Report of the Referee Board, 1954-1955

In the year ending May 31, 1955, 48 laboratories were granted Referee Certificates, of which 30 were issued on Cottonseed, Oil Cake and Meal, and Fatty Oils; 12 were issued Certificates on Cottonseed and Oil Cake and Meal; three were issued Certificates on Oil Cake and Meal and Fatty Oils; two were issued Certificates on Oil Cake and Meal, and one on Fatty Oils only. During the year two of the laboratories having restricted certificates were retired from the field by disposing of their laboratories.

No unusual difficulties or situations confronted the Board this year, enabling it to allot some time to constructive planning of changes in the rules of its conduct and proposals for changes in the constitution and by-laws of the Society. The latter information covering a temporary change in the constitution is already in the hands of a special committee of the Governing Board. Consideration of proposals for a change in the rules by which the Referee Examination Board shall operate is being carefully given by its members with the hope that complete agreement may be reached as quickly as is expedient with good judgment.

The chairman extends his thanks and those of the Referee Board to R. W. Bates, who withdrew from the committee when he returned to Armour and Company. His experience as previous chairman of the Referee Board and as present chairman of the Smalley Committee has been a great help. His place on the committee was taken by E. R. Hahn of the Hahn Laboratories, Columbia, S. C. Thanks are also extended to all members of the regular committee and to C. E. Morris, *ex officio* member, for the efficient and cooperative manner in which they responded to all requests.

R. R. King C. E. Morris
R. C. Stillman E. R. Hahn
N. W. Ziels, chairman

Interaction of Gossypol with the Ferrous Ion[1]

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A basic consideration in the utilization of cottonseed meals has been the inactivation or modification of gossypol so that the meal is rendered nontoxic and has a greater nutritive value when fed to nonruminants as well as to ruminants. Consequently considerable attention has been given to ascertaining information on the chemical properties of gossypol and its reactions (1, 2, 5). Although several investigators have used iron salts to inactivate gossypol and render the meal nontoxic to nonruminants (3, 7, 8, 9), no fundamental investigation of the formation of complexes between gossypol and metallic ions seems to have been undertaken. The objective of the present investigation was to determine the combining ratios for the reaction between gossypol and the ferrous ion as a portion of a program of research on improving the nutritional value and utility of cottonseed meal.

Methods and Discussion

Several independent methods were used to establish the proportion in which gossypol reacts with the ferrous ion. These include conductometric, potentiometric, and spectrophotometric measurements as well as analysis of the isolated reaction product. The dissociation constant was determined from spectrophotometric data. The effect of the anion on the reaction was evaluated by substitution of perchlorate for the chloride ion.

The ferrous ion complex was formed by reacting ferrous chloride with sodium gossypolate. A 0.001 M solution of sodium gossypolate was prepared as follows. A flask was freed of oxygen by flooding with oxygen-free nitrogen prepared by passing tank nitrogen through alkaline pyrogallol. Pure gossypol (0.2 millimole) was introduced into the flask and dissolved by addition of 20 ml. of acetone. While mixing with a magnetic stirrer, 180 ml. of a sodium hydroxide solution, containing 0.4 millimoles, were added. This sodium gossypolate solution was found to be stable for about 5 hrs. in the absence of oxygen. This was established by spectrophotometric measurement of the optical density (1 cm. cell) of a 0.00003 M acetone solution of sodium gossypolate (Table I). Assuming that the absorption at the maximum of 380-382 mμ is a criterion of stability, the solution is stable for sufficient time for the study of the reaction with the ferrous ion.

TABLE I
Stability of Sodium Gossypolate Solution

Hours	Optical density at maximum of 380-382 mμ
0	0.515
1	0.522
2	0.515
5	0.517
7	0.498

During preliminary experiments in which solutions of sodium gossypolate were combined with equal portions of ferrous chloride solution of the same molarity, an immediate color change from yellow to dark amber was noted, indicating that a reaction had occurred.

Measurements of optical density at the 389-391 mμ maximum, similar to those for the sodium gossypolate solution, after addition of ferrous chloride indicated that the ferrous gossypolate formed is stable for at

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[1] Findings reported in this paper were included in a thesis submitted to Tulane University by Robert J. Demint in partial fulfillment of the requirements for the M.S. degree.

[2] One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

least 24 hrs., the optical density at 390 mμ, changing only from 0.482 to 0.474 during this period.

Conductometric and Potentiometric Titration. The reaction of gossypol with the ferrous ion was measured conductometrically by titrating 100 ml. of 0.001 M sodium gossypolate solution with 0.01 M ferrous chloride, using a dip-type conductivity cell. The contents of the titration vessel was covered with dental sheet rubber and flooded with oxygen-free nitrogen.

The reaction was also studied by potentiometric titration of 25 ml. of 0.001 M sodium gossypolate in 10% acetone with 0.001 M ferrous chloride, protecting the reagents from oxygen by flooding with oxygen-free nitrogen. The data for the conductometric titrations are plotted in Figure 1. The break in the curve occurs at a 1:1 mole ratio of the ferrous ion to sodium gossypolate. Similarly the potentiometric titrations gave an inflection at a 1:1 mole ratio.

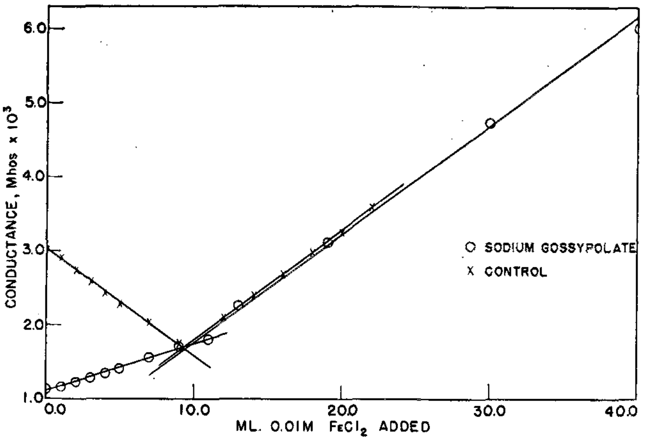

FIG. 1. Conductometric titration of 100 ml. of 0.001 M sodium gossypolate with 0.01 M $FeCl_2$, and control consisting of 100 ml. of 0.002 M sodium hydroxide with 0.01 M $FeCl_2$.

Both conductometric and potentiometric measurements of the reverse titrations also showed inflections when equal mole ratios of gossypolate and the ferrous ion were present. It would appear therefore that one mole of ferrous ion combines with one mole of gossypol.

Absorption Spectra. Absorption spectra of solutions of sodium gossypolate, ferrous chloride, and mixtures of these solutions at concentrations resulting in 1:1, 2:1, 4:1, and 6:1 mole ratios in 0.3% acetone from 220 to 700 mμ were obtained with a recording spectrophotometer, using 1 cm. cells and 0.3% acetone as a reference solution. The same spectra (Figure 2) are obtained for the reaction product even when the ratio of ferrous ion to gossypol was varied sixfold.

Since definite changes in absorption spectra of sodium gossypolate solutions were observed on the addition of ferrous ion, these changes were studied further by Job's method of continuous variations (4). For this purpose increments of sodium gossypolate solution, increasing progressively from 1 to 9 ml., were added to each of nine low actinic 100-ml. volumetric flasks. Suitable increments of a ferrous chloride solution were also added so that a total of 10 ml. of the mixture was present in each flask. The flasks were made to volume to give a series of solutions in 0.4% aqueous acetone with regularly varying ratios of ferrous ion and sodium gossypolate but with the total molarity constant at 0.00004 M. Two additional series of solutions in 0.4% acetone, one with matching concentrations of sodium gossypolate, and the other with matching concentrations of ferrous chloride, were prepared. The optical densities of all three series were measured at 260 and 267 mμ. The increases in optical density resulting from the reaction of ferrous ion with gossypol are plotted in Figure 3. A peak at approximately the 0.5 mole fraction confirms the 1:1 mole ratio found by the conductometric and potentiometric titrations.

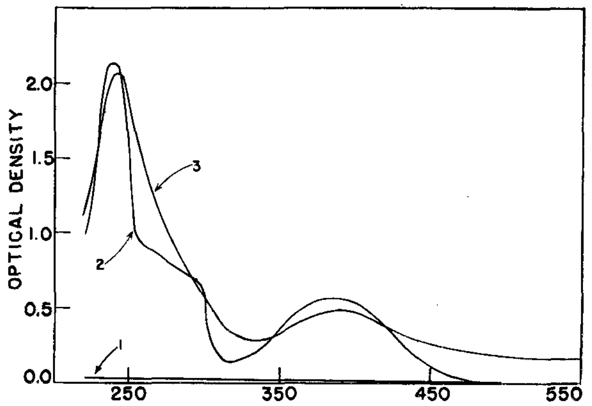

FIG. 2. Absorption spectra of $FeCl_2$, sodium gossypolate, and various mole ratio mixtures.

Curve 1—$FeCl_2$
Curve 2—Sodium gossypolate
Curve 3—1:1, 2:1, 4:1, and 6:1 mole ratio mixtures of $FeCl_2$: sodium gossypolate.

Analysis. Ferrous gossypolate samples were prepared by combining replicate 20 ml. aliquots of a 0.02 M sodium gossypolate solution in 25% acetone with equal portions of 0.12 M ferrous chloride. They were centrifuged, decanted, and washed twice with oxygen-free distilled water. In order to determine their composition the precipitates were transferred to

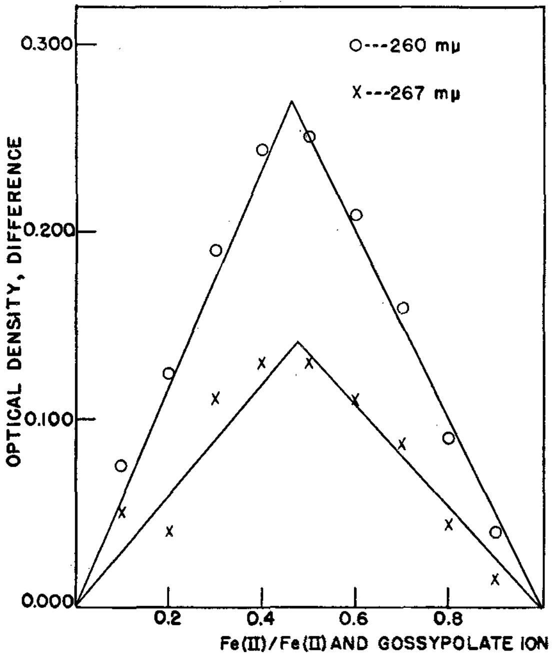

FIG. 3. Changes in optical density with varying mole fractions of ferrous and gossypolate ions.

platinum dishes, dried to constant weight in a vacuum desiccator, ashed in a muffle furnace at 750°C., and weighed as ferric oxide.

The analysis of closely agreeing replicates for iron was 9.69%, compared to the theoretical value of 9.73% for ferrous gossypolate, also supporting the conclusion that ferrous ion combines with gossypol in a 1:1 mole ratio.

Dissociation Constant. Optical densities at 260 mμ of successive dilutions of a 0.00002 M solution at the 1:1 mole ratio of the ferrous ion and sodium gossypolate were determined spectrophotometrically to provide data for calculating the dissociation constant of the ferrous gossypolate by the method of Turner and Anderson (6). This was possible as the data obtained gave a linear curve conforming to Beer's law. Three sets of data obtained from the continuous variation curve were paired with the corresponding data at the same optical density from the dilution curve for this calculation. The average pK value from the three was 7.6 ± 0.3, indicating that ferrous gossypolate is only slightly dissociated in solution.

Effect of the Anion. The conductometric and spectrophotometric studies were repeated, substituting ferrous perchlorate for ferrous chloride in order to evaluate the effect of the anion on the reaction. The curves obtained in each case with ferrous perchlorate were essentially identical to the corresponding ones resulting from the use of ferrous chloride. Therefore these measurements also indicate that gossypol reacts with ferrous ion in a 1:1 mole ratio independently of the anion present. The pK value of 7.0 calculated from the data obtained at 490 mμ, using ferrous perchlorate and 1% acetone as a solvent, is in good agreement with the value of 7.6 based on measurements at 260 mμ where ferrous chloride and 0.4% acetone were used. Consequently there seems to be little if any tendency for the anion to enter the coordination sphere in this reaction.

Structure. According to Roger Adams and coworkers (1), the formula for gossypol must be essentially symmetrical. Based on symmetrical considerations and assuming formula Ia as proposed by Adams, the most plausible site of attachment for the two sodium ions would be at the peri-hydroxyls. If this is the case, it would appear likely that the ferrous ion replaces the two sodium ions of the sodium gossypolate when ferrous gossypolate is formed.

Summary

The preparation of a solution of sodium gossypolate in aqueous acetone has been described. Indications of a 1:1 mole ratio combination of the gossypolate and ferrous ions were established through conductometric and potentiometric studies in the presence of either chloride or perchlorate anion. Application of the method of continuous variations to differences in the absorption spectra of the two species confirmed the 1:1 mole ratio of ferrous ion to gossypol. This finding is supported by analytical data. Values of pK for the dissociation constant of ferrous gossypolate, calculated from spectrophotometric measurement, averaged 7.3.

Since the peri-hydroxyls are the most plausible location of the sodium ions in sodium gossypolate, its reaction with ferrous ion results in displacement of the two sodium ions by the ferrous ion.

Acknowledgments

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Report of the Smalley Committee, 1954-55

FOLLOWING the custom established several years ago the abstracted reports of the five subcommittees will represent the complete report of the Smalley Committee. These reports have been prepared in part by the chairman and the subcommittee chairmen. More than 3,700 samples were distributed this season, representing the tabulation of over 12,000 determinations and calculation of over 800 grades. The subcommittee on drying oils was reactivated, and the subcommittee on glycerine was continued, giving us six active subcommittees. The Smalley activities have grown considerably since this committee was established, at the close of World War I, 37 years ago. It is a most important phase of our Society's activities, and it has been a major factor in establishing oil chemists as one of the most precise groups of analytical chemists.

While all members of our committees have been most cooperative and effective and the chairman expresses his thanks for their support and guidance, there are certain individuals and organizations who deserve special note. The Society is especially indebted to the following:

G. Conner Henry, T. C. Law, and Law and Company for the preparation and distribution of the meal and seed samples;
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T. R. Bresnahan and Darling and Company for the selection of the bulk tallow and grease samples;
K. H. Fink and the Lookout Oil and Refining Company. (a division of Armour and Company) for the preparation and distribution of the tallow and grease samples;
W. D. Pohle and Swift and Company for the complete handling of the glycerine samples;
V. B. Shelburne and Spencer Kellogg and Sons for the preparation and distribution of the drying oil samples;
B. N. Rockwood for tabulating the tallow and grease results and calculating the grades;
F. R. Earle and F. G. Dollear for tabulating and mailing the vegetable oil results;