Low-in-nanoparticulate-impurities sucrose for biopharmaceutical formulations

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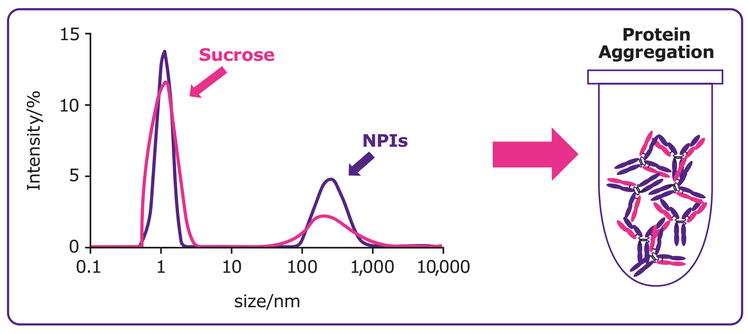
Purpose

Nanoparticle impurities (NPIs) with a size of 100–300 nm have recently been discovered **in pharmaceutical-grade sucrose** (Weinbuch et al., 2015). These can lead to **false analytical results**, as they mimic protein aggregates and can thus cause the potential exclusion of "lead molecules" during early development stages. Studies have also shown that NPIs **reduce the stability** of final protein formulations by inducing **protein aggregation, fragmentation and particle formation** (Weinbuch et al., 2017).



Objective

- Investigating the impact of **NPIs** isolated from **beet- or cane-derived sucrose** on **drug product stability**.
- Developing a **purification process** to reduce the amount of NPIs in sucrose.

Methods

Isolation of NPIs

NPIs were isolated from beet and cane derived sucrose. 50% sucrose solutions (w/v) were prepared in Milli-Q® water and diafiltration was performed against Milli-Q® water (6-fold volume exchange).

NPI Spiking and Forced Degradation Study

Isolated NPIs were spiked into IgG1 antibody mAbC formulation, resulting in a final particle concentration of ~10^{10} particles/mL. The formulations are summarized in Tab. 1.

Sample	pH	Buffer	Tonicity agent	Protein concentration	Amount of spiked NPIs	Surfactant
mAbC w/o spiked NPIs	7.2±0.1	5 mM phosphate	50 mg/mL purified sucrose	2 mg/mL	–	0.005% (w/v) PS80
mAbC + beet derived NPIs	7.2±0.1	5 mM phosphate	50 mg/mL purified sucrose	2 mg/mL	3.5 × 10^{10}/mL	0.005% (w/v) PS80
mAbC + cane derived NPIs	7.2±0.1	5 mM phosphate	50 mg/mL purified sucrose	2 mg/mL	3.5 × 10^{10}/mL	0.005% (w/v) PS80

Table 1: mAbC formulations for spiking studies.

Storage and stress conditions as well as time points for sample analysis are described in Tab. 2.

Sample name	Experimental conditions
T0	Directly after production
2w 25 °C	2 weeks storage at 25 °C
2w 25 °C mech.	2 weeks shaking at 400 rpm and 25 °C
4w 40 °C	4 weeks storage at 40 °C

Table 2: Experimental conditions for forced degradation studies.

The stability of mAbC was assed by using micro-flow imaging (MFI) using an MFI5200 system (ProteinSimple, Santa Clara, CA, USA) equipped with a 100-µm flow cell.

Particle Size Analysis and Quantification

10% sucrose solutions (w/v) were prepared in Milli-Q® water. The solutions were measured before and after sterile filtration through a 0.22 µm PVDF membrane.

For dynamic light scattering **(DLS)**, samples were analyzed with the Zetasizer Nano series (Malvern, Herrenberg, Germany) at 25 °C using automatic attenuation selection and detection via 173° backscatter. Peak size was based on the viscosity of water as dispersant; particle area (%) was based on the intensity. The data processing analysis model was set to general purpose.

Nanoparticle tracking analysis **(NTA)** was performed with a NanoSight LM20 (NanoSight, Amesbury, UK) using a pre-run volume of 0.5 mL and triplicate measurements with 0.1 mL sample volume.

Quantification of β-Glucan contamination

50% sucrose solutions (w/v) were prepared in Milli-Q® water. The (1→3)-β-D-glucan levels were measured by using the **Glucatell® Assay** (Cape Cod, East Falmout, MA, USA), according to the instructions of the manufacturer.

Results

NPIs Have a Negative Impact on Protein Stability

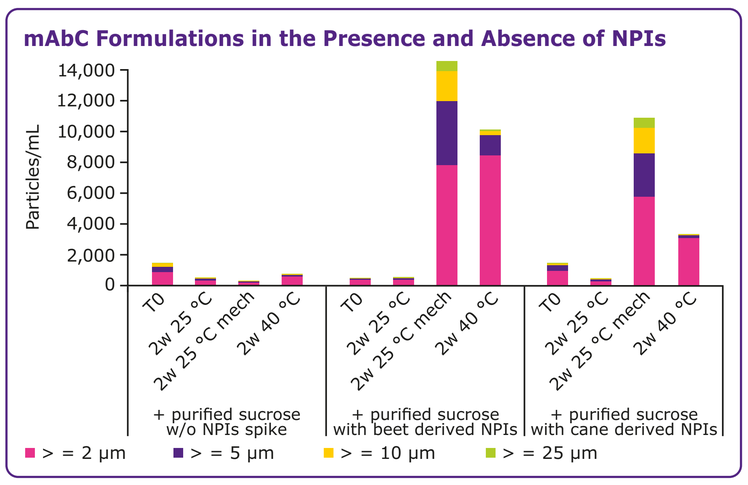


Figure 1: Results of forced degradation studies using mAbC without or with NPIs spiked that were previously isolated from beet- or cane-derived sucrose. Particle concentration was determined using MFI.

Spiking with NPIs in a concentration of ~10^{10} particles/mL induced particle formation in mAbC formulation under stress conditions (Fig. 1). This indicates that NPIs, independent of the sucrose source, can have a **negative impact on protein stability** in final drug product.

Purification Process of Sucrose

In order to mitigate risks during formulation development, nanoparticulate impurities were removed from sucrose in an improved purification process (Fig. 2). This results in the novel grade **Sucrose Emprove® Expert** that is **low in NPI** content. Additionally, the purification leads to **a reduction of bioburden and endotoxin contamination**.

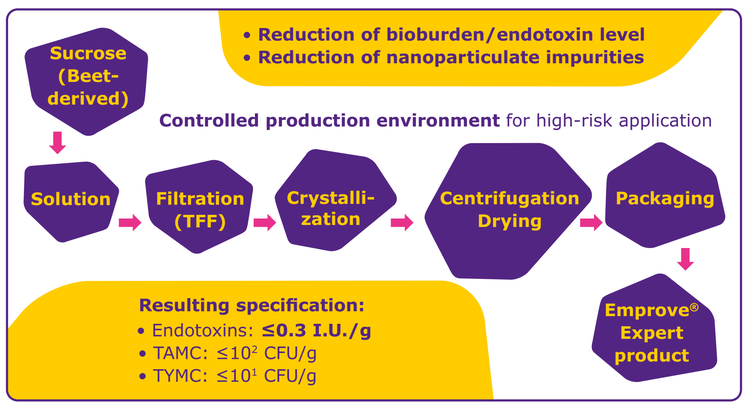


Figure 2: Schematic overview of the improved purification process of sucrose in order to reduce nanoparticulate impurities as well as bioburden and endotoxin content.

Reduction of Nanoparticulate Impurities

- The first peak at about **1–5 nm** is assigned to **sucrose** (Fig. 3). The second peak, with a size distribution of about **100–300 nm**, represents the **NPIs**.
- The decrease in the second peak (Fig. 3) for Sucrose Emprove® Expert proves that the **purification process successfully reduces NPI** contamination in sucrose products.
- The **remaining signal** (Fig. 3) around **100–300 nm** can result from **single larger particles**, since the scattering intensity is proportional to diameter^6 **(I~d^6)**.

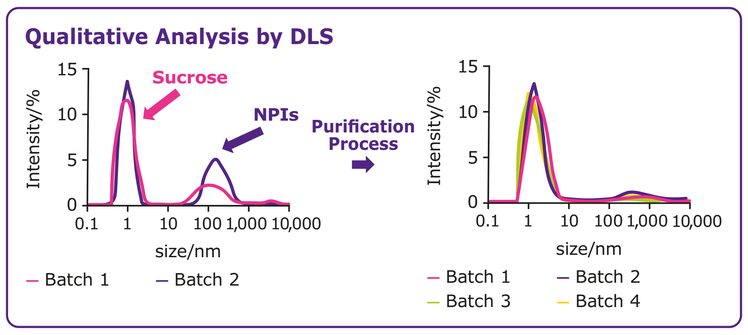


Figure 3: DLS results for different batches of the non-purified sucrose raw material (left), and the purified Sucrose Emprove® Expert (right). Samples were measured in triplicates and error bars represent standard deviation.

The DLS results were confirmed by quantitative NTA measurements, which showed a **significantly lower particle concentration** in the four purified **Sucrose Emprove® Expert** batches compared to the non-purified raw material sucrose (Fig. 4).

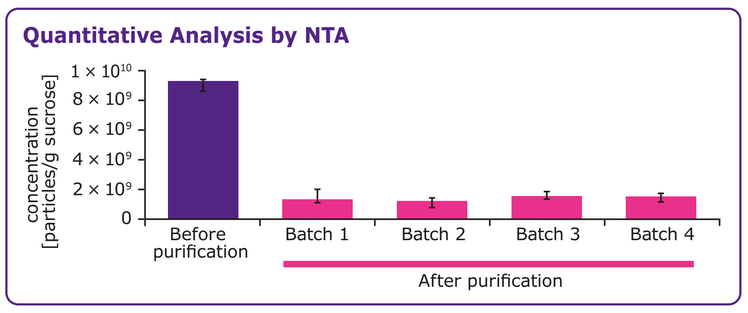


Figure 4: Total particle concentration of non-purified raw material sucrose compared to the means of batches 1–4 of purified, low-NPI Sucrose Emprove® Expert. Error bars represent standard deviation from three replicate measurements.

Reduction of β-Glucan Contamination

(1→3)-β-D-glucans can elicit inflammatory response and are potential contaminants in pharmaceutical products, originating from various raw material (Barton et al., 2016). The (1→3)-β-D-glucan levels were measured by using the Glucatell® assay that is based on a modification of *Limulus* Amebocyte Lysate (LAL) pathway, whereas factor C is eliminated. For this reason, this assay is specific for (1→3)-β-D-glucan (Fig. 5).

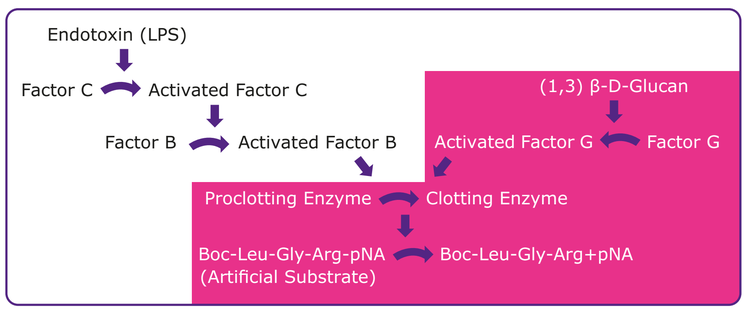


Figure 5: Limulus Amebocyte Lysate (LAL) pathway. Factor C is depleted in Glucatell® assay to ensure specificity for (1→3)-β-D-glucan.

In comparison to the non-purified raw material sucrose, the four purified Emprove® Expert batches contain a significantly lower amount of (1→3)-β-D-glucan, close to the detection limit of the Glucatell® assay. Thus, in addition to NPIs, **(1→3)-β-Dglucan contaminants are also reduced during the purification process** of sucrose.

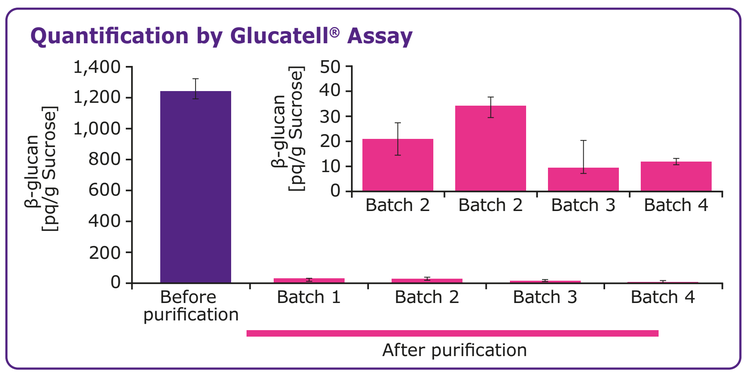


Figure 6: Determination of (1→3)-β-D-glucan amount by using the Glucatell® assay. Duplicate measurements were carried out for each sample. The error bar represents the standard deviation.

Joint Development

Sucrose Emprove® Expert was developed in cooperation with Coriolis Pharma, Munich, Germany.

Coriolis Pharma
Biopharmaceutical Research and Development Service

Summary

Nanoparticle impurities (NPIs) have been discovered in pharmaceutical-grade sucrose in an amount up to 10^{10} particles per gram sucrose, resulting in false analytical results and in protein instability.

A purification process was successfully developed to reduce NPIs in sucrose, which was accompanied with a decrease of endotoxin, bioburden and β-D-glucan. This has enabled the launch of a new grade Sucrose Emprove® Expert Ph Eur, ChP, JP, NF.

References

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