trans bonds; the $k_{10.36\mu}$ constant in Table II is used for corrections of background adsorption due to *cis* bonds when the content of *trans* isomers in mixtures (17) is calculated. GLPC of the methyl esters of these samples show that they are pure C_{18} dienes (Fig. 2d). Because these chromatograms were run at different times (sometimes as much as 3 months apart), elution times are not the same for the same component, e.g., diene ($C_{18}^{2=}$), due to changes in column characteristics. However, this discrepancy does not seriously interfere with qualitative identification.

Isomerization of several samples (Table I) shows all Crystal −55C fractions to be pure linoleic acid. However, when the esters of the isomerized acids were analyzed by GLPC, a small peak occurred in the region where methyl linoleate is usually eluted (Fig. 2e) followed by two well-separated peaks of conjugated esters. The conjugatable and nonconjugatable contents reported in Table II were calculated from the areas under these peaks. The small peak might be due either to esters that were not completely conjugated, or to esters that were nonconjugatable. To clarify this point, isomerizations with tertiary butoxide were run for a longer time, i.e., for 4 hr. The results recorded in Table II give within experimental limits the same values for conjugatable and nonconjugatable acids as found before. It can be concluded therefore that the small peak represents nonconjugatable *cis,cis* dienes. This conclusion explains the disparities referred to earlier between GLPC and tertiary butoxide isomerization for linoleic acid contents of Filtrate 1 −70C fractions. The disparities are obviously due to the presence of such nonconjugatable dienoic acids. The content of nonconjugatable esters is higher when isomerizations are carried by the ethylene-glycol potassium hydroxide procedure (18) (Table II), indicating that with this reagent the isomerization of conjugatable dienoic acids is not complete in 25 min.

To learn more about the isomers that are present, two lines of investigation were chosen, namely, GLPC on capillary columns and oxidative cleavage, followed by analysis of the resulting mono- and dibasic acids. Recent study (19) on the isomeric dienes produced by hydrazine reduction of linolenic acid show that the esters of the *cis,cis* dienes are eluted from a capillary column coated with Apiezon-L in the order: 9,12; 9,15; and 12,15 (Fig. 3a). A comparison of this curve with that for linoleic acid from cottonseed oil (Crystal −55C, Fig. 3c) shows that the major peak is 9,12-diene followed by a shoulder in the 9,15 diene region. Thus there is additional evidence for the presence of nonconjugatable *cis,cis* dienes in these preparations. Crystal −55C fractions of corn and safflower oil acids had similar chromatograms.

LLPC of the dibasic acids formed by oxidative cleavage gave three peaks in the regions of C_8, C_9, and C_{12} dibasic acids with the C_9 being the largest (Fig. 4a). Acidic artifacts have been reported to cause peaks in LLPC analysis of dibasic acids obtained by ozonolysis (20). For this reason the identity of the dibasic acids from Crystal −55C fractions were confirmed by converting them into diethyl esters and by analyzing with GLPC. With the exception of linoleic acid from corn oil in which a C_{11} peak could be identified, only two well-defined peaks, C_8 and C_9, were observed in the other oils (Fig. 4b). Consequently, the C_{12} peak obtained with LLPC is probably due to an artifact.

Since dibasic acids give the position of the double bond nearest the carboxyl group of the parent acid, monobasic acids were analyzed to locate the other bond. Short chain monobasic acids (up to C_9) are volatile; therefore they were applied as acids on the column. Only four monobasic acids C_3–C_6 (Fig. 4c) were detectable, with C_5 and C_6 having measurable peaks. (Fig. 4d is a standard monobasic acid curve.) Since the C_9 dibasic and C_6 monobasic acids were always the major components, consequently linoleic acid, i.e., 9,12-dienoic acid, was the chief component as expected. It is present to the extent of 90–95% in all Crystal −55C fractions, which also contain significant amounts of dienoic acids with the first double bond at the C_8 position (about 5%) and the second bond either at the C_{12} or C_{13} positions as indicated by the presence of C_6 and C_5 monobasic acids. Conceivably, a diene with double bonds at 9,13 positions was also present. The absence of a C_7 monobasic acid precludes the possibility of a 8,11-conjugatable dienoic acid.

Nonconjugatable dienes are present to the extent of 1–2%. Of the possible isomers, the 8,13-diene is the most difficult to isomerize since the double bonds are separated by three methylene groups. The 8,12- and 9,13-dienes are separated by only two methylene groups, and if one of these is attacked by alkali, the resulting rearranged diene offers the readily isomerizable pentadiene system. Because tertiary butoxide is a very strong base, it can conceivably cause the isomerization of double bonds separated by two methylene groups. The content of nonconjugated dienes (Table II) was always higher by the glycol-KOH method than by the tertiary butoxide method, which offers some basis for the belief that isomerization, by tertiary butoxide, of double bonds separated by two methylene groups occurs. With linoleic acid from corn oil, there is evidence that a diene with the first double bond at C_{11} position is present, but the position of the other double bond is not clear.

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An Emulsifier System for Experimental Intravenous Fat Emulsions[1]

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Abstract

An emulsifying system for a fat emulsion which has been very satisfactory for intravenous administration to dogs has been developed. The emulsion contained cottonseed oil (15%), polyethylene glycol monopalmitate (1.2%), tartaric acid ester of monoglycerides (0.3%), and polyoxyethylene-polyoxypropylene (0.3%), in isotonic dextrose solution. The polyethylene glycol monopalmitate was isolated from laboratory and commercial esterification reaction mixtures of monoester, diester, and nonreacted polyethylene glycol. Washing with NaCl solution removed nonreacted glycol, and fractionation in acetone separated the mono- and di-esters, the diester separating as a solid. Analytical methods were applied to characterize all fractions, and to determine the reproducibility of composition of successive batches. The monopalmitates from both laboratory and commercial preparations were similar in properties. The tartaric ester of monoglycerides was washed with NaCl solution and fractionated in acetone. The liquid portion was recovered and used for emulsification. Emulsions prepared with the fractionated emulsifiers appear to give satisfactory physiologic results when infused in dogs.

FAT emulsions suitable for intravenous administration require adequate emulsifier systems, and many such systems have been proposed as are noted in the comprehensive review of the subject by Geyer (4). Many of the proposed emulsifier systems have included soya phosphatides, with but little attention given to synthetic nonphosphatide emulsifiers of known composition and reproducible properties. One emulsifier system of the synthetic type, used in the development of an emulsion of cottonseed oil suitable for prolonged intravenous administration to dogs, has been reported in a previous publication (10). The emulsifier system of the reported emulsion was composed of the three commercial products, polyethylene glycol monostearate, diacetylated tartaric acid ester of monoglycerides, and polyoxyethylene-polyoxypropylene, used as received and also after fractionation. Emulsions prepared with this system were, in general, satisfactory at high dosage levels (40 ml/kg of body weight) when infused in dogs for as many as 30 infusions (2). With continued animal experimentation to improve physiologic response to the emulsion, the emulsifiers of the latter emulsion have been modified to the extent of using the palmitic monoester rather than the stearic monoester of polyethylene glycol, and a nonacetylated tartaric acid ester of monoglycerides rather than the diacetylated emulsifier. These modifications have not changed the chemical types of the respective emulsifiers nor sacrificed the physical quality of the emulsion at a slight reduction in their total concentration, and give more satisfactory physiologic results (6).

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The present investigation describes the methods of preparation of the modified emulsifier system. Methods for separating the components of the emulsifier reaction mixture which results from the esterification of palmitic acid with polyethylene glycol were investigated. Such a mixture contains nonreacted polyethylene glycol and the fatty acid mono- and di-esters of polyethylene glycol (8).

Materials and Methods

Polyethylene Glycol Ester. The principal emulsifier used for the preparation of the improved fat emulsion was polyethylene glycol monopalmitate, prepared in the laboratory from materials of known history, and also obtained from a commercial source. Emulsifiers of this type are prepared either by the addition of ethylene oxide to a hydrophobic base, or by esterification of a hydrophobic base with polyethylene glycol. The latter method was used in preparing the present material.

Polyethylene glycol does not contain a definite number of oxyethylene groups, but is a mixture of molecular entities in which the number of oxyethylene groups follows a statistical distribution, and only the average number of units, or average molecular weight, may be determined. Flory (3) reports that polyethylene glycol with an average of 10 oxyethylene units had 79.6% of the material in the range 8–16 units. For this reason, polyethylene glycol employed in the present study for preparation of the palmitic ester was molecularly distilled to eliminate portions with extremely low and high molecular weights.

The molecular distillation was conducted in a Centrifugal Cyclic Batch Molecular Still, (CMS-5, Distillation Products Industries). Polyethylene glycol 400 (Union Carbide and Carbon Chemical Co.) was degassed by passing over the rotor at room temperature, with mechanical pump vacuum only. With the pump in operation, the material was degassed a second time at 50C. Distillation was begun at 100C, pressure 25 μ, and the distillate collected at progressive temperature increments of 10°. Mol wt of the distillates were determined by the ebullioscopic method (9), and ranged from 241 for the first to 554 for the residue. Those distillates with mol wt in the range 361–448 (approximately 75% of the total distillate) were combined. The mol wt of the combined distillate was 407.

Palmitic acid (Armour Industrial Chemical Co.) was recrystallized three times from acetone and dried with nitrogen at reduced pressure. The acid chloride was prepared by the method of Youngs *et al.* (13).

The molecularly distilled polyethylene glycol was reacted with the palmitoyl chloride in a molar ratio of 1:1, by the method of Youngs (12). The reaction product obtained by this method, or by the ethylene oxide addition method, unavoidably contains not only the monoester but also considerable amounts of diester and nonreacted polyethylene glycol (1, 7). Calculation of the composition of the esterification reaction mixture by the method of Malkemus and Swan (8) gave 15.2% nonreacted polyethylene glycol, 44.9% palmitic monoester, and 40.8% of palmitic diester.

TABLE I

Analysis of Polyethene Glycol Palmitate and Fractions [a]

Product	Saponification value	Hydroxyl value	Mol wt
	mg KOH/g	mg KOH/g	
Lab-prepared ester mixture	90.6	85.3	.....
Lab-prepared ester mixture, washed	106.8	45.9	744
Filtrate fraction	104.1	65.0	734
Precipitate fraction	121.4	6.6	854
Comm. ester mixture	91.2	86.1	.....
Comm. ester mixture, washed	111.3	64.0	723
Filtrate fraction			
lot 1	105.4	61.4	689
lot 2	104.8	64.7	701
lot 3	102.8	54.5	684
Precipitate fraction	123.2	13.2	857

[a] Fractionated in acetone at 6C (100 g esters to 200 ml acetone).

The desired emulsifier was the monoester (10), which therefore was separated from the other two components of the reaction mixture. The nonreacted polyethylene glycol was removed by washing with an aqueous solution containing 20% of NaCl, in which the fatty esters are substantially insoluble, at a ratio of 100 g of product to 300 ml of solution. The reaction product and NaCl solution were heated to 95C, mixed together and vigorously stirred, then transferred to a separatory funnel. The bottom layer (aqueous solution) was removed and discarded after the top layer cooled and cleared. Three such washings were done, withdrawing samples for analysis after the second and third washings. The samples were filtered and dried in a vacuum desiccator prior to analysis (8).

The saponification value (mg KOH/g) of the reaction mixture, originally 88.4, was constant after the second and third washings (108.4 and 106.8, respectively), indicating either removal of the glycol, or reduction of concentration to a constant level. The washed product was dried on a steam bath with nitrogen at reduced pressure, and filtered through a medium porosity glass filter to remove a small amount of solid NaCl. Polyethylene glycol could not be detected in the washed and dried product by a paper chromatographic method (5). The mixed esters were then fractionated in acetone to separate the monoester from the diester, at a ratio of 100 g of esters to 200 ml of acetone. At other ratios in which the amount of acetone exceeded 200 ml per 100 g of mixed esters, the concentration of diester in the filtrate fraction increased, because of increased solubility. For this reason, the ratio of mixed esters to acetone was standardized at 100 g to 200 ml, respectively. The acetone solution was cooled to 6C, allowed to stand at that temperature for 48 hr, then filtered. A cooling period of 48 hr permitted the diester to crystallize as large spherical particles which adhered to the walls of the flask; this did not occur with shorter cooling periods. The filtrate and precipitate fractions were stripped of solvent with nitrogen at reduced pressure, and the saponification and hydroxyl values, and mol wt, determined. The yield of filtrate was approximately 60%.

A commercially prepared polyethylene glycol palmitate, designated as Lipal 4P (Drew Chemical Co.) was obtained for comparison with the laboratory prepared product, and was washed and fractionated as described. Analytical results of both products are given in Table I. It is apparent from these results that fractionation at 6C with the ratio of 100 g of ester to 200 ml of acetone resulted in fairly good separation of the mono- and di-esters. A major portion of the filtrate fraction was the palmitate monoester; the precipitate fraction contained the diester. The efficiency of the diester fraction as an emulsifier was not as good as the monoester fraction. Emulsions prepared with the diester were not as stable to heat as were emulsions prepared with the monoester.

Additional lots of the commercial Lipal 4P were processed as previously described, and the filtrate fractions of each lot were of similar composition, as may be seen from the results given in Table I.

Secondary Emulsifiers. One of two secondary components of the emulsifier system was a tartaric acid ester of monoglycerides of cottonseed oil fatty acids, designated as Drewmulse 5998A (Drew Chemical Co.). The commercial product was washed three times with an aqueous solution containing 25% of NaCl, at a ratio of 100 g of product to 300 ml of solution. The mixture of product and NaCl solution was heated to 95C, vigorously stirred, poured into a separatory funnel, and the bottom layer (aqueous solution) removed and discarded. Unreacted tartaric acid was removed by this procedure. A solution of the washed product in acetone at a ratio of 100 g of product to 100 ml of acetone was cooled to 6C, allowed to stand at this temperature for 24 hr, then filtered. The filtrate fraction was stripped of solvents with nitrogen at reduced pressure. Analysis of the filtrate fraction gave the following results: saponification value 218.8 (mg. KOH/g), and mol wt 657.

Pluronic F 68 (polyoxyethylene-polyoxypropylene, Wyandotte Chemical Corp.) was another secondary component, and was used as received.

Oil. A refined, bleached, winterized, and deodorized cottonseed oil (Southern Cotton Oil Co.), specially selected for resistance to oxidation, was used in all emulsions.

Emulsions

Preparation. The concentrations of emulsifiers required for a sterilizable emulsion containing 15% of c/s oil were determined by preparing several emulsions in which the concentration of each emulsifier was varied in a systematic manner. The range of concentrations tested was based upon previous results with other emulsifiers similar in chemical type to the present emulsifiers. The minimum concentrations capable of producing a physically acceptable emulsion

TABLE II

Appearance of Emulsions Containing 15% of Cottonseed Oil with Different Concentrations of Emulsifiers

Lipal 4P, filtrate fraction	Drewmulse 5998A, filtrate fraction	Pluronic F 68	Remarks
%	%	%	
1.5	0	0.5	Poor storage stability
1.5	0.2	0.5	Particles over 7 μ
1.2	0.5	0.3	Acceptable particle size and stability
1.2	0.3	0.3	Acceptable particle size and stability
1.2	0.3	0	Oil droplets on surface
1.0	0.3	0.3	Particles over 7 μ
0.6	0.15	0.15	Particles over 7 μ
0.5	0.3	1.0	Particles over 7 μ
1.2 [a]	0.3 [a]	0.3	Particles over 7 μ
1.2 [b]	0.3	0.3	Particles over 7 μ

[a] Commercial emulsifiers used as received.
[b] Lipal 4P precipitate fraction.

were desired. The results of these tests are given in Table II.

Based on these results, an emulsion of the oil-in-water type was prepared and designated as SR 695. The concentrations of ingredients, calculated on the weight of the whole emulsion, were as follows:

Cottonseed oil	15.0%
Lipal 4P, filtrate fraction	1.2%
Drewmulse 5998A, filtrate fraction	0.3%
Pluronic F 68	0.3%
Isotonic glucose solution	83.2%

The Pluronic F 68 was dissolved in the isotonic dextrose, and the fractions of Lipal 4P and Drewmulse 5998A were dissolved in the cottonseed oil by warming to about 45C. The aqueous phase was then circulated through a two-stage homogenizer (Superhomo, Cherry-Burrell Co.) at an applied pressure of about 3000 psi. When the circulating aqueous phase reached 40–45C, the warmed oil phase was added, and the pressure was then increased to 4000 psi. The emulsion was completely cycled 5 times through the homogenizer. This procedure produced an emulsion in which the dispersed oil particles were of satisfactory size as observed microscopically with an oil immersion lens.

The emulsion in sealed bottles was sterilized in a steam autoclave at 121C for 17 min, and was removed immediately after slow exhaust of the autoclaving pressue to a walk-in cooler maintained at 4C. During the cooling period the bottles were mechanically rolled by placing them longitudinally on motor-driven rubber-covered rollers rotating at about 18 rpm. This procedure had the effect of constantly renewing the surface of the emulsion, and speeded up the rate of cooling. Emulsions reached room temperature in about 40 min.

Mechanical rolling in a walk-in cooler at 4C was compared with other methods of cooling the sterilized emulsion, which were a) emulsion placed in same cooler, and briefly rotated by hand at 5 min intervals; b) emulsion placed in same cooler, and not disturbed; c) emulsion placed at ordinary room temperature, and not disturbed. All emulsions which were cooled at 4C were acceptable, differing mainly in the number of oil particles 2–4 μ in diam as observed microscopically. The rolled emulsions had 8–10 particles per field in this range, the hand-rotated emulsion about 10–12, and the undisturbed emulsion about 12–15. The emulsions which cooled at room temperature, however, contained 20–30 particles per field in the range 4–7 μ in diam, and was definitely inferior to those cooled at 4C.

Particle Size. Diameters of the dispersed oil particles of the finished emulsions were determined with an oil immersion lens, 950x, using a calibrated eyepiece scale. The bottle of emulsion was inverted 3 times for mixing, and 1 drop placed on a slide for examination. Ten fields were observed on each slide, to get the average number of particles of various diameters. A majority of the particles, termed background particles, were 0.5 μ or less in diam. About 4 particles per field were 2–3 μ in diam, and about the same number 3–4 μ. One particle per field was observed with a diam of 5–6 μ. No particles larger than this were observed. It is generally accepted that emulsions for intravenous alimentation should have particles no larger than about 7–8 μ; the present emulsion may be so described.

Resistance to Mechanical Shock. A measure of the relative resistance of emulsions to mechanical shock as may be encountered during transportation was provided by a shaking test. A 4oz bottle (1⅜ in. in diameter by 5⅜ in. long exclusive of neck) was approximately half-filled with 50 ml of emulsion and clamped in a horizontal position to the platform of a reciprocating shaker. The center line of the bottle was in line with the direction of motion; the horizontal travel was 4 in. at a rate of about 250 cycles per min. A 1-hr period of shaking at room temperature was arbitrarily chosen, with the requirement that the size of the dispersed oil particles of an emulsion should not exceed 7–8 μ afterwards, to be considered satisfactory. Experience with a multitude of emulsions prepared with a variety of emulsifying systems validated the outlined procedure, as emulsions which were satisfactory by this test did not increase in particle size with ordinary handling, whereas emulsions not satisfactory by the shaking test generally were not physically stable.

Concentration of Oil Phase. A physically stable emulsion of 15% oil content could not be produced with an appreciably lower total concentration of emulsifiers than 1.8% (Table II). However, it was possible to increase the oil content of emulsions to 30% and 37.5% with no increase in emulsifier concentration. These were prepared with 1.8% total emulsifier concentration (the same as for emulsions containing 15% of oil) and autoclaved. Dilution of the cooled products with the required volumes of distilled water resulted in emulsions of 15% oil content with the hypertonic glucose phase reduced to isotonic solution. One inversion of the bottle was sufficient for mixing the concentrated emulsions and added water. Both of these emulsions were of acceptable particle size, with no particle over 7 μ in diam. Their stability to shaking both before and after dilution was not as good as the originally prepared 15% emulsion, as both developed a "cream layer" after 1 hour of shaking.

At oil concentrations of 45% and 60%, with 1.8% total concentration of emulsifiers, the emulsions were not satisfactory. There was very little Brownian motion, and many of the particles were 7–14 μ in diam. Floating oil droplets were observed in the emulsion with 60% oil content.

The coefficients of viscosity of all emulsions diluted so as to have an oil content of 15% were the same, within experimental error, as that of an original 15% emulsion, 1.1520 centistokes at 37.5C. This temperature was chosen because of its physiologic significance.

Discussion

The physical stability of autoclaved emulsions depends on the number, type, and concentration of emulsifiers in the emulsifier system (11), and choice of emulsifiers for intravenous emulsions is limited by the requirements that they be nontoxic, free of serious side effects, and otherwise innocuous when used for prolonged periods at the levels employed in emulsion preparation. The 3 emulsifiers used in the present emulsion apparently meet these requirements. The concentration of each was adjusted until the emulsifier system produced an emulsion of satisfactory physical properties such as size of the dispersed oil particles, stability to sterilization, and stability to handling and storage. Each of the emulsifiers performed a specific function. The Lipal 4P fraction provided ease of dispersion or emulsification; Drewmulse 5998A fraction aided dispersion to some extent,

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.

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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-