TECHNICAL NOTE

Purification of Milk-Derived Extracellular Vesicles:

The Challenge of Casein and the Role

1. Introduction

Milk-derived extracellular vesicles (EVs) have emerged as a highly attractive source of bioactive nanoparticles for research, cosmetic, nutraceutical, and therapeutic applications. Bovine milk, in particular, offers a scalable and cost-effective source of EVs, making it an increasingly important raw material for industrial production.

However, obtaining highly purified EV preparations from milk remains challenging due to the presence of one major contaminant: casein micelles.

Why Is Casein a Critical Contaminant?

Casein accounts for more than 80% of the total protein content in bovine milk and forms large supramolecular structures known as casein micelles. Unfortunately, these particles share several physicochemical characteristics with EVs.

Parameter	Extracellular vesicles	Casein Micelles
Size	30–200 nm	100–600 nm
Density	1.08–1.19 g/mL	1.06–1.10 g/mL
Abundance	Moderate	Extremely high

This overlap in size and density makes separation particularly difficult using conventional isolation methods such as differential ultracentrifugation, filtration, or even Size Exclusion Chromatography (SEC).

As a result, casein contamination can interfere with particle characterization, proteomic analysis, RNA profiling, and downstream functional studies.

Strategies for Casein Removal

Current best practices focus on disrupting or removing casein micelles before the final EV purification step.

1. Calcium Chelation

Casein micelles depend on calcium phosphate bridges to maintain their structure. Chelating agents such as EDTA or sodium citrate destabilize these interactions, causing the micelles to dissociate into much smaller submicellar components.

Typical conditions include:

- EDTA: 20–30 mM
- Incubation: 30–60 minutes at 37°C
- Clarification: 5,000–10,000 × g for 10 minutes

This approach effectively reduces casein particle size while preserving EV integrity.

2. Enzymatic Treatment

Chymosin (rennet), commonly used in cheese production, cleaves κ-casein and destabilizes the micellar structure, leading to precipitation of casein aggregates that can subsequently be removed by centrifugation.

3. Isoelectric Precipitation

Lowering the pH to approximately 4.6 causes casein precipitation and efficient removal. While highly effective, this approach may affect EV surface proteins and compromise certain biological functions.

The Role of Size Exclusion Chromatography (SEC)

SEC has become one of the most widely adopted methods for EV purification because it efficiently separates vesicles from soluble proteins, protein aggregates, and lipoproteins while preserving EV integrity.

Numerous studies have demonstrated that SEC provides cleaner EV preparations compared with precipitation-based methods and can significantly reduce protein contamination.

However, when intact casein micelles are present, SEC alone cannot guarantee complete separation due to the extensive size overlap between EVs and casein particles. Therefore, SEC should be viewed as a polishing step, ideally performed after a dedicated casein-removal treatment.

Choosing the Right SEC Column

Once casein micelles have been disrupted or removed, SEC becomes highly effective for obtaining purified EV fractions.

For EV populations centered around 150 nm:

SEC7012

- Optimized for particles above 70 nm.
- Excellent removal of smaller proteins and contaminants.
- Recommended when maximum purity is the primary objective.

SEC3512

- Broader recovery of small EV populations.
- Suitable when maximizing EV recovery is more important than achieving the highest purity.

For most milk-derived EV workflows focused on downstream characterization, therapeutic development, or cosmetic applications, SEC7012 is generally the preferred option following casein removal.

Emerging Industrial Workflows

The rapid growth of milk-derived EV applications has driven the development of scalable manufacturing processes. Recent studies and industrial protocols increasingly combine:

1. Skimming and clarification.

2. Selective casein removal.

3. Tangential Flow Filtration (TFF).

4. Final purification by SEC.

This integrated approach enables the production of highly purified EV preparations suitable for large-scale manufacturing and regulatory development.

Conclusion

Casein removal is the critical step in the purification of milk-derived extracellular vesicles. Due to the substantial overlap in size and density between EVs and casein micelles, no single size-or density-based method can achieve complete separation on its own.

Combining a dedicated casein-removal strategy with Size Exclusion Chromatography provides one of the most effective and scalable solutions currently available, delivering EV preparations with improved purity, reproducibility, and suitability for research, diagnostic, cosmetic, and therapeutic applications.

References

- Marsh SR, Pridham KJ, Jourdan J, Gourdie RG. Novel protocols for scalable production of high quality purified small extracellular vesicles from bovine milk. Nanotheranostics. 2021;5(4):488–498.
- Wu X, Shen J, Zhong Y, et al. Large-scale isolation of milk exosomes for skincare. Pharmaceutics. 2024;16(7):930.
- Hrasnova P. Development of a scalable isolation process for therapeutic applications of extracellular vesicles from human milk. Doctoral Dissertation. Paris-Lodron-University Salzburg; 2026.
- Mata C. Rapid method for the high purity isolation of bovine milk-derived extracellular vesicles via polyester (PET) capillary-channeled polymer (C-CP) fiber columns. Nanotheranostics. 2026;10(1):11–23.