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A Method for Titration of Small Amounts of Iodine in Acetic Acid Medium

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In iodimetric determinations of peroxides in fat the liberated iodine is titrated in a strong acetic acid medium. In Lee's method [1], for instance, the composition of the solution to be titrated is as follows: 20 ml of a mixture of 2 parts of acetic acid and 1 part of chloroform (by volume), 30 ml distilled water, 1 g potassium iodide, and about 1 g of the sample to be tested. The two-layer system obtained is titrated with highly diluted sodium thiosulphate solution until the iodine color has disappeared. Some authors state that the titration can be performed using starch as an indicator. However, starch may be used in the acid medium described above only when larger amounts of iodine are present. With the small amounts of iodine liberated in the determination of peroxides in pure lard (amounting to 10^{-5}—10^{-6} equivalents of iodine per sample) starch is not a suitable indicator in our experience. However, neutralization of the acetic acid solution with sodium bicarbonate increases the sensitivity of starch as to iodine. The use of starch as an indicator in peroxide determinations, as so often mentioned in the literature, may have arisen from experiments with, for example, benzoylperoxide in such high concentrations as seldom correspond to the peroxide content of normal quality fats.

In such solutions, weakly yellow with fat, it is very difficult to titrate iodine without the aid of an indicator. For this reason we have investigated the possibilities of titrating iodine in acid medium with the use of a fluorescent indicator in ultraviolet light. Gotô [2] has earlier shown that iodine can be titrated with thiosulfate in hydrochloric acid solution using α-naphthoflavone or rhodamine B as a fluorescent indicator. For determination of iodine in neutral solution a fluorimetric method has been described by Harlay [3].

The attempts to titrate small amounts of iodine in acid medium with the aid of a fluorescent indicator were made for use in connection with peroxide

determinations. Therefore the titrations were performed in the two-layer system obtained by using Lee's mixture of acetic acid, chloroform, distilled water, and in some cases potassium iodide. As the indicator used by Gotô, α-naphthoflavone, was not obtainable, some 30 other dyestuffs available in our laboratory (including rhodamine BP) were examined with respect to their usefulness in the titration of iodine in ultraviolet light.

The source of light used was a mercury vapour lamp with nickel-glass bulb. The maximum intensity of the light was at 365 mμ.

Among the dyestuffs investigated rhodamine BP, thioflavin, pinacryptol yellow, and dicyanin A showed a distinct change in intensity of fluorescence in the vicinity of the equivalence point when titrating iodine in the described two-layer system. Further experiments showed that the change in fluorescence of thioflavin was more obvious than that of the other dyestuffs. Because of this the experiments were directed to a closer investigation of its usefulness in peroxide determinations and to an attempt to explain its action as an indicator *.

THIOFLAVIN AND ITS BLUE-FLUORESCING COMPONENT

The thioflavin used was obtained from a dye manufacturer in London. Its fluorescence in both water and chloroform solutions was bluish green. In the progress of the experiments the thioflavin proved to have one component with a very strong whitish-blue fluorescence in ordinary organic solvents, and one with a faintly yellowgreen fluorescence. In order to separate the blue-fluorescing component a mixture of 45 ml of 0.1 % aqueous thioflavin solution and 5 ml glacial acetic acid was extracted three times with 15 ml of benzene in a separatory funnel. The benzene extract was filtered and dried over calcium chloride. As the dyestuff was easily absorbed by the drying agent the benzene solution was dried for only one hour. From the filtrate the solvent was evaporated in vacuum on a water bath. The minute residue was dissolved in 1 000 ml of a mixture of 2 parts of acetic acid and 1 part of chloroform (by volume). The solution of the blue-fluorescing thioflavin component thus obtained has been used throughout the experiments described below. The dyestuff solution has been used as solvent in the determination of peroxides in fats and oils according to Lee [1].

* Kocsis, *et al.*[4] earlier made use of thioflavin S as an adsorbtion indicator when performing precipitation titrations on halogen ions with silver nitrate in ultraviolet light.

EXPERIMENTS WITH BENZOYL PEROXIDE

Benzoyl peroxide was recrystallized three times according to Gatterman [5]. From the purified substance a standard solution was prepared containing about 100 mg of benzoyl peroxide dissolved in 100 ml of *n*-propanol. The solution was kept in a glass-stoppered bottle in a dark place.

In order to establish the accuracy in the determination of benzoyl peroxide, samples of the following mixture were titrated:

20 ml of a mixture of HAc + $CHCl_3$ (2 + 1) containing indicator
5.00 ml benzoyl peroxide solution
1 ml saturated KI solution
30 ml distilled water.

When the potassium iodide solution had been added the sample was allowed to stand in the dark for ten minutes. Distilled water was then added and the sample titrated with 0.01 *N* thiosulphate solution. In the two-layer system obtained in this way, the blue-fluorescing thioflavin component dissolved in the chloroform layer only. The end point is reached when the fluorescence in the chloroform suddenly increases to a maximum. This is best observed when the chloroform by gentle agitation is suspended in the water. At this point the chloroform phase, when illuminated from above, appears as a whitish-blue luminous fog separating from the water phase.

In the experiments described above the following relationships prevail:

$$2\ S_2O_3^{2-} \longleftrightarrow I_2 \longleftrightarrow 1/2\ O_2 \longleftrightarrow (C_6H_5CO)_2O_2$$

and the amount of active oxygen is obtained from:

$$\%\ \text{active oxygen} = \frac{8 \times \text{ml thiosulphate sol.} \times \text{its normality} \times 100}{\text{mg benzoyl peroxide per sample}}$$

The results obtained are tabulated below (Table 1).

Nos. 6—8 were titrated four days later than nos. 1—5, the same benzoyl peroxide standard solution being used. The results show that the standard solution is stable during this period of time and that the end-point in titration can be determined with sufficient accuracy.

EXPERIMENTS WITH FATS

In order to estimate the usefulness of this new method in the determination of peroxides in various fats, samples of lard and tallow were titrated in visible light, without indicator, and in ultraviolet light with the thioflavin component

Table 1. Titration of 5.00 ml benzoyl peroxide solution containing 5.44 mg benzoyl peroxide. Theoretically calculated active oxygen = 6.606 %.

Sample no.	Ml. 0.01 *N* thiosulphate sol. used (corr. for blank)	Active oxygen found, %	$\frac{\%\text{ found}}{\%\text{ calc.}} \times 100$
1	4.26	6.62	100.2
2	4.26	6.62	100.2
3	4.26	6.62	100.2
4	4.26	6.62	100.2
5	4.21	6.54	99.0
6	4.26	6.62	100.2
7	4.26	6.62	100.2
8	4.25	6.61	100.1
			Average value: 100.0

as an indicator. These preliminary experiments were performed without using an inert atmosphere.

The samples to be tested were prepared in the following way: The fat was melted on a water bath at 60° C and samples of 1 gram were weighed out in Erlenmeyer flasks. 1 g of potassium iodide and 20 ml of an acetic acid-chloroform-mixture (2 + 1), with and without indicator, were added. The samples were gently boiled over an open flame for 30 seconds. The fat dissolved and the reaction between iodide and peroxides took place. The solutions were immediately cooled to room temperature and 30 ml distilled water added. The samples were titrated with 0.01002 *N* thiosulphate solution.

From Table 2 it is obvious that the values obtained with titrations in visible and in ultraviolet light are not satisfactorily agreeable. The deviations are especially great in the experiment with tallow, probably due to the small amount of thiosulphate required. Considering the experience with benzoyl peroxide, the results obtained from titrations in ultraviolet light are probably more correct than those obtained in titrations in visible light where no indicator was used. In the latter titrations the error is probably due largely to the natural yellowish color of the fat in the chloroform solution which interferes with the end point.

As regards the titration of iodine in ultraviolet light attention has been payed to the possibility that photochemical reactions might interfere with the

Table 2. Determination of peroxides in 1.00 g fat. (1 ml 0.01 N thiosulphate sol. = 0.080 mg active oxygen).

Sample no.	Titration in visible light			Titration in U. V. light in the presence of the blue-fluoresc. compound		
	Ml thiosulphate sol. used (corr. for blank)		% active oxygen × 10^3	Ml thiosulphate sol. used (corr. for blank)		% active oxygen × 10^3
Lard 1	0.24	0.25	1.96	0.26	0.28	2.16
» 2	0.21	0.20	1.64	0.19	0.20	1.56
» 3	0.27	0.28	2.20	0.34	0.35	2.77
» 4	0.29	0.30	2.36	0.29	0.30	2.36
Tallow 1	0.10	0.11	0.84	0.05	0.06	0.44
» 2	0.04	0.07	0.44	0.03	0.00	0.12
» 3	0.04	0.04	0.32	0.06	0.07	0.52

determination. During the time of titration the authors observed no disturbances due to the ultraviolet light. Only after a longer time of exposure to the light an effect was revealed by an increase in the thiosulphate requirement.

THE PRINCIPLE OF THE FLUORESCENCE TITRATION OF IODINE IN THE TWO-LAYER SYSTEM

Experiments to explain the indicator action of the blue-fluorescing thioflavin component showed that neither a reduction-oxidation reaction within the dyestuff molecule, nor the formation of a non-fluorescent complex with the liberated iodine would explain the strong increase in fluorescence when the iodine was transformed into iodide. As the light has to pass through the water layer to reach the dyestuff dissolved in the chloroform layer, the absorption behaviour of the test solutions in ultraviolet light was investigated. In the presence of a large excess of potassium iodide, the water layer contains the iodine in the form of the complex I_n^--ions. It was supposed that the absorption of ultraviolet light by the I_n^- ions was so great that the fluorescent indicator in the chloroform layer was not exposed to the activating light. A simple experiment indicated this to be the case. A long narrow glass tube was filled with a sample of the same composition as used in the experiments with benzoyl peroxide. When the tube was held in slanted position and examined in ultraviolet light, the chloroform layer fluoresced with full brilliance whether the water layer contained large amounts of iodine or excess of thiosulphate.

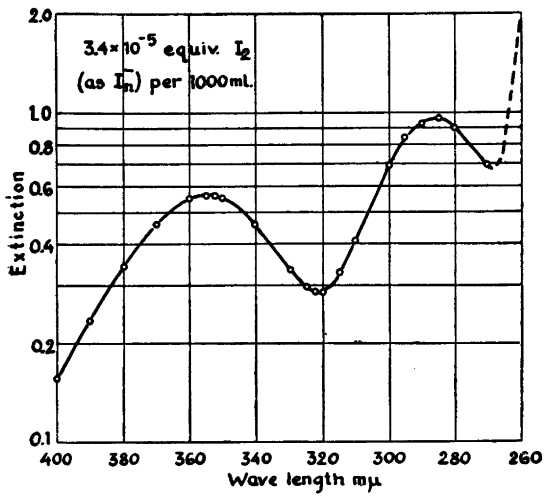

Fig. 1. Absorption curve in ultraviolet light for a solution containing 3.4 × 10^{-5} equivalents I_2 (as I_n^-) per 1000 ml.

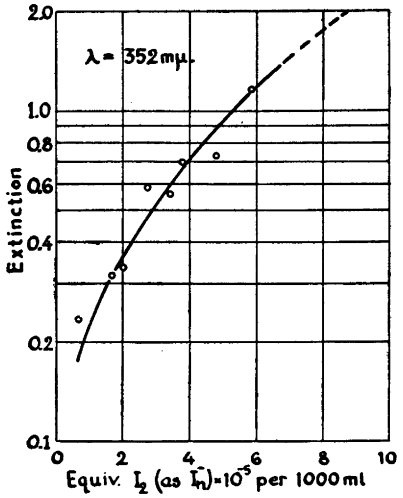

Fig. 2. Extinction at 352 mμ of solutions with varying iodine concentrations.

On this account the absorption curve in ultraviolet light for a solution containing 3.4 × 10^{-5} equivalents of iodine (as I_n^- ions) per 1 000 ml was determined (Fig. 1) and also the extinction at 352 mμ of solutions with varying iodine concentrations (Fig. 2). The apparatus used was the Beckmann spectrophotometer. The measurements were made on the water layer with the same composition as given in the experiments with benzoyl peroxide. The solutions were read against blanks which instead of benzoyl peroxide contained an equivalent amount of benzoic acid.

Fig. 1 shows that the I_n^- ions have an absorption maximum at 350—360 mμ which is very close to the intensity maximum of the mercury-vapour lamp used in the titrations. The results aggree with the findings of Custer and Natelson [6] and of Brode [7]. In Fig. 2 it is shown that the absorption increases very rapidly with increasing iodine concentration. At a concentration of only 10^{-4} equivalents per 1 000 ml the absorption is too high to be measured. The measurements thus confirm the assumption that the I_n^- ions, even in the small concentrations present, *e. g.*, in the determinations of peroxides in fats, absorb the ultraviolet light which activates the fluorescent indicator in the chloroform layer.

In iodine titrations in the presence of fat the impurities in the fat may interfere by absorbing the ultraviolet light. Of the fats examined, the peroxide content could be determined without disturbances in lard, tallow, butter, and

various edible oils of vegetable origin. On the other hand peroxide determinations in strongly colored feeding oils, for example, could not be made. Absorption measurements were made on both layers from solutions of the following composition: 20 ml of a mixture of 2 parts of acetic acid and 1 part of chloroform (by volume), 1 g fat and 30 ml distilled water. The measurements showed that the absorption at around 360 mμ in both the water and the chloroform layers was negligible in all cases except in the case of feeding oils where the absorption was very high in the chloroform layer.

The principle of the method suggested for the determination of small amounts of iodine in acetic acid medium may be of interest. The method has been only briefly described as the dyestuff used at the present time is difficult to obtain. The sensitivity of this method may be improved by using some other indicator. The shape of the titration vessel may also have some effect on the results.

SUMMARY

A new principle for titration of small amounts of iodine has been described. The titration is performed in ultraviolet light with thiosulphate solution in a two-layer system obtained by mixing acetic acid, distilled water, and chloroform. The end point is characterized by the appearance of fluorescence from a dyestuff in the chloroform layer. Because of the strong ultraviolet absorption of the iodine (in the form of the complex I_n^- ions) the chloroform layer does not show fluorescence with full brilliance until all the iodine has reacted with thiosulphate. The sensitivity of the method is about 25 micrograms or 2×10^{-7} equivalents of iodine per sample.

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