Trehalose: A Powerful Excipient in the Formulation Toolbox

In the pharmaceutical industry, products which benefit from the stabilizing effects of trehalose include, but are not limited to: monoclonal antibodies (mAbs), antibody drug conjugates (ADCs), fusion proteins, peptides, stem cells, and vaccines. Table 1 is a brief list of drugs which include trehalose as a component of their formulations.

Table 1: 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

Many of the stabilizing effects of trehalose are derived from its unique properties. The glass transition temperature (T_g) is between 110 and 120°C, which is the highest of the disaccharides. Formulations lyophilized in a glassy amorphous matrix of trehalose can be stored at higher temperatures without concern of negative consequences associated with molecular mobility, since molecular mobility is negligible upon storage 50°C below the T_g of the amorphous matrix. Primary drying during lyophilization should be below the freeze concentration glass transition temperature (T_g') to prevent cake collapse. In comparison to sucrose, the T_g' of trehalose is about 3°C higher, translating to a ~13% reduction in primary drying time for every 1°C difference in T_g'. Trehalose itself is intrinsically stable and is not susceptible to breakdown to reducing sugars, since the free energy of the glycosidic bond is <1 kcal/mol. Formulation at pH below 5 is also possible with trehalose. After one hour in solution at pH 3.5, >99% of trehalose remains compared to ~0% sucrose remaining in the same conditions.

The quality of excipients used should also be of high importance to the formulator since the use of products known to be of the highest purity can mitigate risk from bench top development through commercialization of therapeutics. There are different grades of trehalose available on the market. Pfanstiehl's internal studies have shown (Table 2) that our trehalose contains less impurities and endotoxins, factors of 5 and 12 respectively, than other currently marketed brands.

Table 2: Total Impurities and Endotoxin Levels in Various Trehalose Grades

Grade (# of Lots Tested)	Total Impurities (%)		Endotoxins (EU/g)	
	Data Range	Data Mean	Data Range	Data Mean
Food Grade (6)	0.69 – 1.00	0.84 ± 0.12	0.21 – 2.30	1.17 ± 0.77
"Pharma" Grade (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 assay LOQ (0.05 EU/g). For lots tested <LOQ a value of 0.025 was used in the calculation of the mean.

Stabilization of proteins is best taken on a case by case basis. Proteins that do not vary significantly in primary structure can vary drastically in secondary and tertiary structure, and reactivity to their environment. Formulation components that work well for one system may not work for a similar system. Therefore, formulation scientists must use all of the tools they have at their disposal. Despite being less ubiquitous in the pharmaceutical industry when compared to other excipients such as sucrose and mannitol, trehalose should not be overlooked as a powerful tool for drug product stabilization in both liquid and lyophilized drug product preparations.

For further reading see these review articles on trehalose:

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Glycobiology **2003,** 13, 17R-27R

Food Chem Toxicol. **2002,** 40, 871-898

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