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A PROPOSED METHOD OF ANALYSIS FOR VITAMIN A ACETATE AND VITAMIN A PALMITATE BULK

BY

JULIAN ANTHONY VOLPE

A THESIS
SUBMITTED TO THE FACULTY OF THE
DEPARTMENT OF CHEMICAL ENGINEERING
OF
NEWARK COLLEGE OF ENGINEERING

IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

OF

MASTER OF SCIENCE
WITH A MAJOR
IN CHEMICAL ENGINEERING

NEWARK, NEW JERSEY

1955

ABSTRACT

An ethyl acetate solution of Vitamin A Acetate and Palmitate can be determined colorimetrically with the addition of concentrated hydrochloric acid in the proper ratio; one part of ethylacetate-Vitamin A solution to four parts of acid. A purplish-blue color is produced which reaches its maximum intensity within one minute and lasts for ten minutes. This color can be read in the Klett-Summerson colorimeter using glass filter #54. Like many colorimetric determinations, this method is valid within certain concentration limits of sample, 20-225 milligrams of Vitamin A per final 5 milliliter aliquot.

APPROVAL OF THESIS

FOR

DEPARTMENT OF CHEMICAL ENGINEERING

NEWARK COLLEGE OF ENGINEERING

BY

FACULTY COMMITTEE

APPROVED: ______________________

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NEWARK, NEW JERSEY

JUNE, 1955

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The conduct of this investigation was made possible by the use of Hoffmann-La Roche Pharmaceutical Company Nutley, New Jersey equipment and facilities. The cooperation and advise of Mr. Charles Pifer of Hoffmann La Roche is gratefully acknowledged. The success of this investigation was greatly aided by the counseling of Dr. C. W. Carlson.

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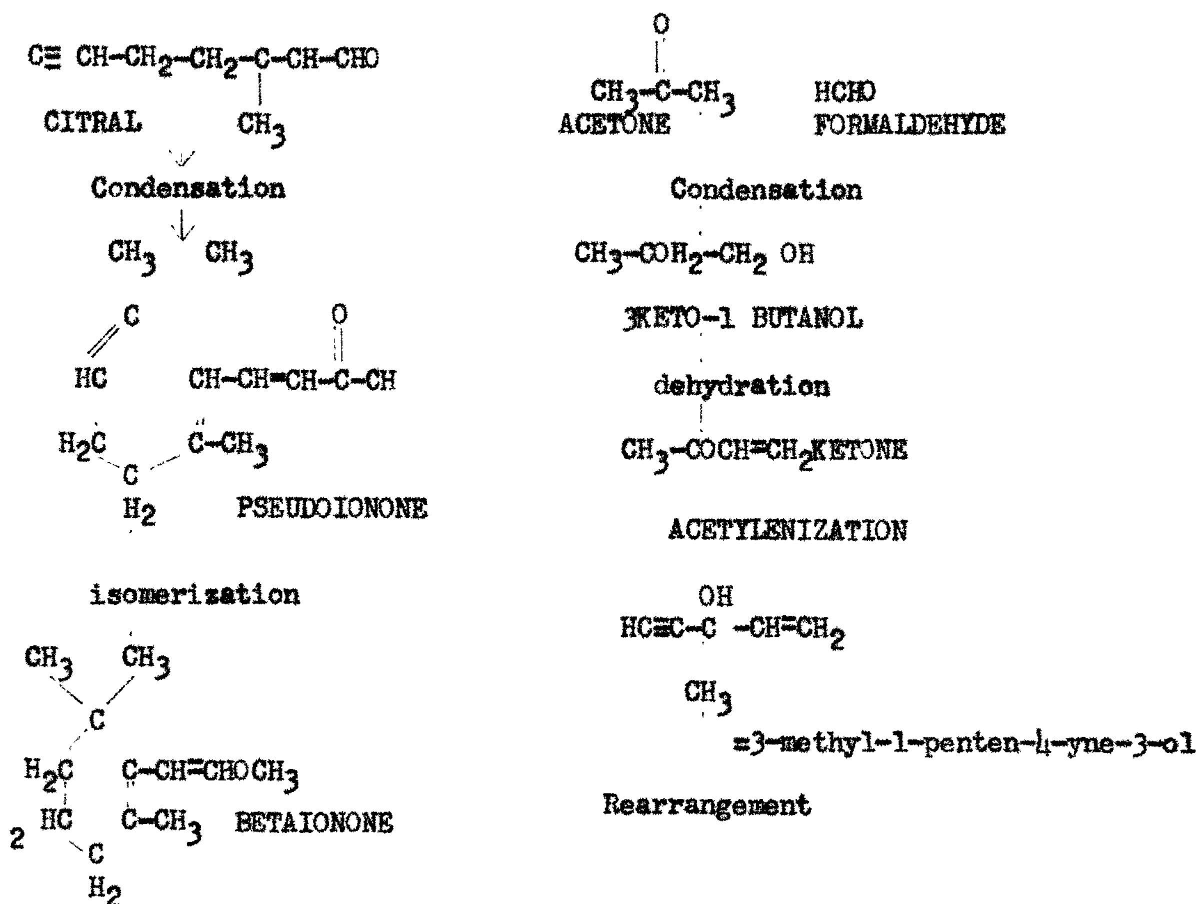
INTRODUCTION

BACKGROUND

Vitamin A, $C_{20}H_{29}OH$, is a polyene alcohol derived from the livers of fish, birds and mammals, and from the lining of the intestines of many fish.

In recent years, many pharmaceutical companies have attempted to synthesize Vitamin A to increase the supply and also to obtain a purer product. Hoffmann LaRoche, of Nutley, New Jersey, and Basle, Switzerland is now a leading producer of synthetic Vitamin A. This synthesis is best shown by the following step-by-step diagram.[1]



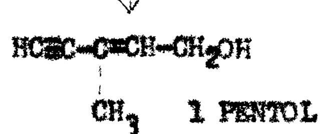
(1) Isler, Huber, Ronco, and Kofler: Helv.Chim. Acta 30, p1911-27 (1947)

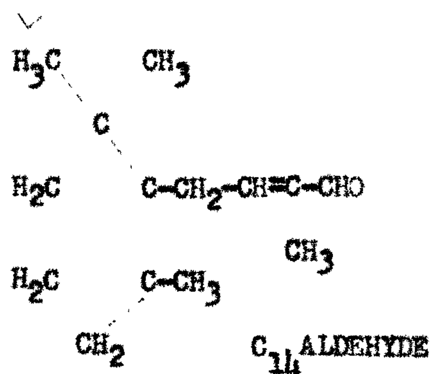
BETAIONONE

Glycidation

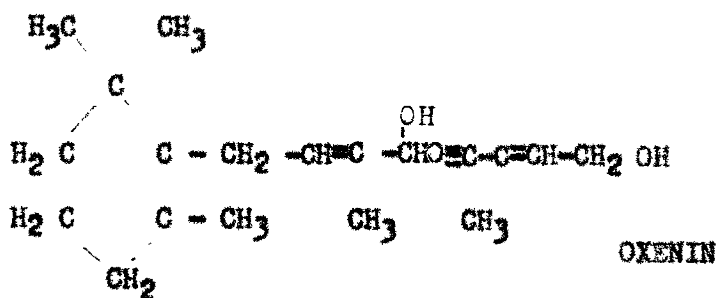
3 PENTOL

REARRANGEMENT

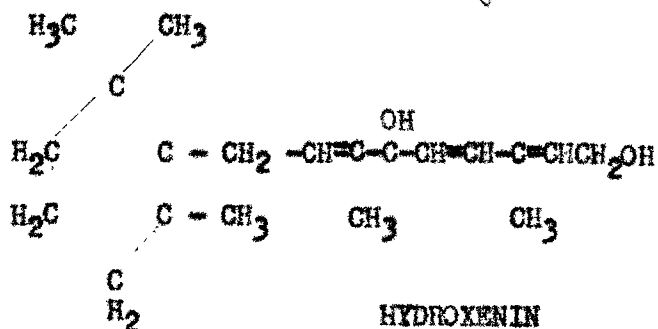




Grignard Reaction

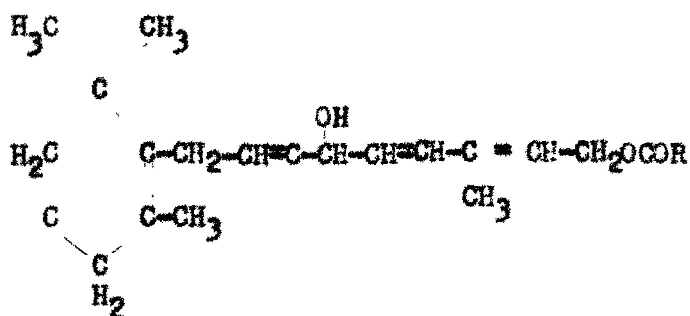


selective hydrogenation

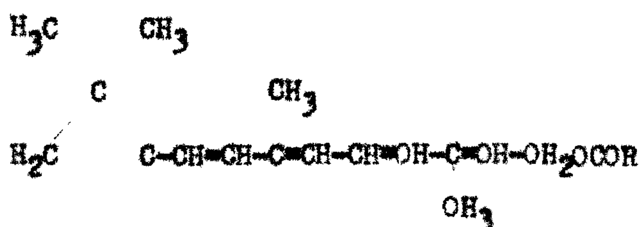


HYDOXENIN

ESTERIFICATION



dehydration and rearrangement



VITAMIN A ESTER

It is the function of the analytical control laboratory to analyze the final products of Vitamin A alcohol, palmitate or acetate and determine its potency. To date, there are three technical methods which serve this purpose, the Morton-Stubbs method, the Direct Spectrophotometric assay and the Carr-Price technique.

THE MORTON STUBBS METHOD (2)

A sample of Vitamin A ester is weighed on an analytical balance in a 10 milliliter amber volumetric flask. (The weight of sample varies according to the approximate potency, for 85% acetate a 50 - 60 milligram sample weight is used.) The sample is dissolved in the volumetric flask to the mark with isopropanol. A 1 milliliter aliquot is pipetted into a ruby 250 milliliter reflux flask, 30 milliliters of ethanol and 3 milliliters of 50% potassium hydroxide solution are added. The reflux flask is connected to a condenser and allowed to saponify for thirty minutes. (This procedure splits the Vitamin A ester and forms Vitamin A alcohol). After the alloted time, the flask is cooled with running water, then 30 milliliters of distilled water is added and the sample is transferred quantitatively into a 250 milliliter ruby Squibb extraction flask. The Vitamin A alcohol, water and salt solution is shaken with four 30 milliliters portions of ether, which has previously been redistilled to eliminate peroxides. These shakings are done by hand and require approximately 500 shakes per 30 milliliter portion of ether. After each extraction, the ether layer is separated from the water-alcohol layer. (The Vitamin A is more soluble in the ether and the byproducts of the saponification remain in the water-alcohol layer.) The ether extracts are combined and washed with three 50 milliliter portions of water, and then run quantitatively into a 250 milliliter

(2) Association of Vitamin Chemists., Methods of Vitamin Assay, p34, 1947

ruby erlenmeyer; placed on a bath of steam and evaporated to a 50 milliliter volume. Ten grams of anhydrous sodium sulfate are added and the solution is allowed to stand for 15 minutes. Water in the ether extract is removed by adsorption. The ether is then funnelled into a 100 milliliter ruby volumetric flask with ether and made to volume. A 25 milliliter aliquot of this solution is pipetted into a 50 milliliter volumetric flask and is evaporated on a steam bath until approximately 5 milliliters of ether remains. Then the remaining 5 milliliters of ether are evaporated under a stream of nitrogen to dryness. The residue which contains the vitamin A is then made to volume with isopropanol. A 2 milliliter aliquot is made to volume in a 10 milliliter volumetric flask with isopropanol and this solution is pipetted into a Beckmann spectrophotometric cell and read in the instrument, at wave lengths of 310 mu, 325 mu, and 334 mu. The 310 and 334 mu readings are points on the sloping sides of the curve, and the 325 mu reading is the peak of the curve. All flasks are flushed with nitrogen gas to avoid any possible oxidation of the Vitamin A. The percent of Vitamin A is calculated in the following manner.[3]

A = corrected reading

$$A_{corr.} = 7\ (A_{325mu.}) - 2.625\ (A_{310mu.}) - 4.375\ (A_{334mu.})$$

$$\text{USP Units/gram} = \frac{A_{corr.} \times 19000}{\text{path length of cell} \times \text{sample weight in grams.}}$$

$$\%\ \text{Vitamin A Acetate} = \frac{\text{USP units/gram} \times 100}{2.9 \times 10^6}$$

$$\%\ \text{Vitamin A Palmitate} = \frac{\text{USP units/gram}}{1.818 \times 1000000} \times 100$$

(3) Pharmacopoeia of the U.S., VITAMIN A ASSAY, 14th revision p.787, 1950

THE DIRECT SPECTROPHOTOMETRIC ASSAY FOR VITAMIN A ACETATE

A sample of Vitamin A acetate (amount determined by the approximate potency) is diluted in a 100 milliliter volumetric flask to the mark with isopropanol, subdiluted, 1 milliliter aliquot to 10 milliliters, and again subdiluted, a 1 milliliter aliquot to 10 milliliters. (All flask are ruby to prevent oxidation by sunlight.) A portion of this sample is pipetted into a cell and read in the Bechmann Spectrophotometer, over a range of wavelengths. Readings are obtained from 260 to 320 mu. by 5 mu increments, from 320 to 330 mu by 1 mu. increments, and 330 to 400 mu. by 5 mu. increments. The complete absorption curve is plotted with wave length on the ordinate and corrected extinction ratios on the abscessa. Percent Vitamin A Acetate is calculated in the following manner: (4)

$$E^{1\%}_{1cm.} = \frac{E_{max.(327mu)} \times 100}{\text{path length of cell} \times \text{sample weight in gms.}}$$

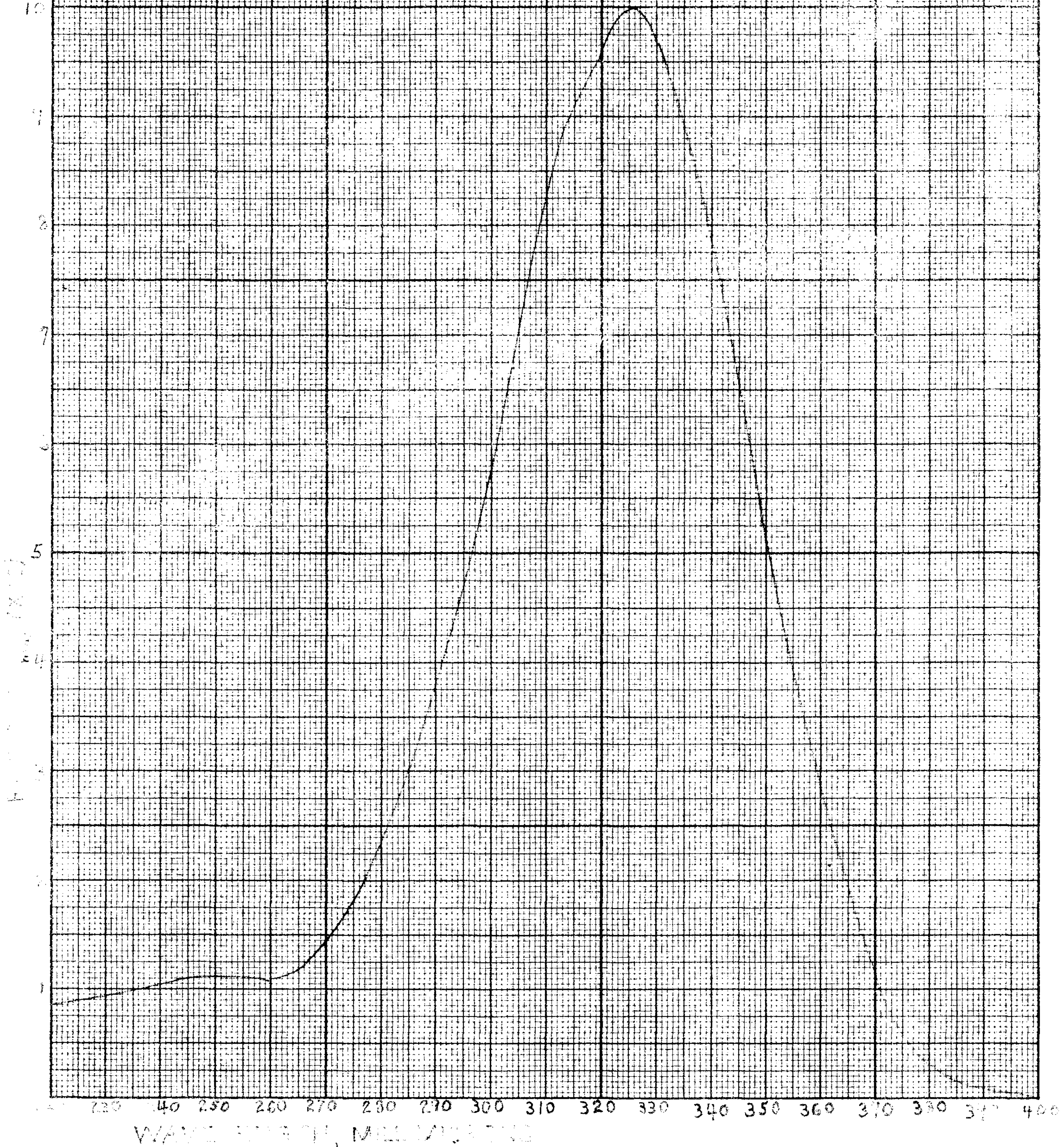
$$\text{USP units/gram} = E^{1\%}_{1cm} \times 19000$$

$$\%\ \text{Vitamin A Acetate} = \frac{\text{USP Units/gram} \times 100}{2.9 \times 10^{6}}$$

The curve is plotted over a mimeographed graph of 100% ideal Vitamin A acetate absorption curve, as indicated in figure #1.

These methods are based on the measurement of light absorption of the vitamin in the solution. The light absorption is equivalent to the concentration of the vitamin. It is most consistent at

(4) Association of Vit. Chemisis, Methods of Vitamin Assay, p24-34, 1947



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MILLIMETER
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that wavelength (324 - 330 mu.) which is maximally absorbed by the Vitamin and applies strictly only for monochromatic light.

THE CARR-PRICE METHOD

This colorimetric determination is similar to the Morton Stubbs method, except the ether residue containing the Vitamin A is dried with a stream of nitrogen and taken up with chloroform and 9 milliliters of Antimony trichloride reagent are added to an aliquot of the chloroform-Vitamin A solution. An unstable blue color is produced. This color must be read within 4 seconds in an Evelyn colorimeter and calculated thusly: (5)

$$L = 2 - \log G$$

then

$$\frac{L_{corr}}{L_{standard} - L_{corr.}} \times C_s$$

G = galvanometer reading

L = optical density

C_s = concentration of standard

This gives the USP units of Vitamin A per milliliter of unknow.
Then the Vitamin A content per gram of sample is calculated from:

$$\text{USP units/milliliter unknown} \times \frac{V}{W}$$

where V = final volume
W = sample weight.

These three methods of analyses, which are reviewed in this paper are reliable and accurate, but require a lengthy and necessary time element. It was the purpose of this paper to obtain a quick and

(5) Gyorgy, Paul: Vitamin Methods, Vol.1, p163, 1950 edition

accurate method ot obtain Vitamin A percentages and concentrations.

THEORY

The concentration of a colored material in solution may be readily determined by the amount of light that is absorbed. The fundamental equation for all colorimetric work involving the transmission of light is the Lambert - Beer's Law:

$$I = I_o e^{-kdc}$$

Where I_o = the intensity of incident light

I = the intensity of transmitted light

c = concentration

e = base of natural logarithims.

Transmittance = $\frac{I}{I_o}$. Transmittance is a relative measurement and is always less than 1.0 if light absorbing material is present. It may be expressed numerically either as a decimal fraction or in terms of percent, e.g., a transmittance of 0.6 or 60%, depending on whether the intensity of the blank is taken as 1.0 or 100%. Another way of expressing the transmittance of a solution is in terms of its negative logarithim (-log T), the value of - log. T is known as the optical density, D, or frequently as the extinction, E.

The obtaining of a suitable standard color is obviously a most important phase of a colorimetric procedure. It may be said that the most satisfactory standard color, and the one which should always be used for accurate results, is that obtained by treating a known concentration of the substance being determined by exactly the same

procedure that is used for the unknown, at the same time and under nearly identical conditions as possible. Thus the final colors in standard and unknown will be due to the same substance, differing if at all only in intensity. It is assumed in the use of a standard color that if the standard and unknown exactly match in color intensity they represent equal concentrations of the substance being determined. In actual practice this may or may not be true. The standard usually contains the substance being determined in relatively pure solution, in the unknown, extraneous factors may be present which may modify color intensity. Other substances than the one being determined may enter in to the color reaction, and results will therefore be too high; the analytical problem under these conditions is to devise either a more specific color reaction or to find methods to eliminate non-specific interfering substances.

In the preparation of this paper two types of colorimeters were used, the Evelyn photoelectric colorimeter (7) and the Klett-Summerson instrument. The Evelyn is a single photocell photoelectric filter photometer, with uniform test tubes customarily employed as solution containers, and with readings made on a sensitive galvanometer which is separated from the rest of the machine. The galvanometer scale is graduated linearly from 0 - 100, so that readings are in percent transmittance. With suitable filter in place, the reference liquid in a special test tube is placed in the instrument and the light intensity adjusted by resistance control until the meter

(7) Gibbs,Thos., Optical Methods of Chemical Analysis, p148, 1st Edition 1942

reads 100. The reference liquid is then replaced by the sample in a similar tube and the galvanometer reading noted. Its value gives the percent transmittance of the sample. To convert transmittance into optical density, the value of 2 - log G is obtained, where G is the galvanometer reading. The light source is a 6 volt bulb, operated from a storage battery to provide constancy of illumination, which is essential with all single cell photometers. The filters used with this instrument are particularly satisfactory from the point of narrowness of the spectral band. The test tubes require a minimum of about 6 milliliters of solution for a reading; a microcolorimeter, requiring much less fluid, is available for use with this instrument. The use of test tubes as solution containers or curvettes has the great advantage that many colorimetric procedures may be carried out partially or wholly in the same tube which will be used for the final reading.

The Klett - Summerson [8] instrument is a photometer with two photocells in a balanced circuit. In operation, with a suitable filter in place, the reference solution contained in a special tube is placed in the path of light striking one of the two photocells, which are arranged in a potentiometric circuit so that the current from one cell is opposed to that from the other through a null point instrument (low sensitivity galvanometer). With the photometer set at zero (corresponding to zero optical density), the current output from the second photocell is adjusted so that it exactly balances that coming from the photocell which is subject to the light emerging from the

(8) Gyorgy, Paul; Vitamin Methods, Vol. 1, 1950 Edition, p. 522

solution. This balance is indicated by a zero reading on the galvanometer. The reference solution is then removed and replaced by the solution under examination. Any light absorption by this solution will throw the two photocells out of balance; the electrical balance is then restored by turning the potentiometer dial until the galvanometer reads zero again. The reading on the potentiometer scale at this point is the measure of the light absorption of the solution. The light source is a 100 watt bulb operated directly from an ordinary power supply; the balanced circuit prevents fluctuations in light intensity from influencing readings. The instrument is designed for use with light filters of relatively narrow spectral transmission (10 millimicrons). The test tubes require about 5 milliliters of solution for a reading; micro tubes requiring about 2 milliliters may be used. The effective solution depth is approximately 1 centimeter so that spectrophotometric data based on this depth of solution are directly applicable to this instrument. The scale on the Summerson is somewhat unusual. It is graduated in units which are proportional to optical density; the actual numerical values represent the optical density divided by two and with the decimal point omitted. Thus a scale reading of 250 corresponds to an optical density of 0.500; of 100 to 0.200, and so on. In general the relation between scale readings and optical density, D, is as follows:

$$\frac{1000}{2} \times D = R$$

The fractional values of optical density have been replaced by whole numbers, to facilitate use in photometric calculations, since the scale readings bear a constant relation to optical density, they may be used directly in place of density values in the calculations of photometric analysis.

Light filters consist of selected glass which is capable of transmitting light over a limited portion of the spectrum only. Thus by placing such a filter in the light path of the photometer, measurements may be made in the spectral region corresponding to the transmittance range of the filter. Various filters differ principally with regard to (a) the spectral region of light transmittance, (b) the width of the spectral band which is transmitted. By suitable selection of various types or combinations of glass it is usually possible to obtain a filter whose transmittance is limited to almost any desired portion of the spectrum. They are sometimes called 'monochromatic' filters, but they are not monochromatic in the sense that light of single wavelength is monochromatic, since they transmit a narrow range of wavelengths rather than a single wavelength. It is customary to designate a filter in terms of the wavelength of peak transmittance, thus a filter called 'no. 540' or 'no.54' has its peak transmittance at a wavelength of 540 millimicrons.

METHOD OF ANALYSIS

The principle of this method is the colorimetric determination of Vitamin A by the formation of a purple color, when an ethyl acetate solution of Vitamin A is treated with a definite amount of concentrated hydrochloric acid. Like most colorimetric determinations, this method follows Beer's Law within certain definite concentration limits. The purple color which is formed lasts for a period of over 10 minutes and reaches its peak intensity within the first minute after the addition of the hydrochloric acid.

At the outset, a plot of concentration of Vitamin A versus Klett - Summerson readings must be made, using the Vitamin A capsules from the National Bureau of Standards. Each capsule contains 2500 units of Vitamin A in oil. With the particular ratios of Vitamin A in ethyl acetate - concentrated hydrochloric that the author selected, a straight line plot on regular graph paper is attained between 0-225 units of Vitamin A per 5 milliliter final aliquot. Table #1 gives the list of dilutions of the standard Vitamin A, the readings obtained and the units per final aliquot. This graph of a standard Vitamin A capsule in solution is used as a reference point in the final calculations, and it should be checked weekly to see that there are no deviations from the usual reading obtained at a particular known concentration.

Preparation of Standard; A gelatin capsule containing 2500 units of Vitamin A is split carefully with a razor blade, the oil

containing the Vitamin A is squeezed carefully onto a funnel leading into a 100 milliliter amber volumetric flask. The oil is washed into the volumetric with ethyl acetate. After carefully washing the outside of the gelatin capsule (to insure quantitative transfer), the capsule is then slit so that it is split in two portions. It is again washed with ethyl acetate while resting in the funnel, and the volumetric is brought to volume. The gelatin shell is then dropped into the ethyl acetate mixture and shaken thoroughly by hand. This is the starting solution for the standard. Each milliliter of the primary dilution of the standard contains 25 units of Vitamin A acetate. Subsequent dilutions were made from the starting solution so that each 5 milliliter aliquot of standard used contained amounts of Vitamin A in the following amounts of units Vitamin A per final 5 ml. 0, 50, 62.5, 125, 150, 200, 225, 250, 350. These 5 ml. aliquots were pipetted into a 25 milliliter flask (amber) and were made to the mark with concentrated hydrochloric acid. The flask was shaken and 10 milliliters pipetted into a Klett-Summerson tube and read in the colorimeter using filter #54, after the instrument was set to zero with a blank consisting of 5 milliliters of ethyl acetate and 20 milliliters of concentrated hydrochloric acid. The readings were recorded and a plot made of concentration versus colorimeter readings.

TABLE I

STANDARD VITAMIN A TABLE

Units Vitamin A per final 5 ml. aliquot	Standard Vitamin A Dilution	Reading
0	——	o
50	2500/100 x 20/50 x 5	21
62.5	2500/100 x 25/50 x 5	27.5
125	2500/100 x 5	54

Units Vitamin A per final 5 ml. aliquot	Standard Vitamin A Dilution	Reading
150	2500/25 x 3/10 x 5	65
200	2500/25 x 4/10 x 5	82
225	2500/25 x 4.5/10 x 5	85
250	2500/25 x 5/10 x 5	89
350	2500/25 x 7/10 x 5	108

Figure #3 is the graphical interpretation of this data, and during the course of this work, the data was checked about three times a week.

Preparation of sample: From experimental determinations, it was found that the starting weights of Vitamin A ester should be used:

TABLE II

WEIGHT OF ESTERS PER ESTIMATED POTENCY

Vitamin A Acetate

Estimated Potency	Weight of sample
75 - 100%	70 - 80 milligrams
50 - 75%	100 - 80
25 - 50%	225 - 100
0 - 25%	300 - 225

Vitamin A Palmitate

Estimated Potency	Weight of sample
75 - 100%	100 - 85 milligrams
50 - 75%	150 - 100
25 - 50%	300 - 150
0 - 25%	400 - 300

The estimated potency can be obtained from the production

chemist, as his tests include refractive indices, color, odor and crystallization techniques, which are accurate usually to within 10 percent. The sample is weighed on an analytical balance (accuracy of 4 decimal places), directly into a 100 milliliter amber volumetric flask. The Vitamin A ester is dissolved with ethyl acetate and made to volume. After thoroughly shaking the sample, to insure complete dissolving and uniform distribution, a 2 milliliter aliquot is withdrawn by pipette and emptied into another 100 milliliter amber volumetric flask. This aliquot is again brought to the mark with ethyl acetate and shaken. A 5 milliliter aliquot is withdrawn by pipette and emptied into a 25 milliliter amber volumetric flask, and made to volume with concentrated hydrochloric acid. Since the purple color developes in a matter of seconds, it is necessary to prepare the blank previous to the final dilution of the sample to be analyzed. In a 25 milliliter amber volumetric flask, 5 milliliters of ethyl acetate is pipetted and made to volume with concentrated hydrochloric acid. Equal amounts of blank and sample are pipetted into 2 Klett - Summerson tubes. The klett colorimeter is adjusted to zero with the blank and filter #54 used as the light wave tranmitter. Then the sample tube is inserted and the galvanometer brought to zero adjustment. The reading of the potentiameter scale is recorded.

Precautions: All flasks should be flushed with nitrogen gas before and after the insertion of the sample to avoid oxidation of the Vitamin A. All pipettes used should be uniform, ie, if blow-out pipettes are used they should be used throughout; or if calibrated

pipettes are used they should be allowed to drain with the tip resting against side of the flask.

Standard chart should be checked weekly.

Equipment needed: 100 milliliter amber volumetric flask with stoppers.

10, 25 milliliter amber volumetric flasks with stoppers.

2 inch outside diameter funnels

2,3,4,5,10,15,20,25 milliliter pipettes.

Reagents needed:

USP reference standard Vitamin A acetate capsules

Ethyl acetate, C.P.

Hydrochloric Acid, C.P.

Nitrogen gas cylinder

Instrument used to measure light absorption:

Klett-Summerson Photometer with special Klett tubes.

SAMPLE CALCULATION

With the readings obtained from the Klett-Summerson photometer and the original sample weight, the calculation becomes a simple straight-forward ratio setup.

$$\frac{\text{Reading of sample x units of Vitamin A in standard x 1000/mgm/gm)}}{\text{Reading of standard x weight in mgms. of sample in final aliquot}}$$

Since the reading of the sample and of the standard are dimensionless, we have:

$$\frac{\text{Units of Vitamin A x milligrams/gram}}{\text{milligrams}} \quad \text{or}$$

Units of Vitamin A/ gram

$$\text{Then } \frac{\text{Units of Vitamin A/gram x 100}}{2.9 \times 10^6} = \text{\% Vitamin A Acetate}$$

$$\text{And } \frac{\text{Units of Vitamin A/gram x 100}}{1.818 \times 10^6} = \text{\% Vitamin A Palmitate}$$

Taking a concrete example of Vitamin A Acetate NP 946-48

Weight of 100 milliliter flask and sample	36.2251
Weight of 100 milliliter flask	36.1482
Sample Weight	.0769 grams

Reading in Klett instrument

Time in seconds	Reading
30	81
60	84
90	84
120	84
150	84
180	84

The standard chart of Vitamin A standard versus Klett readings (Figure #3) is used as a reference point. Select any concentration of standard Vitamin A in units per 5 milliliter aliquot and ascend the graph to the point where the straight line plot cuts the selected concentration value. Then by moving horizontally to the left, the potentiometer reading to be expected at that particular concentration can be found. For example: a concentration of 125 units of Vitamin A standard should have a reading of 54. These values can be used in the calculations.

$$\frac{\text{Reading of sample (84) x units of Vit.A in standard (125) x 1000}}{\text{Reading of standard (54) x Weight in mgms. of sample in aliquot (.0769)}}$$

equals

2.53×10^6 units of Vitamin A per gram.

And $\frac{2.53 \times 10^6}{2.90 \times 10^6} \times 100 = 87.2\ \%$ Vitamin A Acetate.

TABLE III

RESULTS OF INVESTIGATION

Vitamin A Acetate

Plant Identification Number	Proposed Method units/gm.	%	Morton-Stubbs		Direct Spectrophotometric Assay	
NP 977-78	$2.53x10^6$	87.2	$2.64x10^6$	90.9%	$2.61x10^6$	90.1
NP 928-30	$2.60x10^6$	89.7	$2.56x10^6$	88.3	$2.57x10^6$	88.6
NP 979-80	$2.63x10^6$	90.6	$2.60x10^6$	89.7	$2.61x10^6$	90.1
NP 975-76	$2.45x10^6$	84.4	$2.47x10^6$	85.2	$2.47x10^6$	85.2
BN 57	$2.45x10^6$	84.4	$2.47x10^6$	85.2	$2.52x10^6$	86.9
BN 63	$2.77x10^6$	95.5	$2.81x10^6$	96.9	$2.70x10^6$	93.0
NP 973-74	$2.64x10^6$	91.0	$2.61x10^6$	90.1	$2.66x10^6$	91.6
NP 971-72	$2.52x10^6$	86.9	$2.57x10^6$	88.7	$2.55x10^6$	87.9
NP 969-70	$2.52x10^6$	86.9	$2.56x10^6$	88.3	$2.55x10^6$	87.9
NP 967-68	$2.56x10^6$	88.3	$2.56x10^6$	88.3	$2.60x10^6$	89.7
NP 964-66	$2.59x10^6$	89.3	$2.54x10^6$	87.8	$2.62x10^6$	90.3
NP 961-63	$2.62x10^6$	90.3	$2.53x10^6$	87.3	$2.55x10^6$	87.8
NP 958-60	$2.56x10^6$	88.3	$2.59x10^6$	89.4	$2.63x10^6$	90.6
NP 955-57	$2.66x10^6$	91.7	$2.68x10^6$	92.5	$2.63x10^6$	90.6
NP 952-54	$2.63x10^6$	90.6	$2.51x10^6$	86.6	$2.55x10^6$	87.9
NP 949-51	$2.61x10^6$	90.0	$2.55x10^6$	87.9	$2.55x10^6$	87.9
NP 946-48	$2.53x10^6$	87.2	$2.56x10^6$	88.2	$2.54x10^6$	87.7
NP 943-45	$2.51x10^6$	86.5	$2.63x10^6$	90.8	$2.60x10^6$	89.7
NP 1-2	$2.25x10^6$	77.6	$2.25x10^6$	77.6	$2.34x10^6$	80.7
N 197	$2.57x10^6$	88.6	$2.58x10^6$	89.0	$2.65x10^6$	91.5
B 55	$2.59x10^6$	89.3	$2.55x10^6$	88.0	$2.60x10^6$	89.6

TABLE III

RESULTS OF INVESTIGATION

Vitamin A Acetate

Plant Identification Number	Proposed Method units/gm.	%	Morton-Stubbs		Direct Spectrophotometric Assay	
NP 981-82	2.60×10^{6}	89.7	2.65×10^{6}	91.4	2.64×10^{6}	90.9
NP 985-86	2.57×10^{6}	88.6	2.58×10^{6}	89.1	2.60×10^{6}	89.7
NP 983-84	2.51×10^{6}	86.5	2.53×10^{6}	87.2	2.52×10^{6}	86.8
NP 987-88	2.60×10^{6}	89.7	2.62×10^{6}	90.3	2.59×10^{6}	89.2
NP 997-98	2.57×10^{6}	88.6	2.63×10^{6}	90.7	2.53×10^{6}	87.2
B 61	2.71×10^{6}	93.4	2.76×10^{6}	95.2	2.68×10^{6}	92.4
NP 919-21	2.54×10^{6}	87.6	2.59×10^{6}	89.2	2.58×10^{6}	89.1
NP 931-33	2.51×10^{6}	86.6	2.54×10^{6}	87.6	2.59×10^{6}	89.2
NP 925-27	2.59×10^{6}	89.2	2.62×10^{6}	90.3	2.59×10^{6}	89.2

Vitamin A Palmitate

Plant Identification Number	Proposed Method		Morton-Stubbs	
Distilled 223B	1.34×10^{6}	74.0	1.40×10^{6}	77.0
Dist. 248B	1.45×10^{6}	79.7	1.49×10^{6}	81.7
Dist. 290B	1.78×10^{6}	97.9	1.80×10^{6}	99.0
Dist. 291B	1.80×10^{6}	99.0	1.84×10^{6}	101.4
Dist. 324B	1.49×10^{6}	81.7	1.45×10^{6}	80.0
Dist. 321B	1.46×10^{6}	80.5	1.48×10^{6}	81.4
Dist. 320B	1.49×10^{6}	81.7	1.48×10^{6}	81.4
Dist. 316B	1.50×10^{6}	82.5	1.46×10^{6}	80.5

TABLE III

RESULTS OF INVESTIGATION

Vitamin A Palmitate

Plant Identification Number	Proposed Method units/gram	%	Morton-Stubbs	
Distilled 315B	$1.53x10^6$	84.4	$1.51x10^6$	83.1
Dist. 313B	$1.44x10^6$	79.0	$1.42x10^6$	78.2
Dist. 312B	$1.39x10^6$	76.5	$1.43x10^6$	78.5
Dist. 310B	$1.50x10^6$	82.5	$1.52x10^6$	83.5
Dist. 309B	$1.44x10^6$	79.0	$1.48x10^6$	81.4
Dist. 307B	$1.48x10^6$	81.4	$1.51x10^6$	83.1
Dist. 306B	$1.53x10^6$	83.9	$1.50x10^6$	82.5
Dist. 304B	$1.53x10^6$	83.9	$1.53x10^6$	83.9
Dist. 303B	$1.40x10^6$	77.0	$1.45x10^6$	79.6
Dist. 301B	$1.43x10^6$	78.5	$1.42x10^6$	78.1
Dist. 299B	$1.48x10^6$	81.4	$1.50x10^6$	82.5
Dist. 297B	$1.51x10^6$	83.1	$1.51x10^6$	83.1
Dist. 294B	$1.87x10^6$	102.8	$1.93x10^6$	106.5
Dist. 285B	$1.40x10^6$	77.0	$1.42x10^6$	78.0
Dist. 343C	$1.50x10^6$	82.5	$1.50x10^6$	82.5
Dist. 344B	$1.40x10^6$	77.0	$1.44x10^6$	79.0
Dist. 293B	$1.84x10^6$	101.4	$1.87x10^6$	103.0
Dist. 202C	$1.47x10^6$	81.0	$1.48x10^6$	81.4
Dist. 208B	$1.50x10^6$	82.5	$1.46x10^6$	80.5
Dist. 217B	$1.84x10^6$	101.4	$1.87x10^6$	103.0

TABLE III

RESULTS OF INVESTIGATION

Vitamin A Palmitate

Plant Identification Number	Proposed Method units/gram	%	Morton-Stubbs	
Distilled 212B	1.53×10^{6}	83.9	1.49×10^{6}	82.1
Dist. 216B	1.53×10^{6}	83.9	1.51×10^{6}	83.1
Low Potency Vitamin A Acetate				
LR 989-90	0.67×10^{6}	23.0	0.59×10^{6}	20.4
LR 991-92	0.75×10^{6}	25.8	0.65×10^{6}	22.4
LR 995-96	0.74×10^{6}	25.4	0.69×10^{6}	23.8
LR 904-06	0.68×10^{6}	23.4	0.60×10^{6}	20.5
LR 7-8	0.69×10^{6}	23.8	0.68×10^{6}	23.4
LR 3-4	0.73×10^{6}	25.0	0.65×10^{6}	22.5
LR 9-10	0.70×10^{6}	24.2	0.63×10^{6}	21.6

TABLE IV

PERCENT DEVIATION

Plant Identification Number	%Deviation of Proposed Method from Morton Stubbs	%Deviation of Proposed Method from Direct Spectrophotometric Assay	%Deviation of Morton-Stubbs from Direct Spectrophotometric Assay
NP 977-78	- 3.7%	- 2.9	- 0.8
NP 928-30	1.4	1.1	0.3
NP 979-80	0.9	0.5	0.4
BN 57	- 0.8	- 2.5	1.7
BN 63	- 1.4	2.5	- 3.9
NP 975-76	- 2.4	- 0.8	- 1.6
NP 973-74	0.9	- 0.6	1.5
NP 971-72	- 0.8	- 1.0	- 0.8
NP 969-70	- 1.4	- 1.0	- 0.4
NP 967-68	0.0	- 1.4	1.4
NP 964-66	1.5	- 1.0	2.5
NP 961-63	3.0	2.5	0.5
NP 958-60	- 1.1	- 2.3	1.2
NP 955-57	- 0.8	0.9	- 1.9
NP 952-54	4.0	2.7	1.3
NP 949-51	2.1	2.1	0.0
NP 946-48	- 1.0	- 0.5	-.0.5
NP 943-45	- 4.3	- 3.2	- 1.1
NP 1-2	0.0	- 3.1	3.1
M 197	- 1.4	- 1.9	1.5

TABLE IV

PERCENT DEVIATION

Plant Identification Number	%Deviation of Proposed Method from Morton Stubbs	%Deviation of Proposed Method from Direct Spectrophotometric Assay	%Deviation of Morton-Stubbs from Direct Spectrophotometric Assay
B 55	1.3	- 0.3	1.6
NP 981-82	- 1.7	- 0.8	- 0.5
NP 985-86	- 0.5	- 1.1	0.6
NP 983-84	- 0.7	- 0.3	- 0.4
NP 987-88	- 0.6	0.5	- 1.1
NP 997-98	- 2.1	- 0.6	- 3.5
B 61	- 1.8	1.0	+ 2.8
NP 919-21	- 1.6	- 1.5	- 0.1
NP 931-33	- 1.0	- 2.6	1.6
NP 925-27	- 1.1	0.0	- 1.1

Vitamin A Palmitate

Plant Identification Number	%Deviation of Proposed Method from Morton-Stubbs
Distilled 223B	- 3.0
Dist. 248B	- 2.0
Dist. 290B	- 1.1
Dist. 291B	- 2.4
Dist. 324B	1.7
Dist. 321B	- 0.9
Dist. 320B	0.3
Dist. 316B	2.0

TABLE IV

PERCENT DEVIATION

Vitamin A Palmitate

Plant Identification Number	% Deviation of Proposed Method from Morton-Stubbs.
Distilled 315B	1.3
Dist. 313B	1.2
Dist. 312B	- 2.0
Dist. 310B	- 1.0
Dist. 309B	- 2.4
Dist. 307B	- 1.7
Dist. 306B	1.4
Dist. 304B	0.0
Dist. 303B	- 2.6
Dist. 301B	0.4
Dist. 299B	- 1.1
Dist. 297B	0.0
Dist. 294B	- 3.7
Dist. 285B	- 1.0
Dist. 343C	0.0
Dist. 344B	- 2.0
Dist. 293B	- 1.6
Dist. 202C	- 0.4
Dist. 208B	- 2.0
Dist. 217B	- 1.6
Dist. 212B	1.8
Dist. 216B	0.8

TABLE IV

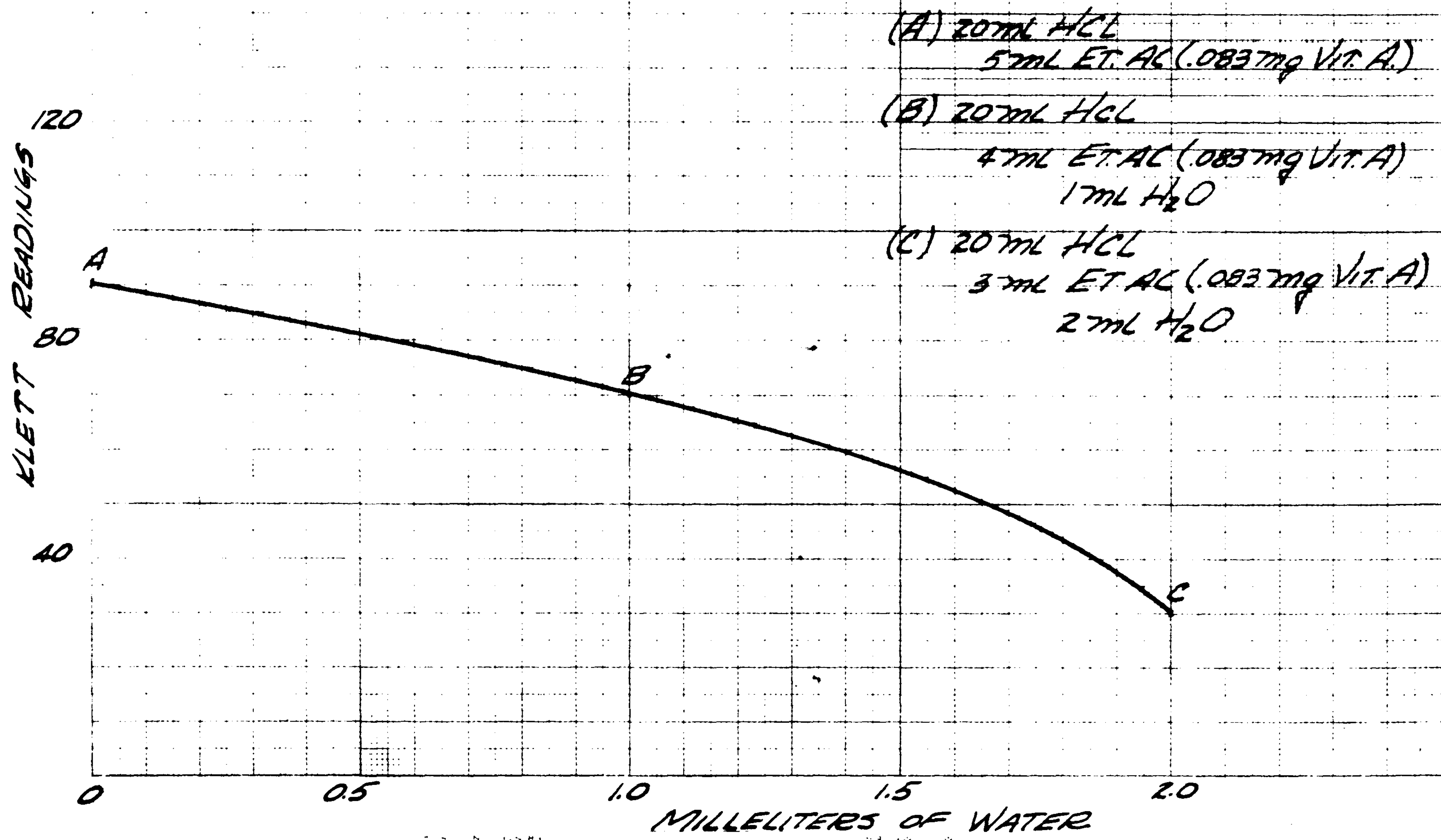
PERCENT DEVIATION

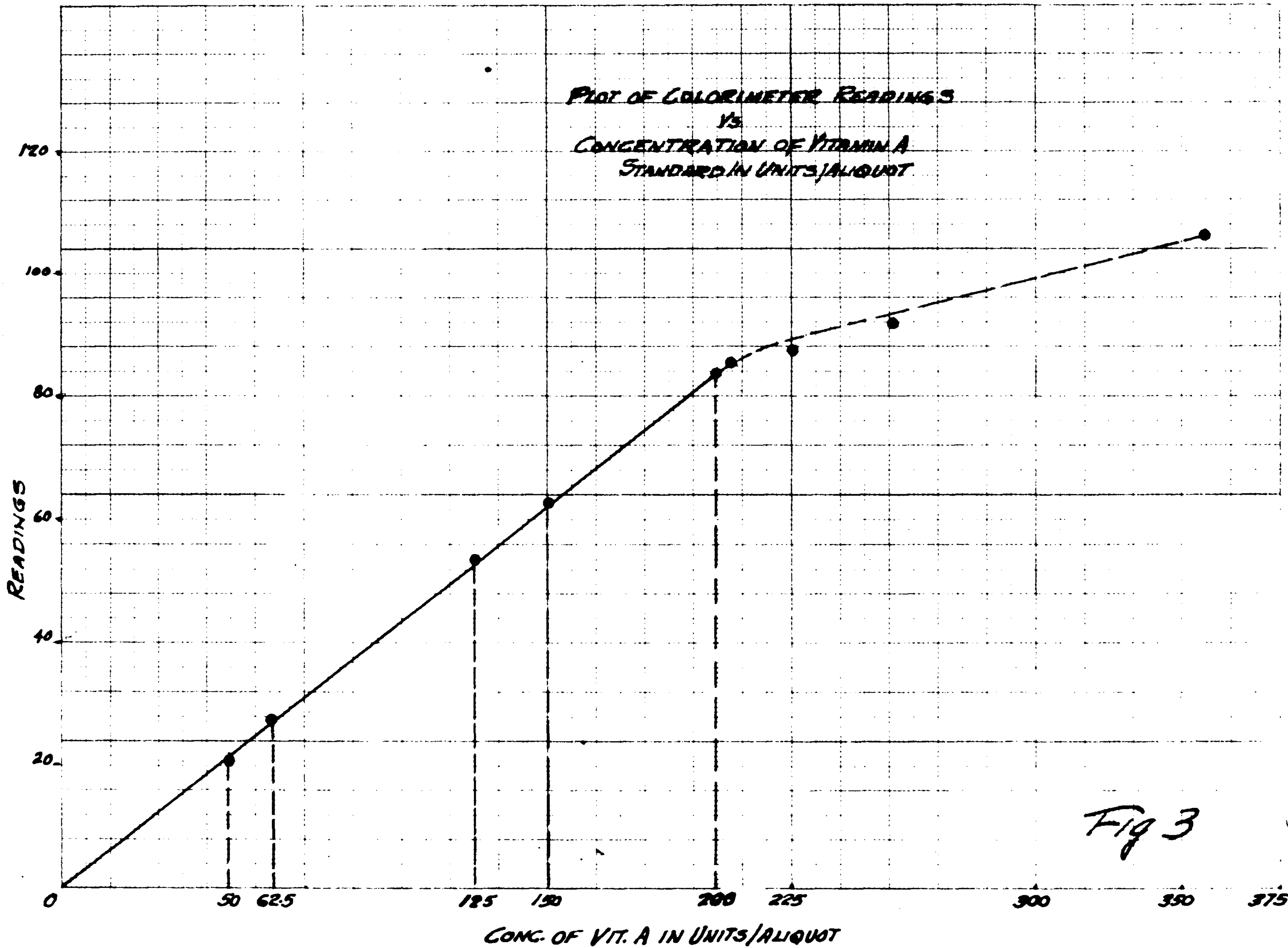
Low Potency Vitamin A Acetate

Plant Identification Number	%Deviation of Proposed Method from Morton-Stubbs.
LR 989-90	2.6
LR 991-92	3.4
LR 995-96	1.6
LR 904-06	2.9
LR 7-8	0.4
LR 3-4	2.5
LR 9-10	2.6

Fig 2

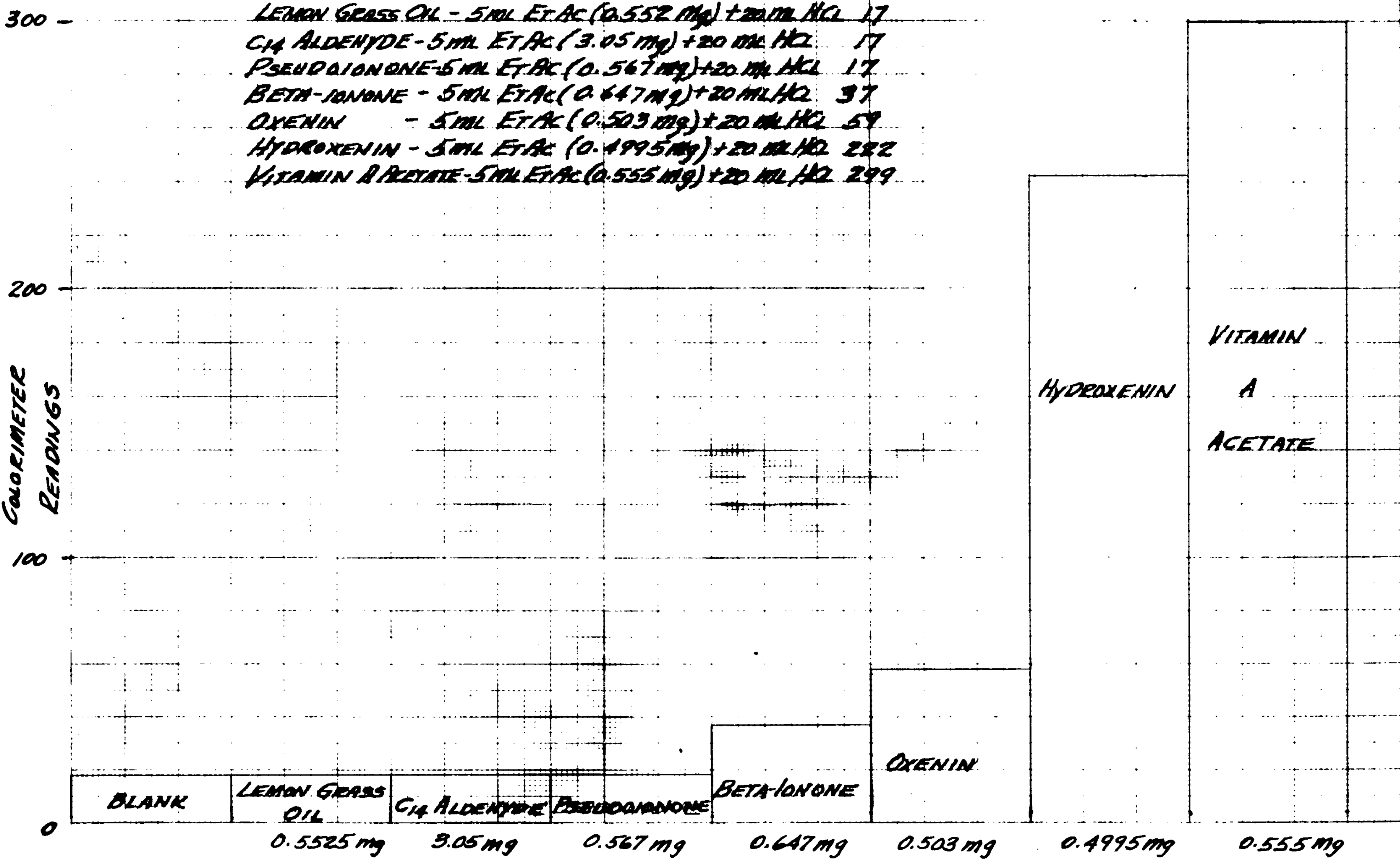
VITAMIN A ACETATE EFFECT OF WATER




PLOT OF COLORIMETER READINGS
VS
CONCENTRATION OF VITAMIN A
STANDARD IN UNITS/ALIQUOT
READINGS
120
100
80
60
40
20
0
50
62.5
125
150
200
225
300
350
375
CONC. OF VIT. A IN UNITS/ALIQUOT
Fig 3

INTERFERENCE BY SYNTHESIS PRODUCTS

Fig. 4



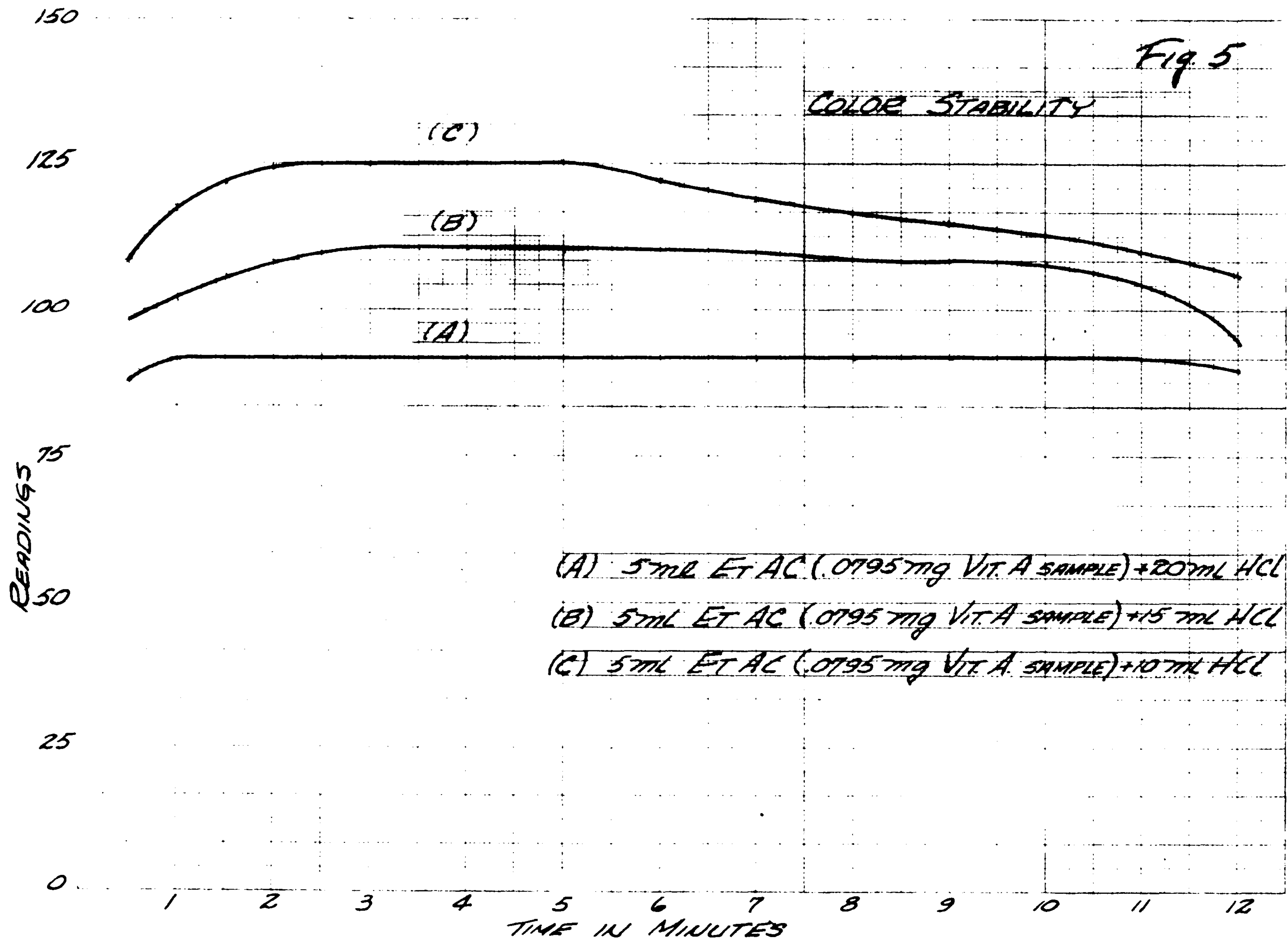


Fig. 5
COLOR STABILITY
(C)
(B)
(A)
150
125
100
75
50
25
0
READINGS
1
2
3
4
5
6
7
8
9
10
11
12
TIME IN MINUTES
(A) 5ml ET AC (.0795 mg VIT. A SAMPLE) +20 ml HCL
(B) 5ml ET AC (.0795 mg VIT. A SAMPLE) +15 ml HCL
(C) 5ml ET AC (.0795 mg VIT. A SAMPLE) +10 ml HCL

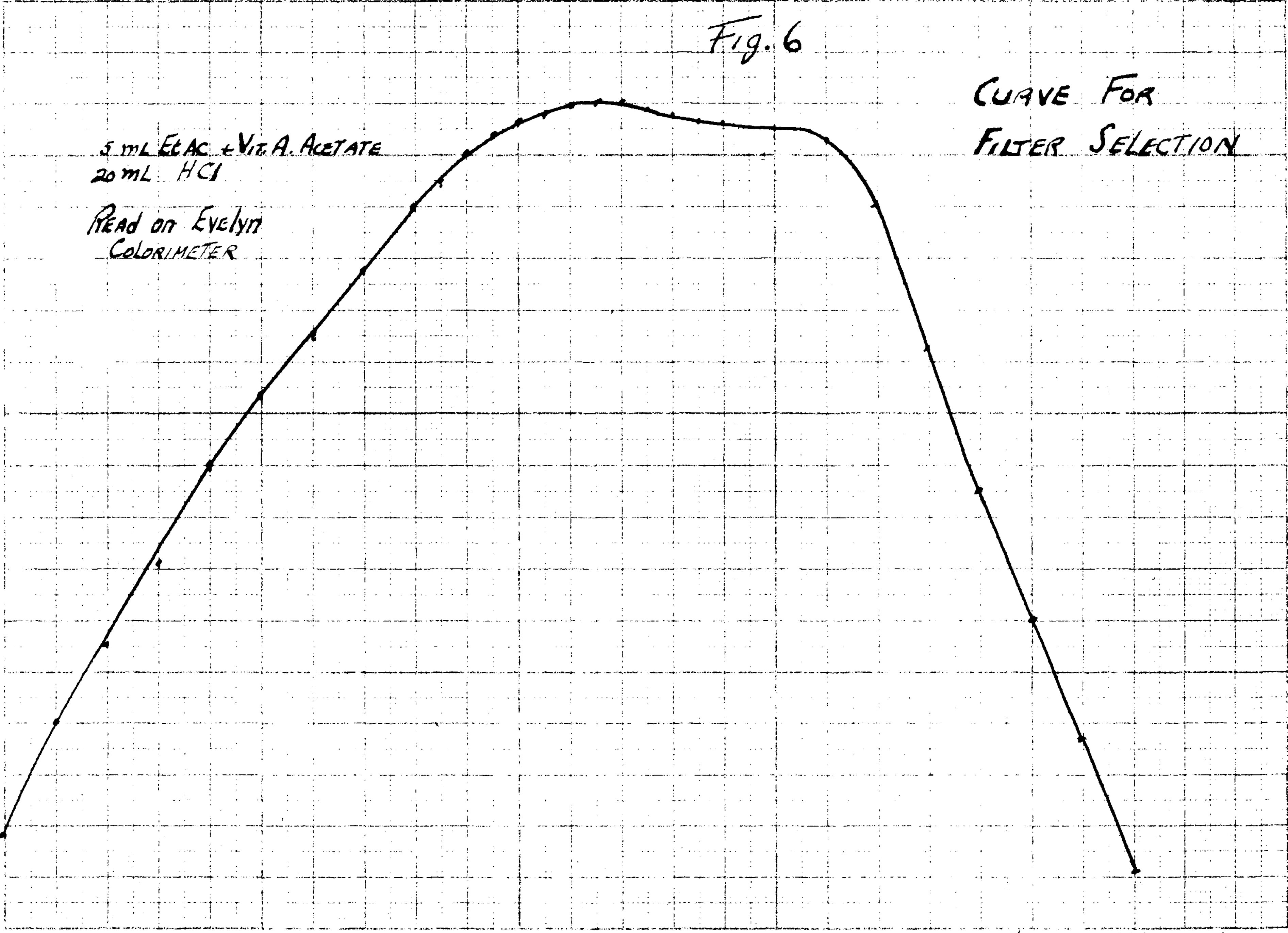


Fig. 6
CURVE FOR
FILTER SELECTION
5 mL EtAc + Vit A. Acetate
20 mL HCl
Read on Evelyn
Colorimeter
ABSORBANCY
0.00
0.25
0.50
0.75
420
440
460
480
500
520
540
560
580
600
620
640
660
WAVELENGTH
(mu)

High Potency Vitamin A Acetate

Range of Deviation of new method from Morton-Stubbs = -4.3% to 4.0%

Median Percentage Deviation = - 0.8%

Average Percentage Deviation = - 0.47%

Range of Deviation of New method from Direct Spectrophotometric Assay

- 3.2% to 2.7%

Median Percentage Deviation = - 0.8%

Average Percentage Deviation = - 0.52%

Range of Deviation of Direct Spectrophotometric Assay from Morton-Stubbs = - 3.9 to 3.1%

Median Percentage Deviation = - 0.05%

Average Percentage Deviation = - 0.03%

High Potency Vitamin A Palmitate

Range of Deviation of new method from Morton-Stubbs = - 3.7% to 2.0%

Median Percentage Deviation = - 0.95%

Average Percentage Deviation = - 0.7%

DISCUSSION

SELECTION OF PROPER FILTER

The most satisfactory filters are those which transmit as narrow a spectral region as possible, since this represents an approach to truly monochromatic light. A good filter for photometric purposes will show a transmittance of about 85 percent or more of the total light over a spectral width of 30 to 50 millimicrons; centered around the wave length of peak transmittance. In Figure 6, a wavelength of 540 millimicrons is shown to be best suited for the blue color developed by this method. A similar curve plotted for Klett-Summerson filters showed the same peak of almost 100 percent transmittance at a wavelength of 540 millimicrons.

INTERFERENCE BY SYNTHESIS PRODUCTS

A plot was made, in Figure 4, of milligrams of synthesis chemicals versus colorimeter readings. The Klett colorimeter was set to zero with ethyl acetate, then the blank consisting of 5 milliliters ethyl acetate and 20 milliliters of hydrochloric acid was inserted and read. Weighed amounts of the chemicals used in the steps of the synthesis were diluted, as per the Vitamin A Acetate, and read in the colorimeter. There is no interference from the lemon grass oil, C_{14} aldehyde, pseudoionone, and very slight interference from betaionone, moderate interference from the oxenin, and a very large amount of interference from hydroxenin. However to date there has never been any contamination of the final product

by oxenin or hydroxenin, as they are eliminated by washing and purifications.

EFFECT OF WATER

Figure 2 shows the effect of water other than that present in the concentrated hydrochloric acid. This was accomplished by keeping the amount of Vitamin A acetate constant (0.083 mgms.) in 5, 4, and 3 milliliter aliquots of ethyl acetate; and adding to the 5 milliliter Vitamin A-ethyl acetate aliquot, 20 milliliters of concentrated hydrochloric acid; to the 4ml. aliquot, 1 milliliter of water and 20 milliliters of the acid; to the 3 milliliter aliquot, 2 milliliters of water and 20 milliliters of acid. The Klett instrument was set to zero with a blank of 5 milliliters of ethyl acetate and 20 milliliters of hydrochloric acid. Then portions of the prepared samples were read. The effect of the water addition is one of color depression. The more the amount of water present the weaker is the color that is produced, and the color fades more rapidly. A similar curve was obtained using Vitamin A Palmitate.

COLOR STABILITY

Figure 5 shows the effect of keeping the amount of Vitamin A Acetate constant (0.0795mgms.) in a 5 milliliter aliquot of ethyl acetate and varying the amounts of hydrochloric acid added from 20 milliliters, to 15 milliliters, to 10 milliliters. With 20 milliliters of hydrochloric acid, the maximum color is reached in less than a minute and maintains its peak for a full 10 minutes. With 15

milliliters of hydrochloric added, the maximum color takes 2 minutes to develop, and lasts for only 6 minutes. With 10 milliliters of hydrochloric acid added, the maximum color develops within 2 minutes, but only lasts for 3 full minutes.

DISCUSSION OF RESULTS

The assays obtained by the proposed method were run in duplicate, and the results shown in Table III are the average. The results obtained by the Morton-Stubbs and Direct Spectrophotometric Assay were run singularly because of the author's long association with these two proven methods. The comparison of percentages between either of the established methods with the proposed assay is almost precisely the same, as seen on page 29. The range of percent deviation between either of the established methods and the proposed one is a range of approximately 8 percent, ie, the greatest negative difference was - 4 percent, and the greatest positive difference was 4 percent. However these large differences were only in isolated plant samples, more than 90 percent of the assays were only 2 percent higher or lower than the established methods and this is considered sufficiently accurate.

The accuracy of this proposed method can be kept within the 2 percent range by observing extreme quantitative care in weighings and making the dilutions to the proper volume. To eliminate any discrepancy due to mechanical difficulties, a voltage rectifier should be connected to the power outlet to correct any fluctuations

in current; also all colorimeter instruments used should be checked at least every three months for mechanical failure of tubes, and just cleaning.

The bluish-purple color developed is probably due to the formation of anhydro Vitamin A, but this is only an assumption. However in many other colorimetric determinations, the authors are at a loss to explain the appearance of their particular color.

This new method is definitely many times faster than the extractive procedures necessary for the Morton-Stubbs method, and since there are no extractions necessary there is less chance for quantitative errors.

APPENDIX

APPENDIX A

EXPERIMENTAL DATA FOR PROPOSED METHOD

APPENDIX A

TABLE V

EXPERIMENTAL DATA FOR PROPOSED METHOD

PLANT IDENTIFICATION NUMBER	SAMPLE WEIGHT IN MGMS.	READING ON KLETT	UNITS/GRAM OF VITAMIN A ACETATE
NP 977-78	74.8	82.0	2.53×10^{6}*
	77.2	84.5	2.53×10^{6}
NP 928-30	73.8	83.0	2.60×10^{6}
	75.0	84.0	2.59×10^{6}
NP 979-80	68.3	78.0	2.64×10^{6}
	73.1	82.5	2.61×10^{6}
NP 975-76	72.2	76.5	2.45×10^{6}
	73.5	77.5	2.44×10^{6}
BN 57	70.0	74.0	2.44×10^{6}
	75.1	80.0	2.46×10^{6}
BN 63	64.8	77.5	2.77×10^{6}
	69.1	83.0	2.78×10^{6}
NP 973-74	65.0	74.5	2.65×10^{6}
	68.3	77.5	2.62×10^{6}
NP 971-72	75.3	82.5	2.53×10^{6}
	73.7	80.0	2.51×10^{6}
NP 969-70	67.5	74.0	2.54×10^{6}
	69.4	75.0	2.50×10^{6}
NP 967-68	70.4	77.5	2.55×10^{6}
	73.2	81.5	2.58×10^{6}
NP 964-66	73.1	82.0	2.59×10^{6}
	74.8	83.5	2.58×10^{6}
NP 961-63	66.0	74.5	2.61×10^{6}
	67.3	76.5	2.63×10^{6}
NP 958-60	73.4	81.0	2.56×10^{6}
	74.1	82.0	2.56×10^{6}
NP 955-57	73.8	84.5	2.65×10^{6}
	70.2	81.0	2.67×10^{6}
NP 952-53	63.0	72.0	2.64×10^{6}
	65.2	74.0	2.63×10^{6}
NP 949-51	73.5	83.0	2.61×10^{6}
	74.3	83.5	2.60×10^{6}
NP 946-48	73.8	81.0	2.54×10^{6}
	73.3	80.0	2.52×10^{6}
NP 943-45	68.9	75.0	2.52×10^{6}
	70.2	76.0	2.50×10^{6}
NP 1-2	80.0	78.0	2.26×10^{6}
	78.3	76.0	2.25×10^{6}

* For calculation, see pages 19 and 20.

APPENDIX A

TABLE V

EXPERIMENTAL DATA FOR PROPOSED METHOD

Vitamin A Acetate

PLANT IDENTIFICATION NUMBER	SAMPLE WEIGHT IN MGMS.	READING ON KLETT	UNITS/GRAM
N 197	75.1	83.0	$2.56x10^6$
	75.6	84.0	$2.57x10^6$
B 55	77.2	86.0	$2.58x10^6$
	77.8	87.0	$2.59x10^6$
NP 981-82	76.5	86.5	$2.62x10^6$
	77.1	86.0	$2.58x10^6$
NP 985-86	70.0	77.5	$2.56x10^6$
	71.2	80.0	$2.59x10^6$
NP 983-84	69.6	75.0	$2.50x10^6$
	69.5	76.0	$2.52x10^6$
NP 987-88	76.2	86.0	$2.61x10^6$
	75.7	85.0	$2.60x10^6$
NP 997-98	66.7	75.0	$2.58x10^6$
	61.8	68.0	$2.56x10^6$
B 61	66.0	78.5	$2.76x10^6$
	64.4	77.5	$2.78x10^6$
NP 919-21	71.8	79.0	$2.55x10^6$
	71.3	78.0	$2.53x10^6$
NP 931-33	75.0	81.0	$2.50x10^6$
	73.5	80.5	$2.53x10^6$
NP 925-27	75.2	84.0	$2.59x10^6$
	71.2	80.0	$2.59x10^6$

Vitamin A Palmitate

Distilled 223B	147.0	84.0	$1.33x10^6$
	137.8	80.5	$1.35x10^6$
Dist. 248B	127.2	80.5	$1.46x10^6$
	122.2	76.0	$1.44x10^6$
Dist. 290B	91.1	70.0	$1.78x10^6$
	102.8	79.0	$1.78x10^6$
Dist. 291B	93.6	73.0	$1.80x10^6$
	87.6	68.0	$1.80x10^6$
Dist. 324B	118.5	77.0	$1.50x10^6$
	120.0	77.0	$1.48x10^6$
Dist. 321B	125.1	78.5	$1.45x10^6$
	129.0	82.0	$1.47x10^6$
Dist. 320B	112.5	72.0	$1.48x10^6$

APPENDIX A

TABLE V

EXPERIMENTAL DATA FOR PROPOSED METHOD

Vitamin A Palmitate

PLANT IDENTIFICATION NUMBER	SAMPLE WEIGHT IN MGMS.	READING ON KLETT	UNITS/GRAM
Distilled 316B	123.3	80.0	1.50×10^{6}
	130.5	84.0	1.50×10^{6}
Dist. 315B	133.0	87.0	1.51×10^{6}
	123.5	82.0	1.54×10^{6}
Dist. 313B	129.8	80.5	1.43×10^{6}
	127.3	80.5	1.46×10^{6}
Dist. 312B	105.7	64.0	1.40×10^{6}
	111.5	66.0	1.37×10^{6}
Dist. 310B	109.1	70.0	1.49×10^{6}
	122.6	80.0	1.51×10^{6}
Dist. 309B	113.5	71.0	1.45×10^{6}
	116.6	72.0	1.43×10^{6}
Dist. 307B	97.5	63.0	1.50×10^{6}
	101.2	64.0	1.46×10^{6}
Dist. 306B	100.5	66.0	1.52×10^{6}
	109.3	72.5	1.53×10^{6}
Dist. 304B	124.3	82.0	1.52×10^{6}
	122.8	82.0	1.54×10^{6}
Dist. 303B	107.2	65.0	1.40×10^{6}
	115.6	70.0	1.40×10^{6}
Dist. 301B	105.5	65.0	1.43×10^{6}
	127.9	79.5	1.44×10^{6}
Dist. 299B	98.2	63.5	1.50×10^{6}
	103.3	65.5	1.47×10^{6}
Dist. 297B	109.9	70.0	1.49×10^{6}
	124.2	82.0	1.52×10^{6}
Dist. 294B	88.0	71.5	1.88×10^{6}
	103.0	83.5	1.88×10^{6}
Dist. 285B	111.0	68.0	1.40×10^{6}
	123.0	75.0	1.41×10^{6}
Dist. 343B	115.0	75.0	1.51×10^{6}
	101.3	65.5	1.49×10^{6}
Dist. 344B	130.3	80.0	1.42×10^{6}
	140.5	84.5	1.39×10^{6}
Dist. 293B	88.0	70.0	1.84×10^{6}
	100.2	79.5	1.84×10^{6}
Dist. 202C	126.8	80.0	1.46×10^{6}
	130.3	84.0	1.49×10^{6}

APPENDIX A

TABLE V

EXPERIMENTAL DATA FOR PROPOSED METHOD

Vitamin A Palmitate

PLANT IDENTIFICATION NUMBER	SAMPLE WEIGHT IN MGMS.	READING ON KLETT	UNITS/GRAM
Distilled 208B	136.3	87.0	1.48×10^6
	134.9	88.0	1.51×10^6
Dist. 217B	91.5	73.0	1.85×10^6
	102.0	80.5	1.82×10^6
Dist. 212B	131.6	87.0	1.53×10^6
	127.9	85.0	1.53×10^6
Dist. 216B	126.6	83.5	1.53×10^6
	113.0	75.0	1.53×10^6

Low Potency Vitamin A Acetate

PLANT IDENTIFICATION NUMBER	SAMPLE WEIGHT IN MGMS.	READING ON KLETT	UNITS/GRAM
LR 989-90	301.0	87.0	0.66×10^6
	274.0	80.0	0.68×10^6
LR 991-92	251.0	81.0	0.73×10^6
	275.0	90.0	0.76×10^6
LR 995-96	263.0	85.0	0.74×10^6
	253.0	79.5	0.73×10^6
LR 904-06	245.0	74.0	0.70×10^6
	248.0	70.0	0.66×10^6
LR 7-8	254.0	76.0	0.69×10^6
	271.0	86.0	0.68×10^6
LR 3-4	254.0	81.0	0.73×10^6
	258.0	80.5	0.72×10^6
LR 9-10	230.0	70.0	0.70×10^6
	238.0	72.0	0.70×10^6

APPENDIX B

EXPERIMENTAL DATA FOR MORTON STUBBS AND DIRECT SPECTROPHOTOMETRIC ASSAYS

APPENDIX B

TABLE VI

EXPERIMENTAL DATA FOR MORTON-STUBBS AND SPECTROPHOTOMETRIC ASSAYS

Plant Identification Number	Weight of sample for Morton-Stubbs in mgms.	Readings obtained on Beckmann Spectrophotometer for 3 wavelengths.*		
Vitamin A Acetate		310 mu	325 mu	334 mu
NP 977-78	55.0	661	784	684
NP 928-30	55.2	655	777	680
NP 979-80	54.9	669	789	690
BN 57	54.2	601	712	618
BN 63	51.3	680	796	695
NP 975-76	54.6	627	739	641
NP 973-74	56.0	684	805	702
NP 971-72	55.0	621	737	637
NP 969-70	55.1	631	750	652
NP 967-68	51.4	589	701	610
NP 964-66	54.5	633	762	670
NP 961-63	54.6	637	752	655
NP 958-60	53.3	602	712	610
NP 955-57	55.0	653	778	674
NP 952-54	56.6	655	777	680
NP 949-51	53.9	613	732	637
NP 946-48	54.3	608	735	644
NP 943-45	55.4	651	777	676
NP 1-2	61.7	638	761	668
N 197	56.1	662	785	679
B 55	53.8	615	731	636
NP 981-82	55.7	660	789	690
NP 985-86	55.1	639	765	670
NP 983-84	54.6	617	740	647
NP 987-88	54.5	632	762	668
NP 997-98	55.1	630	751	650
B 61	53.8	646	771	662
NP 919-21	55.2	661	782	683
NP 931-33	55.0	641	758	661
NP 925-27	54.1	637	758	660

* See page 5 for method of calculation, using the above values at the three wavelengths.

APPENDIX B

TABLE VI

EXPERIMENTAL DATA FOR MORTON-STUBBS AND DIRECT SPECTROPHOTOMETRIC ASSAYS

PLANT IDENTIFICATION NUMBER	VITAMIN A ACETATE WEIGHT OF SAMPLE FOR DIRECT SPEC-TROMETRIC ASSAY MGMS.	READING OBTAINED ON BECKMANN SPECTRO-PHOTOMETER AT A WAVELENGTH OF 327 MILLIMICRONS *
NP 977-78	55.0	754
NP 928-30	55.2	747
NP 979-80	55.2	757
NP 975-76	54.6	711
BN 57	54.2	720
BN 63	51.3	730
NP 973-74	56.0	752
NP 971-72	55.0	742
NP 969-70	55.1	734
NP 967-68	51.4	705
NP 964-66	54.5	758
NP 961-63	54.6	729
NP 958-60	53.3	738
NP 955-57	55.0	773
NP 952-54	56.6	759
NP 949-51	53.9	725
NP 946-48	54.3	726
NP 943-45	55.4	756
NP -1-2	61.7	765
N 197	56.1	788
B 55	53.8	740
NP 981-82	55.7	768
NP 985-86	55.1	752
NP 983-84	54.6	728
NP 987-88	54.5	742
NP 997-98	55.1	729
B 61	53.8	764
NP 919-21	55.2	742
NP 931-33	55.0	744
NP 925-27	54.1	754

* See page 6 for method of calculation using the value at 327 mu.

APPENDIX B

TABLE VI

EXPERIMENTAL DATA FOR MORTON-STUBBS AND DIRECT SPECTROPHOTOMETRIC ASSAYS

PLANT IDENTIFICATION NUMBER	WEIGHT OF SAMPLE FOR MORTON-STUBBS IN MGMS.	READING OBTAINED ON BECKMANN SPECTROPHOTOMETER FOR 3 WAVELENGTHS.		
Vitamin A Palmitate		310 mu	325 mu	334 mu
Distilled 223B	74.3	637	751	652
Dist. 248B	79.0	525	620	637
Dist. 290B	74.7	591	700	604
Dist. 291B	79.3	642	761	658
Dist. 324B	77.4	548	650	577
Dist. 321B	76.2	528	629	551
Dist. 320B	76.9	532	631	554
Dist. 316B	77.3	528	625	544
Dist. 315B	77.6	504	605	524
Dist. 313B	75.6	435	528	460
Dist. 312B	76.5	495	590	516
Dist. 310B	76.7	508	608	529
Dist. 309B	79.4	527	622	538
Dist. 307B	77.1	528	629	548
Dist. 306B	77.5	539	644	568
Dist. 304B	77.8	546	654	575
Dist. 303B	76.8	547	650	577
Dist. 301B	77.3	535	629	557
Dist. 299B	77.4	510	609	529
Dist. 297B	77.3	509	609	529
Dist. 294B	74.5	643	757	652
Dist. 285B	76.1	509	601	527
Dist. 343C	76.0	500	600	523
Dist. 344B	76.2	501	599	526
Dist. 293B	74.3	637	751	652
Dist. 202C	76.3	533	633	558
Dist. 208B	77.5	528	625	548
Dist. 217B	77.6	667	784	679
Dist. 212B	77.8	529	628	548
Dist. 216B	77.0	533	635	546

APPENDIX B

TABLE VI

EXPERIMENTAL DATA FOR MORTON-STUBBS AND DIRECT SPECTROPHOTOMETRIC ASSAYS

PLANT IDENTIFICATION NUMBER	WEIGHT OF SAMPLE FOR MORTON-STUBBS IN MGMS.	READING OBTAINED ON BECKMANN SPECTROPHOTOMETER FOR 3 WAVELENGTHS.		
Low Potency Vitamin A Acetate		310 mu	325 mu	334 mu
LR 989-90	182.0	740	790	685
LR 991-92	180.7	762	818	711
LR 995-96	176.7	786	834	717
LR 904-06	180.1	743	802	708
LR 7-8	172.0	743	785	670
LR 3-4	163.5	680	728	630
LR 9-10	172.9	692	740	639

APPENDIX C

REFERENCES

APPENDIX C

REFERENCES

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