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^{13}C NMR and electrospray ionization mass spectrometric study of sucrose aqueous solutions at high pH: NMR measurement of sucrose dissociation constant

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Abstract

The ^{13}C NMR technique is used for the measurement of the first dissociation constant of sucrose (HL) in highly alkaline solutions. In 1.0 M NaCl/NaOH medium and for 25 °C, the concentration dissociation constant (pK_1) was 13.1 ± 0.3; and, for 60 °C, pK_1 = 12.30 ± 0.05. The β-D-fructofuranosyl ring was found to be responsible for dissociation. The NMR data reveal no clear evidence of the second dissociation step below pH 14, either at 25 °C or at 60 °C. In the solutions with 4–10 mol dm^{-3} NaOH content the ^{13}C NMR technique indicated the chemical shift changes, treated as the second dissociation step of sucrose and a sodium complex formation. A very rough estimation, for variable ionic strength, gives the value: $pK_2 \sim 15.8 \pm 0.8$. The anionic species L^- and $NaH_{-1}L^-$ have been registered by electrospray ionization time-of-flight mass spectrometry (ESI-ToF MS) for 0.01 M sucrose solutions with initial pH 13.

Keywords: Sucrose dissociation constants; NMR ^{13}C; Electrospray ionization time-of-flight spectrometry; Hydroxyl group of sucrose responsible for dissociation

1. Introduction

Many technologically important acid-base equilibria take place in highly basic aqueous solutions. Among these is the sucrose dissociation, which takes place at pH ⩾ 12 (Stability Constants Database, 2003). Knowledge of particular pK values at room and high temperatures is of considerable importance for sugar-beet technology (Beet-sugar Technology, 1971). Unfortunately, the data published to date reveal rather high disparity. This is due to attempts to measure high pK values with the glass electrode technique, which is inadequate for a highly basic medium (Bates, 1973). Recently, the nuclear magnetic resonance (NMR) technique became available as a reliable alternative to potentiometric methods (Popov, Rönkkömäki, Lajunen, Vendilo, & Popov, 2003; Popov, Popov, Rönkkömäki, Vendilo, & Lajunen, 2002). The present paper is focussed on the measurement of sucrose pK by ^{13}C NMR at 25 and 60 °C, with an emphasis on a particular hydroxyl-group responsible for a proton release. An NMR analysis is fortified by a supplementary study of sucrose basic solution with electrospray ionization time-of-flight mass spectrometry (ESI-ToF MS).

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2. Materials and methods

2.1. Reagents and sample preparation

Reagent grade sucrose (Merck) and NaCl (Merck) have been used. Standard 1 mol dm^{-3} NaOH solution was supplied by Baker (DILUT IT^{R}). The series of 0.1 mol dm^{-3} sucrose samples were prepared by adding known variable amounts of NaOH and NaCl to maintain the total ionic strength constant: 1 mol dm^{-3} of NaOH/NaCl. Concentration of NaOH gradually changed between 0.2 and 1.0 mol dm^{-3}. The samples were kept in small tightly closed polyethylene bottles with small air volume, in order to prevent pH drift of basic solution. The sucrose solutions were equilibrated for 10–12 h before NMR measurement.

Since sucrose concentration was significantly less than that of NaOH ($C_{\text{sucrose}} < [OH^-]$), the equilibrium concentration of free [OH$^-$] was taken from the total NaOH amount added and later corrected for sucrose-base interaction, as described in Section 2.3. The corresponding $-\log[H^+]$ value was then derived using the appropriate pK_w at 25 or 60 °C in 1.0 mol dm^{-3} NaCl. For 25 °C pK_w = 13.7 (Kron, Marshall, May, Hefter, & Königsberger, 1995) and, for 60 °C the value measured by DeStefano, Foti, and Sammartano (2001) for 55 °C and 1.0 mol dm^{-3} NaCl (pK_w = 12.8) was used. Therefore, pH is given in terms of [H$^+$]. This approach allows elimination of experimental measurements of pH in highly basic solutions.

2.2. NMR measurement

The ^{13}C NMR spectra of sucrose were recorded with a Bruker DPX400 instrument at 100.61 MHz with a 10-mm diameter sample tube at 60 °C, and with AM 360 (90.56 MHz) and 5 mm diameter sample tube at 25 °C. The external standard, placed in a 2 mm (DPX400) or 0.8 mm (AM 360) coaxial inner tube, represented DMSO-D6, which provided both reference and 'lock'. The samples therefore contained no D_2O internally and no pD/pH scale corrections were required. Chemical shifts are reported in the TMS scale. Downfield shifts are denoted as positive. Susceptibility corrections to the chemical shifts were neglected, being estimated as small for constant ionic strength and a comparatively narrow range of $\log[OH^-]$ variations within the sample series. For thermodynamic experiments, the spectra were measured without ^{1}H decoupling for better temperature control. Experiments intended to provide information on location of a dissociating hydroxyl-group were run with ^{1}H decoupling and 0.2–0.3 mol dm^{-3} sucrose solutions.

2.3. NMR data treatment

As only one hydroxyl-group revealed an outstanding sensitivity towards deprotonation, the sucrose was treated as a monobasic acid HL. Then, the first dissociation step of sucrose can be described by a reaction:

$$HL \leftrightarrow H^+ + L^- \qquad K_1 \tag{1}$$

An experimentally observed single time-averaged ^{13}C NMR chemical shift of "free" (L$^-$) and proton-bonded ligand (HL) δ_{obs} is given by a simple equation,

$$\delta_{\text{obs}} = (\delta_L + K_1^{-1}[H^+]\delta_{HL})/(1 + K_1^{-1}[H^+]), \tag{2}$$

where δ_L – represents chemical shift of a free ligand L and δ_{HL} that of monoprotonated species HL. The dissociation constant (pK_1) was calculated by a non-linear curve-fitting programme, SigmaPlot (SigmaPlot, 1997), operating with 6–10 experimental points for each curve.

For ^{13}C NMR titration, the substrate concentration has to be rather high (ca. 0.1 mol dm^{-3}). Therefore, at pH < 13.5 the equilibrium [OH$^-$] cannot be equal to the total [OH$^-$] added to the system. For this case, the two-step procedure was used. In the first step, the equilibrium [OH$^-$] is taken equal to the total [OH$^-$] added and the full titration curve is plotted, mathematically treated and the pK_1, δ_L and δ_{HL} values are calculated; see for example Fig. 1(a) and (b). The difference between δ_L and δ_{HL} chemical shifts gives the scale of [OH$^-$] consumption by the ligand: 0 mol dm^{-3} (δ_{HL}) and 0.1 mol dm^{-3} (δ_L) for 0.1 mol dm^{-3} solution of L. In the second step, all the experimental values are recalculated. Then the new set of chemical shift – [OH$^-$] data is treated and an improved value of pK_1 is calculated.

The experimental results are presented in Tables 1–5. The typical titration curves are given in Fig. 1. As can be seen, the range of experimental points is limited by the ionic strength (high pH limit) and by a requirement $C_{\text{sucrose}} < [OH^-]$ (low pH limit). The solid line represents the fitted titration curve. For 25 °C, the titration curve included experimental points for p[H$^+$] 7.00, 11.42, 12.62, 12.87, 13.08 and 13.24.

Besides, the semi-quantitative titration curve was obtained with [NaOH] variation from 0 to 10 mol dm^{-3} in 0.2 mol dm^{-3} sucrose solutions. This was done to identify possible dissociation steps for sucrose above pH = 14. The ionic strength was variable in this case, gradually increasing from I = 1 mol dm^{-3} NaOH to $I \sim 10$ mol dm^{-3} NaOH. As the OH$^-$ forms associates with Na$^+$ at pH > 14, with conflicting thermodynamic constants (Stability Constants, 2003), then the exact free hydroxyl ion concentration could not be calculated. Another problem was associated with unknown variations of pK_w as NaOH concentration increased from 1 to 10 mol dm^{-3}. We therefore used chemical shift as a function of total NaOH concentration in the sucrose solution, Fig. 2. For this particular case, the proton

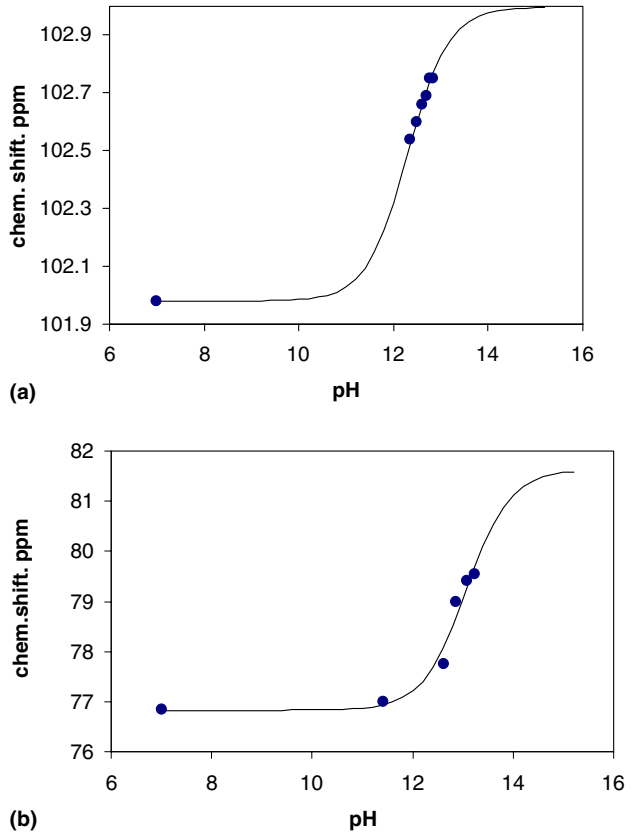

Fig. 1. The typical NMR titration curves of 0.1 mol dm^{-3} sucrose at 60 °C for C7 band (a) and at 25 °C for C8 band (b); I = 1.0 mol dm^{-3} NaCl/NaOH; solid line represents the fitting curve for the second step approximation.

Table 1
ESI-ToF MS experimental conditions

Sample flow-rate, μl min^{-1}	10
Capillary voltage, V	3500.0
Sample cone voltage, V	35
Rf lens, V	200
Extraction cone voltage, V	5
Desolvation temperature, °C	150
Source temperature, °C	120
Resolution	4000.0
Cone gas flow-rate, l h^{-1}	300
Desolvation gas flow-rate, l h^{-1}	1200
Mass range, m/z	100–1000

decoupling was used and the temperature was from 25 to 30 °C. The calculations of pK_2 have been done for the experimental points with p[NaOH] > 0 using Eq. (2) for one step dissociation and assuming pK_w = 14[1]. Although the experimental points fit the curve rather well, the corresponding p$K_2 \sim 15.8 \pm 0.8$ is evidently a very rough estimation.

[1] For 3 M NaCl, pK_w = 14.088; hydrogen electrode (Kron et al., 1995).

Table 2
The 0.2 mol dm^{-3} sucrose ^{13}C NMR chemical shifts in aqueous solutions at 25 °C

Band No.	C_i	δ, ppm, pH 6.0	δ, ppm, pH 13.7	$\Delta\delta$, ppm
1	7	103.54	104.11	0.65
2	1	92.29	93.05	0.97
3	10	81.25	82.96	1.71
4	8	76.55	79.25	2.70
5	9	74.07	75.37	1.30
6	3	72.61	74.30	1.69
7	5	72.32	72.62	0.30
8	2	71.06	72.19	1.13
9	4	69.26	69.98	0.72
10	11	62.43	63.19	0.76
11	12	61.41	63.02	1.61
12	6	60.18	60.64	0.46

2.4. Electrospray ionization mass spectrometry

The ESI-ToF mass spectra have been recorded with a Micromass LCT mass spectrometer equipped with a Z-spray electrospray interface. The 0.01 mol dm^{-3} sucrose aqueous solution, with initial pH 13 (NaOH), was introduced into the spectrometer by a Harvard Apparatus Model 11 syringe pump at a flow rate of 10 μl min^{-1}. The sample cone voltage was 35 V. The negative ion mode was acquired using Masslynx NT software. The experimental parameters are listed in Table 1, and are very close to those used by Sarpola, Hietapelto, Jalonen, Jokela, and Laitinen (2004).

3. Results and discussion

Sucrose (β-D-fructofuranosyl-α-D-glucopyranoside) is one of the most common sugars, occurring widely in plants, fruits and honey. Its structure, and the nomenclature used to number the carbon atoms in the present study, are given in Fig. 3. The NMR line assignment was done according to Bax, Freeman, and Levitt (1981). The obtained spectra (Table 2) are well consistent with those reported earlier. Obviously, the base affects all the resonances. At the same time, the sensitivity of carbon NMR to the proton removal appears to be very different, ranging from 2.7 to 0.3 ppm as the base concentration increases from 0 to 1.0 mol dm^{-3}. The supplementary experiments, including intermediate points, indicated that all sucrose resonances shift in a monotonous and a similar way. Therefore any resonance line can be taken for pK_1 measurements. This is not surprising in as much as both sugar rings of sucrose behave together as a single rigid entity, bound by an intramolecular hydrogen bond and reorienting isotropically in an aqueous solution (Baraguey, Mertens, & Dölle, 2002). Thus, a dissociation of one hydroxyl-group in some way affects the entire molecule.

Table 3
The results of 0.1 mol dm^{-3} sucrose ^{13}C NMR chemical shifts at 25 °C and in 1 mol dm^{-3} NaCl/NaOH aqueous solutions data treatment[a]

C_i	L, ppm (calc.)	HL, ppm (exp.)[b]	HL, ppm (calc.)	pK_1	R	$\Delta\delta$, ppm
7	103.97 (0.18)	103.54	103.00 (0.06)	13.30 (0.25)	0.982	0.97
8	80.60 (0.14)	76.55	76.80 (0.02)	13.30 (0.22)	0.985	3.60
9	76.16 (0.28)	74.07	74.31 (0.10)	13.20 (0.21)	0.987	1.85
10	83.99 (0.55)	81.25	81.52 (0.14)	13.40 (0.25)	0.980	2.47

[a] First step data treatment; values in parentheses represent the standard deviations.
[b] Values for 0.2 M sucrose aqueous solution at pH 6.

Table 4
Dissociation constant of 0.1 mol dm^{-3} sucrose (HL) from ^{13}C NMR-titration at 60 and 25 °C in 1 mol dm^{-3} NaCl/NaOH

T, °C	Calculation procedure	δ_L, ppm	δ_{HL}, ppm	pK_1	R
60	One step	103.00	101.98	12.40 ± 0.05	0.999
	Two steps	102.96	101.98	12.30 ± 0.05	0.999
25	One step	80.6	76.80	13.30 ± 0.22	0.985
	Two steps	81.6	76.83	13.10 ± 0.30	0.982

Table 5
The pK_1 values of sucrose

Method	T, °C	Ionic strength, mol dm^{-3}	pK_1	Reference
^{13}C NMR	25	1.0; NaCl	13.1 ± 0.3	Present work
Unknown	25	Unknown	12.7	Beet-sugar Technology (1971)
gl	Room	0.03; $NaNO_3$	12.4	Safina et al. (1990)
gl	25	1.0; $NaClO_4$	12.6	Coccioli and Vicedomini (1974)
^{13}C NMR	60	1.0; NaCl	12.30 ± 0.05	Present work
Unknown	80	Unknown	11.6	Beet-sugar Technology (1971)

Only one carbon demonstrates much higher shift than the others. Evidently, the largest chemical shift (2.7 ppm) reveals that the dissociation takes place at the fructofuranose ring, at a hydroxyl group attached to the C8 carbon. The second greatest chemical shift change (~1.3–1.7 ppm) occurs at carbons located close to C8: C9, C10, C12. In the glucose ring there are only two carbon atoms (C2 and C3) that reveal a comparable shift upon sucrose deprotonation. At the same time, the farthest atom, from C8, of the glucose ring (C4, C5, C6) show poor NMR sensitivity toward the dissociation process. Thus, there are no indications that some other hydroxyl-groups pass dissociation at pH < 14 and 25 °C. It is reasonable to note, that resonances of both carbon atoms that form oxo-bridges (C1 and C7) are less sensitive to the dissociation process.

Thus, the pK_1 measurements were done at 25 °C, operating with a one dissociation step model and Eqs. (1), (2) for four different NMR lines: C7, C8 C9 and C10. For one-step data treatment the obtained values (13.37, 13.27, 13.30, 13.24) revealed good agreement, Table 3. This is an additional argument in favour of the one proton dissociation model validity for pH < 14. For further calculations at 25 °C, the C8 resonance band, with maximal chemical shift between HL and L, was taken. For 60 °C, the NMR bands resolution and NMR sensitivity decreases, and the best observable line was C7.

An application of the two-step data treatment model demonstrated, that the ignorance of base consumption by sucrose gives a systematic error of 0.1–0.2 in pK_1, Table 4. The pK_1 measured at high temperature is considerably lower than the one at 25 °C.

A comparison of our data with the literature demonstrates that application of the glass electrode evidently leads to a serious underestimation of pK_1, Table 5. At the same time, we found no clear evidence of the second step dissociation of sucrose below pH 14. Therefore, the reported pK_2 values at 25 °C: 13.24 (Safina et al., 1990); 13.45 (Coccioli & Vicedomini, 1974) and 13.1 (Beet-sugar Technology, 1971) are likely to be erroneous. Indeed, the removal of one proton from the molecule makes the second step of dissociation sufficiently more difficult. The corresponding pK is normally shifted, for 0.8–2.0 logarithmic units, to higher values (Stability Constants, 2003). For example, for cyclohexane–1,4-dicarboxylic acid, the difference between pK_1 and pK_2 is around 1.0 log unit while, for 1,8-diaminooctane it ranges between 0.8 and 1.3 (Stability Constants, 2003). Alternatively, we have to assume that both sucrose rings

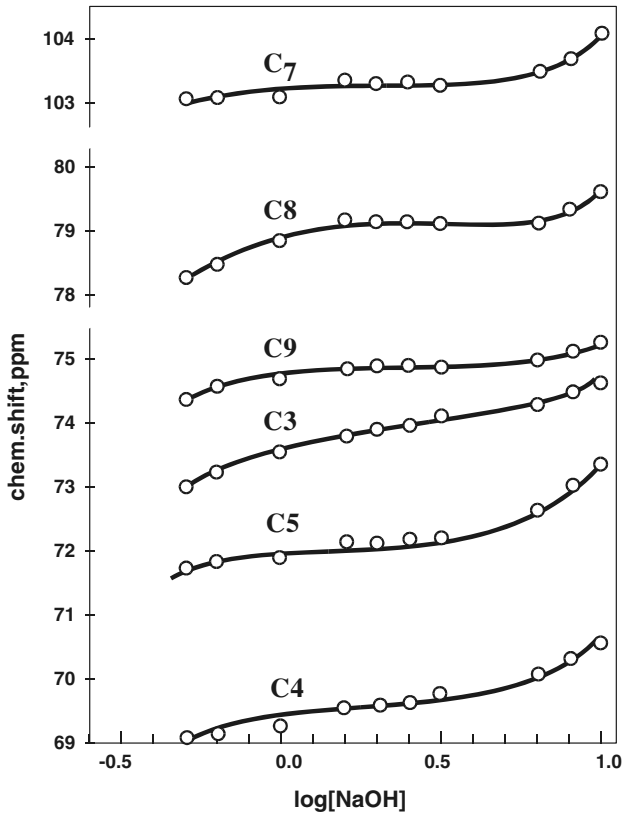

Fig. 2. The 0.2 mol dm^{-3} sucrose NMR chemical shift dependence on the total NaOH concentration at 25–30 °C for C3, C4, C5, C8 and C9 bands.

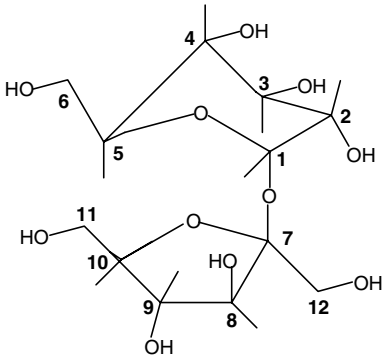

Fig. 3. Labelling of the carbon atoms in the sucrose molecules.

pass dissociation absolutely simultaneously with $pK_1 = pK_2$. Indeed, the calculation of pK values for the most pH-sensitive bands of glucose and fructose rings at pH < 14 gave the same values within the experimental error. Therefore, the pH-dependence of chemical shift at pH > 14 (Fig. 2) should be attributed to pK_3 Although such a possibility could not be completely excluded, we find it to be not realistic.

This conclusion is indirectly supported by the electrospray ionization mass spectrometry, which becomes a powerful tool for investigation of organometallic complexes (Electrospray ionisation mass spectrometry, 1997). The ESI-ToF MS spectrum of 0.01 mol dm^{-3} sucrose solution, with initial pH 13 (NaOH), revealed two dominating peaks of almost equal intensity with m/z 341 and 363. The first one was assigned to sucrose anion L^-, which is expected to be present in a solution at pH close to the pK value in a significant amount. The second peak corresponds to the negatively charged sodium complex with a double ionised sucrose moiety: $[NaH_{-1}L^-]$ (here and elsewhere the removal of n protons besides the first one is indicated as $H_{-n}L^{-(1+n)}$). Thus, the ESI-ToF MS likely demonstrates some of the equilibrium species that could arrive in aqueous solution at pH 15–16 at room temperature. Indeed, the fast evaporation of water molecules at 150 °C, from the surface of a sucrose solution droplet within the ESI-ToF MS experiment, leads to a fast increase of NaOH concentration. Under these rather aggressive conditions, the substitution of a proton by a sodium ion in a sugar hydroxyl-groups becomes possible.

It should be noted that the H/Na substitution degree is not very high, although the initial mole ratio for pH 13 corresponded to [sucrose]:[NaOH] = 1:10. Besides the above mentioned $[NaH_{-1}L^-]$ species, two other sodium complexes have been registered in negligible amounts: $[Na_2H_{-2}L^-]$ (m/z = 385) and $[Na_3H_{-3}L^-]$ (m/z = 407). No indication of species with positive charge or negative charge −2 or higher was found.

As ESI-ToF MS reveals only charged species, it is reasonable to suppose that, at unattainably high pH at room temperature, the following sucrose species are likely to exist in equilibrium with each other: L, L^-, NaL, $[NaH_{-1}L^-]$, $[Na_2H_{-1}L^0]$, $[Na_2H_{-2}L^-]$, $[Na_3H_{-2}L^0]$, $[Na_4H_{-3}L^0]$ and $[Na_3H_{-3}L^-]$. Indeed, in Fig. 2, one can see a definite increase of chemical shifts at NaOH concentrations higher than 7 molar. A very rough estimation for variable ionic strength by SigmaPlot gives the particular value: $pK_2 \sim 15.8 \pm 0.8$. This value is obtained under conventional assumptions that (i) NaOH is completely dissociated up to 10 mol dm^{-3}; (ii) pK_w for 3 mol dm^{-3} NaCl is valid for the whole range 1–10 mol dm^{-3} NaOH. Both these assumptions evidently do not strictly take place. Therefore, the pK_2 value is really a very approximate one and has a semiquantitative character.

The second ionisation centre is likely to be located in another sucrose ring. The ionisation of the third and fourth hydroxy-groups seems to be much less favourable than are the first two, as the corresponding concentrations of $[Na_2H_{-2}L^-]$ and $[Na_3H_{-3}L^-]$ in ESI-ToF MS spectra are an order of magnitude smaller than that of $[NaH_{-1}L^-]$ species. The next dissociation step is likely to take place if the primary ionised hydroxyl group is coordinated by sodium cation or forms an ionic pair with Na^+.

4. Conclusions

Dissociation of the sucrose molecule in aqueous solution takes place at pH > 10.9 at 25 °C and pH > 10.0 at 60 °C and $I = 1.0$ mol dm^{-3} of NaCl. Therefore, interaction of Ca^{2+} with sucrose anions in highly basic solutions should be accounted. The dissociation occurs at a β-D-fructofuranosyl ring with p$K = 13.1$ (25 °C) and 12.3 (60 °C). It is also demonstrated that the most 'acidic' hydroxyl-group is attached to the C8 carbon. The dissociation constants of sucrose, measured by the NMR technique at 25 and 60 °C, revealed that previously found values are quite underestimated due to inadequate methods used.

No clear indication of a second step dissociation of sucrose up to pH 14 was found, either at 25 °C or at 60 °C. NMR data reveal that the second step of dissociation is observed at 25 °C when NaOH concentration exceeds 7 molar. At the same time, the ESI-ToF MS demonstrated that, at elevated temperatures and at pH $\gg$ 14, the further dissociation of L^- really occurs. The following species are registered: $[NaH_{-1}L^-]$, $[Na_2H_{-1}L^0]$, $[Na_2H_{-2}L^-]$, $[Na_3H_{-2}L^0]$, $[Na_4H_{-3}L^0]$ and $[Na_3H_{-3}L^-]$. Tentatively, these further dissociation steps are promoted by sodium complexes formation.

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