# A Determination of the Apparent Molar Absorption Coefficients of the Cobalt Thiocyanate Complexes of Nonylphenol Ethylene Oxide Adducts

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## Abstract

Cobalt thiocyanate is used as a colorimetric reagent to determine traces of polyoxyethylene nonionic surfactants. It has been established that when there is an insufficient number of oxide units per hydrophobe the color intensity is markedly diminished, and with smaller numbers of oxide units the color fails to form altogether. The exact number of oxide units at which the colorimetric procedure fails has not been established previously, due to the unavailability of the pure individual ethylene oxide adducts.

The individual ethylene oxide adducts of high purity were obtained by liquid chromatography on silica gel. The mixed solvent used was originally developed for thin-layer chromatography and was applied without change to column chromatography. The composition of the separated isomers has been determined by infrared, ultraviolet, nuclear magnetic resonance, and mass spectroscopy.

The apparent molar absorption coefficients have been obtained for the individual cobalt thiocyanate complexes both in benzene and chloroform. For the low molecular weight adducts studied, the efficiency of color development and extraction into the organic phase has been found to be dependent on the concentration of the cobalt thiocyanate reagent. A saturated aqueous solution of cobalt thiocyanate was found to be preferable and benzene was found to be a more reliable extractant than chloroform.

The apparent molar absorption coefficients do not vary linearly with chain length at low molecular weights and the minimum number of ethylene oxide units that will form an extractable color with the saturated reagent was found to be 3.

## Introduction

NONIONIC DETERGENTS derived from ethylene oxide and an alcohol or a phenol comprise a large portion of the surfactants marketed in the world today. These detergents have recently undergone a second generation transition from biorefractive to biodegradable surfactants. Biodegradation studies on these materials required the detection and measurement of parts per million nonionic surfactants in water. Procedures that have evolved to fill this need include modifications of the cobalt thiocyanate procedure of Brown (1–3). These procedures are dependent on the measurement of the absorbance of a cobalt thiocyanate complex that has been extracted from an aqueous phase by a suitable organic solvent. All workers recognized that the color intensity was dependent upon the length of the ethylene oxide chain, and consequently the method was calibrated using matching samples of nonionic surfactants as standards.

The universal applicability of the method has been questioned. The uncertainties which have arisen concern the choice of the extractant and the minimum chain length of ethylene oxide thiocyanate complex. These questions are answered in this paper by applying the colorimetric method to the ethoxylates derived from the condensation of ethylene oxide and nonylphenol, and separated into pure individual chain lengths by column chromatography. The single-chain-length ethoxylate isomers were also used to determine the apparent molar absorption coefficients of the aromatic nuclei absorbing at 275 m$\mu$ in methanol solution. The purity of each isomer was determined by NMR, mass spectroscopy, and thin-layer chromatography. Infrared spectra of the homologous series were also obtained and critically evaluated.

The absorption coefficients determined in this study are more appropriately labeled "apparent molar absorption coefficients." This arises because the samples measured were obtained by extraction of the complex from an aqueous phase into an organic phase. The distribution coefficients of the compounds used in this study are unknown. Consequently, the completeness of the extraction is not known, and the data should be labeled "apparent molar absorption coefficients."

## Experimental Procedures

### Isolation of Single-Chain-Length Ethoxylates

The single-chain-length ethoxylates were isolated by column chromatography on 500 g of 0.05 to 0.2 mm silica gel packed from a benzene slurry into a column whose dimensions were 50-mm diameter and 760 mm long. The column temperature was maintained at 19C by circulating an ethylene glycol water mixture through a jacket. Eight grams of sample were chromatographed with a silica gel-to-sample ratio of 62.5:1. The solvent (acetone:benzene:water, 50:50:1 v/v) was chosen based on satisfactory separations obtained by thin-layer chromatography on silica gel. By controlling the solvent at a constant height in the column, a constant flow rate of 1 ml/min was maintained. A drop counter fraction collector was used to collect 645, 10-ml fractions. The solvent was removed by vacuum stripping with a minimum of heat.

### Determination of Apparent Molar Absorption Coefficients of Cobalt Thiocyanate Ethoxylate Complexes

The reagent (280 g $Co(NO_3)_2 \cdot 6\,H_2O$, 620 g $NH_4SCN$, and 850 g water) was pre-extracted with 3 (100-ml) portions of either benzene or chloroform. All extractions of the reaction product were per-

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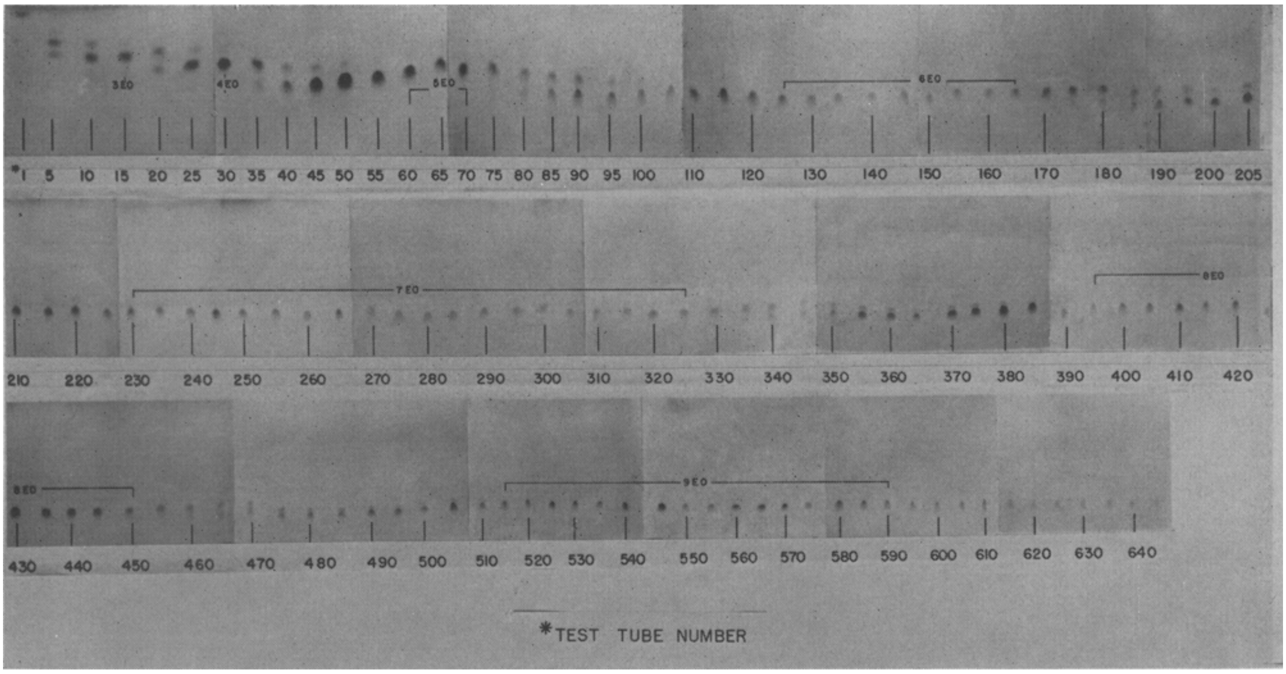

FIG. 1. Mixed Tergitol nonionic NP-14 and NP-16 thin-layer chromatograms of column fractions.

formed using 4 (50-ml) portions of solvent and the 4 fractions were collected and brought to 200 ml with pure solvent. The absorbance measurements were made on a Beckman DK-2A with appropriate dilutions or changes in cell path to provide spectra with an absorbance range between 0.1 to 0.75. A known weight (about 10 mg) of each pure isomer was reacted with 1 ml of cobalt thiocyanate reagent and extracted with either benzene or chloroform.

### Spectral Data

Weighed samples of the pure isomers were brought to a known volume with carbonyl-free methanol and the visible (620 mμ) and ultraviolet (320 mμ) absorbances were measured using a Beckman Model DK-2A spectrophotometer. All infrared spectra were of capillary films and were recorded on a Beckman Model IR-4 spectrometer using a NaCl cell. By controlling the thickness of the capillary film it was arranged that all major absorption bands had greater than 20% transmission. The NMR spectra were obtained using a Varian model A-60 spectrometer, and tetramethyl silane was the reference standard. The mass spectra were obtained using the heated probe attachment of the Bendix Time-of-Flight mass spectrometer. The conditions for obtaining the spectra, and a detailed analysis of these spectra, are to be submitted for publication in a subsequent paper.

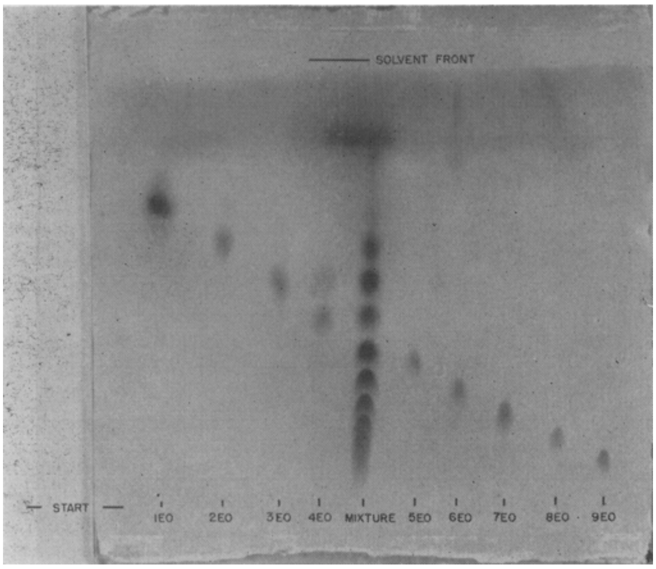

FIG. 2. Thin-layer chromatogram of composited column fractions of pure ethoxylates.

## Discussion

Column chromatographic separation of polyoxyethylene nonionic surfactants into single molecular weight fractions has been attempted by numerous workers with varying degrees of success. Kikolski and Wegrzynowicz (6) have prepared an excellent review of the progress in this field. Fractions containing a single ethoxylate chain have been isolated previously (5–8); however, overlapping of fractions and characterization of the eluate were difficulties inherent in earlier work.

Thin-layer chromatography was used to solve these difficulties and assisted in the choice of the optimum solvent for the separation of alkyl phenol ethoxylates. The solvent was specifically chosen to yield a greater degree of separation of the intermediate molecular weeight ethoxylates. Silica gel having a 0.05- to 0.2-mm particle size was used for the column chromatography. This narrow range in small particle size permitted a direct transition from thin-layer to column chromatography. The same solvent (acetone:benzene:water; 50:50:1 v/v) used in the thin-layer separation was used for the column chromatography. The progress of the column chromatographic separation was followed using thin-layer chromatography as the detector. Following the discharge of the first 150 ml of solvent, every fifth test tube was sampled and chromatographed using the same solvent as described previously. The resulting chromatograms are seen in Fig. 1.

These chromatograms show that for this separation the early fractions eluted as mixtures with some

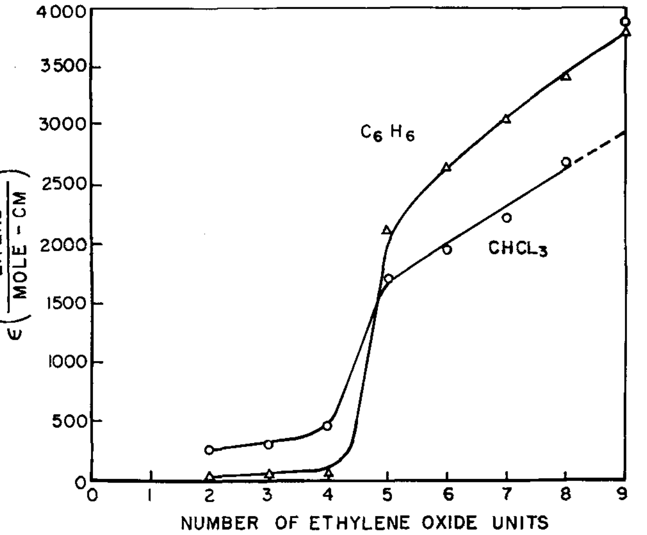

FIG. 3. Apparent molar absorption coefficients of cobalt thiocyanate complexes at 320 $m\mu$ vs. nonylphenol ethoxylate chain length.

fractions containing as many as 3 components, then 2 components, and, finally, fractions were obtained that contained only a single component, as is shown by test tube No. 65. The single component-mixed component-single component eluate pattern was repeated until pure fractions of the 5 to 9 mole adducts had been collected.

Since single molecular weight samples of all the chain lengths were desired, a second column chromatographic separation was carried out using a reduced sample size and a slower solvent flow rate, to obtain the pure lower molecular weight members of the series.

The sample chosen for this study was artificially enriched with the higher molecular weight adducts by addition of 10% Tergitol Nonionic NP-16 (6-mole average) to a commercial sample of Tergitol NP-14 (4-mole average). In addition, the column was intentionally overloaded to a slight degree, to increase the amount of pure isomers collected. Consequently, no attempt was made to determine a molecular weight distribution.

The fractions found to be pure by thin-layer chromatography, Fig. 1, were combined and the solvent removed by vacuum stripping with minimal heat. The largest amount of pure isomer collected was 0.9 g of the 6-mole adduct. Fig. 2 is a thin-layer chromatogram of the original sample and the isolated fractions, showing that the column chromatographic fractions are indeed pure members of the homologous ethoxylate series.

### Cobalt Thiocyanate Complexes

Previous workers have reported a variation of the color intensity with the length of the polyethylene oxide chain; however, these results were obtained using the number average molecular weight of the nonionic surfactant (2,3). Since the color intensity is a function of the ethoxylate chain length, absorbance data are extremely dependent on the molecular weight distribution of the sample chosen for construction of the calibration curve. Therefore, the calibration curve must be constructed from a representative portion of the sample under study. In addition, reports on the minimum number of ethylene oxide units detectable, obtained using samples with an unknown molecular weight distribution, are questionable because of the effect of chain length on color intensity. Inasmuch as the results obtained using the cobalt thiocyanate procedure are based upon a calibration curve, it is important to know the effect of the length of the polyethylene oxide chain on the color intensity.

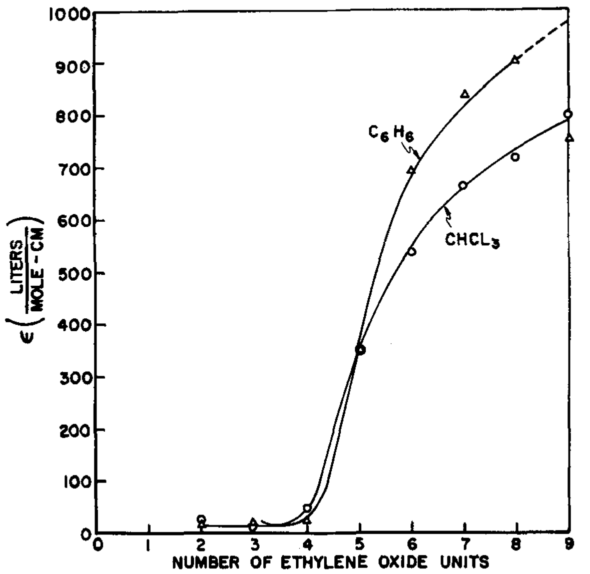

FIG. 4. Apparent molar absorption coefficients of cobalt thiocyanate complexes at 620 $m\mu$ vs. the nonylphenol ethoxylate chain length.

Absorbance measurements made on the cobalt complexes of the pure isomers confirm that the color intensity does vary with the length of the polyoxyethylene chain, but that the absorbance is nonlinear with respect to the number of units in the chain. The data plotted in Fig. 3 and 4 show that at least 5 moles of ethylene oxide are required to produce a significant extractable color; however, lesser numbers of oxide units do produce an extractable color. Since the absorption coefficients of the higher molecular weight complexes are 10 to 15 times greater than those of the lower members, these data illustrate conclusively that a small percentage of a higher molecular weight adduct in a sample of lower molecular weight will yield a cobalt thiocyanate color out of proportion to its concentration. Consequently, data obtained with samples having an unknown molecular weight distribution should be evaluated with these results in mind.

The fact that the color intensity of the cobalt complexes undergoes such a rapid decrease from the 5- to the 4-mole adduct has additional importance. During the course of biological attack on polyoxyethylene nonionic surfactants, the ethoxylate chain is consumed. Measurements of biodegradation based solely on the disappearance of the cobalt thiocyanate complexes can be misinterpreted as complete biodegradation, when in reality the analytical method failed to detect ethoxylate chains less than 5 units long. Consequently, it is advisable that complementary analyses be performed, e.g., Warburg respirometry.

The initial work put forth to obtain Fig. 3 and 4 made use of the cobalt thiocyanate reagent reported by Brown and Haynes (1). These experiments confirmed earlier results, i.e., at least 6 moles of ethylene oxide are required for color development (1,2).

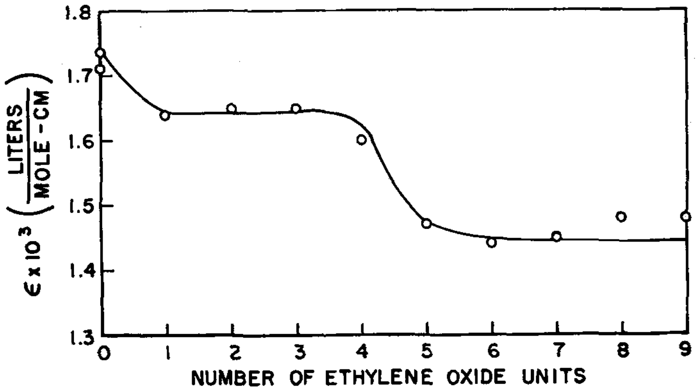

FIG. 5. Molar absorption coefficients of the aromatic content of nonylphenol ethoxylates vs. the ethoxylate chain length.

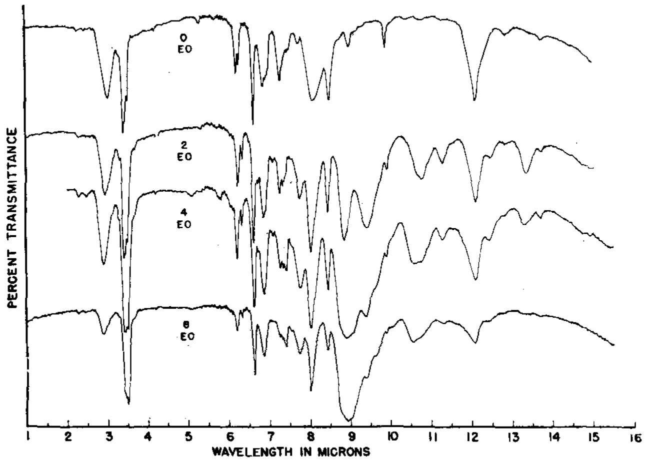

FIG. 6. Infrared scans of some of the pure ethoxylates of nonylphenol.

The data reported in Fig. 3 and 4 were obtained using a saturated aqueous solution of ammonium cobalt thiocyanate, a reagent similar to that reported by Greff et al. (3), except that NaCl was not required, since the reagent used in this study was added to an anhydrous sample. These data confirm earlier reports that the lower limit of detection is 2 or 3 moles of ethylene oxide (3).

The major difference between the reagents of Brown (1) and Greff (3) is the amount of water used to prepare the reagent. The data in Fig. 3 show that only weak or poorly extractable complexes are formed between cobalt thiocyanate and short-chain ethoxylates. Since water is competing with the ethoxylate oxygen for coordination positions on cobalt, it follows that higher concentrations of water will reduce the color intensity and, in fact, when water is added to the extracted complex, the color is discharged immediately in favor of the preferred ammonium hexa-aqua cobalt. In addition, ethoxylates separated by thin-layer chromatography (adducts 1 through 15) can be detected using anhydrous cobaltous thiocyanate dissolved in acetone, illustrating that under nearly anhydrous conditions the 1-mole ethylene oxide adduct should be detectable by cobalt thiocyanate.

These data confirm the findings of Greff, i.e., the least hydrated organic solvent will yield the highest extractable color intensity per gram of sample. Considering the 2 solvents investigated in this study, benzene was apparently superior to chloroform as an extractant, except for measurements made at 320 mμ on polyoxyethylene chains of 4 units or less. This result could arise from baseline corrections necessary in this region and the fact that the small sample absorbance band is superimposed on the broad absorbance band for benzene.

### Spectroscopic Measurements

The NMR spectra of polyoxyethylene nonionic surfactants have been used to determine simultaneously the ethylene oxide and hydrophobic content of nonionic surfactants. Consequently, the hydrophobe-hydrophile ratio can be obtained from the NMR spectra. Since the hydrophobic portion of the molecule was known, the ratio of ethylene oxide to benzene was readily calculated. Nuclear magnetic resonance spectra have been obtained for the pure ethoxylate isomers obtained in this study, which have confirmed the ratio of ethylene oxide to alkylbenzene, a ratio assigned previously by the thin-layer chromatographic separation of Tergitol NP-14 and Tergitol NP-16. These results confirmed that the chromatographic process is separating the ethoxylates in the order of an homologous series, with the lower molecular weight ethoxylates traveling the most rapidly up the plate.

The absorbance of the substituted benzene ring at about 275 mμ has been used by many workers to measure the presence and concentration of alkylphenol ethoxylates. In general, calibration curves are prepared from samples of known number average molecular weight. The molar absorption coefficients of these compounds have been reported to be independent of molecular weights (4).

The molar absorption coefficients determined by the aromatic content of the pure isomers are plotted in Fig. 5. This curve shows that the absorption coefficients are independent of molecular weight beyond the 5-mole adduct, and that calibration for each adduct is not necessary beyond this molecular weight. However, the coefficients for the shorter chain lengths do not behave in a predictable manner. The transition between the 3- and 5-mole adducts was unexpected and is not explainable at this time. These results show that proper calibration curves are required for the lower members of the ethoxylate series, but the ultraviolet absorption coefficient of the common commercial products should be independent of molecular weight.

Infrared spectra of surfactants are obtained in a routine fashion in numerous laboratories for the purpose of identification of surfactants, and frequently are used to determine the amount of surfactant in a sample. Calibration curves are needed for quantitative infrared measurements and are generally obtained from measurements made on samples with a known number average molecular weight but unknown distribution. Quantitative infrared measurements made on polyoxyethylene nonionic surfactants generally use the C–O–C stretching vibrations at about 1,120 $\text{cm}^{-1}$ (8.9 μ). Measurements of this band and also absorption bands associated with the hydrophobic portion of the molecule have been used to follow the course of fractionation experiments (4).

Detailed analyses of the infrared spectra of polyoxyethylene molecules have been made by several authors (13–15). These workers conclude that the lower molecular weight members of the series produce irregularly shaped bands which originate due to the presence of both "gauche" and "trans" isomers. However, the crystalline higher molecular weight members of the series have a helical structure which contains 7 chemical units and 2 turns of the chain in a length of 19.3 A. These optical configurations produce multiple absorption bands in the region of 1,100 cm$^{-1}$.

Fig. 6 shows the transformation in absorption band multiplicity and shape encountered in lengthening the chain by single ethylene oxide units. The spectra of the lower members of the series are sufficiently different from the higher members of the series that an accurate identification without prior knowledge would be difficult.

Since the infrared spectra were not obtained in a cell of fixed thickness, absorption band ratios were made for each scan and the several scans are compared in Fig. 7. These curves, ether/hydroxyl and ether/aromatic, show the influence of the multiple bands obtained for the lower members of the series. The spectra obtained for the more or less fixed configurations found in the longer chains yield useful analytical data, and fortunately this is the ethylene oxide chain length of interest for commercial nonionic surfactants. However, data obtained for the shorter chain lengths are unreliable unless proper calibration standards are available.

The influence of hydrogen bonding is shown in the nonlinear behavior of the hydroxyl/aromatic ratio as a function of the number of ethoxylate units. These results are consistent with additional studies made in this laboratory on the nonlinear behavior of the hydroxyl absorbance in polyoxyethylene polymers. These data are to be reported upon in a future publication.

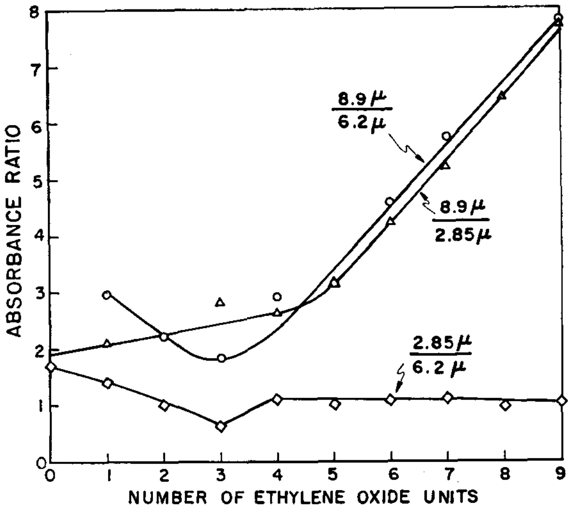

FIG. 7. A plot of selected infrared absorption band ratios vs. the nonylphenol ethoxylate chain length.

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