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OMICS Group is an amalgamation of Open Access Publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 700+ online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 1000+ International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

OMICS International Conferences

OMICS International is a pioneer and leading science event organizer, which publishes around 700+ open access journals and conducts over 500 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 1000+ conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

Purity, Endotoxin Level, and Trace Metal Comparison of Different Grades of Trehalose

Ben Nelson, PhD.

Formulation Solutions Manager

ben.nelson@pfanstiehl.com

http://www.pfanstiehl.com

NON-CONFIDENTIAL PRESENTATION

Protein and Cell Stabilization

Some Functions of Carbohydrates

- Reduction in denaturation caused by heat, stress, oxidation
- Prevention of mAb and protein aggregation
- Improvements in post-thaw cell viability
- Vaccine stabilization, membrane stabilization, phospholipid bilayer protection
- Depression of phase transition temperature of dry lipids, rendering them more resistant to damage during desiccation, maintains liquid crystalline phase in the absence of water (glassy state)

Carbohydrates Used in Biotech Drug Products

- Trehalose
- Sucrose
- Mannitol
- Sorbitol

Marketed Drugs Formulated with Trehalose

Drug	Manufacturer	API Class	Formulation Type
Adcetris	Seattle Genetics	ADC	Lyophilized Powder
Avastin	Genentech/Roche	mAb	Solution
Blincyto	Amgen	Bispecific mAb	Lyophilized Powder
Gazyva	Genentech/Roche	mAb	Solution
Herceptin	Genentech/Roche	mAb	Lyophilized Powder
Lucentis	Genentech/Roche	mAb	Solution

Trehalose and Sucrose

Comparison of Properties

Properties	Trehalose	Sucrose	References
Solubility (g/100 g H_2O, at 20°C)	40.6–68.9[a]	200	27,33
Melting temperature (°C)	210–215[b]	188	27–30
Glass transition temperature (T_g, °C)	110–120	65 –75	28–29,34
Relative viscosity[c]	1.85	1.3	42–43
# Equatorial –OH	8	6–7	44
Diffusion coefficient (cm^2/s)[d]	1.91×10^{-8}	5.89×10^{-8}	45
Density (g/cm^3, at 25°C and 85°C)	1.58, 1.41	1.59, 1.37	46–47
Hydration number[e]	11	8	46
Rate of hydrolysis (s^{-1}, at 25°C)[f]	3.3×10^{-15}	5.0×10^{-11}	38
Stability in extreme pH (% remaining)[g]	>99%	~0% at pH 3–4	27,42
Acrylamide formation[h]	0 mg/mol Asn	98 mg/mol Asn	27
Calcium dissolution in phosphate buffer[i]	24 ppm	6 ppm	27,48–49
Sweetness[j]	45%	100%	27

[a] Wide range of solubility is due to the difference in purity of trehalose used in the studies.

[b] Melting temperature of anhydrate trehalose crystals.

[c] Viscosity of sugar solutions (0.5 M) with respect to water (viscosity = 1) at 25°C, as measured by Cannon-Manning semi-Micro-type capillary viscometer.

[d] Diffusion coefficient of disaccharides (74 wt %) was measured using pulsed-gradient-spin-echo NMR at 50°C.

[e] Hydration number is defined as the average number of water molecules that are hydrogen bonded to the sugar molecule, as computed by molecular simulation. The hydration numbers for sucrose and trehalose were computed for 50 wt % sugar solutions at 80°C and 87°C, respectively.

[f] Hydrolysis rate constants of disaccharides (0.05 M) in potassium phosphate buffer (0.1 M, pH 8.1) were determined using protein NMR at high temperatures and extrapolated to obtain the values at 25°C.

[g] In a pH range from 3.5 to 10 at 100°C for 24 h.

[h] Amount of acrylamide formation in a mixture containing asparagine and disaccharide, both at 0.1 mmol concentration, was measured by GC. The solution was heated at 150°C for 20 min.

[i] In a 50 mM phosphate buffer at pH 6.8 and 10% solution of either disaccharide.

[j] Sweetness with respect to that of sucrose.

Ohtake and Wang, J. Pharm. Sci., 100(6), 2020, (2011)

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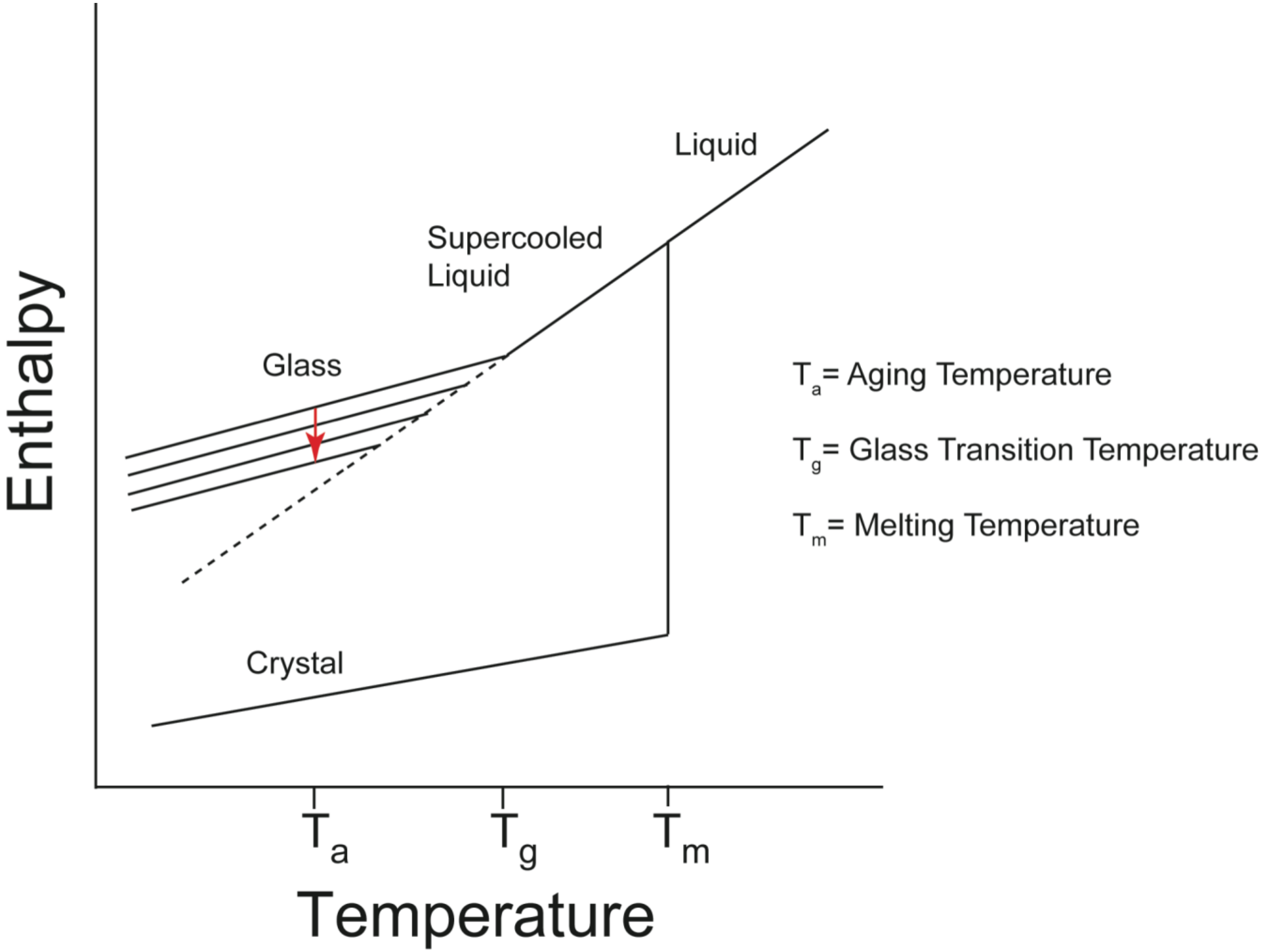
Glass Transition Temperature (T_g)

- The temperature at which and amorphous material transitions between glass and rubber states.
  - Below T_g = glass state – lower mobility
  - Above T_g = rubber state – higher mobility
- The higher the T_g is above the storage temperature the less likely:
  - Re-crystallization
  - Chemical reactions: excipient/excipient and excipient/API (largely due to decreased mobility when in the glassy state)
- Water acts as a plasticizer – the more water present, the lower T_g is.

Enthalpy of the Amorphous State

Diagram depicting the energetics of a crystalline-liquid-amorphous system. As an amorphous material is held below its glass transition temperature, it relaxes to a lower energy state during a process referred to as aging or annealing.



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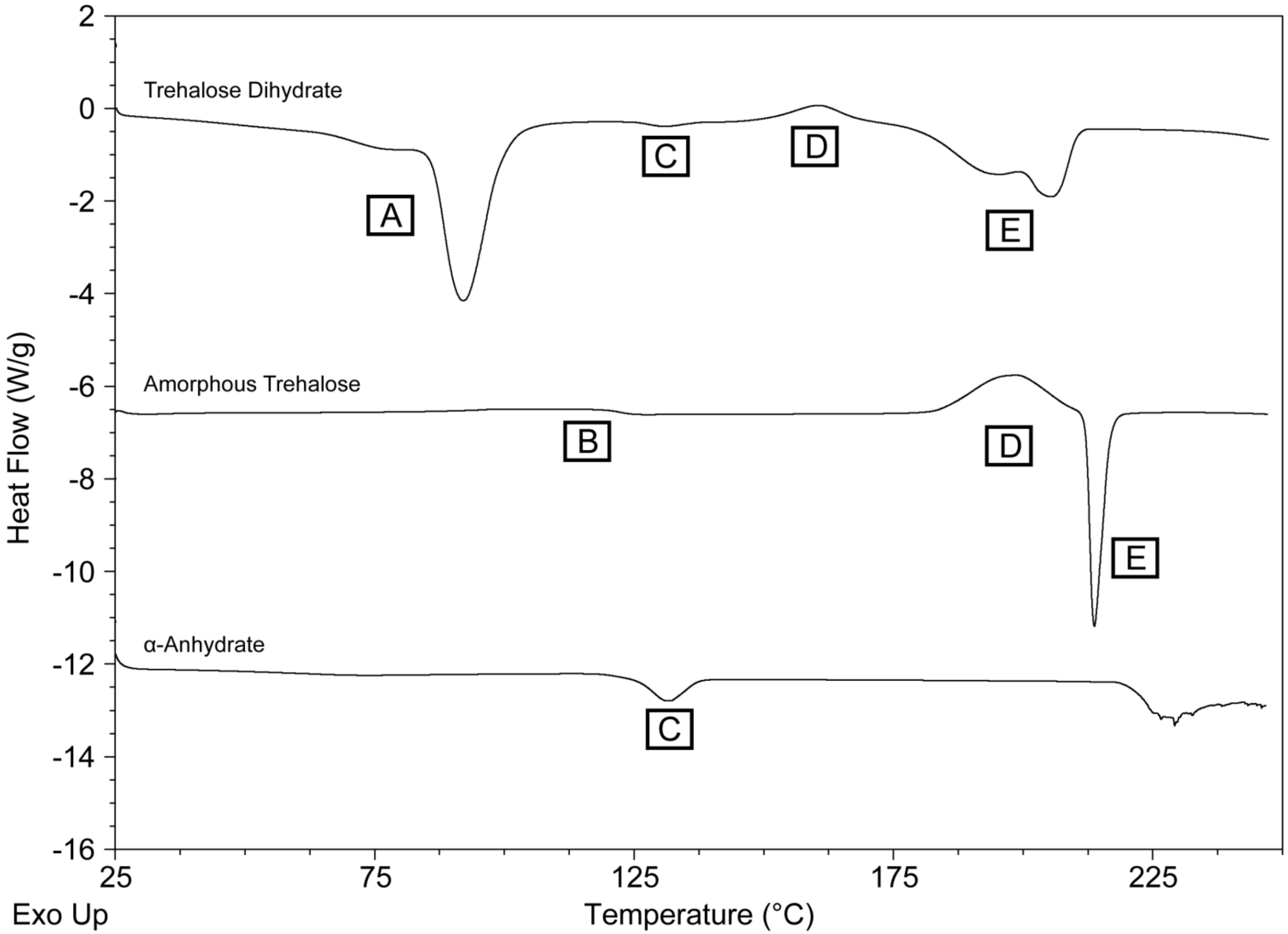
DSC Thermograms of Trehalose Forms



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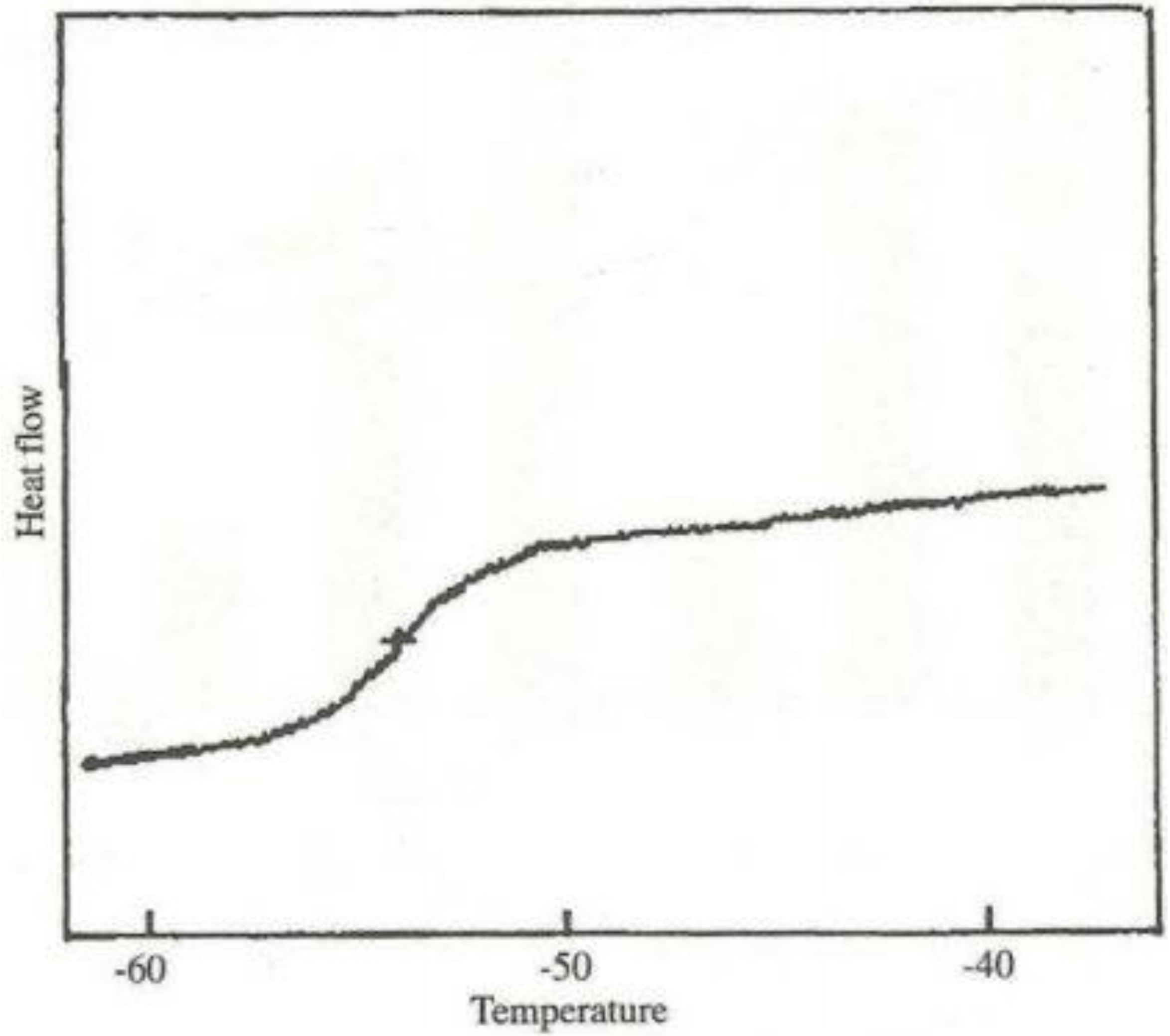
T_g Prime (T_g’)

- In the majority of cases solutes do not crystallize at the solubility limit as the sample is freezing.
- As the temperature is decreased, ice continues to form and the solution continues to concentrate until it is so viscous it turns to a glass.
- This glass transition temperature is denoted T_g’
- The “prime” mark is used to denote this is the glass formed by freeze concentration

T_g' DSC Thermogram



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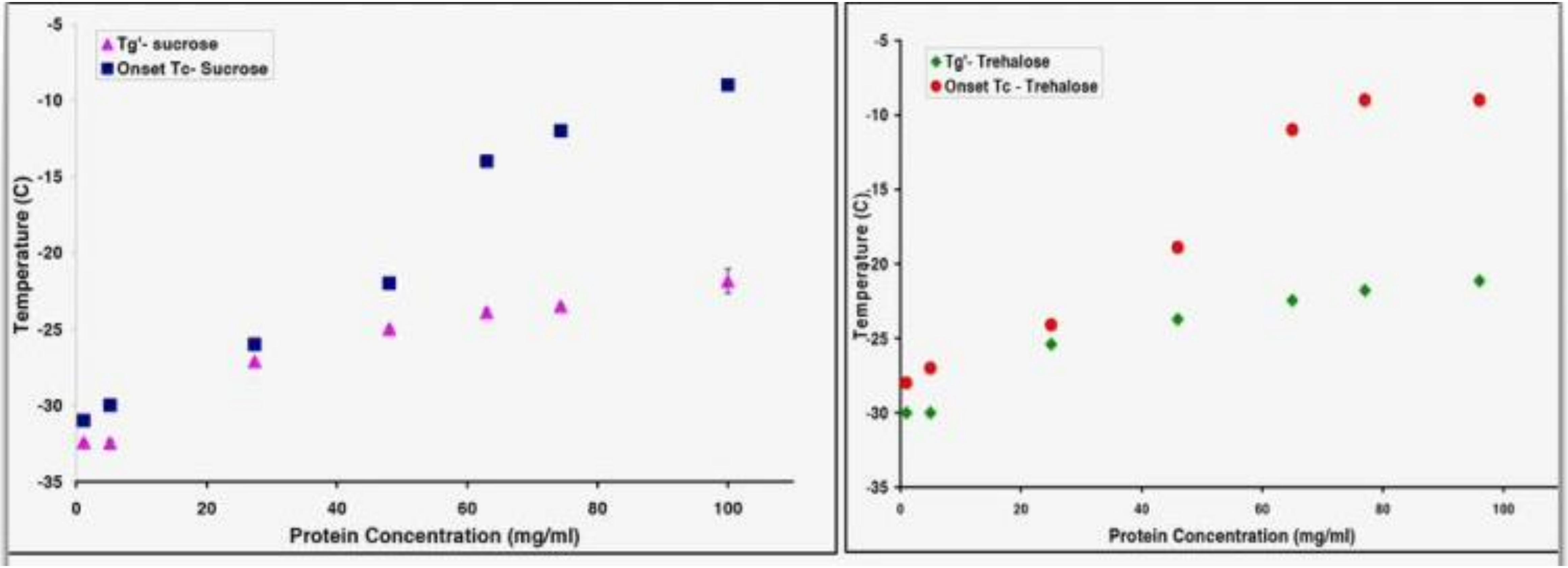
T_g'/T_c vs Protein Concentration



- T_g' measured by DSC
- T_c measured by freeze drying microscopy

- Every 1°C increase in product temperature results in ~13% reduction in primary drying time

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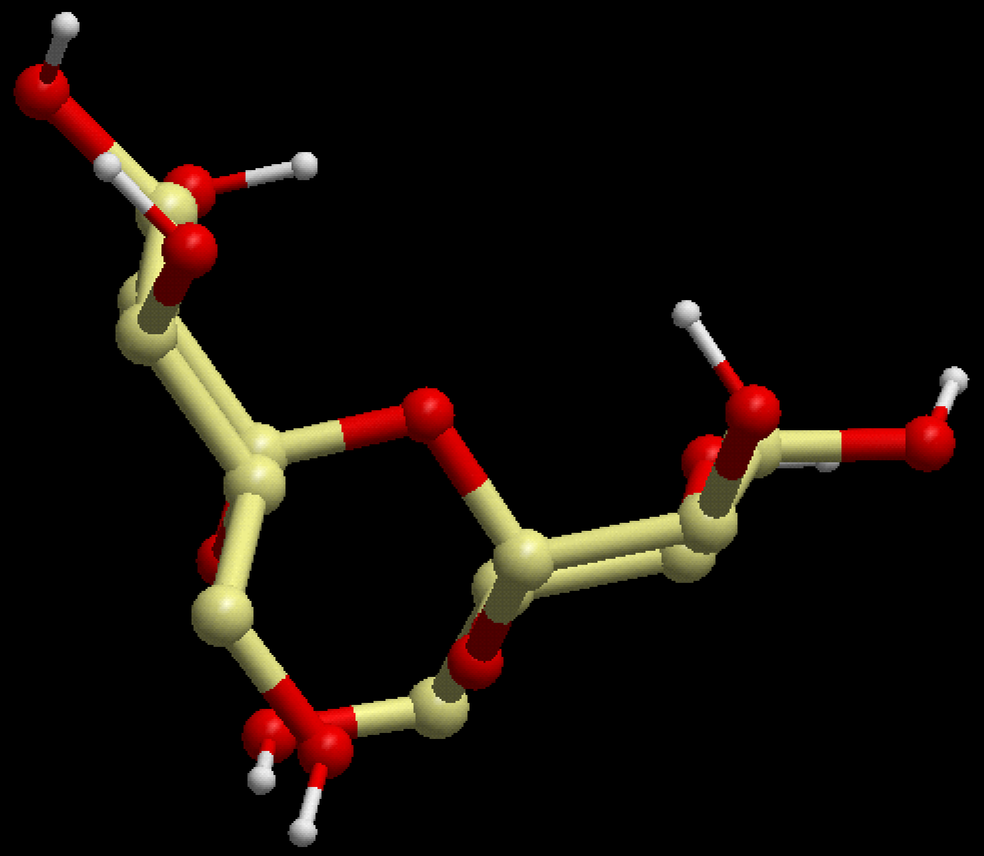
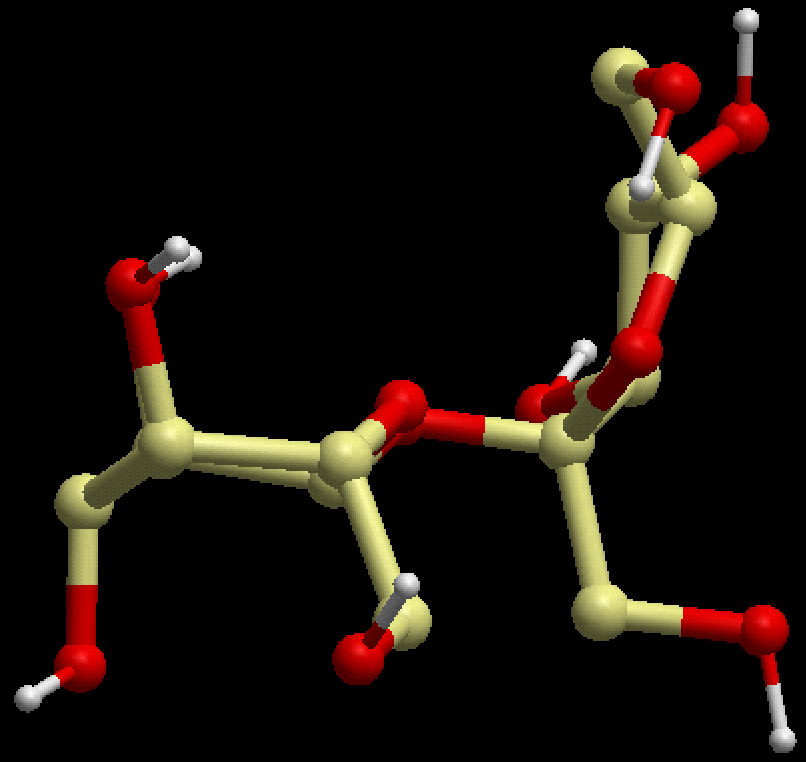
Equtorial OH Groups

- The more equatorial OH groups a disaccharides has the stronger interactions with water
- Helps Trehalose include itself in the water cluster with relative ease
- Sucrose does not integrate into the water cluster creating a larger structure of the sugar-water clusters. This prevents it from getting into smaller places.





Rate of Hydrolysis

- Free Energy of Glycosidic Bonds
  - Trehalose <1 kcal/mol
  - Sucrose = 27 kcal/mol
- Higher free energy bonds renders the disaccharide more susceptible to hydrolysis
  - Non-acid catalyzed hydrolysis rates at 25°C:
    - Trehalose = $3.3 \times 10^{-15}\ s^{-1}$
    - Sucrose = $5 \times 10^{-11}\ s^{-1}$
  - Stability after 1 hour in solution at pH = 3.5:
    - Trehalose remaining >99%
    - Sucrose remaining ~0%

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Hydration Number

- Disaccharides at high concentration have a scarcity of water in their vicinity, thus intramolecular hydrogen bond formation is needed. The arrangement of intramolecular hydrogen bonds results in a folded configuration (around the glycosidic bond) and thus a reduction in hydration number
  - Does this make the glycosidic bond more susceptible to glycolysis, resulting in more reducing sugars?
  - Trehalose exhibits no such configurational changes

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Total Impurities and Endotoxin Levels

Grade (# of Lots Tested)	Total Impurities (%)		Endotoxins (EU/g)	
	Data Range	Data Mean	Data Range	Data Mean
Food (6)	0.69 – 1.00	0.84 ± 0.12	0.21 – 2.30	1.17 ± 0.77
Technical (6)	0.42 – 0.68	0.54 ± 0.11	0.54 – 4.10	0.84 ± 0.12
Pfanstiehl High Purity (16)	0.06 – 0.17	0.14 ± 0.03	<LOQ – 0.33	0.06 ± 0.09*

*Only two lots tested above the LOQ (0.05 EU/g). For lots tested <LOQ a value of 0.025 was used in the calculation of the mean.

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Trace Metal Profile of Pfanstiehl Trehalose

Elements	V	Cr	Mn	Fe	Ni	Cu	Zn	As	Mo	Ru	Rh	Pd	Cd	Os	Ir	Pt	Hg	Pb
Limit (ppm)	1	2.5	25	130	2.5	10	130	0.15	1.0	1.0	1.0	1.0	0.25	1.0	1.0	1.0	0.15	0.5
Limit of Quantitation (ppm)	0.5	0.5	5	26	1	5	26	0.03	0.5	0.2	0.2	0.2	0.05	0.2	0.2	0.2	0.03	0.1
Lot 1	<0.5	<0.5	<5	<26	<1	<5	<26	<0.03	<0.5	<0.2	<0.2	<0.2	0.11	<0.2	<0.2	<0.2	<0.03	<0.1
Lot 2	<0.5	<0.5	<5	<26	<1	<5	<26	<0.03	<0.5	<0.2	<0.2	<0.2	<0.05	<0.2	<0.2	<0.2	<0.03	<0.1
Lot 3	<0.5	<0.5	<5	<26	<1	<5	<26	<0.03	<0.5	<0.2	<0.2	<0.2	<0.05	<0.2	<0.2	<0.2	<0.03	<0.1
Lot 4	<0.5	<0.5	<5	<26	<1	<5	<26	<0.03	<0.5	<0.2	<0.2	<0.2	<0.05	<0.2	<0.2	<0.2	<0.03	<0.1
Lot 5	<0.5	<0.5	<5	<26	<1	<5	<26	<0.03	<0.5	<0.2	<0.2	<0.2	<0.05	<0.2	<0.2	<0.2	<0.03	<0.1
Lot 6	<0.5	<0.5	<5	<26	<1	<5	<26	<0.03	<0.5	<0.2	<0.2	<0.2	<0.05	<0.2	<0.2	<0.2	<0.03	<0.1
Lot 7	<0.5	<0.5	<5	<26	<1	<5	<26	<0.03	<0.5	<0.2	<0.2	<0.2	<0.05	<0.2	<0.2	<0.2	<0.03	<0.1
Lot 8	<0.5	<0.5	<5	<26	<1	<5	<26	<0.03	<0.5	<0.2	<0.2	<0.2	<0.05	<0.2	<0.2	<0.2	<0.03	<0.1
Lot 9	<0.5	<0.5	<5	<26	<1	<5	<26	<0.03	<0.5	<0.2	<0.2	<0.2	<0.05	<0.2	<0.2	<0.2	<0.03	<0.1
Lot 10	<0.5	<0.5	<5	<26	<1	<5	<26	<0.03	<0.5	<0.2	<0.2	<0.2	<0.05	<0.2	<0.2	<0.2	<0.03	<0.1

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Pfanstiehl Know Your Product & Process Initiative

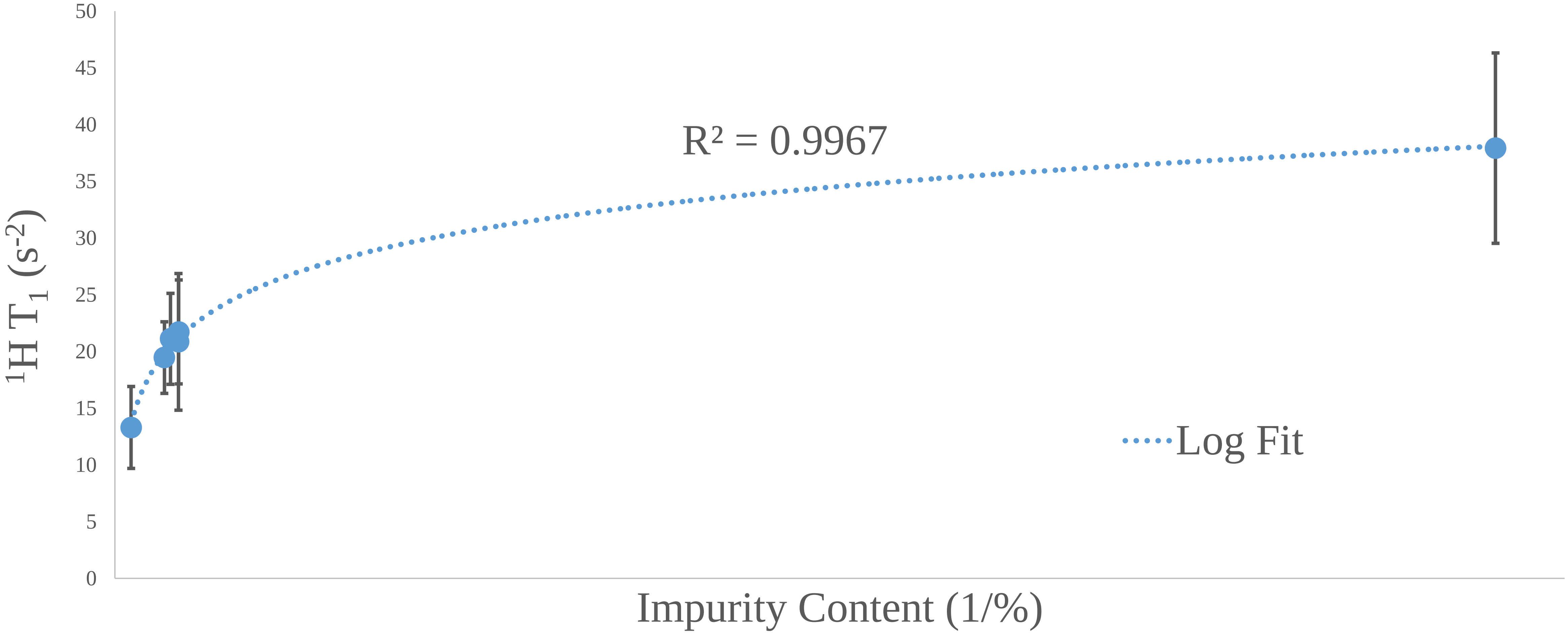
Additional Characterization

- Particle Size
- Polymorph
- DSC
- Residual Elemental Impurities
- Nanoparticulates
- Conductivity
- Dexrtrins
- Glucans
- Impurity Profile
- 2D NMR

Functional Testing

- Dissolution Studies
- Customer Function (Formulation Stability Testing etc.)

$^1H\ T_1$ vs Impurity Content



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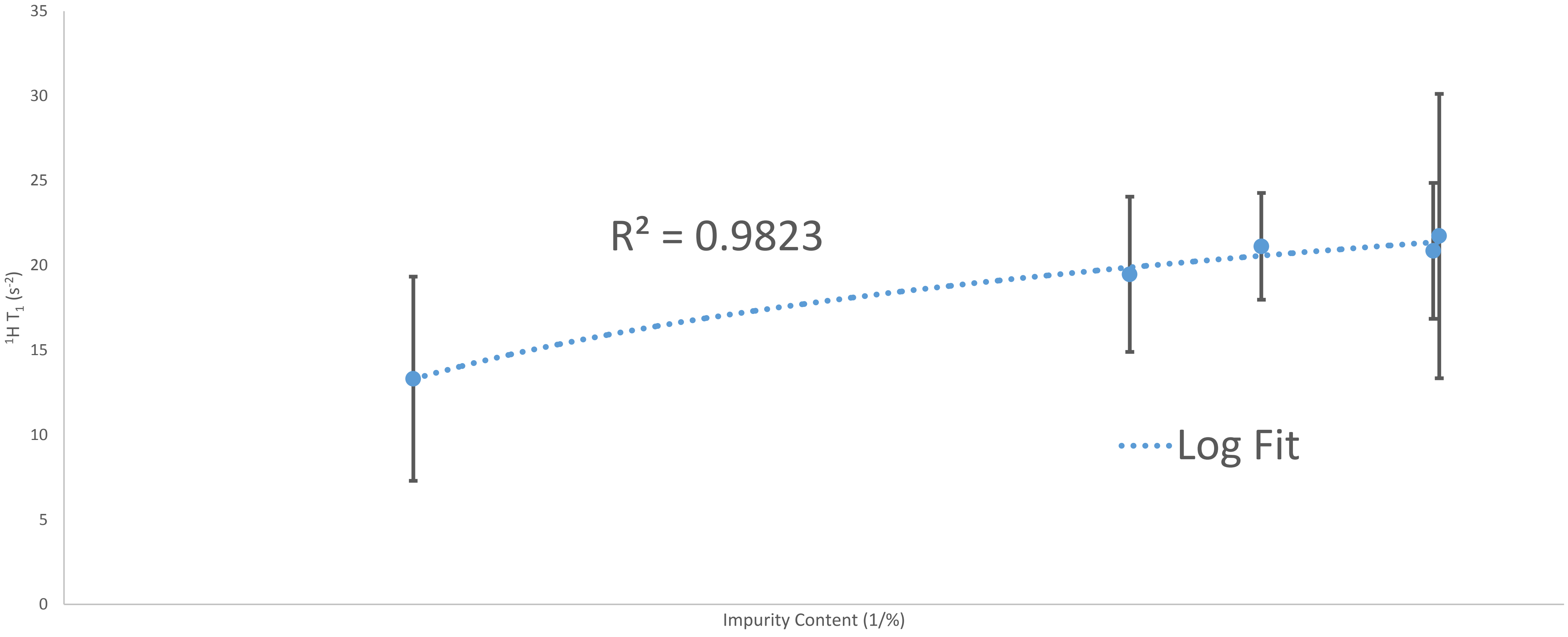
$^1H\ T_1$ vs Impurity Content



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Thank You

Contact Information

Ben Nelson
Pfanstiehl
1219 Glen Rock Avenue
Waukegan, IL 60085

Office: 847-599-5139

Email: ben.nelson@pfanstiehl.com

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