Virus / Prion reduction for Large Plasma-Derived Proteins: Caprylic Acid Treatment and UV-C Irradiation

Biotest
From Nature for Life

The manufacturing process of Trimodulin

Highly Confidential results:

Relevant patent information:

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ViruSure 2nd Virus Safety Workshop, 23-Sep-2022, M. Asper

The manufacturing process of Trimodulin

Highly Confidential results:

Inactivation of HAV and PPV by [redacted] and [redacted]

[redacted] log

Relevant patent informati

Virus		qPCR
[redacted]	$\log_{10}$	[redacted]
	$\log_{10}$	
	$\log_{10}$	

Greetings from the legal department

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From Nature for Life

Many thanks for your *short* attention!

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ViruSure 2nd Virus Safety Workshop, 23-Sep-2022, M. Asper

Virus / Prion reduction for Large Plasma Derived Proteins

Part 1: Manufacturing process of IgM
(Trimodulin)

Part 2: Caprylic acid treatment
Procedure and case study

Part 3: UV-C treatment
Procedure and case study

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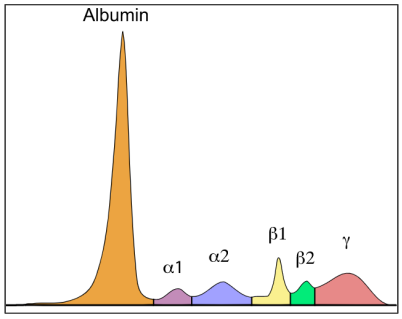
Human Plasma derived proteins

Human Plasma donation

- ≈ 92% water
- ≈ 1% salts
- ≈ 7% proteins (> 120 different proteins)



60%	Albumin
4%	α1-Globulins
8%	α2-Globulins
12%	β1+2-Globulins
16%	γ-Globulins (IgA, IgD, IgE, IgG, IgM)

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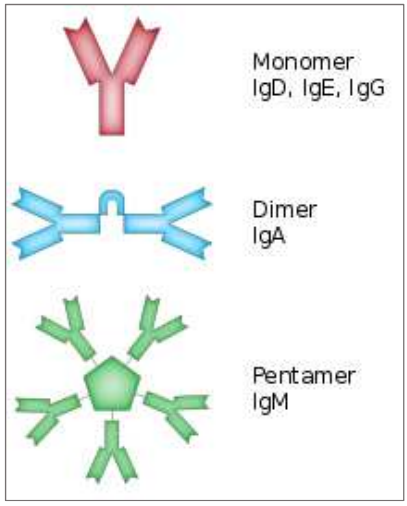
Trimodulin

Biotest Trimodulin (IgM Concentrate)

IgM : IgA : IgG ≈ 1 : 1: 2



Drug application areas of Trimodulin (phase III studies)

- Severe community acquired pneumonia, sCAP
- Severe coronavirus disease 2019 (COVID-19)

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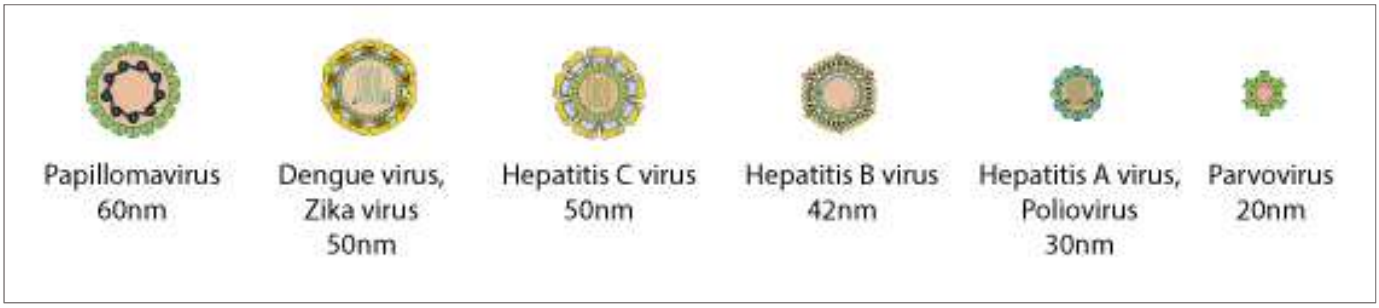
Trimodulin – Virus / Prion Safety

Prion Protein (PrP^{sc}):	35 KDa
IgG:	150 KDa
IgA:	160 KDa
IgM:	960 KDa (≈29-35 nm)



https://viralzone.expasy.org/5216

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Manufacturing process of Trimodulin

Human Plasma

↓

Separation of cryoprecipitate

Cohn Fractionation

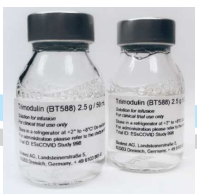
↓

Caprylic Acid precipitation & filtration → Virus / Prion inactivation / removal step

↓

UV-C → Virus inactivation step

↓



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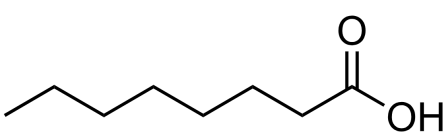
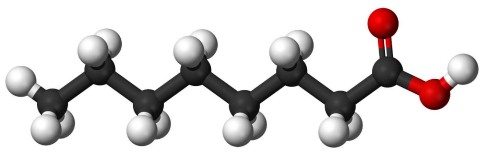
Caprylic Acid

Caprylic Acid
$CH_3(CH_2)_6CO_2H$





$$CH_3(CH_2)_6COOH \underset{pH\downarrow}{\overset{pH\uparrow}{\rightleftharpoons}} CH_3(CH_2)_6COO^- + H^+$$

nonionized form — ionized form

Main contributor to virus inactivation

pK_a 4.89

→ Virus inactivation: pH, temperature and time dependend

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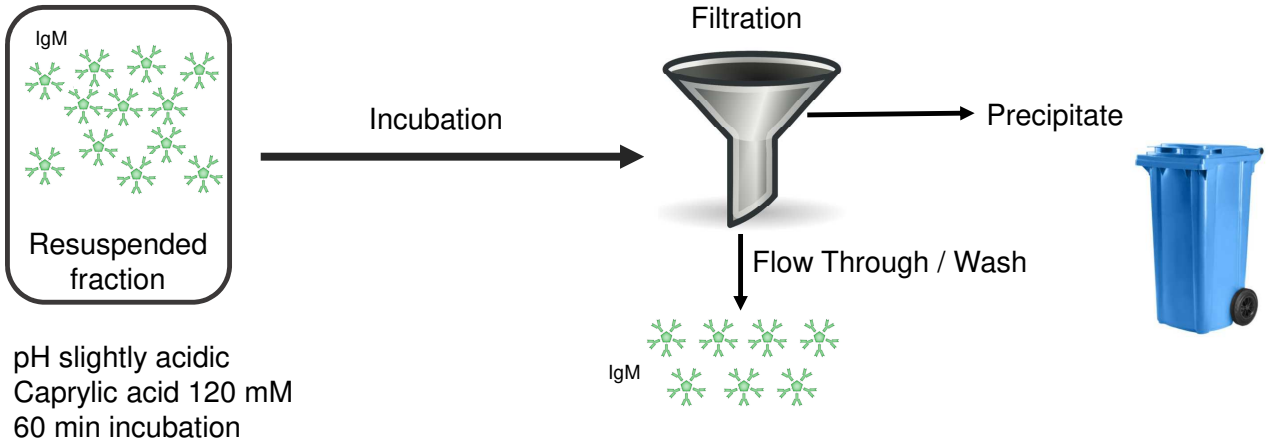
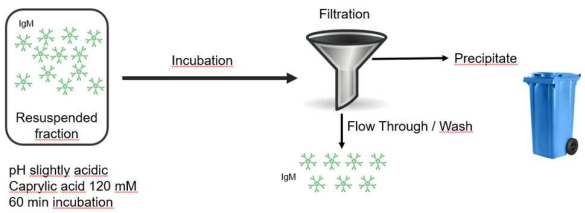
Caprylic Acid treatment



pH slightly acidic
Caprylic acid 120 mM
60 min incubation

Caprylic Acid treatment

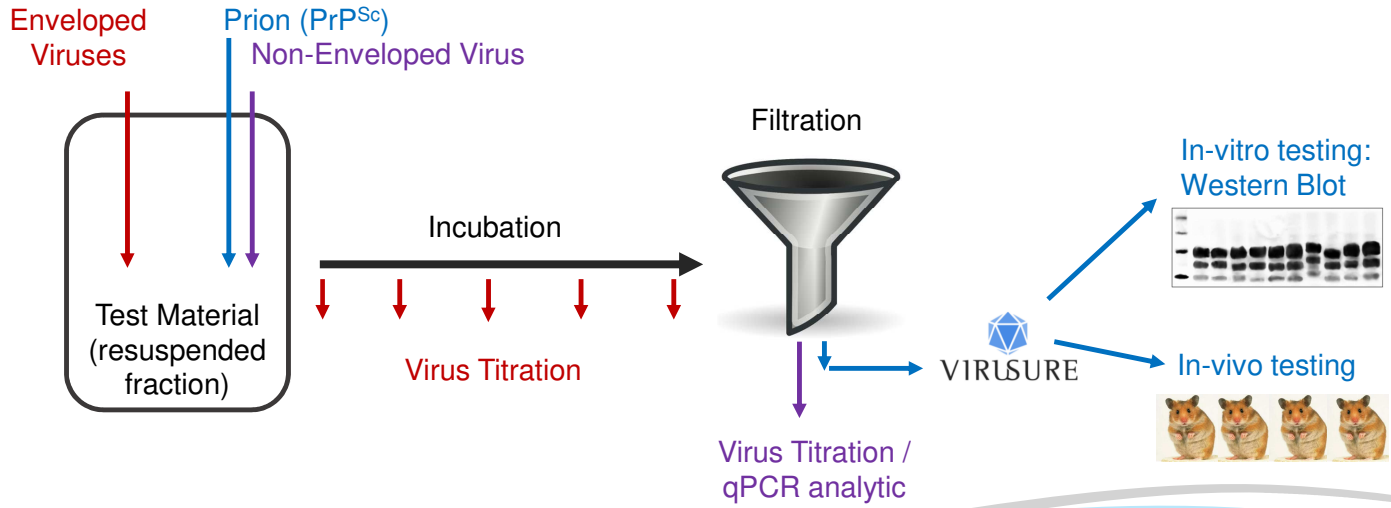


pH slightly acidic
Caprylic acid 120 mM
60 min incubation

Different mechanisms for enveloped / non-enveloped viruses & Prions

- Enveloped Virus *inactivation* by Caprylic Acid
- non-enveloped Virus / Prion *removal* by precipitation with impurities

Case study: Caprylic Acid treatment



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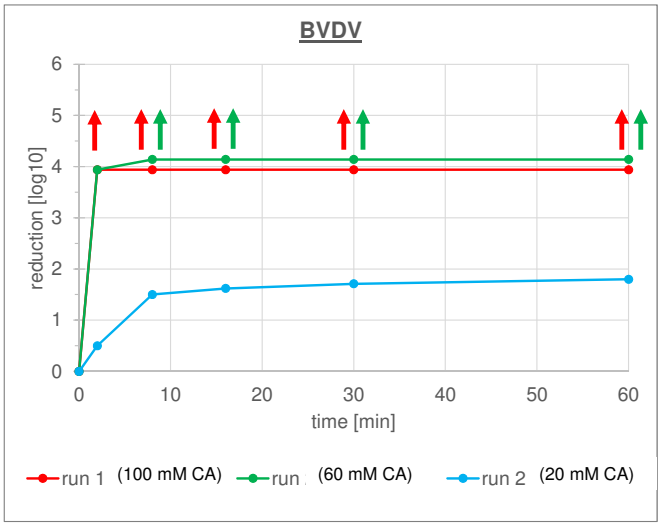
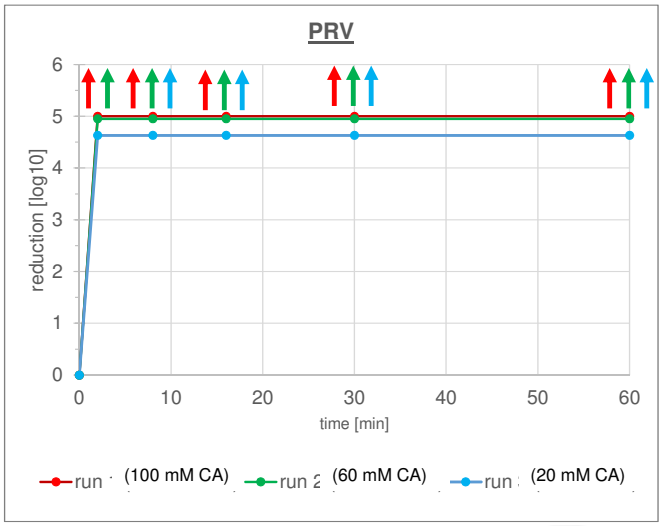
Case Study: Caprylic Acid treatment virus inactivation





(100 mM CA)

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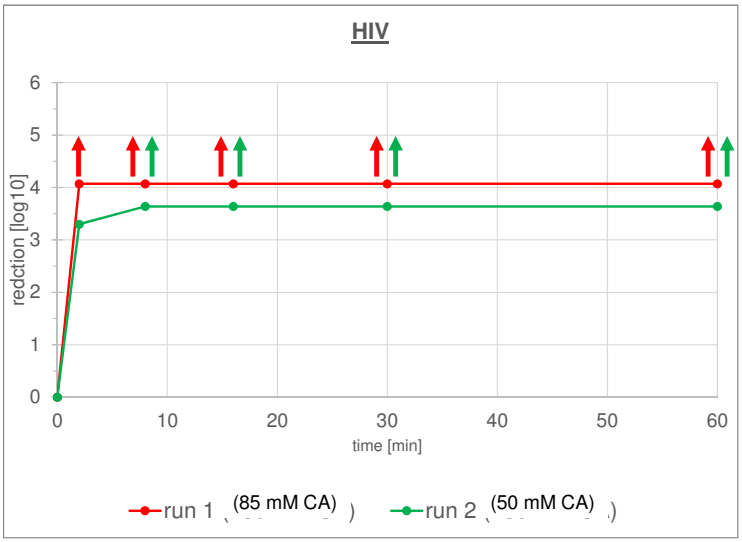
Case Study: Caprylic Acid treatment virus inactivation



Inactivation of enveloped viruses:
Very quick and very effective

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Case Study: Caprylic Acid treatment Virus / Prion removal

Virus	Infectivity Assay	qPCR
HAV	> 3.7 log10	1.9 log10
HEV	---	> 4.8 log10
PPV	1.7 log10	---
B19V	---	2.5 log10

Prion	In-vitro	In-vivo
PrP^{Sc} (263K)	> 3.9 log10	6.0

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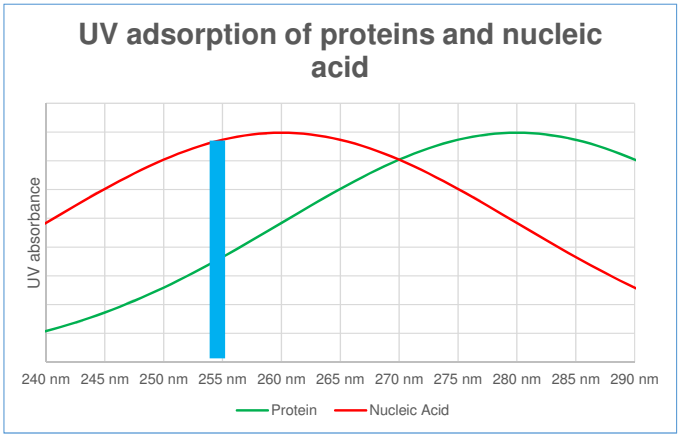
UV-adsorption spectra of protein and nucleic acid

- Adsorption maxima:
  proteins at ≈280 nm,
  DNA/RNA at ≈260 nm

➔ UV-C irradiation at 254 nm is preferentially absorbed by nucleic acids over proteins



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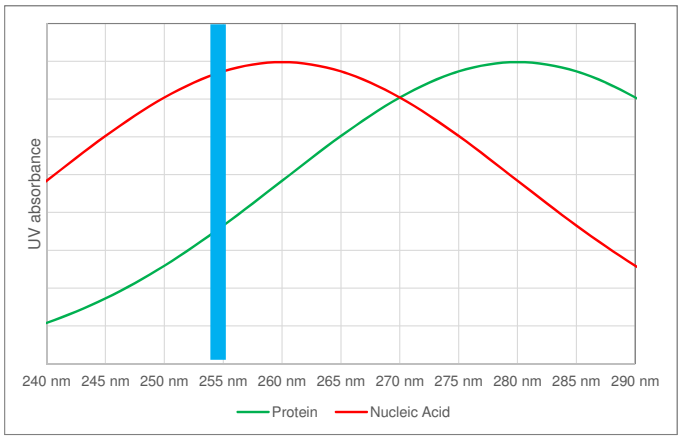
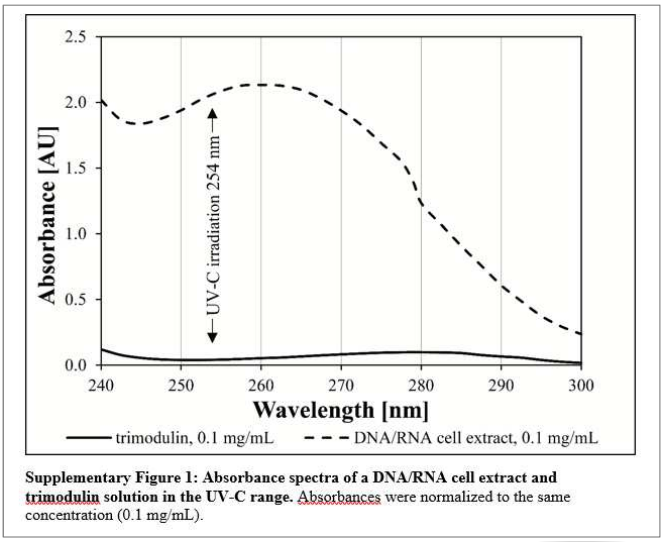
UV-adsorption spectra of Trimodulin





Supplementary Figure 1: Absorbance spectra of a DNA/RNA cell extract and trimodulin solution in the UV-C range. Absorbances were normalized to the same concentration (0.1 mg/mL).

S. Luelf, et al., 2022, submitted

UV-C action on nucleic acids

DNA/RNA damage

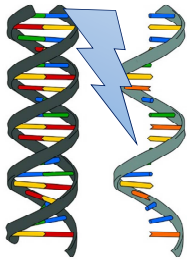
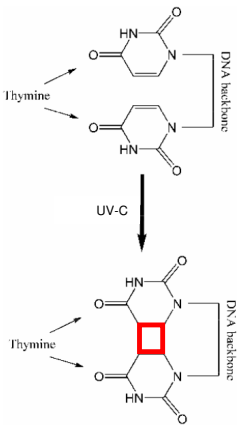


Image: Wikimedia Commons
Public Domain, author garriet41

- Prominent mechanism: direct hits of purine/pyrimidine bases
- UV-C irradiation leads to covalent bonds between neighbouring Pyrimidine nucleotides

➔Formation of cyclobutane „rings“:



UV-C equipment

What is needed?

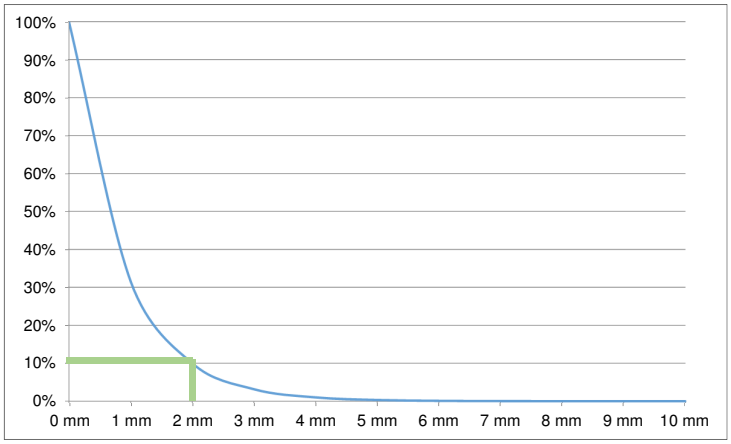
1. **UV-C lamp**
   generating UV-C irradiation

2. **UV-C „reactor“**
   vessel/container where process feed is irradiated

UV-C treatment

- Limited UV-C penetration in protein solutions
  - Exponential decay of UV intensity (W/m^2)
  - E.g. at OD_{254nm} = 5.0 and depth = 2 mm: loss of 90% of UV intensity

OD(254nm) = 5.0 (protein concentration: approx. 9.5 g/l)



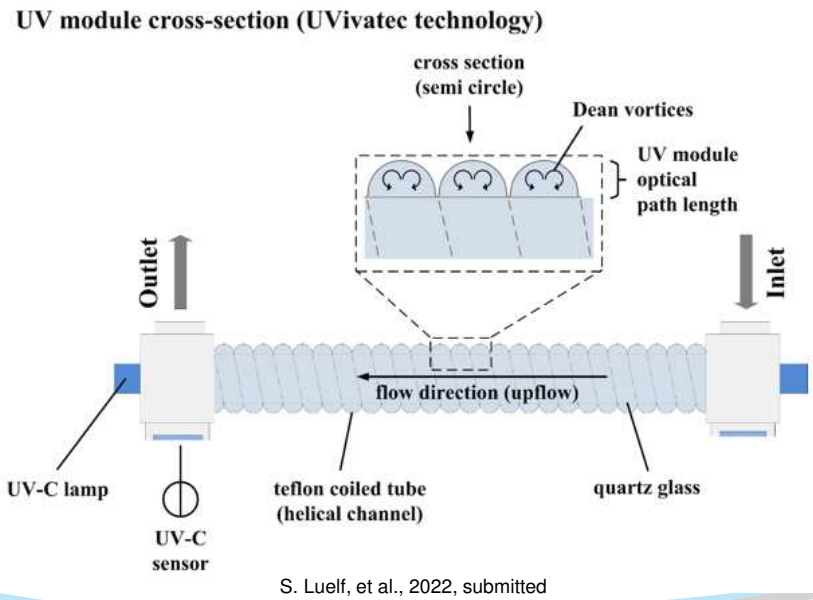
Homogenous mixing

homogenous mixing = lower UV-C dose required

less protein damages + more virus inactivation

UV-C
UVivatec® technology

Dean Vortices



S. Luelf, et al., 2022, submitted

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Dean Vortices

Primary Flow

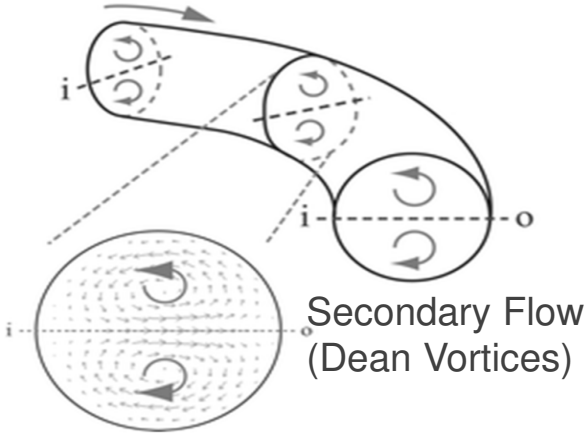
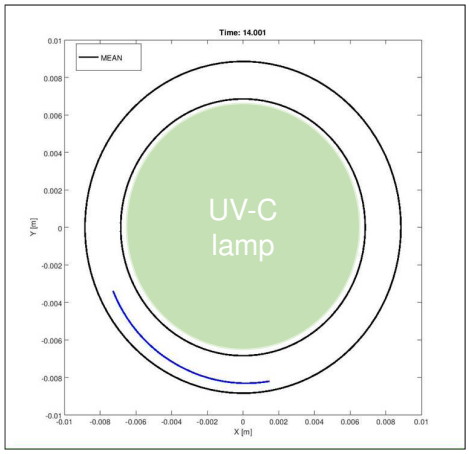


Figure modified after Sudarsan and Ugaz (2006)

Fluid dynamics simulation



ZETA

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Lab scale versus process scale unit

lab scale unit

	lab scale	process scale
Volume of module	24 mL	140 mL
Optical path length	2 mm	3.5 mm
Flow rate	6 – 20 L/h	30 – 120 L/h
Feasable for protein conc.	3-19 g protein per L	6-19 g protein per L

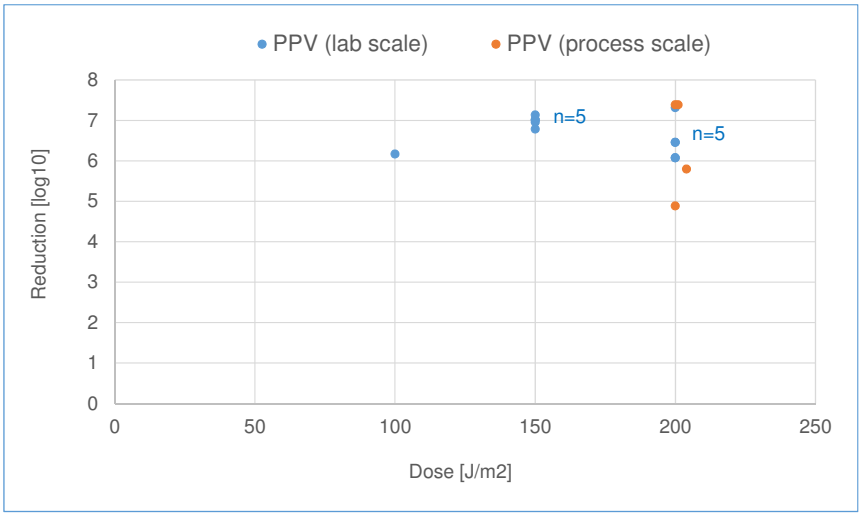
process scale unit

Case study: UV-C irradiation for Trimodulin

UV-C irradiation of Trimodulin:

- OD_{254nm} (approximate): >3 (= protein concentration of > 6 g/L)
- UV-C dose: 100 - 200 J/m²

Case study: PPV in lab / process scale unit



PPV inactivation: very effective

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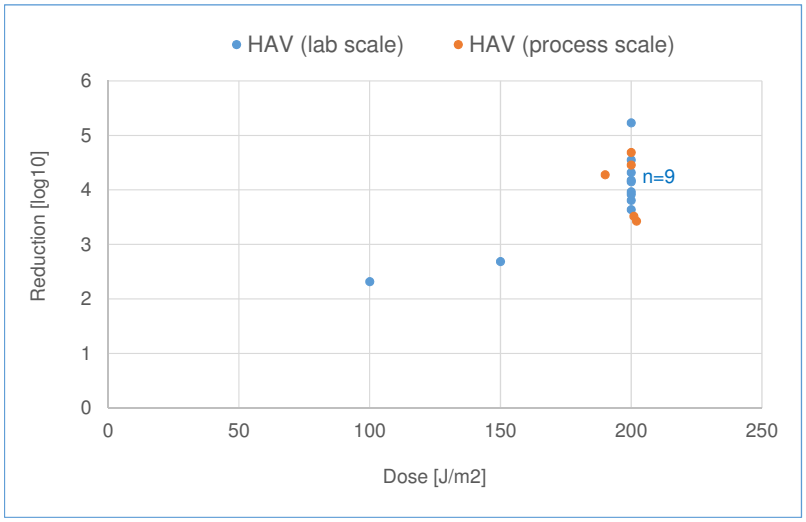
Case study: HAV in lab / process scale unit



HAV inactivation: incomplete inactivation, but effective at ≈200 J/m² (below IgM target UV-C dose)

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Case study: UV-C treatment

Virus	lab scale [log_{10}]	process scale [log_{10}]
BVDV	2.99	---
HIV	0.63	---
PRV	3.76	---
HAV	4.53	4.28
PPV	6.96	6.60

Standard conditions: 200 J/m^2
Mean values of different experimental runs

➔ Equivalence with process scale unit could be demonstrated

Summary

Virus	Envelope	*Caprylic acid treatment*	UV-C *Virus inactivation*
PPV	No	😔	😎
B19V	No	😔	Not performed
HAV	No	😔 😄	😎
HEV	No	😄	Not performed
BVDV	Yes	😄	😔 😎
HIV	Yes	😄	😢
PRV	Yes	😄	😎

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