but primarily acted to provide stability of the emulsion to mechanical shock; and Pluronic F 68 prevented phase separation during sterilization. Emulsions in which the concentration of any of the emulsifiers was less than in No. 695 were inferior to the latter in physical properties.

The polyethylene glycol palmitates were very hygroscopic, and resisted subsequent removal of gained moisture. One sample in an open container was placed in ordinary room atmosphere overnight, and gained 22% in weight. This sample was then placed in a desiccator, and after 16 days had lost only 0.9% of the gained moisture. Determinations of the mol wt of polyethylene glycol esters therefore may be in error unless these materials are thoroughly dried. For example, if the determined mol wt of an incompletely dried polyethylene glycol monopalmitate is 747.9, the removal of only 0.1% of moisture from this material will give a calculated mol wt of 780.0, assuming that the moisture is free and affects the boiling point of the reference solvent as an additive increment. Also, calculation of the composition of polyethylene glycol esters with the use of saponification and hydroxyl values may be inaccurate if the mol wt of the polyethylene glycol is considered to be a constant value, which is 400 in the present instance. These materials actually are esters of polyethylene glycol of variable mol wt. For these reasons, in this work calculations of composition of the various fractions of polyethylene glycol palmitates or of polyoxyethylene materials in general, are not considered as absolute. The analytical results were used mainly as reference properties of successive batches of similar materials. This use is believed to be quite sufficient to the purpose of the present investigation, which was to provide a usable system for emulsions for intravenous administration.

Although the total concentration of emulsifiers in emulsions of 15% oil content could not be reduced below 1.8% without sacrificing physical properties, an effective reduction in emulsifier concentration of 50% per unit of oil content was attained upon dilution of a 30% emulsion, and 60% per unit of oil content upon dilution of a 37.5% emulsion. The latter content of oil apparently was the upper limit of concentration of this phase with 1.8% concentration of emulsifiers, since particle size was not satisfactory at higher concentrations of oil.

The commercial emulsifiers Lipal 4P and Drewmulse 5998A, which are mixtures of reaction products as received, apparently are well tolerated by dogs when fractionated and used as described. Their efficiency as emulsifiers was not decreased by the fractionation procedures employed. No differences in physiologic activity between the commercial and laboratory prepared polyethylene glycol palmitate could be observed, and the commercial product, after fractionation, has been used in all subsequent preparations of emulsions.

Acknowledgment

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Analysis of Nonvolatile Material in Solvent Hexane

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Abstract

The amount and composition of the nonvolatile residue in solvent hexane are important quality criteria. These residue characteristics are particularly significant in the hexane extraction of foods because of the possibility of contaminating edible products. A laboratory evaluation of the residue from a typical hexane was made to find if there was any trace of multi-ring aromatics which might be potential carcinogens.

Samples of hexane directly from a refinery and after storage and transportation to two solvent extraction plants were analyzed. All residues were separated by paper chromatography and analyzed by ultraviolet spectrophotometry. In no case, were they found to contain polynuclear aromatic carcinogenic materials. The test method is sensitive to less than 0.01 ppm.

In order to verify the accuracy of the test, an analysis was made for the quantitative recovery of a known carcinogen. For this purpose, 0.01 ppm of 3,4 benzpyrene was added to the hexane as an internal standard and the analysis conducted as before. In these tests, the benzpyrene was recovered quantitatively.

IN RECENT YEARS there has been an increase in the use of solvent hexane to extract edible oils from various seeds. Solvent hexane is used in the extraction of such materials as cottonseed, soy beans, and peanuts to produce cooking oils, vegetable shortening, and margarine. In addition, the meal remaining after extraction is often used as food. Solvent hexane, used in extraction, contains a very small amount, less than 10 ppm, of nonvolatile material at 212F. There is a growing interest in demonstrating conclusively that this residue does not contaminate food products manufactured by hexane extraction.

No standard techniques for analysis of such nonvolatile material have been established. Neither has a satisfactory definition of "zero" been given in regard to the presence of contaminants. Therefore, an analytical technique was developed to measure the possible presence and level of contaminants (carcino-

gens) (1,2) in the nonvolatile material in solvent hexane. Carcinogens as discussed in this paper refer to those polynuclear aromatic compounds which are generally recognized to be carcinogenic. Three samples of commercial hexane from petroleum sources meeting AOCS specification H-16-56 were analyzed by this technique. Similar studies of wax used in milk cartons were used as a guide to determine the lowest significant level at which contamination could be accurately detected. This level is approximately 0.01 ppm.

Sampling

The first, and often the most critical, step of any analysis is obtaining a proper sample. In the case of solvent hexane, the analysis is being run to detect extremely small quantities of possible contaminants, and sampling is of utmost importance. Sample containers and stoppers must be scrupulously clean. Simply stated, the object is to obtain a true sample of the solvent in question and to avoid contamination from outside sources such as sample pumps, valves, or lines. Although such precautions may seem obvious, they are often not appreciated by those obtaining the samples in the field.

Analytical Procedure

Apparatus. Utmost precaution must be taken during the analysis of the hexane to prevent contamination; only absolutely clean laboratory apparatus and materials should be used. Wash glassware with chloroform, then acetone, and rinse with distilled water. It should then be acid washed, rinsed with distilled water, and dried.

Materials. Benzene, CP, Baker's Analyzed (redistilled, taking 80% heart cut). Cyclohexane, spectroanalyzed, Fisher Certified Reagent. Whatman No. 1 Paper. *N*-Heptane. Dimethylformamide. Activated Alumina (F-20, 80–200 Mesh, Alcoa). Cetane.

Quantity of Nonvolatile Material

Transfer the hexane sample of about 1 gal to clean qt bottles and weigh to the nearest 0.01 g. Fill a 250 ml beaker two-thirds full with the hexane from one of the bottles. Place on a steam bath and direct a gentle stream of clean, dry nitrogen to the surface of the hexane. Add remaining hexane to the beaker in small portions until about 3 l have been evaporated, and about 10–15 ml of liquid remains. Determine the weight of hexane evaporated by difference in weight of the sample bottles. Remove beaker from steam bath, and transfer the remaining liquid to a weighed 50 ml beaker, using four 5 ml portions of cyclohexane as a final rinse. Place this beaker on the steam bath and use nitrogen as before. Continue evaporation until the volatile liquid has just evaporated. Do not allow beaker to remain on the steam bath after it becomes dry. Wipe dry and cool in a desiccator to constant weight. Determine weight of residue.

Identification of Nonvolatile Matter

Dissolve the residue in 1–2 ml of benzene. Spot the total sample on paper and develop by the method of Tarbel *et al.* (3).

Prepare sheets of chromatographic paper according to dimensions shown in Figure 1 and saturate them with dimethylformamide. Dry and condition overnight by hanging in the chromatographic bath with the developer (*n*-heptane saturated with dimethylformamide).

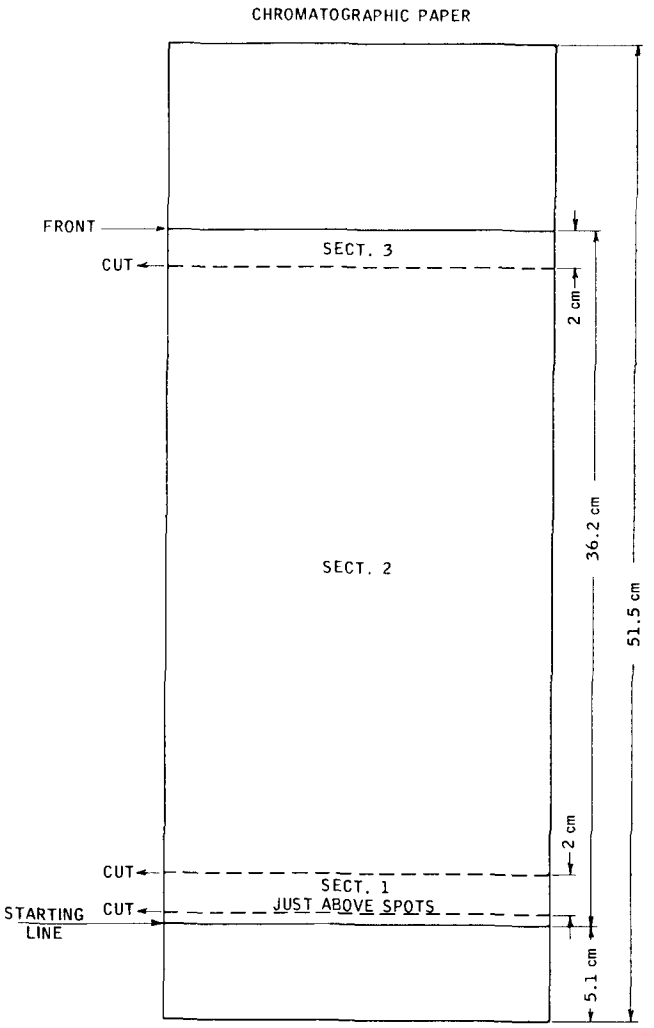

FIG. 1.

Spot this prepared paper with the samples of residue, place in the chromatographic bath and allow to develop for about 6 hr, using the ascending technique. Withdraw the paper from the chromatographic bath, air dry, and examine for fluorescence with a hand UV lamp. Use fluorescence as a guide in cutting the paper for subsequent analysis; but whether fluorescence is observed or not, each sheet of paper should be cut into sections as shown in Figure 1. Elute each section of paper thoroughly with hot benzene. Evaporate this benzene on a steam bath. Dissolve each residue in 5 ml of cyclohexane. Obtain an ultraviolet spectrum of the resulting solution from 400 to 220 mμ. Previous work has shown that this technique will successfully separate mixtures of chrysene, dibenzanthracene, and benzanthracene in the 0.01 ppm range.

Evaporate the cyclohexane solution to about 3 ml and add to a 5 x 3/8 inch column packed with activated alumina. Wash with *n*-heptane until ultraviolet spectra of small fractions show no absorption in the aromatics range of 400 to 220 mμ. This step leaves only the aromatics adsorbed on the alumina. Add benzene to the column to recover the aromatics. Follow the fluorescent bands down the column using a small UV lamp. Collect each band as a small fraction of 1–2 ml. Evaporate this fraction just to dryness on the steam bath and dissolve in cyclohexane. Obtain the UV spectrum of this solution in the 400 to 220 mμ range. Continue this procedure, adding benzene to the column until the ultraviolet spectrum of a final residue

dissolved in cyclohexane has no absorption at 400 to 220 mμ. Finally, add ethyl alcohol to the column and obtain small fractions. Obtain the same UV spectrum on these fractions.

Results and Discussion

Three samples of hexane from petroleum sources were analyzed as above. All were from the same refinery source and represent a commercially fractionated and finished material. Benzene is removed on this type of hexane during refinery processing by Udex extraction. Typical physical inspections of this solvent hexane are shown in Table I. Gross composition data obtained by gas chromatographic procedure are shown in Table II.

TABLE I
Typical Solvent Hexane Inspections

Gravity, °API	76.3
Color, Saybolt	+30
Sulfur, ppm.	4
Bromine No.	0.3
Aniline Point, °F.	145
Refractive Index 20C.	1.3816
Distillation, ASTM	
IBP, °F.	151.0
Dry Point, °F.	157.5

TABLE II
Typical Composition of Solvent Hexane

2,2 Dimethylbutane	Trace
Cyclopentane	Trace
2,3 Dimethylbutane; 2 Methylpentane	16.5
3 Methylpentane	16.6
Normal Hexane	46.2
Methyl Cyclopentane	18.6
2,4 Dimethylpentane	0.1
Cyclohexane	1.8
Benzene	0..2

The three samples of hexane were obtained from different sources. One was from the refinery. The second, originating from the same refinery, was taken as it was being delivered from a tank car to a cottonseed extraction plant. The third was obtained under similar conditions at a soybean extraction plant. The last two samples were taken to see if any difference in residue would result from storage, handling, and shipping. Results are shown in Table III.

TABLE III
Nonvolatile Material in Solvent Hexane

Sample Source	Nonvolatile, ppm
Refinery	0.15
Soybean Extraction Plant	6.3
Cottonseed Extraction Plant	7.4

The nonvolatile from the refinery sample was clear and only slightly fluorescent in UV light. The nonvolatile from other samples was yellow and showed a blue fluorescence in UV light. The ultraviolet spectrum of the nonvolatile in the sample from the soybean plant was similar to that for crude soybean oil. The spectrum from the other was similar to either a vegetable or mineral oil. Although the amount of nonvolatile was small in each case, it was considerably higher than the material found in the sample from the refinery. These data are not conclusive in identifying the source of the additional nonvolatile matter in these last two samples; but the UV spectra indicate that it is probably the vegetable oil. The source of vegetable oil contamination has not been found but it is thought it could have come from the tank car itself, or from manifolded loading and unloading lines. Although hexane tank cars are usually kept in hexane service exclusively, occasionally they are used to ship the extracted product.

The nonvolatile obtained from each sample was analyzed by the methods described. With these techniques, no absorption peaks in the 370 to 260 mμ range were detected in any concentration in the residue samples. Based on our experience with waxes, the sensitivity of the test methods employed would have detected these materials had they been present in concentrations greater than 0.01 ppm.

The choice of 370 to 260 mμ range was based on an examination of published spectrograms (4) and our experience. Aromatics absorb in the range of 400 to 220 mμ. Known common carcinogens indicated a maximum UV absorption spectra between about 370 and 260 mμ. Since no materials in the residues showed absorption peaks in this range, the residues from solvent hexane contain no known common carcinogens in concentrations greater than 0.01 ppm. While Lijinsky and Raha (2) reported finding carcinogens in hexane at levels greater than 0.01 ppm, we found no carcinogens in three samples of commercial solvent hexane. The difference in results probably reflects the difference in sample source, or possible contamination, although somewhat different analytical techniques were used.

Proof of the validity of the analytical procedure employed was made by adding a volatile known carcinogen (3,4 benzpyrene) to a sample of solvent hexane and analyzing for it. These tests were carried out by first obtaining the UV spectrum of cyclohexane containing 0.5 ppm of 3,4 benzpyrene. The absorbance at 385 mμ = 0.063. This was used as the calibration. While 3,4 benzpyrene has a maximum UV absorption in the 370 to 260 mμ range, it also has a specific absorption at 385 mμ. Its presence was determined from absorption at this wave length to avoid possible interference from other compounds. One hundred ml of solvent hexane containing 0.5 ppm of added 3,4 benzpyrene was evaporated slowly to dryness on the steam bath under nitrogen. The residue was then diluted to 100 ml with cyclohexane free of 3,4 benzpyrene, and the UV spectrum was obtained from 400 to 220 mμ. The absorbance at 385 mμ = 0.063. This indicated 100% recovery of the 3,4 benzpyrene under these conditions.

One of the necessary precautions in the evaporation of the hexane to obtain the residue, is the removal of residue from the steam bath as soon as the hexane has evaporated. Previous work indicated that if the beaker is left on the steam bath even a few minutes after the volatile material has evaporated, carcinogenic materials can be lost. One way to prevent the loss is by the addition of cetane. Cetane leaves a liquid film in the beaker and minimizes the loss (5). To determine the usefulness of cetane addition, the procedure outlined above was repeated except that 1 ml of cetane was added to solvent hexane containing 0.5 ppm 3,4 benzpyrene. It was observed that the cetane had no effect on recovery, the UV reading being 0.063 at 385 mμ.

In another modification of the procedure, 3,4 benzpyrene was added to 1000 ml of the hexane in a concentration of 0.01 ppm. This sample was split into two equal parts; one was evaporated without further treatment, and to the other 1 ml of cetane was added before evaporation. The residues were dissolved in 10 ml of cyclohexane instead of 100 ml. In each case the UV absorption at 385 mμ was 0.064, indicating complete recovery.

Two additional checks were made at double the 3,4 benzpyrene concentration. In this instance one sample was prepared by adding 3,4 benzpyrene to 1000

ml of cyclohexane in a concentration of 0.01 ppm. To the second 1000 ml of solvent hexane 0.01 ppm 3,4 benzpyrene was added. Cetane was not used in either solvent. The full 1000 ml was evaporated in each test and the residues were dissolved in 10 ml of cyclohexane. UV analysis showed an absorbance of 0.128 at 385 mμ for the cyclohexane sample, and 0.120 for the solvent hexane sample. Assuming a straight line calibration curve, this would indicate 100% and 94% recovery, respectively.

These results prove the validity of the technique outlined in the procedure section. The techniques present an accurate and valid means of determining the amount of nonvolatile material in solvent hexane, and of analyzing the resulting residue. The addition of cetane is a precautionary measure to eliminate the critical nature of the evaporation step. The use of cetane is recommended as part of the procedure in cases where several samples are being analyzed simultaneously, or when the evaporation cannot be closely watched. However, as the results show, cetane addition is not essential.

It is also noted here that a blank determination should be made on the cyclohexane used for washing. The residue from the blank, after dilution, should be checked for UV absorption. A correction should be made in the final calculations for absorption noted here.

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Vernonia anthelmintica Willd. Seed Oil and Salts of Vernolic Acid as Stabilizers for Plasticized Poly (Vinyl Chloride)[1]

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Abstract

Vernonia anthelmintica seed oil, trivernolin, the main constituent of the oil, barium and cadmium salts of vernolic acid (epoxyoleic acid), and various combinations of the salts have been evaluated as heat and light stabilizers of plasticized poly(vinyl chloride). Evaluation was made at the 1 and 3% levels of the vernonia oil components. Transparent sheets whose color ranged from light amber to colorless were obtained after milling and molding. All compounds studied greatly improved both heat and light stability of the sheets; the 3% stabilizer level gave the better results. In general the stabilizing ability of the compounds studied increased as follows: vernonia oil < trivernolin < barium vernolate < cadmium vernolate and < barium-cadmium vernolate mixtures. Comparisons with 3 commercial stabilizers show that the vernonia oil and derivatives thereof are equal to or better than the comparative commercial controls.

EPOXIDIZED OILS are being used extensively to stabilize poly(vinyl chloride) containing plastics against heat and light degradation. When used in combination with metal salts of fatty acids the stabilizing action is improved through synergism (1,2). Therefore, a study was made of the heat and light stabilizing properties of the natural epoxy oil from the seed of *Vernonia anthelmintica,* trivernolin (the main constituent of the oil), the barium and cadmium salts of vernolic acid (epoxyoleic acid), and combinations of the salts.

Experimental

Materials. The materials used in this investigation are:

	% Oxirane		Iodine number	Hydroxyl %
	Experimental	Theory		
Vernonia oil	4.0	....	107.5	Neg.
Trivernolin	5.0	5.2	82.7	0.1
Barium vernolate	4.2	4.4	72.1	Neg.
Cadmium vernolate	4.4	4.5	73.9	Neg.
Epoxystearate ester (commercial)	3.4	....	1.4	....
Epoxidized soybean oil (commercial)	6.6	....	2.7	....

Preparation of Materials. Vernonia oil was obtained from *Vernonia anthelmintica* seed by the method previously described (3).

Trivernolin, the triglyceride of vernolic acid obtained by crystallization from vernonia oil (3), was a natural plant product, and the vernolic acid used in making metallic soaps was produced by enzymic hydrolysis (3) that occurred in the air-dry plant matter following grinding; therefore, the products were not exposed to artificial oxidation or saponification, which ordinarily are of concern as sources of hydroxylated contaminants of epoxides. Spectra of the materials at 3 μ indicated no significant contamination from hydroxyl. Hydroquinone, 0.03%, was added to prevent gel formation.

Barium and cadmium salts of vernolic acid were prepared as follows: vernolic acid (3) was dissolved in a moderate quantity of warm methanol, which was then mixed into a calculated equimolar proportion of NaOH in sufficient water to give an aqueous soap solution of considerable dilution, 5 g/100 ml or more dilute. This was slowly mixed into an equal volume of an aqueous solution of an equivalent proportion of a soluble salt of the appropriate metal, either the barium chloride or cadmium acetate hydrates. The resulting milky suspension was then frozen, thawed, filtered, washed with water and 80–90% acetone, air dried, ground, and finally vacuum desiccated over P_2O_5 until fresh replacements of it remained little changed.

The epoxystearate ester and epoxidized soybean oil were commercial materials.

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