CONNECTING qEV100 GEN 2 COLUMNS TO AUTOMATED CHROMATOGRAPHY SYSTEMS

A GUIDE TO GETTING STARTED

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SEPARATING EVs FROM LARGE SAMPLE VOLUMES

Growing interest in the therapeutic potential of extracellular vesicles (EVs) has created a need for bioprocesses capable of separating EVs from tens or hundreds of litres of biological samples. To this end, Izon has been working alongside an industry partner to develop a separation process involving a large, customised column coupled to Sepragen's automated chromatography system, QuantaSep® 100. The lessons and key parameters that enabled the desired elution profile to be obtained provide a useful starting point and can be applied to other models and systems.

About Izon Science

Izon Science has been providing solutions for the isolation of nano-sized particles since 2014 (and their analysis since 2008) and this has largely been driven by the growing EV field. Researchers working with diverse sample types require columns suited to different loading volumes, and Izon has met this need by developing a wide range of size exclusion chromatography (SEC) qEV columns. Alongside this, the Automatic Fraction Collector (AFC) continues to provide an element of automation to the processing of sample volumes between 150 µL and 10 mL. Izon has also brought tunable resistive pulse sensing (TRPS) to the EV field, paving the way for researchers who want to obtain precise, single-particle measurements of size, charge and concentration. The insights provided by TRPS measurements continue to shape the evolution of the qEV range, and now, the development of large-scale EV separation processes.

Disclaimer: *The information shared in this document is a guide only, and specific values are derived from a column that is similar (but not identical) to the qEV100 Gen 2. The Gen 2 range of qEV columns is relatively new, and comprehensive work involving the qEV100 Gen 2 column and automated chromatography systems has not been carried out. Therefore, although the information and specific values shared here should provide a highly relevant and helpful starting point for working with the qEV100 Gen 2 column, it is important to acknowledge that the qEV100 Gen 2 has not been tested under these conditions. qEV customers are encouraged to develop their own protocols independently and reach out to an Izon representative for support.*

Figure 1. The qEV100 Gen 2, capable of running 100 mL samples. Like all columns in the qEV range, qEV100 Gen 2 columns are available in two types: the 35 nm Series (left, provides optimal recovery of particles in the range of 35-350 nm) and the 70 nm Series (right, provides optimal recovery of particles in the range of 70-1000 nm).

BACKGROUND INFORMATION ON IZON'S EXPERIENCE IN LARGE-VOLUME SEPARATION

Izon is working alongside an industry partner to establish a method for isolating EVs from their highly concentrated cell culture media. As their end goal is to process thousands of litres each year, they need to have the capacity to process hundreds of litres each month.

Achieving separation on such a large scale requires use of an automated chromatography system, and in this case, involved a large, custom-made chromatography column.

To enable the exploration of large-scale bioprocesses (including pre-SEC clarification and crossflow filtration), a large, customised column (capable of processing sample loading volumes of 100 mL) was coupled to the automated chromatography system, QuantaSep® 100. Column capability and elution profiles were assessed, and a UV chromatogram was used to guide the selection of optimal fractionation. Fractions were assessed via TRPS and bicinchoninic acid assay to determine particle count and protein reduction. As has been established previously (Figure 2), elution profiles were found to be highly consistent with ample separation between EVs and protein.

The lessons and parameters established here provide the groundwork for further process and column development – and provide a useful starting point for others who wish to separate EVs from similar sample volumes. These guidelines will also be applicable to other QuantaSep® models (see notes below) and other automated chromatography systems such as ÄKTA systems by GE Healthcare Life Sciences.

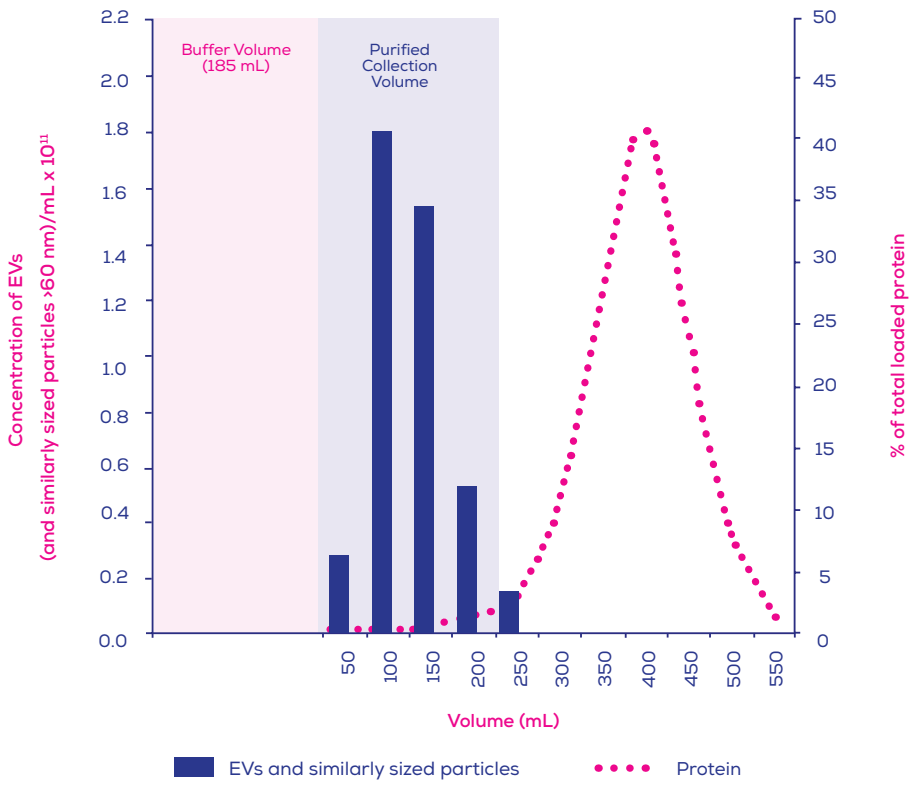


Figure 2. Typical elution profile for qEV100/35 nm Gen 2 columns with 100 mL of human plasma loaded; proteins elute in a later volume than extracellular vesicles (EVs) and similarly sized particles >60 nm. EV concentration was measured using an Exoid and protein levels by bicinchoninic acid (BCA) assay.

TIPS FOR GETTING STARTED WITH YOUR qEV100-AUTOMATED CHROMATOGRAPHY SETUP

As samples will behave differently depending on their complexity, type, prior processing steps, and volume, there is a need to characterise elution profiles to identify fractions of interest. Using a UV chromatogram as an indicator, optimal fractionation can be selected.

Connecting qEV100 columns to QuantaSep® or ÄKTA systems

qEV100 Gen 2 columns can be simply adapted to QuantaSep® or ÄKTA systems using Leur Lock hose barb fittings. Fitting specifications:

For the top of the column: Masterflex® Fitting, Polycarbonate, Straight, Male Luer Lock to Low-Profile Hosebarb, 1/16" ID; 25/PK

For the bottom of the column: Masterflex® Fitting, Polypropylene, Straight, Female Luer to Hosebarb Adapters, 1/16"; 25/PK

Sample loading volume

The qEV100 is suited to sample loading volumes of 100 mL.

Sample flow rate

We recommend using a flow rate of 8-10 mL/min as a starting point. This value can be increased to reduce run time, and for samples with low viscosity. Alternatively, the flow rate should be reduced if excessive pressures are observed.

Compatibility with other systems

Whether an automated chromatography system is compatible with qEV100 Gen 2 columns depends largely on the system's flow rate capability (see above), and capacity for pressure regulation (see below). If you are unsure, discuss with an Izon representative.

Use of pressure regulation mode

We recommend using a system with a pressure regulation mode, as pressure spikes can occur with highly viscous samples, high load volumes, and with column reuse (Figure 3).

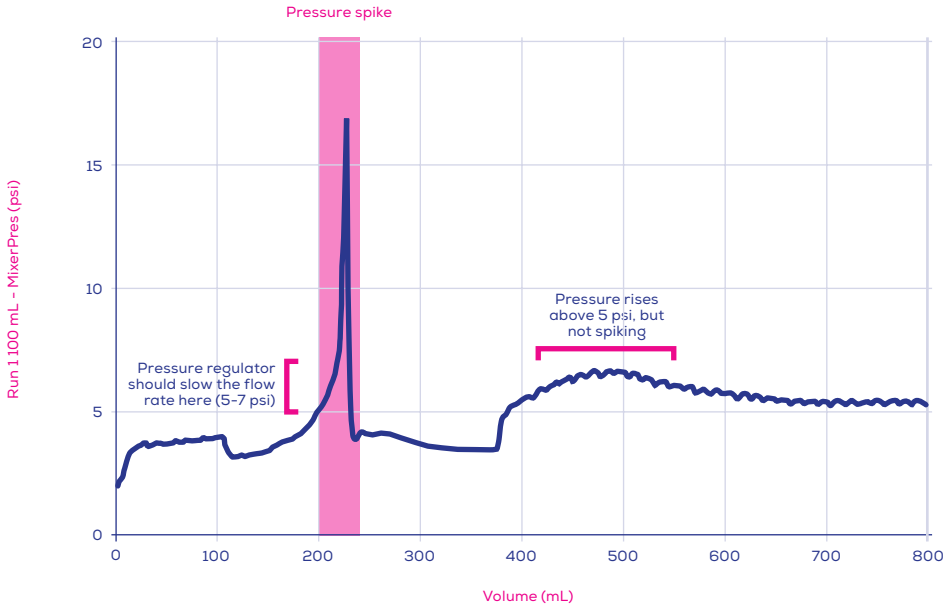


Figure 3. Column pressure without the use of pressure regulation. A pressure spike is observed in the mixer pressure graph during a run of a highly concentrated EV-containing sample derived from cell culture media, loaded on a large, customised column (capable of handling a 100 mL sample loading volume) coupled to an automated chromatography system (QuantaSep® 100, Sepragen).

Although no noticeable changes to elution profiles have been observed in subsequent runs, and pressure (Figure 3) resolves after the flow is adjusted, pressure spiking should be avoided. The effect of these spiking events on column longevity is unknown, therefore pressure spikes should be caught and mitigated. This can be achieved by identifying and setting an upper pressure limit that is appropriate for the sample.

By using the pressure regulator, an upper pressure limit can be set, and the system will compensate by reducing the flow rate, allowing a suitable pressure to be maintained. The optimum upper pressure limit will be sample dependent, with more viscous samples requiring a lower upper pressure limit.

To begin with, we recommend setting an upper pressure limit of 5-7 psi. From here, watch for pressure spiking events. These manifest as a sharp increase in pressure (Figure 3) and are most likely to occur during sample loading and immediately after, when EVs begin to elute. If pressure spikes do occur, note the pressure at which they arise. The upper pressure limit can then be introduced accordingly, to prevent a full spike from occurring.

If none occur, the pressure can be increased to 8 psi or more.

Pressure spiking events differ from the slow, steady rise and plateau which is also shown in Figure 3. Such slow and steady pressure changes are normal and are not a cause for concern.

Identifying fractions of interest

Using a UV chromatogram as an indicator, optimal fractionation can be selected.

Cleaning protocols

Refer to your qEV user manual and get in touch if you have any questions.

Reusability

The limits of column reuse depend on sample complexity, desired purity and yield; this can be discussed with your Izon representative.

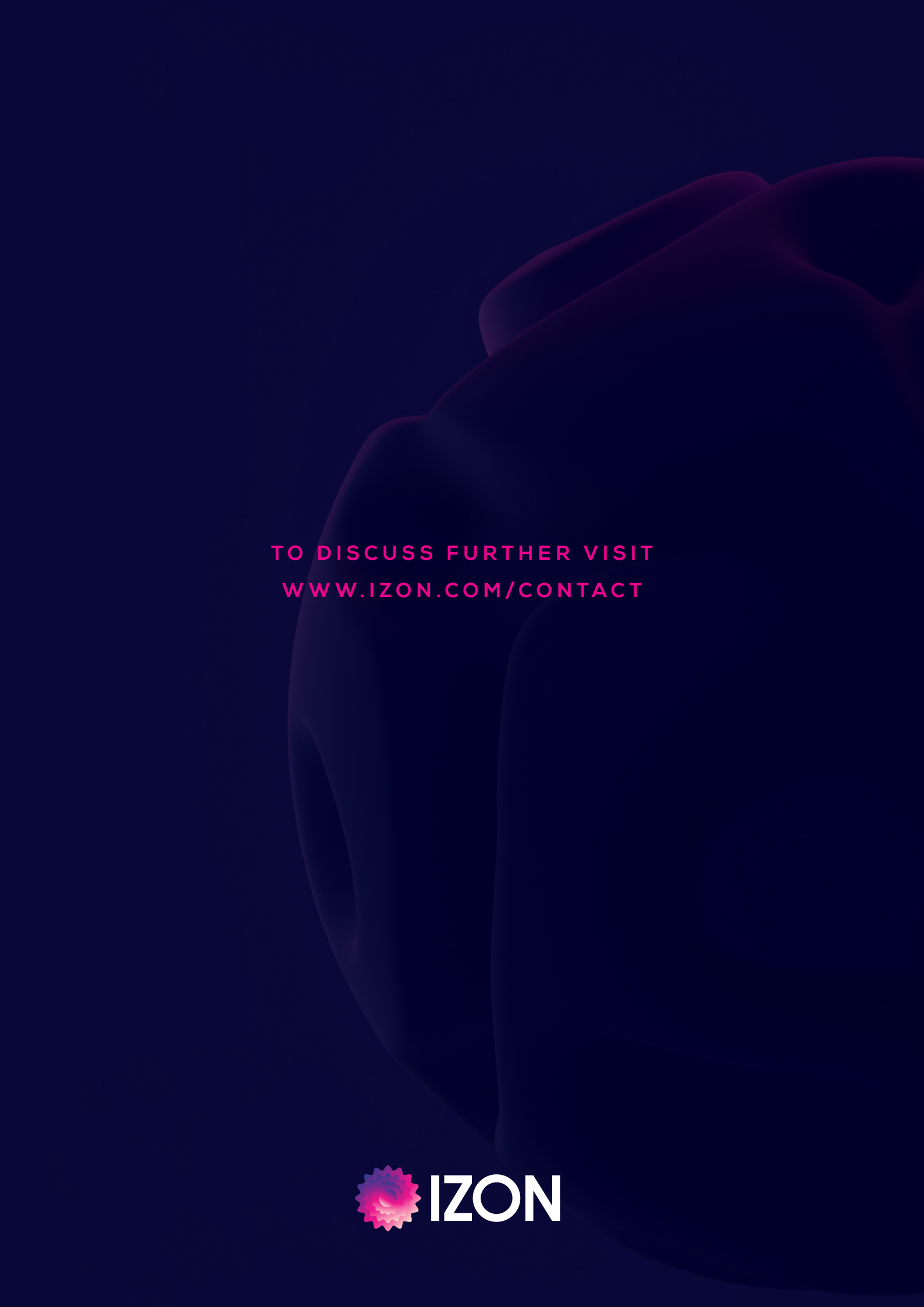
Other considerations

Reminder: Before connecting the column, remember to purge the lines to avoid getting air bubbles in the column.

HOW CAN IZON HELP?

Building on a solid foundation in the EV-separation space, the team at Izon continues to accumulate experience in medium-to-large-scale process development.

If you are interested in large-scale custom columns and/or outsourcing process development and validation, don't hesitate to get in touch to discuss how we can help you on your scale-up journey.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