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COLORIMETRIC MICRODETERMINATIONS OF DIPHENYL IN CITRUS

by

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A micromethod for the determination of diphenyl based on a color reaction, which diphenyl gives together with sulfuric acid and traces of formaldehyde and ferric iron, was described in an earlier paper (6). The purpose of this work is the application of that method to the determination of diphenyl in citrus. The specificity and sensitivity of the reaction make possible the detection of even the smallest quantities of diphenyl in citrus while spectrophotometric methods are limited by an apparent and variable amount of diphenyl due to various impurities.

Materials: Electric blender

Shaking machine

Colorimeter absorptiometer: A Higler absorptiometer type H8 10, with 6 inch x 5/8 inch tubes was used.

Distilling apparatus with ground glass joints consisting of a) flasks with round bottom, short neck, 1 liter, 2 liters; b) condensers - Liebig, length of jacket 25 cm, 10 cm tips; c) 2 cm diameter joined tubes.

Beakers: 250 ml.

Separatory funnels - ground-glass stoppers: 75, 100 and 200 ml.

Volumetric flasks with ground-glass stoppers: 50 ml.

Graduated cylinders with ground-glass stoppers: 50 ml.

Erlenmeyers: 100 ml

Graduated tubes: ten ml tubes graduated at 0.1 intervals with ground-glass stoppers with apparatus for concentration.

Test tubes - 150 mm x 16 mm.

Pipettes-volumetric: 1,5 and 10 ml.

Micropipettes: curved, 0.1 and 0.2 ml pipettes graduated at 0.001 ml intervals.

Filter paper: Wattman No. 42.

Reagents: All reagents should be analytically pure.

Chloroform.

Sulfuric acid: concentrated.

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Sulfuric acid: diluted, made up of 95 volumes concentrated sulfuric acid and 5 volumes distilled water.

Sodium hydroxide: 5% solution.

Sodium metabisulphite: 1% solution.

Bone charcoal powder: check that it does not absorb diphenyl.

Acetic acid reagent: prepared as indicated previously (6).

Sulfuric acid reagent prepared as indicated previously (6).

Determination:

Principle of the method: Diphenyl is extracted from citrus by steam distillation. The distillate is extracted with chloroform; the chloroform extract is purified successively by a sodium metabisulphite solution, sodium hydroxide solution, concentrated sulfuric acid and diluted sulfuric acid. The diphenyl is then determined in the extract colorimetrically.

Sampling procedure: Diphenyl is determined separately in fruit peel and pulp.

Take 70 g of peel and, at the most, 600 g of pulp. For analysis of an average fruit sample, weigh 10 fruits and remove their peel carefully to avoid contaminating the pulp by traces of diphenyl from the peel; from each fruit an average of 7 g of the peel is put aside, making altogether exactly 70 g of peel from 10 fruits. If the pulps weigh more than 600 g an aliquot corresponding to $\frac{1}{2}$ or $\frac{1}{3}$ of the total weight of the pulp may be used, by taking from each pulp the predetermined portion and adjusting the weight of all the fruit pulps.

Sample preparation: Peels: Prepare 500 ml of water in a graduated cylinder. Place the finely chopped peels in an electric blender, add 200 ml of the water from the cylinder and blend the peels to a perfect puree, essential for the complete extraction of diphenyl. Transfer the puree quantitatively into a one liter flask; rinse the blender with part of the water and add the rinse water and the remainder of the original 500 ml to the one liter flask.

Pulp: Prepare 200 ml of water. Place the chopped pulp in an electric blender and mash. Transfer quantitatively into a two liter distillation flask, rinse the blender with part of the water and add the rinse water and the remaining water to the flask.

Distillation: Set up the distillation apparatus (Fig. 1'). Plunge the end of the condenser into the beaker containing 20 ml water, heat the flask on a gas burner and distil. To keep the distillate clear, boil the pulp gently and avoid foam. Collect the first 100 ml of distillate. Stop distillation. Rinse the inner tube and the tip of the condenser carefully with the 10 ml chloroform which is collected in the beaker containing the distillate.

Extraction: Transfer the contents of the beaker to a 200 ml separatory funnel. Rinse the beaker with 5 ml chloroform and add to the funnel. Extract the distillate by shaking for 2-3 min. Decant. Extract 3 more times with 10 ml portions of chloroform. Collect the chloroform. Collect the chloroform layers in a 50 ml volumetric cylinder to complete the volume to 50 with chloroform (these extracts may be kept for some time, preferably in a dark place).

Purification of extracts: The pulp extracts are used as they are; the peel extracts must be diluted. Put 10 ml of peel extract (5 ml in case of lemons) into a 50 ml volumetric flask and complete the volume to 50 with chloroform. Place about 35 ml of the extract to be purified into a 100 ml separatory funnel. Wash the extract successively with 5 ml of 1% sodium metabisulphite solution, 2 ml of 5% sodium hydroxide and 3 times with 5 ml of distilled water. Extract the chloroform layer 3 times with 5 cc concentrated sulfuric acid, shaking each time for 2-3 min. Decant the acid layer, and the traces of acid which adhere to the sides of the separatory funnel, very carefully. Then extract with 10 ml sulfuric acid diluted with 5% water, shaking for 5-10 minutes. Decant the acid layer and repeat the operation several times, until the sulfuric acid remains perfectly colorless (compare the color with pure sulfuric acid). Extract the chloroform layer another 2-3 times, with 5 ml portions of concentrated sulfuric acid. The acid should remain colorless. Decant carefully. Transfer the purified extract into a 100 ml Erlenmeyer flask. Add bone charcoal power, mix and filter through Wattman No. 42 filter paper. Collect the filtrate into a 50 ml graduated cylinder and close carefully. (If the extracts must be kept, record the exact volume, usually about 27 ml, to allow correcting for eventual evaporation of the chloroform).

Concentration of extract for determination: Even minute amounts of diphenyl, of the order of $25 \mu\text{g/ml}$ of extract, may be accurately determined (Fig. 2). The extracts obtained as indicated above usually contain much lower amounts than $25 \mu\text{g}$ of diphenyl/ml and must be concentrated. Generally, 20 ml of pulp extracts must be concentrated to 0.5 ml and 20 ml of peel extract to 0.5-1.0 ml. Concentrate the extract using the 10 ml graduated tube and the apparatus for concentration (Fig. 3). Close the tube with the apparatus slowly, introduce a maximum of 5 ml of extract* measured with a pipette, through the funnel; rinse the funnel with a few drops of chloroform and close. Place the tube in a beaker containing a 2-3 cm thick layer of water kept between 50 and 60°C . Join the apparatus to a water vacuum pump, and carefully reduce the volume of extract to 10-12 ml, under low depression. Close the connecting faucet, open the funnel and add a maximum of 5 ml extract. Continue as above, until all the required volume has been concentrated to about one ml. Remove the tube from the water and reduce the volume to exactly 1 ml or 0.5 ml, according to need (avoid evaporating too much chloroform as this provokes volatilization of diphenyl). Detach the apparatus from the water vacuum pump and allow to cool. Open the tube slightly, rinse the inside and the capillary tube of the concentration apparatus with 2-3 ml of acetic acid reagent which falls into the graduated tube. Remove the apparatus, complete the contents of the tube, with acetic acid reagent, to 10 or 5 ml, depending on whether the final volume of the chloroform extract was 1.0 or 0.5 ml. Close the tube, mix and determine diphenyl.

Determination of Diphenyl: Before making the determination one should check, as indicated previously (6), whether the optical density does not increase with increasing amounts of acetic acid reagent. If it does increase, dilute the mixture as necessary.

Principle of Determination (see (6) for details): Place 0.05, 0.10 and 0.15 ml of mixture (or 0.10, 0.20 and 0.30 ml if the colors are very weak), respectively, into 3 test tubes; add 5 ml of sulfuric acid reagent as indicated in (6) and determine the optical densities using the orange filter $610 \mu\text{m}$. The colors obtained must be pure blue and the optical densities must rise proportionally to the amount of mixture taken for the determination (a violet-like color or a lack of proportionality may be due to a lack of acetic acid reagent).

Compute diphenyl in ppm of peel or pulp.

* Note: Up to 0.5 ml acetic acid reagent may be added to avoid the eventual loss of diphenyl caused by too great an involuntary evaporation of chloroform. This addition must be taken into consideration when obtaining the final volume of concentrated extract.

Example of computation: Given that:

n - μ g of diphenyl found in 0.10 ml of acetic mixture

v - volume of acetic mixture in ml

a - ml of extract taken for concentration.

b - dilution rate of the original extract (5 or 10 times)

p - weight in g of peel or pulp taken for the determination.

$$\text{ppm of diphenyl in the pulp} = \frac{n \times 10 \times v \times 50}{a \times p}$$

$$\text{ppm of diphenyl in the peel} = \frac{n \times 10 \times v \times 50 \times b}{a \times p}$$

Compute rate of diphenyl in the entire fruit from these values.

Sensitivity of the method: Under these described conditions the reaction conforms to the Beer-Lambert law for small quantities of diphenyl. The optical densities corresponding to 0.25 μ g of diphenyl/0.1 ml of acetic acid mixture can be measured quite accurately depending upon the sensitivity of the absorptiometer (Fig. 2).

Admitting that, in the case of the pulp:

$$n = 0.25 \mu\text{g}$$

$$v = 5 \text{ ml}$$

$$a = 20 \text{ ml}$$

$$p = 500 \text{ g}$$

$$\text{ppm of diphenyl} = \frac{0.25 \times 10 \times 5 \times 50}{20 \times 500} = 0.062$$

In the case of lemon peel:

$$n = 0.25 \mu\text{g}$$

$$v = 5 \text{ ml}$$

$$p = 70 \text{ g}$$

$$a = 20 \text{ ml}$$

$$b = 10$$

$$\text{ppm of diphenyl} = \frac{0.25 \times 10 \times 5 \times 50 \times 10}{20 \times 70} = 4.46$$

For orange peels, b = 5 and ppm of diphenyl = 2.23

As the peel represents about 33% of the fruit the sensitivity of the method will be 0.75 — 1.5 ppm of diphenyl for the entire fruit.

Accuracy of method: Diphenyl was determined a) in essential oils, peel and pulp of untreated citrus, to which known amounts of diphenyl had been added (Table 1); b) in several samples originating from the same mixture of peels of fruit treated with diphenyl (Table 2) and c) in several average samples of peels successively removed from the same lot of ten diphenyl treated fruits (Table 3).

Table 1

Determination of diphenyl in products containing known amounts of diphenyl

| Product analyzed | Diphenyl added mg | Diphenyl found mg | Diff. mg | Error % |
|----------------------|----------------------|----------------------|-------------|------------|
| Orange essential oil | 0.00 | 0.00 | 0.00 | 0.0 |
| Orange essential oil | 4.00 | 3.80 | -0.20 | -5.0 |
| Orange essential oil | 17.90 | 17.20 | -0.70 | -4.0 |
| Orange essential oil | 20.40 | 20.00 | -0.40 | -1.9 |
| Orange essential oil | 55.70 | 55.00 | -0.70 | -1.2 |
| Orange peel | 0.00 | 0.00 | 0.00 | 0.0 |
| Orange peel | 5.90 | 5.75 | -0.15 | -2.5 |
| Orange peel | 50.00 | 50.23 | +0.23 | +0.46 |
| Lemon peel | 0.00 | 0.00 | 0.00 | 0.0 |
| Lemon peel | 5.00 | 4.85 | -0.15 | -3.0 |
| Lemon peel | 7.00 | 6.85 | -0.15 | -2.0 |
| Grapefruit peel | 0.00 | 0.00 | 0.00 | 0.0 |
| Grapefruit peel | 7.00 | 6.90 | -0.10 | -1.4 |
| Grapefruit peel | 20.00 | 20.00 | 0.00 | 0.0 |
| Orange pulp | 0.00 | 0.00 | 0.00 | 0.0 |
| Orange pulp | 0.80 | 0.80 | 0.00 | 0.0 |
| Lemon pulp | 0.00 | 0.00 | 0.00 | 0.0 |
| Lemon pulp | 0.50 | 0.48 | -0.02 | -4.0 |
| Grapefruit pulp | 0.00 | 0.00 | 0.00 | 0.0 |
| Grapefruit pulp | 0.40 | 0.412 | +0.012 | +3.0 |

Table 2

Determination of diphenyl in diphenyl-treated orange peels (70 g samples removed from the same mixture of peels)

| Sample | 1 | 2 | 3 | 4 | Average |
|-----------------------------|-------|-------|-------|-------|---------|
| Diphenyl found (mg) | 40.50 | 41.13 | 39.50 | 40.50 | 40.41 |
| Deviation from the mean: mg | +0.09 | +0.72 | -0.91 | +0.09 | |
| % | 0.22 | +1.77 | -2.22 | +0.22 | |

Table 3

Determination of diphenyl in diphenyl-treated citrus peels (70 g average samples taken from the same lot of 10 fruits)

| Sample No. | Diphenyl (mg) | | |
|------------|---------------|-------------|------------------|
| | Orange-peels | Lemon peels | Grapefruit peels |
| 1 | 17.48 | 24.8 | 11.2 |
| 2 | 17.48 | 26.3 | 11.2 |
| 3 | 17.48 | 26.7 | 10.8 |

The tables show that deviations are slight and remain within the limits of experimental errors*.

Justification of extract preparation method

Distillation: The main fraction of diphenyl was found to pass with the first 25 ml of distillate. Subsequent fractions contain decreasing amounts of diphenyl (Table 4). The amount of liquid for distillation, from 300 to 2000 ml, did not affect the results.

* Note: This described method permits the determination of diphenyl in the presence of o-phenyl-phenol.

Table 4

Distillation of a mixture of water and essential citrus oils

| Diphenyl introduced (mg) | Diphenyl found in successive 25 ml portions of distillate (mg) | | | | | | Total |
|--------------------------|--|-------|-------|-------------|------|-----|---------|
| | I | II | III | Fraction IV | V | VI | |
| 20.0 | 18.80 | 0.94 | 0.125 | 0.0 | 0.0 | 0.0 | 19.865 |
| 50.0 | 48.75 | 1.16 | 0.280 | 0.0 | 0.0 | 0.0 | 50.190 |
| 100.0 | 78.75 | 16.25 | 5.000 | 1.0 | 0.08 | 0.0 | 101.700 |

Moreover, it was observed that the amounts of diphenyl liable to be found in the samples being distilled are completely eliminated with the first 100 ml of distillate. This justifies the collection of the first 100 ml from the distillate. Distillation of peel and pulp lasts about 20 and 40 minutes respectively (Table 5).

Table 5

Elimination of diphenyl during distillation

| Product distilled | Diphenyl added (mg) | Diphenyl found in successive 100 ml fractions of distillate | | | |
|-----------------------------------|---------------------|---|--------------|-----|-----------|
| | | I | Fractions II | III | T o t a l |
| <u>Non diphenyl-treated fruit</u> | | | | | |
| Orange pulp | 0.400 | 0.400 | 0.0 | 0.0 | 0.400 |
| Lemon pulp | 0.400 | 0.380 | 0.0 | 0.0 | 0.380 |
| Grapefruit pulp | 0.800 | 0.780 | 0.0 | 0.0 | 0.780 |
| Orange peel | 10.00 | 9.85 | 0.0 | 0.0 | 9.850 |
| Orange peel | 20.0 | 20.0 | 0.062 | 0.0 | 20.062 |
| Lemon peel | 1.60 | 1.66 | 0.0 | 0.0 | 1.66 |
| Lemon peel | 4.0 | 4.0 | 0.0 | 0.0 | 4.0 |
| Grapefruit peel | 1.6 | 1.6 | 0.0 | 0.0 | 1.6 |
| Grapefruit peel | 20.0 | 19.90 | 0.04 | 0.0 | 19.94 |
| <u>Diphenyl treated fruit</u> | | | | | |
| Orange pulp | | 0.450 | 0.0 | 0.0 | 0.450 |
| Lemon pulp | | 0.280 | 0.0 | 0.0 | 0.280 |
| Grapefruit pulp | | 0.120 | 0.0 | 0.0 | 0.120 |
| Orange peel | | 32.60 | 0.094 | 0.0 | 32.694 |
| Orange peel | | 35.44 | 0.0 | 0.0 | 35.44 |
| Lemon peel | | 3.36 | ? | 0.0 | 3.36 |
| Lemon peel | | 10.15 | 0.0 | 0.0 | 10.15 |
| Grapefruit peel | | 5.04 | 0.0 | 0.0 | 5.04 |
| Grapefruit peel | | 6.18 | 0.018 | 0.0 | 6.20 |

Purification of extract: Conflicting opinions exist among research workers (1,4,5)7 8 regarding the loss of diphenyl as a result of purification of diphenyl solution by concentrated sulfuric acid. As previously shown (6), under certain conditions diphenyl disappears quickly under the action of concentrated sulfuric acid, while no losses were observed when 35 ml of the chloroform extract was repeatedly purified with 10 ml concentrated sulfuric acid. Because of these two conflicting results the influence of treatment conditions of the chloroform extract of diphenyl, by sulfuric acid, was investigated and the following conclusions reached:

- 1) The loss in diphenyl depends on the ratio between the volumes of concentrated sulfuric acid and chloroform; it rises with an increase in the ratio (Table 6).

Table 6

Influence of the ratio of $\frac{H_2SO_4}{CHCl_3}$ upon the loss in diphenyl

(300 μ g of diphenyl/ml of concentrated sulfuric acid-chloroform mixture shaken for 15 Minutes)

| | | | | | | | |
|------------------------|-------|-------|------|------|------|-----|-----|
| Volume of H_2SO_4 | 50 | 20 | 10 | 5 | 2 | 1 | 1/3 |
| Volume of $CHCl_3$ | | | | | | | |
| Loss in diphenyl | | | | | | | |
| (% of initial content) | 100.0 | 100.0 | 95.0 | 90.0 | 85.0 | 5.0 | 0.0 |

- 2) The loss in diphenyl increases with lengthening of shaking period and is accelerated by repeatedly replacing the sulfuric acid with fresh acid (Table 7).

Table 7

Influence of length of shaking on loss of diphenyl (10 mg diphenyl, 30 ml chloroform, 10 ml of concentrated sulfuric acid replaced every 10 minutes)

| | | | | |
|---|-----|------|------|------|
| Length of shaking time (hours) | 4 | 8 | 12 | 16 |
| Loss in diphenyl (% of initial content) | 0.0 | 20.0 | 40.0 | 50.0 |

The influence of shaking time on the loss in diphenyl is not constant but depends on other undetermined factors.

3) The loss in diphenyl depends on the water content of sulfuric acid and decreases when the dilution of the acid rises (Table 8).

Table 8

Influence of water content of sulfuric acid on diphenyl loss (10 ml of diphenyl, 30 ml chloroform, 10 ml sulfuric acid replaced every 10 mins. Length of shaking time:

12 hours

| Sulfuric acid solution A: concentrated sulfuric acid (ml) | | | | | | |
|---|------|------|------|------|------|------|
| B: Distilled water (ml) | | | | | | |
| A | 100 | 99.0 | 98.0 | 97.0 | 96.0 | 95.0 |
| B | 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 |
| Loss in diphenyl | 40.0 | 25.0 | 17.0 | 13.0 | 0.0 | 0.0 |

These observations justify the mode of purification by the combined action of concentrated and diluted sulfuric acid. This makes possible a very prolonged purification of the extract.

References

1. Böhme, H., and Bertling L. (1957) Zur Bestimmung von Diphenyl in Zitrusfrüchten. Zeitsch. Lebensm. Unters. u. Forschung. 105 : 311.
2. Cox, H.E. (1945) Spectrophotometric estimation of biphenyl and o-phenyl phenol, Analyst 70 : 373.
3. Gunther F.A., Blinn, R.C. and Barkley J.H. (1959) Procedure for determining amounts of biphenyl and o-phenyl phenol on and in a single sample of citrus fruit. University of California. Citrus Experiment Station, Riverside.
4. Ihloff, M.J. and Kalitzki M. (1957) Zur diphenyl Bestimmung in Zitrusfrüchten. Mitteilungsblatt GDCh. Fachgr. Lebensmittelchem. 11 : 129.
5. Koether, B. (1958) Beitrag zur quantitativen Bestimmung des Diphenyl in Zitrusfrüchten. Zeitsch. Lebensm. Unters. u. Forschung. 108 : 158-163.
6. Rajzman, Anna (1960) A method for the quantitative microdetermination of diphenyl. Analyst. 85 : 116-121.
7. Steyn, A.P. Rosselet, F. (1949) Photometric determination of biphenyl in orange peel. Analyst, 74 : 89-95.
8. Tomkins, R.G. and Isherwood F.A. (1945) The absorption of diphenyl and o-phenyl phenol by oranges from treated wraps. Analyst 70 : 330-333.

Fig. 1: Distilling Apparatus

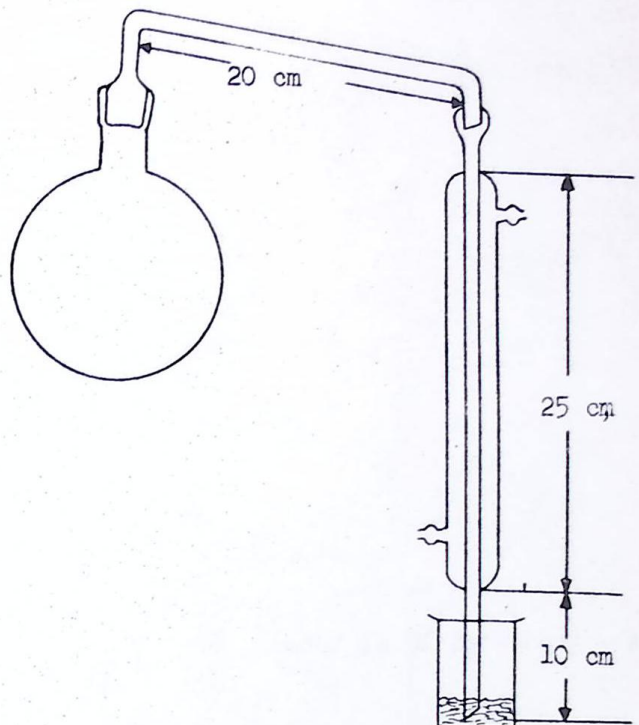


Fig. 2: Optical density curves, μg diphenyl/ml chloroform

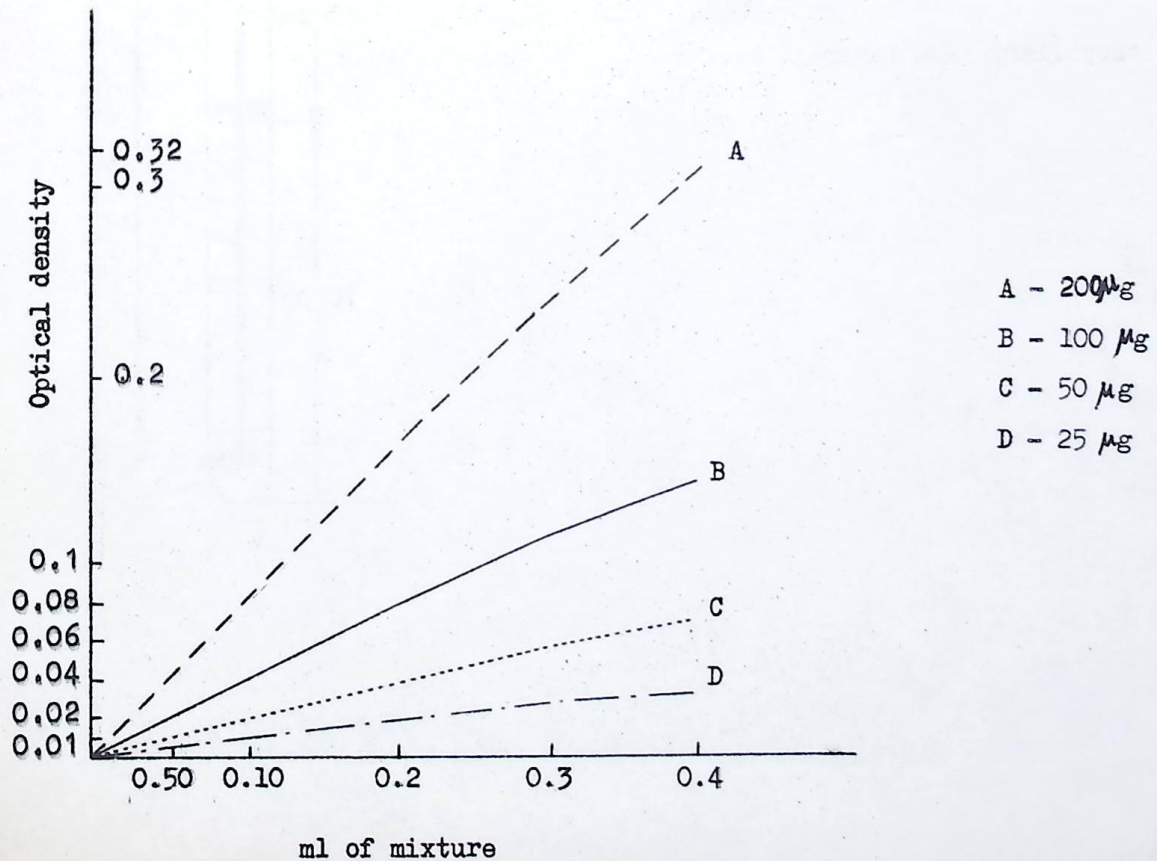


Fig. 3: Concentration Apparatus

A - graduated 10 ml tube

B- concentration disposal apparatus

a) funnel

b) capillary tube raised 1 ml.

