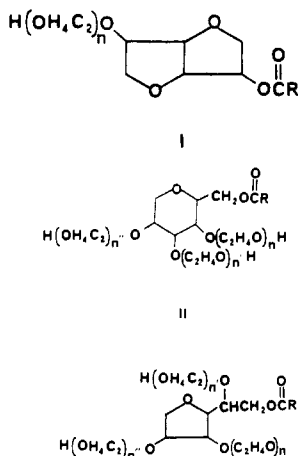


Characterization of Polysorbates by OH⁻ Negative Ion Chemical Ionization Mass Spectrometry

William C. Brumley,* Charles R. Warner, Daniel H. Daniels, Denis Andrzejewski, Kevin D. White, Zhao Min,¹ Jo Y. T. Chen, and James A. Sphon

The characterization of polysorbates, a complex mixture of polyoxyethylated fatty acid esters of sorbitan, by OH⁻ negative ion chemical ionization mass spectrometry is described. OH⁻ mass spectra afforded two ion series representing oligomers of polyoxyethylenes and polyoxyethylenes attached to sorbitans. Distinct ions characteristic of the fatty acids attached as sorbitan esters were also observed. OH⁻ results were then compared with the data available from Cl⁻ attachment mass spectra of polysorbates and materials used in their synthesis. A number of the negative ions produced under OH⁻ conditions were characterized by collision induced decomposition/mass-analyzed ion kinetic energy spectroscopy, and fragmentations typical of these ions are discussed. The OH⁻ spectrum served as a basis for identifying polysorbate 60 isolated from salad dressing.

Polysorbates (I and II), the fatty acid esters of sorbitan that contain polyoxyethylene (POE) units, constitute an important class of surface active agents (Griffith et al., 1975). In application, polysorbates may be compared to other food additives of similar purpose including mono- and diglycerides, Spans, polypropylene glycol esters, and other complex esters (Griffith et al., 1975). In 1977 an estimated 3.8 million pounds of compounds containing POE were used (National Academy of Sciences, 1979).



Polysorbates are regulated in foods according to sections 172.836 and 172.838 of the Code of Federal Regulations (1981) and *Fed. Regist.* (June 13, 1967). Mono- and diglycerides are regulated according to section 172.834 of the Code of Federal Regulations (1981). Polysorbates are primarily used in baked goods, meats, and dairy products.

Relatively few methods for the determination of polysorbates are available, and they focus primarily on characterization of the POE content, either as free oligomers (III) or bound to the sorbitan (I, II, and IV). Thus, complexation was used for the detection of POE by steric exclusion chromatography (Warner et al., 1979) and in thin-layer chromatographic (TLC) determination of polysorbate 60 in salad dressing (Daniels et al., 1982). Earlier work using TLC included that of Murphy and Scott

(1969) and Hodda (1976). Alternatively, chemical degradation for characterization was used by Kaduji and Stead (1976), titrimetry by Siggia et al. (1958), colorimetry by Boyer et al. (1977), and coprecipitation by Smullin (1974). The latter two techniques were reviewed by Longman (1975). Gas chromatography as a characterization technique was based on analysis of fatty acid moieties (Lindner, 1974) or alkyl iodides from hydriodic acid treatment (Ehrenberger, 1965). High-performance liquid chromatography was used by several workers (Bombaugh, 1970; Bombaugh et al., 1970; Huber et al., 1972; Krejci et al., 1974; Allen and Rice, 1975; Cassidy and Niro, 1976). Nuclear magnetic resonance spectroscopy (Crutchfield et al., 1964; Cooks et al., 1974) has also been used.

No mass spectrometry (MS) work has focused on characterization of polysorbates. However, various polymers have been characterized by MS by using field desorption (Schulten, 1979; Matsuo et al., 1979; Lattimer et al., 1980; Neumann et al., 1980; Craig et al., 1981) including a study of POE (McCrae and Derrick, 1983). Chemical ionization (CI) MS was also used in a survey of several polymers by Adams (1983).

The introduction of OH⁻ negative ion (NI) CIMS (Smith and Field, 1977) facilitated the study of a large number of organic functional groups, with a limited amount of fragmentation usually observed. However, considerable fragmentation was found by Brumley et al. (1982) and Dreifuss et al. (1983) in the OH⁻ spectra of polyfunctional molecules. These authors also studied NI fragmentation with collision induced decomposition/mass-analyzed ion kinetic energy spectroscopy (CID/MIKES). Earlier references to NI CID/MIKES and related collisional activation experiments include Kondrat et al. (1978), Hunt et al. (1980), and Burinsky and Cooks (1983). The (M - H)⁻ ions of fatty acids and fatty acid methyl esters were also recently studied by CID/MIKES (Bombagiotti et al., 1982, 1983; Tomer et al., 1983).

In this paper we explore the potential of OH⁻ NICIMS for characterizing polysorbates and certain starting materials used in their production. In addition, mass spectral data from Cl⁻ attachment spectra (Tannenbaum et al., 1975) and CID/MIKES under OH⁻ conditions are discussed. Finally, we apply the OH⁻ NICIMS technique to the confirmation of identity of polysorbate 60 in a salad dressing.

EXPERIMENTAL SECTION

Mass Spectrometry. OH⁻ and Cl⁻ low-resolution mass spectra were obtained on a Finnigan-MAT quadrupole

Division of Chemical Technology (W.C.B., D.A., K.D.W., Z.M., and J.A.S.) and Division of Chemistry and Physics (C.R.W., D.H.D., and J.Y.T.C.), Food and Drug Administration, Washington, DC 20204.

¹Visiting Scientist from the National Institute of Metrology, Beijing, People's Republic of China.

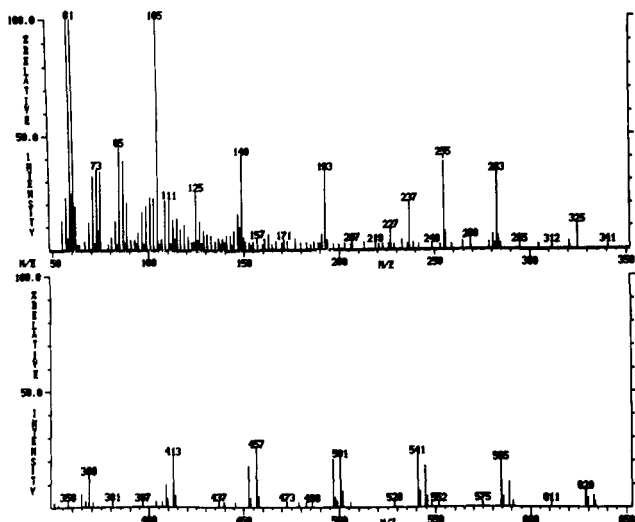


Figure 1. OH^- NICI mass spectrum of polysorbate 60; ion source 130°C ; solid probe introduction. (Normalized to m/z 105.)

mass spectrometer under conditions previously described (Brumley et al., 1982). Data are reported for the mass range of m/z 55–655. Polysorbate and Span 60 were heated from ambient to about 400°C in about 5 min. CID/MIKES experiments were performed on a VG ZAB-2F instrument under conditions described by Brumley et al. (1982). Samples were introduced by a solid probe heated independently of the ion source; ethylene glycol was introduced via the heated inlet on the ZAB-2F instrument.

TLC. A sample of creamy style Italian salad dressing was found by TLC quantitation to contain ca. 0.07% polysorbate 60. A 5-g sample was partitioned between methylene chloride and saline solution. An aliquot of the organic layer was evaporated to dryness and the residue partitioned between hexane and acetonitrile. The acetonitrile layer was streaked on a preparative scale TLC plate and developed as described by Daniels et al. (1982). The plate was scraped at the appropriate R_f and the scrapings were extracted with methylene chloride and filtered through a Millipore Millex filter ($0.5\ \mu\text{m}$).

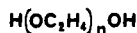
Fourier Transform Infrared (FT IR) Spectroscopy. Digitized FT spectra were obtained on a Digilab FTS-10 spectrometer at a resolution of $4\ \text{cm}^{-1}$ in double precision and a spectral range from 4000 to $600\ \text{cm}^{-1}$. Samples were placed between two NaCl crystals. Data were stored on General Data computer disks and manipulated by using the Digilab user's manual of Fortran application programs.

Chemicals. Polysorbate 20, 40, 60, and 80 were used as received (Sigma Chemical Co., St. Louis, MO).

RESULTS AND DISCUSSION

OH^- Spectra of Polysorbates. A composite OH^- spectrum of polysorbate 60 is given in Figure 1 and represents summed spectra over the entire heating cycle. Although a number of ions are observed in the spectrum, it is relatively simple considering the complexity of polysorbate.

Two major ion series of oligomers are observed. The first occurs at m/z 61, 105, 149, 193, 237, 281, 325, 369, 413, 457, 501, 545, 589, and 633. This ion series derives from POE oligomers (III) represented as $\text{HO}(\text{C}_2\text{H}_4\text{O})_n^-$, where n



III

ranges from 1 to 14. (We have observed the series up to $n = 15$.) We propose that each of the ions of the series

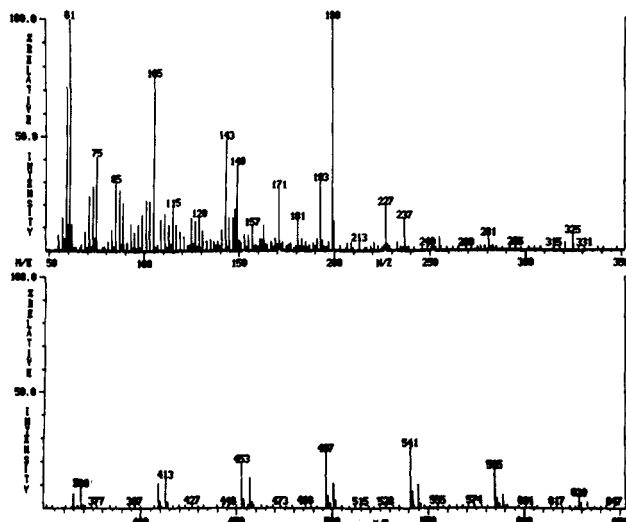
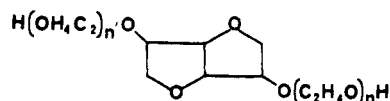


Figure 2. OH^- NICI mass spectrum of polysorbate 20; ion source 130°C ; solid probe introduction. (Normalized to m/z 199.)

corresponds to the $(\text{M} - \text{H})^-$ ion of the appropriate oligomer and, in general, does not arise as a fragment ion of higher molecular weight oligomers. Examination of ion profiles as a function of scan number (increasing probe temperature) provides evidence to support this hypothesis. Maxima in the ion profiles of the various ions appear at increasing probe temperature (scan number) as the masses of the ions under consideration increase. Thus, for example, m/z 369 maximized at a lower probe temperature than did m/z 413, the next member in the homologous series. Additional support for this interpretation comes from Cl^- spectra (see below).

The second ion series occurs at m/z 145, 189, 233, 277, 321, 365, 409, 453, 497, 541, 585, and 629. Ions at m/z 321 and above are the most easily discerned. We propose that this series represents the $(\text{M} - \text{H})^-$ ion of IV with the number of POE units ranging from 0 to 11. (We have observed the series up to $n = 13$.) The structure of the sorbitan unit shown as IV requires substantiation (see below).



IV

Polysorbate 60 is composed of a mixture of esters of stearic, palmitic, and myristic acids. These fatty acid constituents are readily indicated by ions at m/z 283, 255, and 227 representing $(\text{RCO}_2)^-$ of the appropriate acid. Nucleophilic attack of esters in the gas phase was previously shown to afford fatty acid anions (Brumley et al., 1982; Bambagiotti et al., 1982; Driefuss et al., 1983).

The presence of ions indicative of individual fatty acids serves as the chief basis for distinguishing among the various polysorbates by OH^- NICIMS. As an example, the composite OH^- spectrum of polysorbate 20 is shown in Figure 2. The abundant ion at m/z 199 represents $(\text{RCO}_2)^-$ of lauric acid and distinguishes polysorbate 20 from 60. Minor amounts of myristic and palmitic acids are also implied by ions at m/z 227 and 255. The two major ion series discussed already are also observed in the spectrum and in the spectra of the individual polysorbates. In the spectrum of polysorbate 40, predominantly m/z 255 of palmitic acid is observed. In the spectrum of polysorbate 80, m/z 281 of oleic acid is seen, with smaller amounts of palmitic and palmitoleic acids at m/z 255 and 253.

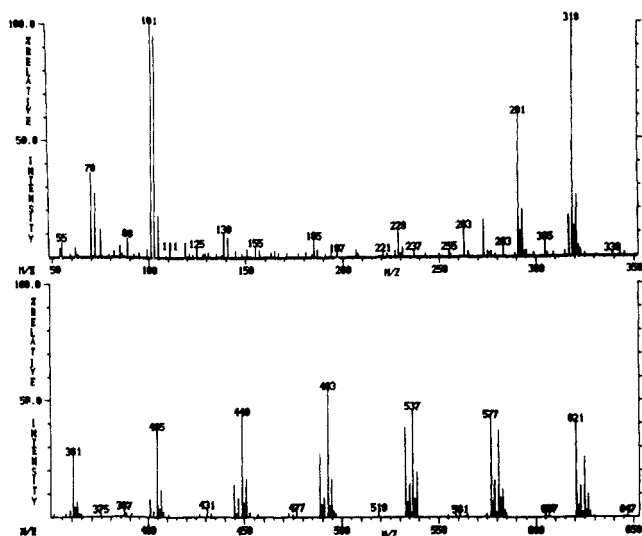


Figure 3. Cl^- NCI mass spectrum of polysorbate fraction from polysorbate 60; ion source 130 °C, solid probe introduction. (Normalized to m/z 319.)

The absence of $(M - H)^-$ ions of I or II prevents direct mass spectral verification of these intact structures. I and II represent generally assumed structural types of polysorbates and many isomeric possibilities exist. The presence of RCO_2^- and ions corresponding to $(M - H)^-$ of III and IV provide a composite identification of polysorbate. The structure of the fatty acid containing moieties is thus not determined under OH^- conditions.

To try to clarify structural issues, chemical separation with the Wiebull procedure (Coupkova et al., 1978) was used to divide polysorbate 60 into two fractions—one containing POE and fatty acid moieties of sorbitan (polysorbate fraction) and one without the fatty acid component (carbowax fraction). The OH^- spectra of both fractions afforded the two major ion series already discussed. In addition, the polysorbate fraction also afforded m/z 227, 255, and 283, indicative of the fatty acid moiety observed in the OH^- spectrum of polysorbate 60. We postulate that the carbowax fraction contains structures III and IV and the polysorbate fraction also gives rise to $(M - H)^-$ ions consistent with structures III and IV as well as the RCO_2^- ions. Again, no direct verification of I and II was obtained.

Our finding of two separate fractions, one containing POE and the other containing POE and fatty acid moieties, is consistent with the work of Murphy and Scott (1969). Because of the presence of free POE, the molecular weights of components with POE incorporated in sorbitans are likely to be substantially less than manufacturers' specifications. We believe that this is the first mass spectral verification that the Wiebull procedure separates carbowaxes and fatty acid containing moieties.

Cl^- Attachment Spectra of Polysorbates. Figure 3 shows the composite Cl^- spectrum of the polysorbate fraction from polysorbate 60. It appears similar in all respects to the Cl^- spectrum of polysorbate 60 itself. The most abundant ions at m/z 291 and 319 correspond to the $(\text{RCO}_2\text{H} + \text{Cl})^-$ ions of palmitic and stearic acids. The presence of these ions suggests that free fatty acid is present before ionization. Two major ion series are again observed. Under Cl^- conditions, they correspond to $(\text{III} + \text{Cl})^-$, at m/z 185, 229, 273, 317, 361, 405, 449, 493, 537, 581, and 625, and $(\text{IV} + \text{Cl})^-$, at m/z 357, 401, 445, 489, 533, 577, and 621. (We have observed the two series to m/z 713 and 709, respectively.) Each of these ions represents an individual oligomer, and these data support our

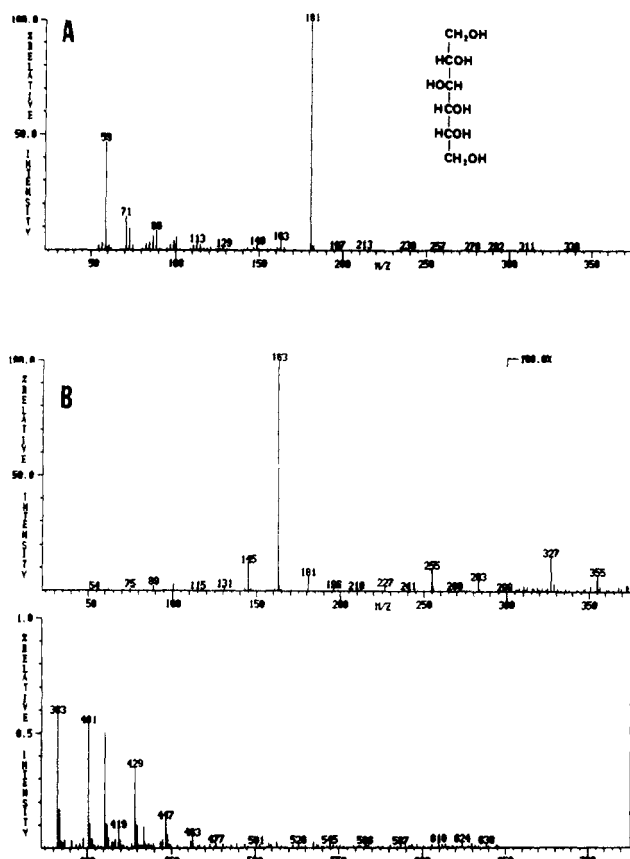


Figure 4. OH^- NCI mass spectra of (A) sorbitol and (B) Span 60; (multiplied by 100 beyond m/z 300); ion source 130 °C; solid probe introduction.

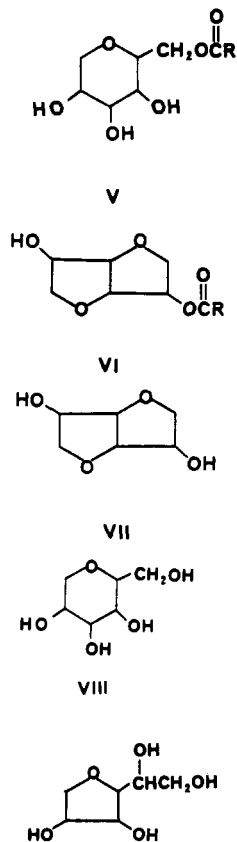
interpretation of the OH^- spectra as revealing oligomers. These results also suggest that neutral species presumably represented by III and IV are present before ionization.

Independent evidence from FT IR spectra indicates that free fatty acid is not confirmed in polysorbate 60 or in the polysorbate fraction. The carbonyl band at 1738 cm^{-1} supports the presence of an ester bond in polysorbate 60. The carbonyl band of free fatty acids occurs at 1711 cm^{-1} . A band at 1110 cm^{-1} confirms the presence of POE units as well. These data are consistent with II. The presence of minor components within a matrix can usually be detected by FT IR at levels approaching 0.1% by using least-squares curve fitting. The situation is complicated with respect to polysorbate 60 because of the inhomogeneity of this material. Therefore, the presence of free fatty acid that is implied by Cl^- spectra is consistent with the findings under OH^- conditions and is not necessarily at variance with the IR data.

OH^- Spectra of Starting Materials for Polysorbates. We obtained OH^- spectra of sorbitol and Span 60 (used in the production of polysorbate 60) to clarify certain aspects of the OH^- spectrum of polysorbate 60. Furthermore, CID/MIKE spectra of negative ions in these spectra and in spectra of polysorbate 60 suggest common ion structures.

The OH^- spectrum of sorbitol (Figure 4) reveals an intense ion at m/z 181, $(M - H)^-$, and numerous fragment ions at odd masses that result from bond cleavage accompanied by rearrangement. The ion at m/z 163 corresponds to $(M - H - \text{H}_2\text{O})^-$. The multiple loss of H_2O from $(M - H)^-$ is not observed (e.g., m/z 145). The substantial carbon-carbon bond cleavage that occurs is significant since it is generally assumed that fragmentation is limited under NCI conditions (Hunt et al., 1980).

The OH^- spectrum of Span 60 (Figure 4) reveals weak $(\text{M} - \text{H})^-$ ions at m/z 429 (stearate ester) and m/z 401 (palmitate ester) derived from V. Ions at m/z 411 and 383 are $(429 - \text{H}_2\text{O})^-$ and $(401 - \text{H}_2\text{O})^-$ and could result from loss of water as fragmentation or as $(\text{M} - \text{H})^-$ ions of VI. (Cl^- attachment spectra are most consistent with the loss of water via fragmentation.) Ions at m/z 283 and 255 represent RCO_2^- of stearic and palmitic acids. Ions at m/z 181, 163, and 145 are consistent with the presence of sorbitol, VII, and VIII. The OH^- spectrum of sorbitol indicates that abundant ions at m/z 163 and 145 from Span 60 are not derived from sorbitol.



CID/MIKE Spectra of Ions Produced by OH^- . CID/MIKE spectra of ions in the OH^- spectra of sorbitol, Span 60, and polysorbates were obtained to study their fragmentations and to identify similar ion structures. The CID/MIKE spectrum of m/z 181 of sorbitol revealed decomposition ions at m/z 163, 149, 131, 119, 115, 113, 101, 89, 71, and 59 (see Hunt et al., 1980, and references therein). The major ions correspond to loss of 32, 62, and 92 u that can be correlated with loss of methanol, ethylene glycol, and glycerol moieties. The MIKE spectrum verifies that fragment ions observed can be derived from the $(\text{M} - \text{H})^-$ ion.

CID/MIKE spectra of m/z 163 from both Span 60 and sorbitol were obtained. Both of the m/z 163 ions revealed major decompositions to m/z 131, 113, 101, 85, and 59. In addition, their similarity in relative abundance suggests derivation from a similar structure such as VIII. The ion at m/z 145 from Span 60 (Figure 5) revealed major decompositions at m/z 127, 115, 85, 73, and 59. The $(\text{M} - \text{H})^-$ ion of a sample of VII gave a similar CID/MIKE spectrum (Figure 5). The results provide a basis for comparing structures and are consistent with V, VII, and possibly VIII in Span 60.

The CID/MIKE spectrum of m/z 283 from Span 60 illustrates its use to further characterize fatty acid components (Bambagiotti et al., 1983; Tomer et al., 1983).

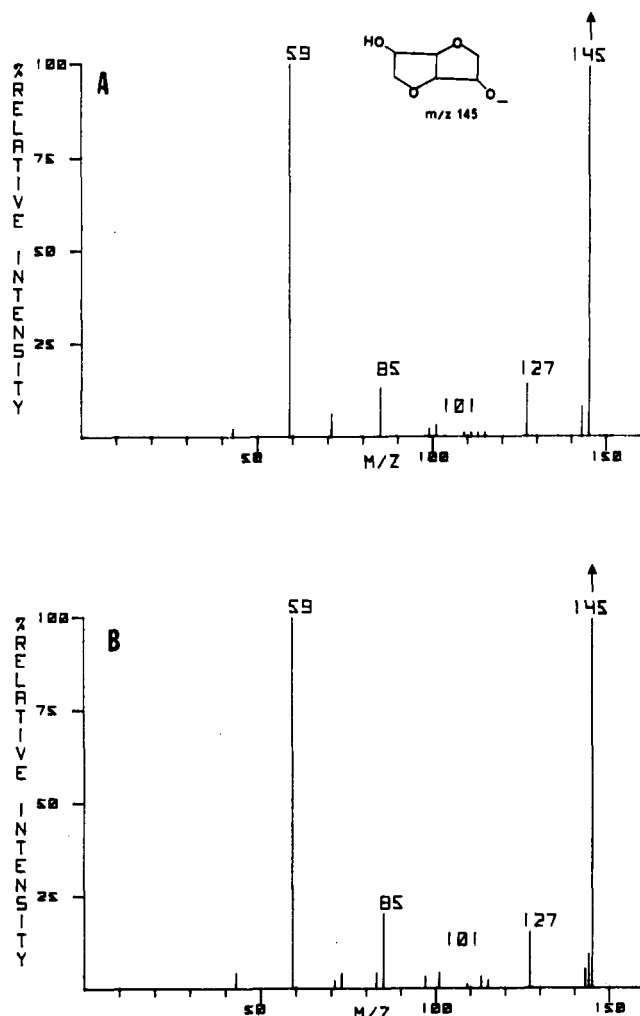


Figure 5. CID/MIKE spectra of (A) m/z $(\text{M} - \text{H})^-$ of 1,4:3,6-dianhydrosorbitol and (B) m/z 145 of Span 60.

Decompositions were observed at m/z 265 and 253, and then at a series of ions differing by 14 u (m/z 239, 225, 211, 197, 183, 169, 155, 141, 127, 113, and 99). Similarly, m/z 255 (palmitic) afforded decomposition ions at 237, 225, 211, 197, 183, 169, 155, 141, and 127. These results are consistent with those of Tomer et al. (1983).

In the CID/MIKE spectra of ions from the two oligomer series afforded in the OH^- spectrum of polysorbate 60, fragmentation occurs predominantly at the ends of the POE chains where the negative charge probably resides. Ions from the POE series show predominantly successive losses of 44 u, with the most abundant metastable ion usually $(\text{M} - \text{H} - 88)^-$. Thus, for example, m/z 237 yields m/z 221, 206, 193, 149, 105, and 61, where m/z 149 is the most abundant metastable ion.

The identification of the ion series derived from III in the OH^- spectrum is thus supported by the data on metastable ions. We speculate that the predominance of 88 u loss can be related to a tendency for the ion to exist in a meander structure (Roesch, 1967) in the gas phase and therefore to preferentially eliminate a dioxane moiety. The ion series derived from IV also shows successive losses of 44 u, with $(\text{M} - \text{H} - 44)^-$ the most abundant metastable ion. For example, m/z 321 shows decompositions to m/z 305, 303, 291, 277, 249, 233, and 145, with m/z 277 the most abundant ion. These data are consistent with IV.

The very large relative abundance of m/z 61 in the OH^- spectrum of polysorbate 60 was of interest to us. Its occurrence could be a result of thermal decomposition and fragmentation processes as well as be indicative of the

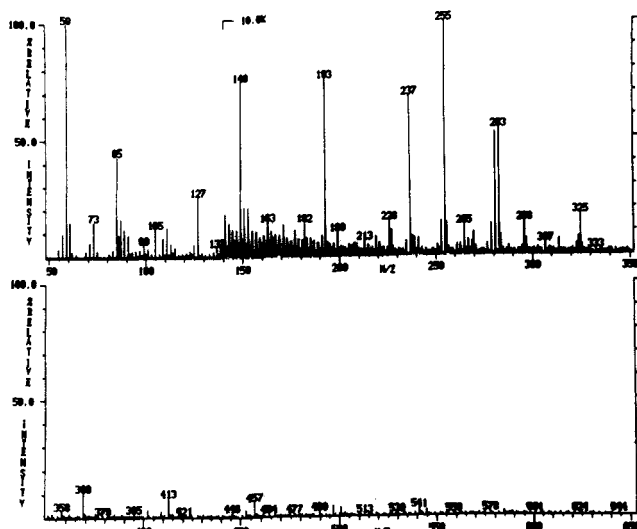


Figure 6. OH^- NCI mass spectrum of polysorbate 60 isolated from salad dressing; ion source 130°C ; solid probe introduction (multiplied by 10 beyond m/z 140).

presence of ethylene glycol. Therefore, further characterization of m/z 61 was obtained via its CID/MIKE spectrum. Major decompositions were m/z 59 and a much smaller m/z 31, both of which occur with similar abundance in the $(\text{M} - \text{H})^-$ ion of authentic ethylene glycol (m/z 61). Thus, the ion structure of m/z 61 is likely that of the $(\text{M} - \text{H})^-$ ion of ethylene glycol.

Confirmation of Polysorbate 60 in Salad Dressing.

The composite OH^- spectrum of polysorbate 60 isolated by TLC from salad dressing is shown in Figure 6. The spectrum reveals the two major ion series represented by III and IV as well as the RCO_2^- ions at m/z 227, 255, and 283. The qualitative agreement with the spectrum of the standard in Figure 1 is apparent. We conclude that the OH^- spectrum serves as a tool for the confirmation of a polysorbate in a food.

Registry No. Polysorbate 60, 9005-67-8; polysorbate 20, 9005-64-5; sorbitol, 50-70-4; Span 60, 1338-41-6; 1,4:3,6-dianhydrosorbitol, 652-67-5.

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