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Mixed-mode and reversed-phase liquid chromatography–tandem mass spectrometry methodologies to study composition and base hydrolysis of polysorbate 20 and 80

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ABSTRACT

Polysorbate 20 (polyoxyethylenesorbitan monolaurate) and polysorbate 80 (polyoxyethylenesorbitan monooleate) used in protein drug formulations are complex mixtures that have been difficult to characterize. Here, two HPLC methods are used with evaporative light scattering detection (ELSD) and mass spectrometry (MS) to characterize polysorbate from commercial vendors. The first HPLC method used a mixed-mode stationary phase (Waters Oasis MAX, mixed-mode anion exchange and reversed-phase sorbent) with a step gradient to quantify both the total polyoxyethylene sorbitan ester and polyoxyethylene sorbitan (POE sorbitan, a non-surfactant) in polysorbate. The results indicated POE sorbitan was present from 16.0 to 27.6 and 11.1 to 14.5% (w/w) in polysorbate 20 and 80, respectively. The second HPLC method used a reversed-phase stationary phase (Zorbax SB-300 C_8) with a shallow gradient to separate, identify, and quantify the multiple ester species present in polysorbate. For all lots of polysorbate 20 analyzed, only 18–23% of the material was the expected structure, polyoxyethylenesorbitan monolaurate. Up to 40% and 70% (w/w) di- and triesters were found in polysorbate 20 and polysorbate 80 respectively. Likewise, polyoxyethylenesorbitan monooleate accounted for only 20% of polysorbate 80. A variability of 3–5% was observed for each ester species between multiple lots of polysorbate 20. The reversed-phase method was then used to determine the rate of hydrolysis for each polyoxyethylene sorbitan ester of polysorbate 20 in basic solution at room temperature. Increasing rates of hydrolysis were observed with decreasing aliphatic chain lengths in polysorbate 20.

1. Introduction

Polysorbate 20 (polyoxyethylene sorbitan monolaurate) and polysorbate 80 (polyoxyethylene sorbitan monooleate) are nonionic surfactants commonly used in the formulation of protein pharmaceuticals. The role of polysorbate in protein formulations is to prevent the formation of aggregates [1,2] and protect the protein from denaturation at liquid-vial and liquid-air interfaces [3]. The polysorbate molecule consists of two parts; the polar head group, polyoxyethylene sorbitan (POE sorbitan), and the hydrophobic ester tail (Fig. 1). Structural variability can occur in both the POE sorbitan and the ester tail. The POE sorbitan in polysorbate 20 and 80 contains approximately twenty ethylene oxide subunits that vary in both total number and positional isomers. Based on the European Pharmacopeia specification, for the content of fatty acids, the laurate ester accounts for 40–60% of esters in polysorbate 20, the remaining esters range from C_8 to C_{18}. The oleate ester accounts for 58–85% of esters in polysorbate 80, the remainder range from C_{14} to C_{18} including stearate, linoleate, and linoleneate esters [4]. This variability is the primary reason characterization of polysorbates has been difficult.

Studies have been performed characterizing polysorbates using matrix assisted laser desorption ionization mass spectrometry [5,6], LC–MS [7], and reversed-phase chromatography [8]. There have also been many assays developed to quantitate polysorbate in various matrices such as plasma [9,10] or drug formulations [11–14]. These studies focused on the identification of the many species in polysorbate or the quantitation of the total polysorbate in a sample, not the identity and abundance of each of the sub-species present in polysorbate. Here, we discuss two HPLC methods developed for the characterization and quantitation of the sub-species present in polysorbate 20 and polysorbate 80. A mixed-mode method previously reported separates POE sorbitan from the POE sorbitan esters in polysorbate [13]. The mixed-mode

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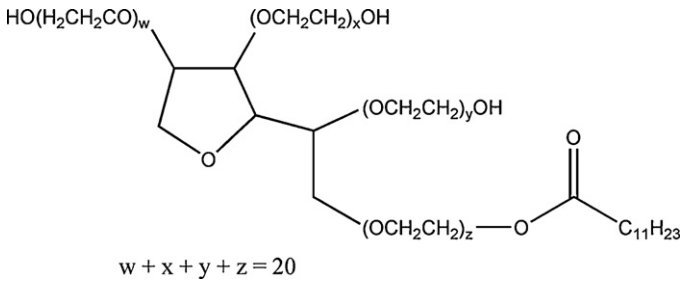

Fig. 1. Theoretical structure of polysorbate 20. Approximately 40–60% of the hydrophobic tail is a laurate ester.

method was used to quantify the total percent mass of POE sorbitan in polysorbate 20 and polysorbate 80, the amount of which has not been reported, and its specification has neither been set by vendor certificate of analysis nor by compendial method. This is important because POE sorbitan is not a surfactant. Second, a reversed-phase (RP) HPLC method used a linear gradient to separate, identify, and quantitate the multiple POE sorbitan esters of polysorbate 20 and 80. The HPLC methods were used in conjunction with evaporative light scattering detection (ELSD) and mass spectrometry (MS) to characterize and quantitate sub-species present in polysorbate obtained from commercial vendors.

The ester linked hydrophobic tail is critical to the surfactant property of polysorbate. Since esters are susceptible to hydrolysis, it is important to understand what effect hydrophobic tail heterogeneity will have on polysorbate hydrolytic stability. Both HPLC methods described in this report are able to monitor the hydrolysis of polysorbate. The mixed-mode method can measure the overall hydrolysis of polysorbate which was previously reported. The RP method can measure the hydrolysis of each POE sorbitan ester in polysorbate. This information will allow scientists to gain insight on the hydrolytic stability of polysorbate lots by obtaining a reversed-phase profile.

2. Experimental

2.1. Reagents and materials

HPLC grade isopropyl alcohol (IPA) (Burdick & Jackson, Muskegon, MI, USA), formic acid (Alfa Aesar, Ward Hill, MA, USA), and water purified using a Milli-Q filtration system (Millipore, Billerica, MA, USA) were used in the HPLC mobile phase. Three grades of polysorbate 20 were evaluated from both standard and "super" refinement processes (Croda, Rancho Cucamonga, CA, USA) and NOF (Tokyo, Japan). Polysorbate 80 samples were obtained from Croda. POE sorbitan standards were obtained from NOF and Croda. These materials were used as received.

30 mg/mL polysorbate or POE sorbitan stock solutions were prepared by accurately weighing 150 mg into a 5 mL volumetric flask, then filled with MiliQ water. Serial dilutions were carried out to reach concentrations outlined later in the communication.

2.2. Chromatographic system

Chromatographic analysis was performed on an Agilent 1200 HPLC system (Palo Alto, CA, USA) equipped with a binary gradient pump, autosampler, a temperature-controlled column compartment, and an evaporative light scattering detector (ELSD, 380-LS, Varian, Palo Alto, CA, USA). The ELSD settings were as follows; gas flow rate 1.0 SLM, nebulizer temperature 45 °C, and evaporation tube temperature 100 °C. Nitrogen gas was provided by an in-house nitrogen gas generator system at 65 psi. The injection volume was 20 μL, and the column temperature was 30 °C.

2.3. Mixed-mode chromatography

The Mixed-mode method has been previously reported [13]. Analytes were separated using an Oasis MAX (20 × 2.1 mm, 30 μm, Waters, Milford, MA, USA) column. Initial conditions were set at 90% solvent A (2% formic acid) and 10% solvent B (2% formic acid in IPA). Solvent B was increased to 20% in the first minute and held for 2.4 min. POE sorbitan esters were eluted using a step gradient of 20% to 100% B over 0.1 min, followed by an equilibration step of 10% B for 0.9 min. The flow rate was kept at 1 mL/min.

2.4. Reversed-phase chromatography

Analytes were separated using a Zorbax SB C8 column (50 × 4.6 mm, 5 μm, Agilent, Santa Clara, CA, USA). Initial conditions were set at 90% solvent A (2% formic acid) and 10% solvent B (2% formic acid in IPA). Solvent B was increased to 20% in the first minute and held for 2.4 min. Separation of polysorbate esters was achieved using a linear gradient of 20% to 100% B over 19.6 min, followed by an equilibration step of 10% B for 5 min. The flow rate was kept at 900 μL/min.

2.5. Mass spectrometric analysis

Mass spectrometric analysis was carried out on a Micromass QTOF-1 (Beverly, MA) mass spectrometer operating in a positive ion mode via electrospray ionization (ESI). The instrument was set to run with a capillary voltage of 3500 V, sample cone voltage of 55 V, source block temperature of 125 °C and desolvation temperature of 200 °C. The mass spectrometer was triggered via a contact closure. Calibration was performed in the *m*/*z* range of 100–3500 using a solution of sodium cesium iodide (NaCsI). Instrument control and data analysis were achieved using Waters MassLynx version 4.0 software package. The MaxEnt 3 program was applied to deconvolute the multiply-charged ions.

2.6. NMR analysis

Data were recorded on a Bruker 500 MHz spectrometer equipped with a 5 mm BBI probe and a Bruker BACS-60 autosampler. The H_2O signal was suppressed by irradiating the sample with a low power saturation pulse at H_2O frequency during the relaxation delay. Prior to the NMR measurement, D_2O was added to all samples to a final concentration of 10%.

2.7. Base hydrolysis of polysorbate 20

Polysorbate 20 (1 mg/mL) was incubated with ammonium hydroxide (200 mM) for 22 h at room temperature. Every 30 min the autosampler injected an aliquot of the reaction mixture into the HPLC reversed-phase method (Section 2.4). For each time point, the concentration of each POE sorbitan ester was calculated from a standard curve generated using the mixed mode method (Section 2.3).

3. Results and discussion

3.1. Characterization of polysorbate using the mixed-mode HPLC method

The mixed-mode HPLC method uses a step gradient to elute all POE sorbitan esters in a single peak. Two peaks are observed in a typical polysorbate chromatogram (Fig. 2). The flow through peak at 0.3 min accounts for approximately 15–20% (w/w) of the total peak area. Both peaks were analyzed using liquid chromatograph

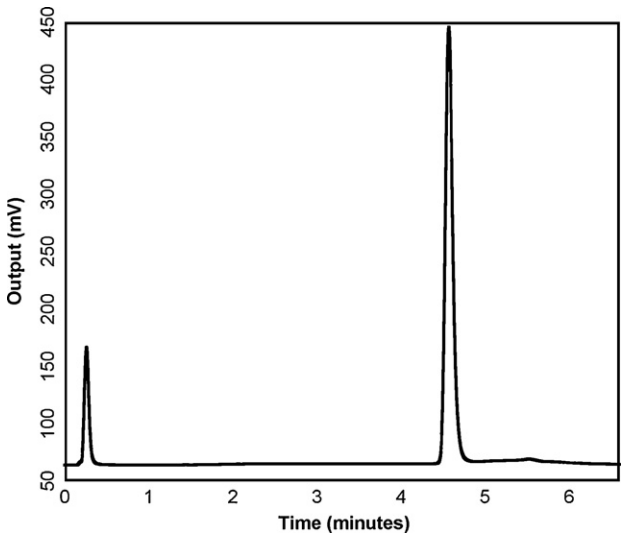

Fig. 2. Typical mixed-mode ELSD chromatogram of polysorbate 20. Peaks at 0.3 min and 4.6 min correspond to POE sorbitan and POE sorbitan ester respectively.

mass spectrometry (LC–MS) and collected for nuclear magnetic resonance spectroscopy (NMR) analysis.

Raw mass data were deconvoluted using the Waters MassLynx MaxEnt 3 software package, yielding the monoisotopic mass of the analytes. Both sodiated and non-sodiated forms of polysorbates were observed in the deconvoluted mass spectra. The deconvoluted mass spectrum for each peak contains two mass envelopes (Fig. 3B). The more abundant higher molecular weight envelope (1100–2200 Da) contains masses consistent with POE sorbitan esters. The masses in each envelope are separated by 44 Da, the mass of one ethylene oxide residue. The most abundant masses observed are 1309.74 Da (Fig. 3A) and 1491.91 Da (Fig. 3B) in the 0.3 min and 4.6 min peaks respectively, a difference of 182.17 Da. The observed monoisotopic mass of 1491.91 Da $[M+H]^+$ correlates well with the theoretical monoisotopic mass (1491.92 Da, $[M+H]^+$) of the POE sorbitan laurate ester containing 26 ethylene oxide residues (within mass error). The expected loss due to hydrolysis of the laurate ester is 183.32 Da. Therefore, the higher molecular weight envelope of the 0.3 min peak was assigned to POE sorbitan and the 4.6 min peak was assigned to POE sorbitan esters. The mass spectrum for the 4.6 min peak is more complex than that of the 0.3 min peak owing to multiple POE sorbitan esters present in polysorbate 20.

The lower molecular weight envelope (400–1000 Da) corresponds to byproducts of polysorbate synthesis, mainly isosorbide polyethoxylates (IPE) [5–7]. The observed mass of 813.53 Da (Fig. 3B) correlates well with the $[M+H]^+$ IPE laurate ester ion containing 11 ethylene oxide subunits, whose theoretical monoisotopic mass is 813.52 Da. Similar to what was found for the high molecular weight envelope, the 587.33 Da mass observed in the 0.3 min peak correlates well with the $[M+H]^+$ IPE ion containing 10 ethylene oxide subunits theoretical monoisotopic mass is 587.32 Da. The loss of aliphatic resonances between 0.5 and 2.5 ppm in the NMR spectrum of the 0.3 min peak confirms these assignments (Fig. 4).

The presence of POE sorbitan in polysorbate is likely a byproduct of polysorbate synthesis, either due to incomplete esterification of sorbitan or as a result of hydrolysis during ethoxylation, the condensation of the sorbitan ester with ethylene oxide, in the presence of an alkaline catalyst [15]. The mixed-mode method was used to determine the amount and variability of POE sorbitan present in commercial polysorbate 20 and 80. A total of nineteen polysorbate 20 lots and four polysorbate 80 lots were analyzed. The POE sorbitan

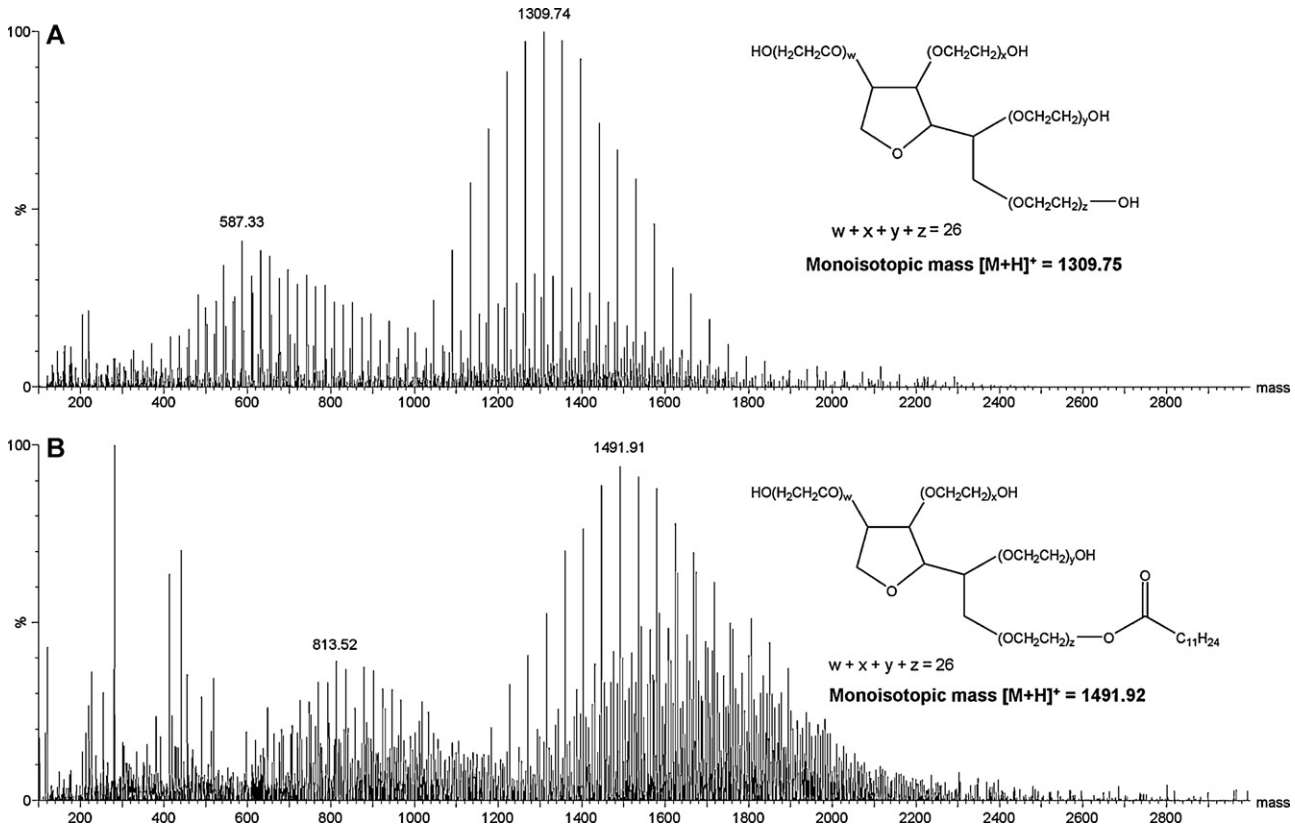

Fig. 3. Deconvoluted mass spectra of the 0.3 min, POE sorbitan peak (A) and the 4.6 min, POE sorbitan ester peak (B) for polysorbate 20 Lot #1 acquired using the mixed-mode HPLC method.

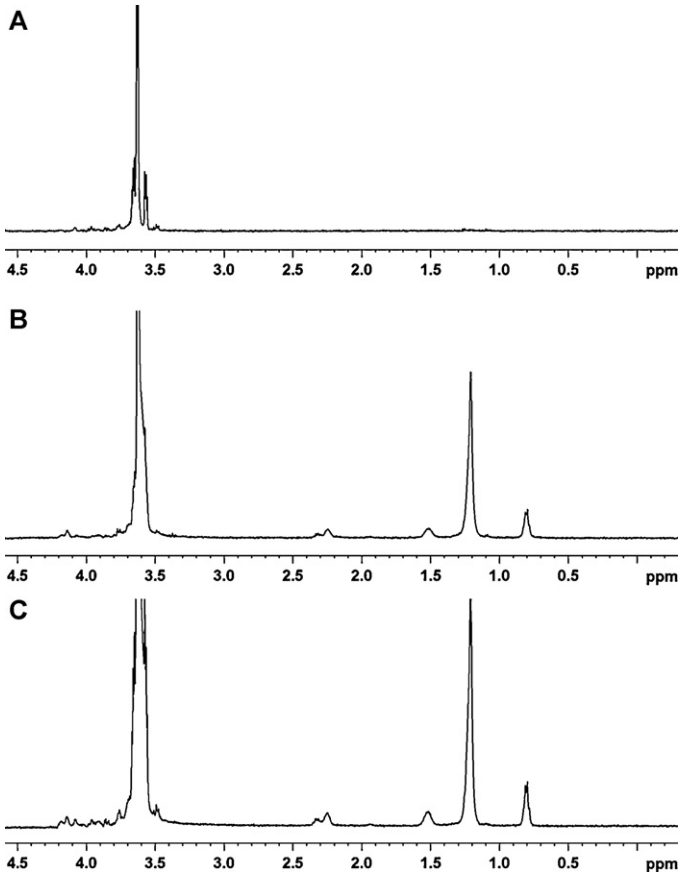

Fig. 4. NMR spectra of fraction collected mixed-mode 0.3 min peak (A), 4.6 min peak (B), and polysorbate 20 control (C).

concentration was calculated from a POE sorbitan standard curve ranging from 10 to 100 μg/mL prepared from material received from Croda. LC–MS data confirmed the POE sorbitan masses found in polysorbate 20 (Fig. 3A) are consistent with those in the standard POE sorbitan (Fig. 5A), and NMR confirms the absence of ester species in the standard (Fig. 5B). Since the ELSD has a logarithmic not linear response [16], a log–log linear standard curve was obtained by plotting the log(peak area) against the log(mg/mL) of POE sorbitan yielding a Pearson correlation coefficient (R) >0.999. The resulting concentration of POE sorbitan ranged from 16.0 to 27.6% (w/w) in polysorbate 20 and 11.1 to 14.5% (w/w) in polysorbate 80 samples (Table 1).

3.2. Characterization of polysorbate using the reversed-phase HPLC method

Polysorbate 20 is made up of 40–60% laurate esters, the remainder is a mixture of esters with chain lengths from C_8 to C_{18} [4]. A reversed-phase HPLC method was used to separate, identify, and quantitate the different POE sorbitan ester species in polysorbate. A similar method was previously reported, but made no attempt to identify or quantitate the species observed [17]. Representative chromatograms acquired for polysorbate 20 and polysorbate 80 using the RP method are shown in Fig. 6. The 2.4 min peak contains non-esterified POE sorbitan, IPE, and polyethylene glycol (PEG). Later in the polysorbate 20 chromatograph, there is a series of eight peaks, followed by a broad tailing peak. The peaks were assigned using LC–MS and are summarized in Table 2. An example deconvoluted mass spectrum used for the assignment of the 10.4 min peak is shown in Fig. 7. The IPE laurate $[M+K]^+$ species is identified in the 719, 763, and 807 series of ions and PEG laurate $[M+K]^+$ species is identified in the 679, 723, and 767 series of ions, both present in relatively low abundance. The POE sorbitan mono-

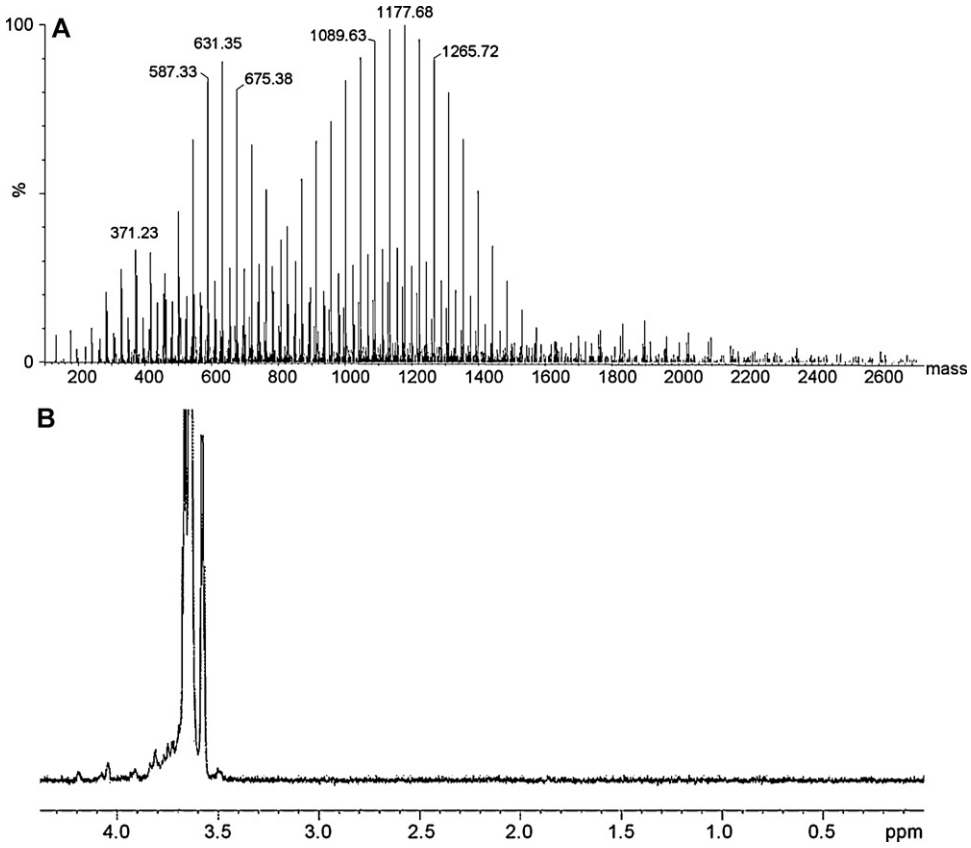

Fig. 5. Deconvoluted mass spectrum (A) and NMR spectrum (B) of the POE sorbitan standard obtained from Croda.

Table 1
POE sorbitan mass percentages in commercial polysorbate lots. Lot numbers were arbitrarily assigned.

Sample	Vendor	Lot number	Refinement process	POE sorbitan (% mass)
Polysorbate 20	Croda	1	Standard	26.4
Polysorbate 20	Croda	2	Standard	18.9
Polysorbate 20	Croda	3	Standard	24.9
Polysorbate 20	Croda	4	Standard	23.8
Polysorbate 20	Croda	5	Standard	21.9
Polysorbate 20	Croda	6	Standard	24.0
Polysorbate 20	Croda	7	Standard	21.2
Polysorbate 20	Croda	8	Standard	26.5
Polysorbate 20	Croda	9	Standard	24.2
Polysorbate 20	Croda	10	Standard	21.9
Polysorbate 20	Croda	11	Standard	23.1
Polysorbate 20	Croda	12	Standard	23.2
Polysorbate 20	Croda	13	Standard	23.2
Polysorbate 20	Croda	14	Standard	24.7
Polysorbate 20	Croda	15	Super refined	27.6
Polysorbate 20	Croda	16	Super refined	17.7
Polysorbate 20	Croda	17	Super refined	18.0
Polysorbate 20	Croda	18	Super refined	16.0
Polysorbate 20	NOF	19	Standard	27.6
Polysorbate 80	Croda	20	Standard	14.2
Polysorbate 80	Croda	21	Standard	14.5
Polysorbate 80	Croda	22	Standard	13.4
Polysorbate 80	Croda	23	Standard	11.1

laurate $[M+Na]^+$ species is identified by the 1381, 1425, and 1469 series of ions. The $[M+H]^+$ adduct is also present in Fig. 6 identified by the 1535, 1579, and 1623 series of ions. The presence of the mass envelopes is reflective of the polydispersity of ethylene oxide subunits in polysorbate. It was interesting to note that the average number of ethylene oxide subunits was twenty-six for each POE sorbitan ester, similar to that observed for the POE sorbitan in the mixed mode method, which is consistent with previously reported results [18]. POE sorbitan esters in polysorbate 20 follow the expected trend where longer hydrophobic esters exhibit later elution times. The broad peak late in the chromatogram is made up of several di- and tri-ester polysorbate species, some of those identified are listed in Table 2. Since true standards could not be readily obtained for the POE sorbitan ester species, a five point calibration curve was constructed from the POE sorbitan ester peak (4.6 min) in the mixed-mode method using the same injection volume, flow rate, and ELSD settings as the reversed phase method. The calibration curve (log(peak area) vs. log(mg/mL)) was corrected to account for the presence of POE sorbitan. This calibration curve was used to calculate the concentration of each ester species observed in the reversed-phase method. The total mass of ester species in the reversed-phase method recovered using this calibration was between 90 and 110% of the expected value for all polysorbate lots

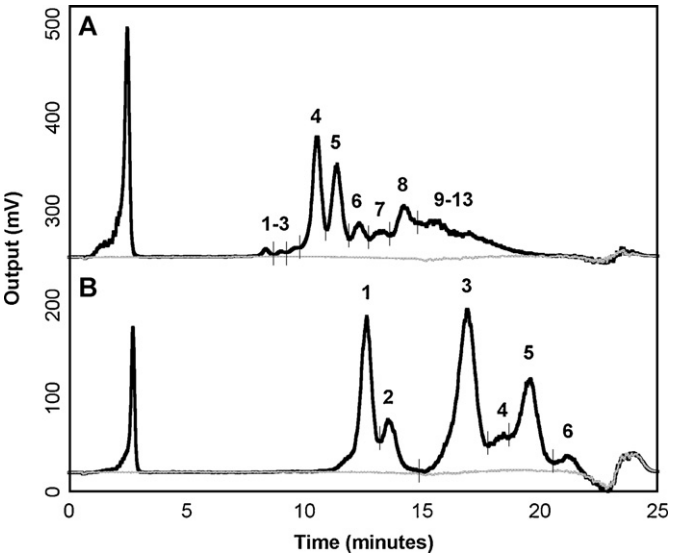

Fig. 6. Representative chromatograms for polysorbate 20 Lot #1 (A) and polysorbate 80 Lot #20 (B) using the reversed-phase HPLC method with ELS detection. Grey traces are water injections demonstrating the baseline of the chromatograms.

Table 2
Reversed-phase method peak assignments for Croda polysorbate 20 Lot #1. The monoisotopic theoretical mass was calculated using the "ethylene oxide subunits reported" column, this value corresponds to the most abundant mass observed. The stearate ester is represented by C_{18} and the oleate ester is represented by $C_{18:1}$. Peak numbers correlate to those designated in Fig. 6.

Peak number	Retention time (min)	Assignment	Ethylene oxide subunit range	Ethylene oxide subunit reported	Monoisotopic theoretical mass $[M+Na]^+$	Observed mass $[M+Na]^+$	ppm
1	8.2	C_8 ester	19–32	26	1457.84	1457.80	31
2	8.8	C_8 IPE	7–16	11	779.44	779.43	13
3	9.7	C_{10} ester	16–34	26	1485.87	1485.82	33
4	10.4	C_{12} ester	17–31	23	1381.83	1381.79	28
5	11.5	C_{14} ester	19–33	26	1541.94	1541.91	19
6	12.2	C_{16} ester	19–29	23	1437.89	1437.87	14
7	13.0	$C_{18:1}$ ester	22–30	24	1507.93	1507.84	59
		C_{18} ester	22–30	24	1509.94	1509.92	13
8	14.2	C_{12} diester	18–33	25	1652.05	1651.99	36
9	15.0	C_{12}/C_{14} diester	21–29	26	1724.11	1724.10	6
10	16.0	C_{12}/C_{16} diester	19–30	24	1664.08	1664.06	12
11	16.9	$C_{12}/C_{12}/C_{12}$ tri-ester	24–32	27	1922.27	1922.15	62
12	17.9	$C_{12}/C_{12}/C_{16}$ tri-ester	22–30	26	1934.30	1934.23	36
13	18.4	$C_{12}/C_{12}/C_{18}$ tri-ester	22–27	25	1918.31	1918.24	36

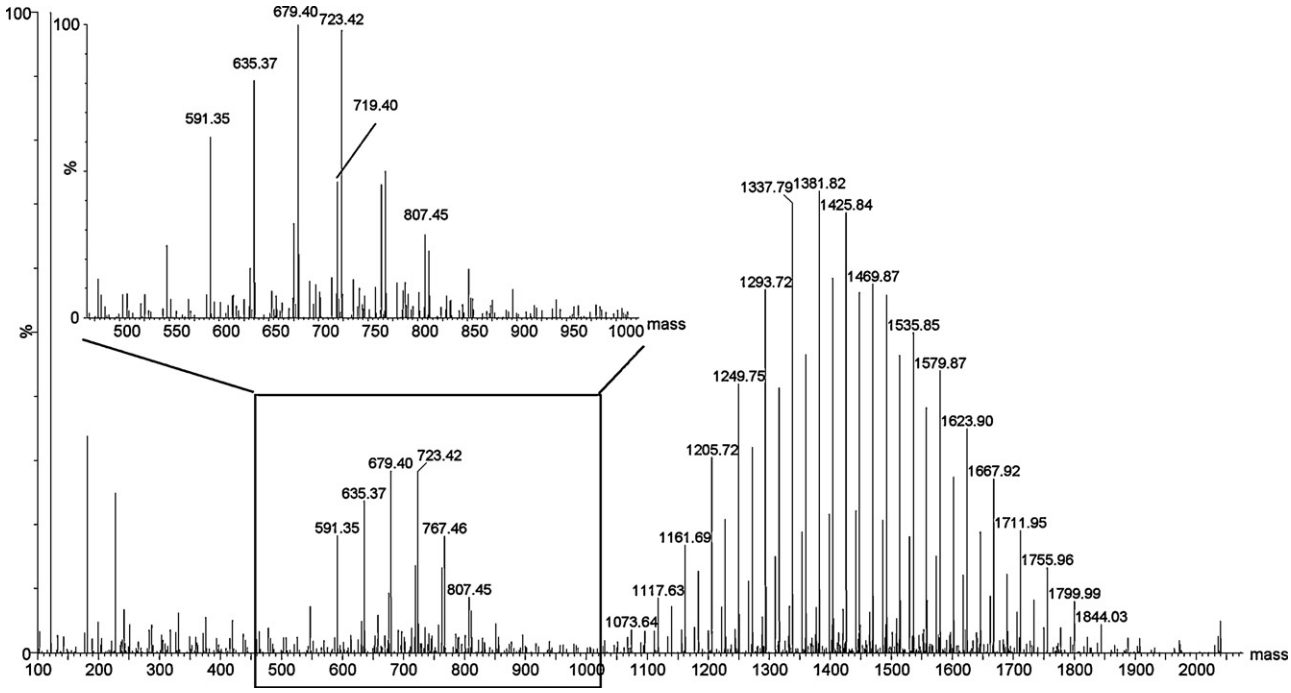

Fig. 7. Deconvoluted mass spectra of the 10.4 min peak observed in polysorbate 20 Lot #1 using the reversed phase method.

tested. Fig. 8 shows a histogram of the percent abundance by mass of each species observed in the polysorbate 20 lots. Surprisingly, in each lot tested the expected structure of polyoxyethylenesorbitan monolaurate only accounts for 18–23% (w/w) of polysorbate 20. The polyoxyethylenesorbitan di-laurate ester makes up 13–16% (w/w) of polysorbate 20, other diester and higher ester species were also observed which have previously been reported [5,18,19]. The total lauric acid content reported as 40–60% in the pharmacopeia arises from the saponification of all mono-, di- and tri-laurate esters of POE sorbitan.

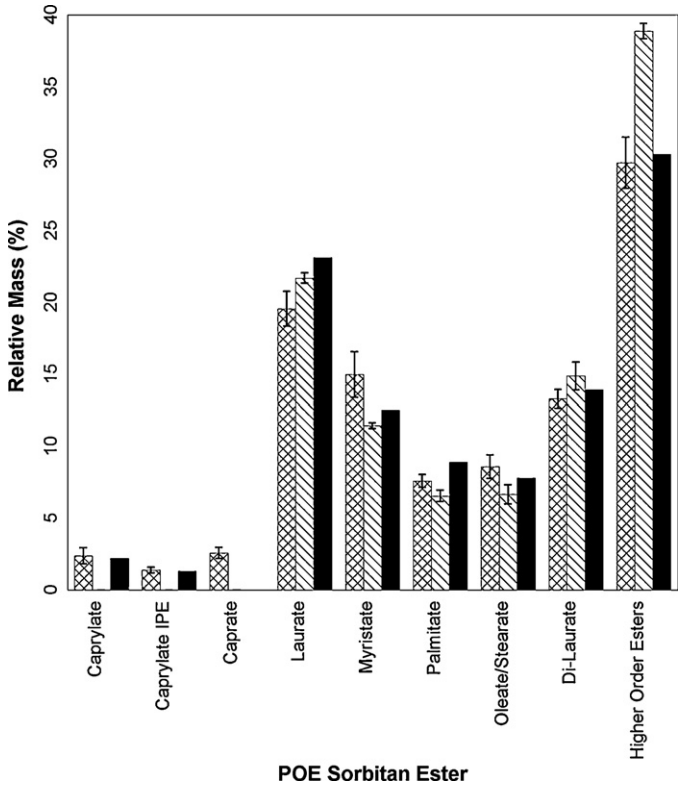

Fig. 8. Histogram of the percent abundance of esters in the Croda standard refinement (crossed bars), super-refined (diagonal bars), and NOF (solid bars) polysorbate 20 lots. Since resolution of di- and tri-esters larger than the C_{12}/C_{12} diester was not achieved, they are reported as "Higher Order Esters". Error bars indicate one standard deviation from the calculated mean of 14 lots and 4 lots of Croda standard and super refined polysorbate 20 respectively.

Polysorbate 80 contains 58–85% oleate esters, the remaining 15–42% is made of myristate, palmitate, linoleate, and stearate esters [4]. The LC–MS assignments of polysorbate 80 are outlined in Table 3. Like polysorbate 20, there was evidence of several di- and tri-ester species. Only 20% of the total polysorbate 80 raw material was the expected sorbitan mono-oleate in the three lots tested. The remaining oleate was present in di- and tri-ester species. It should be noted that these di- and tri-esters, with a total amount up to 30% or more, are deviation from the correct structure shown in pharmacopeia.

The POE sorbitan ester profile can also be evaluated in the presence of protein using this reversed phase method by placing the Oasis MAX column followed by a switching valve before the C_8 column. In this format, the Oasis MAX column acts as a solid phase extraction device, diverting protein to waste during the first 2.4 min of the method, then eluting and separating the ester species using the C_8 column while flow is on line with the detector, similar to our previous work [13]. This will allow formulators to understand the state of polysorbate in drug substance real time and accelerated stability studies. These experiments are currently on-going in our laboratories. It is important to note that POE sorbitan information will be lost in this format.

3.3. Base hydrolysis of polysorbate

The surfactant property of polysorbate in protein formulations helps to stabilize the protein against aggregation and protect protein against denaturation at air and vial interfaces. Upon loss of the ester tail, polysorbate loses its surfactant behavior, rendering it unable to form micelles. For this reason, it is important to understand the hydrolytic stability of POE sorbitan esters. Degradation of polysorbate has been previously described [20–22], but these

Table 3
Reversed-phase method peak assignments for Croda polysorbate 80 Lot #20. The monoisotopic theoretical mass was calculated using the "ethylene oxide subunits reported" column, this value corresponds to the most abundant mass observed. The stearate ester is represented by C_{18} and the oleate ester is represented by $C_{18:1}$. Peak numbers correlate to those designated in Fig. 6.

Peak number	Retention time (min)	Assignment	Ethylene oxide subunit range	Ethylene oxide subunit reported	Monoisotopic theoretical mass $[M+Na]^+$	Observed mass $[M+Na]^+$	ppm
1	12.4	$C_{18:1}$ ester	24–38	31	1816.12	1816.07	28
2	13.4	$C_{18:1}$ IPE ester	9–18	13	983.651	983.623	13
		$C_{18:1}$ PEG monoester	11–20	14	921.61	921.58	32
3	16.8	$C_{18:1}/C_{18:1}$ diester	26–36	30	2036.33	2036.29	19
4	18.2	$C_{18:1}/C_{18:1}$ diester IPE	9–15	12	1225.85	1225.82	24
		C_{18}/C_{18} diester IPE	9–15	12	1229.88	1229.84	32
5	19.3	$C_{18:1}/C_{18:1}/C_{16}$ tri-ester	25–36	30	2256.55	2256.54	4
6	21.0	$C_{18:1}/C_{18:1}/C_{18:1}$ tri-ester	31–43	36	2564.74	2564.63	43

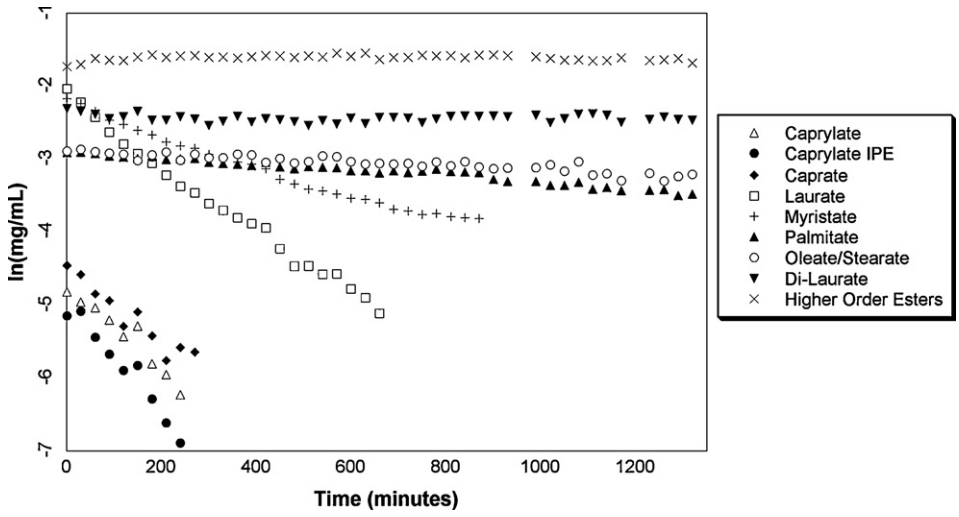

Fig. 9. Pseudo first-order plot of POE sorbitan ester hydrolysis with different ester linkages. Hydrolysis was performed with Croda polysorbate 20 Lot #1.

studies have not included the dependence of the hydrolytic rate on alkyl chain length. Here, the hydrolysis of polysorbate 20 was studied using the RP method. Pseudo first order rate plots were prepared by plotting the log of the POE sorbitan ester concentration (mg/mL) for each peak versus time (Fig. 9). The pseudo first order rate constants obtained indicate that the hydrolytic rate decreased with increasing alkyl chain length (Table 4). No detectable hydrolysis was observed at these experimental conditions for the di- and tri-ester species. These observations may be explained by the work of Aniansson et al. [23]. They determined that the dissociation of a surfactant monomer from a micelle is dependent on the chain length of its hydrophobic tail, where shorter chain lengths have faster dissociation kinetics. Therefore, the shorter chain polysorbate has higher solvent exposure leading to faster hydrolysis when above the critical micelle concentration of the surfactant. The presence of di- and tri-esters may present the following concerns. As additional alkyl chains are present, these esters will exhibit decreased HLB (hydrophylic–lipophylic balance) and resistance to hydrolytic clearance.

Table 4
Pseudo first order rate constants of the basic hydrolysis (200 mM NH_4OH) of the component in polysorbate 20 (Croda Lot #1, 1 mg/mL) at ambient temperature.

Polysorbate ester	k_{Obs} (min^{-1})
Caprylate	5.5×10^{-3}
Caprylate IPE	6.6×10^{-3}
Caprate	4.8×10^{-3}
Laurate	3.6×10^{-3}
Myristate	2.1×10^{-3}
Palmitate	4.0×10^{-4}
Oleate/stearate	3.0×10^{-4}
Laurate diester	1.0×10^{-5}
Higher Order Esters	3.0×10^{-6}

4. Conclusions

Two HPLC methods were used to characterize polysorbate 20 and 80 from commercial vendors. POE sorbitan, a component of no surface activity, was present in polysorbate 20 from 16.0 to 27.6% (w/w) and polysorbate 80 from 11.1 to 14.5% (w/w) in lots tested. The difference in the percent POE sorbitan is likely due to the higher stability of the POE sorbitan oleate ester to hydrolysis as long alkyl chain exhibited greater stability when above its critical micelle concentration. The distribution of esters in polysorbate 20 was characterized using RPLC with ELS detection. Diester and higher ester species were also found in significant amount, ca 26–40% (w/w). The expected structure of POE sorbitan monolaurate only accounted for 18–23% (w/w) of the total polysorbate 20 in lots tested. Likewise, POE sorbitan monooleate accounted for only 20% of the polysorbate 80. The observed variability for each ester species was 3–5% for each commercial vendor, but the ester population between vendors and refinement process had greater variability. The hydrolysis of polysorbate was investigated using a RP-HPLC method yielding increased hydrolysis rates with decreasing POE sorbitan ester chain lengths in 200 mM NH_4OH at room temperature.

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