[151st Contribution from the Color and Farm Waste Division, Jointly with Soil Fertility Investigations, Bureau of Chemistry and Soils, United States Department of Agriculture]

SPECTROPHOTOMETRIC DETERMINATION OF HYDROGEN ION CONCENTRATIONS AND OF THE APPARENT DISSOCIATION CONSTANTS OF INDICATORS.

V. FAST GREEN F C F

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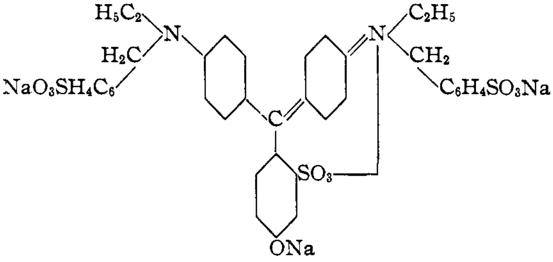
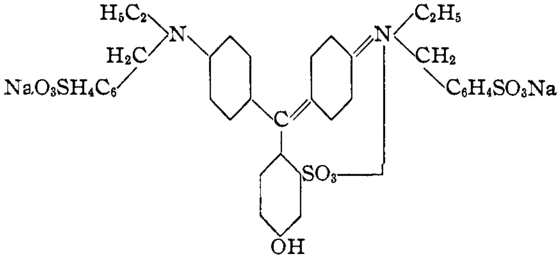
Fast green F C F, or *p*-hydroxyerioglucine A (C. I. No. 671), is the most recent addition to the group of artificial coloring matters, the use of which is sanctioned in coloring foods.[1] It appears probable, also, that it will prove to be a very valuable biological stain.[2] The dye undergoes several color transformations with variation in hydrogen ion concentration. Since these phenomena might, in some measure, affect its utility in the application referred to and might, on the other hand, render it of use in indicator practice, their investigation was undertaken.

The tetrasodium salt of the dye, which is formed under conditions of pronounced alkalinity, is practically colorless. The trisodium salt is blue, having an absorption band with a maximum at approximately 611 mμ. In passing from about *P*H 10 to about *P*H 6, the dye is transformed to the disodium salt which is green and has an absorption band with a maximum at approximately 628 mμ. A further decided modification in color is formed in the region of pronounced acidity, consequent upon acid salt formation at the unsaturated amino group of the





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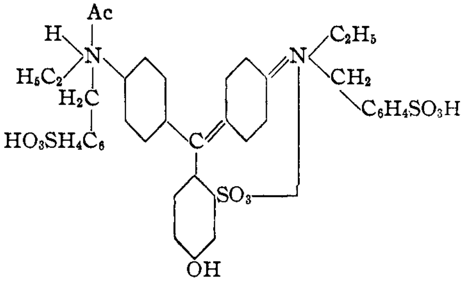
[1] H. Johnson and P. Staub, *Ind. Eng. Chem.*, **19**, 497 (1927).

[2] R. Haynes, *Stain Technology*, **3**, 40 (1928).

dye. The polyacidic salt formed



is yellow and is doubtless to be considered as a yellow of the second order. The two dissociation ranges of possible indicator utility are those of the polyacidic salt and of the phenolic salt.

The acid indicator range of the dye was investigated only in a preliminary manner. It was found that the conversion to the polyacidic dye salt was very nearly but not quite complete at $P_H{}^{-1}$. Spectrophotometric measurements carried out with buffered solutions of the dye over the range between P_H 0.87 and P_H 2.00 gave calculated values for apparent dissociation constants which increased with each step in the reduction of the acidity of the solution. Two intermediate products are formed in passing from the polyacidic salt of the dye to its disodium salt; the color acid and the monosodium salt. It was evident that the dissociation ranges of these various products overlapped and that their dissociation constants could not be determined by the methods at hand. It would have been possible to carry out an arbitrary calibration of a spectrophotometric ratio against hydrogen ion concentration in this region, but this was not undertaken, since the preliminary data indicated that the color of the dye was distinctly less sensitive to minor variation in acidity than is that of sulfonephthaleins available for employment in the same region.

The alkaline indicator range was investigated more thoroughly. The equipment and technique employed were those outlined in previous papers.[3] The ratio of the extinction coefficients of the solutions at 600 mμ to their extinction coefficients at 640 mμ was selected as the standard spectrophotometric ratio. Since a ratio of this type (R_2) is unaffected by variation in indicator concentration, no effort was made to avoid minor variation in that particular. All solutions, however, contained approximately 17.3 parts of dye per million parts of solvent. The temperature at which the spectrophotometric measurements were made was approximately 29°, and the Sørensen values of solutions recorded below have been corrected, when necessary, to conform to that temperature.

The dissociation of the indicator with increasing alkalinity appears normal until approximately P_H 9.8 is reached, at which point the forma-

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[3] Holmes and Snyder, THIS JOURNAL, **47,** 221 (1925).

TABLE I

STANDARDIZATION OF FAST GREEN F C F AGAINST SØRENSEN VALUES

*P*H	*E* at 600 mμ (Av.)	*E* at 640 mμ (Av.)	R_2	*P*H	*E* at 600 mμ (Av.)	*E* at 640 mμ (Av.)	R_2
6.14	1.18	1.50	0.79	8.55	1.425	1.02	1.40
6.68	1.21	1.46	.83	8.81	1.47	.97	1.52
7.12	1.26	1.43	.88	9.02	1.51	.95	1.59
7.62	1.29	1.31	.98	9.53	1.53	.90	1.69
7.82	1.30	1.25	1.04	9.80	1.54	.88	1.74
8.03	1.33	1.18	1.12	10.06	1.52	.85	1.77
8.22	1.37	1.12	1.23	10.41	1.50	.83	1.80
8.41	1.40	1.05	1.34	10.91	1.46	.805	1.81

tion of the trisodium salt is about 98% complete. Beyond that point it is evident that the further formation of the trisodium salt is accompanied by conversion of trisodium salt into the colorless tetrasodium salt. That circumstance is without effect upon spectrophotometric application but renders suitable precautions advisable if the indicator is used in conjunction with drop ratio colorimetric practice. In drop ratio technique it is recommended that the color standards be prepared with buffer solutions of *P*H 9.8 and *P*H 6.5, whereby the indicator will be obtained in approximately 98% of its respective forms. Error will result if the alkalinity specified is exceeded.

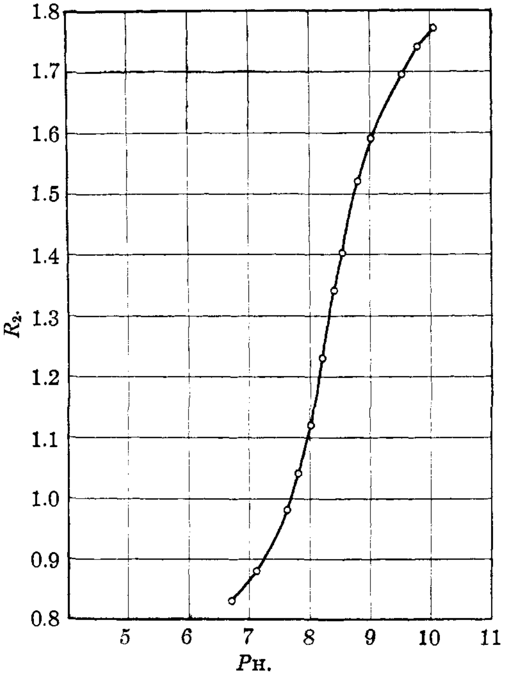

Fig. 1.—Fast green F C F. R_2 = *E* at 600 mμ/*E* at 640 mμ. Temperature, 29° alkaline range.

The apparent dissociation constant of the indicator in this range is approximately 8.1(5). This calculated value was checked by measuring the combined absorptions of two solutions at *P*H 9.8 and *P*H 6.5, respectively, contained in cells of equal thickness.

Spectrophotometric technique in the alkaline range of Fast Green F C F is slightly less accurate than with such other indicators as have been investigated previously. Minor discrepancies were noted in duplicate determinations, which arose, apparently, from readjustments in physical equilibria, and the spectral interval between the bands of the dye forms is smaller than is desirable. Whereas, however, the sulfonephthaleins are to be preferred in general indicator application, Fast Green F C F may well prove useful under some circumstances.

Summary

Spectrophotometric data are supplied for the determination of hydrogen ion concentrations over the *P*H range between 6.7 and 10.0 with Fast Green F C F.

The apparent dissociation constant of the dye in this range is approximately 8.1(5) at 29°.

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NOTE

Note on Colorimetric Estimation of Titanium by Hydrogen Dioxide Method.—The employment of hydrogen dioxide as reagent in the colorimetric estimation of titanium compounds, in acid solution, constitutes an accurate and rapid method for the determination of small quantities of this element.

It has been ascertained[1] that the presence of phosphoric acid produces a slight fading of the yellow to orange coloration, even when present in small amounts, rendering corrections essential to the accuracy of the procedure.

During experimental work undertaken and carried out by the writer, the fact has been observed that the addition of uranium acetate to the solution of titanium salt in solutions containing free hydrogen ions eliminates entirely the tendency to fading, at temperatures ranging from 20 to 50°. The inhibiting effect of the uranium compound upon the $PO_4^{\equiv}$ ion is not so pronounced at temperatures in excess of the maximum figure furnished.

It has been found that the uranium compound, in the proportion of 1 cc. of 0.1% solution to each 0.1 mg. of the element under examination, is most satisfactory in stabilizing the coloration produced by the action of hydrogen dioxide on titanium salts in solutions containing phosphoric acid, thus counteracting the tendency of the acid in question to decrease the intensity of the original coloration obtained.

The effect of the uranium acetate solution is independent of the amount or quantity of phosphoric acid or its compounds present in the solution under investigation.

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[1] P. Faber, *Z. anal. Chem.*, **46**, 277 (1907).