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UNIVERSITY OF COPENHAGEN




Building the quality into manufacturing of macromolecular systems

Jukka Rantanen

JTR/MDR/Jun 2008

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Outline

- * Process analysis – Manufacturing Sciences
- * Source of active compound -> Processing
- * Production of macromolecular drug products, CASES:
 - fermentation
 - freeze drying

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Perspectives


MA

1. Search for new candidates



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Few facts

New treatments – new molecules – new challenges

- * amount of new molecules ↓
- * size/complexity of molecules ↑

Processing

- * mixture of excipients and active
- * recent innovations?

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Few facts



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Source of active compound

Table 1.1
Examples of drugs from different sources: natural products, synthetic chemistry and biopharmaceuticals

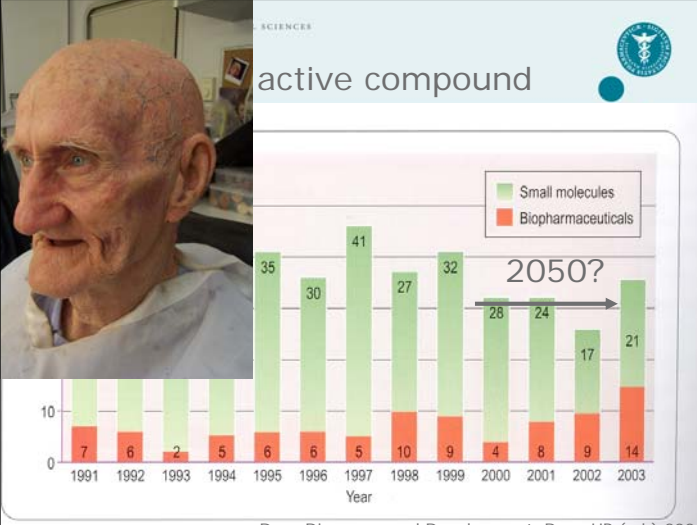
Natural products	Synthetic chemistry	Biopharmaceuticals produced by recombinant DNA technology
Antibiotics (penicillin, streptomycin, tetracyclines, cephalosporins etc.)	Early successes include:	Human insulin (the first biotech product, registered 1982)
Anticancer drugs (doxorubicin, bleomycin, actinomycin, vincristine, vinblastine, taxol etc.)	Antiepileptic drugs	Human growth hormone
Atropine, hyoscine	Antihypertensive drugs	α -interferon, γ -interferon
Cisplatin	Antimetabolites	Hepatitis B vaccine
Cocaine	Barbiturates	Tissue plasminogen activator (t-PA)
Colchicine	Bronchodilators	Insulin
Digitalis (digoxin)	Diuretics	Hirudin
Ephedrine	Local anaesthetics	Blood clotting factors
Heparin	Sulfonamides	Erythropoietin
Human growth hormone*	<i>[Since c.1950, synthetic chemistry has accounted for the great majority of new drugs]</i>	G-CSF, GM-CSF
Insulin (porcine, bovine)*		
Opium alkaloids (morphine, papaverine)		
Physostigmine		
Rosiglitazone		
Statins		
Streptokinase		
Tubocurarine		
Vaccines		

Drug Discovery and Development, Rang HP (ed.) 2006

*Now largely or entirely replaced by material prepared by recombinant DNA technology.

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Source of active compound



Year	Small molecules	Biopharmaceuticals
1991	7	7
1992	6	6
1993	2	2
1994	5	5
1995	6	6
1996	6	6
1997	5	41
1998	10	27
1999	9	32
2000	4	28
2001	8	24
2002	9	17
2003	14	21

2050?

Drug Discovery and Development, Rang HP (ed.) 2006



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Source of active compound

Macromolecules

Small molecules

JT04020/Apr 2008

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Source of active compound

Macromolecules

FERMENTATION

SEPARATION

PURIFICATION

Small molecules

SYNTHESIS

CRYSTALLIZATION

JT04020/Apr 2008

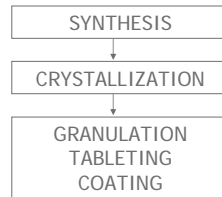
Processing – small molecules



Sophisticated process monitoring and control solutions do exist!

Do we fully utilize them?

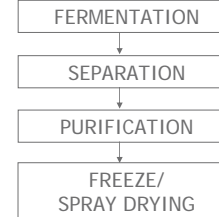
Small molecules



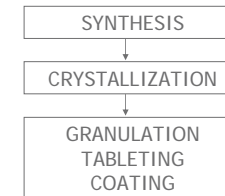
Processing of pharmaceuticals



Macromolecules



Small molecules

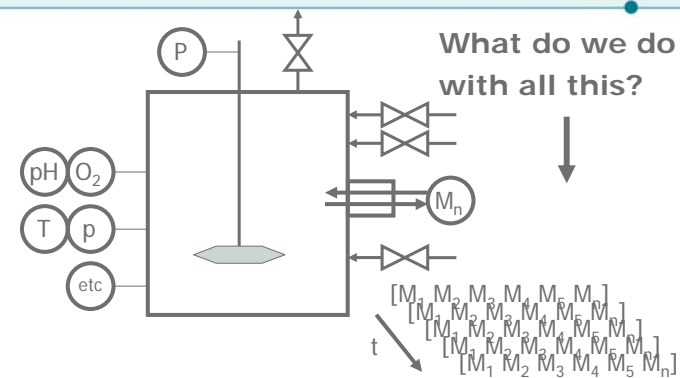


Outline



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Fermentation





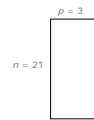
Multivariate Analysis

Principal Component Analysis (PCA)

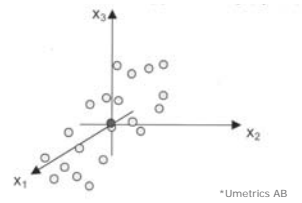
A data mining tool for:

- isolating systematic variance in large data sets
- obtaining overview of large amounts of data

(1) Data matrix (X)



(2) Visual representation (centered data)*



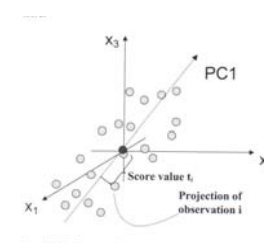
*Umetrics AB



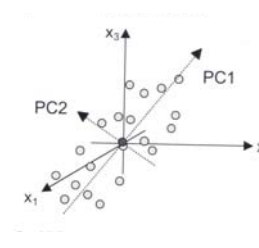
Multivariate Analysis

Principal Component Analysis (PCA)

(3) Fit of PC1*:



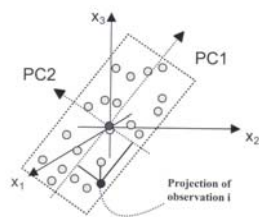
(4) Fit of PC2*:



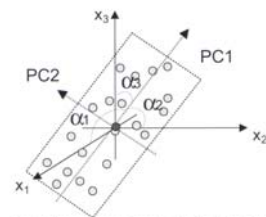
Multivariate Analysis

Principal Component Analysis (PCA)

(5) PC1/PC2 plane*:



(5) Loadings*:

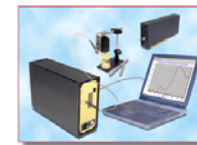


Non-invasive spectroscopy

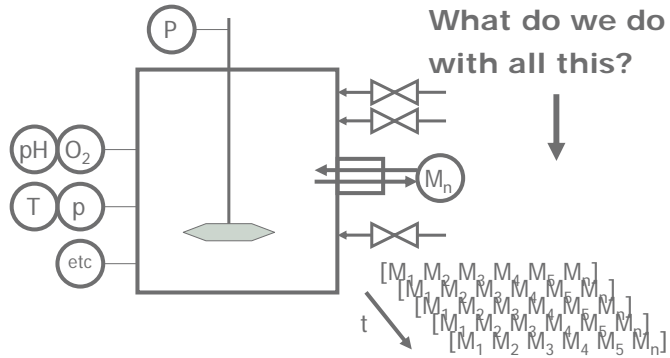


Reflectance measurement
(solids)

Transmission
measurement (liquids)



Fermentation



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Fermentation case

Table 2
The composition of the culture medium employed for the cultivation of *P. chrysogenum* [34]

Ingredients	Concentration (g/l)
Lactose	70.0
Sucrose	10.0
(NH ₄) ₂ SO ₄	7.0
K ₂ HPO ₄	1.6
KCl	0.5
MgSO ₄ ·7H ₂ O	0.1
FeSO ₄ ·7H ₂ O	0.04
CaCl ₂	0.04
MnSO ₄ ·H ₂ O	0.02
ZnSO ₄ ·7H ₂ O	0.02
CuSO ₄ ·5H ₂ O	0.005
pH	5.9 (±0.1)

Vaidyanathan, S. 2001 *Anal. Chim. Acta* 428: 41-59.

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Fermentation case

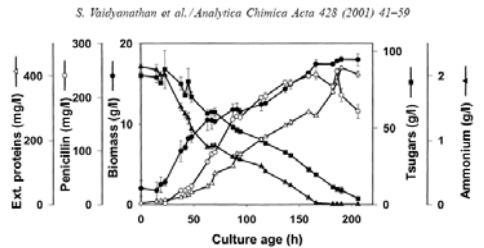


Fig. 1. Time course of variables monitored in a typical *P. chrysogenum* submerged bioprocess.

Vaidyanathan, S. 2001 *Anal. Chim. Acta* 428: 41-59.

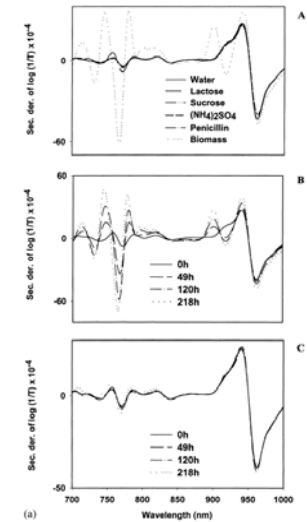
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Fermentation case

NIR spectral information during bioprocessing – Sampling at different time points

Vaidyanathan, S. 2001
Anal. Chim. Acta 428: 41-59.

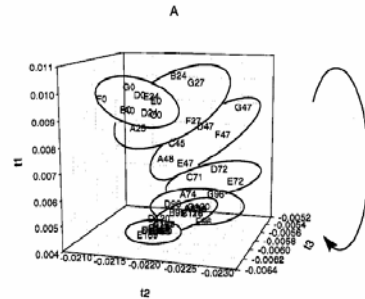
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Fermentation case

NIR spectral information during bioprocessing – process monitoring with PCA



Vaidyanathan, S. 2001 *Biotechnol. Bioeng.* 74: 376-388

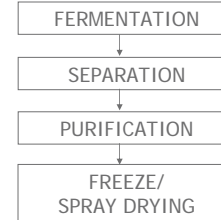
J18408/Jan 2008



Freeze drying



Macromolecules



J18408/Jan 2008



Freeze drying

- Case #1 - Solution for freeze drying:
- liposomal vaccine adjuvant system
 - quantify components (NIR)

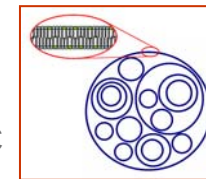
J18408/Jan 2008



Case #1 - vaccine system*

* Christensen, D. et al 2008. Submitted for *EJPB*.

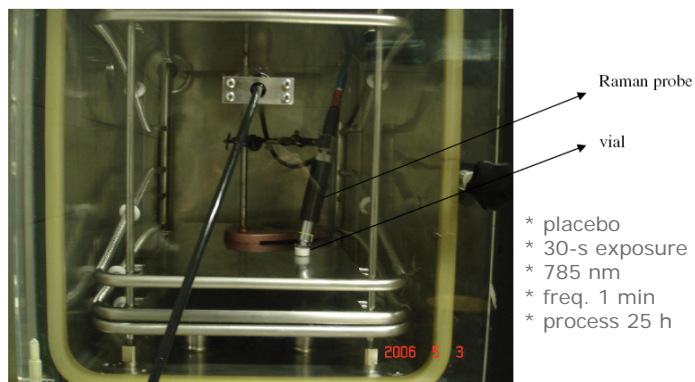
- DDA/TDB adjuvant components
- dimethyldioctadecylammonium bromide (DDA)
 - trehalose 6,6'-dibehenate (TDB)
- DDA/TDB adjuvant formulation
- Multivesicular liposomes
 - Film preparation method
 - Trehalose added as lyoprotector



HPLC
DSC

J18408/Jan 2008

Figure S-1. Experimental setup for the in-line and real-time monitoring of freeze drying processes using Raman spectroscopy



Raman probe
vial

- * placebo
- * 30-s exposure
- * 785 nm
- * freq. 1 min
- * process 25 h

Process monitoring of freeze drying

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Case #2 – Freeze drying

Table 1. Design Space of the Studied Process and Formulation Variables

variable	unit	levels	
		-	+
mannitol concentration (<i>A</i>)	% w/v	5	15
NaCl concentration (<i>B</i>)	% w/v	0	2
volume (<i>C</i>)	mL	2	4
freezing rate (<i>D</i>)	-	slow	fast

+ solid state properties of mannitol



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section	start point (min)	end point (min)	occurring physical phenomenon
1	1	36	unfrozen aqueous mannitol solution
2	37	50	ice crystallization
3	51	61	ice crystallization finished + amorphous mannitol
4	62	105	mannitol crystallization
5	106	175	mannitol crystallization finished (=end of freezing step)
6	175	280	ice sublimation
7	280	400	sublimation finished (=end of primary drying)

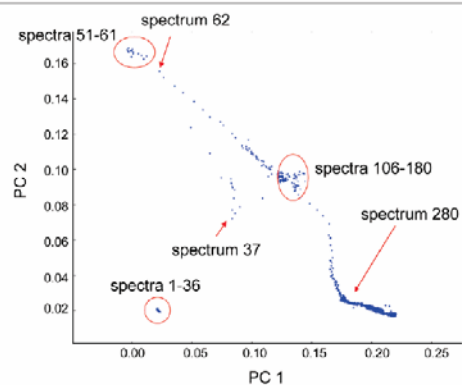


Figure 1. PC 1 vs PC 2 scores plot obtained after PCA analysis on all in-line collected Raman spectra during experiment 1.

Case #2 – Freeze drying

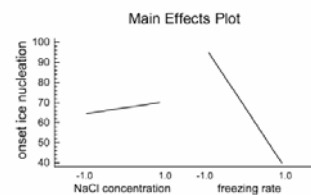
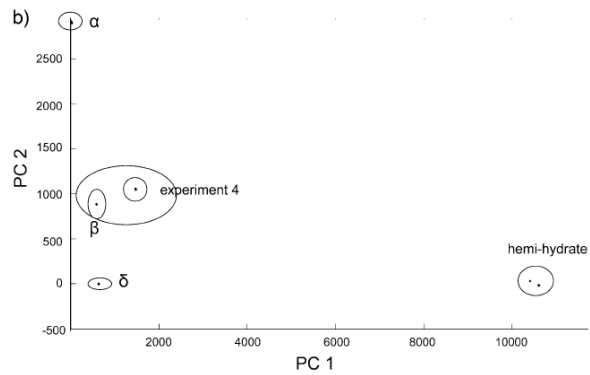


Figure 8. Screening design analysis of onset of ice nucleation. The units of the y-axis in all plots are minutes.

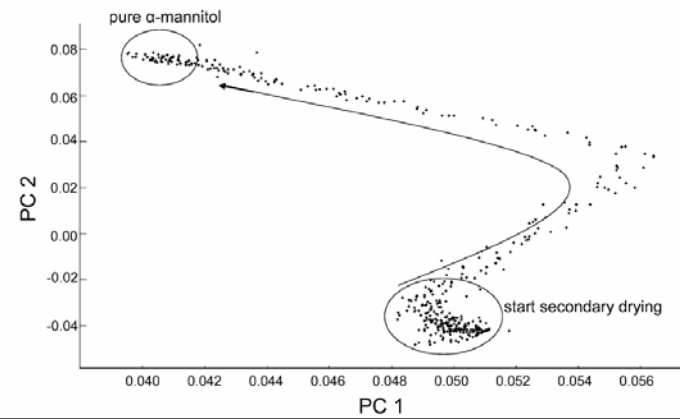
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Case #2 – Freeze drying



Case #2 – Freeze drying



Case #3 - Freeze dried product



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Case #3 - Freeze dried product




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Case #3 - Freeze dried product



2400 vials



mannitol:sucrose

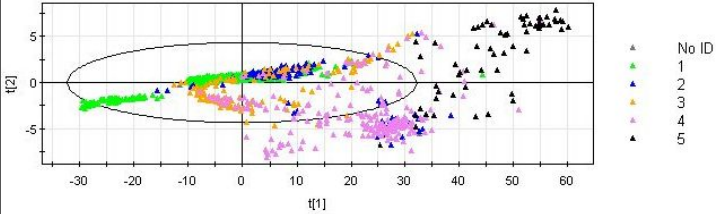


NIR

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Case #3 - Freeze dried product



PCA score plot of PC1 and PC2 of 2400 vials.
Colouring according to visual inspection from 1 (good) to 5 (poor)

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Freeze drying

Case #1 - Solution for freeze drying:

- liposomal vaccine adjuvant system
- quantify components (NIR)

Case #2 - Process monitoring of freeze drying

- placebo mannitol system
- state of matter (Raman)

Case #3 - Freeze dried product

- mannitol:sucrose based system
- fast analysis of all vials (NIR)

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Processing – macromolecules

Sophisticated process monitoring and control solutions do exist!

We have the tools for building the quality into these products.

Do we fully utilize them?



MACROMOLECULES

```

    graph TD
      A[FERMENTATION] --> B[SEPARATION]
      B --> C[PURIFICATION]
      C --> D[FREEZE/SPRAY DRYING]
  
```

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Acknowledgements

* Vaccine case #1:

Statens Serum Institut (Adjuvant Research, Dept. Infectious Disease Immunology)
- Dennis Christensen
- Morten Allesø (PHARMA)

* Freeze drying case #2:

University of Ghent, Belgium
- Thomas de Beer
H. Lundbeck A/S
- Heidi Lopez De Diego
- Morten Allesø (PHARMA)

* Freeze drying case #3:

- Holger Grohganz, Margot Fonteyne (PHARMA)
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- Erik Skibsted, Thomas Falck, Bent Palmqvist



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