Experimental and Predicted Results of Anomeric Equilibrium of Glucose in Alcohols

Georgios Leontarakis, Panagiotis Tsavas,* Epaminondas Voutsas, Kostis Magoulas, and Dimitrios Tassios

Thermodynamics and Transport Phenomena Laboratory, School of Chemical Engineering, National Technical University of Athens, 9 Heroon Polytechniou Street, Zographou Campus, 157 80 Athens, Greece

Total glucose solubility as well as equilibrium concentrations of α- and β-glucose anomers in 1-butanol, *tert*-butyl alcohol, 2-propanol, 1-propanol, ethanol, methanol, and an ethanol (1) + water (2) mixture ($w_1 = 90$ %) at 40 °C and 60 °C were measured. Also, the recently developed mS-UNIFAC model is applied in the prediction of the total glucose solubility in these solvents and of the anomeric distribution in them yielding very satisfactory results.

Introduction

Aqueous solutions of sugars play an important role in biological systems as well as in the food and beverage industries.[1−3] Recent developments in biocatalysis present particular interest in the use of nonaqueous solvents, mainly tertiary alcohols,[4−6] as the media for the enzymatic production of fatty acid sugar esters, which is influenced by sugar solubility and dissolution rate.[7]

Reducing sugars in their solutions are a mixture of all their possible conformations in equilibrium: five-membered (furanose) or six-membered (pyranose) rings, noncircular chains, and simultaneously existing stereoisomers (anomers) of each conformation. Since enzymes are stereoselective catalysts, the conformational equilibrium of sugars affects the yield of the reaction with respect to the desirable product, considering that the reactivity of the hydroxyl groups of sugar molecules is depending on the conformation of the sugar molecule.

When a reducing sugar (e.g., fructose, glucose) is dissolved in a solvent, apart from the solid−liquid-phase equilibrium, a chemical equilibrium (conformational equilibrium) between their isomeric forms is established (Figure 1). The presence of several sugar conformations and hydrogen bonding sites on sugar molecules makes the thermodynamic modeling of sugars and their derivatives in aqueous and nonaqueous mixtures a difficult task. However, there is an increasing need for thermodynamic models applicable to such systems due to their presence in many applications related to the food and cosmetic industry, such as the design and simulation of bioreactors and the development of separation processes (evaporation, crystallization, liquid−liquid extraction).

Experimental phase equilibrium data for sugar solutions, needed for the development of thermodynamic models, are available in the literature mainly for aqueous sugar solutions, while for nonaqueous solvents they are limited. Furthermore, experimental data of conformational equilibrium of sugars in nonaqueous solvents are very scarce.[8,9]

In this work, total glucose solubility and equilibrium concentrations of α- and β-glucose anomers in alcohols and one alcohol + water mixture were measured. Furthermore, total glucose solubility predictions as well as calculations of glucose anomer concentrations in solutions were performed with the recently developed mS-UNIFAC[10] group-contribution model.

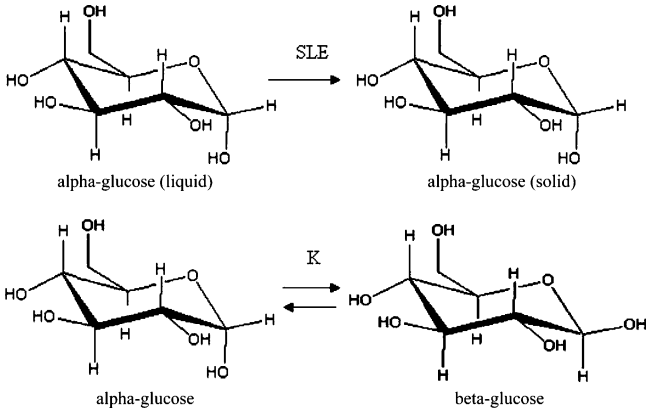

Figure 1. Glucose phase and chemical equilibrium.

Experimental Section

Materials. Crystalline α-D-glucose (99 % purity) was purchased from Aldrich. Crystalline β-D-glucose (99 % purity) was purchased from Sigma. *tert*-Butyl alcohol, 2-propanol, ethanol, and heptane were purchased from Lab-Scan. 1-Propanol and 1-butanol were purchased from Acros Organics, and *tert*-pentyl alcohol was purchased from Fluka. All solvents had over 99 % purity and were dried over molecular sieves (4 Å) for at least 1 week before use. Pyridine (99 % purity) and 1-trimethylsilylimidazol (TMSI) with purity over 98 % were used as purchased from Lab-Scan.

Experimental Procedure. When the solubility of glucose in organic media is measured, it is important to take into consideration that the achievement of equilibrium between the α- and β-glucose anomers is rather slow, so that the total dissolved concentration of α- and β-glucose continues to increase slowly for some time after an initial rapid rise. This effect has been also reported elsewhere.[9,11]

The experimental configuration used in this study has been presented elsewhere.[12] Excess α-D-glucose is added

* Corresponding author. Telephone: +30210 7723137. Fax: +30210 7723155. E-mail: tsavas@chemeng.ntua.gr.

10.1021/je050193b CCC: $30.25
Published on Web 10/11/2005

Table 1. Experimental Equilibrium Concentrations of α-Glucose and *β*-Glucose and Anomeric Ratios in Different Alcohols at 40 °C and 60 °C

solvent	α-glucose/g·L^{-1}	*β*-glucose/g·L^{-1}	anomeric ratio	total solubility/g·L^{-1}
		T = 40 °C		
tert-pentyl alcohol	0.50 ± 0.01	0.58 ± 0.01	0.85	1.08 ± 0.02
tert-butyl alcohol	0.58 ± 0.03	0.66 ± 0.03	0.88	1.24 ± 0.06
1-butanol	0.27 ± 0.00	0.32 ± 0.01	0.87	0.59 ± 0.01
ethanol	1.82 ± 0.09	1.99 ± 0.11	0.91	3.81 ± 0.19
methanol	14.52 ± 0.29	14.24 ± 0.34	1.02	28.76 ± 0.58
ethanol (1) + water (2)[a]	10.72 ± 0.22	10.51 ± 0.17	1.02	21.23 ± 0.38
		T = 60 °C		
tert-pentyl alcohol	1.11 ± 0.03	1.29 ± 0.03	0.86	2.40 ± 0.06
tert-butyl alcohol	1.65 ± 0.07	1.86 ± 0.08	0.89	3.51 ± 0.14
1-butanol	0.69 ± 0.03	0.78 ± 0.04	0.88	1.47 ± 0.07
2-propanol	1.82 ± 0.08	1.96 ± 0.09	0.93	3.78 ± 0.17
1-propanol	1.77 ± 0.11	1.95 ± 0.09	0.91	3.72 ± 0.20
ethanol	4.51 ± 0.06	4.51 ± 0.06	1.00	9.02 ± 0.12
ethanol (1) + water (2)[a]	20.83 ± 0.71	21.26 ± 0.55	0.98	42.09 ± 1.26

[a] w_2 = 10 %.

to jacketed vessels of about 250 cm^3. The vessels are loaded with 150 cm^3 of the solvent and are sealed. The temperature is set at the desired level within 0.1 °C accuracy. The solution is stirred at (600 to 800) rpm at constant temperature for about 48 h with magnetic stirrer until equilibrium is reached. Then the solution is allowed to stand at constant temperature for about 48 h to enable any finely dispersed solids to settle down, and samples are taken out with and without filtering. Sampling procedure is performed using pre-warmed pipets at a slightly higher temperature than the solution temperature in order to avoid any glucose precipitation during sampling. The samples are filtered with 0.22 μm nylon filters (polypropylene housing). Then 4−6 filtered samples (0.5 ± 0.005 mL) are introduced in a vacuum oven at 80 °C under (500 to 700) mbar until all the solvent is evaporated, which is checked by repeating gravitational test. The time needed until equilibrium is reached and excess glucose settles down was measured in similar previous experiments by measuring the solubility of several filtered samples withdrawn at specified intervals with the procedure described below. Equilibrium is considered to have been reached when the difference in the value of solubility (g/L) in 24 h intervals is less than the experimental uncertainty of the analytical method followed. For the gravimetric method, the experimental uncertainty in the solubility measurement is 1 %, while for the gas chromatography method it is up to 5 %.

Analytical Methods. Two analytical methods were used: gravimetric method and gas chromatography (GC). The gravimetric method was used in order to measure the total glucose mass fraction to more than 0.1 %.

The GC analysis method used has the ability to distinguish the two glucose anomers (α- and *β*-) and was used for measuring both the concentrations of both anomers and, consequently, the total glucose solubility as the sum of the concentrations of the two anomers. Finally, the ratio of the concentration of the α-anomer divided by the concentration of the *β*-anomer at equilibrium was defined here as the anomeric ratio.

(a) Gravimetric Method. Gravimetric solubility measurements were performed by drying the solvent from a previously weighted sample of a saturated solution (10 to 25) mL in a vacuum oven [80 °C, (500 to 700) mbar] and by weighting the precipitated glucose until a constant value was achieved.

(b) GC Analysis. Dry samples are re-suspended in 375 μL of pyridine containing 3.3 mg/mL octyl *β*-D-glucopyranoside (as internal standard). After stirring the solution in ultrasonic bath, 375 μL of TMSI is added to the samples. The samples are incubated for 45 min at 70 °C, and the addition of 5 mL of heptane followed. Aliquots of 10 μL are injected into the GC. The temperature profile was as follows: the temperature is kept at 120 °C for 5 min, increases from 120 °C to 135 °C at a rate of 2 °C/min, constant at 135 °C for 1 min, increases from 135 °C to 180 °C at a rate of 10 °C/min, and finally increases from 180 °C to 330 °C at a rate of 40 °C/min. GC grade helium was used as carrier gas. The temperatures of the injector and detector were 270 °C and 350 °C, respectively. The measurements were done in a Shimadzu 800 GC series gas chromatograph equipped with a split/splitless injection port and FID detector. A 10 m sim-dist (simulated distillation) capillary column (Chrompack, UK) was used.

Results and Discussion

The experimental results are presented in Table 1. It is shown that a small temperature increase leads to a significant increase of both the total glucose solubility and the concentrations of the two anomers. Also, the total solubility of glucose decreases with increase in the number of carbon atoms in the solvent when comparing separately normal alcohols (1-butanol < 1-propanol < ethanol < methanol) or branched alcohols (*tert*-pentyl alcohol < *tert*-butyl alcohol < 2-propanol). On the other hand, when comparing alcohols with the same number of carbon atoms, it is noticed that glucose presents higher solubilities in branched alcohols (1-butanol < *tert*-butyl alcohol and 1-propanol < 2-propanol). Furthermore, the solubility of glucose in alcohols increases significantly with the addition of water. There is about a five times increase for a mass fraction of water w_1 = 10 % for the water (1) + ethanol (2) mixture.

Concerning the anomeric ratio, it follows the same trend as the total solubility (i.e., for normal alcohols: 1-butanol < 1-propanol < ethanol < methanol; for branched alcohols: *tert*-pentyl alcohol < *tert*-butyl alcohol < 2-propanol). This increase in the anomeric ratio with polarity would suggest a still larger value for water. Surprisingly, the anomeric ratio of glucose in water is 0.61 at 31 °C,[13] which is lower even than the anomeric ratio in *tert*-pentyl alcohol.

Also, although the effect of temperature on the anomeric ratio is very small, the experiments indicate that the temperature increase leads to the anomeric ratio increase as well. This is in agreement with the equilibrium constant values reported by Goldberg and Tewari,[13] which show that the reaction of α- to *β*-glucose is an exothermic one. An

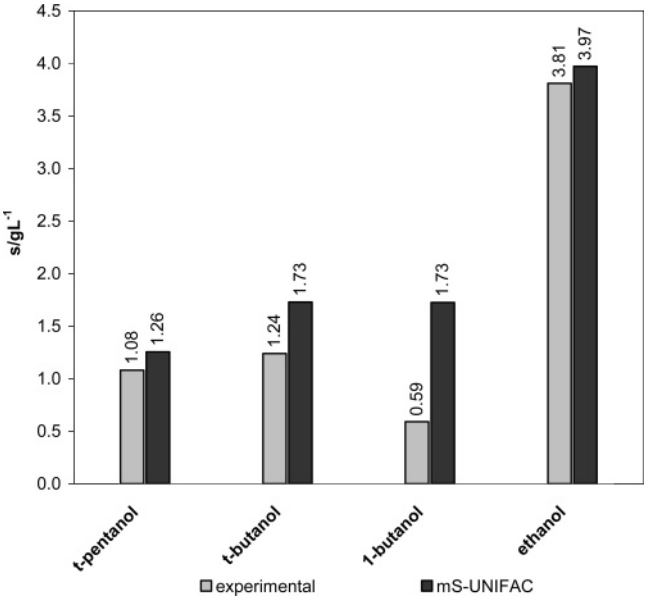

Figure 2. Experimental and predicted glucose solubility (*s*) in alcohols at 40 °C.

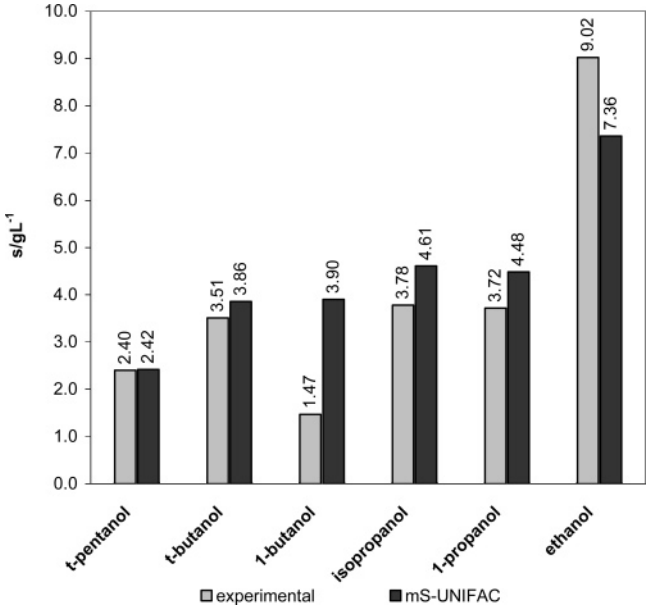

Figure 3. Experimental and predicted glucose solubility (*s*) in alcohols at 60 °C.

exception seems to be the ethanol/water mixture, although no clear conclusion can be derived from these only two measurements.

The mS-UNIFAC[10] model is a group-contribution thermodynamic model suitable for phase equilibrium predictions, like solubility predictions, in mixtures containing sugars and/or sugar derivatives with water, alcohols, acids, and esters. The model was developed considering that only one conformation is present in sugar solutions—without taking into account the chemical equilibrium between the anomers—and more specifically the predominant anomer in aqueous solution. This was done because experimental data of sugar conformational equilibrium are scarce.

Glucose solubilities are predicted using the following standard thermodynamic solid−liquid equilibrium (SLE) expression:

$$\ln(x_{\text{gluc}}\gamma_{\text{gluc}}) = -\frac{\Delta_{\text{fus}}H}{RT}\left(1-\frac{T}{T_{\text{m}}}\right)+\frac{\Delta C_p}{R}\left(\frac{T_{\text{m}}-T}{T}\right)+\frac{\Delta C_p}{R}\ln\left(\frac{T}{T_{\text{m}}}\right) \quad (1)$$

where x_{gluc} is the glucose solubility expressed as mole fraction, γ_{gluc} is the glucose activity coefficient in the solution predicted by the mS-UNIFAC model, T_{m} is the glucose melting temperature, $\Delta_{\text{fus}}H$ is the glucose heat of fusion, and ΔC_p is the difference between the heat capacities of the supercooled pure melt and the pure solid assuming that it is independent of temperature. Since mS-UNIFAC was developed treating pure solid glucose as β-glucose,[10] the corresponding T_{m}, $\Delta_{\text{fus}}H$, and ΔC_p values used in eq 1 was as follows: $T_{\text{m}} = 423$ K,[14] $\Delta_{\text{fus}}H = 32.43$ kJ·mol^{-1},[14] and $\Delta C_p = 66$ J·mol^{-1}·K^{-1}.[10]

A comparison between the experimental and the mS-UNIFAC predicted glucose solubilities is presented in Figure 2 and Figure 3.The obtained results are satisfactory with the exception of 1-butanol. The solubility of glucose in 1-butanol is significantly overestimated by the mS-UNIFAC model. This behavior of the model must be attributed to the well-known problem of UNIFAC type models to accurately predict the isomeric effect. Thus, although the model gives good results for *tert*-butyl alcohol, which has been included in the database used for the mS-UNIFAC model development, this is not the case for 1-butanol. To overcome this problem, different parameters for the different types of hydroxyls (primary, secondary, and tertiary) must be developed.

It would, of course, be desirable to have a model that could predict the total solubility in a rigorous way. Predict first the solubility of the species in excess; in this case α-glucose since the amount of β-glucose formed in solution never exceeds its solubility limit because α-glucose is less soluble than β-glucose.[12] Then predict the concentration of the second species—in this case β-glucose—through the use of the chemical equilibrium relationship:

$$K = e^{\frac{-\Delta G^\circ}{RT}} = K_x K_\gamma = \frac{x_\beta}{x_\alpha}\frac{\gamma_\beta}{\gamma_\alpha} \quad (2)$$

where x_α and x_β are the equilibrium concentrations of α- and β-anomers expressed as mole fraction; γ_α and γ_β are their activity coefficients that would be calculated by the model; K is the equilibrium constant that is the same for all solvents and depends only on the temperature (T); and ΔG° is the Gibbs energy of formation at the temperature (T).

The mS-UNIFAC model, in its present form, cannot be used in such a rigorous way due to the abovementioned constraints involved in its development that were caused by the lack of experimental data like the ones presented in this work. Actually, the development of a rigorous thermodynamic model would rely on a database that involves except from total solubility data, the concentrations of the individual anomer species at equilibrium as well, such as those presented here, and of course, ΔG° values for the mutarotation reactions between the various anomeric forms at equilibrium.

However, since in the database used in the development of the mS-UNIFAC the groups involved in α-glucose were present in other sugars (fructose)—and thus the corresponding interaction parameters were evaluated—we examined the possibility of using it to predict the distribution of these two glucose anomer species from the total glucose solubility known experimentally or predicted. This can be

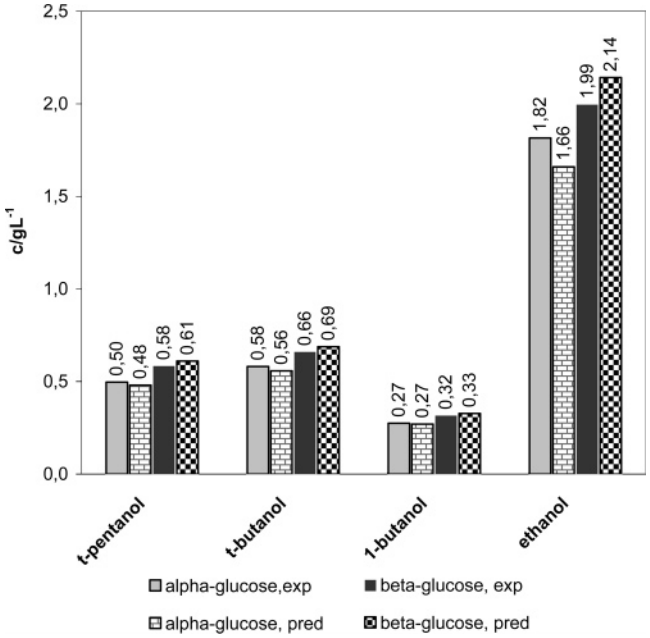

Figure 4. Experimental and predicted glucose equilibrium concentrations (c) in alcohols at 40 °C: exp, experimental; pred, predicted.

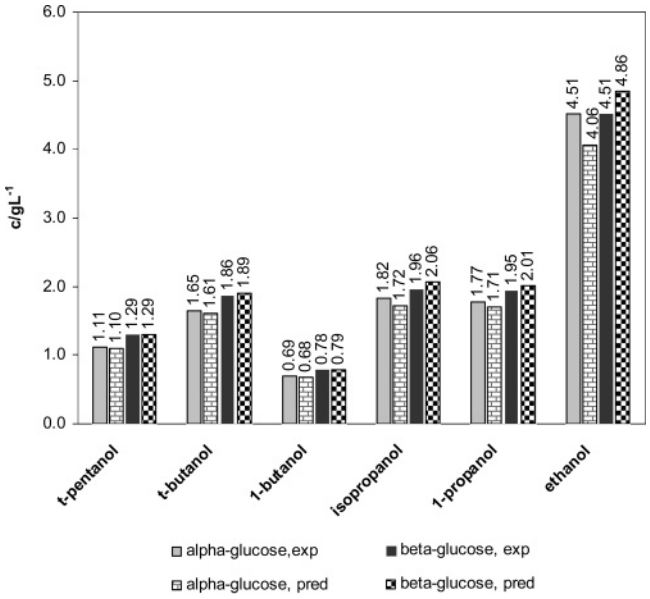

Figure 5. Experimental and predicted glucose equilibrium concentrations (c) in alcohols at 60 °C: exp, experimental; pred, predicted.

done using in eq 2 the values for K equal to 1.57 at 40 °C and equal to 1.49 at 60 °C, which were calculated through the experimental $\Delta G°$ at 298 K and $\Delta H°$ data given by Goldberg and Tewari,[13] combined with the equation:

$$x_\alpha + x_\beta = x_{\text{tot}} \tag{3}$$

where x_α and x_β are the equilibrium concentrations of α- and β-anomers and x_{tot} is the total glucose solubility, all expressed in mole fraction.

The system of eqs 2 and 3 has two unknowns: x_α and x_β. The results obtained following this procedure—and using the experimentally determined total solubility—are presented in Figure 4 and Figure 5 (similar results are obtained if we use the predicted solubility except of course 1-butanol). The satisfactory quality of the obtained results indicate that mS-UNIFAC would be an excellent candidate for the development of a rigorous thermodynamic model that will be able to simultaneously predict the phase and chemical equilibrium present in sugar solutions.

Conclusions

New glucose solubility data, as well as equilibrium concentrations of glucose anomers in *tert*-butyl alcohol, 1-butanol, 2-propanol, 1-propanol, ethanol, methanol, and an ethanol−water mixture are presented. Application of the mS-UNIFAC model gave satisfactory predictions of (a) the total glucose solubility in the solvents examined and (b) the concentrations of the glucose anomers, given the experimentally observed total glucose solubility.

The results indicate that the mS-UNIFAC model can be appropriately modified so as to be able to predict simultaneously the phase and chemical equilibrium present in sugar solutions. This task requires the re-evaluation of the model parameters by utilizing, except from total sugar solubility data, the concentrations of the individual sugar anomer species when, of course, they are present.

Literature Cited

(1) Watanabe, T. Sucrose fatty acid esters: past, present and future. *Foods Food Ingredients J. Jpn.* **1999**, *180*, 18.
(2) Montgomery, R. Development of biobased products. *Bioresour. Technol.* **2004**, *91*, 1.
(3) Riva, S. Biocatalytic modification of natural products. *Curr. Opin. Chem. Biol.* **2001**, *5*, 106.
(4) Akoh, C. C.; Swanson, B G. *Carbohydrate Polyesters as Fat Substitutes*; Marcel Dekker: New York, 1994.
(5) Tsuzuki, W.; Kitamura, Y.; Suzuki, T.; Kobayashi, S. Synthesis of sugar fatty acid esters by modified lipase. *Biotechnol. Bioeng.* **1999**, *64*, 267.
(6) Degn, P.; Pedersen, L.; Zimmermann, W. Lipase-catalyzed synthesis of glucose fatty acid esters in *tert*-butanol. *Biotechnol. Lett.* **1999**, *21*, 275.
(7) Flores, V. M.; Naraghi, K.; Engasser, J. M.; Halling, P. J. Influence of glucose solubility and dissolution rate on the kinetics of lipase catalyzed synthesis of glucose laureate in 2-methyl-2-butanol. *Biotechnol. Bioeng.* **2002**, *78*, 815.
(8) Allavudeen, S. S.; Kuberan, B.; Loganathan, D. A method for obtaining tautomeric mixtures of reducing sugars viaglycosylamines using nonaqueous media. *Carbohydr. Res.* **2002**, *337*, 965.
(9) Flood, A.; Johns, M. R.; Whit, E. T. Mutarotation of D-fructose in aqueous-ethanolic solutions and its influence on crystallisation. *Carbohydr. Res.* **1996**, *288*, 45.
(10) Tsavas, P.; Voutsas, E.; Magoulas, K.; Tassios, D. Phase equilibrium calculations in aqueous and nonaqueous mixtures of sugars and sugar derivatives with a group-contribution model. *Ind. Eng. Chem. Res.* **2004**, *43*, 8391.
(11) Signorella, S.; Lafarga, R.; Daier, V.; Sala, L. F. The reduction of Cr^{VI} to Cr^{III} by a and b anomers to D-glucose in dimethyl sulfoxide. A comparative kinetic and mechanistic study. *Carbohydr. Res.* **2000**, *324*, 127−135.
(12) Tsavas, P.; Polydorou, S.; Faflia, I.; Voutsas, E. C.; Tassios, D.; Naraghi, K.; Flores, M. V.; Halling, P. J.; Engasser, J. M.; Ghoul, M.; Chamouleau, F.; Ferrer, M.; Ferrer, F. J. Solubility measurements of α-glucose in mixtures containing t-pentanol, dimethyl sulfoxide, acids, esters and water. *J. Chem. Eng. Data* **2002**, *47*, 513.
(13) Golberg, R. N.; Tewari, Y. B. Thermodynamic and transport properties of carbohydrates and their monophosphates: pentose and hexoses. *Phys. Chem. Ref. Data* **1989**, *2*, 809.
(14) Reamy, A.; Schweizer, T. F. Thermal behaviour of carbohydrates studied by heat flow conductivity. *J. Therm. Anal.* **1983**, *28*, 9.

Received for review May 15, 2005. Accepted September 6, 2005.

JE050193B