# A selective recovery methodology for the primary purification of lipid envelope virus-like particles from *S. cerevisiae*

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# Presentation outline

## Research Overview

- Background
- Objectives

## Studies & Findings

- Purification potential & product yield
- Impact of homogenisation pressure conditions
- Effect on downstream HIC

## Summary & Future Work

# Virus-Like Particles

## Virus Like Particles (VLPs):

*Virus capsid proteins expressed in the absence of DNA*

## Benefits Vs Challenges

- Better safety profiles
- Higher efficiency
- Lower dosage requirements

- Difficult to characterise
- Sensitive to manufacturing process

*"Process defines product"*
*(Buckland, 2005)*

- Purification involves a complex process stream & high levels of contaminants

Buckland, (2005), Nature, 11, 4, S16-S19

# Project definition

## Objective

To improve process for future generation VLP vaccines

## Focus

Primary purification and process interactions

## Motivation

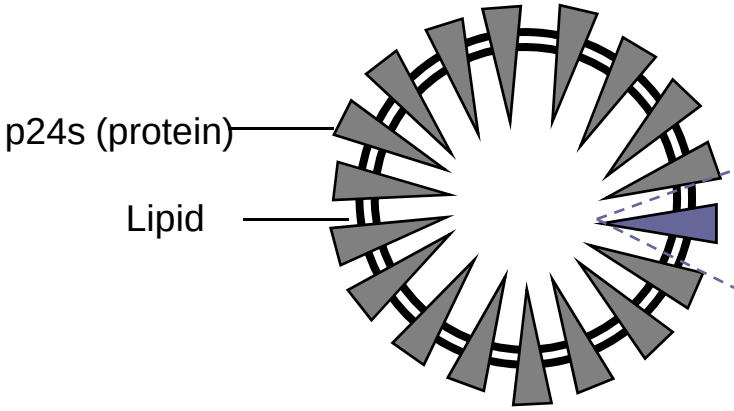
Sets the framework for final product yield & quality

Influences process stream and performance of downstream operations

## Research Material

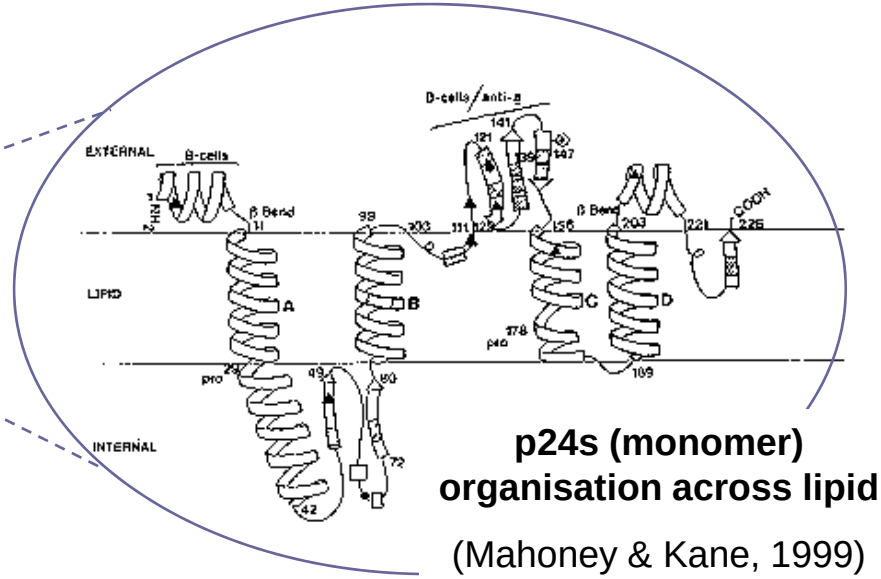
Lipid envelope VLP: Hepatitis B Surface Antigen (HBsAg)

# Hepatitis B Surface Antigen




**Structure of HBsAg**

(adapted from Koistinen, 1980)




**p24s (monomer) organisation across lipid**

(Mahoney & Kane, 1999)

After expression, VLP particles remain localized on the ER (Fu et al, 1995)

Protein transport through the secretion pathway is blocked (Herbert et al, 1956)


- Koistinen, (1980), J. Virol., 35, 1, 20-23
- Mahoney & Kane, (1999), Vaccines, 3rd ed., pp158-182
- Fu et al, (1995), Biotechnol. Bioeng., 49, 578-586
- Herbert et al, (1956), J. Gen. Microbiol., 14, 601-622

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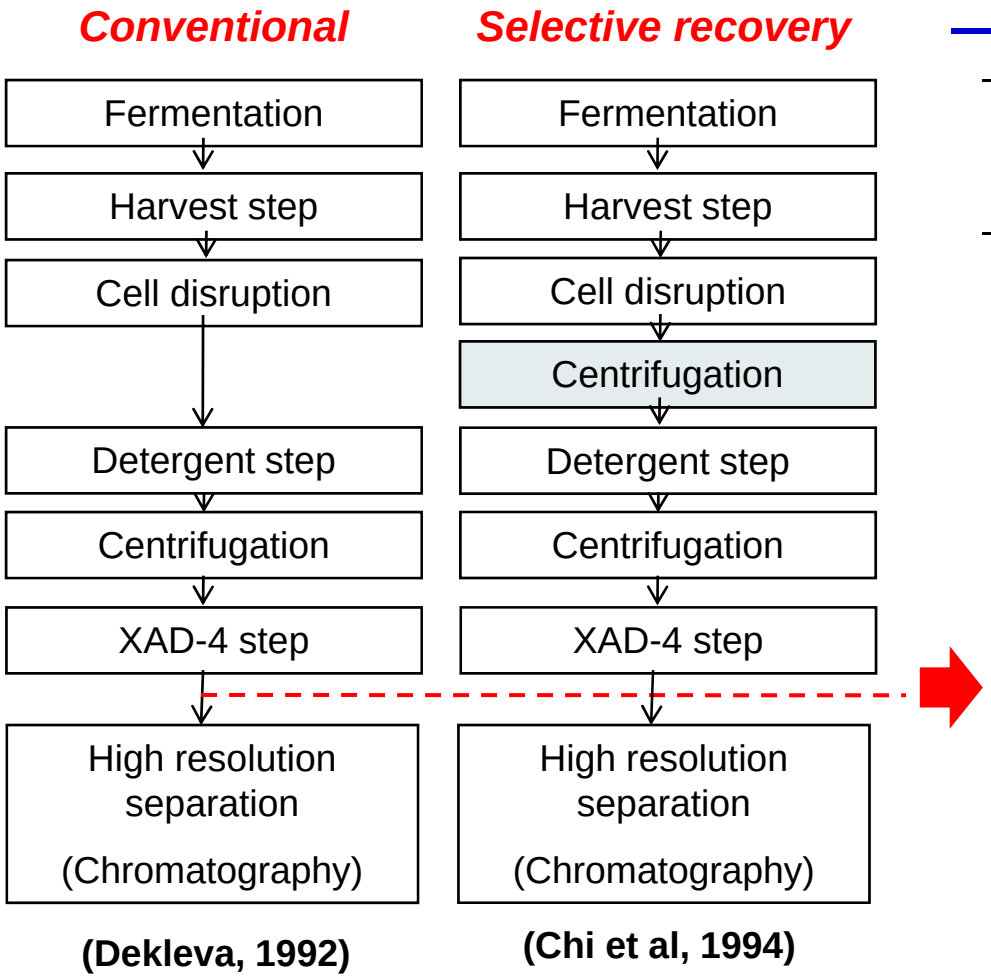
# Research Interests




**(Dekleva, 1992)** **(Chi et al, 1994)**

***HBsAg remain localized on the ER following expression***

Aim: Exploit expression characteristics to impart selectivity to product recovery

**Major contaminants**:

- Host cell proteins & lipids

**Resulting impact**:

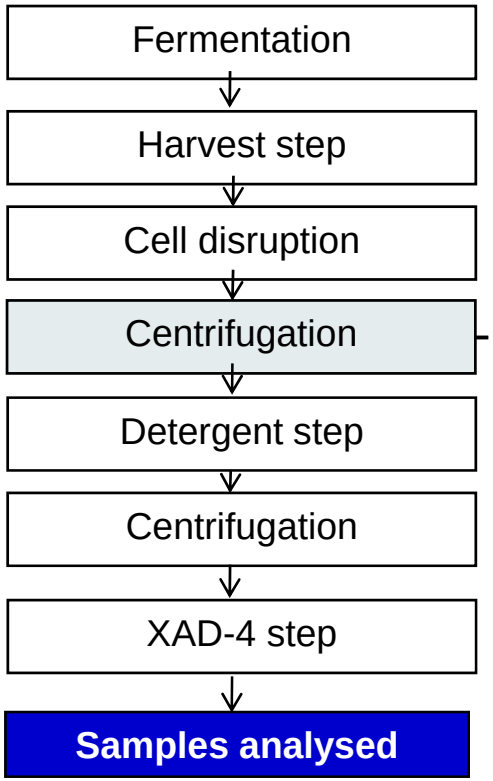
- fouling of membrane / column
- performance affected by non-specific interactions
- proteolysis effects on product

**Dekleva, M. L., (1999),** Vaccine Technology, pp2611-2622, Encyclopedia of Bioprocess Technology

**Chi et al; (1994)**, Ann NY Acad. Sci. 1994, 721(1), 365-373

# Yield & Clarification

***Potentials of selective recovery methodology***

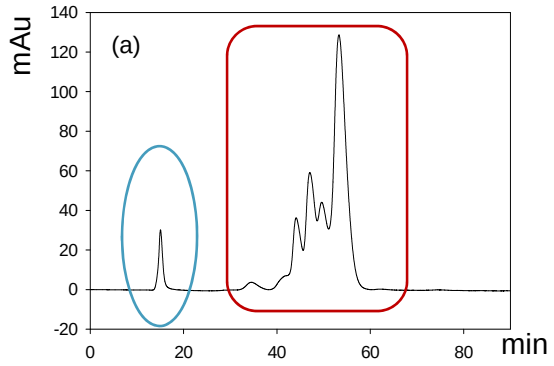



**(Chi et al, 1994)**

| | Supernatant | Solids |
|---|---|---|
| VLP | 17% | 83% |
| Protein contaminants | 91% | 9% |
| Lipid contaminants | 67% | 33% |
| | ***Waste stream*** | ***Product stream*** |

Recovery of VLP from solids fraction allows removal of bulk contaminants with minimal product loss

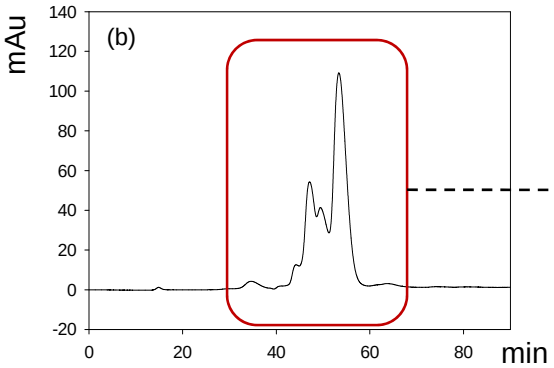
# Protein (SEC) profile




*(a) Conventional method*

Early peak - VLP product

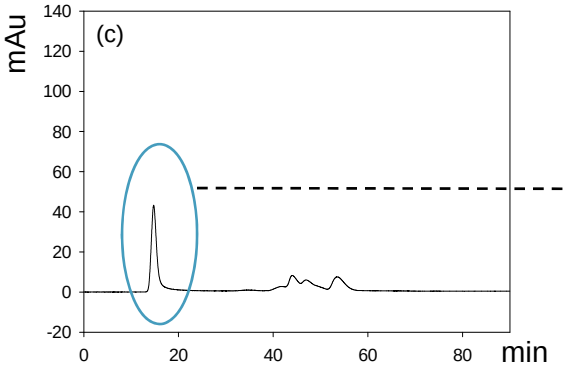
Later peaks – host protein contaminants




*(b) Selective recovery – supernatant*

host protein contaminants

***Waste stream***



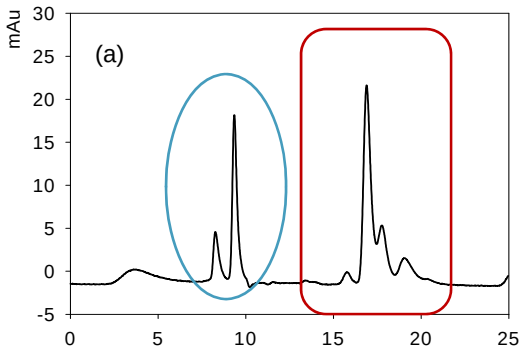

*(c) Selective recovery –solids*

VLP product

***Product stream***

Protein purification factor of > 8

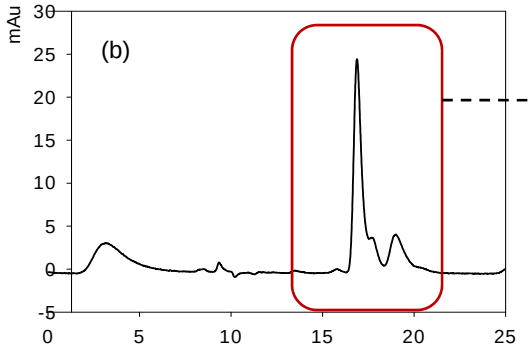
# Lipid HPLC profile




*(a) Conventional method*

Early peak - sterols

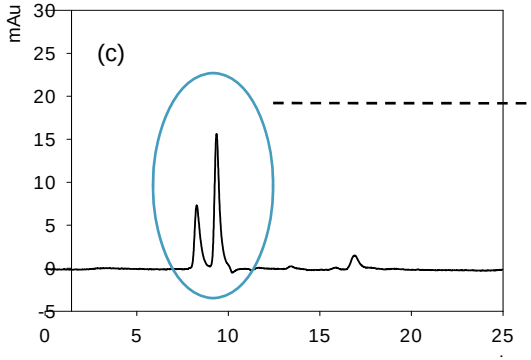
Later peaks – phospholipids




*(b) Selective recovery – supernatant*

Phospholipids (contaminants)

***Waste stream***




*(c) Selective recovery – solids*

Sterols (contaminants)

***Product stream***

Lipid purification factor of ~ 3

# Homogenisation

*"Disruption by a high pressure homogenizer about 10,000 to 20,000 psi (700 – 1400 bar) is preferred because of its rapid and efficient operation."* *(Sitrin & Kubek, US patent 669705 )*

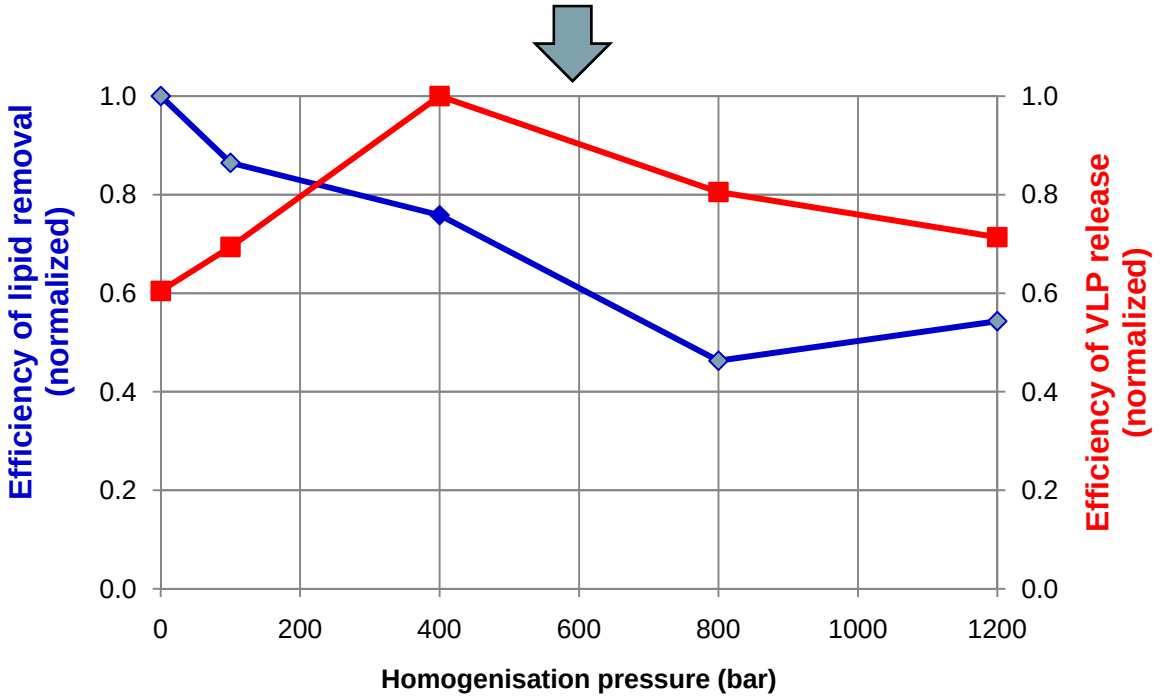
***Impact of varying homogenisation pressure conditions on:***

- *Host protein elimination – **no** significant difference*
- *Host lipid elimination & VLP release*

Analysis of material from **solids fraction** using the selective recovery methodology

NB: # passes kept constant at **4** passes

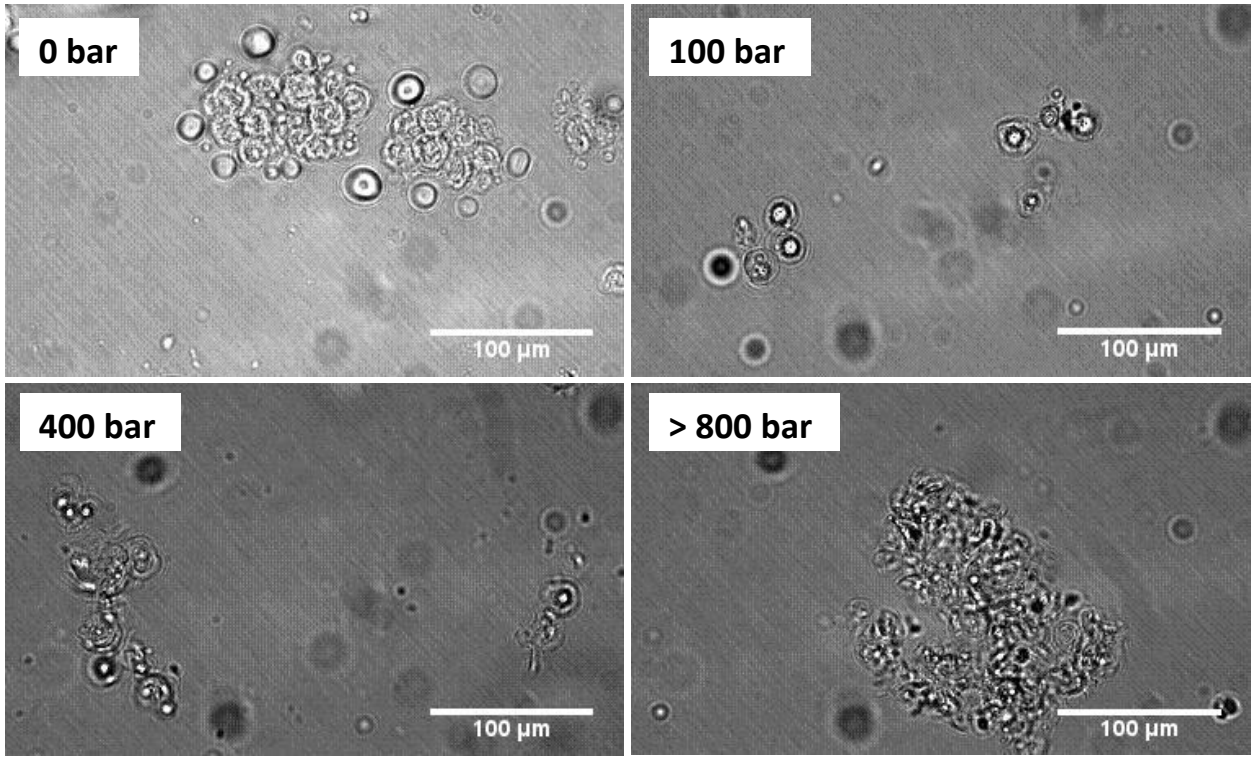
Best trade-off at 400 bar



# Homogenisation

*Microscopy analysis of homogenate under different operating pressures*

***Detergent promotes co-liberation of host cell lipids into process stream (Kee at al, 2008)***




- Greater cell disruption & fragmentation at higher discharge pressures
- Greater surface area for detergent to extract lipids from

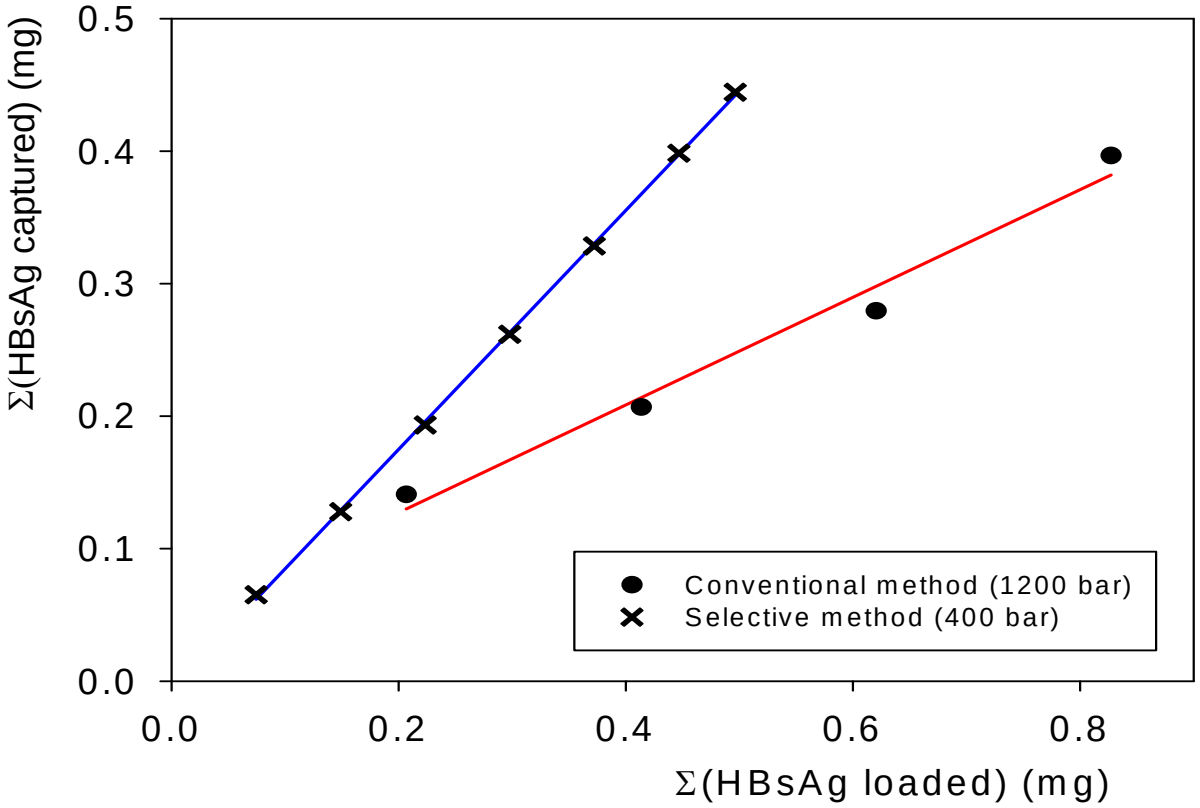
Higher levels of lipid contamination at increased homogenisation pressures



Kee et al, **(2008), Biotech. Prog.** (doi10.1021/bp070472i)

# HIC chromatography

*Evaluating impact on performance of downstream chromatography*




***HIC challenge using Butyl Sepharose (Hi-Trap)***

Higher binding capacity for VLP product for sample from selective recovery method resulted in higher step yield

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# Summary

- Selective recovery method allows the elimination of bulk contaminants originating from cell cytosol.
- Discharge pressures during homogenisation impacts VLP activity as well as the lipid level in the product stream. Best trade-off at 400 bar.

| | Conventional method (1200 bar) | Selective recovery (400 bar) |
|---|---|---|
| **VLP product** | 1 | 1.36 (+36%) |
| **Protein (contaminants)** | 1 | 0.06 (-94%) |
| **Lipid (contaminants)** | 1 | 0.22 (-78%) |

Framework for future VLP process development

# Future Work

## Product characterisation studies

- To validate product quality following selective recovery methodology

## Further homogenisation optimisation

- To study the effect of the number of passes in relation to operating pressure

## Scale up studies & process validation

- To characterise the clarification level and dewatering characteristics upon scale up for the additional centrifugation step
- To ensure that process benefits observed at lab scale are not lost

## Options for subsequent chromatographic operations

- To investigate the possibilities of reducing the number of chromatographic operations

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