

DOWNSTREAM PURIFICATION OF INSULIN AND INSULIN ANALOGUES USING NLAB SAGA® SILICA MEDIA

Olga Krivosheeva, Jan Blid, Katarina Alenäs
Nanologica AB, Forskargatan 20G, SE-15136 Södertälje, SWEDEN



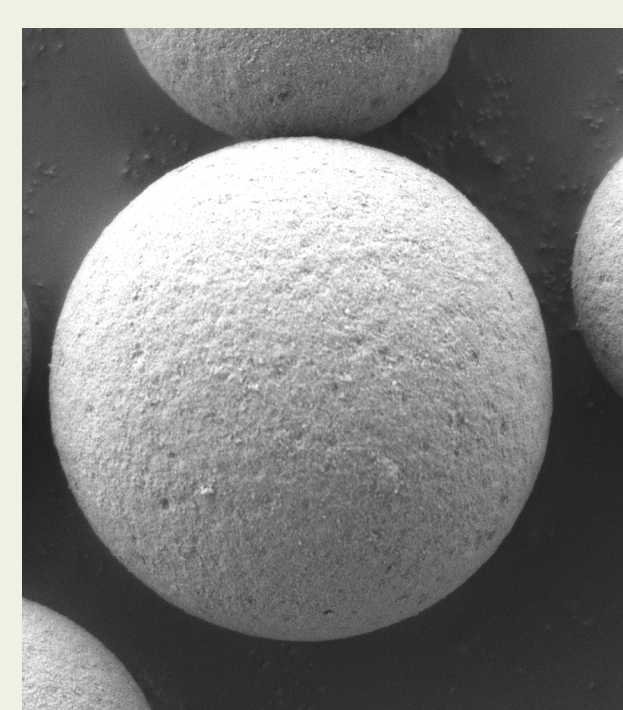
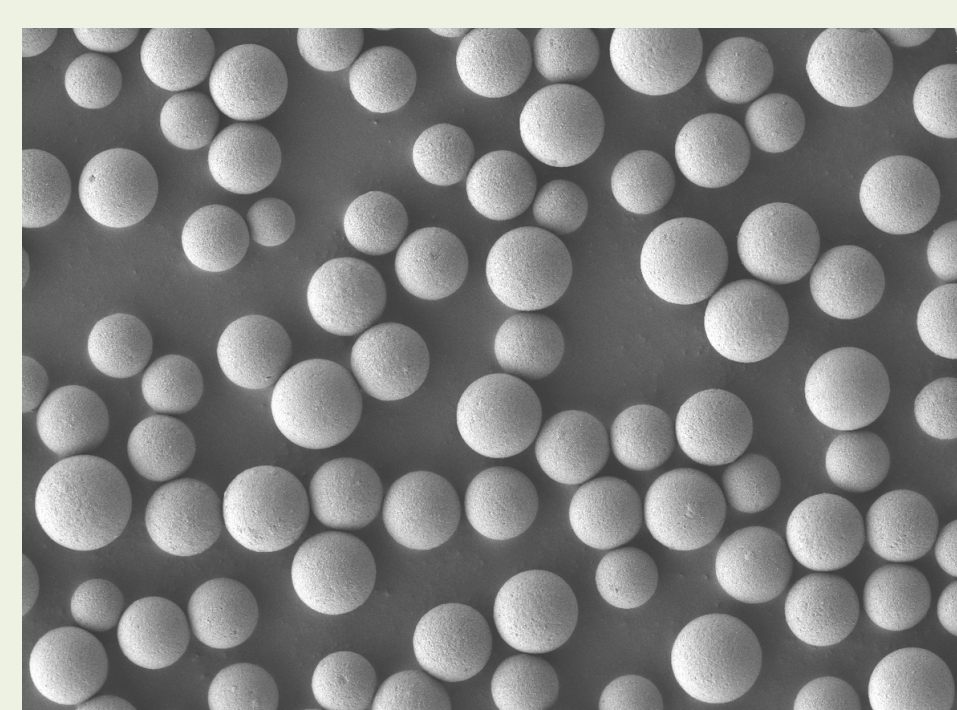
Website

The NLAB Saga® silica media was developed by Nanologica with a clear goal in mind – to increase the availability of better and cheaper peptide and oligonucleotide-based medicines to a larger number of patients across the world, for the betterment of mankind.

Benefits of NLAB Saga® silica for peptide purification

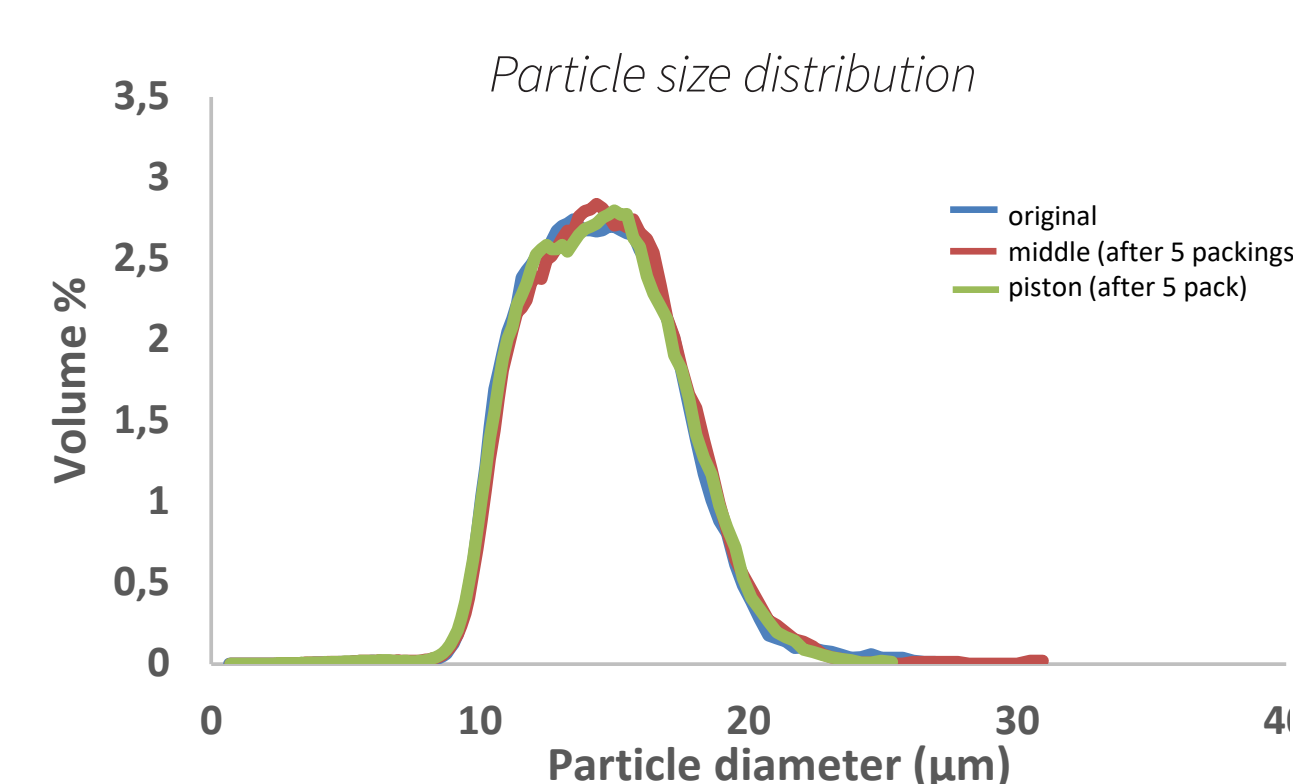
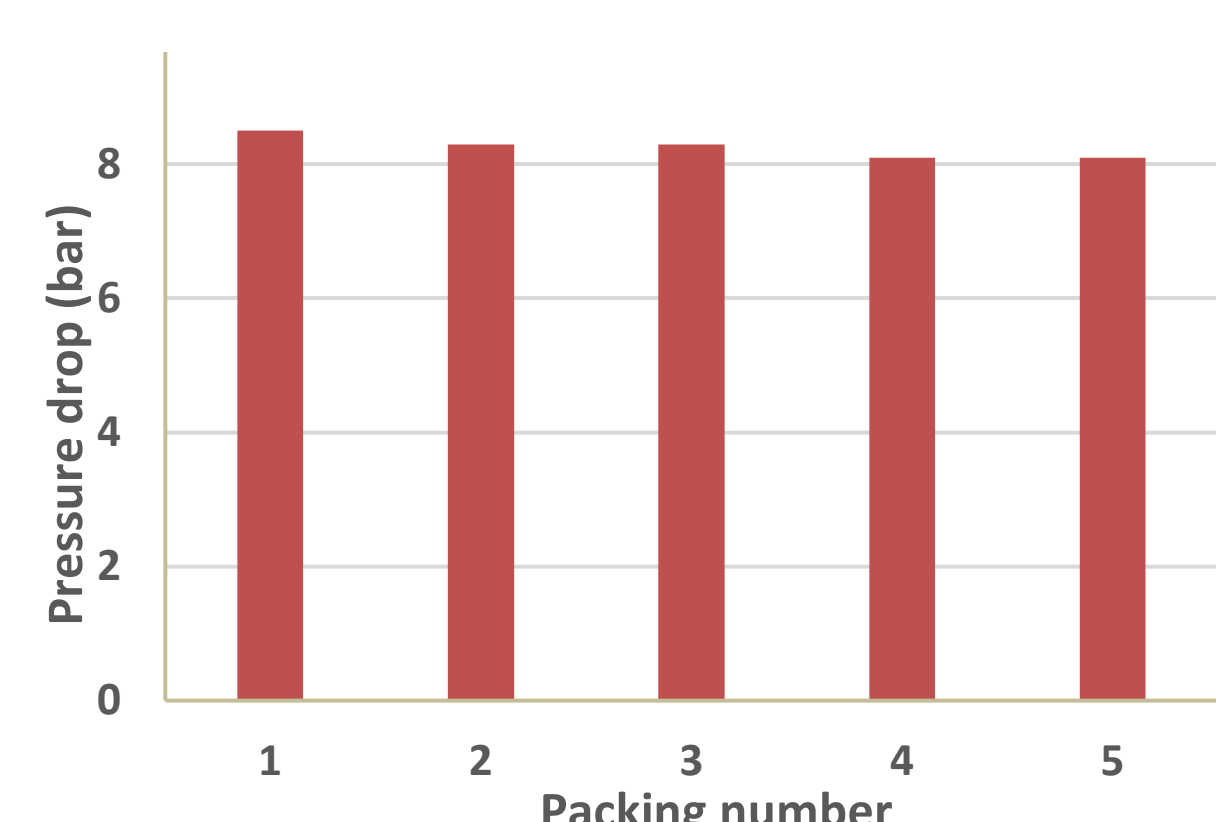
✓ Perfectly spherical particles

NLAB Saga® has been specifically developed to meet the strict requirements of industrial scale purification by chromatography. It has a superior mechanical and chemical stability together with a high available surface area and ligand density, in addition to narrow pore size distribution. This, combined with controlled particle size distribution, results in a silica with a high loading capacity and low back pressure.



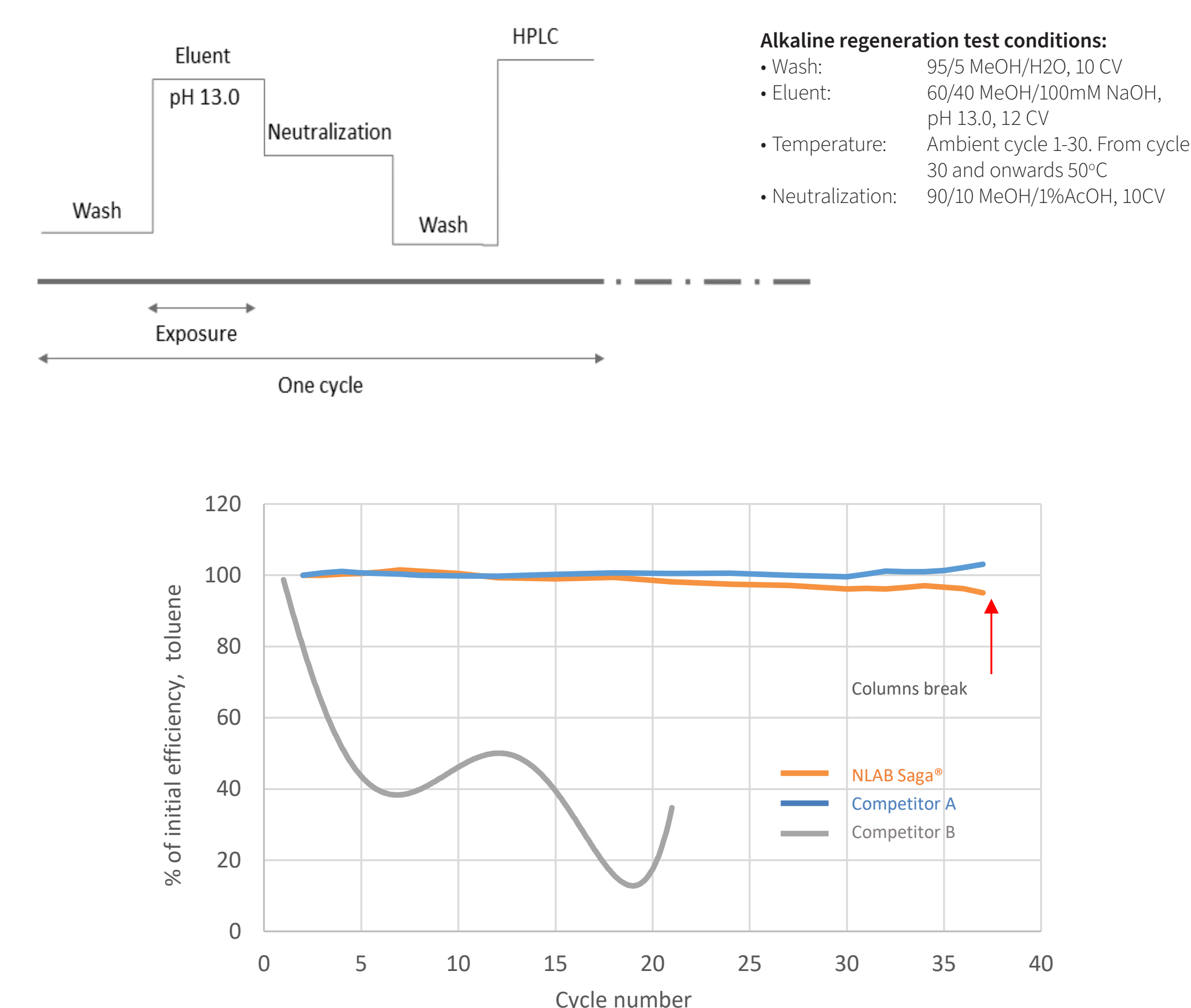
✓ High mechanical stability

Mechanical strength tests were performed in a 50mm ID DAC column at 100 bar piston pressure. Analytical results show no mechanical degradation of the silica under high pressures. NLAB Saga® has an excellent mechanical stability due to proprietary process and pore volume.



✓ Long lifetime

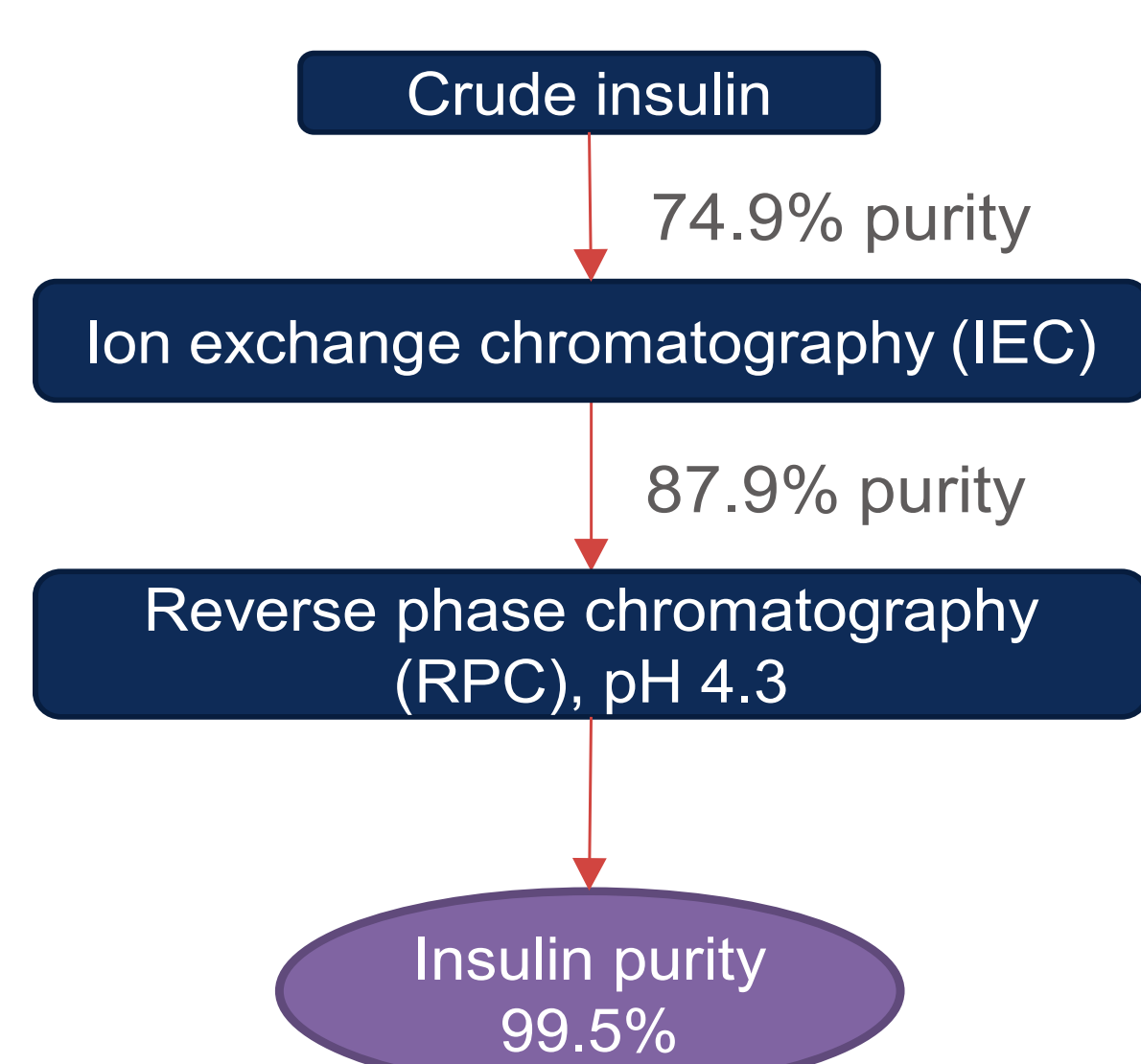
High chemical stability under basic pH is important in insulin manufacturing. During the purification cycles silica gets contaminated by aggregated insulin which results in the increase of the backpressure and a decline in purity/yield. To regenerate the column, CIPs (Cleaning In Place) procedures are used.



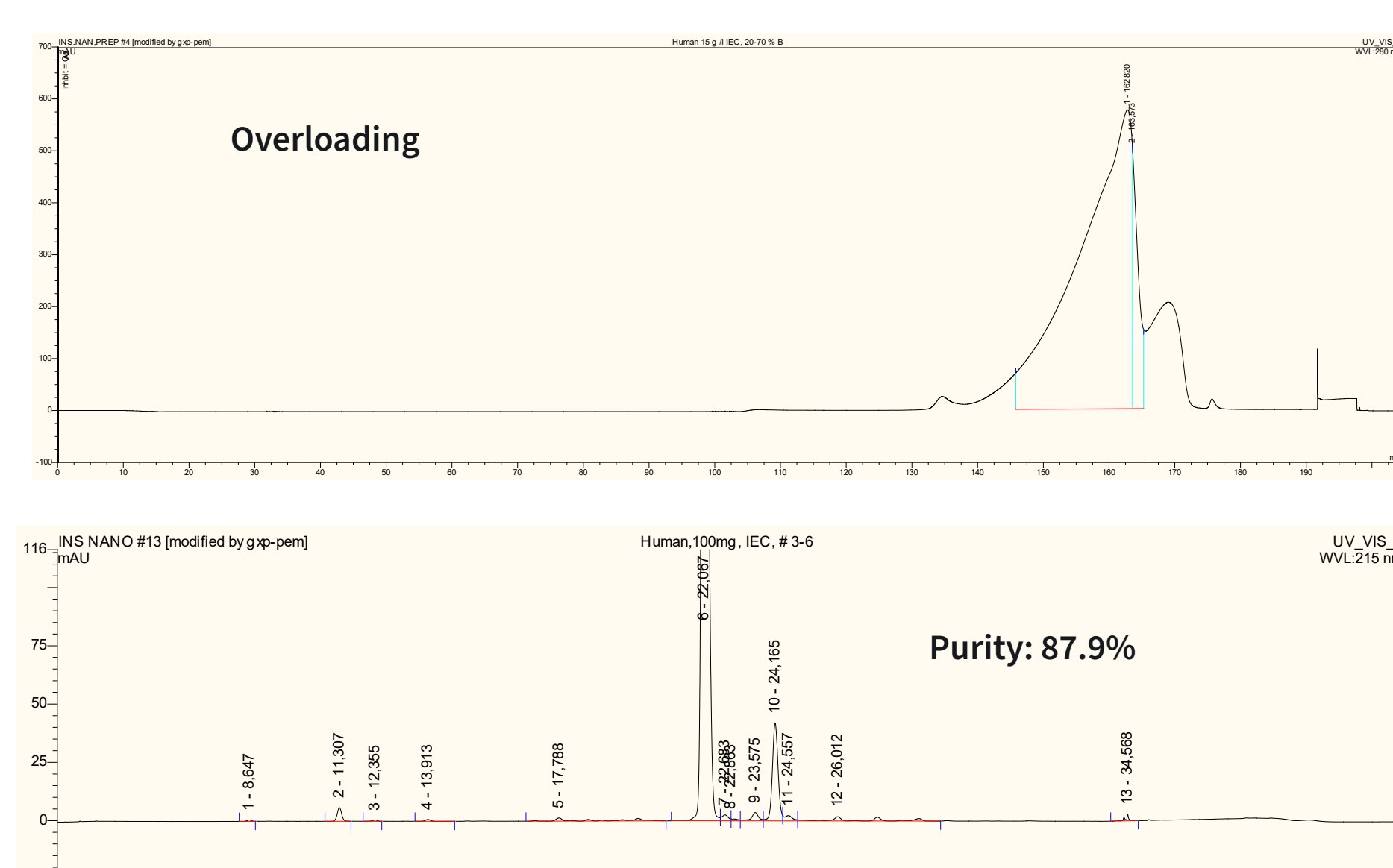
Insulin purification: Comparison of NLAB Saga® silica with other silica media

A comparison experiment between Nanologica's NLAB Saga® C8 13µm and three competitors (A, B, and C) was run. The target for the experiment was to reach the required purity threshold of 99.2% as set per the USP. The same quality of crude insulin was used in all trials. Overloading conditions as well as loading amounts, particle size and functionality were kept the same for all silica materials. As a first step, ion exchange purification was performed. Ion exchange has an orthogonal selectivity to reversed phase chromatography purification and removes impurities, which are difficult or cannot be removed by the latter step.

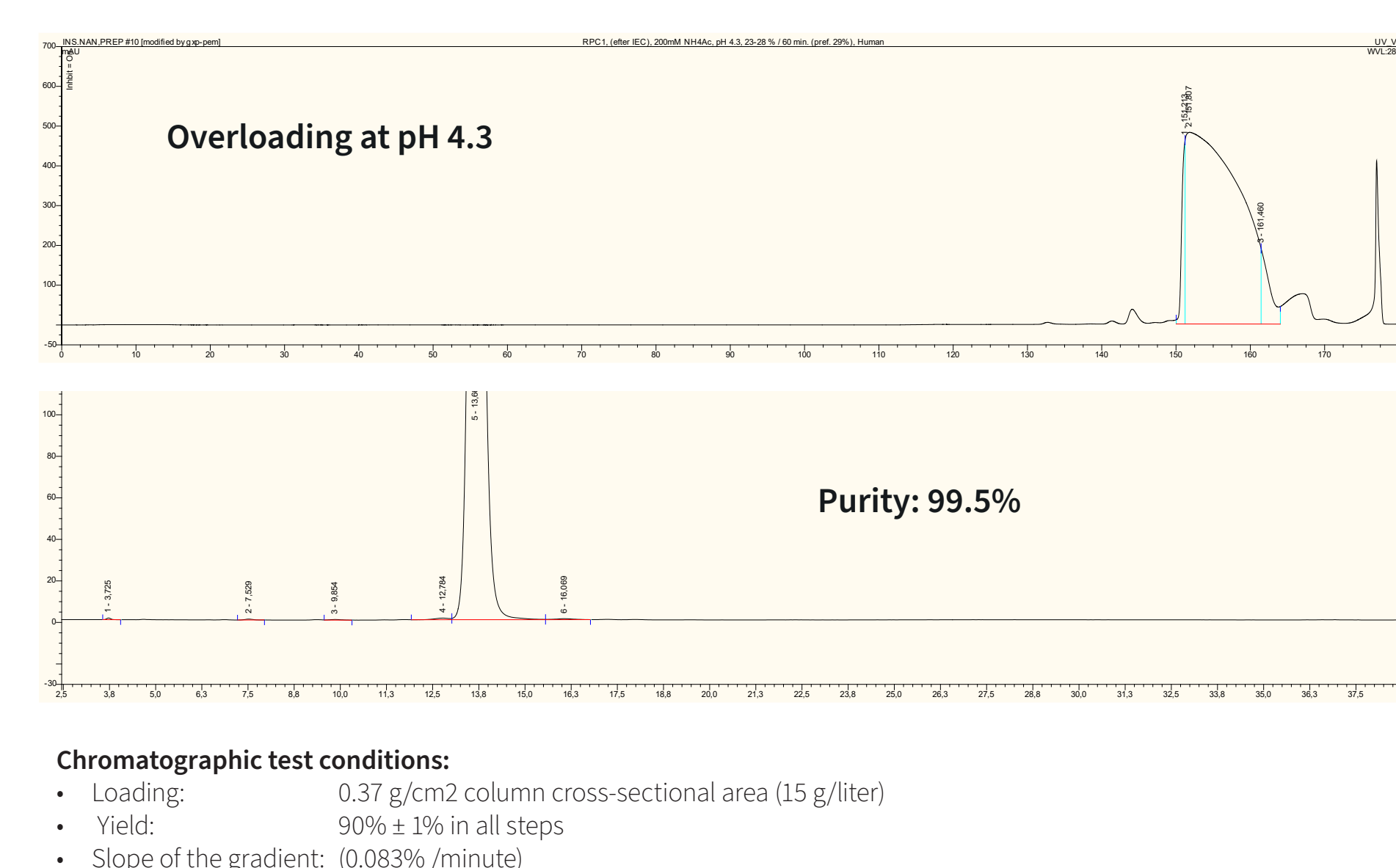
Experiment setup



Ion exchange purification



Reversed phase chromatography pH 4.3



NLAB Saga® silica helps to reduce the manufacturing cost

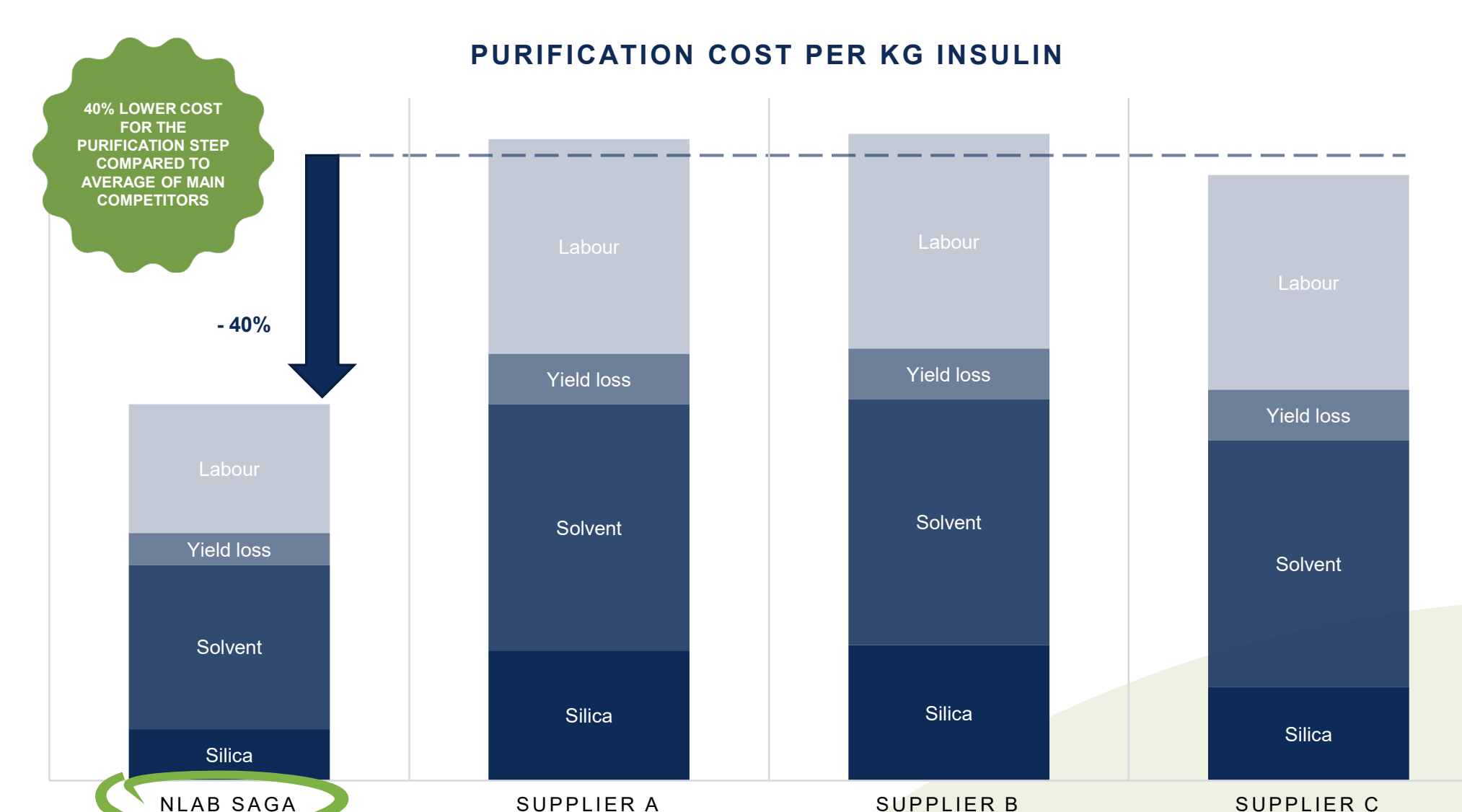
Results of RPC insulin purification experiments showed that by using NLAB Saga®, insulin targeted purity was achieved after one RPC step, whereas for competitors A, B, and C one more RPC step was required. Experimental results were used in the cost calculation model. The cost model has been developed describing the flow in the manufacturing process for the purification of insulin, including use of material (e.g., solvent) and labour cost**. The cost saving in the purification step has then been applied to the total manufacturing cost for insulin to show the overall cost saving potential. Assumptions are based on published*** and internal data.

Phase	NANOLOGICA NLAB® C8	COMPETITOR A C8	COMPETITOR B C8	COMPETITOR C C8
Insulin purity (%), according to USP method	99.50%	97.08%	98.56%	98.82%

Lowering manufacturing costs

Do not reach purity threshold of 99.2%

- ✓ Excellent performance compared to competitors
- ✓ Target purity of final product was reached faster than competitors
- ✓ Higher lifetime and lower manufacturing cost



- Nanologica manufactures NLAB Saga® giving full control in each production step
- All supportive data is available in the NLAB Saga® Regulatory Support File
- NLAB Saga® is the most cost-effective alternative to large scale purification of peptides