

8. Mustard, Margaret J. Ascorbic acid content of some miscellaneous tropical and subtropical plants and plant products. *Food Research*, 17, 31-35, 1952.
9. Mustard, Margaret J. The ascorbic acid content of some malpighia fruits and jellies. *Science*, 104, 230, 1946.
10. Mustard, Margaret J. and Stahl, A. L. The freezing preservation of some tropical and sub-tropical

fruits. *Fla. Sta. Hort. Soc. Proceedings for 1948*, 275-279, 1948.

11. Reasoner, Plinty. *Royal Palm Nursery Catalogue for 1887-88*. Oneco, Fla.

12. Stahl, Arthur L. Commercial possibilities of frozen tropical fruit juice concentrate. *Proc. Fla. State Hort. Soc.* 255-258, 1952.

## APPLICATION OF THE POLAROGRAPH TO THE DETERMINATION OF ACETYLMETHYLCARBINOL IN ORANGE JUICE

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Acetylmethylcarbinol, more commonly known as acetoin, is believed to be either the precursor or an intermediate in the formation of diacetyl in biological processes. The determination of diacetyl has become important in recent years because of the development of the "buttermilk" off-flavor in orange concentrates. Because acetoin is considered as a precursor of diacetyl, it was of interest to investigate methods of its determination. It was decided to explore the possibilities of the polarograph for this analysis, and this paper covers the exploratory work involved.

Kolthoff and Lingane (1) provide an excellent description of the polarograph, its principle, equipment, uses, and limitations. A survey of the literature indicated that Winkel and Proske (2) obtained a wave at  $-1.7$  v., using a  $0.001$  M solution of acetoin in  $0.1$  N ammonium chloride, and that I. Tachi (3) found a wave at  $-1.14$  v., using a  $0.0001$  M solution of acetoin in a mixture of  $0.01$  N hydrochloric acid and  $0.1$  N potassium chloride. Pleticha (4), in a very careful study of both diacetyl and acetoin, stated that acetoin is not reducible but can be oxidized to diacetyl with ferric chloride and determined as such.

The work of Winkel and Proske, and Tachi was repeated by the authors, and, using their conditions, no waves of useable size could be produced. Several other electrolytes were tried, including lithium chloride, tetramethylammonium chloride, and sodium acetate-acetic acid. These were not successful.

Acetone reduced at the dropping mercury electrode with difficulty requiring the use of tetramethylammonium iodide as an electrolyte and voltages of the order of  $2.25$  volts (5). Lupton and Lynch (6) found that the hydrazones and phenylhydrazones of acetone and other ketones and aldehydes were much more easily reduced requiring voltages of the order of  $1.1$  volts for ketones.

The first series of experiments conducted used hydroxylamine as a part of the electrolyte solution with the intention of producing the oxime. A solvent made up of  $50\%$  water and  $50\%$  isopropyl alcohol was first used. The interest in such a solvent stemmed from the ease with which a clear serum could be prepared from a mixture of  $50\%$  orange concentrate ( $42^\circ$  brix) and  $50\%$  isopropyl alcohol. The electrolyte was  $0.1$  M hydroxylamine hydrochloride,  $0.37$  M sodium acetate, and hydrochloric acid to a pH of  $3.5$  in  $50\%$  water and  $50\%$  isopropyl alcohol. A wave was obtained with this solution at about  $1.0$  v., but the wave height was not proportional to concentration. The electrolyte was modified to  $0.1$  M sodium acetate,  $0.1$  M acetic acid, and

TABLE 1.

CONCENTRATION Moles		E <sub>1/2</sub> Volts	CURRENT Microamperes
0		NO WAVE	
0.001	Acetoin	-1.15	0.40
		-1.60	9.6
0.0018	Acetoin	-1.12	1.05
		-1.29	0.37
		-1.60	10.91
0.0025	Acetoin	-1.13	2.23
		-1.25	0.54
		-1.60	24.75
0.0008	Acetoin }	-1.14	0.40
0.001	Diacetyl }	-1.25	0.68
		-1.59	9.05
0.001	Diacetylmonooxime	-1.27	3.44
0.001	Diacetyldioxime	-1.15	2.32

Half wave potentials and currents for acetoin, acetoin and diacetyl, diacetylmonooxime, and diacetyldioxime (dimethylglyoxime) in  $0.1$  M sodium acetate,  $0.1$  M acetic acid (pH 5.5), and 20 times the concentration of acetoin hydroxylamine hydrochloride in  $50\%$  isopropyl alcohol, and  $50\%$  water.

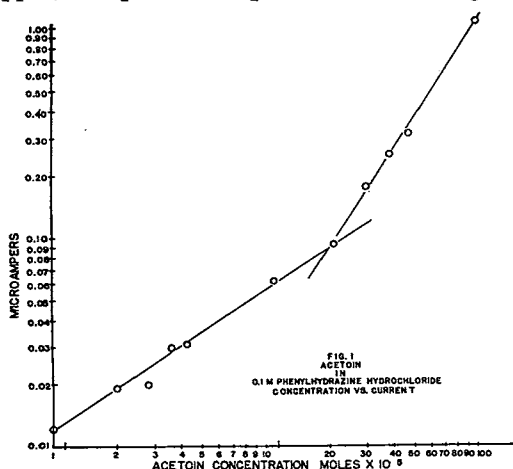
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a concentration of hydroxylamine hydrochloride equal to 20 times the concentration of acetoin. This solution had a pH of 5.5. Table 1 lists the concentrations used and the half wave potentials obtained. It was noted that there were two small waves and one large wave. The same electrolyte containing concentrations of 0.001 M diacetyl and 0.009 M acetoin yielded the same three waves. An attempt was made to identify the waves. A 0.001 M solution of diacetylmonooxime was prepared in the above electrolyte. This had a half wave potential of  $-1.27$  v. corresponding to the middle wave. A 0.001 M solution of diacetyldioxime (dimethylglyoxime) was prepared in the same electrolyte and showed a half wave potential of  $-1.15$  v. which corresponded to the first wave. The last and largest wave represented acetoinoxime. The acetoin solution used oxidized in part to diacetyl.

This electrolyte showed some promise, but as there were several waves close together, two of which were due to diacetyl, it was felt that other derivative forming reagents should be tried.

The next electrolyte investigated was 0.1 M phenylhydrazine hydrochloride. The curves were sharp and clean and produced waves of convenient height for accurate measurement. The concentrations studied ranged from 0.00001 M to 0.001 M (0.0000114 M = 1.0 ppm). A plot of the points obtained (Figure

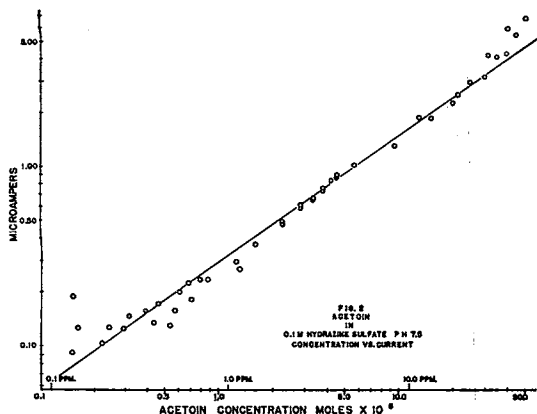


1) indicated two separate curves with a break at 0.0002 M. The solution became cloudy at this concentration, and it was felt that the

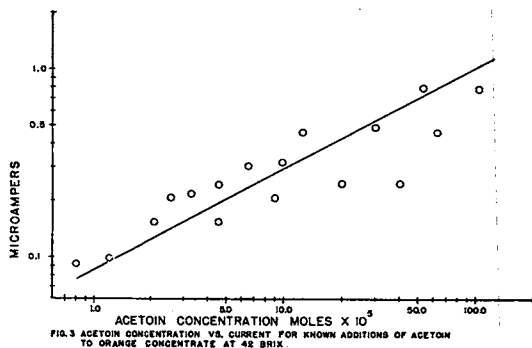
change in slope of the graph was due to the derivative becoming insoluble. There was also change in the half wave potentials found. This was probably due to a change in the pH of the solution, because HCl was released in the reaction and this solution was not buffered. It should be noted at this point that phenylhydrazine is the classical reagent for making solid derivatives of sugars. These derivatives were introduced and studied by Emil Fisher and were called osazones. When this electrolyte was tried with orange juice, solid osazones were formed causing voluminous precipitates, and the polarograms were of such complexity that they were useless. A solution containing 0.001 M acetoin and a concentration of glucose estimated to be equal to glucose in concentrate dissolved in the phenylhydrazine hydrochloride electrolyte caused complete obliteration of the acetoin wave. Similar solutions with the appropriate amount of citric acid or ascorbic acid substituted for the glucose did not interfere with the acetoin wave.

Acetoin was added to samples of orange concentrate to a concentration of 0.0000782 M and 0.000123 M. These samples were extracted with benzene and the benzene extract in turn extracted with 0.1 M phenylhydrazine hydrochloride. This effected a separation of the sugars from the benzene soluble materials. The recovery as indicated by the polarograms and the calibration curve was 0.000038 M for the first sample and 0.000060 M for the second. This represented a recovery of 48.7% in both cases. A sample of concentrate containing 0.0000049 M of diacetyl naturally present and determined by the normal distillation procedure, yielded an extract 0.000015 M in acetoin or 0.0000308 M acetoin in the concentrate.

The last reagent tried was hydrazine sulfate. This was tried at first as a 0.1 M solution of hydrazine sulfate in water. This was not successful, as the hydrogen ion discharges at about 1 v. obscuring the acetoin wave. The pH of the solution was adjusted to 7.5 with potassium hydroxide, and the wave appeared at about 1.2 v. At very low concentrations the wave tended to split into two waves. Due to this fact, the wave height was measured as the rise in the wave between 1.02 and 1.32 volts. This curve is plotted in Figure 2.



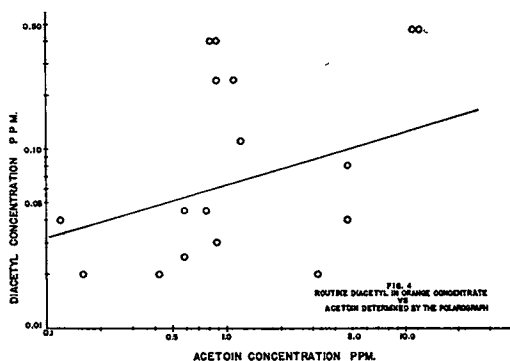
The use of 0.1 M hydrazine sulfate adjusted to a pH of over 7 appeared to be the best electrolyte for use in determining acetoin. With this fact established, a series of known concentrations of acetoin in orange concentrate was run. Extraction of the acetoin from the concentrate with benzene, followed by extraction of the acetoin from benzene with the electrolyte, was necessary to free the solution of unwanted sugars and other interfering materials. A plot of this information is shown in Figure 3.



Samples from the production line that were determined to contain diacetyl by quality control were checked by the above method to determine their acetoin content. The diacetyl content as found by quality control is compared to the acetoin content of the concentrate graphically in Figure 4.

#### Experimental:

The polarograms were run on a manual, indicating, Model III Sargent Polarograph using a Heyrovsky Erlenmeyer Form polarographic



electrolysis cell with a minimum capacity of 10 ml. The samples were purged with the best commercially available grade of nitrogen from high pressure cylinders. All half wave potentials were measured with the potentiometer on the instrument and were not referred to the standard calomel electrode. The best grade of reagents available was used throughout this work without further purification.

#### Preparation of samples:

One volume of orange concentrate (42° brix) was thoroughly mixed with one volume of reagent grade benzene. The mixture was centrifuged and the benzene decanted into one volume of 0.1 M hydrazine sulfate adjusted to a pH of 8.0 with potassium hydroxide. This was then intimately mixed and centrifuged to effect a separation. The aqueous layer was then placed in a prepared electrolysis cell and deaired for 15 minutes with oxygen-free nitrogen. The polarogram was then run.

This is obviously not a finished method of analysis. While purely exploratory in nature, the results indicate that the polarograph is capable of determining acetoin in orange concentrate.

#### LITERATURE REFERENCES

1. I. M. Kolthoff and James J. Lingane, *Polarography*, Vol. I and II Second edition (1952), Interscience Publishers, New York.
2. A. Winkel and G. Proske, *Ber.*, **69**, 1917 (1936).
3. I. Tachi, *Mem. Coll. Agr. Kyoto Imp. Univ.*, **42**, 1 (1938).
4. Pleticha, *Sbornik Mezinarod Polarog. Sjezdu Proze*, 1st Congr. (1951) P and III C. A., **47**, 11080f (1953).
5. M. B. Neiman and Z. V. Markina, *Zavodskaya Lab.*, **13** 1177 (1947); *C. A.*, **43**, 4183 (1949).
6. J. M. Lupton and C. C. Lynch, *J. Am. Chem. Soc.*, **66**, 697 (1944).