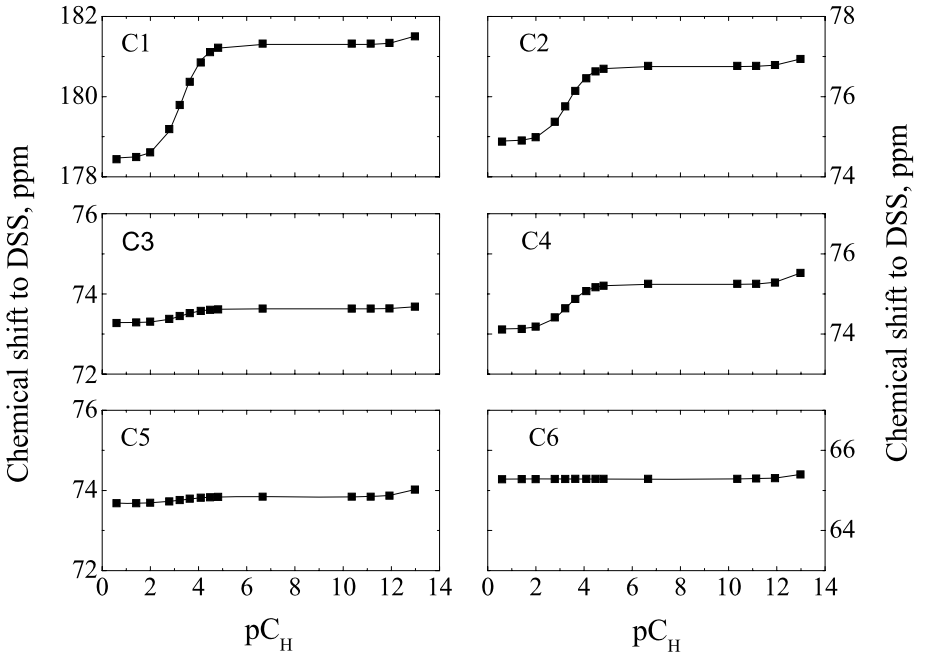


Fig. 2 HYPNMR2000 fitting results for the chemical shifts of the individual gluconate carbons as a function of the pC_H: $C_{NaGH4}{}^0 = 0.1$ mol·L^{-1} and $t = 22°C$ (room temperature). Acid/base concentrations used for the pC_H adjustments are: $C_{DNO3} = 70\%$ w/w in D_2O, $C_{NaOD} = 40\%$ w/w in D_2O. Symbols: (■), experimental data; *solid line*, calculated values

($\log_{10} K_a \approx 3.2$ to 3.3 [24]). This slight increase in acidity might be attributed to the formation of hydrogen bonding between the carboxylate group and the C2/C4 hydroxyl groups, which could stabilize the deprotonated form and result in a lower value for $\log_{10} K_a$.

In the near neutral to basic range of p$C_H = 6.0$ to 13.0, it was observed that the chemical shifts simply show an inflection point near the high pC_H edge. Due to the limited data in this region, the exact reason for this inflection point is not clear, but two different mechanisms could explain this observation. One is that deprotonation occurs on a hydroxyl group. Although it is difficult to determine uniquely which group undergoes deprotonation, the C4 hydroxyl group appears to be a logical choice due to its largest displacement. The corresponding equilibrium and protonation constant ($K_{a'}$) are described as follows:

$$GH_3^{2-} + H^+ \rightleftharpoons GH_4^- \tag{8}$$

$$K_{a'} = \frac{[HGH_4^-]}{[GH_3^{2-}][H^+]} \tag{9}$$

The HYPNMR2000 [22] fitting program yielded $\log_{10} K_{a'} = 12.9 \pm 0.6$. Taking into consideration the high uncertainty introduced by the limited number of data points available for the fitting at high values of pC_H, we decided to increase the uncertainty of this value and express this constant as $\log_{10} K_{a'} = 13 \pm 1$, which is close to the pK_a ($\log_{10} K_a$) range observed for aliphatic alcohols (p$K_a = 15$ to 20) [26].

Another possible reason for this inflection point is the occurrence of an interaction between the sodium (Na^+) and gluconate (GH_4^-) ions. Such complexation has been discussed

Table 1 Experimental data and corrections for the kinetics of the lactonization reaction as determined with the ESI-MS method. Starting solutions: $C_{NaGH4}{}^0 = 50$ mmol·L^{-1}, $V_0 = 10$ mL, p$C_H \approx 6.2$; added acid, $C_{HClO4} = 0.99$ mol·L^{-1}, $V_{HClO4} = 0.05$ mL, and p$C_H \approx 5.0$

Time, t (hr)	Intensity ($I_{m/z}$)		Calibrated I_{373} (I_t)	Extent of equilibrium $(I_e - I_t)/(I_e - I_0)$, %
	I_{195}	I_{373}		
0.00	65101	56	56	100.0
0.13	60110	318	315	97.0
0.30	62802	1090	1035	89.0
0.47	63010	1979	1873	79.6
0.63	63790	2805	2622	71.3
0.80	62900	3130	2967	67.4
0.97	56806	3470	3642	59.9
1.13	58910	3912	3960	56.3
1.56	58205	4750	4866	46.2
2.15	59606	6055	6057	32.9
3.15	57250	7001	7286	19.2
4.15	55500	7412	7945	11.8
36.00	55803	8395	8995	0.1
72.00	55010	8305	9002	0.0

Table 2 Thermodynamic and kinetic properties of the protonation and lactonization of gluconic acid

Reaction	Stability/kinetic constants	Note
Protonation:		
$H^+ + GH_3^{2-} \leftrightharpoons GH_4^-$	$\log_{10} K_{a'} = 13 \pm 1$	NMR: $I = 0.1$ mol·L^{-1}, room temp.
$H^+ + GH_4^- \leftrightharpoons HGH_4$	$\log_{10} K_a = 3.30 \pm 0.02$	NMR: $I = 0.1$ mol·L^{-1}, room temp.
Lactonization:		
$HGH_4 \leftrightharpoons L + H_2O$	$\log_{10} K_L = -(0.54 \pm 0.04)$	Batch titration: $I = 0.1$ mol·L^{-1}, room temp.
$HGH_4 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} L$	$k_1 = 3.2 \times 10^{-5}$ s^{-1}	ESI-MS: p$C_H \approx 5.0$, room temp.
	$k_{-1} = 1.1 \times 10^{-4}$ s^{-1}	

in Cho et al.'s work [24] for ISA. It is expected that the GH_4^- complexation reaction with Na^+ is weak, but its effect can be appreciable only when the concentration of Na^+ is sufficiently high, which is the case when pC_H approaches 13. This complexation reaction may break the inner-molecular hydrogen bonding of gluconate, causing a conformational change and thereby producing the chemical shift displacement seen at pC_H near 13 as shown in Fig. 2.

3.3 Lactonization constant by potentiometry

The batch titrations were made in both directions as is shown in Fig. 3. The titration process was performed in the basic-to-acidic direction first and then in the acidic-to-basic direction. As discussed in the last section, only the carboxylic group deprotonates in this pC_H range (2.5 to 6.0). Given the value of $\log_{10} K_a = 3.30$ obtained from the NMR study, the

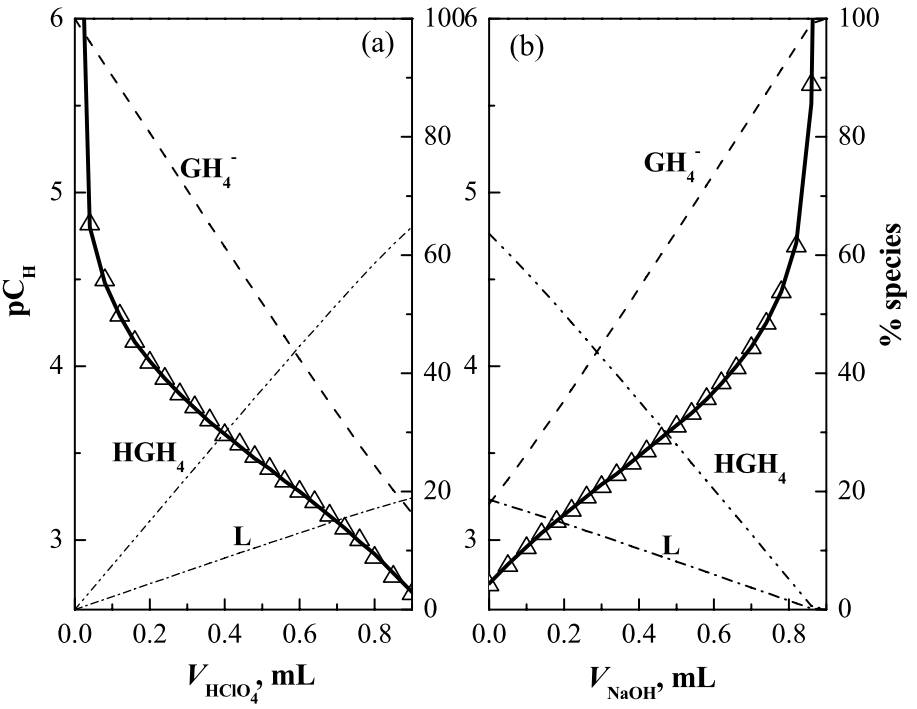


Fig. 3 Batch titrations for the lactonization of gluconic acid at $I = 0.1$ mol·L^{-1} and room temperature. Cup solution: $V^0 = 20$ mL and $C_{\mathrm{NaGH4}}{}^0 = 50$ mmol·L^{-1}. Titrant: $C_{\mathrm{HClO4}} = 0.9893$ mol·L^{-1} for the base-to-acid direction (**a**) and $C_{\mathrm{NaOH}} = 1.0015$ mol·L^{-1} for the acid-to-base direction (**b**). Symbols: (Δ), experimental pC_{H}; *solid line*, fitted pC_{H}

δ-lactonization constant was calculated to be $\log_{10} K_{\mathrm{L}} = -(0.54 \pm 0.04)$ by fitting these titration data with the program HYPERQUAD [19].

Sawyer et al. [10] used pH measurements and the optical rotation method to investigate the deprotonation and lactonization of gluconic acid within the pH range 2.0 to 5.0, and obtained $\log_{10} K_{\mathrm{L}} = -0.86$. Careful inspection of their work suggests that the difference between their result and ours is caused by use of the different protonation constants determined in these two studies. Due to the limitations of the methods and the techniques available forty-five years ago, the protonation constant that they determined likely has a large uncertainty. If their determined protonation constant ($\log_{10} K_{\mathrm{a}} = 3.70$) is substituted by our value ($\log_{10} K_{\mathrm{a}} = 3.30$), then re-evaluation of the lactonization constant yields $\log_{10} K_{\mathrm{L}} = -0.51$, which is the same as ours considering our uncertainty limits. Also, Combes et al. employed an optical rotation method in combination with high-performance liquid chromatography, to investigate the kinetics of the lactonization of gluconic acid and the hydrolysis of the δ-lactone at p$C_{\mathrm{H}} = 2.4$ [11]. From their kinetic results, the stability constant of the lactonization reaction was calculated to be $\log_{10} K_{\mathrm{L}} = -0.65$, which is close to our value.

Assuming that the hydroxyl group deprotonates at high pC_{H} values, and using our determined protonation and lactonization constants, the speciation of gluconic acid in the pH region from 2 to 14 was calculated and is shown in Fig. 4. For solutions with p$C_{\mathrm{H}} < 5$, the two equilibria, protonation and lactonization, are coupled and the ratio of the lactone and

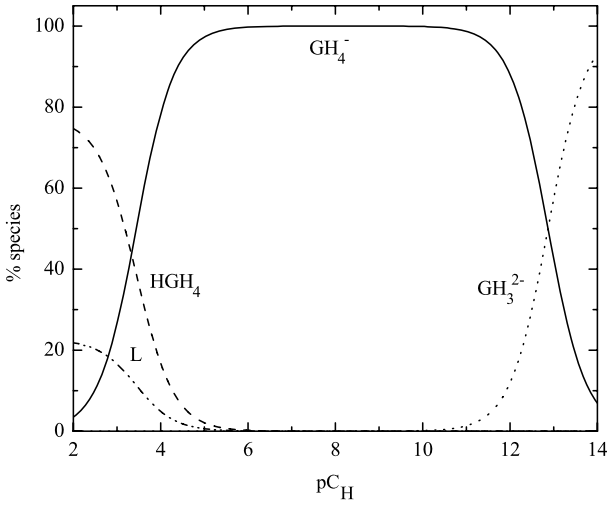


Fig. 4 A speciation diagram for gluconic acid at the constant gluconate concentration (0.1 mol·L^{-1}). Species fraction lines: *solid*—GH_4^-, *dash*—HGH_4, *dash-dot-dot*—L, *dot*—GH_3^{2-}

Scheme 4

$$HGH_4 \underset{k_1-1}{\overset{k_1}{\rightleftharpoons}} L$$

acid concentrations remains constant as the pC_H changes. When p$C_H > 11.0$, the hydroxyl group starts to deprotonate.

3.4 Rate Constants of Lactonization and Hydrolysis

The rate constants of δ-lactonization and hydrolysis are defined as k_1 and k_{-1}, respectively (Scheme 4).

As an essential step for estimating these constants with the ESI-MS method, the mechanism of lactone ionization during the electrospray process needs to be discussed first. Under the negative operation mode of ESI, a lactone could accordingly be ionized in two ways. One mechanism is by the deprotonation of a hydroxyl group of the lactone [27], in which the lactone is directly ionized. The other mechanism is by the adduction of an anion to the lactone [20]. The gluconate ion, a main anion in the sample, could be a good candidate for this adduct. The experimental results (Fig. 5) verify both possibilities. Two types of lactone anions are formed and detected with the ESI-MS method: $m/z = 175$ (deprotonation of hydroxyl group) and $m/z = 373$ (lactone adducted by gluconate). Since the pC_H values of the samples are within the range of 3.0 to 6.5, the detected lactone is likely to be the δ-lactone. Figure 5 also indicates that without acidification, no lactone signals are observed (Fig. 5a), but with the decrease of pC_H, the lactone signals appear (Fig. 5b–c). This observation is in agreement with our potentiometric results, i.e., that the amount of the lactone formed is inversely related to the pC_H of the gluconate solutions. It is therefore suggested that ESI-MS measurements can be used to follow the formation of a lactone. Between these two signals, the one at $m/z = 373$ is more sensitive with a well-defined ionization mechanism, and hence it was chosen to be an indicating signal for the lactone in the kinetics studies.

Using $m/z = 373$ as an indicator for the lactone, we employed the ESI-MS results to estimate the rate constants for lactonization and hydrolysis, taking advantage of this quick measurement method compared to the slow kinetics of the lactonization/hydrolysis, and

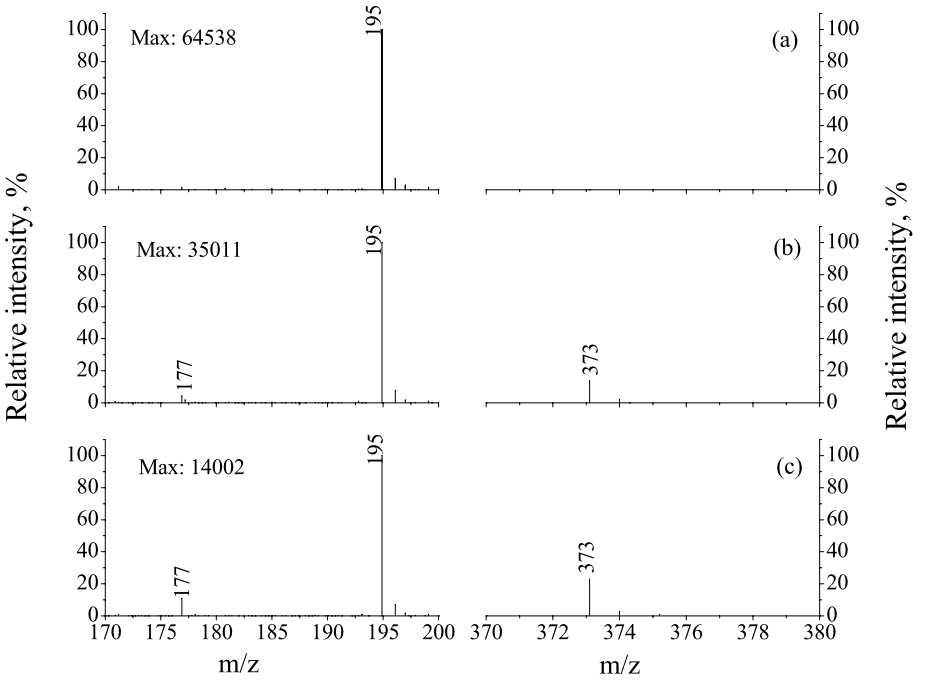


Fig. 5 Mass spectra of acidified gluconate samples with $C_{\mathrm{NaGH4}}{}^{0} = 50$ mmol·L^{-1}: (**a**) 0.0% acidification and p$C_{\mathrm{H}} = 6.2$; (**b**) 50% acidification and p$C_{\mathrm{H}} = 4.3$; (**c**) 100% acidification and p$C_{\mathrm{H}} = 3.3$

its capacity to identify the lactone. Assuming that the δ-lactonization and hydrolysis follow pseudo-first-order reactions with respect to the concentrations of gluconic acid and the δ-lactone, respectively [10, 11], the two rate constants (k_1 and k_{-1}) are thus related to the equilibrium constant (K_{L}) as given by Eq. 10:

$$K_{\mathrm{L}} = \frac{k_1}{k_{-1}} \tag{10}$$

and the corrected lactone signal (I_t) can be described as a function of time (t) by Eq. 11:

$$\ln\left(\frac{I_e - I_t}{I_e - I_0}\right) = \left(k_1 + \frac{K_{\mathrm{L}}}{k_1}\right)t \tag{11}$$

where I_{e} and I_0 indicate the intensities of the lactone signal at equilibrium and at an initial concentration, respectively. The details for deriving Eq. 11 can be found elsewhere [28].

Table 1 lists the measured intensities of the gluconate signal ($m/z = 195$) and the lactone signal ($m/z = 373$) for the kinetic sample at p$C_{\mathrm{H}} \approx 5.0$, as well as the related corrections mentioned in Sect. 2.3. Figure 6 shows the graph of $\ln\{(I_{\mathrm{e}} - I_t)/(I_{\mathrm{e}} - I_0)\}$ versus the time t. The observed linear relationship confirms the assumption of pseudo-first-order reactions, and provides a value of the rate constant, 0.523. Using this value, the δ-lactonization rate constant (k_1) is 3.2×10^{-5} s^{-1} according to Eq. 11, and consequently the hydrolysis rate constant (k_{-1}) is calculated to be 1.1×10^{-4} s^{-1} using $K_{\mathrm{L}} = 0.29$ as described by Eq. 10. All obtained constants are summarized in Table 2.

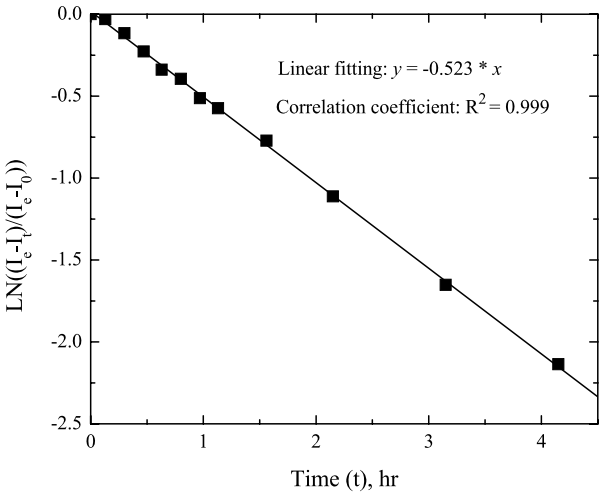


Fig. 6 Kinetic results for lactonization obtained with the ESI-MS method. Starting solutions: $C_{NaGH4}{}^0 = 50$ mmol·L^{-1}, $V^0 = 10$ mL, p$C_H \approx 6.2$; added acid, $C_{HClO4} = 0.99$ mol·L^{-1}, $V_{HClO4} = 0.05$ mL, and p$C_H \approx 5.0$. Symbol (■): experimental data; *solid line*, fitted values

It is interesting to note that lactonization and hydrolysis follow first-order reactions with respect to the concentration of gluconic acid and the lactone, respectively, and the resulting rate constants are close to those reported by Combes et al. [11] ($k_1 = 3.807 \times 10^{-5}$ s^{-1} and $k_{-1} = 1.730 \times 10^{-4}$ s^{-1}).

4 Conclusion

Gluconic acid undergoes two equilibria, carboxylate protonation and lactonization, in acidic media. The variations of the amount of lactonization with the pC_H were investigated using ^{13}C NMR spectroscopy. With a decrease of the pC_H, the amount of lactonization increases but the formation of the two lactones (δ- and γ-lactone) occurs differently. The δ-lactone is generated more readily than the γ-lactone. In 0.1 mol·L^{-1} gluconate solutions where the p$C_H > 2.5$, only the δ-lactone is formed. When the p$C_H < 2.0$, the formation of the γ-lactone is observable although the δ-lactone does predominate.

The thermodynamic properties of these two equilibria were determined by NMR and batch potentiometric titrations. At $I = 0.1$ mol·L^{-1} $NaClO_4$ and room temperature, the carboxylate protonation and δ-lactonization constants were determined to be $\log_{10} K_a = 3.30 \pm 0.02$ and $\log_{10} K_L = -(0.54 \pm 0.04)$, respectively.

Negative-mode ESI-MS measurements were applied to examine the formation of lactones within the pC_H range of 3.0 to 6.5. The δ-lactone species were detected either as an alkoxide anion (deprotonation of hydroxyl group) or as an adducted anion (adduction by gluconate). At p$C_H \approx 5.0$, the ESI-MS method was used to investigate the kinetic properties of lactonization and hydrolysis. The rate constants for the δ-lactonization (k_1) and the hydrolysis (k_{-1}) reactions were estimated to be 3.2×10^{-5} s^{-1} and 1.1×10^{-4} s^{-1}, respectively.

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