CONNECTING qEV10 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 mechanisms and 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. Izon offers a range of size exclusion chromatography columns suited to different sample loading volumes, including the qEV10 Gen 2 column for 10 mL loading volumes. The qEV10 Gen 2 can be coupled to automated chromatography systems, and a UV chromatogram can be used to guide the selection of optimal fractionation. This document shares lessons from previous work and provides guidelines for setting up your qEV10 Gen 2 column with automated chromatography 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 qEV10 Gen 2. The Gen 2 range of qEV columns is relatively new, and comprehensive work involving the qEV10 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 Gen 2 column, it is important to acknowledge that the Gen 2 column 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. qEV10 Gen 2 columns. Left: qEV10 Gen 2 (35 nm Series), optimum recovery range 35–350 nm. Right: qEV10 Gen 2 (70 nm Series), optimum recovery range 70–1000 nm.

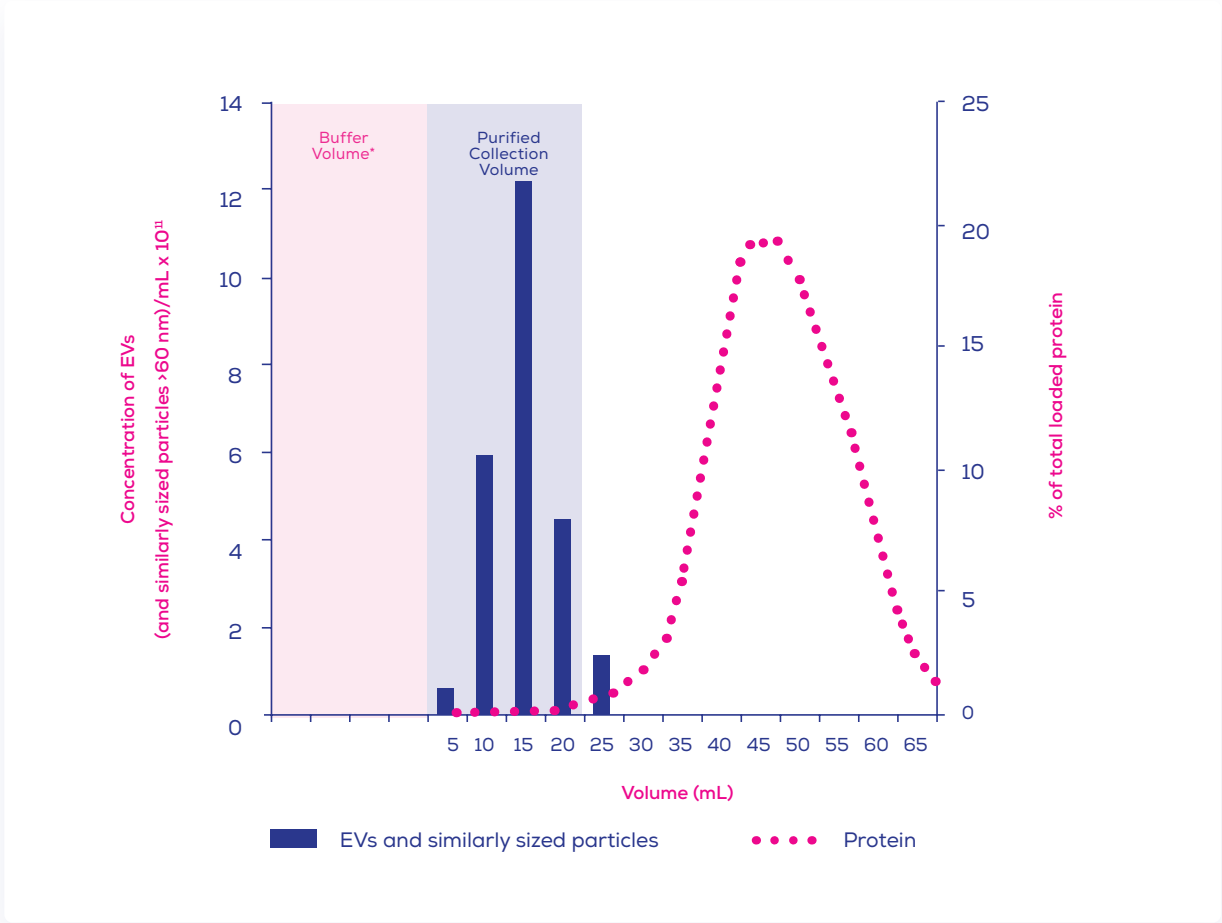


Figure 2. Typical elution profile for qEV10/35 nm Gen 2 columns with 10 mL of human plasma loaded; proteins elute at a later volume than extracellular vesicles and similarly sized particles >60 nm. EV concentration was measured using an Exoid and protein levels by bicinchoninic acid (BCA) assay. *Recommended buffer volume values vary for qEV10/35 nm and qEV10/70 nm columns: refer to your qEV User Manual for guidance.

TIPS FOR GETTING STARTED WITH YOUR qEV10-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 qEV10 Gen 2 columns to QuantaSep® or ÄKTA systems

qEV10 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 qEV10 Gen 2 has an optimal sample loading volume of 10 mL.

Sample flow rate

We recommend using a flow rate of 4 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 qEV10 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.

Identifying the buffer volume

To identify the point at which EVs begin eluting and fractions of interest, we recommend you start collecting fractions as soon as the sample has been loaded onto the column. Results from the UV chromatogram can be used to determine subsequent fractionation schedules.

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. A pressure spike is illustrated in Figure 3.

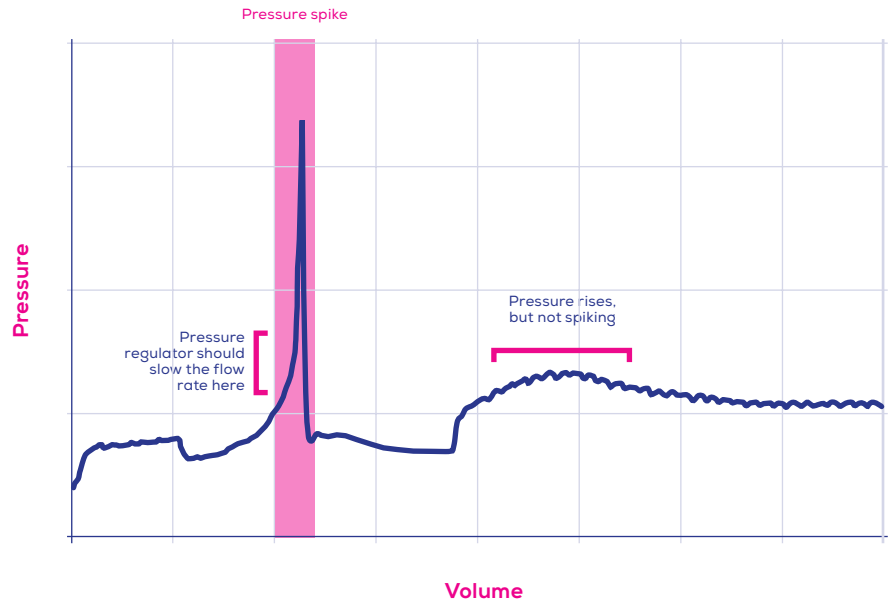


Figure 3. Column pressure without the use of pressure regulation.

Although no noticeable changes to elution profiles have been observed in subsequent runs, and pressure 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 3 psi. From here, watch for pressure spiking events. These manifest as a sharp increase in pressure (above) 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 6 psi or above.

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.

Accounting for hold-up volume

There is a volume of tubing that exists between the UV detector and the fraction outlet. This hold-up volume will vary depending on the system setup and will need to be accounted for when determining which volumes to collect or discard. The hold-up volume can be established through in-house testing or by contacting the chromatography system manufacturer.

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.

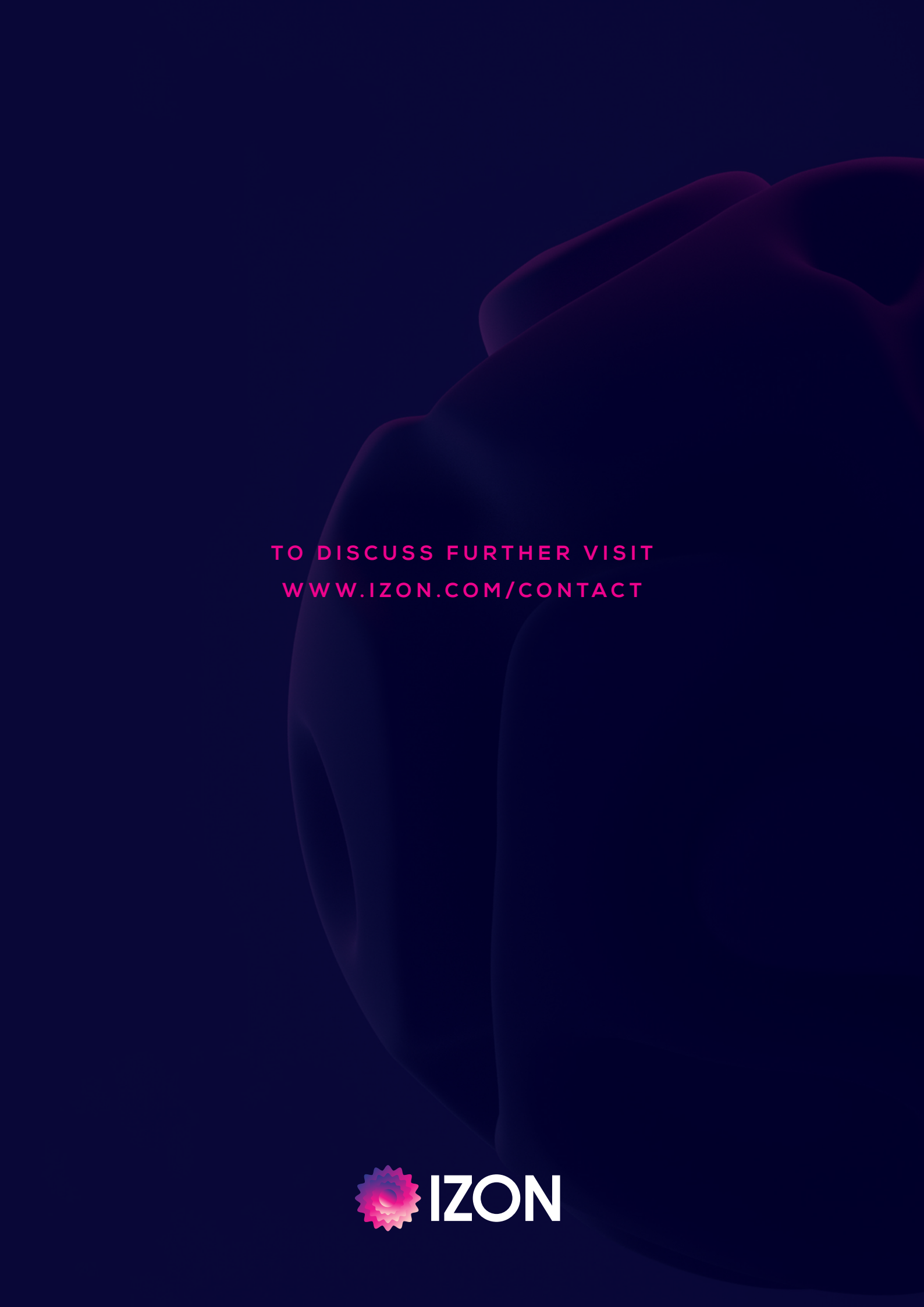
Other considerations

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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