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White Paper

Protecting Protein Stability with a Novel Grade of Sucrose

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Ensuring the chemical and physical stability of the therapeutic protein is critical to the safety and efficacy of biopharmaceuticals and presents one of the major challenges in formulation. A number of excipients, including sugars, can be used to solve this problem.[1] Sugars are stabilizers that maintain conformational stability by preferential exclusion[2] and function as cryo- and lyoprotectors in lyophilized formulations. Given these properties, it is not surprising that sucrose is one of the most widely used stabilizers in marketed drug products.[3]

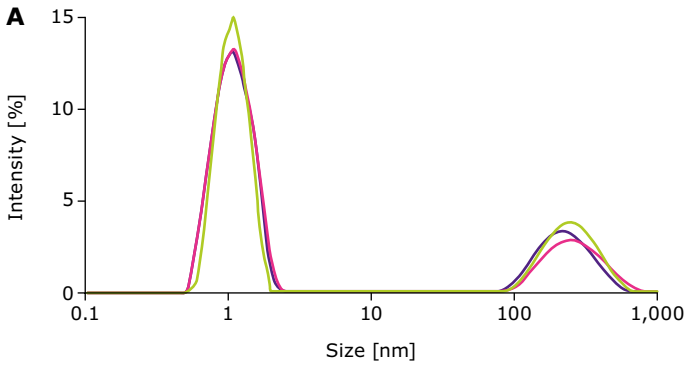
A challenge with the use of sucrose as an excipient, however, is that nanoparticle impurities (NPI) in a size range of 100–200 nm have been detected in pharmaceutical grade sucrose.[4] These impurities originate from the raw materials or can be introduced during production, and are not entirely removed during the sugar refinement process. NPIs can lead to false analytical results as they mimic protein aggregates. They can also induce protein aggregation, fragmentation and particle formation, resulting in reduced stability of therapeutic protein formulations.[5] While the quality of pharmaceutical-grade sucrose is regulated by pharmacopeias, the pharmacopeial monographs do not demand testing for NPIs. As such, there is no way of knowing which batch of sucrose might have a low or high NPI concentration, thus presenting a challenge for its use as an excipient in formulations.

This white paper provides an overview of NPIs found in commercially available sucrose, their origin and impact on protein stability and describes a novel sucrose purification process designed to minimize the presence of NPIs.

Types and Origins of NPIs in Sucrose

When measured by dynamic light scattering (DLS), monomeric sucrose has a size of approximately 1 nm; a second peak around 100–200 nm is frequently visible in DLS data of sucrose (Figure 1A). While the size of the particles remains within the range of 100–200 nm, the intensity can vary between different sucrose batches.[4] In the past, the presence of this second peak has falsely been attributed to collective diffusion of the sucrose molecules as an intrinsic phenomenon.[6] A recent study has now demonstrated that nanoparticles, which are not completely removed during sugar refinement, were responsible for this signal.[4]

Dynamic Light Scattering (DLS)



Nanoparticle Tracking Analysis (NTA)

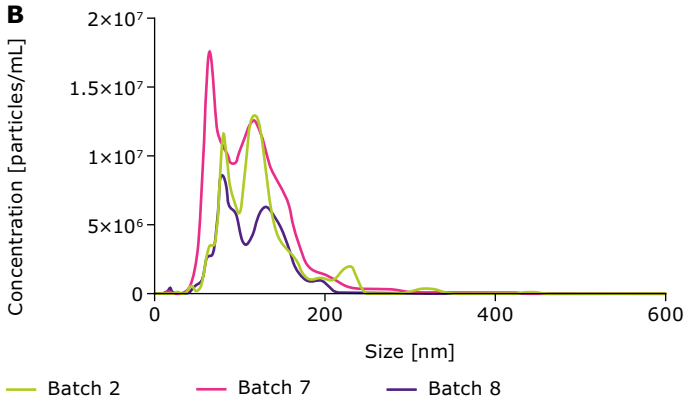


Figure 1.

Size distribution of a 10 % (w/v) sucrose solution prepared from different batches measured by using DLS (A) and NTA (B).

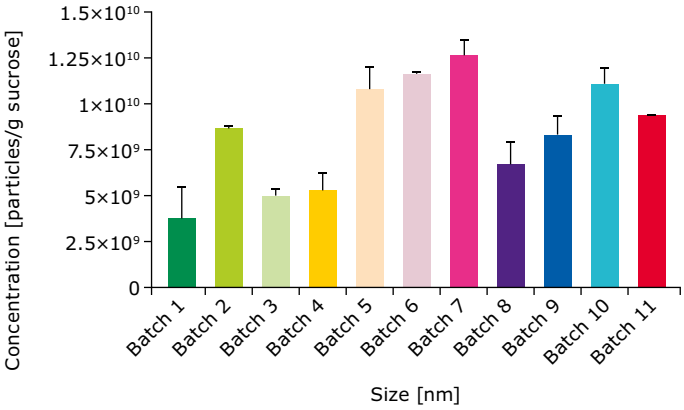


Figure 2.

Particle concentration in a 10 % (w/v) sucrose solution prepared from different batches determined using NTA measurements.

The second peak is also visible by using an orthogonal technique called nanoparticle tracking analysis (NTA), which can be used to determine particle size and quantify particles down to approximately 50 nm (Figure 1B). Depending on the sucrose batch, the quantity of NPIs can be up to 10^{10} particles per gram of sucrose (Figure 2).

Previous studies by Weinbuch *et al.* have shown that these NPIs consist partly of dextran.[4] Dextran can be produced by *Leuconostoc* bacteria, which mainly enter the sugar cane or beet during harvesting, cutting and grinding, or can be introduced in later production steps; the amount present depends on the time period between storage and harvesting. Moreover, a combination of elements has been found that matches the description of ash. Ash consists of solid oxide that can be introduced through the soil of the sugar cane or beet raw material or via dirt and trash. Varying amounts of fluorescent impurities of different compositions have also been found in sugar products including catechols and species similar to tryptophan and tyrosine. All the impurities and elements in NPIs isolated from sucrose are known to the sugar industry and are difficult to remove during the refinement process. As explained above, these contaminants are mainly introduced by the raw material, which is why the amount of NPIs in sucrose varies depending on the source of the raw material and capability of the production process to remove them from the final sucrose product.[4]

Impact of NPIs on Protein Stability

As sucrose is used as an excipient in therapeutic formulations, it is important to understand the impact of any impurities on the proteins it is intended to protect. Weinbuch *et al.* demonstrated that NPIs have a negative impact on the stability of different monoclonal antibody (mAb) formulations currently on the market.[5] Protein degradation and aggregation was induced by NPIs at concentrations that could be found in sucrose-containing drug products. However, the extent of degradation depends on the nature of the protein as has been shown for the different mAbs used in this study. Spiking NPIs into these mAb formulations have resulted in the formation of protein aggregates of various sizes ranging from nm to µm. In contrast, NPIs seem to have no influence on the conformational stability and charge variants.[5] The results for the different mAbs are summarized in Table 1.

Table 1.

Impact of NPIs on protein stability. The extent of protein degradation and aggregation depends on the nature of the protein.

Attribute	Method	Trastuzumab	Rituximab	Infliximab	Cetuximab
Visible particles	Visual inspection	+	-	+	-
Turbidity	Visual inspection	++	-	++	+
µm-particles	MFI	++	+	++	+
nm-particles	NTA	-	++	++	++
HMW species	SEC	-	-	-	+
Sample recovery	SEC	+	-	+	-
LMW species	SEC	-	+	+	-
Conformational instability	nDSF	-	-	-	-
Charge variants	cIEF	-	-	-	-

- was not affected (relative to control); + was affected, but only at high concentrations of NPIs and/or not immediately (relative to control); ++ was highly and immediately affected and/or affected even at NPIs concentrations potentially present in drug products (relative to control)

Development of an Improved Sucrose Grade

The presence and unpredictable impact of NPIs on protein stability, combined with variability in the type and concentration from batch to batch and among suppliers presents a problem for formulators. A solution was needed to mitigate the risk of this important and valuable excipient in pharmaceutical formulations. This led to the development of a novel, improved grade of sucrose (Sucrose Emprove® Expert).

As a first step in development, the impact of NPIs isolated from the two major sources of sugar (beet or cane) on protein stability was evaluated. NPIs were spiked at a concentration of approximately 10^{10} particles/mL into a formulation of a mAb and the formation of protein aggregates was assessed using Micro-Flow Imaging (MFI), which measures concentration of particles in the µm size range. The samples were analyzed directly after spiking (T0) and after 2 weeks at 25 °C. In addition, the samples were subjected to a forced degradation study at elevated temperature (4 weeks at 40 °C) and mechanical stress (2 weeks shaking at 400 rpm at 25 °C). MFI data (Figure 3) show that the particle concentration remains constantly low in the absence of NPIs at all experimental conditions. However, a significant increase in the particle concentration was observed for the mAb formulations containing NPIs under stress conditions. Overall, these results confirmed the earlier study[5] that NPIs isolated from sucrose have a negative impact on protein stability.

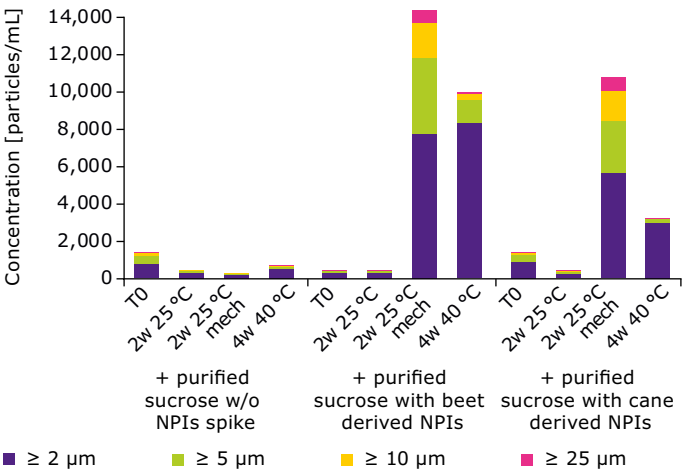


Figure 3.

Particle concentration determined by MFI showing the impact of beet- and cane-derived NPIs on protein stability.

A purification strategy including a filtration step was developed to minimize the NPI content in sucrose. An additional benefit of this improved purification process was reduction of bioburden and endotoxin levels to less than 0.3 I.U./g (Figure 4). Using this approach, the novel grade Sucrose Emprove® Expert was developed, featuring a low NPI content as well as reduced bioburden and endotoxin levels. This high-quality excipient offers the potential to mitigate risks during drug product development and reduce side effects in patients.

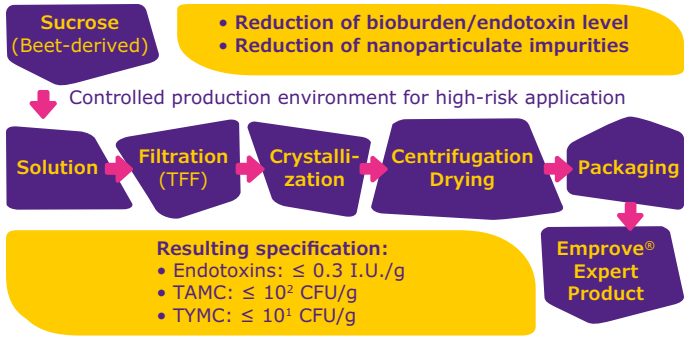


Figure 4.

Process used for production of a novel grade of sucrose.

DLS measurements of the novel grade sucrose demonstrated the improved quality as evidenced by the clear reduction in NPIs (Figure 5). The signal at about 100–200 nm is markedly reduced but not eliminated, because in DLS, intensity is proportional to the diameter to the power of six. As a result, the presence of just a few bigger nanoparticles results in a very high signal in the intensity. The actual number of particles in the novel grade sucrose is comparatively lower as can be seen in the NTA results (Figure 6).

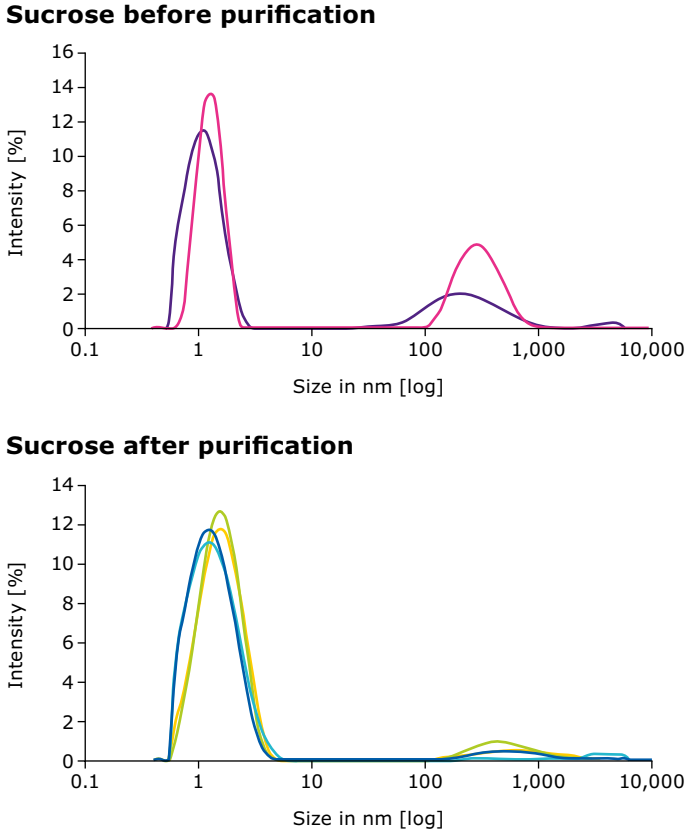


Figure 5.

DLS results for different batches of the non-purified sucrose raw material and the purified Sucrose Emprove® Expert.

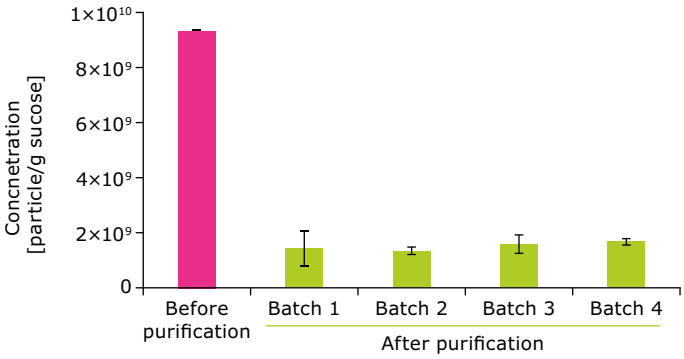


Figure 6.

Total particle concentration of non-purified raw material sucrose compared to different batches of purified Sucrose Emprove® Expert.

Conclusion

NPIs are present in pharmaceutical grade sucrose and other sugars. They originate from raw materials and can be introduced through production processes. These impurities are not entirely removed during sugar refinement, have a demonstrated effect on the stability of therapeutic proteins and interfere with analytical methods. Currently, the pharmacopeias do not require testing for NPIs which is why formulators face the risk of variations in NPI concentration between excipient batches. These variations directly affect stability of the biologic API. As a result, performance and stability of the formulation as well as the therapeutic effect may be reduced.

To address the challenge presented by existing pharmaceutical-grade sucrose, a novel purification process was developed to reduce NPI content, which was further accompanied by a reduction of bioburden and endotoxin. As a result of this improved grade, Sucrose Emprove® Expert can be used as excipient for biopharmaceutical products, even for high-risk applications, to mitigate the risks and safety concerns during the formulation development.

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