Separation of Virus Like Particles and Nano-Emulsions for Vaccine Development by Capillary Zone Electrophoresis

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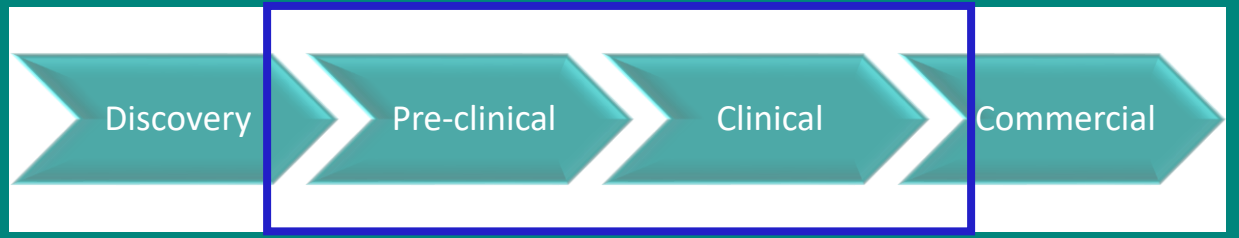
Analytical Research & Development



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Types of Vaccines

Polysaccharide-based Vaccines

1977

1990

Vaxneuvance™ Pneumococcal 15-valent Conjugate Vaccine

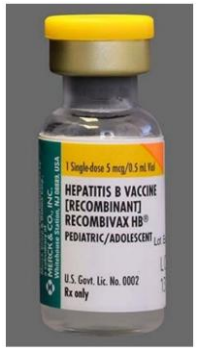
2021

CAPVAXIVE™ Pneumococcal 21-valent Conjugate Vaccine

2024

Recombinant Virus-like Particle Vaccines

Hepatitis B



1986

HPV

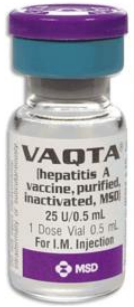
2006

Attenuated Live Virus Vaccines

Measles, Mumps, Rubella, Varicella

1971

Hepatitis A



1995

Rotavirus

2006

Ebola

2019

Components of Vaccines

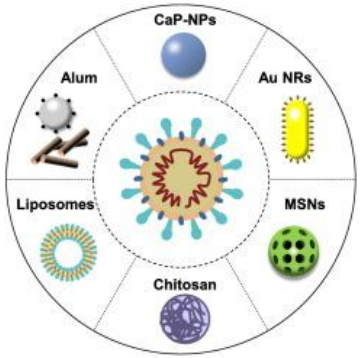
Active Components

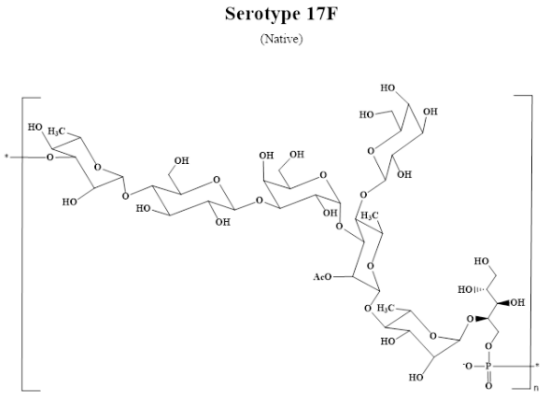
- Polysaccharides 50k – 2 000 kg/mol
- Proteins and Virus Like Particles (VLPs) 50-70 nm
- Viruses 30-400 nm in size

Excipients

- Buffer components
- Salts
- Stabilizers (eg polysorbates)

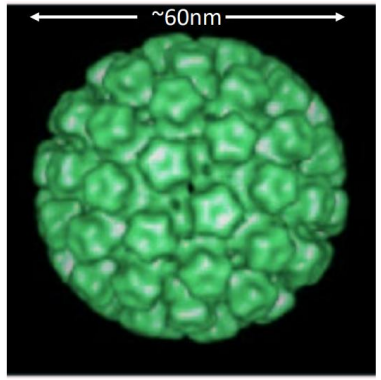
Adjuvants



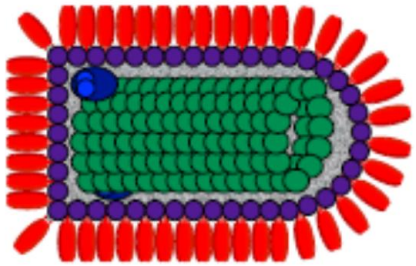


C. Jones et al. (2000) Carbohydrate Research 325, 192–201

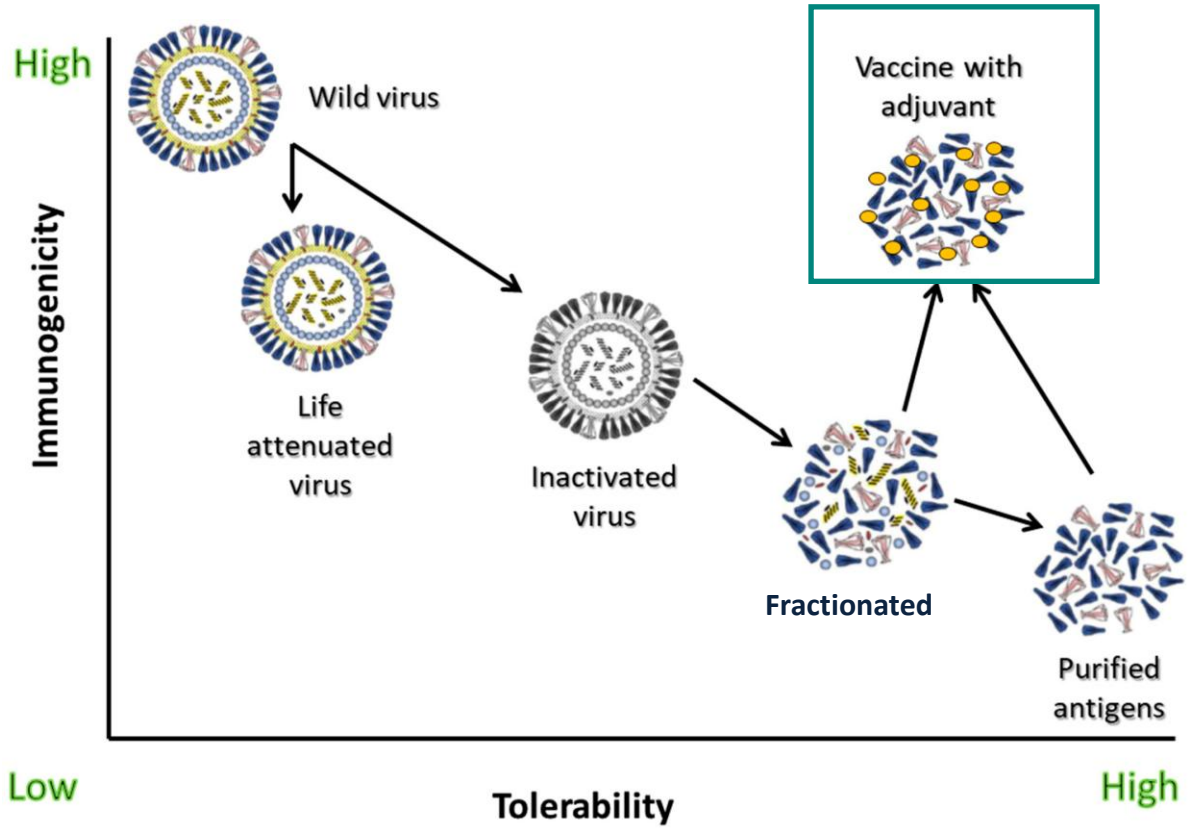
VLP



Indiana Vesiculovirus



Why are Vaccine Adjuvants Important?



Goal
More purified antigens but same immunogenicity

Vaccine Adjuvants: from 1920 to 2015 and Beyond, *Vaccines* **2015**, 3, 320-343; doi:10.3390/vaccines3020320

Vaccine Adjuvants

Questions about vaccines with adjuvants:

1. Can we monitor both the adjuvant and the antigen at the same time?
2. Are they associated together?
3. How can we do this with multiple antigen types and multiple adjuvant types?

Lichun Mao, Ziwei Chen, Yaling Wang, Chunying Chen, Design and application of nanoparticles as vaccine adjuvants against human corona virus infection, *Journal of Inorganic Biochemistry*, 219, **2021**, 111454, https://doi.org/10.1016/j.jinorgbio.2021.111454

What kind of Method(s) do we need?

Something that can:

1. Separate all the antigens, excipients and adjuvants at the same time

2. Distinguish if an antigen and adjuvant are associated together

3. Be compatible with multiple antigen and adjuvant types

Methods normally used for separating macromolecules and nanoparticles:

- SEC
- AF4
- AUC
- ELISA/Simple Western

1. Not useful if they are the same size or antibodies are not available

2. What if an association is perturbed?

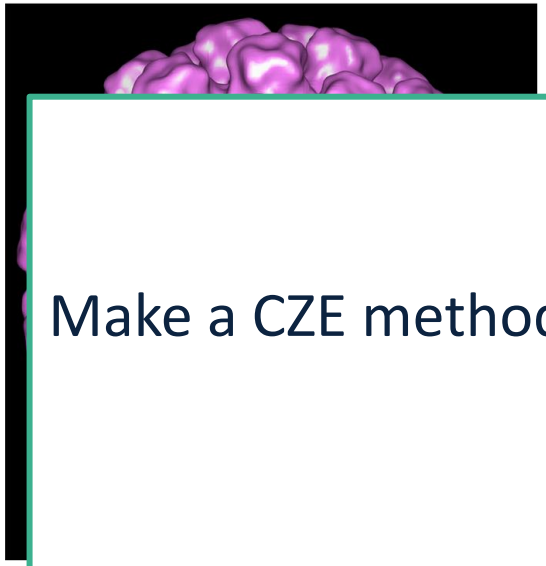
Potential Methods

Capillary Zone Electrophoresis – charge to friction ratio

ACE and FACCE for weak associations

Step 1 – Proof of Concept with Virus-Like Particles and Emulsion Adjuvant

Human Papilloma Virus
Virus-Like Particle (VLP)



pI value of 8.35

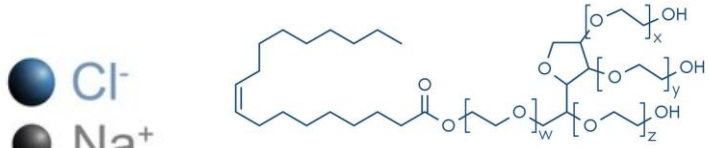
Squalene NanoEmulsion (SNE)
adjuvant

n® 80)

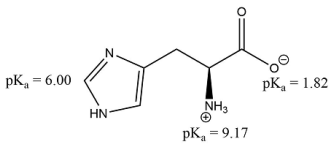
DL-α-tocopherol)

Goal

Make a CZE method to separate and quantitate all components in one analysis



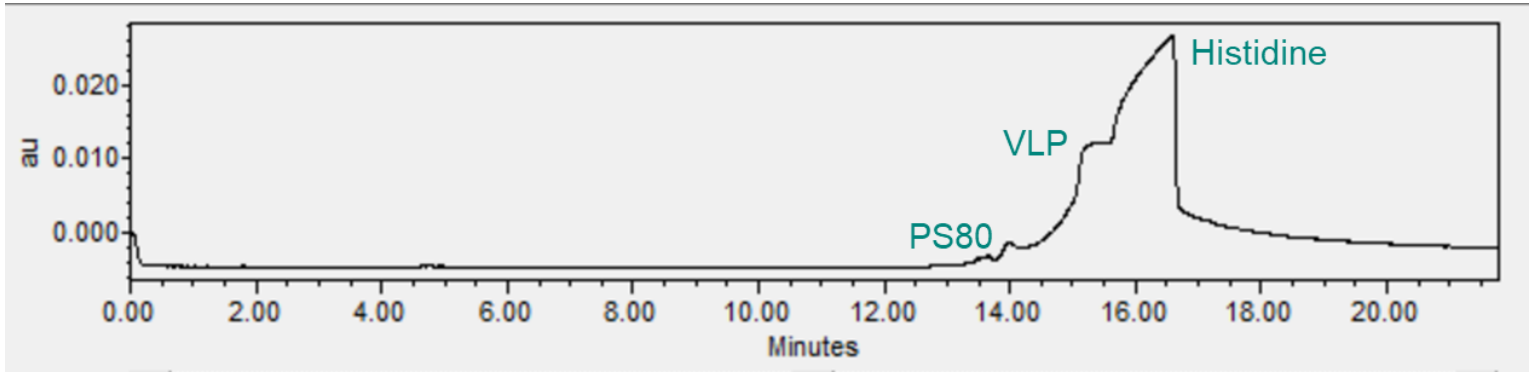
polysorbate 80



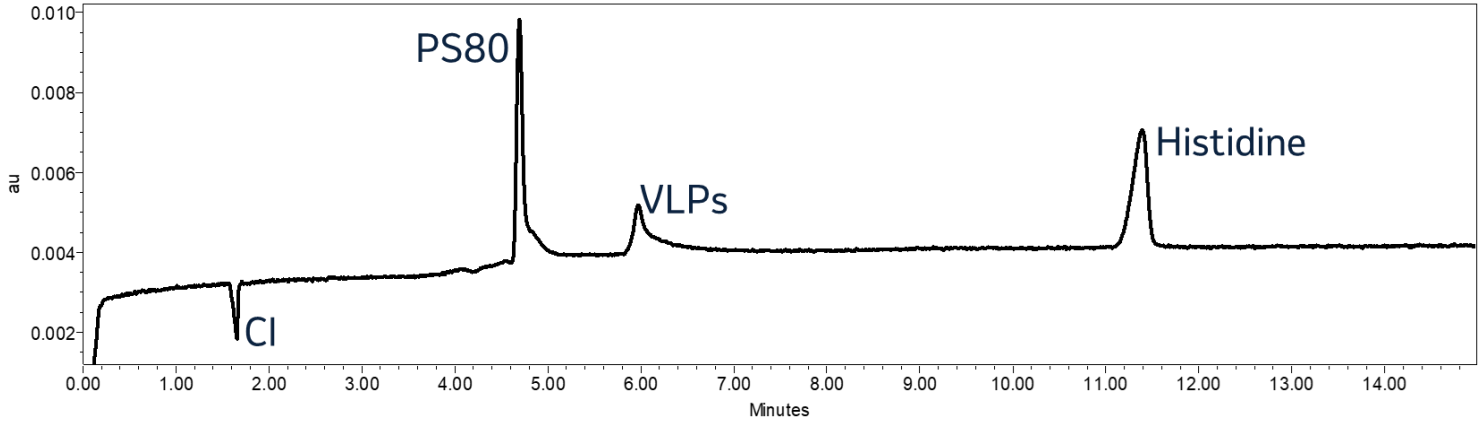
Histidine

Separation of VLPs at different pH values

VLPs separation (Tris Buffer pH 7.5)



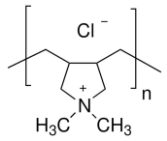
VLPs separation (Na acetate pH 5.2)

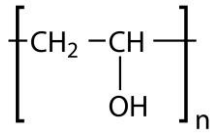


Provides resolution but makes the species positively charged so a coating is needed

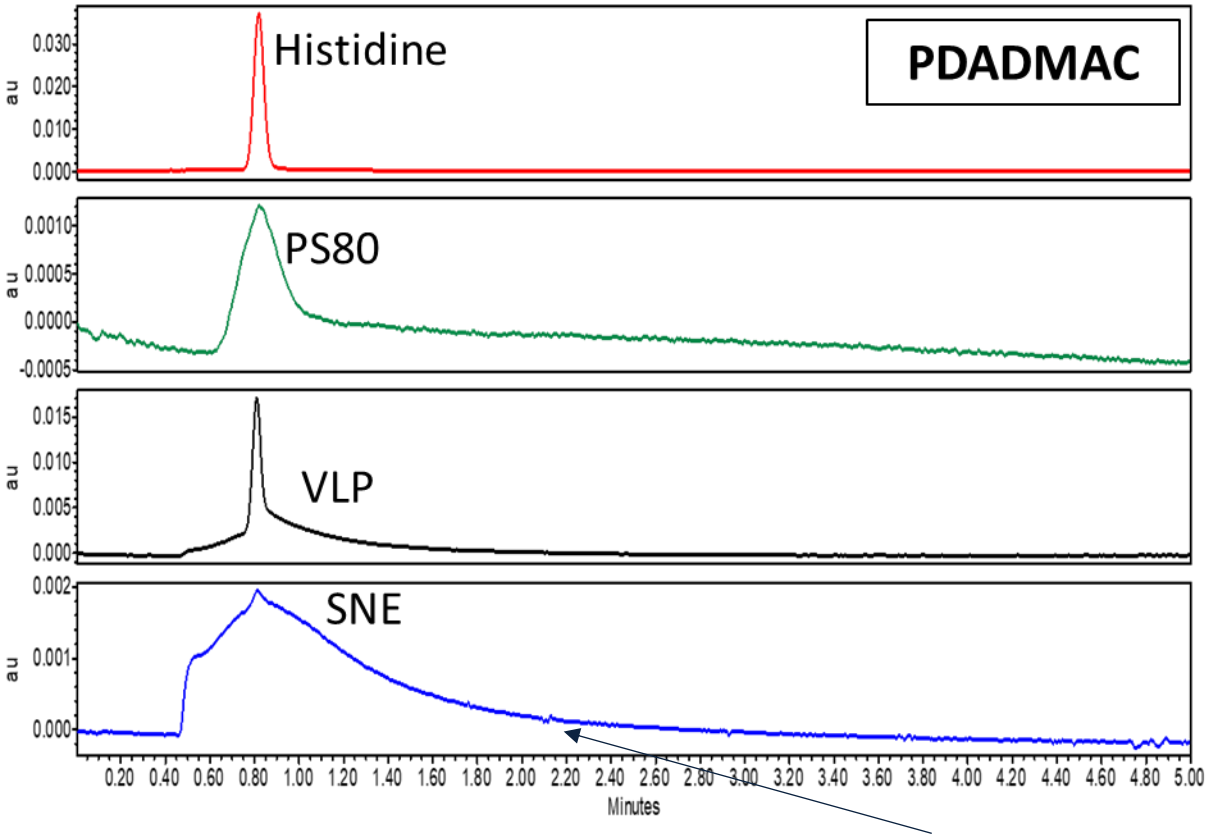
Choosing ideal coating for separation

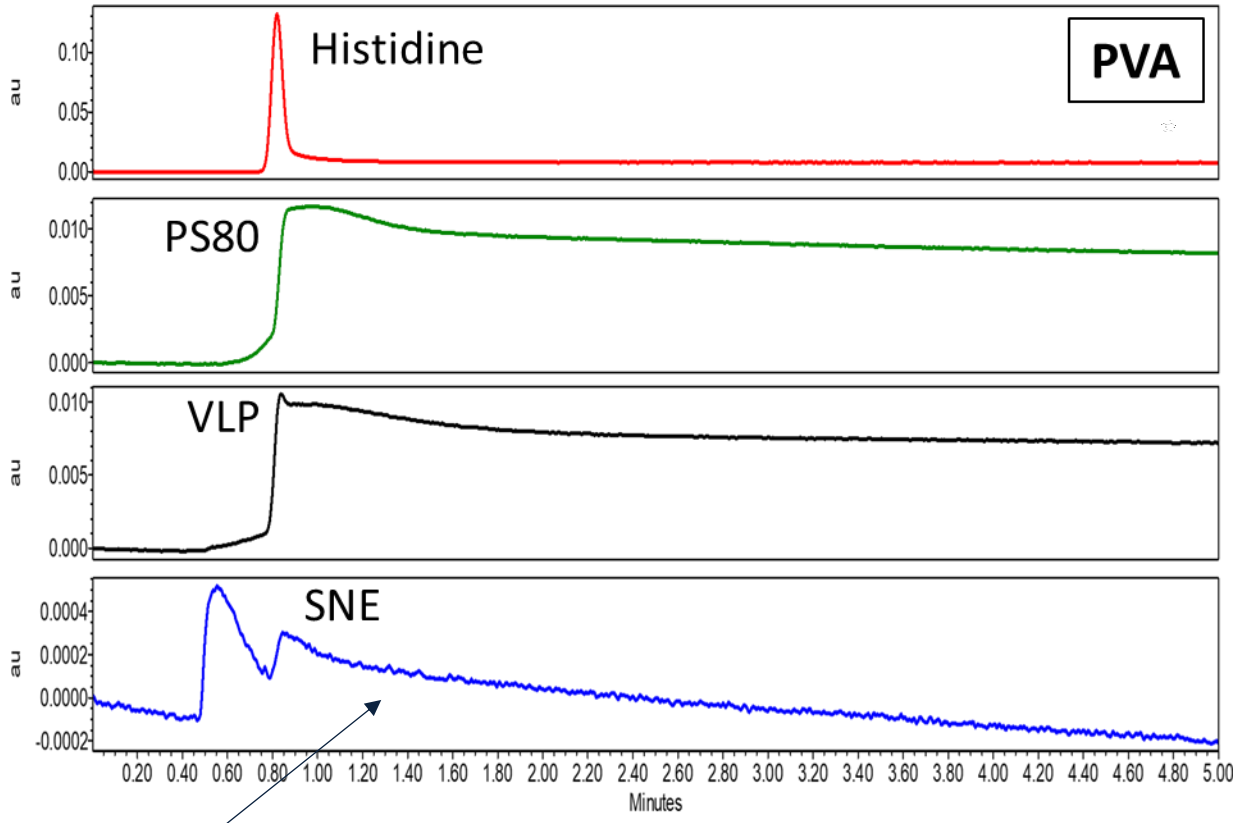
Pressure mobilization study





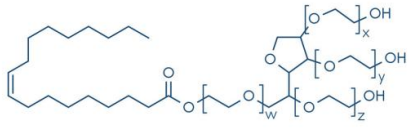
Commercial from Agilent

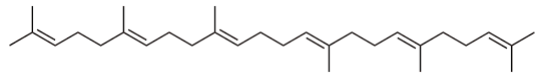


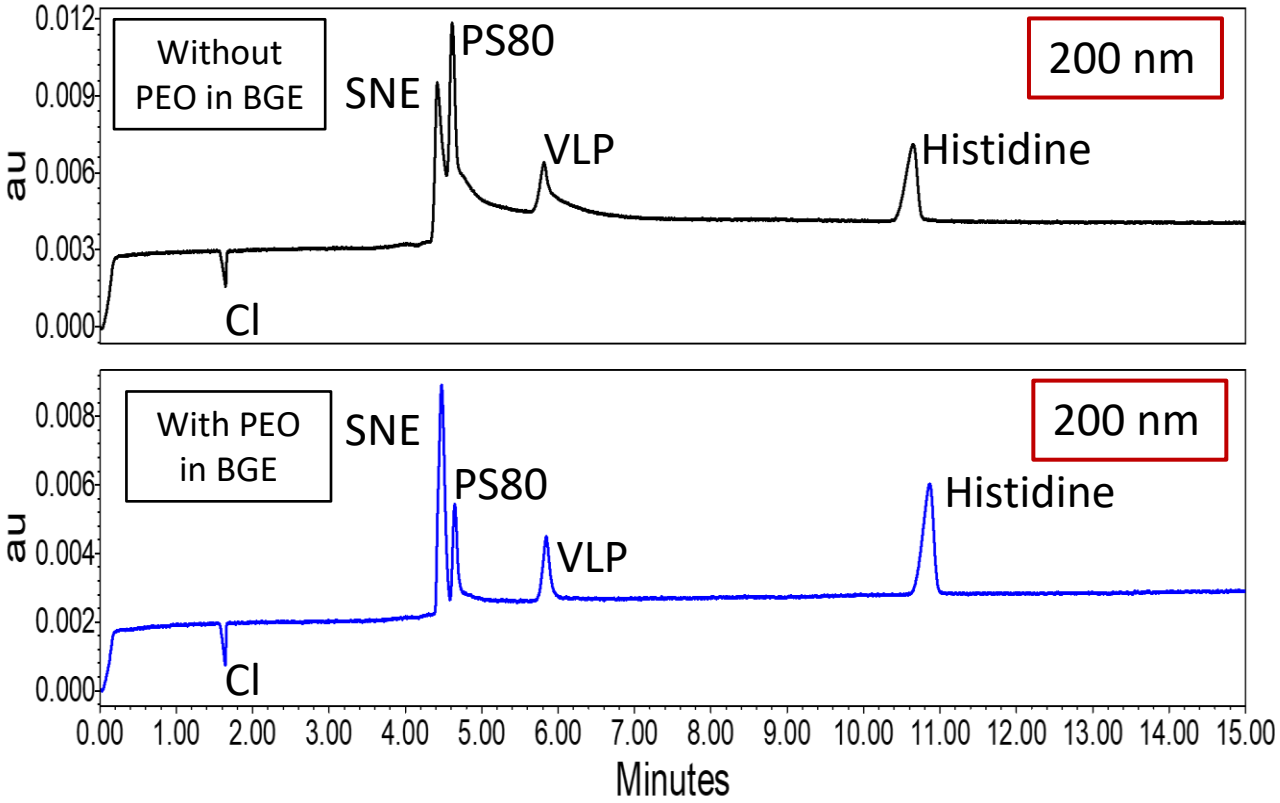


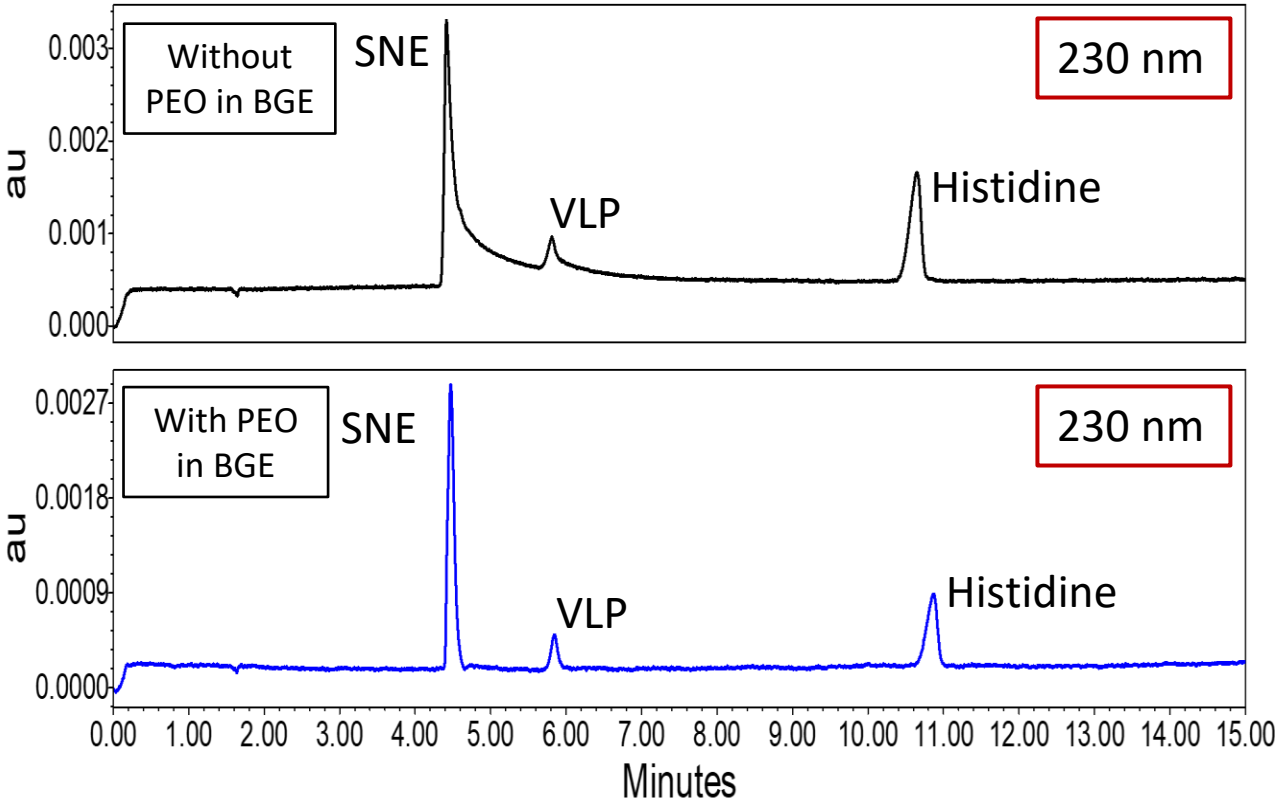
Tailing indicates adsorption to the capillary wall

Achieving a Suitable Separation









- PEO reduces peak tailing from adsorption to the capillary
- Using 230 nm to measure SNE without PS80 interference
- Quantitate 4 components at the same time

Precision and accuracy

Table. Precision and accuracy of the proposed method

	Chloride	SNE	VLP	Histidine
Within-day precision (RSD%)	2-3%	0.5 - 3%	1 - 4%	0.9 – 2%
Between days precision (RSD%)	3 - 9%	4 – 6%	4 - 20%	2 – 10%
Intermediate precision (RSD%)	6 - 20%	15 - 20%	8 - 20%	5 - 15%
Accuracy (%Recovery)	98 – 109%	90 – 97%	87 – 100%	100 – 101%

Measurements are based on n = 6 for precision studies and n = 4 for accuracy study

- RSD% observed to be ≤ 4 for within-day precision, ≤ 20% between-day precision, and intermediate precision (2 analysts)
- The accuracy of all analytes ranges from 87 – 109%

The Linearity, LOD, LOQ Determination

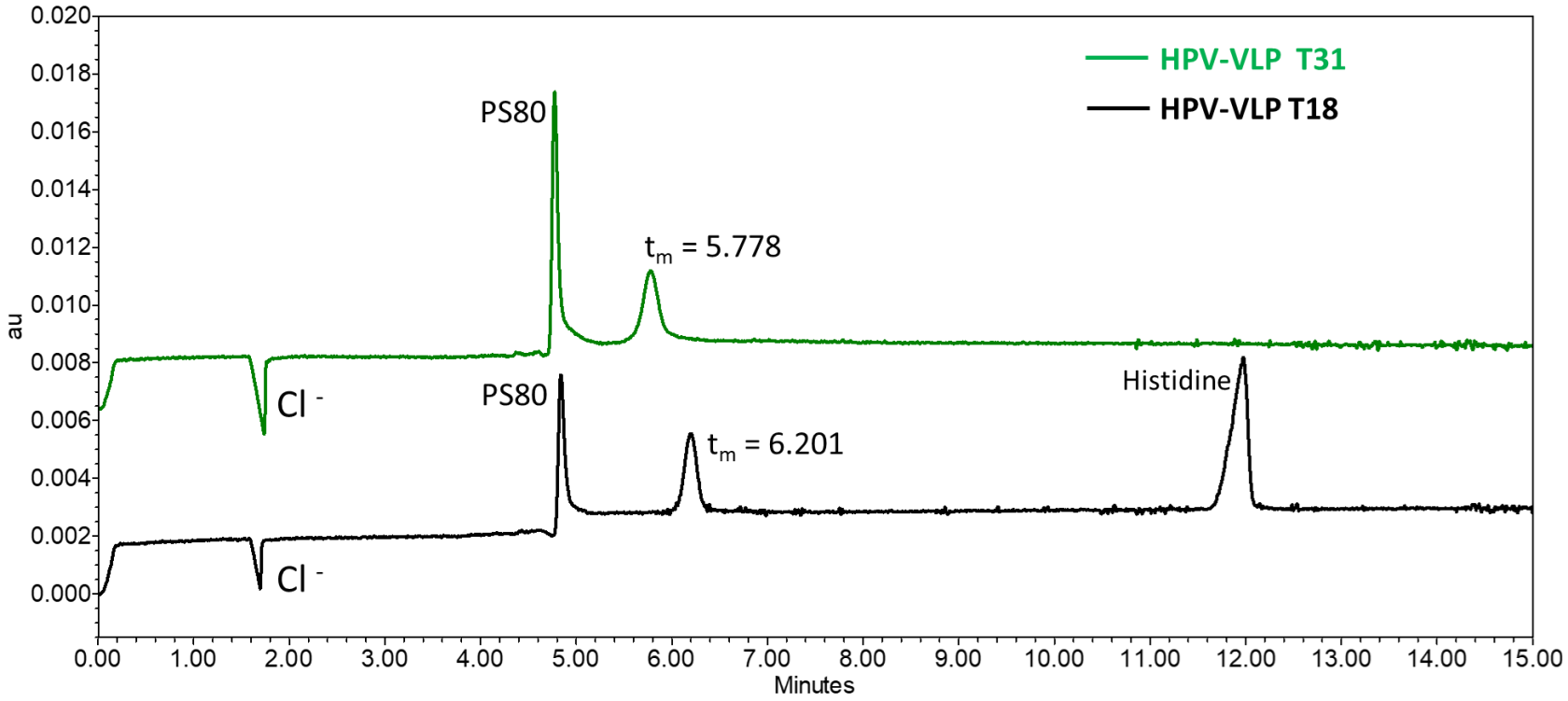
Table. Analytical parameters for the determination of chloride, SNE, VLP, and histidine of the proposed method

	Chloride	SNE	VLP	Histidine
Linear range*	7 – 70	20 – 200	20 – 200	0.7 - 7
Linearity (R^2)	0.990	0.990	0.989	0.986
S_b%	4.00	3.17	4.27	4.72
Limit of Detection (LOD) *	0.7	0.3	2	0.04
Limit of Quantification (LOQ) *	2	1	6	0.1

* Concentration expressed in µg/mL for SNE and VLP, and in mM for chloride and histidine
* LOD is calculated as 3 x (blank signal/slope), and LOQ is calculated as 10 x (blank signal/slope)

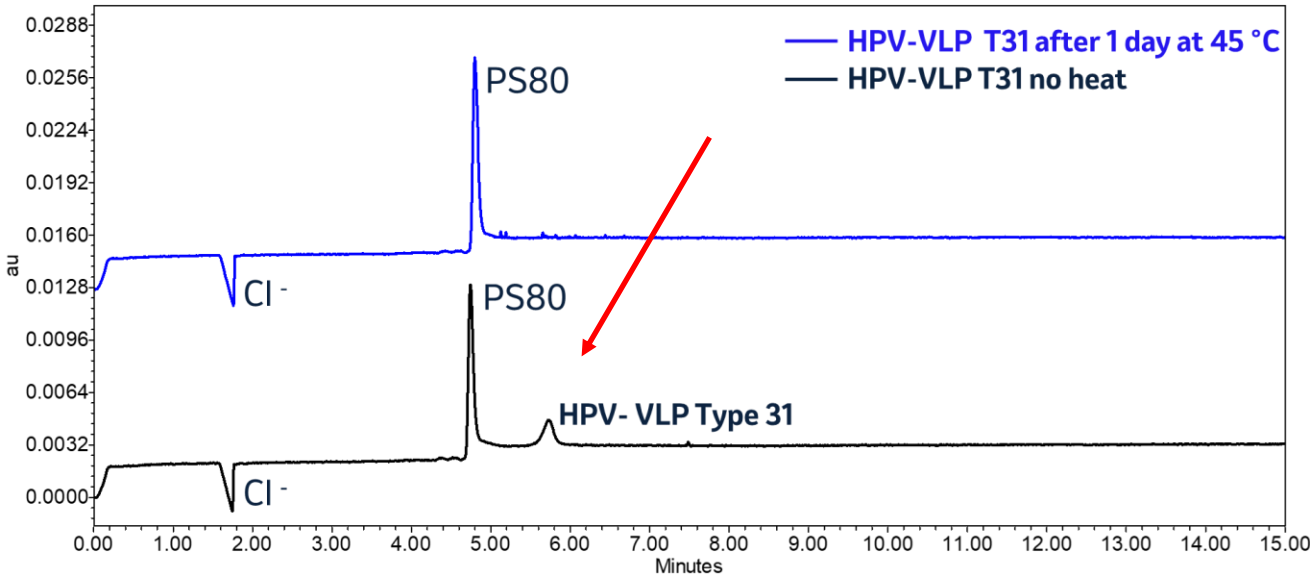
- The correlation coefficient (R^2) values ≥ 0.98
- RSD% of slope values (S_b%) ≤ 5%.

Comparing different HPV types



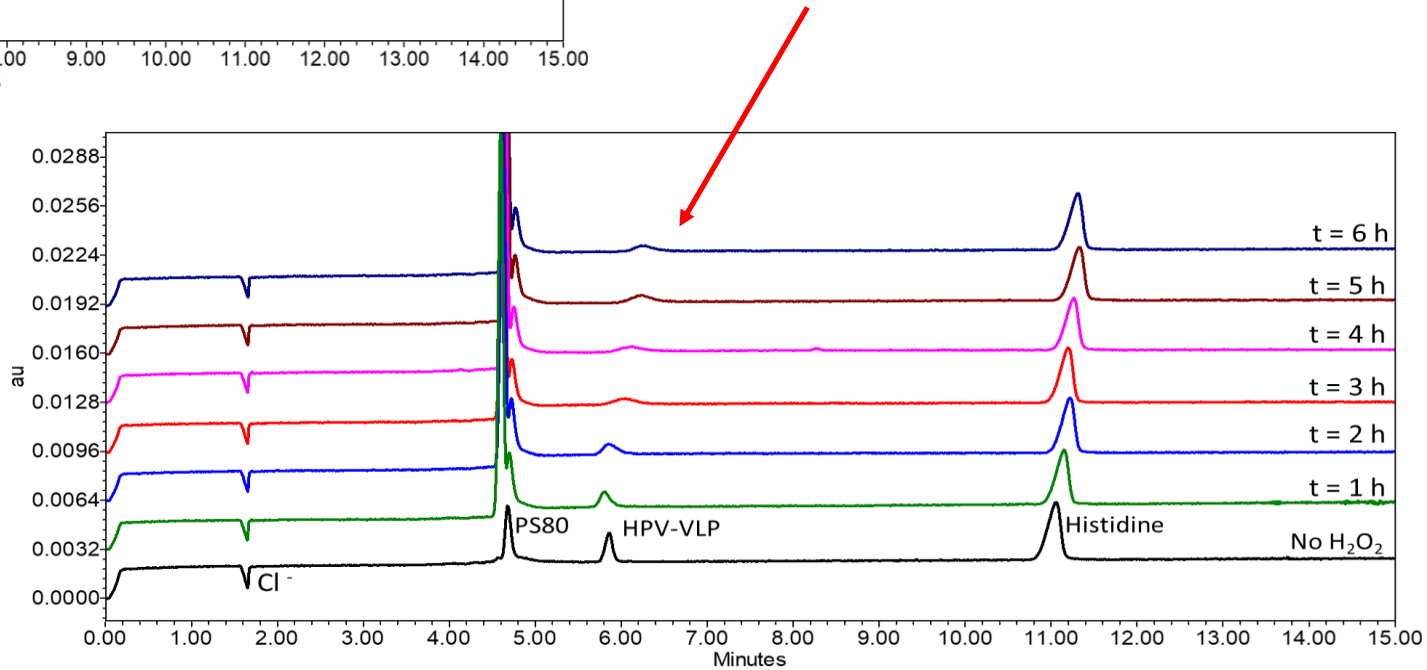
% difference in electrophoretic mobility is 22 %

Detecting Degradation in VLPs

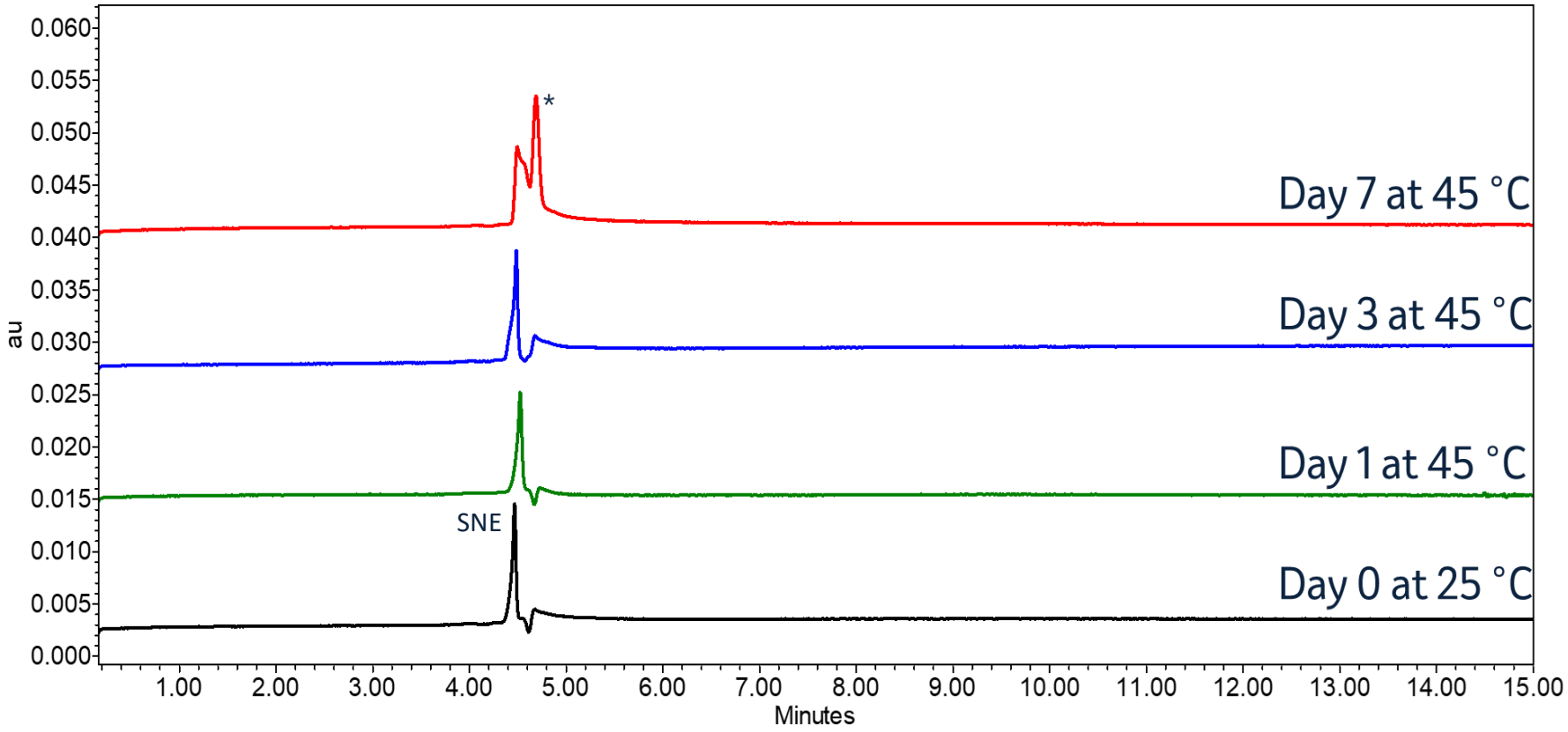


Temperature

Oxidation with hydrogen peroxide



Stability of the Squalene NanoEmulsion (SNE)



*At Day 7: a new peak, likely PS80 from the nanoemulsion appears.

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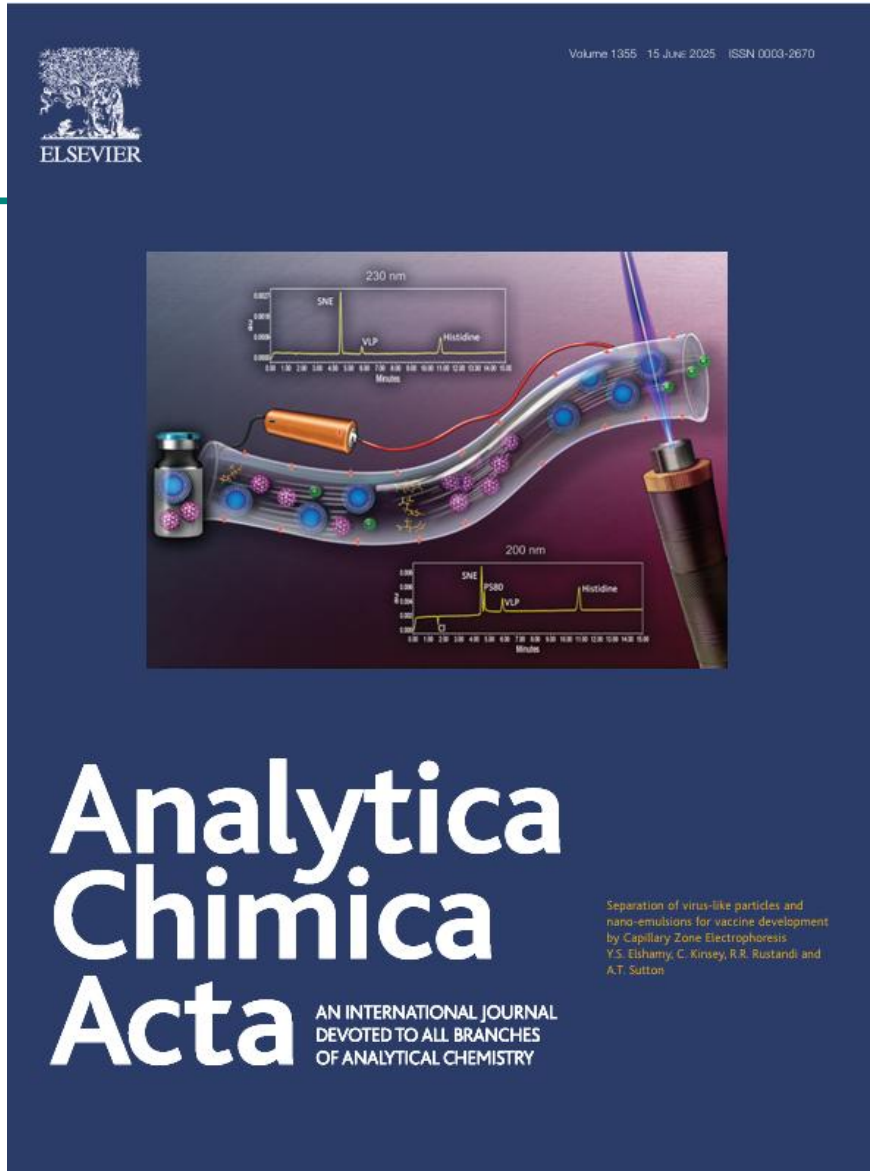
Conclusions

- A CZE method with a simple background electrolyte (BGE) can separate the entire mixture in a single run to monitor all components simultaneously under stressed conditions
- CZE is demonstrated to separate two nano species of similar sizes
- Poly(ethylene oxide) (PEO) in the BGE reduced peak tailing to a cationic coating
- Confirmation that there is no strong association between antigen and adjuvant

Future Directions

- Expand to other antigen and adjuvant types
- Investigating other capillary coatings and additives
- Explore other CE methods to detect weaker associations



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