pothesis that the absorption of dyes is dependent upon the distribution of residual affinity in the molecule, and that with solutions of dyes the interplay of the residual affinities of the solvent and solute may bring about rearrangement in the distribution of affinity of the dye.

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EVIDENCE CONCERNING THE CONSTITUTION OF GUINEA GREEN B

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Although many articles have appeared on the use of the spectrophotometer for identifying dyes, little has been said as to its quantitative application.

From Beer's law the concentration of any solution of a dye can be determined if we know the amount of absorption of light at any given wave length for some definite concentration of this dye.[1] The method of establishing the "standard" absorption of a dye is comparatively simple. The absorption spectra (extinction coefficient-wave length) are plotted for three or four samples of the same dye from different batches, all of which have been carefully analyzed and the color content determined by chemical means. From these figures the absorption curve for a 100% sample is calculated. For example, if a 0.002% solution of a sample containing 85% of dye shows an extinction coefficient at some definite wave length of 1.02, the extinction coefficient for a 0.002% solution of the pure dye would be 1.20. By determining the extinction coefficients for several samples and averaging their 100% curves, errors due to observation are practically eliminated from the standard. This method has been used to obtain the absorption constants for the eleven permitted food colors. Analyses based on these figures have proved entirely satisfactory in every way for all except three dyes, Light Green S F Yellowish, Guinea Green B and Indigotin. The last named dye offers entirely different difficulties from the greens and will be dealt with at another time.

Three methods are in general use for determining the exact color content of the permitted food dyes: color by difference, sulfated ash, and titration with titanium trichloride.

The color content can be found by determining all the possible impurities, such as inorganic salts and uncombined intermediates, and subtracting their total from 100%. In the cases of the permitted greens the "color

[1] The variations in Beer's law are negligible over the concentrations which can be used in the standard 1-cm. cell.

by difference" method cannot be used as there is always present some material for which no method of detection or determination is known and which is called "organic material not dye." It is said that any attempt to purify these dyes will produce more of this material.

With water-soluble dyes the color can also be computed from the sulfur content or sulfated ash (sodium content), provided that the necessary corrections are made for the inorganic salts present. Inasmuch as the sodium content of these green dyes varies widely, the practice has been to base the strength of the color on the titanium trichloride titration figure and the requirements for certification specify that 1 g. of dye shall require not less than a given amount of 0.1 *N* titanium trichloride to destroy the color.

In the course of establishing standard absorption curves for the two green dyes, it became apparent that there was something wrong with the usual methods of titration. For example, a sample of Guinea Green B which titrated 85.7% gave an extinction coefficient, in a 0.002% solution, using a 1-cm. cell, of 2.10 at 6200 Å., while one titrating 81.5% gave an extinction coefficient of 1.88 at the same point. These are equivalent to 2.44 and 2.30 for a 100% sample. To complicate matters further one of the manufacturers, having produced a sample of this dye which was low in color strength, evolved a new method of titration with titanium trichloride which gave him much higher results. As this method really indicated the solution to the problem it will be given here in detail.

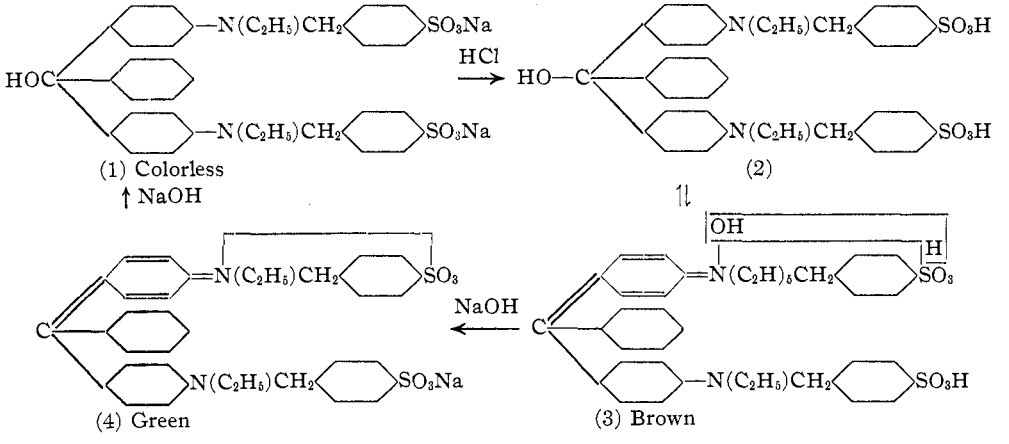
A 0.25g. sample of Guinea Green B was dissolved in 150 cc. of distilled water, and 75 cc. of 35% hydrochloric acid was added. The volume was then increased to 250 cc. with water and the mixture was heated at 50° for 30 minutes. It was then cooled to room temperature in an ice-bath and 10 g. of sodium acetate was added. Just sufficient 50% sodium hydroxide solution to destroy the acidity to congo red paper was then run in. Finally 5 g. of Rochelle salt was added and the solution was heated to boiling in a current of carbon dioxide. It was then titrated with 0.1 *N* titanium trichloride until all green color was destroyed.

Believing that some reducible material was formed by this process and that the actual color content was lowered, this procedure was carefully followed and a sample was withdrawn before titrating, for spectrophotometric observations. A blank for the optical work was made by not adding the 0.25 g. of Guinea Green B until after neutralization. The purpose of this was to correct for any buffer action due to the high concentration of inorganic salts present. Contrary to expectation, the spectrophotometric determinations showed an increase in color exactly equal to that found with titanium trichloride.

At this point in the work a discovery was made, more or less by accident, which furnished the key to the problem. When a solution of Guinea Green B was poured into a hot solution of sodium carbonate it was completely decolorized. This reaction is not quantitative and reversing

the process—pouring the sodium carbonate into hot Guinea Green B solution—reduces the color only to a pale green. It does not seem possible to go beyond this point with carbonate, although a small quantity of sodium hydroxide completely decolorizes the dye. On the addition of a slight excess of acid over that required to destroy the carbonate, the green color is restored. This bleaching process also takes place very slowly in the cold.

Guinea Green B is the sodium salt of diethyl-dibenzyl-diamido-triphenyl-carbinol-disulfonic acid. The free acid is brown, as is shown by the addition of an excess of hydrochloric acid to the aqueous solution. It is therefore apparent from these experiments that the monosodium salt of this compound is green and the disodium salt is colorless, although most authors express the dye as the completely neutralized base. The procedure of analysis described above converts the disodium salt into the free acid and only enough alkali is added to form the monosodium salt. The heating is necessary to hasten the molecular rearrangement as represented by the following formulas, from (2) which is probably colorless to the quinoid structure (3) which is brown.



It has often been noticed that a very slow titration of Guinea Green B with titanium trichloride will give a higher figure than a more rapid one. This is easily explained in view of the above. The customary method is to titrate a boiling solution of the dye with titanium trichloride dissolved in hydrochloric acid. Under these conditions the disodium salt is slowly converted into the monosodium salt which, experiments indicate, is the first to be reduced. The free acid is reduced so slowly that it cannot be successfully titrated with titanium trichloride.

In the certification of a food dye only the actual color content can be considered. It therefore became necessary to devise some method of

titrating only the monosodium salt. It was found that this could be accomplished by dissolving 0.5 g. of dye in 250 cc. of distilled water which had been previously boiled and cooled in an atmosphere of carbon dioxide, and titrating at a temperature of 60° to 70° after adding 30 g. of sodium acid tartrate. A blank must be titrated on the sodium acid tartrate and correction made on the dye titration figure.[2]

Several samples of Guinea Green B were titrated according to this method. Other samples from the same batches were then treated with hydrochloric acid and sodium hydroxide as described above and titrated a second time. The difference between these two titrations represents the Guinea Green B which has been produced from the disodium salt. If this value is converted into the equivalent figure for the disodium salt and added to that obtained from the original titration, the result agrees fairly well with that required by the analysis.

TABLE I

ANALYTICAL DATA

	Sample A %	Sample B %	Sample B %
Titration before treatment	68.8	69.5	69.5
Titration after treatment	84.5	90.4	85.6
Difference	15.7	20.9	16.1
Difference computed as disodium salt	16.6	22.1	17.1
Dye + disodium salt	85.4	91.6	86.6
Color by difference	91.4	90.6	90.6
Sulfated ash from disodium salt (computed)	3.23	4.29	3.31
Sulfated ash from first titration (computed)	7.07	7.14	7.14
Total ash (computed)	10.30	11.43	10.45
Sulfated ash by analysis (corrected for inorganic salts)	10.69	10.90	10.90

These results indicate that the "organic material not dye" already mentioned is, in reality, mostly the colorless salt of Guinea Green B. The agreement of the results is not all that could be desired but is probably within the experimental error. The accuracy of the titanium trichloride titrations for this particular dye is not known but is probably within 1 or 2%. The largest error is in the neutralization of the hydrochloric acid solution with sodium hydroxide, using congo red paper as an indicator. It is very difficult to determine whether the paper is the blue color of acid congo red or the green color of the dye. No more satisfactory indicator has been found and determination of the exact Sörensen (P_H) value for the complete formation of only the monosodium salt was impossible with the apparatus available, owing to the ease of reduction of the dye.

An unsuccessful attempt was made to determine with the spectrophotometer the correct Sörensen value for the production of the greatest amount

[2] This method was devised by W. F. Clarke, of this Laboratory.

of color. To accomplish this a fixed amount of Guinea Green B was added to buffer solutions of different hydrogen-ion concentrations and the height of the absorption maxima measured. These measurements were repeated after the solutions had stood for several hours to allow for any slow change in constitution. Although the results indicated that the change from the di- to the monosodium salt was accomplished at about P_H 4.5, in the congo red range, the increase in color was much smaller than that required by analysis. Samples of the dye dissolved in the disodium phosphate-acetic acid buffer solution (P_H 6.0) which has been used in this Laboratory for many years[3] gave the same percentage of color by the spectrophotometer that was obtained by W. F. Clarke's modified titanium trichloride method and further proved the value of this buffer for quantitative dye analysis.

An examination of the figures obtained in the two determinations on Sample B shows the effect of errors due to the neutralization. These were run under identical conditions and differed only in the quantity of alkali required to destroy the acidity to congo red paper. The variation in the computed sulfated ash content from that found by experiment is the same in both, though opposite in sign, while the variation in percentage of dye plus disodium salt from that calculated is widely different in the two cases.

In the case of Light Green S F Yellowish it can also be shown that the highest or trisodium salt is colorless. Both the mono- and disodium salts are probably colored. At present no method of distinguishing between them has been devised.

The author wishes to thank O. E. May of this Laboratory for his assistance in making the titanium trichloride titrations.

Summary

It has been shown that Guinea Green B is the monosodium salt of diethyl-dibenzyl-diamido-triphenyl-carbinol-disulfonic acid and that the disodium salt is colorless. A method of titrating only the colored component has been devised and checked by means of spectrophotometric observations.

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[3] Mathewson, *J. Assoc. Official Agr. Chem.*, **2**, 164 (1916).