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QUANTIFICATION AND CHARACTERIZATION OF THE TRIFLUORO-ACETIC ANHYDRIDE DERIVATIVES OF N,N'-ETHYLENEBISSTEARAMIDE AND N,N'-ETHYLENEBISOLEAMIDE

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SUMMARY

An analytical technique for the measurement of low levels (0.1–3.0%) of N,N'-ethylenebisstearamide (EBS) and N,N'-ethylenebisoleamide (EBO) in polymeric matrices has been developed. The method involves reaction of the secondary bis-amides with trifluoroacetic anhydride (TFAA) followed by high-performance liquid chromatographic (gel permeation chromatographic) separation and quantification of the N-trifluoroacyl derivatives. The method is linear in ranges typical of polymer compounding applications and offers a direct measurement of EBS and EBO without the need for prior extraction. The TFAA derivatives were isolated and characterized by infrared and direct-probe mass spectrometry.

INTRODUCTION

Trifluoroacetic anhydride (TFAA) is a powerful electrophilic acylating reagent that has been shown to react with primary and secondary amines[1–3], amino acids[4], amides[5] and polyamides[6], as well as a number of other nucleophiles[7]. Historically, TFAA derivatizations have been employed in gas–liquid chromatography (GC) to improve the volatility and chromatographic behavior of otherwise non-volatile or difficult to separate compounds[8]. Recently, TFAA has been employed as a derivatization reagent for gel permeation chromatography (GPC) of polymers such as polyamides[9–11] and polyurethanes[12]. N-Trifluoroacylation of these polymers improves their solubility in common GPC solvents allowing characterization of the polymer based upon the chromatographic distribution of the TFAA analogue.

N,N'-Ethylenebisstearamide (EBS) and N,N'-ethylenebisoleamide (EBO) are high-melting aliphatic amides that are used as additives in a variety of polymers, functioning as lubricants, mold release agents and slip agents. EBS is widely used in acrylonitrile–butadiene–styrene (ABS) and other styrene co-polymers[13]. EBO has shown utility as a slip agent and a dispersant for other additives including flame retardants, pigment and colorants in polyolefins[14]. In addition, numerous patents have been issued for the application of EBS and EBO in a varity of thermoplastics, including polyurethane elastomers, polyacetals, nylons and acrylates. For these

applications, compounders require accurate quantitation of additive loadings, particularly where low levels of additives are used. Large variations in polymer performance are known to occur with relatively small differences in additive loadings.

This paper describes an analytical derivatization–GPC procedure for the measurement of low levels (0.1–3.0%) of EBS and EBO in polymer compounds. The derivatization procedure involves reaction of EBS and EBO with TFAA followed by GPC analysis of the N-trifluoroacyl derivatives. In addition, qualitative data are presented on the isolated TFAA derivatives, confirming the N-trifluoroacylation reaction.

EXPERIMENTAL

Reagents

EBS and EBO were prepared in-house and are commercially available. ABS, styrene–acrylonitrile (SAN) and low-density polyethylene (LDPE) were obtained from commercial sources. Reagent-grade TFAA, trichloroacetic anhydride and acetic anhydride were purchased from Aldrich (Milwaukee, WI, U.S.A.). High-performance liquid chromatography (HPLC) grade chloroform, dichloromethane, carbon tetrachloride and tetrahydrofuran (THF) were obtained from J. T. Baker (Philipsburg, NJ, U.S.A.). ACS reagent-grade heptane was purchased from EM Science (Cherry Hill, NJ, U.S.A.). All other incidental chemicals were ACS reagent grade.

Apparatus

The HPLC system used for the GPC separation of EBS-TFAA and EBO-TFAA derivatives consisted of a Model 510 pump (Waters Assoc., Milford, MA, U.S.A.), a Model 7125 injector equipped with a 100-μl loop (Rheodyne, Cotati, CA, U.S.A.) and a Model LC-100 column oven, maintained at 60 $\pm$ 1°C (Perkin-Elmer Corporation, Norwalk, CT, U.S.A.). Separations were performed at a flow-rate of 1.0 ml/min, with THF as the mobile phase. The gel permeation column set consisted of two μStyragel® 100 A and two μStyragel 500 A columns (Waters). Detection was performed with a Model 410 differential refractive index detector (Waters), with the internal temperature maintained at 50 $\pm$ 1°C. Chromatographic recording, integration and data handling were performed with a Model 4270/Chromatation AT system (Spectra-Physics, Santa Clara, CA, U.S.A.).

GC separations of the TFAA derivatives of EBS and EBO were performed on a Model 5890 GC system (Hewlett-Packard, Palo Alto, CA, U.S.A.). The gas chromatograph was equipped with a 3.5 m × 0.53 mm I.D. SPB-5 (d_f 1.0 μm) fused-silica column (Supelco, Bellefonte, PA, U.S.A.). Split injections (5:1) of 1.0 μl were performed with helium as the carrier gas at 65 cm/s. The column inlet temperature was 300°C. The flame ionization detector was set at 325°C. The column temperature program was from 280 to 320°C at 5°C/min with a final hold time of 5 min.

For identification of the N-trifluoroacyl derivatives a Model 4021 gas chromatograph–mass spectrometer was used (Finnigan, Sunnyvale, CA, U.S.A.). The gas chromatograph was equipped with a 12 m × 0.20 mm I.D. cross-linked methyl silicone (d_f 0.33 μm) fused-silica column (Hewlett-Packard). Split injections (20:1) of 1.0 μl were performed with helium as the carrier gas at 40 cm/s. The column inlet was set at 300°C and the column temperature program was from 280 to 320°C at 5°C/min with

a hold time of 15 min. Direct-probe mass spectral (MS) sampling was performed with ballistic heating from 50 to 300°C. In all experiments the mass spectrometer was used in the electron-impact (EI) mode with the ionization energy set at 70 eV. The scan rate was one scan per 3 s over a mass range of 35–950 a.m.u.

Infrared (IR) spectra were recorded on a Model 281 IR spectrophotometer (Perkin-Elmer) using a thin film preparation on a thallium bromide window (Model KRS-5; Foxboro Company, North Haven, CT, U.S.A.).

SAN and LDPE were compounded in a Model EPL-V3302 Brabender Plasticorder (C.W. Brabender, Hackensack, NJ, U.S.A.) equipped with a No. 6 roller head and roller blades. LFPE was pelletized in a 3/4-in. single screw extruder. ABS was powder blended with EBS and melt blended in an Instron Model 1123 universal testing instrument (Instron Corp., Canton, MA, U.S.A.) equipped with a Model 3210 capillary rheometer.

Procedure

TFAA derivatization of EBS in SAN was performed by transferring 5.000 g (± 5 mg) of SAN containing between 0.3 and 3.0% EBS into a 100-ml pressure-tight reaction vessel (Model 3-3110, Supelco). To the reaction vessel were added 50.00 ml of chloroform and 2.00 ml of TFAA. The vessel was sealed and heated on a hot plate (115°C) for 1.5 h. After reaction, the sample was cooled to ambient temperature, diluted ten-fold in THF and chromatographed by GPC with THF as the mobile phase at 1.0 ml/min. Multi-level calibrations were performed by following the same procedure with the addition of 15.0 (± 0.5), 75.0 (± 0.5) and 150.0 mg (± 0.5 mg) of EBS to each of three reaction vessels containing 5.000 g SAN with no additives.

TFAA derivatization of EBS in ABS was performed as described above for SAN samples. Multilevel calibrations were carried out in the same manner, with the same EBS standard levels.

TFAA derivatization of EBO in LDPE was performed by transferring 1.000 g (± 5 mg) of LDPE containing between 0.2 and 0.5% EBO to a 100-ml pressure tight reaction vessel. To the vessel were added 30.00 ml of chloroform plus 2.00 ml of TFAA. The vessel was sealed and heated for 1.5 h on a hot plate (115°C). After reaction, the sample was cooled to ambient temperature, during which time the LDPE precipitated. The resulting mixture was centrifuged at 3070 *g* for 10 min (Model HN-SII; International Equipment Company, Needram Heights, MA, U.S.A.). The lighter polyethylene gel separated from the reaction mixture and migrated to the top of the vessel. A 10-ml luer lock syringe, with a 3-in. No. 17 gauge needle, was used to penetrate the LDPE layer and collect the chloroform layer for HPLC analysis. The collected portion was chromatographed directly by GPC without further dilution. Multilevel calibrations of EBO in LDPE were performed by following the same procedure as above with the addition of 2.00 (± 0.05), 3.50 (± 0.05) and 5.00 mg (± 0.05 mg) of EBO to each of the reaction vessels containing 1.000 g LDPE containing no additives.

TFAA derivatives of EBS and EBO were prepared for the purpose of isolation and identification by reacting 0.150 g (± 5 mg) of the bis-amide with 2 ml TFAA in 50 ml of chloroform in a 100-ml reaction vessel. The mixture was heated for 1.5 h at 115°C, and then dried under a stream of high-purity nitrogen. The EBS-TFAA derivative was recrystallized twice from scrupulously dried diethyl ether. The
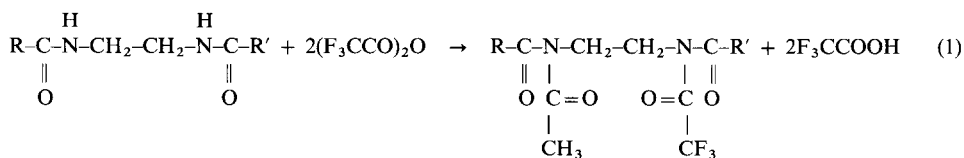
EBO-TFAA formed a yellow oil that would not recrystallize. The latter was analyzed without further purification. The derivatives were stored over desiccant prior to GC, IR and GC–MS analysis.

RESULTS AND DISCUSSION

HPLC analysis of EBS and EBO in polymer compounds is made difficult by the fact that neither the polymers nor the aforementioned additives is readily soluble in routine HPLC solvents. GC analysis is limited by both the solubility and volatility of the fatty secondary amides and the polymer matrix. Edwards[15] has reported an HPLC method for the analysis of amide slip agents in polyethylene and polyethylene–vinyl acetate copolymers. However, the slip agents reported were the more soluble primary amides. The latter were readily extracted from resin granules and thin film strips with methanol. Additives in polyolefins have also been analyzed by capillary GC with on-column injection of dichloromethane–methanol (1:1) extracts[16]. In-house procedures for separation and analysis of the less soluble secondary bis-amides, EBS and EBO, in various polymer compounds historically involved high temperature Soxhlet extractions (6–24 h), followed by total nitrogen (Kjeldahl) analysis. These methods are time consuming, require significant amounts of sample handling and lack specificity and sensitivity. This lab has also investigated dispersive IR and Fourier transform (FT)-IR analysis of polymer thin film for EBS and EBO using reflectance and attenuated total reflectance accessories. While IR spectroscopy shows promise for higher levels of these additives (1–5%)in a limited number of polymer compounds, it does not represent a universal analytical approach. Furthermore, IR techniques have been hampered by the ability to prepare and/or reproduce thin films. Differential scanning calorimetry (DSC) has also been investigated. Preliminary results indicate that subtle changes in the polymer glass transition state may be correlated to additive loading levels.

TFAA derivatization of EBS and EBO in polymer compounds followed by HPLC (GPC) analysis of the N-trifluoroacyl derivatives allows direct measurement of low levels of these additives without the need for extraction.

Eqn. 1 shows the reaction of TFAA with EBS/EBO:

$$
\begin{array}{c}
\quad\ \text{H}\qquad\qquad\ \text{H}\\
\text{R–C–N–CH}_2\text{–CH}_2\text{–N–C–R}' + 2(\text{F}_3\text{CCO})_2\text{O} \ \rightarrow\ \text{R–C–N–CH}_2\text{–CH}_2\text{–N–C–R}' + 2\text{F}_3\text{CCOOH} \qquad (1)\\
\ \ \|\qquad\qquad\qquad\ \|\qquad\qquad\qquad\qquad\qquad\ \|\ |\qquad\qquad\ |\ \|\\
\ \ \text{O}\qquad\qquad\qquad\ \text{O}\qquad\qquad\qquad\qquad\qquad\ \text{O C=O}\quad\text{O=C O}\\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad\ |\qquad\qquad\ |\\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad\text{CH}_3\qquad\quad\ \text{CF}_3
\end{array}
$$

Although both N- and O-trifluoroacetylation reactions have been reported[7] and EBS/EBO derivatization may proceed through the enolic intermediate, no evidence of O-trifluoroacetylation has been found. Other anhydrides were also tested for their ability to derivatize EBS and EBO. Trichloroacetic anhydride, chloroacetic anhydride and acetic anhydride, respectively, were less reactive towards the secondary bis-amides. The latter two anhydrides did not react with EBS or EBO in polymer matrices. TFAA not only reacts with the secondary bis-amides, but significantly enhances polymer solubility in the reaction media.

TABLE I

TYPICAL ALKYL DISTRIBUTION OF COMMERCIAL STEARIC AND OLEIC ACIDS

Commercial acid	Component (%)								
	C_{14}	$C_{14:1}$	C_{16}	$C_{16:1}$	C_{17}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Stearic	5	—	45	—	1.5	48	0.5	—	—
Oleic	2	2	6	6	—	3	72	8	1

Table I lists the typical fatty acid distributions of the aliphatic portion of EBS and EBO. Although the aliphatic portion of these compounds shows no apparent effect upon the reaction with TFAA, the chain length distribution does effect chromatographic behavior of the resulting derivatives, and was a determining factor in choosing the GPC column set and GC capillary column.

Fig. 1A is a chromatogram showing the GPC separation of the N-trifluoracyl derivative of EBS from SAN copolymer. Fig. 1B is a chromatogram showing the GPC separation of the N-trifluoroacyl derivative of EBO from LDPE. In both separations the derivative peak is well resolved from the polymer distribution (or residual polymer in the case of LFPE) and the reaction solvent and by-products. The latter elute near the total permeation volume of the GPC column set. The negative deflection in both chromatograms at 32–34 min is due to residual TFAA. In some cases, low levels of other additives such as stabilizers and antioxidants may be compounded along with
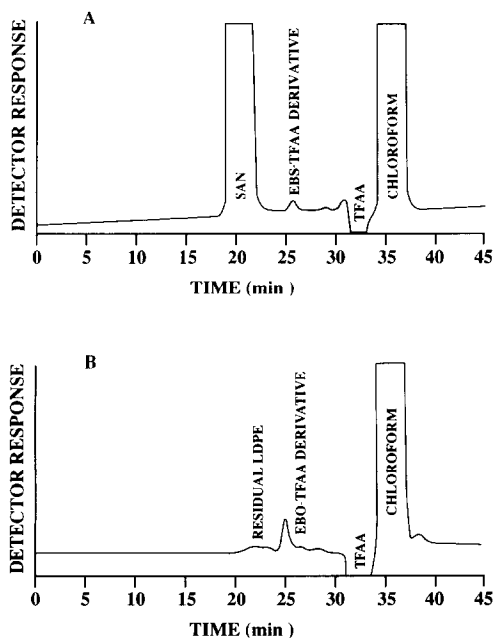

Fig. 1. Gel permeation chromatogram of (A) TFAA derivatized EBS (3%) in SAN copolymer and (B) TFAA derivatized EBO (1%) in LDPE. Instrument operating conditions given in the Experimental section.

TABLE II

LINEAR RANGES AND MINIMUM DETECTION LIMITS FOR EBS AND EBO DERIVATIVES

Sample	Working range (%,w/w)	Linearity, R^2	MDL^a
EBS in SAN	0.3–3.0	0.9999	0.01
EBS in ABS	0.3–3.0	0.9996	0.02
EBO in LDPE	0.2–0.5	0.9989	0.05

^a MDL = Minimum detection limit, based on a signal-to-noise ratio of 2:1.

[a] MDL = Minimum detection limit, based on a signal-to-noise ratio of 2:1.

EBS or EBO. Such components may present a problem in quantitation of EBS or EBO if they fall in the same molecular-weight range as the respective TFAA derivatives. In this study, the EBS and EBO derivatives were sufficiently resolved from any other interferences to allow for good quantitation. Preliminary results obtained in this laboratory for complex polymer blends indicate that quantitation by the method of standard addition may be used where additives of similar molecular weight interfere with EBS or EBO measurement. Also, changing data handling from peak area to peak height improves quantitation when EBS and EBO derivatives are not completely resolved from interfering peaks.

Table II lists the linear ranges and minimum detection limits for EBS and EBO compounded in three polymers, ABS, SAN and LDPE. The secondary bis-amide/polymer combinations and the corresponding working ranges are consistent with specific plastic applications. EBS and EBO response linearity are based on a three point standardization. The minimum detection limits have been obtained experimentally and represent that quantity of analyte that produces a signal equal to twice the baseline noise.

Table III lists the precision and accuracy of the method for EBS and EBO measurement in the respective polymer matrices. The standard deviation is less than 2% relative for both standards and samples ($n = 5$ determinations). Good agreement was found between the measured level and the actual compounded amounts of EBS and EBO. The accuracy of the determination is largely dependent upon the method of standardization. A multipoint external standard calibration of the secondary bis-amide in the polymer matrix was found to give the best results.

Fig. 2 shows the effect of the polymer matrix upon the standardization of EBS in SAN. It is evident that the polymer matrix affects the standardization, decreasing the

TABLE III

PRECISION AND ACCURACY FOR EBS AND EBO USING TFAA DERIVATIZATION METHOD

Sample	$R.S.D.^a$ (%)		Accuracy	
	Standards	Samples	% Compounded	% Found
EBS in SAN	1.42	1.88	2.00	2.08
EBS in ABS	1.69	1.98	1.00	0.96
EBO in LDPE	1.35	2.00	0.40	0.41

^a R.S.D. = Relative standard deviation ($n = 5$).
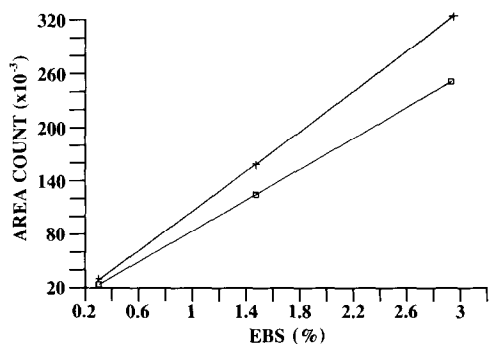
[a] R.S.D. = Relative standard deviation ($n = 5$).

Fig. 2. Comparison of SAN copolymer matrix (□) and non-matrix (+) three-point calibration of TFAA-derivatized EBS (range 0.3–3.0%).

slope of the standard curve. This trend was consistent, regardless of the secondary bis-amide or polymer matrix studied. Table IV lists calibration data for polymer matrix and non-matrix standardization of EBS and EBO in the three polymers studied. The response is always greater for the analyte in the matrix-free environment. However, the polymer matrix does not appear to affect the linearity of response as evidenced by the correlation coefficients shown. These results indicate that improved accuracy may be expected when standards are prepared in the polymer matrix in which they intend to be measured. However, it may be possible to apply a calculated correction factor to non-matrix standardization results based on the differences between the two calibration curves.

As noted previously, TFAA addition to chloroform greatly improved the solubility of otherwise insoluble or very slow to dissolve polymers. Hence, some controversy arose as to whether EBS and EBO were in fact derivatized, or perhaps more readily dissolved with the aid of TFAA. Four pieces of evidence indicate the formation of bis-amide -N-trifluoroacyl derivatives: IR spectral data, melting point data, MS analysis and GC analysis.

Fig. 3 shows IR spectra of EBO and the isolate from the reaction of EBO with TFAA, respectively. Strong bands at 3350 cm^{-1} (N–H stretching vibrations) and 1565 cm^{-1} (N–H bending vibrations) characteristic of secondary amides were not present in the TFAA derivative isolate spectrum. Furthermore, the shift of the amide band in the derivative spectrum from 1640 to 1720 cm^{-1} is consistent with the formation of

TABLE IV

EFFECT OF POLYMER MATRIX ON EXTERNAL STANDARDIZATION

Sample	Slope[a]		y Intercept		R^2	
	Matrix	Non-matrix	Matrix	Non-matrix	Matrix	Non-matrix
EBS in SAN	$4.97 \cdot 10^{-6}$	$3.63 \cdot 10^{-7}$	0.010	0.002	0.9999	1.0000
EBS in ABS	$1.23 \cdot 10^{-5}$	$9.48 \cdot 10^{-6}$	0.190	0.001	0.9996	0.9999
EBO in LDPE	$2.3 \cdot 10^{-7}$	$1.37 \cdot 10^{-8}$	0.001	0.001	0.9989	0.9997

[a] Slope based on a three-point external standardization.
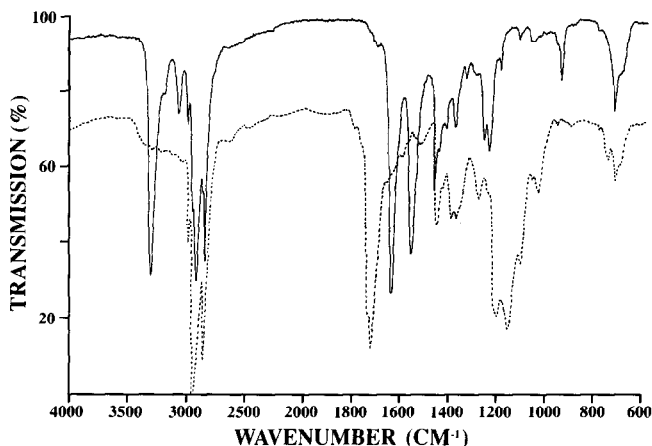
Fig. 3. Infrared spectrum of (———) EBO and (--------) the isolate from the reaction of EBO with TFAA.

a tertiary amide and with the addition of an electron withdrawing group to the nitrogen. Analogous IR spectral differences between EBS and the EBS/TFAA isolate were observed. These results are consistent with those published previously for the N-trifluoroacyl derivatives of polyamides[12].

The EBS and EBO derivative isolates also displayed different melting ranges than their respective starting materials. The EBS derivative isolate displayed a melting range of 61–69°C. The EBS starting material melts between 140 and 145°C. Recrystallization of the EBS-TFAA isolate was performed in scrupulously dried solvents since the N-trifluoroacyl derivatives favor hydrolysis back to the secondary amide. The EBO starting material had a melting range of 110–117°C, the EBO-TFAA derivative isolate was a liquid at room temperature. Repeated attempts to recrystallize this isolate, even under cryogenic conditions (-72°C), were unsuccessful. Decreased melting ranges are consistent with the formation of tertiary amide derivatives, due to loss of available protons for inter-molecular bonding.

Fig. 4 and 5 are direct-probe EI mass spectra (DP-EI-MS) of EBS and the EBS-TFAA derivative isolate, respectively. The molecular ion (M^+) at $m/z = 784$ (Fig. 5) represents the derivatized bis-amide with two stearate groups making up the aliphatic portion of the molecule. As shown in Table I, the aliphatic portion of EBS is derived form a complex distribution of fatty acids. Although the molecular ions throughout the probe profile were weak ($<0.1\%$ total ion current), M^+ ions of $m/z = 770, 756, 742$ and 728 representing other possible combinations of fatty amide derivatized species were found in other MS runs. Molecular ions and high-molecular-weight fragments were als quite weak in the DP-EI-MS of the EBS starting material. However, some common low-molecular-weight fragments ($m/z = 43, 57, 71, 84$ and 98) were found in both this spectra and the EBS/TFAA derivative isolate spectra. These data indicate that the isolate is likely a derivative of the EBS starting material. No clear assignment could be made for fragments associated with the trifluoracyl fragment. Similar DP-EIMS results were obtained for the EBO-TFAA derivative, with weak M^+ ions of $m/z = 780, 778, 766, 754, 752$ and 750 being observed.
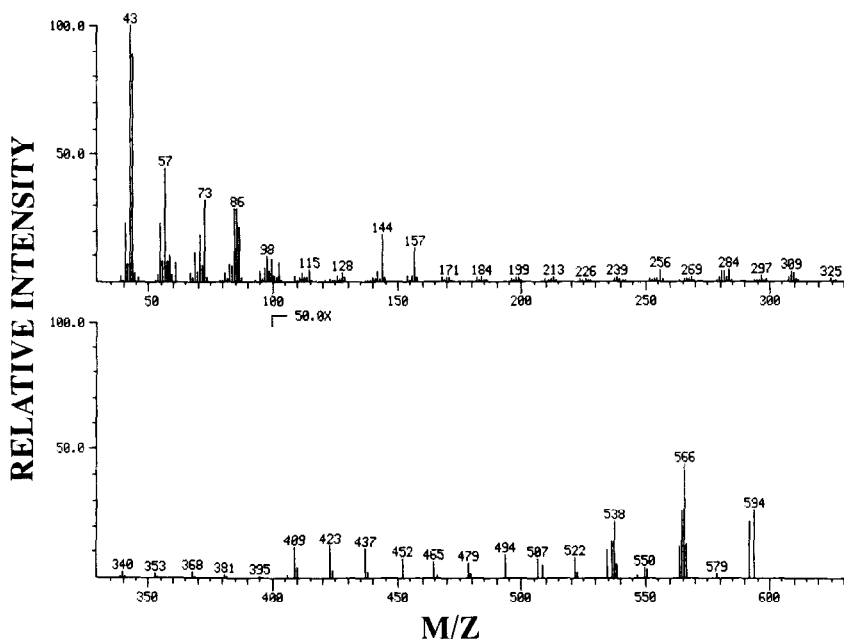
Fig. 4. Direct-probe EI (70 eV) mass spectrum obtained by analysis of underivatized EBS. Instrument operating conditions given in the Experimental section.
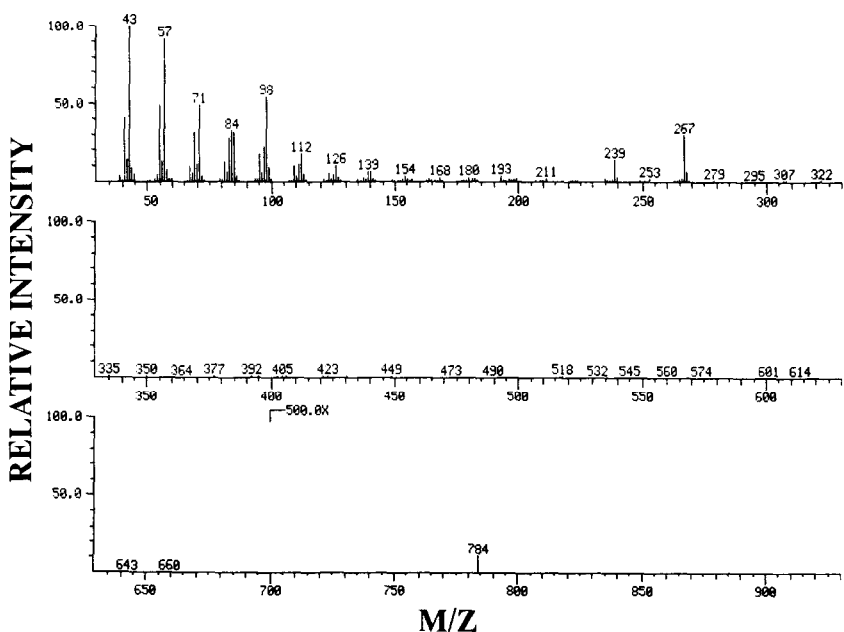

Fig. 5. Direct-probe EI (70 eV) mass spectrum obtained by the analysis of TFAA derivatized EBS, showing the weak molecular ion (*m/z* 784) for the distearate bis-amide derivative. Instrument operating conditions given in the Experimental section.

GC–EI-MS analysis of both the EBO-TFAA and EBS-TFAA derivatives was unsuccessful, presumably because not enough material was ionized to produce a strong mass spectrum. In either case, no molecular ions were observed for the N-trifluoroacyl derivatives. A softer ionization mechanism, such as chemical ionization (CI) or fast atom bombardment (FAB) may have given improved results, especially with respect to generating stronger molecular ions. However, these techniques were not available during this study. The use of ammonia reagent gas in desorption CI-MS has been reported to be a useful technique for obtaining strong pseudo molecular ions (M + NH$_4$) in high-molecular-weight compounds containing long chain aliphatic functions[17]. This technique may be successful for MS analysis of the TFAA derivatives.

The TFAA derivatives of EBS and EBO have also been analyzed by GC on a short non-polar stationary phase column (3.5 m, SPB-5) with high carrier gas flow-rate (65 cm/s). This fact alone supports the formation of the N-trifluoroacyl derivative, since EBS and EBO are themselves not sufficiently volatile to undergo GC. While TFAA derivatization of EBS or EBO followed by GC would not be applicable to polymer analysis, it may be an attractive approach for product characterization. The TFAA derivatives are readily formed at room temperature and the GC separation may be fine-tuned to give a distinct "finger print" of the final product distribution. An important consideration in the GC technique is the removal of excess TFAA and trifluoroacetic acid by evaporation or neutralization prior to GC analysis. Residual acid and anhydride are highly corrosive and may damage stainless steel surfaces on the GC injector and detector, and may also depolymerize the stationary phase.

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