TABLE 5

Iodine Numbers and Indices of Refraction of the Several Oil Specimens Obtained From a Given Lot of Dehulled Cottonseed

Fraction	Iodine No.	Refractive Index
Diethyl Ether	84.4	1.47601
Chloroform	99.0	1.47523
Acetone	98.8	1.47476
Carbon Tetrachloride	.....	1.47414
Petroleum Ether (40-55°)	103.1	1.47087
Autoclaved + cold pressed	104.5	1.47039
Cold pressed	104.5	1.47029

It should perhaps be reported that some of these several extracts are not homogeneous; crystalline material separates from the chloroform and acetone extracts, and with petroleum ether it has been noted that a brown substance, presumably a polymer, is deposited on the upper part of the stripping column of the Frampton-Giles extraction apparatus.

The results which have been obtained in this study are in keeping with those which have been reported for other oil-bearing seeds (3, 4, 5, 6, 7, 8, 9, 10, 11).

Summary

1. Consistent results are obtained with the refractometric method of oil assay, but they are not in agreement with those obtained by the official methods involving extraction with diethyl ether or petroleum ether.

2. The composition of the extract obtained with cottonseed using petroleum ether varies from specimen to specimen.

3. The yield of lipid-soluble materials obtained on extraction of cottonseed with several solvents is different with each solvent.

4. Heat treatment of cottonseed reduced the quantity of lipids which could be extracted with petroleum ether.

5. Absorption spectra of chloroform solutions of cottonseed extracts obtained with several solvents show qualitative differences in the material obtained with the different solvents. Iodine numbers and indices of refraction are also different.

6. Distinct qualitative differences are noted in the different fractions obtained on the extraction of cottonseed with chloroform. These differences are apparent in the absorption spectra and iodine numbers of the fractions.

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The Spectrophotometric Determination of Glycerol*

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A SPECTROPHOTOMETRIC method based upon the measurement of the characteristic blue color of the sodium cupri-glycerol complex is presented for the determination of glycerol in various glycerol-containing materials.

A simple, rapid, and reliable method for the estimation of glycerol has continued to be a much desired development, especially by most producers and processors of this commodity. The two officially accepted analytical procedures at the present time are the international acetin and dichromate oxidation methods. Neither of these methods is entirely desirable since they both lack a satisfactory degree of specificity, the former method constituting a measure of the reactive hydroxyl radicles and the latter of the total oxidizable material, expressed in terms of glycerol. Glycerol values obtained by the acetin method tend to be low due to the following inherent and varying stoichiometrical errors:

1. Failure of the acetylation equilibrium to attain 100%.
2. Partial premature saponification of the ester during the neutralization phase.

The applicability of the acetin method is also limited to glycerol concentrations in excess of about 60%. Other methods exist involving the oxidation of glycerol with periodic acid (1, 2), based upon the work of Malprade (3). The use of this reagent provides a higher degree of specificity and therefore is useful to the organic analyst.

A fourth method, that of Bertram and Rutgers (4), depends upon the formation of a sodium cupri-glycerol compound and its subsequent estimation by iodimetry. The reagent employed forms complexes only with compounds possessing hydroxyl groups attached to adjacent carbon atoms, and, therefore the method is characterized by a degree of specificity rendering its application to most glycerol-containing materials very satisfactory. The method is extremely rapid and relatively simple.

In presenting this spectrophotometric development founded upon the aforementioned procedure of Bertram and Rutgers, the author feels that previously encountered anomalies manifested in the original method have been eliminated, and the rapidity and simplicity of the determination very considerably enhanced.

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Apparatus

A filter type photometer (Fisher) and a Beckman spectrophotometer were utilized in the development of this analytical procedure.

Reagents

Cupric chloride solution, 10.0 grms. of cupric chloride dihydrate per 100 ml. of ethyl alcohol.

Sodium hydroxide solution, 21.0 grms. ± 0.1 grms. per 100 ml. of distilled water.

Ethyl alcohol, formula 3A.

Procedure

Into a previously weighed precision grade 100 ml. glass-stoppered volumetric flask introduce a quantity of the solution to be analyzed of such a size as to contain 0.12-0.17 grm. of glycerol (in the case of samples containing very high concentrations of glycerol, e.g. crudes and distillates, gravimetrically prepared 5-10% solutions of the material should be used as the basis for the analytical sample). If the material evidences unusually high alkalinity or acidity, adjust the sample in the reaction flask to neutrality using hydrochloric acid or sodium hydroxide with methyl orange as indicator. Then add sufficient distilled water to bring the volume up to 10.0 ml. Alternatively, a sample of the material of appropriate size may be taken and transferred to a 250-ml. precision grade, volumetric flask, adjusted to neutrality, where necessary, then made up to volume with distilled water. A 10-ml. aliquot sample of this solution is introduced by means of a precision grade transfer pipette into a 100-ml. precision grade glass-stoppered volumetric reaction flask, no further addition of water being made. Now, add 10.0 ml. of the sodium hydroxide solution followed by 60.0 ml. of ethyl alcohol. From a convenient burette drop into the flask small increments (about 0.25 ml.) of the cupric chloride solution, replacing the stopper and shaking vigorously between each addition, until the point is reached where, after continued shaking for at least 1¼ minutes, a permanent, distinctly perceptible turbidity exists. Add a 1.0 ml. excess of cupric chloride solution and agitate vigorously for a period of two minutes. A chemical blank is similarly prepared, except that 1.0 ml. of cupric chloride solution is used and agitation continued for one minute. Bring the contents of the flasks up to volume with alcohol. Transfer part of the well-mixed contents of the flasks to dry centrifuge tubes, and centrifuge using a force of F/G = 500 for a period of 12-15 minutes (increasing the centrifugal force would curtail this period). Transfer some of the supernatant liquid derived from each respectively into optical cells. Employing a Corning filter No. 2412 (H. R. Lantern Red) photometer, and with a null setting for the blank, determine the optical density of the sample. Ascertain the glycerol content of the sample by means of the previously constructed absorption concentration curve, or calculate directly by use of the established calibration factor.

Calculations

% Glycerol in Sample

$$\text{From Curve} = \frac{\text{Conen. (Mgs./liter)} \times 0.01}{\text{Wt. of Sample (grms.)}}$$

$$\text{From Calibration Factor (C.F.)} = \frac{\log I_o/I \times \text{C.F.} \times 0.01}{\text{Wt. of Sample (grms.)}}$$

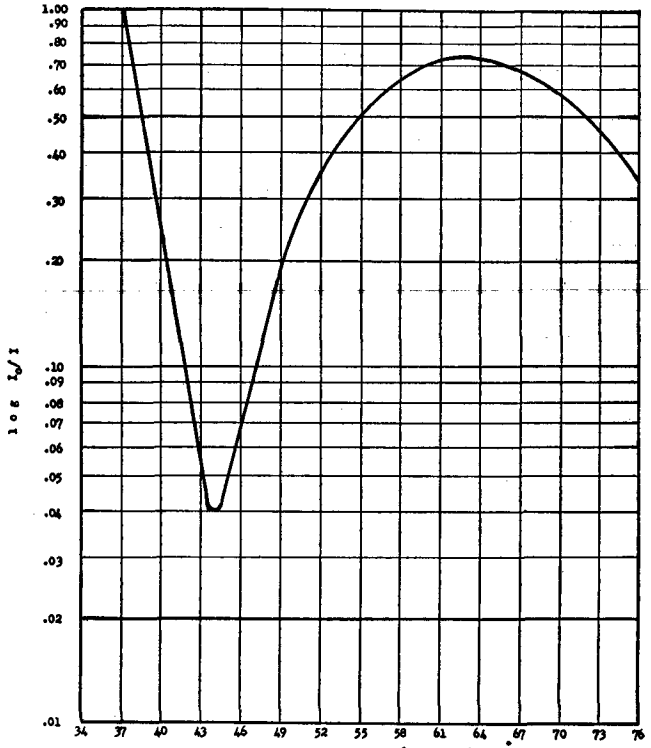

Figure 1

Absorption Spectrum of the Sodium Cupri-Glycerol Complex

If a Beckman or other spectrophotometer be employed, calculations may be made from the extinction coefficient ($E^{1\%}_{1cm.}$) of the glycerol complex at a wavelength of 6300 Å.

$$\% \text{ glycerol in sample} = \frac{a\,(100)}{E^{1\%}_{1cm.} \times 2.201}$$

a = specific extinction of the sample at λ = 6300 Å

2.201 = glycerol conversion factor

Mol. Wt. of Complex ($C_3H_5O_3$ Cu Na 1.5 H_2O) = 202.661

Experimental

The absorption spectrum of the sodium cupri-glycerol complex was determined with a Beckman spectrophotometer, Figure I. It will be noted that a broad absorption band is exhibited in the red region of spectrum with its maximum lying at about 6300 Å. The solution was found to conform closely to the Lambert-Beer law over the prescribed concentration range.

The subsequent experimental work was performed using a filter-type photometer equipped with a Corning filter No. 2412 (H. R. Lantern Red) and 3 ml. round optical cells. Samples of high grade chemically pure glycerol were carefully assayed by specific gravity determinations (pycnometer). These samples were then used as the bases for the accurate gravimetric preparation of 5-10% glycerol solutions in distilled water. Analytical samples of this dilute material such as to contain 0.12-0.17 grm. of glycerol were introduced into weighed precision grade 100-ml. glass-stoppered volumetric flasks. In each case distilled water was added to yield a volume of 10 ml. The modus operandi from this point was according to that previously described under Procedure.

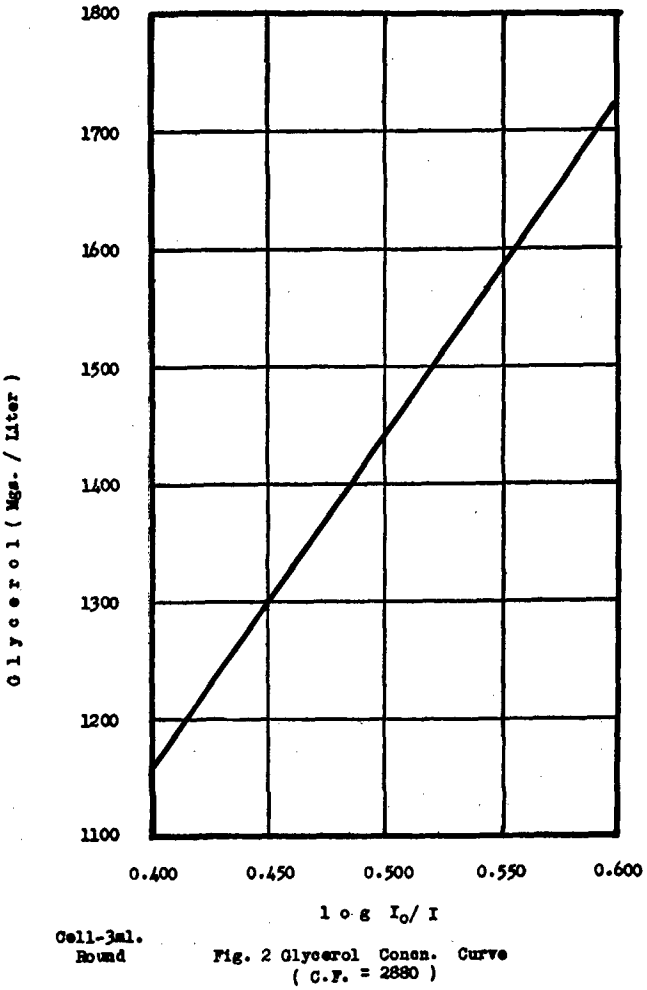

Fig. 2 Glycerol Concn. Curve (C.F. = 2880)

The average value of the concentration-absorption ratios obtained was adopted as the calibration factor (C.F.).

$$C.F. = \frac{\text{Glycerol Concn. (Mgs./liter)}}{\log I_0/I} = 2880$$

The foregoing concentration-absorption relationship is depicted graphically in Figure 2, the absorption ($\log I_0/I$) and the concentration (Mgs./liter) appearing along the abscissa and ordinate respectively.

The calibration factor of 2880 derived from the foregoing experimental work is cited merely for interest, since its value is dependent upon specific conditions obtaining to the photometer employed, it cannot arbitrarily be universally adopted.

Applications

Photometric glycerol determinations were made on samples of various glycerol-containing materials, such as, crudes, lyes, evaporator salts and filter-press cakes, etc., Table 1. The corresponding dichromate and acetin glycerol values are furnished where possible. In view of the fact that by far the most significant organic contaminating substance present in glycerine materials in-process is trimethylene glycol (1,3-propanediol), it was considered interesting to include analyses obtained on gravimetrically prepared solutions of glycerol and trimethylene glycol (Practical Grade—Eastman Kodak Co.), and also data covering the estimation of this substance in two impure distillate fractions, which were derived very rapidly by

TABLE 1

Material	Percent Glycerol		
	Photometric	Dichromate	Acetin
Soap Lye			
No. 1	9.44	9.60	.....
No. 2	11.23	11.51	.....
No. 3	8.41	8.66	.....
1 T	8.65	8.92	.....
2 T	10.18	10.49	.....
4 T	9.96	10.13	.....
Evap. Salt			
No. 1	0.34	0.34	.....
No. 2	0.81	0.79	.....
Press Cake	1.20	1.29	.....
Still Foots (Soln.)	7.16	10.67	.....
Soap Lye Crude			
No. 1 (AOCS, 1929)	83.95	84.25	83.40
No. 2	82.84	83.59	82.19
Refined Glycerine	98.98	98.97 [1]	98.54
Oils			
Tallow	10.38	10.46	.....
Grease	8.33	8.50	.....

[1] 99.02% by Sp. Grav.

calculation from the photometric and dichromate analyses, Table 2.

There is, of course, in the case of most in-process glycerol materials derived from fats and fatty oils by the usual saponification procedures, adequate analytical evidence regarding their nature to justify the assumption of the presence of trimethylene glycol as the major organic impurity, and therefore its subsequent approximate assay by calculation from the photometric-dichromate or acetin-dichromate differential. Obviously this procedure cannot be applied ad libertatem as an analysis for trimethylene glycol in materials whose origin lies outside the aforementioned classification.

Summary

The described spectrophotometric method for the determination of glycerol is based upon the blue solution developed by the formation of the sodium cupri-glycerol complex. A recommended analytical procedure and the formulae employed in the computation of the percentage glycerol present in various samples have been furnished. Standards containing various amounts of chemically pure glycerol were prepared as the bases for the establishment of a calibration curve showing the relationship of glycerol concentration to the optical density of the sodium

TABLE 2

Material		Percent present	Percent Glycerol (Apparent)		
			Photometric	Dichromate	Acetin
Synthetic Samples					
No. 1	Glycerol	6.34	6.36	7.78 [1]	.....
	T.M.G.	1.08			
	Water	92.53			
	Undetd. Matter	0.05			
No. 2	Glycerol	4.90	4.90	6.39 [2]	.....
	T.M.G.	1.10			
	Water	93.95			
	Undetd. Matter	0.05			
Special Distillate					
No. 1		.....	48.33	104.80	81.61
No. 2		.....	75.53	81.40 [3]	79.10

	Percent T.M.G. (Apparent)	
	Photometric/ Dichromate	Acetin/ Dichromate
Special Distillate		
No. 1	40.83	40.24
No. 2	4.24	3.99

[1] Calcd. 7.84.
[2] Calcd. 6.42.
[3] 71.60%—periodate.

cupri-glycerol complex under prescribed conditions of the procedure. Results covering the application of the method to various and typical glycerol-containing materials have been tabulated. It has been shown that trimethylene glycol, present as a major organic impurity in glycerine materials in-process, can be very rapidly estimated by simple calculation from the photometric glycerol-dichromate oxidation differential.

Notes

It may be remarked that most spectrophotometric analysis in the past has been confined to the determination of relatively small quantities of some compound present in the material under investigation. This is readily understandable. In this case, the application of spectrophotometry to the estimation of a compound with acceptable precision even when present in very high concentrations is probably attributable to two factors: first, the relatively low extinction coefficient of the glycerol complex at a wave-length of 6300 Å; and second, to the control of the analytical sample size so as to yield optical densities from 0.4-0.6.

The method is exceedingly simple, nevertheless the procedure recommended should be adhered to fairly closely. In the course of regular routine analysis it is not necessary continually to run chemical blanks with each group of samples; however, it would be advisable to prepare a blank when a new supply of reagents is being employed; simply determine the optical density of the blank against distilled water in the reference cell, then subsequently determine the optical density of all samples using distilled water in the reference cell and apply the necessary correction to the readings obtained.

No correction for the volume of the cupric hydroxide precipitate is necessary, since the established absorption-concentration relationship is empirically based upon the supernatant liquid, whose ratio to the volume of precipitate is essentially constant under the conditions of the procedure.

It is observed that the transient and final turbidity caused by the cupric hydroxide precipitate is far more readily discernible in the photometric than in the original volumetric method, attributable to the lower concentrations of the colored complex involved.

The Cl, SO_4, and Fe ions are non-interfering providing the solubility limits of their compounds in the medium in the reaction flask are not exceeded. The inherent brown hue imparted by organic impurities, etc., to most in-process glycerol materials is of no consequence since the characteristic absorption of these substances lies in another region of the spectrum.

The contents of the reaction flask prior to the addition of the cupric chloride solution preferably should be clear (only a barely perceptible turbidity is permissible). When considered necessary, appropriate measures may be taken to ensure this requisite.

The non-reactivity of ethyl alcohol with the reagent involved is a very important asset, since it renders possible its easy and useful employment as an extractive solvent in the preliminary preparation of certain materials for analysis.

Conclusions

The method, possessing adequate specificity, can be recommended for the direct determination of the actual glycerol content of most commonly encountered glycerol materials. In conjunction with the dichromate analysis, the trimethylene glycol content of the usual in-process glycerol materials can be very rapidly ascertained (approximately three hours). A satisfactory degree of precision, generally well within $\pm$ 0.35%, was obtainable. In view of the fact that apparently the magnitude of the deviation is for the most part a direct function of the sensitivity and reproducibility of the spectrophotometer employed, limits of $\pm$ 0.2% could confidently be expected with an instrument of high precision, such as the Beckman spectrophotometer.

Other existing analytical methods do not approach the photometric in facility and rapidity—determinations not requiring preliminary sample-treatment may be completed within twenty-five minutes.

Preliminary purification for most samples is unnecessary; existing volumetric analytical procedure is eliminated, and concomitantly the tedious preparation of accurately standardized volumetric solutions.

Acknowledgment

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