Non-Invasive Raman Spectroscopy – Ready for Continuous Monitoring of Extracellular Vesicle Purification

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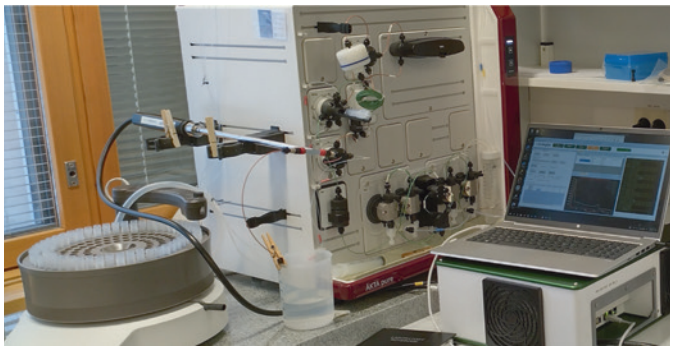
1. | Introduction

- Due to their natural functions, **extracellular vesicles (EVs)** are being explored as **drug carriers and diagnostic or therapeutic tools.** However, production, purification, and characterization of these nanocarriers remains challenging. One of the main difficulties is monitoring chromatography-based purification in-situ, which requires **selectively measuring** key analytes because EVs are intrinsically heterogeneous. **Industrial-scale production** requires continuous monitoring of EV quantity and quality throughout the purification process.

- Although **Raman spectroscopy** is **well suited** for this task, its practical suitability depends on the ability to mount and unmount the spectrometer probe during the process without compromising sterile integrity.

- To solve this problem, SCHOTT developed a **novel flow cell** that acts as an interface between the product flow an spectrometer probe, making it possible to perform advanced in-situ monitoring of bioprocesses through a hermetically sealed optical window.

2. | Test Setup and Assembly



- **Comparison measurement:** Cytiva Chromatography system with 280nm UV sensor

- **In-situ measurement set-up:** Novel ViewCell® (SCHOTT, Germany) and time-gated PicoRaman M3 spectrometer with 532nm pulsed laser (Timegate, Finland)

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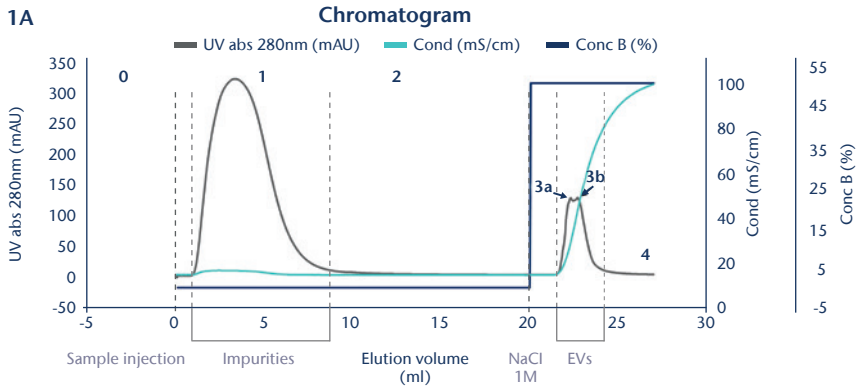
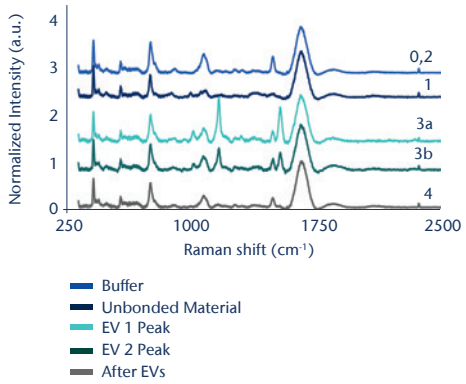
3. | USE CASE: Continuous monitoring of EV purification

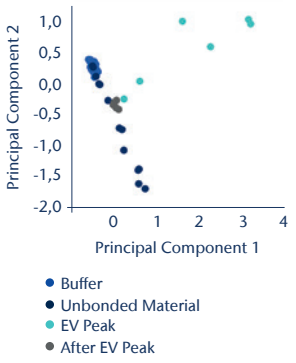
1A



1B



1C Principal Component Analysis (PCA)



- **In-situ measurement:** Two measurements per minute
- Eluent Tris 50 mM (buffer A) and Tris 50 mM NaCl 2M (buffer B) for ion exchange chromatography; elution rate: 1 min/ml
- Fig. 1A: Chromatogram, incl. UV absorption at 280nm (**grey**), mobile phase conductibility (**turquoise**), and buffer composition (**dark blue**); phases: 1 – conditioning prior sample injection, 2 – impurity removal by unbonded material elution, ~1 column volume after sample injection, 3 – washing step (buffer A), 3a/3b – elution step (buffer B) with EV peak, 4 – post EV elution and end of run
- **UV signal:** Two unresolved peaks of similar UV absorbance intensity vs. **Raman spectroscopy:** Selective analysis of two EVs with different chemical compositions (Fig. 1B)
- **Principal component analysis (PCA)** (Fig. 1C): Clear differences between EV and non-EV fractions

4. | Results

- Results demonstrate that a time-gated Raman spectrometer combined with the novel flow cell can **detect different EV chemical compositions** that are **not resolvable with a UV sensor** due to similar absorbance intensities.
- By enabling specific analysis, the new approach allows **optimization of EV purification processes** while **maintaining sterile integrity** during the entire purification process (e.g., if the probe head needs to be unmounted for calibration or reference measurements).
- With the new approach, PCA can be used to separate the EV-containing fractions, ultimately enabling optimization of the purification process.

5. | Summary

Perform high-quality measurements without penetrating the **sterile** boundary of the bioreactor

Enhance **automation** by comprehensive system solution design (sensor + reactor interface)

Flexibly exchange measurement system – ViewCell® is the only **wetted part**

Reduce effort and costs with **in-situ** process control

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