HAYASHIBARA
NAGASE Group

TREHALOSE SG JP, USP-NF, Ph. Eur., CP

High-purity and low endotoxin injectable grade trehalose

TREHALOSE SG is an injectable grade pharmaceutical excipient and is monographed as being low endotoxin and high purity. It is supplied as powdered dihydrate crystals produced by the enzymatic saccharification of starch. Hayashibara has achieved exceptionally high quality and stable supply by production of trehalose and removal of endotoxin in our manufacturing plant in Japan. TREHALOSE SG is stable to heat and acid, and as a non-reducing disaccharide, does not participate in the Maillard reaction, so it stabilizes biopharmaceutical formulations.

Name	Trehalose dihydrate
Formula	$C_{12}H_{22}O_{11} \cdot 2H_2O$
Formula Weight	378.33
Endotoxin	＜0.3 EU/g
Purity	≧99.0 %
Quality Level	JP, USP-NF, Ph. Eur., CP

Introduction

Biopharma consists of molecules from organisms that can be used as active pharmaceutical ingredients (APIs). Many modalities such as antibodies, peptides and nucleic acids have been developed, and their market is rapidly expanding every year. In general, biopharmaceuticals are considered to be more specific and safer than small molecule drugs. However, most biopharmaceuticals are administered as injectable drugs due to their lower stability and absorption compared to small molecules. Currently, there are many studies being conducted with the aim of improving the precision of quality control, extension of storage periods, and simplifying storage conditions.

A typical biopharmaceutical formulation contains various excipients including osmotic regulators, buffers, and surfactants. Among them, saccharides are added to many products to regulate the osmolality and stabilize the API[1,2]. Some of the saccharides (especially sucrose and trehalose) are also used as API protectants during cryopreservation and lyophilization[1,3]. In this white paper, Hayashibara proposes the use of trehalose as a strategy to improve the storage stability of antibodies, nucleic acids and exosomes. In the case of antibodies, Hayashibara introduces data to not only improve stability during storage, but also provides benefits during manufacturing processes such as culture and purification.

Trehalose For Biopharmaceuticals

Trehalose is a non-reducing disaccharide consisting of two glucose molecules linked by an α,α−1,1 bond (Figure 1), and its unique molecular structure gives it properties such as low-reactivity and acid resistance. Trehalose is known to stabilize proteins, especially with regards to antibodies, and both its anti-aggregation effect and function as a chemical chaperone have been widely reported[4,5]. Further, it is commonly used as a cryoprotectant, and many reports have shown that it protects biomolecules such as proteins and lipid bilayers from physical stress during lyophilization[3,5,6].

TREHALOSE SG JP, USP-NF, Ph. Eur., CP

TREHALOSE SG is an injectable grade pharmaceutical excipient and is monographed as being low endotoxin (<0.3 EU/g) and high purity (≧99.0 %). It is supplied as powdered dihydrate crystals produced by the enzymatic saccharification of starch. As shown in Figure 2, unlike Maltose and Sucrose, TREHALOSE SG does not participate in the Maillard reaction and is stable to heating such as autoclave. Thus, TREHALOSE SG can contribute to quality stabilization of various biopharmaceutical formulations.

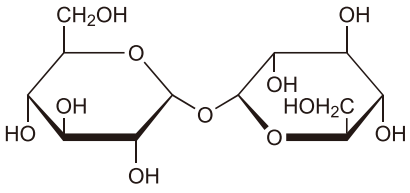

Figure 1 Structure of trehalose

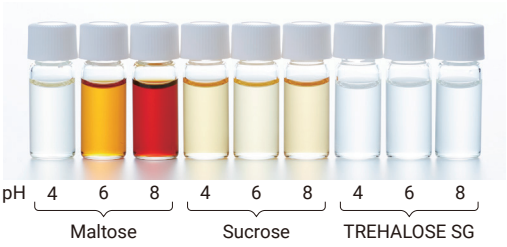

Figure 2 Reaction of glycine with saccharide solutions
Saccharides (12.5%) with glycine (0.5%) at 120°C for 1 hour

For Antibody Stabilization

During antibody production and storage, physical and chemical stress can cause denaturation and/or aggregation, but trehalose has been reported to protect antibodies from the stress[4,7]. TREHALOSE SG not only contributes to the stabilization of final formulations as an excipient, but also contributes to quality improvements in each upstream to downstream manufacturing process.

Inhibition of aggregation during upstream processing

Chinese hamster ovary (CHO) cell lines are widely used as a host cell for antibody production. While CHO cells have a high antibody producing ability, the high antibody concentrations tend to aggregate in the culture medium. In a study adding TREHALOSE SG to a medium of bispecific single-chained diabody (scDb-Fc)-producing CHO cells, antibody aggregation was suppressed (Figure 3).

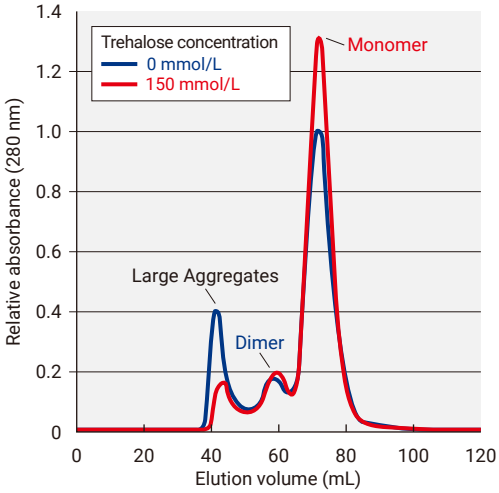

Figure 3 Gel filtration column chromatogram

Table 1 Ratio of aggregates

Trehalose concentration	Large Aggregates	Dimer	Monomer
0 mmol/L	17.5%	10.3%	72.2%
150 mmol/L	5.9% ↓	8.7%	85.4% ↑

After culturing scDb-Fc-producing CHO cells in a medium containing 150 mmol/L trehalose and purifying the harvested culture medium, the distribution (Figure 3) and ratio (Table 1) of monomers, dimers, and large aggregates were assayed when separated by gel filtration column chromatography.

Inhibition of aggregation during downstream processing

During downstream processing of antibodies, there are various physical or chemical stressors which can cause aggregation. Common aggregation stressors include acidic buffers and high salt concentrations. In a study, monoclonal antibody (mAb-TNF-β) was incubated with TREHALOSE SG, Sucrose, D-Mannitol or Sorbitol in glycine-HCl buffer (0.1 mol/L, pH 2.7) or lithium chloride buffer (5 mol/L) at 25°C for 30 min. TREHALOSE SG showed the highest anti-aggregation effect of all the carbohydrates (Figure 4, 5).

Acidic buffer (glycine-HCl pH2.7)

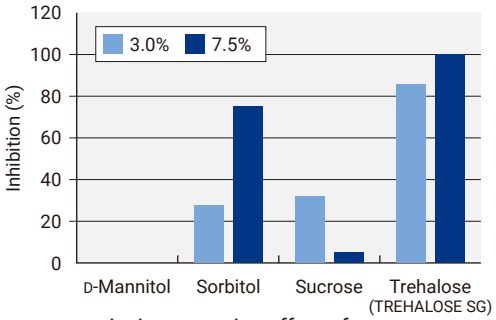

Figure 4 Anti-aggregation effect of each saccharide in glycine-HCl buffer

Salt (5 mol/L lithium chloride)

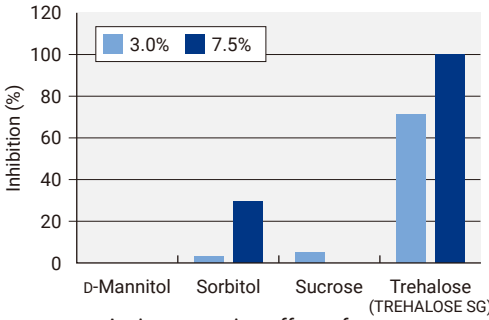

Figure 5 Anti-aggregation effect of each saccharide in lithium chloride buffer

Stabilization of antibody formulations

Trehalose is used in many antibody formulations as a stabilizer or lyoprotectant. Figure 6 shows that TREHALOSE SG maintained the activity of the monoclonal antibody (mAb-IFN-γ) in liquid phase more effectively than Sucrose or D-Mannitol. It has been suggested that trehalose contributes to the maintenance of the high-order structure of proteins under the stress of freezing or drying by forming hydrogen bonds with the protein instead of water molecules, or that trehalose becomes amorphous reducing the mobility of molecules[4]. After lyophilizing a TNF-β neutralizing antibody (mAb-TNF-β) using the same carbohydrates as above, the samples were stored for 6 months. The sample lyophilized with TREHALOSE SG maintained almost all of its neutralizing activity (Figure 7). These data demonstrate that TREHALOSE SG suppresses aggregation which can occur at various upstream to downstream antibody production steps and improves the storage stability of both liquid and lyophilized preparations.

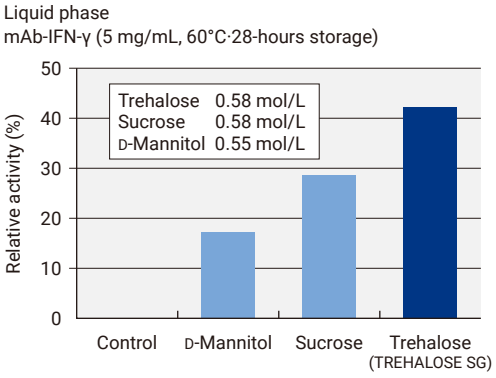

Figure 6 Storage stability under liquid phase

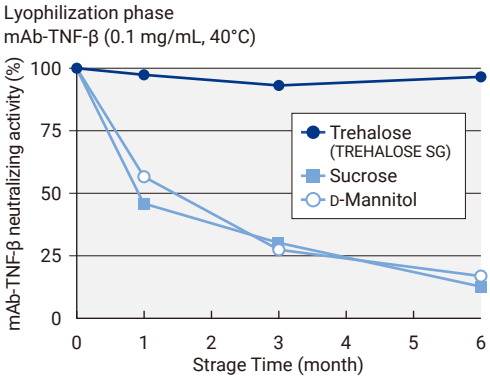

Figure 7 Storage stability under lyophilization phase
Saccharides in solution 80 mg/mL

For RNA Stabilization

Stable storage of nucleic acids from clinical samples or reagents is essential for accurate diagnosis and analysis. In particular, RNA is much more unstable than DNA due to its molecular structure, making the processing and storage of RNA especially important. It was found that TREHALOSE SG protects RNA extracted from mouse liver during the stress of dry storage (Figure 8, 9). Additionally, this protective effect was also observed for DNA. Thus, it was demonstrated that TREHALOSE SG is suitable for use as a stabilizer for nucleic acid samples and reagents.

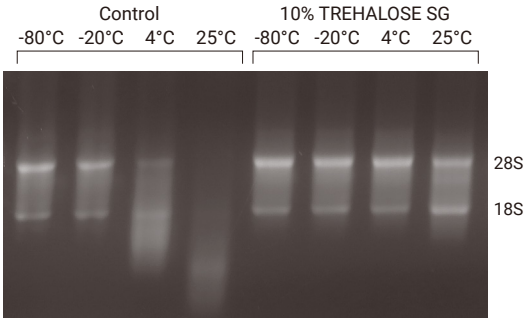

Figure 8 28S, 18S rRNA Electrophoresis

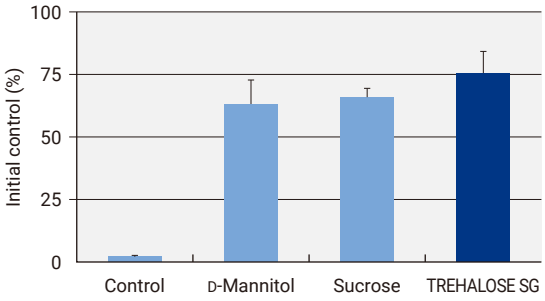

Figure 9 18S rRNA quantification (qPCR)
Saccharides in solution 10%

For Exosome Storage

Exosomes are 50-150 nm extracellular vesicles with a lipid bilayer derived from the endosomal membrane. Exosomes are expected to have a wide range of potential applications in regenerative medicine, drug delivery systems (DDS), diagnostic tests and cosmetic products. Exosomes are mainly composed of lipids, proteins, and nucleic acids, which need to be protected from various stresses during storage, such as heat and freeze-thaw, in order to maintain their function. Many reports have shown the usefulness of trehalose for the preservation of cells, liposomes and exosomes composed of a lipid bilayer[8-12]. In a study using mesenchymal stem cell (MSC)-derived exosomes, TREHALOSE SG showed a significant protective effect during liquid storage at 4°C for 2 weeks, as compared to Glucose and Sucrose (Figure 10). Further, the addition of TREHALOSE SG also showed a protective effect during 3 freeze-thawing cycles (Figure 11).

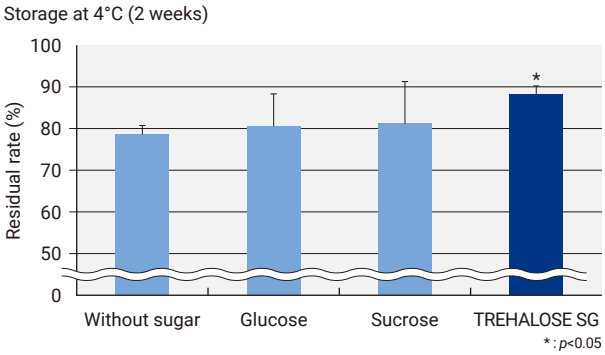

Figure 10 Stability during 4°C storage
Saccharides in solution 50 mmol/L

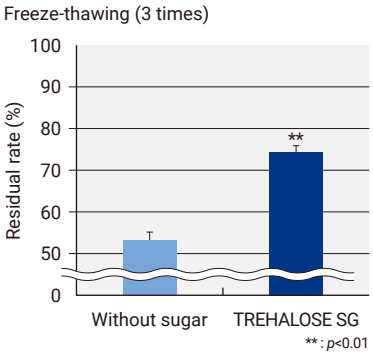

Figure 11 Stability against freeze-thawing
Saccharides in solution 50 mmol/L

Further Prospects

As described above, TREHALOSE SG is useful for improving the stability of antibodies, nucleic acids and exosomes, and is being incorporated into expanding uses in biopharmaceuticals. In a recent study, Hayashibara has also demonstrated that TREHALOSE SG promotes MSC-derived exosome production and inhibits aggregation during purification. As with antibodies, the data suggests that TREHALOSE SG can be effectively used during the manufacturing process to increase the yield of functional exosomes.

Published reports demonstrating the usefulness of trehalose for cell cryopreservation has been reported by several research teams, with potential applications in regenerative medicine and cell therapy[8,13]. Furthermore, the usefulness of trehalose in the lyophilization of platelets and blood cells has been reported many times and is expected to contribute to successful medical outcomes in areas of the world where storage conditions are not optimal[14-16]. Hayashibara continues to investigate the effects of trehalose on various medically relevant modalities, and hopes to contribute to improving the quality, production efficiency and storage stability of biopharmaceutical formulations.

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