H A E M A T E I N A N D I T S A P P L I C A T I O N

T O F I B R E S

A T H E S I S

SUBMITTED BY

JAYANT NANALAL DESAI

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

A C K N O W L E D G M E N T

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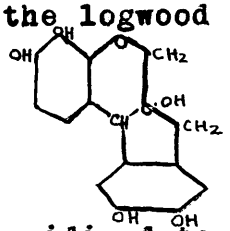
Royal Technical College,
Glasgow.

INTRODUCTION.

Haematein (Colour Index No. 1246) is the product of a logwood tree growing in the West Indies. Formerly the wood itself, in the form of chips, was used by dyers, but now a crystalline extract of the wood is commonly used. The colouring matter in the logwood tree is haematoxylin (I) which is



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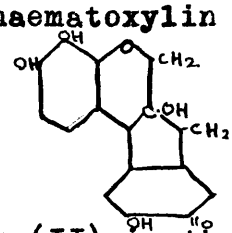


(II)

partially oxidised to haematein (II) in the dyeing process. Logwood extract contains varying amounts of (I) and (II) according to its degree of oxidation. Logwood has remained in use and is the only natural dye of any great commercial importance at the present time. The history of this natural dye is very fascinating and is included in Part I of this thesis.

Although haematein has been in use for dyeing wool for a long time, it is only during the last two to three years that a renewed interest has developed amongst the manufacturers and users of this dye. This is due to its cheapness, better feel and hue of the dyeings and many uses, including the dyeing of nylon and leather and the coloration of nitrocellulose lacquers. Since 1908, when W.H.Perkin established the structure of haematein, no systematic investigation has been carried out either with regard to its affinity towards fibres or with regard to its fastness/

fastness properties. Since no literature is available, the problem is studied against a background of the theoretical aspects of the fundamental properties of the parent substances, like haematein and haematoxylin, together with present knowledge regarding wool and nylon and theories of dyeing and lake formation Part II of the thesis has been divided into several sections in order to present a clearer picture about present knowledge and the different angles from which the problem has been viewed.

The author is fully conscious that some of the suggested explanations of the experimental observations are highly speculative; this applies particularly to Part IV, where an attempt has been made to suggest the possible mechanism of dyeing. This was inevitable in view of the complex nature of haematein and absence of any other evidence. More detailed investigations with similar simpler substances would help to reduce the complexity of the problem.

PART I

HISTORICAL INTRODUCTION.

The Natural Colouring Matters have been used for dyeing for thousands of years. For instance, chemical analysis of ancient Egyptian mummy cloths shows that their makers were acquainted with Indigo and Madder. Indigo has been known in Asia from a remote period of antiquity and there exist very ancient records in Sanskrit describing the methods of its preparation. Its employment in Europe was very limited until 1616, when it began to be imported from India, but it was not imported in large quantities until after 1600. The discovery of America resulted in the introduction into Europe of many new natural colouring matters, two of the most important of which were logwood and brazilwood. Logwood has remained in use and is the only natural dye of any great commercial importance at the present time.

It is said that just prior to the introduction of the various dyes from the new world, there were more than two hundred dyeing firms in the city of Florence alone, which gives some idea of the possibilities of commercial exploitation during the early part of the 16th Century. Knowledge of dyeing gradually extended from Italy, through Germany into Flanders and thence to Britain. King Edward III first caused dyes to be brought into Britain from Flanders and in 1472 a Dyers' Company was incorporated in the City of London.

The name logwood, as applied to the wood of the tree botanically/

botanically known as Haematoxolon Campechianum, did not appear in print until 1581, when the importation and use of logwood were forbidden in Britain by a Parliamentary Statute (Act. 23. Elizabeth) which reads in part as follows:

> "There hathe byn brought from beyonde the seas a certain kinde of ware or stuffe called Logwood alias Blockewood... As the colours made with the said stuffe be false and deceiptful... all such logwood shal be forfeyted and openly burnt."

The word Blockewood as it appeared in the Parliamentary Statute is sometimes corrupted into blackwood; blockewood originated among the early privateers who captured a number of Spanish ports. Bloc and Blocke are forms of a continental word meaning a log of wood. Although the name blockewood remained in use for many years, particularly among sailors and merchants, the consumers of the wood and the dye, soon abandoned the term in favour of its English equivalent "Logwood," which has since continued in use. In Spanish literature this wood is called "Brasilwood," a general term meaning a hard, heavy and reddish wood affording a reddish dye. The Portugese imported brasil woods from Brazil as early as 1504 and maintained a monopoly of them for many years.

Logwood entered all the main consuming centres of Europe, but principally the Spanish ports, e.g. Seville and Cadiz by direct shipment from America in Spanish ships. The Crown of Spain had declared Logwood to be an article of contraband.

These conditions obtained during the hundred years between 1500 and 1600. During this period Britain obtained the Island of Jamaica from Spain and was thus placed in a favourable position to gain control of the logwood cutting business. This made Port Royal (Kingston) the principal logwood shipping centre in the West Indies. At that time the demand for dyes was materially greater in Continental Europe than in Britain. Logwood shipments were mainly consumed in Spain and in important dye-consuming centres of Europe.

After the discovery of America, many new dyes became known to Spanish explorers, who withheld facts and figures about them from the rest of the world. The right of exploitation was granted by the King of Spain to only a few traders, who thus earned fortunes. It is said that the passing of the Act of Parliament mentioned above was not solely due to the fugitive character of logwood; during that time there were strained relations between Spain and Britain and prohibition of logwood was a step towards lessening trade with Spain. Also the introduction of logwood into the country was a hard blow to the woad growers.

Britain did not permit the entry of logwood until 1662, eighty-one years after the passage of the Act. During this time, British dyers continued to use logwood by capturing logwood cargoes under different names from Spanish ships. Before the introduction of indigo, woad was used extensively throughout the dyeing/

dyeing industry for dyeing blue. Since indigo was a competator of woad, it was discouraged and in 1577 the German Diet issued a decree declaring indigo to be a "pernicious, deceitful... corrosive dye."

Thus the importation and commercial use of the two principal raw products yielding blue dyes was being discouraged by legislative measures. After the defeat of the Spanish Armada in 1588, Spain lost the logwood trade and also her grip on Northern Europe, which afforded Flanders, Holland and parts of Germany and France much greater opportunities for developing their own resources.

Logwood slowly began to be approved of and employed by British and European dyers. In 1655 Cromwell took Jamaica and this date marks the beginning of Britain's entry into the ultimate control of the logwood business. By an Act of Parliament of 1688, Charles II declared it lawful to import logwood.

When Britain took over Jamaica, the only logwood producing centres were Campeche (Mexico) and the Bay of Honduras. By the end of the 17th century, two well defined grades of logwood from these two regions had become well established in the European markets and in 1715 logwood was introduced into Jamaica.

Edward Long says:- (History of Jamaica) "It was first propagated in this island in 1715 from seed brought from the bay Campeche/

Campeche, with design to establish it as an article of export and prevent the necessity of forming settlements, upon the bays on the Spanish Main, where the cutters were liable to great risks by working to their knees in water and where constantly harassed by the stings of mosquito gnats." The geographical distribution of the logwood tree was greatly extended by cultivation throughout all of the West Indian islands.

Soon after 1753, when several important works dealing with the systematic arrangement of plants according to the natural scheme of classification were published, a greatly increased interest was evidenced in the botanical source of numerous raw vegetable products. Interest in the chemistry of logwood was also developed during this period. The use of logwood became an important industry in Germany and the Netherlands. This resulted in increased demand with a consequent rise in prices. The services of many chemists on the Continent were utilised for studying logwood and in 1810 Chevreul succeeded in separating in crystallised form one of its colouring principles. In 1842 Erdmann produced for the first time the true colouring matter, named haematoxylin. Chemists and dyers' devised better methods for extracting and purifying the dye and for making it more permanent.

In 1856, W.H.Perkin discovered the first synthetic dye and a large number of new and useful synthetic dyes began soon to be prepared by many European chemists. While these coal/

coal tar dyes were being investigated and developed, chemists such as Erdmann, Rein, Meyer and Perkin continued their investigations upon haematoxylin. Competition soon developed between vegetable dyes and the new synthetic dyes, but a few vegetable dyes, especially logwood, remained in favour. It is reported that the United Kingdom imported in 1890 about 60,000 tons of logwood and even to-day logwood is consumed to an appreciable extent in Britain (70,000 tons p.a.).

Production of logwood.

The growth of the logwood tree is so quick that it will rise in proper soil to the height of ten feet in three years. The seed, which is best sown in swampy ground, is very perishable soon losing its power of germination. The colouring principle of logwood is haematoxylin. This body on oxidation yields haematein, which is the real colouring matter in prepared logwood Haematoxylin is the result of the decomposition of a glucoside which exists in the fresh wood. Regarding the glucoside itself little is known except that under the influence of a species of fermentation it is decomposed with the formation of sugar and haematoxylin.

In order to bring about decomposition of the glucoside and the conversion of haematoxylin into colouring matter - haematein, the wood is chipped and after 25% water addition, placed in heaps about 20 ft. long, 10 - 12 ft. broad and/

and 3 - 4 ft high. The heaps are frequently turned over in order to allow the air to act upon the haematoxylin and also to prevent a too great elevation of temperature, which would cause excessive fermentation, resulting in the complete destruction of the colouring matter. This operation, the so-called "ageing" or "maturing" of logwood, requires effective control. The state of the atmosphere has great effect upon this process of fermentation. When the fermentation of wood proceeds in a satisfactory manner, the haematoxylin is oxidised to haematein. The ammonia formed by decomposition of the nitrogenous matters present no doubt plays an important part in the production of the colour. In order to accelerate the operation, the wood is sometimes watered with a solution of glue, a highly nitrogenous body, but the practice is attended with considerable risk of spoiling the colouring matter. Prepared and matured logwood contains from 20-30% more water than the original wood. During ageing, wood changes from yellow brown or rich red brown to dark crimson. A decoction of matured logwood contains both haematoxylin and haematein, since the former is not entirely converted into haematein during fermentation.

Formerly the colouring matter present in logwood was usually extracted by the dyer himself by simply boiling the wood in water and using this solution as a dye bath. Under these conditions, however, the colouring matter so obtained was never in a pure form but was contaminated with tannins and resinous matter. At the present time, it is customary for the manufacturer to market

a/

a ready made product prepared by extracting logwood chips with boiling water, filtering the solution thus obtained and concentrating it in vacuum. Extracts are sold in both liquid and solid forms. The solid form, known as "crystals" is obtained by oxidising the extract and concentrating it until it sets to a solid mass on cooling; it consists chiefly of haematein. Liquid extracts contain a considerable quantity of haematoxylin, the preparation depending upon the degree of oxidation. As a general rule, not more than 20 per cent. of haematoxylin is oxidised in the liquid extract, while in solid forms 80 to 90 per cent. of the colouring matter is present as haematein.

Liquid extracts are sometimes adulterated with extracts of other woods such as chestnut and their specific gravity is increased by the addition of glucose, salt or, more generally, molasses, which makes a convenient, cheap and difficultly detectable adulterant. Solid extracts are often diluted with salt and sometimes with farina and various tannin extracts. Methods for detecting and estimating various impurities in logwood extract are given by L. Bruchi (J.S.D.C. 1889, 6, 111, 122).

Logwood has been used for dyeing many types of substances. Suitable mordants give different shades on cotton, wool, silk, leather and nylon. Logwood products have also found application in dyeing rayon, bone, wood, also in the preparation of logwood pigments for nitro cellulose lacquers and as biological stains/

stains and indicators for pH values.

Dyeing on mordanted wool.

Chromium - A black of excellent shade and very good fastness to wet treatment is readily obtained with about 85% exhaustion of the dyebath by dyeing chrome mordanted wool with haematein. Its drawback is that its fastness to light on prolonged exposure is about Grade 5 on the Soc.Dyers Colourists Scale compared with 7.8 for the best synthetic chrome blacks. As with other types of dyes, fading is no doubt due to oxidation since it does not occur in absence of air. Once the fading of a chrome logwood black has commenced, it continues at an increasingly rapid rate.

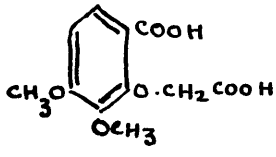
Iron-copper mordant - Blacks of good shade and fastness properties including fastness to light (6-7) can be obtained on wool mordanted with iron or iron and copper, using oxalic acid or cream of tartar (both of which are rather expensive) as the assistant. Compared with blacks on a chrome mordant, the iron or iron-copper blacks might be expected to show rather poorer fastness to rubbing, especially if the wool contained residual soap or grease.

PART II

Section 1.

* CHEMISTRY OF HAEMATOXYLIN AND HAEMATEIN.

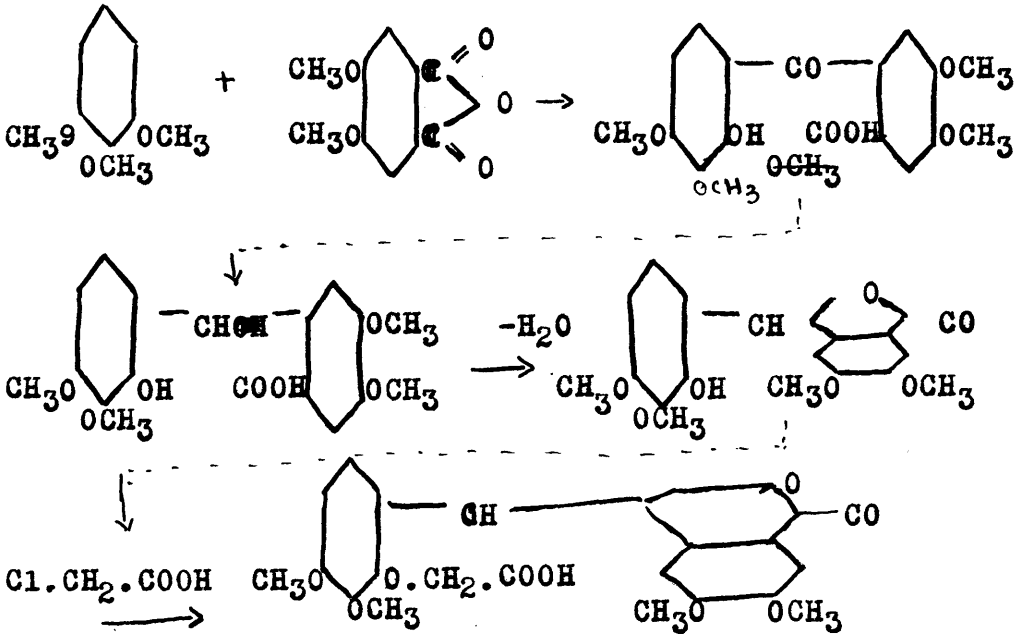
Haematoxylin may be obtained from logwood in the crystalline condition and forms colourless needles with the empirical molecular formula $C_{10}H_{14}O_6$. It takes up five acetyl groups and therefore contains five hydroxyl groups, of which one is alcoholic in character. It was quite early postulated that brazilein and haematoxylin possess closely related constitutions, when the extra oxygen atom in the haematoxylin formula would be accounted for by an additional hydroxyl group. Support for this supposition was found in the fact that on fusion with potash, haematoxylin yielded pyrogallol instead of the resorcinal obtained from brazilein. Moreover, the oxidation of tetramethylhaematoxylin with potassium permanganate to hemipinic acid and a second fragment 1 carboxy - 4 5 dimethoxy - 6-phenoxy acetic acid was also in agreement with this view.



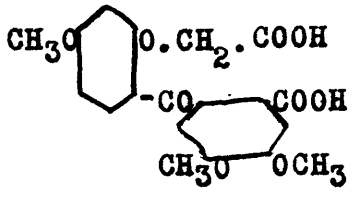
Haematoxylinic acid $C_{20}H_{20}O_{10}$ appeared to provide still another oxidative fragment in which the original skeleton had undergone less modification. This acid was reduced to a further acid $C_{20}H_{20}O_9$ which was characterised as a monobasic lactonic acid - the constitution of which was finally established by/

* "NATURAL ORGANIC COLOURING MATTER" by Perkin and Everest.

by the following synthesis:-



The constitution of haematoxylinic acid must therefore be (I)



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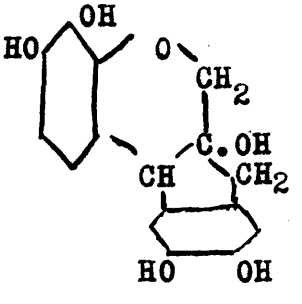

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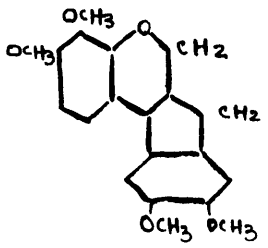
Further elucidation of the constitution and discussion of the origin of the fission products brought forward no further arguments beyond those already elaborated on the basis of research on brazilein, since all the products obtained differed from those of brazilein only by virtue of the additional hydroxyl group.

Thus the formula (II) as above corresponding to that of brazilein was finally proposed first by Pfeiffer (Chem. Zeit., 1904, 3, 380), and supported by Perkin, while other workers such as V.Kostanecki and Herzig associated with the investigation, proposed constitutions corresponding to their formulas for brazilein. As with brazilein itself, the formation of a brazane derivative was of some significance but more convincing are the synthesis of tetramethylanhydrohaematoxylin (III) and tetramethyldesocyhaematoxylin (IV).



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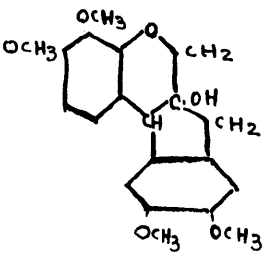

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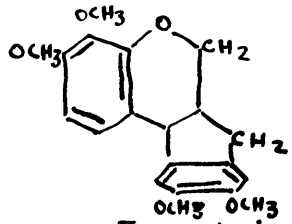

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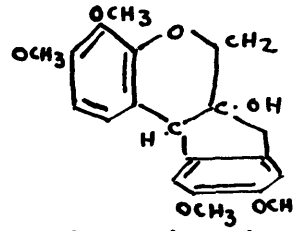
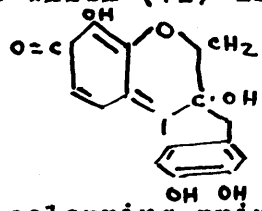


(IV)

Haematein is formed by oxidation of haematoxylin according to the equation

$$C_{16}H_{14}O_6 + O = C_{16}H_{12}O_6 + H_2O$$

and possesses by analogy with brazilein one of the structures (V) or (VI) of which (VI) is preferred



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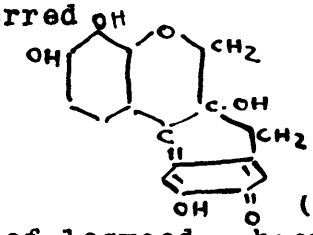

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Properties.

The colouring principle of logwood - haematoxylin was first isolated in a crystalline condition by Chevreul (Ann.Chim. Phys (ii) 82, 53, 126), who obtained it by extracting the wood with ether, evaporating the extract and digesting the residue with alcohol. After distilling off the alcohol, the residue was allowed to stand in contact with water when haematoxylin separates in crystals.

It may also be prepared by similarly treating commercial logwood extract which has been incorporated with a large quantity of sand (C.L. Erdmann, Annalen, 44, 292, J. pr. Chem., 26, 193; 36, 205, 75, 318). For this purpose, ether containing water is preferable. It is, however, more easily obtained from the dark coloured crusts which slowly separate when concentrated logwood liquor stands for some time in a cool place. The crude mass is ground to a fine powder, extracted repeatedly with ether, the ethereal solution evaporated and the residue left in contact

with/

with water when dark coloured crystals separate which by recrystallisation from water containing a small quantity of sodium bisulphite may be obtained colourless. (W.H. Perkin and Yates. Chem. Soc. Trans 1902, 81, 236).

Pure haematoxylin crystallises in prisms with 3 H_2O and is sparingly soluble in cold, readily so in hot water. Its aqueous solution is coloured purple with alkalis and this on exposure to air eventually assumes a brown tint. Haematoxylin is dextro rotatory, a 1% aqueous solution having a rotation of 1.85 in a 20 m.m. tube. It readily reduces salts of silver and gold, gives with alum a rose red coloration, with iron alum a violet black precipitate and with neutral and basic lead acetate at first a colourless and then a blue deposit which darkens by air oxidation.

When oxidised under suitable conditions haematoxylin readily passes into the colouring matter haematein according to the equation:

$$C_{16} H_{14} O_6 + O = C_{16} H_{12} O_6 + H_2O$$

Haematein was first produced by C.L. Erdmann by passing air through an ammonical solution of haematoxylin and subsequently acidifying with acetic acid. Erdmann and Schultz who adopted a somewhat similar procedure succeeded in isolating haematein in leaflets which possessed a metallic lustre. Reim, on the other hand (Ber., 1871, 4, 331), treated an ethereal solution of haematoxylin with a little concentrated nitric acid.

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By extracting 'aged' logwood with ether, Halberstadt and Reis (Ber., 1881, 14, 611) obtained 1% of a very pure crystalline haematein. An ammonical solution of logwood extract was exposed to air for two or three days by Hummel and A.G.Perkin (Chem. Soc. Trans., 1882, 41, 367). The precipitated ammonia compound of haematein was collected, dissolved in water, dilute acetic acid added and the mixture digested on the water-bath to dissolve as much as possible of the suspended amorphous haematein. The clear liquid after partial evaporation deposited crystals of the colouring matter, possessing a yellowish-green incidence and having the composition $C_{16}H_{12}O_6$.

According to Mayer (Chem. Zentr., 1904, (1), 228), haematein may also be prepared by oxidising haematoxylin in aqueous solution with sodium iodate.

Engels, W.H. Perkin and Robinson (Chem. Soc. Trans., 1908, 93, 1140) passed air for six hours through a solution of 15 grams of haematoxylin dissolved in a solution of 15 c.c. of concentrated ammonia in 150 c.c. of water. The product of the oxidation was added in a thin stream to dilute acetic acid (250 c.c. of 10%), heated on the water-bath, when haematein separated in crystals.

Haematein is very sparingly soluble in water and the usual solvents. Alkalis dissolve it readily. Ammonia dissolves it with a brown-violet colour whilst its strong alkaline solution has a rich purplish blue colour. On exposure to air, the colour

of/

of these alkaline solutions gradually becomes red and finally brown, the colouring matter being destroyed. By addition of potassium acetate to the boiling alcoholic solution of haematein, the monopotassium salt $C_{16}H_{11}O_6K$ is deposited. (A.G.Perkin, Chem. Soc. Trans., 1899, 75, 443).

Sulphurous acid or sodium bisulphite solution converts haematein into a colourless addition product, readily soluble in water but no reduction thereby appears to occur as on boiling the solution or by addition of acid haematein is precipitated. With zinc and hydrochloric acid or with stannous chloride and caustic soda, a solution of haematein is decolourised but on standing the liquid regains its former tint.

Various Compounds.

E. Erdmann and Schultz (Ann., 1883, 216, 234) re-examined Reim's compound $C_{16}H_8O_6(C_2H_8O)_6$ and showed that this was in reality a penta-acetyl haematoxylin $C_{16}H_9O_6(C_2H_3O)_5$, melting point 165-166°C.

Herzig (Monatsh, 1894, 15, 143) by the action of sodium methoxide and methyl iodide on haematoxylin, obtained the tetramethyl derivative $C_{16}H_{10}O_2(O\,Me)_3$, melting point 139-140° a substance which still contains the hydroxyl group, since on treatment with acetic anhydride, it yields acetyltetramethyl-haematoxylin $C_{16}H_9O_2(O\,Me)_3(C_2H_3O)$, melting point 178-180° C.

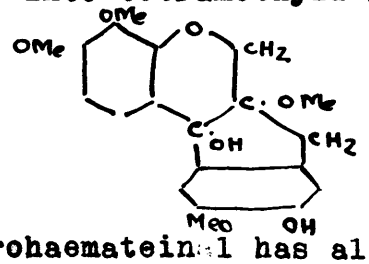
Other derivatives reported in the literature are:

Pentamethylhaematoxylin C_{16} H_9 0 (0 CH_3)$_5$ plates, melting point 144-147° C. dibromohaematoxylin C_{16} H_{12} Br_2 O_6 (Dralle, Ber., 1884, 17, 373); penta-acetyl bromohaematoxylin C_{16} H_8 Br O_6 (C_2 H_3 0)$_5$, needles, mepting point 110°C. (Buchka., Ber., 1884, 17, 685); penta-acetyl tetrabromohaematoxylin C_{16} H_5 Br_4 O_6 (C_2 H_3 0)$_5$ (Dralle); and haematoxylinphthalein C_{40} H_{30} O_{14} (Lelts, Ber., 1879, 12-1632).

W.H. Perkin and his co-workers (Chem. Soc. Trans., 1902, 31, 1059) prepared tetra-methylhaematoxylin by treating the solution of haematoxylin in alcoholic potash with methyl sulphate, a method which had previously been found serviceable by V. Kostanecki and Lampe (Ber., 1902, 35, 1669) for the production of trimethyl brazilein from brazilein.

When haematein is methylated by means of alkali and methyl sulphate, the product consists essentially of tetra-methyl haematein and pentamethyldehydrohaemateinal.

Tetramethylhaematein crystallises in amber prisms and when digested with dilute potassium hydroxide is converted by the addition of water into tetramethyld hydrohaematein.l, yellow prisms:-



Pentamethyld hydrohaematein.l has also been prepared by further methylation with methyl sulphate and alkali.

Haematein and brazilein are converted by the action of mineral acids into orange or red salts from which the original

colouring matters could not be regenerated.

When haematein is dissolved in cold sulphuric acid a reddish brown solution is produced from which by cautious addition of acetic acid minute orange-coloured prisms of acid Iso-haematein sulphate $C_{16} H_{12} O_5 SO_4$ are deposited. The reaction may be represented thus:-

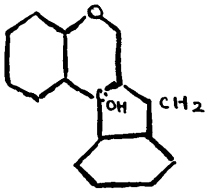
$$C_{16} H_{12} O_6 + H_2 SO_4 = C_{16} H_{12} O_5 SO_4 + H_2O$$

This substance when left in contact with 80% alcohol is gradually transformed with loss of sulphuric acid into a new compound crystallising in orange-red plates possessing a strong metallic lustre and to which the formula $(C_{16} H_{12} O_6)_2 C_{16} H_{12} O_5 SO_4$ was assigned. On the other hand, hydrochloric and hydrobromic acids in a sealed tube at 100°C. convert haematein respectively into Iso-haematein chlorohydrin $C_{16} H_{11} O_5 Cl$ and Iso-haematein bromhydrin $C_{16} H_{11} O_5 Br$, which crystallise in orange-red needles.

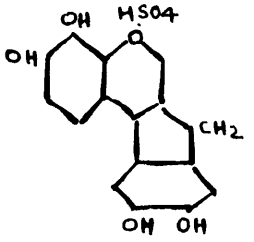
If to an aqueous solution of Iso-haematein chlorohydrin or bromhydria, silver hydroxide is added to remove the halogen, a solution of Iso-haematein is obtained which, on evaporation, leaves this substance as an amorphous mass possessing a green metallic lustre. Iso-haematein and its salts dissolve in solutions of the alkaline hydroxides with a red-violet colour which is easily distinguished from the corresponding blue-violet solution of haematein; moreover, the dyeing properties of these compounds and haematein differ considerably.

Haematein salts (W.H.Perkin, Chem.Soc.Trans., 1908, 93, 1085) are derivatives of 4:3-indeno-benzopyranal (i) and

Iso-haematein (ii) Sulphate

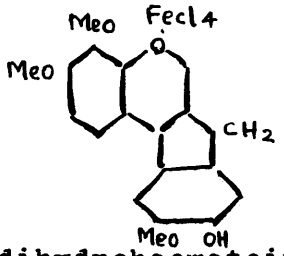


(i)

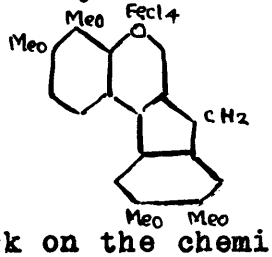


(ii)

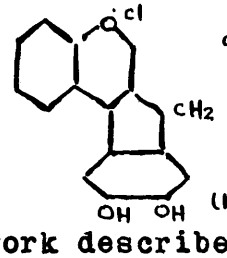
Engels and Perkin prepared from tetramethyl haematein-5^1 hydroxy-7:8:4^1-trimethoxy-4:3-idenebensopyranal-1:4-anhydro ferri chloride which crystallises from acetic acid in well defined elongated prisms



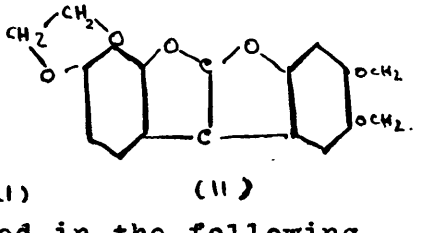
Pentamethyldihydrohaemateinal, on the other hand, gives 7:8 : 4^1:5^1:- Tetramethoxy-4:3-idenbenzopyranal-1:4-anhydro ferri chloride



The main work on the chemistry of haematoxylin and haematein as seen in the foregoing pages was carried out by Perkin and Robinson in the period 1900-1910; while the assigned structure has never been confirmed by synthesis; it is well supported by the more recent work of Robinson who has successfully synthesised the closely related Iso-haematein chloride (i) and o-diethylene haematoxylene (ii).



(i)

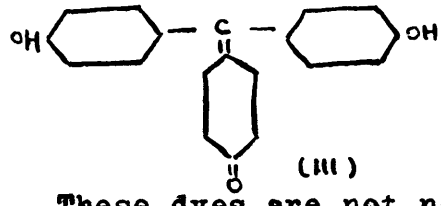


(ii)

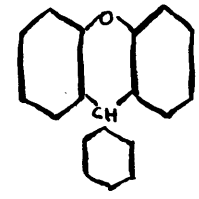
In the experimental work described in the following pages, it has been assumed that the structure assigned by these authors/

authors is correct. * The results of recent work in this laboratory are in agreement with the above formula.

It will be readily seen that the compound is closely related to two classes of synthetic dyes, the diphenyl methane dyes (iii) typified by aurine and the xanthenes (iv).



(III)



(IV)

These dyes are not noteworthy for their light fastness and therefore it would at first sight seem likely that fastness of haematein would be low. It has been confirmed that haematein dyed on wool, gives a red shade of very poor light fastness; the blue-black shade obtained on chrome-mordanted wool has better light-fastness, which, however, is still not equal to that of synthetic chrome blacks. *

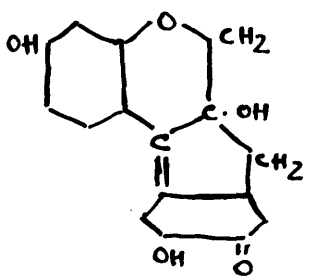
* D.J.Duff, private communication.

Chemistry of Brazilein.

Brazilwood, meaning soluble red wood, yield its colouring matter easily to boiling water. The colouring principle is Brazilin, $C_{16}H_{14}O_5$ and was first isolated in a crystalline condition by Chevreul (Ann. Chim. Phys., (1), 66, 225). The crude substance is purified by two or three crystallisations from water, to which a little Sulphurous acid has been added (Gilbody and W.H.Perkin, and Yates, Chem.Soc. Trans., 1901, 79, 1396). When Brazilin the colouring principle is oxidised under suitable conditions, it is converted into Brazilein, the true colouring matter

$$C_{16}H_{14}O_5 + O = C_{16}H_{12}O_5 + H_2O$$

Perkin (Chem.Soc.Trans., 1882, 42, 367) prepared brazilein by adding excess of ammonia to the extract of the wood and passing air through the solution. Impure ammonium salt separates, which is collected, dissolved in hot water, and acetic acid (Sp. gravity 1.04) is added to it. A brown viscous precipitate of the crude colouring matter is thus obtained which is extracted with hot dilute acetic acid and the extract evaporated on the water bath. Crystals of brazilein separate, which are collected and washed with acetic acid.



Brazilein.

PART II.

Section 2.

CHEMISTRY OF WOOL.

Wool fibre has been found to consist of five chemical elements - carbon, hydrogen, oxygen, nitrogen and sulphur. The empirical composition is as follows:-

C	52.7 - 54.9%	N	15.4 - 17.6%
H	6.90 - 7.3%	S	0 - 5.0%
O	20.9 - 23.5%		

Keratine, the essential structural component of wool, is a protein. By hydrolysis, e.g. with 20% hydrochloric acid or 35% sulphuric acid for 8 hours, it gives mixtures of α amino acids

$$R - CH \begin{matrix} \nearrow NH_2 \\ \searrow COOH \end{matrix}$$

in which R may be a basic or acidic group or a simple hydro carbon chain. Fischer by his polypeptide synthesis gives a clue as to how the different amino acids are linked together to form the protein:-

$$CH_3\ Br\ CH\ COCl + NH_2.CH_2.COO\ Et \rightarrow CH_3.CH.Br.CO.NH\ CH_2.COO\ Et$$

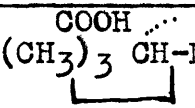
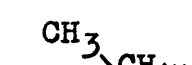
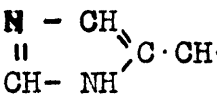
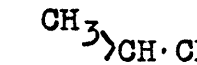
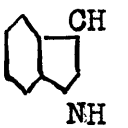
$$\downarrow \text{Hydrolysis ammonia}$$

$$CH_3\ CH\ (NH_2)\ CO\ NH\ CH_2\ COOH$$

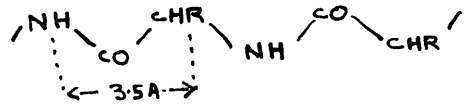
The product resembles proteins in some respects. Hydrolysis of wool gives the following products of the general formula

$$R.-CH \begin{matrix} \nearrow NH_2 \\ \searrow COOH \end{matrix}$$

Hydrolysis of wool gives the following products.

R =	Name	R =	Name	R =	Name
H ····	Glycine		phenylalamine		Proline
CH_3 ····	Alamine	$NH_2(CH_2)_4$	Lysine		
	Valine	$NH_2 \cdot C \cdot NH(CH_2)_3$ NH	Arginine		Histidins
	Leucine	$COOH \cdot CH_2$	Aspartic acid		trypto-phine
~~Ph. CH_2 · OH~~ Ph. CH_2 ····	Serinic	$COOH \cdot CH_2CH_2$	glutanic acid		
$CH_3CH \cdot OH$	Threonine			$(S \cdot CH_2)_2$ ::::	Cystine
OH  ···	Tyrosine			$CH_3 \cdot S \cdot CH_2 \cdot CH_2$	Methio-nine

X-ray analysis and chemical and physico-chemical evidence (Astbury and Speakman, J.S.D.C., Jubilee issue, 1934, 24), shows that keratine has the following basic structure.



This is characteristic of all proteins.

The chains in wool are 9.8°A apart. The molecular weight is about 68,000. It swells in water fifteen times as much in diameter as in length. Hence it is presumed that the molecule must be a long one. The rigidity of the structure is due to cross links between molecules. These links may be postulated in the following ways:-

(1) -N H C O
↓
-N H C O

(2) - N H_3...O O C

(3) -OH
↓
OH -

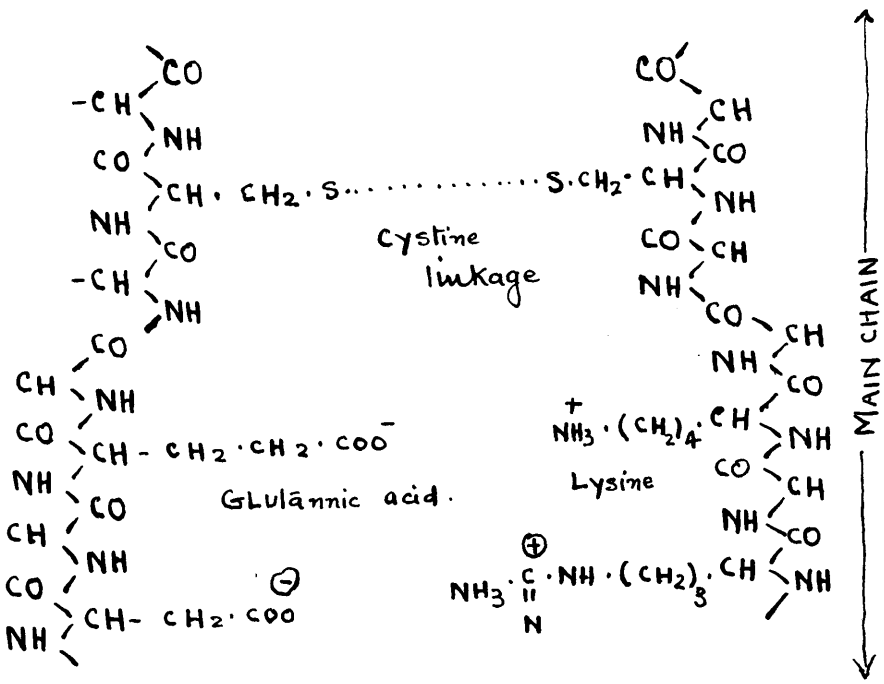
(4) - N H_2
↓
H O -

(5) -S-S-

(6) -O H + O H -
↓
-O- (-H_2O)

(7) - N $H_3^{\oplus}$ + $^{\ominus}$O O C -
↓ (- N_2O)
-N H CO-

Astbury has suggested that the structure of wool fibre reduced to its simpler terms consists of long polypeptide chains bridged by cystine and salt linkages as shown in the following formula:-



Salt linkage

<---------- Side Chains ----------->

The/

The molecular structure of the wool fibre being composed of long peptide chains bridged by cystine and salt linkages is compact and offer resistance to the penetration of large molecules. In acid solution, however, the salt linkages are broken up, resistance inside the structure is reduced and against this reduced resistance, operates the increased swelling pressure developed in accordance with the Proctor-Wilson theory of Swelling (Chem.Soc.Trans., 1916, 109, 307) The fibre swells to an extent depending on the pH of the solution and the strength of the acid. Hence, acids besides determining the extent to which wool combined with dye in accordance with the pH of the dyebath, facilitate combination by increasing the accessibility of the fibre to large molecules. As regards changes of temperature, a variety of experiments point to the occurrence of important structural changes in wool in water at temperatures above 40°C. (Speakman, Stott and Chang, J. Textile Inst., 1933, 34, T 273). It has been noticed by these authors that swelling of wool below 37°C. first decreases with rise in temperature, but above 37°C., it increases with rise in temperature.

The explanation offered by these authors for increased swelling in hot water is due to the breakdown of sulphur linkages. Whether or not hydrolysis of the sulphur linkages is reversible, its occurrence in hot water leads to a reduced cohesion within the structure, increased swelling and greater accessibility to dye/

dye. It is, however, difficult to refer the increased swelling of wool fibres in hot water solely to hydrolysis of sulphur linkages because cotton shows a minimum swelling in water at 45°C. Hence the increased swelling of both wool and cotton in hot water is probably due to a common mechanism, supplemented in the case of wool by hydrolysis of sulphur linkages.

X-ray photographs show that wool fibre consists of amorphous material containing regions of crystallinity, which are termed crystallitesor micelles. On these micelles, the long chain molecules are arranged parallel to one another and are held together by crystal forces and by definite cross-linkages between chains. The edges of the micelles are not well defined but merge gradually into amorphous material, which is possibly unoriented material of the same chemical constitution as the crystallites. This material links the micelles together to form the fibre. One long chain molecule may form a part of two or more micelles. The crystallites are arranged with their long axes lying approximately along the length of the fibre, but the degree of orientation varies in different fibres. The attraction between the peptide chains which form the micelles is due to Van der Waal's forces and separation can occur if a sufficient swelling pressure is developed in concentrated solutions of weak acids.

It is thus clear that both acids and hot water swell the wool fibre in such a manner as to increase the accessibility to large dye molecules. When acid and hot water act together, as/

as in a boiling acid dyebath, wool should approximate to a condition of uniform accessibility to dye, so far as its internal structure is concerned, the degree of approximation being least where the structure was originally most crystalline. Because the approximation is imperfect, the gross structure as well as the fine structure of animal fibres, plays a part in determining their resistance to dye penetration.

Speakman and Smith (J.S.D.C., 1936, 52, 121) have found that the amount of dye absorbed by animal fibres, from a circulating dyebath, is a linear function of the square root of time, despite the falling concentration of the dye present. This is because the dye absorption takes place by diffusion from a film of approximately constant concentration on the surface of the fibres. The scale structure of animal fibres offers considerable resistance to the penetration of dye molecules, so that de-scaled fibres and fibres cut to short lengths show an increased rate of absorption over normal fibres. Differences between the rates of absorption of dye by different animal fibres are determined mainly by variations in fibre diameter. The finer the fibres, the greater is the speed of the process. When corrected for variations in external surface, the differences between fibres as regards accessibility to dye are small.

Skinner and Vickerstaff (J.S.D.C., 1945, 61, 193) suggest that swelling of the fibre in water is due to the osmatic pressure developed internally. X-Ray analysis shows, however, that the/

the inter-atomic spacing of the crystalline portion is unchanged, from which it follows that the osmotic forces are insufficient to separate the chains in the crystallites and that all the swelling must take place in the amorphous inter-micellar material. This results in moving apart of micelles, to a point at which osmotic pressure is balanced by elastic forces tending to restore the fibre to its original state. In this swollen condition, the inter-micellar material forms open networks or pores between crystallites. The diameter of these pores is not known with certainty, but is probably about 60 A. in wool. In any case it seems probable that the pores are sufficiently large to permit the passage of dye molecules.

If the cross-section of a normal dyed fibre is examined, it is seen to be uniformly coloured. The dye has penetrated to the centre and since it cannot have passed through the crystalline portions, which are unswollen, it must have diffused along the inter-micellar pores. In other words, in placing the fibre in the dyebath, swelling takes place, resulting in the formation of pores along which dye can diffuse. Once inside the fibre, the dye must be fixed in some way in order to account for the progressive increase in exhaustion of the dyebath.

PART II

Section 2.

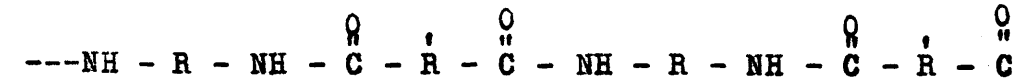
CHEMISTRY OF NYLON.

Nylon is a generic term coined to describe synthetic super-polyamides formed by condensation of diamines and di-carboxylic acids, followed by polymerisation. . The ordinary commercial nylon is formed by condensing stoichiometric proportions of hexamethylenediamine and adipic acid, the reaction being:-

$$HOOC\cdot(CH_2)_4\cdot COOH + NH_2\cdot(CH_2)_6\cdot NH_2$$
$$\downarrow$$
$$HOOC\cdot(CH_2)_4\cdot CONH\cdot(CH_2)_6\cdot NH_2 \text{ etc.}$$

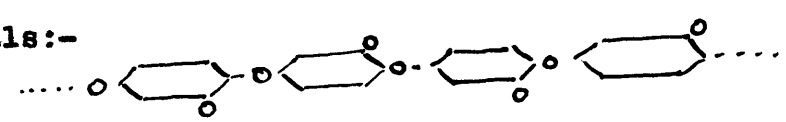
The reaction proceeds until a long chain is built up with a molecular weight of 10,000 to 12,000, leaving a carboxyl group at one end and an amino group at the other. By altering the nature of the diamine and the di-carboxylic acid, any number of super-polyamides may be obtained.

Structurally and in chemical properties the nylon



are closely related to the natural protein fibres such as silk and wool, which also are polyamides -

than they are to the cellulosic fibres such as cotton and flax which are polyacetals:-



In the case of silk R and $\dot{R}$ represent principally the $-CH_3$ group. In wool R and $\dot{R}$ represent the residue of alpha-amino/

amino acids such as cystine, glutamic acid, lysine and many others, which may have acidic, basic or non-polar end-groups.

Mechanism of dyeing.

Stott (Amer. Dyestuff Rep., 1940, 29, 646) has investigated the dyeing of nylon with acid dyes and has noted that their 'affinity' for nylon is less than for silk and wool. This low 'affinity' is the major difficulty in the application of acid dyes. Elod and Schachowsky (Melliand Textilber, 1942, 23, 437) studied the mechanism of dye absorption and drew the following conclusions:-

(a) The amount of acid dye absorbed by nylon is dependent on the pH of the dyebath.

(b) The swelling of nylon is independent of the pH of the dyebath.

(c) No maximum acid absorption is obtained with decreasing pH of the dyebath, which represents a marked deviation from the behaviour of acid dyes on wool.

In order to explain these facts, they suggested that not only are the amino groups at the ends of chains involved, as in dyeing wool, but other forms of linkage must be considered, although they did not draw any conclusions about the nature of these additional linkages. The limited number of amino groups in nylon has an important effect on the practical dyeing of/

of this fibre, for 2-4% of the commercial dye may represent saturation.

The iso-electric fibre contains an equal number of negatively charged carboxyl and positively charged amine groups together with a number of non-ionised carboxyl or amine groups depending on which type is present in excess. In the case of nylon the iso-electric point and analytical data show that the carboxyl groups are in excess. Accordingly, when nylon is placed in acid solution hydrogen ions are absorbed by ionised carboxyl groups to give non-ionised groups. The total number of hydrogen ions, or milliequivalents of acid, which can be absorbed in this way is determined by the number of amine groups in the fibre, but the pH at which the ionisation of the acidic groups is repressed depends on the dissociation constant of the acid groups. Carlene, Fern and Vickerstaff (J.S.D.C. 1947, 63, 388) have observed that the acid groups are stronger in wool than in nylon.

Peters (J.S.D.C., 1945, 61, 95) states that the dyeing of nylon with acid dyes takes place in two ways. At normal dyebath pH values, viz., above pH.3 combination between dye and end amino groups occurs. The amount of dye which can combine in this way is strictly limited. At lower pH values, combination with the weakly-basic amino groups can take place and the amount of dye which can be bound in this way is very great.

The/

The dyeing of nylon fibre differs from the dyeing of wool at normal dyebath acidities merely in the greatly reduced number of basic sites available (about one sixteenth of those in wool) and in the greater affinity of the dye anions for nylon. This second factor is shown by Peters by the displacement of the titration curve to higher pH values and in practical dyeing by the greater fastness of dyes in nylon as compared with wool.

Nylon fibre as a whole thus absorbs hydrogen ions at a higher pH than wool which means that the affinity of hydrogen ions for nylon is greater than for wool. Using the equation derived by Gilbert and Ridsal (Proc. Roy. Soc. 1944, A. 182, 335) for the absorption of acids by wool, the affinity of hydrogen ions for nylon may be calculated as -10 Kg. Cal. as compared with -6.4 Kg.Cal. in wool and this increased affinity, contributing to the total affinity of the dye acid may be one of the reasons why acid dyes are faster to washing on nylon than on wool.

PART II.

Section 3.

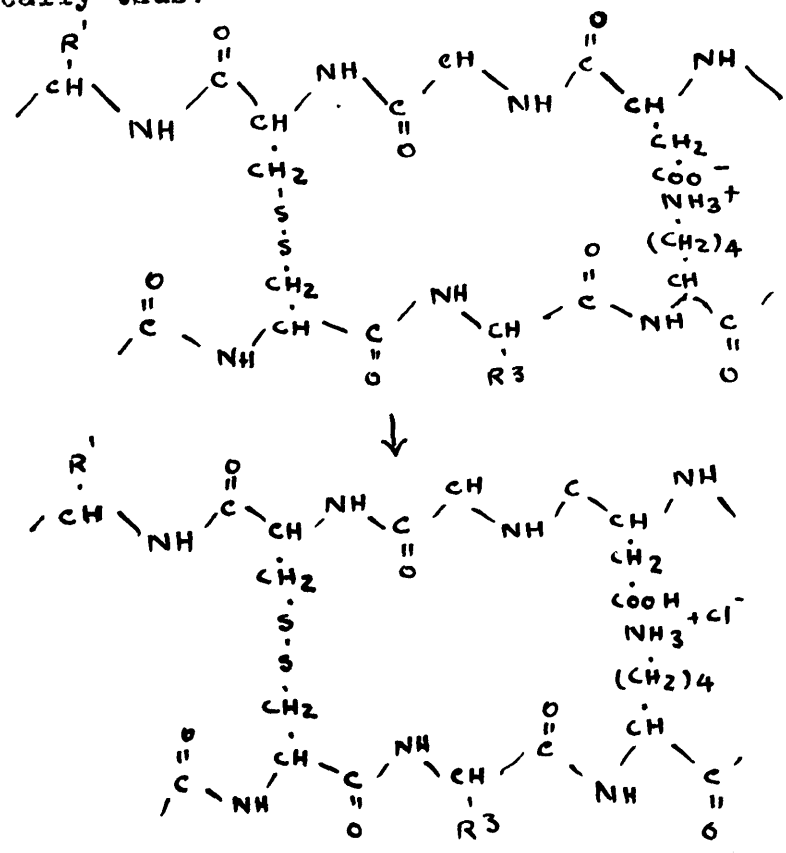
THEORY OF DYEING WOOL.

Wool is a fibrous protein formed by the condensation into long chains of a variety of α-amino-carboxylic acids of general formula R·CH·(NH_2)·COOH where R may be an aliphatic, aromatic or heterocyclic residue or may even contain another carboxyl or amine group.

During the last fifty years this subject has attracted much attention. Various theories which have been put forward, e.g. the mechanical, chemical, solution, adsorption, colloidal and electrical theories have been summarised by Wood. (The Chemistry of Dyeing, Gurney & Jackson, 1926). Since then knowledge of the chemistry of wool has increased enormously and it is now possible to formulate a satisfactory theory of dyeing of this fibre.

Speakman (J.S.D.C., 1924, 40, 408; 1925, 41, 172) first showed that acids combine with wool chemically and not by adsorption. He also showed that "deaminated" wool can be dyed with acid dyes, because it is very difficult to deaminate wool completely. Deamination breaks the salt links in wool and free carboxyl groups are then present to combine with basic dyes. If amino-groups are acetylated the affinity of wool for acid dyes is greatly reduced and its affinity for basic dyes greatly increased. When wool is immersed in solutions of hydrochloric acid, the acid combines with the wool through back-titration of the salt links which bridge the main peptide chains, shown diagrammatically/

diagrammatically thus:-



The amount of acid absorbed at room temperature when combination with all the salt links has taken place is about 80 ml. of N-acid per 100 grams of dry wool. This figure, which is reached at about pH 1, is in good agreement with the percentage of amino groups in wool and corresponds to an equivalent weight of 1250 approximately

The/

The fixation of the dye is attributed to the amino and carboxyl groups carried on the side chains of a keratin molecule. In the neutral fibre an internal salt is formed by the transfer of a hydrogen ion from a carboxyl group to give a positively charged substituted ammonium ion and a negatively charged carboxyl ion. The electrostatic attraction between these two oppositely charged groups forms the so-called salt linkage which helps to hold adjacent main chains together and thus plays an important part in the physical properties of the fibre. When placed in an acid solution, hydrogen ions diffuse rapidly into the fibre, entering because of their small size, even into the unswollen crystalline portions and neutralising the charges on the weakly acidic carboxyl groups. Thus the fibre is left with a positive charge and attracts any anions, i.e. sulphate or dye anions which are present in the solution. These anions diffuse into the fibres through the pores and approach as closely as possible to the ionised amino groups of the protein. In the main amorphous portion of the fibre the anions can approach very closely to the substituted ammonium groups and chemically the process is equivalent to the formation of a salt between the dye acid and the basic group of the protein. It has been shown that combination of wool with acid dyes is stoichiometric in character, i.e. the combining capacity of wool for acid dyes is the same as for simple acids, e.g. hydrochloric acid. At a particular temperature the amount of acid absorbed by wool at equilibrium is a function of the concentration of protons and anions in solution as shown in Figure [1] which is a titration/

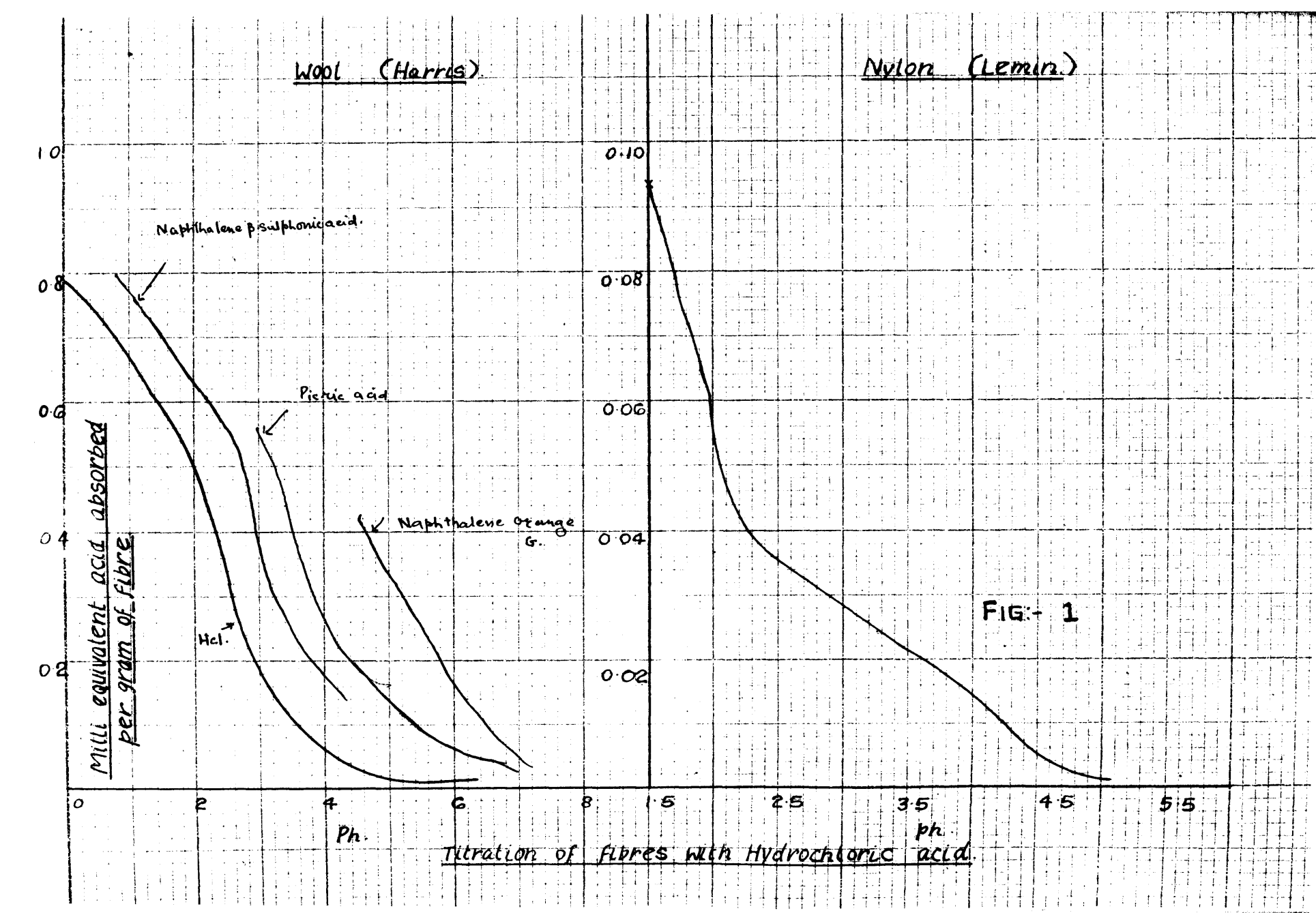


FIG:- 1

Titration of fibres with Hydrochloric acid.

titration curve of wool with hydrochloric acid, the amount of acid bound being plotted against the pH of the solution in equilibrium with the wool. As the acidity of the solution increases the amount of acid bound rises slowly, then more rapidly and finally approaches a limiting value which is determined by the number of amine groups in the fibre.

Steinhardt, Fugitt and Harris (Bur.Stand.J. Res. 1941, 26, 293) have published accurate titration data for a number of acids varying in complexity from hydrochloric through naphthalene-Sulphonic to the free acid of a dye, Orange II (colour index No. 151). These curves are shown in Figure 1. Where it is evident that as the complexity of the anion increases, the titration curve of the acid is displaced towards higher pH values, so that at any pH, amount of acid bound by the fibre increases as the complexity of the anion increases. Dye anions, because of their size, are unable to penetrate the crystalline portions of the fibre as is shown by the X-Ray analysis of Astbury and Dawson and it is possible that they collect on the surface of the micelles in numbers corresponding with the number of ionised groups inside the micelles.

According to the theory of the dyeing of wool with acid dyes, the cause of dyeing is in effect the formation of a salt between wool and dye. This is supported by many experiments in which the maximum amount of acid which the wool fibre can absorb has been determined. The acid combining capacity has been determined/

determined most frequently and with greatest accuracy in the case of hydrochloric acid and it has been shown that 1 g. of wool will absorb 0.82 milli equivalent of hydrochloric acid. Many experiments with acid dyes have given saturation values corresponding fairly closely with this figure and hence the evidence in favour of salt formation is very strong.

Elod (Trans.Faraday Soc. 1933, 29, 327) has explained this process of dyeing wool with acid dyes in presence of a strong acid on the basis of the Donnan theory. This theory can be explained by considering the following equations:-

$$-\overset{+}{N}H_3 \quad \bar{O}OC- \quad + HCl \rightleftarrows -\overset{+}{N}H_3 \; \bar{C}l \; + \; HOO\bar{C} \quad \ldots\ldots\ldots(1)$$

$$\overset{+}{N}H_3 \; C\bar{l} \; + \; \bar{D} \rightleftarrows -\overset{+}{N}H_3 \; \bar{D} \; + \; C\bar{l} \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

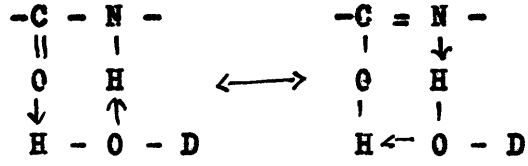
$$-\overset{+}{N}H_3 \quad \bar{O}OC- \quad + HD \rightleftarrows -\overset{+}{N}H_3 \; \bar{D} \; + \; HOOC- \quad \ldots\ldots\ldots(3)$$

as reversible and applying the law of mass action.

Thus the picture provided for the dyeing of wool in a strongly acid dyebath with acid dyes of the equalising type is fairly complete, but many acid dyes dye wool from neutral or slightly alkaline baths.

Valko (J.S.D.C. 1939, 55, 173) has put forward an explanation for neutral dyeing with concentrations of sulphate ions and dye ions (under the conditions of acid dyeing) practically only the dye ions are absorbed by wool. Since the electrical attraction of the wool towards the sulphate ion is no less than that/

that towards the dye ion and since dyes are not aggregated at the boil Valko postulates non-electric specific attraction. He suggests that attraction is due to hydrogen bond formation between the peptide chains of the wool and hydroxyl and amino groups in the dye. The type of bonding may be in accordance with Eisterts conception of two mesomeric forms corresponding with the keto and enol forms of the peptide groups, e.g. in the case of a dye molecule containing hydroxyl groups -



where D = Dye residue. Arrow = Hydrogen bond.

The formation of hydrogen bonds between wool and acid dyes accounts for the small and varying residual affinity of deaminated wool for acid dyes. These bonds are weak and hence account for poor fastness to water. This ability to form hydrogen bonds is shown not only when dyes combine with wool but also in the tendency of dye molecules to aggregate together in solution the two phenomena being different aspects of the same fundamental property. Dyes which aggregate in solution are in general also capable of dyeing wool from neutral bath, i.e. they exhibit strong residual valency forces and strong affinity for wool in consequence they are also unlevel dyeing.

Ender and Muller (Melliand Textilber, 1937, 18, 633) consider the dissociation constant of the dye acid to be very important/

important in determining its fastness to water when dyed on wool, i.e. the ease of hydrolysis of wool-dye-acid salt, although other factors, e.g. molecular weight and speed of diffusion are also involved.

Skinner and Vickerstaff (J.S.D.C. 1945, 61, 193) found that if high acidities are employed, wool is partially hydrolysed during dyeing, leading to increased dye absorption. They consider it probable that an ionic mechanism is the governing factor in determining the extent of dye absorption and that any other forces which are involved merely assist in fixation of the dye to the fibre on sites other than ionic sites.

Gilbert and Rideal (Proc. Roy. Soc., 1944, A 182, 335; A 183, 167) treated this subject more theoretically and derived the expression

$$\log \frac{\theta_H}{1-\theta_H} - pH = \log \frac{(\Delta\mu^0_H + \Delta\mu^0_x)}{2RT}$$

where R is the gas constant in calories per molecule.

T absolute temperature of the experiment

θ_H fractional saturation by protons of the available proton sites in the fibre

$\Delta\mu^0_H$ and $\Delta\mu^0_x$ protons and amins affinity respectively.

affinity of the acid is the sum of these two.

All the quantities on the right of this expression are capable of direct measurement so that the affinity of an acid may be estimated at any temperature T, the result being expressed in calories per molecule.

Lemin/

Lemin and Vickerstaff (Society of Dyers' and Colourists' Recent Advances in Dyeing Symposium, 1947, 41) have extended the Gilbert-Rideal treatment by modifying it. They used in addition to free-acid titration, methods in which (a) one free acid was allowed to compete with another for the available sites in the fibre, (b) wool previously dyed in presence of hydrochloric acid in such a way that all the sites were occupied by hydrogen chloride or dye ions was treated in solutions of sodium chloride and hydrochloric acid. The amount of dye 'desorbed' from the fibre at equilibrium was estimated colorimetrically and the quantity $\Delta\mu^{o}_{D} - \Delta\mu^{o}Cl$ which is the difference in affinities of the dye and chloride ion calculated by means of the equation:-

$$\log_e \frac{(\Delta\mu^{o}_{D} - \Delta\mu^{o}Cl)}{RT} = \log_1 \frac{\theta_D}{1 - \theta_D} - \log \frac{D}{Cl}$$

θ = proportion of available sites for anions occupied by the dye.

D and Cl are concentrations of dye and chloride ions in desorbing solution.

This technique provides the most convenient and accurate method of estimating affinities according to the Gilbert-Rideal treatment and has the advantage of being applicable to temperatures as low as 20°C. since equilibrium is established very rapidly.

PART II

Section 4.

THEORY OF DYEING NYLON.

The dyeing of nylon has been investigated fairly fully and it has been found possible to dye it with dispersed acid, direct cotton, solacet and basic dyes. Stott (Amer.Dyestuff Rep., 1940, 29, 646) has investigated the dyeing of nylon with acid dyes but his results were not completely satisfactory. Elod and Schachowsky (Melliand Textilber., 1942, 23, 437) studied further the mechanism of dye absorption and noted that acid dye absorbed by nylon depends on pH of the bath, and swelling of nylon is independent of the pH of the dyebath. This represents a marked deviation from the behaviour of acid dyes on wool. The most important contribution in the study of the mechanism of the dyeing process with acid dyes was due to the work of Peters (J.S.D.C., 1945, 61, 95).

Peters showed very clearly that the dyeing of nylon with acid dyes takes place primarily on the amino groups which terminate the polyamide chain. At low pH further combination can occur, probably on the weakly basic amide groups, since these are the only remaining potentially basic groups in the fibre. This view is supported by the observations of Harris and Sookne (Bur.Stand.J.Res., 1941, 26, 289) on the electrophoresis of particles of nylon and dibenzyldiketopiperazine in solutions of varying pH. Both kinds of particle become positively charged

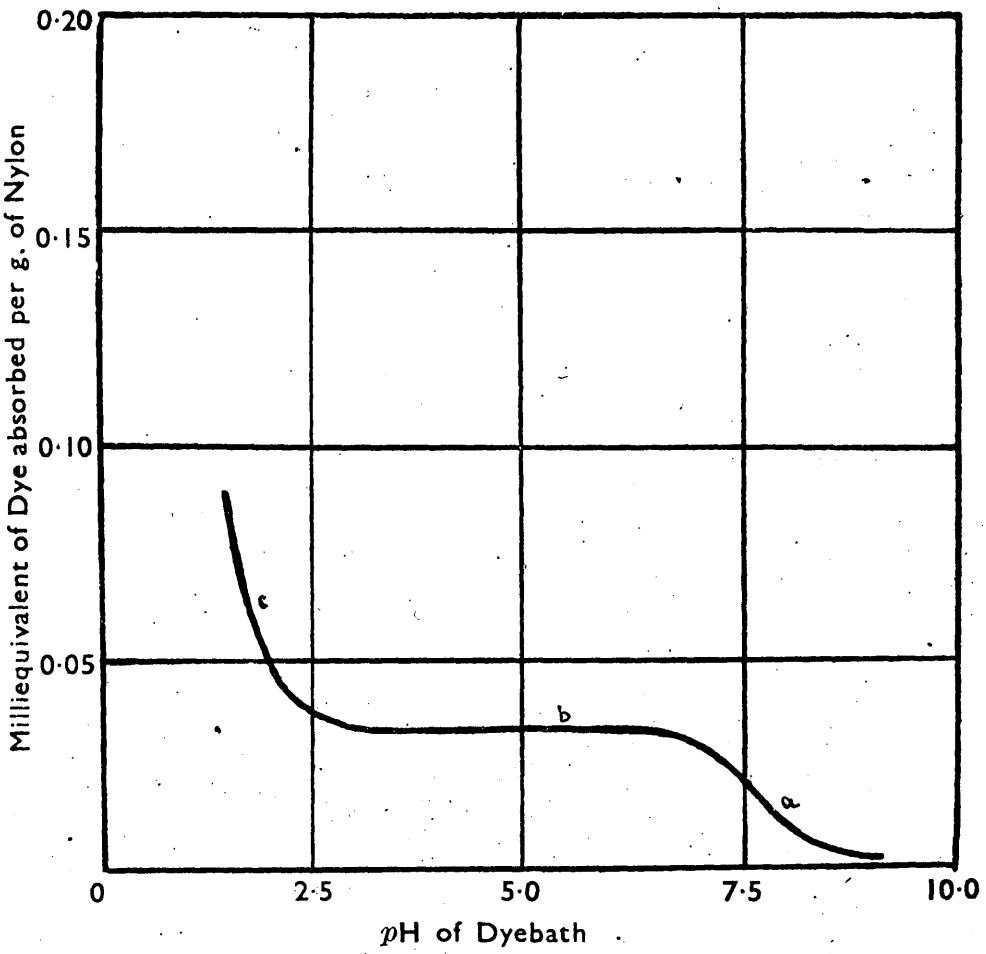


FIG. 1A– Titration of Nylon Fibre with Solway Blue B

Figure 1. A.

at pH values below 2.5 owing to the absorption of hydrogen ions which in the latter case must take place on the amide groupings. By analogy, a similar process must occur with nylon, so that positively charged amide sites will be available for dye absorption below pH 2.5. The effect of the two kinds of groups are well displaced in the typical titration curve of nylon with Solway Blue B shown in Figure 1a., which is taken from Peter's paper.

The curve obtained in Figure 1a, by plotting the amount of dye absorbed against pH is divided into three sections. The first part of the curve labelled as 'a' is very similar to the wool titration curves, obtained by Harris, Figure 1. The curve is displaced towards higher pH values. It is considered that this portion of the curve represents combination of dyes with the dyeing of wool. The evidence for this view is first that the limiting dye absorptiondye absorption reached in this pH region corresponds closely to a value of 0.05 milli equivalents per g. of fibre which is the same as the number of free amino groups at the ends of the fibres as determined by direct titration of the nylon with acid in m-cresol solution. This proof was further substantiated by acetylation of the amino groups and noting the reduction in dye absorption.

In the second section of this curve 'b'(Figure 1a) the absorption of dye is affected only very slightly, by decreasing pH clearly; in this region all the amino groups are combined with dye and no further combination can be promoted by increased acidities.

Finally/

Finally, in the third section 'C' (Figure 1a), another critical pH value is reached, at which the adsorption of dye again begins to rise and so far as the present data go, this rise appears to continue indefinitely, with decreasing pH. This critical pH value seems to be that at which the amido groups become positively charged. It is suggested therefore, that in this region 'C', hydrogen ions are adsorbed on the weakly basic amido groups and are cocompanied by an equivalent number of dye anions to maintain electrical neutrality.

Peters and Carlene, Fern and Vickerstaff's (J.S.D.C. 1947, 63, 388) work has provided enough evidence about the existence of amine group and its extreme importance in practical dyeing. The amine groups are active in providing dye sites between pH 3.0 and pH 6.0 where dyeing with farmic acid and acetic acid is normally carried out.

PART II.

Section 5.

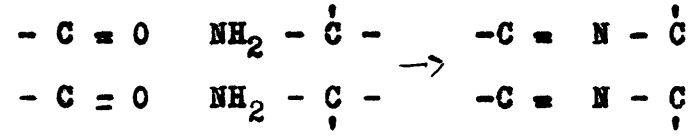
QUINONES AND WOOL.

In the quinones there are two carbonyl groups, the carbon atoms of which are part of the aromatic nucleus and are situated either ortho- or para- to one another; meta-quinones are unknown. One striking characteristic of quinones is colour and a usual, though not invariable, differentiation between simple para- and ortho- quinones is that most of the former are yellow and the majority of the latter are orange or red. Particularly beautiful colour phenomena of value both for identification and analysis of specific members of the series often are observed on dusting a few crystals of a quinone on the surface of a dilute aqueous dolution of alkali or on a drop or two of concentrated sulphuric acid. Hydroxyquinones form intensely coloured alkali salts and many other quinones, particularly those with no substituents in the position α and β with

with respect to one of the carbonyl groups are highly sensitive to alkali and give characteristic colour tests. Fully substituted quinones are more stable to basic reagents but dissolve in concentrated sulphuric acid by virtue of oxonium-salt formation with the production of vivid colours.

Amines, particularly aromatic amines, react with p.benzoquinone in a manner analogous to hydrogen chloride or benzene sulphuric acid to yield either 2-arylaminoquinols or 2:5-di arylaminoquinols which are oxidised by the unchanged quinone in the reaction mixture to the corresponding quinones.

Orthoquinones condense with diamines containing primary amino-groups on adjacent carbon atoms. Cyclic compounds containing the following grouping are formed:-



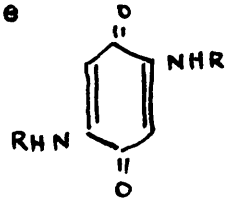
Wurster and Raciborski (Chem. Zentr. 1889, 1, 392) and Raciborski (ibid., 1907, 1, 1595) first showed that quinones give a red coloration with proteins, proteoses and amino acids. Meunier (Compt. Rend.,1908, 146, 987) observed that tanning of hide was due to the reaction between quinones and the amino groups of the tissues. Scharvin (Angew. Chem., 1913, 26, 254) concluded that the product formed is a substituted aminoquinone and that the displaced hydrogen of the amino groups reduces further molecules of quinone to hydroquinone. Suida (Z. Physiol.Chem., 1913, 85, 308) pointed/

pointed out that quinones active in dyeing all contained the grouping - CO CH : CH CO- and suggested that reaction occurred with the amino group of wool.

However, the first positive evidence regarding the linkages between quinone and wool was obtained by Speakman and Coke (Trans.Faraday Soc., 1939, 35, 258) by determining the increase in weight of wool after treatment with aqueous solutions of benzoquinone. They concluded from this experiment that reaction between wool and benzoquinone is not restricted to basic side chain and suggested the formation of additional cross-linkages from acid amides, tyrosine and tryptophane.

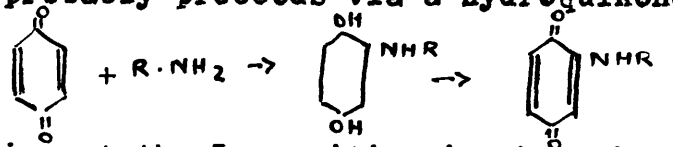
It is a well known fact that light and heat cause polymerisation of benzoquinone in aqueous solution. Speakman' found a large increase in weight in benzoquinone-treated wool which might be chiefly due to deposition of polymerised quinone within the fibres. To investigate this further, Stoves (J.Textile Inst., 1943, 39, 301) carried out systematic studies upon the conditions governing the formation of new linkages. He concluded that formation of new linkages occurs best at pH 7. At about pH 8 rapid polymerisation of quinone reduces the number of new linkages.

The reaction of benzoquinone with the amino group of the Keratin may be of the type



the/

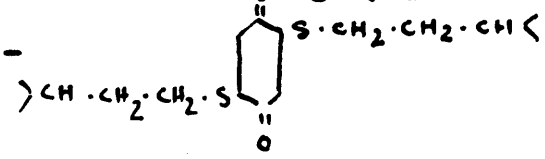
the reaction probably proceeds via a hydroquinone derivative:



Further reaction at the 5- position in the substituted quinone would give rise to the linkage described above. This view has been put forward by Stoves (Thesis: University of Leeds, 1938). The reaction of benzoquinone with Keratin is complicated, due to polymerisation reactions. The constitution of the polymer is not known but Stecker (Chemistry & Leather Manufacture, New York, 1945) has observed that it depends upon temperature, i.e. it decreases with increasing temperature.

The reaction between amino groups of the fibre and the carbonyl groups of benzoquinone is not possible as the main reaction almost certainly occurs at the 2:3- or 3:6- positions, but in 2:3:5:6- tetrachloro-benzoquinone, reaction is limited to the carbonyl groups. This is supported by the fact that a smaller number of new linkages are formed.

The reaction between cystine disulphide linkages and quinones has been examined by several workers and from the general chemistry of quinone it seems highly probable that these linkages are of the type:-



It has been observed that at the same time linkages may be formed by reaction with amino groups, although in simpler compounds containing both thiol and amino groups, e.g. homocystein and reduced glutathione, the amino groups remain unaltered.

E X P E R I M E N T A L

PART III. Section 1.

PRELIMINARY INVESTIGATIONS OF COMMERCIAL LOGWOOD PRODUCTS.

In earlier days the colouring matter present in logwood was usually extracted by the dyer himself by simply boiling the wood in water and using the liquor as a dyebath. Under these conditions however the colouring matter so obtained was never in a pure condition but was contaminated with tannin and resinous substances. At the present time it is customary for the manufacturer to market a ready-made product prepared by extracting logwood chips with boiling water, filtering the solution thus obtained and concentrating it in vacuo. Extracts are sold in both liquid and solid forms. The solid form, known as "crystals" is obtained by oxidising the extract and concentrating it until it sets to a solid mass on cooling; it consists chiefly of haematein. Liquid extracts contain a considerable quantity of haematoxylin, depending upon the degree of oxidation. As a general rule, not more than 20 per cent. of haematoxylin is oxidised in the liquid extract, while in solid forms eighty to ninety per cent. of the colouring matter is present as Haematein.

Liquid extracts are sometimes adulterated with extracts of other woods, such as chestnut, and their specific gravity is raised by the addition of glucose, salt, or, more generally, molasses, which makes a convenient, cheap and difficultly detectable adulterant. Solid extracts are often diluted with salt and sometimes with farina and various tannin extracts.

Methods/

Methods for detecting and estimating various impurities in logwood extract are given by L. Bruehl (J.S.D.C. 1889, Vol.6, 111, 112).

Three commercial samples supplied by the British Dyewood Company Limited (Glasgow) marked as haematein crystals 100% ZA, 50-60% M and 15% NAD were used in the present work.

Several dye liquor exhaustion trials of haematein were carried out on woollen fabric in order to find out the rate of exhaustion and also to make preliminary observations of impurities present in the substance.

During the course of these dipping tests at various time intervals, it was noticed that after a two minutes' time interval in cold in a 1% solution of the 15% haemateim sample, a blue compound was absorbed by the woollen fabric, which on standing gradually faded away and turned greyish. This blue compound had great affinity for wool but it could not be exhausted completely from the bath; when dyed in presence of a little dilute sulphuric acid or dilute acetic acid, a yellow colour was obtained on the fabric instead of blue.

The presence of this blue compound was also noticed in the 100% and 50-60% haematein samples and it was thought necessary to investigate it further.

Various organic solvents were tried to extract the blue component from the fabric but with little success. Finally, it was decided to use the chromatographic method for separation of various components, present in the haematein sample.

Chromatographic Separation.

Solutions of commercial haematein (0.5 g./1.) were prepared and chromatographic separation was carried out in the following three different ways:-

(i) Filter paper method.

(ii) Glass plate method

(iii) Column method.

In all the three methods a suitable absorbent and solvent is absolutely necessary. The absorbent must be insoluble in the solvent, must not react with the substances filtered, must be sufficiently active, colourless, cheap and reconvertible. Besides there are points such as viscosity of solvent, height of column and size of particles of absorbent, which require the most careful attention.

There are many absorbents on the market, both organic and inorganic. Amongst the most important and widely used are sucrose, starch, calcium carbonate, slaked lime, alumina, activated charcoal, etc.

The solvents of the non-polar type commonly used are petrol, ether or benzene and of the polar type, carbon tetrachloride, acetone, pyridine, water, organic acids etc.

In the case of haematein, all attempts to separate the components using the above absorbents were unsuccessful. The metallic compounds formed lakes with the colouring matter. Silica, however, was found to be a satisfactory material for separation.

Chromatograms were prepared for the three commercial samples and the presence of a bluish-grey substance was found to be common to all the three samples.

Two glass tubes as shown in the diagram were prepared and a silica column was made in each tube by slowly pouring in a thin stream of silica paste from the top, carefully tapping each time to avoid air gaps, with slow suction at the bottom. The solution of haematein was poured in drops over the silica column. The dye solution slowly resolved into three distinct colours over the column as shown in the figure 22. This method was tried for all three commercial samples and the presence of a distinct blue in the lowest zone of the column was confirmed.
This blue component when separated from the column lost its colour, probably due to air oxidation.

Dyeing trials with the three components obtained were carried out. It was found that the top and the bottom components had no dyeing properties. The middle zone which contained pure haematein gave a black dyeing but it was not a full black as obtained by ordinary haematein crystals.

The blue components may be tannins or oxidised tannins or glucasodes which help the formation of a full black on the fabric.

The blue component was tested for tannin as follows:-

The portion containing the blue component was removed from the column and extracted with dilute acetic acid. To the extract/

extract a 1% solution of ferric ammonium sulphate was added, which gave a blue black colour, indicating the presence of pyrogallol tannine.

Further investigation was not continued on these lines as the main object was to find analytical methods for estimating haematein and haematoxylin quantitatively.

A chromatographic method was found to be impracticable for obtaining haematein in a pure state on a large scale, due to difficulties of separating the absorbent and crystallising the dye again.

PART III.

Section 2.

ANALYSIS OF HAEMATEIN.

Since commercial logwood contains several coloured substances, it is impossible to determine the haematein or haematoxylin content by any colorimetric process. The analytical method used commercially at present is a form of dye trial made under practical conditions. It is essential that strict attention be paid to details, because the practical value of any logwood sample depends largely upon the process used in applying it. If only haematein is present, no oxidation is necessary and hence a basic mordant such as chromium sulphate may be used.

The following are examples of analytical methods which have been suggested:-

(a) By dyeing trial. Two skeins of wool are mordanted, one with reduced chromium mordant and the other with oxidised chromium mordant. These are then dyed under similar conditions with the logwood/

logwood extract. The skein mordanted with reduced chromium mordant will be dyed only by the haematein present in the bath, but the one mordanted with the oxidising mordant will oxidise any haematoxylin present to haematein and this, together with the haematein originally present, will be fixed on the wool. If only haematein is present, both skeins will be dyed to the same depth of colour, but if haematoxylin is also present, the skein mordanted with oxidising mordant will be dyed deeper than the other. The proportions of the two substances may be judged from the difference in shade.

(b) Rawson and Trimble (J.S. D.C. 1885, 2, 92) have made use of an alcoholic solution of the aluminium and copper lakes of haematein for colorimetric analysis. Several other soluble coloured lakes can be prepared similarly and tested on a colorimeter. These methods are not nearly so reliable, however, as carefully conducted dye trials and at present there is no satisfactory analytical procedure for the quantitative determination of haematoxylin and haematein in solid or liquid logwood extract. Experiments were therefore commenced in order to device a satisfactory method.

The reaction between haematein and haematoxylin is reversible if carried out in presence of oxidising or reducing agents under controlled conditions. Hence the deeply coloured haematein should readily be reduced by a suitable reducing agent to give colourless haematoxylin. The reducing agents at present employed/

employed in volumetric analysis include ferrous salts, stannous chloride, arsenious oxide, sodium thiosulphate, sodium hydrosulphite and hydrazine and its derivatives. Of these only thiosulphate and arsenious oxide are stable in aqueous solutions when exposed to air and their application is almost entirely restricted to Iodimetry.

Volumetric analytical methods based upon the use of reducing agents are relatively few. The only sufficiently powerful reducing agents which can be maintained at constant strength for a reasonable period are titanous chloride and titanous sulphate. These have been much used in the estimation of reducible dyes. The solution of these salts take up oxygen rapidly when exposed to air even at ordinary temperatures and they must therefore be stored and employed in an inert atmosphere. Volumetric and potentiometric titrations of various samples of commercial and pure haematein were carried out with titanous chloride and the methods and results are described below.

EXPERIMENTAL.

Direct and indirect volumetric titration methods with titanous chloride were employed and their accuracy checked potentiometrically. Details are given below.

Preparation of Pure haematein (Colour Index No. 1246).

Haematein was prepared by different methods from haematoxylin (American Dyewood Company), Logwood chips and haematein/

haematein paste.

Haematoxylin was dissolved in water and made alkaline with ammonia liquor. Air was bubbled through this solution for 6-8 hours in the cold. This solution was poured into an acetic acid bath at 65°C. in a thin stream very slowly. Haematein crystals begin to appear and, on standing for a longer time, haematein separates out.

Logwood chips were extracted with water and the extract concentrated. This was filtered and purified further by treatment with charcoal. The purified extract was made alkaline by adding ammonia liquor and oxidised as before.

Haematein was also obtained from haematein-paste in a pure form. Haematein paste was filtered to remove water and dried at a low temperature. This was dissolved in ethyl alcohol by warming it up on a hot water-bath. When the paste had dissolved completely and the solution cooled down, ether was added until all the tarry matter separated out. It was allowed to settle and filtered. The filtrate was distilled to remove ether and on concentrating the alcoholic solution, haematein crystallised in a characteristic crystalline form. It was dried at 110°C. The purity of haematein obtained from the above methods was checked by optical density variation and melting point.

Haematein solutions from the above were made by dissolving 0.005 gram. in 100 ml. of boiling distilled water. Solutions were allowed to cool down and examined on the "Spekker"

for/

for their optical density, using Ilford Spectrum Violet 601. Purification of haematein, was continued until there was no change in the optical density observed. Melting point for haematein from haematoxylin, logwood chips and haematein paste was 207°C, 210°C and 210°C. respectively.

Commercial samples of "haematein crystals" are easily soluble in water. The material is pasted with a few drops of ethylene glycol mono ethyl ether ("cellosolve"). It is then dissolved with hot distilled water, free from dissolved oxygen and diluted to the required volume.

Preparation of Titanous Chloride Solution:

50 ml. of titanous chloride solution (15%) is boiled for a few minutes with 100 ml. concentrated hydrochloric acid. The mixture is cooled and made up to two litres in a storage bottle designed to keep the solution in an inert atmosphere.

Standardisation:

Pure ferric ammonium sulphate (3.5 grams) is dissolved in water and about 100 ml. of 5 N Sulphuric acid is added. The solution is then diluted to 250 ml. An aliquot (25 ml.) portion of this solution is carefully oxidised with potassium permanganate solution ($N/50$) until a faint persistent coloration is obtained. A large excess of potassium sulphycyanide, as indicator is then added and the ferric ion is titrated with titanous chloride until the red coloration entirely disappears. A large excess of potassium sulphocyanide must be used to clarify the/

the end point. The iron equivalent of titanous chloride is then calculated. Hendrixson and Verbeck (J.A.C.S., 1922, p.2382) have described an electrometric method for the standardisation of titanous sulphate.

Volumetric analysis.

(a) Direct method: To 25 ml. of a 0.01% solution of haematein, 10 ml. of a 10% Rochelle salt solution is added and air-free carbon dioxide is continually bubbled through the liquid which is heated to 60° to 70°C. and titrated with titanous chloride solution, drop by drop, until a distinct colour change from red to very faint yellow is obtained. It is extremely difficult to judge the end point accurately. However, a number of observations were made and a mean value calculated. The result compared favourably with that obtained by the indirect method.

Commercial samples of haematein were subjected to analysis by following the above procedure. In Column 1 of Table I different solvents such as Cellosolve and triacetin have been shown. Haematein is sparingly soluble in water, the solubility being of the order of 0.1 grams per litre. Solvents help to keep the haematein in suspension. Low results obtained without using solvents indicate that part of the haematein remains in an insoluble state and is not completely reduced. Rochelle salt maintains the pH of the titrant and thus avoids the lake-formation of haematein with Titanium.

Estimation/

ESTIMATION OF COMMERCIAL LOGWOOD SAMPLES.

TABLE I

Direct Method

Sample.	15%	50-60%	100%
Solvent	14.20%	24.70%	50.50%
	14.30%	24.20%	50.80%
Water	13.80%	24.90%	50.65%
	14.10%	23.80%	50.70%
	13.20%	24.35%	50.80%
Mean Value	13.92%	24.39%	50.69%
	18.80%	34.80%	60.80%
	19.70%	33.70%	60.50%
Cellosolve	18.50%	33.90%	60.00%
	19.10%	34.00%	60.98%
	20.30%	34.50%	60.00%
Mean value	19.30%	34.18%	60.45%
	20.50%	34.20%	60.50%
	20.35%	33.90%	60.80%
Triacetin	20.00%	34.80%	60.75%
	19.85%	33.90%	60.30%
	20.00%	34.50%	59.80%
Mean value	20.30%	34.28%	60.43%

Temperature 60° - 70°C.
Titration carried out in air-free carbon dioxide.

(b) Indirect method: Haematein solution was prepared as before and heated to 60°-70°C. in an inert atmosphere with 10 ml. of a 10% Rochelle salt solution and 20 ml. of excess titanous chloride solution added. The red solution of haematein is immediately reduced to a faintly yellow colour. The excess of titanous chloride is determined by titrating against standard iron alum solution, using ammonium sulphocyanide as indicator.

Results obtained by this method have been given in Table II.

It was noted that indirect titrations showed good consistency in results. End point was obtained fairly sharp.

Analysis of Pure Haematein.

The direct and indirect titrations of pure haematein with titanous chloride solution was carried out as follows:- Procedure:- Haematein obtained from red paste by the method described on page 61 was used for the analysis. The moisture content was determined and was found to be 0.33%

Haematein crystals 0.005 g. was accurately weighed and transferred in a beaker and crystals were parted with 10 ml. of cellosolve and warmed; the solution was made up to 100 ml. by adding the required quantity of boiled distilled water. The direct and indirect titrations were carried out as described on page 64 by using Rochelle salt and maintaining an inert atmosphere. The indirect method shows a higher degree of purity for the sample used. Results are described in Table III.

Use of Rochelle Salt.

When haematein solution is treated with titanous chloride solution in presence of saturated solution of Rochelle salt, decolourisation takes place but the solution becomes turbid owing, probably, to separation of an insoluble titanium compound. In consequence of this, the end reaction is indistinct. By adding an equal colume of cellosolve, acetine (mixed glyceryl acetates) or alcohol to the solution before titrating, a definite end reaction is obtained and the solution becomes yellow on reduction. The use of a saturated solution of Rochelle salt (potassium sodium tartrate $C_4H_5O_6K$ Na, 4 H_2 O, was unsatisfactory due to the separation of potassium tartrate (C_4 H_5 O_6 K) caused by the presence of free mineral acid in the titanous chloride solution. This often caused the results to be erratic. The presence of Rochelle salt in the titrant, removed the free mineral acid from titanous chloride by forming sodium and potassium salts of hydrochloric acid and liberating weak organic acid. Piccard (Ber. 1909, 42, 439) thinks that tartaric acid exerts a catalyric action on reduction. Sodium tartrate ($C_4H_4O_6Na_2$, 2 H_2O) was found to be equally satisfactory.

Effect of Impure carbon dioxide.

It was observed during the titrations for the standardisation of titanous chloride and the analysis of pure haematein that readings showed gradual drift downwards. Several attempts were made to find the reason for this peculiar change. It was discovered that the carbon dioxide used for the purpose, which was drawn from the cylinders, contained air. The cylinder was therefore replaced by a generator containing solidified carbon dioxide. Results in the estimation of pure haematein improved by as much as 2.0% and became consistent over a series of readings.

ESTIMATION OF COMMERCIAL LOGWOOD SAMPLES.

TABLE II.

Indirect Method.

Solvent	15%	50-60%	100%
Water	13.95%	25.45%	54.95%
	14.05%	25.10%	35.50%
	14.25%	24.95%	54.80%
	14.35%	24.90%	54.20%
	14.45%	25.65%	56.50%
Mean value	14.21%	25.21%	55.19%
Cellosolve	19.95%	35.55%	60.45%
	18.99%	36.50%	60.10%
	19.90%	34.50%	59.85%
	20.50%	34.15%	61.50%
	20.15%	34.25%	59.95%
Mean value	19.88%	34.99%	60.37%
Acetin	20.20%	34.0%	60.85%
	20.55%	34.25%	60.55%
	20.35%	34.55%	60.40%
	20.45%	35.25%	60.05%
	20.60%	35.05%	60.95%
Mean value	20.41%	34.62%	60.56%

Temperature 60° - 70°C.

Titrations carried out in air-free carbon dioxide.

ESTIMATION OF PURE HAEMATEIN.

TABLE III.

Solvent.	Direct	Indirect
Cellosolve	99.15%	98.50%
	99.35%	99.55%
	99.40%	99.05%
Mean value	99.30%	99.03%
Tri-acetine	99.55%	99.60%
	99.40%	99.75%
	99.05%	99.55%
Mean value	99.33%	99.63%

Moisture content of pure haematein sample 0.33%

PART III.

Section 2b.

THE ELECTROMETRIC TITRATION OF HAEMATEIN.

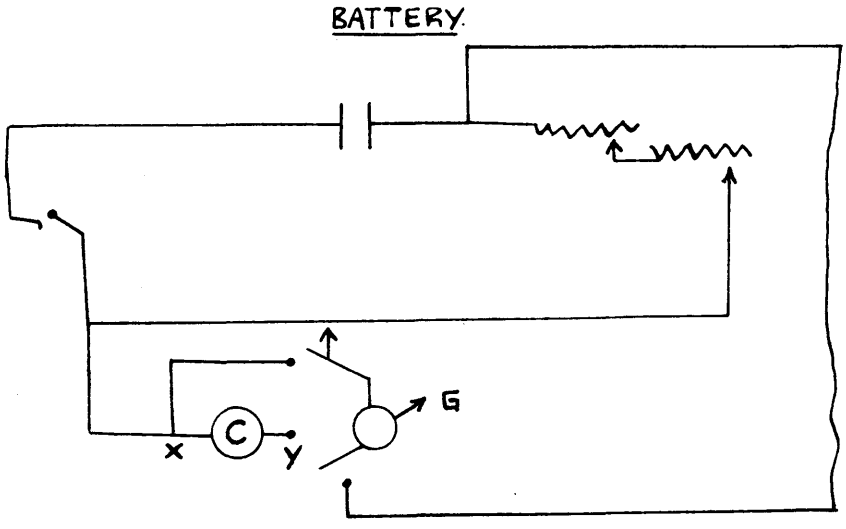
Electrometric methods of analysis have in recent years come into quite general use, particularly in the field of inorganic chemistry. Knecht and Hibbert ("New reduction methods in Volumetric Analysis") originally suggested titanous chloride for estimation of organic and inorganic compounds. In the volumetric analysis of haematein with titanous chloride, the end point was difficult to determine with accuracy on account of the turbidity of the solution and masking of the end point by the colour of the solution itself.

Titanous chloride used in an electrometric method for determining the end-point of the titration has been successfully employed in the following experiments for the estimation of haematein. The reduction was made with an excess of standard titanous chloride and the excess was determined by titration with ferric alum.

Volumetric analytical methods for haematein with titanous chloride were unsatisfactory due to colour reactions between the metal salt and the colouring matter. These colours are not as a rule alike under all conditions and special care is necessary to get an accurate end point.

Standard solutions.

A 0.25 N $TiCl_3$ solution was used for the analysis of haematein and 0.05 N ferric alum solution for the back titration. The methods of preparing, storing and using the solutions/



ELECTROMETRIC TITRATION CIRCUIT.

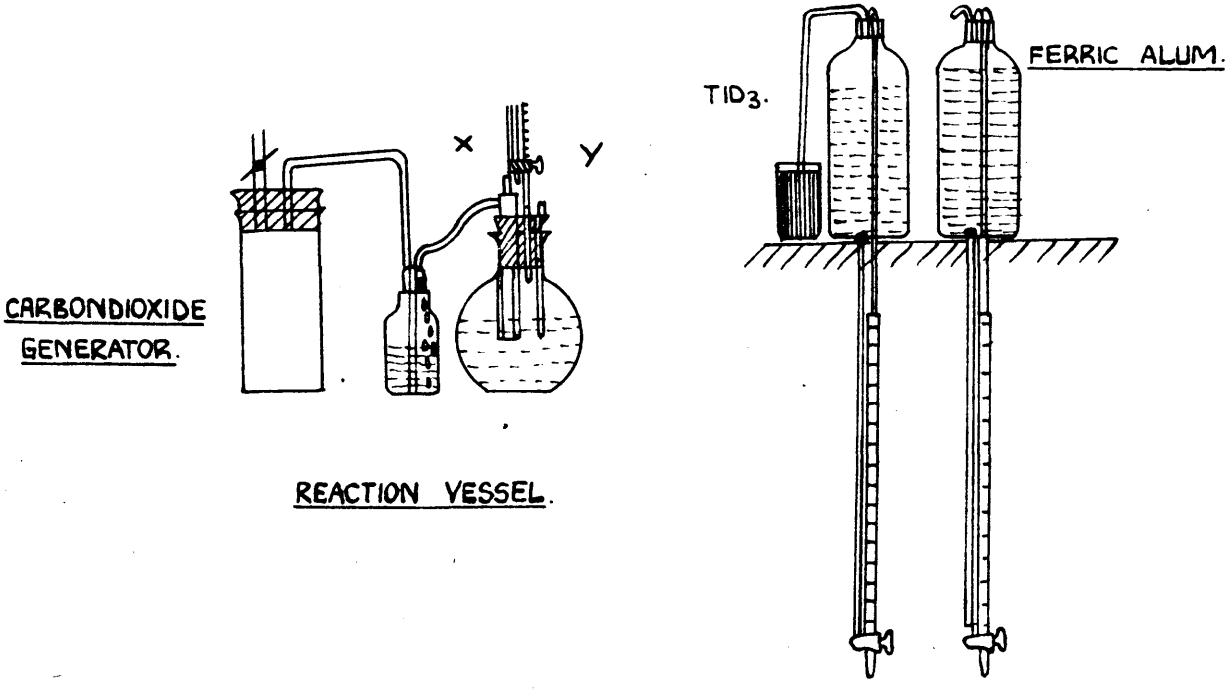


FIG:- 3

solutions have been described on page 63

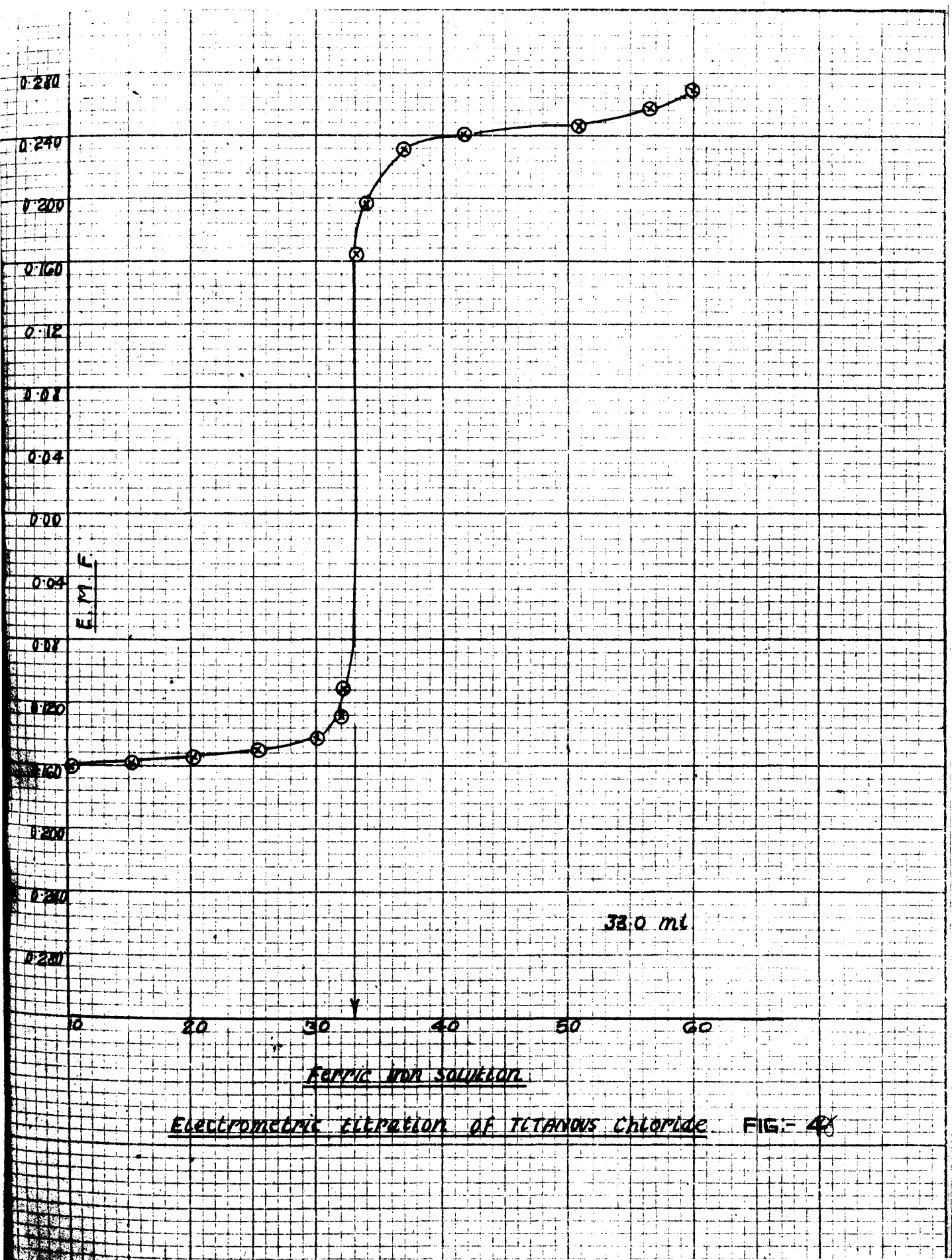
Apparatus.

For the application of the electrometric method to oxidation reactions, reference is made to the work of Hostelter and Roberts (J.Amer.Chem.Soc. (1921) 43, 91). The electrometric apparatus used in conjunction with titanous chloride is shown in the diagram. Figure 3. Carbon dioxide from the thermos flask specially arranged for the purpose was used for maintaining an oxygen-free atmosphere in the reaction flask, which consisted of a 250 c.c. flask with a rubber stopper having openings for admitting burette, thermometer and electrodes. In latter experiments, arrangements for the reaction flask were slightly modified by connecting the carbon dioxide inlet to the end of the burette and replacing the bridge type calomel electrode with a Cambridge type Calomel electrode.

Titanous chloride was standardised as before with ferric ammonium sulphate.

Analytical procedure.

Haematein (0.5 gram) was accurately weighed and pasted with cellosolve until dissolved. The solution was made up to 250 ml. with previously boiled distilled water. 50 ml. of this solution was tranferred into the reaction flask and 10 ml. of 10% Rochelle salt solution was added. The reaction vessel was stoppered and a current of carbon dioxide passed through the solution/



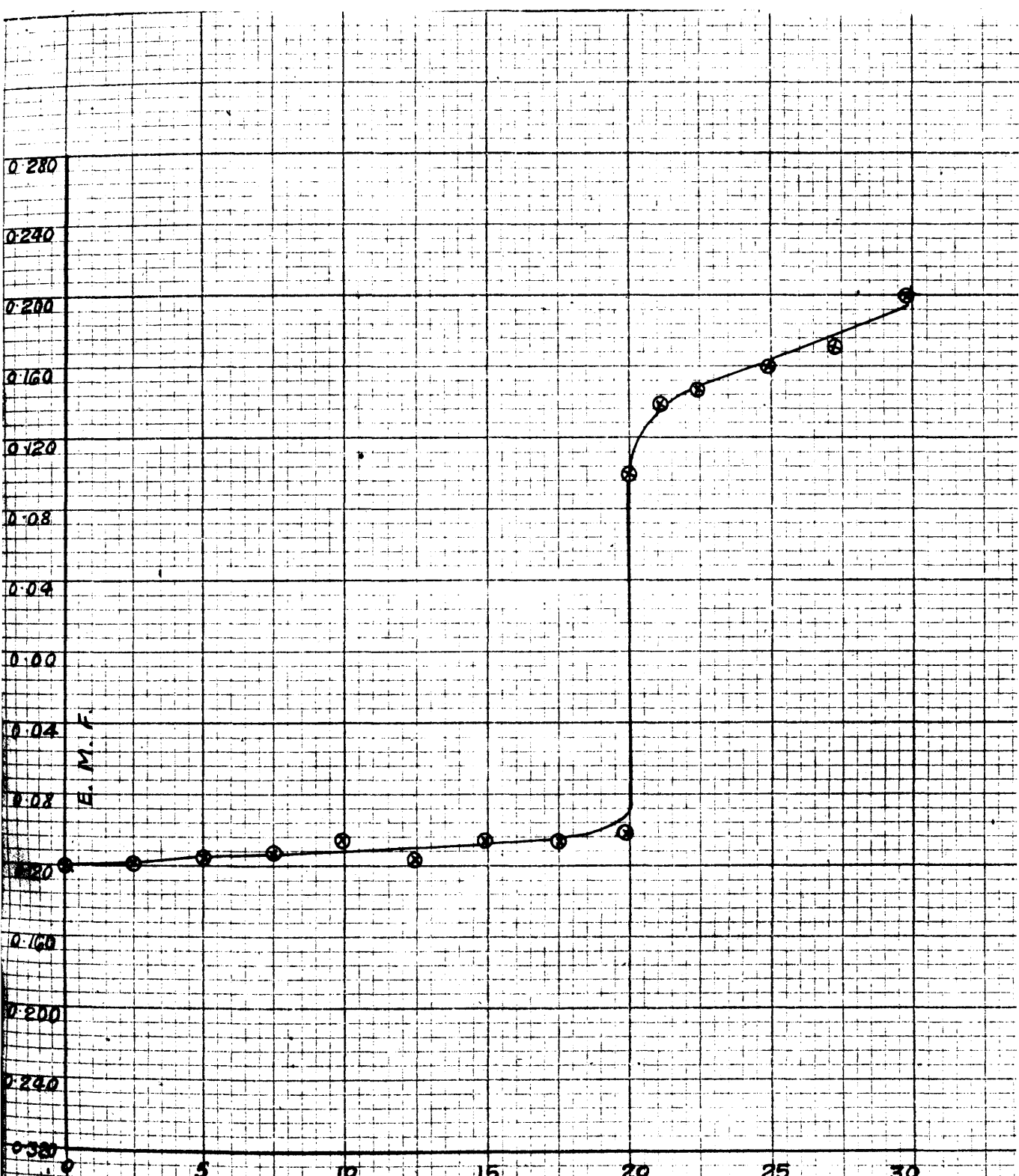
Ferric iron solution

Electrometric titration of Titanous chloride FIG:- 4

TABLE IV.

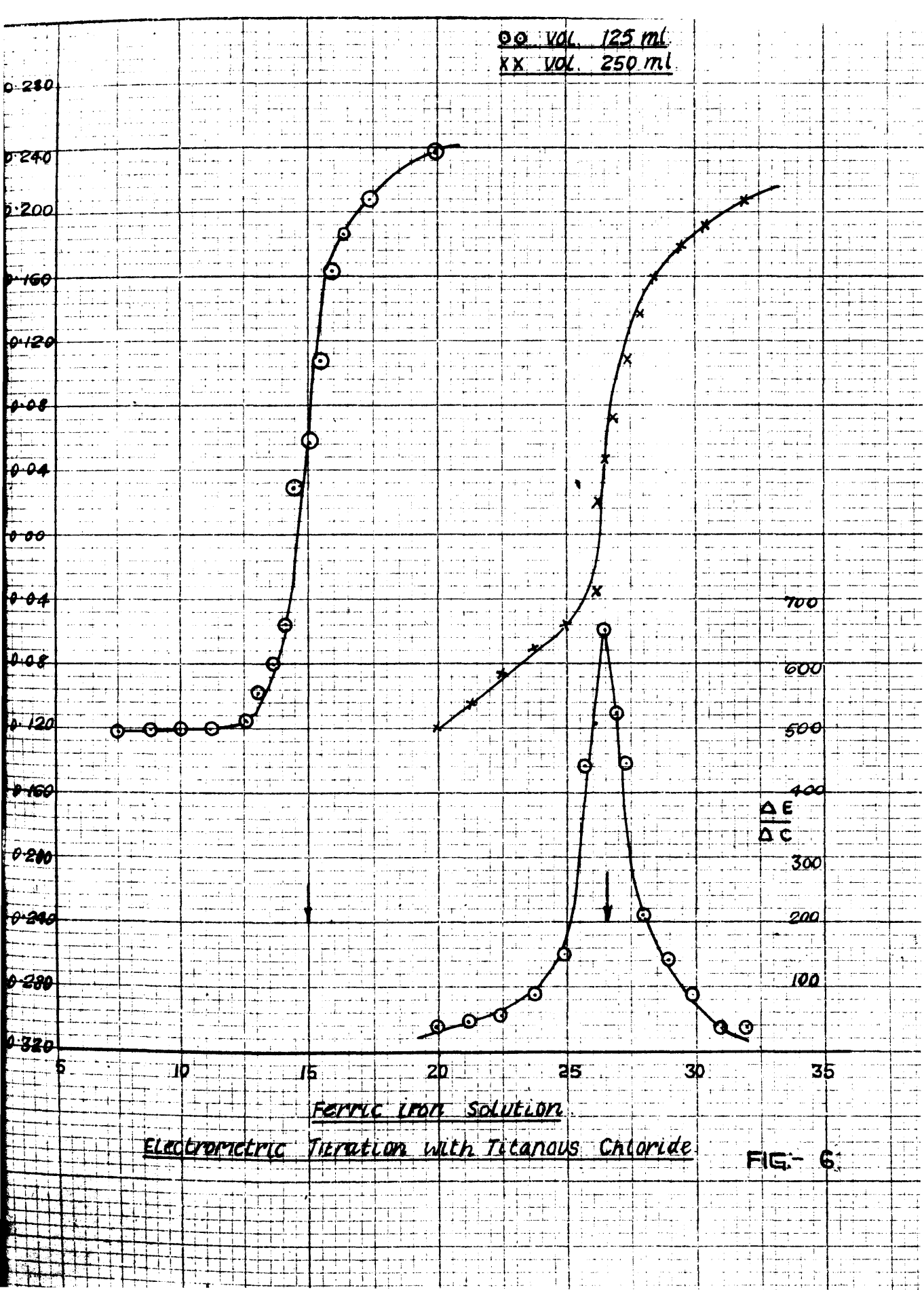
Titration of 50 ml of 0.1 % haematein in presence of titanous chloride, 10 ml of 10% Rochelle salt with E.M.F. measured against saturated calomel electrode.
1 ml of $TiCl_3$ = 0.00118 g.Fe.

"15%"				"50%"				"100%"				pure haematein			
ml $TiCl_3$	E.M.F.	$\frac{E}{C}$	Per cent haematein	ml $TiCl_3$	E.M.F.	$\frac{E}{C}$	Per cent haematein	ml $TiCl_3$	E.M.F.	$\frac{E}{C}$	Per cent haematein	ml $TiCl_3$	E.M.F.	$\frac{E}{C}$	Per cent haematein
0.0	0.425			0.0	0.425			0.0	0.425	30		0.0	0.425	20	
		60				40		0.5	0.410	20		0.5	0.415	15	
0.5	0.350			0.5	0.405			1.0	0.400	15		1.0	0.407	10	
		30				30		1.5	0.392	20		1.3	0.402	10	
1.0	0.335			1.0	0.390			2.0	0.382	10		2.0	0.397	15	
		10				20		2.5	0.377	10		2.5	0.390	10	
1.5	0.330		20.0%	1.5	0.380		34.5%	3.0	0.375	30	60.5%	3.0	0.385	20	99.5%
		150				15		3.5	0.360	30		3.5	0.375	10	
2.0	0.250			2.0	0.378			4.0	0.340	15		4.0	0.370	10	
		80				20		4.5	0.332	10		4.5	0.305	10	
2.5	0.210			2.5	0.332			5.0	0.327	175		5.0	0.360	10	
		40				140		5.5	0.312	30		5.5	0.355	10	
3.0	0.190			3.0	0.262			6.0	0.300	25		6.0	0.350	20	
						20		6.5	0.292	15		6.5	0.340	15	
				3.5	0.220			7.0	0.287	10		7.0	0.332	15	
						10		7.5				7.5	0.324	90	
				4.0	0.200							8.0	0.297	20	
												8.5	0.269	15	
				4.5								9.0	0.260		
1.60 ml				2.75 ml				5.19				7.95			



Ferric iron solution.

Electrometric titration with Titatanous chloride FIG:- 5



Ferric iron solution.

Electrometric Titration with Titanous Chloride

FIG.- 6.

used by Hostelter and Roberts in which the coincidence of the curves obtained by plotting E/V against V indicates the true end point.

Analysis of commercial and pure haematein samples carried out by following the procedure described on page 72 shows that values obtained by volumetric method are nearly the same. Detailed results are given in Table IV.

This method gives a satisfactory means of estimating the haematein present in a logwood sample.

Indirect Method.

The haematein and titanous chloride solutions were of the same strengths as used in the previous experiments. Titanous chloride solutions (20 ml.) was added to 25 ml. of 0.1% haematein solution in presence of 10 ml. of 10% Rochelle salt at room temperature and the excess of titanous chloride was then determined by titration with ferric alum, using ammonium sulphocyanide as indicator. Results are shown in Table V.

TABLE V.

Solvent	15%	50-60%	100%	Pure Haematein
Cellosolve	20.50%	34.5%	60.0%	99.7%
Acetine	20.42%	34.25%	60.35%	99.65%

This method gives good results. Near the end point longer intervals are required for the potential to become steady. Titration in presence of Rochelle salt at room temperature showed an inflection-potential of 0.250 volt against the saturated calomel electrode.

Purification of Haematoxylin.

Commercial haematoxylin was supplied by the Yorkshire Dyeware & Chemical Co.Ltd. About 100 gram of the material was dissolved in 250 ml. of water at boil. 5 grams of norit,

5/

5 ml. of sulphurous acid and 10-15 ml. of alcohol was added to the solution. Solution was filtered and haematoxylin was allowed to crystallise. Sulphurous acid prevented the oxidation of haematoxylin and alcohol kept the colouring matter in solution. Crystals were collected and further purified by following the above procedure. Purity of haematoxylin is difficult to determine as there is no record of its melting point in literature. The anhydrous haematoxylin gave M.P. 240°C. and hydrated one gave 138°C. It is difficult to keep anhydrous variety free from oxidation, since it oxidises even in a sealed air-tight glass tube; probably alkalinity of silica in glass acts as a catalytic agent for oxidation Haematoxylin used for the analysis was of hydrated variety.

Estimation of Haematoxylin.

Haematoxylin can be determined by iodine, potassium dichromate or hydrogen peroxide. In the present work it has been found that potassium dichromate gives satisfactory results by the potentiometric method and that the other two reagents are best used for the volumetric method.

Volumetric method.

(a) Iodine:- Haematoxylin reacts with iodine as follows:-

$$2C_{16}H_{14}O_6 + 2I \rightarrow 2C_{16}H_{12}O_6 + 2HI$$

As haematoxylin is extremely susceptible to oxidation, the utmost care is necessary to ensure that titration is carried out in an inert atmosphere and the haematoxylin must be dissolved in oxygen-free water. Several direct and indirect titrations with

commercial samples as well as pure haematoxylin were carried out. The method employed was to add iodine-KI solution containing sodium bicarbonate in slight excess to haematoxylin solution and then to determine excess of iodine by sodium thiosulphate. The quantity of iodine utilised by haematoxylin can thus be estimated.

To 50 ml. of 0.1% solution of logwood extract is added 2 ml. of 4 N acetic acid, 20 ml. of 2 N sodium acetate solution and excess (15 ml.) of 0.1 N iodine solution, under an inert atmosphere. The liquid is then titrated with good agitation against standard sodium thiosulphate solution, using starch as indicator. The haematoxylin solution is acidified to reduce its tendency to oxidise. Results obtained by the volumetric method are given in Table VI.

TABLE VI.

Determination of haematoxylin.

15%	50-60%	100%	Pure haematoxylin
40.5%	23.8%	0.5%	92.0%
35.85%	24.0%	0.95%	95.5%
38.75%	23.8%	0.20%	91.5%

Moisture content of pure haematoxylin - 2.5%

Potassium/

Potassium dichromate.

A 0.1% haematoxylin solution (50 ml.) at 25°C. in presence of 10 ml. of 10% Rochelle salt solution was titrated with 0.1 N potassium dichromate solution in presence of 20 ml. of 4 N sulphuric acid in an inert atmosphere. The solution becomes yellow, then dark green and, at the end point, violet. This change is very slow, particularly at the end point, and depends upon the temperature and concentration of the solutions used. Results obtained with pure haematoxylin are given in Table VII.

The volumetric method is not very satisfactory on account of the difficulty of judging the end point and a potentiometric method is found to be better.

TABLE VII/

TABLE VII

Potentiometric determination of Pure Haematoxylin with Potassium dichromate.

ml of $K_2Cr_2O_7$	E.M.F. against std. Cal. el.	$E/_C$	Percentage.
0.0	0.650	40	
0.5	0.630	30	
1.0	0.615	30	
1.5	0.600	40	
2.5	0.580	20	96.55%
3.0	0.570	20	
3.5	0.560	25	
4.0	0.448	20	
4.5	0.403	20	
5.0	0.393	20	
5.5	0.278	30	

Temperature 25°C. Inflection potential 0.488

Hydrogen Peroxide.

A weighed quantity of haematoxylin crystals is added to a known volume of hydrogen peroxide in an inert atmosphere together with potassium iodide and sulphuric acid. After shaking the flask for a minute or two the liquid is titrated against standard thiosulphate solution, using starch as indicator.

Details are given in Table VIII.

TABLE VIII

Pure haematoxylin taken	Hydrogen peroxide volume used up.	Percentage Calculated
25 ml. 0.1%	1.50 ml.	81.0%
25 ml. 0.1%	1.58 ml.	85.2%
25 ml. 0.1%	1.70 ml.	92.0%
25 ml. 0.1%	1.80 ml.	97.50%

Results in Table VIII show gradual drift in the volume of hydrogen peroxide used up each time. Drift is upward. It was found that in transferring hydrogen peroxide solution to haematoxylin solution, the latter was partly oxidised in air, giving low results. With careful and quicker transfer of the solutions and properly bubbling carbon dioxide from solidified material, this drift was checked with improved results.

In Tables I to VI, results of analysis with Titanous chloride for the commercial samples of haematein have been described. The 15%, 50-60% and 100% brands of commercial haematein show on analysis 20.4%, 34.6% and 60.5% of haematein.

Practical dyeing trials as described before show that 100% haematein crystals correspond only to about 30% pure haematein. The high results obtained on analysis of commercial haematein crystals indicate that some of the impurities present in the product must also have been reduced by titanous chloride.

Hence/

Hence it seems that the haematein content of commercial products of logwood, containing impurities, are difficult to estimate by this method. The method, however, is satisfactory as far as pure haematein and haematoxylin are concerned.

The Spectrographic analysis, as described in the following pages, throws some light on the percentage purities of commercial samples of haematein.

PART III

Section 2c.

SPECTROGRAPHIC ANALYSIS.

It was thought that a comparison of the analysis of pure haematein with titanous chloride and by the Spectrographic method would be of interest. Titanous chloride method of analysis gives a definite percentage purity of haematein, but it can never entirely displace the practical dyeing test, as it gives no information upon the quality of the colouring matters present, that is, upon the ultimate shade obtained by dyeing. The Spectrographic method, on the other hand, has the possibility of not only giving the strength of haematein but also indicating variations in quality. The subsidiary substances, which exist as impurities in commercial haematein, are the cause of variations in due and brightness on the fibre and might be expected to show up in Spectrographic measurements of solutions. The absorption spectra of pure and commercial samples of haematein in cellosolve, water and alcohol, have been examined (pH 7.0). Solutions of three commercial haematein crystals have been measured in the same way in distilled water at pH 7. The information obtained has been studied to determine whether spectral transmission values for solutions of haematein can be translated into ordinary comparative dyeing terms of strength and quality.

Materials.

The commercial samples of haematein supplied by the British Dyewood Company Limited (Glasgow), marked as haematein crystals 100% 2A, 50-60% M and 15.0% NAD were used for this purpose/

purpose.

Pure haematein was prepared as described before. Purity was determined by noting its M.P. (210°C) and optical density.

Procedure:

0.008% solutions of each sample were prepared in distilled water (pH 7.0). The absorption spectrum was taken of each, using a 1 cm. cell or 2 cm. cell as found convenient. The description of the instrument and its working are as follows:-

Apparatus:

A Hilger E.498 medium quartz spectrograph was used for all the Ultra violet absorption spectra recorded. The light incident on the slit is collimated by a quartz lens and then passes through a quartz prism of two halves, each having opposite optical rotation, to annul double refraction of the quartz. It then passes through a focussing quartz lens system and forms a spectrum on the photographic plate.

The width of the slit is controlled by a screw mechanism, carrying a graduated scale and the slit width used throughout the investigation was 0.02 mm. A shutter is situated between the slit and the collimating lens.

The photographic plate on which the spectra are recorded is 10 in. x 4 in. in size and is supported in a plate holder fitted with a shutter. The plate holder is moved in a vertical direction by means of a special mechanism, the portion

of/

of the plate undergoing exposure being shown on an external scale. An interior wavelength scale is fitted and it is possible to print the scale on the negative at any desired position.

For absorption spectra measurements, a Hilger Spekker photometer is used in conjunction with the quartz spectrograph. The photograph on page 9c illustrates the light path within the photometer which is place in front of the quartz spectrograph and is so constructed as to fit the base bar of the spectrograph.

A Spark discharge between tungsten steel electrodes supplies the ultra violet light, the electrodes being enclosed in a sound insulating box and the light emerging via a quartz window in the box. The nitric acid vapour formed inside the box during the passage of a high voltage current is removed by drawing a current of air through the sound box by means of a vacuum pump, and then through a bubbler containing alkali solution.

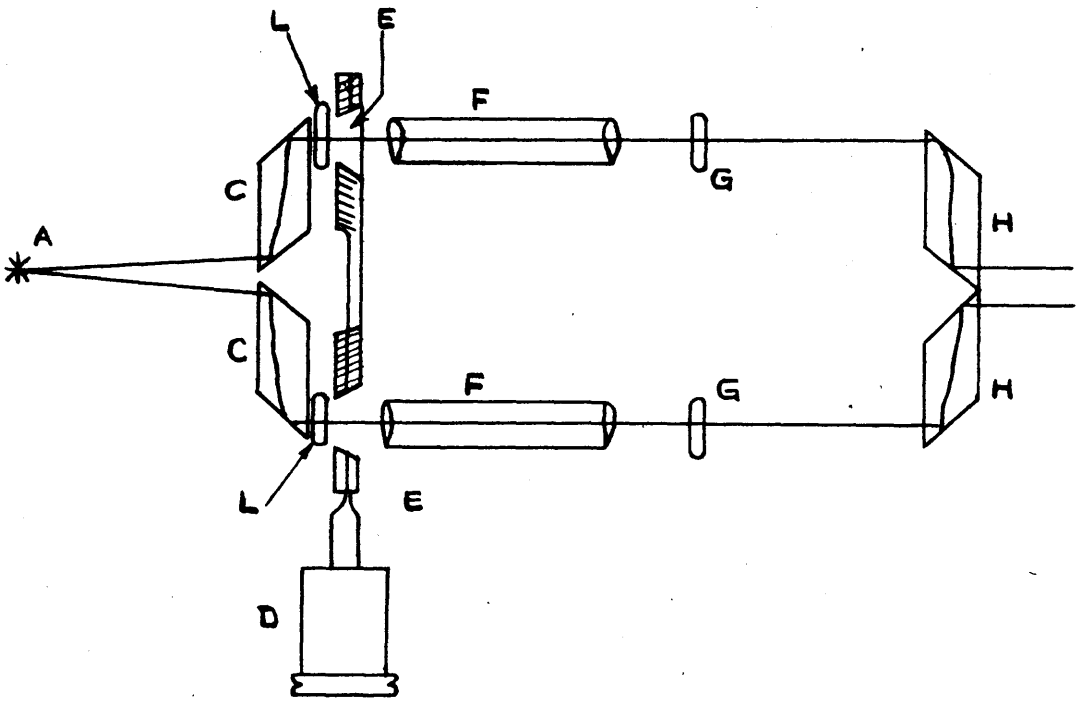
A current supply of 240 volts is stepped up to 12,000 volts by a 0.25 Kilowatt transformer, the electrical circuit including a condenser of capacity 0.005 micro-farads, connected in parallel with the Spark gap and an induction coil of 0.06 milli-henries. The electrods are filed to a wedge sharp and are placed with their edge collinear with the optical axis of the spectrophotometer and exactly 4 m.m. apart. The self inductance in the secondary circuit materially reduces the intensity of air lines/

lines due to nitrogen and oxygen.

The spark discharge gives a spectrum extending to 1850 A at the source and with many lines so that the spectrum is almost continuous. The light passes towards the inner edges of two quartz rhombs C C from which beams are diverted upwards and then downwards to be then reflected forward. The beams from the rhombs pass, one through a fixed rectangular aperture and the other through a variable aperture. The variable aperture is governed by a micrometer screw and has a direct reading scale attachment. The beams then pass through tubes F.F. in the upper of which is placed the solution and in the lower the solvent. This system eliminates erros due to air absorption and the reflection of the end plates. After passing through the solution and solvent respectively, the light beam pass through a lens G.G. of quartz, whose focal length is such that an image of the light source is formed on the face of the Spectrograph slit. A second pair of quartz rhombs H,H, are arranged to bring the beam of light together on to the slit in such a way that the image from the top rhomb and that from the bottom rhomb falling on the slit forms a complete image of the light source. The beams from the slit pass on without any vignetting into the interior of the Spectrograph, to form an image of the aperture at the prism and to be concentrated as monochromatic images of the slit at the photographic plate.

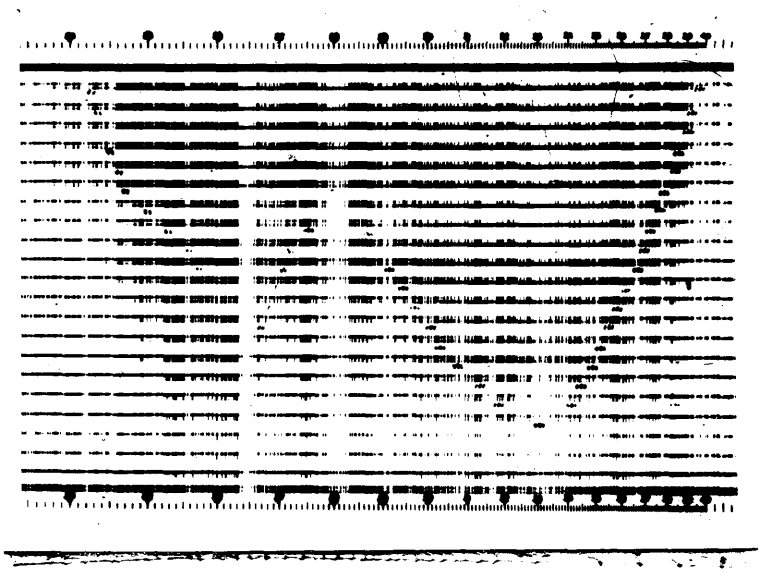
The optical construction of the Spekker photometer is

such/



A DIAGRAM OF THE LIGHT PATH IN THE SPEKKER PHOTOMETER.

Fig :- 7



ABSORPTION SPECTRA OF HAEMATEIN.

Figure 8.

such that at E,E, there are equal fluxes of uniform radiation which may be represented by I per unit area. Let E_1 and E_2 be the areas of aperture corresponding to the solution and solvent respectively, then the quantities of radiation transmitted through the aperture are E_1 I and E_2 I. After transmission through the solution and solvent, these become $(E_1 I)\ 10^{-d_1}$ and $(E_2 I)\ 10^{-d_2}$, where d_1 and d_2 are the optical densities of the solution and solvent respectively. On examination of the section of the photographic plate illustrated on page , it can be seen that each double spectrum has one or more white spots marked in it, corresponding to equal opacity of the spectra representing the solution (lower) and the solvent (upper). If E_1 is the area of fixed aperture and E_2 the area of variable aperture, then at the match point, since equal capacity indicates equal amounts of light reaching the plate, we have the relationship:

$(E_1 I)\ 10^{d_1} = (E_1 I)\ 10^{d_2}$ where d_1 and d_2 are the densities of a solution and a solvent respectively.

Then $\log 10 \frac{E_1}{E_2} = d_1 - d_2$

The drum reads $\log 10 \frac{E_1}{E_2}$ whence we get a direct reading of the density of the solution, i.e. $\log 10 \frac{I_o}{I}$ with reference to the solvent and corresponding to the wavelength at which the match point is found. From the value of $\log 10 \frac{I_o}{I}$ we can calculate the molecular extinction coefficient of each double spectrum and these values are plotted against the

wavelengths/

wavelengths of the corresponding match points.

The Hilger Spekker photometer therefore provides by simple adjustment to the proper value on the scale, a direct reading of the density of the solution corresponding to the wavelength value of the match point at that reading.

Each photographic plate conveniently records twenty-one double spectra and a comparison spectrum and a scale at top and bottom. For each plate exposed, a record of all the details is kept on special tables. The relative exposure times for each value of $\log \frac{I_o}{I}$ are as follows:

$\log \frac{I_o}{I}$	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Exposure (seconds)	0.5	0.5	0.5	0.5	0.75	0.75	1.0	1.0	1.5
$\log \frac{I_o}{I}$	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7
								1.8	1.9
Exposure (seconds)	2.0	2.5	3.0	3.5	3.5	5.0	6.5	8.0	10.0
							12.5	15.0	20.0
$\log \frac{I_o}{I}$	2.0	0							
Exposure (seconds)	25.0	0.5							

This basic scale of exposure is calculated from the expression

Exposure = "A" antilog d. Where "A" = suitable exposure for initial value of $\log \frac{I_o}{I}$ and "d" = reading on the density scale of the photometer. "A" depends on the light source, photographic/

photographic plate, the region of spectrum where the match point of the photograph in question is expected to lie and on the development conditions. The exposure required may be a simple multiple of the basic exposure scale.

Ilford auto-filter plates were used, developed with developer I.D.2 for 3.5 minutes at 18°C., then fixed, washed and dried.

The plates were spotted with the help of a specially constructed ground glass screen box, illuminated by direct light. The match points are then marked in ink on the glass side of the plate and below each double spectrum with the aid of a hand magnifying glass. A pale blue filter decreases the eye strain involved in reading the match points by this method.

Absorption spectra of pure and commercial haematein have been determined in cellosolve, ethanol and water. In Table IX are given the data for estimating the strength of a commercial sample, from the value of molecular extinction. Pure haematein representing 100% has been taken as standard for comparison with the peak of molecular extinction of other commercial haematein samples. The comparison of the strength of a dye could be done in two ways, either by plotting the transmittancy of a dye against the wavelength or by plotting molecular extinction of a dye against the wavelength.

The transmittancy of a dye solution is the quantity obtained from actual measurements on a solution of given thickness and/

and concentration at any wavelength, i.e. the ratio of the light transmitted by the solution to that transmitted by the solvent. It is used in the form of its negative logarithm because this quantity is on the basis of Beer's law, directly proportional to the product of concentration and thickness, computations being thus simplified. Curves plotted with this quantity as also represent the absorption more nearly as it appears to the eye.

Molecular extinction of a dye in different solvents would vary although the wavelength of absorption may be the same. In the present investigations, graphs have been plotted of molecular extinction of a dye against the wavelength of absorption We know that $D = E.C.1$ where D = Density

E = Molecular extinction

C = Concentration. Molar.con/lit.

1 = length of the cell.

$$\therefore \quad E = \frac{D}{C\ 1}$$

Assuming that Beer's law holds over the range in concentration at which these measurements were made, the percentage purity of a dye is calculated as follows:-

$D = E\ C\ 1$ If absorption spectra of a pure substance is taken and using the same cell and same concentration absorption spectra of a commercial sample be taken, the peak of the molecular extinction values for both would form a simple ratio giving the idea of the concentration.

TABLE

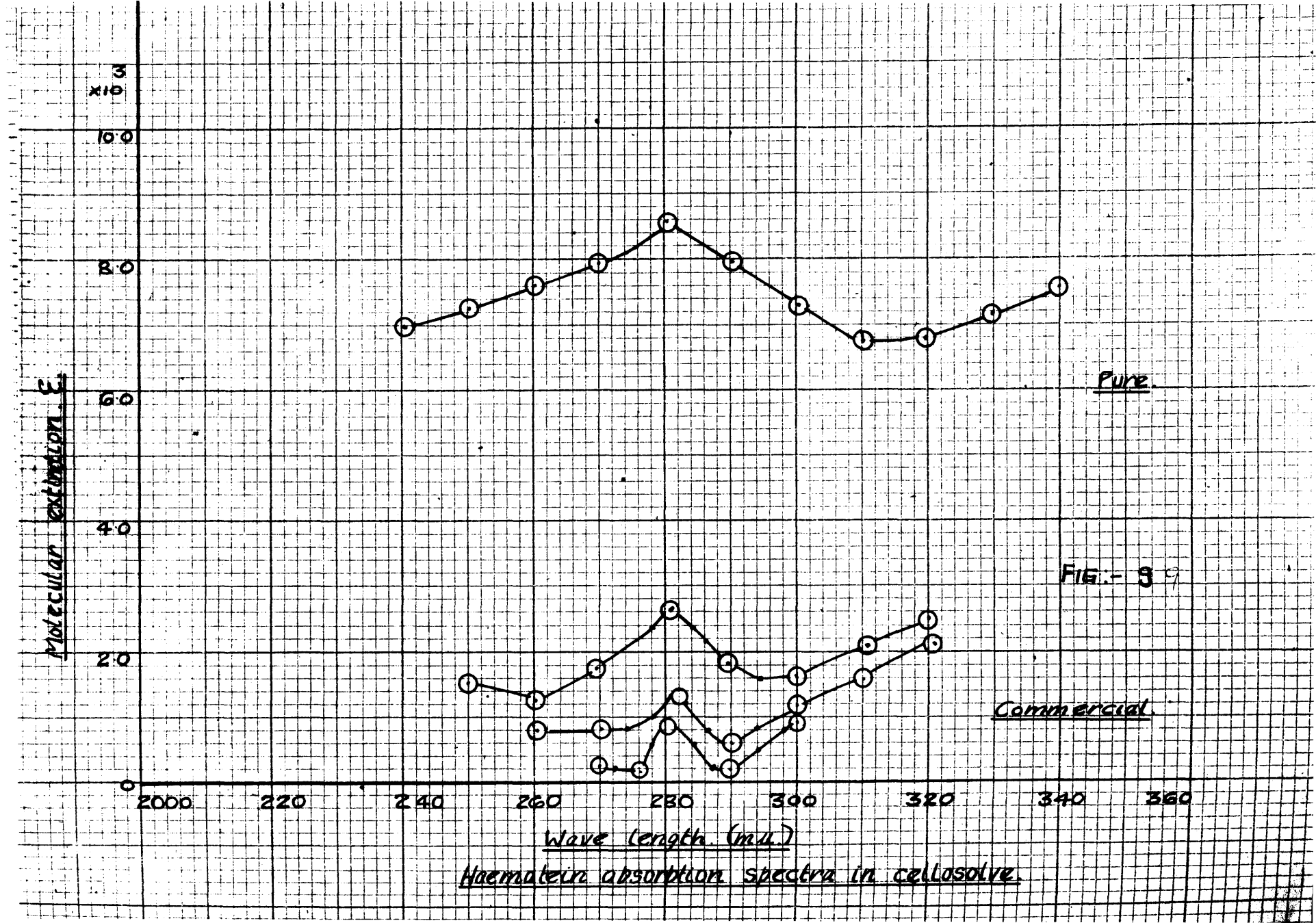


FIG.- 3

Haemalein absorption spectra in cellosolve.

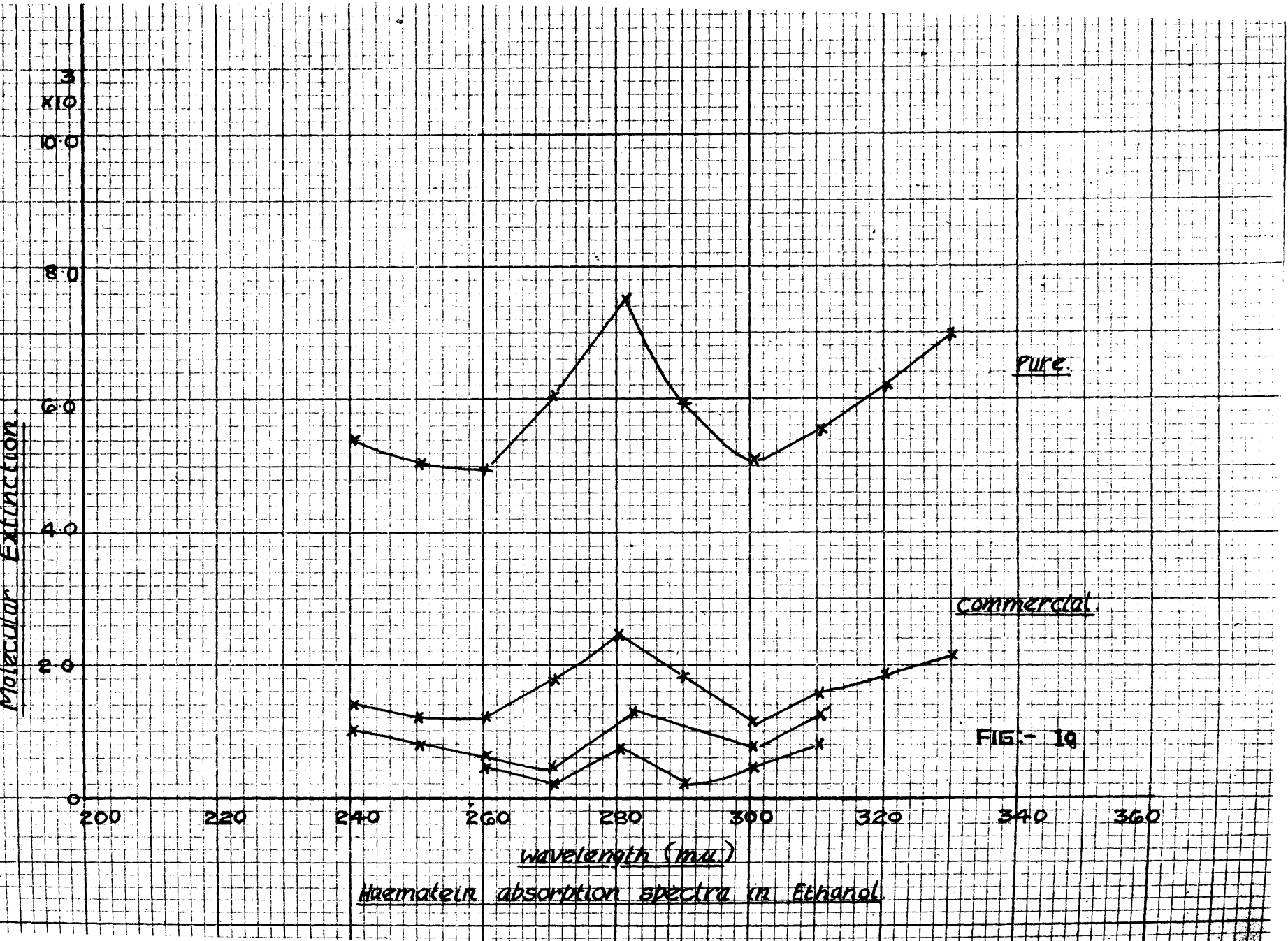


FIG:- 10

Haematein absorption spectra in Ethanol.

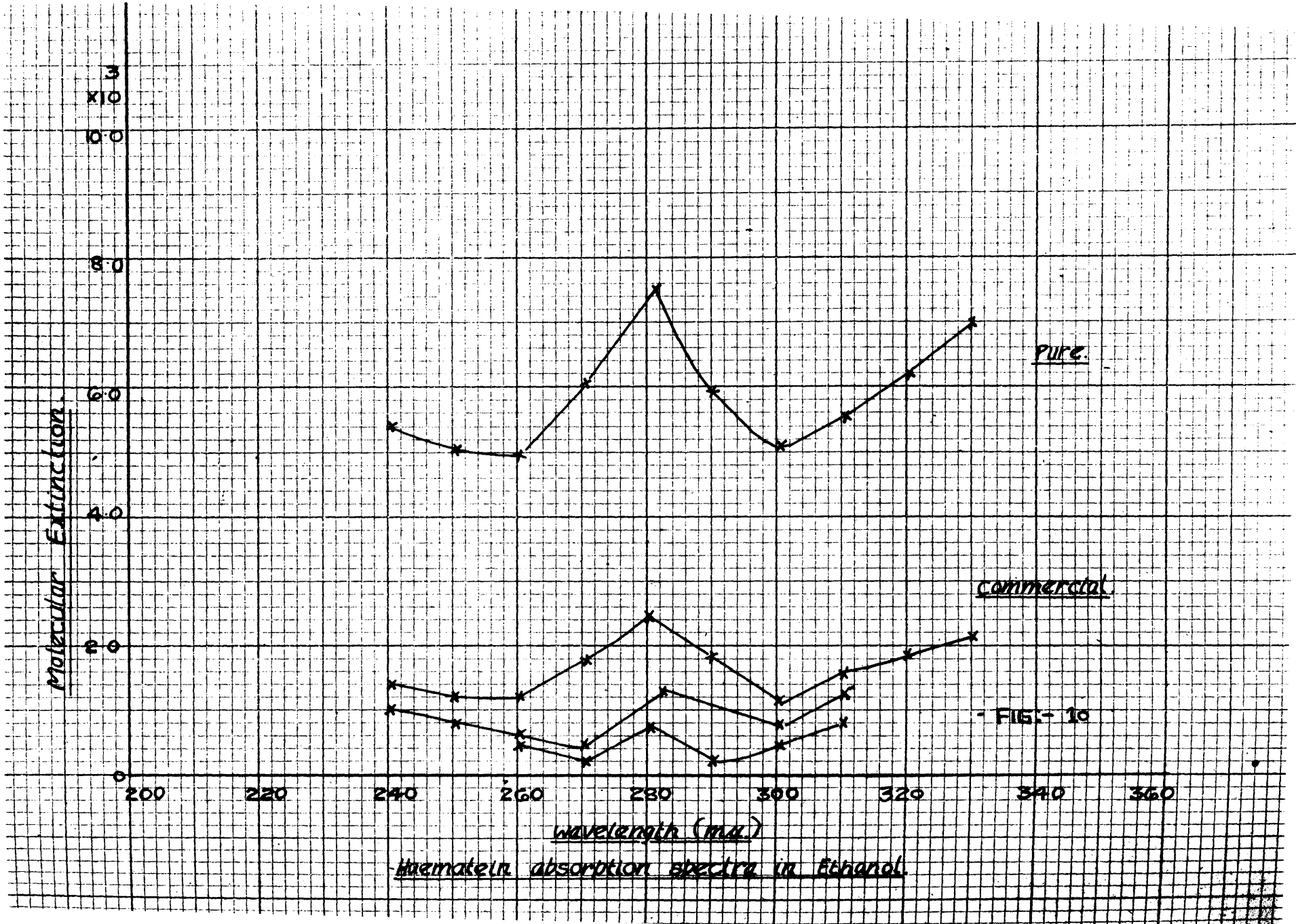


FIG:- 10

Haematein absorption spectra in Ethanol.

TABLE IX

Plate No.	Substance.	Solvent.	Conc. g./litre	Maximum λ m.μ	Molecular extinction (peak values).
1	Pure haematein	Cellosolve	0.0314	28.0	8675.0
2	15%	"	0.0314	28.2	860.0
3	50-60%	"	0.0314	28.4	1287.0
4	100%	"	0.0314	28.2	2659.0
5	Pure haematein	Ethanol	0.0237 %	28.1	7597.0
6	15%	"	0.0237 "	28.0	760.0
7	50-60%	"	0.0237 "	28.0	1140.0
8	100%	"	0.0237 "	28.0	2355.0
9	Pure haematein	Neutral water	0.08 "	28.0	1875.0
10	15%	"	0.08 "	28.0	150.0
11	50-60%	"	0.08 "	28.0	188.0
12	100%	"	0.08 "	28.0	363.0

In Table IX molecular extinctions for pure and commercial haematein have been tabulated. Since molecular extinction is directly proportional to density, which means concentration, we can write C_1 for pure haematein and C_2 for any commercial sample of haematein and compare the ratio of their molecular extinctions. The percentage purity calculated from this experiment are given in Table X.

PART III.

Section 2d.

DISCUSSION.

The commercial samples of haematein supplied by the British Dyewood Co.Ltd. (Glasgow) were used for the estimation of haematein. The haematein solution was prepared from these commercial samples and 'dipping tests' with wool fibre were carried out at different time intervals. It was observed that colour on the fabric, dipped in the haematein solution, showed a series of different colours. This observation showed the presence of impurities present in the commercial haematein and its further investigations were undertaken with a view to separating them and analysing the pure colouring matter present in the samples.

The qualitative separation of impurities and pure substances in the samples was obtained by following a chromatographic technique. This technique, however, is not satisfactory for quantitative estimations of the substances. There is a need for a suitable method of quantitative analysis of haematein in view of the importance of logwood. Results described in Section 2 can be considered under the following two topics:-

(1) Difficulties in quantitative analysis of haematein.

(2) Variation of results obtained by different methods.

(1) Difficulties in quantitative analysis of haematein.

Haematein in a pure state is sparingly soluble in water. Hence, in order to keep it in a perfectly soluble state for analysis, solvent is essential. In the present investigations, triacetin and cellosolve have been frequently used. Haematein has a complicated chemical structure with four hydroxyl groups and a quinonoid structure at the bax of the molecule. The hydroxyl groups present in the molecule forms easily a lake with the metal used for reduction. The lakes formed are coloured, and hence interfere with the end point. As haematein reduces to a pointed out colourless haematoxylin by Wainon's chloride solution, the reduction is smooth, but the reduced product oxidises with the slightest trace of air to a coloured substance. This difficulty, together with the usual property of haematein of changing colour at different pH, makes it extremely difficult for noting the correct end point.

In devising suitable titration technique with Wainon's chloride, these observations have been considered and it was found that the best results could be obtained by employing an indirect electrometric titration of haematein solution with titanous chloride, in an inert atmosphere. Use of Rochelle salt and acetine or cellosolve in the solution helps to keep the haematein in solution. The results obtained by different methods are explained in the following pages.

(2)/

(2) Variation of results obtained by different methods.

The results of the analysis of commercial and pure haematein by different methods are given below for convenience.

TABLE XI.

Estimation of haematein with titanous chloride.

Sample	Substance Haematein	Dyeing tests percentage	Methods					
			Volumetric		Electrometric	Spectrographic		
			Direct. %age	Indirect %age	Indirect %age	Water.	Eth. anol	Cell solve
1	100% comm.	28-30.0	55.0	60.0	60.2	19.4	32.1	30.2
2	50-60% "	15-17.0	25.0	34.0	34.5	10.0	14.9	15.1
3	150% "	10.0-12.0	14.0	20.0	20.1	12.5	10.0	9.97
4	Pure	100.0%	99.5	99.6	99.5	100.0		

Pure haematein was prepared by oxidising haematoxylin with air, in an alkaline condition. The estimation of haematein was carried out by four different methods.

Assuming the results of the estimation of pure haematein with titanous chloride by volumetric and electrometric titrations to be correct, the dyeing trial tests for commercial samples showed 28-30% for 100% commercial sample. The results for the other two samples/

samples were much lower. The volumetric analysis by direct titration for the same samples gave 55.0%, 25.0% and 14.0% respectively. The results are higher as compared to dyeing trial tests. More estimations were carried out by indirect titrations and although the results nearly agreed with the direct titration results, they were very much higher as compared with the dyeing trials tests. Repeated experiments by carefully controlling the inert atmosphere, during titration, keeping haematein in perfectly soluble state and adding sufficient rochelle salt to maintain the pH of the solution, showed more consistent readings for indirect titration. A further check was made by an electrometric titration of haematein solution by an indirect method. Results were found to be in good agreement. Hence it was concluded that the method was satisfactory for analysis of haematein. The dyeing trials of commercial samples of haematein showed much lower results than the titration method. This was taken to be due to some reducible impurities present in the commercial samples of haematein. Hence the method, although satisfactory for the analysis of haematein, fails for commercial samples, due to the nature of the impurities. The observation that the results are higher because of the impurities, was confirmed by taking several absorption spectra of the haematein samples. The percentage strength of haematein calculated from these results, agree well with the dyeing trials.

PART III.

Section 3

OXIDATION OF HAEMATEIN DURING NORMAL DYEING PROCESS.

Oxidation of haematein has been studied by using Spekker absorptiometer and U.V. Spectrograph. Both these instruments work on the principle of light absorption. A short theory of light absorption is included in this section.

I. SPEKKER-ABSORPTIOMETER.

In the investigations to be described, measurements were made of the optical density variations, of haematein and haematein compounds on the Spekker absorptiometer. This was carried out by studying changes in conditions such as concentration, temperature, pH, in presence and absence of inert atmosphere.

II. U.V. SPECTROGRAPH.

The degree in which the intensity of the absorption of solutions of dyes is modified by variations in concentration, solvent, alkalinity, acidity, and other conditions, is dependent upon the degree of alteration of the dye in molecular form or aggregation, which in turn, is conditioned upon constitutional differences. The quantitative measurement by Spectrograph of intensity of absorption under such variation in conditions, accordingly affords an important clue. Haematein and haematein compounds have been subjected to such spectro-analysis.

PART III.

Section 5a

THEORY OF LIGHT ABSORPTION.

Absorption or emission of light was at one time thought to take place by reason of a correspondence between the period of vibration peculiar to the molecule and the light absorbed or emitted. After the development of quantum theory and wave mechanics, considerable progress was made in interpreting spectra in the ultra violet, visible and infra red regions.

In the present, use has been made of two instruments, the photoelectric absorptiometer and the quartz spectrograph, working on the principles of light absorption.

The two fundamental laws of light absorption can be briefly formulated as follows:

Lambert's law stated that the proportion of light absorbed by a substance is independent of the incident light intensity. By light intensity is meant the quantity of light energy incident in unit area in one second (erg/cm^2/sec) or in terms of the quantum theory, the intensity of a given wavelength is the number of quanta of corresponding frequency reaching 1cm^2 in one second.

If I_0 = intensity of incident light entering the medium

I = intensity of light remaining after passing through a path of unit length and path length

$1/K$ = passage through which reduces the light intensity to one tenth of its original value

then/

$I = I_0 \ 10^{-K\ell}$

K = a constant, called the extinction coefficient, depending on the medium, and ℓ is in centimetres.

Beer's law states that absorption is proportional to the number of molecules of absorbing substance through which the light passes, i.e. to the concentration of the solution, if the solvent is assumed to be non-absorbing.

If I_0 = light entering the solution

I = light after passing through a length of solution of ℓ cm. of concentration then

$I = I_0 \ 10^{-\ell EC}$

When C is expressed in gram.molecules of absorbing substance per litre of solution and ℓ is in centimetres, E becomes a measure of absorption due to a single molecule or the molecular extinction coefficient. The expression log 10 $\frac{I_n}{I}$ is known as the density of the absorbing medium.

Deviations from Beer's law usually arise from the formation or disintegration of molecular aggregates, interaction between solute and solvent or disturbance of equilibrium between two types of absorbing molecule present in the system.

In practice, as most solutions deviate from the Beer-Lambert law, a calibration curve is prepared for the "Spekker" by taking several different concentrations of the substance and plotting against the corresponding density difference readings.

The/

The theory of Ultra-Violet Absorption Spectra.

The correlation between the colour of an organic compound and its structure has interested chemists almost from the beginning of organic structural formulae. It was very early recognised that some sort of unsaturation was necessary, in order that a compound may be coloured, i.e. absorb visible light. (Graebe, C., and Liebermann, C. Ber., 1868, 1, 106). This idea was extended by O.N.Witt (Ber, 1876, 9, 522) who called certain unsaturated groups chromophores, with the stipulation that their presence was required for a compound to be coloured. Further, he designated another set of groups generally saturated, i.e. containing no double bonds, as auxochromes and their presence enhanced the chromphoric properties of the chromophores. These ideas of unsaturated chromophoric and auxochromic character, have undergone many modifications and developments, until in recent years, they have been combined in the theories of Dilthey and Wizinger. (Wizinger, R.K. Organische Farbstoffe Ferd. Dummlers, Verlag. Berlin V. Bonn, 1933).

It is now known that the absorption of light by organic compounds in any region but the far ultra violet, is certainly associated with the phenomenon of resonance in systems containing multiple bonds (Bury, C.R. K.Amer.Chem.Soc. (T)$\frac{51}{A}$ 1935, 2115). This is especially true of dyestuffs which contain many double bonds and aromatic groups, in conjugation and a number of attempts have been made to calculate the absorption spectra.

PART III.

Section 3b.

STUDY IN THE OXIDATION OF HAEMATEIN DURING THE NORMAL DYEING PROCESS WITH SPEKKER-ABSORPTIOMETER.

The absorption of haematein by wool and nylon can be measured by determining the optical density of the dyebath, before and after dyeing. The change in the optical density measured on a Spekker-absorptiometer is thus a measure of percentage dye absorption. The optical density of the dyebath should remain constant, if the dye is not absorbed by the fibres.

During the course of investigations regarding the absorption of haematein by wool and nylon, it was observed that the optical density had changed and it gradually increased with the increase of time and temperature. This effect was noticed, in spite of pH remaining constant. This increase in the optical density of the dyebath -liquor showed anomalously high values.

In order to determine the cause of this peculiar behaviour of haematein, standard haematein solutions, at various pH values, were prepared with buffer solutions. These solutions of haematein at different pH were kept under reflux for several hours at 95°C. in a water-bath. The optical density of each of these solutions was determined initially on the photoelectric absorptiometer and then measured at five-hourly intervals. The experiment was continued for 48 hours.

A/

bubbling through the liquor. Samples from each of these flasks were taken out every five hours, and their optical density immediately examined by the Spekker absorptiometer, using the filter Ilford No. 608. Readings are given in Table XIII.

Experiments were similarly carried out with brazilian, *Iso-haematein, *Chloro-haematein and *bromo haematein. Readings obtained on the photoelectric absorptiometer have been given in Tables XIV to XVII, Figures 13-16.

* Compounds were prepared by Mr. D.J.Duff in this laboratory.

SPEKKER-ABSORPTIOMETER WITH ROTARY CONVERTER FROM D.C. TO A.C.

Figure 11a.

TABLE XI I

CHANGE OF HAEMATEIN SOLUTIONS WITH TIME IN PRESENCE OF AIR.

	Optical Density.					
Time hr.	pH 10.13	pH 8.91	pH 7.20	pH 6.10	pH 4.46	pH 2.18
0	0.250	0.200	0.050	0.130	0.180	0.170
2	0.800	0.550	0.130	0.180	0.200	0.181
4	1.230	0.800	0.190	0.190	0.215	0.191
6	1.190	0.950	0.230	0.210	0.220	0.210
8	1.020	1.020	0.270	0.200	0.230	0.200
10	0.860	1.000	0.310	0.210	0.240	0.180
12	0.720	0.910	0.320	0.215	0.245	0.185
14	0.550	0.750	0.330	0.225	0.250	0.188
16	0.410	0.610	0.340	0.230	0.261	0.192
18	0.280	0.420	0.340	0.245	0.272	0.199
20	0.210	0.230	0.339	0.260	0.280	0.220
22	0.130	0.150	0.338	0.260	0.285	0.219
24	0.080	0.120	0.339	0.259	0.285	0.220
26	0.040	0.080	0.340	0.258	0.290	0.221
28	0.040	0.050	0.338	0.260	0.289	0.219
30	0.040	0.040	0.340	0.250	0.290	0.220
40	0.040	0.040	0.339	0.249	0.310	0.170

Concentration: 0.05 gram per litre.
Spekker absorptiometer: Spectrum Red No.608
Temperature 22°C.

TABLE XII

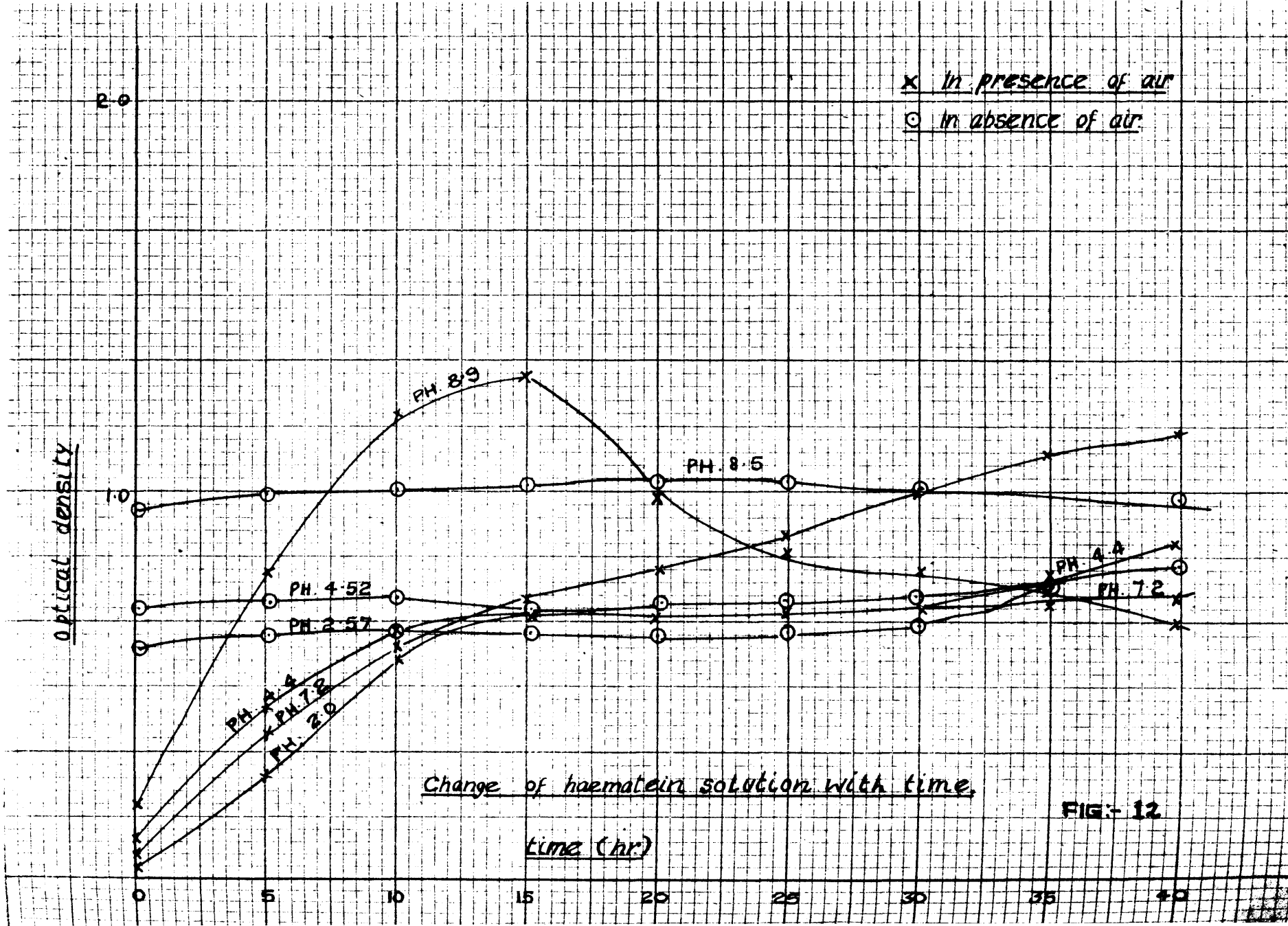
CHANGE OF HAEMATEIN SOLUTION WITH TIME IN PRESENCE OF AIR.

	Optical Density			
Time hr.	pH 8.9	pH 7.2	pH 4.4	pH 2.0
0	0.200	0.070	0.100	0.050
5	0.810	0.370	0.460	0.280
10	1.210	0.620	0.650	0.600
15	1.320	0.680	0.690	0.730
20	0.990	0.680	0.700	0.800
25	0.850	0.695	0.720	0.900
30	0.820	0.701	0.740	1.00
35	0.750	0.715	0.800	1.10
40	0.680	0.730	0.850	1.15
45	0.580	0.750	0.930	1.170
48	0.530	0.770	0.950	1.180

Concentration of haematein solution: 0.01 g. per litre.

Spekker absorptiometer: Spectrum Red No. 608

Temperature 95°C.



Change of haematein solution with time.

FIG:- 12

TABLE XIII.

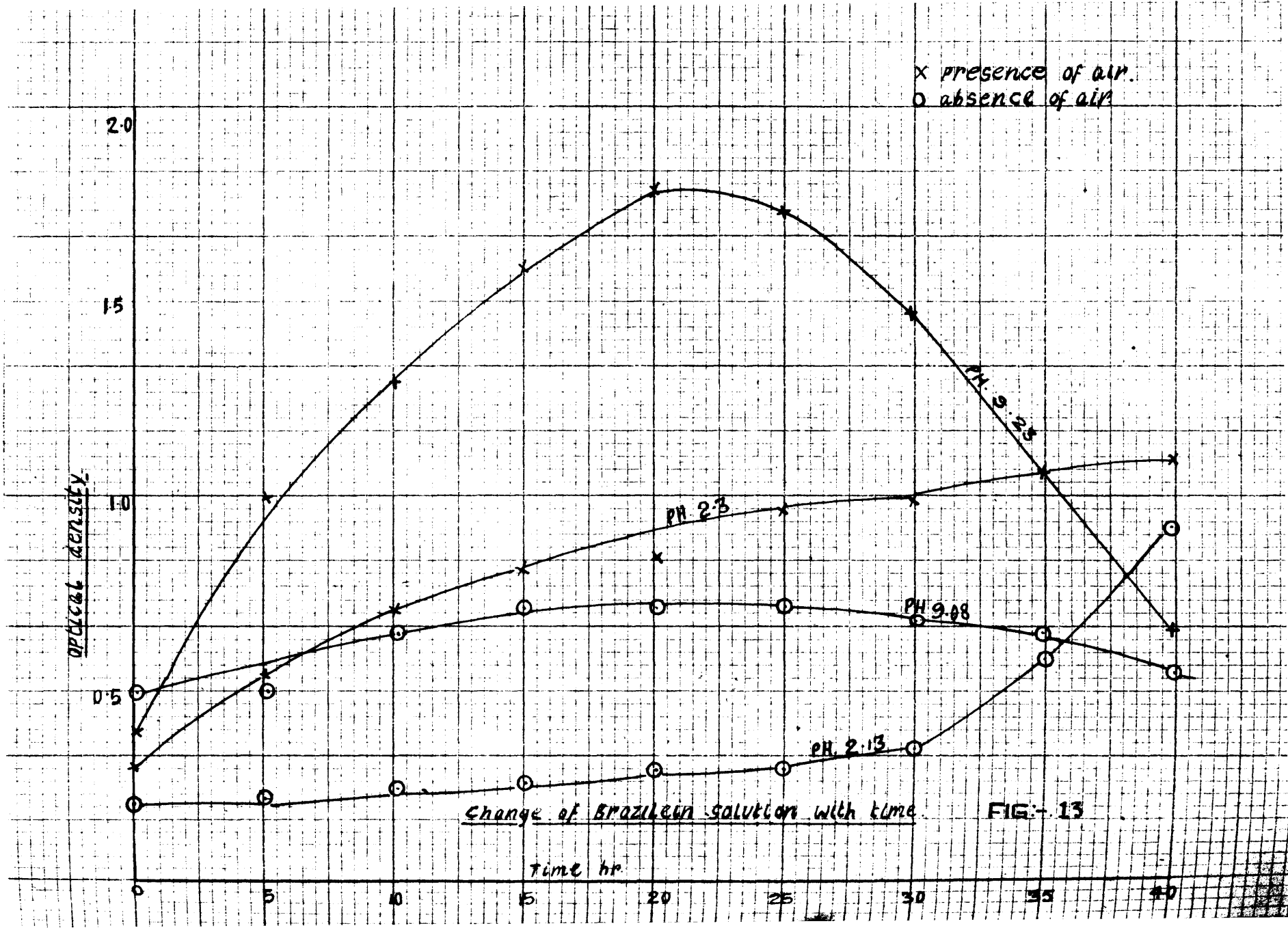
CHANGE OF HAEMATEIN SOLUTION WITH TIME IN ABSENCE OF AIR.

	Optical Density		
Time hr.	pH 8.5	pH 4.52	pH 2.57
0	0.970	0.720	0.610
5	1.00	0.750	0.670
10	1.02	0.770	0.710
15	1.035	0.780	0.730
20	1.05	0.785	0.751
30	1.05	0.785	0.751
35	1.04	0.788	0.760
40	1.015	0.850	0.810
50	0.790	0.960	0.940

Haematein solution concentration: 0.1 g. per litre.

Spekker absorptiometer: Spectrum red No. 608.

Temperature 90°C. to 95°C



Change of Brazilein solution with time. FIG:- 13

TABLE. XIVA

CHANGE OF BRAZILEIN SOLUTION WITH TIME IN PRESENCE OF AIR.

Time hr.	Optical Density pH 9.23	pH 2.3
0	0.4I0	0.3I0
5	I.050	0.55I
I0	I.350	0.720
I5	I.60I	0.823
20	I.8I0	0.85I
25	I.750	0.98I
30	I.49I	I.030
35	I.090	I.083
40	0.690	I.I2
45	0.350	I.I4
50	0.I70	I.I5

Brazilein solution concentration: 0.05 gram/litre

Spekker absorptiometer: Spectrum Red No. 608.

Temperature 95° C.

TABLE XV.

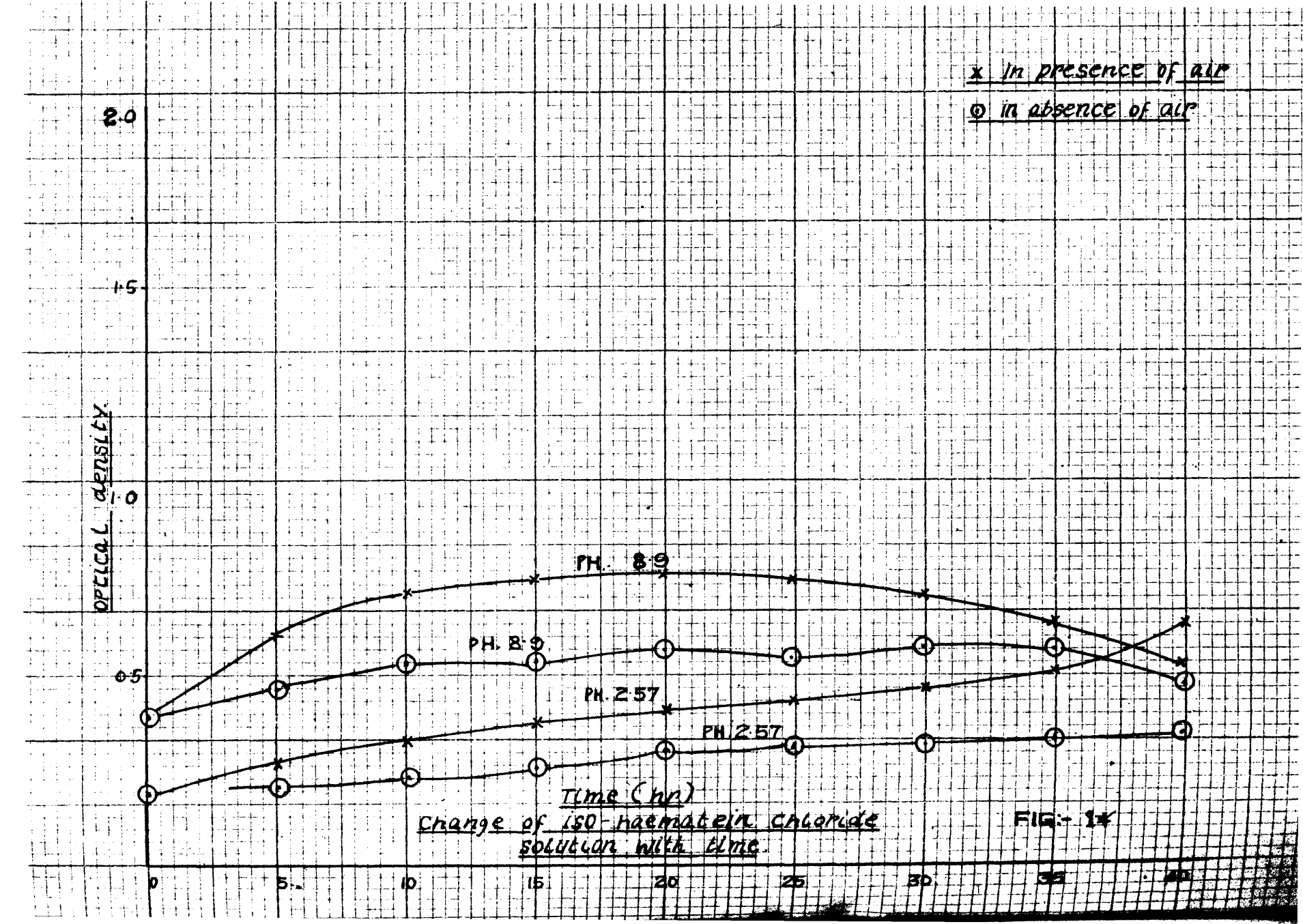
CHANGE OF ISO-HAEMATEIN CHLORIDE SOLUTION WITH TIME.

Time hr.	Optical density			
	In presence of air.		In absence of air.	
	pH 8.9	pH 2.57	pH 8.9	pH 2.57
0	0.401	0.203	0.401	0.203
5	0.666	0.266	0.504	0.204
10	0.730	0.330	0.533	0.266
15	0.810	0.401	0.541	0.264
20	0.801	0.403	0.533	0.301
25	0.733	0.409	0.535	0.300
30	0.700	0.503	0.540	0.330
35	0.608	0.508	0.530	0.320
40	0.503	0.609	0.466	0.330
45	0.450	0.710	0.466	0.330

Iso-haematein chloride solution: 0.05 g. per litre.

Spekker absorptiometer Spectrum Red 608.

Temperature 85°C.



Change of iso-haematein Chloride solution with time.

FIG:- 1*

TABLE XVI

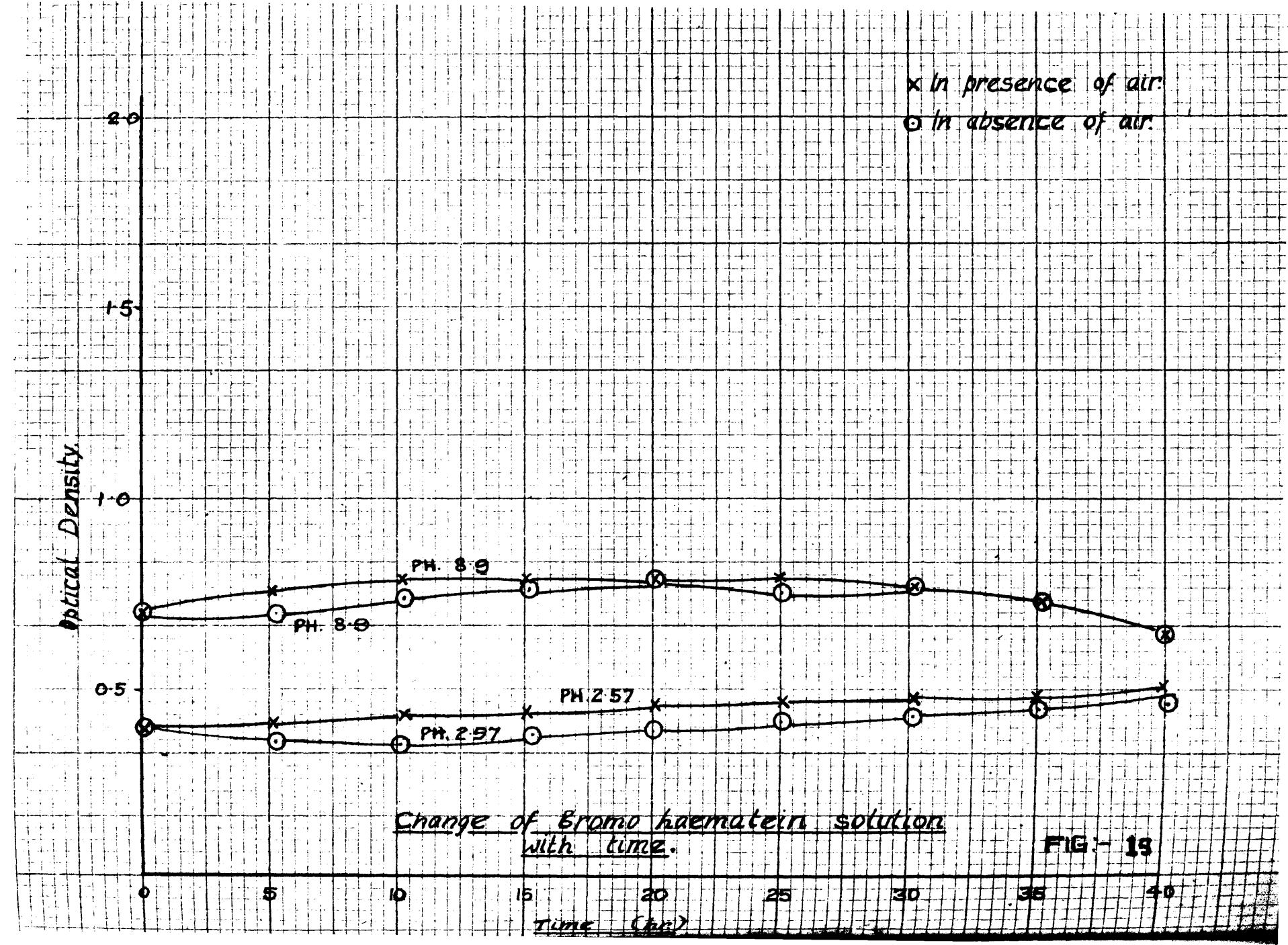
CHANGE OF BROMO-HAEMATEIN SOLUTION WITH TIME.

Time Hr.	Optical Density			
	In presence of air.		In absence of air.	
	pH 8.90	pH 2.57	pH 8.90	pH 2.57
0	0.710	0.408	0.710	0.408
5	0.720	0.410	0.712	0.360
10	0.715	0.430	0.730	0.330
15	0.800	0.435	0.720	0.350
20	0.801	0.460	0.810	0.401
25	0.800	0.465	0.733	0.408
30	0.715	0.468	0.715	0.433
35	0.720	0.466	0.725	0.460
40	0.668	0.530	0.670	0.531

Bromo-haematein solution: 0.05 gram per litre.

Spekker absorptiometer. Spectrum red 608.

Temperature: 85°C.



Change of Bromo haematein solution with time.

FIG:- 19

TABLE XVII

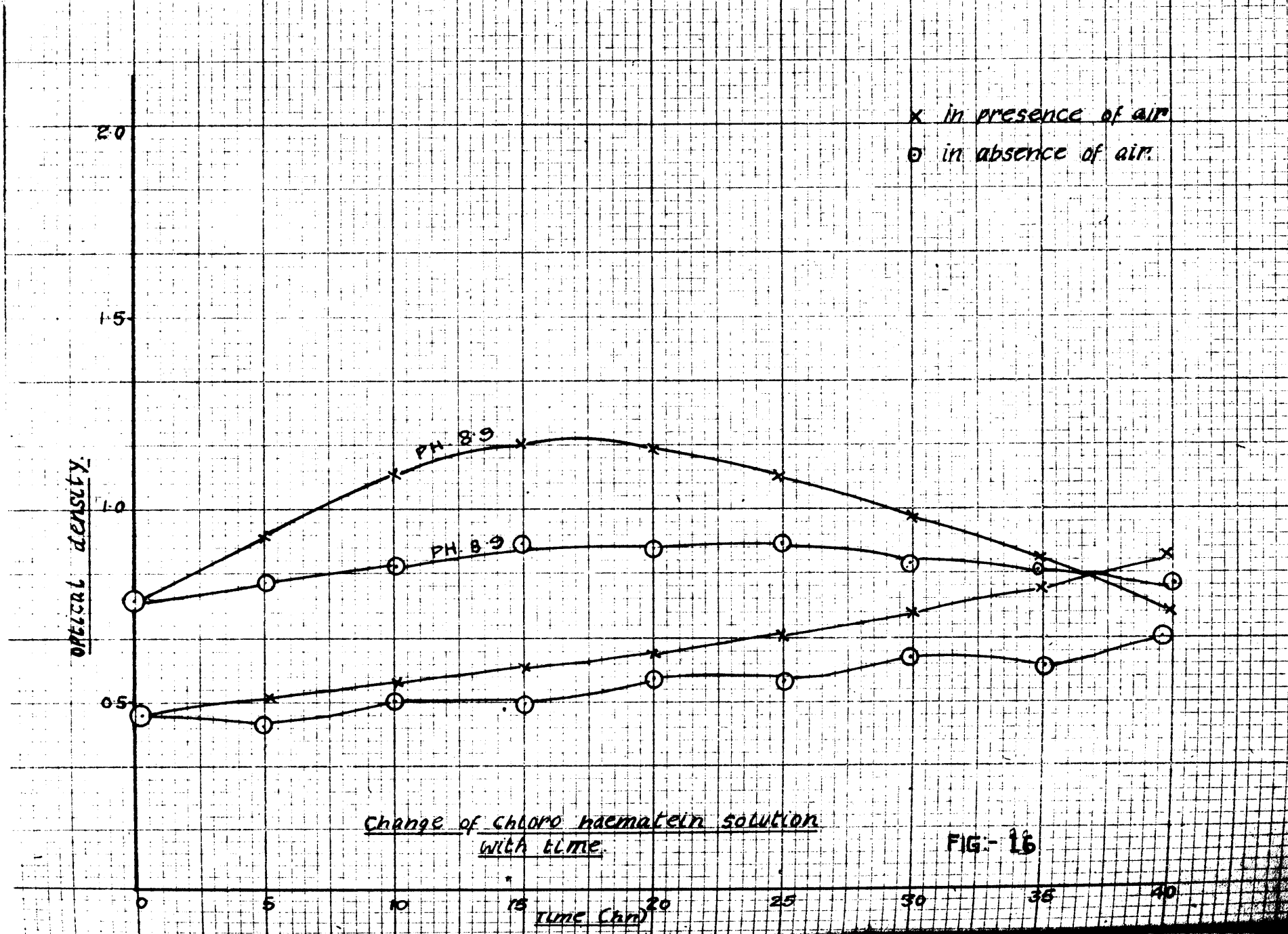
CHANGE OF CHLORO-HAEMATEIN SOLUTION WITH TIME.

Time hr.	Optical Density			
	In presence of air.		In absence of air.	
	pH 8.90	pH 2.57	pH 8.90	pH 2.57
0	0.760	0.503	0.760	0.501
5	0.930	0.533	0.830	0.433
10	1.105	0.550	0.886	0.510
15	1.200	0.600	0.900	0.520
20	1.160	0.633	0.903	0.560
25	1.10	0.660	0.901	0.533
30	1.013	0.733	0.866	0.600
35	0.860	0.833	0.833	0.540
40	0.730	0.910	0.800	0.633

Chloro-haematein solution: 0.05 gram per litre.

Spekker absorptiometer. Spectrum red. 608.

Temperature: 85°C.



Change of chloro haematein solution with time.

FIG:- 16

PART III.

Section 3c.

OXIDATION OF HAEMATEIN AND HAEMATEIN COMPOUNDS.

Ultra Violet Spectrograph.

A Hilger E.498 medium quartz spectrograph was used for all the absorption spectra recorded. A description of the apparatus and its working has been given on page 87 .

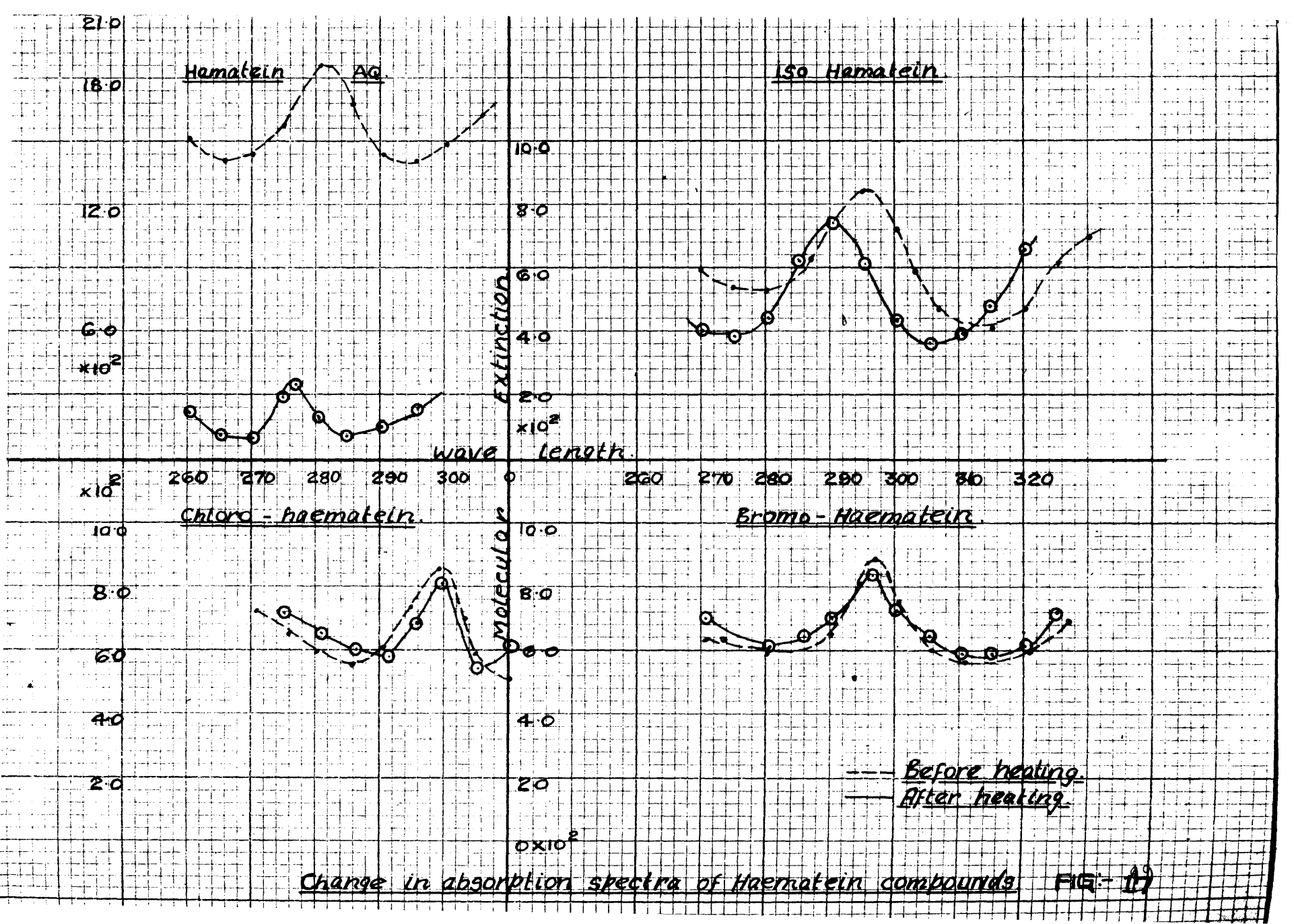
Method of preparation:

0.008 gram of the substance was dissolved in 100 ml. of boiling distilled water. 50 ml. of this solution was placed in a 250 ml. round bottomed flask fitted with a ground glass condenser. The solution was heated to 85°C. for 50 hours in a thermostat. The absorption spectram of the solution was recorded before and after heating.

The compounds were all examined under the same conditions of pH, temperature and volume.

Absorption spectra of the substances have been recorded in Table XVIII, illustrated by Figure 17.

TABLE ___/



Change in absorption spectra of Haematein compounds FIG:- 17

TABLE XVIII

CHANGE IN ABSORPTION SPECTRA OF HAEMATEIN AND HAEMATEIN COMPOUNDS WITH TIME.

Spectra Number	Substance	Before heating.		After heating	
		Wave Length - A	Molecular Extinction Coeff.	Wave Length - A	Molecular Extinction Coeff.
1	Haematein aq.	2800	1875.0	2758	365.0
2	Haematein Alcoho.	2856	7597.0	2790	2100.0
3 *	Iso-haematein	2950	8215.0	2900	7500.0
4 *	Chloro-haematein	2990	8532.0	2900	8148.0
5 *	Bromo-haematein	2965	8950.0	2960	8798.0

* Compounds were prepared by Mr. D.J. Duff.

CONCLUSIONS.

Study in the oxidation of haematein by the Spekker absorptiometer and U.V. Spectrograph suggest that haematein is most susceptible to degradation under normal dyeing conditions. Experiments on the haematein compounds show marked stability under similar condition.

Bromo-haematein, whose molecular extinction coefficient remains practically unaltered even after prolonged heating, shows good resistance to degradation. This is also shown on Spekker where bromo-haematein has been examined for different pH values.

Iso-haematein shows change in molecular extinction coefficient which means change in the concentration. The absorption spectra is displaced towards the lower wavelength.

PART III.

Section 3d.

DISCUSSION.

The experimental observations recorded in this section could be considered under the following three topics:-

(1) Effect of oxidised haematein on dyeings.

(2) Behaviour of haematein solution under inert atmosphere.

(3) Improved stability to oxidation of haematein derivatives.

(1) Effect of Oxidised haematein on dyeings.

The change in colour on heating of haematein solution was noticed while carrying out experiments of quantitative absorption of haematein on wool. This change was measured on Spekker-absorptiometer. The usual colour change, due to variation in pH of the haematein solution, was checked by maintaining the pH of the solution. It was observed that the change in colour of the solution continued, in spite of pH remaining constant. However, this variation was retarded by heating the solution in an inert atmosphere. It was concluded therefore, that the change in colour of the solution of haematein on heating was due to the gradual oxidation of haematein, giving rise to some coloured products.

It will be seen (Section 4) that the dyeings of haematein/

haematein on wool, when dyed in a normal way, differ in shade and light fastness from that of one dyed in an inert atmosphere. The improvement in light fastness, noticed when dyed as above, has a great practical significance.

The brightness and hue of haematein dyed fibres surpasses that of practically every synthetic dye, but the low light fastness of haematein has been the main cause of its gradual fall in the dyeing trade. Further investigations of this observation would have a great effect on the current method of dyeing haematein;

(2) Behaviour of haematein solution under an inert atmosphere.

The behaviour of haematein solution to heating was studied by Spekker-absorptiometer and U.V. Spectrograph. It will be seen from the results described in this section that the haematein solution changes colour on heating. The change is not uniform as seen from Table XII. In case of solution having higher pH, i.e. on the alkaline side, the colour changes to a maximum value in about ten hours and then rapidly falls. This type of change occurs for pH 8-10. The change is slow for a neutral or acidic solution. There is no rapid fall of the optical density for an acid solution. The effect of colour change in the solution has been observed at lower temperature. The change has been found to be lower than at higher temperature. The variation in optical density has been descr ibed in Table XI.

However/

However, when the solutions are examined for the same temperature, pH and concentration, under an inert atmosphere, the variation in the optical density, becomes steady and is practically uniform for a heating period of 30-35 hours continuously. These observations are illustrated by Figures 11 and 12, where the changes have been shown in presence and absence of air. The optical density of a solution, heated for different lengths of time, at a fixed pH, has been found to be uniform, when examined under an inert atmosphere. The results of U.V. Spectrograph have shown that molecular extinction coefficient of the oxidised haematein at a fixed pH decreases, and thus the strength of haematein goes dowm. The absorption of oxidised haematein takes place at a lower wavelength as compared with haematein which is absorbed at 28 m . The results of absorption spectra are given in Table XVIII for haematein and haematein derivatives.

(3) Improved stability to oxidation of haematein derivatives.

The main defects of haematein has been its poor light-fastness as compared with synthetic chrome blacks. This fact has attracted attention of several workers. Duff prepared several haematein derivatives, such as Iso-haematein chloride, chloro haematein and bromo-haematein with the hope of examining the light fastness properties of their derivatives and comparing them with haematein. The author has taken the opportunity of studying/

studying them on Spekker absorptiometer and U.V. Spectrograph for their stability towards oxidation under normal dyeing conditions. The absorption spectrographs of these derivatives are given in Table XVIII illustrated by Figure 16.

It will be seen from the tables XV-XVII that iso-haematein, chloro-haematein and bromo-haematein are more stable than haematein under similar conditions. In Figure 15, stability of bromo-haematein has been illustrated. It will be seen that it is extremely stable. The results of absorption spectra confirm this observation. It can be expected from this behaviour of bromo-haematein that its light fastness would be better than haematein dyed under normal dyeing conditions. The dyed samples of these derivatives are being subjected to exhaustive light-fastness tests.

PART III.

Section 4.

DYEINGS WITH HAEMATEIN AND ITS LIGHT FASTNESS.

Preliminary observations:

The observations made in this section are of a qualitative nature and are supposed to give only a guidance to the quantitative experiments carried out in the following sections. The dyeings have been carried out with wool fibre.

Haematein is sparingly soluble in water. Freshly prepared solution of haematein in water (0.01%) when left overnight forms an aggregation of the dye particles with slight turbidity. The capilliary rise (Garner, J.S.D.C., 1933, 346), of a dye from an aqueous solution on a filter paper gives an idea of the particle size. This filter paper test was carried out for haematein solution. It was found that capillary rise of a haematein solution was much lower than a capilliary rise noted for water on filter-paper. This is supposed to be due to bigger size of the dye particles. When haematein is pasted with cellosolve, warmed and then dissolved in water, the solution of haematein keeps clear for a longer time. Wool fibre dyed in haematein solution at boil retains haematein which is not removed even by pyridine extraction.

Several rough experiments were carried out by dyeing haematein on wool with a view (a) to show the nature of haematein affinity/

affinity towards fibres and (b) to show the properties of various groups present in the haematein molecule..

(a) In order to get an idea about the affinity of haematein towards wool, some typical dyeings were carried out by following the procedure below.

Procedure: 0.5 g. of pure haematein was pasted in cellosolve and the solution made to 100 ml. with distilled water. 5.0 g. of wool fibre (pre-treated as described on page 140) was placed in a 500 ml. flask and the dye solution was added to it. The addition of acid, tannin etc. was made and the total volume of the bath was brought to 250 ml. The time of dyeing was varied and after the dyeing was over, sample of the dyebath liquor was taken out, brought to an alkaline pH value and measured on the Spekker. The samples of wool taken out from the bath were rinsed and mordanted with 5.0% potassium dichromate and 6.0% tartaric acid and washed thoroughly. The experiments carried out by the above procedure showed the results below:

(1) Haematein can be dyed on wool from a neutral bath but the degree of exhaustion of the bath is poor.

(2) Exhaustion of the bath can be improved by increasing the pH.

(3) Better exhaustion can be obtained by dyeing for a longer time and at a higher temperature, i.e. 60°C.

(4) Chlorinated wool has a better affinity for haematein than normal wool.

(5)/

(5) Affinity of haematein is less for bleached wool or with the addition of tannin in the bath.

(b) In order to get an idea about the groups responsible for dyeing properties in the haematein molecule, typical dyeings were carried out following the procedure given below.

Procedure:

0.5 g. of substance was dissolved in water and transferred to a 500 ml. conical flask containing 5.0 g. of pre-treated wool (page 140). The total volume in the flask was brought to 250 ml. at pH 2.5, by addition of water and acid. Dyeing continued for one hour at boil. The fabric after dyeing was rinsed and mordanted with 3.0% potassium dichromate, 6.0% tartaric acid, washed and dried.

It was observed from the experiments carried out by following the above procedure that patterns after dyeing showed different shades. Haematein dyed in a normal way showed reddish deep blue colour while haematein dyed in a carbondioxide atmosphere showed much deeper colour. Haematoxylin dyed in carbondioxide atmosphere showed no colour, while one dyed on an oxidised mordant showed colour. Unmordanted wool showed no absorption towards crude methylated haematein, whereas mordanted wool showed absorption. The colour on the fibre obtained after mordanting was found to be bluish black.

It was concluded from the above series of experiments that/

that:

(1) Haematein dyed on wool in a normal way has a different shade in reflected light from that dyed in an inert atmosphere.

(2) Haematoxylin and tetra methyl haematocylin are not absorbed by wool.

(3) Crude methylated haematein is not absorbed by unmordanted wool but dyes mordanted wool.

These observations were useful in planning the experiments carried out in the following sections.

Recently a series of light-fastness tests on haematein obtained from different sources have been carried out. The results are of particular interest for the present investigations as it is interesting to note the improvements in light-fastness properties of haematein dyed under different conditions. The results of light-fastness for the other haematein compounds studied in Section 3 are still awaited.

"Fastness" is a term inseparably connected with textile finishing and applied more especially to "fastness to light." The degree of fading of a dyeing, apart from air moisture, is dependent upon the quantity of light that falls upon it. The fastness to light is standardised by denoting (1) the lowest and (8) the best fastness to light.

The figures given in Table XIX are interpreted as follows:-

1/

1	- poor	6	- very good
2-3	- moderate	7	- excellent
4-5	- good	8	- maximum.

* FASTNESS TESTS.

Tests were carried out by exposing the dyed samples to direct sunlight in Jamaica for 250 hours and fastness measured on fastness scale designed by Society of Dyers' and Colourists.'

TABLE XIX.

Sample	5.0 g. of wool fibre mordanted with 3.0 potassium dichromate, 6.0 tartaric acid and dyed with the following at boil for 1 hour.	Fastness.
1	0.5 g. haematein, 1 hr.boil passing carbon dioxide	8.0
2	" (from pure haematoxylin by H_2O_2 oxidation)	7.0
3	" (from red paste)	7.5
4	" + 0.2 g. tannin	6.5
5	" 4 hr.at boil	6.5
6	" haematoxylin	3.0
7	" 100% oxidised logwood extract	4.5
8	" crude methylated haematein(CH_2N_2)	2.0
	Unmordanted wool	
9	0.5 g. haematein (frem red paste)	1.75
10	" haematoxylin	2.0
11	1.0 g. crude methylated haematoxylin + 1.5 ml. 10V H_2O_2	2.25
12	0.5 g. crude methylated haematein (CH_2N_2)	2.0

* D.J.Duff. Private communication.

It is seen from Table XIX that pure haematein has an excellent fastness to light when dyed under normal conditions and it is improved when dyed in a carbon dioxide atmosphere. This suggests that during the normal dyeing process the oxidised product formed has a deteriorating effect. The commercial haematein has a moderate fastness as seen for sample number 8.7.

Haematein dyed on an unmordanted wool has a poor fastness but when dyed on a mordanted wool, the fastness is excellent. The presence of tannin and fustic which are very often associated in commercial dyeings to improve shade, lowers the fastness value.

UNMORDANTED FIBRE.

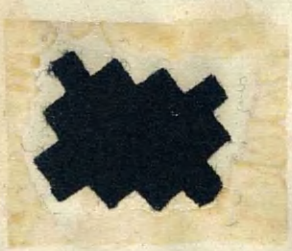
Haematein on nylon.

Haematein on wool

Haematoxylin on wool

Methylated haematein on wool

MORDANTED WOOL.



Haematein dyed in a normal way

Haematein dyed in an inert atmosphere.

Iso-haematein chloride

Bromo haematein.

DYEINGS WITH HAEMATEIN. Figure 18.

PART III.

Section 5.

EXPERIMENTAL.

EFFECT OF pH ON THE ABSORPTION OF HAEMATEIN AND BRAZILEIN BY WOOL AND NYLON.

Experiments were carried out to find the amount of haematein and brazilein absorbed by wool and nylon with variation in pH value at a fixed concentration and temperature.

Materials:

(i) Woollen fabric was scoured in a 3% solution of commercial soap flakes in distilled water at 65°C. for half an hour. It was then rinsed, first in hot water and then in cold water and allowed to condition at laboratory temperature for a week.

(ii) Nylon fabric was scoured in a bath containing 3 g. soap and 1 cc. ammonia per litre in distilled water at 65°C. and then thoroughly rinsed first in hot water and then in cold water and allowed to condition at laboratory temperature for a week.

Dye:

Haematein and Brazilein were examined for their purity by optical density and by titanous chloride. Purity found: 99.5%.

Distilled water was used throughout the work.

Procedure:

Woollen fabric (1 g.) and Nylon (0.5 g.) was cut into small pieces and weighed out. The two fibres were placed respectively in two 500 ml. flasks fitted with reflux air condensers. To each flask was added 250 ml. of haematein solution, prepared/

prepared by accurately weighing 1 g. of haematein (99.5%) and pasting it with cellosolve (ethylene glycol monoethyl ether), then warming and diluting the solution to one litre with hot distilled water. Varying amounts of N. Sulphuric acid and water were added to the dyebath, making the total volume to 400 ml in each flask. The flasks were kept in a water-bath at 95°C. ± 3°C. The dyeing in the case of woollen fabric was continued for 6 hours and in the case of Nylon, which takes a longer time to reach equilibrium, it was continued for 18 hours.

The pH of the dye bath in each flask was determined at the end of the experiment by the Cambridge pH meter, using a standard saturated Calomel electrode and a glass electrode.

The amount of haematein on the woollen fabric could not be determined by ordinary stripping methods because a small amount of haematein still remained attached to the woollen fabric even after extraction with alcohol, pure pyridine or aqueous pyridine. The haematein present in the dyebath was therefore determined by the Spekker absorptiometer after dyeing and the amount present on the fabric was calculated by difference.

The absorptiometer was calibrated by preparing standard haematein solutions of different concentrations and making these solutions slightly alkaline by the addition of dilute ammonia. This was necessary because the colour of haematein varies with the pH of the solution. Calibration curves for these standard

solutions/

solutions were then obtained using the violet filter Ilford.601.

Woollen fabric was removed from each dyebath by filtering through glass wool, then washed with distilled water, the dye liquor and washings collected and made just alkaline by the addition of dilute ammonia. These solutions were then examined on the Spekker using filter 601. The concentration of the haematein present in each dyebath was calculated from the Calibration curve. The amount of dye absorbed by the fibre was then determined by difference. With Nylon the difficulty of extraction did not exist. Haematein absorbed by Nylon could easily be determined by examining the solution of the dyed nylon in m -cresol. A comparison of this value with the optical density of a solution containing a known amount of haematein then gave the quantity of haematein present on the fibre. The pH of the dyebath was determined as in wool after dyeing. Standard haematein solution for calibrating the Spekker was made with "Analar" m -cresol and the Calibration curve was obtained using filter 608.

The full results are given in Table XX-XXIII illustrated by figures 19-20 The initial concentration of the dye in the dye bath is expressed in grams of standard dye per litre, the residual dye in gram molecules of pure dye per litre of liquor, and the dye on the fibre in milli molecules of pure dye per gram of the fibre.

TABLE /

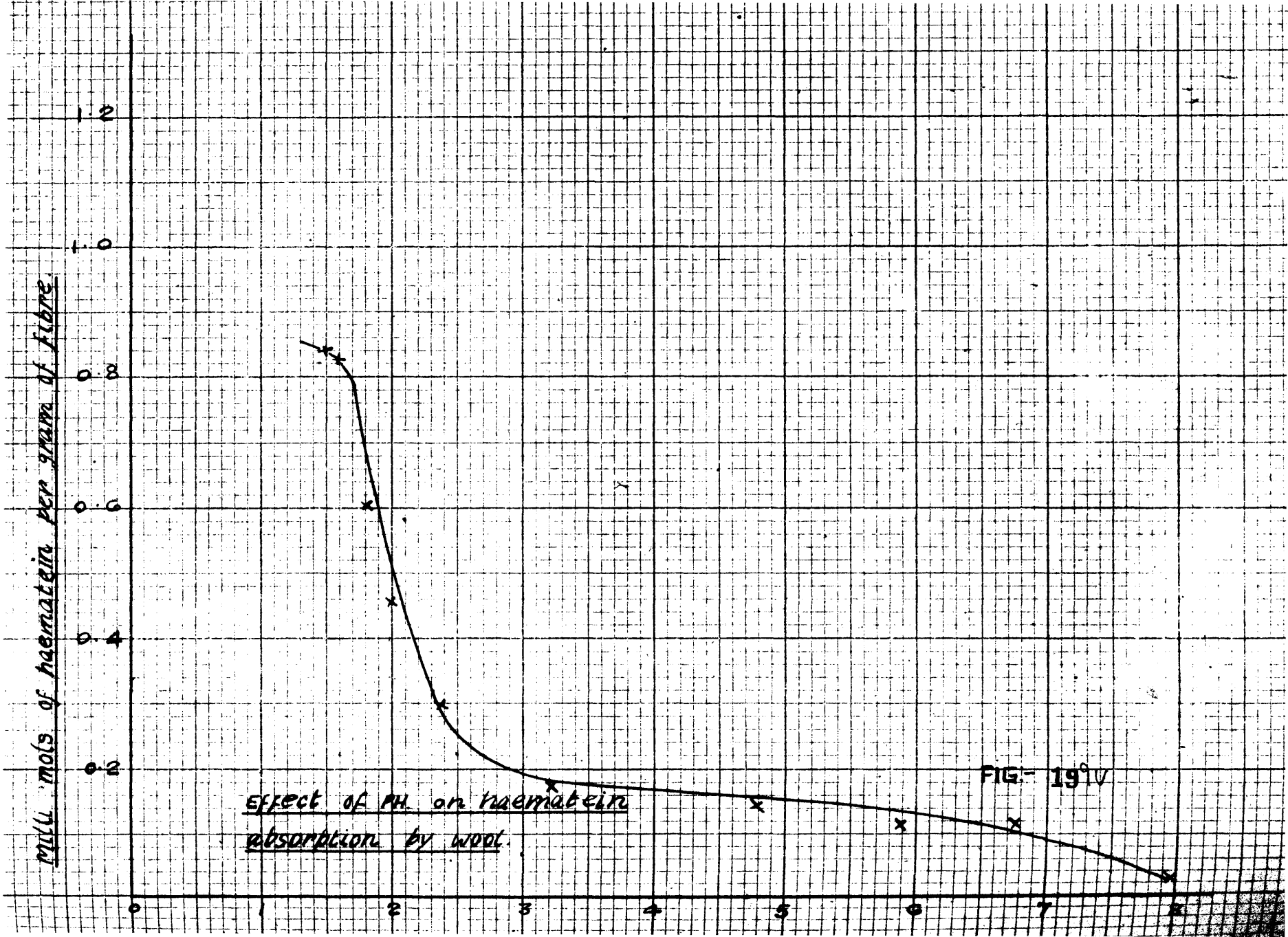


FIG:- 19 V

Effect of PH on haematein absorption by wool.

TABLE XXI.

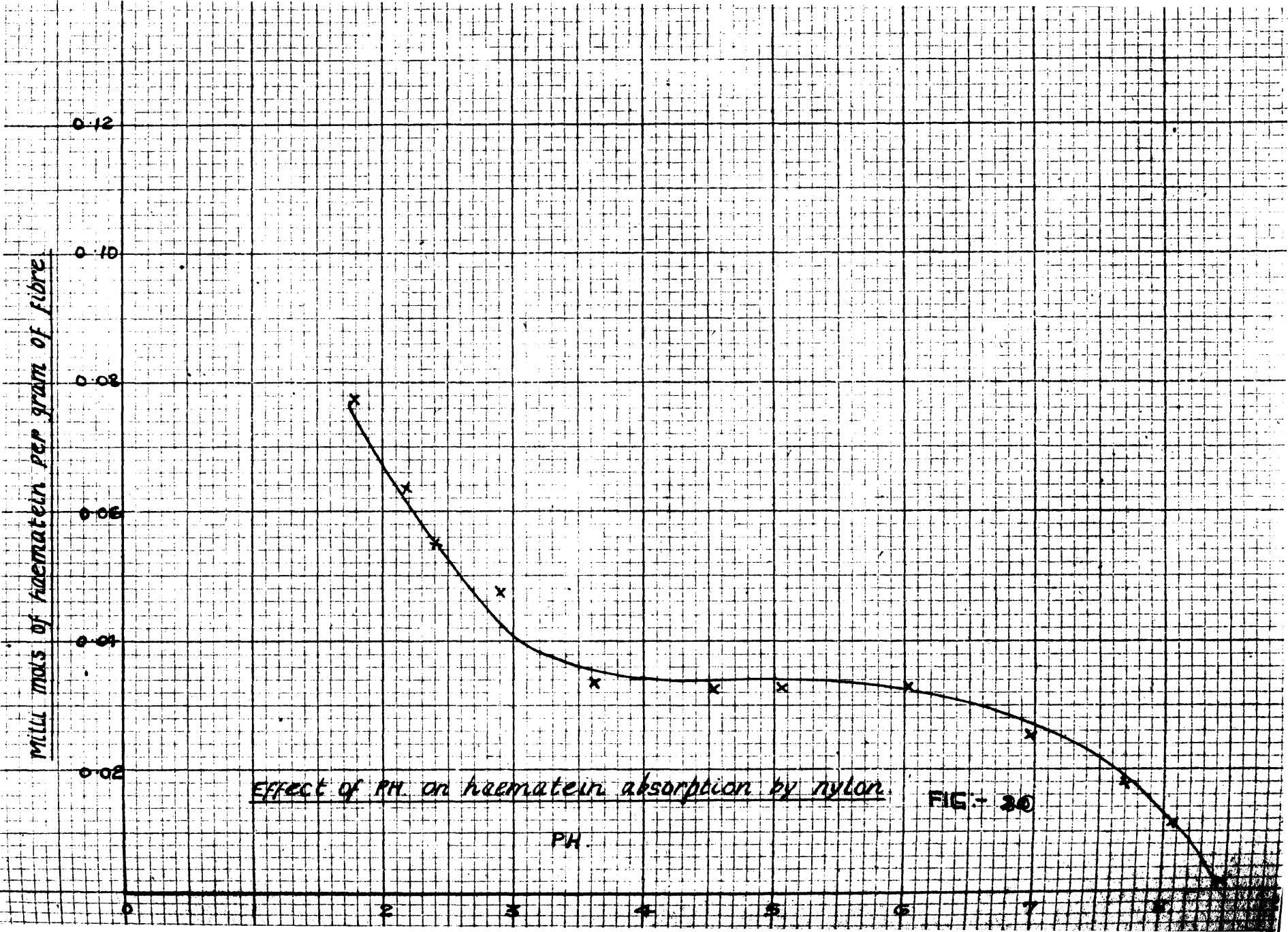
EFFECT OF pH ON HAEMATEIN ABSORPTION BY NYLON.

Final pH of the dye bath.	Spekker Readings	Haematein in the bath.	Haematein on the fibre(Fig).	Milli mols of haematein per gram. of fibre.	Gram mols per litre in the bath
8.51	0.170	0.249	3×10^{-4}	0.002	3.32×10^{-3}
8.12	0.242	0.248	18×10^{-4}	0.012	3.31×10^{-3}
7.71	0.368	0.247	28×10^{-4}	0.019	3.29×10^{-3}
7.01	0.486	0.246	39×10^{-4}	0.026	3.28×10^{-3}
6.10	0.620	0.245	50×10^{-4}	0.033	3.27×10^{-3}
5.13	0.620	0.245	50×10^{-4}	0.033	3.27×10^{-3}
4.51	0.620	0.245	50×10^{-4}	0.033	3.27×10^{-3}
3.62	0.620	0.245	50×10^{-4}	0.033	3.27×10^{-3}
2.91	0.841	0.243	72×10^{-4}	0.048	3.24×10^{-3}
2.41	0.940	0.242	82×10^{-4}	0.055	3.23×10^{-3}
2.21	1.070	0.240	94.5×10^{-4}	3.063	3.20×10^{-3}
1.8	1.290	0.238	117.0×10^{-4}	0.078	3.17×10^{-3}

Concentration: 1 g. per litre. 0.5 gram of fibre in 250 ml. of dye solution.

Dyeing time: 18 hours.

Temperature: $95^{\circ}C \pm 3^{\circ}C$.



Effect of PH on haematein absorption by nylon.

FIG:- 30

TABLE XXII

EFFECT OF pH ON HAEMATEIN ABSORPTION BY WOOL.

Final pH of the dye bath	Spekker Readings	Dye in the bath from Calibration curve.	Dye on the fibre.	Milli mols of dye per gram. of fibre.	Gram mols per litre in the dye bath
8.63	0.920	0.485	0.015	0.05	6.47×10^{-3}
8.21	0.710	0.404	0.096	0.32	5.39×10^{-3}
7.62	0.650	0.338	0.162	0.54	4.51×10^{-3}
6.93	0.530	0.281	0.219	0.73	3.75×10^{-3}
6.18	0.432	0.218	0.282	0.94	2.91×10^{-3}
5.12	0.351	0.179	0.321	1.07	2.39×10^{-3}
4.11	0.280	0.149	0.351	1.17	1.99×10^{-3}
2.71	0.250	0.125	0.375	1.25	1.67×10^{-3}
1.84	0.190	0.086	0.414	1.38	1.15×10^{-3}
1.25	0.080	0.035	0.465	1.55	0.47×10^{-3}
1.05	0.020	0.002	0.498	1.66	0.027×10^{-3}

Initial Concentration: 2 g. 1 litre. 1 gram of fabric in 250 ml. of dye liquor.

Dyeing time: 8.5 hours.

Temperature: $95^{\circ}C \pm 3^{\circ}C$.

EFFECT OF pH ON THE ABSORPTION OF HAEMATEIN BY WOOL AND NYLON IN AN INERT ATMOSPHERE.

Results obtained for the absorption of haematein by wool and nylon in Tables XXII show anomalously high values. These high values were due to the gradual oxidation of haematein during the normal dyeing process. In the experiments to be described below, oxidation was avoided by maintaining an inert atmosphere during dyeing.

Procedure:

Materials were prepared as stated before. For each experiment 1 gram of the fibre was accurately weighed and placed in a 500 ml. flask fitted with a water condenser and an inlet tube for carbon dioxide to bubble through the liquor. To the flask was added 400 ml. of haematein solution prepared in previously boiled water, varying amounts of N.Sulphuric acid were added to each flask and the total volume of liquor was made 400 ml. The flasks were kept in a thermostat at 85°C. Dyeing was continued for 8 hours in the case of wool and for 18 hours in the case of nylon. Air-free carbon dioxide from a supply of the solid was continuously bubbled through the flasks during the experiment.

After dyeing was over, the pH of each bath determined on a Marconi pH meter, and the dye on Nylon was estimated after rinsing the fabric completely, drying at laboratory temperature and/

and dissolving in m-cresol. The optical density of this solution was determined on the Spekker absorptiometer. Haematein left in the bath was calculated by difference and the results obtained have been given in Table XXIV, illustrated by Figures 20. In the case of wool, haematein was estimated in the bath and not in the fibre as stated previously. Detailed results are given in tables XXVI, illustrated by figures 21. Results for Brazilein are given in table XXV illustrated by figures 23 and 24.

TABLE /

TABLE XXIII

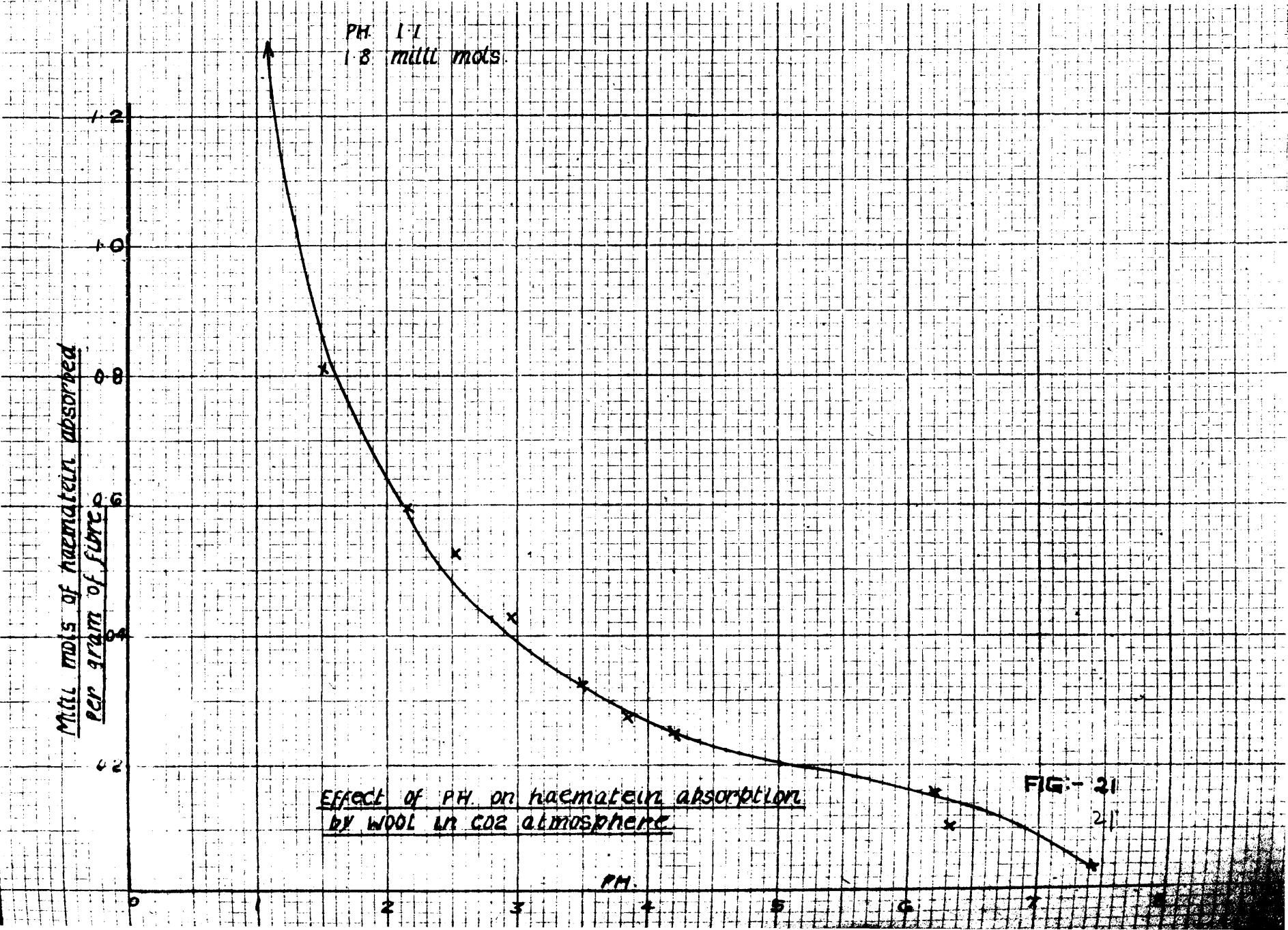
EFFECT OF pH ON HAEMATEIN ABSORPTION BY WOOL IN CO_2 ATMOSPHERE.

Final pH	Spekker Readings	Grams per litre from Calibration	Dye in bath	Dye on fibre	Milli mols per gram of fibre	Gram mols per litre
7.45	0.912	1.97	0.788	0.012	0.04	65.7×10^{-4}
6.30	0.900	1.92	0.768	0.032	0.110	64.0×10^{-4}
6.20	0.880	1.88	0.752	0.048	0.160	62.7×10^{-4}
4.20	0.852	1.81	0.724	0.076	0.250	60.3×10^{-4}
3.86	0.850	1.79	0.716	0.084	0.280	59.7×10^{-4}
3.50	0.820	1.75	0.700	0.100	0.330	58.3×10^{-4}
2.97	0.780	1.68	0.672	0.128	0.430	56.0×10^{-4}
2.49	0.750	1.60	0.640	0.160	0.530	53.3×10^{-4}
2.15	0.720	1.55	0.620	0.180	0.600	51.7×10^{-4}
1.46	0.640	1.35	0.540	0.260	0.870	45.0×10^{-4}
1.10	0.340	0.65	0.260	0.540	1.80	21.7×10^{-4}

Concentration 2 g. 1 litre. 1 gram of fibre in 400 ml. of dye liquor.

Dyeing time: 8 hours.

Temperature: $85^{\circ}C \pm 0.5^{\circ}C$.



Effect of PH on haematein absorption by wool in CO2 atmosphere.

FIG:- 21

TABLE XXIV.

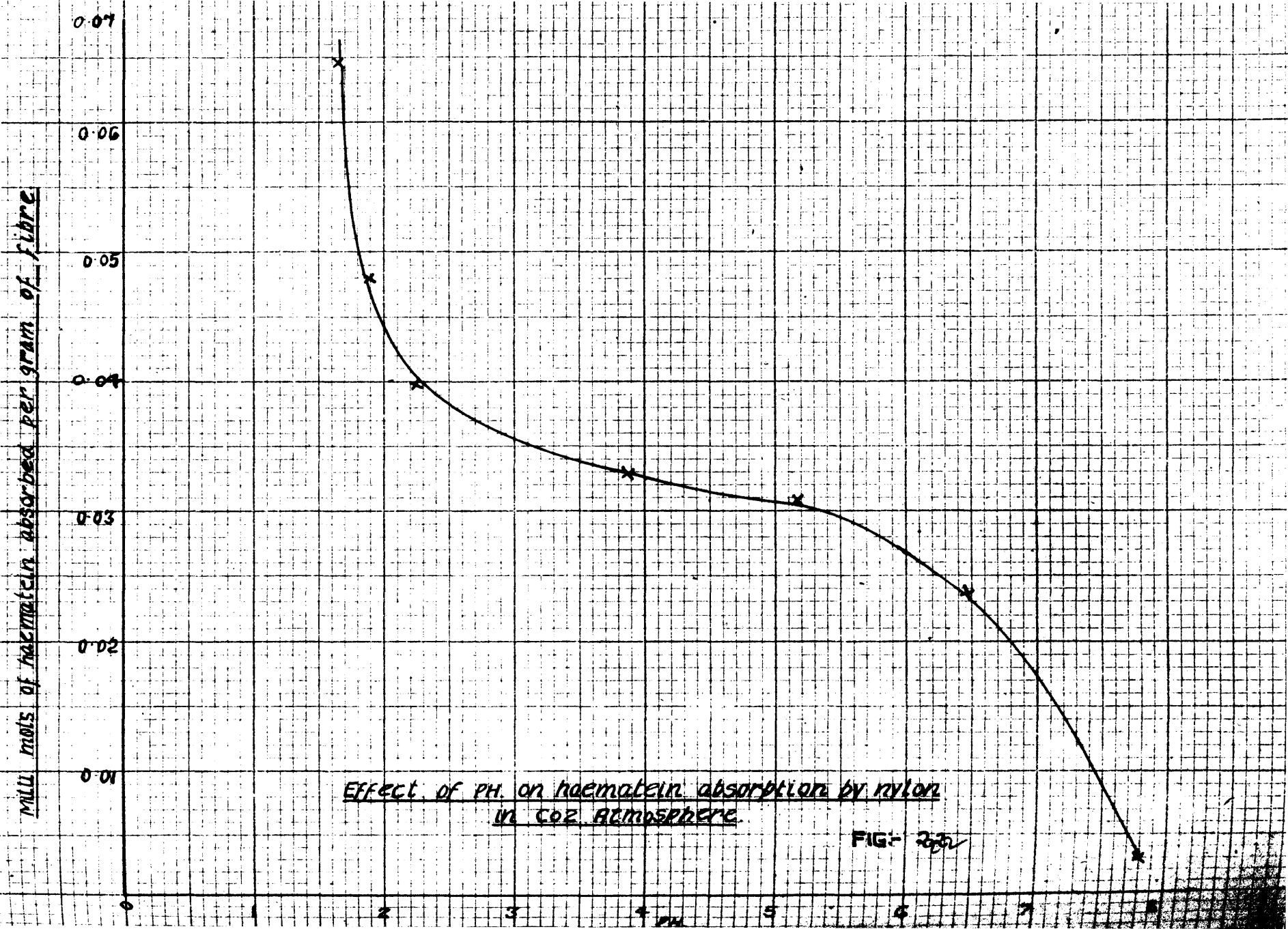
EFFECT OF pH ON HAEMATEIN ABSORPTION BY NYLON IN CO_2 ATMOSPHERE.

Final pH	Spekker readings (Dye on fibre).	Grams per litre from Calibration	Dye on the fibre	Dye in the bath	Milli mols per gram of fibre	Gram mols per litre in bath.
7.85	0.140	0.0015	0.0006	0.396	0.002	33.0×10^{-4}
6.50	0.560	0.018	0.0072	0.393	0.024	32.7×10^{-4}
5.19	0.680	0.023	0.0092	0.391	0.031	32.6×10^{-4}
3.91	0.720	0.025	0.010	0.390	0.033	32.5×10^{-4}
2.25	0.870	0.030	0.012	0.388	0.040	32.3×10^{-4}
1.90	1.020	0.036	0.0144	0.385	0.048	32.1×10^{-4}
1.66	1.33	0.0488	0.0195	0.381	0.065	31.7×10^{-4}

Concentration 1 g. litre. 1 gram of fibre in 400 ml. of dye liquor.

Dyeing time: 18 hours.

Temperature: $85^{\circ}C. \pm 0.5^{\circ}C.$



Effect of PH. on haematein absorption by nylon in CO_2 Atmosphere.

FIG:- 22b

TABLE XXV.

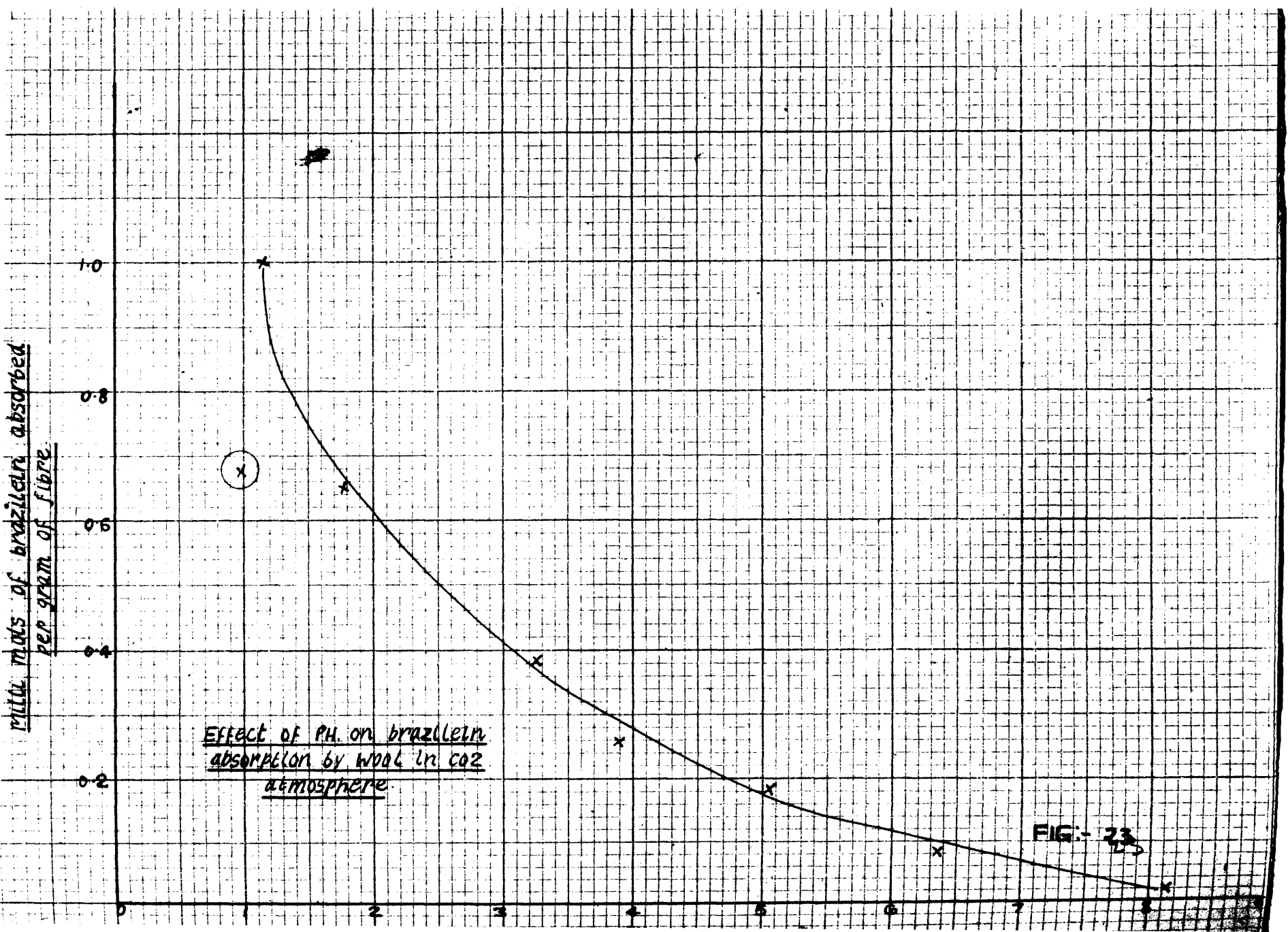
EFFECT OF pH ON BRAZILEIN ABSORPTION BY WOOL AND NYLON IN CO_2 ATMOSPHERE.

Final pH	Spekker Readings	Dye in bath.	Dye on fibre. (Difference)	Milli mols per gram of fibre
8.23	0.06	0.00072	0.00028	2×10^{-3}
6.40	1.31	0.089	0.011	78×10^{-3}
5.15	1.06	0.073	0.027	190×10^{-3}
3.97	0.95	0.065	0.035	250×10^{-3}
3.30	0.69	0.045	0.055	390×10^{-3}
1.80	0.151	0.007	0.093	654×10^{-3}
1.10	0.121	0.005	0.095	670×10^{-3}
		NYLON		
8.95	-	-	-	-
7.10	0.16	0.00072	0.0002	1.4×10^{-3}
5.54	0.29	0.0089	0.00113	8.0×10^{-3}
3.43	0.56	0.0972	0.0028	20.0×10^{-3}
2.91	0.84	0.0955	0.0045	32.0×10^{-3}
1.95	1.19	0.0934	0.0066	47.0×10^{-3}
1.8	1.23	0.0931	0.0069	49.0×10^{-3}
1.53	1.28	0.0928	0.0072	51.0×10^{-3}

Concentration 2 g. litre. 0.5 gram of fibre in 50 ml. of dye liquor.

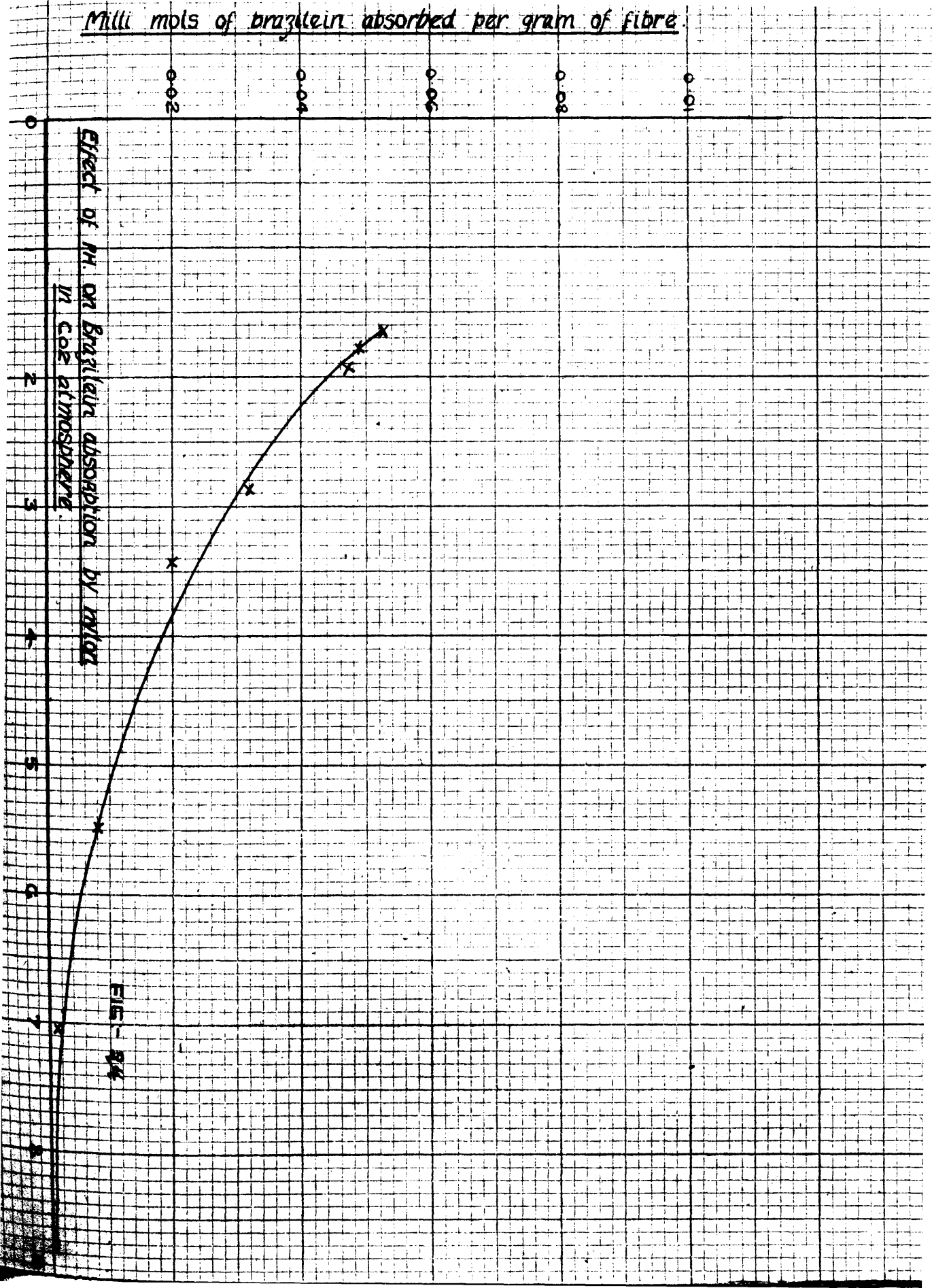
Dyeing time 8 hours. wool
18 hours. nylon.

Temperature $85^{\circ}C. \pm 0.5^{\circ}C.$



Effect of PH. on brazilein absorption by wool in CO2 atmosphere.

FIG:- 23


Milli mols of brazilein absorbed per gram of fibre.
0·02
0·04
0·06
0·08
0·1
0
2
3
4
5
6
7
8
Effect of RH. on Brazilein absorption by wool in CO2 atmosphere.
FIG 84

PART III

Section 6.

ABSORPTION ISOTHERMS OF HAEMATEIN AND BRAZILEIN ON WOOL AND NYLON IN AN INERT ATMOSPHERE.

In carrying out the experimental work needed to determine the isotherms, it was decided to use a fairly low pH value in the dyebath. The object of this was to ensure that all the amino-groups were liberated from the internal salt linkages so that the saturation value of the fibre should be independent of the dissociation constant or affinity of the dye used.

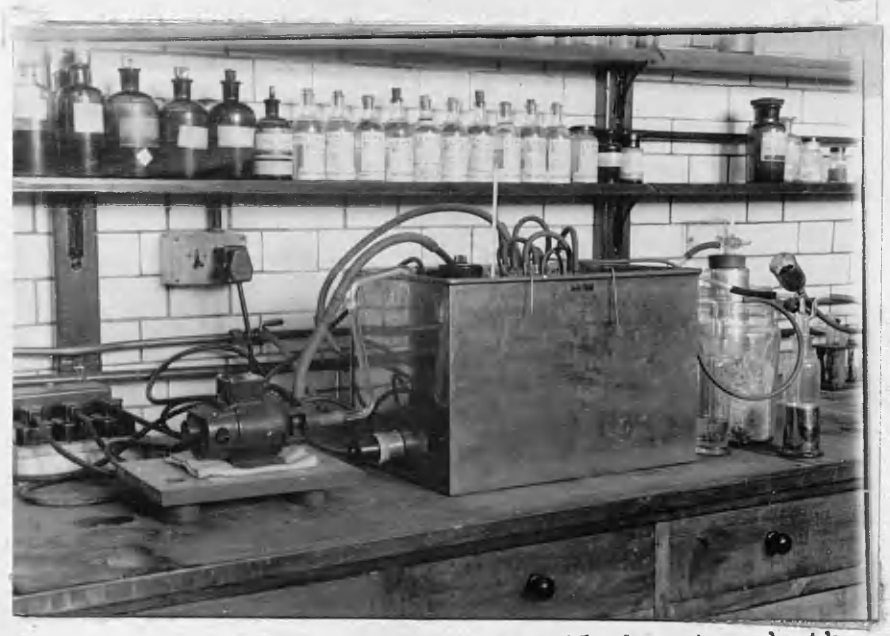
The experiments which have already been described suggest that the amount of haematein and brazilein absorbed at different pH values by wool and nylon increases up to a more or less fixed saturation value, which occurs between pH 1 and 2. Isotherms were therefore determined at pH 1.8.

Materials were prepared as described previously. 1 g. of fabric was weighed out, cut into pieces, and placed in a 800 ml. flask fitted with a water condenser and an inlet tube for carbon dioxide. To the flask was added 500 ml. of dye liquor. This was prepared by dissolving the necessary amount of dye in 400 ml. of water, adding sufficient sulphuric acid to bring it to the correct pH (1.8-2.0) and making up to 500 ml. with water. The flasks were kept in a water-bath maintained at 85°C. for 8 hours (for wool) and 18 hours (for nylon). Carbon dioxide was continuously bubbled through the 8 dyeing solutions. After the required dyeing time, the fabric was separated by filtration through glass wool, rinsed with cold water and dried at laboratory temperature. As stated

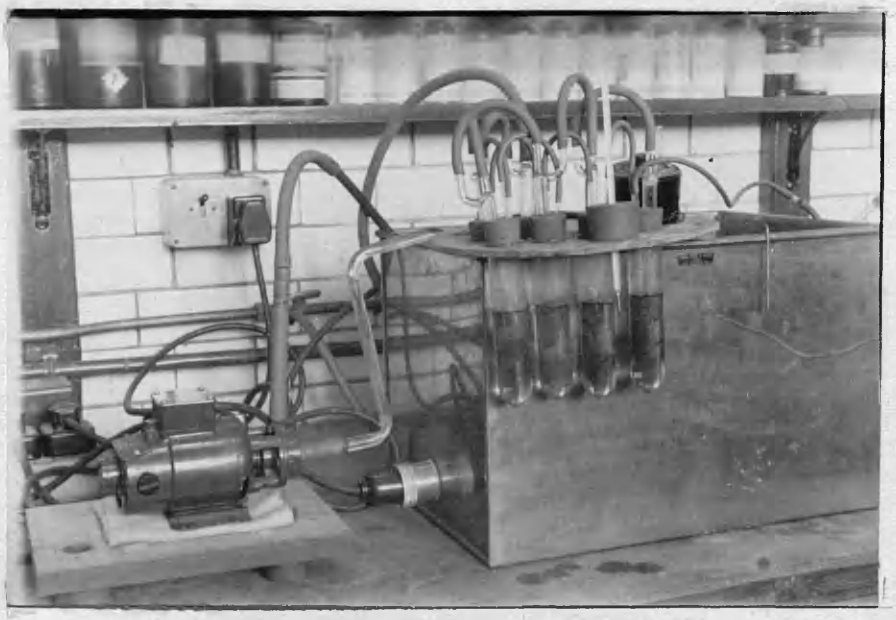
previously, wool could not be completely extracted free from dye and hence the dye was estimated from the dye left in the bath after dyeing. Nylon was dissolved in m-cresol and estimated colorimetrically as before.

Experiments were repeated for nylon at lower temperature for longer dyeing periods in a specially fabricated apparatus shown in figures 25 . Results are given in Tables XXVI-XXIX, illustrated by figures 27-29.

TABLE ___/



A: Thermostatically controlled water-bath with solid carbon dioxide generator.



B: Inside tubes with water and carbon dioxide circulation arrangements.

ABSORPTION APPARATUS. Figure 25.

TABLE XXVI

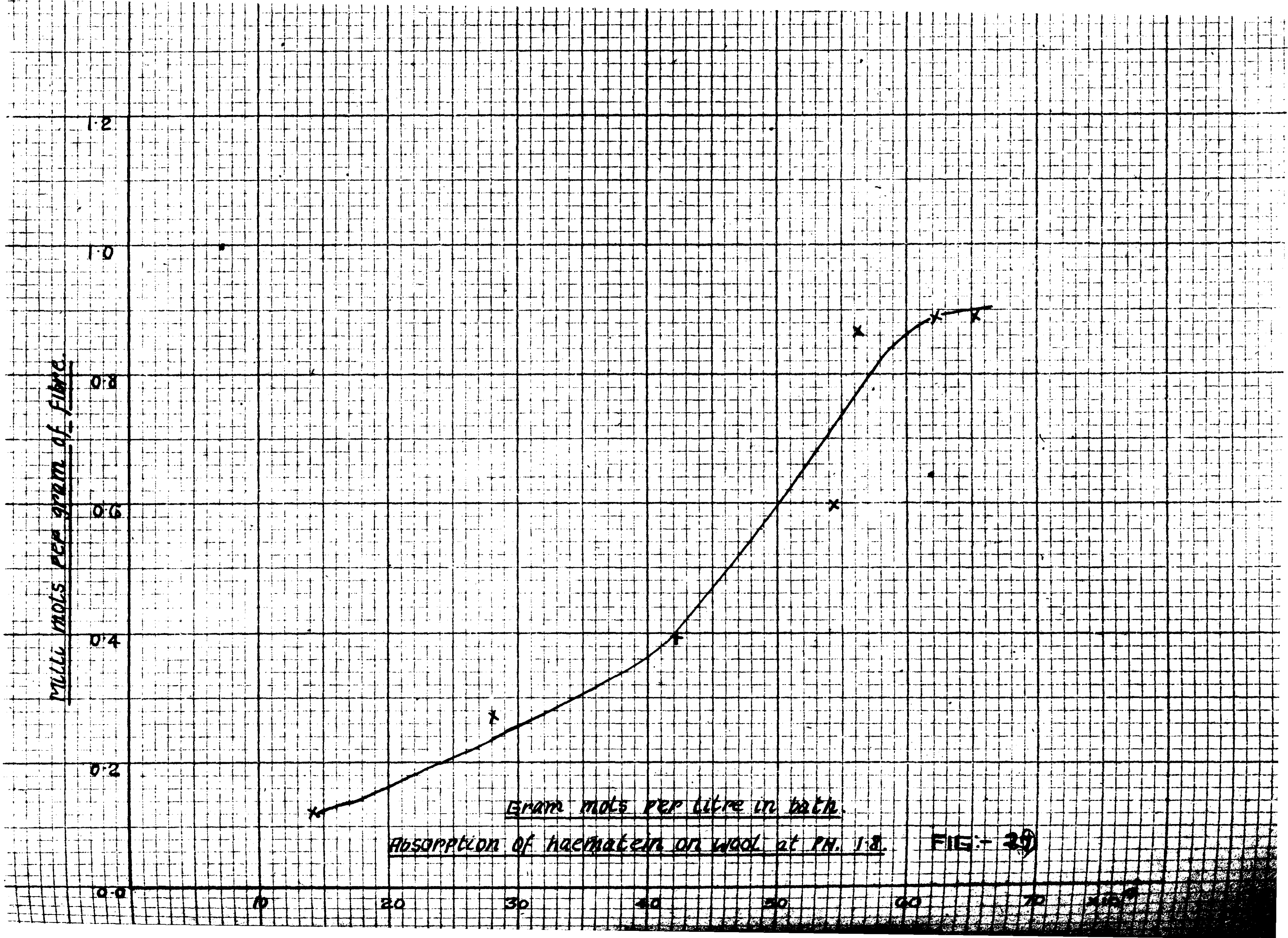
ABSORPTION ISOTHERM OF HAEMATEIN ON WOOL.

Initial conc. in bath gm/litre	Spekker readings 0.D.	Haematein in bath. Estimated.	Haematein on fibre. (Difference)	Milli mols of Haematein per gr. of fibre.	Gram mols. of Haematein in bath
0.5	0.220	0.212	0.038	0.127	14.1×10^{-4}
1.0	0.430	0.420	0.084	0.270	28.0×10^{-4}
1.5	0.610	0.631	0.119	0.397	42.1×10^{-4}
2.0	0.770	0.820	0.180	0.600	54.7×10^{-4}
2.2	0.790	0.839	0.261	0.870	55.9×10^{-4}
2.4	0.870	0.933	0.267	0.890	62.2×10^{-4}
2.5	0.920	0.982	0.268	0.893	65.5×10^{-4}

pH 1.8 1 g. of fibre in 500 ml. of dye liquor

Dyeing time 8 hours.

Temperature 85°C.



Absorption of haematein on wool at PH. 1·8.

FIG:- 29

TABLE XXVII

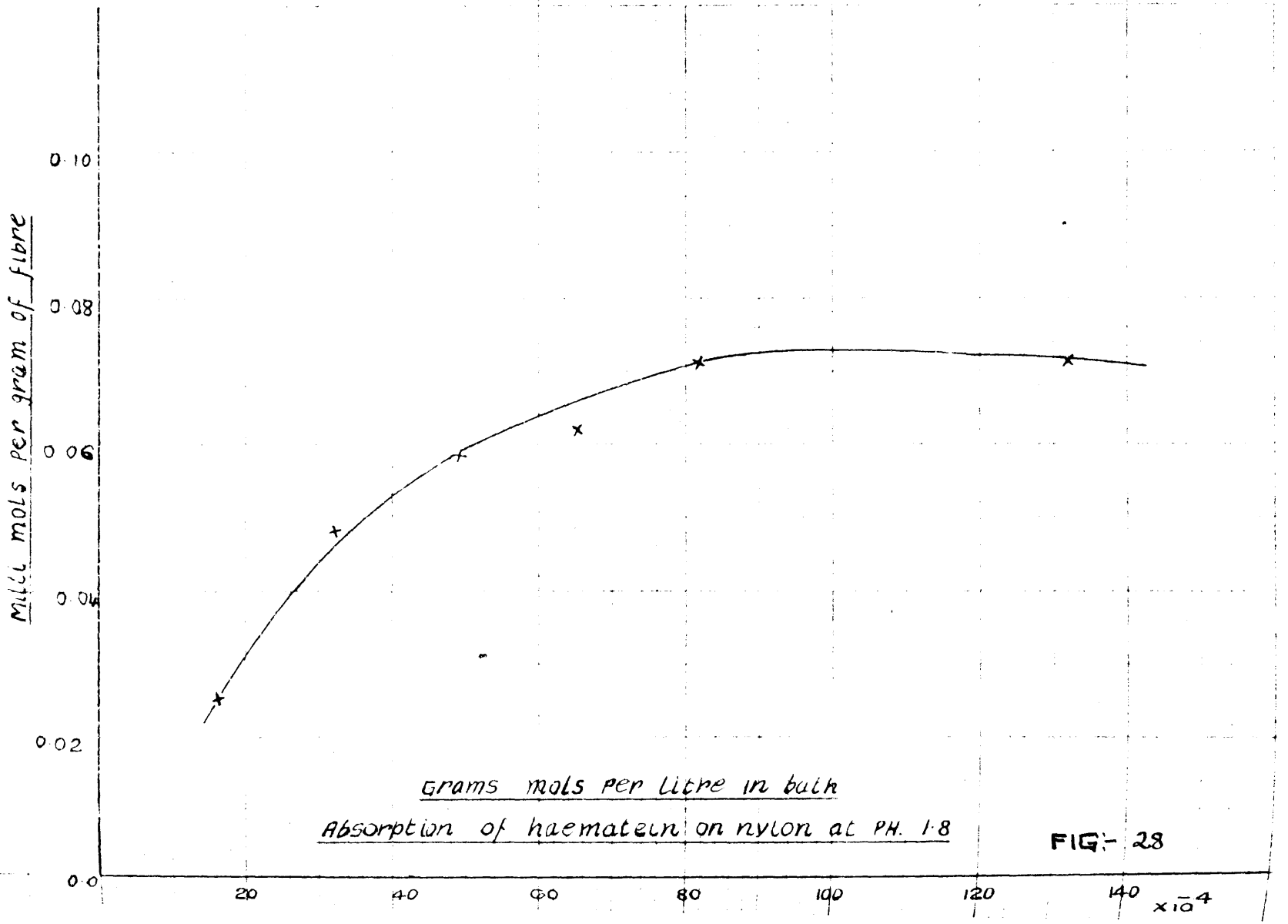
ABSORPTION ISOTHERM OF HAEMATEIN ON NYLON.

Initial conc.	Haematein on fibre. (Estimated)	Haematein in bath. (Difference)	Milli mols of haematein per gm. of fibre.	Gram. mols. of haematein per litre.
0.5	0.0075	0.243	0.025	16.2×10^{-4}
1.0	0.0144	0.486	0.048	32.4×10^{-4}
1.5	0.0174	0.733	0.058	48.9×10^{-4}
2.0	0.0186	0.981	0.062	65.4×10^{-4}
2.5	0.0213	1.230	0.071	82.0×10^{-4}
4.0	0.0214	1.980	0.071	132.0×10^{-4}

pH 1.8 — 1 gram of fibre in 500 ml. of liquor.

Dyeing time — 18 hours.

Temperature — 85°C.



Grams mols per litre in bath

Absorption of haematein on nylon at PH. 1·8

FIG:- 28

TABLE XXVIII

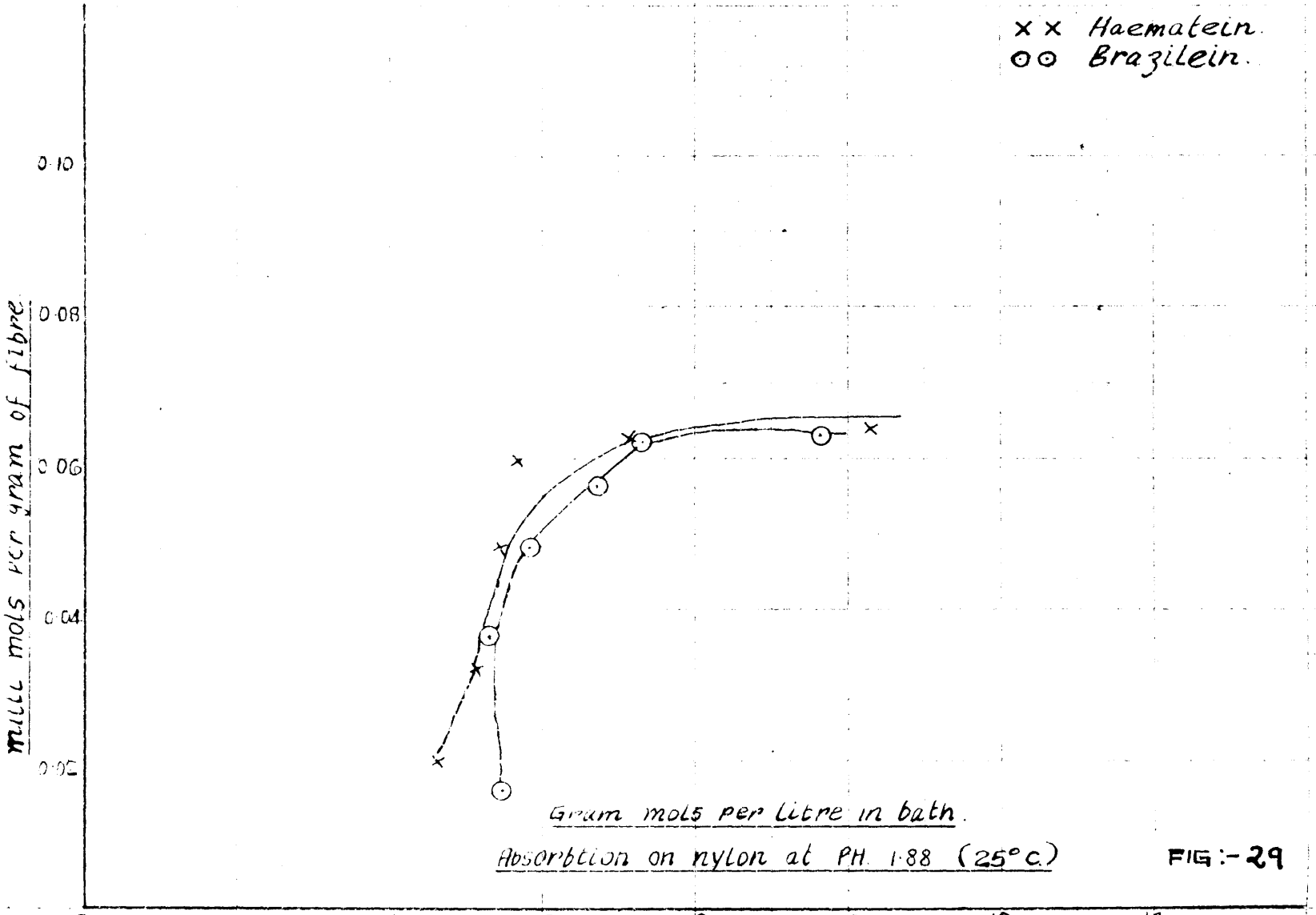
ABSORPTION ISOTHERM FOR HAEMATEIN AND BRAZILEIN ON NYLON.

Initial conc. in grams per litre.	Haematein				Brazilein			
	Fibre (Est.)	Bath (Diff)	Milli mols per gram.	Gram mols per litre.	Fibre (Est.)	Bath (Diff.)	Milli mols per gram.	Gram mols per litre.
0.20	0.003	0.007	0.02	4.6×10^{-4}	0.00227	0.00773	0.016	5.44×10^{-4}
0.25	0.0048	0.0077	0.032	5.1×10^{-4}	0.00497	0.00753	0.035	5.30×10^{-4}
0.30	0.0072	0.0078	0.048	5.2×10^{-4}	0.0068	0.0082	0.048	5.8×10^{-4}
0.35	0.0091	0.0084	0.061	5.6×10^{-4}	0.0079	0.0096	0.056	6.75×10^{-4}
0.40	0.0094	0.0106	0.063	7.1×10^{-4}	0.0088	0.0112	0.062	7.19×10^{-4}
0.45	-------Failed----------				0.00887	0.01363	0.063	9.6×10^{-4}
0.50	0.0096	0.0154	0.064	10.3×10^{-4}	0.0085	0.0165	0.060	11.6×10^{-4}

Temperature: $25^{\circ}C$

Dyeing time: 6 days

pH 1.88 0.5 g. of fibre in 50 ml. dye liquor.



Absorbtion on nylon at PH 1·88 (25°C.)

FIG:-29

TABLE XXIX

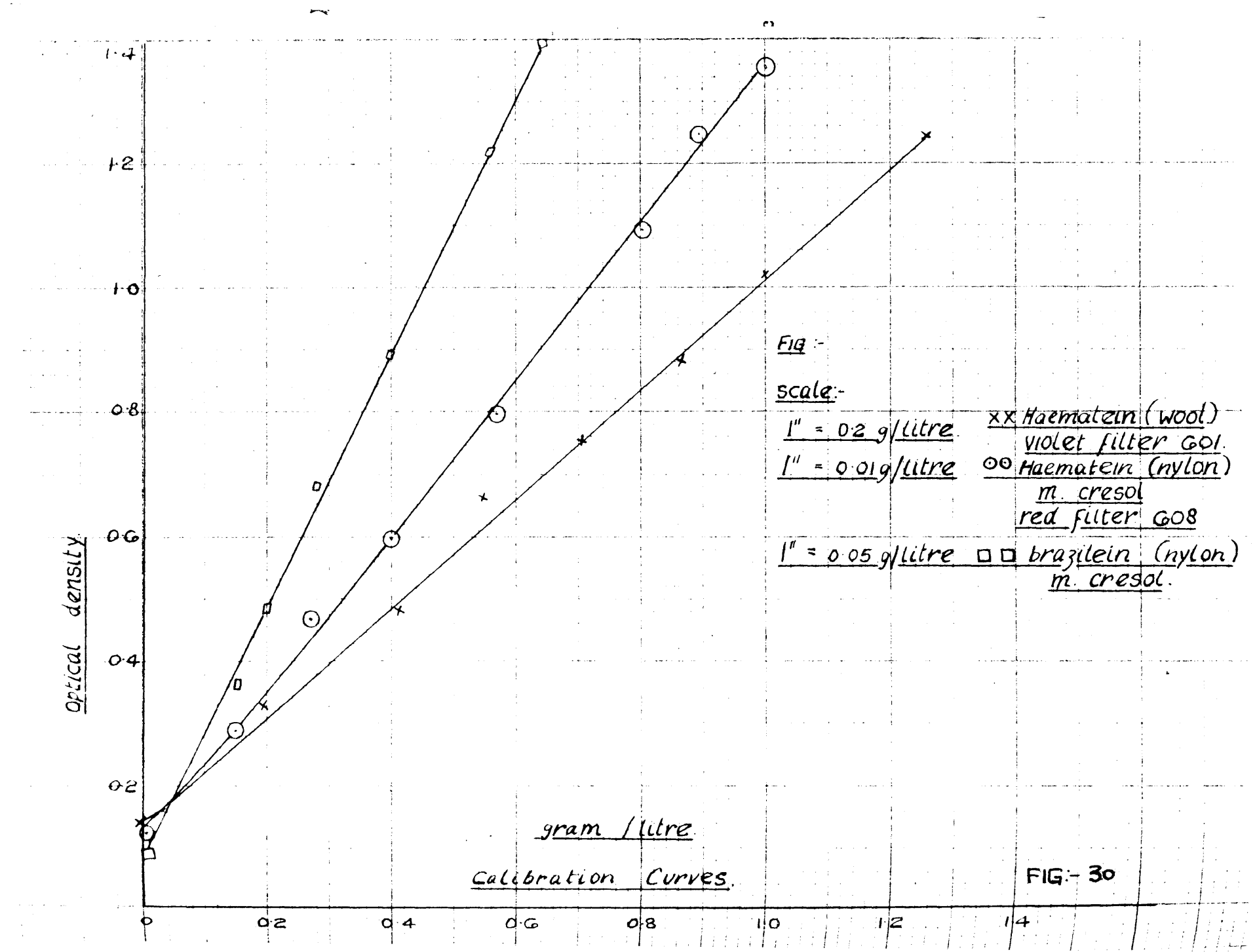
ABSORPTION ISOTHERM FOR BRAZILEIN ON WOOL.

Initial Conc. grams per litre	Spekker Readings	Readings from Calibration graph.	Brazilein in bath (Estimated)	Brazilein in fibre (Difference)	Milli mols per gram of fibre	Gram mols per litre.
0.5	0.380	0.474	0.0237	0.0013	0.09	11.65×10^{-4}
1.0	0.350	0.44	0.022	0.0280	0.20	15.5×10^{-4}
1.5	0.520	0.681	0.034	0.041	0.29	23.9×10^{-4}
2.0	0.350	0.441	0.22	0.078	0.550	15.5×10^{-4}
2.2	0.291	0.342	0.017	0.093	0.657	10.2×10^{-4}
2.4	0.302	0.361	0.018	0.102	0.710	12.65×10^{-4}
2.5	0.231	0.263	0.013	0.1121	0.720	9.15×10^{-4}

pH 1.88 — 0.5 g. of fabric in 50 ml. of dye liquor.

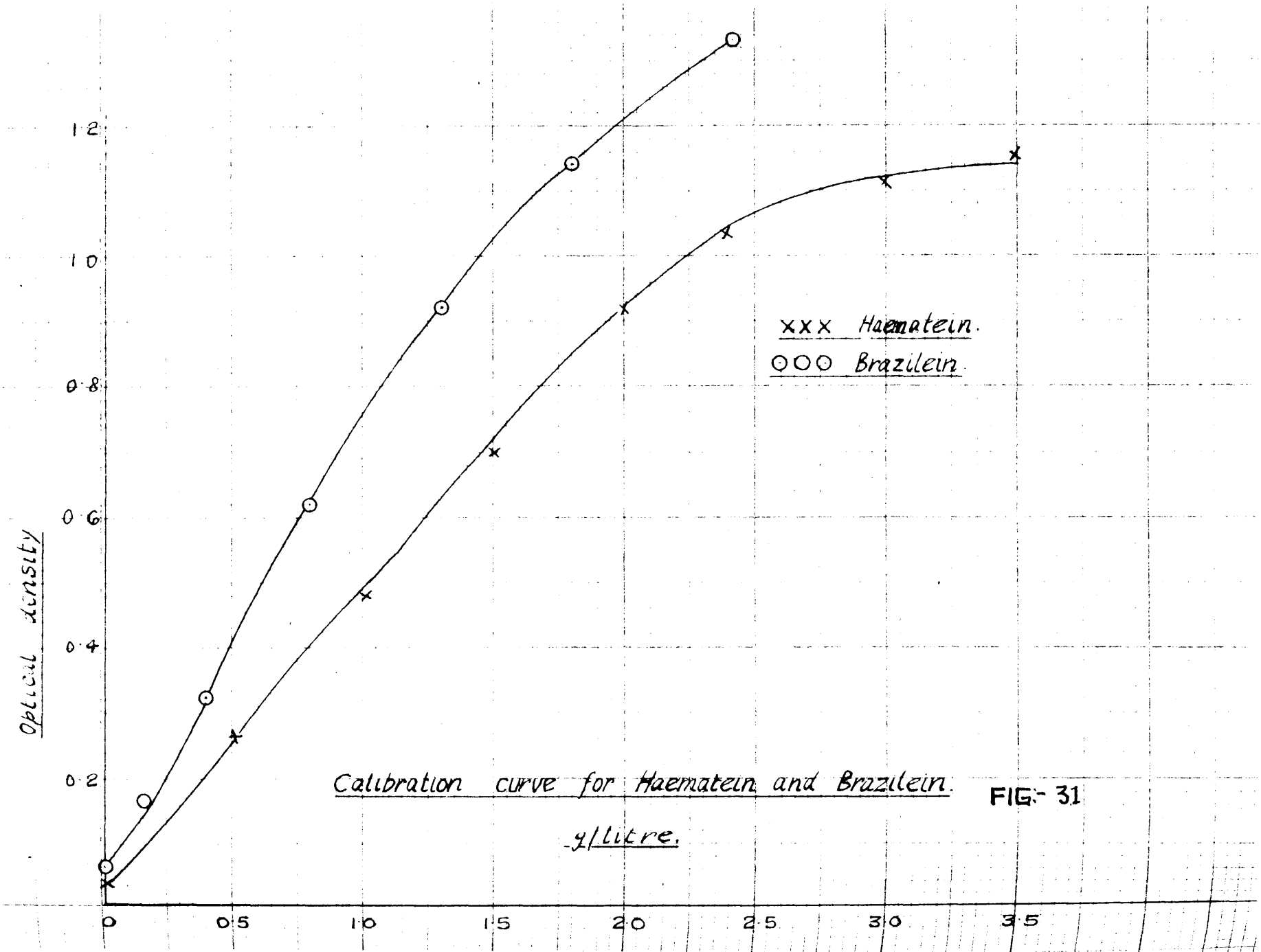
Dyeing time — 8 hours.

Temperature — 85°C.



Calibration Curves.

FIG:- 30



Calibration curve for Haematein and Brazilein.

FIG.- 31

PART III.

Section 7.

ABSORPTION OF HYDROXY COMPOUNDS BY FIBRES.

The study of substances like haematein and brazilein alone is not adequate to formulate any idea about their linkage with fibres. It was thought that if simple hydroxy compounds like phenol, resorcinol, catechal and hydroxyquinone were studied similarly, the nature of their linkages with the fibres would be clarified.

The work described below is divided into two parts:-

(1) Experiments to show the absorption of phenol, resorcinol, catechal and haematoxylin.

(2) Experiments to determine the activity of various hydroxyl groups in haematein and brazilein molecules by measuring the dissociation constants.

ABSORPTION OF PHENOL BY NYLON.

The absorption of phenol from aqueous solution by nylon was studied by V.B. Chipalkatli. * It was observed that in neutral and acidic media phenol is absorbed, whereas there is no absorption in alkaline medium. A similar observation has been made by Marsden and Urquhart (J. Textile Institute, 1942, 33, T. 105) in a study of the absorption of phenol by cellulose acetate from aqueous solutions. The absorption increases with increasing concentration of phenol. The relationship between the concentration and absorption is shown in Table XXXI , illustrated by figure 34 .

TABLE ____/

* V.B.Chipalkatli. Private communication.

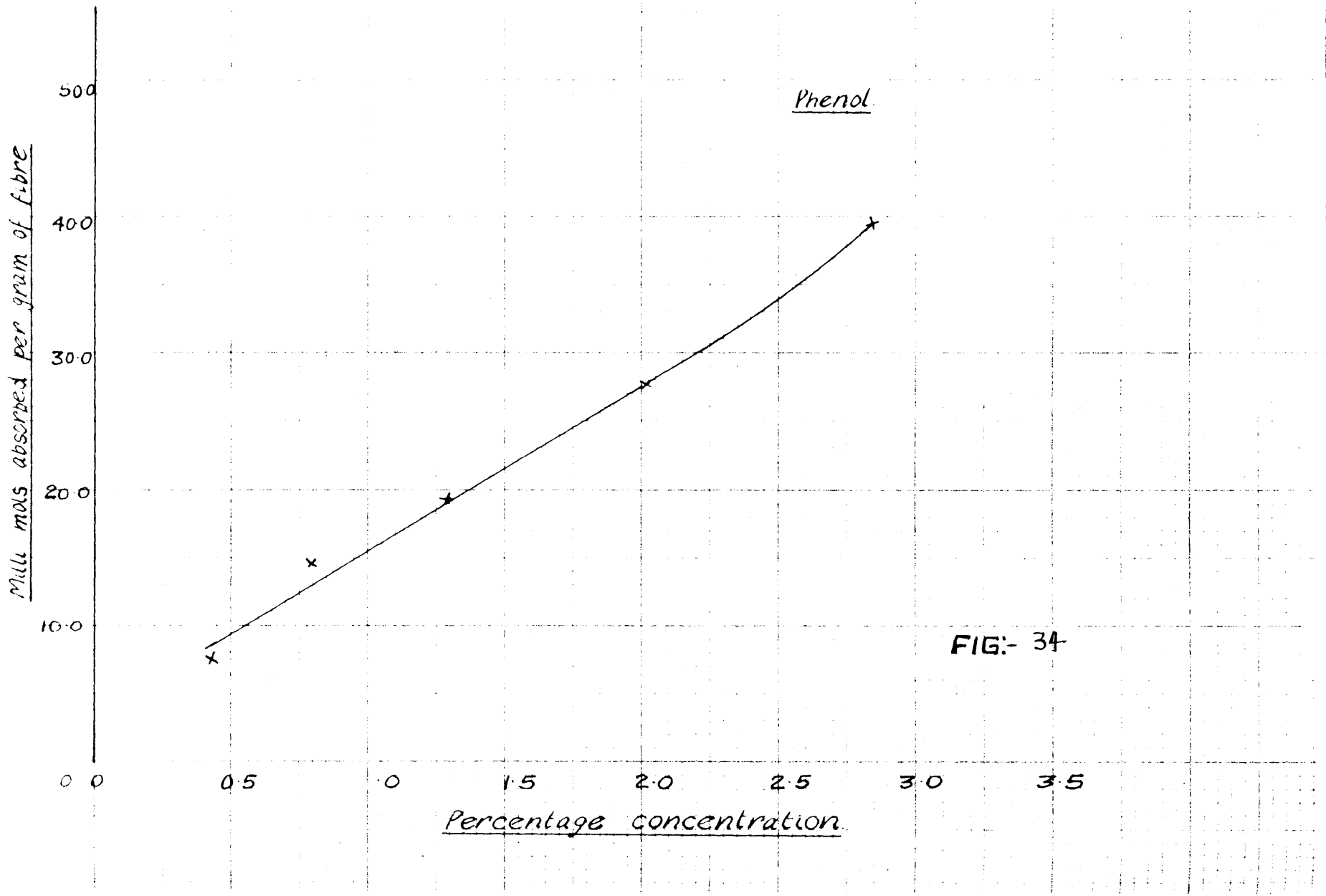

Phenol
Milli mols absorbed per gram of fibre
500
400
300
200
100
0 0
0·5
1·0
1·5
2·0
2·5
3·0
3·5
Percentage concentration

FIG:- 34

TABLE XXXI

Initial Conc. gram/litre.	Milli mols of phenol absorbed per gram.	% Equilibrium Concentration
4.8	7.5×10^{-4}	0.4319
9.7	10.45×10^{-4}	0.7940
19.4	17.7×10^{-4}	1.3396
29.1	27.6×10^{-4}	2.060
48.6	39.7×10^{-4}	2.870

Time: 24 hours.

Temperature:

ABSORPTION OF HYDROXY COMPOUNDS BY WOOL AND NYLON.

Resorcinol (M.P.111°C.) and Catechal (M.P.105°C.) were purified and used for the experiment. Wool and Nylong were purified as described before.

Procedure: 0.5 gram of the fibre was accurately weighed and placed in a 500 ml. flask. Resorcinol and Catechal solutions (1.gm. per litre) were prepared and 200 ml. of each was transferred to the respective flasks containing the fibre. The pH of each flask was adjusted by adding different quantities of sulphuric acid. The total volume in each was 400 ml. The flasks fitted with a condenser, were placed in a water-bath maintained at 60°C. The experiment was continued for 8 hours. At the end of the experiment, the flasks were cooled, woollen fabric removed and/

and the concentration of the resorcinol and catechal left in the bath was estimated by colorimetry following coupling with diazo sulphanilic acid. The pH of each bath was determined at the end of the experiment.

ESTIMATION OF RESORCINAL AND CATECHAL.

Preparation of diazo sulphanilic acid:-

To react with 1 gm/litre solution of Resorcinal or Catechal, an $N/50$ sulphanilic acid solution is required. The following quantities were used - 10 ml. of $N/2$ sulphanilic acid and 5 ml. of $N/1$ Caustic soda were mixed, diluted and boiled. The solution was cooled to 5°C. and 1.5 ml. of hydrochloric acid (conc.) was added, followed by 10 ml. of $N/2$ sodium nitrate. The solution was made up to 250 ml. The coloured solutions were measured on the photo electric absorptiometer.

5 ml. of the solution left in the bath after the experiment was treated with 5 ml. of diazo sulphanilic acid solution of equivalent strength to the resorcinal or catechal put into the baths, i.e. 1 gm. per litre. Before coupling, 1 ml. of a buffer solution of pH 7 was added to the solution. Calibration curves for these compounds were first determined and the amounts left in the bath read directly from them. Results are given in Tables XXXII-III, illustrated by figures 35

TABLE /

TABLE XXXII

ABSORPTION OF HYDROXY COMPOUNDS BY WOOL.

Substance.	Final pH of the bath.	Present in the bath	Gram of substance absorbed per g. of fibre.	Milli mols of substance per gram of fibre.
Resorcinal	1.01	0.181	0.0385	0.35
	2.06	0.180	0.0410	0.37
	2.95	0.185	0.0310	0.28
	4.03	0.193	0.014	0.13
	5.10	0.194	0.012	0.103
	6.25	0.196	0.0078	0.07
	7.18	0.195	0.0082	0.075
	7.9	0.199	0.002	0.02
Catechal	0.45	0.169	0.0627	0.520
	1.5	0.171	0.058	0.531
	2.1	0.175	0.051	0.450
	2.23	0.181	0.041	0.362
	2.95	0.184	0.032	0.291
	3.71	0.189	0.022	0.201
	4.35	0.191	0.0176	0.160
	5.71	0.196	0.007	0.065
	7.18	0.197	0.0045	0.041
	8.10	0.199	0.0001	0.001

Concentration 1 g. litre 0.5 g. of fibre in 200 ml. of liquor.

Dyeing time 8 hours.

Temperature 60°C.

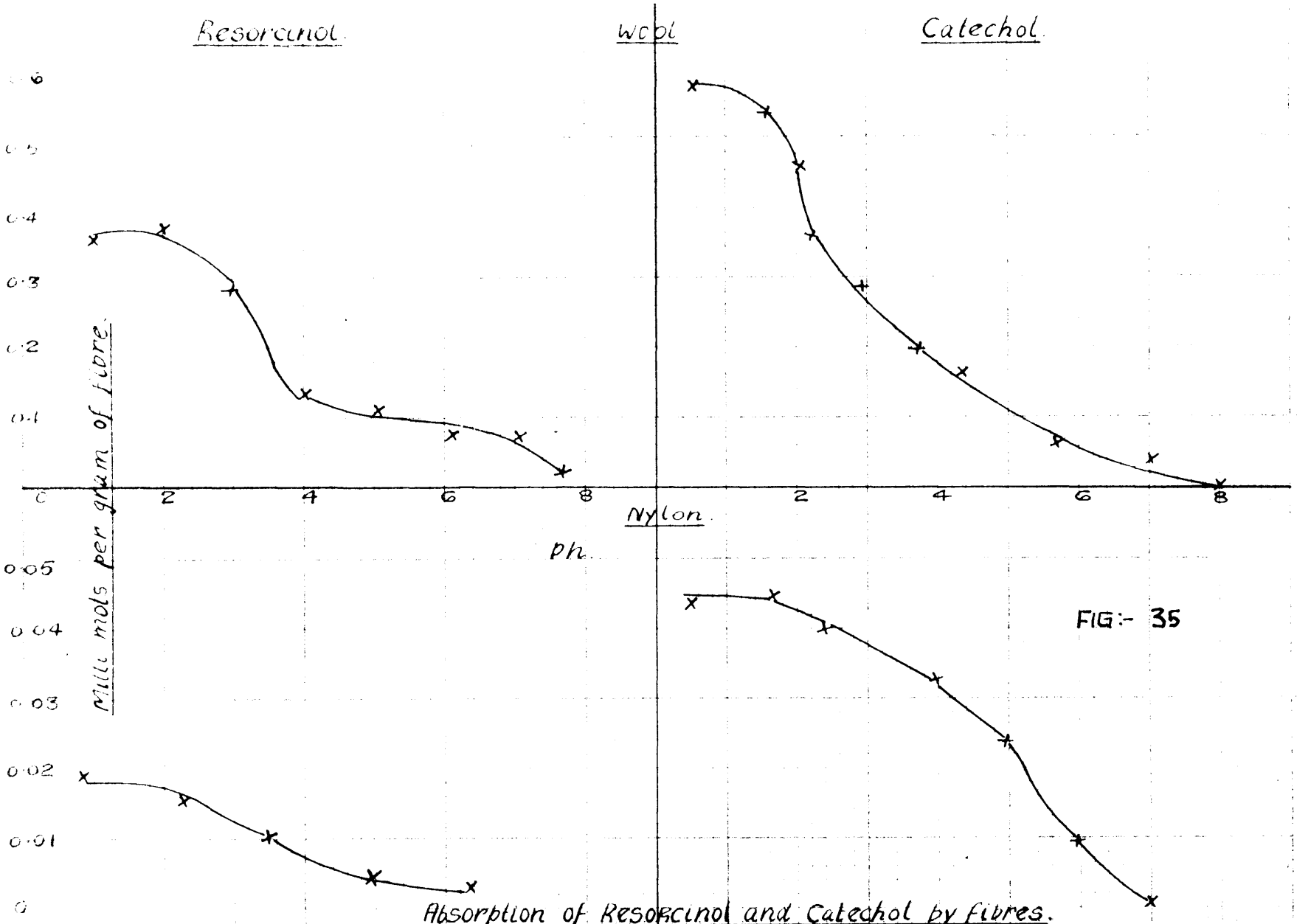


FIG:- 35

Absorption of Resorcinol and Catechol by fibres.

TABLE XXXIII

ABSORPTION OF HYDROXY COMPOUNDS BY NYLON.

Substance.	Final pH of the bath	Present in the bath	Gram of substance absorbed per gram of fibre.	Milli mols of substance absorbed per g. of fibre.
Resorcinal	0.91	199.0×10^{-5}	1×10^{-5}	0.019
	2.30	199.2×10^{-5}	0.8×10^{-5}	0.0015
	3.53	199.5×10^{-5}	0.5×10^{-5}	0.01
	5.01	199.78×10^{-5}	0.22×10^{-5}	0.004
	4.35	199.8×10^{-5}	0.19×10^{5}	0.0035
Catechal	0.51	197.7×10^{-5}	2.3×10^{-5}	0.043
	1.71	197.6×10^{-5}	2.4×10^{-5}	0.045
	2.31	197.8×10^{-5}	2.2×10^{-5}	0.041
	4.10	199.2×10^{-5}	1.8×10^{-5}	0.033
	5.10	198.7×10^{-5}	1.3×10^{-5}	0.024
	6.0	199.5×10^{-5}	0.5×10^{-5}	0.01
	7.02	199.1×10^{-5}	0.99×10^{-5}	0.0018

Concentration: 1 g. litre. 0.5 g. of fibre in 200 ml. of liquor.

Dyeing time: 18 hours.

Temperature: 60°C.

PART III

Section 8.

ABSORPTION OF HAEMATOXYLIN BY NYLON BY MEASUREMENT OF DIELECTRIC CONSTANT.

Haematoxylin is extremely difficult to handle as an unoxidised product. During the course of investigations, it was thought that if the mechanism of dyeing with haematein has to be clearly understood, it would be necessary to study the application of haematoxylin to fibres, as it is the parent substance of haematein, though it is colourless by itself.

Haematoxylin being easily susceptible to oxidation in air, it was difficult to handle. Further, since it is colourless, ordinary colorimetric analysis is not applicable. It would thought that if it could be coupled with Sulphanilic acid to give a coloured solution, the amount of haematoxylin left in the bath after the completion of the experiment could easily be determined. However, coupling was not satisfactory either with Sulphanilic acid, p-nitroaniline, -naphthalamine, H-acid or picranic acid, probably owing to further oxidation of the product by nitrous acid.

Recently,* G.B.Murray has studied the dieletric behaviour of nylon in presence of acids and bases. The method consists of measurements of dielectric constant of solutions of nylon and various dyestuffs. It was thought that if nylon was dissolved in m-cresol and if varying quantities of haematein and haematoxylin solutions in m-cresol were added to the nylon solution respectively, the dielectric constant would be expected

to/

to show a change. The amount of haematein and haematoxylin absorbed by nylon solution could then be calculated from the graph obtained. Since it is a new approach to the problem it would be better for our understanding if it is viewed under the following four headings:-

(1) Theoretical approach.

(2) Theory of Dielectric constant.

(3) Description of apparatus.

(4) Dielectric behaviour of haematein and haematoxylin.

Theoretical approach.

If the dielectric constant of nylon is considered it can be seen that the polarity of the molecule

$$\underline{\text{HOOC}} \cdot (CH_2)_4 \left[\underline{\text{CONH}} \, (CH_2)_6 \ldots\ldots \right] (CH_2)_6 \cdot \underline{\overset{+}{N}H_2}$$

must be due in part to the ionised end amino group, and carboxylic groups. If the fibre is dyed with an acid dye, the positive charge on the amino groups should be discharged in proportion to the amount of dye combined and hence a change in dielectric constant should take place. Thus the measurements of dielectric constant of solutions of nylon dyed to different extents should offer a means of estimating the amount of dye necessary to saturate these groups.

Theory of dielectric constant.

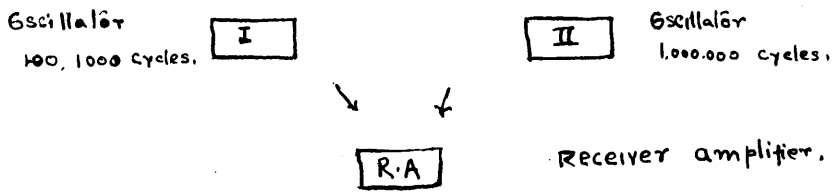
The dielectric constant is generally familiar in the expression/

expression for the force "f" between two charges "e" and "e'" separated by a distance "d":- $f = \frac{ee'}{kd2}$ in which "K" is a constant characteristic of the medium between the charges, i.e. the dielectric constant. It is also familiar as a quantity to which the capacity C of a condenser is proportional, i.e. $C = KC_0$ where C_0 is the capacity of a condenser with a vacuum between the plates and "K" is the dielectric constant of the medium between its plates when C is measured. It is by means of this latter relationship that "K" is commonly obtained experimentally.

The basis of most methods is thus the measurement of the capacity of a condenser when there is nothing (or usually air) between the plates, and when the substance being investigated fills the space between the plates. There are various methods of capacity measurement but the method selected in this investigation is the Heterodyne Beat method.

This method takes advantage of the dependence of the frequency of a thermionic valve oscillator upon the resistance, inductance and capacity of its circuits. Referring to diagram: If oscillator I generates it 1,001,000 cycles and oscillator II

<u>Block circuit of apparatus</u>

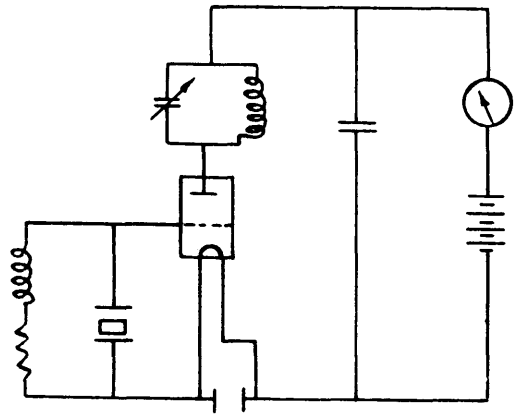


generates/

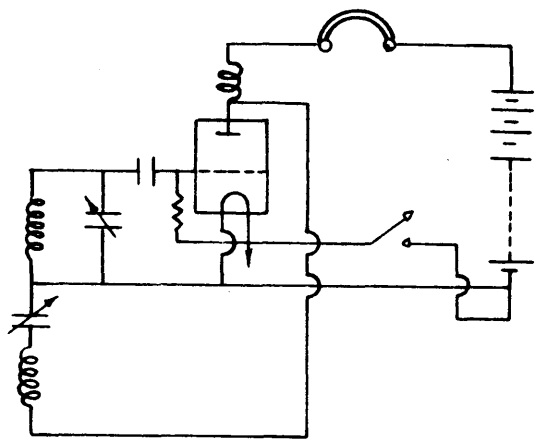
generates 1,000,000 cycles and the two are loosely coupled, beats of a frequency of 1,000 cycles occur. This 'beat frequency' is in the audible range and may be heard in a telephone as a musical note. If the capacity in Oscillator I is increased the frequency is lowered and the frequency of the beat note is also lowered until it becomes in-audible. Further lowering of the capacity in oscillator I eventually lowers the frequency below that of Oscillator II and the beat note becomes audible again. If an amplifier is used in the receiver circuit the region of inaubility is very narrow and corresponds to a point on the variable condenser scale.

With such a system the dielectric cell or condenser is placed in one oscillator circuit in parallel with a calibrated condenser. The system is tuned to 'zero beat' C in audibility with dielectric cell empty. The cell is then filled with the liquid under investigation which, by altering the capacity of the cell, changes the frequency of the oscillator. The system is again tuned to zero beat by the calibrated condenser. The difference in readings of the condenser gives the change in capacity of the dielectric cell and enables the dielectric constant of the liquid to be calculated from the relationship previously mentioned.

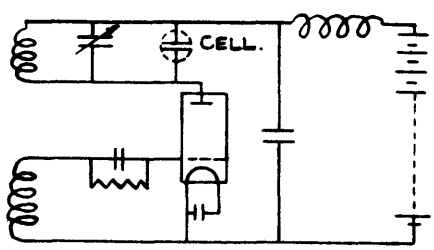
The method is extremely sensitive as it is possible to measure approximately 50 cycles in several million. If the electrical stability of the circuits is good and temperatures variations/



CRYSTAL OSCILLATOR



RECEIVER - AMPLIFIER.



HARTLEY OSCILLATOR.

CIRCUIT DIAGRAM OF APPARATUS

FIG. 32A.

variations take place and the resistance and inductance of the circuits remain unchanged. It can be seen that if the liquid used has any appreciable conductivity, inaccuracies will occur and in extreme cases, the energy absorbed by the cell may stop the system oscillating. The method is therefore limited chiefly to organic liquids.

Various designs and apparatus are described in the literature and the subject is treated in detail by Smyth (Dielectric constant Molecular Structure.") (The Chemical Catalogue Ch. III).

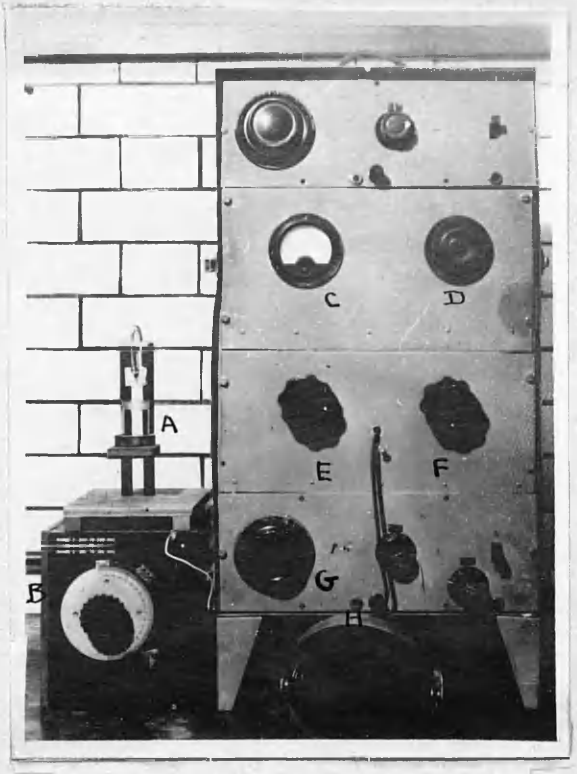
Description of electrical circuits.

As already described the electrical design includes two oscillators and a receiver-amplifier. These will be described separately.

(a) First Oscillator.

To ensure good frequency stability this oscillator was constructed to operate on one frequency only and includes a quartz crystal in the grid circuit. The well known properties of quartz in frequency control are described by Vigoreux (Quartz Resonators and Oscillators H.M.S.O. 1931. "Quartz Oscillators and their application. H.M.S.O. 1939). The circuit of the Oscillator is a modified Pierce circuit, which combines simplicity with good stability. The frequency used was 3.25 megacycles.

(b)/



Front view of the apparatus.

A:- Dielectric cell.

B:-Micrometer condenser

C:-Anode current (Quartz oscillato

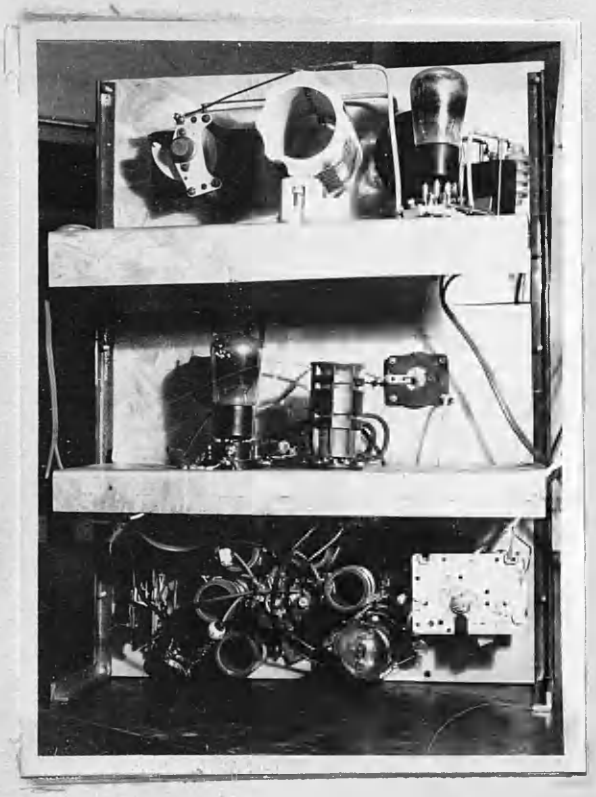
D:-Anode tuning(Quartz oscillator)

E:-Reaction control(Receiver).

F:-Tuning control(Receiver).

F:-Main tuning control(V. oscillator)

H:-Head phone.



Interior of apparatus

ABSORPTION OF HAEMATEIN BY MEASUREMENT OF DIELECTRIC CONSTANT.

Figure 32.

(b) Second Oscillator.

This variable oscillator was adapted from Ex.W.D. equipment and is a single value Hartley circuit. A switching arrangement permits a frequency range of 1400 Kilocycles to 22000 Kilocycles. A simple ladder type alternator controls the outfit. The main tuning condenser is semi-circular plate type which gives a linear change of capacity with dial reading.

(c) Receiver Amplifier.

The receiver is a simple one valve circuit with reaction control constructed on standard lines. Each unit described was constructed separately on a metal chassis of heavy gauge tinned iron with due consideration to mechanical rigidity. The three sections were bolted to a welded frame of half each angle iron and enclosed in a sheet metal cabinet. The arrangement is shown in the drawings and photographs.

To test the apparatus it was switched on and after a 'warming up' period of thirty minutes tuned to zero beat. No appreciable frequency drift was observed over a period of an hour.

The dielectric cell.

The cell was designed with platinum plates. The platinum wires supporting the plates were attached by oxy-hydrogen welding. The only moving portion is the glass vessel and this is held rigidly on its platform by a retaining ring as shown in the figure 32. The leads to the main circuit from the cell were

made/

made with "twinfeed" cable. This is a special type of radio frequency twin conductor with polythene insulation and very low electrical losses. The cable is mechanically strong and rigid, and prevents capacity changes which might be caused if ordinary wire were used.

Micrometer Condenser.

The main tuning condenser in the variable oscillator was of too large a capacity to measure small changes and a micrometer condenser was constructed. The design consists basically of two circular metal plates, one of which is attached to a threaded rod and enables it to be moved at right angles to the plane of the plate. The instrument suffered from a slight amount of back-lash but was calibrated against the main condenser, with the results shown. It was decided, however, to use a small variable condenser with semi-circular plates to obtain a linear change of capacity against dial readings. This enabled experimental results to be plotted directly without conversion. The condenser obtained was of 10 micro micro farads Maximum capacity and had silvered plates mounted in ceramic insulation. The condenser was fitted in a separate box with a vernier dial and in dielectric cell mounted on top of the box.

* G.B.Murray - B.Sc Thesis 1948, University of Glasgow.

HAEMATEIN ABSORPTION.

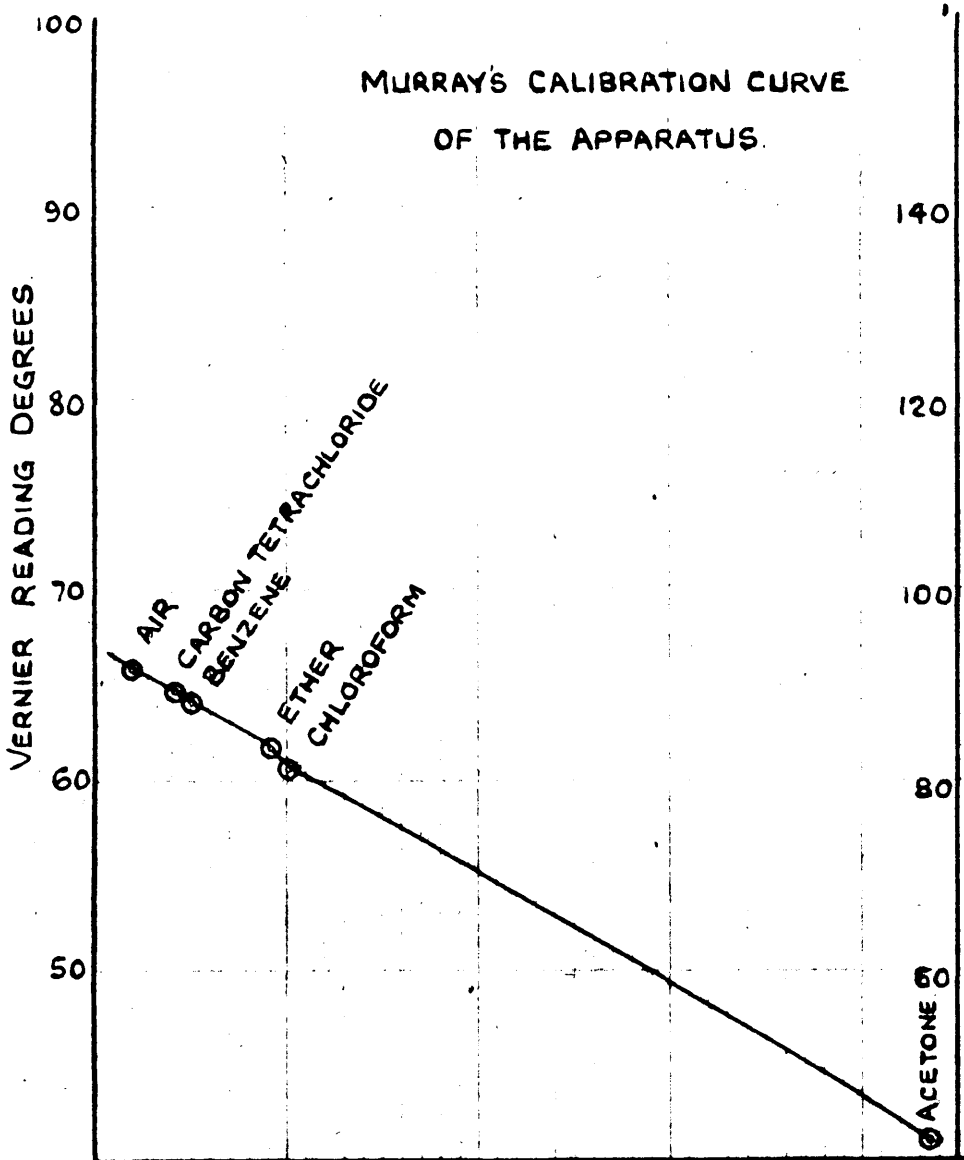
0.5 g. of purified nylon was dissolved in 100 ml. m-cresol (100%) by slightly warming it on a water-bath. Haematein crystals (99.8%) 0.05 g. were accurately weighed and dissolved in 100 ml. of m-cresol.

Several 20 ml. portions of nylon solution were placed in 50 ml. conical flasks and varying amounts of haematein solution from a micro burette were added to each flask. The volume in each flask was brought to 30 ml. by adding required quantity of m-cresol. The dielectric constants of the solutions were then measured and graphed against the concentration of the dye.

Great variations were noticed if two electrodes were not properly cleaned. After each reading the electrodes were cleaned first with m-cresol to remove any sticking nylon solution, then with ether at least twice to ensure complete cleanliness and dryness of the electrodes.

Readings showed steadiness in 10 minutes. If the solution is kept for a longer time, (40 mins.) in contact with the electrodes, a brownish substance seems to precipitate, probably due to oxidation of haematein. However, for the absorption measurements solutions could be properly mixed, corked and left overnight to reach an equilibrium between the nylon and haematein solution.

Results are described in Table XXXIV illustrated by figures 33 .


MURRAY'S CALIBRATION CURVE
OF THE APPARATUS.
VERNIER READING DEGREES.
100
90
80
70
60
50
AIR
CARBON TETRACHLORIDE
BENZENE
ETHER
CHLOROFORM
ACETONE
4·00
5
10
15
20
22·5
DIECTRIC CONTANTS.

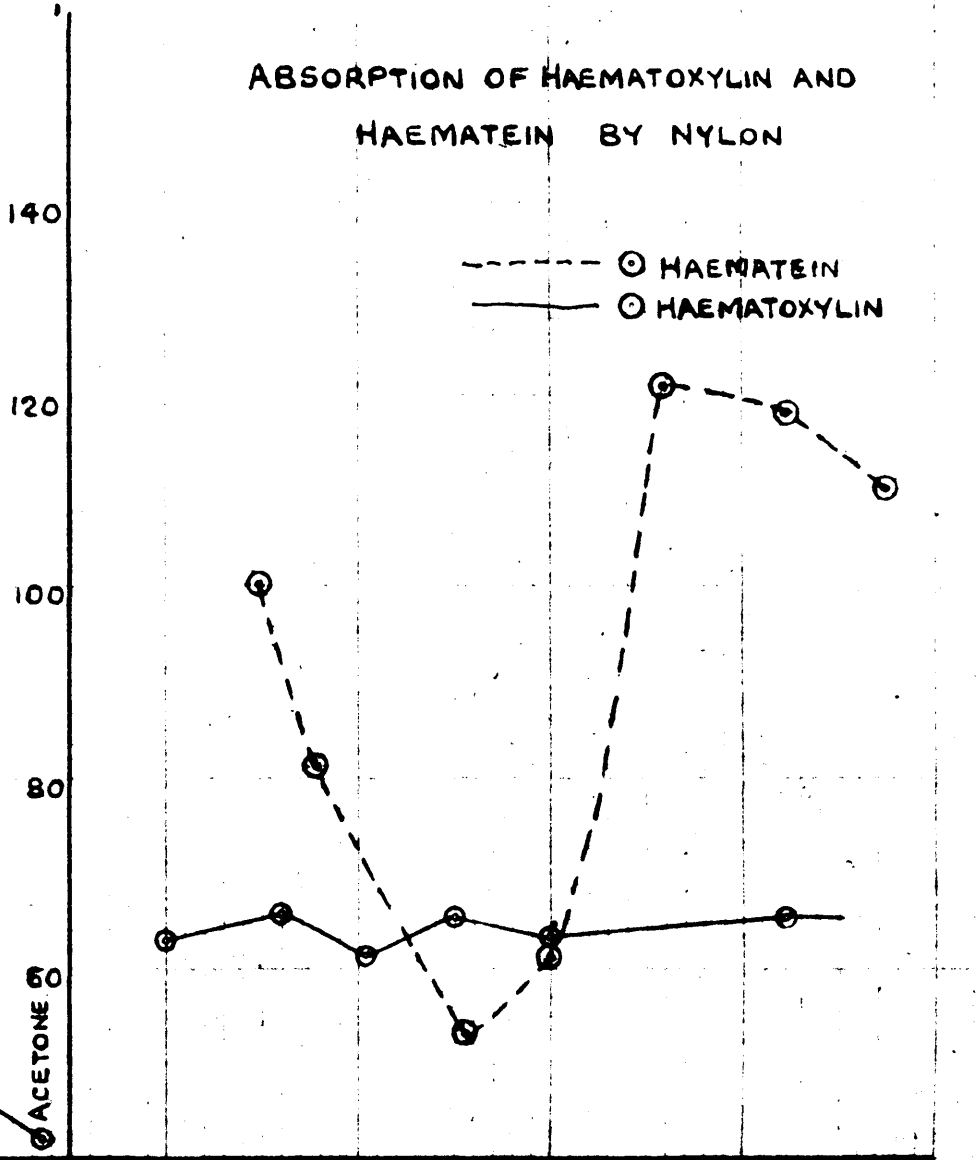

ABSORPTION OF HAEMATOXYLIN AND
HAEMATEIN BY NYLON
HAEMATEIN
HAEMATOXYLIN
140
120
100
80
60
0·02
0·04
0·06
0·08
0·085
MILLIMOLS PER G. OF NYLON.

FIG 32

HAEMATOXYLIN ABSORPTION.

A solution of nylon previously purified as described before was used for this experiment. 0.5 g. of nylon was dissolved in 100% m-cresol in 100 ml. Haematoxylin (98% purity) 0.053 g. was accurately weighed and dissolved in 100 ml. of m-cresol.

20 ml. of nylon solution was placed in a 50 ml.conical flask and 2, 5, 8, 9, 10 ml. of haematoxylin solution were added from the micro burette to each of these flasks respectively. The total volume in each flask was made up to 30 ml. The solutions were well shaken and allowed to stand overnight in corked flasks. The dielectric constants of the solutions were then measured and graphed against concentration of the dye.

It was noticed that haematoxylin takes a much longer time to reach an equilibrium with nylon solution in m-cresol. At least half an hour is required before each reading stabilises itself. The longer contact of haematoxylin solution with the dielectric plates was found to have an oxidising effect on the solution, turning it to red from faintly yellowish. This was avoided by maintaining an inert atmosphere just above the dielectric cell. Readings obtained have been described in Table XXXIV illustrated by Figures 33 .

TABLE

TABLE XXXIV

Number	Haematein		Haematoxylin	
	Millimols per gram. nylon	Vernier reading degrees	Millimols per gram nylon.	Vernier reading degrees
1	0.02	100.2	0.01	62.5
2	0.025	80.5	0.025	64.2
3	0.041	53.1	0.031	61.0
4	0.05	61.3	0.04	64.15
5	0.062	120.15	0.05	63.0
6	0.075	118.1	0.075	64.25
7	0.085	110.5		

Nylon 0.5 g. in 100 ml. m-cresol

Haematein 0.05%

Haematoxylin 0.053%

PART III

Section 9

ELECTROMETRIC TITRATION OF HAEMATEIN AND BRAZILEIN WITH DILUTE CAUSTIC SODA SOLUTION IN AN INERT ATMOSPHERE.

The presence of three and two phenolic hydroxy groups in haematein and brazilein respectively was confirmed by an electrometric titration of the respective solutions with dilute sodium hydroxide. The purpose of this experiment was twofold. It was intended to show the acidic nature of the molecule together with a means of determining the possible chromium linkage with the hydroxyl group in the molecules. (Section 10b)

Initial difficulties in Titrations.

The apparatus used for this experiment has been described in the analytical section, page , when dilute caustic soda solution was gradually added to a cold solution of haematein or brazilein the e.m.f. readings were found to be fluctuating even after allowing the circuit to stabilise. Several trials by varying concentrations of caustic soda etc. showed little improvement. It was noticed that if instead of aqueous solution of haematein, a solution of haematein in cellosolve and water (1:4) was prepared and a proper oxygen free nitrogen atmosphere maintained, steadier results were obtained.

Experiment.

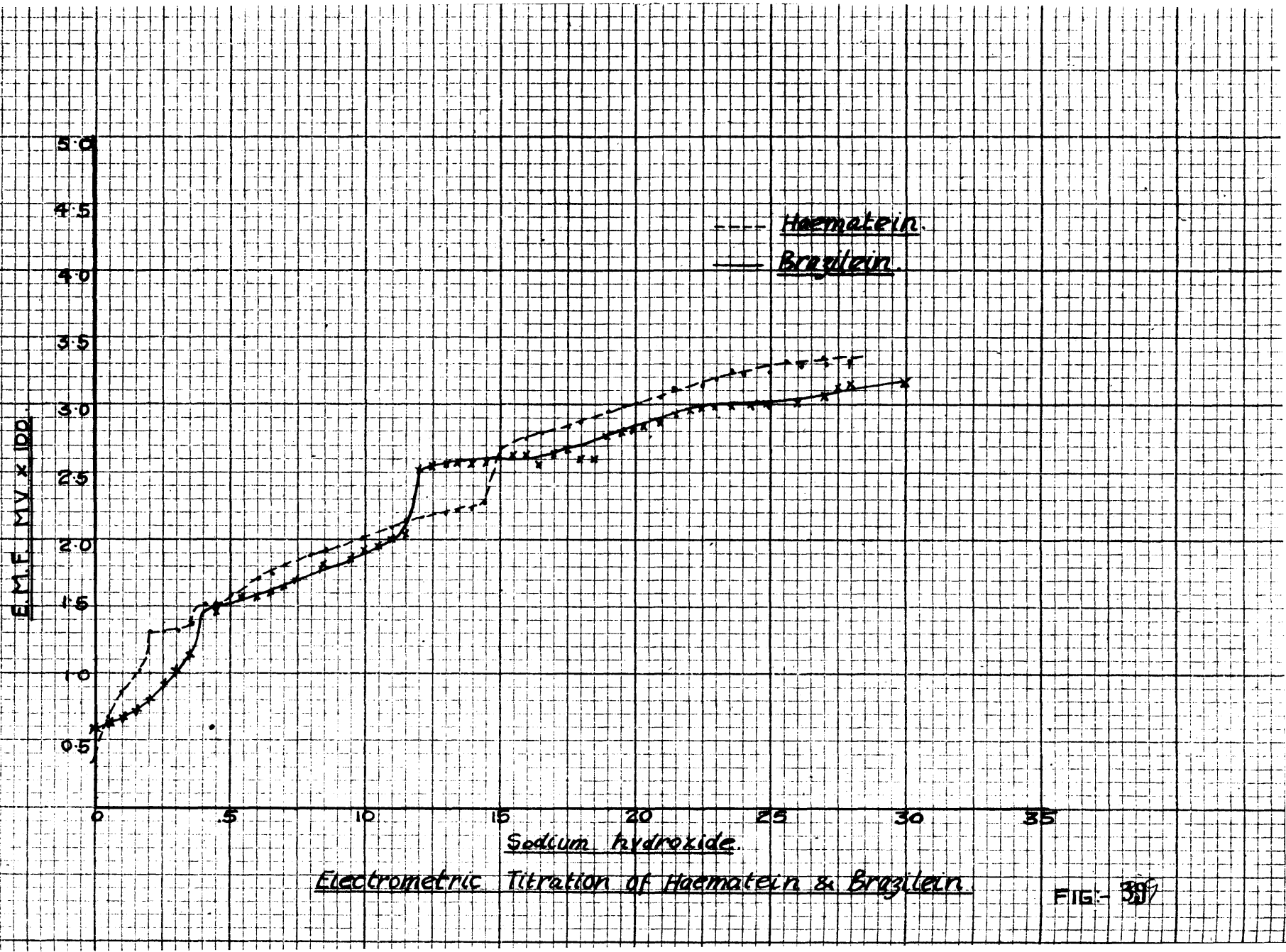
0.3 g. haematein (99.8%) was accurately weighed, pasted with 25 ml. of cellosolve and the solution made up to 100 ml. by

adding/

adding boiled distilled water. Caustic soda solution (0.0145 N) was taken in a burette. Temperature of the solution was 22.5°C. A steady stream of nitrogen was passed throughout the experiment. Since the variations in readings were extremely small, a number of readings had to be taken to note the steady readings in c.m.f. Readings are recorded in Table 35 illustrative by figure 39 .

TABLE XXXV

Sodium hydr-oxide	MVx100	Sodium hydr-oxide	MVx100	Sodium (ml)	MVx100	Sodium hydr-oxide (ml)	MVx100
0	0.350	6.5	1.75	15.0	2.630	21.0	3.10
0.5	0.650	7.0	1.80	15.5	2.650	22.0	3.15
1.0	0.860	8.0	1.89	16.0	2.70	23.0	3.20
1.5	1.050	9.0	1.90	16.5	2.75	25.0	3.25
2.0	1.310	10.0	2.01	17.0	2.8	28.0	3.310
2.5	1.310	11.0	2.11	17.5	2.83	30.0	3.320
3.0	1.310	11.5	2.13	18.0	2.86		
3.5	1.350	12.0	2.15	18.5	2.90		
4.0	1.502	12.5	2.18	19.0	2.95		
4.5	1.504	13.0	2.23	19.5	2.99		
5.0	1.503	13.5	2.28	20.0	3.03		
5.3	1.550	14.0	2.30	20.5	3.03		
6.0	1.710	14.5	2.35				



Electrometric Titration of Haematein & Brazilein.

FIG:- 39

Experiment.

0.3 g. Brazilein was accurately weighed and pasted with cellosolve (10 ml.) and warmed to dissolve. The solution was made up to 100 ml. by adding distilled water and was titrated against 0.015 N sodium hydroxide, standardised against oxalic acid. The change in e.m.f. was noted on a Marconi pH-meter with glass and calomel electrodes. Nitrogen atmosphere was maintained as in the previous experiment. Temperature of the solution was 23°C.

Results are described in Table XXXVI illustrated by Figure 39.

It is seen from Figure 39 that haematein gives three distinct inflections for 2.0, 3.5, 14.75 ml. of sodium hydroxide, whereas brazilein shows only two for 4.0 and 11.75 ml. and caustic soda.

TABLE XXXVI

Sodium hydroxide	MV x 100	Sodium hydroxide	MV x 100	Sodium hydroxide	MV x 100
0	0.60	8.5	1.81	17.0	2.70
0.5	0.610	9.5	1.85	19.0	2.78
1.0	0.630	10.0	1.91	19.5	2.80
1.5	0.710	10.5	1.95	20.0	2.82
2.0	0.803	11.0	2.00	20.5	2.84
2.5	0.913	11.5	2.05	21.0	2.86
3.0	1.03	12.0	2.51	21.5	2.95
3.5	1.150	12.5	2.51	22.0	2.97
4.0	1.45	13.0	2.51	22.5	2.98
4.5	1.48	13.5	2.51	23.0	2.99
5.0	1.51	14.0	2.51	24.0	2.995
5.5	1.55	14.5	2.51	25.0	3.00
6.0	1.58	15.0	2.51	26.0	3.01
6.5	1.62	15.5	2.55	27.0	3.10
7.0	1.65	16.0	2.61	28.0	3.18
7.5	1.71	16.5	2.65	29.0	3.19
				30.0	3.195
				32.0	3.20

PART III

Section 9a

MEASUREMENT OF THE DISSOCIATION CONSTANTS OF HAEMATEIN AND BRAZILEIN.

The following experiments were carried out to determine the relative strength of the various hydroxyl groups in haematein and brazilein in order to throw some light on the mechanism of their linkage with the fibre.

Procedure:

Neutral aqueous haematein and brazilein solutions of different molar concentrations (1×10^{-4} M, 1×10^{-3} M) were prepared. The solutions were titrated with $N/1000$ potassium hydroxide solution, noting the change in pH on a marconi pH meter.

The potentiometric titrations (page 189) of haematein and brazilein by addition of dilute caustic soda in nitrogen atmosphere had clearly indicated the presence of three distinct inflections, which probably correspond to the two hydroxyl groups in the ortho position and one hydroxyl group near the carboxyl group.

The titration with potassium hydroxide was carried out at room temperature. It is seen from Table XXXVIE that with haematein there are three neutralisation stages as compared to two for brazilein. This agrees with the respective numbers of phenolic hydroxy groups known to be present in these two compounds. Results in Table XXXVIE has been illustrated by figures 38 In Table XXXVII the calculated dissociation constants are tabulated. The value of first dissociation constant of each compound is nearly same. This may well be that of the hydroxyl group near the carboxyl group in both compounds.

TABLE XXXIVII

TITRATION OF HAEMATEIN AND BRAZILEIN WITH POTASSIUM HYDROXIDE.

pH	Haematein		pH	Brazilein	
	KOH stages			KOH stages	
4.4	0.0		5.8	0.0	
4.8	2.0		6.4	2.0	
5.2	4.0		6.9	4.0	
5.9	6.0		7.58	6.0	
6.6	8.0		8.15	8.0	
7.2	10.0		8.5	10.0	
7.7	12.0		8.61	12.0	
7.9	14.0	14.2	8.71	13.0	
8.1	16.0		8.81	14.0	14.8
8.2	17.0		8.85	15.0	
8.5	18.0		9.0	16.0	
9.0	20.0	20.0	9.2	17.0	
9.1	21.0		9.5	18.0	19.32
9.3	22.0		11.5	19.5	
9.4	23.0				
9.8	24.0	24.05			
12.0	25.0				

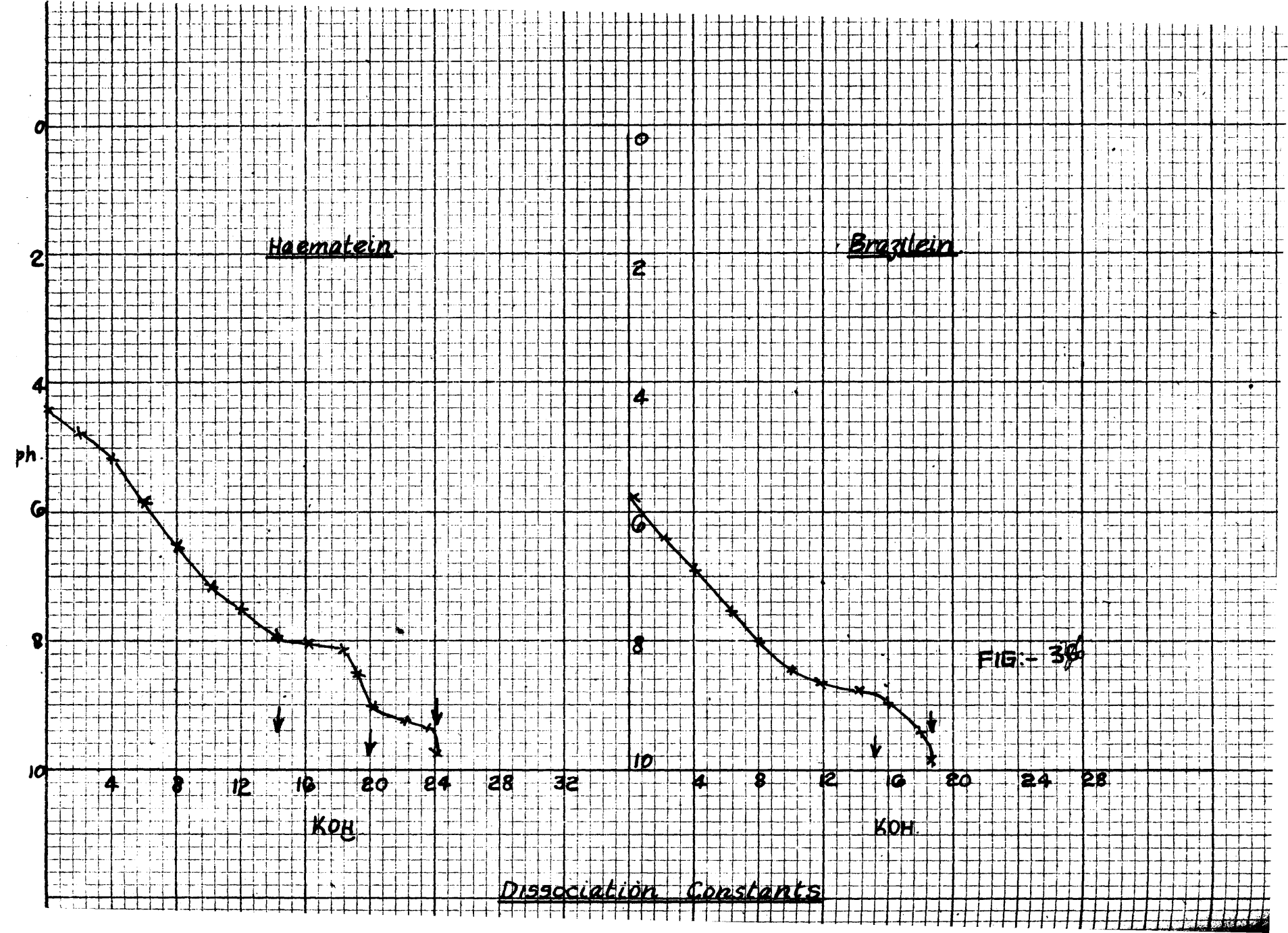


FIG:- 36

Dissociation Constants

TABLE XXXVII

DISSOCIATION CONSTANTS.

Compound	Mean	Ka	Kb	Kc
Haematein M/1000	5.012×10^{-8}	2.089×10^{-9}	8.318×10^{-12}	5.75×10^{-14}
Brazilein M/100	8.53×10^{-8}	3.502×10^{-9}	1.455×10^{-10}	

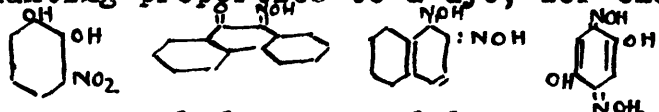
PART III

Section 10

THEORY OF LAKE FORMATION.

The term "mordant dye" was first used in 1887 by Kostanecki (Ber., 1887, 20, 3146). Mordant dye possesses the property of combining with a number of metallic oxides or hydroxides to form coloured "lakes." The salts of aluminium, chromium, copper, iron, etc., which give very sparingly soluble hydroxides are suitable as mordants.

Kostanecki found that hydroxy carbonyl and oximino groups impart mordanting properties to a dye, for example:-



As the structural features of dyes capable of being mordanted became more apparent, theories on the structure of the metal-dye complex began to be advanced.

Liebermann (Ber., 1893, 26, 1374) suggested that in mordanting alizarin with iron salts, a cyclic compound is formed in which metal and dye are united by normal valency bonds.

Werner (Ber., 1908, 41, 1062) applied his theory of co-ordination to the structure of metal-dye complexes. He concluded that for a dye to have mordanting properties, its constitution must be such that it can form both a salt-building link and a co-ordinative link with the metal.

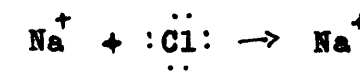
This work was extended by Morgan and his co-workers (J.C.S., 1915, 107, 645; 1919, 115, 1126; J.S.D.C., 1921, 37,43) to the dye lakes with the aid of the electronic theory of valency and he coined the word "chelation" to describe the process of

lake/

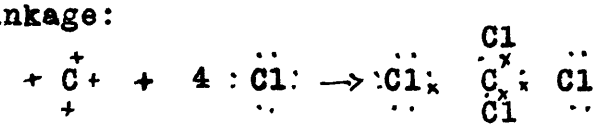
lake formation.

According to the electronic theory of valency, combination between atoms takes place in one of three ways:-

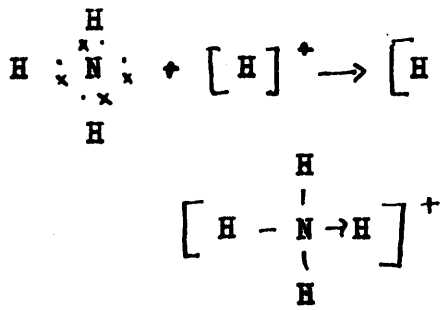
(1) By the transfer of one or more electrons from one atom to another. This combination is termed an electrovalent linkage,



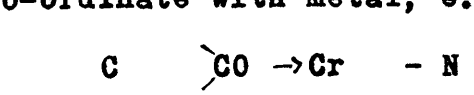
(2) By the sharing of one or more electrons between two or more atoms, e.g. in carbontetra chloride. This is termed a covalent linkage:



(3) By one atom supplying two electrons which are shared by both partners in the bond, e.g. in the formation of an ammonium ion from neutral ammonia and a hydrogen ion:



This type of combination is termed a co-ordinate linkage. The atoms providing the two electrons is termed the "donor" and atoms sharing them the "acceptor," the linkage being indicated by an arrow pointing from the donor to the acceptor. Nitrogen and oxygen are important donor atoms whilst many metals are acceptors. Thus the oxygen in the carbonyl group and nitrogen in an azo group will co-ordinate with metal, e.g. chromium

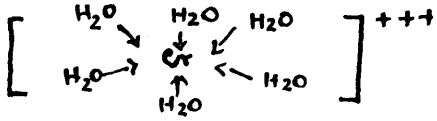


A special case of the co-ordinate linkage is provided by hydrogen, which when already joined in a stable manner by a pair of electrons to another atom is capable under certain conditions of accepting another pair of electrons. Thus water molecules combine together

$$H - \overset{H}{O} \rightarrow H - \overset{H}{O} \rightarrow H - \overset{H}{O} \quad \text{etc.}$$

So water is both a donor and an acceptor. Other compounds containing the hydroxyl -(OH) group behave similarly and when hydrogen and oxygen are involved in this way, the linkage is known as a hydrogen bond.

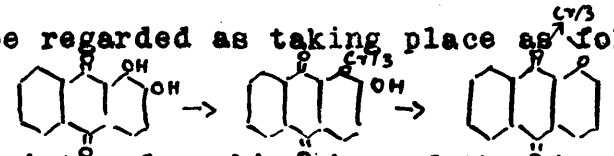
The chromium atom can co-ordinate a maximum of twelve electrons in its outer shell, i.e. four more than carbon, oxygen, sodium or chlorine, which require eight electrons for stability. Thus the following ion can exist:



Twelve electrons are supplied by the six water molecules whilst the loss of the three valency electrons makes the complex a tervalent ion.

Structure of Lakes.

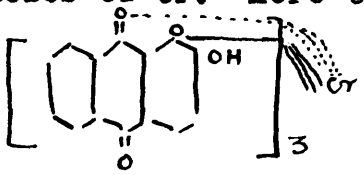
The mechanism of lake formation with a chromium salt and alizarine may be regarded as taking place as follows:-



The first stage consists of combination of the chromium with alizarine in a similar manner to the combination of sodium hydroxide with phenol to give sodium phenate. Since chromium is tervalent

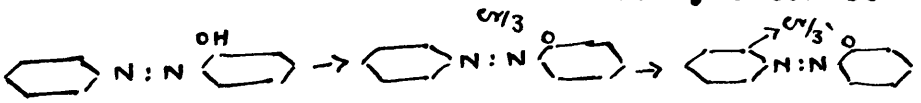
it/

it combines with three molecules of alizarine. For simplicity only one molecule is shown combined with $Cr_{/3}$, i.e. with one of the three primary valencies of Cr. More complete representation of this structure is:

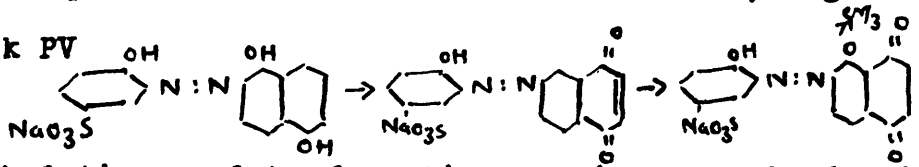


Where the dotted lines represent the co-ordinate links and the unbroken lines the primary valencies.

The mechanism of lake formation with azo dyes can be represented as:



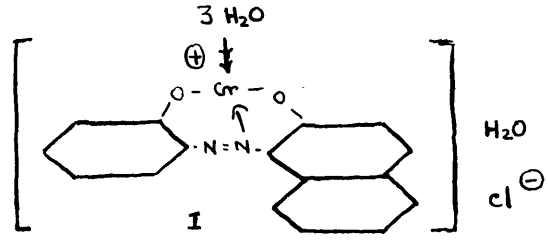
In the case of certain azo dyes which require oxidation for complete development of the shade, Morgan considered that oxidation to a quinone form precedes lake formation, e.g. with Diamond Black PV



Thus chelation or lake formation requires a salt forming group which is usually hydroxyl, and a co-ordinately unsaturated atom, such as oxygen or nitrogen, present in groups such as Keto (C = O), carboxyl (-C$\overset{OH}{\underset{O}{\diagup\!\!\diagdown}}$) and azo (-N=N-) and acting as an electron donor to the central metallic atom of the lake, thus completing its co-ordination number, which in the case of chromium is six, contributed by three molecules of dye, each of which donates two electrons to the chromium atom by means of a co-ordinate link. Since the other three links are of the covalent type, each contributes one electron and the chromium atom/

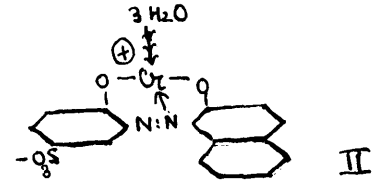
atom, therefore, gains nine additional electrons, thus completing its stable outer shell of twelve electrons.

A rather different type of structure was suggested in the more recent work of Drew and Fairbairn (J.Chem.Soc.1939, 823) By heating chromium chloride with azo mordant dyes, a lake of the type I is formed.



I

Here the chromium atom is combined with one molecule of dye instead of three molecules as in Morgan's structures, combination taking place through the two hydroxyl groups in the dye and involving two of the chromium atom's three primary valencies. The other valency is shown electrovalently linked to a chloride ion, which may be substituted by an ionised sulphonic group, if, as is the case with most commercial dyes, the dye molecule contains one or more sulphonic groups, the resulting lake (II) then being soluble in water. This electrovalent link involves the loss of an electron by the chromium and this is made up by means of an additional co-ordinate link, i.e. there are four of these links instead of three as shown previously.

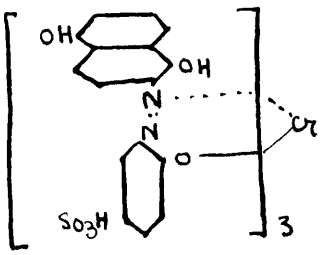


II

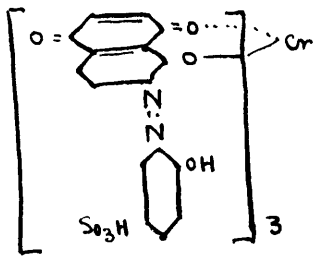
Of these links, three are provided by molecules of water, the oxygen atoms acting as electron donors; the fourth link is provided by one of the azo nitrogen atoms. Under suitable conditions, lakes of the above types I and II can be condensed together/

together to give an acidic complex.

A number of mordant dyes form a normal type of lake, with chromium salts, but the action of chromic acid produces a lake derived from an oxidised (quinoid)form, which is of an entirely different shade from the unoxidised lake. Morgan and Mainsmith gave the following constitutions for the normal (III) and oxidised (IV) lakes of Diamond Black PV, which are purple and black respectively



III



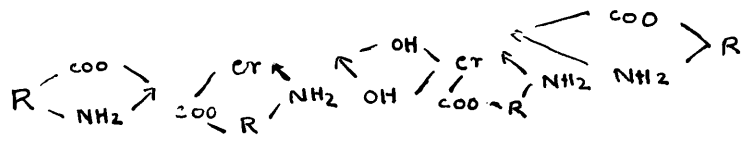
IV

Drew and Fairbairn considered that lakes of the above types, but containing sulphonic groups, represent essentially structures which are actually present in chrome dyed fibres, although they believed that a mixture of lakes may be present in many cases.

Boyle, Cumming and Steven (J.R.T.C., 1940, 4, 617) prepared and examined copper and chromium complexes derived from certain O-substituted dyes. The effect of group influence in the formation of complexes shows that O-amine, O-carboxy and O-hydroxy groups assume a role similar to that of the O-hydroxy in lake formation.

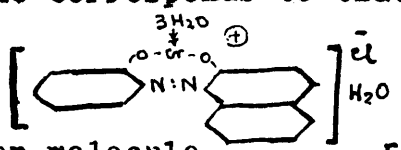
The Nature of the linkage binding fibre, dye and mordanting metal.

It is now generally accepted that acid dyeing of wool, at least with dyes which are not aggregated in solution, takes place through ionic links, formed between basic (NH_3^+) groups in the protein chains, with acidic groups in the dye, the mode of linking of dyes when a mordant is present, has not been fully worked out. The action of chromic hydroxide on amino acids results in the formation of red or violet complexes of the following type:-

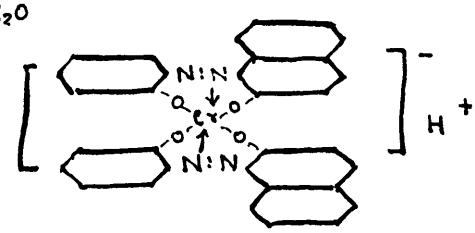


Whether the relatively inaccessible chromium in a dye-chromium lake, particularly of the type of the acid complex, could combine with protein in this way and remain attached to the azo residue appears doubtful.; Lieben (Nature, 1938, 142, 463) thought that coloured complexes of metal salts and proteins are formed by reaction, with the nitrogen atoms of the protein in alkaline solution and with the carboxyl groups of the protein in acid solution.

Giles (J.S.D.C., 1944, 60, 303-315) suggests that the structure of the lake corresponds to that of the compounds below



and not to the larger molecule



because/

because there is normally not a shortage of chromium relative to dye present during the mordanting process. It may then be assumed that when the lake is formed on the fibre, the water molecules co-ordinated with the metal atom are replaced by co-ordinated linkages with nitrogen or oxygen atoms of the main protein chains of the fibre. The sulphonic groups of the dye and perhaps the ionic valency of the chromium atom form salt links with ammonium and carboxyl ions respectively in the protein side chains.

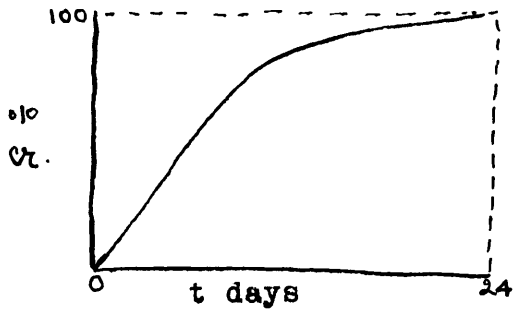
The Palatine fast and Neolan dyes also appear to exist with one metal atom per dye molecule. For instance, Palatine fast blue G.G.N. (Valko. Osterr. Chem. zt_2. 1937, 40, 465; Kalloidchemisce Grundlagen der Textilveredlung J. Sprunger, 1937) Here also, a mechanism of replacement, of co-ordinated water molecules or covalently linked hydroxyl groups, attached to the metal atom by groups in the wool molecule, can be postulated. Ender and Muller have supposed that such a combination takes place with Palatine fast dyes. Haller (Kolloid. 1942, 100, 121) has outlined similarly possible fibre-dye mordant lake. Potentiometric titration measurements of free acids of such disulphonic acid molecules as Palatine fast blue G.G.N. were made by Ender and Muller, who showed thereby that one sulphonic group is freely titratable as a strong acid and the other forms a Zwitterion with the positively charged chromium complex. In experiments involving measurements of the total acid combining capacity and wool both undyed and dyed with increasing amounts of

Palatine/

Palatine fast dye acids, the same authors obtained evidence that the chromium complex is capable of uniting through the metal atom, with only about 10% of the total number of basic groups in the wool. If quantities of dye are used in excess of the amount needed to saturate these specific groups in this way, then the excess is absorbed and fixed on the fibre, just as an ordinary acid dye. These workers were also able to demonstrate by the colour change of a chromable dye, applied to a palatine fast dyed pattern, that the chromium in the latter is still able to co-ordinate with further dye molecules and is thus present in the primary complex.

Race, Rowe and Speakman (J.S.D.C., 1946, 62, 375) has recently been able to determine the structure of chromium lakes actually present on wool. In Carlene, Row and Speakman (J.S.D.C. 1946, 62, 337) continuous extraction of chromed wool with pH 1 exalic acid has been described. A sample of conditioned wool was mordanted, with potassium dichromate, for two hours in standard beaker apparatus, washed in distilled water and then sealed between two glass wool plugs in a wide glass tube connected to a syphoner apparatus. pH 1 oxalic acid solution at 22.2°C. was syphoned through the wool into a flask during 48 hours, and the chromium in the extract estimated. Successive extractions with fresh pH 1 oxalic acid solution were made at 22.2°C. for 24 hours in each, until the chromium content of the extract had fallen to a small amount, after which each portion of pH 1 oxalic acid solution was used/

used three times for 24 hours each time before estimating chromium. In this way, chromed wool was extracted for 23 days.



$$\frac{d\ Cr}{d\ t} = K\ (100 - Cr\ t)$$

Cr is % total chromium removed in T time days

There is no doubt that the extraction of chromium from wool is governed by a simple exponential law. Exact agreement between the experimental and theoretical curves is not to be expected, because the rate of stripping at any given moment depends, to some extent, upon the rate of diffusion of the complex chromium compound into the extraction liquor. Since k remains substantially constant, it is clear that only one chromium compound is stripped for chromed wool. There is no evidence to suggest that the small amount of chromium remaining on the wool, after extraction with pH 1 oxalic acid solution is attached in a manner significantly different from the remainder. When chromed wool is treated with oxalic acid solution at room temperature, a comparatively high percentage of the chromium is removed, presumably as chromi-oxalic acid:

If, therefore, a limited amount of dye, which exhausts completely/

completely from the dye-bath, is applied to wool by the onchrome, metachrome or afterchrome process, under such conditions that the wool contains an excess of chromium can afterwards be removed by extraction with oxalic acid solution, if the latter does not decompose, the dye-chromium fibre complex and if it does not dissolve the dye-chromium complex. The residual amount of chromium in association with the fibre can then be estimated and related to the known amount of dye present in the wool. As a further check on this procedure, the amount of chromium in the oxalic acid extracts can be determined and related to the total amount of chromium found on the wool after dyeing.

PART III.

(Section 10b)

PREPARATION, ANALYSIS AND STRUCTURE OF HAEMATEIN LAKES.

There are several metallic derivatives of haematein but the chromium one is of great importance because of its exceptionally good shade and fastness properties. It is well known that the chroming of many dyes improves their fastness to washing, milling and to light and that it has an important effect in modifying the shades.

During the last thirty years, important developments in the use as dyes of chromium lakes themselves have taken place. The method of dyeing with a chromium lake leads to the introduction into the fibre of a practically uniform colouring lake. Hardly any information is available as to the structure of the colouring lakes of chromium produced with haematein.

It would clearly be of great advantage in considering the mechanism of dyeing with haematein on various fibres and the factors which control the degree of fastness to several agencies, to have an outline of the structure and properties of the chromium lakes of haematein. The object of the work to be described here was to prepare and examine chromium derivatives of haematein and brazilein and compare them with the products of the normal dyeing operation on wool and nylon.

Haematein and brazilein were purified as described before. All substances used in the preparation of lakes were of Analar quality.

Experimental/

EXPERIMENTAL.

Lakes of haematein were prepared using the following:-

(1) Chromic Chloride tetrahydrate. $CrCl_3 \cdot 4\ H_2O$

(2) Chromic fluoride. CrF_3

(3) Hexa-Amino Chromic nitrate. $[Cr\ (NH_3)_6]\ (NO_3)_3$

(4) Hexa Amino Cobaltic Chloride. $[Co\ 6\ NH_3]\ Cl_3$

Haematein: Haematein (2.63 g.) suspended and partly dissolved in boiling ethyl alcohol (80 ml.) (99%) was treated with a solution of Chromic Chloride tetrahydrate (1 g.) in warm alcohol (50 ml.) and the mixture boiled under reflux in a flask with a ground glass joint for 15 hours. The colour of haematein changes to purple after half an hour of refluxing but the complete formation of lake takes a long time. The solution was cooled and allowed to evaporate at 40°C. to a solid mass. This was to be further purified to remove free haematein and free chromic chloride. It was found that iso-propyl alcohol dissolves haematein and chromic chloride at a low temperature. The mass was therefore subjected to repeated extraction with iso-propyl alcohol until the extract was free from any colouration. The residue was washed with ether and dried on a tile. (Yield 3.1.g). Six preparations following the above procedure were carried out and the results of the analysis for chromium showed that in six different preparations

Found: Haematein Cr = 6.325%*, 6.18%*, 5.43%* respectively
Compound Cr = 5.68%, 5.48%, 5.61% respectively

Theoretical :- $(C_{16}\ H_{11}\ O_6)_3\ Cr$ - 5·51% Cr. chlorine = absent.

$(C_{16}\ H_{11}\ O_6)_2\ Cr$ - 8·00 % Cr.

* These compounds were extracted with iso-propyl alcohol for 12 h

The estimation of chromium was carried out by ashing a weighed quantity of the substance to a constant weight, fusing with sodium peroxide/

peroxide in a platinum crucible dissolving in hydrochloric acid and making up the solution to a known volume. It was then titrated against standard sodium thiosulphate solution.

The higher percentages of chromium in the first two samples can be explained as follows. Firstly, the ashing was not satisfactory and it may be that some of the product had not ashed completely. Secondly, the extraction of Chromic Chloride with Iso-propyl alcohol may not be perfect due to a shorter time allowed for the first three samples.

These analyses therefore show that three molecules of haematein combine with one molecule of chromium. The probable structure of the lake is represented by (I) and (II) in Fig.39. In order to examine whether the lake formed by the above procedure was the same as that which is formed by dyeing haematein on Chrome mordanted fibre, the properties of the lakes prepared were examined.

It was found to be insoluble in most of the organic solvents. Glacial acetic acid and mineral acids dissolved it. It was soluble in water. A dyeing on woollen fabric showed a brownish colour. The normal shade of the lake formed on the fabric is dark blue or black, which suggests that the lake obtained is different from that on the fibre.

Chromic fluoride.

Haematein (1.5 g.) was dissolved in aqueous ethyl alcohol (80 ml.) and 0.5 g. of chromic fluoride dissolved in 30 ml. of water was added to it. The solution was refluxed for 24 hours

on/

on a water-bath. After the experiment was complete, the solution was cooled and evaporated to dryness at low temperature. The solid mass was dissolved in slightly warm cellosolve. It was filtered and the residue was further purified by treatment with cellosolve. It was washed with ether and dried. Chromium was estimated as before.

Found Chromium = 5.85, 6.31, 5.98 per-cent. Fluorine = absent.

This again corresponds to one Chromium atom for three molecules of Haematein. The probable structure of the lake is represented by (I) in Figure 38.

The compound was insoluble in water, organic solvents and dilute organic acids. It was soluble in mineral acids, probably with decomposition as on addition of 2 N sulphuric acid it gave an orange colour and a fine suspension of the metal. It could not be dyed on the fibre owing to this difficulty.

Hexa Aminochromic nitrate.

This reagent was employed in order to test the existence of chelate groups. It is a co-ordination compound of chromium containing ammonia in the co-ordinated sphere. When hexamino-chromic nitrate is added to haematein in aqueous solution at ordinary temperature, practically no change takes place. However, if the temperature is raised to 60°C. formation of a lake begins, which is blue in colour and insoluble in water and organic solvents. Although the lake obtained by this method appears to be similar to that formed on the fibre, due to the hydrolysis of the co-ordinated compound, the lake formed is not stable. This is shown by dyeing

a/

a woollen fabric at a lower temperature about 45°C. and by dyeing at higher temperature 95°C. Piece dyed at a lower temperature shows a blue colour whereas the one dyed at a higher temperature shows yellowish colour.

~~Possible structure is represented in figure~~

Chromium = 9.3% 8.9% 9.1%

Nitrogen = Absent

Chromium atoms: Haematein molecules.

Chromic Chloride tetrahydrate.

Brazilein was purified as described before and partially dissolved in ethyl alcohol to form a suspension. Chromic chloride tetrahydrate solution in alcohol was added to it. The mixture was refluxed for 10 hours and the lake formed after the completion of refluxing, was allowed to dry and the solid mass was further purified by cellosolve extraction. The purified product was partly soluble in water and gave a purple shade when dyed on wool which is similar to that obtained when Chrome-mordanted wool is dyed with brazilein. (Ratio of brazilein - chromium used was same as in haematein.)

Found. Chromium

$(C_{16}H_{11}O_5)_3$ Cr requires Cr = 5.8%, 5.85%, 5.82%

Theoretical

$(C_{16}H_{11}O_5)_3Cr$ " Cr = 5·70 %

$(C_{16}H_{11}O_5)_2Cr$ " Cr = 8·40 %

Possible structure is represented in figure. 39 (same as haematein)

Hexamino Cobaltic Chloride.

Lakes of haematein and brazilein were prepared using

hexa/

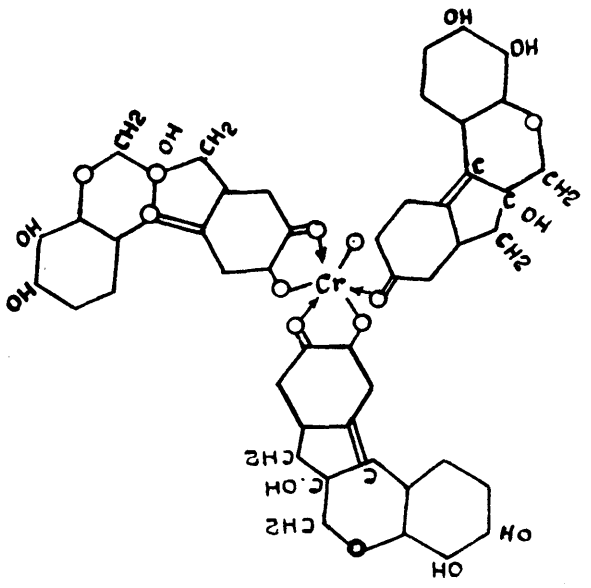
hexamino Cobaltic Chloride. This reagent was used because of its stability and the ease with which it differentiates between mordanting and salt-forming groups in a molecule. It was thought that if lakes of haematein and brazilein could be prepared using the same amount of dye and reagent and if their Cobalt and Nitrogen contents were estimated, it might give some indication regarding the nature and position of Chromophoric and auxochromic groups in the molecule.

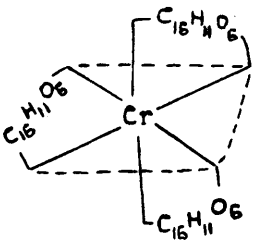
Preparation of lakes.

Haematein (2.3 g.) was pasted with cellosolve and the volume made up to 75 ml. Hexamino cobaltic chloride (3.0 g.) was dissolved in warm water (25 ml.) and both the solutions were mixed together and refluxed on a boiling water-bath for 24 hours, cooled and allowed to settle. A bluish precipitate settled, leaving a clear supernantant liquor. The residue was filtered and washed repeatedly with water until no yellowish tinge was seen in the filtrate. This residue (4.31 g.) was found to be insoluble in water, organic solvents, dilute acids, except that dilute sulphuric acid partially dissolved it. It was further purified with water, dried and analysed.

Haematein Compound.	Found	Co = 14.8%;	N = 11.85% Chlorine - absent
Brazilein Compound		Co = 11.52%;	N = 6.83%

Possible structure is represented in figures 38-III & IV. for haematein and brazilein respectively.





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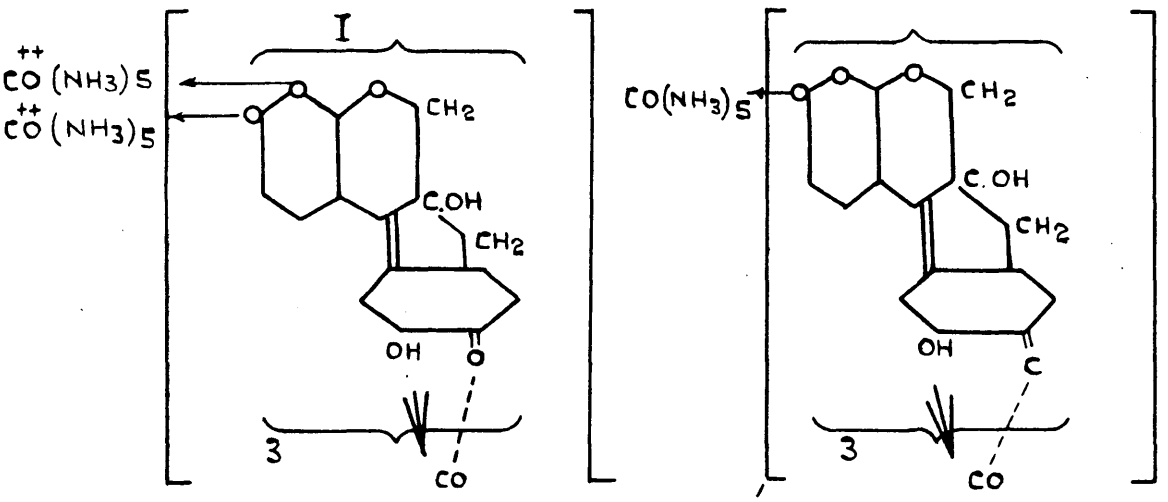
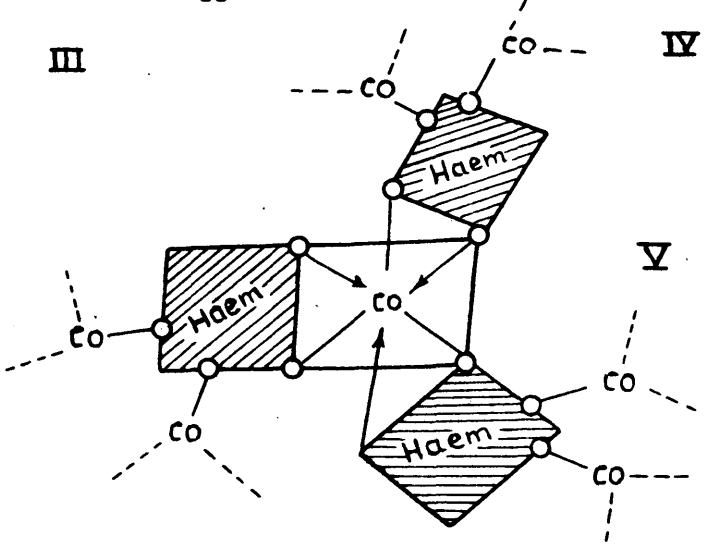


III IV



V

FIG. 38.

Since there are no negative ions present in the haematein complex, the cobalt in the haematein-hexamino cobalt chloride complex must become trivalent by cross-linking to haematein molecules $CO(Haem)_3$ groups, as in Figure 39.

Thus each peripheral cobalt would contribute 1/3 to the central group $CO(Hm)_3$. There are six cobalt atoms contributing to three haematein molecules.

Therefore Empirical ratio is $CO(Hm)_3\ CO_2$

Now each peripheral cobalt could take 3 NH_3, a total of 18 NH_3 for 6 cobalt atoms, or 6 NH_3 for 2 peripheral cobalt atoms giving the empirical ratio:-

$$CO\ (Hm)_3\ \ CO(NH_3)_6 \text{.....A}$$

In the structures III and IV in Figure 38, we have an empirical ratio:-

$$CO\ \ (Hm)_3\ \ CO\ \ (NH_3)_5 \text{.....B}$$
$$2$$

The difference in the empirical ratios of A and B may be due to two reasons:

(i) Ammonia might be lost in drying.

(ii) Water might replace ammonia

($H_2O = 18$; $NH_3 = 17$).

Assuming the nitrogen estimation to be correct, the difference before and after heating in the mol.wt. of the heavy molecule would be $(NH_3)_6$ instead of $(NH_3)_5$, Since the molecular weights of ammonia and water are almost equal, there would be no change in the percentage of other constituents.

PART IV.

DISCUSSION OF RESULTS.

The experimental observations recorded in part III of this thesis may be considered in relation to four main topics:

(1) The activity of the phenolic hydroxy groups in the haematein molecule.

(2) The occurrence of maximum absorption of haematein at pH 3.2 on nylon and pH 1.1 on wool fibre.

(3) The properties of haematein with respect to light fastness.

(4) Significance of the results in relation to the theory of dyeing haematein on wool and nylon fibre.

(1) The activity of phenolic hydroxyl groups in the haematein molecule.

The experimental observations presented in various sections of Part III will already have indicated that system under consideration can hardly be regarded as a simple one. Hence, in attempting to offer a reasoned explanation of the observed phenomena, it seems all the more desirable to obtain all the help possible about our present knowledge regarding the chemistry of haematein, wool and nylon molecule. This will be considered in the following way:-

(a) Asmumption that structure of haematein is correct.

(b) Activity of amine, amido groups in nylon and salt linkage in wool fibre.

(c) Lake forming properties of haematein and brazilein.

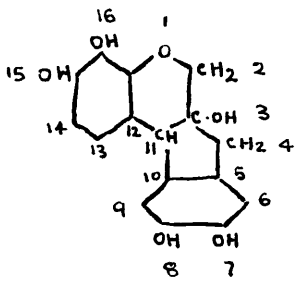
(d)/

(d) Titration of haematein solution with dilute sodium hydroxide solution and measurement of the dissociation constants.

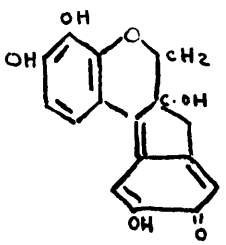
(e) Absorption of haematein and brazilein on wool and nylon fibre.

(f) Haematoxylin is not absorbed by wool and nylon fibre.

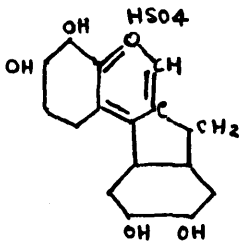
(a) As previously stated in Part II, the following constitutions have been assigned to haematoxylin (I), haematein (II), Iso-haematein Sulphate (III), and Brazilein[3] (IV) by Perkin and Robinson.



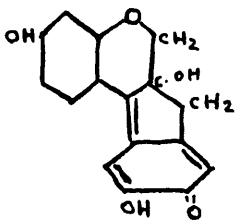
I



II



III



IV

Haematoxylin. Haematein. Iso-haematein sulphate. Brazilein

It can beseen that haematoxylin in (I) contains five hydroxyl groups[4] of which one is alcoholic in nature.[5] Main knowledge about the chemistry of haematoxylin was obtained by study on oxidation products of haematoxylin[6] tetramethyl ether. This substance on oxidation yields compounds containing pyrogallal and catechal structure. Hence, our present knowledge about/

about the chemistry of haematoxylin, the parent substance of haematein, shows the presence of four phenolic hydroxyl groups and one alcoholic hydroxyl group. Haematein contains three phenolic[4] hydroxyl groups and one alcoholic hydroxyl[5] group. Haematein tetra methyl[7] ether combines with formic acid; yielding a formic acid derivative, probably indicating the presence of a carbonyl group.

(b) The activity of amine and amido groups in nylon has been fairly well established due to the work of Peters[8]; Carlene, Fern and Vickerstaff[9]; Harris and Sookne[10] and others. Nylon molecule could be represented as follows:-

$$\underline{HOOC}.(CH_2)_4\left[\underline{CO.NH}.(CH_2)_6.\underline{NH.CO}.(CH_2)_4\right]_n\underline{CONH}(CH_2)_6.\underline{NH_2}$$

The underlined groups in the above molecule are considered to be active with regard to nylon's behaviour towards acid. Typical titration curve of nylon fibre[11] with hydrochloric acid is given on page 39 which shows that a distinct inflection corresponding to the absorption of 0.036 milliequivalents of hydrochloric acid per g. of nylon fibre is obtained, which corresponds to the number of primary amine groups in the fibre. At pH values below 2.0, a very great increase in acid absorption occurs, without reaching any limit in the pH range studied. This acid must be attached to the amido groups. A titration curve of this type is an expression of the number of hydrogen ions bound by the fibre at varying pH values. The isoelectric nylon fibre contains an equal number of negatively/

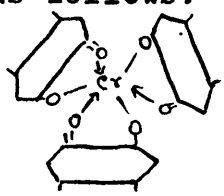
negatively charged carboxyl and positively charged amine groups, together with a number of non-ionised carboxyl or amine groups depending on which type is present in excess. In the case of nylon, the isoelectric point and analytical data show that the carboxyl groups are in excess. Accordingly, when nylon is placed in an acid solution, the hydrogen ions are absorbed by ionised carboxyl groups to give non-ionised groups. Thus the total number of hydrogen ions are milli equivalents of acid which can be absorbed in this way, determined by the number of amine groups in the fibre, but the pH at which the ionisation of the acidic groups is repressed, depends on the dissociation constant of the acid. It has been calculated that dissociation constant of acidic groups in nylon is 3.8 compared with dissociation constant of 2.1 for fibre and titration with potassium hydroxide showed 0.78 milliequivalents per g. of fibre. Edner and Muller[16] showed that combination of wool with acids is of the same nature as its combination with simple dye acids, differing only in degree of affinity of the acid anion for the wool. Skinner and Vickerstaff[17] found that at pH 1.2 wool can absorb more dye than is needed to saturate all the basic groups in the wool fibre.

(c) It will be seen from Section 10 of Part III that both haematein and brazilein form lakes with chromium compounds. Considering the structures of haematein (II) and brazilein (IV) on page 218 , the linkages of chromium can take place at 7-8, and 13-15; in formula (II) and 7-8 in formula (IV) as chelation or lake formation requires a salt forming group which is hydroxyl

and/

and a co-ordinating unsaturated atom such as oxygen. In formula (II) and formula (III), position number seven has an unsaturated oxygen atom present in the >C=O group.

From the experimental observations (Section 10), it has been found that the value for the ratio of chromium to haematein and chromium to brazilein is practically the same. It would seem that the hydroxyl group number 8 in formulas II and IV would form a linkage with chromium as follows:-



(d) Haematein solution when titrated with dilute sodium hydroxide solution (~~Figure 38~~ page. 191) shows three distinct inflection points whereas in brazilein only two such points are noticed. The two points in the case of haematein titration are near each other, probably indicating the proximity of hydroxyl groups at 15 and 16 positions in the haematein molecule. The dissociation constants of haematein and brazilein have been determined (Table XXXVII) and it has been found that the first value of K_a is very nearly the same for both the compounds. The value shows the more acidic nature of hydroxyl group number 8 in the haematein molecule. This acidic nature may be due to the presence of the adjacent carbonyl group.

(e) The activity of the hydroxyl group (position 8 in formula II) near the carbonyl group in haematein is observed in the/

the various absorption experiments carried out on wool and nylon. The results described (Tables XXIV-V) in Section 5 of Part III, shows that absorption values for haematein and brazilein on wool are nearly of the same order. If the active positions of haematein could be 1, 7, 8, 15 and 16. But it has been found that haematoxylin is not absorbed by wool when dyed under an inert atmosphere. Nylon also does not absorb haematoxylin as measured by dielectric measurements (Section 8). This shows that positions 1, 15 and 16 are not responsible in the affinity of haematein towards fibres. It can be concluded from these observations that positions 7 and 8 might be responsible for linkages with the fibre.

(2) <u>The occurrence of maximum absorption of haematein at pH 3.2 on nylon and pH 1.1 on wool fibre</u>.

(a) Variation in absorption of haematein under normal dyeing conditions.

(b) Absorption of haematein with variations in pH, temperature and concentration on wool and nylon fibre in an inert atmosphere.

(c) Absorption of catechol and resorcinol on wool and nylon fibre.

(d) Absorption of haematein at pH 3.2 on nylon and at pH 1.1 on wool fibre.

(a) Variation in absorption of haematein, under normal dyeing conditions, has been noticed (Part III, Section 5) and the effect of/

of pH has been studied. It was observed that absorption of haematein on wool at 95°C. and pH 1.6 was 0.83 millimol. of haematein per g. of fibre. It can be seen from Figure 19 (p.144) that below pH 3.5 for wool fibre there is a sudden rise in the absorption of haematein. In the case of nylon (Figure 20 (p.146)) this change is noticed below pH 3.2. However, the values were found to be varying a great deal and it was discovered that the haematein was being gradually oxidised in the bath, during the experiment. Hence, the experiments were repeated under an inert atmosphere to carry out similar observations.

(b) Effect of an inert atmosphere, maintained during absorption of haematein on wool, is shown in Tables ~~XX and~~ XXIII. Absorption is found to be higher in an inert atmosphere. The variation in absorption with change in pH is illustrated by Figure 20 (p.151). The curve is divided into four parts. The first part 'A' shows the lowest absorption, probably indicating the poor ionisation of haematein and its difficulty to penetrate the miscelle structure, due to the size of the particle. Part 'b' shows increase in absorption with increase in H ion concentration, showing the ionisation of haematein. Part 'c' shows rapid absorption and below pH 1.1 absorption increases much higher. The rapid absorption noted at pH 1.1 suggests that due to the particle size of the haematein, it penetrates into Keratine structure and forms some aggregates which presumably disrupts the fibre and liberates more amino groups. This in turn takes more dye acid present in the bath.

In/

In the case of nylon, this rapid absorption begins at pH 3.2. The absorption of haematein on nylon is shown in Figure 22. The curve shows three distinct phases of absorption. The gradual absorption takes place between pH 5-8, becomes steady between pH 3-5 and increases to a high value below pH 2.0. Experimental observations for the absorption of brazilein on wool and nylon fibre are given in Table XXV. Results show that absorption values are practically of the same order as haematein. Typical curves are represented in Figure 23-24 for brazilein and haematein. It is seen from the figure that absorption rate is slower for brazilein than haematein. Absorption of haematein increases at pH 1.1 for wool.

p·153

Nylon has a much smaller uptake of brazilein at pH 5 as compared to haematein. This means that affinity of haematein towards nylon is greater than that of brazilein. This affinity increases below pH 3.2 for haematein on nylon. Effect of pH on the absorption of haematein on wool showed that below pH 1.1 there was a rapid rise of absorption. In order to determine the maximum value of absorption on the fibre, isotherms have been determined and saturation values for haematein and brazilein have been determined by keeping pH constant and varying the concentration of haematein in the dyebath. The acidity of the dyebath was maintained at pH 1.8, where it is generally recognised[17] that wool does not decompose. The saturation value obtained at 85°C. for wool (Figure 25) is 0.893 millimols of haematein/

haematein per g. of wool. The saturation value for brazilein on wool is 0.72 millimols per g. of wool. Results in Table XXVIII gives the saturation values obtained at 25°C. for nylon with haematein and brazilein. It is seen from the figure 29 that the rate of absorption on nylon at lower temperature is practically the same for haematein and brazilein.

(e) Absorption of catechol and resorcinol on wool and nylon fibre.

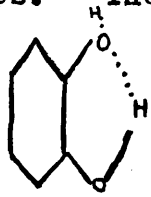
Haematein, as pointed out before, is a complex molecule. In order to simplify the study of its mechanism of absorption on fibres, it was thought that if simpler substances like phenol, quinone, catechol and resorcinol be studied on fibres, the complexity of haematein might be simplified. Action of quinone[19,22] and phenol[20,21,23] has been studied on protein and cellulosic fibres by several workers. It is known that quinone is capable of forming cross-linkages between peptide chains of animal fibres and it has often been used to prevent and repair damage in the fibre. Cathechol is the simplest oxidation product of haematoxylin in tetramethyl ether and its behaviour towards wool and nylon (Figure 35)[(p.174)] has been studied. It is seen from Table XXXII that wool at 60°C. absorbs 0.35 millimols of resorcinol and 0.32 millimols of catechol per g. Nylon at 60°C. (Table XXXIII) absorbs 0.019 millimols of resorcinol and 0.043 millimols of catechol per g. of nylon.

The absorption of these hydroxyl compounds on protein fibres could be explained on the basis of hydrogen bond formation.

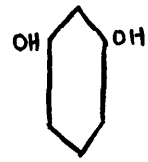
Catechol, which has two ortho hydroxyl groups, forms[24] a

hydrogen/

hydrogen bond between the two hydroxyl groups and leaves one hydrogen ion free to combine. In resorcinol, the hydroxyl groups are in meta position and no hydrogen bond formation takes place between the two groups. The structures could be represented as follows:-



Catechol.



Resorcinol.

Catechol forms a hydrogen bond[24] and makes the structure more stable. Examination of values obtained for resorcinol and catechol for nylon show that they are practically one half of the other. The absorption on fibre seems to be a function of pH. It is difficult to explain why phenol acts as a solvent to nylon and dihydroxyl compounds like catechol do not attack nylon. Catechol and resorcinol appear to be linking with the fibres in a loose form. This is seen by rinsing the fibre twice in hot water and trying to couple the resorcinol or catechol left on the fibre by sulphanilic acids. The hydrogen bond formation between the -CO group of polyamide, are polypeptide and hydroxyl group of resorcinol or catechol has been visualised. In the case of resorcinol, there are two hydrogen ions to combine with hydroxyl group and there is only one hydrogen ion for catechol. Hence the value of resorcinol is half that of catechol.

(d) <u>Maximum absorption of haematein at pH 3.2 on nylon and at pH 1.1 on wool</u>.

In order to show the comparative affinities of haematein, brazilein, catechol and resorcinol towards wool and nylon fibre, at/

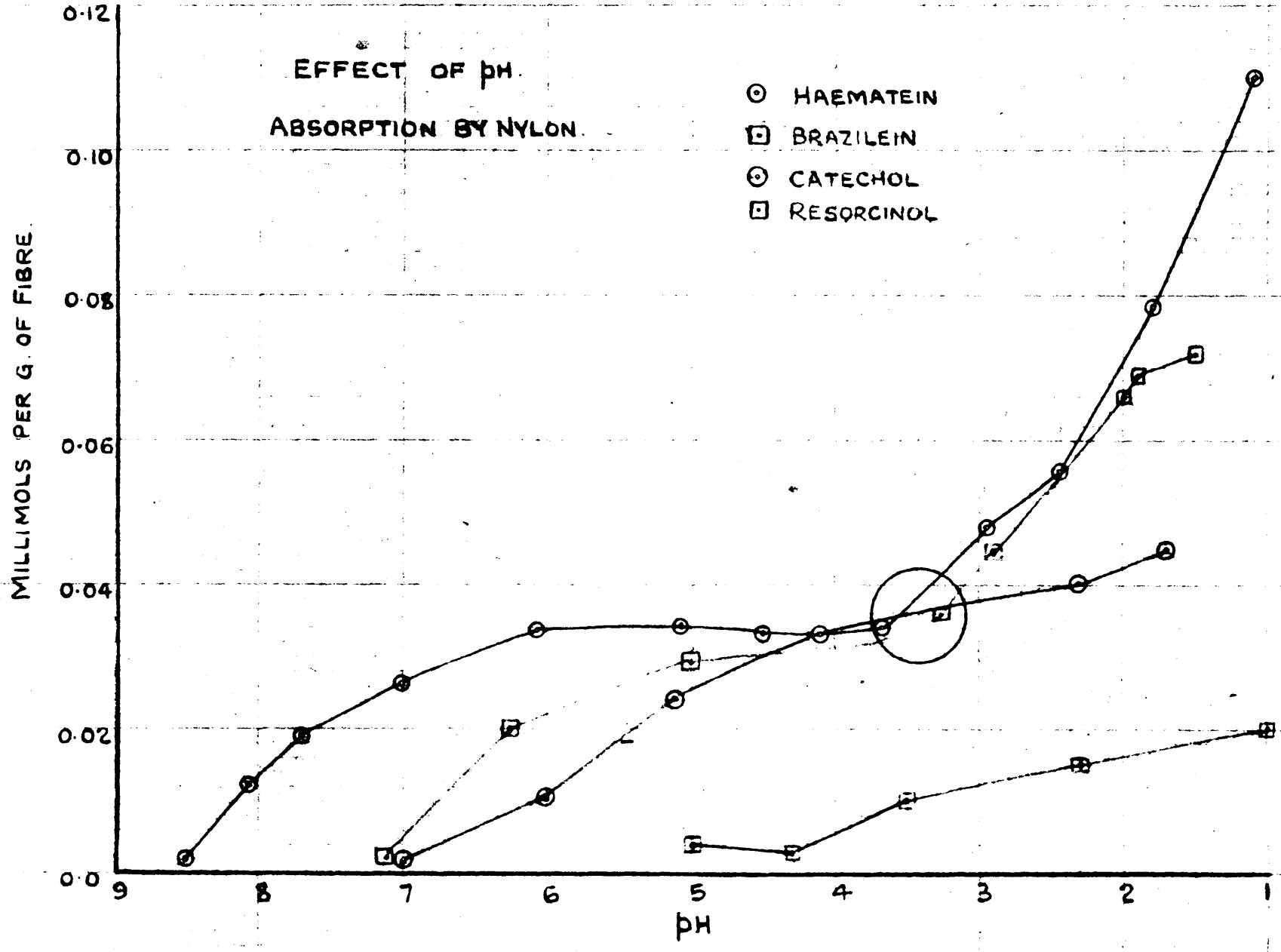

EFFECT OF pH.
ABSORPTION BY NYLON.
HAEMATEIN
BRAZILEIN
CATECHOL
RESORCINOL
MILLIMOLS PER G. OF FIBRE.
0·12
0·10
0·08
0·06
0·04
0·02
0·0
9
8
7
6
5
4
3
2
1
pH

FIG. 40

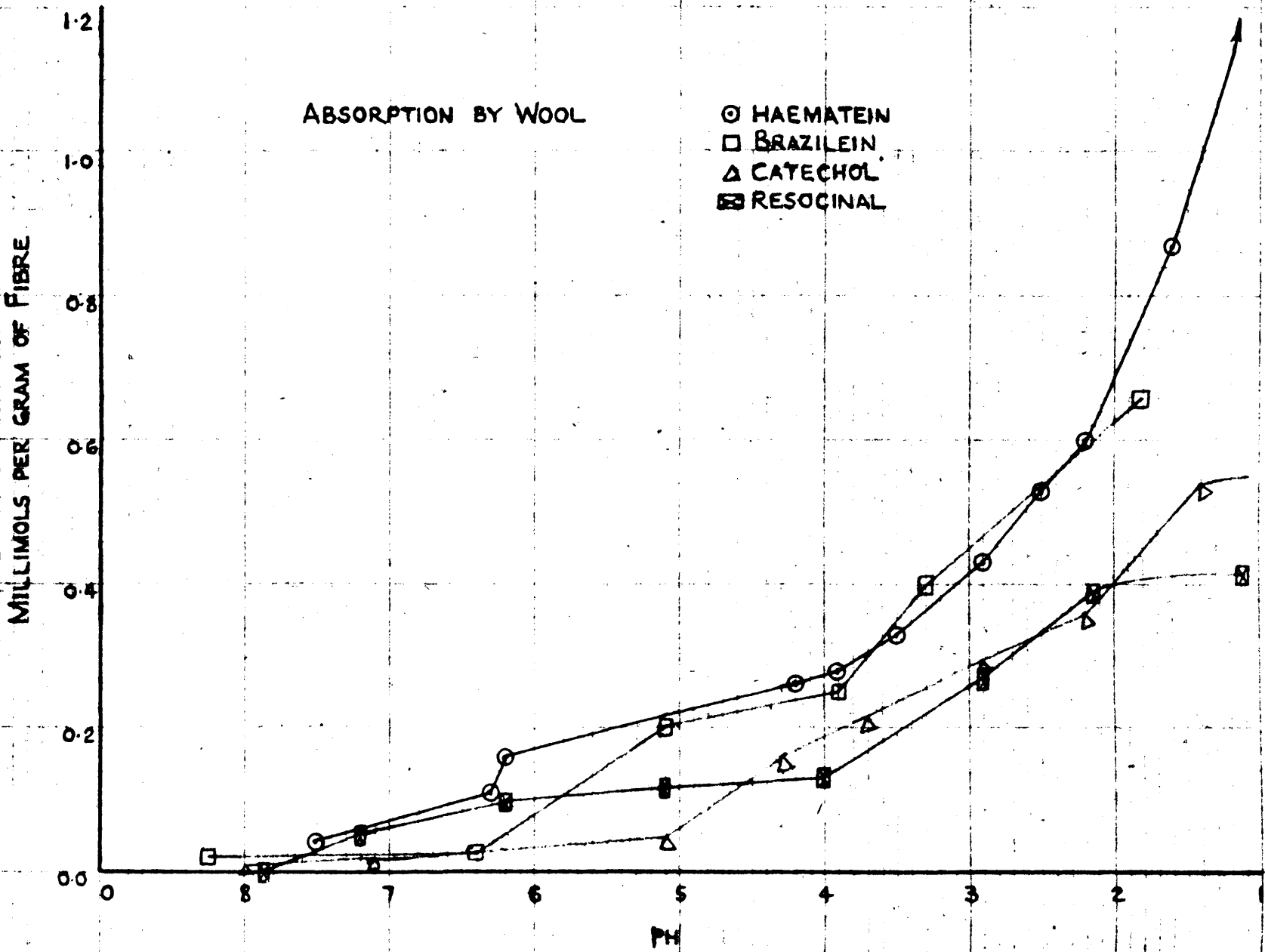

ABSORPTION BY WOOL
HAEMATEIN
BRAZILEIN
CATECHOL
RESORCINAL
MILLIMOLS PER GRAM OF FIBRE
1·2
1·0
0·8
0·6
0·4
0·2
0·0
0
8
7
6
5
4
3
2
PH

FIG. 41

at different pH values, curves have been drawn which are illustrated in Figures 40 and 41. It is seen from Figure 40, that below pH 3.2, absorption value increases. The absorption of 0.036 millimols haematein, brazilein and catechol per g. of nylon fibre corresponds to Lemin's titration[11] curve for nylon with hydrochloric acid. This value has been confirmed by dielectric measurements of haematein on nylon fibre (~~Figure 28~~ page 186). The high absorption of haematein - 1.8 millimols per g. of fibre may be due to the decomposition of the fibre at higher pH. The decomposition of the fibre may be due to aggregation of haematein inside the fibre at higher pH. Aggregation of haematein was noticed (Part III, Section 4) during the experiments of dyeings with haematein. It may also be due to the formation of additionall cross-linkage with cystine, as the haematein has been found to be more firmly attached to wool than nylon.

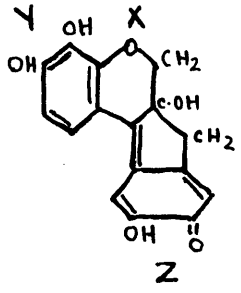
(3) Light Fastness properties of haematein:

The light fastness tests of haematein have been carried out by D.J.Duff and have been described on page 137 . It is concluded from these observations that haematein dyed on an unmordanted wool, has a fastness value of 1.75 whereas the fastness is considerably increased when it is dyed on a chromium mordant. The commercial samples of haematein when dyed on a mordanted wool, show fastness 4.5, which is lower than fastness obtained with pure haematein.

It is interesting to note that light fastness of

methylated/

with X, Y, Z to consider the various forms of linkages with the fibre.



Haematein.

The results and observations seen so far could be considered under the following three headings:-

(a) Oxonium salt formation.

(b) Hydrogen bond formation.

(c) Complex salt formation.

(a) Oxonium salt formation.

It is well known that oxygen atom in the pyron ring[28] and C=O[29] group forms a compound with sulphuric acid, usually known as oxonium compound or salt. It may be imagined that since haematein is dyed on fibre in presence of acid, such salt formation might be taking place, which might be responsible for its attraction into the fibre. But oxonium salts formed in the above way, are unstable and are quite different from that formed in the case of iso-haematein (III), Where the stability of the oxonium salt is due to the conjugated unsaturated system of the pyran ring, such salt formation would take place only in a highly acidic medium. The carbonyl group in the haematein is capable of forming salts as it is seen from the work of W.H.Perkin that haematein/

nylon, we are led to visualise the existence of some non-electric specific attraction. The author believes that this is a matter of the formation of 'hydrogen bond' between the peptide groups of wool on one side and the hydroxyl group of the haematein on the other side. In the case of nylon, similar 'hydrogen bond' formation is visualised between the polyamide group and the hydroxyl group of the haematein molecule.

It is clear from the discussion in the foregoing pages that the only hydroxyl group active in forming a hydrogen bond are forming a linkage with the fibre, would be the one at position 8 in the haematein molecule. If this conception is correct, then the results obtained could be examined as described below. The saturation values, obtained for haematein, brazilein, catechol and resorcinol on wool and nylon are given below:

TABLE. XXXIX

SATURATION VALUES FOR FIBRES.

Substance	pH	Millimols per gram of fibre.	
		Wool	Nylon.
Haematein	1.88	0.893	0.078
Brazilein	"	0.720	0.071
Catechol	"	0.570	0.043
Resorcinol	"	0.350	0.019

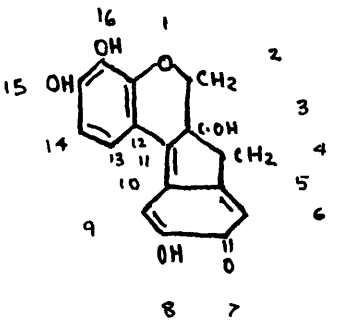
The formation of hydrogen bond between the hydroxyl group at position 8 in the haematein molecule on one side and polyamide/

free to combine. Resorcinol has two groups but are not connected by hydrogen bond and would be capable of uniting by a hydrogen bond independently. But the results, although very nearly the same for haematein and brazilein, are not nearly half for catechol to resorcinol. However, it may be that such a mechanism may not be impossible to visualise. As far as present investigations are concerned, nothing definite can be said, due to lack of sufficient evidence.

(4c) Complex salt formation.

The combination of a carboxyl and a hydroxyl group is considered as a barboxyl function and is associated with acidic characteristics.

In the haematein molecule which is shown below for easy reference:-



positions 7 and 8 have a carbonyl and hydroxyl group. The dissociation constant of haematein (Part III, Section 8a) has been measured and it has been found that the acidic nature of hydroxyl group at position 8 is more than at position 15 or 16. The results show that haematein and brazilein are more acidic than phenol and less acidic than acetic acid. The absorption spectra of

haematein/

haematein is indicative of unsaturation corresponding to a carbonyl group. This is shown in figure 8 page 91 where the maximum absorption of haematein takes place at 28A°.

Thus the mechanism of haematein absorption by wool and nylon in an acidic medium has been found possible to explain on a 'salt-forming theory.'

The saturation values for haematein and brazilein on wool and nylon are very nearly the same. This value compares well with the value obtained for the titration of wool (Part II, Section 3) with the hydrochloric acid. This suggests that haematein has formed a dye acid in presence of mineral acid, which penetrates into the keratine molecule and neutralises the weakly acidic negative charge on carboxyl ion leaving positive charge on ammonium ion. This attracts the haematein from the bath. This is represented as follows:-

$$H.\ Hm + H_2SO_4 \longrightarrow H^{+} + Hm\ SO_4$$

$$R.\ NH_3 - COOR' + Hm\ SO_4^{-} \longrightarrow \underset{\text{Wool.}}{R.NH_3\ Hm\ SO_4.} + R' - COOH.$$

But the mechanism is not so simple as described above. It has been observed that haematein could be dyed from a neutral bath although the exhaustion of the dyebath is not very good. Again the dyeing of haematein on wool and nylon is a function of pH. The absorption of haematein on wool reaches maximum at pH 1.1 and at pH 3.2 on nylon. Haematein is difficult to strip off from the fibres with pyridine and other organic solvents.

At/

At lower temperature its affinity towards the fibres has been found to be poor, but it increases with rise in temperature.

In order to explain the above behaviour of haematein, it is necessary to understand the effect of water and acid on wool fibre, wool contains micelles which are arranged lengthwise along the fibre, are probably lamellar in shape and are about ten times as long as they are thick. The micelles consist of long folded polypeptide chains in parallel, linked together in one place by cystine and salt linkages. Such linked planes are held together along their length by Van der Waal's forces.

Speakman[30] has demonstrated the probability that only the edges of the superimposed sheets of linked peptide chains are exposed to external attack by acids, alkalies or dyes which must therefore find some means of penetrating the fibre, before any form of internal combination, whether physical or chemical, can take place. This penetration with consequent physical or chemical combination is achieved by the swelling of the fibres in contact with aqueous media. Swelling in water alone increases the maximum diameter of the pores while swelling in water or acid at temperatures above 40°C. causes much greater efforts, which however, are complicated by disturbances in the micelle itself, so rendering it impossible to measure the exact change in para size under these conditions.

The disturbances within micelle, arising from rise in temperature/

temperature with dilute acid or water, are due to action on the salt linkage, hot water alone causes a hydrolytic decomposition. Alkali or acid enters into a form of double decomposition with this linkage. In either event, the fibre in a hot dye-bath, whether neutral or acidic, must be visualised as containing side chains in a labile or highly reactive state. The above explanation is necessary in order to explain the behaviour of dyeing haematein from a neutral or acidic bath.

Elod[3] has showed the use of 'Donnan equilibrium theory' for explaining the acid absorption by wool. According to this theory, the distribution of diffusible cations between the fibre and the bath is always a function of the ionisable protein and the pH of the bath and any other disturbing ion. This is explained by the formula:-

$$\lambda = 1 + \frac{C_1}{C_2 + C_2} \quad \ldots\ldots\ldots I$$

where λ = Distribution ratio, i.e. the ratio at equilibrium of the concentration of any diffusible univalent cation outside the fibre to the concentration of the same cation in the fibre.

i.e. $$\lambda = \frac{[H]^+ \text{ outside}}{[H]^+ \text{ inside}} \quad \ldots\ldots\ldots II$$

In the expression I C_1 is the concentration of protein ion in the fibre, C_2 is the acid concentration outside the fibre and C_3 is the concentration of haematein.

It has been observed that haematein forms aggregates and

is/

is not in a true dispersion state due to its sparing solubility. If expression I were to be applied to haematein dyeing, C_3 would represent the haematein solution in a truly molecular dispersion state and dissociated into anions and cations.

To explain some of the behaviour of haematein dyeing from an acidic and a neutral bath, put $C_2 = 0$, i.e. no acid in the bath than $C_1 = 0$ and the protein will not be ionised. Hence, there will be no absorption as the haematein is not in a truly molecular dispersed state. The first addition of acid to the system are rapidly and largely absorbed by wool and hence C_1 is large in comparison with C_2 but as there is a limit to the increase of C_1 on account of the nature of protein, the value of λ must, with increasing addition of acid, rise to maximum. In the case of haematein in acid bath, this value is found to be pH 1.1 as shown in the ~~results~~ graph on page 228. Since the ionisation of protein (C_1) is a function of pH of the bath (C_2) and $C_3 = 0$ in case of acid-wool system, the distribution ratio is fixed if C_2 is fixed. This means that number of positively charged protein ions in the fibre is dependent on the value of the external acid concentration conveniently measured by the pH of the dyebath.

The poor exhaustion of the haematein solution at low temperature could be explained as follows:-

Wool fibre when immersed in water, is swollen. Below 40°C. the haematein particles due to their aggregation, can do little more than enter these pores whilst above 40°C., owing to hydrolytic/

hydrolytic action on salt, and cystine linkages, the micelle itself is opened up sufficiently to permit entry of haematein particles of a size which, whilst indeterminate at the moment, must necessarily be small. This is in a neutral solution. The conditions of haematein in acid solution is better dispersion of haematein and due to the presence of acid, the salt linkages are not only broken, but an increased amount of ionisation of protein occurs, to an extent dependent on the pH of the acid liquor present in the bath. This is a possible explanation on the basis of results obtained so far.

SUMMARY AND CONCLUSIONS.

The Commercial samples of legwood products were used for preliminary investigations. Experiments of a qualitative nature were carried out to find the exhaustion of haematein from a dyebath by dipping at different time intervals a piece of woollen fabric. These experiments were carried out with variations in pH, time and temperature. During these experiments, presence of impurities in these samples were noticed. The separation of these impurities was effected by employing a chromatographic technique on a silica column. Pure haematein and haematoxylin were separated from the other products present in the commercial sample. One of the products present as an impurity, was confirmed as tannin.

Preparation of pure haematein, haematoxylin and brazilein from the crude products was carried out by crystallisation technique and their purity determined by following a change in the optical density following successive crystallisation and determining the melting points. Moisture contents of the pure products was estimated by drying in air at 110°C.

Analysis of haematein was carried out by reduction with titanous chloride in presence of cellosolve (Ethyleneglycol monoethyl ether) or acetine and rochelle salt using volumetric, electrometric, direct and indirect titration methods. These results/

results were checked by quantitative estimation of haematein by spectrographic method and dyeing trials. It was found that consistent results could be obtained by following an indirect electrometric titration of haematein with titanous chloride solution under carefully controlled conditions and the percentage of haematein content in the commercial samples was found to be higher by reduction method than that obtained by dyeing trial or spectrographic method. It is concluded therefore that some reducible impurities present in the commercial sample of haematein affect the results obtained by reduction method. Reduction method is unsatisfactory when appreciable quantity of reducible impurities are present. Spectrographic method and dyeing trial tests are satisfactory means of estimating the haematein present in a logwood product.

When a solution of pure haematein at constant pH is heated on a water bath, it is found that its optical density is changed. However, no change in the optical density is observed when the experiment is carried out in an inert atmosphere. This is concluded to be due to oxidation of haematein by air during the normal dyeing process. The effect on the variation of optical density of pure haematein solution at different pH temperature and concentration has been examined on Spekker absorptiometer and U.V. Spectrograph. It is concluded from the results that the optical density of pure haematein solution when heated in a normal way shows rapid increase with rise in temperature and pH, but remains constant/

constant when heated in an inert atmosphere.

Haematein derivatives such as iso-haematein chloride chlorohaematein and bromo haematein have been examined in a similar way. It has been found that the stability to normal oxidation by air of the haematein molecule is improved by the introduction of the halogen radical in the molecule. Results of Spekker-absorptiomster and U.V. Spectrograph show that bromo-haematein is the most stable of these derivatives to normal heating in an ordinary dye bath. The improved stability to normal oxidation by air of the bromo-haematein solution might show improved light fastness.

The light fastness tests of haematein under different conditions have been carried out. It is concluded from the results that light fastness of haematein on unmordanted wool is very poor but when it is dyed on a chromic mordanted wool under an inert atmosphere, the light fastness is found to be of good standard. The presence of impurity such as tannin is haematein has shown to lower its light fastness value.

Dyeing experiments show that the rate of absorption of pure haematein on wool increases with rise in temperature, and that absorption takes place from a neutral or acidic dyebath. The increase in absorption increases with increase in acidity of the dye bath. Pure haematein is sparingly soluble in water and forms aggregates at lower temperatures and pH.

The/

The effect of pH on the absorption of haematein and brazilein by wool and nylon has been studied in detail. It is found that maximum absorption of haematein and brazilein in an inert atmosphere takes place between the pH values 1-2 and 3-4 on wool and nylon, respectively. The types of curves obtained for haematein and brazilein are similar to those obtained for an acid dye by several workers. The absorption isotherms of haematein and brazilein on wool and nylon have been determined and the saturation values have been calculated. The results are compared with the acid-wool and acid-nylon titration figures obtained by several workers.

Haematein has a complex chemical structure and its study on fibres has been simplified by a similar study of simpler substances such as phenol, catechol and resorcinol. It is concluded from the results obtained that behaviour of catechol is comparable with that of haematein towards fibres. Resorcinol differs in its behaviour towards fibres from catechol.

Electrometric titration of pure haematein and brazilein solutions with dilute caustic soda in an inert atmosphere has been carried out; from the curve so obtained the presence of three pehnolic hydroxy groups in haematein and two phenolic hydroxy groups in brazilein has been confirmed. The relative acidity of these phenolic hydroxy groups has been worked out by measurements of the dossociation constants of haematein and brazilein/

brazilein molecules. It has been found that pehnolic hydroxy groups near the carbonyl group in the molecules show greater acidity than the other hydroxy groups. It is concluded from these observations that the activity of the haematein molecule with regard to its mode of attachment to the fibres lies at the position 7 and 8 in the haematein molecule. (See pg.)

In considering the mechanism of dyeing with haematein on various fibres and the factors which control the degree of fastness to several agencies, it is of great importance to have an outline of the structures and properties of chromium lakes of haematein. There are several metallic derivatives of haematein but the chromium one is of great importance because of its exceptionally good shade and fastness properties.

Chromium lakes of haematein and brazilein have been prepared and analysed. Lakes have been prepared by use of simple chromium compounds and co-ordination compounds of chromium containing ammonia in the co-ordination sphere. Use has also been made of hexamino-cobaltic chloride (Co.6 NH_3) Cl_3 to prepare metallic lake of haematin so as to differentiate between the functions of the mordanting and salt forming groups in the molecule. It has been found that one molecule of chromium combines with three molecules of haematein. Chromium lake of haematein in solution is different from that which is present on the fibre. Suggestions for possible structures of such lakes and their properties have been described.

SUGGESTIONS FOR FURTHER WORK.

Further work is suggested on the following lines:-

(1) Determination of structure of the lakes of haematein on the fibre.

(2) Correlation of theory of dyeing of haematein and other phenolic and quinonic bodies.

(3) Further investigations of oxidation products of haematein in dyebath.

(1) Investigations of the chromium lakes of haematein show that lake formed on the fibre is different from that obtained in the solution. In the present work, structures have been worked out of chromium lakes of haematein in solution.

The structure of haematein-chromium fibre complex is of importance if some insight is to be gained on its light fastness properties.

The nature of the haematein chromium wool complex could be established by applying a technique (Race, Rowe, Speakman, 1946, 62, 372) of dyeing wool with a limited known amount of pure haematein by the metachrome or afterchrome processes and removing excess of chromium from the wool by continuous extraction with N-oxalic acid solution at room temperature and relating the residual chromium on the fibre to the known amount of pure haematein on the fibre. The constitution of the haematein-chromium-wool complex could be worked out.

(2) The mechanism of dyeing wool and nylon with haematein has been suggested in the light of the results so far obtained. The study/

ABBREVIATIONS EMPLOYED IN THE REFERENCES.

(1)	Annalen.	Justus Liebig's Annalen der Chemie.
(2)	Ber.	Berichte der deutschen chemischen Gesellschaft.
(3)	Bur.Stand.J.Res.	Bureau of Standards Jounnal of Research.
(4)	C.I.	Colour Index. published by Society of Dyers' and Colourists.
(5)	Chem.Soc.Proc.	Chemical and Colourists Proceedings.
(6)	Compt.Redd.	Comptes Rendus hebdomadaires des seances de'l Academie des Sciences.
(7)	Ind.Eng.Chem. Anal.	Industrial and Engineering Chemistry. Analytical edition.
(8)	J.Amer.Chem. Soc.	Journal of the American Chemical Society.
(9)	J.Chem.Soc.Trans.	Journal of the Chemical Society Transactions
(10)	J.Phys.Chem.	Journal of Physical Chemistry.
(11)	J.R.T.C.	Journal of the Royal Technical College, Glasgow.
(12)	J.S.D.C.	Journal of the Society of Dyers' and Colourists
(13)	Melliand Textilber.	Melliand Textilberichte A.G. Heidelberg.
(14)	J.Text.Inst.	Journal of the Textile Institute.
(15)	Monatsh	Monatshefte fur chemic und Verwandte Teile anderer Wissenschaften.
(16)	Trans.Faraday Soc.	Transactions of the Faraday Society.

R E F E R E N C E S

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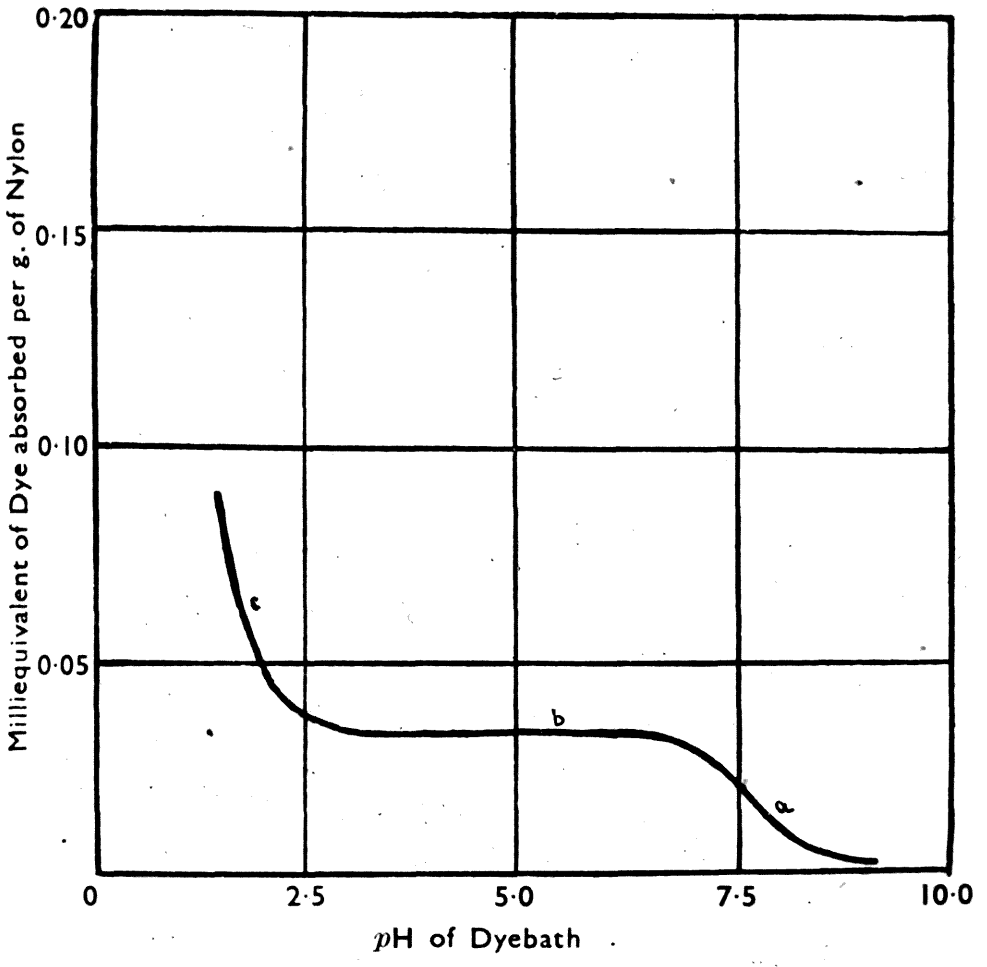


FIG. 1A– Titration of Nylon Fibre with Solway Blue B

Figure 1. A.

CHROMATOGRAPHIC METHODS

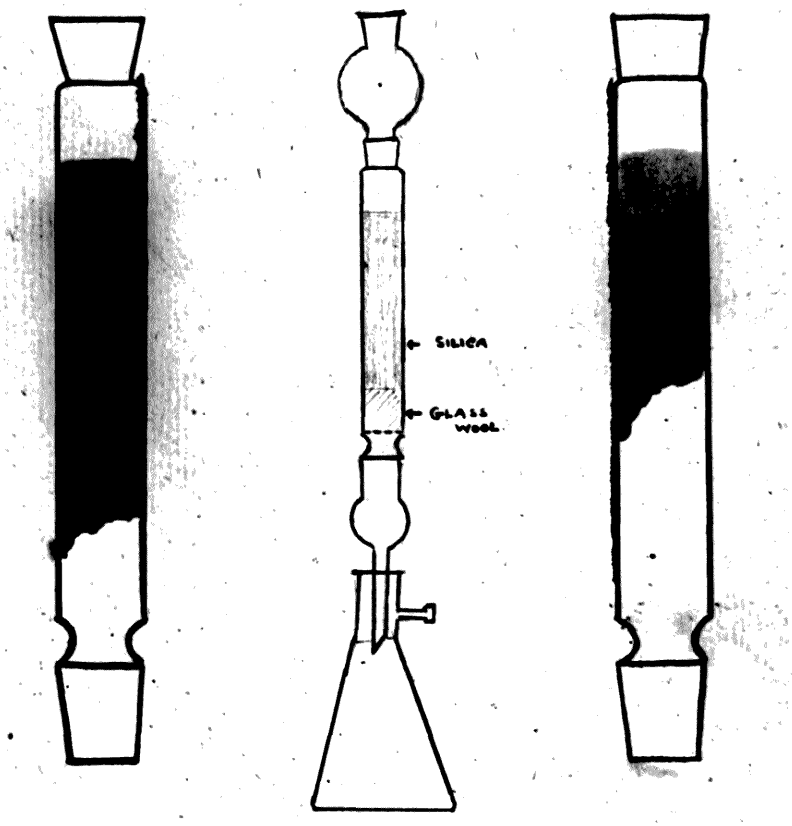
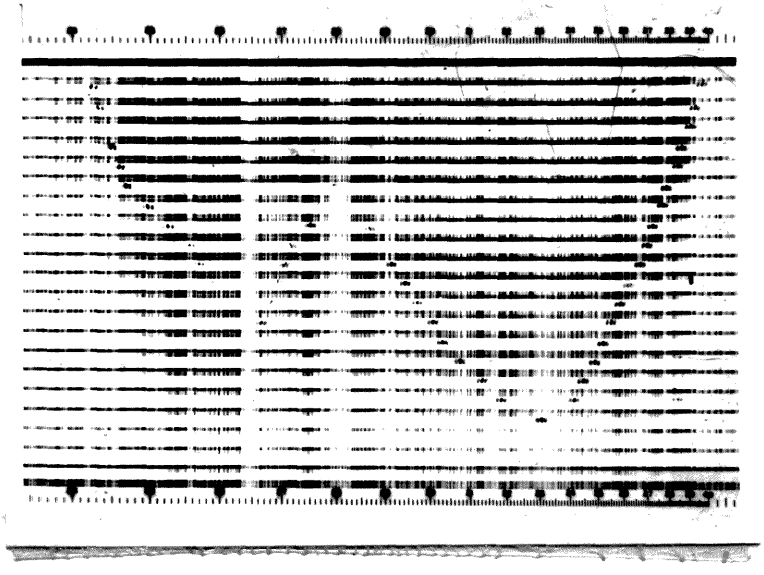


Figure 2.

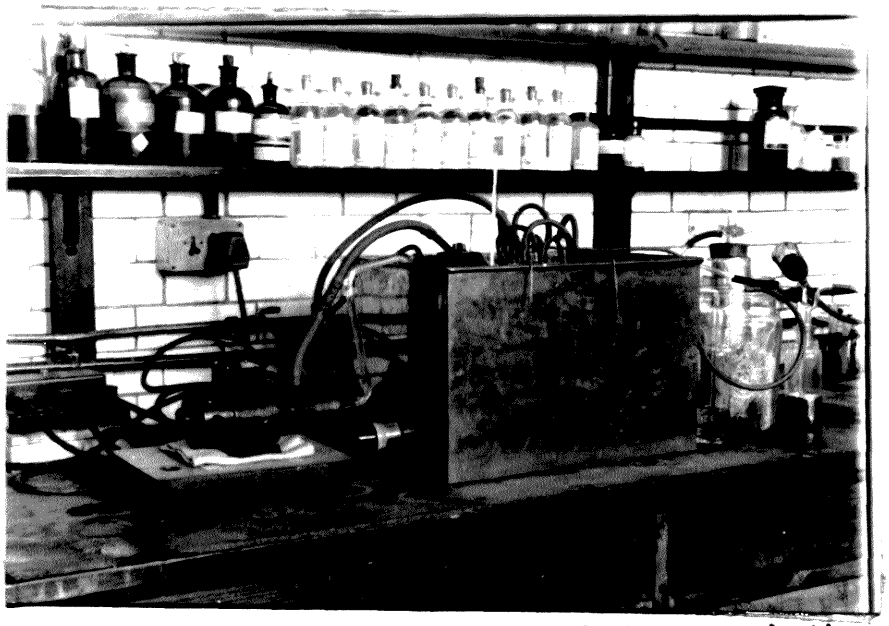


ABSORPTION SPECTRA OF HAEMATEIN.

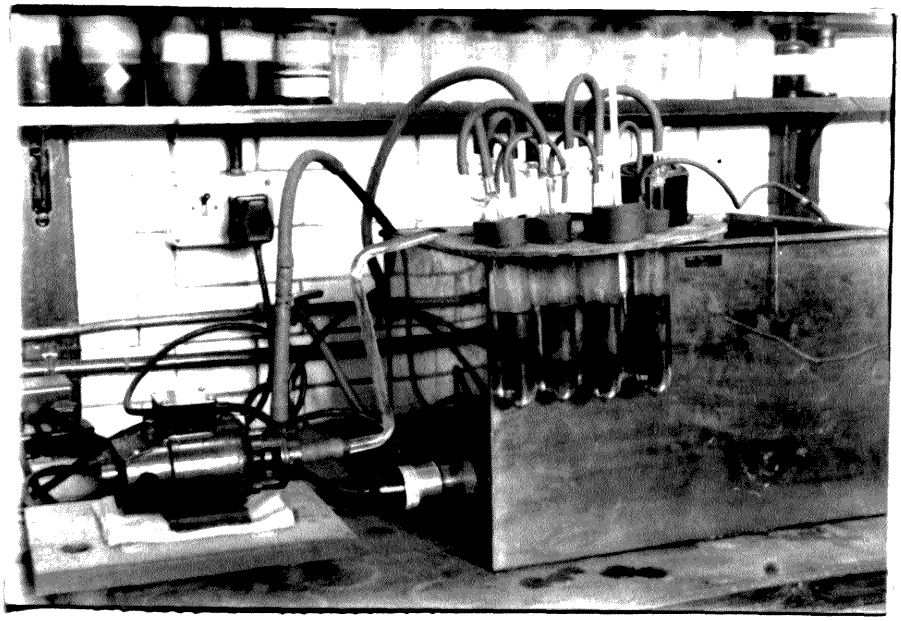
Figure 8.

SPEKKER-ABSORPTIOMETER WITH ROTARY CONVERTER FROM D.C. TO A.C.

Figure 11a.

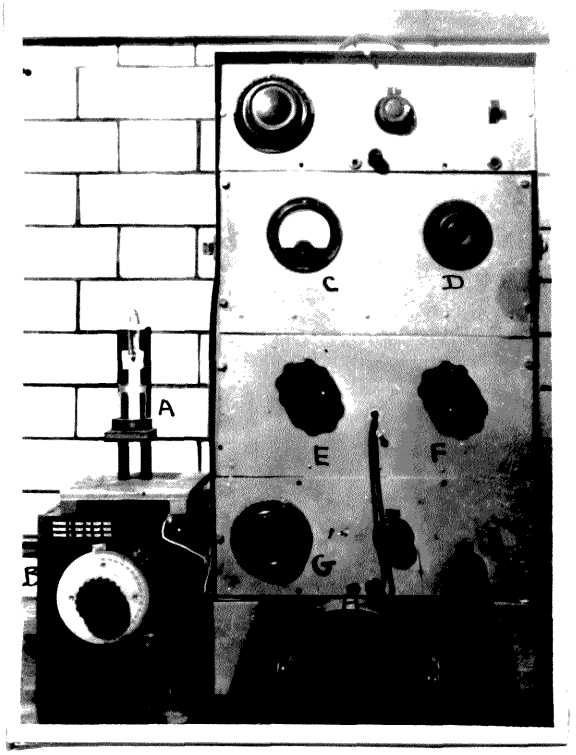


A: Thermostatically controlled water-bath with solid carbon dioxide generator.



B: Inside tubes with water and carbon dioxide circulation arrangements.

ABSORPTION APPARATUS. Figure 25.



Front view of the apparatus.

A:- Dielectric cell.

B:-Micrometer condenser

C:-Anode current (Quartz oscillator)

D:-Anode tuning(Quartz oscillator)

E:-Reaction control(Receiver).

F:-Tuning control(Receiver).

F:-Main tuning control(V. oscillator)

H:-Head phone.

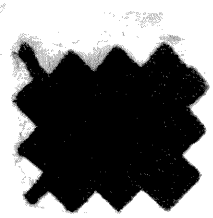
Interior of apparatus

ABSORPTION OF HAEMATEIN BY MEASUREMENT OF DIELECTRIC CONSTANT.

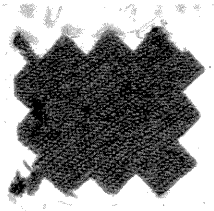
Figure 32.

UNMORDANTED FIBRE.

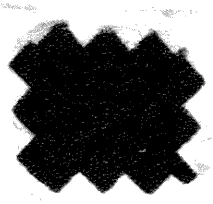
Haematein on nylon.



Haematein on wool

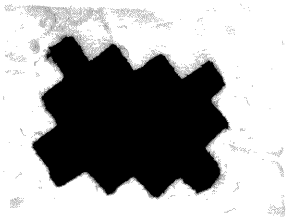


Haematoxylin on wool

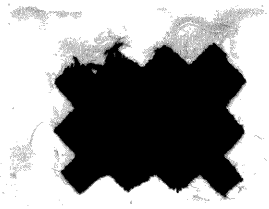


Methylated haematein on wool

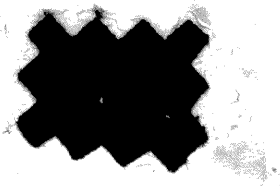
MORDANTED WOOL.



Haematein dyed in a normal way



Haematein dyed in an inert atmosphere.



Iso-haematein chloride

Bromo haematein.

DYEINGS WITH HAEMATEIN. Figure 18.