

Electronic Supplementary Information (ESI) for

Mechanisms of crystallisation in polysorbates and sorbitan esters

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Table S1: Pharmacopoeia, Ph. Eur 9.0, specifications for PS20, PS40, PS60 and PS 80, as well as USP35-NF30 specifications for PS 80.

Fatty acid	PS20	PS40	PS60	PS80
Caproic acid	≤ 1.0 %	---	---	---
Caprylic acid	≤ 10.0 %	---	---	---
Capric acid	≤ 10.0 %	---	---	---
Lauric acid	40.0-60.0 %	---	---	---
Myristic acid	14.0-25.0 %	---	---	≤ 5.0 %
Palmitic acid	7.0-15.0 %	≥ 92.0 %	+ (*)	≤ 16.0 %
Palmitoleic acid	---	---	---	≤ 8.0 %
Stearic acid	≤ 7.0 %	---	40.0-60.0 %	≤ 6.0 %
Oleic acid	≤ 11.0 %	---	---	≥ 58.0 %
Linolenic acid	---	---	---	≤ 4.0 %
Linoleic acid	≤ 3.0 %	---	---	≤ 18.0 %

(*) Sum of Palmitic acid and Stearic acid ≥ 90.0 %.

Table S2: Definitions of samples tested in this study.

Product	Manufacturer	Purity Grade	Batch number
PS80	KLK OLEO	ST	2427146
	CRODA	ST	0501UC1909
	CRODA	HP	1176143
	CRODA	SR	1186606
	NOF	UP	704352
PS60	CRODA	ST	MKBX0810V
PS40	CRODA	ST	MKCD4849
PS20	KLK OLEO	ST	2431131
	CRODA	ST	0502TD1419
	CRODA	ST	1607SP1139
	CRODA	HP	1052445
SPAN80	KLK OLEO	ST	2424145
	CRODA	ST	1239340
	NOF/NOFABLE	UP	173502
SPAN60	CRODA	ST	SLBT7599
SPAN40	CRODA	ST	MKCD1354
SPAN20	KLK OLEO	ST	2430784
	CRODA BATCH 1	ST	1067455

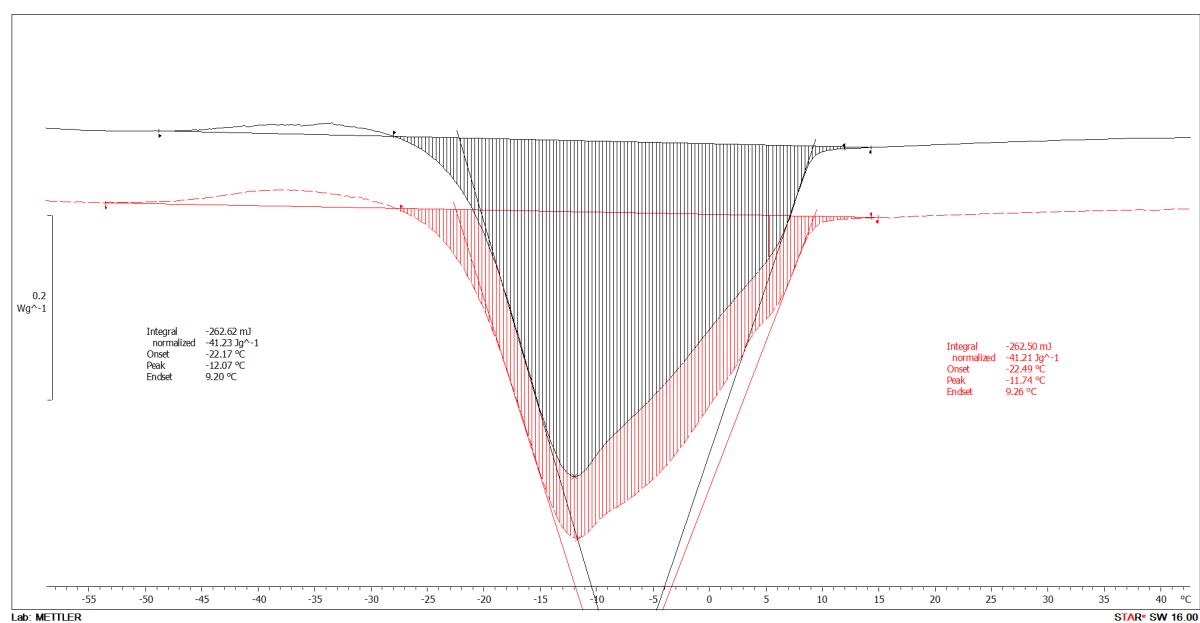


Figure S1: Schematic of the integration of DSC peaks, show integration of PS80 Croda HP, with baseline adjustment and integration limits.

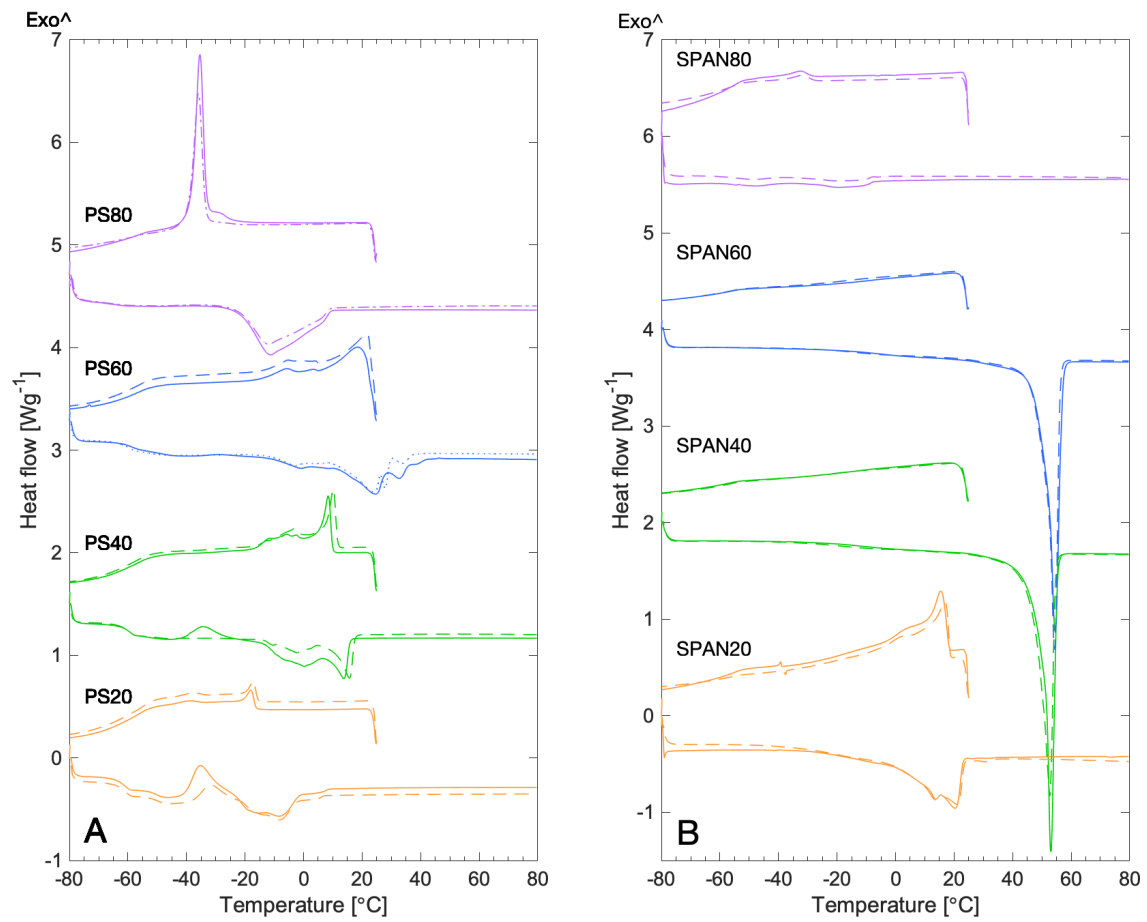


Figure S2: DSC thermograms, displaying the first thermal cycle, of PS and SPAN products measured without drying (dashed line) and after drying (solid lines).

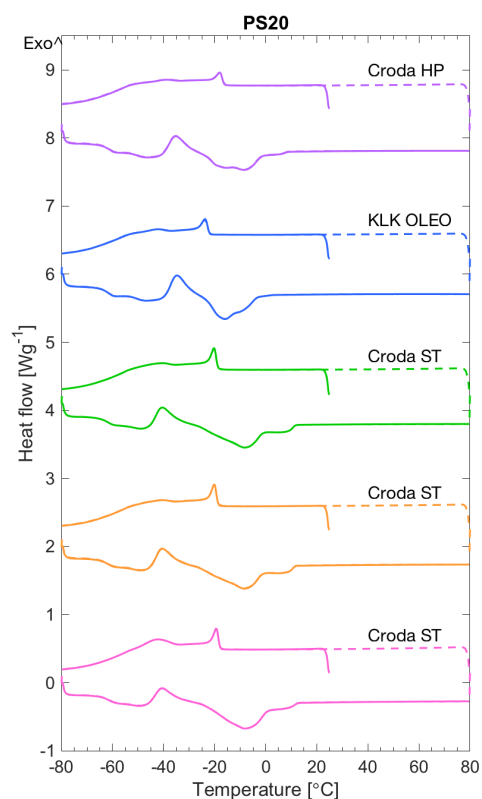


Figure S3: First (solid line) and second (dashed line) thermal cycle for PS20 samples of different purity grades, including three different grades for PS20, and three different batches of PS20 Standard from Croda showing the batch-to-batch difference.

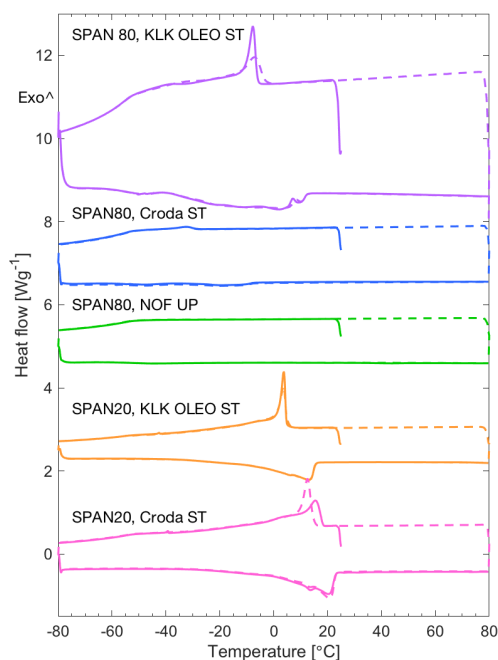


Figure S4: First (solid line) and second (dashed line) thermal cycle for SPAN samples with different purity grades, including three different grades of SPAN80 and two different grades for SPAN20.

Table S3: Values from individual DSC runs of all samples presented in the paper.

SAMPLE	Sample no.	SAMPLE WEIGHT [mg]	1st cycle				2nd cycle			
			Melting peak temperature [°C]	Heat of melting [J/g], 1st cycle	Melting peak height [W/g]	Melting peak FWHM [°C]	Melting peak temperature [°C]	Heat of melting [J/g], 2nd cycle	Melting peak height [W/g]	Melting peak FWHM [°C]
PS 80 OLEO ST	1	8.88	-10.51	41.75	0.60	10.51	-10.50	41.41	0.59	10.58
PS 80 OLEO ST	2	7.60	-10.31	43.27	0.63	10.28	-10.31	43.21	0.62	10.30
PS 80 OLEO ST	3	6.68	-10.46	43.76	0.64	10.23	-10.46	44.17	0.64	10.27
PS 80 OLEO ST	4	5.77	-10.44	43.63	0.63	10.26	-10.44	43.54	0.63	10.27
	Average	7.23	-10.43	43.10	0.63	10.32	-10.43	43.08	0.62	10.36
	Standard deviation	1.15	0.07	0.80	0.02	0.11	0.07	1.03	0.02	0.13
PS 80 Croda ST	1	8.27	-6.80	49.00	0.47	15.98	-6.63	48.70	0.48	15.47
PS 80 Croda ST	2	7.27	-6.95	48.93	0.47	16.04	-6.78	48.84	0.47	15.62
PS 80 Croda ST	3	6.67	-7.44	50.57	0.48	16.05	-7.11	50.54	0.49	15.62
PS 80 Croda ST	4	5.58	-7.25	50.48	0.48	16.10	-7.09	50.13	0.49	15.42
	Average	6.95	-7.11	49.75	0.48	16.04	-6.90	49.55	0.48	15.53
	standard deviation	0.97	0.25	0.78	0.01	0.04	0.20	0.80	0.01	0.09
PS 80 Croda HP	1	5.34	-11.74	45.16	0.41	18.25	-11.57	45.45	0.40	18.65
PS 80 Croda HP	2	7.49	-11.78	45.32	0.41	18.43	-11.61	45.59	0.40	18.81
PS 80 Croda HP	3	8.29	-11.46	50.05	0.45	18.45	-11.29	50.22	0.44	19.07
PS 80 Croda HP	4	5.88	-11.91	45.90	0.41	18.54	-11.91	45.95	0.40	19.02
	Average	6.75	-11.72	46.61	0.42	18.42	-11.60	46.80	0.41	18.89
	standard deviation	1.19	0.16	2.01	0.02	0.11	0.22	1.98	0.02	0.17
PS 80 Croda SR	1	3.79	-8.07	44.33	0.60	11.49	-8.07	44.45	0.60	11.49
PS 80 Croda SR	2	7.15	-7.97	44.47	0.59	11.69	-7.97	44.96	0.59	11.72
PS 80 Croda SR	3	7.58	-7.31	46.70	0.63	11.44	-7.31	47.05	0.63	11.42
PS 80 Croda SR	4	5.97	-7.44	46.26	0.62	11.42	-7.44	46.90	0.63	11.44
	Average	6.12	-7.70	45.44	0.61	11.51	-7.70	45.84	0.61	11.52
	standard deviation	1.47	0.33	1.05	0.02	0.11	0.33	1.15	0.02	0.12
PS 80 NOF UP	1	9.77	-6.69	40.55	0.55	11.63	-6.69	40.36	0.56	11.46
PS 80 NOF UP	2	8.81	-7.00	40.77	0.56	11.56	-7.00	40.38	0.56	11.44
PS 80 NOF UP	3	9.11	-6.51	42.12	0.59	11.40	-6.34	42.30	0.59	11.32
PS 80 NOF UP	4	5.68	-7.27	41.98	0.58	11.54	-7.27	42.13	0.58	11.47
	Average	8.34	-6.87	41.36	0.57	11.53	-6.83	41.29	0.57	11.42
	standard deviation	1.58	0.29	0.70	0.02	0.08	0.35	0.92	0.01	0.06
PS 60 Croda ST	1	22.35	24.09	44.28	0.35	12.00	25.89	49.56	0.43	11.69
PS 60 Croda ST	2	8.67	23.83	44.08	0.46	10.91	24.17	48.90	0.45	10.43
PS 60 Croda ST	3	3.98	24.42	47.25	0.48	11.05	25.41	50.21	0.51	9.50
PS 60 Croda ST	4	6.49	23.87	46.98	0.47	10.85	25.36	50.95	0.49	9.89
	Average	10.37	24.05	45.65	0.44	11.20	25.21	49.91	0.47	10.38
	standard deviation	7.11	0.23	1.47	0.05	0.47	0.63	0.76	0.03	0.83
PS 40 Croda ST	1	7.43	13.70	40.42	0.39	23.00	13.53	40.35	0.42	22.00
PS 40 Croda ST	2	8.64	13.86	39.15	0.38	22.02	13.86	39.24	0.39	22.00
PS 40 Croda ST	3	22.84	14.12	39.90	0.36	22.63	14.12	39.86	0.38	22.18
PS 40 Croda ST	4	6.16	13.72	40.67	0.41	23.50	13.55	40.83	0.43	23.00

	Average	11.27	13.85	40.04	0.39	22.79	13.77	40.07	0.41	22.30
	standard deviation	6.74	0.17	0.58	0.02	0.54	0.24	0.59	0.02	0.41
<i>PS 20</i> <i>OLEO ST</i>	1	4.55	-16.40	26.97	0.34	13.93	-16.40	27.19	0.34	14.01
<i>PS 20</i> <i>OLEO ST</i>	2	8.67	-16.28	26.84	0.34	13.95	-16.28	26.96	0.34	13.96
<i>PS 20</i> <i>OLEO ST</i>	3	7.26	-16.26	26.20	0.33	13.93	-16.26	26.30	0.33	14.00
<i>PS 20</i> <i>OLEO ST</i>	4	6.60	-16.58	24.07	0.31	13.80	-16.58	24.06	0.31	13.83
<i>PS 20</i> <i>OLEO ST</i>	5	8.42	-16.11	27.42	0.34	13.90	-16.11	27.47	0.34	13.94
	Average	7.10	-16.33	26.30	0.33	13.90	-16.33	26.40	0.33	13.95
	standard deviation	1.48	0.16	1.18	0.01	0.05	0.16	1.23	0.01	0.06
<i>PS 20</i> <i>Croda ST</i> <i>batch 2</i>	1	1.69	-8.53	38.23	0.33	16.40	-8.53	37.89	0.33	16.23
<i>PS 20</i> <i>Croda ST</i> <i>batch 2</i>	2	10.30	-7.97	35.79	0.32	15.97	-7.97	35.87	0.32	15.97
<i>PS 20</i> <i>Croda ST</i> <i>batch 2</i>	3	7.93	-8.11	35.01	0.31	15.72	-8.11	35.05	0.31	15.76
<i>PS 20</i> <i>Croda ST</i> <i>batch 2</i>	4	6.20	-8.41	36.63	0.33	15.78	-8.24	36.62	0.32	15.92
	Average	6.53	-8.26	36.42	0.32	15.97	-8.21	36.36	0.32	15.97
	standard deviation	3.15	0.22	1.19	0.01	0.27	0.21	1.04	0.01	0.17
<i>PS 20</i> <i>Croda ST</i> <i>batch 3</i>	1	4.42	-8.23	36.12	0.32	15.71	-8.57	36.40	0.32	15.83
<i>PS 20</i> <i>Croda ST</i> <i>batch 3</i>	2	7.00	-8.26	35.72	0.32	15.81	-8.26	35.71	0.32	15.89
<i>PS 20</i> <i>Croda ST</i> <i>batch 3</i>	3	6.75	-8.25	34.67	0.31	15.44	-8.08	35.02	0.31	15.58
<i>PS 20</i> <i>Croda ST</i> <i>batch 3</i>	4	8.73	-8.28	36.93	0.33	15.82	-8.28	37.25	0.33	15.96
	Average	6.73	-8.26	35.86	0.32	15.70	-8.30	36.10	0.32	15.82
	standard deviation	1.53	0.02	0.81	0.01	0.15	0.18	0.83	0.01	0.14
<i>PS 20</i> <i>Croda HP</i>	1	5.19	-8.57	22.90	0.24	16.18	-8.57	24.10	0.24	16.64
<i>PS 20</i> <i>Croda HP</i>	2	9.30	-8.45	27.83	0.26	17.72	-8.45	27.47	0.25	17.67
<i>PS 20</i> <i>Croda HP</i>	3	8.01	-8.60	27.95	0.26	17.68	-8.60	27.89	0.26	17.62
<i>PS 20</i> <i>Croda HP</i>	4	9.35	-8.61	27.91	0.26	17.67	-8.61	27.96	0.26	17.64
	Average	7.96	-8.56	26.65	0.26	17.31	-8.56	26.86	0.25	17.39
	standard deviation	1.69	0.06	2.16	0.01	0.65	0.06	1.60	0.01	0.43
<i>SPAN 80</i> <i>OLEO ST</i>	1	4.91	1.96	15.08	0.0785	33.29	2.13	14.74	0.0723	33.91
<i>SPAN 80</i> <i>OLEO ST</i>	2	12.55	2.22	15.88	0.0795	34.15	1.89	15.90	0.0775	34.24
<i>SPAN 80</i> <i>OLEO ST</i>	3	12.12	1.89	15.47	0.0809	33.14	2.23	15.31	0.0760	33.75
	Average	9.86	2.02	15.48	0.0796	33.53	2.08	15.32	0.0753	33.97
	standard deviation	3.50	0.14	0.33	0.0010	0.44	0.14	0.47	0.0022	0.20
<i>SPAN 80</i> <i>Croda ST</i> <i>batch 2</i>	1	2.77	-18.86	9.41	0.0601	17.47	-19.36	10.29	0.0596	19.90
<i>SPAN 80</i> <i>Croda ST</i> <i>batch 2</i>	2	3.91	-19.86	8.59	0.0510	20.25	-19.86	8.44	0.0496	19.99
<i>SPAN 80</i> <i>Croda ST</i> <i>batch 2</i>	3	10.43	-19.57	8.88	0.0501	20.22	-19.74	8.87	0.0504	20.40
	Average	5.70	-19.43	8.96	0.0537	19.31	-19.65	9.20	0.0532	20.10
	standard deviation	3.37	0.42	0.34	0.0045	1.30	0.21	0.79	0.0045	0.22

<i>SPAN 80 NOF UP SPAN 80 NOF UP SPAN 80 NOF UP</i>	1	6.34	-45.71	2.26	0.0206	17.81	-45.71	2.39	0.0214	18.06
	2	2.24	-44.85	2.24	0.0213	17.10	-45.01	2.25	0.0207	17.32
	3	8.22	-46.05	2.57	0.0235	17.35	-46.38	2.37	0.0213	17.66
	Average	5.60	-45.54	2.36	0.0218	17.42	-45.70	2.34	0.0212	17.68
	standard deviation	2.50	0.50	0.15	0.0012	0.29	0.56	0.06	0.0003	0.30
<i>SPAN 60 Croda ST SPAN 60 Croda ST SPAN 60 Croda ST</i>	1	8.29	53.88	98.12	2.82	3.72	52.5	94.16	1.71	6.1
	2	2.12	53.09	99.04	3.7	2.76	52.04	97.05	1.9	5.55
	3	3.43	53.61	103.3	3.54	2.95	52.27	98.69	1.93	5.56
	Average	4.61	53.53	100.15	3.35	3.14	52.27	96.63	1.85	5.74
	standard deviation	2.65	0.33	2.26	0.38	0.42	0.19	1.87	0.10	0.26
<i>SPAN 40 Croda ST SPAN 40 Croda ST SPAN 40 Croda ST</i>	1	8.07	52.63	100.95	2.57	3.93	50.15	95.88	1.15	10.58
	2	9.65	53.63	103.29	2.73	4.21	50.41	96.58	1.18	10.02
	3	6.20	52.38	102.1	3.1	2.8	50.23	95.28	1.2	9.9
	Average	7.97	52.88	102.11	2.80	3.65	50.26	95.91	1.18	10.17
	standard deviation	1.41	0.54	0.96	0.22	0.61	0.11	0.53	0.02	0.30
<i>SPAN 20 OLEO ST SPAN 20 OLEO ST SPAN 20 OLEO ST</i>	1	3.90	13.1	45.54	0.42	14.73	12.77	45.72	0.42	14.96
	2	3.82	12.77	46.12	0.42	14.83	12.76	43.82	0.42	14.57
	3	4.38	12.92	45.61	0.43	14.5	12.92	45.25	0.43	14.34
	Average	4.03	12.93	45.76	0.4233	14.69	12.82	44.93	0.4233	14.62
	standard deviation	0.25	0.13	0.26	0.0047	0.14	0.07	0.81	0.0047	0.26
<i>SPAN 20 Croda ST batch 1 SPAN 20 Croda ST batch 1 SPAN 20 Croda ST batch 1</i>	1	3.47	20.25	52.66	0.52	14.08	20.57	52.79	0.64	10.17
	2	7.36	20.5	56.37	0.57	13.2	20.65	56.48	0.67	10.17
	3	8.23	20.48	55.13	0.56	13.02	20.8	57.54	0.66	10.38
	Average	6.35	20.41	54.72	0.5500	13.43	20.67	55.60	0.6567	10.24
	standard deviation	2.07	0.11	1.54	0.0216	0.46	0.10	2.04	0.0125	0.10

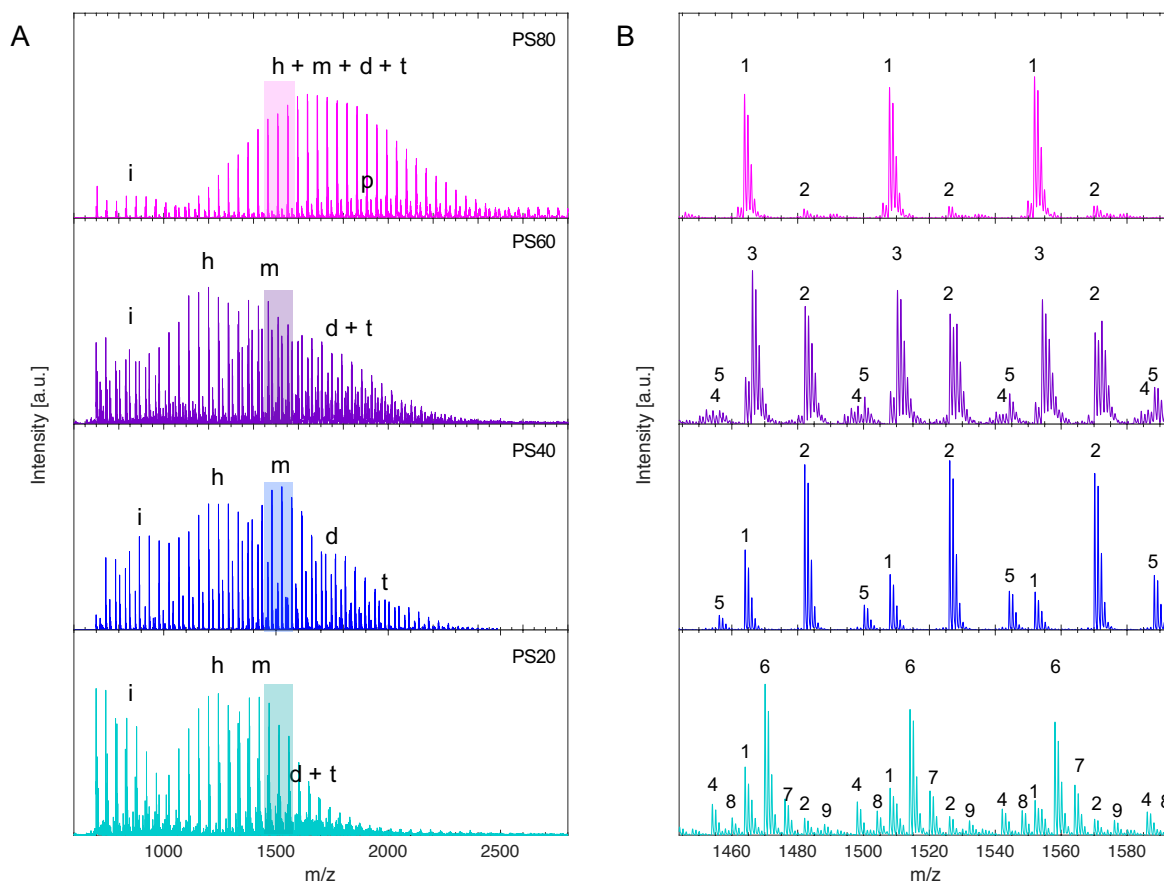


Figure S5 A: MALDI-TOF of polysorbate samples of different main tail composition: PS80, PS60, PS40, and PS20. For PS80 the spectrum shows less peaks because of overlapping distributions of isobaric molecules. The letters denote, i) PEO isosorbide and PEO ester species, h) non-esterified headgroups, m) the main monoester distribution, d) PEO sorbitan diester species, t) PEO sorbitan triester species, p) PEO sorbitan palmitates in PS80. B: zoom of the peaks for all products as marked by the transparent box in A. The peak distribution assignments 1-9 can be found in Table S4.

Table S4: molecular species in PS products as assigned by MALDI-TOF. The distribution numbers 1-9 refer to the peaks assigned in Figure S5.

D	Molecular species assigned in Figure S5B
1	PEO sorbitan (headgroups), PEO sorbitan oleates (all)
2	PEO sorbitan monopalmitate
3	PEO sorbitan monostearate
4	PEO sorbitan monomyristate
5	PEO sorbitan dipalmitate
6	PEO sorbitan monolaurate
7	PEO sorbitan dilaurate
8	PEO sorbitan laurate-myristate
9	PEO sorbitan Laurate-palmitate

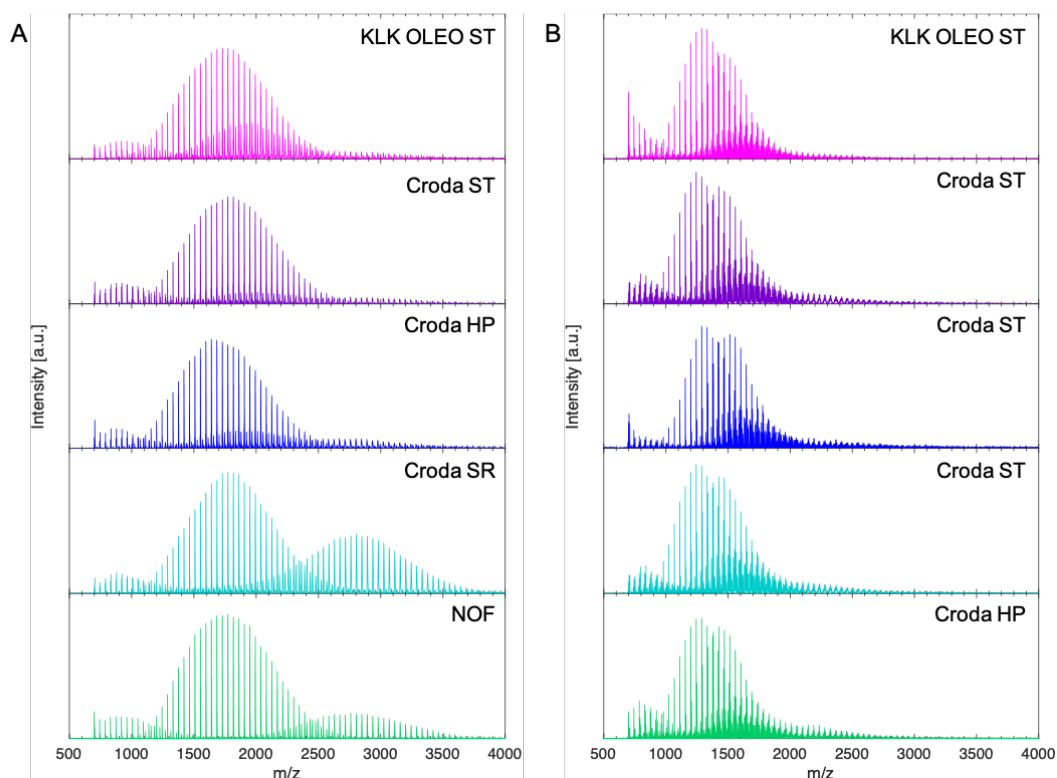


Figure S6 A: five different mass spectra are shown that depicts standard grade (ST) PS80 from KLK OLEO and Croda, high performance (HP) and super refined (SR) from Croda and a NOF (UP) sample. Isosorbide ester species and PEO monoesters showed up as low molecular mass distributions (~700-1500 Da) and PEO sorbitan oleates as a broad distribution from 1000 Da to approximately 2500 Da. Within this broad distribution a smaller distribution of PEO sorbitan palmitate could be seen for all samples apart from NOF and SR, which has 99.3 % and 87.2 % oleic acid, respectively. High mass distributions (2000-3000 Da) pertaining to PEO sorbitol esters were found for Croda (SR) and NOF (UP). B: five different mass spectra of PS20 from KLK Oleo (ST) and Croda (ST and HP). Several more mass distributions were distinguished due to the primary ester being laurate, which is not isobaric with six EO units like oleate. Again, isosorbide ester species and PEO monoesters (~700-1500 Da) were found at low mass. The two largest distributions correlated with free PEO sorbitan and PEO sorbitan monolaurate and smaller distributions with various distributions of PEO sorbitan monomyristate, monopalmitate and diester species of different combinations.

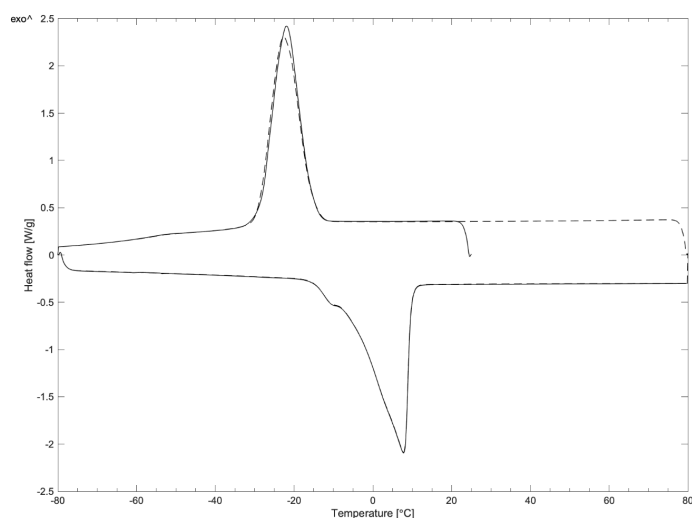


Figure S7: DSC thermogram showing the first (solid line) and second (dashed line) thermal cycle for PEG 400.