Novel in Situ Device for Measuring Solubilities

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A novel in situ device for measuring solubilities of moderately soluble substances such as high-molecular-weight carbohydrates is described. A known sample mass is added to a thermostated cell that is agitated and heated at a steady rate, and the refractive index is monitored until a sudden change in slope is observed, signaling total dissolution of all solids. Two versions of the device are described: one contained about 30 mL of solution, and the second one contained about 3 mL of solution for use with small amounts of solids (30−200 mg). Solubilities measured with both in situ devices are virtually identical and agree closely with literature data; the average deviation from literature values is less than ±0.2 wt % for both devices. Data, collected at two heating rates (3.9 ± 1.7 and 10.2 ± 2.1 °C/h), indicate that the fast heating rate gives solubilities statistically identical with those given by the slow heating rate, meaning that reliable data can be collected at the fast rate within 3 h. This new device requires significantly shorter times and less solid consumption than the classical batch sampling approach. It is also better suited for high-temperature measurements than the classical batch sampling approach commonly used to determine carbohydrate solubilities.

Introduction

The solubilities of solids in liquids are important to many processes. For example, food industry extraction processes require solubility information to determine the best conditions for separations.[1] In the pharmaceutical industry, it is very important to know the solubility of drugs in the body for pharmakinetic studies.[2] Our research is focused on converting lignocellulosic biomass into valuable products, and understanding the solubility of sugar oligomers would help us determine the role they play in hydrolysis of carbohydrate polymers.[3] Unfortunately, these oligomers are commercially available only in small amounts and are difficult to prepare from biomass, so it is necessary to perform experiments with a minimum amount of sugar.

There are two classes of experimental methods for determining the solubility of sparsely soluble materials: analytical and synthetic methods. In an analytical method, excess solute is added to the solvent and the system is kept at a constant temperature. Then the system is sampled periodically until equilibrium is reached, i.e., the sample concentration no longer changes with time.[4] An example of this approach was used by Peres and Macedo to measure the solubility of D-glucose in water and water/ethanol mixtures. In their study, 80 cm^3 glass cells were filled with a known volume of solvent, and glucose was added in excess of the expected solubility. The cells had a thermostatically controlled water jacket to maintain temperature, and the sugar and solvent mixture was agitated by magnetic stir bars and a stir plate for 48 h. Then the mixture was left to settle for 24 h. During sampling, heated pipets were used to remove samples that were dried to determine the concentration.[5]

This common approach to measuring solid/liquid solubilities has important limitations. For example, a sugar/water mixture must be held at a given temperature and sampled repeatedly to determine when equilibrium is reached. Unless the experiment is carried out under aseptic conditions, microbes may consume sugar. Thus, it is desirable to collect data as quickly as possible. Sampling requires three main steps: removing an aliquot, separating the solution from residual solids in that aliquot, and measuring the concentration of the solution in that aliquot. Each step introduces opportunities for error. In the first step, the mixture can cool as an aliquot is extracted on a pipet tip unless the tip is at the same temperature. If the pipet tip is heated, it could heat some of the mixture and dissolve more solids. In addition, if the mixture is hot, liquid in the aliquot may evaporate slightly, increasing the sugar concentration relative to that of the solution it should represent. Furthermore, it is necessary to separate the liquor from residual solids by filtration, centrifugation, or settling. The first two of these methods are likely to cool the aliquot, precipitating dissolved solids, and cooling is likely to be more of a problem at higher temperatures. These temperature effects are expected to be larger for solubility measurements conducted in small-volume instruments because small aliquots have a greater surface-area-to-volume ratio and will cool more rapidly. Thus, the analytical method is difficult to miniaturize.

The second approach to determining solubility is the synthetic method. In one version of this method, known quantities of solute and solvent are added to a vessel, and the temperature is increased until the solids dissolve. Sousa used this approach to measure the solubilities of potassium hydrogen tartrate in water/ethanol mixtures. In this study, a known amount of solute and water was added to a closed vessel which was immersed in a water bath and the temperature was slowly increased until the solute crystals disappeared, signaling the complete dissolution of the solids.[6] In a second version of the synthetic method, a known amount of a solute is kept at a constant temperature and solvent is slowly added until all the solids dissolve. Ngo used this approach to measure solubilities for various organic components in supercritical CO_2 coupled with IR, UV,

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and fluorescence spectroscopy.[7] Carbon dioxide was added to a known amount of solute in a pressure chamber while spectroscopic measurements were taken, and solubility was established on the basis of the CO_2 partial pressure at the time the signal no longer changed. Another version of the synthetic method could involve adding solute to a known amount of solvent at constant temperature; however, no examples of this technique were found in the literature. Advantages of the synthetic method compared to the analytical method are that it is faster because it does not require an analytical step and it eliminates the experimental error associated with that step.

Refractive index provides a good in situ measurement of sugar concentrations during solubility testing. For example, Prasil[8] used refractometry to measure the kinetics of sucrose dissolution near solubility. In this procedure, an isothermal solution of sucrose was made with 5−10 wt % less sugar added than the expected solubility. Then a known amount of sugar was added and the refractive index response monitored. Saturation was defined at the point when the RI signal no longer changed with sugar addition. At that point, a sample was taken to verify the concentration by drying.

The present study introduces a novel in situ device to measure the solubility of solids in liquids on the basis of measuring the change in refractive index of a solid/liquid mixture as the temperature is slowly increased. This procedure uses a synthetic measurement method and gives solubility temperatures at different concentrations instead of concentrations at different temperatures. To determine the solubility at a given temperature, it is necessary to measure solubilization temperatures at different solids loadings and interpolate. β-Cyclodextrin (cycloheptaamylose), a cyclic starch oligomer consisting of seven glucose chains linked by α-1−4 bonds, was used to validate the accuracy of our in situ device because it is inexpensive, solubility data are available in the literature, and it has a limited solubility similar to those of the sugar oligomers of interest to us. A larger device was developed first to test the procedure, and then a smaller in situ device was constructed to minimize the amount of sugar required for measurements. Last, we studied the effect of the heating rate to shorten the time required for measurements.

Experimental Section

Materials. β-Cyclodextrin was obtained from Sigma Aldrich (St. Louis, MO). Initially the sugar was stored in a sealed container and kept in a cabinet. However, over time the solubility values measured for this sugar differed significantly from initial measurements due to hydration of the sugar. Once this problem was identified, the sugar was then kept in a vacuum oven at 50 °C. To make sure that the sugar never became hydrated again, a dry mass analysis was performed periodically and was always found to be greater than 98%. The water used was deionized.

Apparatus and Procedure. A traditional analytical method was used to gather control data for comparison to our new in situ approach. A known amount of β-cyclodextrin was combined with a known amount of deionized water in a 60 mL Fisher Scientific (Pittsburgh, PA) serum bottle in a ratio of about 2.5 wt % sugar in excess of the expected solubility, which was between 800 and 2500 mg of sugar per bottle in this temperature range. At least three bottles were run simultaneously. The bottles were sealed with 20 mm Fisher Scientific (Pittsburgh, PA) stoppers and 20 mm Fisher Scientific (Pittsburgh, PA) aluminum crimp seals. They were then fixed to a 30 cm diameter, 10 cm wide plastic wheel containing a groove around its circumference 4.4 cm from the rim. The wheel was mounted on a steel structure and connected by a chain to a Dayton DC (Niles, IL) gear motor which turned the wheel at 50 rpm. Up to nine bottles were fastened to each side of the wheel using plastic ties. The wheel was then immersed in a 30 cm × 61 cm × 52 cm water bath containing a Fisher Scientific Isotemp 2100 immersion circulator (Pittsburgh, PA) providing a temperature stability of ±0.1 °C and a pumping rate of 14 L/min.

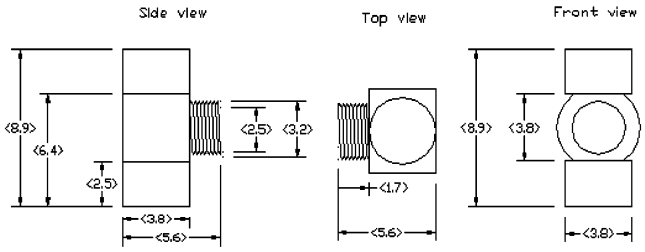

Figure 1. Schematic representation of the large in situ device including a side view, top view, and front view. All the units are in centimeters.

To take samples, the motor was stopped periodically and the bottles were removed. After the caps were removed, a sample was extracted with a 3 mL Luer-Lock, Beckon Dickinson syringe (Franklin Lakes, NJ). A 25 mm, 0.5 μm Millipore filter (Bedford, MA) was then fastened to the end, and the liquor was pushed onto a VWR aluminum weighing dish and quickly weighed on an OHAUS AS120 balance (Pinebrook, NJ). The samples were dried in a convection oven at 100 °C overnight, and after drying, the samples were placed in a desiccator to cool to room temperature. Next, they were weighed, and concentrations (as mass fractions) were calculated as the ratio of the dry mass (the mass of sugar) to the original mass of the liquor. At each temperature, samples were collected until equilibrium was established, and an unpaired *t*-test was performed to determine when the change in concentration with time was undetectable. Solubility testing by this method took between 41 and 55 h.

In Situ Device. Our novel device consisted of an inline refractive index detector and an adapter, both from AFAB Enterprises (Eustis, FL). It was calibrated by the manufacturer with xylose to operate over a temperature range of 30−80 °C at sugar concentrations of 0−80% by mass. Two different adapters were used, the larger of which was a 2.5 cm i.d. NPT female stainless steel pipe tee with an internal volume of 35 mL, as shown in Figure 1. The inline refractive index detector screwed into the side arm of the tee, and the bottom was plugged with a 2.5 cm NPT male brass stopper. During operation, the top was plugged with a size 8 silicone stopper. The smaller adapter is shown in Figure 2 and is essentially a 3.8 cm × 3.8 cm × 5 cm block of stainless steel containing a 9 mm diameter by 3.65 cm deep chamber with an internal volume of about 3 mL. The top is hinged on one side and contains a screw top on the other. The side contains a 2.5 cm NPT female thread that screws into the refractometer. Either refractometer and adapter assembly was placed on a programmable hotplate/stirrer to heat and mix the

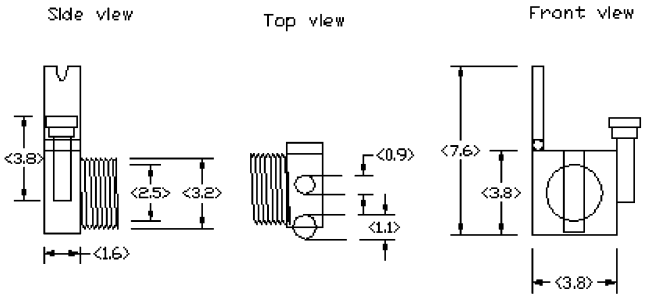

Figure 2. Schematic representation of the small in situ device including a side view, top view, and front view. All the units are in centimeters.

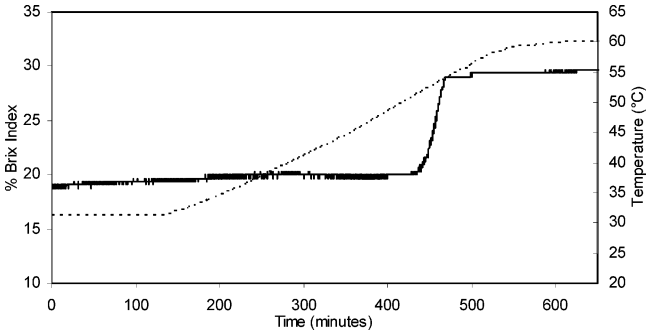

Figure 3. Continuous recording of the sample refractive index (solid line) and temperature (dotted line) versus time for the larger in situ device. The initial solids loading was 0.067 27 g of sugar/g of total mass.

contents. For the large adapter, the contents were stirred with a cross-shaped magnetic stir bar, and for the small adapter a 9 mm magnetic stir bar was used. Styrofoam insulation from The Dow Chemical Co. (Midland, MI) was placed around both devices to minimize heat losses. The refractometer apparatus provided real time data of the refractive index and temperature at its lens, and analog signals from the refractomer were digitized and recorded on a model OM-SL-L320 and a model OM-SL-L430 data logger from OMEGA (Stamford, CT). The temperature signal was calibrated using an NIST-certified thermometer from Cole-Palmer (Vernon, Hills, IL) over the temperature range of 25−55 °C.

Results

Operation. Either in situ cell was loaded with a known mass of β-cyclodextrin and deionized water. For the large device, one measurement required 0.6−2.1 g of sugar, and for the small device 90−300 mg of sugar was needed. The contents were allowed to equilibrate at room temperature for 40 min to 1 h until the signal was steady, and then the device was heated at a known rate by the hotplate/stirrer. The temperature and refractive index were recorded over time. At first, the RI signal displayed substantial scatter because of the presence of solids, but as more and more sugar dissolved with increasing temperature, the scatter was reduced. Figure 3 demonstrates that the RI signal increased with both dissolved solids concentration and temperature, and as complete dissolution was approached, the RI signal increased even faster until dissolution was complete, after which point the RI signal climbed much more slowly with temperature. The RI data for this approach are illustrated in Figure 3, in which β-cyclodextrin and water were added to the large in situ device

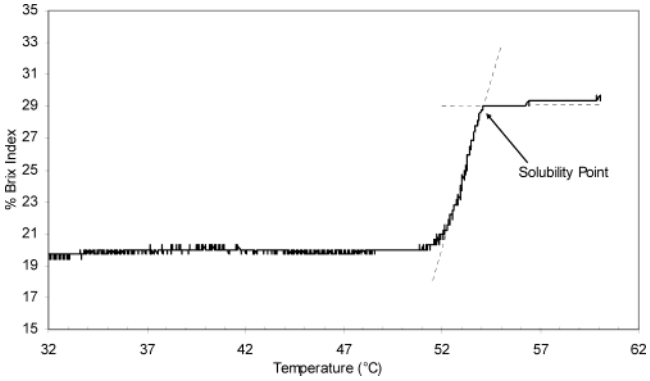

Figure 4. Change in refractive index with temperature for the larger in situ device with an initial solids loading of 0.067 27 g of sugar/g of total mass. The solubility point is the point at which the line changes from a sharp climb to being almost horizontal, indicating the dissolution of all solids.

Table 1. Summary of the Analysis of Variance Statistical Test[a]

	sum of squares	no. of degrees of freedom	mean square	f_0	$f_{0.05,1,8}$
total	64.68	11			
solids effect	54.19	1	54.19	65.7482	5.32
heating rate effect	3.10	1	3.10	3.76239	5.32
interaction effect	0.80	1	0.80	0.97169	5.32
error	6.59	8	0.82		

[a] The low solids loading is 5.6 ± 0.17% solids, and the high solids loading is 6.6 ± 0.21% solids. The slow heating rate is 3.9 ± 1.7 °C/h, and the fast heating rate is 10.2 ± 2.1 °C/h. The test statistic is f_0, and $f_{0.5,1,8}$ is the criterion for rejection at 95% confidence with 1 and 8 degrees of freedom.

at a solids loading of 0.067 g of sugar/g of total mass, and the cell was heated at a rate of 4.5 °C/h.

Once the test was completed, the RI vs time and temperature vs time data were converted into a plot of RI vs temperature as shown in Figure 4. The first half of this graph showed an exponential increase in the RI signal as the solubility temperature was approached, but after this point, the RI versus temperature signal became nearly flat. The solubility point was determined from the point where the slope of the RI vs temperature line changes.

Heating Rate. While analytical methods for measuring solubility often take days to complete, the in situ device can perform the same measurement in significantly less time. However, there is an important limitation: if the heating rate were too fast, then the temperature at which the solids dissolve could be higher than the actual solubility temperature; thus, for the in situ device to provide accurate data, the rate of dissolution must be fast compared to the heating rate. Thus, a two-level factorial experiment was performed to determine the effect of solids loading and heating rate on the final solubility value. In particular, solubility temperatures were measured at two solids loadings (5.6 ± 0.17% and 6.6 ± 0.21% solids) and two heating rates (3.9 ± 1.7 and 10.2 ± 2.1 °C/h), and three repeats were run for each combination of these variables. Table 1 shows the results of the analysis of variance (ANOVA) to determine the relative effects of each factor, and it was revealed that only the solids loading significantly affected the solubility value. There was no significant difference between the saturation temperatures measured at the slow and fast heating rates at the 95% confidence level. Also, there was no significant interac-

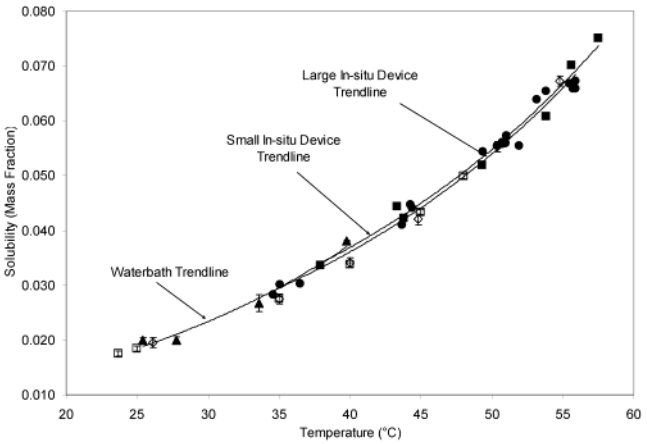

Figure 5. Solubility results as determined by the large-chamber in situ device (●), the small-chamber in situ device (■), the water bath (▲), Jennings and Rousseau[10] (◇), and Jozwiakowski and Connors[9] (□). Exponential equations fit to the experimental data are also plotted for each data set.

tion between heating rate and solids loading, meaning that there should not be a heating rate effect at another solids loading. Consequently, solubility data can be collected in a matter of hours instead of days. For instance, the saturation temperature of β-cyclodextrin at 0.55 g of sugar/g of water could be measured in 4 h, including 1 h for equilibrating the sample at room temperature and 3 h to heat the sample from room temperature to a little more than saturation temperature (50 °C).

Solubility Results. The solubility values initially measured with the large in situ device were substantially greater than the literature values, and we discovered two sources of error: (1) the stock sugar used in this experiment became partially hydrated while being stored in a cabinet, and (2) the initial calibration of the inline refractometer's temperature was inaccurate. The first problem was overcome by storing the sugar in a 50 °C vacuum oven and transferring it to a desiccator to cool to room temperature before the solubility tests were performed. Then a known amount of sugar was weighed and placed in the in situ device quickly to avoid hydration from moisture in the air. The second problem was addressed by regularly calibrating the thermister with an NIST-certified thermometer. Both of these steps were necessary to obtain good data.

Figure 5 shows the solubility of β-cyclodextrin as a function of temperature for the large and small in situ devices and the analytical approach along with literature values. Because the data were collected at different temperatures, solubility data from the literature were interpolated to compare them to experimental data. All of the solubility data sets displayed an exponential dependence on temperature. Table 2 shows the exponential functions fit to describe each data set with the coefficients determined by minimizing the sum of squares of the difference between the data and predictions along with the deviation from previously reported values and 95% confidence intervals. The regression coefficients are all very close to 1.0, indicating that all the data are well described by exponential equations. In addition, all of the experimental data reported in this work agree well with literature data. Data from the in situ devices agree slightly better with the values reported by Jozwiakowski and Conners[9] than the values reported by Jennings and Rousseau.[10] Results from the small in situ device agree with interpolated values from Jozwiakowski and Connors's data with a maximum deviation of 0.15% and an average deviation of 0.08%. The large device has a maximum deviation of 0.29% and an average deviation of 0.12%. The data from the water bath approach differ from both literature data sets by a similar amount, with a maximum deviation of 0.36% and an average deviation of 0.15%. Thus, all of the data are in very close agreement.

Determining the variability of the procedure is a bit more complicated. The literature values were determined from multiple samples tested at identical conditions with only the mean solubilities reported along with their standard deviations. The water bath data are reported in the same way; the 95% confidence intervals were similar to those reported in the literature. The maximum confidence interval was 0.16%, less than Jozwiakowski and Conner's maximum of 0.18%.[9] Because it is difficult to weigh out exactly the same amount of sugar repeatedly for the in situ approach due to the small quantities of sugar used and the fact that the sugar is hygroscopic, it was not possible to repeat each measurement exactly. Thus, statistical methods were employed to determine 95% confidence intervals for the data at every point,[11] and Table 2 shows the maximum and average 95% confidence intervals for the devices. Both devices have small confidence intervals, indicating good reproducibility. The larger device has smaller confidence intervals than the small device throughout because it has more data. Nonetheless, the maximum 95% confidence interval for the small device is only 0.53%, indicating that both the large and small devices have very good repeatability.

Conclusions

A novel device was developed to measure the solubility of solids in liquids by monitoring the change in refractive index as temperature is ramped up. Solubility data for β-cyclodextrin from both the large device holding 30 mL and a smaller 3 mL system were in close agreement, proving that this in situ device could be successfully miniaturized and that data could be collected using small quantities of sugar. A factorial test revealed that it gave accurate solubility values at a heating rate of 10 °C/h, meaning that measurements could be made in a few hours. The measurements

Table 2. Comparative Summary of Data from the in Situ Devices, the Water Bath, and the Literature

exponential solubility equation	no. of data points	temp range (°C)	R^2	max dev from ref 10 (%)	av dev from ref 10 (%)	max dev from ref 9 (%)	av dev from ref 9 (%)	max 95% confidence interval (%)	av 95% confidence interval (%)	source
$y = 0.0059 \exp(0.0442x)$	12	14.7−84 .8	0.9994					0.18	0.04	ref 10
$y = 0.0063 \exp(0.0427x)$	6	23.7−48	0.998					0.05	0.02	ref 9
$y = 0.0057 \exp(0.047x)$	4	25.4−39 .8	0.972	0.36	0.15	0.36	0.15	0.16	0.07	water bath
$y = 0.0067 \exp(0.0417x)$	19	27.1−55 .9	0.9915	0.37	0.15	0.29	0.12	0.39	0.24	large in situ device
$y = 0.0062 \exp(0.0426x)$	7	37.9−57 .5	0.9973	0.40	0.18	0.15	0.08	0.53	0.43	small in situ device

obtained by both devices agreed with data obtained by the analytical solubility method and literature data within 0.4 wt %, demonstrating the ability of the in situ devices to gather accurate solubility data quickly using a small amount of solid. Unlike the analytical solubility methods, this device can be used at high temperatures and pressures because sampling is unnecessary. In addition, this approach is more rapid than the commonly used batch sampling method (often requiring days or weeks).

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