Chiesi

People and ideas for innovation in healthcare

Case study: salt and polymorph screening and crystallization study of an M3 antagonist for inhalation

Fausto Pivetti

Aschimfarma, Milano 23 April 2013

Inhalation Therapy

- Inhalation therapy is widely used for the direct administration of drugs to the respiratory tract for local treatment of diseases such as asthma and COPD

- The range of product available is broad encompassing
  - Inhalers (metered-dose, dry powder and aqueous droplet)
  - Nebulisers (jet, ultrasonic and vibrating mesh)
  - Nasal (aqueous based, dry powder and propellant based)

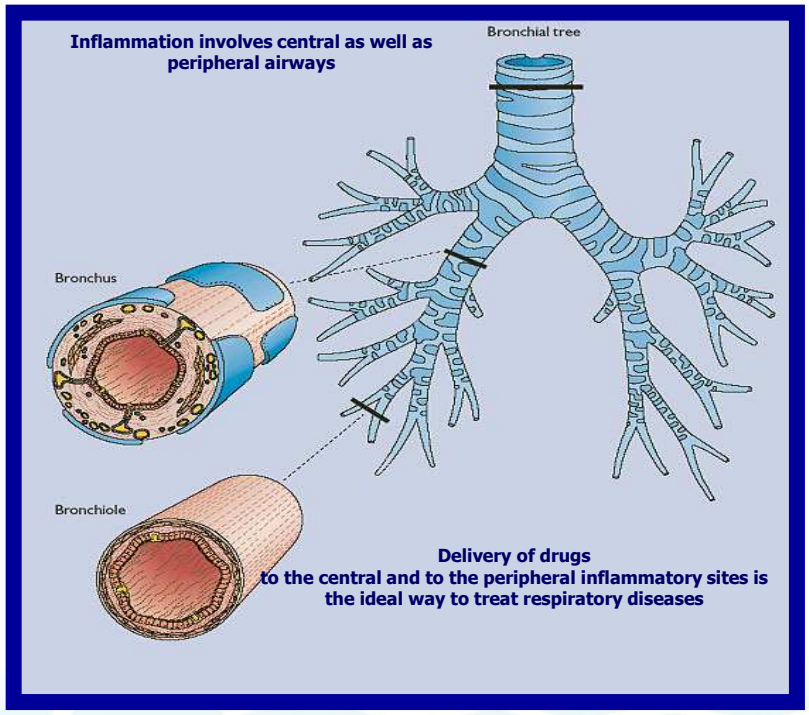
- Compared to systemic therapy, aerosols are effective at a much lower dose, provide a rapid onset of action and have fewer side effects (i.e. limiting systemic exposure)

- Combination therapy: more than one API administered in one shot

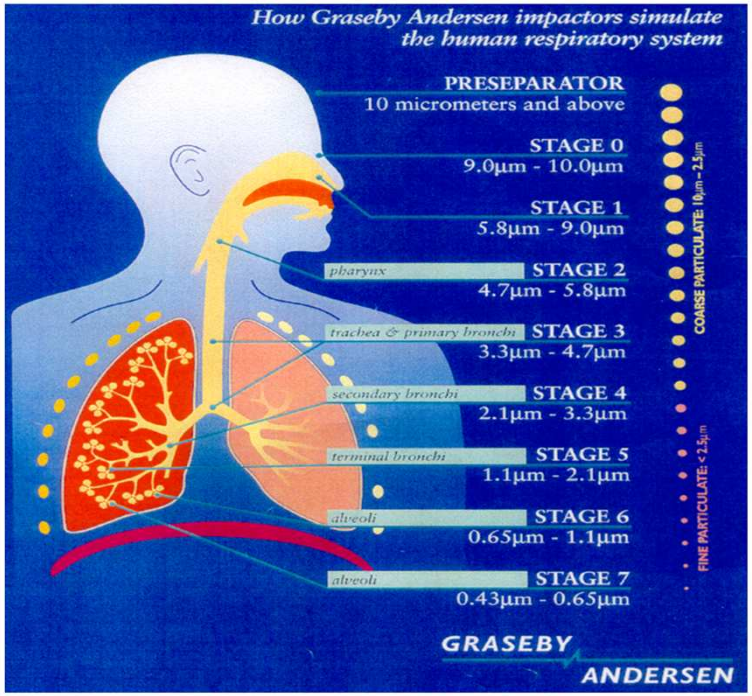
Inhalation Therapy

To develop an efficient and fine/extra-fine formulation in order to ensure consistent and accurate lung distribution and deposition.

- Efficient : able to deliver to the respiratory tract an high amount of medication
- Fine / extra-fine : most of the respirable particles able to reach the peripheral airways



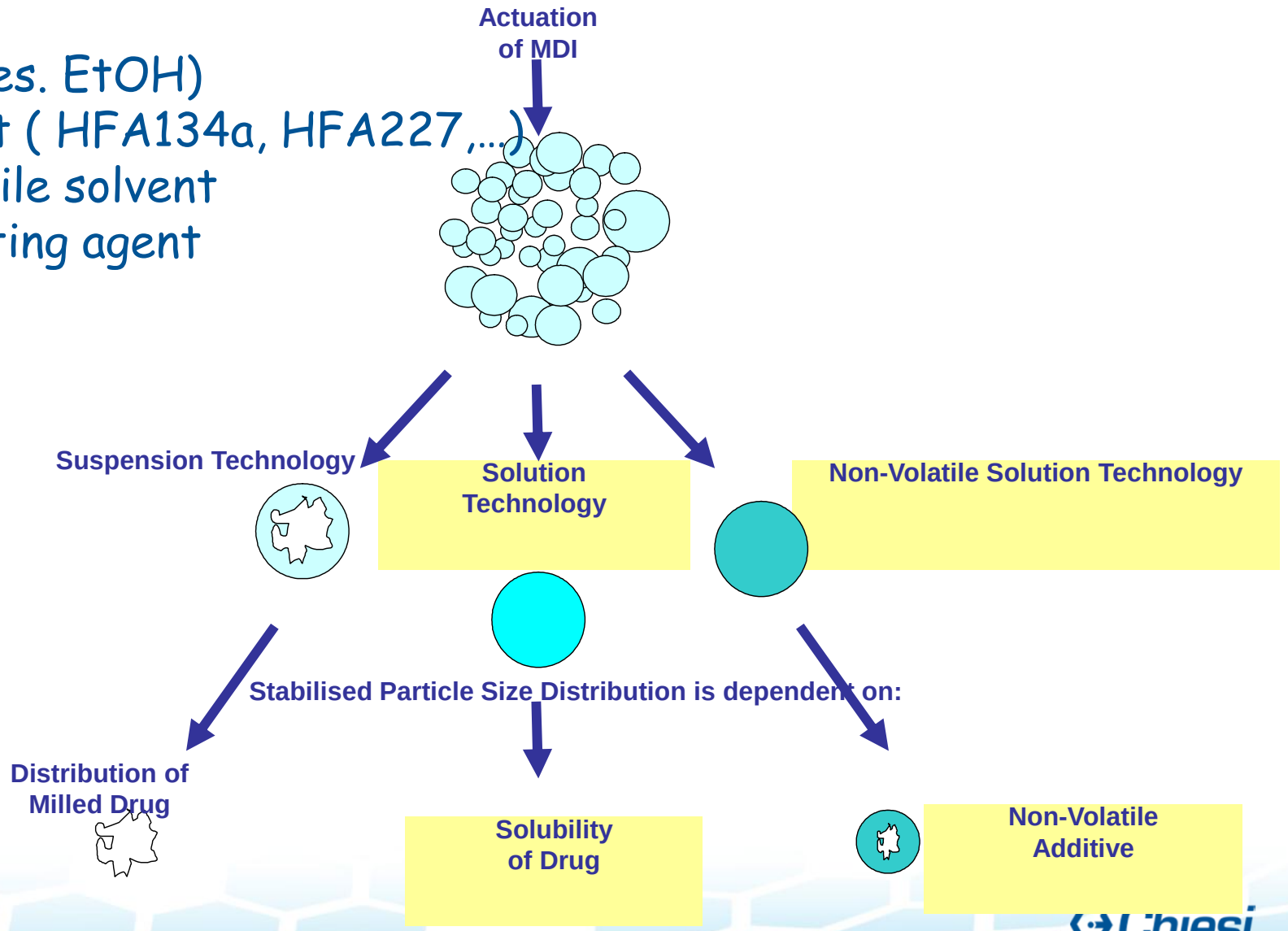
After T. Hansel and PJ Barnes, 2004



Chiesi

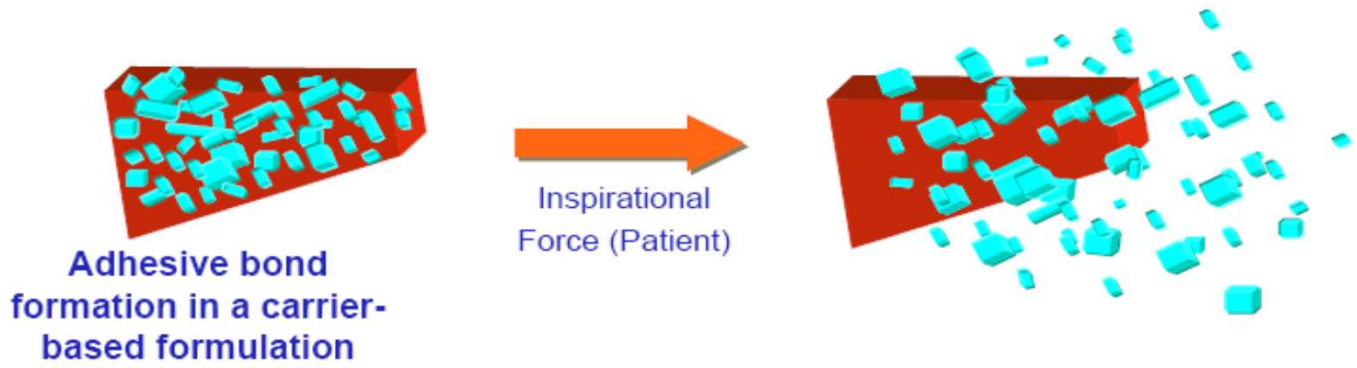
Metered dose Formulations

Solvent (es. EtOH)
Propellant (HFA134a, HFA227,...)
Non volatile solvent
pH adjusting agent



Dry Powder Inhaler Formulation

During the inhalation, the small drug particles de-aggregate and detach from the surface carrier particles and penetrate into the lungs, while the particles of the carrier are usually swallowed



Adhesive bond formation in a carrier-based formulation

De-aggregation depends on particle size, particle shape, surface characteristics, adhesion, cohesion and friction forces

General Features of inhalation

Physical & pharmaceutical properties

- Solubility, crystallinity, polymorphism, hygroscopicity
- Salt form frequently major issue

Dosing & exposure

- Very low dose, limit systemic exposure further
- Intra-tracheal, nebulised, pMDI, dry powder (device issues)

Safety

- Irritancy (taste?)
- Exposure & methodology

Combination opportunities & issues

New LAMA

TPP: once daily M3 antagonist
to be formulated as pMDI
in combination with an existing LABA in development

consider also formulation as DPI in combination with other products (es. PDE4, …)

Critical quality aspects:

- Purity and impurities profile
- Solubility
- Dissolution rate
- Polymorphism
- Compatibility with other API (consider counter-ions)

Why examine different salts?

- Alter physicochemical & biological properties of drug without modifying chemical structure

Improved properties

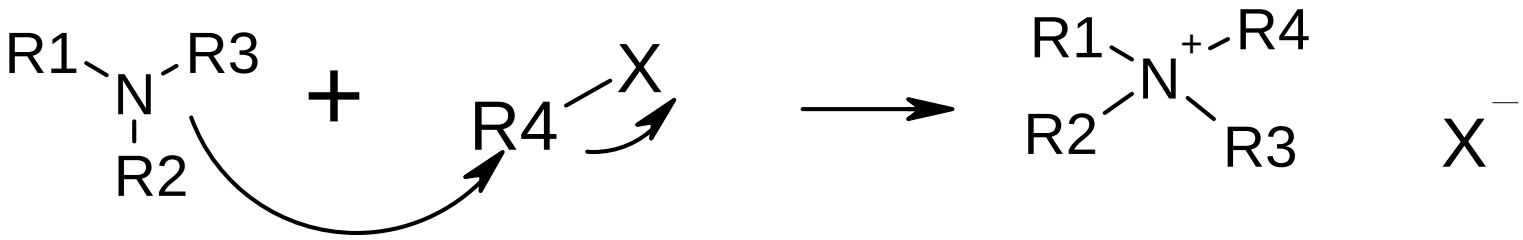
- Crystallinity,
- Dissolution rate,
- Stability (Phys., Chemical),
- Bioavailability,
- Polymorphism.
- Hygroscopicity,
- Processability (needles vs. plates),
- Flow, filterability,
- Irritancy, taste.

Diversity in inhalation not simply a matter of primary chemical structure alone

NEW LAMA quaternary ammonium salt

M3 antagonists generaly are quaternary ammonium salts : es. ipratropium, tiotropium,glycopyrrolate, aclidinium

For quaternary ammonium salts the anion corresponds to the leaving group of the alkylating agent in the quaternization reaction



Alternative routes via ion exchange mechanisms are possible (ion exchange resins, silver salts, etc…)

Chiesi

Salt screening

Salt screening identified candidates:

1. Chloride
2. Bromide
3. Iodide
4. Mesylate

- Isolated as crystalline powders
- Other tested salts (hydrogensulfate or sulfate, tosylate, napsylate,etc…) were oily or amorphous

Salt screening: preliminary characterization

Counter ion	Chloride	Bromide	Iodide	Mesylate
Appearance	White powder	White powder	White powder	White powder
XRPD spectrum	crystalline	crystalline	crystalline	crystalline
m.p., ° C (DSC)	238-241	248-252	217-219	199-201
Loss on drying, % (TGA)	< 0,1	< 0,1	< 0,1	< 0,1
Purity, % (HPLC)	98.0	97.8	98.1	98.1

Salt screening: Solubility assessment (mg/ml; T=20-25°)

Counter ion	Chloride	Bromide	Iodide	Mesylate
Water	9.0	1.2	0.9	86.8
Acetone	1.9	1.6	16.3	6.0
THF	0.6	0.3	5.0	7.5
EtOAc	0.1	0.1	0.4	0.5
MeCN	11.9	9.1	47.9	44.6
MeOH	≥ 100	38.8	15.4	≥ 100
EtOH	≥ 100	4.9	2.4	33.7
2-prOH	8.1	0.6	0.4	4.3
HFA/EtOH 95/5	0.8	0.1	0.1	0.8
HFA/EtOH 90/10	2.2	0.2	0.1	2.4
HFA/EtOH 85/15	8.7	0.6	0.4	7.0

Salt screening: fast polymorph screening

	Chloride	Bromide	Iodide	Mesylate
XRPD	All spectra comparable	Most spectra comparable sample from CHCl3/MeOH showed different spectrum	All spectra comparable	All spectra comparable
FT-Raman	All spectra comparable	All spectra comparable with baseline drift	All spectra comparable with baseline drift	All spectra comparable
DSC	All thermograms comparable: sharp melting peak 237-241° C	All thermograms comparable: front shoulder in the melting peak 248-256° C	All comparable: endo at 102-110° C; melting peak at 216-222° C	All thermograms comparable: sharp melting peak 219-202° C
Conclusion	**No evidence of polymorphism**	**Suspect polymorphism; evidence of solvate**	**Suspect polymorphism**	**No evidence of polymorphism**

Salt screening: effect of common ion in combination with B2-API hydrochloride

Solvent mixture	Solubility of β2-agonist HCl (mg/ml,20° C)	Margin factor in case of chloride	Solubility of β2-agonist HBr (mg/ml,20° C)	Margin factor in case of bromide	Solubility of β2-agonist Mesylate (mg/ml,20° C)	Margin factor in case of mesylate
Ethanol	14.2	-	8.2	-	6.1	-
HFA/EtOH 95/5 v/v	0.005	-	0.001	-	0.003	-
HFA/EtOH 90/10 v/v	0.056	**0.96**	0.021	**0.17**	0.032	**0.4**
HFA/EtOH 85/15 v/v	0.254	**12.7**	0.089	**3**	0.0141	**7.0**

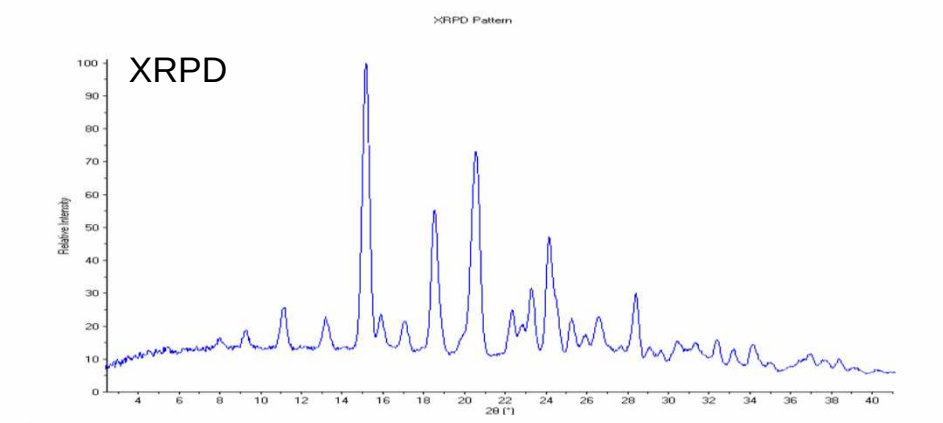
Margin factor: solubility of B2-API salt / cincentration required to deliver the dose

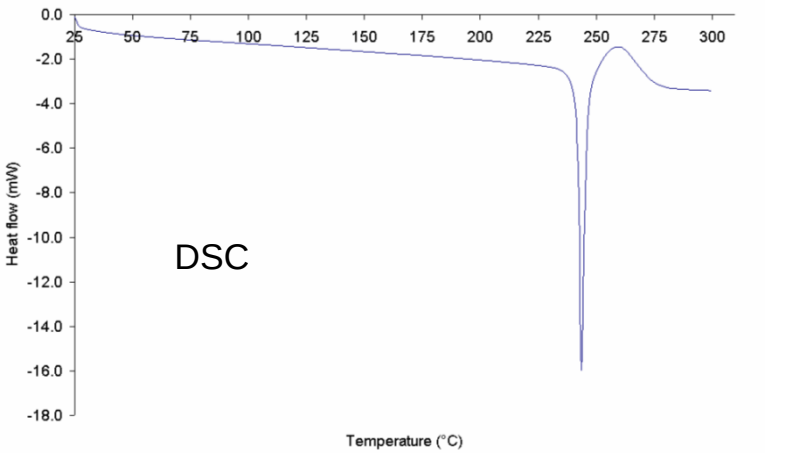
Sal screening: safety considerations

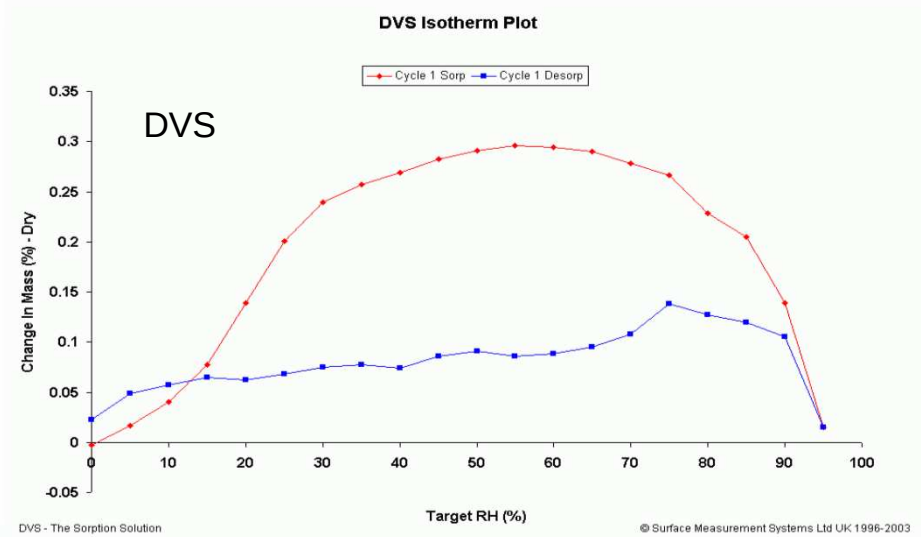
- **Chloride** abundant constituent of the body, well tolerated.
- **Bromide**, even if still abundant in drug salts (ipratropium, glycopyrrolate,…), should be avoided whenever possible because of its accumulation in the “chloride-environment” (bromism).
- The physiological significance of the **iodide** ion greatly limits its general use as a salt former.
- **Mesylate** creates concerns: in fact in alcoholic solutions it could form the alkyl-sulfonate esters, recognized genotoxic impurities

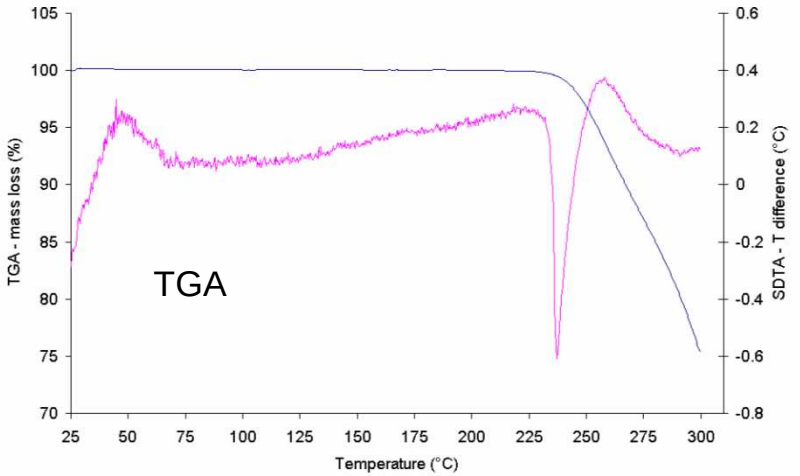
RANKING	SALT	REASON
1	CHLORIDE	Stable and soluble enough, no polymorphism, no safety concerns
2	MESYLATE	Stable and soluble enough, no polymorphism, safety concerns for PGI, difficulties in synthesis of suitable intermediate
3	BROMIDE	Stable, solubility in water and HFA formulations limited, warnings about polymorphism, minor safety concerns
4	IODIDE	Stable, solubility in water and HFA formulations not adequate, warnings about polymorphism, safety concerns

API solid state characterization



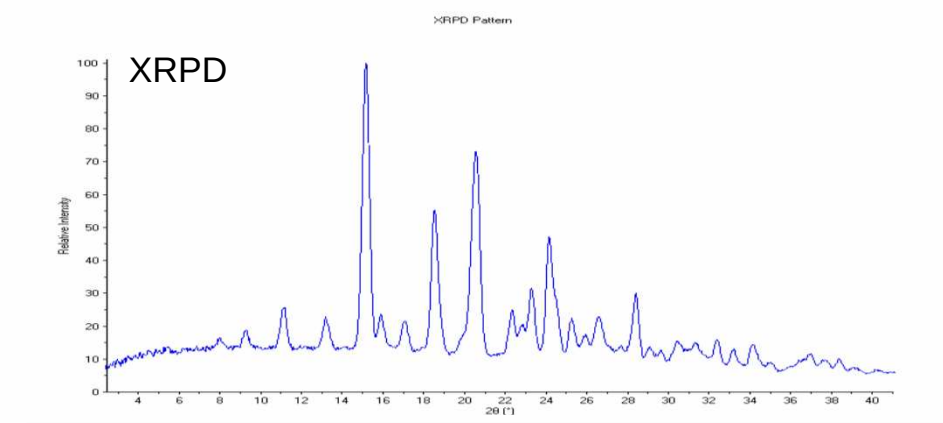


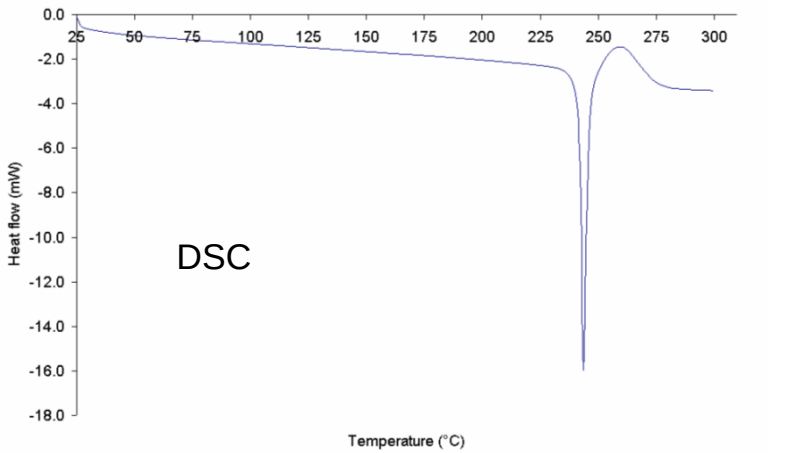


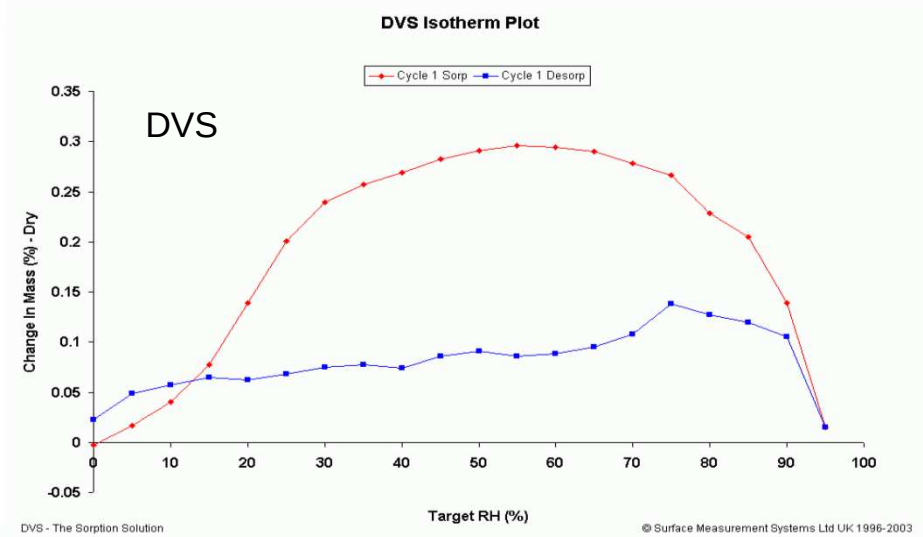


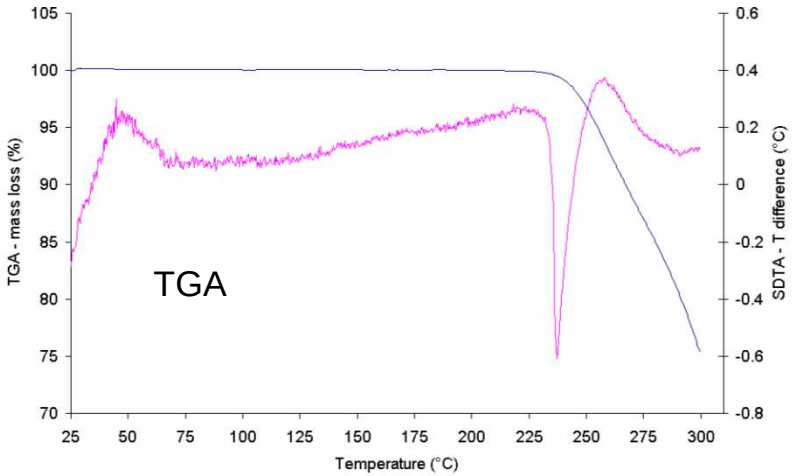
Chiesi

API solid state characterization



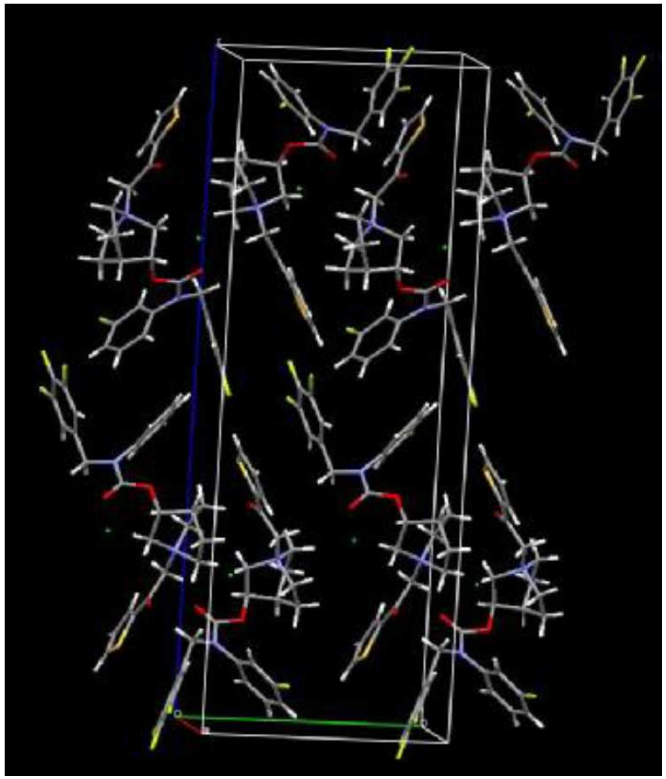






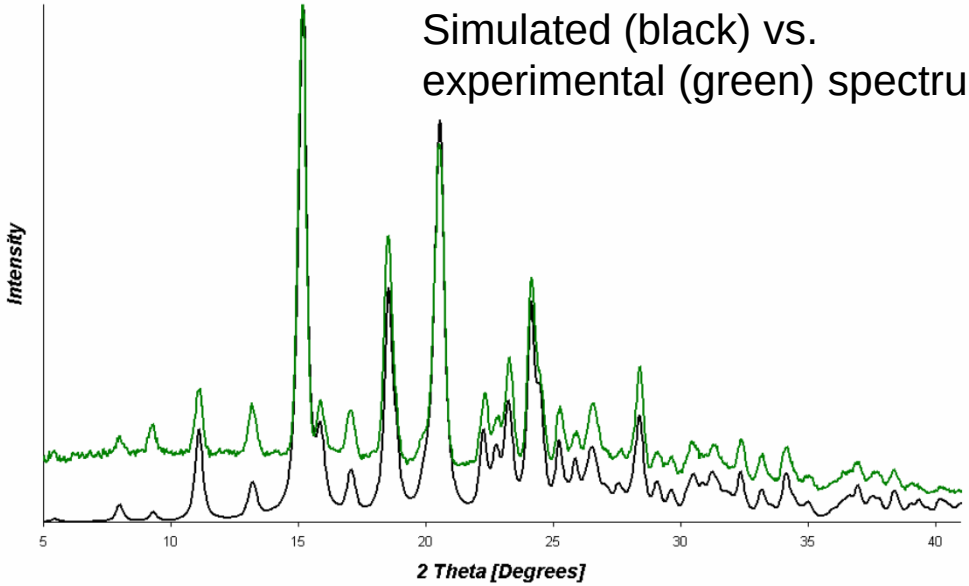
Chiesi

Single crystal XRD analysis: form A



Crystal system Orthorhombic
Space group P 212121
Unit cell dimensions:6.8800(1);
11.7670(2); 32.2920(6)
Z=4

Simulated (black) vs.
experimental (green) spectrum



API polymorph screening

HTPS evaporation-crystallization experiments

- 48 different solvents and solvent mixtures
- 2 concentrations (60mg/ml and 120mg/ml)
- 1 initial temperature (60° C)
- 2 cooling rates (2 and 30° C/h)
- 2 end temperatures (5 and 25° C)
- 2 ageing times (10 and 72 h)

Total: 576 experiments in 6 well-plates * 96 wells

Samples characterization by XRPD, digital images and DSC

RESULT: all solid samples were form A, no polymorphs were found

API polymorph screening

Additional experiments

- Room temperature recrystallizations (RT, 3-7 days, 13 solvents)
- Low temperature recrystallizations (5° C, 1-2weeks, 13 solvents)
- High temperature recrystallizations (60° C, 2-3 days, 13 solvents)
- Slurry experiments (RT, 1 month)
- Crystallization by precipitation (solution at 60° C→-5° C in 24hr)
- Crash cooling (solution at 40° C→-20° C instantaneous)
- Grinding
- Humidity up-take (80%RH, 25° C, 1 week)

Samples characterization by XRPD

RESULT: all solid samples were form A, in case of crash cooling and grinding amorphous material initially obtained spontaneously converted to form A

API crystallization: solvent selection

API should be at least slightly soluble at room temperature, but not very soluble (es. > 50mg/ml) so as to guarantee adequate crystallization yield.

Solubility should strongly increase with increasing temperature, so as to allow complete dissolution of the crude product in a reasonable volume of hot solvent.

The API should be stable in the solvent (mixture) in the T range of interest for adequate time

The solvent should be quite selective versus most of the impurities, so that the crystallization procedure could be effective in improving API purity.

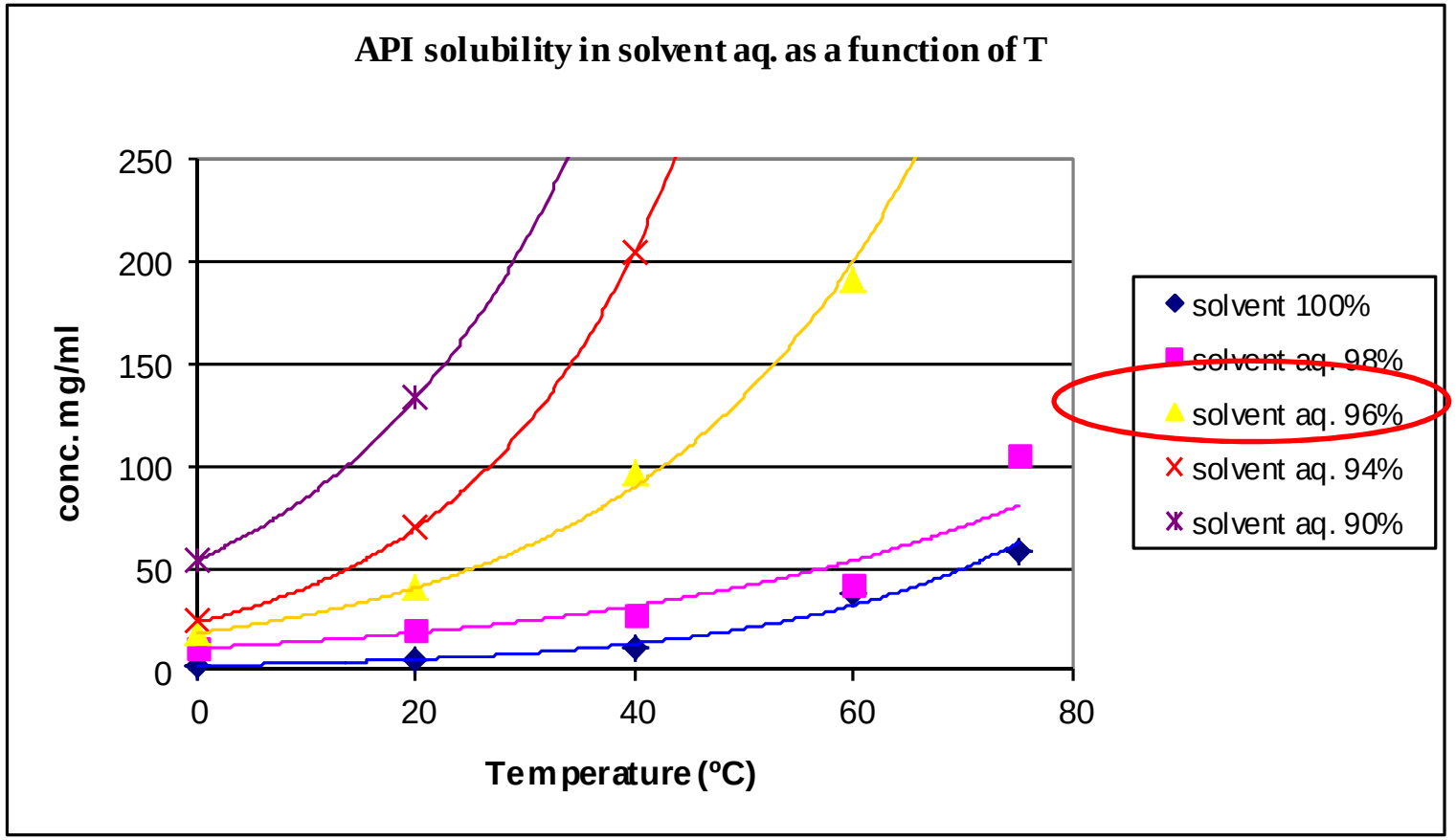
The solvent should possibly be included in the list of class 3 of guideline ICHQ3C so as to minimize toxicity potential.

API crystallization: solubility and solution stability assessment

solvent	Solubility (mg/ml) at T					Purity (60° C, 2hrs)
	0° C	20° C	40° C	60° C	80° C	
water	9,1	18,1	28,0	138,6	na	98,1
2-propanol	2,1	5,7	11,0	37,7	57,8	97,8
1-propanol	79,2	111,7	113,7	138,8	na	
EtOAc / EtOH 90/10 v/v	23,9	23,5	24,3	25,0	26,7	97,9
EtOAc / EtOH 80/20 v/v	120,1	113,5	127,6	138,3	149,2	
acetone/water 97,5/2,5 v/v	112,0	106,5	124,1	141,8	na	
MEK / EtOH 90/10 v/v	98,8	94,4	107,0	114,1	123,6	97,9
MEK / EtOH 80/20 v/v	204,6	217,2	251,4	264,5	323,8	
MTBE / MeOH 90/10	n.a.	5,0	5,4	7,2	na	

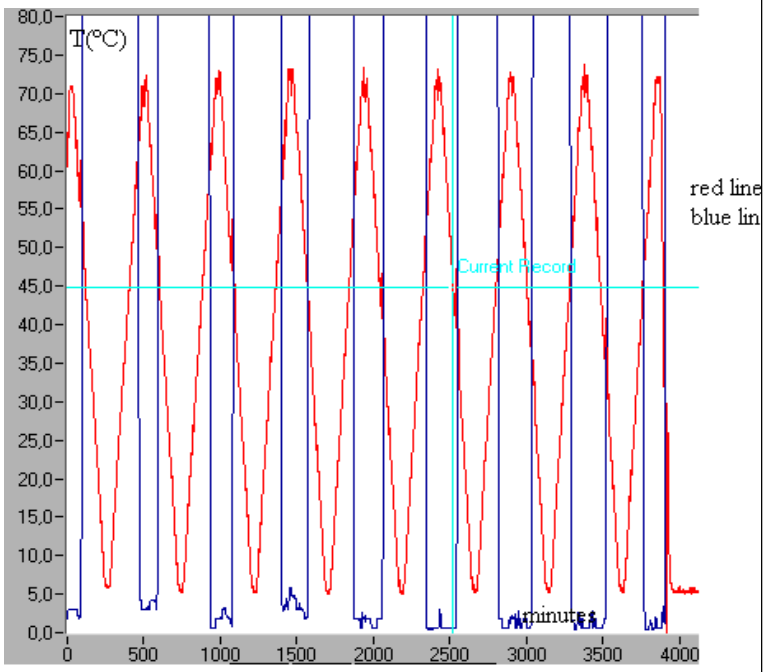
API crystallization: solubility as a function of temperature

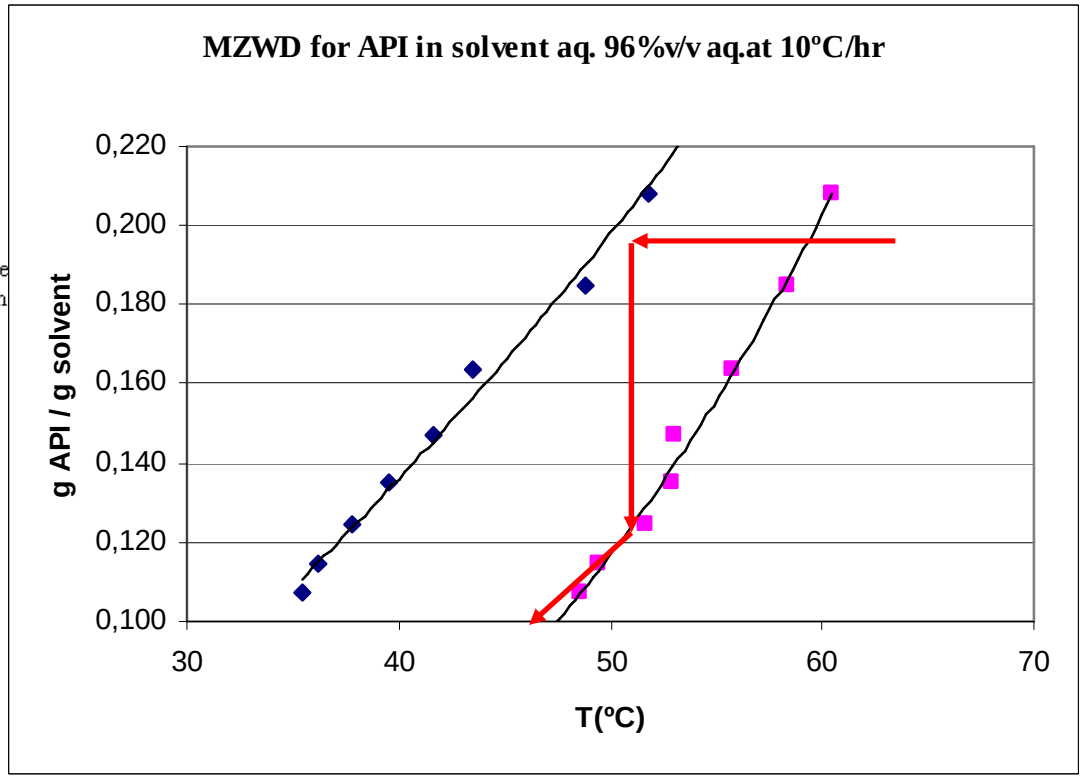
API solubility in 2-propanol/water system



Chiesi

API crystallization: metastable zone width determination



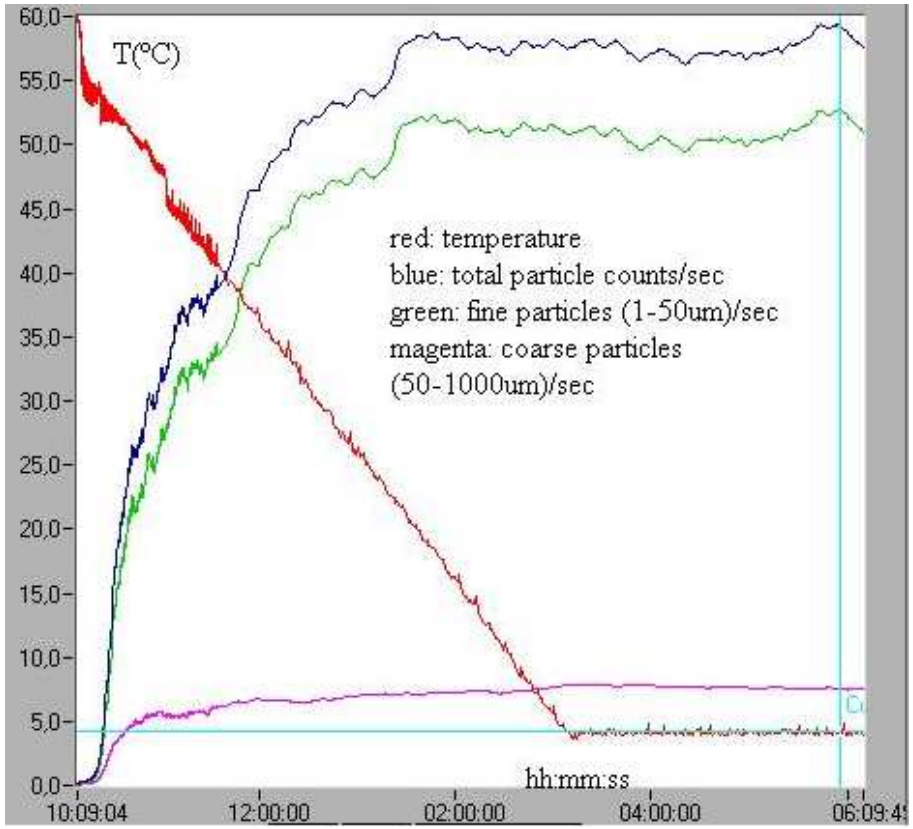


Solvent: 6 volumes of 2-propanol aq. 96%, dissolution complete at T>60° C, cool at T=50-55° C

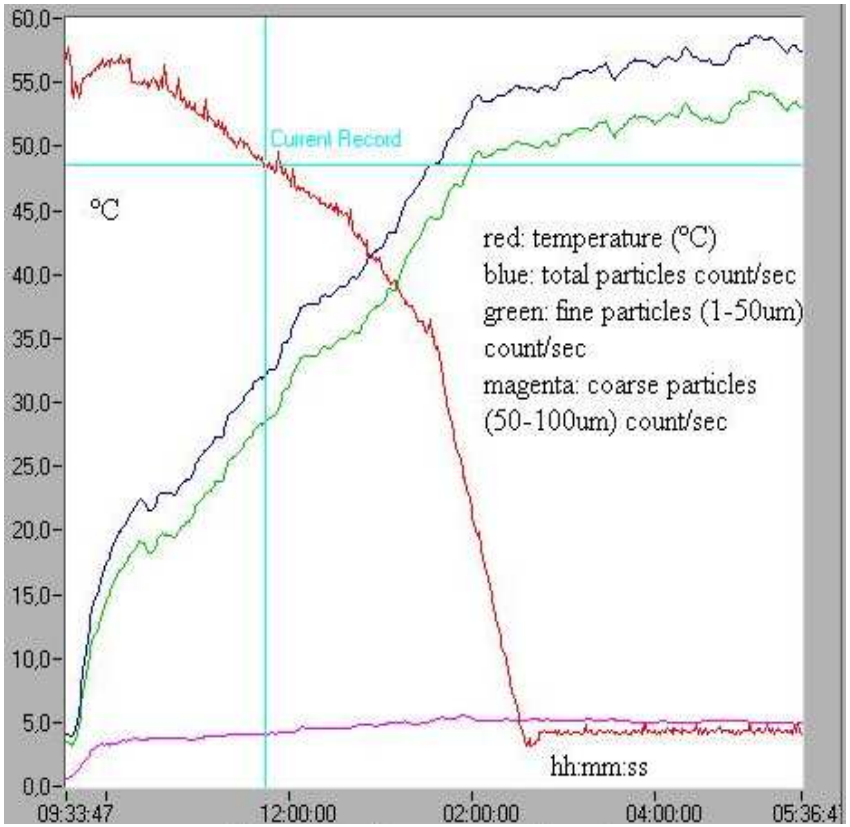
Chiesi

API crystallization: cooling profile, tests with Lasentec FBRM®

1: slow (10° C/hr) linear cooling



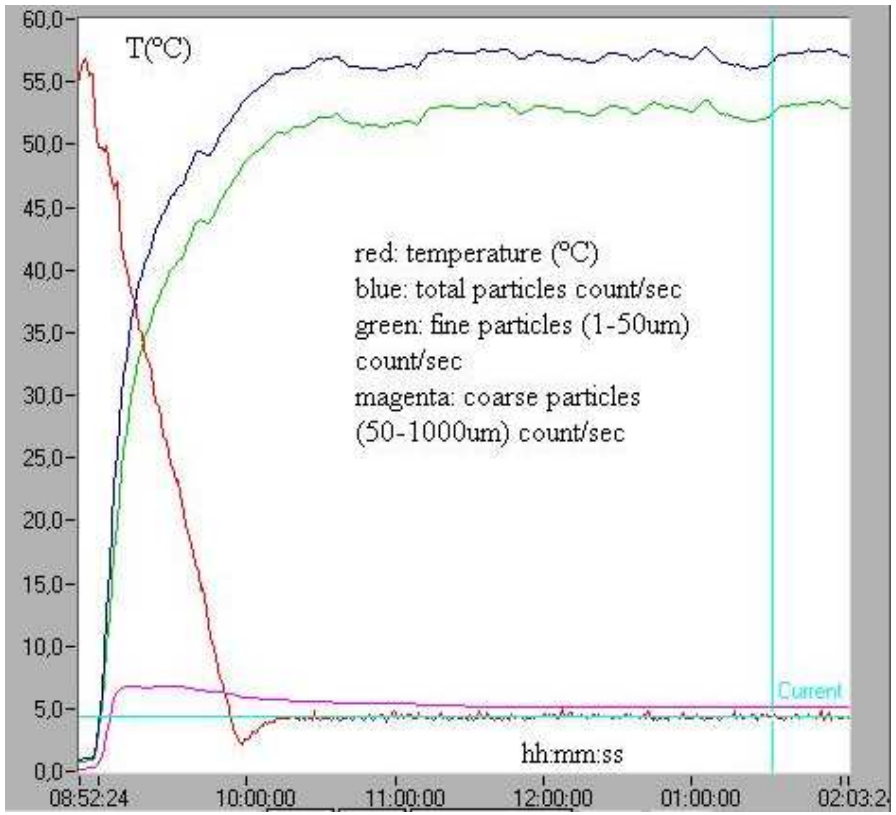
2: "cubic", plateau at 40-50° C



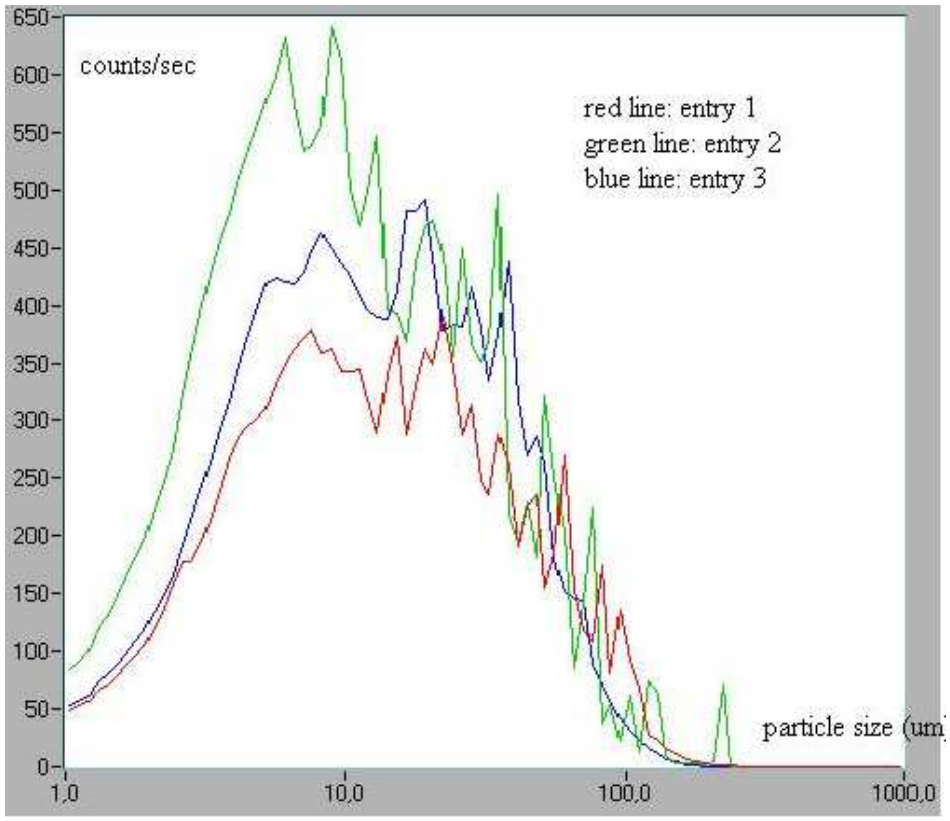
Chiesi

API crystallization: cooling profile, tests with Lasentec FBRM®

3: fast (50° C/hr) linear cooling



Final particle size distributions



Chiesi

API crystallization: final tests

entry	Cooling profile	Filtration (fast/slow)	solvent retained in wet solid	T drop at drying	Time needed to reach 50° C u.v.	yield
1	slow (10ºC/hr)	Fast	8,1 %	6 ° C	90 min	86,4
2	"cubic"	Fast	0,03 %	0 ° C	45 min	85,8
3	fast (50ºC/hr)	fast	4,5 %	2 ° C	90 min	87,6

entry	cooling profile	ref.	API purity	impA	int 3	impB	impC	impD	impE	imp F
crude API		K097560	98,1	1,1	n.d.	0,12	0,05	0,20	0,36	n.d.
1	slow (10ºC/hr)	Cryst API *(mother liq.)*	99,0 *90,0*	0,38 *8,0*	n.d. *n.d.*	n.d. *1,2*	0,04 *0,12*	0,21 *0,18*	0,38 *0,13*	n.d. *n.d.*
2	"cubic"	Cryst API *(mother liq.)*	99,0 *91,0*	0,33 *7,2*	n.d. *n.d.*	n.d. *1,0*	0,04 *0,11*	0,21 *0,18*	0,40 *0,13*	n.d. *n.d.*
3	fast (50ºC/hr)	Cryst API *(mother liq.)*	98,9 *92,3*	0,50 *6,1*	n.d. *n.d.*	0,01 *1,0*	0,04 *0,10*	0,21 *0,18*	0,40 *0,12*	n.d. *n.d.*

Final prescription: keep at 50° C for 1 hr or until nucleates (seeding), then cool to 5° C in 2 hrs