Evaporation-controlled Automated Embedding and Polymerization

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Traditional embedding

Standard Aldehyde and OsO_4 fixed specimen

Replace water with solvent

Freeze sub specimen

Replace solvent with resin

Refresh resin

Polymerize resin

Time involved at least 24-48hrs

Considerations

- resins are ‘sticky’
- exposure to hazardous/carcinogenic chemicals
- tedious handling # specimens (a.o. duplicates)
- cross contamination risk
- interrupts other activities
- boring, yet requires attention
- risk of specimen loss in final steps
- hazardous waste

How to improve?

Don’t exchange dilute resin for more concentrated mixtures/pure resin

but instead

Concentrate the resin by evaporating solvent

Until all solvent is removed: pure resin

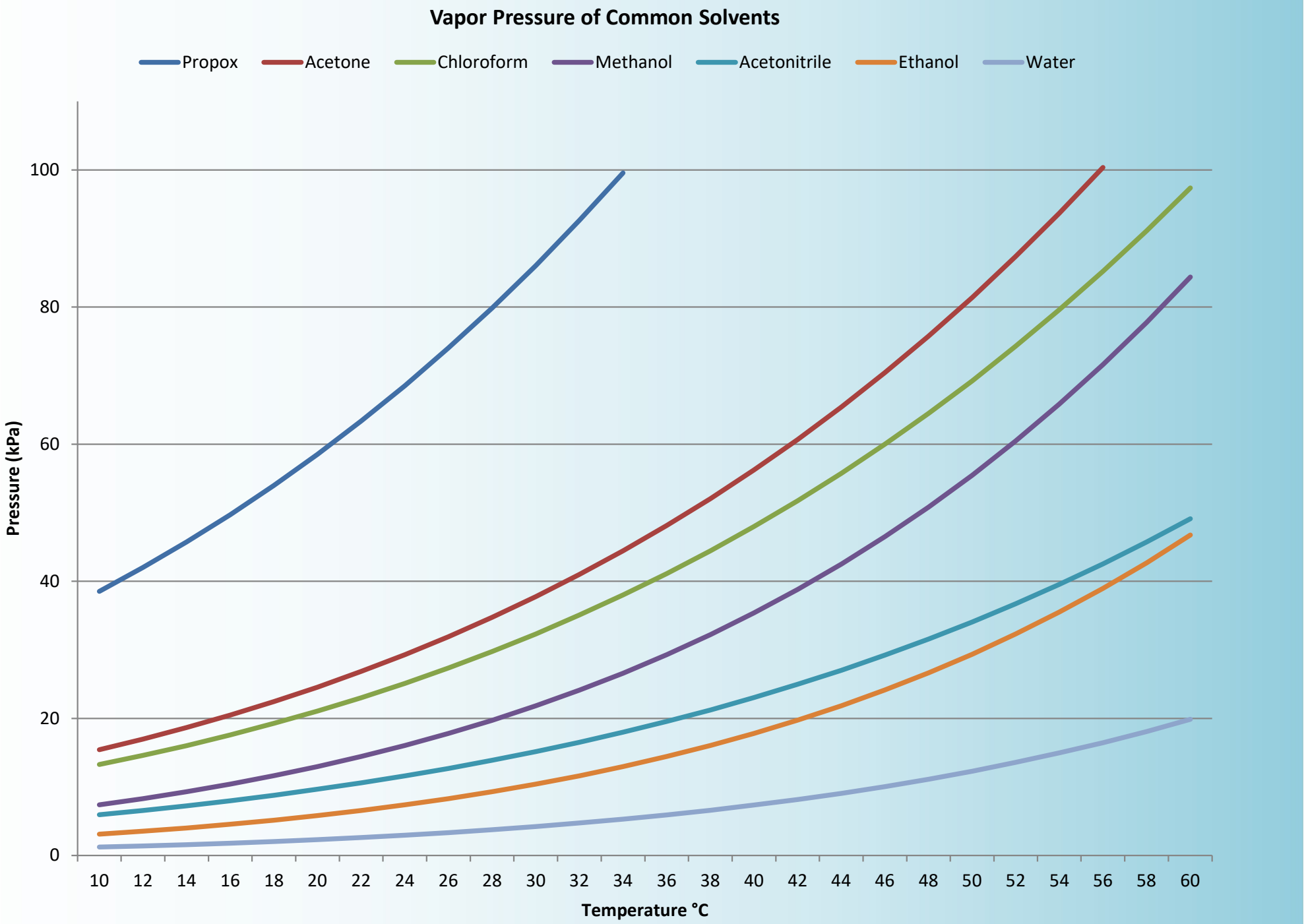
Set Temperature – Set Vacuum – Air/N2 Flush

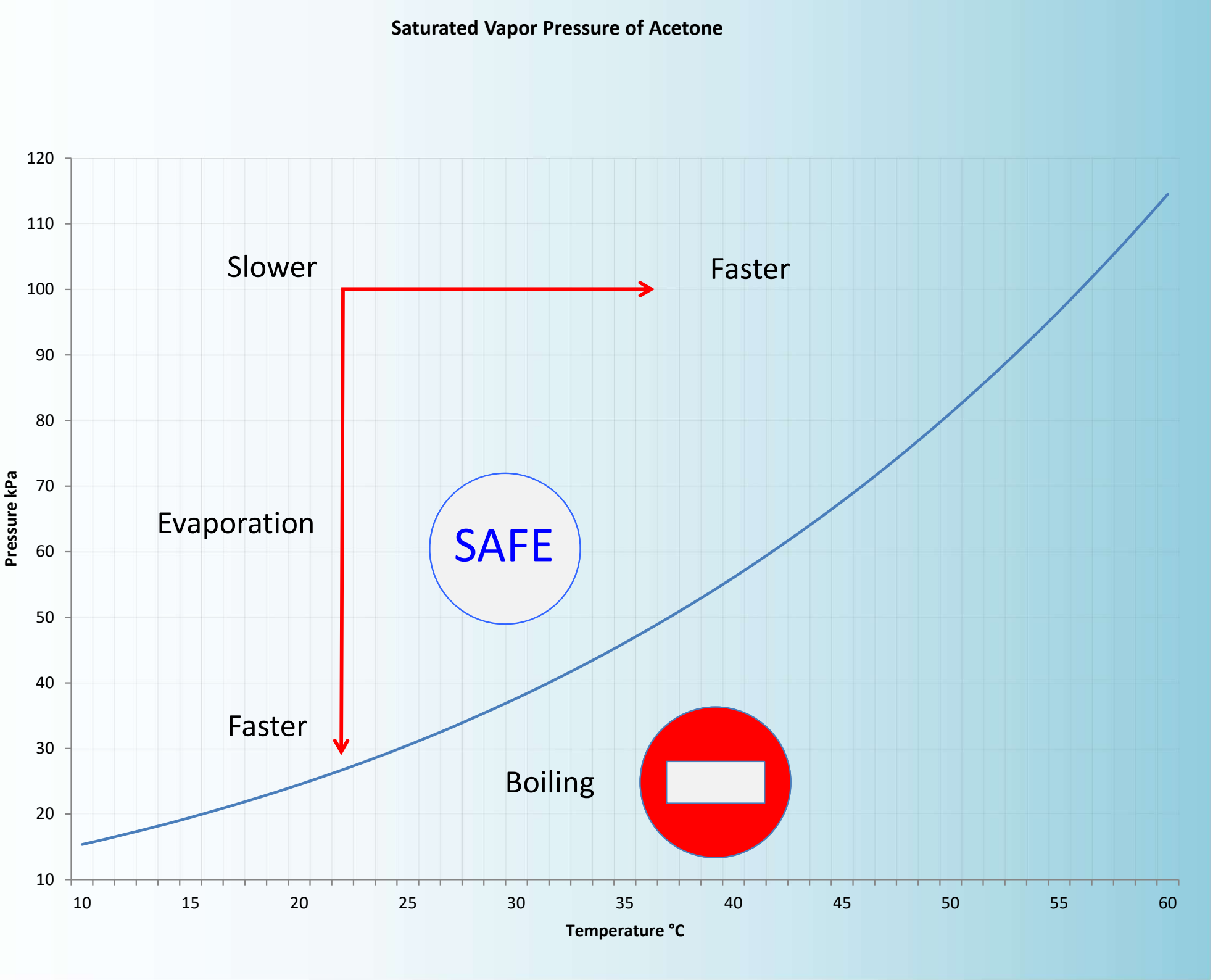
Critical: Vacuum Pressure

!!At pressures below the saturated vapor pressure the solvent will boil!!

Therefore at any given temperature:

the vacuum pressure should NEVER be significantly lower than the saturated vapor pressure at that temperature


Vapor Pressure of Common Solvents
Propox
Acetone
Chloroform
Methanol
Acetonitrile
Ethanol
Water
Pressure (kPa)
0
20
40
60
80
100
10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
Temperature °C


Saturated Vapor Pressure of Acetone
Pressure kPa
120
110
100
90
80
70
60
50
40
30
20
10
10
15
20
25
30
35
40
45
50
55
60
Temperature °C
Slower
Faster
Evaporation
SAFE
Faster
Boiling

While we're at it…can we…

(i) automate embedding from the first solvent/resin step up to pure resin, and polymerization,

(ii) reduce solvent and resin use with no waste, except what is left in the mixing vials,

(iii) minimize exposure to hazardous and irritating chemicals,

(iv) reduce hands-on time to the bare minimum,

(v) facilitate the processing of a sufficiently large number of samples in one instrument run and

(vi) prevent cross-contamination/specimen loss.

EMS poly III

Evaporation-Controlled Automated Embedding and Polymerization

EMS poly III

Evaporation-Controlled Automated Embedding and Polymerization

3 programs for embedding (solvent parameters)
2 programs for polymerization (resin parameters)

Fully user programmable

All programs:
5 steps with both global
and individual parameter settings

EMS poly III

Evaporation-Controlled Automated Embedding

Overview of Program Steps

Step 1 (S1): removal of gas bubbles, moderate vacuum, short time
Step 2 (S2): removal of bulk of solvent,
when done auto-proceed to next step
Step 3 (S3): removal of trace amounts of solvent,
using a fixed number (10) of flushing cycles
with fresh air/N2 gas
Steps 4 (S4) and 5: as Step 3, especially for less volatile solvents

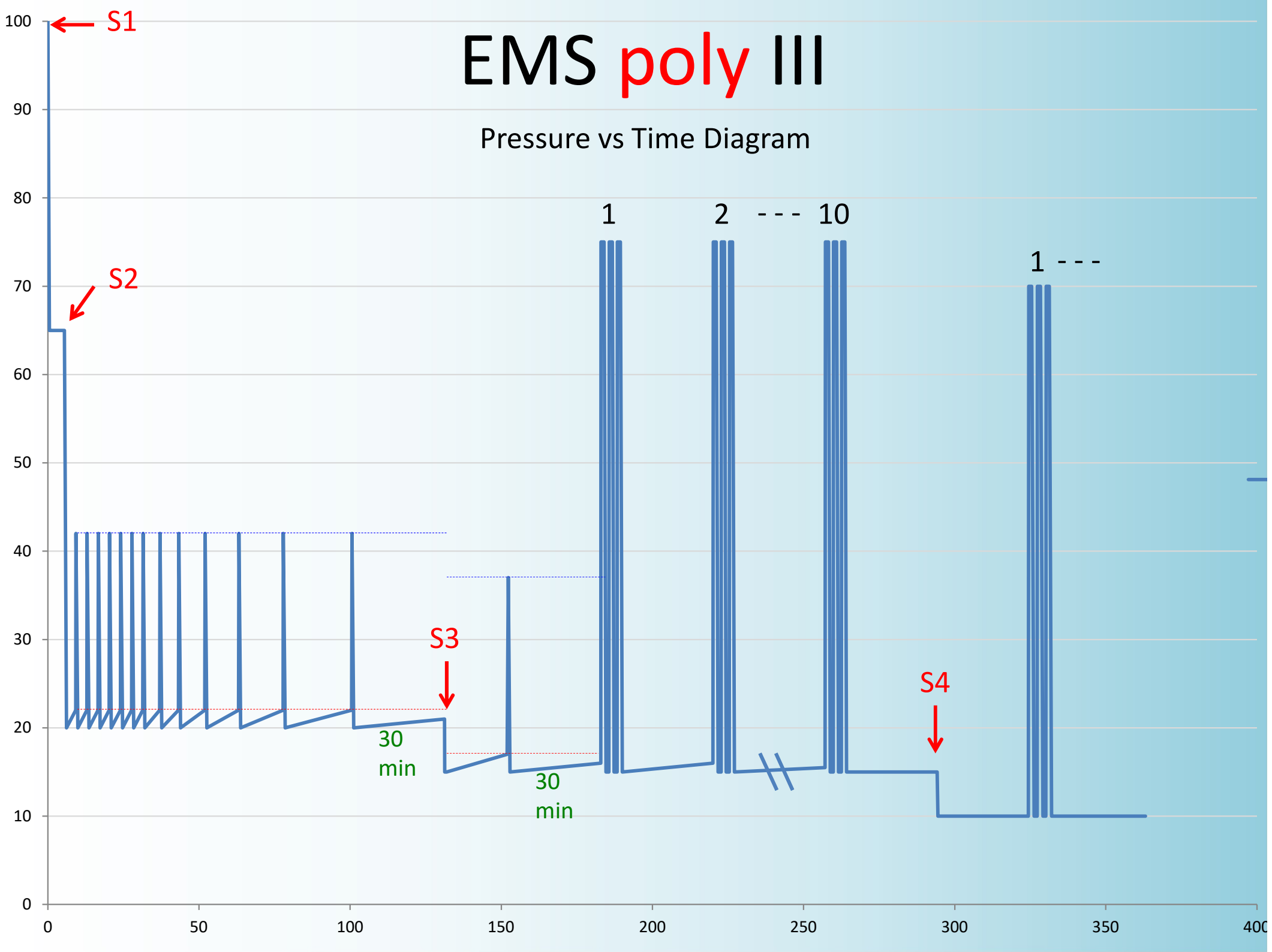
EMS poly III

Evaporation-Controlled Automated Embedding

Parameters

Global: user programmable pressure triggers
for gas atmosphere cleanup by venting (ΔP1)
for pumping ON and diluted vapor removal (ΔP2)
next Step trigger (IT>30min) is factory set
flushing cycles with fresh air/N2 gas, factory set

Individual: temperature, vacuum pressure, time


EMS poly III
Pressure vs Time Diagram
S1
S2
S3
S4
1
2 - - - 10
1 - - -
30 min
30 min
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
400

EMS poly III

In detail Step 2

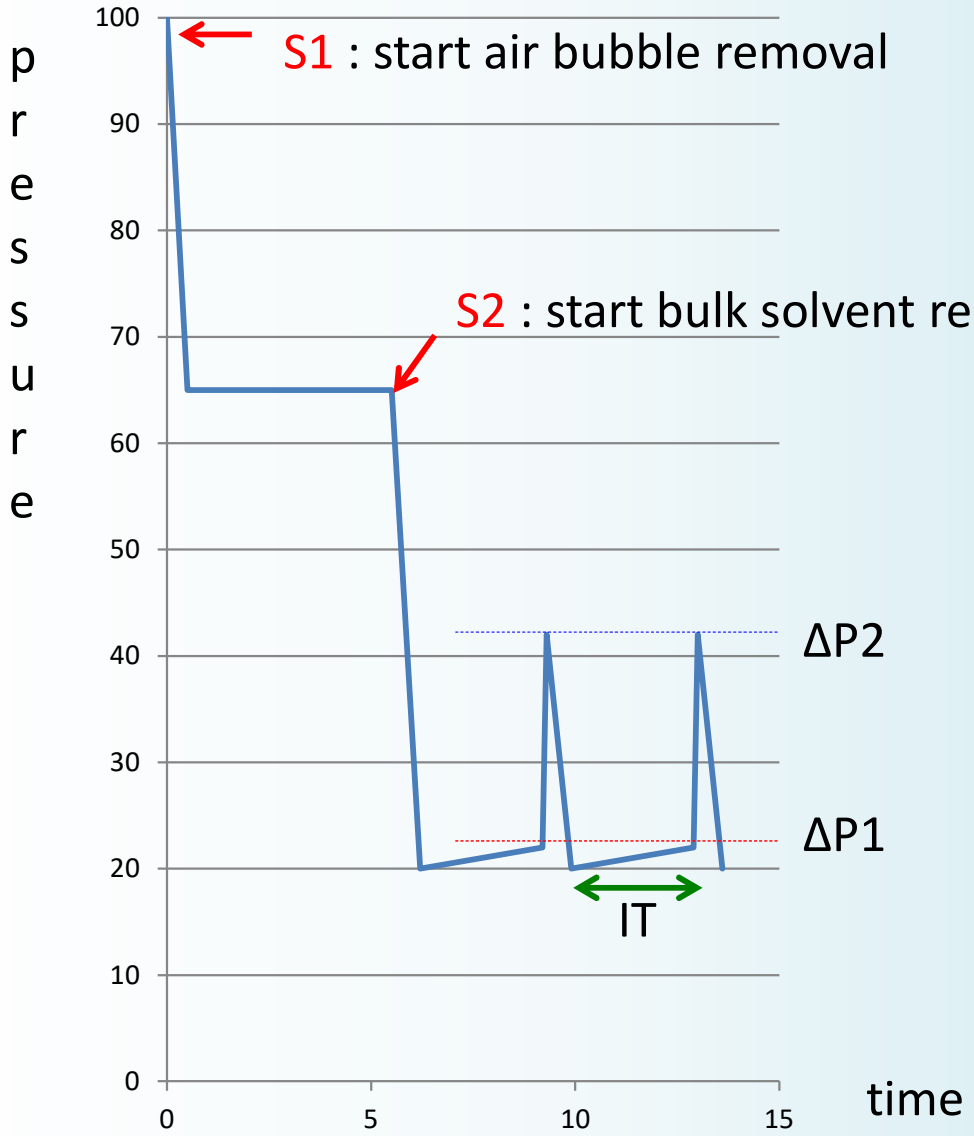
Step 2 (S2): remove bulk of solvent, then auto-proceed to next step

Set temperature (T)
Pump down to pressure P just above the saturated vapor pressure (no boiling!)
Solvent evaporates → Pressure increases
When pressure increase exceeds a preset value ΔP1
(indicated by red dotted line in previous slide)
Air is admitted (solvent gas is diluted) until a preset value ΔP2
(indicated by blue dotted line in previous slide)
And the pump is activated until pressure is P

This procedure is repeated
until the waiting time between pump cycles exceeds 30 min
then proceed automatically to Step 3 (S3)

EMS poly III

In detail Step (1 and) 2



! Only the first two evaporation cycles are shown in the graph!

When IT > 30 min
Proceed to Step 3

EMS poly III

In detail Step 3 (4 and 5)

Step 3 (S3): removal of trace amounts of solvent, then auto-proceed to next step

Temperature and Pressure setting (P) as close as possible to saturated vapor pressure,
or slightly below, to facilitate evaporation
Setup as Step 2
Continue until the interval between pump cycles exceeds 30 min….
Start Flush cycle:
[Air admit while evacuating (flush, gas ballast) for 45 secs
with intermediate vacuum for 45 secs]
3 cycles, then allow ~60 min evaporation at chosen pressure P]

Total number of gas ballast cycles: 10

When finished auto-proceed to next Step or Finish

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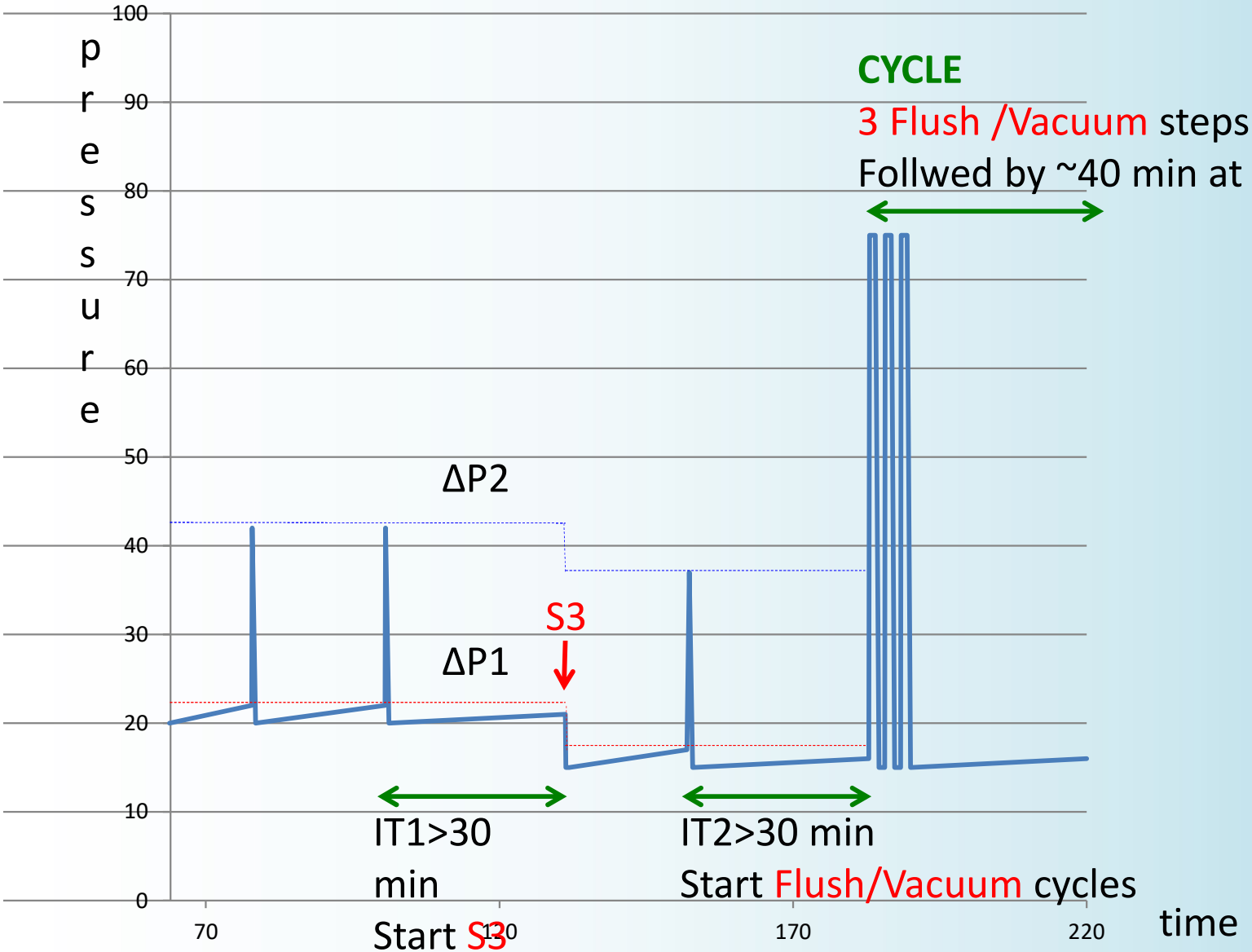
In detail Step 3 (4 and 5)



CYCLE

3 Flush /Vacuum steps

Follwed by ~40 min at S3 Pressure

The cycles are repeated 9 times to remove residual solvent

↓

Step 4 (optional)

↓

Step 5 (optional)

EMS poly III

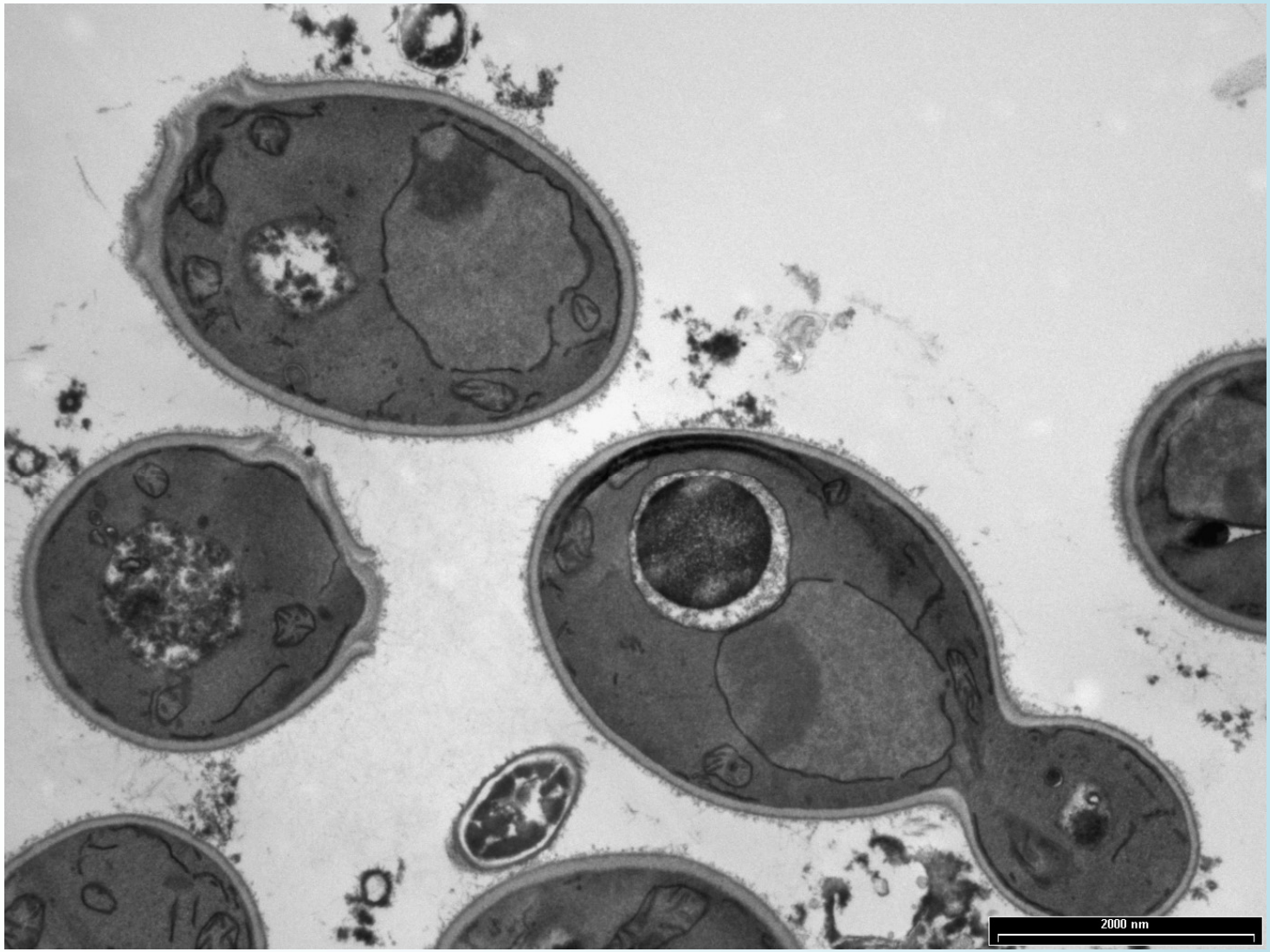
Polymerization

At end of embedding program 1, 2 or 3:

top up BEEM capsules with pure resin
or
transfer tissue blocks into silicone molds with resin
or
leave as small resin volume

Polymerize using programs 4 or 5

Cider yeast processed with EMS polyIII

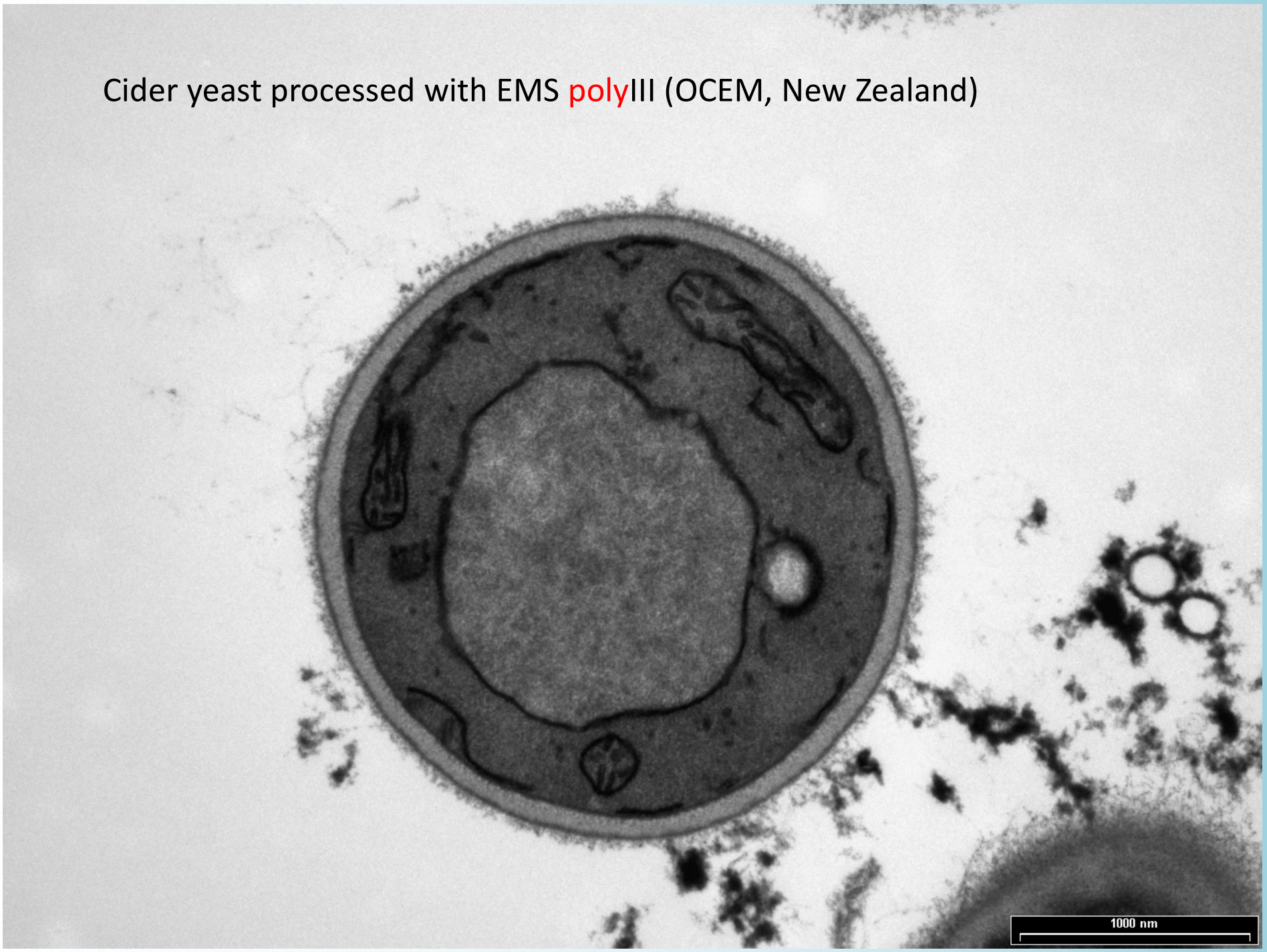


1% GA/caco
1% KMnO4/dw

Acetone dehydration

EMS polyIII
Embedded
In epoxy resin

Cider yeast processed with EMS polyIII (OCEM, New Zealand)



EMS poly III

- No exchanges of resin or solvent/resin mix
- Fully automated, except top-up with resin
- Minimum solvent and resin use
- Environment-conscious: no resin waste
- Minimal exposure to chemicals
- Minimum hands-on time
- Up to 52 specimens in one go
- For BEEMS and Silicon molds
- No specimen loss
- Reproducible results
- Ideal for multi-user facility