Crystallization Screening and Theoretical Studies on Molecular Recognition of D-glucose Molecules

***Luca Sironi*[1], *Leonardo Lo Presti*[1], *Silvia Rizzato*[1]**

1. Department of Chemistry, Università degli Studi di Milano, Via Golgi 19, 20133, Milano (Italy)

Contact:
luca.sironi4@studenti.unimi.it

INTRODUCTION

Industrial crystallization of glucose is still today one of the most important and widely studied crystal growth processes, such that its world-wide annual production reaches the millions of tons. D-glucose takes part in an anomerization equilibrium in solution and crystallizes in three main crystalline forms, namely, α-D-glucose monohydrate, α-D-glucose anhydrous, and β-D-glucose anhydrous (***Tables 1-2***)[1].

The investigation of molecular recognition is fundamental for the understanding of nucleation process and to try to explain why this kind of molecules with 5 almost free to rotate hydroxyl groups[2] don't produce a large number of conformational polymorphs. Thus, we performed an extensive and systematic study of crystallization conditions through solvent-solvent and solvent-gel counter-diffusion experiments, looking for new polymorphs, and we outlined a reproducible procedure to obtain high quality single crystals of α-D-glucose, the most studied and common form.

In addition, in order to achieve a deep understanding of chemical bonding and intermolecular interactions, we determined electronic properties in solid state through high resolution single-crystal XRD at 90 K, determination of charge density using multipolar expansion and DFT quantum mechanical calculations.

	α-D-glucose monohydrate	α-D-glucose	β-D-glucose
Temperature	-4 – 52°C	52 – 91°C	> 91°C
Mass concentration	31 – 71%	71 – 87%	> 87%

***Table 1**: Experimental conditions for obtaining the different forms of glucose through slow cooling of an aqueous solution (boundary values between α-D-glucose and β-D-glucose are extrapolated)*

	α-D-glucose monohydrate	α-D-glucose	β-D-glucose
Crystal system	monoclinic	orthorhombic	orthorhombic
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
Chemical formula	$C_6H_{12}O_6 \cdot H_2O$	$C_6H_{12}O_6$	$C_6H_{12}O_6$
Z	2	4	4
a / Å	8.803(1)	10.3662(9)	9.205(4)
b / Å	5.085(1)	14.8506(16)	12.640(5)
c / Å	9.708(2)	4.9753(3)	6.654(3)
α / °	90	90	90
β / °	97.67(1)	90	90
γ / °	90	90	90
V / Å³	430.674	765.919	774.201

***Table 2**: Crystallographic information of the different forms of glucose. Refcodes: GLUCMH11, GLUCSA10, GLUCSE01*

RESULT: Crystallization of α-D-glucose

An experimental investigation on the solubility of glucose was performed. It was found to be very soluble in water and DMSO, quite soluble in DMF, methanol and ethanol and insoluble in some of organic solvents. A crystallization screening was carried out by using the antisolvent counter-diffusion method. Methanol and ethanol were selected as solvents and hexane and pentane as co-solvents. A series of experiments were performed as a function of glucose concentration. Ethanol/hexane was proven to be the best solvent/antisolvent couple.

All crystallizations were carried out at 26±1°C in vials with a diameter of 13 mm using 2 mL of solution and 4 mL of co-solvent. Results of crystallizations experiments by using ethanol as solvent are reported in ***Table 3*** and ***Figure 1***.

	Pentane	Hexane
Saturated EtOH	Many aggregates, few large single crystals	Few aggregates, many large single crystals
EtOH diluted 1 : 1	Some aggregates, many single crystals	Few aggregates, many large single crystals
EtOH diluted 1 : 1.5	Many small single crystals	Few single crystals
EtOH diluted 1 : 3	Few small single crystals	Very few single crystals

***Table 3**: Comparison between ethanol solution crystallizazions as a function of concentration with pentane and with hexane as co-solvents*

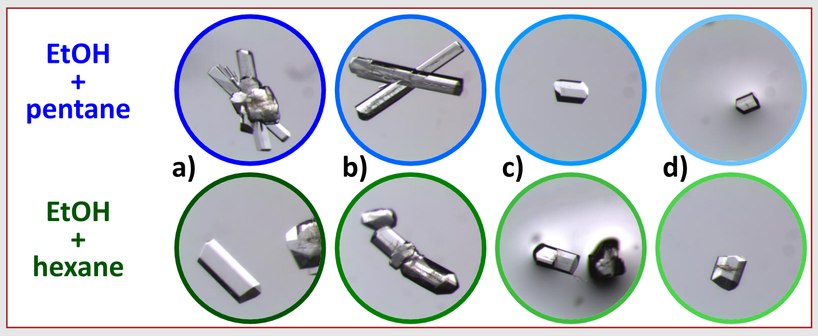

***Figure 1**: Selection of representative crystals isolated from ethanol solution crystallizations with pentane and with hexane at different concentrations: **a)** saturated; **b)** 1 : 1; **c)** 1 : 1.5; **d)** 1 : 3*

RESULT: New solvated form of β-D-glucose with acetonitrile

We obtained millimetric single crystals (***Figure 3***) of a new solvated form of β-D-glucose with acetonitrile from both solvent-gel and solvent-solvent counter-diffusion crystallization experiments at room temperature:

solvent-gel: 4 mL of saturated MeOH solution of α-D-glucose + 0.5 mL water + 0.5 mL TMOS (10%) → 1 week → Addition of 5 mL CH_3CN → 4 weeks → Crystals

solvent-solvent: 2 mL saturated MeOH + 4 mL CH_3CN → 1 week → Crystals

The crystallographic asymmetric unit contains one solvent molecule and one β-D-glucose molecule that interact through a relatively weak H-bond (***Figure 2*** and ***Table 4***). It is important to highlight that the β anomer is mainly crystallized from water at high temperature (> 91°C) but, in our case, the presence of acetonitrile stabilized it in solid state at room temperature.

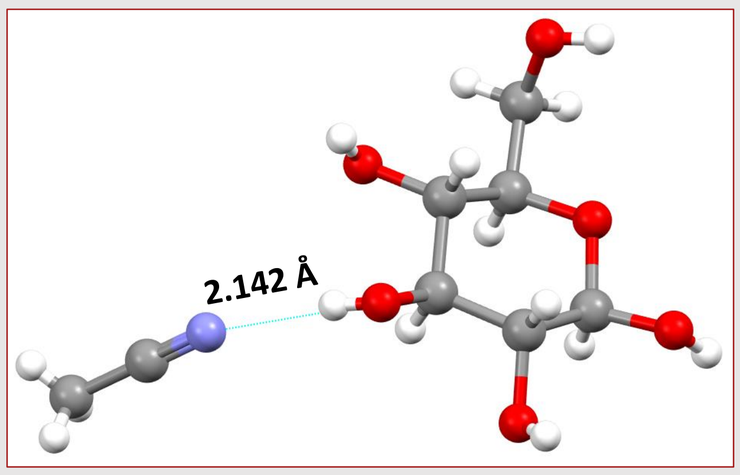

***Figure 2**: Interaction between acetonitrile and β-D-glucose in the new structure*

Crystal system	Monoclinic
Space group	$P2_1$
Chemical formula	$C_6H_{12}O_6 \cdot CH_3CN$
Z	2
a / Å	8.8196(3)
b / Å	6.6223(2)
c / Å	9.8994(3)
β / °	116.3200(11)
V / Å³	518.25(3)

***Table 4**: Crystallographic information of the new solvate of β-D-glucose and acetonitrile*

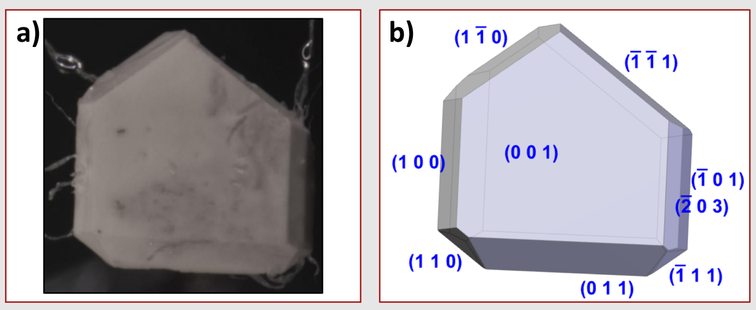

***Figure 3**: **a)** Crystal of β-D-glucose and acetonitrile isolated from solvent-gel crystallization; **b)** Crystal model with Miller indices*

RESULT: High resolution XRD of α-D-glucose at 90 K

Single-crystal XRD study at 90 K of α-D-glucose didn't show any dynamic disorder of hydroxyl groups but surprisingly showed the co-presence of β anomer with an extent of more than 5%, not detectable at low resolution (***Figure 4***). Hamilton test[4] performed on two models including or not disorder of O(1)-H(8) and H(1) demonstrated that the lowering of R factor due to the refinement of disordered atoms has a significance level of more than 99.5% (***Table 5***).

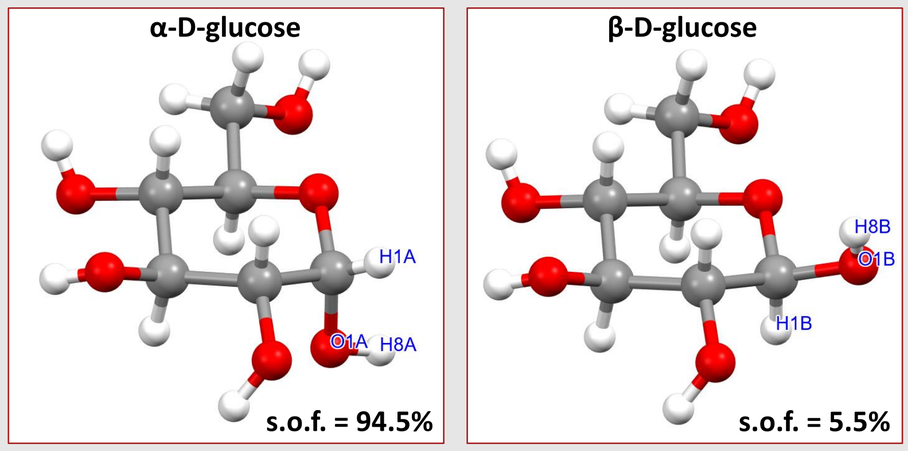

***Figure 4**: Comparison between disordered atoms and s.o.f. of **a)** α-D-glucose and **b)** β-D-glucose*

	Ordered	Disordered
Reflections	8182	8182
Parameters refined	145	155
R all	3.57%	3.44%

***Table 5**: Parameters used for Hamilton test*

Despite the presence of disorder, thanks to the great quality of the data set, we managed to determine crystal charge density through multipolar refinement using Hansen & Coppens formalism[4] and to integrate Bader's atomic basins. We had to limit multipole expansion and to constrain poles population of disordered atoms of molecule β to be equal to those of molecule α.

We also determined structure factors from DFT calculations using Crystal software[5] to make a comparison between multipolar models based on observed and calculated structure factors and we calculated molecular dipole moments for the α anomer (***Table 6***).

	H-C, Fo	H-C, Fc	Bader, Fo	Bader, Fc
μ_x / D	2.53	0.97	1.31	1.74
μ_y / D	-0.25	0.88	-3.84	-1.25
μ_z / D	3.75	0.70	5.02	2.19
|μ| / D	4.53	1.48	6.46	3.06

***Table 6**: Molecular dipole moments obtained from Hansen & Coppens monopoles population (**H-C**) or integration of Bader's atomic basins (**Bader**) based on observed (**Fo**) or calculated (**Fc**) structure factors.*

CONCLUSIONS AND FUTURE WORK

Solvent screening and crystallizations allowed us to devise a simple and reproducible procedure to obtain single crystals of α-D-glucose from EtOH and hexane and to discover a new solvated form of β-D-glucose with acetonitrile. We also showed that, in some cases, low resolution XRD is not sufficient to detect the presence of disorder and we managed to determine charge density of a disordered structure.

At the moment, we are performing complementary studies to XRD in order to have a full view of molecular recognition process, performing molecular dynamics simulations at different temperatures with MiCMoS[6] and GROMACS[7] software, analyzing radial distribution functions, dihedral angles and self-diffusion coefficients for α-D-glucose in crystalline state, in melted state and in aqueous solution at different concentrations.

REFERENCES

1) Young, *J. Phys. Chem*. **1957**, 61, 616
2) Zuccarello, Buemi, *Carbohydr. Res.* **1995**, 273, 129
3) Hamilton, *Acta Cryst*. **1965**, 18, 502
4) Hansen, Coppens, *Acta Cryst*. **1978**, A34, 909-921
5) Dovesi *et al*, *Comput Mol Sci*. **2018**, 8, e1360
6) (a) Gavezzotti, Lo Presti, *J. Appl. Crystallogr.*, **2019**, 52, 1253; (b) Gavezzotti, Lo Presti, *New J. Chem*., **2019**, 43, 2077; (c) Gavezzotti, Lo Presti, Rizzato, *CrystEngComm*, **2020**, 22, 7350; (d) Rizzato, Gavezzotti, Lo Presti, *Cryst. Growth Des*. **2020**, 11, 7421
7) Bekker *et al*., *Physics computing*, **1993**, 92, 252