

# Downstream Processing and Clearance of Impurities for Bio-pharmaceuticals

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# Outline Presentation

- ◆ Products
- ◆ Synthesis of the products
- ◆ Specifications, identity, purity, and biological activity
- ◆ Stability
- ◆ Impurities
- ◆ Production process, removal impurities, validation process
- ◆ Conclusions



# Bio-pharmaceutical Products represent a diverse group of Products that includes:

- ◆ (glyco)proteins
- ◆ peptides
- ◆ nucleic acids
- ◆ whole cells
- ◆ viral particles
- ◆ vaccines



# Some of these Bio-pharmaceutical Products

belong to the group of  
well-characterised biologicals.

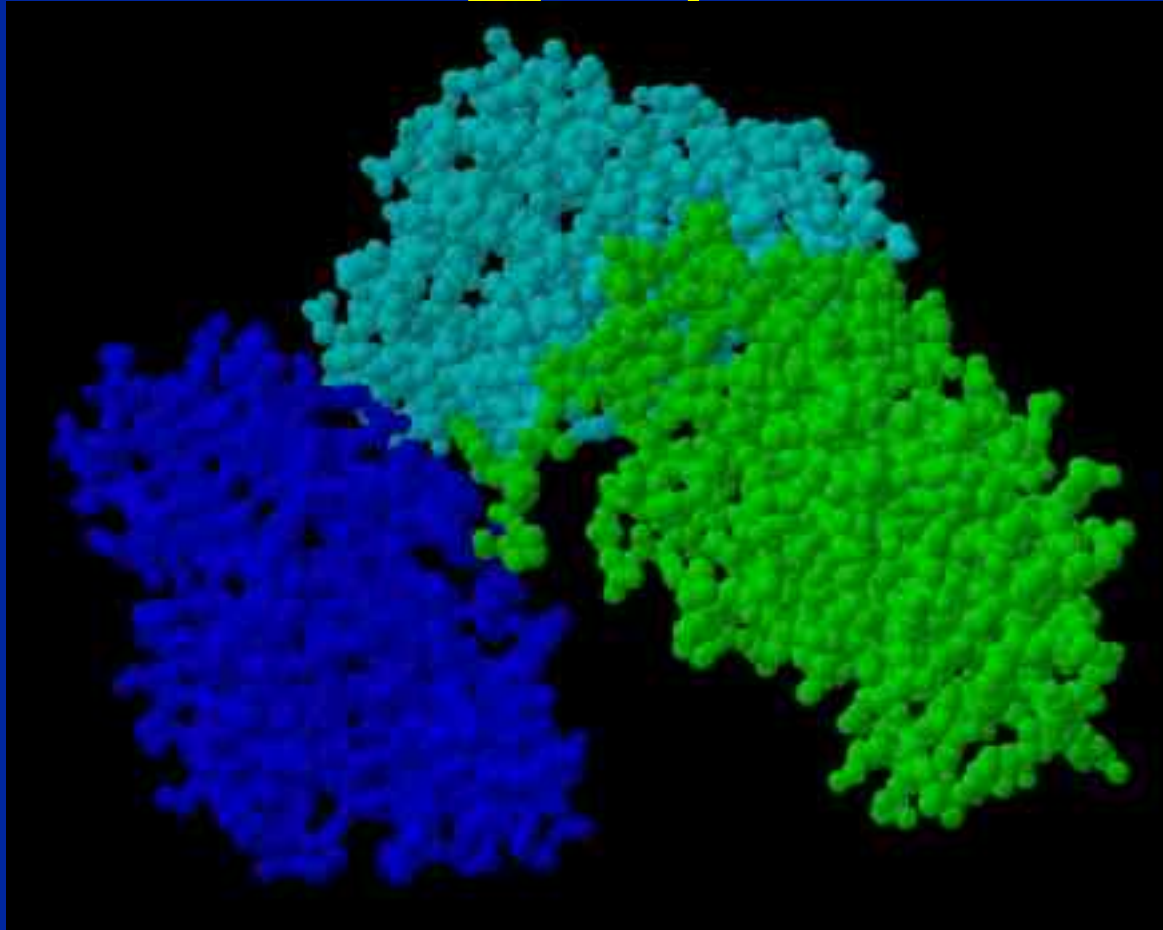
The major characteristics of this group are:

Proteins whose identity, purity,  
impurities, potency, and quantity  
can be determined.

*This presentation deals with  
this group of biologicals*



# 3-D structure of Diphtheria



# First well-defined Bio-pharmaceutical product:

- ◆ *Insulin* approved by FDA in 1982
  - ◆ Insulin is applied for the treatment of insulin-dependent diabetes mellitus
- ◆ r-DNA technology was applied
- ◆ Insulin is produced in *E.coli*
  - ◆ Awareness of impurities from the producer cells (ao LPS and host cell proteins)



# Outline of a Production Process of a Bio-pharmaceutical Product

Upstream part (cultivation steps)

Downstream part (Purification steps)

Active Pharmaceutical Ingredient (API)

Formulation

Final Product



# Post-translational Modification after Biosynthesis

- ◆ Acylation of the N-terminus
- ◆ Phosphorylation of serine, threonine and tyrosine
- ◆ Oxidation of methionine
- ◆ *Glycosylation of serine and asparagine*
- ◆ Cleavage of N- and/or C-terminal residues by proteolytic enzymes



# Specifications are the Crucial Characteristic of Well-Defined Biologicals

- ◆ Production process
- ◆ Stability profile
- ◆ Qualities of materials used in preclinical and clinical trials
- ◆ Statistical validity of data analysis

From: Murano (1997)



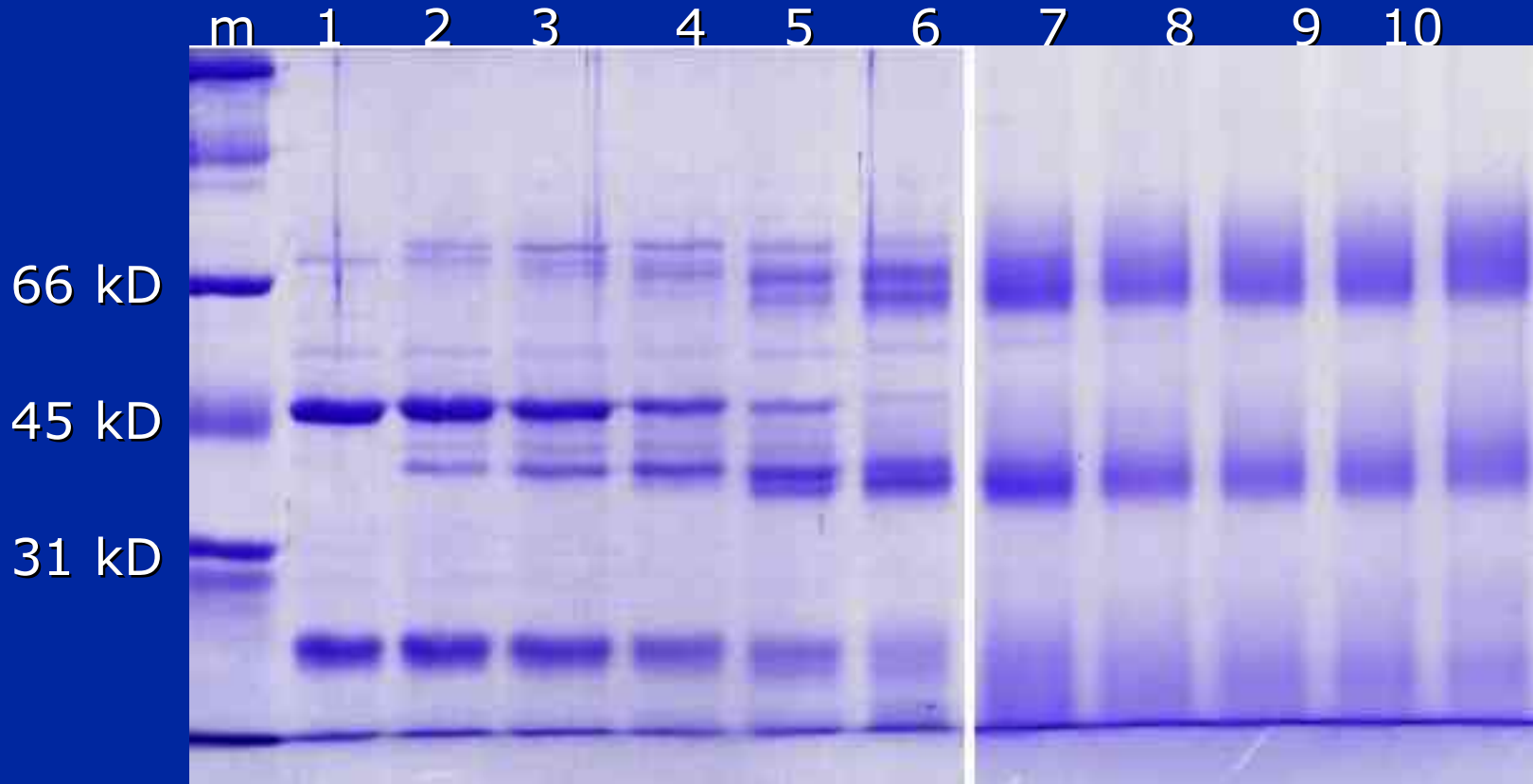
# Product Characterisation

- ◆ Physical properties (MW, OD, IEP)
- ◆ Composition/structure (amino acid composition and sequence; glycosylation, disulphide bridges)
- ◆ Subunit structure (SDS-PAGE, under reducing and non-reducing conditions)
- ◆ 3-D structure (UV, fluorescence, CD, infrared, NMR and crystal)
- ◆ Identify indicating patterns (peptide map, sugar map, chromatography profiles, IEF, SDS-PAGE, CE, MS, NMR and SPR)

Adapted from: Murano (1997)



# Treatment of Diphtheria Toxin with Formaldehyde (SDS-PAGE)



0-128  
mM

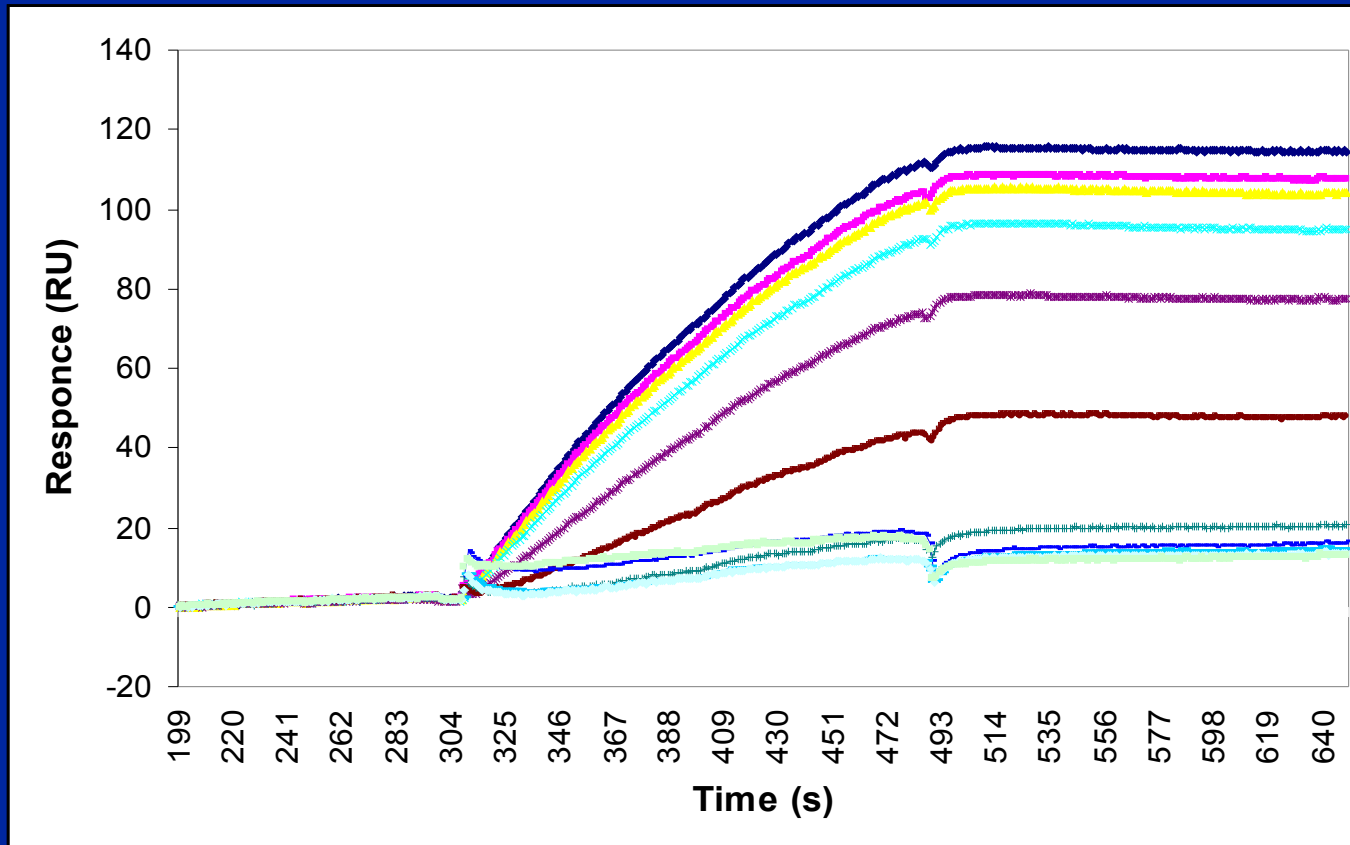


# Major characteristics of SPR- technology

- ◆ Real time binding!
- ◆ Identification of API and impurities
- ◆ Quantification
- ◆ Affinity (as an alternative for the bioassay)
- ◆ Structure/function relations
- ◆ Binding site analyses (e.g. epitope mapping for vaccines)



# Treatment of Diphtheria Toxin with formaldehyde (SPR)



# Identity in relation with API and Final Product

- ◆ Tests uniquely identify molecular structure
  - ◆ Qualitative measure
  - ◆ Favours specificity over sensitivity
  - ◆ Not complete molecular characterization
- ◆ For bulk product
  - ◆ Performed at appropriate points in manufacture
  - ◆ may differ depending on stage
  - ◆ Complete id on all lots
- ◆ For final product
  - ◆ Specific/adequately id as designed on label
  - ◆ Distinguish from other products from the same facility

Adapted from Murano (1997)



## Stability of a bio-pharmaceutical product is defined to be:

“.... The capacity... to remain within specifications established to ensure its identity, strength, quality, and purity.”

Stability is interpreted as the length of time under specific conditions of storage that the product will remain within predefined limits for all important characteristics.



# Purity

- ◆ Determined by two methods defined by principles of separation
- ◆ One should be quantitative
- ◆ Specific limits set for each method based on
  - ◆ Analytical data from clinical trial lots
  - ◆ Manufacturing capabilities
  - ◆ Method variability
  - ◆ Stability experience
- ◆ Explanation of calculations

Adapted from Murano (1997)



# Chemical degradation of proteins

- ◆ Disulphide bond interchange (may lead to aggregation)
- ◆ Deamidation (hydrolysis of amide of glutamin and asparagin)
- ◆ Oxidation of the side chains of tryptophan, methionine and cysteine
- ◆ Degradation side chain of tryptophan by light
- ◆ Cleavage of lys-thr bonds by copper ions
- ◆ Cleavage of asp-pro and of asp-tyr bonds at low pH



# Impurities (product related)

- ◆ Efforts to identify and investigate toxicity of all detectable impurities
- ◆ Use of standards for quantification
- ◆ Lot release (determination of purity and impurities)
  - ◆ incomplete post-translation modification
  - ◆ degradation products based upon stability studies



# Impurities (process related)

- ◆ Starting materials (including medium components, viruses and BSE)
- ◆ Host cell proteins
- ◆ DNA



# Biological activity

## Bioassay

- ◆ Assures the expected pharmacological/biological response
- ◆ Surrogate for 3-D structure

## Methodologies

- ◆ Bio(immuno)chemical assay (e.g. SPR)
- ◆ cell-line based assay
- ◆ animal assay

*If assay variability exceeds lot-to-lot variation,  
quantify based upon mass*



# Steps in Manufacture: Cultivation

Major activity	Goal
<ul style="list-style-type: none"><li>◆ Proliferation</li><li>◆ Maintenance of cells</li></ul>	<ul style="list-style-type: none"><li>◆ Formation of the active component</li><li>◆ Prevention of lysis of the cells</li></ul>



# Steps in Manufacture:

## Cultivation - Critical Issues (i)

- ◆ Use of a well-characterised cell substrate (free from adventitious agents, fi viruses)
- ◆ Use of defined media free from adventitious agents (fi viruses) or to use vegetable derived component(s)
- ◆ Proper control of culture conditions in order to obtain a reproducible post-translational modification
- ◆ Secretion of the active component in the medium
- ◆ Minimal release of DNA and (host) cell proteins



# Steps in Manufacture:

## Cultivation - Critical Issues (ii)

- ◆ Use of a well-characterised cell substrate (free from adventitious agents (fi viruses))
- ◆ Use of ill-defined media containing serum and/other proteins; risk introduction adventitious agents (fi viruses and BSE); introduction of impurities
- ◆ Proper control of culture conditions in order to obtain a reproducible post-translational modification
- ◆ Secretion of the active component in the medium
- ◆ Minimal release of DNA and (host) cell proteins



# Steps in Manufacture: Harvest

Major activity	Goal
◆ Removal of particles and cell debris	◆ DNA ◆ Lytic enzymes



# Steps in Manufacture: Harvest- Critical Issues

- ◆ In case active components is secreted:  
prevention of lysis
- ◆ Lysis results in release of host cell proteins,  
proteases and glycosidases



# Steps in Manufacture: Purification A

<b>Major activity</b>	<b>Goal</b>
◆ Product separation from proteins with chemistry similar to product	◆ Removal non-active product related proteins



# Steps in Manufacture:

## Purification A - Critical Issues

- ◆ Knowledge of inadequate post-translational modification as result of the cultivation conditions
- ◆ Stability active component during processing
- ◆ Knowledge of cell substrate (eg formation of cytokines by hybridoma cells, urokinase by CHO cells)
- ◆ Knowledge of starting materials (presence of IgG in albumin)
- ◆ Analytical capabilities



# Steps in Manufacture: Purification B

Major activity	Goal
<ul style="list-style-type: none"><li>◆ Product separation from process related impurities</li></ul>	<p>Biological (risk) factors:</p> <ul style="list-style-type: none"><li>◆ Viruses</li><li>◆ LPS</li><li>◆ DNA</li><li>◆ Host cell proteins</li></ul>



# Steps in Manufacture:

## Purification B - Critical Issues

- ◆ Knowledge of process related impurities
- ◆ Analytical capabilities (host cell proteins, DNA, LPS)
- ◆ Validation the removal of potential contaminating viruses



# Steps in Manufacture: Formulation

## Major activity

- ◆ Avoidance of hazardous milieu, addition of additives for protection

## Goal

- ◆ Stability
- ◆ Compatability for use in patients



## Steps in Manufacture:

### Formulation - Preventive measures to keep the integrity of the active component

- ◆ Aggregation: Addition of surfactants and other excipients
- ◆ Deamidation: pH 3-5
- ◆ Oxidation: pH <7
- ◆ Oxidation of met: protect from oxygen
- ◆ Photodecomposition: protect from light
- ◆ Copper induced cleavage: chelating agents
- ◆ Hydrolysis of peptide bonds: pH >7



# Steps in Manufacture:

## Formulation - Excipients to keep the integrity of the active component

- ◆ Amino acids (e.g. glycine)
- ◆ Sugars
- ◆ Surfactants
- ◆ Salts
- ◆ Polyols (e.g. cyclodextrin)
- ◆ Antioxidants (e.g. ascorbic acid)
- ◆ Polymers (e.g. PEG)
- ◆ Chelating agents (e.g. EDTA)



# Process validation and controls (1)

- ◆ Process validation and controls constitute key elements of the overall strategy in assuring product quality.
- ◆ Documented evidence proves that the process is effective and reproducible and yields a product that consistently meets pre-determined quality specifications and attributes.

Adapted from Murano (1997)



# Process validation and controls (2)

- ◆ Purity of the active component is reproducible
- ◆ Level of impurities is reproducible

*If the formation of aggregates or the occurrence of protein fragments is found inconsistently it is viewed as a lack of control of the process*



# Conclusions

- ◆ Well-defined bio-pharmaceuticals need a well-defined process and analytical capabilities
- ◆ Sound science is needed to realise this approach



# Acknowledgement:

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