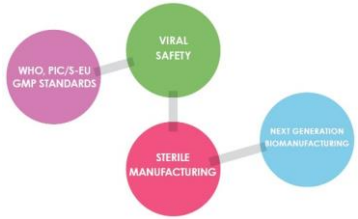
Anil Kumar Chawla, PhD, India/Switzerland

- Chairman and Managing Director of Protaccine Biotec Sarl, Switzerland

GaBI
Educational
Workshops

18 December 2019, Hotel Gran Mahakam, Jakarta, Indonesia

2nd ASEAN Educational Workshop on
GMP FOR BIOLOGICALS/BIOSIMILARS



Purification of vaccines and biologicals

Anil Kumar Chawla, PhD
18 December 2019

GaBi
GENERICS AND BIOSIMILARS INITIATIVE
Building trust in cost-effective treatments

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PURIFICATION OF VACCINES & BIOLOGICALS

DR ANIL KUMAR CHAWLA
PROTACCINE BIOTEC SWITZERLAND

PURIFICATION OF VACCINES & BIOLOGICALS

SYNOPSIS

- Manufacturing Process
- Harvest/Clarification
- Purification
- Techniques Used For Purifications
- Advances in Purification
- Critical and Major Observations- By Regulatory Agencies

SIMPLE EXAMPLE OF PRODUCTION-AGRICULTURE/FARMING





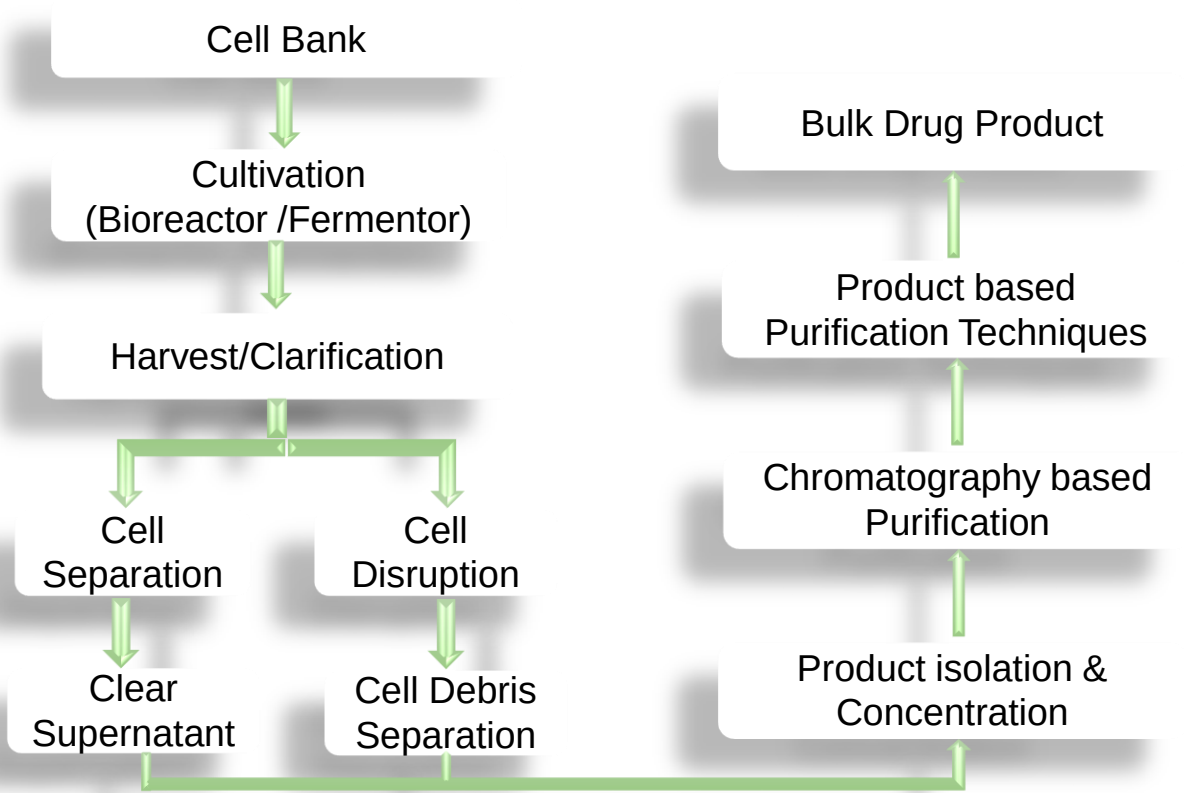

VACCINES & BIOLOGICALS

Biological products include a wide range of products such as vaccines, mono/polyclonal antibodies, gene therapy human tissue & cellular products, cytokines, growth factors, enzymes, immunomodulators blood and blood components, allergenics, somatic cells & recombinant therapeutic proteins.

Basically Biomolecules, Group of Biomolecules, Live or Inactivated viruses, Whole cells etc desirably **purified to high purity level of more than 99.9?????%**

Manufacturing Process

VACCINES & BIOLOGICALS
GENERAL MANUFACTURING PROCESS



Harvest/Clarification

HARVEST/CLARIFICATION

- Preliminary step for separation of product from a bioreactor/fermentor broth having process impurities is the first step in a downstream process.

- Primary step that can introduces a significant risk to product degradation or increase in bioburden.

HARVEST / CLARIFICATION

- Centrifugation
- Microfiltration

(Tangential Flow Filtration)

- Combination Technique (i.e. centrifugation and depth filtration)

Continuous Centrifugation

Microfiltration

Purification

PURIFICATION

Process to remove all possible impurities to acceptable level to avoid any adverse event.

- Intracellular products are difficult to purify

  Required cell disruption, removal of cell debris, DNA, RNA, lipid etc…

- Extracellular products are easier to purify
  - No cell disruption and low level of intracellular impurities

TECHNIQUES USED FOR PURIFICATIONS

TECHNIQUES USED FOR PURIFICATIONS

- Cell Disruption for intracellular products
- Precipitation
- Adsorption
- Membrane Separation
- Chromatography

Cell Disruption

CELL DISRUPTION

Disruption: Cell disruption is a method or process for releasing biological molecules from inside a cell.

- **Ultrasonication** (sonicators) can be applied at lab scale for disruption of Bacteria & Viruses
- **Mechanical oscillation**: by a titanium probe immersed in a cell disrupter equipment.

This method is usually used in combination with a chemical method.

CELL DISRUPTION CONTI..

Milling:

Continuous operation at low

temperature.

The System with mechanical agitator & beads in horizontal grinding container for dispersion and wet grinding in a completely enclosed system. Generally used for

- Bacteria and fungi
- At Industrial, Pilot & Lab scale

CELL DISRUPTION CONTI..

Other Method

- Drying
- Heat or Osmotic Shock
- Freeze Thaw
- Organic Solvent
- Chaotropic agents
- Enzymes
- Surfactant

CELL DISRUPTION CONTI..

Homogenization

Process whereby a biological sample is brought to a state such that all fractions of the sample are equal in composition.

CELL DISRUPTION CONTI..

Issues/Challenges of Disruption :

Damage to the product

- Denaturation
- Oxidation
- Unwanted intracellular byproducts

SEPARATION OF SOLUBLE PRODUCTS

SEPARATION OF SOLUBLE PRODUCTS

- Precipitation
- Adsorption or Absorption Techniques
- Membrane Purification Technologies
- Chromatography

Precipitation

SEPARATION OF SOLUBLE PRODUCTS

Precipitation

Concentration of proteins of biological products in order to and purify them from various contaminants present in broth by precipitation.

SEPARATION OF SOLUBLE PRODUCTS CONTI..

Precipitation Methods:

- **Salting-out;** by adding inorganic salts such as ammonium sulfate, or sodium sulfate to increase high ionic strength.
- **Isoelectric precipitation;** The isoelectric point (pI) is the pH of a solution at which the net primary charge of a protein becomes zero. The pI of most proteins is in the pH range of 4–6.
- **Precipitation with miscible solvents;** Addition of miscible solvents such as ethanol or methanol to a solution may cause proteins or polysaccharides in the solution to precipitate.
- **Non-ionic hydrophilic polymers;** Polymers, such as dextrans and polyethylene glycols, are frequently used to precipitate proteins
- Temperature based precipitation

Adsorption

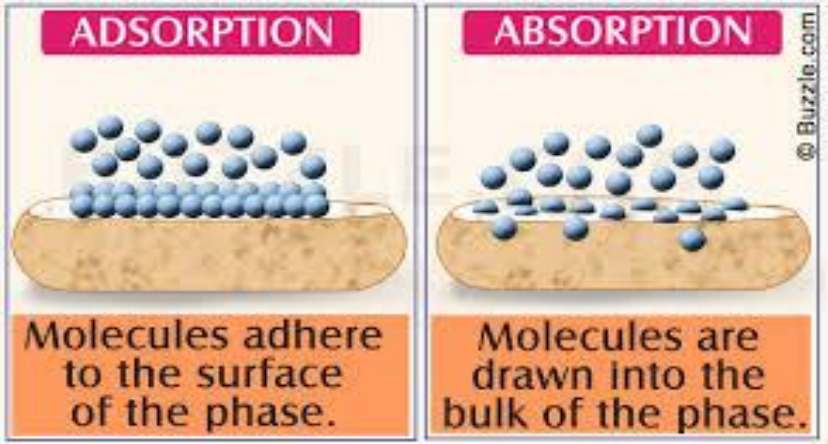
SEPARATION OF SOLUBLE PRODUCTS CONTI..

Adsorption

Adsorption & desorption is simple, low-cost and scalable unit operations. Its a traditional protein separation approaches with low protein purity. e. g. Silica for rHBsAg

Purification of the molecule is depends upon

- ✓Functional groups
- ✓Surface properties
- ✓pH
- ✓Ionic strength
- ✓Temperature



Membrane Separation

SEPARATION OF SOLUBLE PRODUCTS CONTI..

Concentration & Diafiltration by Tangential Flow Filtration: Pressure driven separation process that uses membranes to separate components in a liquid solution or suspension based on their size.

Technique	Microfiltration	Virus Filtration	Ultra Filtration	Nano Filtration
Approximate membrane cut of range	0.05 µm – 1 µm	100 kD – 0.05 µm	1 kD – 1000 kD	<1 kD

Chromatography

SEPARATION OF SOLUBLE PRODUCTS CONTI..

Chromatography

Chromatography is a technique used for separation of a mixture. The mixture is dissolved, called *mobile phase,* which carries it through another material called the *stationary phase.*

SEPARATION OF SOLUBLE PRODUCTS CONTI..

- Ion exchange chromatography (Anion vs. Cation) - Based on Charge
- Affinity chromatography - Specific binding
- Hydrophobic interaction chromatography - Hydrophobic/hydrophilic characteristics
- Gel filtration (Size exclusion chromatography) - based on Molecular Weight

SEPARATION OF SOLUBLE PRODUCTS CONTI..

- Column Chromatography is the most common approach to purifying larger amounts of proteins
- It achieves highest level of purity and used at industrial scale.
- It can be operated at atmospheric pressure (gravity out) or at high pressure

Advances In Purification

ADVANCES IN PURIFICATION

Biological industry is challenged to develop safe & quality products at decreased cost with shortest possible time results in new development strategies and techniques.

High Throughput Technology (HTT) to Accelerate Biological Purification Process Development

In HTT a large number of experimental conditions can be evaluated simultaneously in small space with screening of chromatography condition and software assisted data evaluation.

ADVANCES IN PURIFICATION conti..

- Single-use filters
- Automated buffer dilution systems
- Single-use tangential flow filtration
- Single Use Chromatography Systems
- Enhanced capacity adsorption systems

ADVANCES IN PURIFICATION conti..

- High-capacity chromatography resins with short residence time
- Continuous Chromatography System
- Simulated moving bed chromatography systems
- Cast-in-place "monolithic" chromatography media
- Improved Analytical techniques

CRITICAL AND MAJOR OBSERVATIONS-

MATURE REGULATORY AGENCIES

CRITICAL AND MAJOR OBSERVATIONS

- Storage condition of cassettes, buffers and mediums
- Bioburden analysis
- CIP Validation ; product testing
- Trend and analysis of data along with reference to previous year and batches used in clinical trial and initial batches
- Leachable and Extractable

Protaccine Biotec

Thank You