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Composition analysis of two batches of polysorbate 60 using MS and NMR techniques

Hoang Vu Dang [a], Alexander I. Gray [a], David Watson [a], Catharine D. Bates [a], Peter Scholes [b], Gillian M. Eccleston [a,*]

[a] *Department of Pharmaceutical Sciences, Strathclyde Institution of Biomedical Sciences, University of Strathclyde, Glasgow, UK*
[b] *Department of Pharmaceutics, 3M Health Care Limited, Loughborough, Leicestershire, UK*

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Abstract

Batch variation in Tween 60 has shown to influence the rheological properties of semisolid emulsions. MS (LC–MS, GC–MS, MS^n) and NMR (^{13}C, ^1H, ^1H COSY and HMBC) techniques were used to analyze and compare the composition of two batches of Tween 60 with particular emphasis on the number of POE groups and their distribution within the molecule. Acid and saponification values were also determined. The batches contained different proportions of components (sorbitan polyethoxylates, sorbitan monoester–diester-polyethoxylates and isosorbide monoester–diester-polyethoxylates). The number of POE groups were averaged over the four sites in sorbitan and the two sites in isosorbide molecules. The batches differed from each other in terms of (i) the POE sorbitan stearate/POE sorbitan palmitate ratios (batch 1, 3:2 and batch 2, 4:5), (ii) the ratio of sorbitans to isosorbides (batch 1, 2:3; batch 2, 7:13); and (iii) the acid values (batch 1, 3.1; batch 2, 0). It is concluded that liquid chromatography combined with electrospray mass spectrometry and ion trap separation is a useful tool for establishing the compositional profile of different batches of Tweens. ^1H NMR could provide a simple and rapid pharmacopoeial test for the ratio of sorbitan to isosorbide in Tweens.

Keywords: Tween 60; Polyoxyethylene (POE); Sorbitan; Isosorbide; Stearate; Palmitate; MS; NMR; Semisolid emulsions

1. Introduction

Polysorbate 60 (Tween 60) is a polyoxyethylene (POE) type non-ionic surfactant commonly employed as emulsifier, dispersant and stabilizer in a wide variety of cosmetic and pharmaceutical products [1]. The surfactant consists primarily of partial fatty acid esters (stearate and palmitate) of sorbitol-derived cyclic ethers (sorbitans and sorbides) polymerized with approximately 20 molecules of ethylene oxide per molecule of Tween.

Fig. 1 shows the two slightly different routes used to synthesize Tween 60 [2,3] containing approximately 20 POE groups per molecule (BP 2004). In the preliminary steps of polysorbate manufacture, the dehydration of sorbitol is performed, generally at 225–250 °C, in the presence of a catalyst to yield isomers of sorbitol monoanhydrides (sorbitans) and/or sorbitol dianhydrides (isosorbides). Following cyclization, the mixture of sorbitans and isosorbides is condensed with ethylene oxide (polymerization) and then reacted with complex mixture of C15–C17 fatty acids (esterification), route 1. On the other hand, this mixture can be reacted with fatty acids and then condensed with ethylene oxide, route 2, to produce commercial polysorbate formulations. This means that although Tween 60 is mainly composed of polyoxyethylene sorbitan monoester, it is in fact a complex mixture of polyoxyethylene sorbitan monoester and other intermediates that can be chromatographically separated using evaporating light scattering detection [4]. Fig. 2 summarizes possible polyethoxylated intermediates.

The emulsifying capacity of Tween 60 will be dependent upon its chemical nature, as governed by the structure of sorbitol derivative core (i.e. sorbitan and isosorbide), the alkyl chain lengths of fatty acids (i.e. palmitate and stearate), the degree of esterification, the number of POE groups and their distribution within the molecule. Thus, understanding Tween 60 chemical composition would help formulators not only in forecasting properties of Tween 60 emulsified products but also in choosing Tween 60 concentrations to achieve desired applications.

* Corresponding author. Tel.: +44 141 5482510; fax: +44 141 5526443.
E-mail address: g.m.eccleston@strath.ac.uk (G.M. Eccleston).

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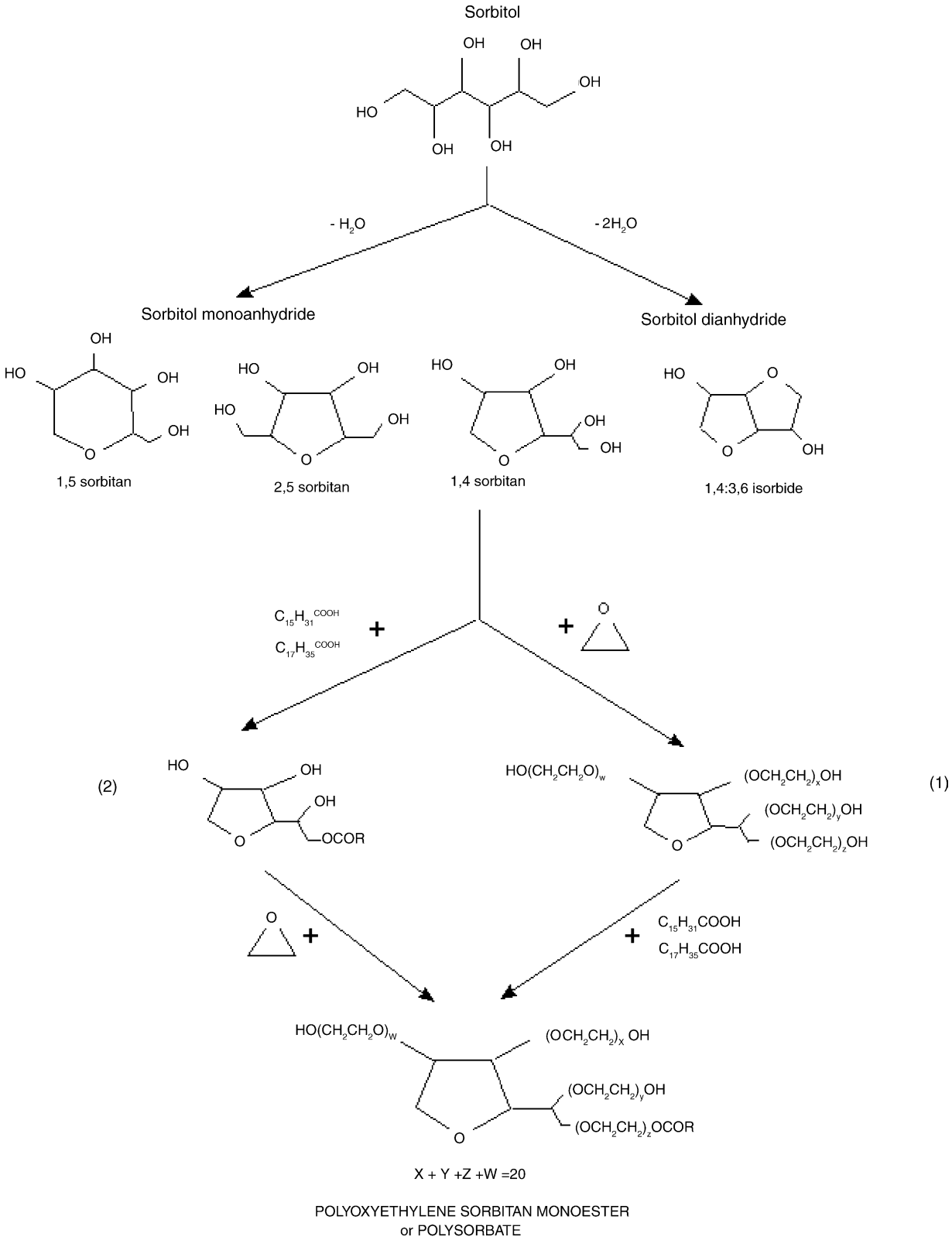


Fig. 1. Tween 60 synthesis routes.

In the past, although the possibility of using NMR techniques as useful analytical tool in the field of polyoxyethylene-type substances was considered [5–7], the NMR exploitation in analyzing structure of polysorbate emulsifiers is still limited quantitatively and qualitatively. For instance, ^{1}H spectra of Tween 20 only picked up NMR signals arising from the sorbitan skeleton, lauric acid tail and polyoxyethylene protons, respectively [7]. In addition, this method did not show the complex nature of Tween

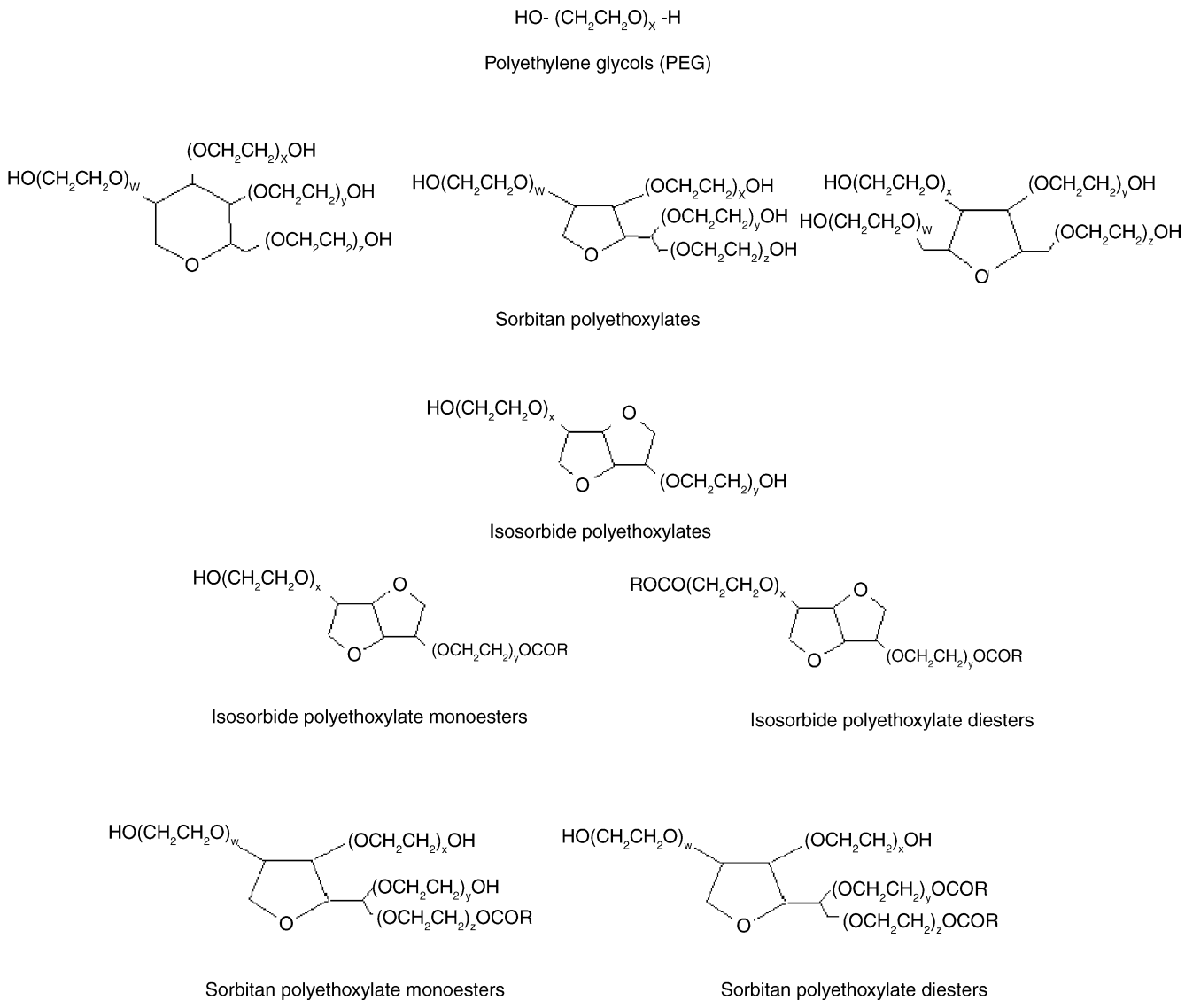


Fig. 2. Proposed major polyethoxylate components and their esters in Tween 60 formulations.

20 nor did it consider the length and distribution of POE groups within its molecules.

MALDI MS (matrix-assisted laser desorption/ionization mass spectrometry) and tandem mass spectrometry have been used to analyze polyoxyethylene-type non-ionic surfactants including Tween 20, Brij 35 and Triton X-100, -114 [8] and Tween 80 [9]. Although these studies mostly detected very heterogeneous mixtures of polymer species containing different numbers of ethoxy groups, their spectra were of poor quality and peak identity was not addressed. The compositions of a series of polysorbate formulations (Tween 20,40,60,80) have also been analyzed using MALDI-TOF-MS (matrix-assisted laser desorption/ionization-time of flight-mass spectrometry) [2]. The mass spectra for these formulations revealed a greater degree of complexity than previous results by indicating the existence of all the intermediates such as polyethylene glycols, sorbitan polyethoxylates, isosorbide polyethoxylates, and polysorbate esters (e.g., monolaurates, monomyri states, monopalmitates in Tween 20, mono/dipalmitates in Tween 40, monopalmitate and monostearate in Tween 60, mono/dioleates in Tween 80). The molecular complexity of polysorbate emulsifiers (Tween 60, 80) was further confirmed by Frison–Norrie and co-workers using MALDI-TOF-MS [3].

Despite the fact that MALDI-TOF-MS possesses some methodological advantages i.e. ease of sample preparation, speed of analysis, high sensitivity and minimal fragmentation allowing direct access to molecular weight, complete compositional analysis of polysorbate emulsifiers is unachievable due to its inherent limitation of inability to differentiate among components of the same molecular weights. Moreover, none of the above studies determined the number of POE groups and their distribution within the four possible sites on the Tween molecule. The consistency and rheological properties of semisolid oil-in-water emulsions containing mixed emulsifiers of fatty alcohols and straight chain POE alkyl ether surfactants (e.g. Cetomacrogol 1000) is due to the swelling properties of a lamellar gel network phase formed when the non-ionic surfactant is incorporated amongst alcohol molecules to form bilayers separated

by layers of water. The thickness of the interlamellar water is approximately twice the length of a fully hydrated POE chain. Batch variations in the number of POE groups on the molecule thus markedly influence the rheological profile of the emulsion [10]. We are currently investigating systems containing fatty alcohols and Tween 60, and have shown that batch variations in Tween 60 also influence rheological profiles of creams prepared with them (to be published). Although lamellar phases also form in these systems, it is not clear whether batch variation on the distribution of the POE groups across the four sites available as well as the number of POE groups may be implicated in this.

The objective of this study was to compare the chemical heterogeneous nature of two batches of Tween 60 utilizing a combination of composition and molecular structure analyzing techniques, liquid chromatography–mass spectroscopy and nuclear magnetic resonance, respectively which have not been explored together. In particular, we aim to investigate the distribution of POE chains within the molecule with the use of fragmentation experiments (MS^n).

2. Experimental

2.1. Materials

Two batches of Tween 60 synthesized by route 2, bovine (batch 1) and non-bovine (batch 2) provided by 3M Health Care limited UK, were used. The following chemicals were of analytical grade and purchased from Sigma, UK: acetonitrile (CH_3CN), formic acid, acetyl chloride, HCl, KOH, methanol, hexane, ethanol, deuterated solvent (CD_3OD).

2.2. LC–MS

Tween 60 samples were analyzed using LC–MS technique as follows. A Thermo-separations P2000 HPLC was fitted with a PLRP-5 column (150 mm × 4.6 mm, 5 μm 100 Å, Polymer Labs, Shropshire, UK). Twenty microlitres of sample (ca. 2 mg/ml in water) was injected, the mobile phase was programmed: flow rate 0.7 ml/min; CH_3CN/0.1% formic acid (20:80) to CH_3CN/0.1% formic acid (30:70) at 10 min, to CH_3CN (100%) at 40 min, to CH_3CN (100%) at 60 min. The column was then equilibrated with starting conditions for 5 min prior to the next injection. The HPLC was interfaced without splitting to a LCQ instrument (Thermo Finnigan, Luton, UK), which was operated in Electrospray Ionisation (ESI) +ve mode with a needle voltage of 4.5 kV and was scanned between 200 and 2000 amu. In addition, MS^n were also performed on selective ions with collision energy 50–80 and activation Q 0.25.

2.3. GC–MS

In order to analyse the fatty acids in the two Tween 60 batches, 200 mg of Tween was weighed into a screw cap glass tube, dissolved in 5 ml of 2 M KOH and then heated in a heating block at 100 °C for 30 min. The sample was cooled and 2 ml of concentrated hydrochloric acid was added. The sample was then extracted with 5 ml of hexane and 0.5 ml of the hexane layer was blown to dryness under a stream of nitrogen. The residue was heated with 0.5 ml of ca. 1% (w/v) hydrochloric acid in methanol (prepared by carefully adding 3 ml of acetyl chloride to 100 ml of methanol) in order to form methyl ester. The sample was heated in a sealed tube at 70 °C for 30 min and the methanol/HCl was then evaporated under stream of nitrogen. The residue was re-dissolved in 3 ml of hexane and 0.5 μl of the solution were injected in an Automass GC–MS system (Thermo Finnigan, Luton, UK). The GC was fitted with a 30 m × 0.5 mm i.d. × 0.5 μm film Rtx-1 Column (Restek, Windsor UK). The oven was programmed as follows: 100 °C (1 min) then 20 °C/min to 200 °C then 10 °C/min to 320 °C. The mass spectrometer was operated in electron impact mode at 70 eV.

2.4. Determination of acid and saponiflcation values

The acid and saponification values were determined according the British Pharmacopeoial monograph for Polysorbate 60 [11].

2.5. NMR

All NMR spectra were determined using 400 MHz instruments either Bruker AMX 400 or DPX 400 spectrometers (Coventry, UK): ^1H at 400.13 MHz and ^{13}C at 100.61 MHz. The samples (ca. 40 mg/ml) were dissolved in deuterated solvent (CD_3OD) at 25 °C. Data were obtained and interpreted in ^1H, ^{13}C, ^1H COSY and HMBC spectra.

3. Results and discussion

3.1. GC–MS analysis, acid and saponification values

Table 1 displays the composition of fatty acid in each batch and their acid and saponification values. The results indicate that batch 1 differed from batch 2 in terms of relative percentages of fatty acids and acid values. On the other hand, their saponification values were similar.

3.2. LC–MS analysis

Fig. 3 shows total ion current (TIC) traces obtained from the two batches of Tween 60. Both batches were composed of approximately seven different species (labeled A–G) with their identities shown in Table 2. The results indicate that polar components (peak A) i.e. sorbitan polyethoxylates had a lower response in ESI mode than the esters (peaks B–F), which ran later in the chromatogram. Compounds with high surface activity (i.e. esters) do tend to give strong responses in ESI mode [12].

The absence of standards to determine response factors hindered absolute quantitative analysis of different species present in the Tween 60 batches. However, the relative proportions of main species could be approximated by comparing the peak areas/heights in Fig. 3a and b. The results show that the ratio of POE sorbitan stearate/POE sorbitan palmitate in batch 1 (3:2) was higher than that in batch 2 (4:5). This reflects the GC–MS

Table 1
Relative fatty acid percentages, and acid and saponification values of the two batches of Tween 60

Sample	C12 (%)	C14 (%)	C16 (%)	C17 (%)	C18 (%)	Acid value	Saponification value
Batch 1	0.7	1.9	46.4	1.0	50.0	3.1	51.30
Batch 2	–	–	64.3	–	35.7	–	50.76

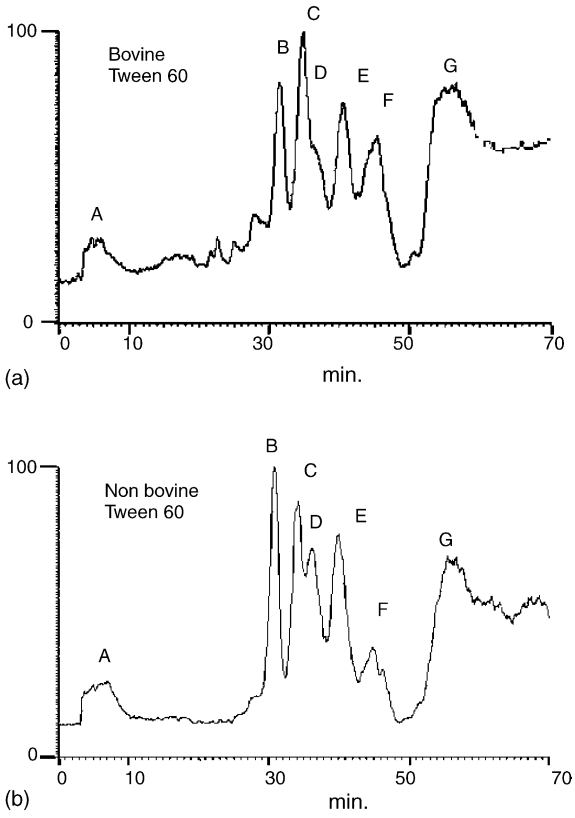


Fig. 3. Total ion current (TIC) traces obtained from two different batches of Tween 60: (a) batch 1, (b) batch 2.

analysis (Table 1) where batch 1 was found to contain proportionately more C18 chain lengths. Furthermore, batch 1 also contained more diesters i.e. POE sorbitan palmitate/stearate and POE isosorbide palmitate/stearate than batch 2. It is emphasized that although there were some differences in the proportions of the various components present in two batches of Tween 60, there were no clear differences between the types of components.

A probable explanation for the series of ions present in peak A at around 8 min is that they were due to polyoxyethylene sorbitan species. Fig. 4a shows that this peak contained two envelopes. The ions in the higher mass group were due to sorbitan-POE Na^+ adducts containing ca. 20–32 POE groups. For instance, the ion at 1331 is due to the Na^+ adduct of sorbitan + 26 POE units. If POE chains are evenly distributed on each of the four

Table 2
Peak identities of the seven species in the Tween 60 batches

Peaks	Identities
A	Sorbitan-POE (20–32)
B	Sorbitan-POE (18–34)-monopalmitates
C	Sorbitan-POE (15–34)-monostearates
D	Isosorbide-POE (11–15)-monopalmitates
E	Isosorbide-POE (11–15)-monostearates
F	Sorbitan-POE (20–30)-palmitate/stearates
G	Isosorbide-POE (9–15)-palmitate/stearates

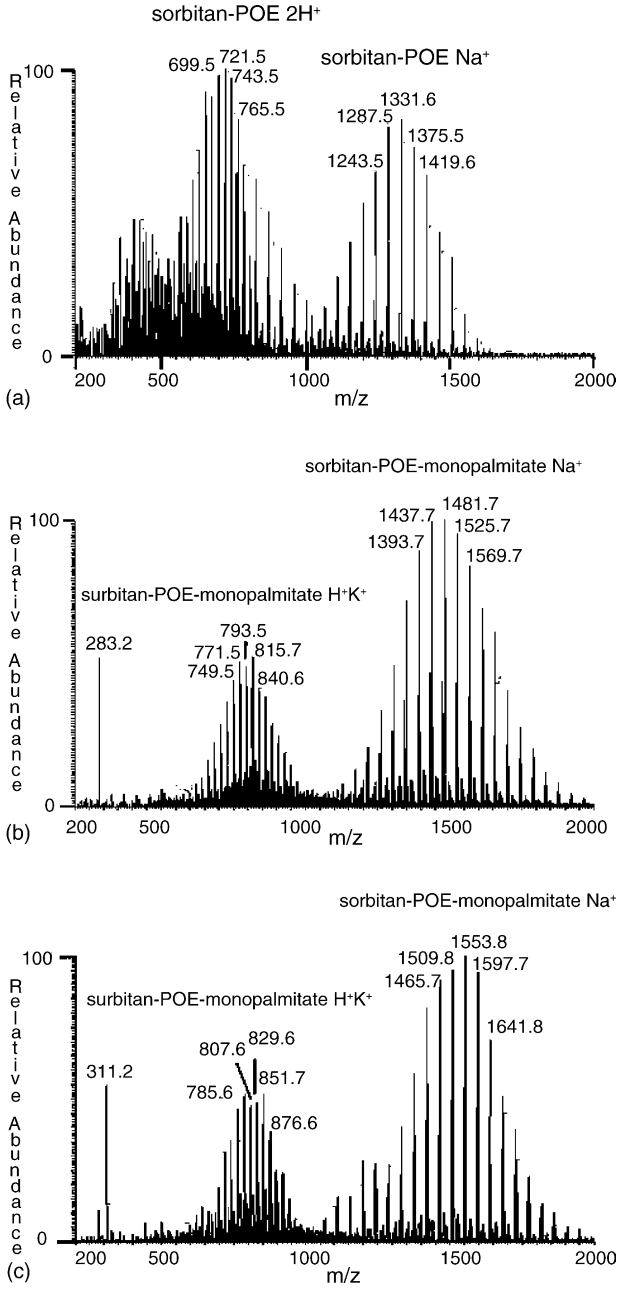


Fig. 4. (a) Sorbitan-POE (20–32) species, peak A; (b) sorbitan-POE (18–34)-monopalmitate species, peak B; (c) sorbitan-POE (15–34)-monostearate species, peak C.

sites then the strongest ions containing 24–28 POE groups must have six to seven POE groups per chain. The second envelope of ions in the spectrum of this peak around *m/z* 699 was due to doubly charged ions bearing two protons. In this envelope, the series of ions separated by 22 amu also represented 20–32 POE sorbitans.

The major peak in the chromatogram around 30.8 min (peak B) was composed of Na^+ adducts of sorbitan POE monopalmitate esters. This peak also contained two envelopes (Fig. 4b). The ions present in the first envelope showed a range of POE chains between 19 and 34 (e.g. the strongest ion at *m/z* 1481.7 was due to sorbitan + 24 POE units-monopalmitate). In the other peak, the spectrum contained doubly charged ions bearing a potassium ion and a proton. For example, the ion at 749.5 when doubled gave a mass of 1499 that differed from the ion at 1481.7 by $(K^+ + H^+) - Na^+$.

The replacement of palmitate by stearate could be seen in peak C at 34.2 min (Fig. 4c). The pattern described above was repeated for peak C and was related to sorbitan POE monostearate species; the strongest ions again being due to sorbitan POE monostearate with 24 and 25 POE units, respectively. For example, the *m/z* 1553.8 was equivalent to the ion at *m/z* 1525.7 in the sorbitan POE monopalmitate species (c.f. Fig. 4b and c).

The peak D centred at 36.1 min, which had only one envelope, was attributable to Na^+ adducts of POE isosorbide monopalmitate species (Fig. 5a). The strongest ion at *m/z* 934 was due to an isosorbide monopalmitate with 12 POE units.

Since isosorbides have only two sites for POE groups, if the POE groups are evenly distributed there are six per site. This is consistent with peak A as previously discussed where 24–28 POE groups are equally distributed over four sites. To check the assumption of equal distribution, further fragmentation on this isosorbide species was performed as shown in Scheme 1.

The high mass parent in the series was at 520 *m/z*, which incorporated the average chain length of six POE units. This fragmentation was repeated up the final ion in the series at 283 *m/z*, which incorporated the fatty acid + a monomer unit, supporting the fact that there are at least six POE groups per chain.

Similar data obtained from peak D was repeated for peak E observed at 40.1 min, which was composed of Na^+ adducts of isosorbide POE monostearate species (Fig. 5b). In this case, the biggest ion at *m/z* 918 was due to an isosorbide monostearate with 11 POE units i.e. five to six POE groups per chain if evenly distributed. When further fragmented, an ion series similar to that for the monopalmitates was observed starting at *m/z* 311 and ending at a chain length of four POE units with the ion at *m/z* 443. This agrees with the assumption.

The peak F centred at 40.5 min fitted the K^+ adducts of sorbitan POE palmitate/stearate. For instance, the ion at *m/z* 1780 was due to sorbitan palmitate/stearate with 25 POE units i.e. approximately six POE groups per chain if evenly distributed (Fig. 6a).

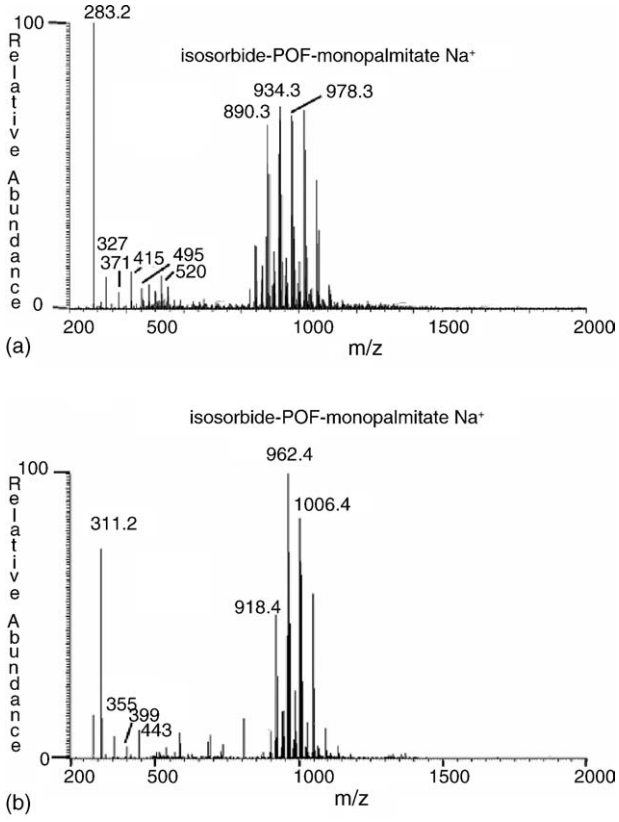
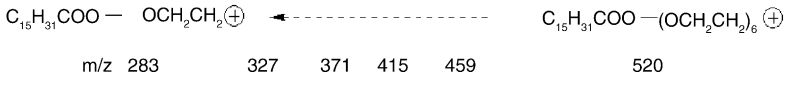


Fig. 5. (a) Isosorbide-POE (11–15)-monopalmitate, peak D; (b) isosorbide-POE (11–15)-monostearate, peak E.

This peak also contained ions at *m/z* 283 and 311 derived from palmitate and stearate moieties, respectively.

The broad indistinct peak G eluting towards the end of the run time was due to stearate/palmitate esters of isosorbide POE (Fig. 6b). For example, the ion at *m/z* 1156 was due to an isosorbide POE stearate/palmitate with 11 POE units i.e. approximately five to six POE groups per chain if evenly distributed. There were probably some ions due to isosorbide POE dipalmitate in this envelope.

Although the strongest ions in peaks A–G imply possibility of equal distribution of POE groups, fragmentation experiments were performed to confirm this assumption. As outlined above there was some evidence from spontaneous in source fragmentation the chain length on the isosorbides was around 6 (c.f. peak D and Scheme 1). In order to investigate the POE chain length attached to the sorbitan rings a series of three fragmentation experiments were carried out on POE sorbitan palmitate 32 POE units attached (*m/z* 1833.8). This ion was chosen as its average POE chain length of 8 would be expected if evenly distributed. Possible fragments obtained were shown in Scheme 2. Fig. 7a shows the MS^2 spectrum derived from the ion at *m/z* 1833.8 in batch 2. The most readily formed fragment arises from loss of



Scheme 1. Series of ions produced by loss of POE units from the acylated side chain of isosorbide-POE-monopalmitate.

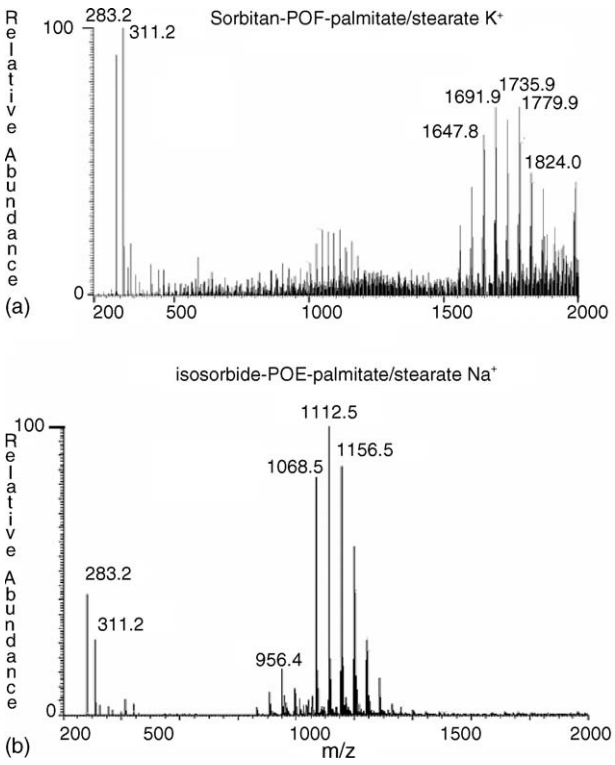


Fig. 6. (a) Sorbitan-POE (20–30)-palmitate/stearate, peak F; (b) isosorbide-POE (9–15)-palmitate/stearate, peak G.

palmitic acid as shown in Scheme 2, which yields an ion at *m*/*z* 1579.3. This fragment dominates MS^2 spectra of the acylated POE sorbitans (i.e. major components of Tween 60). However, another abundant ion can be seen at *m*/*z* 1552.2. This ion arises via the loss of six POE units (rather than eight as expected) from a chain. Although it is not clear why fragmentation should occur six POE units from the end of the chain, there may be an energetically favourable dioxan ion formed from two POE units, which repeated three times may account for the loss of six PEO units. The corresponding ion due to the loss of six POE units occurred when most of the sorbitan palmitate species above *m*/*z* 1500 were fragmented but its intensity varied according to the POE chain lengths attached to the molecule. This fragmentation experiment suggests that it is most abundant for the molecular species with one or more eight POE units attached to the sorbitan ring. It is very weak or non-existent where there is an average of six or less POE units attached to the four positions on the sorbitan ring.

If MS^3 is carried out on the ion at 1833.8 *m*/*z*, it yields the spectrum shown in Fig. 7b that comprises an ion at *m*/*z* 1296.4 due to the loss of palmitic acid (as shown in Scheme 2). Thus, it is evident that the ion at *m*/*z* 1552.2 still bears the palmitate group and results from the loss of a six POE unit.

It is also possible to observe an ion at *m*/*z* 1269.3 in the MS^3 spectrum of *m*/*z* 1833.8, which corresponds to the loss of a second six POE unit chain (Fig. 7c). If an MS^4 spectrum is acquired by fragmenting the ion at *m*/*z* 1269 loss of palmitate is again observed giving an ion at *m*/*z* 1013.2 but in this case the spectrum is too weak to see any evidence of the loss of a third six POE unit chain, which would give an ion at *m*/*z* 986. There were

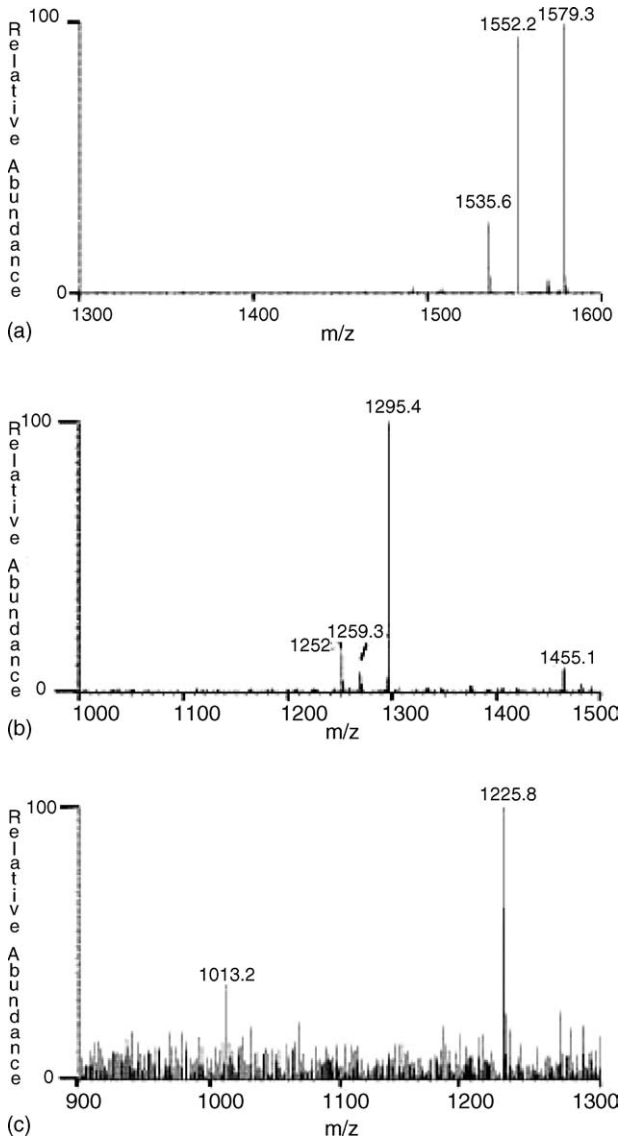
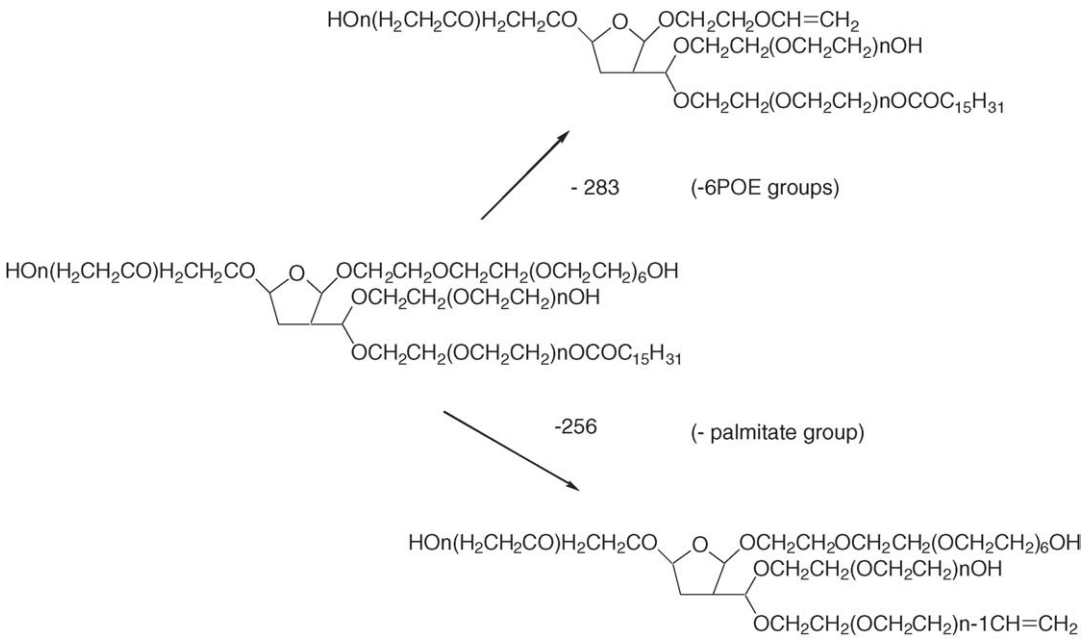


Fig. 7. (a) MS^2 (collision energy 80 activation Q 0.25), (b) MS^3 (collision energy 60, activation Q 0.25), (c) MS^4 (collision energy 50, activation Q 0.25) spectra of *m*/*z* 1833.8.

no appreciable differences between batch 1 and batch 2 Tween 60 with regard to the fragmentation of the sorbitan palmitates. Both batches gave ions of similar intensity corresponding to losses of six POE units thus probably indicating that it is very unlikely that they contain a different balance of POE chain lengths.

3.3. NMR analysis

Fig. 8a and b shows the normalized ^1H NMR spectra for batches 1 and 2. The spectra have been normalized by dividing by the area of the CH_3 group, which is present at the end of the fatty acid acyl chains, this group and the CH_2–CH_2–CO in the acyl chains retain a 3:2 ratio in both spectra since they are not strongly affected by changes in chemical environment. The CH_2–CO at δ 2.34 differs between batches 1 and 2. In batch 2 this group has close to the expected 3:2 relative response but in batch 1 the relative response is lower. In batch 1 there is an



Scheme 2. Series of ions produced by tandem mass experiments with sorbitan 32 POE palmitate.

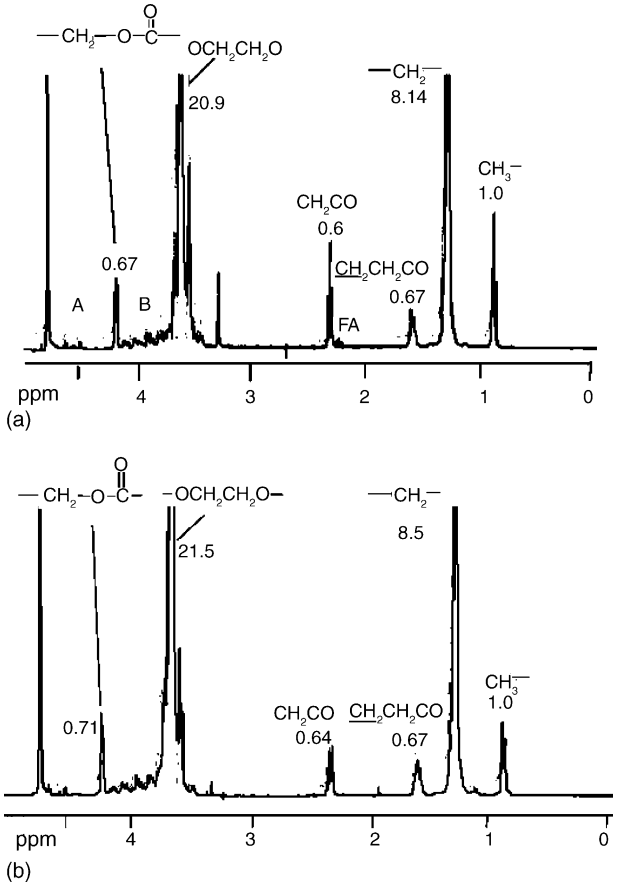


Fig. 8. Tween 60 ^{1}H spectra (a) batch 1, (b) batch 2.

additional small peak slightly up field from the peak at δ 2.34 and this signal is probably due to a small amount of free unesterified fatty acid (in this case at higher field since polyethylene glycol as an esterifying group will have a slight deshielding effect) and this is consistent with the acid value for batch 1 which was found to be 3.1 (equivalent to about 14 mg/g of free acid in the sample). Thus batch 1 contains about 8% less ester than batch 2.

In batch 1, the area for the –CH_2– envelope of the fatty acid side chain gives an average of 24.4 protons. In combination with the two –CH_2– and the CH_3 groups outside the main envelope, this gives an average number of protons of 31.4 per chain (confirmed by the calculation from the GC–MS data in Table 1, which gives an average of 32.6 protons per chain). The reason for the low value given by the NMR is not clear. In the case of batch 2 the same calculation indicates that there are an average of 32.5 protons per chain (calculation of the average chain length from the LC–MS analysis in Table 2 also gives 32.5). However, the bovine Tween (batch 1) has a saponification value of 51.3 and the non-bovine (batch 2) a saponification value 50.76 indicating that their fatty acid content in terms of chain length is similar, a high content of shorter chain fatty acids would give a higher saponification value (c.f. Table 1). There are 10 small –CH–O peaks in the ^{13}C spectrum between 80 and 90 ppm indicating the likely presence of all of the sorbitol-derived cyclic nuclei shown in Fig. 9.

Most of the polyoxyethylene protons occur in an envelope between 3.7 and 3.75 ppm in the ^{1}H domain (Fig. 8a and b) and between 70 and 71 ppm in the ^{13}C domain (Fig. 9). However, one of the CH_2 groups is clearly shifted downfield and occurs at ca. 4.12 ppm. This is the group adjacent to the fatty acid side chain. The identity of this group was confirmed by HMBC (hetero nuclear multiple bond coherence) experiments (Fig. 10). The proton (from POE group) at ca. δ_H 4.12 coupled (^{3}J) to the acyl

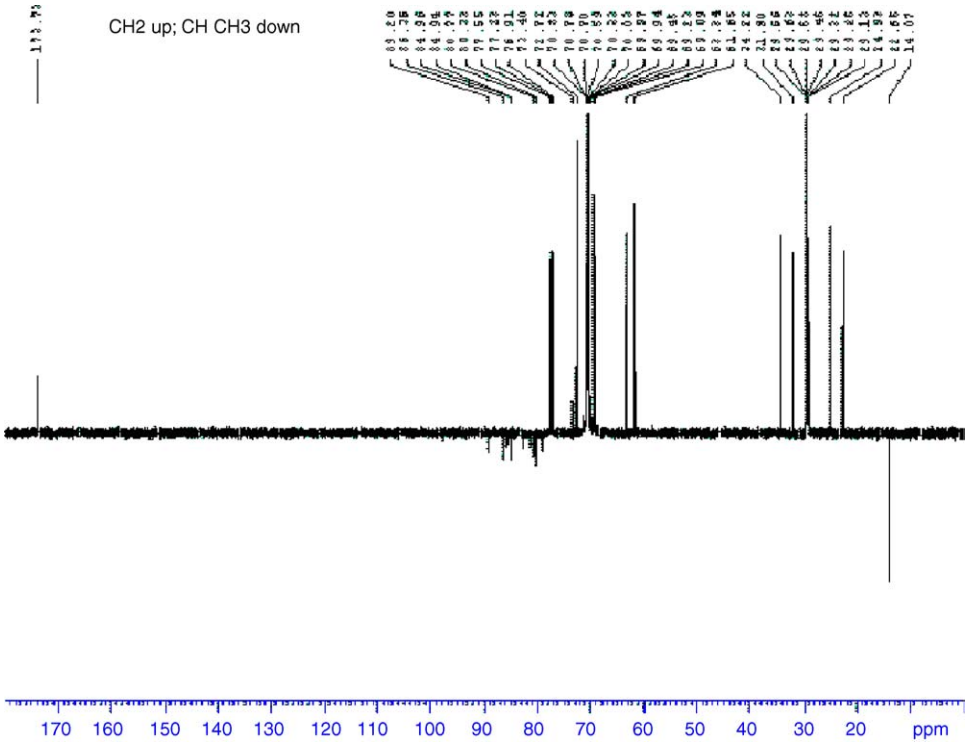


Fig. 9. Tween 60 ^{13}C spectrum.

carbonyl δ_C 173.7 and the latter showed further correlation (^2J) with the acyl α-CH_2 at δ_H 2.26 (triplet). This is because other equivalent polyoxyethylene groups were relatively shielded i.e. they showed the same shift in ^{13}C domain between 70 and 71 ppm and in ^{1}H COSY domain between $\delta = 3.5$–3.6 (Fig. 11). The relative intensity of this signal and the fatty acid CH_3 in batch 1 was 2:3, however, this cannot be correct since about 8% of the fatty acid in the sample is not esterified. This suggests that there may be an additional signal underneath the signal due to –CH2–OCO– that contributes to its intensity. In batch 2 the signal due to –CH_2–OCO– has an intensity ratio of 2.13:3 in relation to the fatty acid CH_3 and this again suggests that there

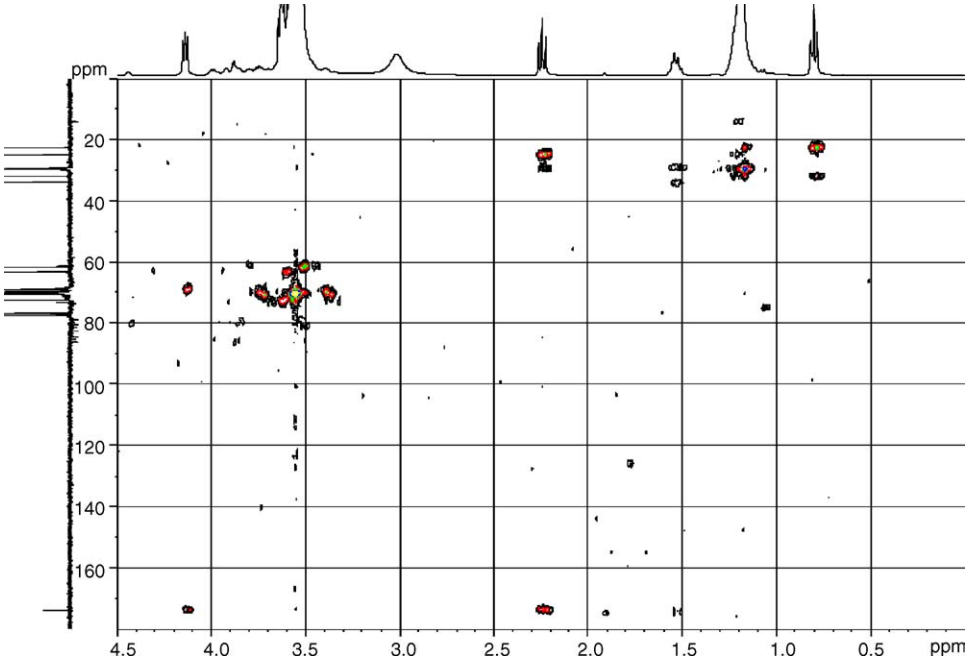


Fig. 10. Tween 60 hetero nuclear multiple bond coherence (HMBC) spectrum.

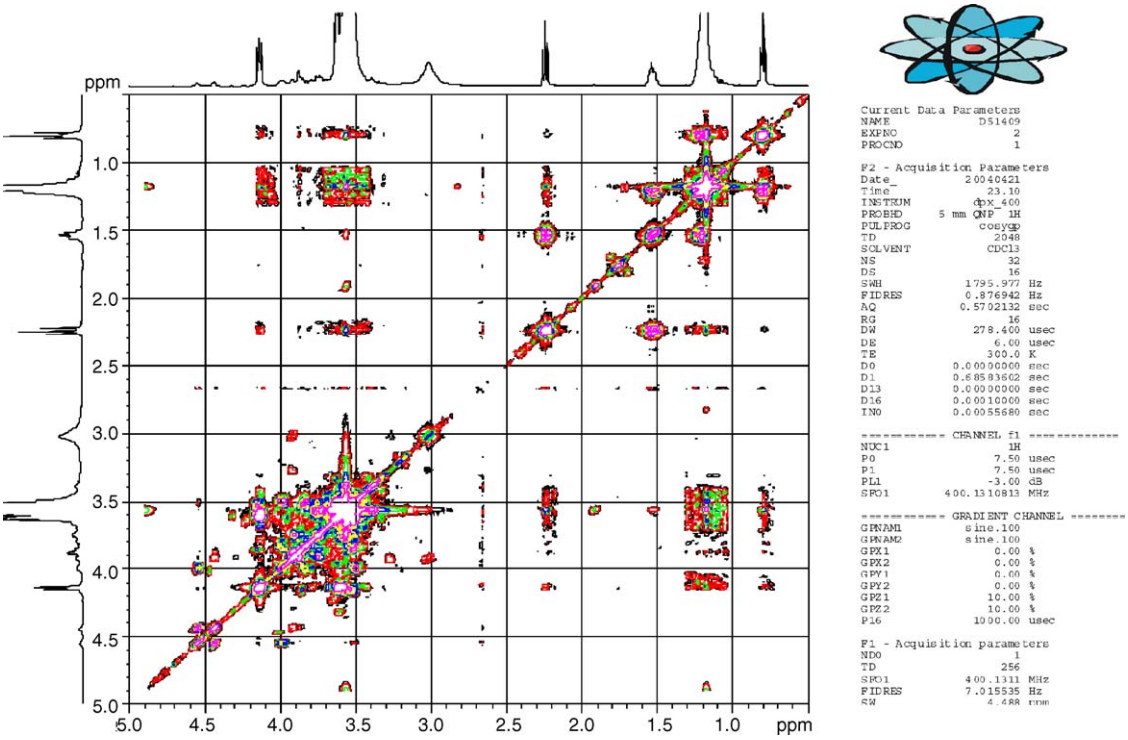


Fig. 11. Tween 60 H correlated spectroscopy (COSY) spectrum.

is an additional signal contributing to the signal at 8 4.12 since in this case the fatty acid is fully esterified and the ratio should be 2:3, even if some diester occurs. The signal of the polyoxyethylene envelope in batch 2 indicates the presence of an average of 16.2 polyoxyethylenes per molecule. The expected average if all the molecules were sorbitans would be 24 i.e. six per site of substitution; whereas, the average for substitution of isosorbide would only be 12 polyoxyethylenes per molecule. Thus, the average of 16.2 POE groups per molecules possibly indicates that batch 2 contains 35% sorbitans and 65% isosorbides. In batch 1, the estimate for polyoxyethylenes per molecule in batch 1 is slightly low due to the presence of free fatty acid in the sample contributing to the CH_3 signal. Possibly, batch 1 contains more sorbitans than batch 2. The ^{1}H NMR of Tween 60 is simply obtained and provides an indication of both fatty acid content and the content of isosorbide in the sample. Thus, the obtaining the ^{1}H NMR spectrum of a batch of Tween could provide a simple pharmacopoeial test.

4. Conclusions

1. LC–MS and ^{13}C NMR confirmed that Tween 60 was a complex mixture of polymeric species containing POE groups. The surfactant contained not only POE sorbitan monostearate but also a number of POE intermediates (POE sorbitan- and isosorbide- mono–di-esters of palmitic and stearic acids).
2. Although both batches contained similar components as reflected in both LC–MS and ^{1}H NMR experiments, LC–MS indicated that batch 1 differed from batch 2 in terms of the proportions of POE sorbitan palmitate/POE sorbitan stearate and amount of POE mono and/or diester-related substances.
3. NMR and acid values indicated that only batch 1 contained free fatty acid (∼8%). NMR also confirmed the ratio of sorbitans to isosorbides.
4. MS^n fragmentation experiments indicate that both batches have chains composed of a more or less equal distribution of POE units. The major ions present in the envelopes arising from the isosorbide and sorbitan species indicate that in both cases an average of six POE units is attached to two and four positions, respectively. This was confirmed by ^{1}H NMR.
5. Batch 1 contained more sorbitan esters than batch 2, and also free fatty acids. This may be responsible for the rheological properties of semisolid emulsions rather than the distribution of POE groups.
6. The information obtainable from ^{1}H NMR could form the basis for a straightforward pharmacopoeial test for the polyoxyethylene and fatty acid content in batches of Tween 60.

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