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Saturation molalities and standard molar enthalpies of solution of α-D-xylose(cr) in H_2O(l); standard molar enthalpies of solution of 1,4-β-D-xylobiose(am), and 1,4-β-D-xylotriose(am) in H_2O(l) ☆

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ABSTRACT

The saturation molality of α-D-xylose(cr) in water was measured by using HPLC and is $m(\text{sat}) = (8.43 \pm 0.42)\ \text{mol} \cdot \text{kg}^{-1}$ at $T = 298.15$ K. It was also established that the anhydrous form of α-D-xylose(cr) is the crystalline form that is in equilibrium with the aqueous solution at $T = 298.15$ K. Solution calorimetry was used to measure the following standard molar enthalpies of solution at $T = 298.15$ K: $\Delta_{\text{sol}}H^{\circ}_{\text{m}} = (12.10 \pm 0.12)\ \text{kJ} \cdot \text{mol}^{-1}$ for α-D-xylose(cr); $\Delta_{\text{sol}}H^{\circ}_{\text{m}} = -(8.1 \pm 2.7)\ \text{kJ} \cdot \text{mol}^{-1}$ for 1,4-β-D-xylobiose(am); and $\Delta_{\text{sol}}H^{\circ}_{\text{m}} = -(24.1 \pm 6.4)\ \text{kJ} \cdot \text{mol}^{-1}$ for 1,4-β-D-xylotriose(am). It was observed that both 1,4-β-D-xylobiose(am) and 1,4-β-D-xylotriose(am) were amorphous substances and that they form thick gels in water in which no solid phase is present. Consequently, it is not possible to measure m(sat) for these two substances. All substances were carefully characterized by using both HPLC and Karl Fischer analysis. NMR was used to measure the anomeric purity of the α-D-xylose(cr). Thermodynamic network calculations were used to calculate standard molar formation properties for the aforementioned substances.

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1. Introduction

The efficient conversion of lignocellulosic biomass to useful products is an important practical problem [1–3]. The solution of this problem, however, can be significantly aided by having reliable property values available for design and engineering calculations. The xylose-containing or C5 fraction comprises an important part of lignocellulosic biomass. However, while some property values, specifically saturation molalities m(sat) and standard molar enthalpies of solution $\Delta_{\text{sol}}H^{\circ}_{\text{m}}$ have been reported for α-D-xylose(cr), the existing values in the literature show more scatter than is desirable. Most notably, the results of recent solubility measurements by Zhang *et al.* [4] for α-D-xylose(cr) in H_2O(l) differ significantly from the results of several other studies [5–10], including one unpublished study from NIST [7]. There is a complete absence in the literature of property values for 1,4-β-D-xylobiose, for 1,4-β-D-xylotriose, or for higher oligomers. Indeed, Gray *et al.* [5] have pointed out that "oligomer solubility could potentially play an important role in controlling the rates and yields in the thermochemical hydrolysis of hemicellulose as a pretreatment for subsequent enzymatic conversion of cellulose." In this study, we measured values of $\Delta_{\text{sol}}H^{\circ}_{\text{m}}$ for α-D-xylose(cr), 1,4-β-D-xylobiose(am), and 1,4-β-D-xylotriose(am) in H_2O(l) at $T = 298.15$ K. We also measured the saturation molality m(sat) of α-D-xylose(cr) at this temperature. We observed that both 1,4-β-D-xylobiose and 1,4-β-D-xylotriose exist in the solid state as amorphous substances. These substances form a thick gel in water in which no solid phase is present. Thus, it is not possible to measure m(sat) for these two substances. Nevertheless, as a part of measuring $\Delta_{\text{sol}}H^{\circ}_{\text{m}}$, we performed careful characterizations of all three substances (α-D-xylose, 1,4-β-D-xylobiose, and 1,4-β-D-xylotriose) by using HPLC to detect impurities, and Karl Fischer to determine the mass fractions of water in the samples. NMR was used to measure the anomeric purity of the α-D-xylose(cr). Finally, thermodynamic network calculations were used to calculate standard molar formation properties for these substances.

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TABLE 1
Principal substances used in this study with their Chemical Abstracts Service (CAS) registry numbers, empirical formulae, relative molecular masses M_r, mass fraction moisture contents w of the samples as determined by Karl Fischer analysis, supplier (M = Megazyme, N = NIST, S = Sigma, and U = US Biological), and estimated mole fraction purities x as determined by HPLC.

Substance	CAS No.	Formula	M_r	w	Supplier	x^a
Aldose 1-epimerase[b]	9031-76-9				U	
Water	7732-18-5	H_2O	18.0153		N	[c]
α-D-Xylose	58-86-6	$C_5H_{10}O_5$	150.13	0.00060	S	0.998[d]
1,4-β-D-Xylobiose[e]	6860-47-5	$C_{10}H_{18}O_9$	282.25	[f]	M	[f]
1,4-β-D-Xylotriose[e]	47592-59-6	$C_{15}H_{26}O_{13}$	414.36	0.123	M	0.962

[a] The estimated mole fraction purities are exclusive of the amounts of water in the samples and are based on the chromatographic methods used in our laboratory as described in Section 2.2.
[b] This enzyme is also known as mutarotase (EC 5.1.3.3). It was supplied as a suspension in 80% saturated $(NH_4)_2SO_4$(aq) at a mass concentration of 5 mg · cm^{-3}. The specified activity was $5.84 \cdot 10^3$ units · mg^{-1}, where one unit is defined as causing an increase in the rate of spontaneous mutarotation of 1 mol of α-D-glucose to β-D-glucose per minute at T = 298.15 K and pH 7.0.
[c] The mass fraction of organic substances in the distilled water was less than $2 \cdot 10^{-9}$. The conductivity of this water was ≈12.6 MΩ.
[d] Based on the retention time of the chromatographic peak that corresponds to the impurity in the sample of D-xylose, the most likely impurity in this sample is D-glucose.
[e] These substances were prepared from wheat arabinoxylan by controlled hydrolysis. Since the xylose residues in arabinoxylan are β-1,4-linked, it is assumed that the inter-glycosidic linkages in the samples of xylobiose(am) and xylotriose(am) have the same β-1,4-linkage. However, the anomeric configurations of the reducing moieties in xylobiose and xylotriose have not been established. In figure 1, both xylobiose(am) and xylotriose(am) are shown as having the α anomeric configuration.
[f] Three samples of D-xylobiose were used in this study. Their respective mass fraction moisture contents were w = {0.027, 0.033, and 0.045}. Their estimated mole fraction purities were x = (0.890, 0.970, and 0.890).

2. Experimental

2.1. Materials

Pertinent information on the substances (see figure 1) used in this study is given in table 1.[1] The purities of the samples were assessed by using high-performance liquid-chromatography (HPLC) (see Section 2.2). The mass fractions of water in these samples were measured by performing Karl Fischer analyses with a Metrohm 795 KFT Titrino automatic titrator and a Metrohm 831 KF Coulometer. Hydranal Composite 2 and Hydranal Coulomat AG solutions, respectively, were used as the solvents for the aforementioned instruments. Calibration of the Karl Fischer apparatus was done by using a water-saturated octanol solution [11]. It was observed that the sample of α-D-xylose was crystalline and that the samples of 1,4-β-D-xylobiose and 1,4-β-D-xylotriose were amorphous. Additionally, the 1,4-β-D-xylobiose and 1,4-β-D-xylotriose were found to be hygroscopic.

2.2. Chromatography

This study utilized a Dionex DX 500 Ion Chromatograph with an ED50 amperometric detector (AgCl cell set at T = 303 K), a Carbopac A20 column (3 mm i.d., 150 mm long), and a Carbopac A20 guard column (3 mm i.d., 50 mm long). Both the column and the guard column were thermostatted in an LC25 chromatography oven at T = 303 K. The data collection rate was set at 2.0 Hz and the waveform potentials were: 0.10 V at time t = 0.0; 0.10 V at t = 0.20 s; 0.10 V at t = 0.40 s; −2.00 V at t = 0.41 s; −2.00 V at t = 0.42 s; 0.60 V at t = 0.43 s; −0.10 V at t = 0.44 s; and −0.10 V at t = 0.50 s. The volume of the injection loop was 0.010 cm^3. For α-D-xylose, analyses were performed isocratically using a mobile phase consisting of NaOH(aq) at a concentration c = 0.010 mol · dm^{-3}. After 12 min, the mobile phase was changed to NaOH(aq) at c = 0.40 mol · dm^{-3}. The flow of this solution was continued for 10 min in order to recondition the column. After that time, the mobile phase was changed to NaOH(aq) at c = 0.010 mol · dm^{-3} and continued for at least 10 min. For 1,4-β-D-xylobiose and for 1,4-β-D-xylotriose, analyses were also performed isocratically as was done for α-D-xylose, except that the concentration of NaOH(aq) was 0.049 mol · dm^{-3}. Under these conditions, the approximate retention times of α-D-xylose, 1,4-β-D-xylobiose, and 1,4-β-D-xylotriose were, respectively, 7.5, 6.8, and 8.6 min.

The response factor of xylose was determined on a daily basis. In doing this, only the value of the area of the integrated peak corresponding to xylose was used to calculate the response factor. Also, corrections were made for the mass fractions of both water and the impurity, which was assumed to be D-glucose (see table 1), in the sample of α-D-xylose. This procedure serves to minimize possible systematic errors due to impurities in the samples. The integration of the chromatograms was done as consistently as possible in order to minimize any possible differences between calibration and analyte measurements.

2.3. Solution calorimetry

The solution calorimeter and the associated measurement procedures have been described previously [12]. In test tube experiments, the samples of α-D-xylose(cr), 1,4-β-D-xylobiose(am), and 1,4-β-D-xylotriose(am) were observed to dissolve completely and almost instantaneously when placed in H_2O(l). In this study, additional attention was paid to the change in enthalpy associated with breaking the glass sample bulb into water. Specifically, six measurements of the enthalpy of breaking an empty bulb into water were performed with the following results: (−0.013, −0.195, −0.690, 0.088, −0.027, and −0.011) J. Since, in the third experiment, the bulb was very difficult to break, the value obtained (−0.690 J) is judged to be an outlier. The average of the remaining five measurements is −(0.03 ± 0.09) J. The uncertainty is equal to two estimated standard deviations of the mean. However, there is also some vaporization of water in the solution calorimeter due to the space that is made available by the breaking of the bulb. The enthalpy of vaporization $\Delta_{vap}H$, accompanying the breaking of a bulb is calculated as $n(H_2O) \cdot \Delta_{vap}H^\circ_m$, where $\Delta_{vap}H^\circ_m$ = 44.012 kJ · mol^{-1} [13] is the standard molar enthalpy of vaporization of H_2O(l) at T = 298.15 K. The amount of H_2O(l) that is vaporized is $n(H_2O) = \Delta(pV)/(RT)$ where p is pressure, V is volume, and R is the molar gas constant (8.314472 J · K^{-1} · mol^{-1}). The quantity $\Delta(pV)$ is equal to $p_w \cdot (1.0 - r) \cdot V$(bulb), where p_w is the vapor pressure of H_2O(l) at the temperature at which the experiment is performed, r is the relative humidity of the room (expressed as a fraction) at the time the bulb is sealed, and V(bulb) is the volume of the glass bulb. For the

[1] Certain commercial equipment, instruments, or materials are identified in this paper to specify the experimental procedures adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology (NIST), nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose

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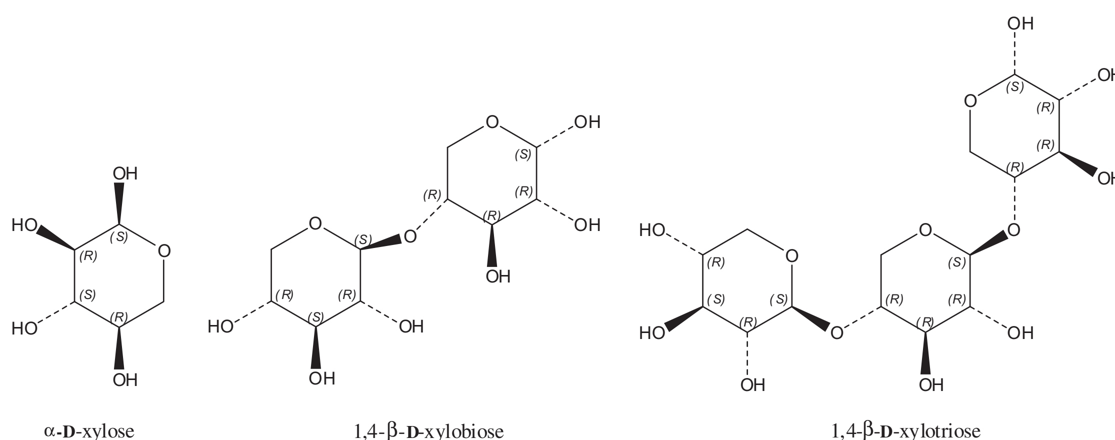

FIGURE 1. The structures of the substances studied herein.

bulb-breaking measurements discussed above, $\Delta_{vap}H = 0.048$ J. Thus, the enthalpy change, after inclusion of the vaporization correction for breaking a bulb, is −(0.08 ± 0.09) J. Note that there is a separate correction that must be applied for the change in the volume and the change in the vapor pressure that accompany the solution process [12].

2.4. Microcalorimetry

Attempts were made to use microcalorimetry to perform enthalpy of solution measurements. The microcalorimeters and the associated measurement procedures have been described previously [14,15]. These calorimeters were calibrated electrically by using a high stability d.c. power supply, calibrated digital voltmeter, standard resistor, and time-interval counter. The electric potential differences U of the thermopiles in the microcalorimeters are measured with Agilent model 34420A Nanovolt Meters. The values of U are then recorded on a microcomputer and the areas A (units of $J \cdot V^{-1} \cdot s^{-1}$) of the thermograms are calculated by numerical integration.

The sample vessels were fabricated from high-density polyethylene. Each vessel had two compartments that hold, respectively, 0.55 cm^3 and 0.40 cm^3 of solution. In order to measure enthalpies of solution, a known mass of α-D-xylose was introduced via a funnel into the 0.40 cm^3 side of the sample vessel. Then, a known mass of water was introduced into the 0.55 cm^3 side of the sample vessel. The sample vessel was then placed in an aluminum block maintained at $T = 298.15$ K for 5 min, as a first step in equilibration, and then into the microcalorimeter. An equilibration time of 60 min in the microcalorimeter was allowed. Following this equilibration, drifts (electric potential difference across the thermopile as a function of time) were taken for 2 min to establish the fore period baseline. This was followed by ten complete rotations of the calorimeter to dissolve the sample in the water. In some experiments, electrical energy was introduced to compensate for the endothermic enthalpy of solution. Approximately, 60 min was allowed for the establishment of a final or after period baseline.

"Blank" enthalpy changes $\Delta_{mix}H$ were measured in control experiments in which water was mixed in a sample vessel that did not contain a solid sample. The average of four measurements gave $\Delta_{mix}H = (2.9 \pm 1.9)$ mJ. Here, the uncertainty is equal to two estimated standard deviations of the mean. Since the measured reaction enthalpy changes Δ_rH were in the range 1.27 J to 1.40 J, the uncertainty in the blank enthalpy change leads to an uncertainty of $0.0015 \cdot \Delta_rH$ in the final results.

Pertinent to the measurement of the enthalpy of solution of α-D-xylose by microcalorimetry is the matter of absorption of water by the α-D-xylose prior to the solution process. Thus, a control experiment was performed in which a sample of α-D-xylose(cr) was allowed to stand over H_2O(l) for 60 min. The mass fraction of H_2O(l) in this sample of α-D-xylose(cr), as measured by Karl Fischer analysis, was $w = (6.0 \pm 0.2) \cdot 10^{-4}$. This is in agreement with the value $w = (6.0 \pm 0.4) \cdot 10^{-4}$ that corresponds to the original sample of α-D-xylose(cr) that had not been allowed to stand over H_2O(l) for 1 h. Similar experiments performed with 1,4-β-D-xylobiose(am) and 1,4-β-D-xylotriose(am) demonstrated that these substances were hygroscopic. This prevents the use of microcalorimetry for the accurate measurement of the enthalpies of solution of these two substances.

2.5. Saturation molality measurements

Saturation molalities of α-D-xylose were measured by preparing two separate solutions each consisting of (crystalline sample + water). The bottles used to hold these solutions had a volume $V \approx 20$ cm^3 and were sealed with Teflon-lined caps. One of these two solutions was placed in a bath thermostatted at $T = 293.15$ K. The other solution was placed in a bath thermostatted at $T = 303.15$ K. The solutions were allowed to equilibrate with a gentle lateral shaking motion (≈100 shakes $\cdot$ min^{-1}). After 5 d, the two bottles were transferred to a thermostat set at $T = 298.15$ K (approximate temperature control ± 0.001 K) and allowed to equilibrate for an additional 9 d to 10 d. In both cases, sufficient crystalline sample was present to insure that a solid phase was always present. The temperature of the bath was measured with a Hewlett-Packard Model 2804A Quartz Thermometer, which had been calibrated against a NIST calibrated platinum resistance thermometer. The possibility of temperature gradients within the bath was examined by placing the quartz thermometer at a few different locations. It was found that any gradients were less than the quality of the temperature control of the bath.

After the final equilibration, a portion of the clear, supernatant solution was taken from each sample bottle and diluted with H_2O(l) in order to avoid any precipitation of the sample, and also to bring the concentrations into a suitable range for measurement by HPLC. The shaking of the samples was stopped at least 1 h prior to the removal of supernatant solution, in order to allow any finely dispersed solids to settle. The removal of supernatant solution was done very carefully by using pipette tips. For α-D-xylose(cr), a set of measurements was also performed in which supernatant solution was first removed with a syringe, and then delivered to the bottle used for the analysis, by passing the solution through a 0.2 μm Millipore Millex-LG hydrophilic polytetrafluoroethylene (PTFE) filter attached to the syringe with a Luer Lock connection. This control

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experiment was performed to minimize the possibility of there being any particles in the analyte solution, and to see if there was any difference between the results obtained using this method of delivering the supernatant solution for analysis, and the method where just a pipette tip was used. The pipette tips, syringes, and Millipore filters used for the sample removal were warmed to ≈320 K in order to avoid the possibility of any precipitation during the removal of the supernatant solutions. The molinities[2] c' of xylose in the aqueous solutions were determined chromatographically (see Section 2.2). In performing these analyses, all solutions were diluted precisely by using gravimetric procedures. The imprecision of the mass differences was $\approx 2 \cdot 10^{-5}$ g. Also, both the calibrant solutions and the analyte solutions were prepared by appropriate dilutions with $H_2O(l)$ so that their respective chromatographic areas were close to each other. Specifically, the maximum difference in the chromatographic areas corresponding to the calibrant and analyte solutions was $0.08 \cdot A$, where A is the chromatographic area corresponding to the calibrant solution(s). Calibration experiments, done with gravimetrically prepared solutions of α-D-xylose in water, showed that the response factor f varied with the xylose concentration. This variation was essentially linear. Since only the chromatographic area A is measured for the analyte solution, one needs to know how the response factor changes with the measured area. Thus, the slope $(\Delta f/\Delta A)$ was also measured and used to obtain values of the response factor f that corresponded to the measured chromatographic areas of the analyte solutions. The molinities of xylose in the analyte solutions were then calculated as $c' = f \cdot A$, where A is the area that corresponds to the analyte solution. The molinities c' of the solutions that had been thermostatted for 9 d to 10 d were then calculated by using the measured molinities of the diluted solution(s) and the known, gravimetrically determined dilution factors. The saturation molalities $m(\text{sat})$ [units of $\text{mol} \cdot (\text{kg} \cdot H_2O)^{-1}$] were then calculated from the measured molinities by first calculating the mass of water $w(H_2O)$ in 1 kg of solution, which is equal to $[1.0\ \text{kg} - c'M_r \cdot (1\ \text{kg solution})]$, where M_r is the relative molar mass of xylose in $\text{kg} \cdot \text{mol}^{-1}$. Then, $m(\text{sat})$ is equal to $c' \cdot (1\ \text{kg solution})/w(H_2O)$.

Control experiments were performed on a daily basis in which distilled water was injected into the HPLC. The areas corresponding to these injections were less than $0.0015 \cdot A$ where A is the chromatographic area associated with either the analyte or calibrant solutions. Control experiments using distilled water that had passed through the 0.2 μm Millipore Millex-LG hydrophilic PTFE filter also gave very small chromatographic areas ($<0.005 \cdot A$).

Following the completion of a saturation molality measurement, the solid phase was removed from its respective solution by filtration with a sintered glass crucible attached to the house vacuum line. The vacuum was continued overnight to remove any excess water from the crystals. These air-dried crystals were then collected and Karl Fischer analyses were performed on them. The results of the Karl Fischer analyses of the air-dried samples permit the calculation of the amount of water in a mole of each sample.

Attempts were made to measure the saturation molalities of 1,4-β-D-xylobiose and of 1,4-β-D-xylotriose at $T = 298.15$ K. After mixing 100 mg of 1,4-β-D-xylobiose and 180 mg of $H_2O(l)$ in a small bottle, it was observed that there was no solid phase present. In an attempt to remove excess water, this solution was allowed to air dry at room temperature ($T \approx 295$ K) for 26 d. This was followed by placement over Drierite for 13 d, followed by storage in a vacuum desiccator for 4 d, and, finally, desiccation over P_2O_5 for 6 d. After this period of time, only a very thick gel was present, but no solid phase. Similarly, for 1,4-β-D-xylotriose, 9 mg of $H_2O(l)$ were added to 7 mg of 1,4-β-D-xylotriose. After equilibration for 2 d with shaking in the constant temperature bath at $T = 298.15$ K, it was observed that there was no solid phase present. The solution was then placed in a vacuum desiccator over P_2O_5. After 6 d, only a very thick gel was present, but no solid phase. A sample of each of these gels was examined using the HPLC methods described above (see Section 2.2). The respective retention times obtained with these samples were the same as those obtained with respective samples of 1,4-β-D-xylobiose and 1,4-β-D-xylotriose that were taken directly from the bottles received from the vendor. Thus, it is concluded that, under the conditions used, it is not possible to obtain a solid phase of either 1,4-β-D-xylobiose or of 1,4-β-D-xylotriose that is in equilibrium with its aqueous solution. The fact that crystals do not form for 1,4-β-D-xylobiose or for 1,4-β-D-xylotriose is consistent with the absence of any crystallographic data for these substances in the literature [17].

2.6. NMR

NMR was used to measure the fractions of the anomeric ring forms in the samples of D-xylose(cr). ^{1}H NMR spectra were recorded at $T = 300$ K, using a Bruker DRX-500 spectrometer equipped with a HCN cryoprobe (o.d. 4.970 ± 0.013 mm). Solutions containing ≈0.030 g of crystalline D-xylose in methylsulfoxide-d_6 (volume $V = 0.5\ \text{Cm}^3$, atom fraction deuterium = 0.999) were used, with tetramethylsilane as an internal chemical shift reference at $\delta = 0$. Sample tubes from the same batch were used for all analyses, and the solvent was stored in a scintillation vial capped with a septum valve. An initial test run was performed for the purpose of probe tuning and magnetic field shimming for a typical sample. The NMR data were acquired by means of the Bruker Topspin program, version 1.3, and processed in version 3.0. The ^{1}H NMR spectra were acquired as 32,768 data points, using 64 scans, a spectral width of 4.01 kHz, a 90° pulse with a time duration of 8.0 μs, and a pulse recycle time of 6 s. The total experiment time was 6 min 31 s. Free induction decays (FIDs) were processed by linear prediction in the forward, complex mode to 65,536 points, and exponential multiplication using a line-broadening of 0.25 Hz. Average times were used for data analysis, which were calculated by adding 3 min and 15.5 s to the NMR experiment start times, which typically fell in the range of 2.5 min to 4.0 min after dissolution of the sample. A further five to seven spectra were acquired during the first 90 min after dissolution, followed by an additional spectrum after 22 h to 24 h.

The HO-1 protons of the various ring forms of D-xylose resonated as doublets at low field, where they were well separated from other proton signals. Therefore, these doublets were used to quantify the ring forms of the sugar. A small, manual fifth-order polynomial baseline correction was applied to the $7.0 \cdot 10^{-6}$ to $5.5 \cdot 10^{-6}$ region, which displayed a strong doublet at $6.13 \cdot 10^{-6}$ due to α-D-xylopyranose, a weak doublet at $6.54 \cdot 10^{-6}$ for β-D-xylopyranose, and even weaker doublets at $5.83 \cdot 10^{-6}$ and $5.79 \cdot 10^{-6}$ that were assigned as D-xylofuranose forms A and B, respectively. The total integral of the four doublets was calibrated as 100%. A local, polynomial baseline correction was then performed on the HO-1 β-D-xylopyranose region to remove the underlying, left-hand wing of the α-D-xylopyranose doublet, thus allowing a more accurate integration of the β-D-xylopyranose doublet. The entire spectrum was then reversed to permit application of the correct, polynomial baseline correction profile to the region containing the D-xylofuranose doublets, thus allowing removal of the underlying, right-hand wing of the α-D-xylopyranose doublet from these signals. The spectrum was then reversed again to its original display, and the D-xylofuranose doublets were integrated. The mole fractions of the β-D-xylopyranose and D-xylofuranose forms A and B at various average times in the first 93 min were then subjected to either linear, or second-order, or fifth-order polynomial regression, depending on the curvature

[2] The term molinity $\{\text{mol} \cdot (\text{kg solution})^{-1}\}$ is very useful for calculations that involve solutions where dilutions have been performed gravimetrically. This quantity has been used in oceanography [16].

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observed in the plots of the fraction of the ring form as a function of time. The intercepts of the fitted straight lines or polynomial curves were then used to determine the concentration of each minor ring form at zero time, which was taken to be a measure of the composition of the D-xylose crystals. Because the HO-1 doublet of α-D-xylopyranose was not integrated separately, its proportion was obtained by difference.

With the knowledge that HPLC had detected D-glucose as a possible impurity in the D-xylose(cr) (see table 1, footnote d), the ^{1}H NMR data acquisition was optimized for detection of this impurity. It had been established previously [18] that the interconversion of α-D-glucopyranose and β-D-glucopyranose does not proceed to a measurable extent during the first 1.5 h after dissolution of either pure, crystalline anomer in methylsulfoxide-d_6. In the present work, at a Larmor frequency of 500 MHz, the ^{1}H chemical shifts of the HO-1 protons of these ring forms were re-measured from a solution of α-D-glucopyranose(cr) (0.030 g, Corn Sugar, L. D. Carlson Co., Kent, Ohio) in methylsulfoxide-d_6 (0.5 cm^3) and were found to be $6.20 \cdot 10^{-6}$ for the major proportion of α-D-glucopyranose present, and $6.57 \cdot 10^{-6}$ for the minor percentage of β-D-glucopyranose. These chemical shifts are in perfect agreement with the values (6.20 and 6.57) $\cdot 10^{-6}$, respectively, determined previously at 90 MHz [18]. The analytical conditions for ^{1}H NMR detection of D-glucose in the vendor's sample of D-xylose(cr) were now modified by increasing the number of signal-averaged scans to 896, corresponding to a total data acquisition time of 90.17 min. Based on start times of 2 min and 5 min after dissolution of the D-xylose(cr) in methylsulfoxide-d_6 for two different runs, an average data acquisition mid-point of 47.6 min was calculated. Improvement of the signal-to-noise ratio permitted some resolution enhancement to be applied, which was implemented by Gaussian multiplication of the FID, using a line-broadening of −0.1 Hz, and an FID truncation fraction of 0.3. As before, local polynomial baseline corrections were applied as needed to the spectral regions of interest. These conditions led to the detection of a weak HO-1 doublet for α-D-glucopyranose at $6.19 \cdot 10^{-6}$ on the side of the very strong doublet for α-D-xylopyranose at $6.13 \cdot 10^{-6}$, as well as an even weaker doublet at $6.23 \cdot 10^{-6}$ that appears to be a very minor impurity of unknown structure.

2.7. Measurement of mass and pH

A Sartorius R160P balance (readability of $1 \cdot 10^{-5}$ g) was used for measurements of mass. The calibration of the balance was checked by using a NIST calibrated set of Class M weights. A radioactive source (NRD Staticmaster, ^{210}Po, 500 μCi), which helps to dissipate electrostatic charge on the object being weighed is kept in the balance during all mass measurements. Also, all objects were allowed to equilibrate for at least 10 min in the balance case prior to use. In making buoyancy corrections, a density $\rho = 1.617\ \mathrm{g \cdot cm^{-3}}$ was used for α-D-xylose(cr) [8] and a density $\rho = 0.997\ \mathrm{g \cdot cm^{-3}}$ was used for H_2O(l) [19].

Measurement of pH was done with a ThermoOrion Model 420 pH meter and a Radiometer combination glass micro-electrode at the temperature at which experiments were performed. The pH meter was calibrated with Radiometer standard buffers that bracketed the pHs of the solutions used in this study. The pHs of the solutions were calculated by using interpolation together with the measured electric potential differences of the electrode and the pHs of the standard buffers.

3. Results and discussion

3.1. Results of saturation molality measurements

The results of the saturation molality measurements are given in table 2. It should be noted that there is agreement in the values of m(sat) obtained from measurements in which the solutions were initially thermostatted at two different temperatures, namely 5 K above and 5 K below the final temperature. This agreement of results obtained by using two different approaches to equilibrium is excellent evidence that equilibrium has been reached. Also, the values of m(sat) obtained with and without the use of the PTFE filter are also in agreement. This lends confidence to the reliability of the removal of the supernatant solution solely by use of a pipette tip. The pooled value of m(sat) is $(8.43 \pm 0.41)\ \mathrm{mol \cdot kg^{-1}}$. The uncertainty in this value is equal to two estimated standard deviations of the mean and is based solely on the random errors associated with the chromatographic measurements. We now consider possible systematic errors in the measurements. Since the HPLC was used solely as a device for comparison of the calibrant and analyte solutions, we believe that all errors that are associated with the chromatography are included in the random error estimate. Other possible systematic errors include weighing errors, sample impurities, and any error in the assignment of the temperature. The smallest critical mass determination involved a mass difference of 0.10 g. If we assume this mass difference to be uncertain by $4 \cdot 10^{-5}$ g, this propagates to an error of 0.007 in the value of m(sat). Similarly, if we assume that our estimate of sample purity (including the moisture determination) is in error by $0.002 \cdot x$, where x is the mole fraction purity of α-D-xylose, this propagates to an error of 0.037 in the value of m(sat). We assume a possible systematic error of 0.01 K in the assigned temperature of 298.15 K. Use of the mole fraction solubilities obtained by Gray *et al.* [5] at T = 293.15 K and T = 303.15 K, leads to a possible systematic error of 0.002 in the value of m(sat) due to a possible error of 0.01 K in the assigned temperature. These estimates of possible systematic error are combined in quadrature together with the statistical uncertainty in the measured value of m(sat), expressed as one estimated standard deviation of the mean, to obtain a combined standard uncertainty [20]. This combined standard uncertainty is then multiplied by two to arrive at the final result: $m(\mathrm{sat}) = (8.43 \pm 0.42)\ \mathrm{mol \cdot kg^{-1}}$. The sensitivity of the value of m(sat) to sample purity and to small weighing errors should be noted. More importantly, an error of only $0.01 \cdot c'(\mathrm{sat})$, where c'(sat) is the molinity at saturation, corresponds to an error of 0.20 in the value of m(sat). Clearly, the major source of error is attributable to the random errors associated with the chromatographic measurements. Expressing the solubility in two other commonly used measures, the mass fraction solubility is (0.559 ± 0.012) and the mole fraction solubility is (0.1318 ± 0.0057). The mass fraction moisture content of the crystals in equilibrium with the saturated solution (see Section 2.5) was w = (0.0024 ± 0.0007). On this basis, we conclude that the stable phase of α-D-xylose(cr) in equilibrium with the saturated solution is the anhydrous form.

TABLE 2
Saturation molalities m(sat) of α-D-xylose(cr) at T = 298.15 K[a].

$m(\mathrm{sat})^b/(\mathrm{mol \cdot kg^{-1}})$	$m(\mathrm{sat})^c/(\mathrm{mol \cdot kg^{-1}})$	$\langle m(\mathrm{sat})\rangle^d/(\mathrm{mol \cdot kg^{-1}})$
8.37 ± 0.52 [e]	8.42 ± 0.34 [e]	8.40 ± 0.43 [e]
8.61 ± 0.43	8.29 ± 0.32	8.45 ± 0.38
$\langle m(\mathrm{sat})\rangle = (8.43 \pm 0.41)\ \mathrm{mol \cdot kg^{-1}}$		

[a] The uncertainties are equal to two estimated standard deviations of the mean and are based solely on the random errors associated with the chromatographic measurements.
[b] Solution initially thermostatted at T = 303.15 K. The final pH of this solution was 3.25.
[c] Solution initially thermostatted at T = 293.15 K. The final pH of this solution was 3.23.
[d] Average values obtained from results in columns 1 and 2.
[e] Result obtained from experiments in which a 0.2 μm Millipore filter was used for the removal of the supernatant solution above the α-D-xylose crystals (see Section 2.5).

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3.2. Results of calorimetry measurements

The results of the solution calorimetry measurements are shown in table 3. Two series of experiments were performed involving α-D-xylose(cr), the principal difference between them being the presence of aldose 1-epimerase in series no. 2. This enzyme was added to accelerate the conversion of the α-D-xylose(aq) to the equilibrium mixture of the α- and β-forms [22]. Some information on the rate of conversion of the α- to the β-form was obtained by using microcalorimetry. Specifically, figure 2 shows a plot of the electric potential difference U of the microcalorimeter's thermopile as a function of time. This plot corresponds to the enthalpy of solution of α-D-xylose(cr) in H_2O(l). It is seen that the large endothermic peak, due to the enthalpy of solution, dwarfs (see figure 2a) the hardly visible small peak due to the enthalpy change for the mutarotation of α- to β-D-xylose(aq). Upon expansion of the scale (see figure 2b), the exothermic peak is seen. Additional microcalorimetry experiments were carried out in which the enzyme aldose 1-epimerase was present at a mass fraction of $8.6 \cdot 10^{-6}$ and the molality of D-xylose(aq) was $m = 0.21\ \text{mol} \cdot \text{kg}^{-1}$. The exothermic peak was not seen in these experiments.

For α-D-xylose(cr), the results obtained using the microcalorimeters are: $\Delta_{sol}H_m = (11.39 \pm 0.23)\ \text{kJ} \cdot \text{mol}^{-1}$ for $\langle m \rangle = 0.2010\ \text{mol} \cdot \text{kg}^{-1}$ and pH 4.63 in the absence of aldose 1-epimerase; and $\Delta_{sol}H_m = (11.19 \pm 0.30)\ \text{kJ} \cdot \text{mol}^{-1}$ for $\langle m \rangle = 0.2127\ \text{mol} \cdot \text{kg}^{-1}$ and pH 5.56 in the presence of aldose 1-epimerase. The results obtained by using the solution calorimeter are: $\Delta_{sol}H_m = (12.21 \pm 0.09)\ \text{kJ} \cdot \text{mol}^{-1}$ for $\langle m \rangle = 0.0347\ \text{mol} \cdot \text{kg}^{-1}$ and pH 4.91 in the absence of aldose 1-epimerase; and $\Delta_{sol}H_m = (12.00 \pm 0.20)\ \text{kJ} \cdot \text{mol}^{-1}$ for $\langle m \rangle = 0.0172\ \text{mol} \cdot \text{kg}^{-1}$ and pH 5.53 in the presence of aldose 1-epimerase. The uncertainties given here are based on two estimated standard deviations of the mean and do not include any allowance for possible systematic errors. However, the solution calorimeter has the clear advantage that the solid sample is completely isolated from the solution prior to sample introduction. Also, the mixing is much more complete than in the microcalorimeters. In fact, incomplete mixing might explain the lower values of $\Delta_{sol}H_m$ obtained with the microcalorimeters. On this basis, we judge the measurements obtained with the solution calorimeter to be more accurate than those obtained with the microcalorimeters, and will use only the solution calorimeter results in the discussion that follows.

TABLE 3
Molar enthalpies of solution of α-D-xylose(cr), 1,4-β-D-xylobiose(am), and 1,4-β-D-xylotriose(am)[a,b,c,d].

Experiment no.	w/g	T/K	ΔT(corr)/K	$E_s/(\text{J} \cdot \text{K}^{-1})$	ΔH(elect)/J	$\Delta_{vap}H$/J	$\Delta_{sol}H$/J	$m/(\text{mol} \cdot \text{kg}^{-1})$	$\Delta_{sol}H_m(T)/(\text{kJ} \cdot \text{mol}^{-1})$	$\Delta_{sol}H_m(T = 298.15\ \text{K})/\text{kJ} \cdot \text{mol}^{-1}$
					α-D-Xylose(cr) – series 1					
1	0.52485	298.09	0.010801	445.53	47.269	0.031	42.559	0.03507	12.174	12.179
2	0.52128	298.13	0.011270	445.52	47.273	0.031	42.295	0.03483	12.198	12.200
3	0.50410	298.03	0.012833	445.16	47.275	0.031	41.604	0.03368	12.409	12.420
4	0.53730	298.13	0.008138	445.15	47.290	0.031	43.709	0.03590	12.23	12.232
5	0.53020	298.18	0.011132	445.43	47.309	0.031	42.393	0.03542	12.021	12.018
6	0.50230	298.09	0.014590	446.59	47.317	0.031	40.843	0.03356	12.226	12.231
7	0.51512	298.14	0.012686	443.33	47.315	0.031	41.733	0.03442	12.181	12.182
					$\langle \Delta_{sol}H_m(T = 298.15) \rangle = (12.21 \pm 0.09)\ \text{kJ} \cdot \text{mol}^{-1}$ for $\langle m \rangle = 0.0347\ \text{mol} \cdot \text{kg}^{-1}$ and pH 4.91					
					α-D-Xylose(cr) – series 2[b]					
1	0.26617	298.04	0.004426	445.29	23.859	0.031	21.930	0.01777	12.403	12.414
2	0.25160	298.07	0.00887	444.81	23.882	0.031	19.979	0.01680	11.957	11.965
3	0.25801	298.07	0.007894	444.40	23.869	0.031	20.403	0.01722	11.907	11.915
4	0.25323	298.09	0.009065	443.63	23.593	0.031	19.614	0.01691	11.664	11.669
5	0.25677	298.12	0.007411	443.62	23.875	0.031	20.629	0.01714	12.097	12.100
6	0.25430	298.14	0.007747	444.25	23.589	0.031	20.189	0.01698	11.955	11.955
					$\langle \Delta_{sol}H_m(T = 298.15) \rangle = (12.00 \pm 0.20)\ \text{kJ} \cdot \text{mol}^{-1}$ for $\langle m \rangle = 0.0172\ \text{mol} \cdot \text{kg}^{-1}$ and pH 5.53					
					1,4-β-D-Xylobiose(am)[b]					
1	0.03142	298.26	0.002081	442.54	0	0.044	−0.832	0.00112	−7.47	−7.48
2	0.03294	298.19	0.001743	443.83	0	0.044	−0.684	0.00117	−5.86	−5.87
3	0.05063	298.15	0.004143	443.62	0	0.044	−1.748	0.00180	−9.75	−9.75
4	0.06556	298.34	0.005645	444.11	0	0.044	−2.418	0.00233	−10.41	−10.43
5	0.06975	298.41	0.003935	444.54	0	0.044	−1.660	0.00248	−6.72	−6.74
					$\langle \Delta_{sol}H_m(T = 298.15) \rangle = -(8.1 \pm 1.8)\ \text{kJ} \cdot \text{mol}^{-1}$ for $\langle m \rangle = 0.0018\ \text{mol} \cdot \text{kg}^{-1}$ and pH 6.23					
					1,4-β-D-Xylotriose(am)[b]					
1	0.02192	298.39	0.002897	444.28	0	0.044	−1.198	0.000530	−22.64	−22.66
2	0.02873	298.40	0.004387	444.39	0	0.044	−1.860	0.000695	−26.83	−26.85
3	0.02725	298.44	0.003478	444.49	0	0.044	−1.457	0.000659	−22.15	−22.18
4	0.02916	298.40	0.004144	443.33	0	0.044	−1.748	0.000705	−24.84	−24.86
5	0.03454	298.40	0.004705	444.21	0	0.044	−2.001	0.000836	−24.00	−24.03
					$\langle \Delta_{sol}H_m(T = 298.15) \rangle = -(24.1 \pm 1.7)\ \text{kJ} \cdot \text{mol}^{-1}$ for $\langle m \rangle = 0.00069\ \text{mol} \cdot \text{kg}^{-1}$ and pH 5.15					

[a] The quantities are: w is the mass (in vacuum) of the sample used in the experiment after correction for moisture; T is the assigned reaction temperature; ΔT(corr) is the corrected temperature change; E_s is the energy equivalent; ΔH(elect) is the electrical energy introduced into the calorimeter during the dissolution reaction; $\Delta_{vap}H$ is the enthalpy change for the (vaporization + condensation) of water; $\Delta_{sol}H$ is the measured enthalpy change; m is the final molality of the solution; $\Delta_{sol}H_m(T)$ is the molar enthalpy of solution at T; and $\Delta_{sol}H_m$(298.15 K) is the molar enthalpy of solution at T = 298.15 K.

[b] Aldose 1-epimerase was used in the measurements involving α-D-xylose(cr) (series 2), 1,4-β-D-xylobiose(am), and 1,4-β-D-xylotriose(am). In each case, 0.070 cm^3 of the solution containing aldose 1-epimerase was added to the 100 cm^3 of H_2O(l) in the calorimeter. In the final solutions, the mass fraction of aldose 1-epimerase was $3.5 \cdot 10^{-6}$ and the molality of $(NH_4)_2SO_4$ was $0.0028\ \text{mol} \cdot \text{kg}^{-1}$.

[c] The following values (all are at T = 298.15 K) and sources of the auxiliary data used in making various corrections are: density of H_2O(l) ($\rho = 0.99702\ \text{g} \cdot \text{cm}^{-3}$) and vapor pressure of H_2O(l) (p = 0.031687 bar), Haar *et al.* [19]; density of α-D-xylose(cr), ($\rho = 1.617\ \text{g} \cdot \text{cm}^{-3}$), Goldberg and Tewari [8]; standard molar enthalpy of vaporization of H_2O(l) ($\Delta_{vap}H^{\circ}_m = 44.012\ \text{kJ} \cdot \text{mol}^{-1}$), Wagman *et al.* [13]; osmotic coefficient of D-xylose(aq) (ϕ = 1.0005 at $m = 0.035\ \text{mol} \cdot \text{kg}^{-1}$), Uedaira and Uedaira [21]; and the change in the standard molar volume ($\Delta_r V^{\circ}_m = -3.96\ \text{cm}^3 \cdot \text{mol}^{-1}$) and the change in the standard molar heat-capacity ($\Delta_r C^{\circ}_{p,m} = 95\ \text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$) for the dissolution of α-D-xylose(cr) in H_2O(l), Goldberg and Tewari [8].

[d] The uncertainties in the values of $\langle \Delta_{sol}H_m(298.15\ \text{K}) \rangle$ are equal to two estimated standard deviations of the mean.

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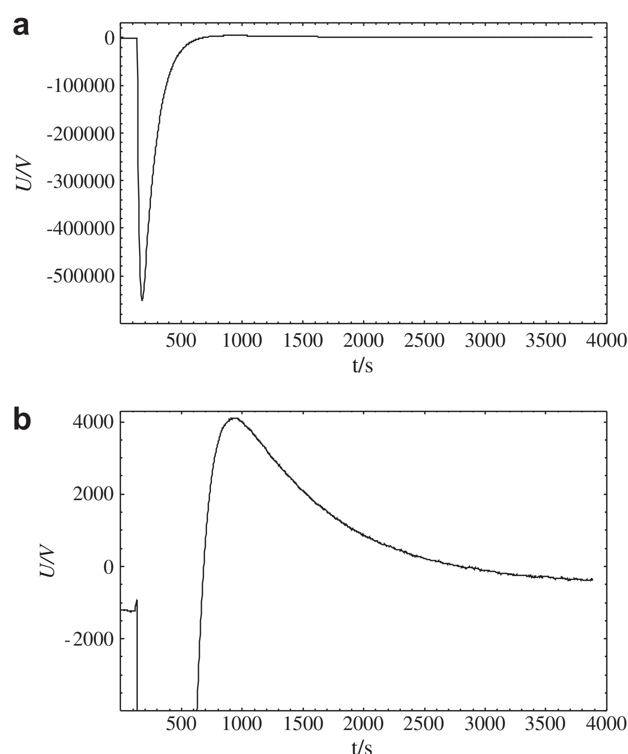

FIGURE 2. Plot "a" shows the electric potential difference U of the microcalorimeter's thermopile as a function of time for the enthalpy of solution of D-xylose(cr) in $H_2O(l)$. Plot "b" is the same as plot "a" except that the "U/V" axis has been changed to show additional detail.

We judge that reasonable estimates of possible systematic error in the value of $\Delta_{sol}H_m$ at $T = 298.15$ K for α-D-xylose(cr) are: $\pm 0.0001 \cdot \Delta_{sol}H_m$ due to weighing; $\pm 0.002 \cdot \Delta_{sol}H_m$ due to sample impurities including water; $\pm 0.0007 \cdot \Delta_{sol}H_m$ due to the calorimetry; and $\pm 0.002 \cdot \Delta_{sol}H_m$ due to the vaporization and bulb breaking corrections. For 1,4-β-D-xylobiose(am) and for 1,4-β-D-xylotriose(am), we judge that reasonable estimates of possible systematic error in the value of $\Delta_{sol}H_m$ at $T = 298.15$ are: $\pm 0.0001 \cdot \Delta_{sol}H_m$ due to weighing; $\pm 0.10 \cdot \Delta_{sol}H_m$ due to sample impurities including water; $\pm 0.001 \cdot \Delta_{sol}H_m$ due to the calorimetry; and $0.08 \cdot \Delta_{sol}H_m$ due to the vaporization and bulb breaking corrections. These estimates of possible systematic error are combined in quadrature together with the statistical uncertainties in the value of $\Delta_{sol}H_m$, expressed as one estimated standard deviation of the mean, to obtain combined standard uncertainties [20]. These combined standard uncertainties are then multiplied by two to arrive at the following values for α-D-xylose(cr): $\Delta_{sol}H_m = (12.21 \pm 0.10)$ kJ $\cdot$ mol^{-1} (series 1) at $T = 298.15$ K, $m = 0.0347$ mol $\cdot$ kg^{-1}, and pH 4.91; and $\Delta_{sol}H_m = (12.00 \pm 0.21)$ kJ $\cdot$ mol^{-1} (series 2) at $T = 298.15$ K, $m = 0.0172$ mol $\cdot$ kg^{-1}, and pH 5.53. It is seen that essentially all of the uncertainty in the measurements involving α-D-xylose(cr) is attributable to the random errors in the solution calorimetry measurements.

The result for 1,4-β-D-xylobiose(am) with expanded uncertainties is $\Delta_{sol}H_m = -(8.1 \pm 2.7)$ kJ $\cdot$ mol^{-1} at $T = 298.15$ K, $m = 0.0018$ mol $\cdot$ kg^{-1}, and pH 6.23. The result with expanded uncertainties for 1,4-β-D-xylotriose(am) is $\Delta_{sol}H_m = -(24.1 \pm 6.4)$ kJ $\cdot$ mol^{-1} at $T = 298.15$ K, $m = 0.00069$ mol $\cdot$ kg^{-1}, and pH 5.15.

3.3. Results of NMR measurements

The mole fractions x of the anomeric ring forms in the D-xylose samples were measured by ^{1}H NMR spectroscopy of methylsulfoxide-d_6 solutions at 500 MHz, by using the HO-1 signals of these forms as analytical handles (see Section 2.6). Interconversion (mutarotation) of the anomeric pyranose forms is slow in this solvent, as exemplified by little observable change in the proportion of β-D-xylopyranose during the first 1.5 h after dissolution of the D-xylose crystals. However, the HO-1 signals of α- and β-D-xylofuranose were also detected during this period, and their mole fractions increased quite rapidly to $x = 0.001$ to 0.002, with the species having an HO-1 doublet at higher field predominating at longer times. Nevertheless, extrapolation of the concentrations of these furanose forms to zero time indicated that they start from very low levels ($x \leqslant 0.0001$). Therefore, we conclude that they are not present in the D-xylose crystals to any significant extent. The mole fraction of β-D-xylopyranose was $x = (0.0062 \pm 0.0004)$ for the sample of D-xylose(cr) as received from the vendor and $x = (0.0050 \pm 0.0005)$ for the sample of D-xylose(cr) that had been equilibrated with a saturated, aqueous solution of the sugar. For the vendor's sample of D-xylose(cr), optimization of the NMR analysis for detection of a low-level α-D-glucopyranose impurity led to the following mole fractions obtained by signal averaging during the first 94 min after dissolution (mid-point 47.6 min): β-D-xylopyranose (0.00641 ± 0.00002); unknown impurity (0.00015 ± 0.00002); α-D-glucopyranose (0.00032 ± 0.00003); D-xylofuranose A (see Section 2.6), 0.00094 ± 0.00003; and D-xylofuranose B (0.00032 ± 0.00001). A data acquisition during the second 94 min after dissolution (mid point 138.75 min), yielded the following mole fractions: β-D-xylopyranose (0.00648 ± 0.00008); unknown impurity (0.00016 ± 0.00004); α-D-glucopyranose (0.00037 ± 0.00003); D-xylofuranose A (0.00248 ± 0.00005); and D-xylofuranose B (0.00143 ± 0.00004). Comparison of the two sets of data indicates that the proportions of β-D-xylopyranose, unknown impurity, and α-D-glucopyranose do not change significantly from the first data acquisition period to the second, but that D-xylofuranoses A and B increase substantially.

These results allow us to assign α-D-xylose(cr) as the form to be specified in writing the solution process. For the aqueous solution, D-xylose is a mixture of α-pyranose, β-pyranose, α-furanose, and β-furanose forms. At $T = 304.15$ K, the mole fraction of α-pyranose(aq) is 0.365 and the mole fraction of β-pyranose(aq) is 0.63. The mole fraction of the aqueous furanose forms is less than 0.01 [22].

3.4. Standard state quantities

For the solution process

$$\alpha\text{-D-xylose(cr)} = \text{D-xylose(aq)}, \tag{1}$$

the standard molar Gibbs free energy of solution

$$\Delta_{sol}G^{\circ}_{m} = -RT\ln\{m(\text{sat})\cdot\gamma\}, \tag{2}$$

where R is the gas constant and γ is the activity coefficient of D-xylose(aq). In reaction (1), D-xylose(aq) is taken to denote the equilibrium mixture of the various aqueous forms of D-xylose (see Section 3.3). The standard state is taken to be the hypothetical solution of unit molality ($m^{\circ} = 1$ mol $\cdot$ kg^{-1}). The standard pressure $p^{\circ} = 0.1$ MPa. Since the pK of D-xylose(aq) is 12.29 [23] at $T = 298.15$ K, there is no need to consider any aqueous species other than xylose^0(aq) at the pHs used in this study. We estimate the value of γ by using the parameter of the excess Gibbs free energy ($g_1 = 81.523$ J $\cdot$ kg^{-1} $\cdot$ mol^{-1}) in the equation given by Goldberg and Tewari [8] (see Section 3 in reference [8]) for G^{ex}_2, the excess Gibbs free energy of the solute, and the relation $\gamma = \exp[G^{ex}_2/(RT)]$. The value of g_1 was calculated by Goldberg and Tewari [8] (see their table 6) from the osmotic coefficients of D-xylose(aq) measured by Uedaira and Uedaira [21]. This estimation of γ involves a long extrapolation from the highest molality ($m = 3.467$ mol $\cdot$ kg^{-1}) used by Uedaira and Uedaira [21] to the saturation molality

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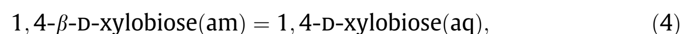
$m = 8.43\ \mathrm{mol \cdot kg^{-1}}$. By using equation (2), we obtain $\Delta_{sol}G^{\circ}_{m} = -6.66\ \mathrm{kJ \cdot mol^{-1}}$. A major uncertainty in this value lies in the extrapolated value of γ, which we assume to be uncertain by ±0.5. This uncertainty together with that in the value of m(sat) leads to $\Delta_{sol}G^{\circ}_{m} = -(6.7 \pm 0.9)\ \mathrm{kJ \cdot mol^{-1}}$ for the solution process (1) at $T = 298.15$ K.

The standard molar enthalpy of solution is given by

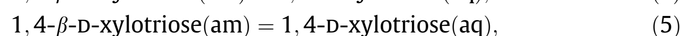
$$\Delta_{sol}H^{\circ}_{m} = \Delta_{sol}H_{m} - L_{\phi}, \quad (3)$$

where L_{ϕ} is the relative apparent molar enthalpy at the molality of interest. We calculate $L_{\phi} = 12\ \mathrm{J \cdot mol^{-1}}$ for $m = 0.0347\ \mathrm{mol \cdot kg^{-1}}$ and $L_{\phi} = 6\ \mathrm{J \cdot mol^{-1}}$ for $m = 0.0172\ \mathrm{mol \cdot kg^{-1}}$ by using the parameters of the excess enthalpy calculated by Goldberg and Tewari [8] (see their table 6) from the enthalpies of dilution of D-xylose(aq) measured by Barone *et al.* [24]. By using equation (3), we obtain $\Delta_{sol}H^{\circ}_{m} = (12.20 \pm 0.10)\ \mathrm{kJ \cdot mol^{-1}}$ (experiments without aldose 1-epimerase) and $\Delta_{sol}H^{\circ}_{m} = (11.99 \pm 0.21)\ \mathrm{kJ \cdot mol^{-1}}$ (experiments with aldose 1-epimerase). We adopt the average of these results, $\Delta_{sol}H^{\circ}_{m} = (12.10 \pm 0.12)\ \mathrm{kJ \cdot mol^{-1}}$. Combination of the values of $\Delta_{sol}G^{\circ}_{m}$ and $\Delta_{sol}H^{\circ}_{m}$ leads to the standard molar entropy change $\Delta_{sol}S^{\circ}_{m} = (63.0 \pm 2.9)\ \mathrm{J \cdot K^{-1} \cdot mol^{-1}}$ for the solution process (1).

For the solution processes:



$$1,4\text{-}\beta\text{-D-xylobiose(am)} = 1,4\text{-D-xylobiose(aq)}, \quad (4)$$



$$1,4\text{-}\beta\text{-D-xylotriose(am)} = 1,4\text{-D-xylotriose(aq)}, \quad (5)$$

the values of L_{ϕ} are negligible in comparison to the values of $\Delta_{sol}H_{m}$. Therefore, we take $\Delta_{sol}H^{\circ}_{m} = \Delta_{sol}H_{m}$.

3.5. Comparison with results from the literature

Results from the literature that lead to values of m(sat) for α-D-xylose(cr) are shown in table 4. Gabas *et al.* [6] and Gray *et al.* [5] used refractometry in their measurements. Tewari [7] and Zhang *et al.* [4] used HPLC. Jónsdóttir *et al.* [10] relied on evaporation to dryness. Jacobsen [9] used HPLC and evaporation to dryness for her measurements. It appears that all of these earlier studies relied upon waiting for a sufficiently long time in order to assert that equilibrium had been achieved. Zhang *et al.* [4] reported their solubilities in units of $\mathrm{g \cdot dm^{-3}}$. However, in the absence of the densities of the saturated solutions, it is not possible to rigorously calculate values of m(sat) from their [4] results. Thus, values of solubilities s in units of $\mathrm{mol \cdot dm^{-3}}$ that have been calculated from their [4] results are also given in table 4. However, densities of aqueous D-xylose solutions have been reported by Uedaira and Uedaira [21] up to $m = 2.9975\ \mathrm{mol \cdot kg^{-1}}$. We obtain an estimated density $\rho = 1.16$ by extrapolation of the densities reported by Uedaira and Uedaira [21]. This estimated density is then used to calculate the value $m(\mathrm{sat}) = 4.44\ \mathrm{mol \cdot kg^{-1}}$ at $T = 298$ K from the value of the solubility reported by Zhang *et al.* [4]. This value is in discord with the other values of m(sat) given in table 4. In any case, it is seen that our result $m(\mathrm{sat}) = (8.43 \pm 0.42)\ \mathrm{mol \cdot kg^{-1}}$ is in good agreement with the results obtained by Gabas *et al.* [6], Tewari [7], and Gray *et al.* [5]. If one assumes that the activity coefficient of saturated solutions of D-xylose(aq) is independent of temperature, one can calculate $\Delta_{sol}H^{\circ}_{m}$ from the temperature dependence of m(sat). Performing this calculation, we obtain the following values of $\Delta_{sol}H^{\circ}_{m}$: $(16.4 \pm 1.7)\ \mathrm{kJ \cdot mol^{-1}}$ from the study of Jacobsen [9]; $(16.5 \pm 1.7)\ \mathrm{kJ \cdot mol^{-1}}$ from the study of Jónsdóttir *et al.* [10]; $(15.8 \pm 0.2)\ \mathrm{kJ \cdot mol^{-1}}$ from the study of Jacobsen [9]; and $(15.9 \pm 0.4)\ \mathrm{kJ \cdot mol^{-1}}$ from the study of Zhang *et al.* [4]. In calculating a value of $\Delta_{sol}H^{\circ}_{m}$ from the data of Zhang *et al.* [4], it was also necessary to assume that the density of the saturated solution is independent of temperature.

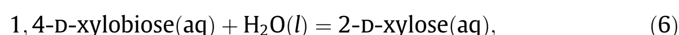
The only result in the literature for $\Delta_{sol}H_{m}$ for α-D-xylose(cr) is that of Jasra and Ahluwalia [25] who reported $\Delta_{sol}H_{m} = (11.98 \pm 0.05)\ \mathrm{kJ \cdot mol^{-1}}$. They [25] also reported $\Delta_{sol}H_{m} = (12.19 \pm 0.08)\ \mathrm{kJ \cdot mol^{-1}}$ for L-xylose(cr). Both values pertain to $T = 298.15$ K. Their measurements were made at low molalities and no dilution corrections were applied to their values. Their results are in excellent agreement with the value of $\Delta_{sol}H^{\circ}_{m} = (12.07 \pm 0.12)\ \mathrm{kJ \cdot mol^{-1}}$ at $T = 298.15$ obtained in this study. The results for 1,4-β-D-xylobiose and for 1,4-β-D-xylotriose are the first to be reported in the literature.

TABLE 4
Saturation molality m(sat) and solubility s results for α-D-xylose(cr) from the literature.

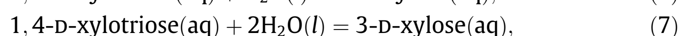
Workers	T/K	$m(\mathrm{sat})/(\mathrm{mol \cdot kg^{-1}})$
Gabas *et al.* [6]	298.15	8.18
Tewari [7]	298.15	8.3
Jacobsen [9]	298.15	9.11
	310.65	11.86
	316.15	12.69
	320.65	14.85
Jónsdóttir *et al.* [10]	298.15	9.23
	308.15	9.89
	318.15	11.88
	328.15	14.58
	338.15	18.34
	348.15	23.65
Gray *et al.* [5]	293.15	7.40
	298.15	8.26
	303.15	9.16
This study	298.15	8.43
	T/K	$s/(\mathrm{mol \cdot dm^{-3}})$
Zhang *et al.* [4]	298	3.08
	315	4.32
	335	5.68
	356	7.59
	377	10.9
	396	13.9
	417	17.5
	438	24.0
	456	28.2

3.6. Calculation of standard molar formation properties

The standard molar enthalpy of formation $\Delta_{f}H^{\circ}_{m}$ for α-D-xylose(cr) can be calculated by using Domalski's [26] selected value for the standard molar enthalpy of combustion $\Delta_{c}H^{\circ}_{m} = -559\ \mathrm{kcal \cdot mol^{-1}} = -2338.86\ \mathrm{kJ \cdot mol^{-1}}$ and which is based on the value obtained by Skuratov *et al.* [27]. Then, with the standard molar entropy $S^{\circ}_{m} = 143.5\ \mathrm{J \cdot K^{-1} \cdot mol^{-1}}$ obtained by Miller [28] from heat-capacity measurements performed from $T = 100$ K to $T = 298$ K, one obtains the standard molar Gibbs free energy of formation $\Delta_{f}G^{\circ}_{m}$ for α-D-xylose(cr). By using our measured values of $\Delta_{sol}H^{\circ}_{m}$ and $\Delta_{sol}G^{\circ}_{m}$ for α-D-xylose(cr), the values of $\Delta_{f}H^{\circ}_{m}$, $\Delta_{f}H^{\circ}_{m}$, and S°_{m} for D-xylose(aq) can be calculated. Next, with the values of $\Delta_{r}H^{\circ}_{m}$ and of $\Delta_{r}H^{\circ}_{m}$ obtained by Tewari *et al.* [29] for the reactions



$$1,4\text{-D-xylobiose(aq)} + H_2O(l) = 2\text{-D-xylose(aq)}, \quad (6)$$



$$1,4\text{-D-xylotriose(aq)} + 2H_2O(l) = 3\text{-D-xylose(aq)}, \quad (7)$$

one obtains values of $\Delta_{f}G^{\circ}_{m}$, $\Delta_{f}H^{\circ}_{m}$, and S°_{m} for 1,4-D-xylobiose(aq) and 1,4-D-xylotriose(aq). In these calculations, we used the standard molar formation properties of H_2O(l), O_2(g), and CO_2(g) from the CODATA Tables [30]. Finally, use of the values of $\Delta_{sol}H^{\circ}_{m}$ for 1,4-β-D-xylobiose(am) and for 1,4-β-D-xylotriose(am) obtained in this study yields values of $\Delta_{f}H^{\circ}_{m}$ for these latter two substances. The calculated values of the standard molar formation properties are given in table 5. Clearly, there is a need for a modern determination of the third-law entropy of α-D-xylose(cr). Also, a modern,

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TABLE 5
Selected values of the standard molar enthalpies of formation $\Delta_f H^\circ$, standard molar Gibbs free energies of formation $\Delta_f G$, standard molar entropies S°_m, standard molar heat capacities $C^\circ_{p,m}$, and relative molecular masses M_r for the substances of interest to this study at T = 298.15 K and p = 0.1 MPa.[a]

Substance and state	Formula	M_r	$\Delta_f H^\circ/(kJ \cdot mol^{-1})$	$\Delta_f G^\circ/(kJ \cdot mol^{-1})$	$S^\circ_m/(J \cdot K^{-1} \cdot mol^{-1})$	$C^\circ_{p,m}/(J \cdot K^{-1} \cdot mol^{-1})$
α-D-Xylose(cr)	$C_5H_{10}O_5$	150.13	−1057.84	−744.35	143.50	184.0
D-Xylose(aq)	$C_5H_{10}O_5$	150.13	−1045.74	−751.05	206.56	279.0
1,4-β-D-Xylobiose(am)	$C_{10}H_{18}O_9$	282.25	−1797.68			
1,4-D-Xylobiose(aq)	$C_{10}H_{18}O_9$	282.25	−1805.78	−1246.90	282.21	
1,4-β-D-Xylotriose(am)	$C_{15}H_{26}O_{13}$	414.36	−2541.53			
1,4-D-Xylotriose(aq)	$C_{15}H_{26}O_{13}$	414.36	−2565.63	−1742.75	358.47	

[a] The standard state for the solute is the hypothetical ideal solution of unit molality (m° = 1 mol · kg^{-1}) and the standard state for the solvent is the pure solvent; the standard pressure p° = 0.1 MPa. Additional numbers of significant figures are included in the values of the formation properties in order to preserve the integrity of the values of calculated properties. The values of $C^\circ_{p,m}$ are from Tewari *et al.* [29].

careful measurement of $\Delta_c H^\circ_m$ for α-D-xylose(cr) should be superior to the earlier studies done by Skuratov *et al.* [27], Karrer and Fioroni [31], and Berthelot [32].

Acknowledgment

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