Solubility of Glucose in Mixtures Containing 2-Methyl-2-butanol, Dimethyl Sulfoxide, Acids, Esters, and Water

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Solubility measurements of glucose in a variety of binary, ternary, and multicomponent mixtures containing 2-methyl-2-butanol, dimethyl sulfoxide, acids, esters, and water at different temperatures are presented. The solubilities of crystalline β-glucose, amorphous β-glucose, and amorphous β-glucose in 2-methyl-2-butanol at 60 °C have also been measured. The results show that the solubilities of the amorphous forms in 2-methyl-2-butanol are higher than that of the corresponding crystalline form. The presence of dimethyl sulfoxide significantly increases glucose solubility in 2-methyl-2-butanol. Finally, the presence of glucose ester increases glucose solubility in 2-methyl-2-butanol, while the presence of fatty acid has the opposite effect.

Introduction

Sugar fatty acid esters are widely used as surfactants and emulsifiers in the pharmaceutical, cosmetic, and food industries.[1] However, most of the emulsifiers manufactured by chemical methods using unprotected sugar moieties and fatty acids may not be used in food applications, because toxic organic solvents such as tetrahydrofuran or dimethylformamide are required for their solubilization and removal from the reaction mixture.

The synthesis of sugar esters using lipases has been studied extensively in recent years.[2−4] Enzymatic sugar esterification reaction mixtures contain sugar, fatty acid, solvent, product esters, and enzymes (immobilized). For the proper modeling of the reaction thermodynamics and kinetics, which is required for the design of bioreactors and the development of processes for the separation of the products from the reaction mixture, accurate thermodynamic properties are needed. Although there is a sufficient amount of thermodynamic data for aqueous solutions of sugars, only a few pieces are available for nonaqueous solutions,[5−7] and some reported values do not seem to be consistent with each other.

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This work presents glucose solubility measurements in a variety of binary, ternary, and multicomponent mixtures. The solubility of crystalline α-glucose was measured in (a) 2-methyl-2-butanol at different temperatures, (b) mixtures of 2-methyl-2-butanol with dimethyl sulfoxide (DMSO) at 60 °C, (c) mixtures of short chain acids (acetic and propionic acid) with water at 30 °C, (d) mixtures of octanoic acid with 2-methyl-2-butanol at 60 °C, and (e) multicomponent mixtures containing glucose laurate, lauric acid, water, and 2-methyl-2-butanol at 60 °C. The solubilities of crystalline β-glucose, amorphous α-glucose, and amorphous β-glucose in 2-methyl-2-butanol were also measured at 60 °C.

Experimental Section

Materials. Crystalline α-glucose (99% purity), 2-methyl 2-butanol (99% purity), dimethyl sulfoxide (99+% purity), crystalline β-glucose (99%), and dodecanoic (lauric) acid (99.5%) were purchased from Aldrich. Acetic acid, propionic acid, and water (HPLC grade) were purchased from Fluka. Octanoic acid (99% purity) was purchased from Sigma.

Glucose 6-*O*-monolaurate was synthesized by adapting a method reported in the literature.[8] D-(+)-Glucose (5.4 g, 0.3 M) was dispersed in 100 mL of 2-methyl-2-butanol. The biocatalyst, 10 g of granulated lipase from *Thermomyces*

10.1021/je0102457 CCC: $22.00
Published on Web 04/25/2002

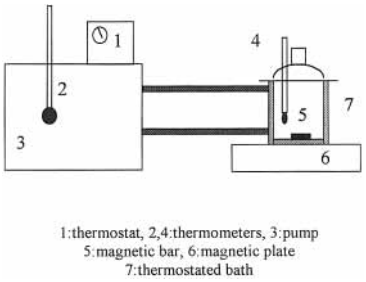

Figure 1. Schematic diagram of the experimental setup used for all glucose solubility measurements except those in the multicomponent mixtures.

lanuginosus prepared as described in the literature,[9] and 3 Å molecular sieves (10 g) were then added, and the suspension was maintained at 40 °C for 30 min with magnetic stirring. Then, lauric acid vinyl ester (7.8 mL, 0.3 M, dried overnight with molecular sieves) was added. After 14 h, when the conversion of glucose to monoester reached the maximum value (determined by HPLC), the reaction mixture was filtered, washed with 2-methyl-2-butanol (100 mL), and evaporated under vacuum. The solid (white powder) was recrystallized in acetone and dried in a vacuum, yielding a white crystalline solid (9.0 g, 88%). The purity was higher than 99% by HPLC. ^{1}H NMR analysis confirmed that the compound was 6-*O*-lauroylglucose.

Experimental Procedure. (a) Binary and Ternary Mixtures. The experimental setup used for the measurements of the binary and ternary mixtures is presented in Figure 1. One to four jacketed vessels of about 150 cm^3 were loaded with the solvent mixture. Each solvent mixture was prepared by weighing the desired amount of each solvent on a 0.1 mg precision balance. The temperature was set at the desired level, and the solvent was added to the vessels. Once the desired temperature was reached, excess glucose (compared with the expected solubility) was added to the solution. At constant temperature, the solution was stirred at 600 rpm to 800 rpm with a magnetic stirrer, until equilibrium was reached. Samples of the solution were withdrawn at several time intervals using pipets with a slightly higher temperature than the solution temperature in order to avoid any precipitation. The samples were then filtered with prewarmed 0.22 μm Nylon filters (polypropylene housing) and analyzed. When the difference in the value of glucose solubility between measurements was less than 2%, it was considered that equilibrium had been reached. In all cases, it was found that after 24 h of stirring both dissolution and anomerization (transformation of α- to β-glucose and vice versa) had been completed. Measurement of water content in all mixtures, except those of acetic or propionic acid and water, was performed using the Karl Fischer method either by titration or coulometrically. In all these measurements, the water content, possibly coming from the solvent and the sugar, was found to be in the range 0.05 to 0.2 mass %. It was found that these low water concentrations had no effect on glucose solubility.

(b) Multicomponent Reaction Mixture. For the measurements on the complete multicomponent reaction mixture, the following procedure was used. All components were first dried over molecular sieves (4 Å) for at least 1 week. A solution of known concentrations of lauric acid and glucose laurate in 2-methyl-2-butanol was placed in a vial with excess solid glucose and a magnetic stirrer bar. The vial (with an open top) was then placed inside a jar containing an agent to control humidity. The jar was then sealed with a lid and placed in a glove chamber with a

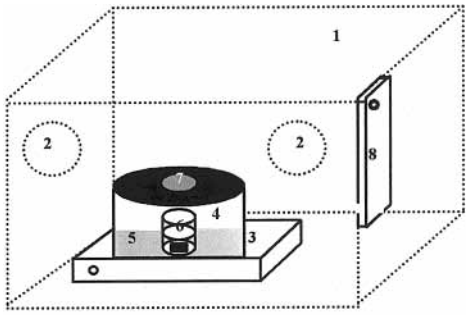

Figure 2. Schematic diagram of the experimental setup used for glucose solubility measurements in the multicomponent mixtures.

controlled air temperature of 60 °C. A magnetic stirrer underneath drives the bar in the test mixture. The experimental setup used is presented in Figure 2. The materials used to control humidity were molecular sieves for achieving water activity, a_w, less than 0.001, and saturated solutions in water (solid-rich slush) of LiCl for achieving $a_w = 0.1$. To avoid temperature changes during sampling, this was done inside the glovebox, with all apparatus prewarmed at 60 °C. Samples were filtered through 0.22 μm nylon filters. For water measurements a syringe was used to inject 50−100 μL directly and immediately into a coulometric Karl Fischer instrument. Samples of 100 μL for analysis were transferred to vials, which could then be allowed to cool. Samples for HPLC analysis were diluted with the mobile phase, to keep all components in solution.

Analysis of samples at various times showed that 24 h of stirring was normally necessary for equilibration.

Analytical Methods. The analytical methods used for glucose analysis depended on the system and involved high performance liquid chromatography (HPLC), gas−liquid chromatography (GLC), the enzymatic method, and the gravimetric one.

(a) HPLC Analysis. The HPLC setup used consisted of a liquid-chromatography (LC) pump, a refractive index (RI) detector, and a Lichrosorb NH_2 column (250 mm × 4 mm). The mobile phase used was a mixture of acetonitrile + water (90/10 volume %) or ethanol + water (90/10 volume %). The flow rate was 1 mL/min at 30 °C for the acetonitrile + water mobile phase or 1 mL/min at 40 °C for the ethanol + water mobile phase.

(b) GC Analysis. The GC measurements were done in a Perkin-Elmer gas chromatograph equipped with a split/splitless injection port and an FID detector. A 10 m simdist (simulated distillation) capillary column (Chrompack, U.K.) was used. Before the silylation treatment, and immediately after the withdrawal, samples (typically 50 μL or 100 μL) were flushed with a stream of nitrogen to evaporate the solvent. Dry samples were resuspended in 75 μL of pyridine containing 3.3 mg mL^{-1} octyl β-D-glucopyranoside and 3.3 mg mL^{-1} stearic acid ethyl ester (as the internal standards), and 75 μL of TMSI was added to the samples. After incubation for 45 min at 70 °C, 1 mL of *n*-heptane was added. Aliquots (0.5 μL) were injected into the gas chromatograph. The temperature profile was as follows:[10] the temperature was kept at 90 °C during 2 min and then increased from 90 °C to 250 °C at a rate of 15 °C min^{-1} and from 250 °C to 350 °C at a rate of 40 °C min^{-1}. The temperatures of the injector and detector were

Table 1. Total Solubility, *s*, of Crystalline α-Glucose in 2-Methyl-2-butanol at Different Temperatures

t/°C	*s*/(g L^{-1})	±SD[a]
30	0.60	0.013
40	1.06	0.053
60	2.40	0.045
80	6.83	0.342
102	9.00	0.450

[a] SD is the standard deviation of the experimentally measured solubility.

270 °C and 350 °C, respectively. Solutions of α- and β-glucose were dissolved in pyridine and used as standards for the calibration curves.

(c) Enzymatic Analysis. The glucose solubility was also determined with the well-established enzymatic method[11] using a glucose oxidase−chromogen reagent (Sigma Chemical).

(d) Gravimetric Analysis. Gravimetric solubility measurements were performed by drying the solvent mixture from a previously weighed sample of saturated solution (5 mL to10 mL) in a vacuum oven (80 °C, 800 mbar) and by weighing the precipitated sugar regularly until a constant value is achieved. Using this method, the solubility of glucose in acetic or propionic acid/water mixtures, where high solubilities are observed, was determined.

All methods give similar results, as verified by analyzing a solution of glucose in 2-methyl-2-butanol of known concentration.

Results and Discussion

When the solubility of glucose in organic media is being measured, it is important to bear in mind two special factors. First, the achievement of equilibrium between the α- and β-anomers may be rather slow, so that the total dissolved concentration continues to increase slowly for some time, after an initial rapid rise. This effect has been reported before[12,13] but has been neglected in some studies. Second, the system can show pronounced supersaturation, which can persist for days even in the presence of excess solid crystals.[14] Thus, methods employing temperatures higher than the equilibrium values to promote rapid dissolution may give erroneously high results. These factors may account for literature values of glucose solubility in 2-methyl-2-butanol that appear to be (after allowing for temperature differences) lower[6] and much higher[7] than those reported here.

Solubility of Crystalline α-Glucose in 2-Methyl-2-butanol. The total glucose (α- plus β-) solubility after 24 h in 2-methyl-2-butanol was measured at five different temperatures in the range 30 to 102 °C. The results are presented in Table 1. At 60 °C the ratio of α- to β-glucose was also measured and it was found to have a value of 0.86.

Solubility of Crystalline α-Glucose in 2-Methyl-2-butanol in the Presence of DMSO. The use of mixtures of 2-methyl-2-butanol with DMSO as solvent instead of pure 2-methyl-2-butanol has been shown to increase the yields of the enzymatic reactions of glucose with fatty acid, because of the increase of glucose solubility. Total glucose solubility measurements were performed in 2-methyl-2-butanol + DMSO mixtures containing up to 40 vol % DMSO. The results are presented in Table 2 and show that there is a significant increase of glucose solubility with increasing DMSO concentration. For example, use of 20 vol % DMSO increases glucose solubility more than 5 times with respect to the solubility in pure 2-methyl-2-butanol, while the presence of 40 vol % DMSO increases the solubility 32 times.

Table 2. Total Solubility, *s*, of Crystalline α-Glucose in Mixtures of 2-Methyl-2-butanol (1) + DMSO (2) at 60 °C

x_2	*s*/(g L^{-1})	±SD
0	2.40	0.045
0.0756	3.62	0.181
0.1473	4.89	0.245
0.2798	13.14	0.657
0.3998	42.46	2.123
0.5089	83.31	4.166

Table 3. Total Solubility, *s*, of Crystalline α-Glucose in 2-Methyl-2-butanol (1) + Octanoic Acid (2) Mixtures at 60 °C

x_2	*s*/(g L^{-1})	±SD
0	2.40	0.045
0.1482	1.16	0.018
0.3170	0.38	0.004
0.5108	0.15	0.002

Table 4. Total Solubility, *s*, of Crystalline α-Glucose in Mixtures of Water (1) + Acetic Acid (2) or Propionic Acid (2) at 30 °C

x_2	*s*/(g L^{-1})	±SD	x_2	*s*/(g L^{-1})	±SD
Acetic Acid			Propionic Acid		
0.8060	44.92	0.143	0.5656	44.28	0.137
0.8618	34.87	0.072	0.6614	33.92	0.084
0.9065	23.36	0.011	0.7523	20.63	0.128
0.9433	15.00	0.028	0.8389	8.98	0.017
0.9740	3.58	0.002			

Solubility of Crystalline α-Glucose in 2-Methyl-2-butanol in the Presence of Octanoic Acid. Total glucose solubility measurements in 2-methyl-2-butanol in the presence of different octanoic acid concentrations were performed at 60 °C. The results are presented in Table 3.

As shown, there is a significant effect of fatty acid on glucose solubility. For example, use of 20 mass % octanoic acid decreases the glucose solubility more than 2 times with respect to the solubility in pure 2-methyl-2-butanol, while use of 40 mass % octanoic acid decreases solubility about 7 times.

Solubility of Crystalline α-Glucose in Mixtures Containing Short Chain Acids and Water. For the extension of the glucose solubility database, total glucose solubility measurements were performed in mixtures containing a short chain length acid (acetic or propionic acid) and water at 30 °C. The results are presented in Table 4. As expected, acetic acid is a better solvent for α-glucose than propanoic acid but, as the water content increases, it dominates the solubility and the difference between the two acids diminishes.

Solubility of Amorphous α- and β-Glucose in 2-Methyl-2-butanol at 60 °C. Amorphous α- and β-glucose were prepared as follows. Crystalline glucose (α- or β-) was melted by heating the crystals to 150 °C, which is above their melting point temperatures. The melting point temperature of α-glucose is 146 °C, and that of β-glucose is 150 °C. The sample was held at this temperature for as short a time as possible to avoid glucose decomposition (caramelization). Then it was cooled to room temperature, which is below the glass transition temperature, that is, 35 to 39 °C. The resulting materials are nearly colorless, transparent glasses.

The measured solubilities of both crystalline and amorphous forms of α- and β-glucose in 2-methyl-2-butanol at 60 °C are presented in Table 5.

Table 5. Total Solubility, *s*, of Crystalline (1) and Amorphous (2) α- and *β*-Glucose in 2-Methyl-2-butanol at 60 °C

sugar	s_1/(g L^{-1})	±SD	s_2/(g L^{-1})	±SD
α-glucose	2.40	0.045	3.60	0.170
β-glucose	3.60	0.170	4.14	0.195

Table 6. Total Solubility, *s*, of Crystalline α-Glucose in Mixtures Containing 2-Methyl-2-butanol (1) + Glucose Laurate (2) + Water (3)

c_2/(g L^{-1})	$100w_3$/(mass %)	s/(g L^{-1})	±SD
	$a_w < 0.001$		
0	0.04	2.38	0.118
18.1	0.06	2.36	0.119
36.2	0.08	2.32	0.116
54.3	0.11	3.22	0.161
	$a_w = 0.1$		
0	0.51	2.40	0.120
18.1	0.56	2.73	0.137
36.2	0.54	3.41	0.171
54.3	0.69	3.56	0.178

Table 7. Total Solubility, *s*, of Crystalline α-Glucose in Mixtures Containing 2-Methyl-2-butanol (1) + Glucose Laurate (2) + Water (3) + Lauric Acid (0.5 M)

c_2/(g L^{-1})	$100w_3$/(mass %)	s/(g L^{-1})	±SD
	$a_w < 0.001$		
0	0.03	1.49	0.075
18.1	0.04	1.41	0.071
36.2	0.04	1.23	0.062
54.3	0.04	1.49	0.075
	$a_w = 0.1$		
0	0.51	1.70	0.085
18.1	0.58	1.99	0.100
36.2	0.54	1.84	0.092
54.3	0.60	1.76	0.088

As shown, the solubility of crystalline *β*-glucose is higher that that of crystalline α-glucose, and the same stands for their amorphous forms. In all cases, the equilibrium ratio of α- to *β*-glucose was also measured. For crystalline α-glucose, as already mentioned, it was 0.86; for crystalline *β*-glucose, 0.95; for amorphous α-glucose, 1.0; and for amorphous *β*-glucose, 1.09.

Solubility of Crystalline α-Glucose in Mixtures Containing 2-Methyl-2-butanol, Water, Lauric Acid, and Glucose Laurate. Crystalline α-glucose solubility measurements were performed in mixtures similar to those encountered in enzymatic reaction mixtures. Two different mixture water activity (a_w) values were examined: near to zero and equal to 0.1.

Two sets of experiments were performed. In the first set no acid (lauric acid) was present and the results are tabulated in Table 6. The ratio of α- to *β*-glucose was also measured, and the average value was found to be about 1.0. In the second set a constant acid concentration of 0.5 M was used and the results are tabulated in Table 7. The average value of the ratio of α- to *β*-glucose was about 2 in this case.

The following comments summarize the obtained results:

(a) The solubility of glucose increases with increasing ester concentration, which indicates that ester acts as a cosolvent for glucose. This effect of the reaction product may be important for the correct understanding of the kinetics of enzymatic synthesis and optimal process design.

(b) The presence of acid results in a decrease in glucose solubility, which is in agreement with the results presented for the solubility in short chain length acid-containing mixtures.

The presence of acid affects the anomerization equilibrium between the α- and *β*-forms. In the absence of acid the ratio of α- to *β*-glucose is about 1, but in the presence of acid the ratio changes to about 2. This explains the decrease of glucose solubility in the presence of acid. The concentration of α-glucose in solution (in equilibrium with solid α-glucose) is little changed. Acid must make solvation of *β*-glucose less favorable, so its concentration falls, and hence the total concentration of α- plus *β*-glucose.

Conclusions

Solubility measurements of crystalline α-glucose, crystalline *β*-glucose, amorphous α-glucose, and amorphous *β*-glucose were performed in a variety of binary, ternary, and multicomponent mixtures and at different temperatures. The results show that the solubilities of the amorphous forms in 2-methyl-2-butanol are higher than those of the corresponding crystalline forms. The presence of dimethyl sulfoxide significantly increases glucose solubility in 2-methyl-2-butanol. Moreover, the presence of glucose ester increases glucose solubility in 2-methyl-2-butanol, while the presence of fatty acid has the opposite effect.

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Received for review September 4, 2001. Accepted March 14, 2002. This research was supported by EC Biotechnology Project Nº PL98-0363.

JE0102457