

**Implementation of a Process Analytical Technology (PAT) System in a Freeze Drying  
Process Using Raman Spectroscopy for In-Line Process Monitoring**

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**SUPPORTING INFORMATION**

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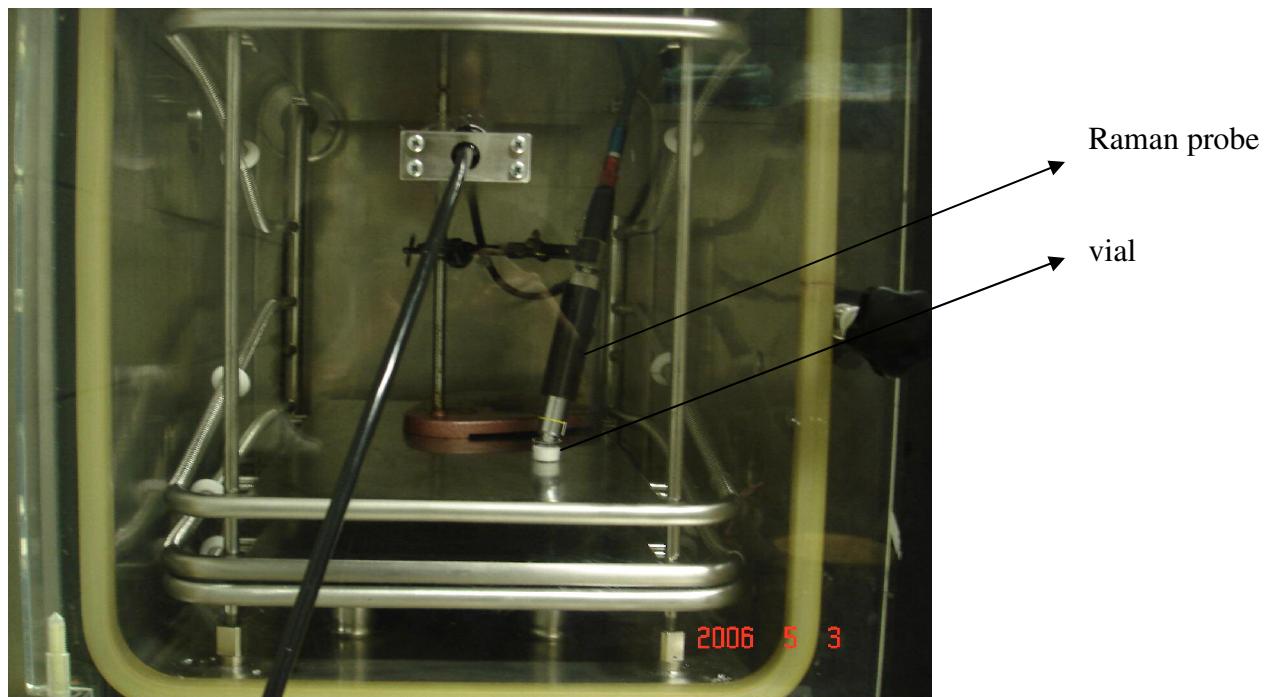
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**Supporting Information Figures: 7 (figure S-1 to figure S-7)**

**Supporting Information Tables: 3 (table S-1 to table S-3)**

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



**Figure S-2.** Raman spectra of mannitol recorded during the freezing stage of a freeze drying process (experiment 1)

Figure S-2a: spectra 1-36

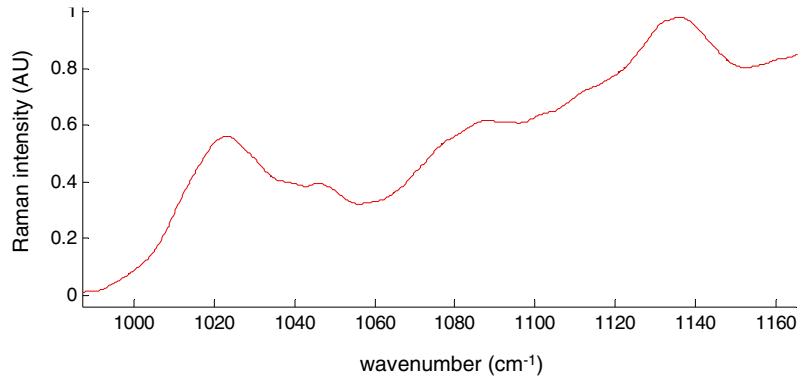


Figure S-2b: spectra 37-51

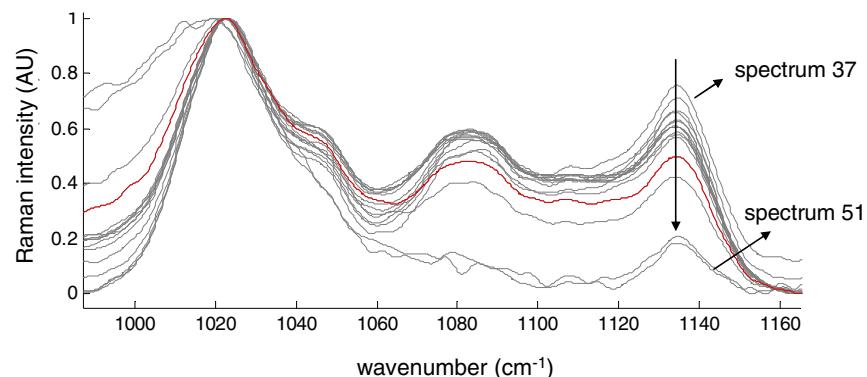


Figure S-2c: spectra 51-61

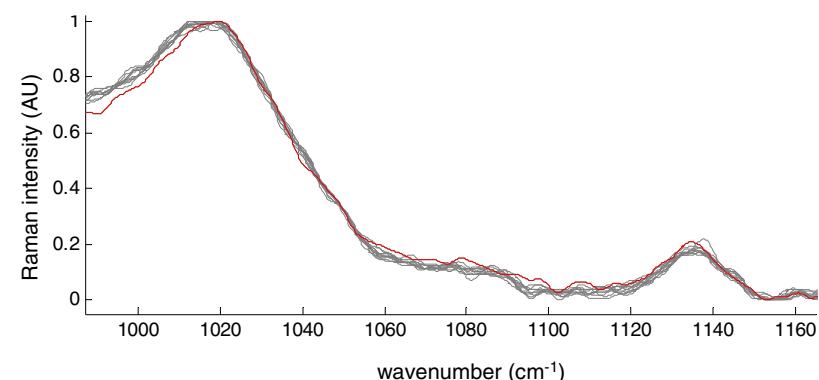


Figure S-2d: spectra 62-106

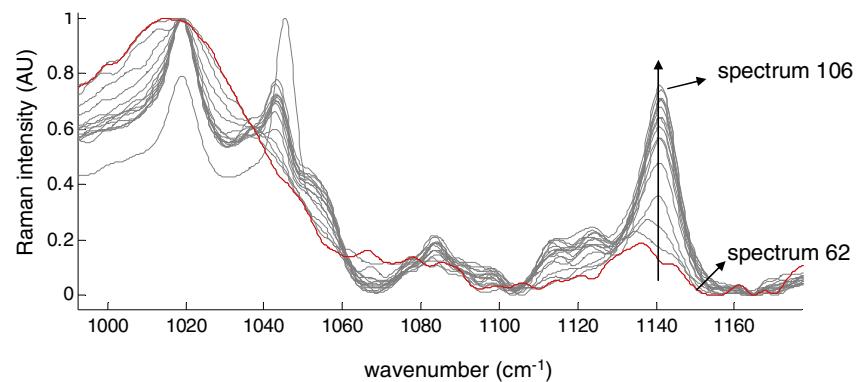
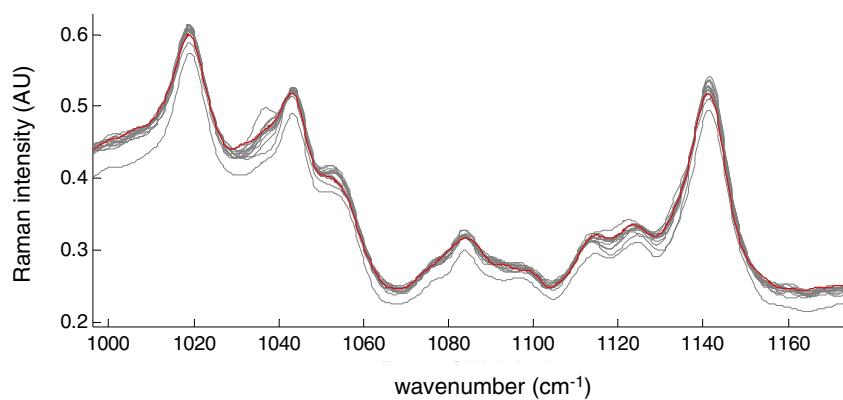


Figure S-2e: spectra 107-175



**Figure S-3.**

Fig. S-3a: Ice produces Raman signal at  $215\text{ cm}^{-1}$

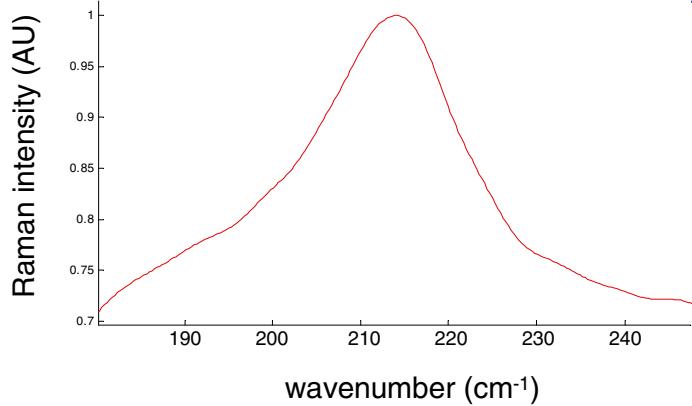
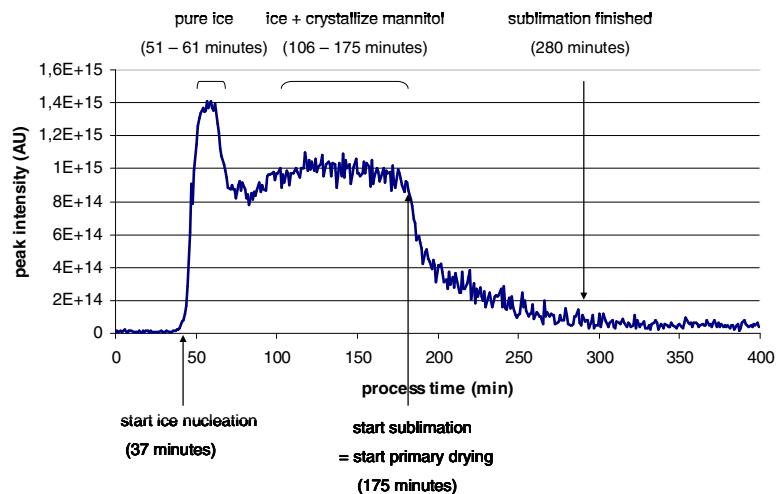
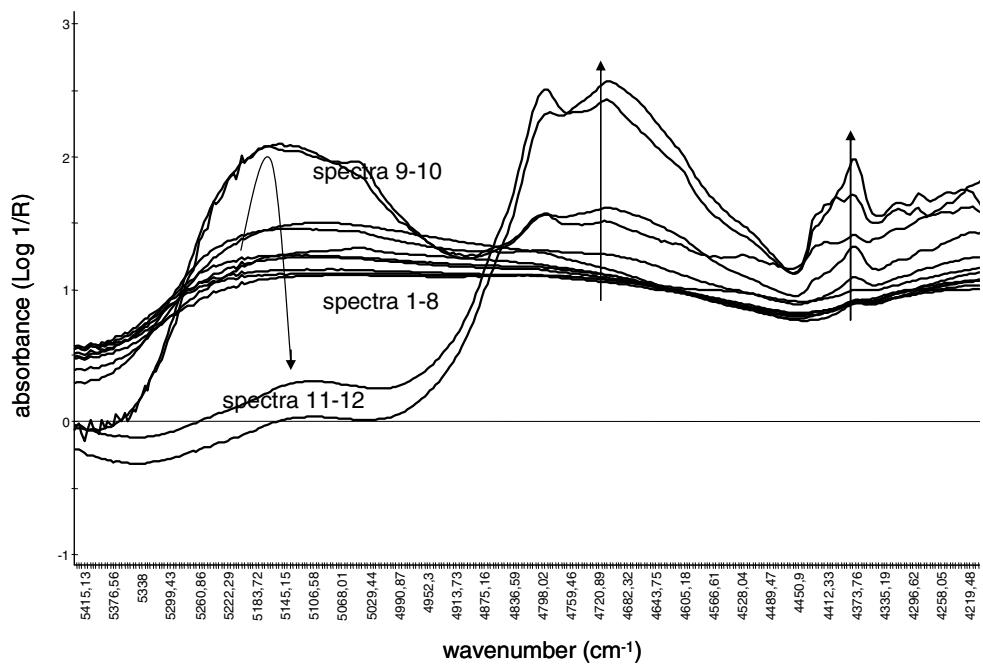


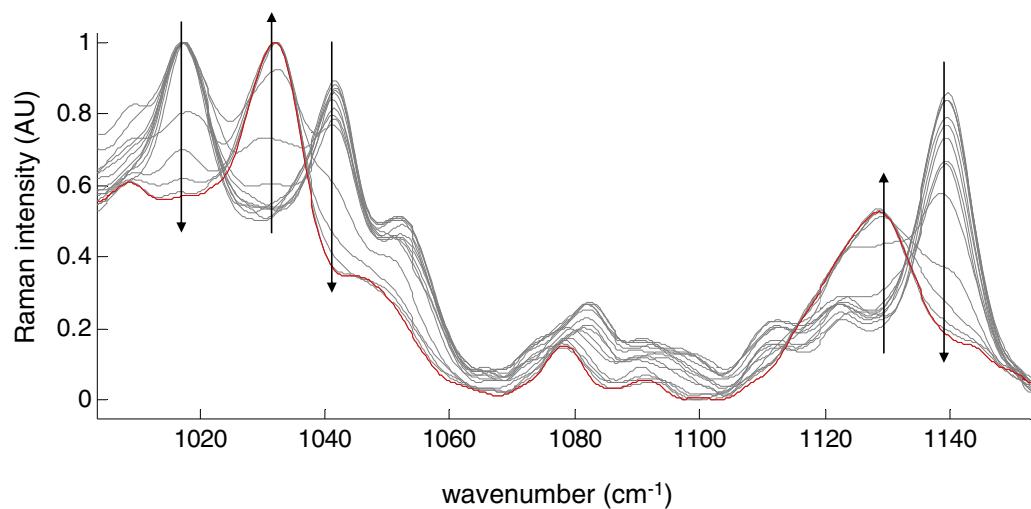
Fig. S-3b: peak intensity ice band vs. process time



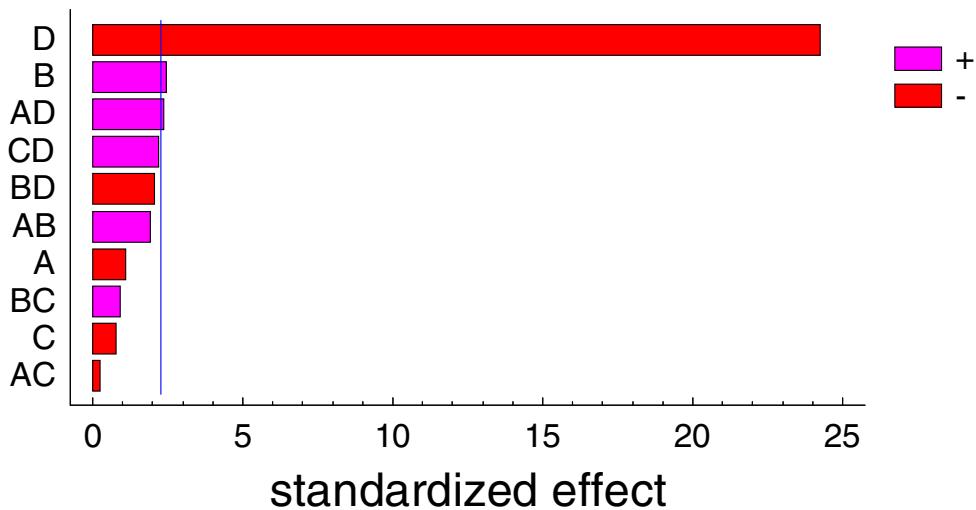
**Figure S-4.** NIR spectral transformations during ice sublimation



**Figure S-5.** Raman spectral transformation to  $\alpha$ -mannitol during secondary drying at 30°C in experiment 9



**Figure S-6.** Standardized Pareto chart for the response variable ‘onset ice nucleation’ ( $\alpha = 0.05$ )



*A = concentration of mannitol*

*B = concentration of NaCl*

*C = volume*

*D = freezing rate*

**Figure S-7.** Screening design analysis. The units of the y-axis in all plots are minutes.

Figure S-7a: duration of ice crystallization process

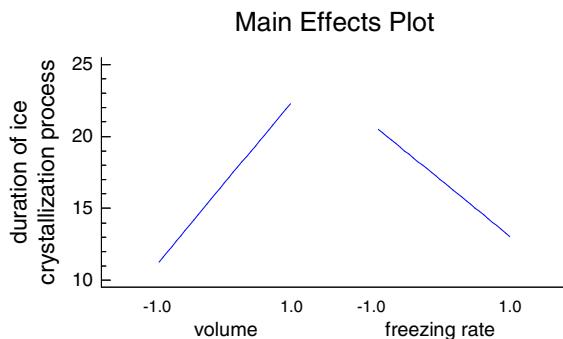


Figure S-7b: onset of mannitol crystallization

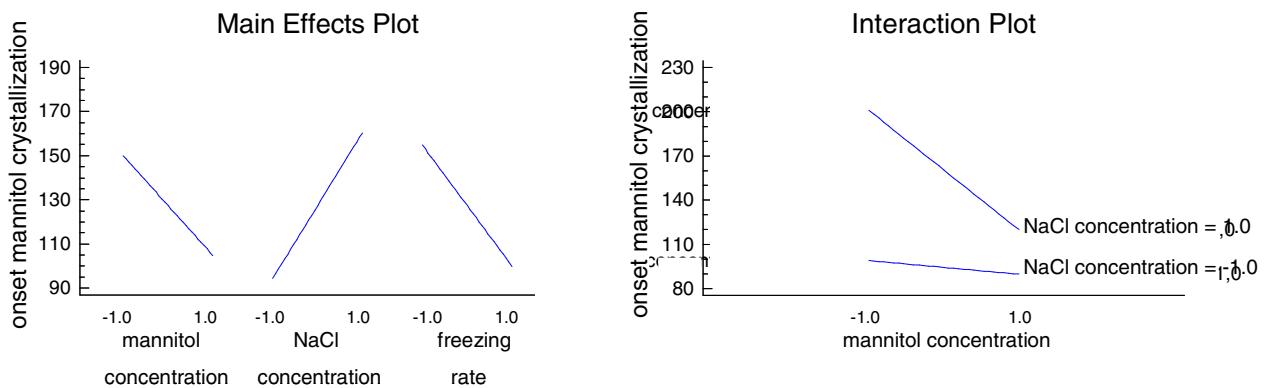


Figure S-7c: duration of mannitol crystallization process

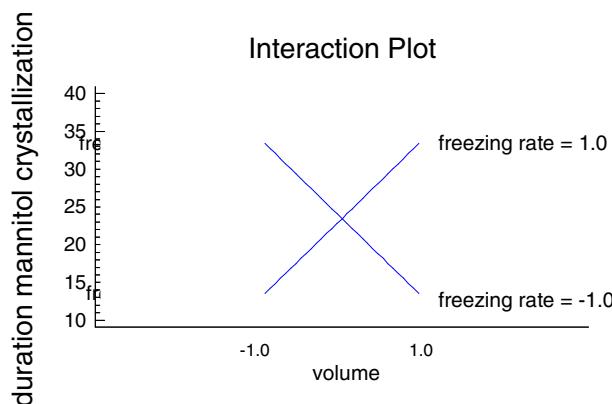


Figure S-7d: duration of sublimation process

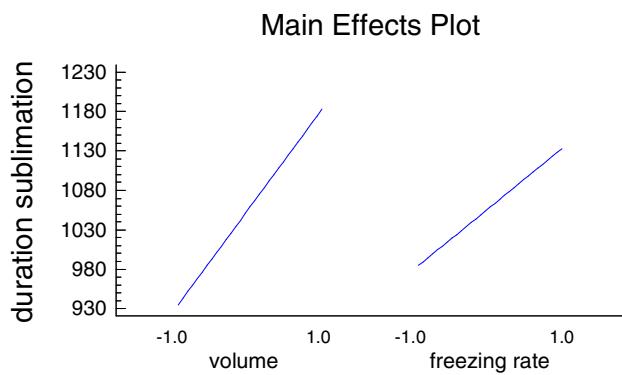
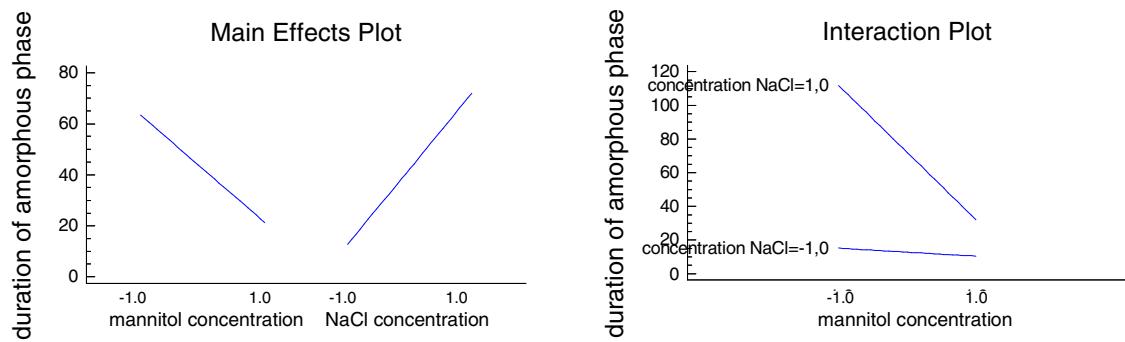


Figure S-7e: duration of amorphous mannitol state



**Table S-1.** Freeze drying programs (fast and slow freezing) used for the experimental design experiments

process phase	time (min)		temperature shelves in freeze drier (°C)		pressure in freeze drier (mbar)
	slow freezing	fast freezing	slow freezing	fast freezing	
freezing	0	0	20	20	1000
	5	5	2	2	1000
	25	25	-5	-5	1000
	120	30	-25	-45	1000
	145	145	-40	-45	1000
	165	165	-45	-45	1000
primary drying	175	175	-15	-15	0.8
secondary drying	977	977	0	0	0.1
	1097	1097	10	10	0.1
program end	1517	1517	10	10	0.1

**Table S-2.** Overview of screening design experiments. The experiments in bold were repeated one time.

experiment number	mannitol concentration (%)	NaCl concentration (%)	volume (ml)	freezing rate
<b>1</b>	<b>5</b>	<b>0</b>	4	fast
2	15	0	4	slow
3	5	2	4	slow
4	15	2	2	slow
5	15	2	4	slow
<b>6</b>	<b>15</b>	<b>0</b>	4	fast
7	<b>5</b>	<b>0</b>	4	slow
<b>8</b>	<b>15</b>	<b>2</b>	4	fast
9	5	0	2	slow
10	15	0	2	slow
11	15	0	2	fast
12	5	0	2	fast
13	5	2	2	fast
14	5	2	2	slow
15	5	2	4	fast
16	15	2	2	fast

**Table S-3.** Overview of the mannitol solid states in the end products from all screening design experiments

experiment number	Raman analysis	NIR/XRPD analysis
1	$\beta$ + hemi-hydrate	$\beta$ + $\delta$ + hemi-hydrate
2	$\delta$ + hemi-hydrate	$\beta$ + hemi-hydrate
3	$\beta$ + $\delta$ + hemi-hydrate	$\beta$ + $\delta$ + hemi-hydrate
4	$\beta$	$\delta$ + hemi-hydrate
5	$\beta$ + hemi-hydrate	$\beta$ + $\delta$ + hemi-hydrate
6	$\beta$ + hemi-hydrate	$\beta$ + hemi-hydrate
7	hemi-hydrate	hemi-hydrate
8	$\beta$ + hemi-hydrate	$\delta$ + hemi-hydrate
9	hemi-hydrate	hemi-hydrate
10	hemi-hydrate	$\beta$ + hemi-hydrate
11	$\beta$ + hemi-hydrate	$\beta$ + $\delta$ + hemi-hydrate
12	$\beta$ + $\delta$ + hemi-hydrate	$\beta$ + $\delta$ + hemi-hydrate
13	$\delta$ + hemi-hydrate	$\delta$ + hemi-hydrate
14	$\beta$ + $\delta$	$\alpha$ + $\delta$ + hemi-hydrate
15	$\delta$	$\beta$ + $\delta$ + hemi-hydrate
16	$\beta$	$\beta$ + $\delta$ + hemi-hydrate
6 BIS	$\beta$ + hemi-hydrate	$\beta$ + hemi-hydrate
7 BIS	hemi-hydrate	hemi-hydrate
1 BIS	$\beta$ + $\delta$ + hemi-hydrate	$\beta$ + $\delta$ + hemi-hydrate
8 BIS	$\beta$ + hemi-hydrate	$\beta$ + $\delta$ + hemi-hydrate