From synthesis to bio-transformation: control and management of the quality of APIs

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Qualità di un principio attivo

Nuovi farmaci:

EMEA (ICH Guide line)

Farmaci Generici:

ICH Guide line

Farmacopea

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

IMPURITIES IN NEW DRUG SUBSTANCES
Q3A(R2)

Current *Step 4* version

dated 25 October 2006

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

2. CLASSIFICATION OF IMPURITIES

Impurities can be classified into the following categories:

- Organic impurities (process- and drug-related)
- Inorganic impurities
- Residual solvents

Organic impurities can arise during the manufacturing process and/or storage of the new drug substance. They can be identified or unidentified, volatile or non-volatile, and include:

- Starting materials
- By-products
- Intermediates
- Degradation products
- Reagents, ligands and catalysts

Inorganic impurities can result from the manufacturing process. They are normally known and identified and include:

- Reagents, ligands and catalysts
- Heavy metals or other residual metals
- Inorganic salts
- Other materials (e.g., filter aids, charcoal)

Relevance of the impurity content in APIs

Thresholds

Maximum Daily Dose[1]	Reporting Threshold[2,3]	Identification Threshold[3]	Qualification Threshold[3]
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

Isolation could be impossible:
levels should be reduced by improving purification processes

Identification by:
GC or HPLC-MS analysis (hypothesis of the possible structure/s)
Chemical synthesis (structure correlation)

General monograph “Substances for pharmaceutical use” (2034)

- To be applied to “all” substances for pharma use
- Describes the general requirements with regards quality and purity
- In particular when a specific monograph is not in compliance with its principles
- Most sections are applicable to fermentation products and products obtained by bio-processes

General monograph 2034 (cont)

- the Related substances Section does not apply to fermentation products, but gives principles to be applied, namely:
  - Qualify and specify main impurities
  - As much as possible identify impurities and specify them
  - Set a limit of « other impurities »

General monograph (2034)

Related substances. Unless otherwise prescribed or justified and authorised, organic impurities in active substances are to be reported, identified wherever possible, and qualified as indicated in Table 2034.-1.

Table 2034.-1. – *Reporting, identification and qualification of organic impurities in active substances*

Use	Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
Human use or human and veterinary use	≤ 2 g/day	> 0.05 per cent	> 0.10 per cent or a daily intake of > 1.0 mg (whichever is the lower)	> 0.15 per cent or a daily intake of > 1.0 mg (whichever is the lower)
Human use or human and veterinary use	> 2 g/day	> 0.03 per cent	> 0.05 per cent	> 0.05 per cent
Veterinary use only	Not applicable	> 0.1 per cent	> 0.2 per cent	> 0.5 per cent

Specific thresholds may be applied for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects.

If the individual monograph does not provide suitable control for a new impurity, a suitable test for control must be developed and included in the specification for the substance.

The requirements above do not apply to biological and biotechnological products, peptides, oligonucleotides, radiopharmaceuticals, products of fermentation and semi-synthetic products derived therefrom, to crude products of animal or plant origin or herbal products.

Peptides

- Generally used at low daily dose
- Many potential related substances: isomers, results of failures in sequences, cleavages or coupling
- Requirements for related substances included in the general monograph 2034 from 1/07/2009:

Table 2034.-2. – *Reporting, identification and qualification of organic impurities in peptides obtained by chemical synthesis*

Reporting threshold	Identification threshold	Qualification threshold
> 0.1 per cent	> 0.5 per cent	> 1.0 per cent

Qualification of Impurities

The level of any **impurity present in a new drug substance** that has been adequately tested in safety and/or clinical studies would be considered qualified.

Impurities in known active ingredient can be qualified comparing the impurity profile with existing commercial products

New impurity in known active ingredient (as reported in Eur. Ph) should be adequately tested in safety and/or clinical studies for qualification.

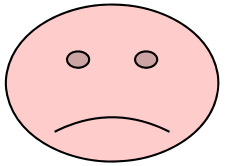
- Genotoxicity studies (point mutation, chromosomal aberration)
- General toxicity studies (one species, usually 14 to 90 days)
- Other specific toxicity endpoints, as appropriate

Time consuming
Delayed registration and commercialization of the product

PROBLEMS OF THE CHEMICAL SYNTHESIS

- Use of toxic solvents and reagents
- Time-consuming multi-step procedures
- Formation of by-products
- residual solvents

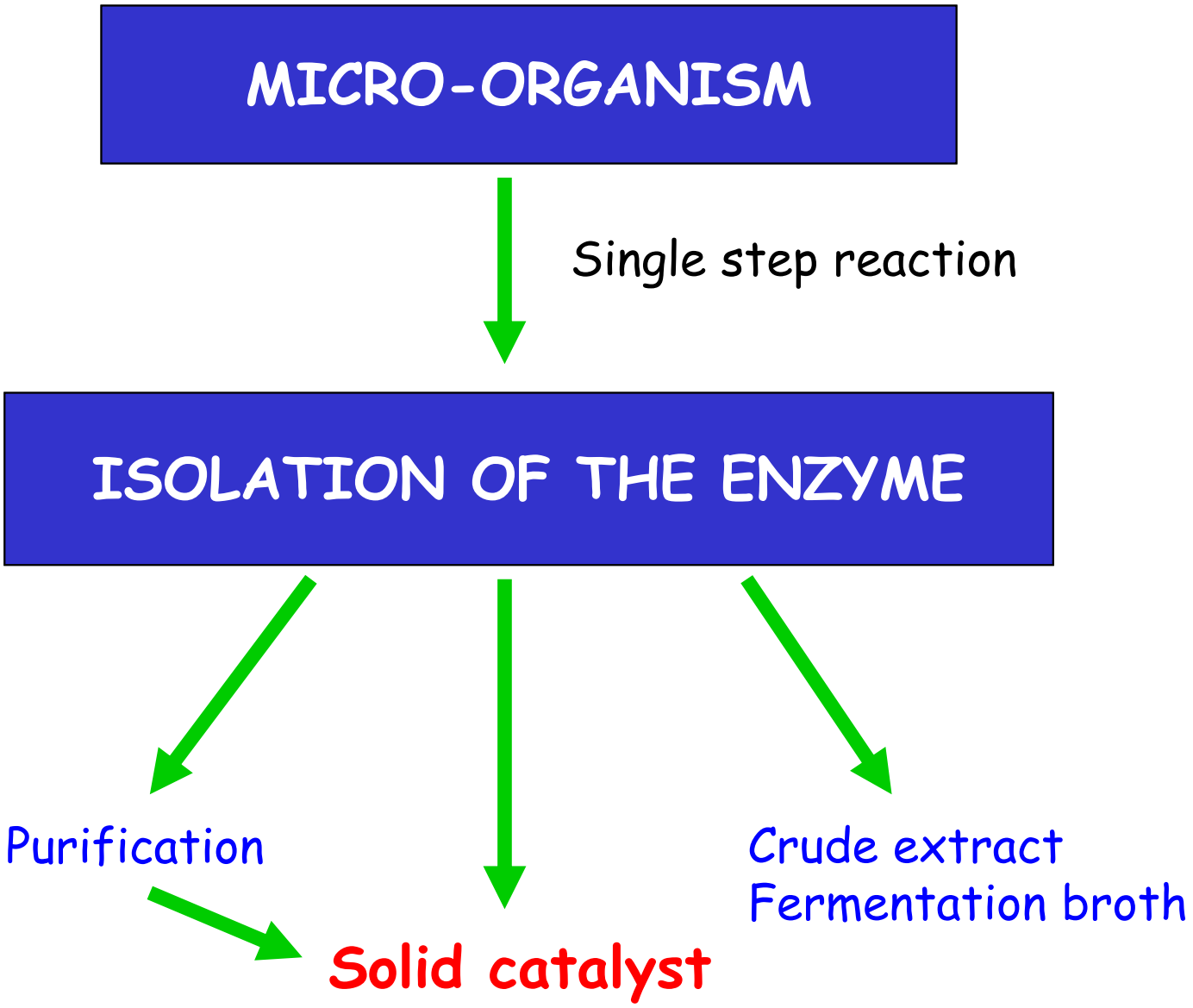
} polluting waste and sometimes low yields



Use of enzymes in the synthesis of active ingredients

i. Reaction in aqueous medium (low environmental impact);
ii. Use of no toxic and no pollutants reagents;
iii. Soft reaction conditions;
iv. Avoiding of protection and deprotection steps;
v. Reduction of the purification steps;
vi. High purity of the final product.

- Lipases
- Esterases
- Amidases
- Glycosidases
- Reductases
- Oxidases
- Transferases



ENZYME PREPARATION

Free soluble enzymes

Insoluble preparations of pure or almost pure enzymes

Enzymes immobilized on solid supports

Isolated Enzymes

- **Fermentation Broth**
- **Crude extract**

Mixture of several proteins

Fermentation-like

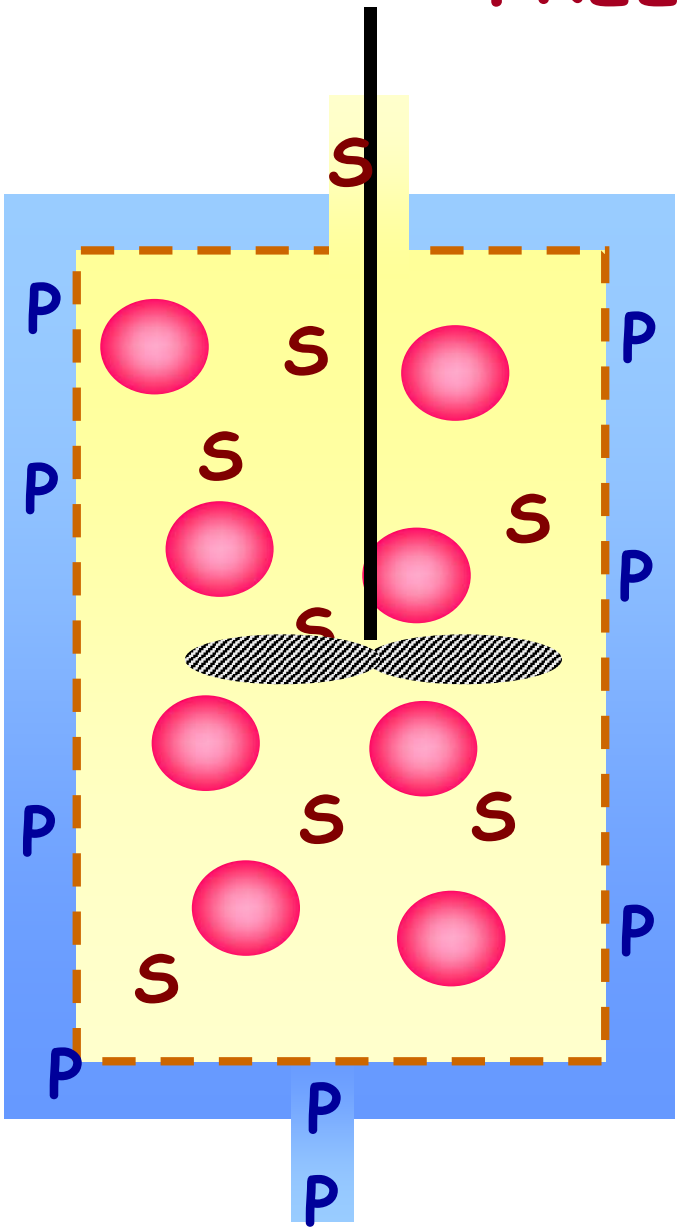
- Residuals arising from micro-organism
- Complex downstream

- **Purified Enzymes**
- **Solid or Immobilised Enzymes**

Potential Residual Proteins

No use of animal origins raw material in the production of the enzyme

FREE SOLUBLE ENZYMES



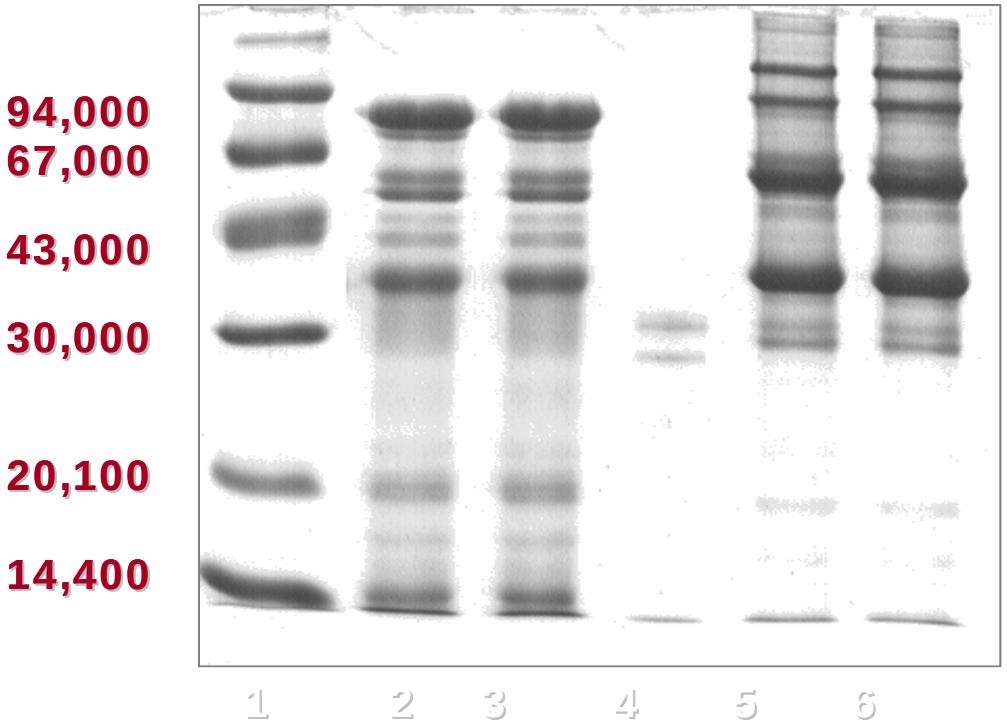
REACTORS WITH ULTRAFILTRATION

- SOLUBLE ENZYMES CAN BE RE-USED
- ALL INACTIVATION MECHANISMS ARE POSSIBLE:

  aggregation
  proteolysis
  interaction with interfaces

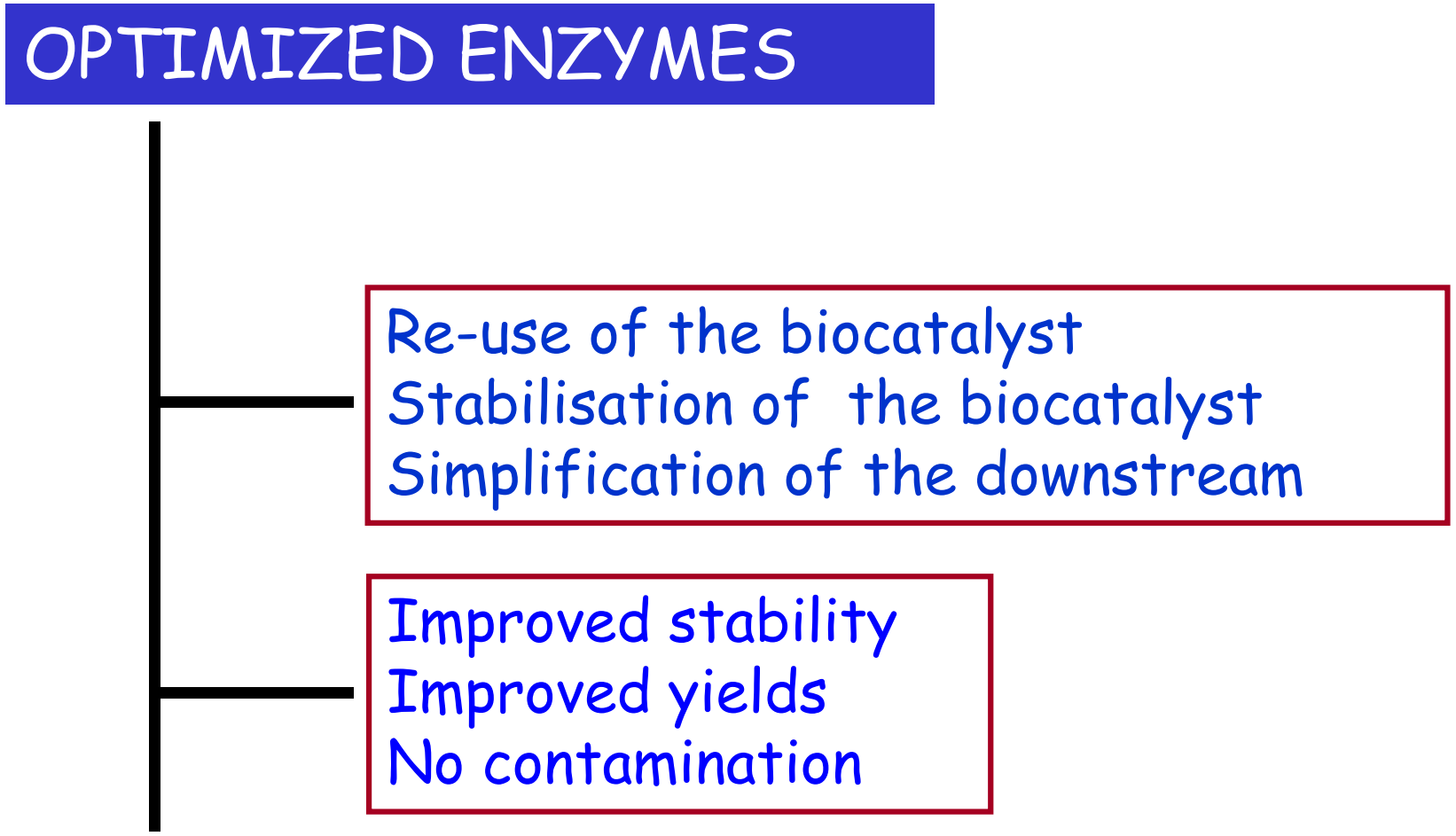
FREE ENZYME ARE OFTEN COMPLEX MIXTURES

SDS-PAGE RML



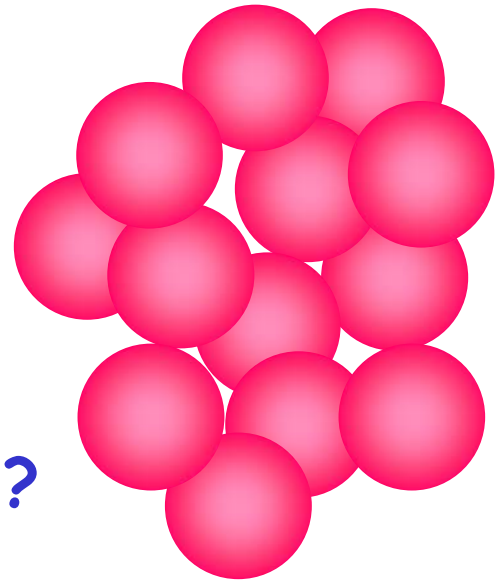
- Contamination by different proteins
- Side reactions catalyzed by different enzymes
- Other contaminants contained in the crude extract

OPTIMIZATION OF THE BIO-CATALYST: SOLID AND INSOLUBLE ENZYMES

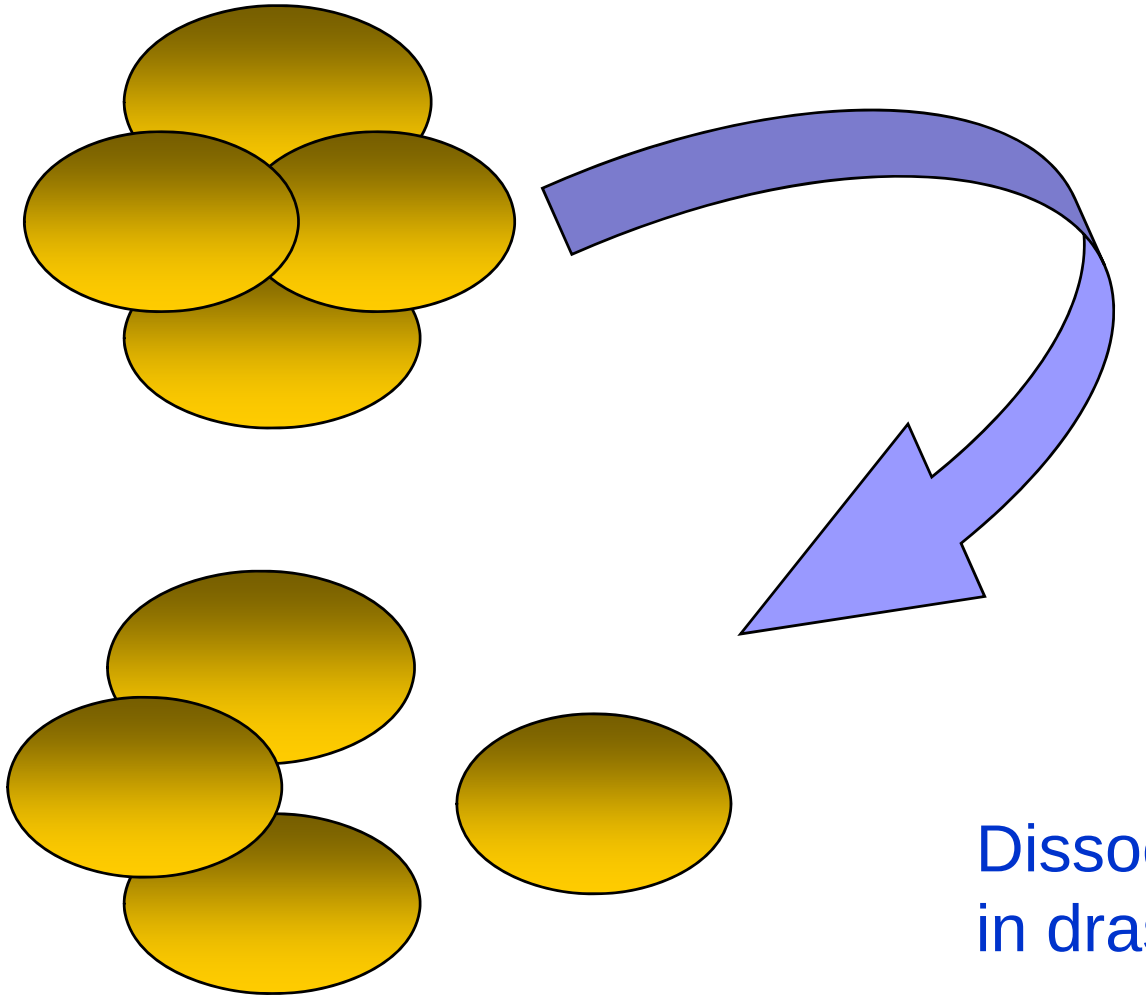


ENZYME DERIVATIVES WITHOUT SUPPORT

- All the solid is enzyme
- No cost of the support
- mechanical stability
- Rigidificaction of the enzyme ?
- Micro-environment ?



Stabilization of Multimeric Enzymes



High pH
High temperature

Problem

Dissociation of the subunits
in drastic reaction conditions

POSSIBILITIES

Crosslinked Enzyme Crystals

CLECs

Pure crystalline enzyme:

requirement of extreme pure enzyme

Stabilization of Multimeric enzymes

Crosslinked Enzyme Aggregates

CLEAs

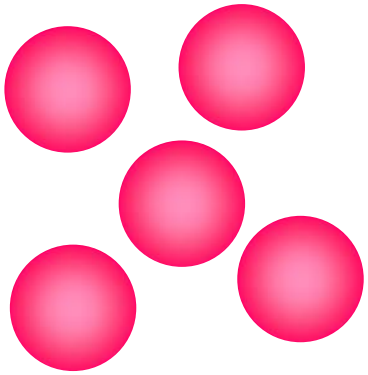
Crude enzyme:

Co-aggregation of enzyms

Aggregation of enzyme and polymer

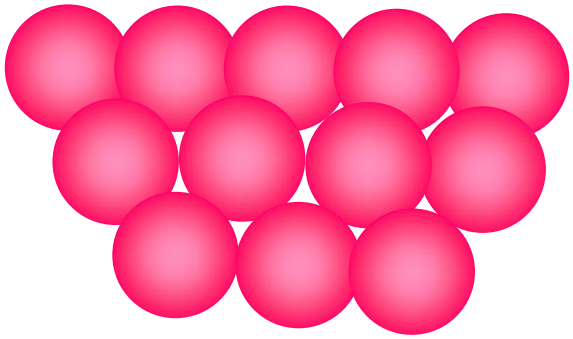
Stabilization of multimeric enzymes

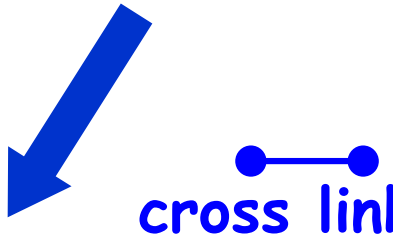
Preparation of CLEAs



+

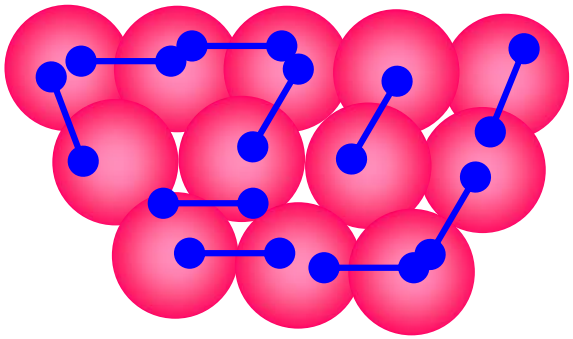
Floculating agent:
PEG
Salts, solvents





cross linking agent

CLEAs

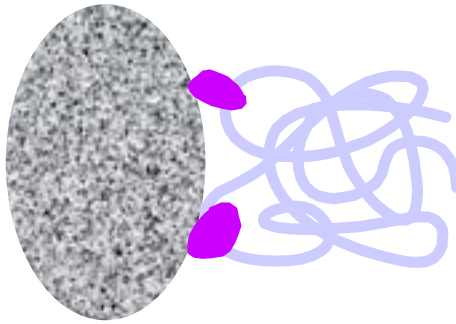
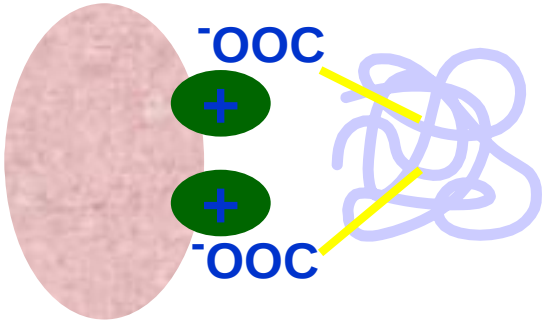
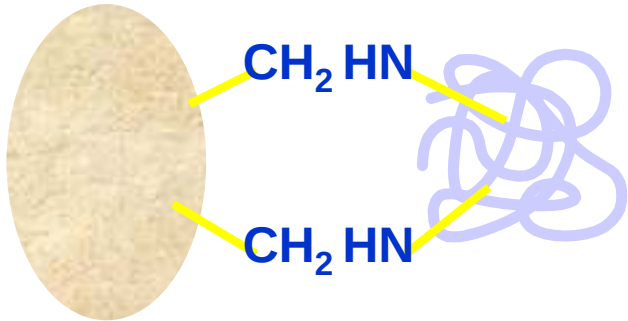


Cao L. et al; Org. Lett (2000) 2: 1361-64

IMMOBILIZATION OF ENZYMES ON PREEXISTING SUPPORTS

- covalent multi-point attachment
- reversible immobilization by adsorption

- cost of the support
- residues after inactivation of the catalyst (covalent derivatives)
- possible release of protein in the reaction medium

Extreme temperature and presence of co-solvents	**HYDROPHOBIC ADSORPTION**	
Extreme temperature and pH	**IONIC ADSORPTION**	
No release; Special case: multimeric enzymes	**COVALENT ATTACHMENT**	

Optimisation of the catalyst

Design of the enzyme derivative

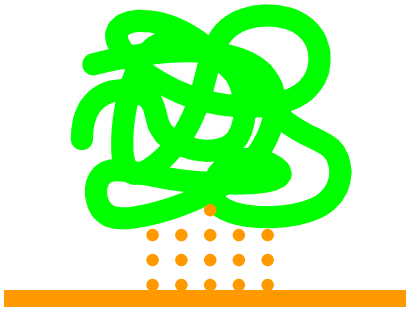
- Stability
- Catalytic properties

Avoid the Release of protein in the reaction medium

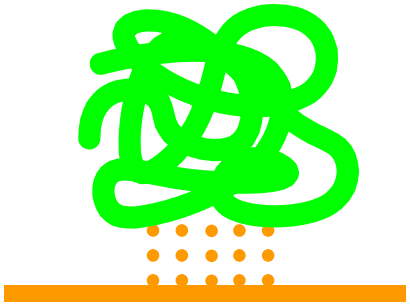
IMMOBILIZATION BY ADSORPTION

- Easy protocols of immobilization
- The support can be re-used after enzyme inactivation

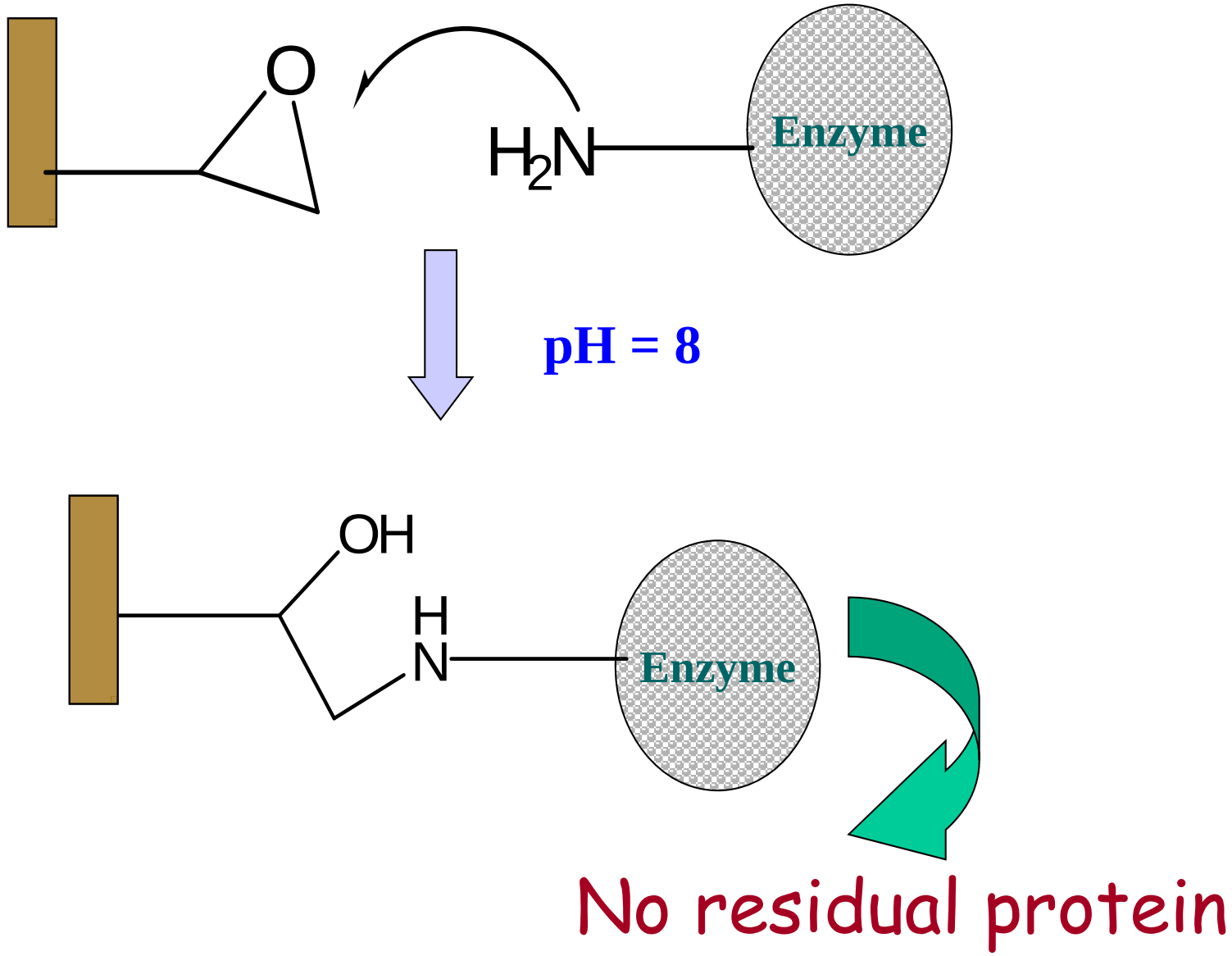
👍Reduction of residues



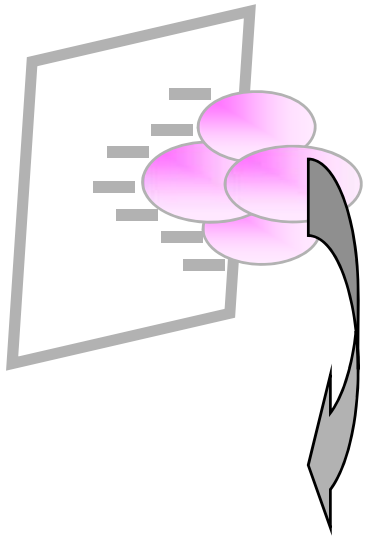
- Possible product contamination with the enzyme



Covalent Immobilisation on Epoxidic Supports



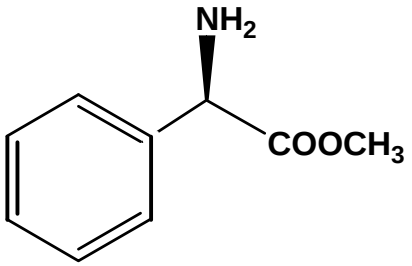
Special case: Multimeric enzymes

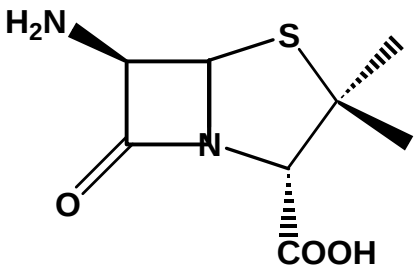


Covalent immobilisation

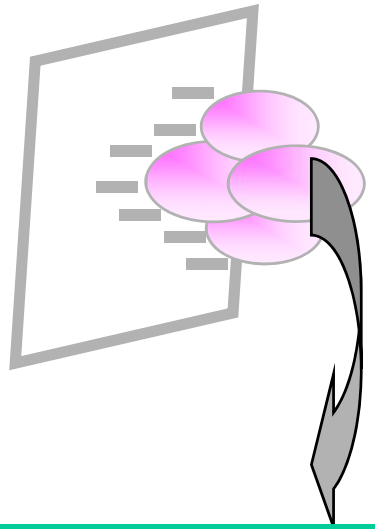
Dissociation of the subunits:
- **Low stability of the enzyme**
- **Residual protein in the final product**

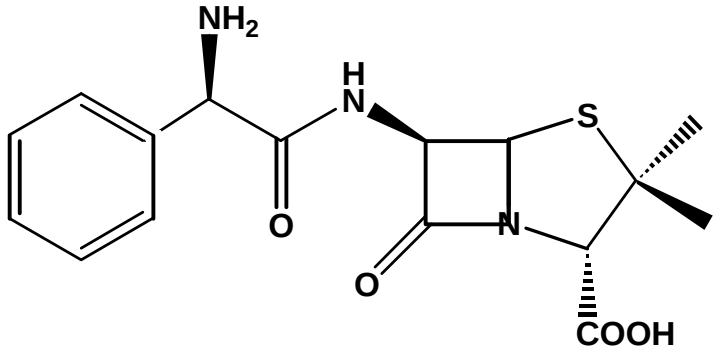
Enzymatic synthesis of Ampicillin





40 % of methanol;
Phosphate buffer at low concentration;



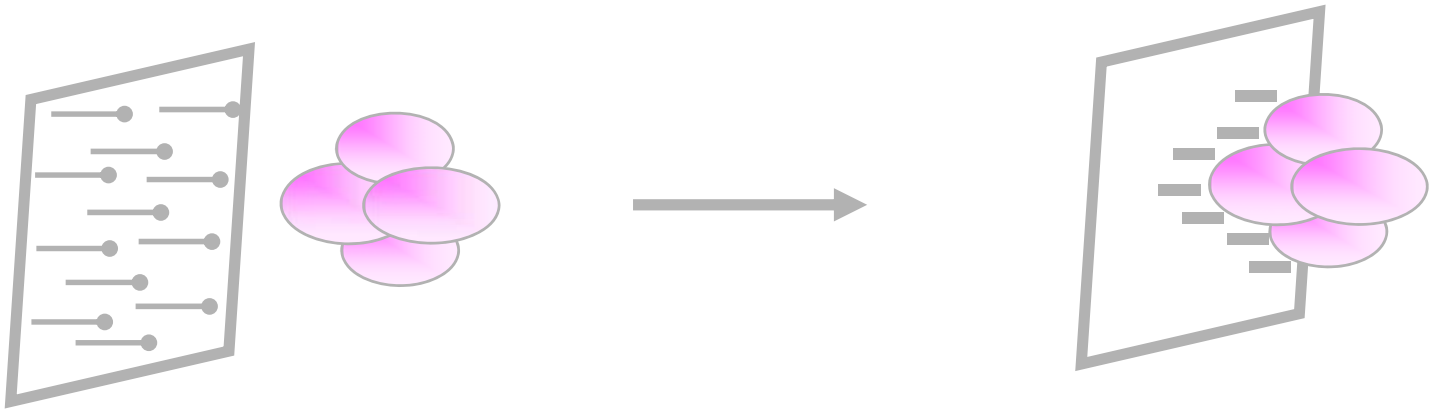


Dissociation of the subunits:
Low stability of the enzyme
In the synthetic conditions

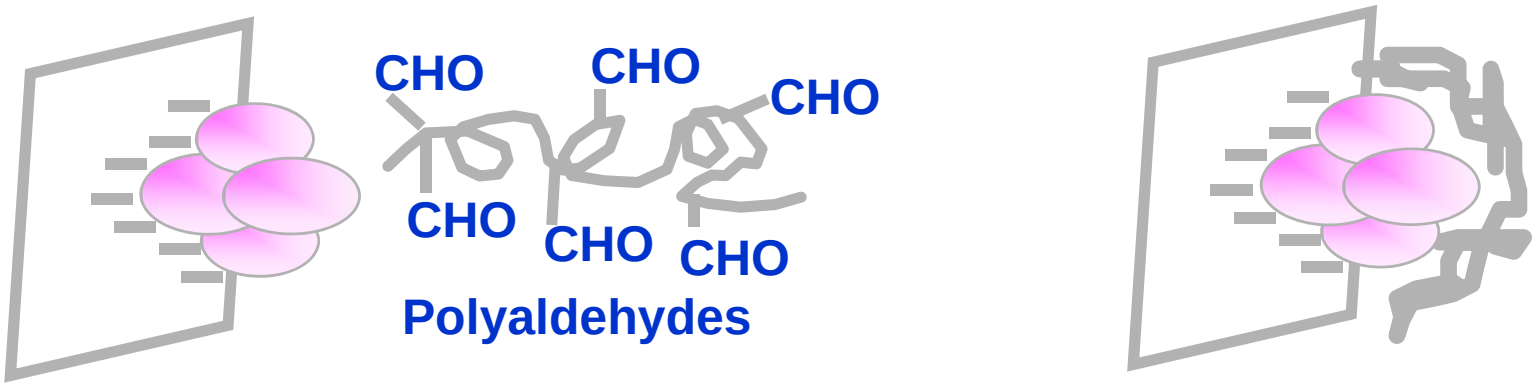
Enz. Microb. Technol. 25 (1999) 336-343

AN INTEGRATED APPROACH TO THE STABILIZATION OF MULTIMERIC ENZYMES

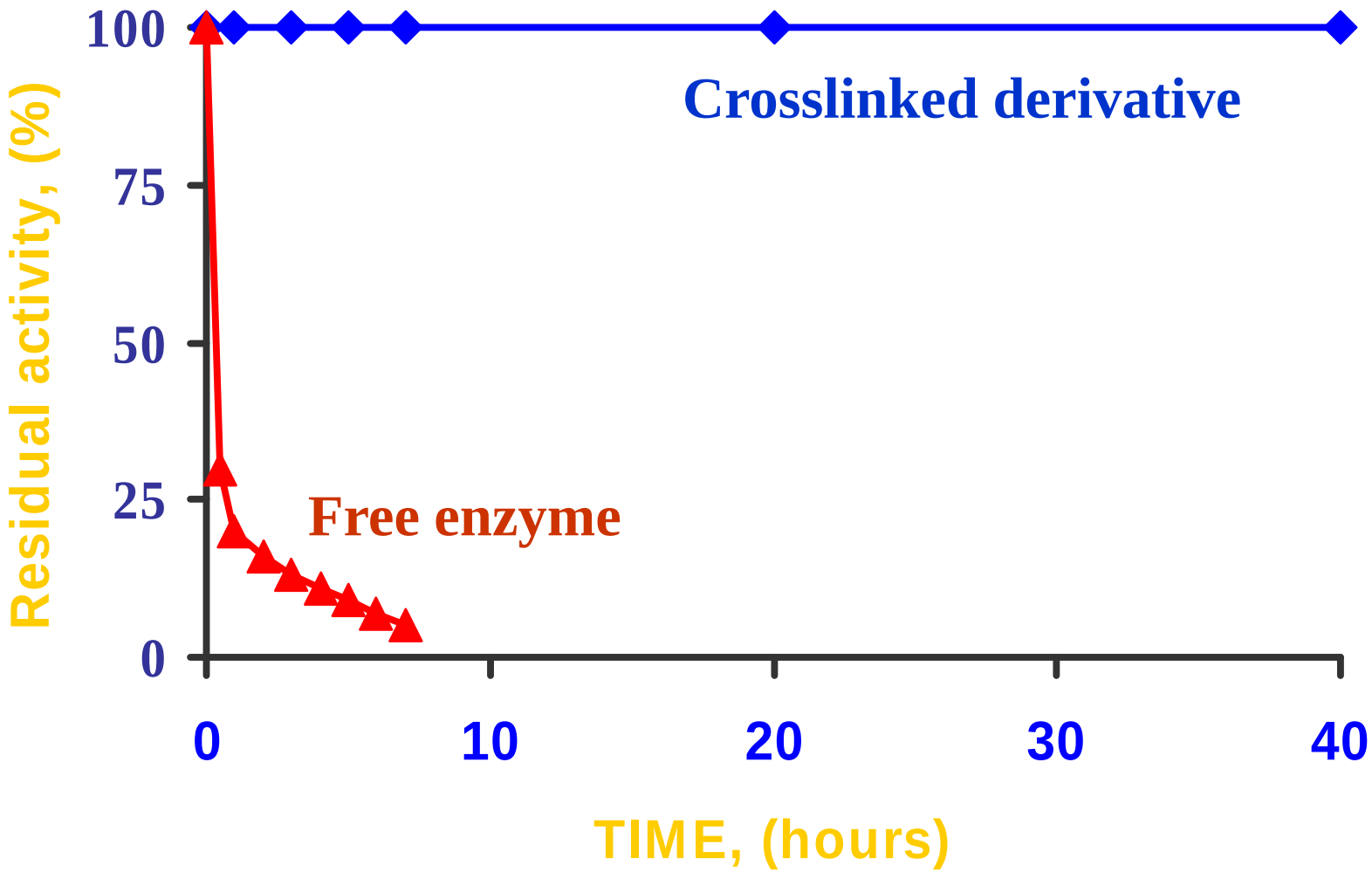
1. MULTI - SUBUNIT COVALENT IMMOBILIZATION



2. SUBUNIT CROSSLINKING WHIT POLYFUNCTIONAL MOLECULES



Stability of the enzyme derivatives



Immobilised Enzyme

Control of residual proteins

- Knowledge of the catalyst engineering
- Suitability of the downstream process
- Test for release of proteins in the reaction condition
- Test for residual proteins in the final product

1. Fermentation Processes

01/2008:1468

PRODUCTS OF FERMENTATION

Producta ab fermentatione

This monograph applies to indirect gene products obtained by fermentation. It is not applicable to:

- *monographs in the Pharmacopoeia concerning vaccines for human or veterinary use;*
- *products derived from continuous cell lines of human or animal origin;*
- *direct gene products that result from the transcription and translation from nucleic acid to protein, whether or not subject to post-translational modification;*
- *products obtained by semi-synthesis from a product of fermentation and those obtained by biocatalytic transformation;*
- *whole broth concentrates or raw fermentation products.*

General monograph « Products of fermentation » (1468)

- Scope:
  - « Indirect » gene products obtained by fermentation
- Out of the scope:
  - Vaccines, products from continuous cell lines, of animal/human origin
  - Direct gene products
  - Semi-synthetic products, biocatalytic process
- Provides general requirements for manufacture of fermentation products. Compliance mandatory for these products

FERMENTATION

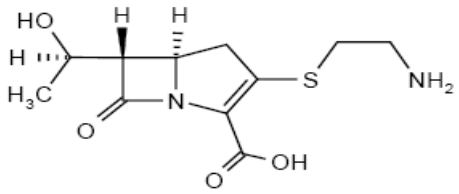
Advantages

- One step reaction: synthesis of complex molecules
- Low costs

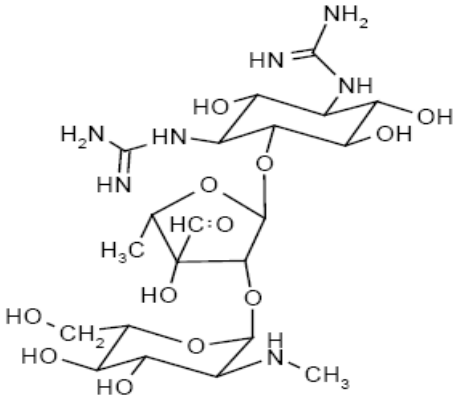
Disadvantages

- Complex mixtures
- Contaminants with different structures
- Residuals arising from micro-organisms
- Complex downstream
- Complex separation and analytical procedure for control of impurities

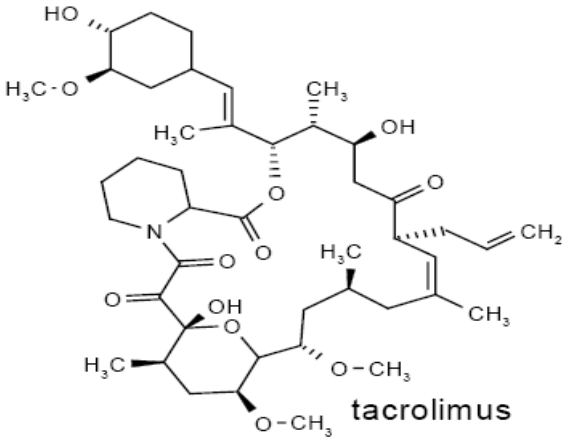
Product of fermentation Complex structures



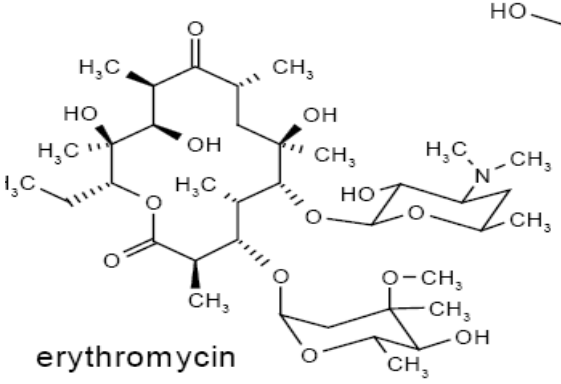
thienamycin



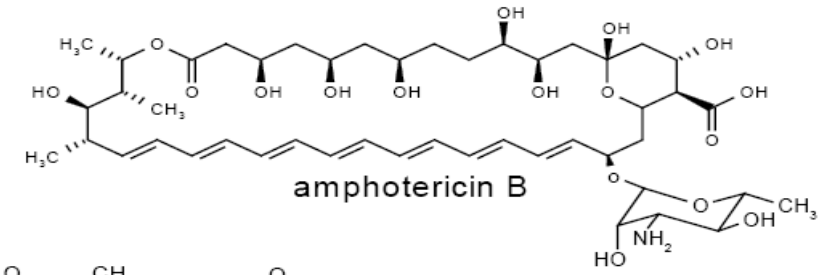
streptomycin



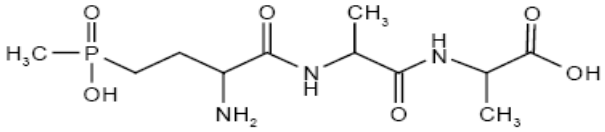
tacrolimus



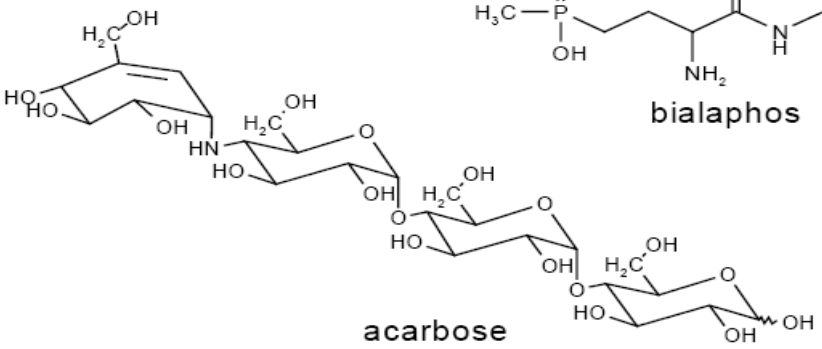
erythromycin



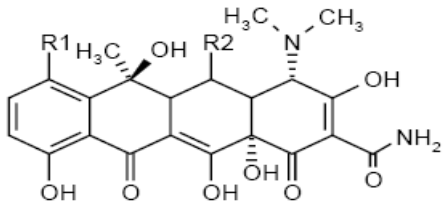
amphotericin B



bialaphos



acarbose



tetracycline

Simple compounds, normally prepared by chemical synthesis, are more and more prepared by fermentation.
For example:

- Aminoacids
- Nucleosides and nucleotides
- Vitamins

Control of the quality is depending from the process used.

Requirements

- Source and history of the producer micro-organism
- Characterisation, stability, of the producer micro-organism
- Detailed description of fermentation, incl materials, preparation of media, downstream processing
- In-process controls at all stages
- Purity of the final substance: impurity profile and specification

Impurity profile

- Impurities (3.2.S.3)
  - Describe all potential related substances (sometimes complex), focus on actual impurities
  - Address residual solvents according to European guidelines
  - Discuss particular impurities arising from fermentation: residues of substrates, cells residues, proteins,…

Limits for related substances

- Set limits according to the monograph
- Set limits for the other known impurities
  - Qualify impurity profile by comparison with products already on the market, or tox data
  - In line with levels found in batches
- Set limits for unknown impurities + total impurities

Limits for residual solvents

- **According to ICH and CHMP guidelines** on Residual solvents
- Show absence of the **solvents used during purification**
- **Set limits and propose methods** for solvents used during purification

Other impurities

- Demonstrate that there are no **residues from fermentation**
- **Proteins**: not a concern for oral use (a lime proposed), to be addressed if parenteral use (absence should be demonstrated)
- **DNA**
- The **Ph. Eur** provides **general methods** which can be used

downstream:
Elimination of residuals from micro-organism

- **Extraction with organic solvents**

allows complete elimination of residuals. Presence of residual indicate a not well separation of phases.

- **Crystallization in organic solvent**

Most adequate for residuals elimination

- **Ultra filtration**

Residuals of low molecular weight proteins and peptides

- **Chromatographic columns**

Complete elimination of protein is ensured depending from the chromatographic conditions

Problems for high water soluble products:

- Peptides
- Amino acids
- Oligosaccharides
- Nucleosides and nucleotides

Evaluation of residuals arising from the micro-organism

- Absence of residuals from the microorganism should be demonstrated during process validation
- Product for injection should be carefully controlled

Nucleic acids

- Absorbance tests
- RT-PCR or PCR

Proteins

- Colorimetric tests: Bradford, Lowry or other according to the *Monograph for Assay of Total Protein 2.5.33* (01/2008:20533)
- Electrophoresis

Example of fermentation product: L-serine

Isolation from fermentation broth:

- Filtration of biomass
- Anionic and cationic exchange resins

Purification:

- Ultrafiltration of water solution (cut-off m.w.>6000)
- Crystallization from water

Possible impurities:

Aminoacids. Analysis performed with A.A. analyzer (each NMT 0.2%; total NMT 1.0%).

Absence of protein demonstrated by Bradfford assay (colorimetric: LOD 1ug/mL)

Example of Enzymatic bioprocess: L-serine

Enzyme Isolation from fermentation broth:

- Filtration of biomass
- Solution is directly used without purification of the enzyme

Purification:

- Ionic exchange colums
- Crystallization from organic solvent/water solution
- Ultrafiltration of water solution (cut-off m.w.>5000)
- Crystallization from water

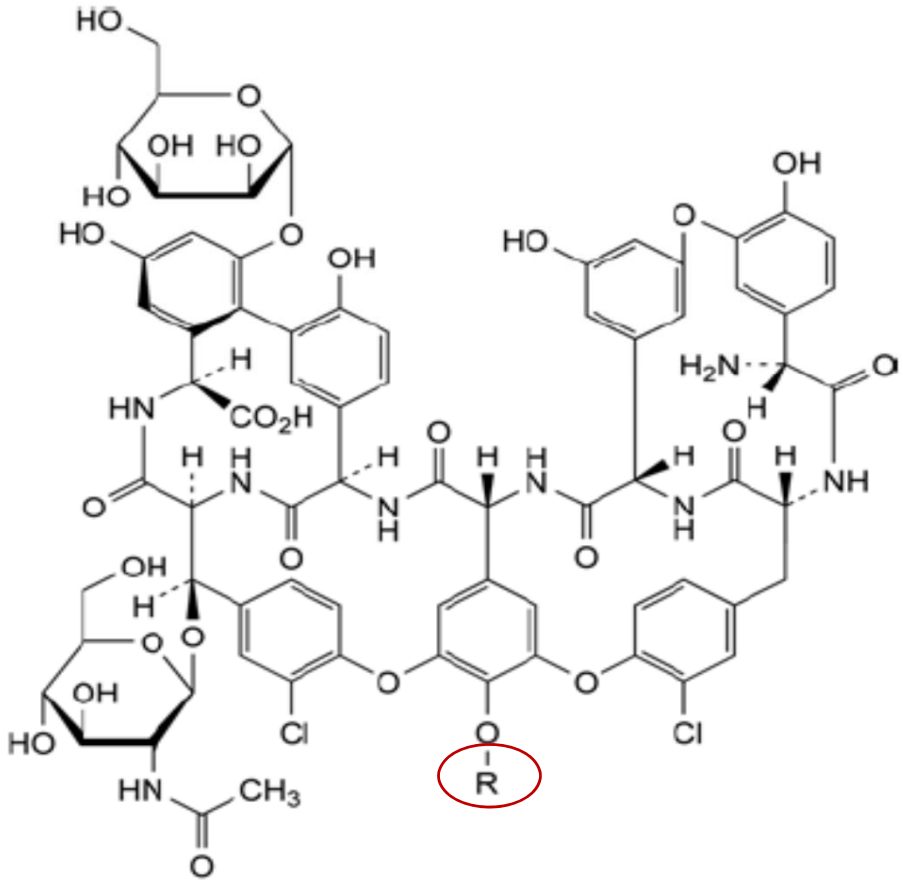
Possible impurities:

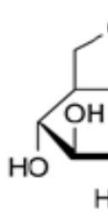
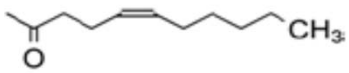
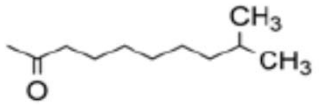
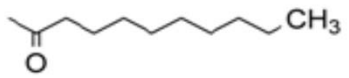
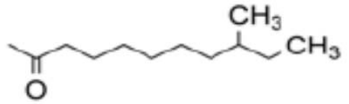
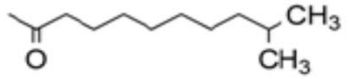
Glicine (starting material) and other A.A. Analysis performed with A.A. analyzer (each NMT 0.5%; total NMT 1.0%).

Absence of protein demonstrated by adsorbance at 650 nm (LOD 40 ppm)

Example of fermentation product: Teicoplanine….a long story

Teicoplaninum



Teicoplanin	R	R'
A_{2-1} $C_{88}H_{95}Cl_2N_9O_{33}$ M. W.: 1878		
A_{2-2} $C_{88}H_{97}Cl_2N_9O_{33}$ M. W.: 1880		
A_{2-3} $C_{88}H_{97}Cl_2N_9O_{33}$ M. W.: 1880		
A_{2-4} $C_{89}H_{99}Cl_2N_9O_{33}$ M. W.: 1894		
A_{2-5} $C_{89}H_{99}Cl_2N_9O_{33}$ M. W.: 1894		
A_{3-1} $C_{72}H_{68}Cl_2N_8O_{28}$ M. W.: 1564	H	

Is a Complex mixture of products. Problems related to:.

- Identification
- Quality

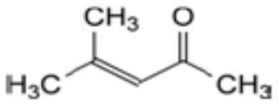
Teicoplanine

Limits:

- *teicoplanin A_2 group*: minimum 80.0 per cent;
- *teicoplanin $A_{2\text{-}2}$*: 35.0 per cent to 55.0 per cent;
- *teicoplanin $A_{2\text{-}1}$ group*: maximum 20.0 per cent;
- *teicoplanin $A_{2\text{-}3}$ group*: maximum 20.0 per cent;
- *teicoplanin $A_{2\text{-}4}$*: maximum 20.0 per cent;
- *teicoplanin $A_{2\text{-}5}$ group*: maximum 20.0 per cent;
- *teicoplanin A_3 group*: maximum 15.0 per cent;
- *total of impurities other than mesityl oxide with a relative retention more than 1.25*: maximum 5.0 per cent;
- *disregard limit*: the area of the peak due to teicoplanin $A_{2\text{-}2}$ in the chromatogram obtained with reference solution (b) (0.25 per cent).

IMPURITIES

Specified impurities: A.



A. 4-methylpent-3-en-2-one (mesityl oxide).

Composition of the "Complex"
and related substances are strictly dependent from:

- Microorganism
- Control of the Fermentation process
- Raw material used for fermentation process

Teicoplanine New Producer
Japan Pharcopoea (JP)

JP Specification	Teico Batch A	Teico Batch B	Teico Batch C
TA2 Group NLT 80%	95.24	94.04	90.50
TA3 Group NMT 15%	3.71	4.45	8.28
Other NMT 5%	1.05	1.51	1.22

Commercial Medicinal product (Tangosit) and the new Teicoplanine API meet specification for JP

Csilla FrankK; Controllo della qualità di un prodotto di fermentazione: aspetti tecnici e regolatori
Thesis "Master in Discipline Regolatorie 2008"; University of Pavia; Pavia; Italy

Teicoplanine: satisfaction of EP Specification

Out of Specification

EP Specification	Teico Batch A	Teico Batch B	Teico Batch C
TA2-1 Group NMT 20%	10.65	6.32	8.62
TA2-2 Group NMT 35-55%	**59.54**	**56.54**	**55.01**
TA2-3 Group NMT 20%	9.04	10.69	8.95
TA2-4 Group NMT 20%	4.27	7.51	5.05
TA2-5 Group NMT 20%	2.79	5.26	3.57

Conform with the Specification

EP Specification	Teico Batch A	Teico Batch B	Teico Batch C
TA2-1 Group NMT 20%	9.3	8.3	7.7
TA2-2 Group NMT 35-55%	**47.7**	**47.7**	**48.7**
TA2-3 Group NMT 20%	8.2	7.5	7.0
TA2-4 Group NMT 20%	14.8	14.1	13.5
TA2-5 Group NMT 20%	6.7	7.1	7.1

Modulation of fermentation condition
Selection of the ingredient used during fermentation

Csilla FranK; Controllo della qualità di un prodotto di fermentazione: aspetti tecnici e regolatori
Thesis "Master in Discipline Regolatorie 2008"; University of Pavia; Pavia; Italy

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

30 June 2012
EMA/CHMP/CVMP/QWP/199250/2009 corr
Committee for Medicinal Products for Human Use (CHMP)/ Committee for Medicinal Products for Veterinary Use (CVMP)

Guideline on setting specifications for related impurities in antibiotics

5.1. Active substances manufactured by semi-synthesis

Semi-synthetic substances are obtained from a fermented starting material by a process involving at least cleavage and formation of covalent bonds and including extraction/purification steps. Acceptance criteria for related impurities should be set in accordance with the thresholds given below.

The ICH Q3A thresholds for reporting, identification and qualification apply.

Reporting threshold: 0.05%/0.03%
Identification threshold: 0.10%/0.05%
Qualification threshold: 0.15%/0.05%

5.2. Active substances manufactured by fermentation, single compound

Acceptance criteria for related impurities should be set in accordance with the thresholds given below.

Reporting threshold: 0.10%
Identification and qualification thresholds: 0.15%

5.3. Active substances manufactured by fermentation, family of compounds

Acceptance criteria for related impurities should be set in accordance with the thresholds given below.

Reporting threshold: 0.10%
Identification threshold: 0.15%
Qualification threshold: 0.50%/0.2%

The qualification threshold of 0.50% for structurally closely related impurities (see definition) is combined with a qualification threshold of 0.2% for other related impurities. Justification for claiming that a related impurity (compound not defined to be included in the active substance) is structurally closely related to the parent compounds should at least be based on evidence such as HPLC/mass spectrometry or the use of reference materials. The proposed 0.50%/0.2% limits are suggested to apply even for daily doses of ≥2 g, which may be relevant for some of these antibiotics.

6.3.3. Existing active substances subject to Ph. Eur. monograph, without transparency statement

The impurity profile should be characterised and individual impurities identified, when necessary to comply with this guideline.

Impurities should be qualified, when necessary to comply with this guideline as described in the General requirements section.

6.3.4. Revision of Ph. Eur. monographs

A revision of the Ph. Eur. monograph should be initiated when:

- The means of identification of known impurities have been established
- New impurities have been identified or qualified

According to Directives 2001/82/EC and 2001/83/EC as amended, the Pharmacopoeia should be informed by the relevant authority when a monograph is insufficient to control the quality of a substance.

Limit for impurities in Teicoplanin?

According to Guide line on setting specification for related impurities in antibiotics

Proposed limits for Active Substances manufactured by fermentation: Fanmily of compound

Reporting threshold:	0.10%
Identification threshold	0.15%
Qualification threshold unknown	0.15%
Qualification threshold related	**0.50%**

Identification at least by HPLC-MS

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Assessment report

Review under Article 5(3) of Regulation (EC) No 726/2004

Teicoplanin

Additional limits and tests proposed for the active substance subcomponents, based on the batch results and proposal for the Ph. Eur. monograph revision

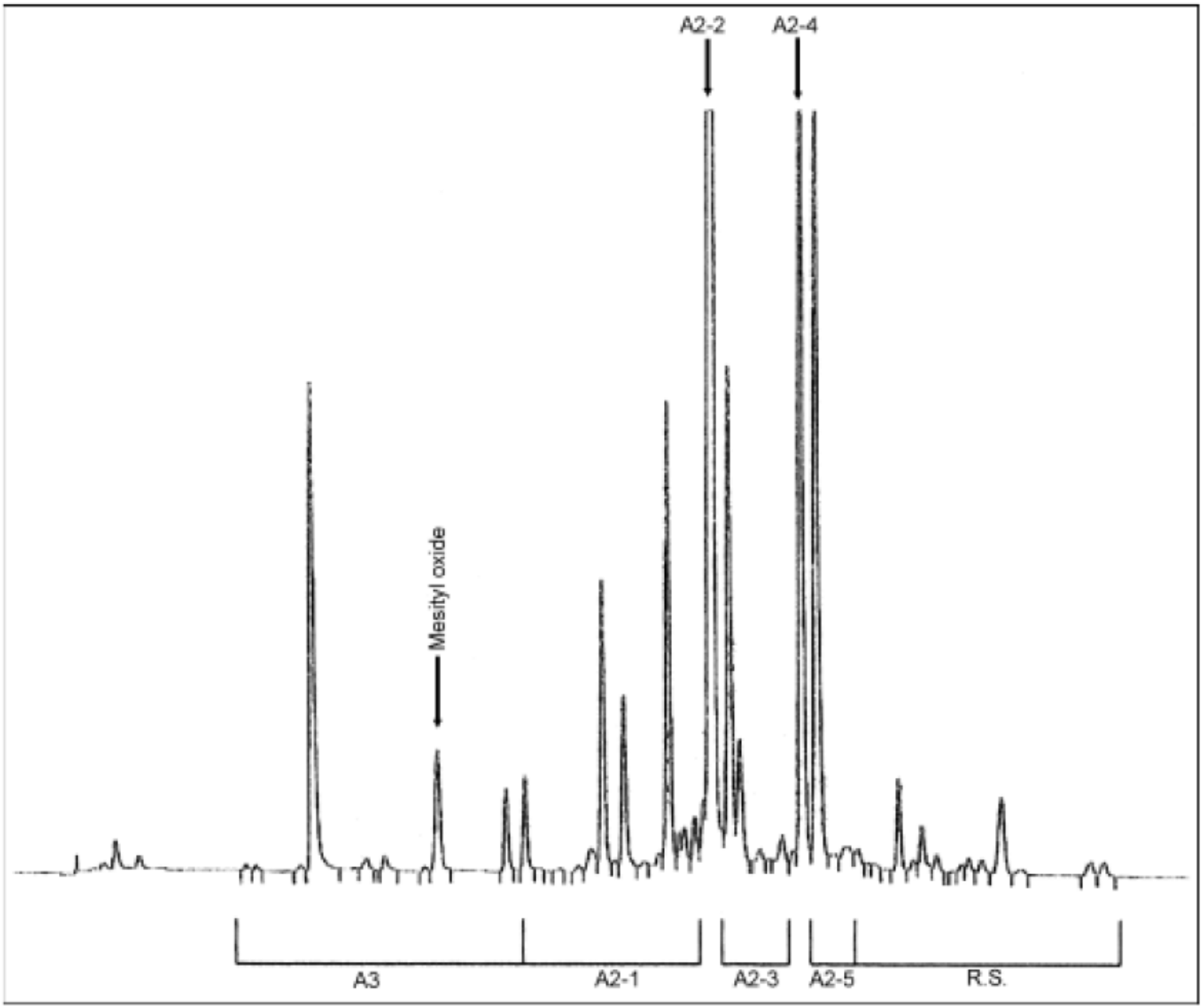
Teicoplanin A3 group	**4.0 - 12.0%**
Teicoplanin A2 group	**84.0 - 93.0%**
Teicoplanin A2-1 group	**10.0 - 19.0%**
RRT about 0.85 (RS3):	0.5-5.5%
RRT about 0.88 (RS4)	0.5-4.0%
RRT about 0.93 (A2-1)	2.0-7.0%
Teicoplanin A2-2	**37.0% - 50.0%**
Teicoplanin A2-3 group	**5.0 - 11.0%**
RRT about 1.03 (A2-3)	4.0-8.5%
Teicoplanin A2-4	**7.0 - 15.0%**
Teicoplanin A2-5 group	**7.0 - 17.0%**
RRT about 1.15(A2-5)	7.0-15.0%

Related substances	**NMT 5.0%**
RRT about 1.25 (RS1):	NMT 1.5%
RRT about 1.30 (RS2)	NMT 1.5%
RRT about 1.38	NMT 2.5%
Any non-teicoplanin like impurity	**NMT 0.5%**

Any other peak in the chromatogram should be identified and confirmed to have a teicoplanin-like structure.

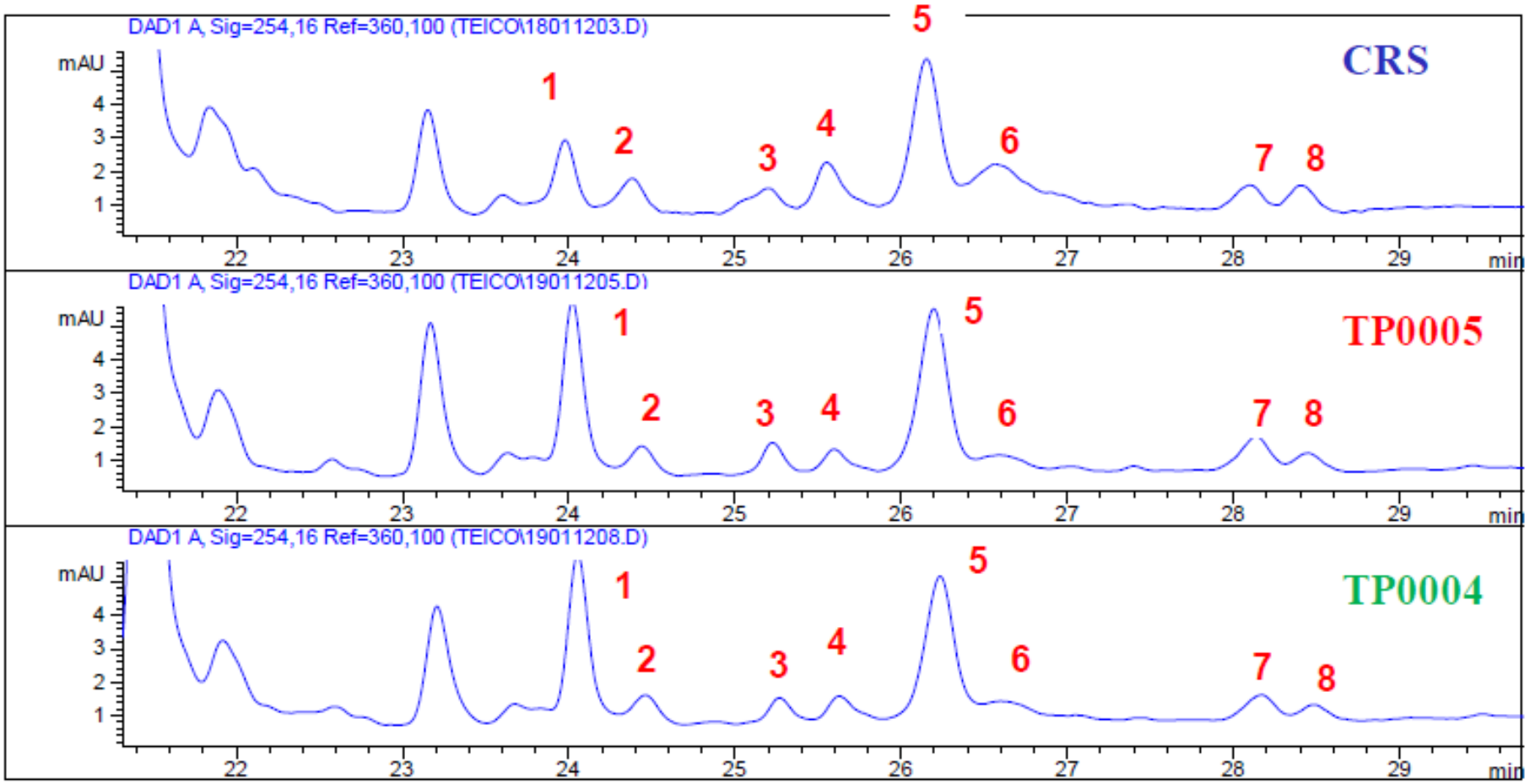
Analysis of teicoplanin chromatogram available (EDQM web site)

The following chromatogram is available at the EDQM website, knowledge database:



EVALUATION OF TEICOPLANINE QUALITY

1. Evaluationof the complex according to the EMA guide line.
2, Compariosion of the new product with EP-CRS and commercial product



3. Confirmation of Teicoplanine-like impurities by HPLC-MS analysis
4. Specification for impurities >0.5% specified by RRT
5. Revision of the EP monograph under evaluation accordingly

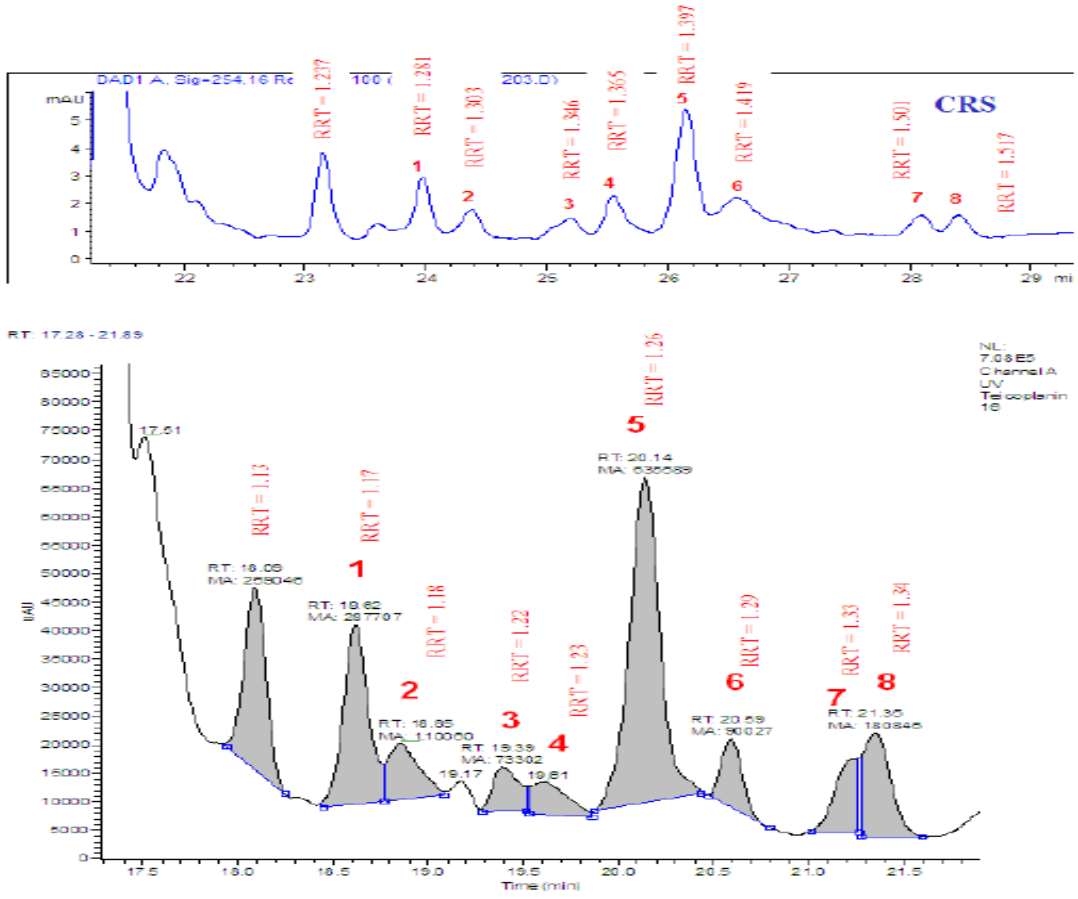


Figure 9: UV traces of the unknown impurity region obtained with method D (top) and method E (bottom). Peak with RRT = 1.13 belongs to A2-5 impurities. Unknown impurities with RRT>1.25 are labeled from 1-8.