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Note

Evidence of two-step deprotonation of D-mannitol in aqueous solution

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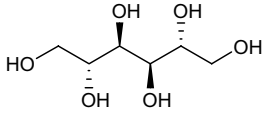
Abstract—Deprotonation of D-mannitol was studied in aqueous basic solutions by means of potentiometry and ^{13}C NMR spectroscopy. Two-step dissociation in the pH range from 12 to 13.8 was shown, and successive dissociation constants K_{a1} and K_{a2} were determined. In a solution with ionic strength $I = 1.0$ M (NaOH + $NaNO_3$) $pK_{a1} = 13.1 \pm 0.1$ and $pK_{a2} = 13.8 \pm 0.2$. With increasing ionic strength from 0.75 to 3.0 M, both pK_{a1} and pK_{a2} values decrease. Deprotonation-induced chemical shifts in pH-variable ^{13}C NMR spectra show that the OH-groups next to internal carbon atoms C-3 and C-4 dissociate to a greater extent compared to OH-groups next to external carbon atoms C-1 and C-6.

Keywords: pK_a; ^{13}C NMR; Potentiometry; Metal ion chelation

D-Mannitol (**1**) is a widespread hexitol found in a variety of plants, algae and fungi. It is endogenous in humans[1–3] and is a widely accepted food additive.[4] Although its biological role is not fully understood, evidence suggests that D-mannitol is an important intermediate in the physiology of plants,[5,6] animals[7,8] and humans.[9–12] In aqueous solution D-mannitol adopts a flat zigzag conformation,[13] and sequesters metal ions forming surprisingly stable chelates.[14] D-Mannitol complexes with oxometallates are stable in a wide pH range,[14] however, complexes with simple non-oxo metal ions are rather weak in acidic and neutral media[15] owing to the weak acidic D-mannitol properties.[16,17] Stable non-oxo metal complexes with D-mannitol exist only in strongly basic solutions where hexitol deprotonation takes place.[14,18,19] Acidic properties of D-mannitol were shown,[20–25] and one-proton per D-mannitol molecule deprotonation is widely accepted.[22–25] Reported pK_{a1} values[20,22–25] range from 13.1[24] to 13.7.[25] Two-step deprotonation was also suggested,[21] however, no pK_{a2} value was reported. Series of studies have shown that D-mannitol chelates metal ions in bidentante, tetradentate and even hexadentate fashion,[26–30] suggesting that deprotonation of multiple D-mannitol OH-groups is possible in strongly basic solution. Since no step-wise deprotonation constants for D-mannitol were known, D-mannitol deprotonation in strongly basic solutions by means of potentiometry and ^{13}C NMR spectroscopy was investigated in the present study.



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Deprotonation of D-mannitol in basic media was studied potentiometrically, and the average number of dissociated OH groups per one D-mannitol molecule (N) was determined as a function of pH (Fig. 1). The N values larger than 1 clearly show that at least two-proton dissociation per one molecule takes place at high pH. The average number of dissociated group (N) for two deprotonating sites with successive dissociation constants K_{a1} and K_{a2} depends on solution pH according to Eq. 1. The experimental data points were fitted to Eq. 1 by a generalized reduced gradient procedure,[31] and although high dispersion is observed for individual data points, the entire data set of 20–40 points allowed both K_{a1} and K_{a2} value determination with relatively small error. pK_a values obtained in solutions of various ionic strength are summarized in Table 1. With increasing ionic strength both dissociation constants increase, showing the propensity of D-mannitol to dissociate into ions in solutions with higher ionic strength. This trend is typical for weak acids.[32,33] The experimental data points were also fitted to the simple one-proton dissociation model ($N = K_a/(K_a + 10^{-pH})$), however, the misfit indicated that at least two-step dissociation has to be considered to describe D-mannitol deprotonation at high pH.

$$N = \frac{10^{-pH}K_{a1} + 2 \cdot K_{a1}K_{a2}}{10^{-2pH} + 10^{-pH}K_{a1} + K_{a1}K_{a2}} \quad (1)$$

Table 1. The step-wise pK_{a1} and pK_{a2} values for D-mannitol dissociation in solution with various ionic strength (I)

I (M)	pK_{a1}	pK_{a2}
0.75	13.3 ± 0.2	13.9 ± 0.3
1.0	13.1 ± 0.1	13.8 ± 0.2
2.0	13.0 ± 0.2	13.7 ± 0.2
3.0	13.0 ± 0.1	13.5 ± 0.2

The ^{13}C NMR spectrum of D-mannitol obtained in aqueous solution is shown in Figure 2. It exhibits three signals at 63.9, 71.46 and 69.86 ppm, which were readily assigned to C-1,C-6, C-2,C-5 and C-3,C-4 carbon atoms, respectively. Observed chemical shifts are similar to the previously published values,[34] with small differences caused by the solvent. Identical chemical shifts were observed for all signals in the pH range from 8 to 12, indicating that the D-mannitol molecule is not affected in neutral and weakly basic media. This observation supports our potentiometric study (Fig. 1). Upon further

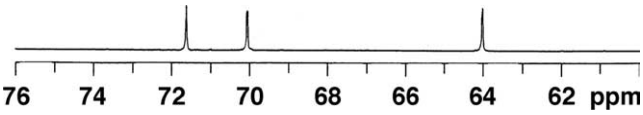

Figure 2. The ^{13}C NMR spectrum of aqueous 0.3 M D-mannitol solution (pH 11.5).

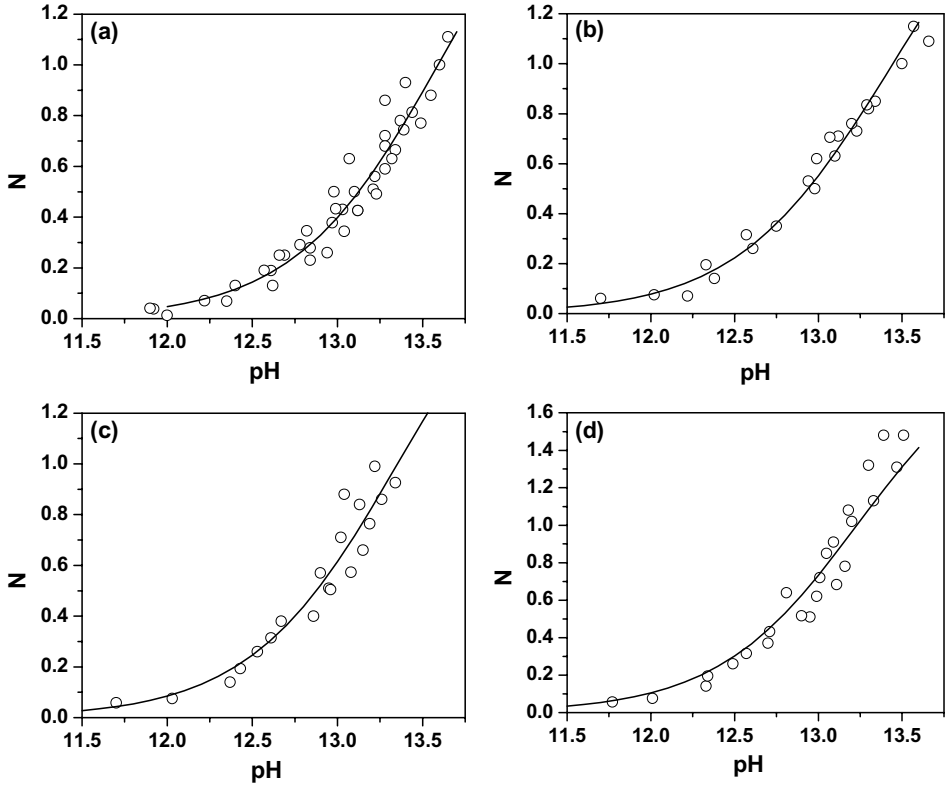

Figure 1. The average number of dissociated OH groups per one D-mannitol molecule (**N**) as a function of pH. The ionic strength (NaOH+$NaNO_3$) is equal to 0.75 M (a), 1.0 M (b), 2.0 M (c) and 3.0 M (d). Open circles represent experimental data points, and solid lines are best fits to Eq. (1).

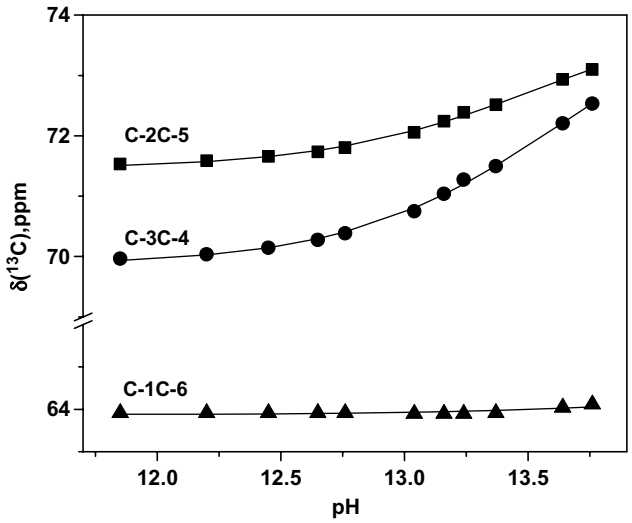

Figure 3. The ^{13}C NMR chemical shift as a function of pH for aqueous D-mannitol solution.

increase in solution pH all three signals shift downfield, showing that hydroxyl groups adjacent to carbon atoms are deprotonating (Fig. 3).

The deprotonation-induced chemical shift (DIS = $\delta_{high\,pH} - \delta_{low\,pH}$), similarly to coordination induced shift (CIS),[35–38] allows determination of the most affected sites in a molecule. The pH increase has significantly higher effect on internal atoms C-2,C-5 and C-3,C-4 than on terminal atoms C-1,C-6 (Fig. 3). For instance, the DIS in solution with pH 13.8 compared to pH 11 solution is equal to 0.20, 1.57 and 2.57 ppm for C-1,C-6, C-2,C-5 and C-3,C-4 atoms, respectively. We conclude that dissociation degree of OH-groups decreases in the following order: C-3(OH)–C-4(OH) > C-2(OH)–C-5(OH) > C-1(OH)–C-6(OH). The consistent downfield shift was observed for pH values above 13.8 (data not shown), and all resonances continuously moved downfield without reaching the limiting value $\delta_{high\,pH}$. This observation suggested the deprotonation of multiple alkoxide groups.

Although there is a large repulsion in the D-mannitol molecule between two neighbouring C–O^- moieties, dianion Man^{2-} also forms in aqueous solution. The fast proton exchange among all OH-groups in the hexitolate anion, and the possible structures of deprotonated D-mannitol species Man^- and Man^{2-} are shown in Figure 4. Based on our pH-variable NMR study the form **III** is the most likely structure for Man^- anion, whereas forms **I** and **II** represent possible, but less likely structures. Respectively, Man^{2-} dianion form **IV** is expected to be more favourable than eight other possible forms (only two of them, **V** and **VI** are shown in Fig. 4). The dissociation impact on the chemical shift of C-1,C-6 and C-2,C-5 pairs can be attributed to the influence of minor forms **I** and **II** in monoanion, and forms **V** and **VI** in dianion (Fig. 4). Electrostatic shielding and conformational changes in the ionized molecule are also possible reasons of deprotonation-induced chemical shift.

Both potentiometry and pH-variable ^{13}C NMR spectroscopy supports two-proton per D-mannitol molecule deprotonation in aqueous solution. The well-known formation of metal complexes with multiply deprotonated hexitols is usually referred to metal-promoted deprotonation,[39–44] where negatively charged alkoxide groups are stabilized by transition metal vacant d-orbitals. We show that D-mannitol dianion forms in aqueous solution, where dissociation is caused solely by strong nucleophilicity of hydroxide ion.

1. Experimental

D-Mannitol (Aldrich) was dried under vacuum for 48 h. Aqueous CO_2-free standard solutions of NaOH (1.0 and 10 M) were prepared from 50% NaOH stock. The NaOH concentration was determined by diluted sample titration with strong acid. $NaNO_3$ (5 M) stock solution was prepared from high purity solid salt. Potentiometric titrations were carried out as follows: to the known amount of D-mannitol, NaOH and $NaNO_3$ stock solutions were added, and the pH values of resulted

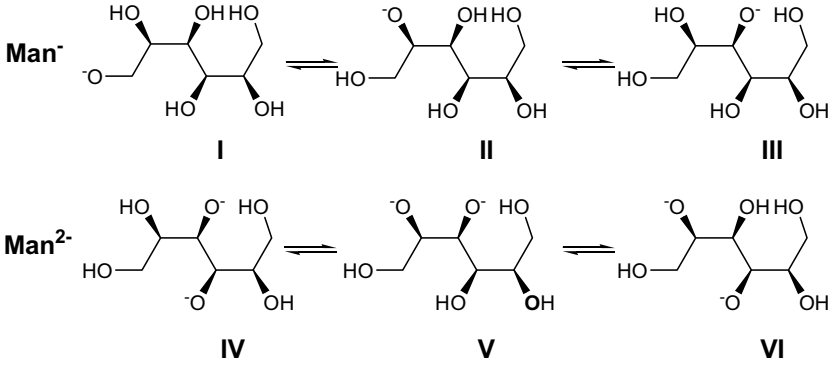

Figure 4. Possible structures of D-mannitol monoanion Man^- (**I**, **II** and **III**) and dianion Man^{2-} (**IV**, **V** and **VI**). See the text for details.

solutions were measured. The total molar concentration of NaOH and $NaNO_3$ was kept constant. The difference between initial and final NaOH concentrations yielded the amount of NaOH reacted with D-mannitol, and the molar ratio of reacted NaOH to D-mannitol yielded the average number of dissociated OH-groups per one hexitol molecule.

$^{13}C\{^1H\}$ high-resolution NMR spectra were acquired on a Varian Inova 400 spectrometer operating at 100.6 MHz and temperature 22 ± 0.5 °C. Ethanol methylene signals at 58.05 ppm[45] were used as internal reference. All chemical shifts were converted to TMS (tetramethylsilane) scale, with an estimated precision of ±0.01 ppm. Samples contained 0.3 M of D-mannitol, 0.1 M of ethanol, NaOH with total concentration from 0.01 to 9.2 M and 10 vol % of D_2O (lock). The pH values were measured directly with Orion 427 pH-meter equipped with Corning glass electrode. The electrode was calibrated in pH region from 10 to 14 using three standard solutions. The sample pH was checked before and after a spectrum was recorded, and the difference between obtained values was 0.02 pH units or less. The spectra were run within 30 min after solution preparation.

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