THE KINETICS OF TEA INFUSION

BY

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

First of all I would like to express my gratitude and thanks to my supervisor Dr. M. Spiro for his assistance and guidance throughout this research project. I would also like to extend my thanks to Cadbury Typhoo Ltd. Birmingham for financing this project in conjunction with Science Research Council and in particular Dr. R.F. Pugh for his valuable ideas during my visits to Birmingham. Last but not least I would like to thank all my past and present colleagues for participating in helpful discussions.

A B S T R A C T

In the present research the kinetics of the extraction into water of polyphenols (theaflavins and thearubigins) and of caffeine in Koonsong B.P. tea and of polyphenols in Assam B.O.P. tea were investigated from the early stages to equilibrium. Experiments were carried out at 25°C (where there was interference from cream formation), 80°C and 95°C. The techniques employed included spectrophotometry, chromatography, solvent extraction and freeze drying. The results were fitted to a simple model for tea infusion and various parameters were calculated. Equilibrium measurements were also carried out to find the partition coefficients of the three soluble constituents. From the temperature variation of the equilibrium and kinetic parameters it was possible to calculate enthalpy changes and energies of activation. The latter are surprisingly small.

C O N T E N T S

AT 25°C

DISCUSSION

INTRODUCTION

I.0 TEA AND TEA MANUFACTURE

Tea is a processed vegetable material used to prepare a delicately flavoured beverage which is widely consumed in the world. Tea plants are grown in many tropical and sub-tropical regions around the world, namely India, China, Sri Lanka and Africa. The tea plant is usually described as Camelia Sinensis L (I).

Hours after harvesting the fresh tea leaves, they are sent to the nearby factory on the tea estate for manufacture. The shoot tips or the green leaves form the initial raw material; the harvestable portion is called the tea flush. Manufacturing leads to the formation of commercial tea products such as green tea (not fermented), oolong tea (partially fermented) or black tea (fully fermented). The tea manufacturing process is a biochemical one and is outlined in Figure I. The traditional process for making black tea may be divided into the following stages: (a) Plucking or harvesting the tea flush fixes the biochemical potential of the tea leaf material to make a tea product (I) and initiates (b) withering. This is essentially a drying process which usually takes 4 - I8 hr during which the moisture content of the tea flush is reduced from 75% to about 65 - 55%. The withered tea leaves are (c) macerated in machines designed to break up the flush, causing a great deal of damage to the tissues of the leaves. This action initiates the (d) tea fermentation process by causing the tea flavanols to come into contact

with endogenous tea catechol oxidase enzymes [the tea flavanols and the tea catechol oxidase are spatially separated in the tea leaf tissues (6 - 8)] .

Tea fermentation, the most important and most characteristic step in tea manufacture, normally takes from I to 4 hr. The primary reactions outlined in Figure 2 involve the oxidative transformation of the tea flavanols (I - IV) (9, I0). About I5 - 25% of the dry weight of the tea leaf tissues are made of flavanols, and they are transformed to colour compounds named theaflavins (IX - XII) and thearubigins (9) which contribute to the appearance (colour characteristic) and taste of black tea; these reactions are depicted in Figure 3. Theaflavins have been isolated as pure substances and structures have been suggested for them (9, II - I4). Thearubigins, characterised as polymeric proanthocyanidins (I5 - I8), are a complex mixture of substances with molecular weights ranging from 700 - 40,000 (I9). The thearubigins have fairly strong acidic properties and a considerable amount of them are present as potassium and calcium salts in tea liquors. Unlike theaflavins, no pure substances have been isolated from the thearubigin mixture although Roberts et al. (20) separated the mixture into two fractions, namely, SI and SII. The two fractions differ in their solubility relationships and their chromatographic behaviour.

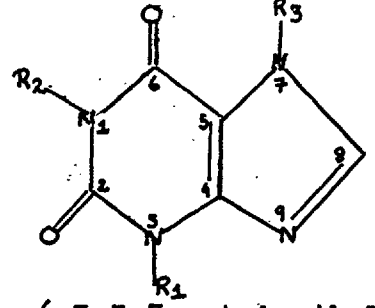
The final stage in the tea manufacturing process is (e) firing (drying) the fermented tea flush. The drying temperature increases from 65°C to 95°C during a drying cycle of period I8 -

22 minutes, during which the moisture content of the tea is reduced to about 2 - 5%. The firing treatment finishes the conversion of the tea flush to a black tea product. Sorting and packing of the tea product is a mechanical process although it does separate the particles of tea into different grades of tea with differing qualities. A more detailed account of the tea manufacturing process can be found in references 2 - 5.

The estimated chemical composition of black tea components soluble in water is listed in Table (I) (2I).

I.I CAFFEINE AND OTHER ALKALOIDS

Apart from the theaflavins and thearubigins in black tea whose contribution towards the colour and taste of tea has been described above, the popularity of tea as a beverage must be accounted for by its alkaloid content. Caffeine, whose structure is given below, was discovered in tea by Oudry in 1827 (22) and is by far the most dominant alkaloid, being present in concentrations as high as 4% in black tea leaf (2I).



(I) = caffeine (1,3,7 - trimethylxanthine): $R_I = R_2 = R_3 = CH_3$

(II) = theobromine (3,7 - dimethylxanthine): $R_2 = H$; $R_I = R_3 = CH_3$

(III) = theophylline (1,7 - dimethylxanthine): $R_I = R_2 = CH_3$; $R_3 = H$

Two other alkaloids, theobromine (II) and theophylline (III)

were found in tea by Zoller and Libich in I87I (23) and Kossel in I889 (24), respectively. These alkaloids are present in tea in trace amounts (25).

I.2 THE PRESENT WORK

No systematic physicochemical measurements have yet been carried out on tea infusion processes. It was the aim of the present research to investigate the kinetics of dissolution of three main constituents (theaflavins, thearubigins, and caffeine) of black tea leaves in aqueous solutions, and also the effect of temperature on the rate constant. So far %theaflavins and %thearubigins (I5) and %caffeine (26, 27) have been determined from equilibrium measurements. Natarajan et al. (28) did study the rate of extraction of different constituents of tea with different brewing temperatures but they did not calculate any parameters.

In the present research the rate constants and partition coefficients and percentages for the infusion of theaflavins and thearubigins from black tea were studied at 25°C, 79.5°C, and 94°C. For the infusion of caffeine the above parameters were studied at 80°C and 94.5°C, the work at 25°C being discarded because of the formation of cream at this temperature (see later). The activation energies and enthalpies for the processes involving these three constituents were evaluated as well as the composition of these constituents in the tea leaf.

FIGURE I

OUTLINE OF BLACK TEA MANUFACTURING PROCESS FROM CULTIVATION OF FRESH GREEN TEA FLUSH TO BREWING OF BLACK TEA

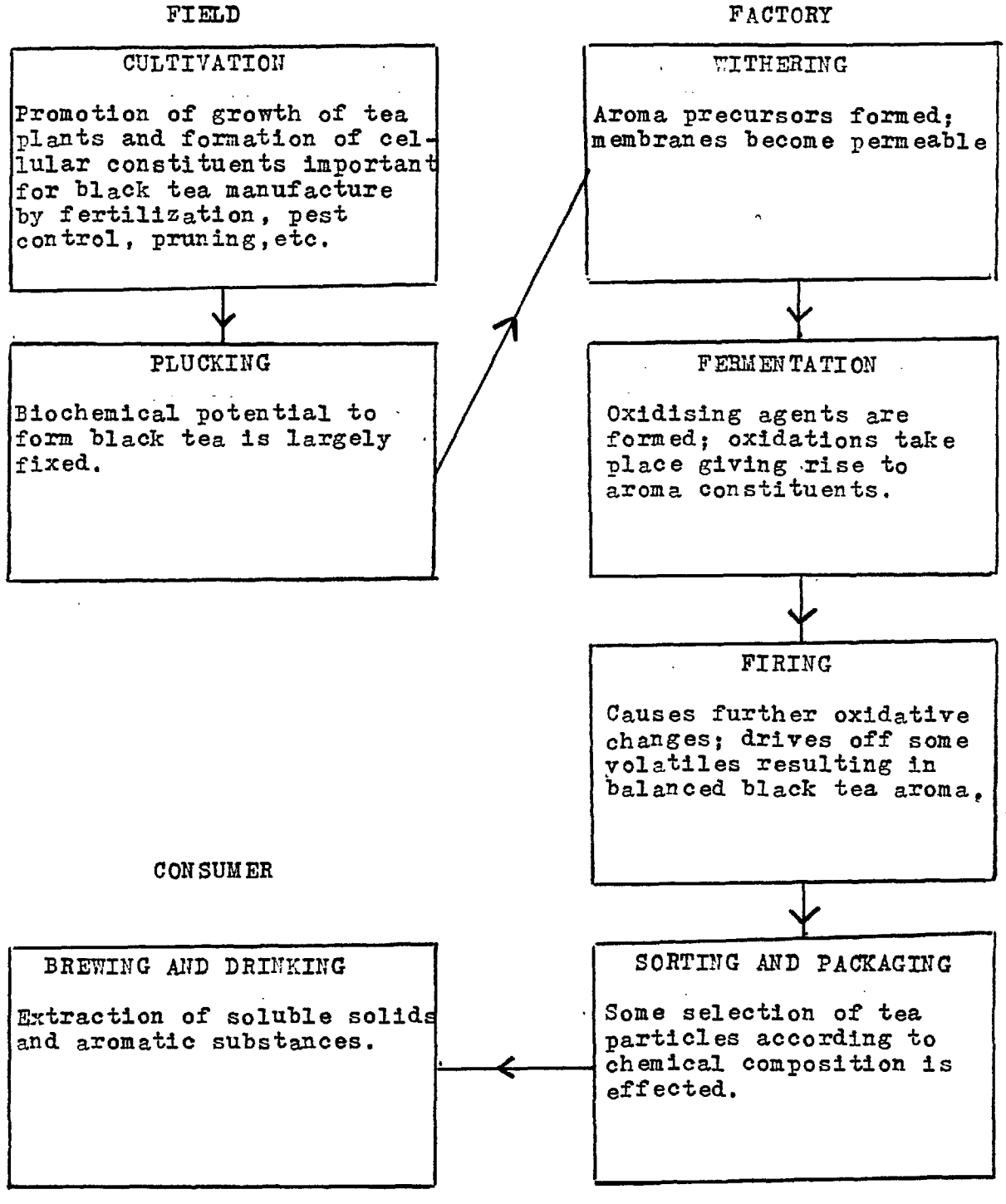


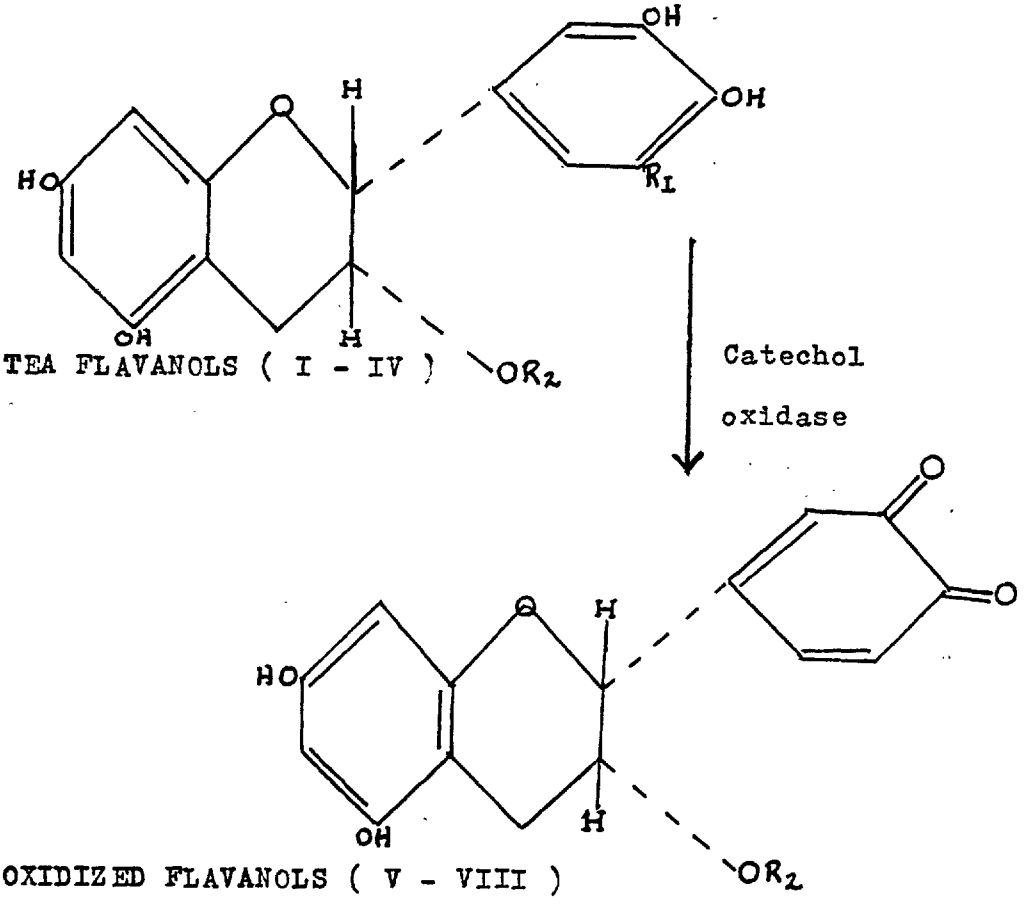
TABLE (I)

ESTIMATED COMPOSITION OF BLACK TEA COMPONENTS SOLUBLE IN WATER (2I)

	% dry wt. of leaf	% of total soluble solids
Flavanols (mainly epi - gallocatechin gallate)	I - 3	3 - 8
Flavanols and flavanol glycosides	2 - 3	6 - 8
Phenolic acids and depsides	4	II
Theaflavins	I - 2	3 - 6
Nondialyzable phenolic substances	I.5	4
Other phenolic substances (bisflavanols, dialyzable thearubigins, other fermentation products)	2 - 4	6 - I0
Caffeine	3 - 4	8 - II
Amino acids and peptides	5	I4
Simple carbohydrates	4	II
Organic acids	0.5	I.5
Partially soluble substances		
Protein ca.	I5	I
Polysaccharide	I4	4
Nucleic acid	0.09	0.I
Mineral salts (ash) ca.	5	ca.I0

FIGURE 2

THE PRIMARY REACTION OF TEA FERMENTATION, NAMELY THE CATECHOL OXIDASE - CATALYSED OXIDATION OF THE TEA FLAVANOLS (I - IV)



TEA FLAVANOLS (I - IV)

OXIDIZED FLAVANOLS (V - VIII)

I = (-) - epicatechin; $R_I = R_2 = H$

II = (-) - epicatechin - 3 - gallate; $R_I = H$, R_2 = 3,4,5 - trihydroxybenzoyl

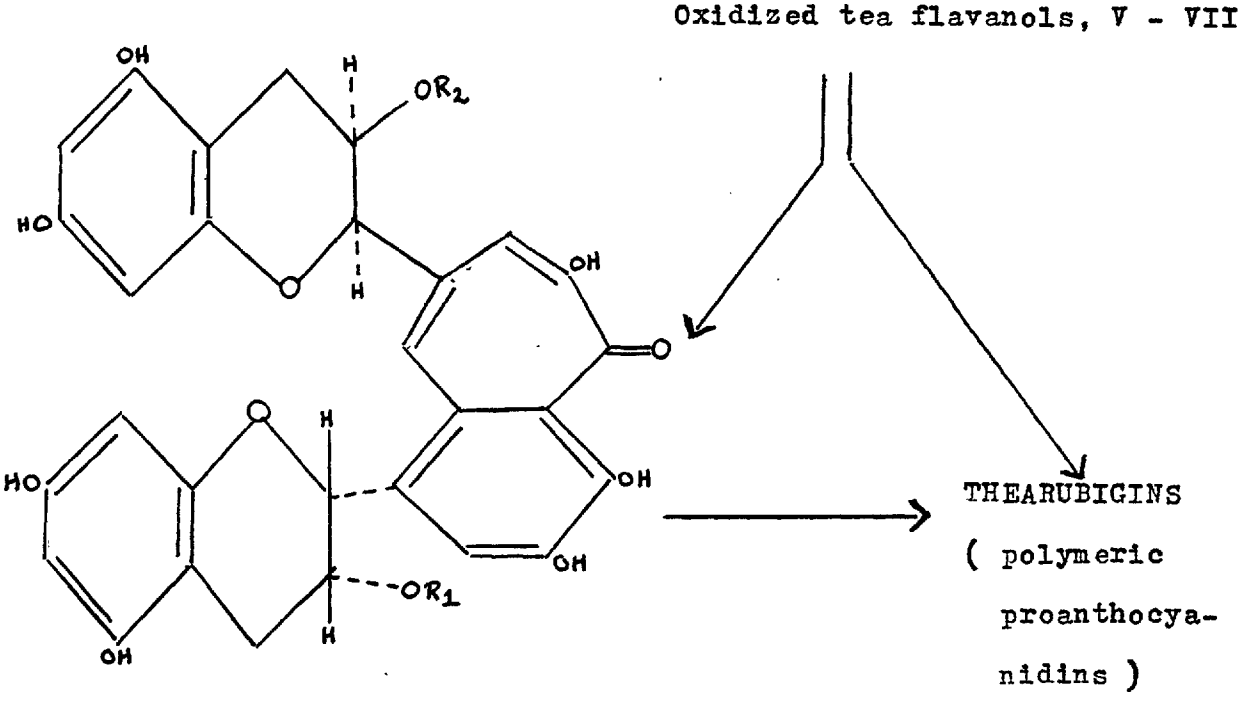
III = (-) - epigallocatechin; $R_I = OH$, $R_2 = H$

IV = (-) - epigallocatechin - 3 - gallate; $R_I = OH$, R_2 = 3,4,5 - trihydroxybenzoyl

V-VIII = 3', 4' - orthoquinones of I - IV, respectively

FIGURE 3

CONDENSATION OF OXIDIZED TEA FLAVANOLS (V - VIII) PRODUCES THEAFLAVINS (IX - XII) AND THEARUBIGINS [POLYMERIC PROANTHOCYANIDINS;BROWN ET AL., 1969a,b (16,17)] WHICH ARE THE PRINCIPAL COLOURED SUBSTANCES OF BLACK TEA



THEAFLAVINS, IX - XII

IX = theaflavin; $R_I = R_2 = H$

X = theaflavin gallate A; $R_I = H$, R_2 = 3,4,5 - trihydroxybenzoyl

XI = theaflavin gallate B; R_I = 3,4,5 - trihydroxybenzoyl, $R_2 = H$

XII= theaflavin digallate; $R_I = R_2$ = 3,4,5 - trihydroxybenzoyl

CHAPTER I

THEAFLAVINS AND THEARUBIGINS

I.0 METHODS OF ANALYSIS

Most analysis of the polyphenols (theaflavins and thearubigins) of black tea have been performed with hot aqueous extracts of the tea leaf, at a concentration similar to that used by Roberts and Smith (20). These workers used 9g of black tea in 375 ml boiling water as a standard brew. This infusion was maintained at 100°C in a boiling water bath for 20 min and resulted in almost complete extraction of the polyphenols. Hilton and Ellis (29) kept the temperature of the infusion close to the boiling point by extracting the black tea in a vacuum flask. This method is simple and quite a number of samples can be infused in a short time. The reproducibility is also good.

The theaflavins and thearubigins in black tea extract were first separated by solvent extractions with isobutyl methyl ketone (IBMK) or ethyl acetate and analysed spectrophotometrically (30). Details of this procedure are given in the next section. Theaflavins have also been determined spectrophotometrically by a method devised by Hilton (31). Diphenyl boric acid ethanolamine complex ("Flavognost", Heyl and Co; Berlin) is used as a specific colorimetric reagent for theaflavins. This reagent does not react with flavanols or any other constituent of fresh green leaf being specific for the benzotropolone nucleus of theaflavins. The reagent produces a green colour complex with theaflavins which gives a broad maximum absorption at 600 nm. This value is well separated from the absorption maxima of other pigments which may be present in the tea extract.

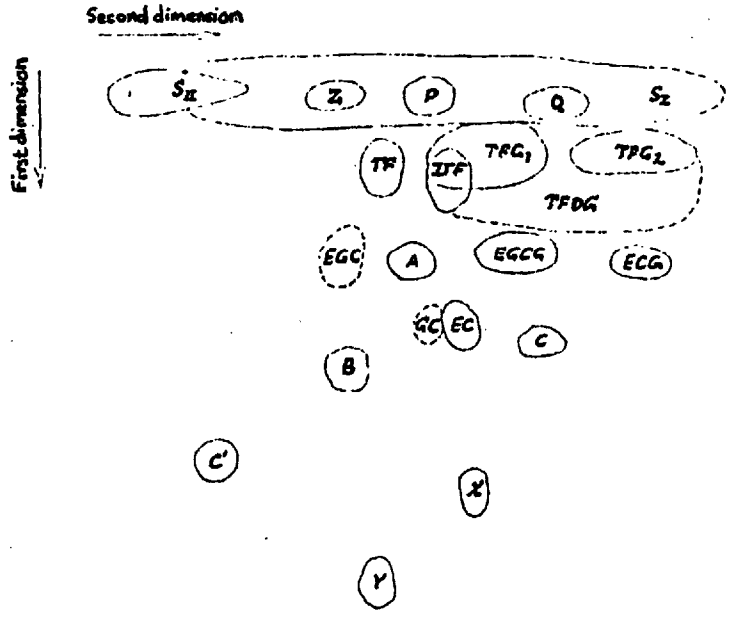
The development of partition chromatography as a preparative method enabled Bradfield and co - workers to elucidate the nature of the polyphenols of tea. They isolated and characterised seven catechins in Ceylon green tea (32, 33). The success of preparative partition chromatography suggested that useful information might be obtained by applying the methods of paper chromatography to a study of catechins and other polyphenols in tea (34, 35). Later Roberts and co - workers fractionated the phenolic substances of manufactured black tea qualitatively by paper chromatography (II, 36). Qualitative separation of black tea polyphenols can also be achieved by thin layer chromatography (37, 38). Of the two chromatographic methods thin layer chromatography is the preferred one, having the advantages over paper chromatography of high resolution, sensitivity, speed of development and ease and efficiency of elution from the layers (37). Two dimensional plates spread with cellulose powder MN - 300 made by Macherey, Nagel and Co. Duren, Germany, to a thickness of 0.25 mm are normally used for thin layer chromatographic work. Essentially, tea infusions are applied to the plates with micropipettes. The plates are developed in the first dimension with water and the second dimension with the top phase 4 : I : 5 butanol - I - acetic acid - water mixture. The chromatograms are dried and examined under daylight and ultraviolet light after fuming with ammonia. The spots can also be examined after spraying with I% ethanolic aluminium chloride solution , vanillin reagent, or 0.5 N sodium hydroxide solution. Figure 4 indicates

a typical chromatogram (39) and the individual components may be identified by their colour reactions given in Table (2).

In recent years liquid column chromatography methods using modified dextran gel (Sephadex LH-20) have been introduced to analyse the theaflavin fractions separately (40) and in general these methods have provided useful information concerning the thearubigin complex (4I). Gas - liquid chromatography has also been used to determine theaflavins in tea brew. Collier and Mallows (42) have estimated theaflavins in tea by gas - liquid chromatography of their trimethyl silyl ethers. Details of the methods used in the present work will be given in the following experimental sections.

FIGURE 4

THIN LAYER CELLULOSE CHROMATOGRAM OF PHENOLIC COMPONENTS OF BLACK TEA



Only those components not found in unfermented tea are shown, except the flavanols, which are shown for orientation purposes. Spots: C, catechin; EG, epicatechin; GC, gallocatechin; EGC, epigallocatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate; S_I and S_{II}, components of thearubigin; TF, theaflavin; ITF, isotheaflavin; TFG, theaflavin - 3 - gallate; TFG_2, theaflavin - 3' - gallate; TFDG, theaflavin - 3,3' - digallate; A, digallolylbisepigallocatechin; B, gallolylbisepigallocatechin; C', bisepigallocatechin; P, Q, X, Y, Z, are of unknown identity (P may be a pentahydroxy flavylium salt, and the Q the oxidation product of epicatechin and gallic acid (9).

TABLE (2)

COLOUR REACTIONS OF PHENOLIC SUBSTANCES IN AN AQUEOUS EXTRACT OF BLACK TEA (45).

Spot code	Colour	UV fluorescence + NH_3 vapour	Spray reagents		
			$AlCl_3$	Vanillin	NaOH
TF	orange	chocolate	pink	pink	purple
ITF	orange	chocolate	pink	pink	purple
TFG_I	orange	chocolate	pink	pink	purple
TFG_2	orange	chocolate	pink	pink	purple
TFDG	orange	chocolate	pink	pink	purple
SI	orange-brown	dark	no change	dark-brown	darkens
SII	brown	dark	no change	dark-brown	darkens
A	colour-less	violet	nil	pink	faint brown
B	colour-less	violet	nil	pink	faint brown
CC	colour-less	nil	nil	pink	faint brown
P	pink	dark	no change	no change	blue
Q	orange	dark brown	deeper orange	darkens	mauve
Z	orange	yellow	no change		
X	yellow	dark	no change	pink-brown	darkens
Y	yellow	dark	no change	pink-brown	darkens

I.I PRELIMINARY WORK

(a) SEPARATION OF THEAFLAVINS AND THEARUBIGINS ON SEPHADEX LH - 20

Of all the methods mentioned in section I.0, the liquid column chromatographic method on Sephadex LH - 20 for the determination of theaflavins and thearubigins and the complex formation method for the determination of theaflavins were first tried in preliminary experiments carried out at the Cadbury Typhoo Laboratories.

Sephadex LH - 20 gels separate a mixture of compounds according to their molecular size. These gels also absorb aromatic and heterocyclic compounds from aqueous solutions and this adsorption effect is frequently used to separate a mixture of similar molecular weights. For example, phenols which strongly interact with these gels are separated on Sephadex LH - 20. Determann and Walter (43) have shown that adsorption occurs via the hydroxy - ether groups which cross - link the dextran chains. The bonding of any aromatic compound to Sephadex is thought to arise from the interaction of the π electrons with the gel matrix. However, Woof and Pierce (44) have noted that the adsorption of a substituted phenol depends on whether the substituent donates or withdraws electrons from the aromatic ring. This suggests that the hydroxyl group takes part in the interaction with the gel.

EXPERIMENTAL PROCEDURE

Freeze dried extract of the tea was prepared by infusing 90g of black tea (Assam Broken Orange Pekoe,B.O.P.) in 375 ml of boiling distilled water. The infusion was stirred for 20 min and filtered through cotton wool. The clear solution (filtrate)

was frozen on a Roneo Vickers Freeze Drier. The yield was approximately 30g of brown hygroscopic powder. 500mg of the freeze dried extract was weighed and suspended in 2 ml of 60 vol% acetone - water solution. Precipitated polysaccharide material was centrifuged off and the supernatant layer added to the top of a column (30 cm x 2.5 cm) containing Sephadex LH - 20 previously equilibrated and eluted with 60% acetone - water. Separation took place over approximately 6 hr. Photograph I shows the column of Sephadex LH - 20. The top band is the theaflavin one and the rest of the bands contain the thearubigin complex. Thearubigins were first eluted with 60% acetone - water and then the bright orange band of theaflavins collected as 40 ml eluent in a flask. The different components can be identified by their elution profile. The eluent can be monitered at 380 nm by passage through a flow cell contained in a photometer or a spectrophotometer connected to a logarithmic scale recorder. Since the monitoring system was not available at the time of the experiment the elution profile was not recorded. However, a typical elution profile is depicted in Figure 5. Acetone was removed from the eluted theaflavins by vacuum distillation. Theaflavins were extracted into ethyl acetate which was then evaporated off leaving a brown/orange solid. This solid was dissolved in approximately 3 ml of 35% acetone - water and added to the top of a (30 cm x 2.5 cm) column of Sephadex LH - 20 previously equilibrated and eluted with 35% acetone - water. In about 5 hr theaflavins separated out into three

distinctive orange bands (Photograph II). The three bands correspond to theaflavin isomers, theaflavin monogallates, and theaflavin bisgallate. Here again due to the unavailibility of the monitoring system the elution profile was not recorded. These compounds can easily be identified by comparison of their absorption spectra at 380 nm with authentic synthetic samples (45). A typical elution profile is shown in Figure 6.

The above method can also be performed by a less time consuming way (40). Instead of separating the theaflavins from the tea extract by liquid column chromatography, one could simply extract the theaflavins from the filtered tea infusion by means of solvent extraction with ethyl acetate and wash briefly with 2.5% aqueous sodium bicarbonate solution followed by removal of ethyl acetate by rotary evaporation. The brownish powder obtained after evaporation of ethyl acetate is dissolved in 35% acetone - water. The mixture can be added to the top of a column (IO cm x I cm) of Sephadex LH - 20 equilibrated in 35% acetone - water. It takes about 5 hr for the theaflavins to separate out into three distinctive components.

FIGURE 5

ELUTION OF AN INFUSION OF BLACK TEA FROM A COLUMN OF SEPHADEX LH - 20. SOLVENT: 6:4 ACETONE WATER. COMPONENTS I - 7, THEARUBIGINS.

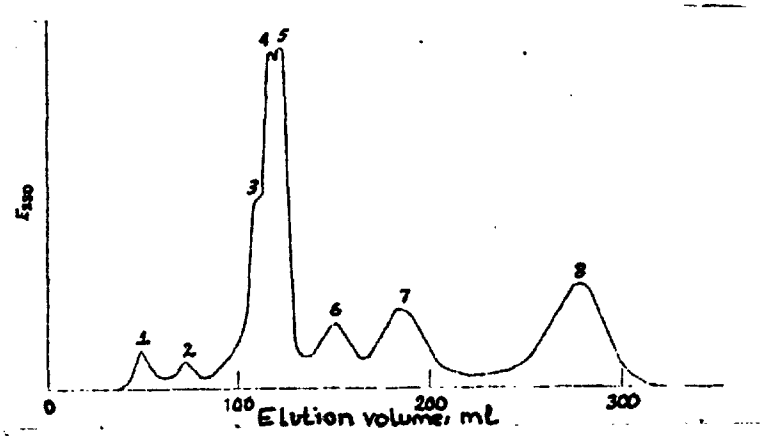
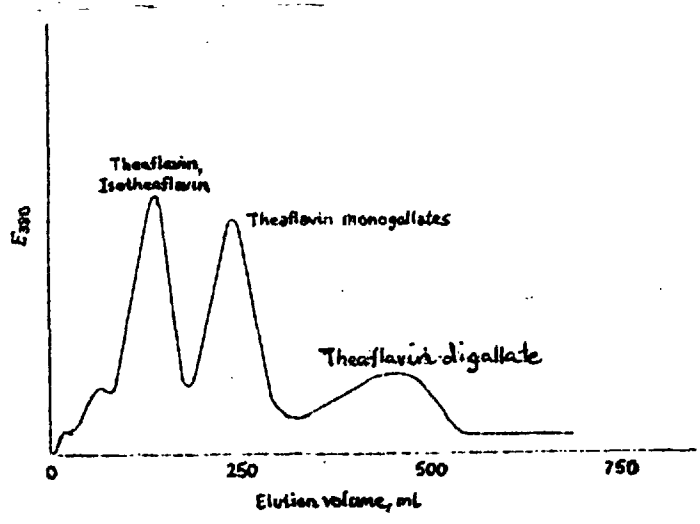
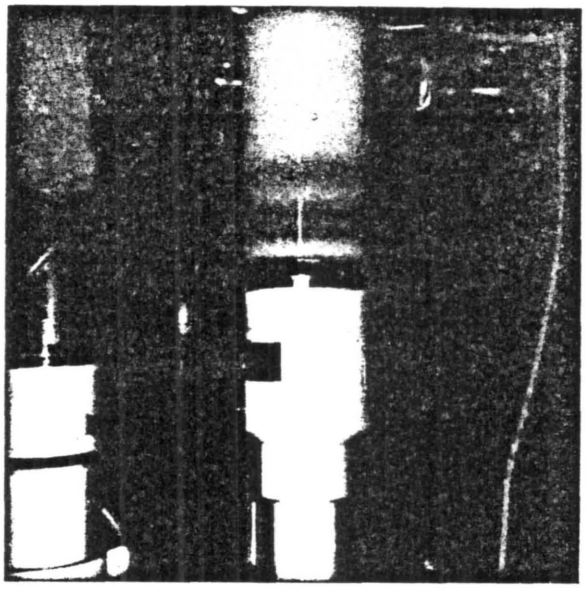


FIGURE 6

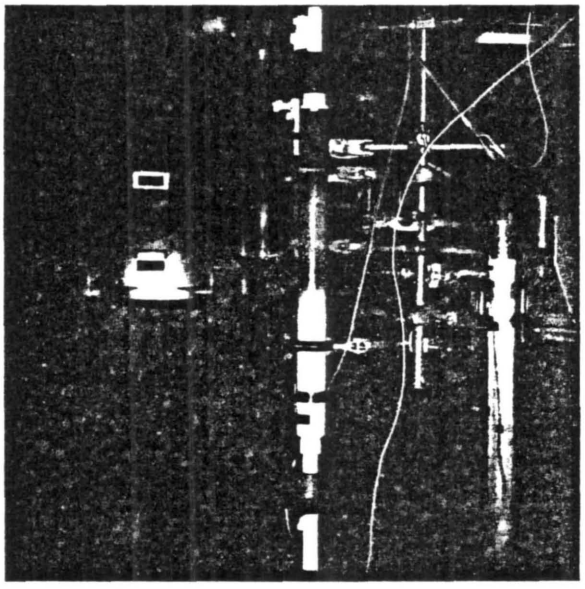
ELUTION OF THEAFLAVINS FROM A COLUMN OF SEPHADEX LH - 20. SOLVENT: 35:65 ACETONE - WATER.



PHOTOGRAPH I



PHOTOGRAPH II



(b) DETERMINATION OF THEAFLAVINS IN BLACK TEA EXTRACT BY A COLORIMETRIC METHOD

This experiment was carried out at Cadbury Typhoo Ltd, Birmingham, in order to make a correlation with the method of Roberts and Smith.

EXPERIMENTAL PROCEDURE

375 ml of boiling distilled water were poured onto 9g of black tea (B.O.P.,Broken Orange Pekoe) in a vacuum flask. The flask was placed in a mechanical shaker and the infusion shaken for 20 min. It was then filtered through a cotton wool plug in a filter funnel. 5 ml aliquots of the filtered tea infusion were placed into three different bottles (25 ml capacity) and to each of these bottles 5 ml aliquots of iso-butyl methyl ketone (BuCoMe) were added. In a fourth bottle 5 ml distilled water and 5 ml BuCoMe were placed to serve as a blank solution. All four bottles were stoppered and shaken in a mechanical shaker for I5 min to ensure complete extraction of the theaflavins from the tea infusion. I ml of the top BuCoMe layer was pipetted into a test tube followed by the addition of I ml absolute ethyl alcohol and I ml 2% (weight / volume) ethanolic flavognost. This reagent had been prepared by dissolving Ig of diphenyl boric acid - 2 - amino ethyl ester (Sigma Chemical Company) in 50 ml absolute ethyl alcohol. After the mixture had stood for 5 min a green coloration developed. The absorbance of this solution was measured at 600 nm in a Hitachi Perkin - Elmer I24 Double Beam Spectrophotometer where it exhibited a broad maximum,

a typical spectrum is depicted in Figure 7. The same procedure was applied to the replicate samples.

RESULTS AND CALCULATION

Baseline blank readings of 0.01 were subtracted from all absorbance values. The absorbances of the three replicate solutions after correction were, 0.290, 0.290 and 0.287 giving an average value of 0.289. The following approximate conversion factor given in the Encyclopedia of Industrial Chemical Analysis (45) for determining percentage theaflavin can be used.

$$\text{Theaflavin, w/w in black tea} = 4.28\ (A_{600})$$
$$= 4.28 \times 0.289 = 1.24\%$$

Table (3) compares the Roberts and Smith and the Hilton method for tea infusions prepared at 25°C. Except for run I, the optical absorbances (A) of the solutions at 600 nm were found to be virtually the same as those of solution C at 380 nm in the Roberts and Smith method. However, the percentage theaflavin is quite different between the two methods. The appropriate factor for determining percentage theaflavin (solution C) of Roberts and Smith's method is 2.25. This value is arbitrary because Roberts and Smith were not aware of the existence of theaflavin gallates or their formulae.

FIGURE 7

SPECTRUM OF THEAFLAVIN DIPHENYL BORIC ACID COMPLEX

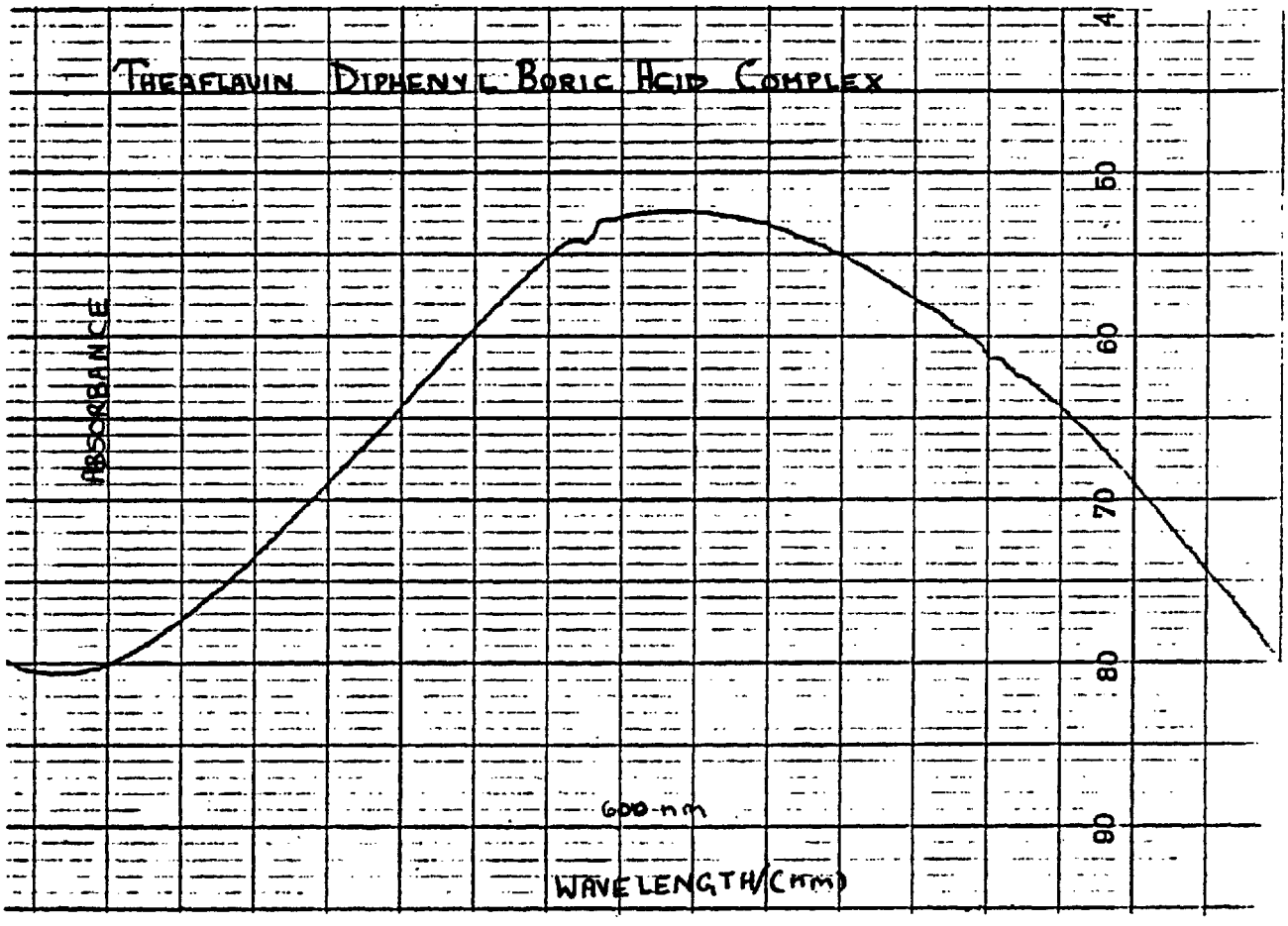


TABLE (3)

COMPARISON BETWEEN ROBERTS AND SMITH'S AND HILTON'S METHOD FOR THE DETERMINATION OF THEAFLAVINS IN BLACK TEA EXTRACT

Runs	T/°C	Time /min	A_c^x	A_c^y	$\%TF^x$	$\%TF^y$
I	25	5	0.030	0.0I5	0.I60	0.064
2	25	5	0.027	0.025	0.I34	0.I07
I	25	24-25	0.090	0.092	0.480	0.394
2	25	24-25	0.089	0.075	0.478	0.32I
I	25	60	0.II0	0.I02	0.590	0.437
2	25	60	0.I00	0.I00	0.537	0.428
3	25	60	0.I05	0.I00	0.560	0.428

x = Roberts and Smith's method

y = Hilton's method

(c) ROBERTS AND SMITH'S METHOD FOR THE DETERMINATION OF THEAFLAVINS AND THEARUBIGINS

Roberts and Smith's method for the determination of theaflavins and thearubigins is based upon the fact that the theaflavins are almost quantitatively extracted from a tea infusion by one extraction with ethyl acetate or IBMK. These solvents do not extract thearubigins of the SII type, although SI type thearubigins present in the form of free acid are partially extracted. Those thearubigins which are extracted by ethyl acetate or IBMK are soluble in aqueous hydrogen carbonate, whereas the theaflavins are insoluble. Therefore theaflavins and SI thearubigins can be almost quantitatively separated by shaking the organic tea extract with an aqueous solution of sodium hydrogen carbonate. Theaflavin and its gallate have well defined absorption maxima at 380 nm and 460 nm (46, 47) where other substances present in tea do not absorb. The extractable thearubigins can be determined by the decrease in optical density which occurs after washing with sodium hydrogen carbonate. The residual SII thearubigins in the aqueous layer, after extraction with ethyl acetate or IBMK, are largely present in the anion forms which are more deeply coloured than the acid forms. Hence an excess of aqueous oxalic acid is added to the aqueous layer in order to convert all thearubigins to the acid form and reduce the colour intensity. Direct spectrophotometric analysis thus becomes possible after acidification. The recommended scheme

of analysis is conveniently summarized in the block diagram depicted in Figure 8.

Given below is the general experimental procedure which was used for work done at three different temperatures, namely, 25°C, 80°C and 95°C.

EXPERIMENTAL PROCEDURE

A glass stoppered 250 ml flat-bottomed flask containing a bar magnet stirrer was placed in a thermostatic water bath maintained at the appropriate temperature. X ml of distilled water at the same temperature were added to the flask and the immersible magnetic stirrer (Rank Bros; Bottisham, Cambs.) turned on and left for 5 min to ensure that the water in the flask attained the temperature of the surrounding bath. Ygm of tea were added to the flask, and the initial temperature of the infusion was recorded with a thermometer (the actual amount of distilled water and tea used are tabulated in the results section). The tea solution was stirred for the desired period and the final temperature was recorded. At the end of the desired time a 10 ml sample of the tea infusion was removed by means of a 10 ml syringe (Beacon Interchangeable Syringe) fitted with a hypodermic needle (metal tip) to ensure that no tea leaves entered the syringe. In Robert's method (15) the tea infusion together with the tea leaves are filtered through a cotton wool plug; such a method of removing tea leaves was not appropriate in the present work with dilute solutions since tannins were adsorbed by the cotton wool plug. The 10 ml tea

infusion was shaken with IO ml IBMK solution in a closed quickfit glass tube (30 ml capacity) for 5 min taking care (by not shaking too vigorously) to avoid the formation of a tea cream as mentioned in Chapter I. However, a brownish precipitate did form at and around the interface itself. The precipitate was very slight at low tea concentrations and high temperatures but more evident at high tea concentrations and low temperatures. After being shaken the two layers were allowed to settle. 4 ml of the top IBMK layer was placed in a 25 ml volumetric flask and the volume was made upto the mark with methanol (B.D.H., Analar) to form solution A. Another 5 ml of the IBMK layer was shaken vigorously in a 30 ml quickfit glass tube with freshly prepared 5 ml of aqueous 2.5% (W/V) sodium hydrogen carbonate (B.D.H., Analar) for 30 sec. The layers were allowed to separate and 4 ml of the top washed IBMK layer was placed in a 25 ml volumetric flask and diluted to the mark with methanol to form solution C. Then, to a 2 ml portion of the aqueous layer left from the first extraction with IBMK were added 2 ml of a saturated aqueous solution of oxalic acid (B.D.H., Analar) and 6 ml of distilled water in a 25 ml volumetric flask, made upto the mark with methanol to form solution D. The optical densities a_A, a_B, and a_C of solutions A, C, and D respectively were measured in I cm cells at 380 nm in a Hitachi Perkin - Elmer I24 Double Beam Spectrophotometer.

EVALUATION OF THE THEAFLAVIN AND THEARUBIGIN CONTENT

The content of theaflavins (as anhydrous theaflavin gallate,

since there is always considerably more theaflavin gallate, than theaflavin in tea) and thearubigins in the tea solutions are calculated by the following formulae given by Roberts and Smith.

$$\% \text{ Theaflavin} = \frac{25}{4} a_C f_I = 6.25\, a_C f_I$$

$$\% \text{ Thearubins} = \left[\frac{25}{2} a_D - \frac{25}{4} (a_A - a_C) \right] f_2$$

$$= 6.25 (2 a_D - a_A - a_C) f_2$$

The factor 25/4 arises from the dilution of solutions A and C, and 25/2 is the dilution factor of solution D. The symbols f_I and f_2 represent spectrophotometric conversion factors.

$$f_I = \frac{(0.02) (856.7) (375)}{(2.225) (892.7) (9)} = 0.36$$

where, 2.225 = $E_{I\ cm}^{0.02\%}$ value of theaflavin gallate dihydrate at 380 nm

856.7 = gram - molecular weight of anhydrous theaflavin gallate

892.7 = gram - molecular weight of theaflavin gallate dihydrate

375 = volume of the black tea infusion in ml (as used by Roberts)

9 = weight of tea leaves in grams (as used by Roberts)

The value of f_2 is necessarily arbitrary, due to incomplete knowledge of the chemistry and degree of hydration of the thearubigin complex. The formula given by Roberts and Smith is:

$$f_2 = \frac{(0.02)(375)}{(0.733)(9)} = 1.13$$

where, 0.733 = $E_{I\ cm}^{0.02\%}$ of thearubigins at 380 nm.

This method only gives a measure of the total theaflavin content of a tea infusion without taking into consideration the existence of different theaflavin fractions. This total %theaflavin is arbitrary, for it is calculated on the basis of mean extinction coefficients which are assumed to be independent of the composition of the theaflavin fraction. Collier and Mallows (42) proved the assumption to be false and showed that though the molar extinction coefficients (ε) of the theaflavins are closely similar, their gram - concentration extinction coefficients ($E_{I\ cm}^{0.02\%}$) differ considerably, as shown in Table (4). Therefore it is much more accurate to determine the molar concentration of theaflavins in a tea infusion rather than the total %theaflavin as by Roberts and Smiths' method. The molar concentration in the present method was determined by using the Beer - Lambert equation. This can written as:

$$\log_{10}(I_0/I) = Ecl = a$$

where I_0 = the intensity of the incident light

I = the intensity of the transmitted light

l = path length of light (length of cell)

c = concentration of the absorbing material

a = absorbance of the soluble constituent

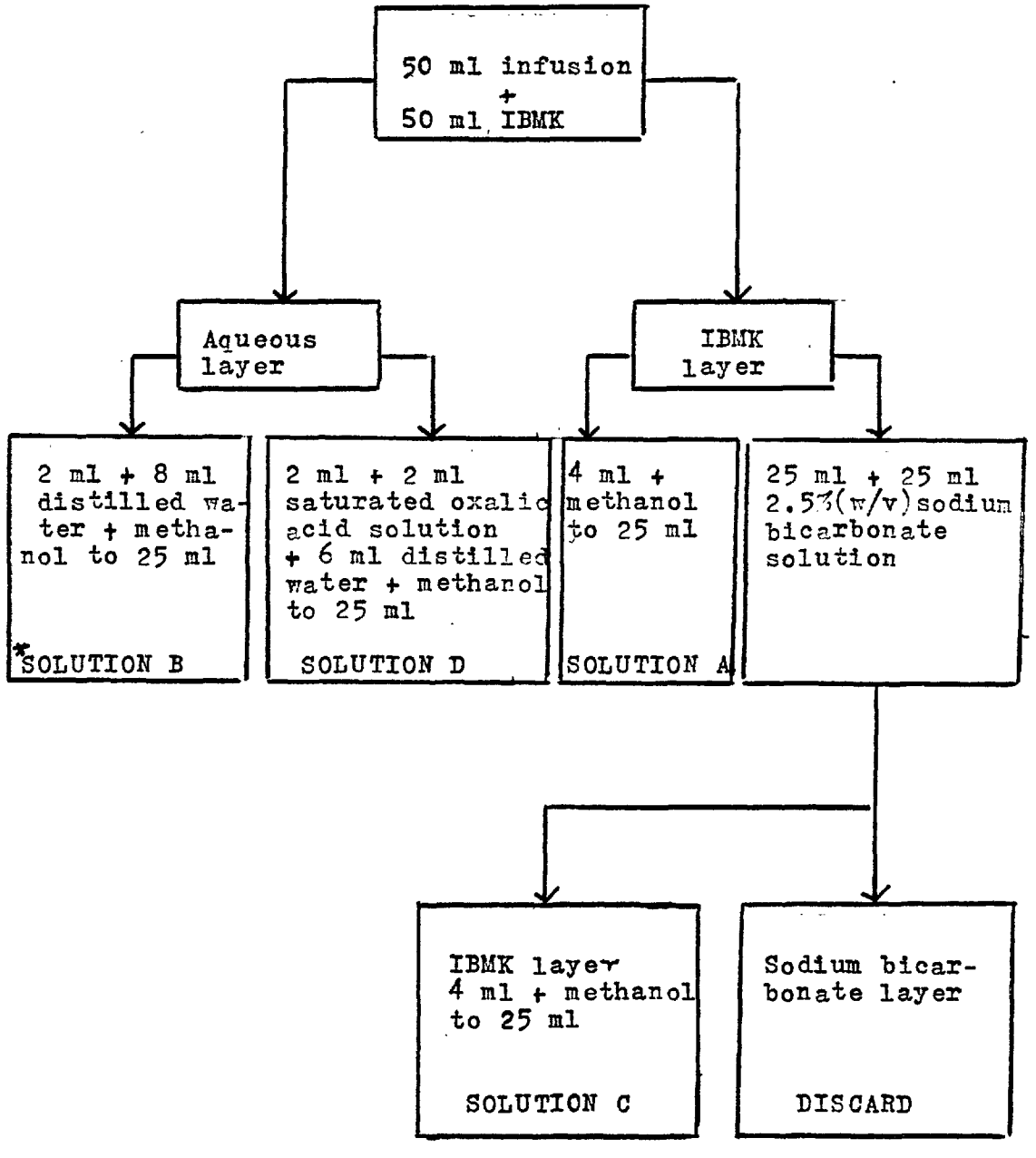
For theaflavins the average extinction coefficient value obtained from Table (4) was used in the present work in order to find the concentration of the theaflavin fractions.

The factor for calculating percentage thearubigins from optical densities is arbitrary due to the uncertainty of the exact structure of the thearubigins as mentioned in the Introduction . Since no extinction coefficients of the thearubigin complex are available we had to resort to Roberts and Smith's formula for calculating percentage thearubigins.

In the present work the general procedure of Roberts and Smith was used but with different experimental conditions. In order to find out whether this method works for low concentration tea infusions, a standard Roberts and Smith run was carried out at 95°C. Absorbances of solutions A, C, and D respectively were measured taking hot infusion / cold IBMK Table (5) . Hot infusion / cold IBMK was preferred to cold infusion / cold IBMK because the later gave slightly lower theaflavin values (could have been due to the formation of cream) as shown in Table (I8), however, the thearubigin values remained practically the same in both cases. Then a certain volume of the infusion (i.e, the hot infusion) was diluted with an equal volume of distilled water and again the absorbances of the diluted solutions were measured. The values of the absorbances are recorded in Table (6). From the present results it appeared that Roberts and Smith's method is feasible for diluted tea infusions.

FIGURE 8

FLOW CHART FOR THE ANALYSIS OF THEAFLAVINS AND THEARUBIGINS BY THE METHOD OF ROBERTS AND SMITH



*In the present research solution B was not made since it is used to measure total colour which was not required in the present work.

TABLE (4)

EXTINCTION COEFFICIENTS OF THE THEAFLAVINS IN METHANOL SOLUTION (42)

	377 nm		459 nm	
	$E^{0.0I\%}_{Icm}$	ε	$E^{0.0I\%}_{Icm}$	ε
TF_I	I.9I	I0800	0.730	4060
TF_2A	I.39	I0004	0.546	39I0
TF_2B	I.45	I0038	0.570	408I
TF_3	I.I8	I023I	0.407	3540

TABLE (5)

EFFECT SHOWING THE DIFFERENCE BETWEEN EXTRACTING HOT INFUSION/ COLD IBMK AND COLD INFUSION/COLD IBMK.

Type of tea used	Weight of tea leaves (g)/ml of distilled water	a_A	a_C	a_D	concn. of TF /10^{-5} mol l^{-1}	%TR
Assam B.O.P.	4/200 hot infusion /cold IBMK	0.844	0.558	0.555	5.43	0.238
Assam B.O.P.	4/200 cold infusion /cold IBMK	0.844	0.522	0.557	5.10	0.245
Koonsong B.P.	4.8/200 hot infusion /cold IBMK	0.856	0.518	0.779	5.04	0.323
Koonsong B.P.	4.8/200 cold infusion /cold IBMK	0.854	0.496	0.772	4.83	0.324

TABLE (6)

TEST OF ROBERTS AND SMITH'S METHOD USING TEA INFUSIONS AT 95°C

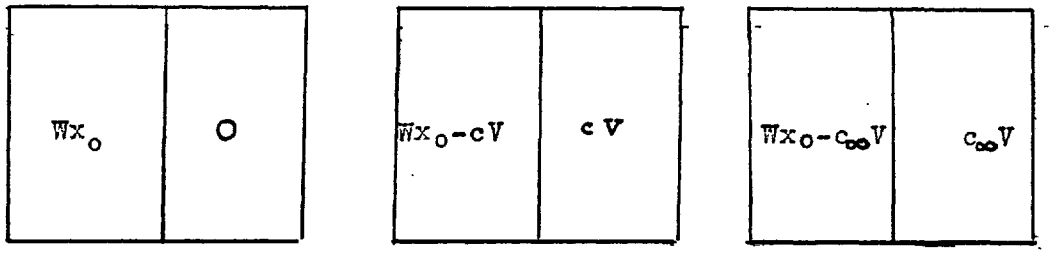
Weight of tea leaves (g)/ml of distilled water	Dilution factor	a_A	a_C	a_D	concn. of TF /10^{-5} mol l^{-1}	%TR
4.8/200	-	0.881	0.500	0.782	4.87	0.331
4.8/200	2	0.440	0.245	0.390	2.385	0.166

CHAPTER 2

PRESENT RESEARCH

I.0 SIMPLE MODEL FOR TEA INFUSION

THEORY



$t = 0$ $t = t$ $t = \infty$

If W g of tea leaves initially contains a weight fraction x_o of a soluble (partitionable) constituent (e.g., theaflavin, thearubigins or caffeine) at a given temperature, and a volume V of distilled water is added, then at a time $t = \infty$;

$$\frac{\text{concn. in water}}{\text{concn. in tea}} = \frac{c_\infty}{(Wx_o - c_\infty V)/W} = \frac{c_\infty}{x_o - c_\infty V/W} = K \qquad (I)$$

where the constant K is the partition coefficient for that constituent.

$$\therefore c_\infty = Kx_o - \frac{Kc_\infty V}{W}$$

$$c_\infty(I + KV/W) = Kx_o \qquad (2)$$

$$\frac{I}{c_\infty} = \left(\frac{V}{W}\right)\frac{I}{x_o} + \frac{I}{Kx_o} \qquad (3)$$

A plot of I/c_∞ versus I/W should produce a straight line of slope V/x_o and with intercept I/Kx_o. Since V is known x_o and the partition coefficient K can then easily be calculated.

KINETICS

The above model also allows the formulation of kinetic equations to describe the rate of tea infusion. It seems likely that the extraction of a soluble constituent will be a zero order process, with the back reaction of readsorption by the leaves following first order kinetics. Thus :

$$dc/dt = k_o \left(\frac{Wx_o - cV}{W} \right) A - k_{-I} c \qquad (4)$$

$$= k_o A x_o - \left(\frac{k_o AV}{W} + k_{-I} \right) c$$

$$= k_o' - k_{-I}' c$$

where A = surface area of the tea leaves ~~per gram~~

k_o' = the effective zero order rate constant

and k_{-I}' = an effective first order rate constant

At $t = \infty$, $dc/dt = 0$, so that:

$$k_o' = k_{-I}' c_\infty \qquad (5)$$

$$\therefore \frac{dc}{dt} = k_{-I}' c_\infty - k_{-I}' c$$

$$= k_{-I}' (c_\infty - c)$$

This equation can be integrated:

$$\int_0^c \left(\frac{dc}{c_\infty - c} \right) = \int_0^t k_{-I}' \, dt$$

$$\therefore k_{-I}' t = \ln \left\{ \frac{c_\infty}{c_\infty - c} \right\} = 2.303 \log_{10} \left\{ \frac{c_\infty}{c_\infty - c} \right\} \qquad (6)$$

Therefore a plot of $\ln [c_\infty / (c_\infty - c)]$ versus t should be

linear with a slope from which k'_{-I} can be evaluated with c known, k'_o is then given by $k'_{-I}c_\infty$.

In the early stages of the infusion, where t is small and $c << c_\infty$, $\ln \left[c_\infty/(c_\infty - c) \right]$ can be expanded as a series :

$$\ln \left\{ \frac{c_\infty}{c_\infty - c} \right\} = -\ln \left(I - \frac{c}{c_\infty} \right) = \frac{c}{c_\infty} + \frac{I}{2} \left(\frac{c}{c_\infty} \right)^2 + \text{--------} \quad (7)$$

The reaction is therefore zero order at small times. From the initial slope of c versus t it might be possible to obtain $c_\infty k'_{-I} = k'_o$.

At infinite time (equilibrium) , $dc/dt = 0$. It then follows from the basic kinetic equation (4) that:

$$k_o A x_o = \left(\frac{k_o AV}{W} + k_{-I} \right) c_\infty$$

Combination with equation (2) gives:

$$k_o A x_o = K x_o \frac{\left(\frac{k_o AV}{W} + k_{-I} \right)}{\left(I + \frac{KV}{W} \right)}$$

$$\therefore \frac{KV}{W} + I = K \frac{\left(\frac{k_o AV}{W} + k_{-I} \right)}{k_o A} = \frac{KV}{W} - Kk_{-I}/k_o A$$

whence $k_o A = Kk_{-I}$ (8)

From the definition of k'_o it also follows that

$$K = k_o A/k_{-I} = k'_o/k_{-I} x_o \quad (9)$$

From the definition of k'_{-I} and k'_o, and equation (8), we have:

$$k'_{-I} = \frac{k_o AV}{W} + k_{-I} \quad (10a)$$

$$= \frac{k_o AV}{W} + \frac{k_o A}{K} = k_o A \left\{ \frac{I}{K} + \frac{V}{W} \right\} = \frac{k'_o}{x_o} \left\{ \frac{I}{K} + \frac{V}{W} \right\} \qquad (10)$$

Thus k'_I should vary linearly with V/W.

I.I THE TEA HOLDER DEVICE

At first all the measurements at 25°C were done by simply pouring the weighed tea leaves from the weighing bottle into the flask containing a known volume of distilled water at the desired temperature. This method was not thought to be accurate for the higher temperature measurements. Here the hot water vapour rising through the falling tea leaves is likely to take with it some of the volatile components which are thereby lost to the atmosphere. Experiments confirmed this view, for when the tea leaves were poured onto the flask from the weighing bottle at 95°C, the points on the kinetic plot were fairly scattered indicating irreproducibility. At this stage it was decided to construct a tea holder device which could be inserted into the flask a quarter of the way down and which would drop all the tea leaves simultaneously. Figure 9 illustrates this device, which was made of stainless steel. A weighed amount of tea leaves was placed into the holder by means of a narrow glass funnel with the cone part pulled tight against the cylinder as shown in Figure 9a . Manoeuvering of the cone at the bottom was done by means of a movable spring system as shown in Figure 9b.

In order to drop the tea leaves into the flask, the ring holder at the top was taken out of its position and the cone lowered. This device proved particularly useful at higher temperatures, and the points on the kinetic plots became much more consistent.

FIGURE 9

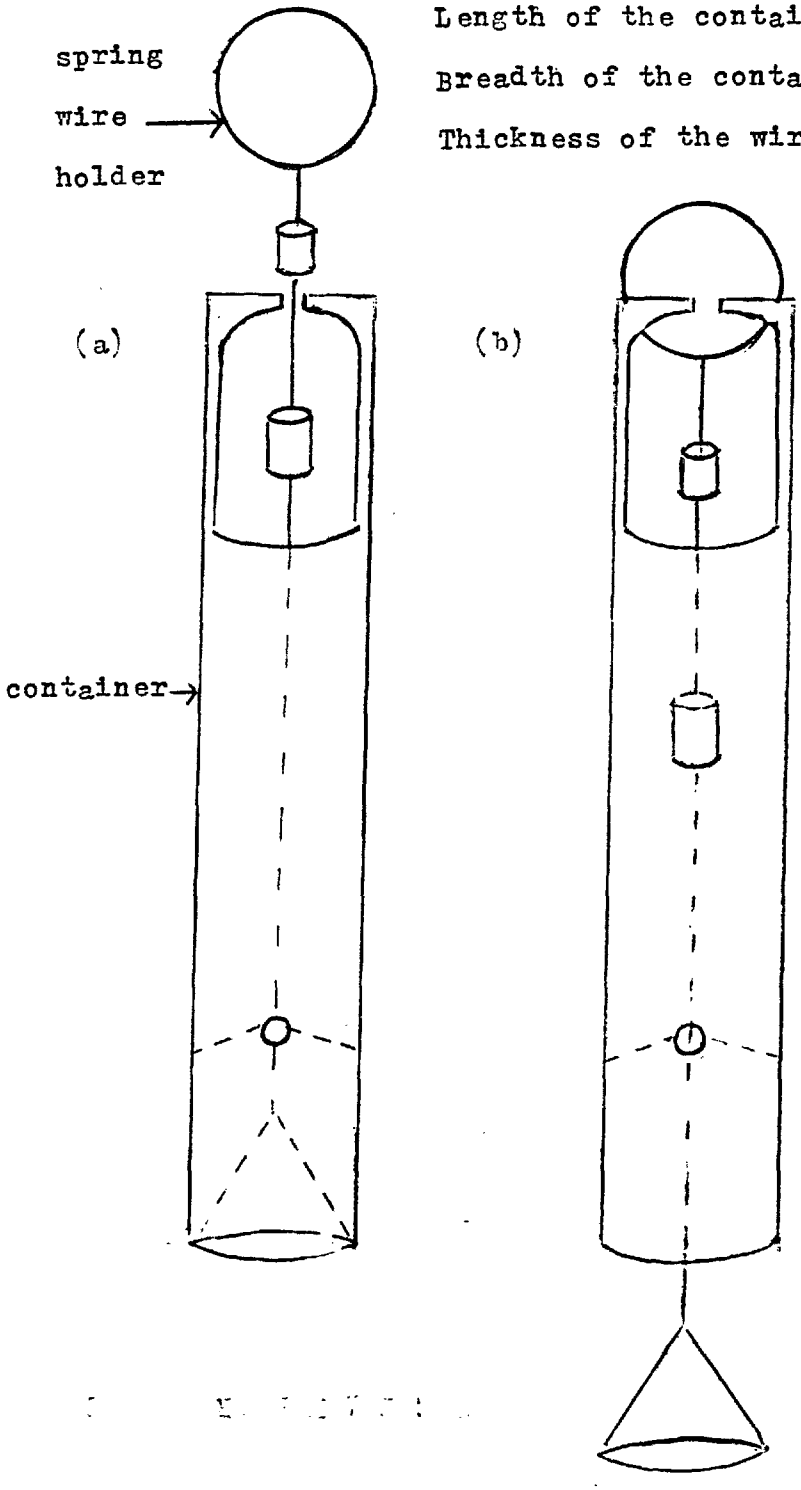
THE TEA HOLDER DEVICE

Thickness of the material (stainless steel), used for the container and the end piece = 0.05 cm

Length of the container = I0.5 cm

Breadth of the container = I.0 cm

Thickness of the wire = 0.I cm



I.2 DETERMINATION OF THE PERCENTAGES, PARTITION COEFFICIENTS AND RATE CONSTANTS OF THEAFLAVINS AND THEARUBIGINS AT 25°C

The experimental procedure is in Chapter I , section I.I (c). These sets of run were done without the tea holder device. The particular brand of tea used was Assam Broken Orange Pekoe (unsieved) kindly supplied to us by Cadbury Typhoo Ltd.

(a) RESULTS AND TYPICAL CALCULATIONS

The results are tabulated for both equilibrium and kinetic measurements [Tables (7) - (II)].

EQUILIBRIUM MEASUREMENTS FOR THEAFLAVINS

Calculations were done by the least squares method. A graph of I/c_∞ versus I/W is plotted in Figure IO, and the points seem to fall on a straight line. A least squares program of the data was run on an HP - 25 calculator. The program used is given on page (49). From the least squares plot the slope equals $0.947 \times IO^5$ g l mol^{-I} and the intercept $0.I7I \times IO^5$ l mol^{-I}. According to the model theory for tea extraction (page 33) and equation (3), the slope equals V/x_o. Thus:

$$x_o = V/\text{slope}$$
$$= 2.II \times IO^{-6} \text{ mol } g^{-I}$$

Relative molecular weight of theaflavins is 562 g

$$x_o = 2.II \times IO^{-6} \times 562 \times I00\%$$
$$= 0.II9 \text{ wt\%}$$

The intercept equals I/Kx_o. Thus:

$$K = \frac{1}{2.11 \times 10^{-6} \text{ mol g}^{-1}} \times \frac{1}{0.171 \times 10^{5} \text{ l mol}^{-1}}$$

$$= 27.7 \text{ g l}^{-1} \times 11/10^{3} \text{g}$$

$$= 2.77 \times 10^{-2} = \frac{\text{molality of TF in water}}{\text{molality of TF in tea leaf}}$$

Thus the partition coefficient K was found to be 2.77×10^{-2}.

KINETIC MEASUREMENTS FOR THEAFLAVINS

These were carried out with 2g of Assam B.O.P. tea-in 200 ml distilled water. A graph of $\log_{10}\left(\frac{c_\infty}{c_\infty - c_t}\right)$ versus time is plotted in Figure II ; and again a straight - line plot results. The fact that the line does not pass through the origin is discussed in the next section. A least squares program of the data was run on the calculator, giving a slope of 22.6 $\times 10^{-2}$ min^{-1}. Thus:

$$k'_{-1} = 2.303 \times 2.26 \times 10^{-2} \text{ min}^{-1} = 5.20 \times 10^{-2} \text{ min}^{-1}$$

From equation (10):

$$k'_{-1} \times x_o = k'_o \left(\frac{1}{K} + \frac{V}{W}\right)$$

$$5.20 \times 10^{-2} \text{ min}^{-1} \times 2.11 \times 10^{-6} \text{ mol g}^{-1} = k'_o \left(\frac{1}{27.7} + \frac{200 \times 10^{-3}}{2}\right) \frac{1}{g}$$

$$1.097 \times 10^{-7} \text{ min}^{-1} \text{ mol g}^{-1} = 0.136\, k'_o \text{ l g}^{-1}$$

$$k'_o = 8.07 \times 10^{-7} \text{ mol l}^{-1} \text{min}^{-1}$$

Check:

According to equation (5) of the theory:

$k_o' = k_{-I}' c_\infty$

$= 5.20 \times 10^{-2}\ min^{-1} \times 15.7 \times 10^{-6}\ mol\ l^{-1}$

$= 8.16 \times 10^{-7}\ mol\ l^{-1}\ min^{-1}$

Furthermore, from the definition of k_o'

$k_o A = k_o'/x_o$

$= \frac{0.807 \times 10^{-6}\ mol\ l^{-1}\ min^{-1}}{}$

$= 2.11 \times 10^{-6}\ mol\ g^{-1}$

$= 0.382\ g\ l^{-1}\ min^{-1} \times (11/10^3 g) = 3.82 \times 10^{-4}\ min^{-1}$

$\frac{k_o AV}{W} = 3.82 \times 10^{-4}\ min^{-1} \times \frac{200}{2\ g} \times 10^{-3}\ l \times (10^3 g/11)$

$= 3.82 \times 10^{-2}\ min^{-1}$

Thus from the definition of k_{-I}',

$k_{-I} = k_{-I}' - \frac{k_o AV}{W}$

$= (5.20 \times 10^{-2} - 3.82 \times 10^{-2})\ min^{-1} = 1.38 \times 10^{-2}\ min^{-1}$

Check:

$k_o A = K k_{-I}$

$= 0.0277 \times 1.38 \times 10^{-2}\ min^{-1} = 3.82 \times 10^{-4}\ min^{-1}$

EQUILIBRIUM MEASUREMENTS FOR THEARUBIGINS

A graph of I/%TR versus I/W is shown in Figure 12 . A least squares program of the results gave a slope of 19.78 g $(\%)^{-1}$ and an intercept of 1.50 $(\%)^{-1}$. Thus from equation (3):

$x_o = V/slope = 200 \times 10^{-3}\ l/19.78\ g\ (\%)^{-1} = 0.0101\ (\%)\ l\ g^{-1}$

$= 0.0101\ \%\ l/g \times (10^3\ g/l) = 10.1\ \%$

The intercept equals $1/Kx_o$, whence

$K = 1/1.50\ (\%)^{-1} \times 10.1\ (\%) = 0.066$

KINETIC MEASUREMENTS FOR THEARUBIGINS

The calculations will be illustrated for 2 g of Assam B.O.P. tea in 200 ml distilled water. Figure I3 shows a plot of $\log_{10}\left(\frac{\%TR_\infty}{\%TR_\infty - \%TR}\right)$ versus time; the non - zero intercept will be discussed later. Least squares calculations give a slope of 0.0256 min^{-1}.

$$k'_{-1} = 2.303 \times 0.0256\ min^{-1} = 5.89 \times 10^{-2}\ min^{-1}$$

From equation (10):

$$k'_{-1} \times x_o = k'_o \left(\frac{1}{K} + \frac{V}{W}\right)$$

$$5.89 \times 10^{-2}\ min^{-1} \times 0.101 = k'_o \left(\frac{1}{0.066} + \frac{200 \times 10^{-3}\ l \times \frac{10^3}{1}\ g}{2\ g}\right)$$

$$0.595 \times 10^{-2}\ min^{-1} = (0.11 5.15)\ k'_o$$

whence

$$k'_o = 5.17 \times 10^{-5}\ min^{-1}$$

Check:

$$k'_o = \%TR_\infty k'_{-1}.$$

$$= 8.77 \times 10^{-2} \times 5.89 \times 10^{-2}\ min^{-1}$$

$$= 5.165 \times 10^{-5}\ min^{-1}$$

From the definition of k'_o,

$$k_o A = k'_o/x_o = 5.17 \times 10^{-5}\ min^{-1}/0.101 = 5.12 \times 10^{-4}\ min^{-1}$$

$$\frac{k_o AV}{W} = \frac{5.12 \times 10^{-4}\ min^{-1} \times 200 \times 10^{-3}\ l \times \frac{10^3\ g}{1}}{2\ g}$$

$$= 5.12 \times 10^{-2}\ min^{-1}$$

Thus from the definition of k_{-I}

$$k_{-I} = k'_{-I} - \frac{k_o A V}{W}$$

$$= 5.89 \times 10^{-2} \text{ min}^{-I} - 5.12 \times 10^{-2} \text{ min}^{-I}$$

$$= 0.77 \times 10^{-2} \text{ min}^{-I}$$

Check:

$$k_o A = K k_{-I} = 0.066 \times 0.77 \times 10^{-2} \text{ min}^{-I} = 5.08 \times 10^{-2} \text{ min}^{-I}$$

All subsequent calculations on equilibrium and kinetic runs were carried out in the same way.

Another set of experiments was done at 25°C using the tea holder device. The particular brand of tea used here was Koonsong Broken Pekoe (sieved through I6 = I.00 mm), and again kindly supplied to us by Cadbury Typhoo Ltd. The results and calculations are given in Tables (I2),(I3) and (I4). Table (I5) shows the calculated percentages and partition coefficients for theaflavins and thearubigins for all work done at 25°C. Tables (I6) and (I7) show the calculated rate constants for all work done at 25°C. Figures I4 and I5 show the equilibrium plots of theaflavins and thearubigins respectively. Figures I6 and I7 respectively show the kinetic plots of theaflavins and thearubigins.

The equilibrium time for maximum extraction of theaflavins and thearubigins was found to be 60 min for work done at 25°C. The tea leaves which were used throughout this research were stored in 2.5 l capped glass bottles. The bottles were kept in a cool dark place in the research laboratory in order to ensure minimum loss of constituents through evaporation.

I.3 EFFECT OF THE FORMATION OF CREAM ON THE COMPOSITION OF THEAFLAVINS AND THEARUBIGINS IN A TEA INFUSION AT 25°C

It was found that at 25°C the kinetic plots of theaflavins and thearubigins did not pass through the origin as expected from equation (6) in the model theory. Furthermore doubling the mass of the tea leaves - and hence their surface area A should have doubled k_0A and k_0', and left k_{-I} and k_{-I}' uneffected. The first three predictions were roughly met but not the fourth. These discrepancies probably arose from the fact that tea forms a"cream" in certain tea liquors when they cool. This cream is a finely dispersed precipitate which is essentially formed between caffeine and polyphenols, namely theaflavins and thearubigins. Cream may either be separated from the liquor after cooling by centrifugation (48), or by the process of"creaming down". This is the enhancement of the formation of cream by acidification of the solution in the proportion I:99 sulphuric acid (49a); the flocculent precipitate so obtained is easier to separate. Neither of these processes for removing cream was suitable for the present work where cream had already formed at the time of sampling whereas Roberts (49a) and Smith (48) had sampled clear hot infusions as well as the solution left after centrifugation of the cream in order to find %cream. To overcome this problem of cream in the present work, it was decided to dilute the tea infusions (so as to decrease the extent of association between caffeine and the polyphenols)

at the end of the desired infusion period (equilibrium time). The cream seemed to form very slightly with low concentration tea infusions but quite markedly with concentrated infusions.

Solutions A, C and D respectively were prepared with the initial concentrated tea infusions and their absorbances measured. Then a certain volume of the concentrated tea solution was diluted with an equal volume of distilled water and the corresponding solutions A', C' and D' were prepared immediately after dilution and their absorbances measured. In the third set of analyses the diluted tea infusion was left to stand for an hour before solvent extraction and the preparation of solutions A", C" and D" whose absorbances were then measured. All the results are recorded in Table (I8). After dilution the concentration of theaflavins seem to be as often higher as lower which suggests irreproducibility. The %thearubigins seem to be as high in about half the diluted samples, again showing irreproducibility; however, most thearubigin results could be accommodated by a 6% increase within experimental error on diluting diluting two - fold and measuring absorbances immediately. All the diluted absorbance and concentration readings in Table (I8) have already been multiplied by the appropriate dilution factor.

CONCLUSION

The results are not constructive enough to arrive at a definite conclusion about how to remove the cream in the tea infusion. A theoretical approach to the cream problem was attempted but the resulting equations proved too unwieldy for practical use.

I.4 DETERMINATION OF THE PERCENTAGES, PARTITION COEFFICIENTS AND RATE CONSTANTS OF THEAFLAVINS AND THEARUBIGINS AT 79.5°C AND 94°C

(a) EXPERIMENTAL PROCEDURE

The distilled water for this purpose was heated to the appropiate temperature and the hot water was measured and transferred to the glass stoppered 250 ml flat - bottomed flask contained in the thermostatic water bath. The magnetic stirrer was switched on and the water was stirred until it attained the required temperature. The rest of the experimental procedure is the same as before. All runs were carried out using the tea holder device. The thermostat was well insulated for these high temperature experiments, and a booster heater was used to raise the temperature of the bath together with the two electrical immersion rod heaters. The temperature was regulated by an electrical thermometer relay system. The initial and final temperatures of the infusions were recorded with a thermometer ranging from 50°C - I00°C (subdivisions: 0.I0). This thermometer was also checked with boiling water at I00°C. The tea used here was Koonsong B.P., sieved through I6 # = I.00 mm (# = mesh sieve)

(b) RESULTS AND CALCULATIONS

Equilibrium data were analysed by a least square program. Kinetic data were analysed graphically and by least squares program. When plots were made graphically with the kinetic data,

the straight line plot could be made to pass through the origin, but the least squares program of the kinetic data produced a negative intercept as shown by Tables (31,33), and (32,34) for theaflavins and thearubigins respectively at high temperatures. The equation for a straight line is:

$$y = mx + c$$

where y and x are the variables and m is the slope of the line with intercept c. The purpose of the least square program is to find the constants m and c

In order to overcome the problem of the negative intercept, it was decided to solve the equation, $y = mx$, where c is assumed to be zero. To minimize the errors in x and y values one takes the sum of the square of the equation $y = mx$. All summations are performed for n number of readings. Thus:

$$\sum_n (y - mx)^2 = \sum_n (y^2 - 2mxy + m^2x^2) = S$$

Differentiate S with respect to m:

$$\frac{dS}{dm} = \sum (-2xy + 2mx^2)$$

At minimum, $\frac{dS}{dm} = 0$

$$\sum 2xy = 2m \sum x^2$$

$$m = \frac{\sum xy}{\sum x^2} = \text{the slope}$$

The least square program for work at 25°C using the equation $y = mx + c$ is given on page (49). A different program (for only kinetic work at 79.5°C and 94°C) is given below.

PROGRAM ENTRY IN PROGRAM MODE

LINE	CODE	KEY ENTRY
00		f, PRGM
0I	25	-
02	I300	GTO 00
03	24 05	RCL 5
04	24 06	RCL 6
05	7I	
06	I300	GTO 00

NOTE: Y DATA MUST BE ENTERED BEFORE X DATA

KEY ENTRY				DISPLAY	
[f]	[PRGM]	[f]	[REG]		
Y_I	[↑]	X_I	[R/S]	I.00	} Displays number of points entered
Y_2	[↑]	X_2	[R/S]	2.00	
Y_n	[↑]	X_n	[R/S]	n.00	
[GTO]	[0]	[3]	[R/S]		- Displays the best fit gradient for equation: $m = \Sigma xy / \Sigma x^2$

The activation energy and enthalpy calculations for the infusion of theaflavins and thearubigins are described in Chapter 5.

This least square program was used throughout this research except where otherwise stated.

PROGRAM ENTRY IN PROGRAM MODE

LINE	CODE	KEY ENTRY	LINE	CODE	KEY ENTRY
00			23	24 07	RCL 7
0I	3I		24	6I	x
02	I5 02	gx^2	25	32	CHS
03	23 5I 02	STO + 2	26	24 04	RCL 4
04	22	R	27	5I	+
05	2I	X⇌Y	28	24 03	RCL 3
06	25	Σx	29	7I	÷
07	I3 00	GTO 00	30	23 00	STO 0
08	24 05	RCL 5	3I	74	R/S
09	24 07	RCL 7	32	24 0I	RCL I
I0	24 04	RCL 4	33	74	R/S
II	6I	x	34	2I	X⇌Y
I2	24 03	RCL 3	35	22	R
I3	7I	÷	36	6I	x
I4	4I	-	37	24 02	RCL 2
I5	24 06	RCL 6	38	24 04	RCL 4
I6	24 07	RCL 7	39	I5 02	gx^2
I7	I5 02	gx^2	40	24 03	RCL 3
I8	24 03	RCL 3	4I	7I	÷
I9	7I	÷	42	4I	-
20	4I	-	43	7I	÷
2I	7I	÷	44	I3 00	GTO 00
22	23 0I	STO I			

The results are tabulated for both equilibrium and kinetic measurements [Tables (I9) - (30)] at 79.5°C and 94°C. Tables [(3I) - (34)] compare the results obtained with the three different methods of calculation (graphical and the two different least squares method) for kinetic measurements at both the temperatures. Figures I8a and I8b represent the equilibrium plots of theaflavins at 79.5°C and 94°C respectively. Similarly Figure I9 show the equilibrium plots of thearubigins at the two different temperatures, while Figures 20 and 2I show the kinetic plots of theaflavins and thearubigins respectively, again at two different temperatures. Figures 2Ia, 2Ib and 2Ic show spectrums of solution A, solution C and solution D respectively. These are typical spectrums of these solutions.

TABLE (7)

EQUILIBRIUM RESULTS AT 25°C AFTER 60 MIN INFUSION WITH ASSAM B.O.P. TEA (UNSIEVED) / 200 ML DISTILLED WATER.

W/g	I/W	a_A	a_C	a_D	$c_\infty^a / 10^{-5}$ mol l^{-1}	10^{-5} mol l^{-1} / c_∞	$\%TR_\infty^b / 10^{-2}$	$I/\%TR_\infty$
2	0.500	0.2535	0.1161	0.2111	1.568	0.640	8.77	11.40
4	0.250	0.4115	0.245	0.367	2.387	0.419	15.40	6.49
6	0.1167	0.535	0.305	0.5115	2.960	0.337	21.48	4.65
8	0.125	0.695	0.372	0.555	8.622	0.275	24.43	4.09

[a] concn. of TF, c_∞ = $\frac{\text{absorbance of solution c}}{\text{Extinction coefficient of TF} \times I}$ (see page 27)

[b] $\%TR_\infty = 6.25 \left(2a_D - a_A - a_C \right) \frac{0.02}{0.733}$ (see page 26)

TABLE (8)

KINETIC RESULTS AT 25°C

2g OF ASSAM B.O.P. TEA (UNSIEVED) / 200 ML DISTILLED WATER

Infusion time/min	a_A	a_C	a_D	Mean a_A	Mean a_C	Mean a_D
2	0.048	0.025	0.045	0.048	0.025	0.045
5	0.090	0.055	0.065			
5	0.080	0.040	0.055	0.070	0. 045	0.06I6
5	0.065	0.040	0.065			
I0	0.I39	0.070	0.092			
I0	0.I40	0.075	0.II0	0.I37	0.073	0.I0I
I0	0.I32	0.075	0.I00			
20	0.I90	00.II0	0.I44	0.I90	0.I075	0.I45
20	0.I90	0.I05	0.I45			
30	0.225	0.I30	0.I70	0.2245	0.I30	0.I70
30	0.224	0.I30	0.I70			

TABLE (9)

KINETIC CALCULATIONS FOR THEAFLAVINS AND THEARUBIGINS AT 25°C

$c_\infty = 2.385 \times 10^{-5}$

$\%TR_\infty = 15.40 \times 10^{-2}$

Infusion time/min	c_t /10^{-5} mol l^{-1}	$(c_\infty - c_t)$ /10^{-5} mol l^{-1}	$\log_{10}\left(\frac{c_\infty}{c_\infty - c_t}\right)$	$\%TR_t$ /10^{-2}	$(\%TR_\infty - \%TR_t)$	$\log_{10}\left(\frac{\%TR_\infty}{\%TR_\infty - \%TR_t}\right)$
2	0.487	1.898	0.099	0.0305	12.35	0.096
5	0.8276	1.557	0.185	5.83	9.57	0.206
10	0.1266	1.119	0.329	8.70	6.70	0.36
20	0.1753	0.632	0.577	12.1	3.30	0.669
30	0.1996	0.389	0.787	13.73	1.67	0.965

TABLE (I0)

KINETIC RESULTS AT 25°C

4g OF ASSAM B.O.P. TEA (UNSIEVED) / 200 ML DISTILLED WATER

Infusion time/min	a_A	a_C	a_D
2	0.089	0.050	0.070
5	0.I57	0.085	0.I35
I0	0.230	0.I30	0.205
20	0.3I0	0.I80	0.290
30	0.330	0.205	0.340

TABLE (II)

KINETIC CALCULATIONS FOR THEAFLAVINS AND THEARUBIGINS AT 25°C

$c_\infty = 2.385 \times 10^{-5}$ mol l^{-1}

$\%TR_\infty = 15.40 \times 10^{-2}$

Infusion time/min	c_t /10^{-5} mol l^{-1}	$(c_\infty - c_t)$ /10^{-5} mol l^{-1}	$\log_{10}\left(\frac{c_\infty}{c_\infty - c_t}\right)$	$\%TR_\infty$ /10^{-2}	$(\%TR_\infty - \%TR_t)$ /10^{-2}	$\log_{10}\left(\frac{\%TR_\infty}{\%TR_\infty - \%TR_t}\right)$
2	0.487	1.898	0 099	3.05	12.35	0.096
5	0.8276	1.557	0.185	5.83	9.57	0.206
10	0.1266	1.119	0.329	8.70	6.70	0.36
20	0.1753	0.632	0.577	12.1	3.30	0.669
30	0.1996	0.389	0.787	13.73	1.67	0.965

TABLE (I2)

EQUILIBRIUM RESULTS AT 25°C AFTER 60 MIN INFUSION WITH KOONSONG BROKEN PEKOE TEA (SIEVED) / 200 ML DISTILLED WATER USING THE TEA HOLDER DEVICE.

W/g	a_A	a_C	a_D	c_∞ /10^{-5} mol l^{-1}	10^{-5} mol l^{-1}/c_∞	$\%TR_\infty$ /10^{-2}	$1/\%TR_\infty$
2	0.209	0.111	0.2306	1.08	0.026	9.54	10.48
3	0.297	0.157	0.353	1.53	0.654	14.4	6.93
4	0.378	0.194	0.475	1.89	0.529	18.6	5.38
6	0.530	0.287	0.540	2.79	0.358	22.55	4.43
8	0.547	0.342	0.781	3.33	0.300	30.1	3.32

TABLE (I3)

KINETIC RESULTS AT 25°C

4g OF KOONSONG B.P. TEA (SIEVED) / 200 ML DISTILLED WATER (TEA HOLDER DEVICE WAS USED HERE).

Infusion time/min	a_A	a_C	a_D	c_t /$I0^{-5}$ mol l^{-I}	$\%TR_t$ /$I0^{-2}$
2.5	0.070	0.03I	0.079	0.302	3.36
5	0.II5	0.057	0.I45	0.555	5.90
I0	0.I75	0.088	0.224	0.857	9.20
I5	0.2I5	0.I06	0.275	I.032	II.20
20	0.2545	0.I245	0.3I8	I.2I2	I3.05

TABLE (I4)

KINETIC CALCULATIONS FOR THEAFLAVINS AND THEARUBIGINS AT 25°C

c_∞ = I.890 x $I0^{-5}$ mol l^{-1}

$\%TR_\infty$ = I8.6 x $I0^{-2}$

Infusion time/min	c_t /$I0^{-5}$ mol l^{-I}	$(c_\infty - c_t)$ /$I0^{-5}$ mol l^{-I}	$\log_{I0}\left(\frac{c_\infty}{c_\infty - c_t}\right)$	$\%TR_t$ /$I0^{-2}$	$(\%TR_\infty - \%TR_t)$ /$I0^{-2}$	$\log_{I0}\left(\frac{\%TR_\infty}{\%TR_\infty - \%TR_t}\right)$
2.5	0.302	I.588	0.0756	3.36	I5.24	0.0865
5	0.555	I.335	0.I5I	5.90	I2.70	0.I66
I0	0.857	I.033	0.262	9.20	9.40	0.290
I5	I.032	0.858	0.343	0.II2	7.40	0.400
20	I.2I2	0.678	0.445	0.I305	5.55	0.525

TABLE (I5)

PERCENTAGES AND PARTITION COEFFICIENT COEFFICIENTS OF THEAFLAVINS AND THEARUBIGINS AT 25°C

slope /$I0^5$ g l mol^{-I} (TF)	Intercept /$I0^5$ l mol^{-I} (TF)	x_o /$I0^{-6}$ mol g^{-I} (TF)	TF /wt% (TF)	K /$I0^{-2}$ (TF)	slope /g(%)$^{-I}$ (TR)	Intercept /(%)$^{-I}$ (TR)	x_o /% (TR)	K (TR)
[a] 0.947	0.I7I	2.II	0.II9	2.77	I9.78	I.50	I0.I0	0.066
[b] I.685	0.098	I.I9	0.067	9.25	I8.62	0.999	I0.74	0.0935

a = experiments done without the tea holder device

b = experiments done with the tea holder device

TABLE (I6)

RATE CONSTANTS FOR THEAFLAVINS AT 25°C

Type of tea used	g of tea /200ml distilled water	Fig( ) slope /$I0^{-2}$ min^{-I}	k'_{-I} /$I0^{-2}$ min^{-I}	k'_o /$I0^{-7}$ mol $l^{-I}min^{-I}$	k_oA /$I0^{-4}$ min^{-I}	$\frac{k_oAV}{W}$ /$I0^{-2}$ min^{-I}	k_{-I} /$I0^{-2}$ min^{-I}
[a]Assam B.O.P.	2	2.29 (x)	5.27	8.I7	3.87	3.87	I.40
[a]Assam B.O.P.	4	2.46 (y)	5.67	I3.90	6.59	3.30	2.38
[b]Koonsong B.P.	4	2.05 (z)	4.72	9.24	7.76	3.88	0.84

a and b are the same as in Table (15)

(x): the intercept in this case at t = 0 is 0·025

(y): the intercept in this case at t = 0 is 0·065

(z): the intercept in this case at t = 0 is 0·040

TABLE (I7)

RATE CONSTANTS FOR THEARUBIGINS AT 25°C

Type of tea used	g of tea /200ml distilled water	slope $/10^{-2}$ min^{-1}	k'_{-1} $/10^{-2}$ min^{-1}	k'_o $/10^{-5}$ min^{-1}	k_oA $/10^{-4}$ min^{-1}	$\frac{k_oAV}{W}$ $/10^{-2}$ min^{-1}	k_{-1} $/10^{-2}$ min^{-1}
[a]Assam B.O.P.	2	2.56 (x)	5.89	5.17	5.12	5.12	0.77
[a]Assam B.O.P.	4	3.08 (y)	7.09	10.99	10.88	5.44	1.65
[b]Koonsong B.P.	6	2.46 (z)	5.67	10.00	9.31	4.66	1.01

a and b are the same as in Table (15)

(x): the intercept in this case at t = 0 is 0.040

(y): the intercept in this case at t = 0 is 0.048

(z): the intercept in this case at t = 0 is 0.035

TABLE (I8)

EFFECT OF DILUTION ON TF AND TR ANALYSES OF INFUSIONS OF ASSAM B.O.P. AT 25°C WHICH SHOWED CREAM FORMATION

Weight of tea leaves (g)/ml of distilled water	Dilution factor	a_A	a_C	a_D	concn. of TF/10^{-5} mol l^{-1}	%TR
8/200:I	-	0.692	0.432	0.554	4.20	0.233
IIa	2	0.680	0.420	0.606	4.09	0.25I
IIb	2	0.804	0.478	0.544	4.65	0.24I
8/200: I	-	0.632	0.372	0.527	3.62	0.224
IIa	2	0.620	0.372	0.550	3.62	0.229
IIb	2	0.664	0.366	0.580	3.56	0.249
8/I00: IIa	2	0.625	0.425	0.479	4.I4	0.I97
IIb	2	0.638	0.4I4	0.469	4.03	0.I98
III	4	0.664	0.4I2	0.470	4.0I	0.203
I2/I50:I	-	0.7I0	0.45I	0.784	4.39	0.3I2
IIa	2	0.800	0.444	0.780	4.32	0.372
IIb	2	0.788	0.446	0.824	4.34	0.340
III	4	0.852	0.448	0.852	4.36	0.359
4/200: I	-	0.440	0.260	0.300	2.53	0.I33
IIa	2	0.408	0.244	0.338	2.38	0.I43
IIb	2	0.408	0.232	0.3I2	2.26	0.I36

I = Readings taken with the initial concentrated solution

TABLE (I8) CONTD.

IIa = Readings taken immediately after the dilution of the concentrated solution (concentrated solution diluted twice with distilled water).

IIb = Readings taken an hour after dilution of the concentrated solution.

III = Readings taken immediately after dilution of the diluted solution (diluted solution diluted twice with distilled water).

All absorbances and concentrations of diluted solutions in the Table have already been multiplied by the appropriate dilution factor.

TABLE (I9)

EQUILIBRIUM RESULTS AT 80°C (BATH TEMPERATURE) AFTER 20 MIN INFUSION WITH KOONSONG B.P. TEA (SIEVED) / 200 ML DISTILLED WATER.

W/g	Initial T of infusion /°C	Final T of infusion /°C	a_A	a_C	a_D	$c_\infty^{(TF)}$ /10^{-5} mol l^{-1}	10^{-5} mol l^{-1}/c_∞	$\%TR_\infty$	$I/\%TR_\infty$
2	79.0	79.5	0.4I3	0.250	0.335	2.434	0.4II	0.I42	7.04
3	79.0	79.5	0.582	0.320	0.485	3.II6	0.32I	0.2Ic	4.80
4	79.0	79.5	0.672	0.402	0.639	3.9I4	0.256	0.264	3.79
6	78.0	79.5	0.875	0.503	0.903	4.900	0.204	0.373	2.68
8	78.0	79.5	I.05	0.580	I.I7	5.65	0.I77	0.480	2.08

TABLE (20)

KINETIC RESULTS AT 80°C (BATH TEMPERATURE)

4g KOONSONG B.P. TEA (SIEVED) / 200 ML DISTILLED WATER.

Infusion time/min	Initial T of infusion /°C	Final T of infusion /°C	a_A	a_C	a_D	c_t /10^{-5} mol l^{-1}	ΣTR_t
2	78.5	79.0	0.382	0.219	0.359	2.132	0.150
3.5	78.5	79.5	0.500	0.292	0.469	2.843	0.195
5	79.0	79.5	0.565	0.330	0.545	3.213	0.226
7	79.0	79.5	0.642	0.372	0.605	3.622	0.252
10	79.0	79.5	0.670	0.391	0.623	3.81	0.260
15	79.0	79.5	0.679	0.401	0.634	3.91	0.2635

TABLE (2I)

KINETIC CALCULATIONS FOR THEAFLAVINS AND THEARUBIGINS AT 80°C (BATH TEMPERATURE) .

$c_\infty = 3.9I4 \times I0^{-5}$ mol l^{-I}

$\%TR_\infty = 0.264$

Infusion time/min	c_t /$I0^{-5}$mol l^{-I}	$(c_\infty - c_t)$ /$I0^{-5}$mol l^{-I}	$\log_{I0}\left(\frac{c_\infty}{c_\infty - c_t}\right)$	$\%TR_t$	$(\%TR_\infty - \%TR_t)$	$\log_{I0}\left(\frac{\%TR_\infty}{\%TR_\infty - \%TR}\right.$
2	2.I32	I.782	0.342	0.I50	0.I44	0.365
3.5	2.843	I.07	0.563	0.I95	0.069	0.583
5	3.2I3	0.70I	0.747	0.226	0.038	0.842
7	3.622	0.292	I.I3	0.252	0.0I2	I.34
I0	3.8I	0.I04	I.58	0.260	0.004	I.82
I5	3.90	0.0I4	2.45	0.2635	0.0004	2.82

TABLE (22)

EQUILIBRIUM RESULTS AT 95°C (BATH TEMPERATURE) AFTER 20 MIN INFUSION WITH KOONSONG B.P. TEA (SIEVED) / 200 ML DISTILLED WATER

W/g	Initial T of infusion /°C	Final T of infusion /°C	a_A	a_C	a_D	c_∞ /10^{-5} mol l^{-1}	10^{-5} mol l^{-1}/c_∞	$\%TR_\infty$	$1/\%TR_\infty$
2	93.5	94.0	0.469	0.250	0.362	2.43	0.412	0.161	6.20
3	94.0	94.0	0.600	0.334	0.531	3.25	0.307	0.230	4.35
4	93.5	94.0	0.765	0.453	0.675	4.41	0.230	0.283	3.53
6	94	94.0	0.965	0.550	0.954	5.36	0.187	0.396	2.53
8	94	94.0	1.148	0.705	1.242	6.86	0.146	0.500	2.00

TABLE (23)

KINETIC RESULTS AT 95°C (BATH TEMPERATURE)

4g KOONSONG B.P. TEA (SIEVED) / 200 ML DISTILLED WATER

Infusion time/min	Initial T of infusion /°C	Final T of infusion /°C	a_A	a_C	a_D	c_t /10^{-5}mol l^{-1}	%TR_t
1.75	94.0	94.0	0.421	0.230	0.369	2.24	0.158
3	94.0	94.0	0.558	0.314	0.498	3.06	0.211
5	94.0	94.0	0.680	0.395	0.598	3.85	0.253
7	94.0	94.0	0.735	0.421	0.638	4.10	0.271
10	94.0	94.0	0.749	0.444	0.660	4.32	0.280

TABLE (24)

KINETIC CALCULATIONS FOR THEAFLAVINS AND THEARUBIGINS AT 95°C (BATH TEMPERATURE).

$c_\infty = 4.41 \times 10^{-5}$ mol l^{-1}

$\%TR_\infty = 0.283$

Infusion time/min	c_t /10^{-5} mol l^{-1}	$(c_\infty - c_t)$ /10^{-5} mol l^{-1}	$\log_{10}\left(\frac{c_\infty}{c_\infty - c_t}\right)$	$\%TR_t$	$(\%TR_\infty - \%TR_t)$	$\log_{10}\left(\frac{\%TR_\infty}{\%TR_\infty - \%TR_t}\right)$
1.75	2.24	2.17	0.310	0.158	0.125	0.355
3	3.06	1.35	0.514	0.211	0.072	0.594
5	3.85	0.560	0.900	0.253	0.030	0.975
7	4.10	0.310	1.15	0.271	0.012	1.37
10	4.32	0.090	1.69	0.280	0.003	1.97

TABLE (25)

RATE CONSTANTS FOR THEAFLAVINS AT 79.5°C

g of tea /200 ml distilled water	Fig(20) slope /10^{-2} min^{-1}	k'_{-1} /10^{-2} min^{-1}	k'_o /10^{-6} mol l^{-1} min^{-1}	k_oA /10^{-3} min^{-1}	$\frac{k_oAV}{W}$ /10^{-2} min^{-1}	k_{-1} /10^{-2} min^{-1}
4	16.3	37.5	14.54	4.60	23.0	14.5

TABLE (26)

RATE CONSTANTS FOR THEARUBIGINS AT 79.5°C

g of tea /200 ml distilled water	Fig(21) slope /10^{-2} min^{-1}	k'_{-1} /10^{-2} min^{-1}	k'_o /10^{-3} min^{-1}	k_oA /10^{-3} min^{-1}	$\frac{k_oAV}{W}$ /10^{-2} min^{-1}	k_{-1} /10^{-2} min^{-1}
4	18.4	42.4	1.13	7.43	37.2	5.25

TABLE (27)

RATE CONSTANT FOR THEAFLAVINS AT 94°C

g of tea /200 ml distilled water	Fig(20) slope /10^{-2} min^{-1}	k'_{-1} /10^{-2} min^{-1}	k'_o /10^{-6} $mol\ l^{-1} min^{-1}$	k_oA /10^{-3} min^{-1}	$\frac{k_oAV}{W}$ /10^{-2} min^{-1}	k_{-1} /10^{-2} min^{-1}
4	17.0	39.2	16.39	5.79	28.95	10.25

TABLE (28)

RATE CONSTANTS FOR THEARUBIGINS AT 94°C

g of tea /200 ml distilled water	Fig(21) slope /10^{-2} min^{-1}	k'_{-1} /10^{-2} min^{-1}	k'_o /10^{-3} min^{-1}	k_oA /10^{-3} min^{-1}	$\frac{k_oAV}{W}$ /10^{-2} min^{-1}	k_{-1} /10^{-2} min^{-1}
4	19.6	45.1	1.31	7.29	36.5	8.60

TABLE (29)

PERCENTAGES AND PARTITION COEFFICIENTS OF THEAFLAVINS AND THEARUBIGINS AT 79.5°C

Fig(18) slope /10^5gl mol^{-1} (TF)	Fig(18) Intercept /10^5l mol^{-1} (TF)	x_o /10^{-6} mol g^{-1} (TF)	TF /wt% (TF)	K /10^{-2} (TF)	Fig(19) slope /$g(\%)^{-1}$ (TR)	Fig(19) intercept /$(\%)^{-1}$ (TR)	x_o /% (TR)	K (TR)
0.633	0.100	3.16	0.183	3.17	13.16	0.466	15.2	0.141

TABLE (30)

PERCENTAGES AND PARTITION COEFFICIENTS OF THEAFLAVINS AND THEARUBIGINS AT 94°C

Fig(18) slope /10^5gl mol^{-1} (TF)	Fig(18) Intercept /10^5l mol^{-1} (TF)	x_o /10^{-6} mol g^{-1} (TF)	TF /wt% (TF)	K /10^{-2} (TF)	Fig(19) (TR)	Fig(19) intercept /$(\%)^{-1}$ (TR)	x_o /% (TR)	K (TR)
0.707	0.0625	2.83	0.159	5.65	11.13	0.669	17.97	0.0832

TABLE (3I)

TF AT 79.5°C

Intercept	Slope /I0^{-2} min^{-I}	k'_{-I} /I0^{-2} min^{-I}	k_o' /I0^{-6} mol l^{-I} min^{-I}	k_oA /I0^{-3} min^{-I}	$\frac{k_oAV}{W}$ /I0^{-2} min^{-I}	k_{-I} /I0^{-2} min^{-I}
0	[a] I6.5	37.5	I4.54	4.60	23.0	I4.5
0	[b] I6.I	37.I	I4.67	4.50	22.5	I4.6
-0.0I5	[c] I6.3	37.5	I4.88	4.56	22.8	I4.7

TABLE (32)

TR AT 79.5°C

Intercept	Slope /I0^{-2} min^{-I}	k'_{-I} /I0^{-2}	k_o' /I0^{-3} min^{-I}	k_oA /I0^{-3} min^{-I}	$\frac{k_oAV}{W}$ /I0^{-2} min^{-I}	k_{-I} /I0^{-2} min^{-I}
0	[a] I8.4	42.4	I.I3	7.43	37.2	5.25
0	[b] I8.5	42.6	I.I2	7.4I	37.I	5.50
-0.058	[c] I9.I	43.9	I.I6	7.67	38.45	5.45

a = values obtained by graphical method

b = values obtained by least squares method (using $y = mx$)

c = values obtained by least squares method (using $y = mx + c$)

TABLE 33

TF AT 94°C

Intercept	Slope /10^{-2} min^{-1}	k'_{-1} /10^{-2} min^{-1}	k'_o /10^{-6} $mol\ l^{-1} min^{-1}$	k_oA /10^{-3} min^{-1}	$\frac{k_oAV}{W}$ /10^{-2} min^{-1}	k_{-1} /10^{-2} min^{-1}
0	[a]17.0	39.2	16.39	5.79	28.95	10.25
0	[b]16.9	38.9	16.40	5.795	28.97	9.93
-0.026	[c]16.6	38.2	16.13	5.64	28.20	10.0

TABLE 34

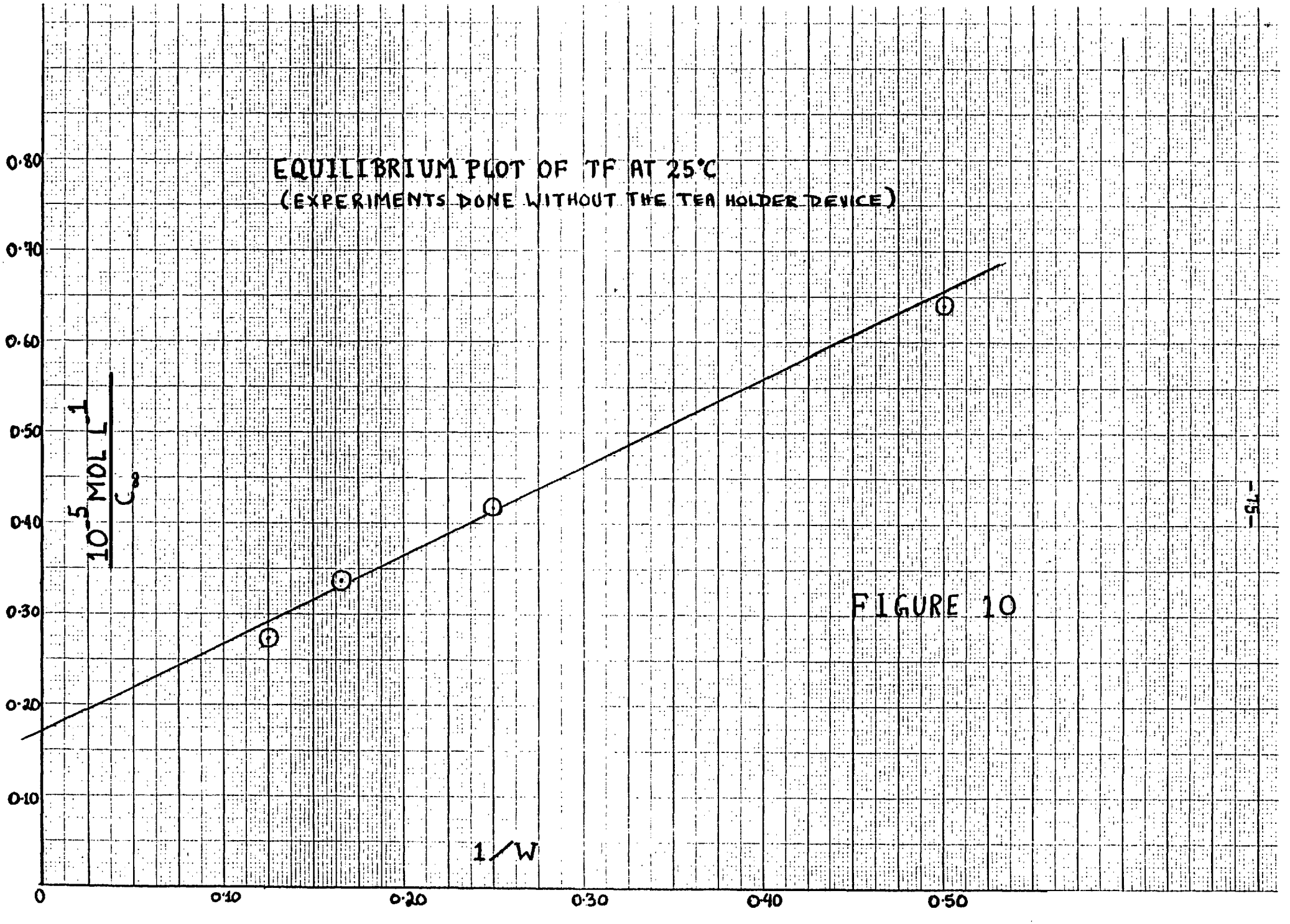
TR AT 94°C

Intercept	Slope /10^{-2} min^{-1}	k'_{-1} /10^{-2} min^{-1}	k'_o /10^{-3} min^{-1}	k_oA /10^{-3} min^{-1}	$\frac{k_oAV}{W}$ /10^{-2} min^{-1}	k_{-1} /10^{-2} min^{-1}
0	[a]19.60	45.1	1.31	7.29	36.5	8.60
0	[b]19.65	45.2	1.31	7.29	36.5	8.70
-0.006	[c]19.60	45.1	1.31	7.29	36.5	8.60

a = values obtained by graphical method

b = values obtained by least squares method (using $y = mx$)

c = values obtained by least squares method (using $y = mx + c$)


EQUILIBRIUM PLOT OF TF AT 25°C
(EXPERIMENTS DONE WITHOUT THE TEA HOLDER DEVICE)
0·80
0·70
0·60
0·50
0·40
0·30
0·20
0·10
0
0·10
0·20
0·30
0·40
0·50
1/W
$\frac{1}{10^{-5}\ \text{MOL L}^{-1}}$
C_s
FIGURE 10

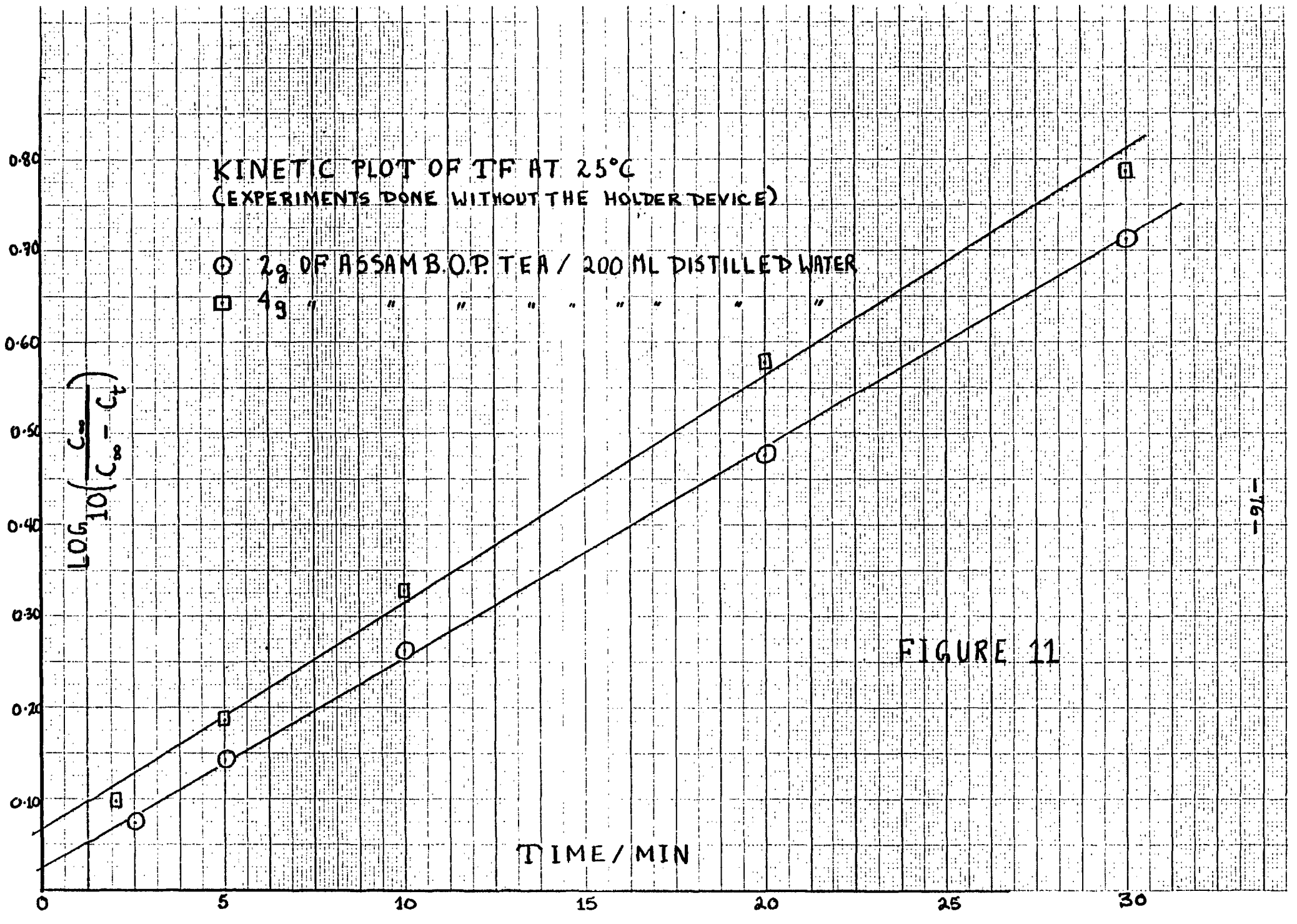

KINETIC PLOT OF TF AT 25°C
(EXPERIMENTS DONE WITHOUT THE HOLDER DEVICE)
○ 2g OF ASSAM B.O.P. TEA / 200 ML DISTILLED WATER
□ 4g " " " " " " " " " "
$LOG_{10}\left(\frac{C_\infty}{C_\infty - C_t}\right)$
0·80
0·70
0·60
0·50
0·40
0·30
0·20
0·10
0
5
10
15
20
25
30
TIME / MIN

FIGURE 11

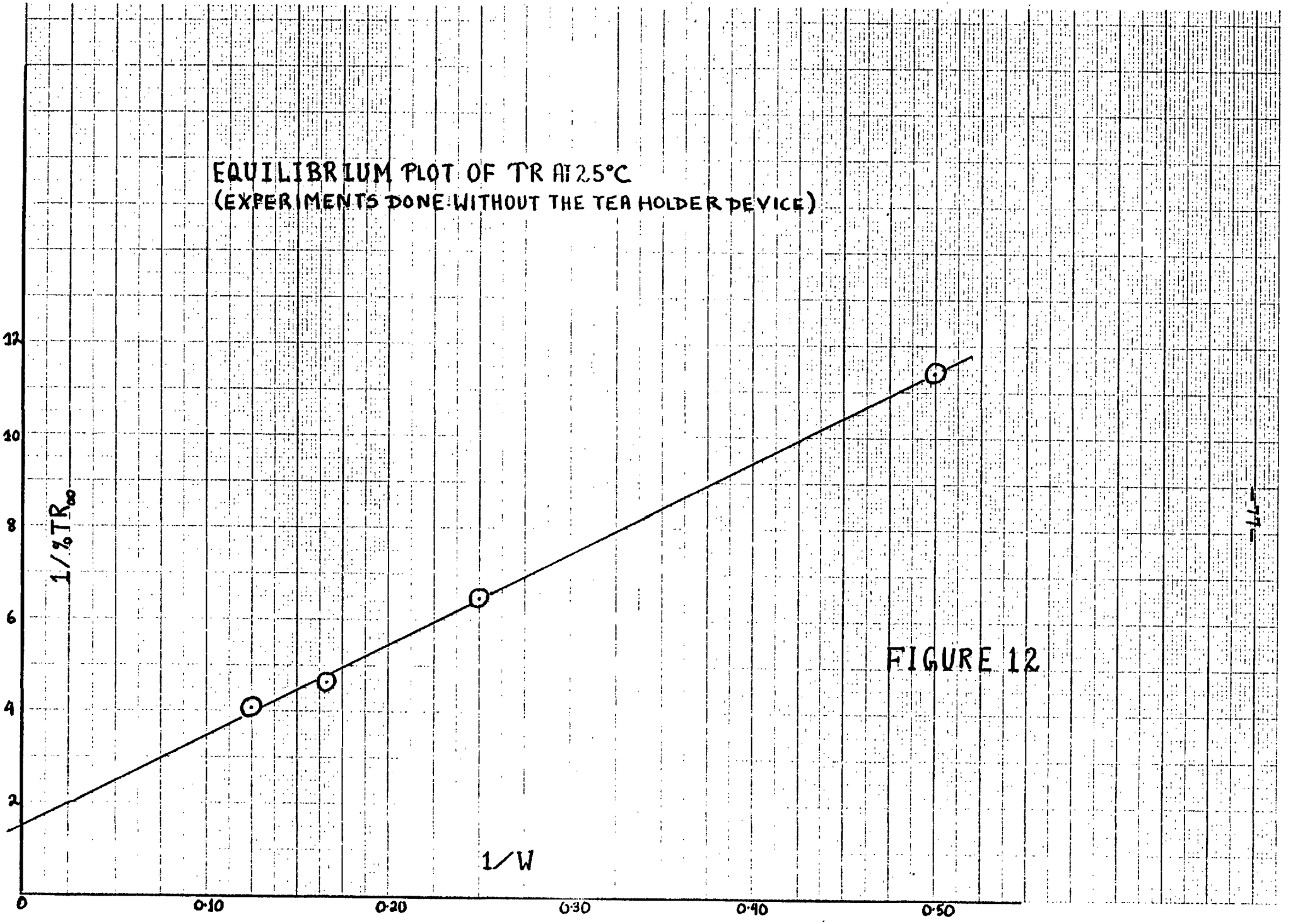

EQUILIBRIUM PLOT OF TR AT 25°C
(EXPERIMENTS DONE WITHOUT THE TEA HOLDER DEVICE)
1/%TR∞
12
10
8
6
4
2
0
0·10
0·20
0·30
0·40
0·50
1/W

FIGURE 12

FIGURE 13

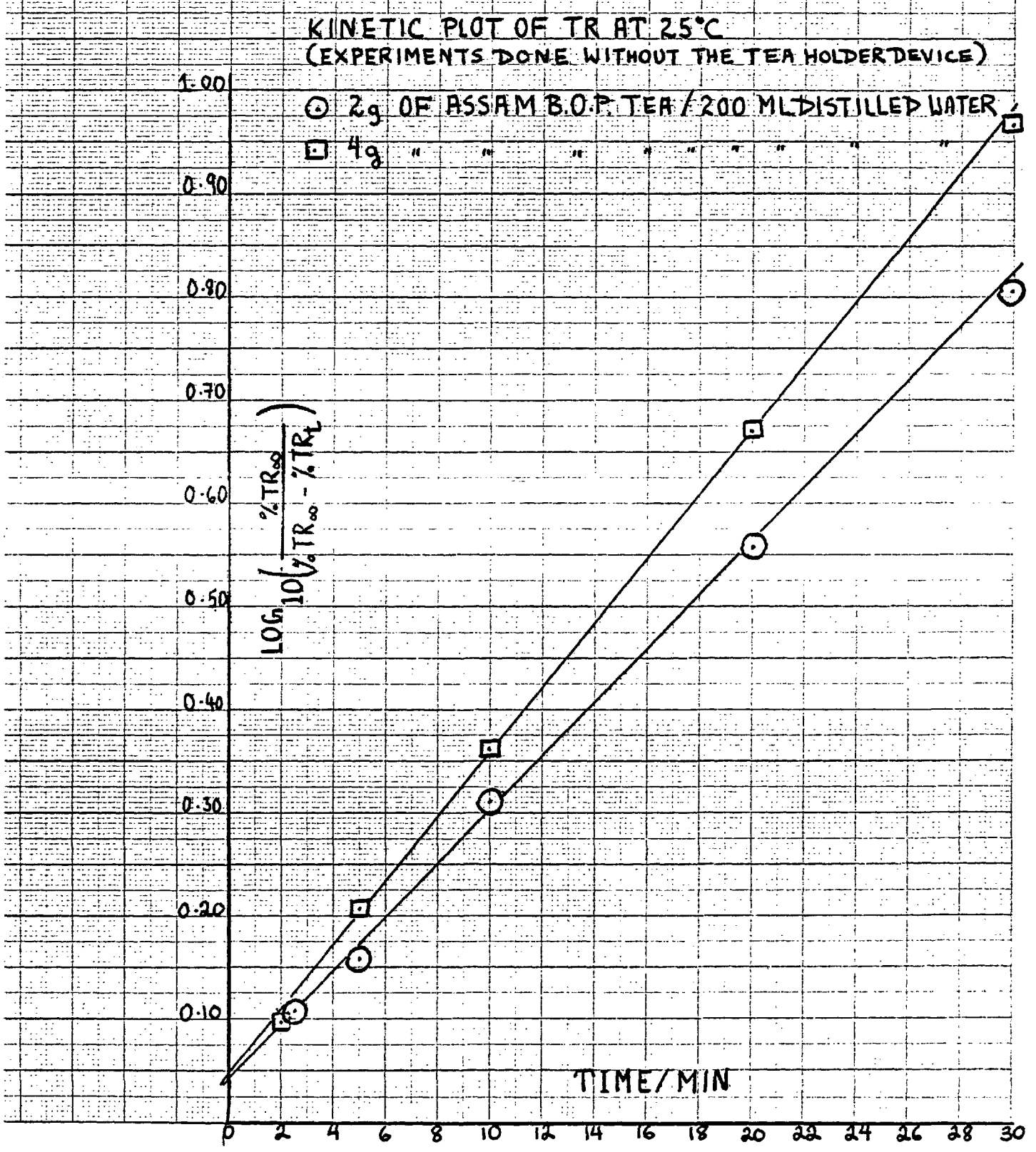

KINETIC PLOT OF TR AT 25°C
(EXPERIMENTS DONE WITHOUT THE TEA HOLDER DEVICE)
⊙ 2g OF ASSAM B.O.P. TEA / 200 ML DISTILLED WATER
⊡ 4g " " " " " " " " " "
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
$\log_{10}\left(\frac{\%TR_\infty}{V_o TR_\infty - \%TR_t}\right)$
TIME/MIN
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

FIGURE 19

EQUILIBRIUM PLOT OF TF AT 25°C

(EXPERIMENTS DONE WITH THE TEA HOLDER DEVICE)

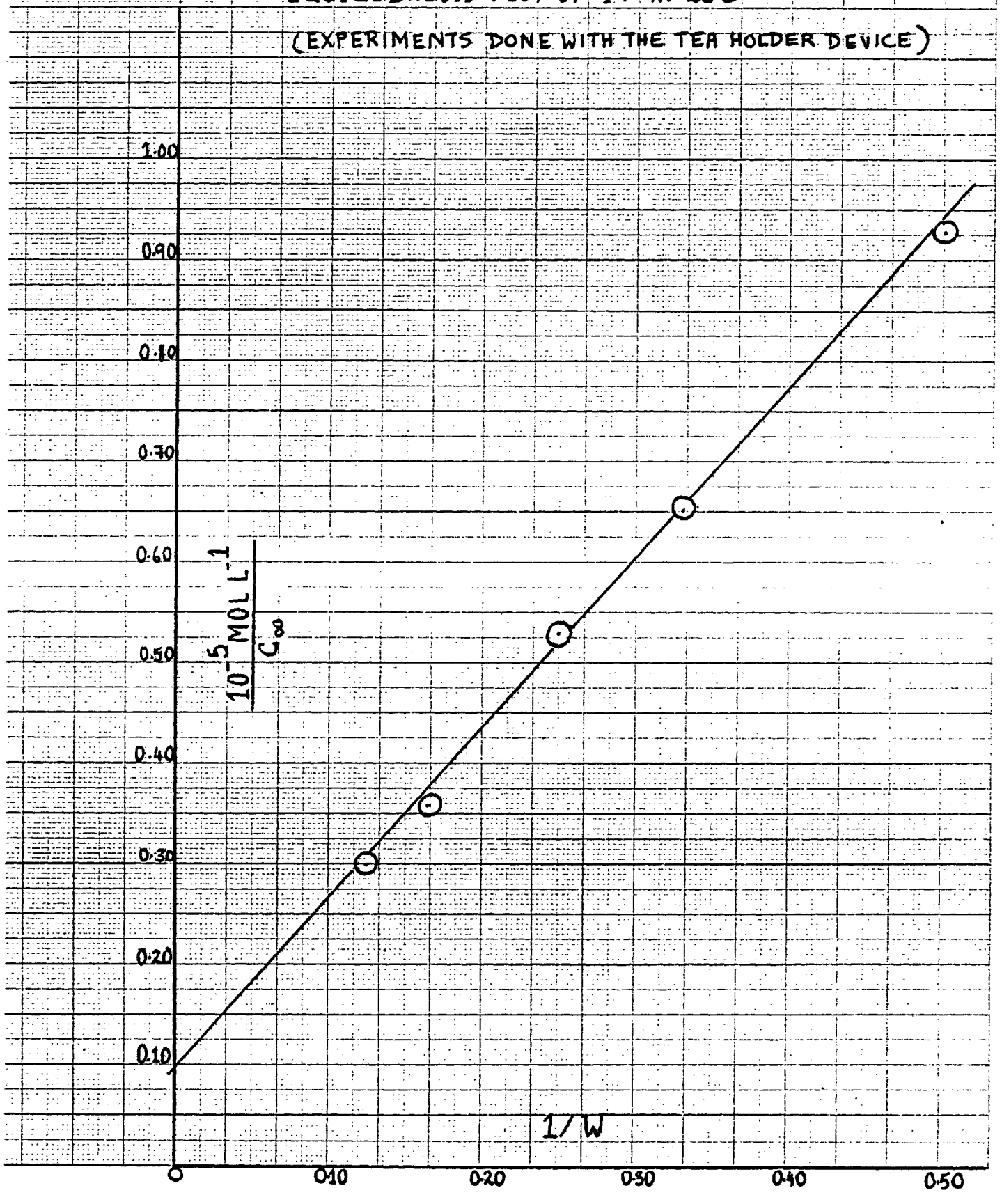
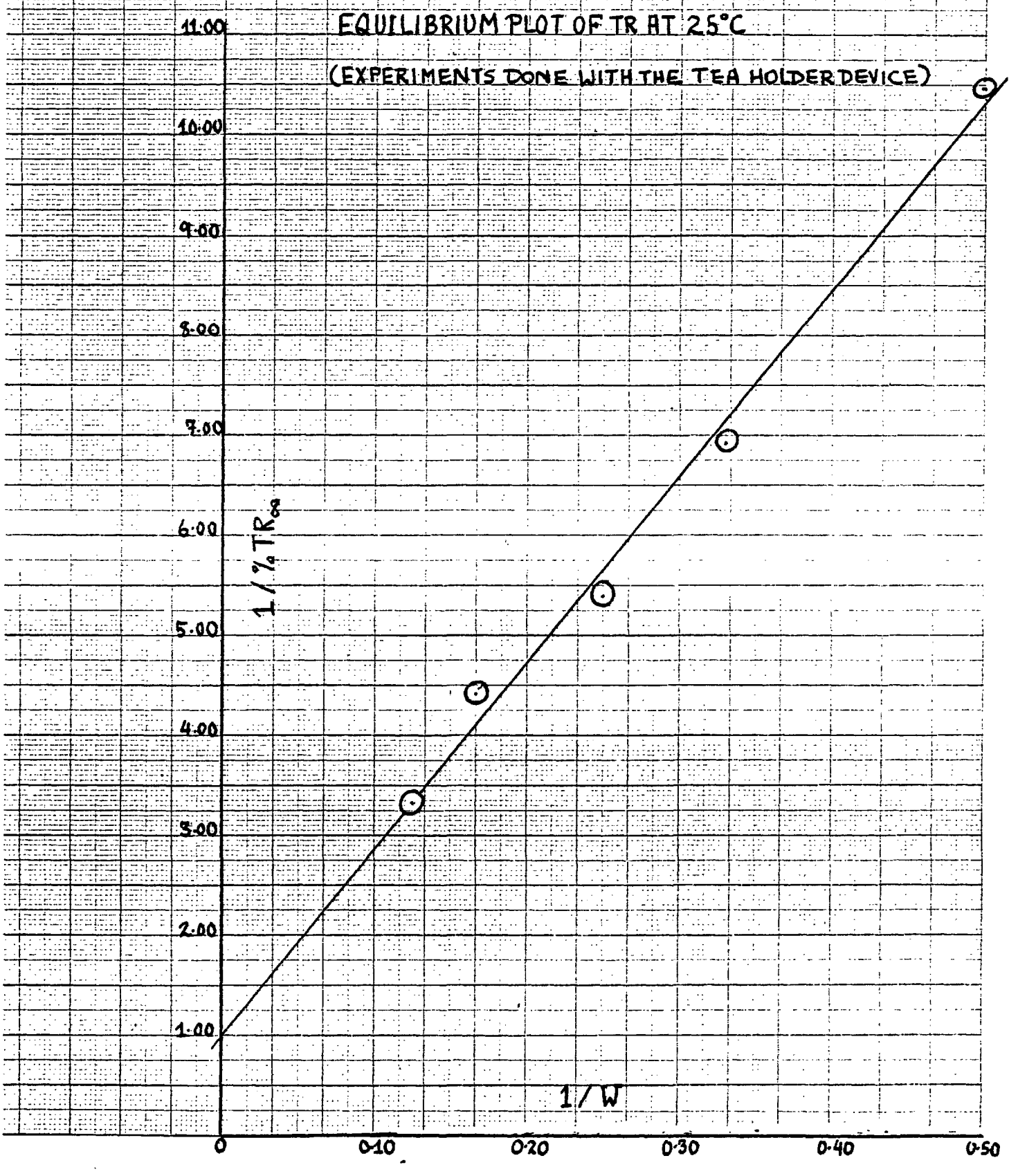


FIGURE 15


EQUILIBRIUM PLOT OF TR AT 25°C
(EXPERIMENTS DONE WITH THE TEA HOLDER DEVICE)
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
1/%TR∞
1/W
0
0·10
0·20
0·30
0·40
0·50

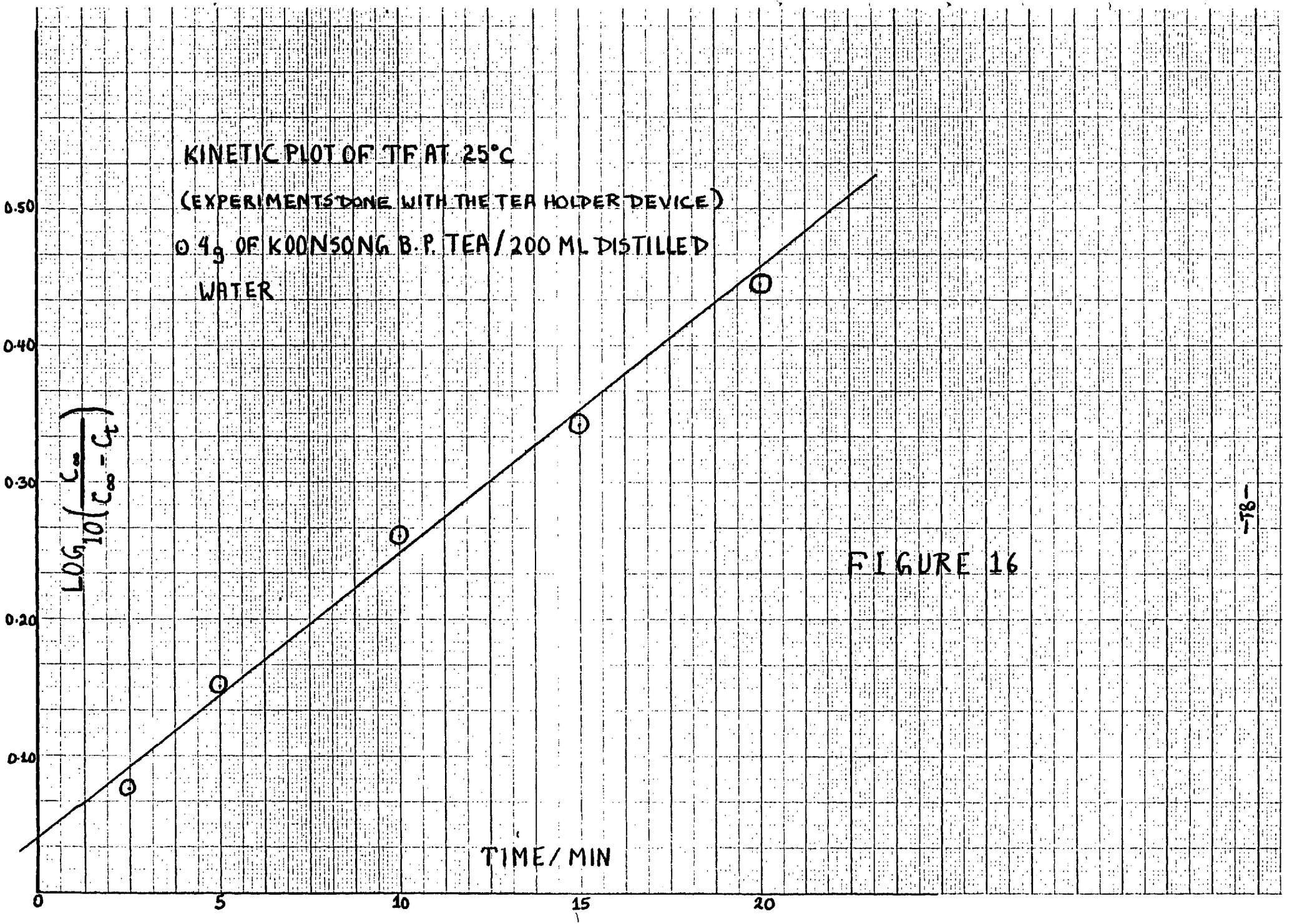

KINETIC PLOT OF TF AT 25°C
(EXPERIMENTS DONE WITH THE TEA HOLDER DEVICE)
○ 4g OF KOONSONG B.P. TEA / 200 ML DISTILLED WATER
$\log_{10}\left(\frac{C_\infty}{C_\infty - C_t}\right)$
0.50
0.40
0.30
0.20
0.10
0
5
10
15
20
TIME / MIN

FIGURE 16

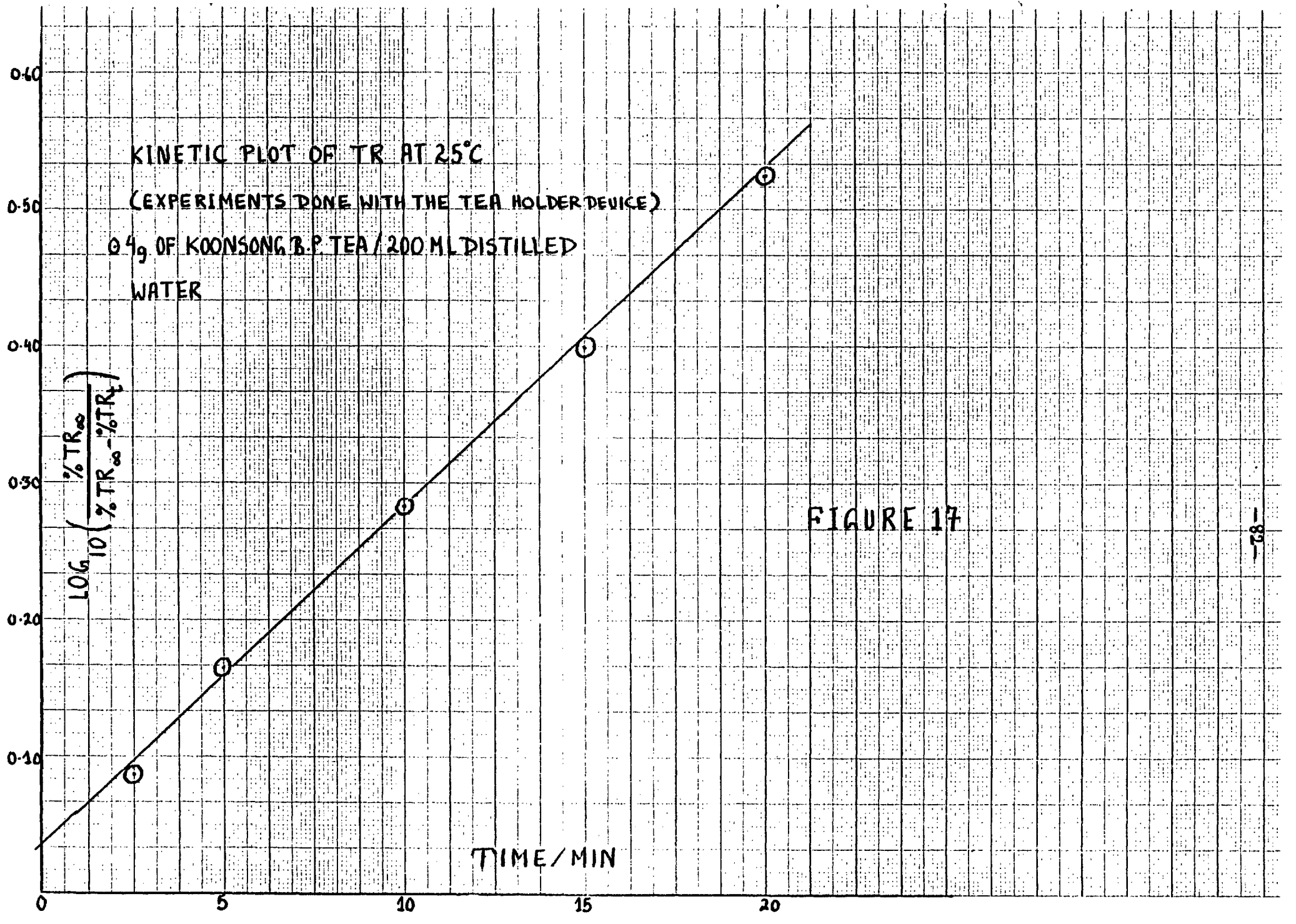

KINETIC PLOT OF TR AT 25°C
(EXPERIMENTS DONE WITH THE TEA HOLDER DEVICE)
0.4g OF KOONSONG B.P. TEA / 200 ML DISTILLED WATER
$\log_{10}\left(\frac{\%TR_\infty}{\%TR_\infty - \%TR_t}\right)$
TIME / MIN
0.60
0.50
0.40
0.30
0.20
0.10
0
5
10
15
20

FIGURE 17

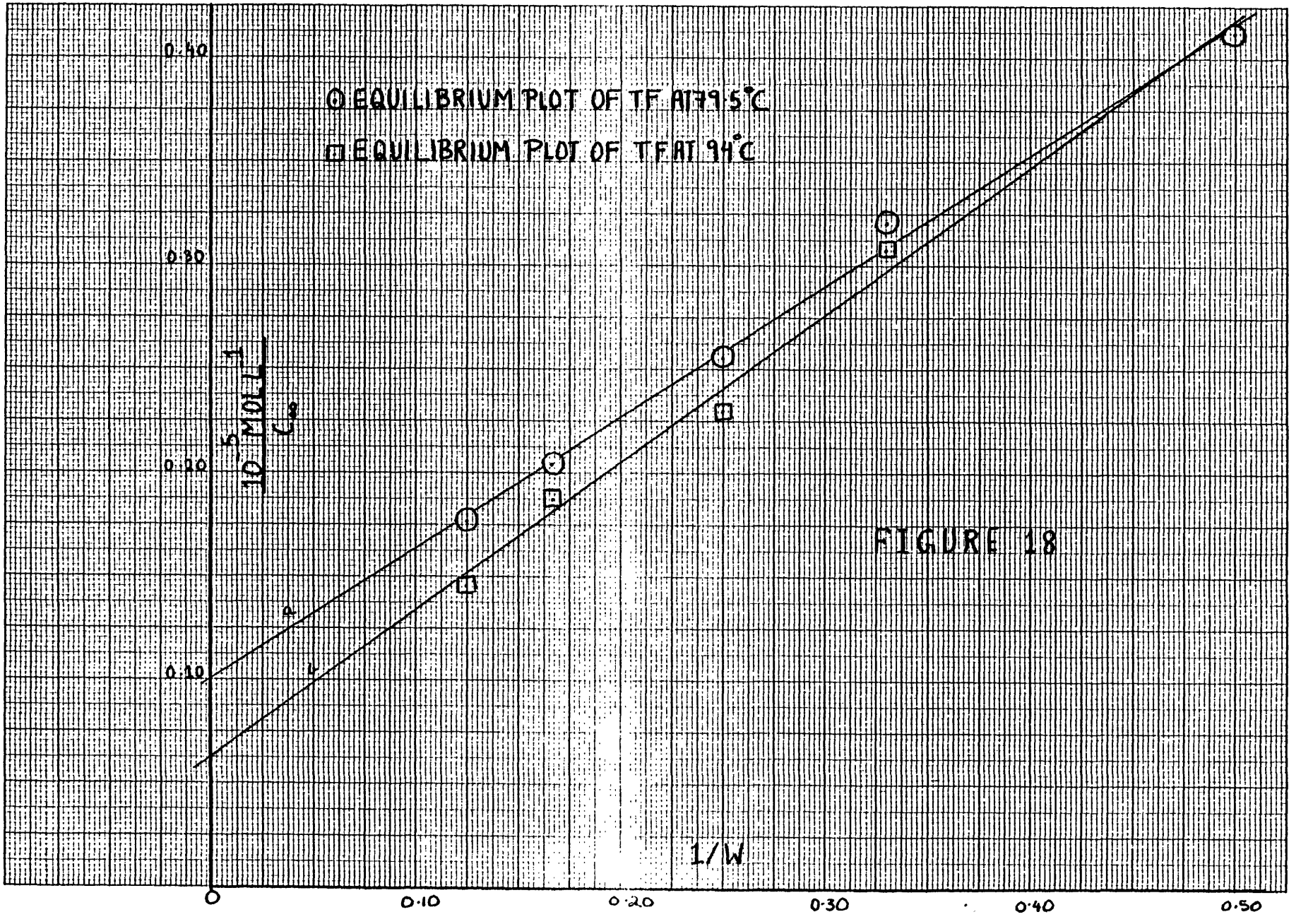

⊙ EQUILIBRIUM PLOT OF TF AT 79·5°C
□ EQUILIBRIUM PLOT OF TF AT 94°C
0·40
0·30
0·20
0·10
10^{-5} MOL L^{-1}
C_{aq}
A
B
0
0·10
0·20
0·30
0·40
0·50
1/W

FIGURE 18

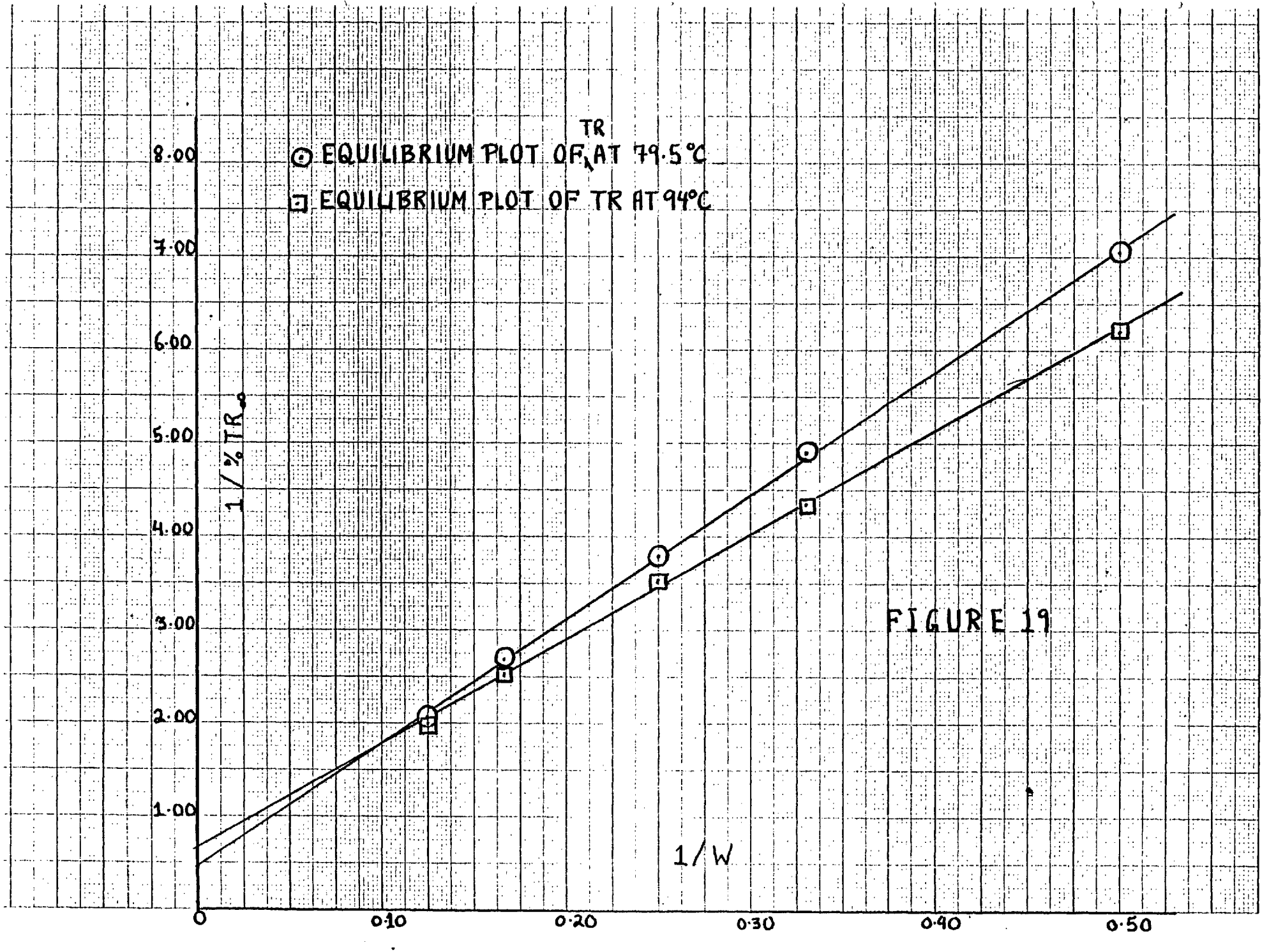

EQUILIBRIUM PLOT OF TR_1 AT 79.5°C
EQUILIBRIUM PLOT OF TR AT 94°C
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
1/%TR
0
0.10
0.20
0.30
0.40
0.50
1/W

FIGURE 19

FIGURE 20

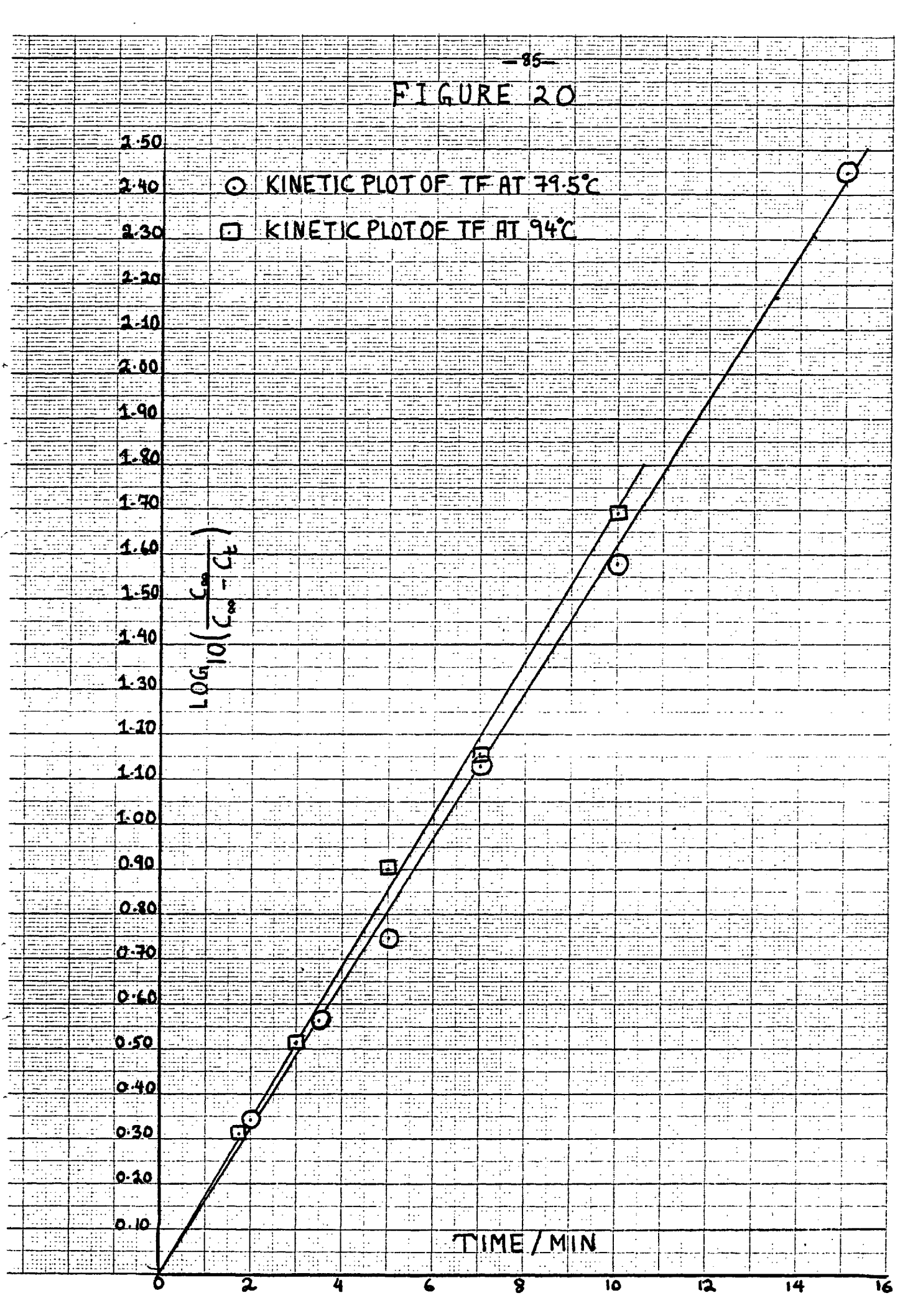

KINETIC PLOT OF TF AT 79·5°C
KINETIC PLOT OF TF AT 94°C
$\log_{10}\left(\frac{c_0}{c_0 - x}\right)$
TIME / MIN
2·50
2·40
2·30
2·20
2·10
2·00
1·90
1·80
1·70
1·60
1·50
1·40
1·30
1·20
1·10
1·00
0·90
0·80
0·70
0·60
0·50
0·40
0·30
0·20
0·10
0
2
4
6
8
10
12
14
16

FIGURE 21

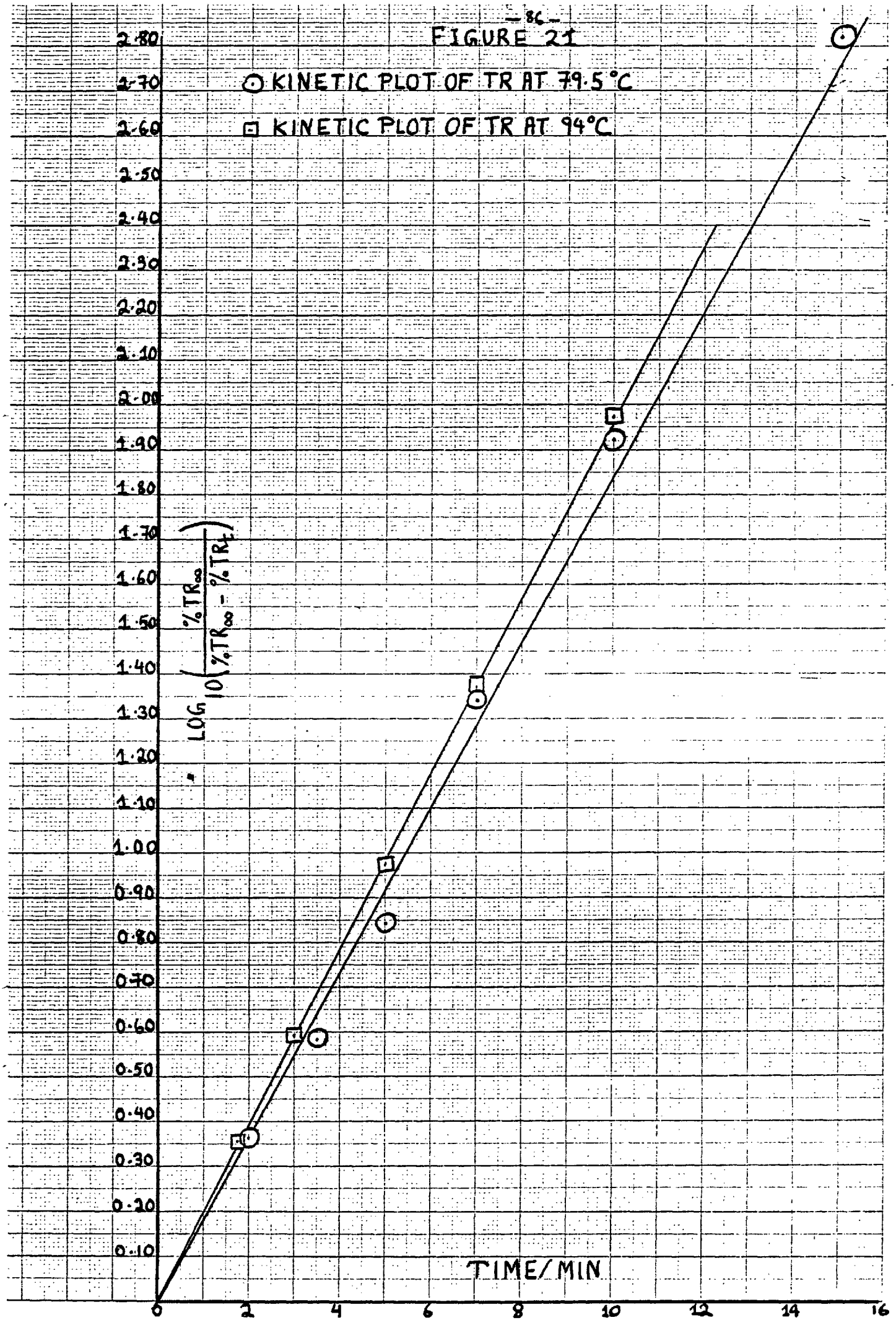

⊙ KINETIC PLOT OF TR AT 79.5 °C
⊡ KINETIC PLOT OF TR AT 94°C
2·80
2·70
2·60
2·50
2·40
2·30
2·20
2·10
2·00
1·90
1·80
1·70
1·60
1·50
1·40
1·30
1·20
1·10
1·00
0·90
0·80
0·70
0·60
0·50
0·40
0·30
0·20
0·10
LOG 10 (%TR∞ / %TR∞ - %TRt)
TIME/MIN
0
2
4
6
8
10
12
14
16

FIGURE 2Ia

4g of Koonsong B.P. tea in 200 ml distilled water were used here. The infusion time was 5 min and the infusion temperature was 95°C. This Figure represents the spectrum of solution A. Figures 2Ib and 2Ic represent the spectrums of solutions C and D respectively.

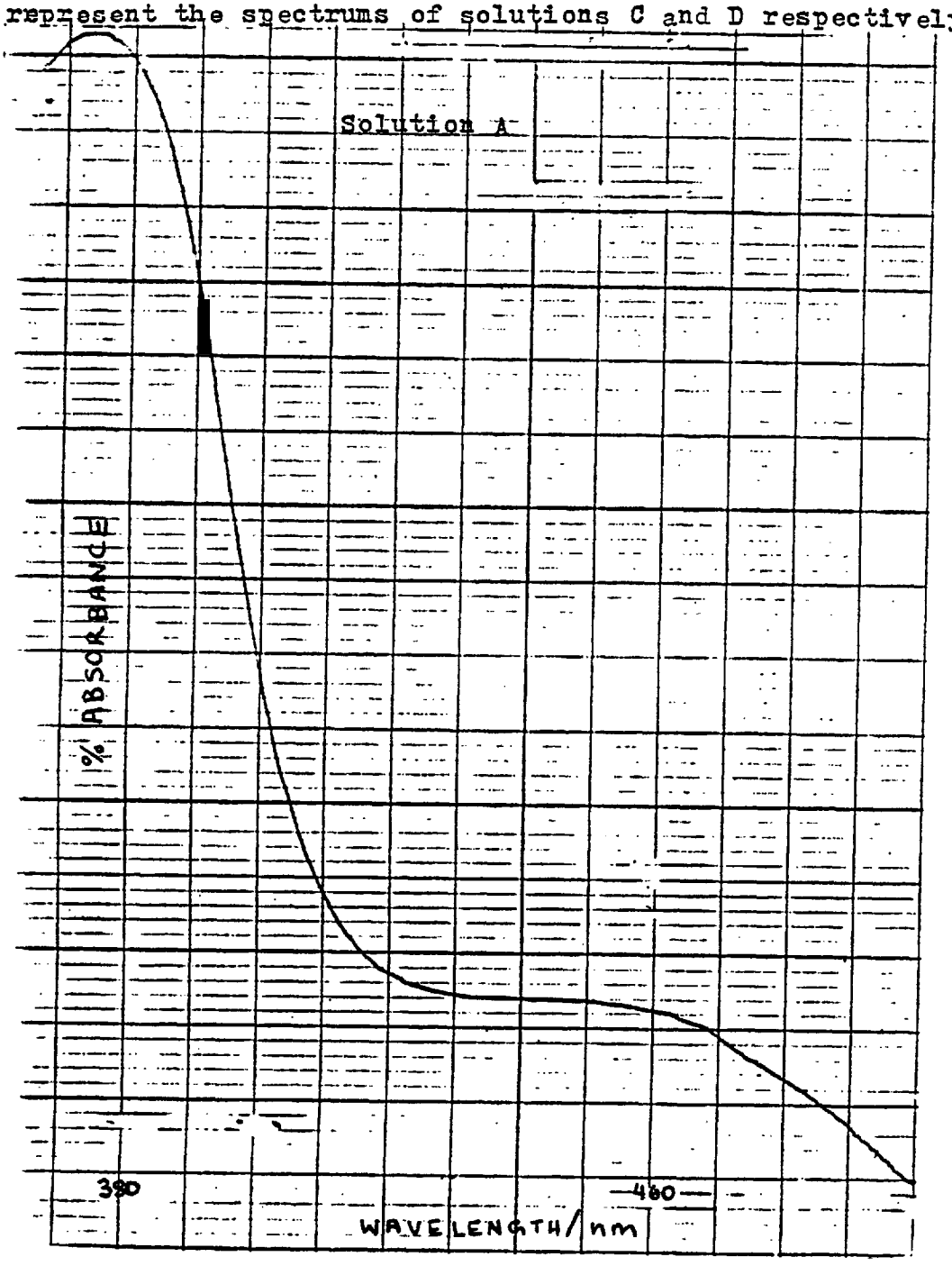


FIGURE 2Ib

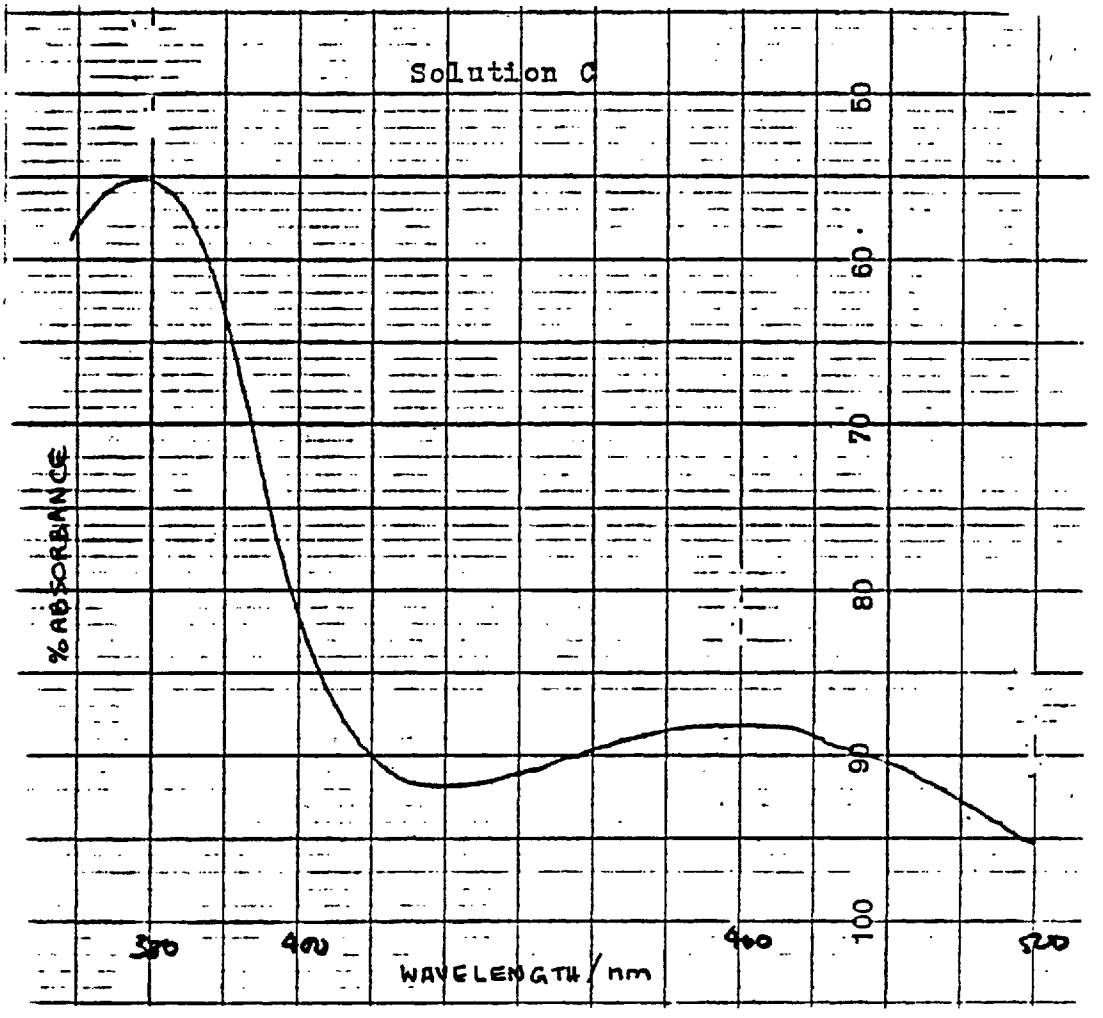
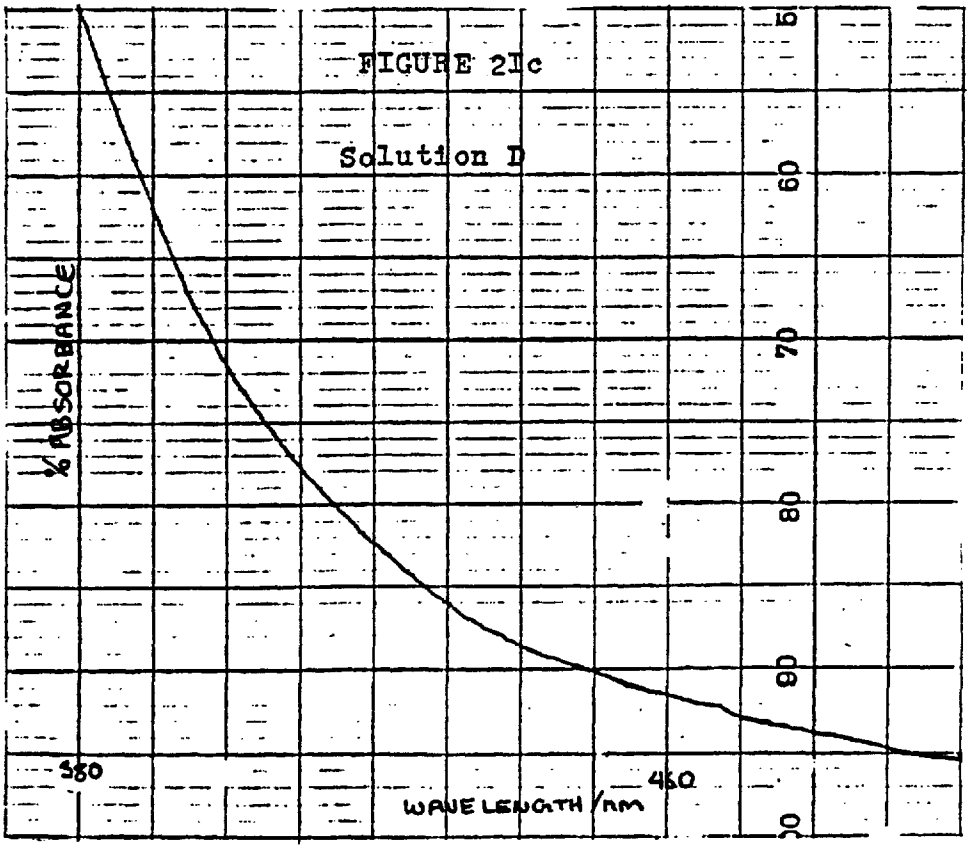


FIGURE 2Ic



CHAPTER 3

CAFFEINE

I.0 METHODS OF ANALYSIS

Most analyses of caffeine in black tea have been performed by taking hot aqueous extract of tea and extracting the caffeine in the aqueous solution with chloroform. However, as mentioned earlier, caffeine forms chemical complexes with polyphenolics in tea leaf which hinders chloroform extraction. Hot water (49b, 26, 50 - 52), dilute acid (53), or a basic additive, such as magnesium oxide (50, 52) or ammonia (53, 54) help in isolating caffeine from the caffeine - polyphenolic complex. Chloroform is used as extractant because it is selective in extracting caffeine but no polyphenols. Caffeine also exhibits a high solubility in chloroform (20g/I00ml at room temperature). The extracted caffeine is either determined through nitrogen analysis by the Kjeldahl method (55), by a spectrophotometric method at 273 nm (52, 54) or by gas - liquid chromatography (56, 57). The following two methods are standard ones for the determination of caffeine:

(i) The modified Bailey - Andrew Method (52) employs magnesium oxide to free chemically bonded caffeine. The caffeine is extracted with chloroform, and estimated from its nitrogen content as determined by the Kjeldahl method. Smith and Rees (26) also used a magnesium oxide filter aid column to release bonded caffeine, extracted the caffeine with chloroform and determined it by spectrophotometry at 273 nm.

(ii) The second method, devised by Borkar and Sloman (58)

and modified by John (59), uses ammonia to release chemically bonded caffeine and alternate basic and acidic columns of Celite as the purification procedure. Caffeine may be analysed by ultraviolet spectrophotometry or gas chromatography.

Apart from the above methods another rapid way of determining caffeine black tea extract was devised by Lehmann and Moran (27). They used polyamide powder as the column material to separate polyphenolic hydroxy compounds. The polyphenols are absorbed by the polyamide powder. Purines (e.g., caffeine) by contrast pass through the polyamide column. Hence, a direct spectrophotometric determination is possible as there are no interferences from the polyphenolic compounds.

In the present work Smith and Rees's method was tried out initially to determine caffeine in black tea extract. It was soon abandoned since it was lengthy and not suitable for kinetic determinations. The polyamide method seemed to be the most appropriate for kinetic measurements.

I.I PRELIMINARY WORK

SPECTROPHOTOMETRIC DETERMINATION OF CAFFEINE IN TEA BY THE SMITH AND REES METHOD

The tea sample was first extracted with boiling water and filtered on a magnesium oxide filter - aid column. The percentage caffeine in the tea sample was calculated from the difference in absorbances at 273 nm of the clear tea infusion before and after extraction with chloroform. This experiment was carried out at the Cadbury Typhoo Laboratories.

EXPERIMENTAL PROCEDURE

To prepare the column (180 mm x 22 mm) in a Soxhlet filter tube with a sintered glass plate (porosity approx. 2 - 3), a suspension in distilled water of a mixture of 2.5g of heavy magnesium oxide and 2.5g of Celite 545 (a diatomaceous filter aid) was sucked through under reduced pressure (water pump). Both heavy magnesium oxide and Celite 545 were obtained from Hopkin and Williams Ltd. To ensure that the column was not sucked dry during the process, distilled water was continuously added to the top.

0.5g of Assam B.O.P. tea were weighed and placed in a beaker followed by 3g of heavy magnesium oxide and 15 ml of boiling water. The beaker was left standing on the boiling water bath for 15 min and the contents were stirred from time to time with a glass rod. The contents were then transferred to the Soxhlet filter tube with boiling water (distilled water) until about 150 ml of the filtrate was obtained. This was placed into

a 500 ml conical flask and 5 ml of I0% (volume/volume) sulphuric acid was added. This solution was then boiled for a few minutes in order to reduce the volume to about I50 ml. The solution was cooled, transferred to a 200 ml calibrated flask and diluted to the mark with distilled water to form solution A.

PREPARATION OF THE SOLUTIONS FOR SPECTROPHOTOMETRIC MEASUREMENTS

I0 ml of solution A were pipetted into a 50 ml calibrated volumetric flask and diluted to the mark with distilled water to form solution B. 20 ml of solution A were pipetted into a 250 ml separating funnel and extracted 5 times with I5 ml of chloroform (analytical reagent) each time. The aqueous solution in the separating funnel was transferred to a beaker with distilled water and boiled to expel any residual chloroform till the volume was reduced to less than 50 ml. The contents of the beaker were then transferred to a 50 ml volumetric flask and diluted to the mark with distilled water to form solution C.

RESULTS AND CALCULATIONS

The values of $E_{1cm}^{0.001\%}$ for a solution of pure caffeine were determined at the wavelength of maximum absorption (273 nm) and of points on the ascending (250 nm) and descending (296 nm) portions of the curve equidistant from the maximum (60,26). E values or the absorbance of the tea sample containing caffeine were also obtained in this manner. The caffeine content, wt%C, of the sample was obtained from the equation used by Roberts and Smith (26) and by Lee Kum - Tatt (60):

$$\text{wt } \%C = \frac{(2E_B - E_C)}{2\,E^{0.001\%}_{\text{caffeine}}} \times \frac{0.001}{2} \times \frac{50}{20} \times \frac{200}{20} \times \frac{100}{W}$$

where, E_B = absorbance of solution B

E_C = absorbance of solution C

$E^{0.001\%}_{\text{caffeine}}$ = absorbance of 0.001% pure caffeine solution

W = weight of tea sample used

50/20, 200/20 are dilution factors,

In the present research:

$$E_B = E_{273} - \left(\frac{E_{250} - E_{296}}{2}\right)$$

$$= 0.983 - \left(\frac{0.38 - 0.200}{2}\right) = 0.692$$

$$E_C = E_{273} - \left(\frac{E_{250} - E_{296}}{2}\right)$$

$$= 0.290 - \left(\frac{0.21 - 0.23}{2}\right) = 0.070$$

$$E^{0.001\%}_{\text{caffeine}} = 0.385$$

$$W = 0.50$$

$$\text{wt } \%C = \left(\frac{1.384 - 0.07}{385}\right) \times 1250$$

$$= 4.27$$

The caffeine content obtained by this method seems fairly reasonable since caffeine is present in concentrations as high as 4% in black tea leaf (21).

Because of the many steps involved, this method is prone to many errors. Also the sample is boiled several times which in effect means loss of caffeine through evaporation. Therefore for the present research it was decided to discard the above method and follow the rapid method for determining caffeine by Lehmann and Moran (27). A few modifications were made to this method.

CHAPTER 4

PRESENT RESEARCH ON CAFFEINE

The same model theory which was used for determining theaflavins and thearubigins in tea infusions applies to the determination of caffeine.

I.0 EXPERIMENT TO DETERMINE THE EFFECT OF HEAVY MAGNESIUM OXIDE ON PURE CAFFEINE SOLUTION

A solution of 0.003% caffeine (BDH Chemicals Ltd.) was prepared (0.003% is an arbitrary value) and its absorbance was recorded. 20 ml of this solution and 3g of heavy magnesium oxide (Hopkin and Williams Ltd) were placed in a I00 ml flat - bottomed quickfit flask and weighed. The flask was then fitted with a water cooled reflux condenser and boiled gently for 5 min (solution AI). The flask was left standing with the water cooled reflux condenser for 5 min at room temperature and then the flask plus the contents were reweighed. Loss in weight was compensated for by the addition of an appropriate volume of distilled water (solution A2). 4 ml of solution A2 was passed through a pre - packed polyamide (see section I.2, page 99) column, followed by two 3 ml and one I0 ml portions of distilled water, the eluent was collected in a 25 ml volumetric flask made upto the mark with distilled water (solution A3). Absorbance of solution A3 was recorded at 273 nm. 20 ml of solution A3 was then placed in a 250 ml separating funnel and extracted 5 times with I5 ml chloroform (Analytical Reagent, obtained from B.D.H. Chemicals Ltd.) each time. The chloroform layer was discarded and the absorption

of the aqueous layer was recorded at 273 nm (solution A4). Results are recorded in Table (35).

The experiment was repeated but this time without heavy magnesium oxide [Table (36)]. Both the experiments were done twice.

The dilution factor used in Tables (35) and (36) arises from the corrected or calibrated volumes of the volumetric flask in which the eluent was collected and the syringe pipette from which the sample was introduced into the column. Hence:

$$\frac{24.952\ (\text{corrected volume of the volumetric flask})}{3.984\ (\text{corrected volume of the syringe pipette})}$$

From the results it appears that heavy magnesium oxide has no apparent effect on a solution of pure caffeine. However, there is an increase in the absorbance on boiling the caffeine solution on both occasions, i.e., with or without heavy magnesium oxide.

I.I EXPERIMENT TO DETERMINE THE EFFECT OF HEAVY MAGNESIUM OXIDE ON THE DETERMINATION OF CAFFEINE IN BLACK TEA EXTRACT BY THE POLYAMIDE METHOD

The experimental procedure was the same as described in section I.2. After recording the absorbance of solution B at 273 nm, 20 ml of solution A and 3g of heavy magnesium oxide were placed in a 100 ml quickfit conical flask and the flask was weighed. The flask was then fitted with a water cooled reflux condenser and the contents of the flask were boiled gently for 5 min. Then the flask plus the contents were cooled and re-

weighed. Loss in weight was compensated for by the addition of an appropriate amount of distilled water to form solution D. The colour of solution D was much lighter than that of solution A, probably due to the absorption of the coloured polyphenols by the heavy magnesium oxide.

4 ml of solution D was passed through the pre - packed polyamide column (see section I.2) followed by two 3 ml portions and one IO ml portion of distilled water. The eluent was again collected in a 25 ml volumetric flask and made upto the mark with distilled water. The absorbance of the resulting solution was recorded at 273 nm.

Another 20 ml portion of solution A was placed in IOO ml quickfit conical flask and the flask weighed. The same procedure as above was carried out but this time without heavy magnesium oxide. After polyamide separation, the absorption of the resulting solution (solution E) was recorded at 273 nm. The whole procedure was repeated twice (with freshly prepared solution A each time), obtaining two more values for solutions B, D and E respectively. The results are recorded in Table (37).

Table (37) shows that the absorbance of solution D compared to the absorbance of solution B is low in the first case, but in the other two cases it is low within experimental error. A test using more MgO was considered but it would have been difficult to separate the tea infusion from MgO for column analysis. Boiling solution A by itself seemed to increase the absorption after polyamide separation. Extraction with chloroform of the

solution treated with MgO yielded the same results as the solution not treated with MgO. Thus apart from the discrepancy in absorbance values after boiling, there seemed to be no apparent effect on treating the tea infusion with MgO. From these results it can be deduced that it is not necessary to treat the tea infusion with MgO prior to the polyamide separation of the polyphenols.

I.2 RAPID METHOD FOR THE DETERMINATION OF CAFFEINE IN TEA LEAF BY POLYAMIDE SEPARATION OF THE POLYPHENOLS; DETERMINATION OF THE PERCENTAGES, PARTITION COEFFICIENTS AND RATE CONSTANTS OF CAFFEINE AT 80°C AND 94.5°C

The method by Lehmann and Moran (27) was employed but changes were made (e.g., chloroform extraction) in order to suit the present research.

EXPERIMENTAL PROCEDURE

A glass stoppered 250 ml flat - bottomed flask containing a bar magnet stirrer was placed in a thermostatic water bath maintained at the appropriate temperature. The thermostatic set up was the same as for the determination of theaflavins and thearubigins at higher temperatures. All runs were carried out using the tea holder device. X ml of distilled water heated to the appropriate temperature were added to the flask and the immersible magnetic stirrer turned on; the water was stirred until it attained the required temperature. Yg of Koonsong B.P. tea (sieved) were added to the flask. The initial and the final temperatures of the infusion were recorded with the same type of thermometer as before. At the end of the required infu-

sion period a 10 ml sample of the infusion was removed by means of a 10 ml syringe (of the same type as used for previous experiments). The sample was placed in a 100 ml volumetric flask and made upto the mark with distilled water to form solution A.

At first it was decided to use pasteur pipettes manufactured by John Poulten Ltd. as columns. But it was soon discovered that these pipettes were not at all suitable for separation purposes since the rate of elution was too fast for any separation to take place. Therefore it was decided to construct a glass microchromatographic tube which has a constriction at the top (Figure 22) so that the rate of elution is slowed down to about one drop/2 sec. The tubes were made by the glass workshop in the Chemistry Department of Imperial College. The tube (15 cm x 0.8 cm) was closed at the narrow end with a small piece of glass wool and about 3 mm of sea sand (acid purified) deposited on top of the glass wool.

Polyamide CC6 powder for column chromatography obtained from Camlab, supplied to them by Macherey Nagel + Co., was allowed to swell in distilled water several hours before use. The chromatographic tube was filled with polyamide powder suspended in distilled water by means of a wide bore plunger pipette of 10 ml capacity (Gallenkamp) as shown in Figure 22 . Care was taken not to let the column run dry.

4 ml of solution A was passed through the polyamide column followed by distilled water in two 3 ml and one 10 ml portions. The eluent was collected in 25 ml volumetric flask and made upto

the mark with distilled water to form solution B. The absorbance of solution B was recorded at 273 nm in a I cm quartz cell, distilled water being used as the blank solution.

Another 20 ml of solution B was placed in a 250 ml separating flask and was extracted 5 times with I5 ml of chloroform each time. The chloroform layer was discarded after extraction and the absorbance of the aqueous layer (solution C) was recorded at 273 nm. Lehmann and Moran did not perform the chloroform extraction, however, this step was found to be necessary in the present research because after extraction of caffeine with chloroform the spectrophotometer did detect some absorption as seen from the results.

(a) RESULTS AND CALCULATIONS

Tea leaves were stored in 2.5 litres capped glass bottles as mentioned earlier. Work at 80°C was done with tea kept in a bottle numbered I. For work at 94.5°C tea from a bottle numbered 2 had to be used since tea in bottle I had almost finished. Therefore, in order to detect any difference in the caffeine determination the absorbances of caffeine obtained from both the bottles at 80°C were compared. All the experiments were done with the tea holder device.

4g Koonsong B.P. Tea (used from bottle I)/200 ml Distilled Water.

Absorbance of caffeine solution = 0.692

4g Koonsong B.P. Tea (used from bottle 2)/200 ml Distilled Water.

Absorbance of caffeine solution = 0.660

$\therefore$ Difference in absorbance value = 4.62%

Hence this factor of 4.62% was added to all absorbance values at 94.5°C.

The factor A used in the Tables is obtained from (B - C) and is the real absorbance of the solution containing caffeine. The value of the extinction coefficient was obtained from the literature (60) as 9900 l mol^{-I} cm^{-I} and was used throughout the caffeine calculations. This value was also checked in the present research. A known concentration of pure caffeine solution (pure caffeine was obtained from B.D.H. Chemicals Ltd.) was diluted several times and absorbance values at 273 nm were recorded Table (38) . A curve of absorbance versus concentration was plotted (Figure 22a) and the gradient (extinction coefficient) was calculated. This value was found to be 9960 l mol^{-I} cm^{-I} which is in good agreement with the literature value. The concentration of caffeine in the sample solutions were calculated from the Beer - Lambert equation

$$\text{concentration of caffeine in sample solution} = \frac{A}{9900} \text{ mol } l^{-I}$$

The percentages, partition coefficients and rate constants for the infusion of caffeine at 80°C and 94.5°C were calculated in the same manner as with the theaflavins and thearubigins. All results and calculations are tabulated [Tables (39 - 48)] . The dilution factor 62.5 is obtained from:

$$\frac{100}{10} \times \frac{25}{4} = 62.5$$

Kinetic graphs were plotted graphically (Figures 24, 25) as well as by both the least squares methods (as a means of comparison) used previously in the case of theaflavins and thearubigins

at higher temperatures. All equilibrium calculations for the infusion of caffeine were done by the least squares method. The plots are depicted in Figures 23a and 23b. The spectrum of caffeine is illustrated in Figure 26 and the spectrum of tea sample containing caffeine is shown in Figure 27. The activation energies and enthalpy calculations are described in the discussion section.

I.3 EXPERIMENT TO DETERMINE WHETHER TEA / WATER SYSTEM REPRESENTS A DIFFUSION CONTROLLED PROCESS

The rate determining step in the dissolution of the soluble constituent in a tea infusion could be either the surface reaction:

X (tea leaf) $\longrightarrow$ X (solution) (a)

or the diffusion controlled process:

X (concentration at the surface) $\longrightarrow$ X (concentration in the bulk) (b)

The rate constant for the surface reaction (a) will be a function of the structure of the tea leaf (which is not known). For reaction (b) the dissolved X must diffuse through the Nernst layer and hence, from Fick's first law of diffusion:

$$\frac{d[X]}{dt} = \frac{D_X A ([X]_{surface} - [X]_{bulk})}{\delta} \qquad (c)$$

where, X = concentration of X (soluble species)

t = the time

D_X = the diffusion coefficient of X in solution

A = the surface area of the tea leaves

δ = the thickness of the Nernst layer

Expression (c) is a simple correction to the kinetic equations if diffusion is slow enough to matter. The rate of diffusion can be enhanced considerably by vigorous stirring, which decreases the thickness of the diffusion layer.

EXPERIMENTAL PROCEDURE

The tea / water system used was the same as before (4g of Koonsong B.P. tea / 200 ml distilled water). The infusion was

stirred at a very slow rate by adjusting the speed of the magnetic stirrer. A 10 ml sample of the tea infusion was extracted after 5 min with the aid of a syringe as before and diluted to a 100 ml with distilled water. After polyamide separation the absorption of caffeine in the solution was recorded. The experiment was repeated but this time the stirring speed was increased to a maximum. Results are incorporated in Table (49). From this Table it appears that more caffeine is extracted when the stirring speed is at a maximum. According to Fick's law this suggests diffusion of the soluble constituent from the tea leaf into the water. Thus the tea / water system can be described as at least partly diffusion controlled.

TABLE 35

EXPERIMENTS DONE WITH HEAVY MAGNESIUM OXIDE

Absorbances of 0.003% caffeine solution (AI) = I.445

Absorbance of solution A3		Absorbance of solution A4	
(I) 0.236	*I.478	0.032	*0.200
(2) 0.240	*I.50	0.034	*0.2I0

TABLE 36

EXPERIMENTS DONE WITHOUT HEAVY MAGNESIUM OXIDE

Absorbance of solution A3		Absorbance of solution A4	
(I) 0.240	*I.50	0.03I	*0.I94
(2) 0.233	*I.459	0.030	*0.I87

*Values are multiplied by the dilution factor: 24.952/3.984

TABLE 37

THE EFFECT OF HEAVY MAGNESIUM OXIDE ON CAFFEINE DETERMINATION BY THE POLYAMIDE METHOD

4g OF KOONSONG B.P. TEA (SIEVED) / 200 ML DISTILLED WATER

Solution	Weight of flask + contents before heating/g	Weight of flask + contents after heating /g	Loss in weight /g	Absorbance at 273 nm
B	-	-	-	(I) 0.820 (2) 0.848 (3) 0.852
D	(I) 79.736 (2) 79.757 (3) 79.874	(I) 79.48I (2) 79.544 (3) 76.378	(I) 0.255 (2) 0.2I3 (3) 0.35I	(I) 0.74I (2) 0.830 (3) 0.843
E	(I) 82.0I9 (2) 76.946 (3) 79.874	(I) 8I.6I0 (2) 76.479 (3) 79.874	(I) 0.409 (2) 0.467 (3) 0.255	(I) 0.860 (2) 0.909 (3) 0.889

TABLE 38

BEER - LAMBERT DETERMINATION OF THE EXTINCTION COEFFICIENT OF CAFFEINE

*Absorbance of I.545 x $I0^{-4}$ mol l^{-I} caffeine solution = I.495

Dilution factor (a/b)	^{c}concn. /$I0^{-4}$ mol l^{-I}	Absorbance at 273 nm
4/25	0.247	0.257
5/25	0.309	0.32I
5/20	0.386	0.398
5/I5	0.5I5	0.528
5/I0	0.7725	0.78I
I0/I5	I.03	I.04I

* 0.03g of pure caffeine dissolved in Il distilled water (0.003% caffeine solution)

a is the volume of 0.003% caffeine solution

b is the total volume to which a is diluted with distilled water

c is the concentration of diluted caffeine solutions:

$$\frac{a}{b} \times I.545 \times I0^{-4} \text{ mol } l^{-I}$$

TABLE 39

EQUILIBRIUM RESULTS FOR CAFFEINE AT 80°C (BATH TEMPERATURE) AFTER 20 MIN INFUSION WITH KOONSONG B.P. TEA (SIEVED) / 200 ML DISTILLED WATER

W/g	Initial T of infusion /°C	Final T of infusion /°C	B	C	(B-C) x dilution factor. (A)	c_∞ /10^{-3} mol l^{-1}	10^{-3}mol l^{-1}/c_∞
2	79.8	80.0	0.406	0.060	21.63	2.19	0.458
3	80.0	80.0	0.625	0.110	32.19	3.25	0.308
4	79.8	80.0	0.852	0.160	43.25	4.37	0.228
5	80.0	80.0	1.017	0.210	50.44	5.09	0.196
6	80.0	80.0	1.255	0.265	61.88	6.25	0.160
8	80.0	80.0	1.53	0.330	75.00	7.58	0.132

B is the absorbance of solution before extraction with chloroform

C is the absorbance of solution after extraction with chloroform

$c_\infty = \frac{A}{9900}$ mol l^{-1}

TABLE 40

KINETIC RESULTS AND CALCULATIONS AT 80°C (BATH TEMPERATURE)

4g KOONSONG B.P. TEA (SIEVED) / 200 ML DISTILLED WATER

Infusion time /min	Initial T of infusion /°C	Final T of infusion /°C	B	C	(B-C) x dilution factor	c_t /10^{-3} mol l^{-1}	$(c_\infty - c_t)$ /10^{-3} mol l^{-1}	$\log_{10}\left(\frac{c_\infty}{c_\infty - c_t}\right)$
1.5	80.0	80.0	0.405	0.066	21.187	2.14	2.23	0.292
2.5	80.0	80.0	0.585	0.100	30.31	3.06	1.31	0.523
5.0	79.8	80.0	0.733	0.130	37.69	3.81	0.56	0.892
7.0	80.0	80.0	0.804	0.145	41.19	4.16	0.21	1.320
10.0	80.0	80.0	0.820	0.140	42.63	4.31	0.06	1.86
12.0	80.0	80.0	0.845	0.158	42.94	4.34	0.03	2.16

B is the absorbance of solution before extraction with chloroform

C is the absorbance of solution after extraction with chloroform

$c_t = \frac{A}{9900}$ mol l^{-1}

TABLE 4I

EQUILIBRIUM RESULTS FOR CAFFEINE AT 95°C (BATH TEMPERATURE) AFTER 20 MIN INFUSION WITH KOONSONG B.P. TEA (SIEVED) / 200 ML DISTILLED WATER

W/g	Initial T of infusion /°C	Final T of infusion /°C	B	C	(B-C) x dilution factor. (A)	c_∞ /10^{-3} mol l^{-1}	10^{-3} mol l^{-1}/c_∞
2	94.5	94.5	0.445 0.435	0.090 0.088	22.19 21.69	2.317	0.432
3	94.3	94.5	0.620	0.109	31.94	3.38	0.296
4	94.5	94.5	0.885 00.888	0.160 0.158	45.31 45.31	4.79	0.210
5	94.5	94.5	1.04	0.200	52.50	5.54	0.181
6	94.5	94.5	1.315 1.320	0.320 0.320	62.19 62.50	6.59	0.152
8	94.5	94.5	1.65	0.352	81.75	8.64	0.116

$c_\infty = \frac{\text{average (A)} - 4.62\% \times \text{average (A)}}{9900}$ mol l^{-1} (see page 101)

TABLE 42

KINETIC RESULTS AND CALCULATIONS AT 95°C (BATH TEMPERATURE).

4g KOONSONG B.P. TEA (SIEVED) / 200 ML DISTILLED WATER

Infusion time /min	Initial T of infusion /°C	Final T of infusion /°C	B	C	(B-C) x dilution factor. (A)	c_t /10^{-3} mol l^{-1}	$(c_\infty - c_t)$ /10^{-3} mol l^{-1}	$\log_{10}\left(\frac{c_\infty}{c_\infty - c_t}\right)$
2.0	94.5	94.5	0.555	0.105	28.13	2.97	1.82	0.420
3.0	94.5	94.5	0.669	0.110	34.94	3.69	1.10	0.639
5.0	94.5	94.5	0.800	0.155	40.31	4.26	0.53	0.956
7.0	94.3	94.5	0.855	0.160	43.44	4.59	0.20	1.38
10.0	94.5	94.5	0.865	0.150	44.69	4.72	0.07	1.84
12.0	94.4	94.5	0.881	0.160	45.06	4.76	0.03	2.20

$$c_t = \frac{(A) - 4.62\%(A)}{9900} \text{ mol } l^{-1}$$

TABLE 43

PERCENTAGE AND PARTITION COEFFICIENT OF CAFFEINE AT 80°C

Fig(23) slope/10^{3} gl mol^{-1}	Fig(23) intercept /10^{3} mol^{-1}	x_o /10^{-4} mol g^{-1}	Caffeine /wt%	K
0.879	0.0167	2.28	4.42	0.263

TABLE 44

RATE CONSTANT OF CAFFEINE AT 80°C

g of tea /200 ml distilled water	Fig(24) slope /min^{-1}	k'_{-1} /10^{-2} min^{-1}	k'_o /10^{-3} mol l^{-1} min^{-1}	k_oA /10^{-3} min^{-1}	$\frac{k_oAV}{W}$ /10^{-2} min^{-1}	k_{-1} /10^{-2} min^{-1}
4	0.185	42.6	1.80	7.89	39.5	3.1

TABLE 45

PERCENTAGE AND PARTITION COEFFICIENT OF CAFFEINE AT 94.5°C

Fig(23) slope/10^{3}gl mol^{-1}	Fig(23) intercept /10^{3}lmol^{-1}	x_o /10^{-4}mol g^{-1}	Caffeine /wt%	K
0.848	0.009	2.36	4.58	0.471

TABLE 46

RATE CONSTANT OF CAFFEINE AT 94.5°C

g of tea /200 ml distilled water	Fig(25) slope /10^{-2} min^{-1}	k'_{-1} /10^{-2} min^{-1}	k'_o /10^{-3} mol l^{-1} min^{-1}	k_oA /10^{-3} min^{-1}	$\frac{k_oAV}{W}$ /10^{-2} min^{-1}	k_{-1} /10^{-2} min^{-1}
4	18.9	43.5	1.974	8.36	41.8	1.70

TABLE 47

CAFFEINE AT 80°C

Intercept	Slope /10^{-2} min^{-1}	k'_{-1} /10^{-2} min^{-1}	k'_o /10^{-3} mol l^{-1} min^{-1}	k_oA /10^{-3} min^{-1}	$\frac{k_oAV}{W}$ /10^{-2} min^{-1}	k_{-1} /10^{-2} min^{-1}
$^a0$	18.5	42.6	1.80	7.89	39.5	3.1
$^b0$	18.4	42.4	1.797	7.88	39.4	3.0
^{c}-0.043	17.86	41.1	1.740	7.63	38.1	3.0

TABLE 48

CAFFEINE AT 94.5°C

Intercept	Slope /10^{-2} min^{-1}	k'_{-1} /10^{-2} min^{-1}	k'_o /10^{-3} mol l^{-1} min^{-1}	k_oA /10^{-3} min^{-1}	$\frac{k_oAV}{W}$ /10^{-2} min^{-1}	k_{-1} /10^{-2} min^{-1}
$^a0$	18.9	43.5	1.974	8.36	41.8	1.70
$^b0$	18.7	43.1	1.951	8.27	41.3	1.80
^{c}-0.093	17.6	40.5	1.835	7.78	38.8	1.70

a = values obtained by a graphical method

b = values obtained by a least squares method (using $y = mx$)

c = values obtained by a least squares method (using $y = mx + c$)

TABLE 49

THE EFFECT OF STIRRING SPEED ON THE EXTRACTION OF CAFFEINE

g of Koon-song B.P.tea /200 ml dis-tilled water	Infusion temperature /°C	Infusion time /min	Stirring speed	Absorbance
4	80.0	7	fast	0.790
4	80.0	7	medium	0.774
2	94.5	5	fast	0.430
2	94.5	5	slow	0.410
4	94.5	5	fast	0.868
4	94.5	5	slow	0.822

FIGURE 22

This diagram shows the experimental set up of the apparatus for the determination of caffeine by the separation of the polyphenols.

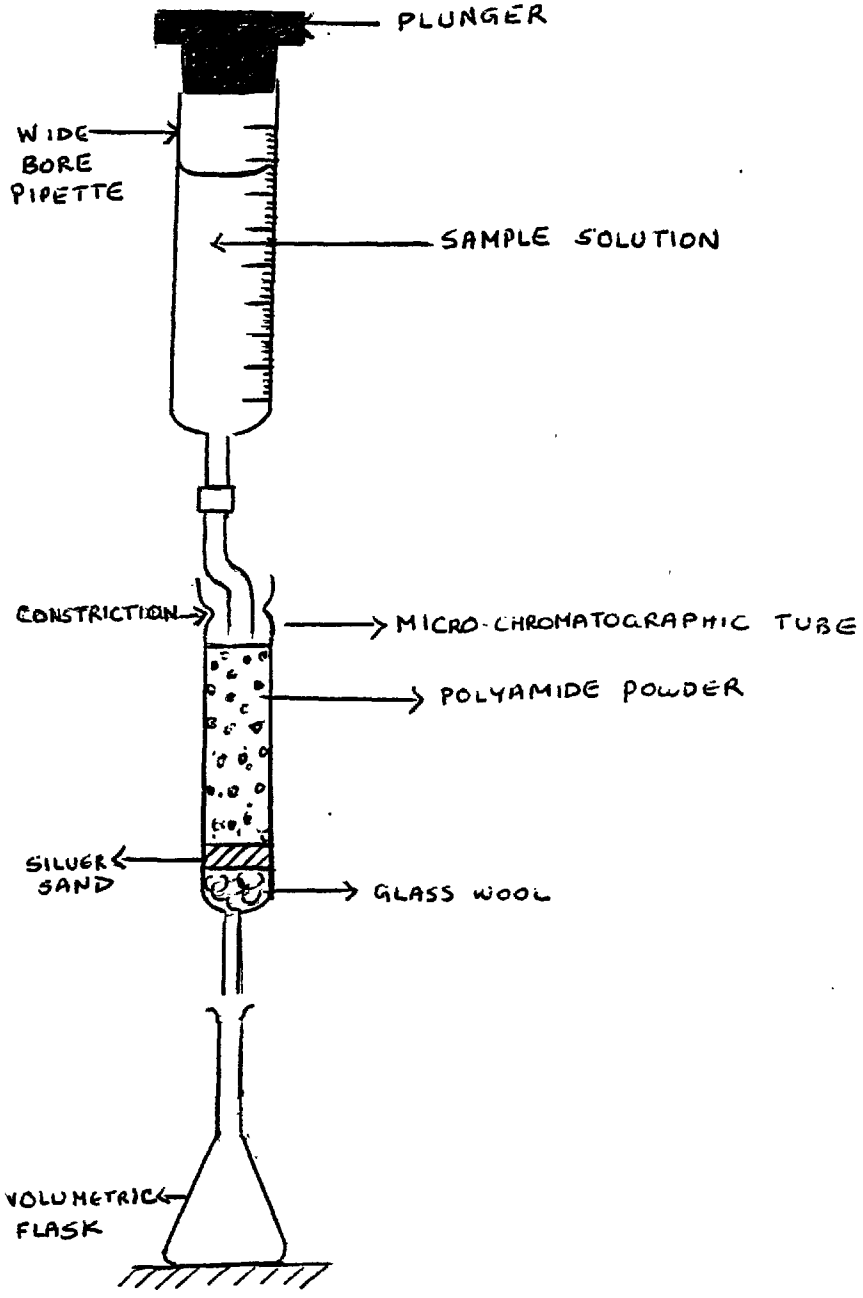


FIGURE 22a

BEER-LAMBERT PLOT OF PURE CAFFEINE SOLUTION

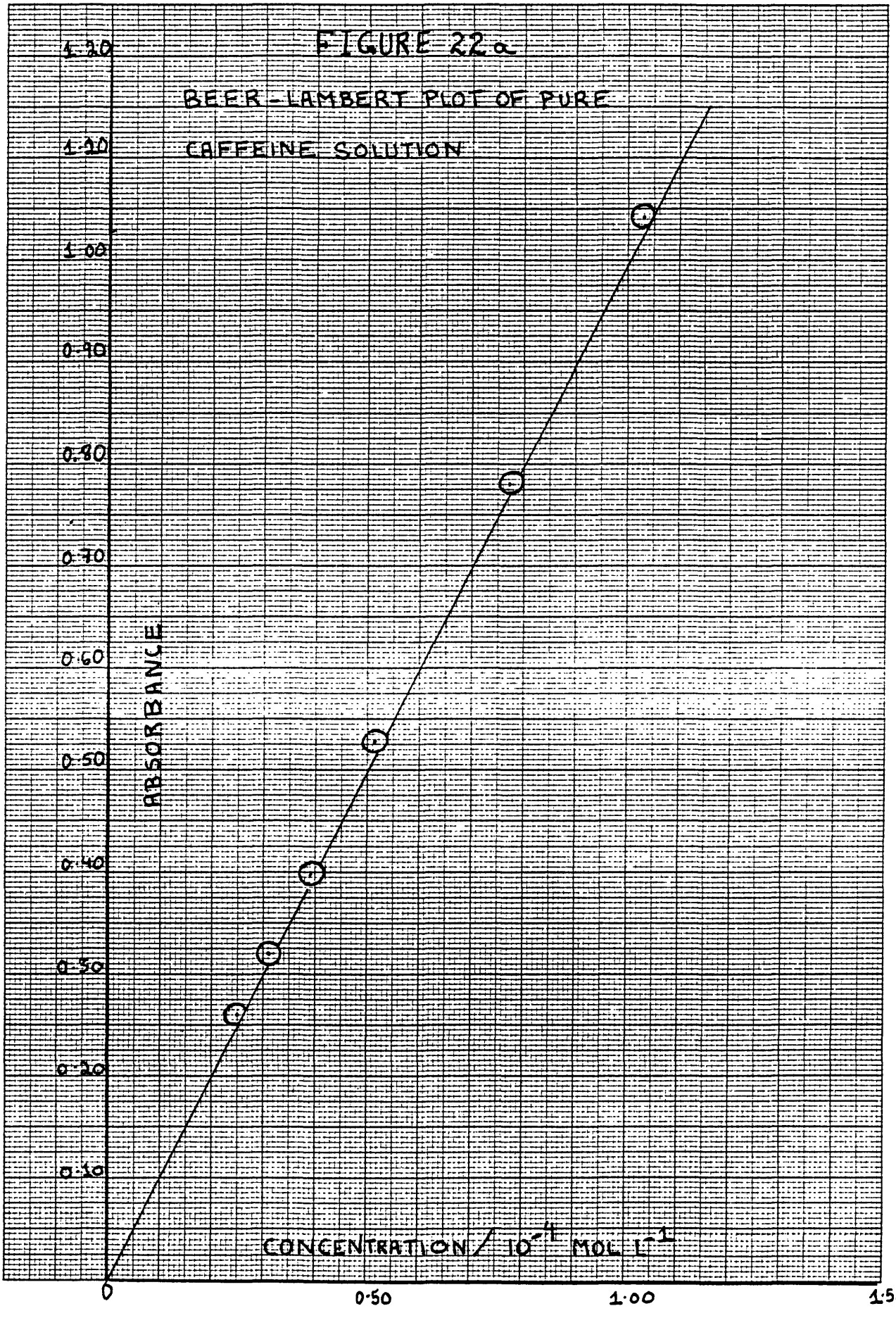


FIGURE 23

(a) ⊙ EQUILIBRIUM PLOT OF CAFFEINE AT 80°C

(b) ⊡ EQUILIBRIUM PLOT OF CAFFEINE AT 95°C

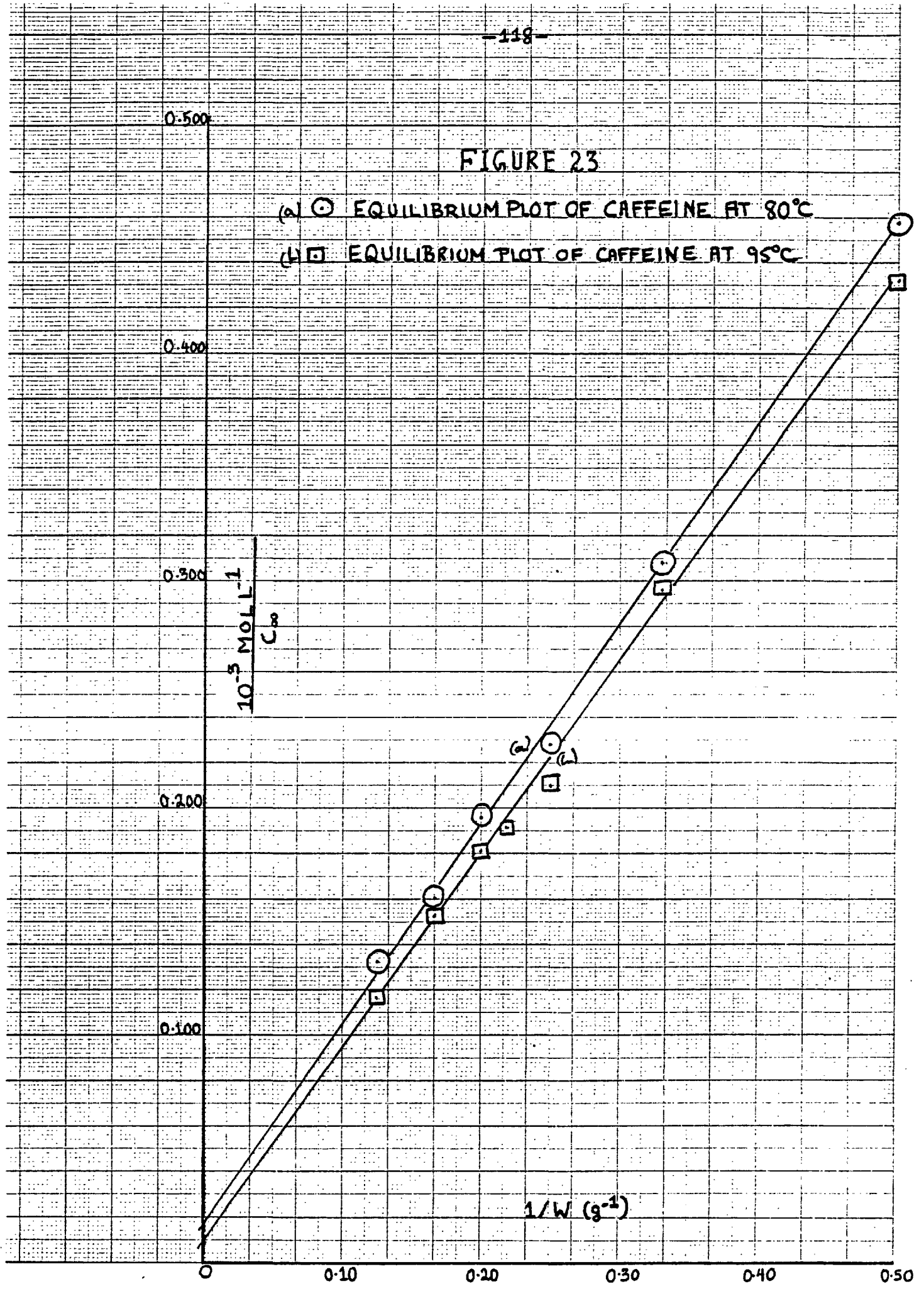


FIGURE 24

KINETIC PLOT OF CAFFEINE AT 80°C

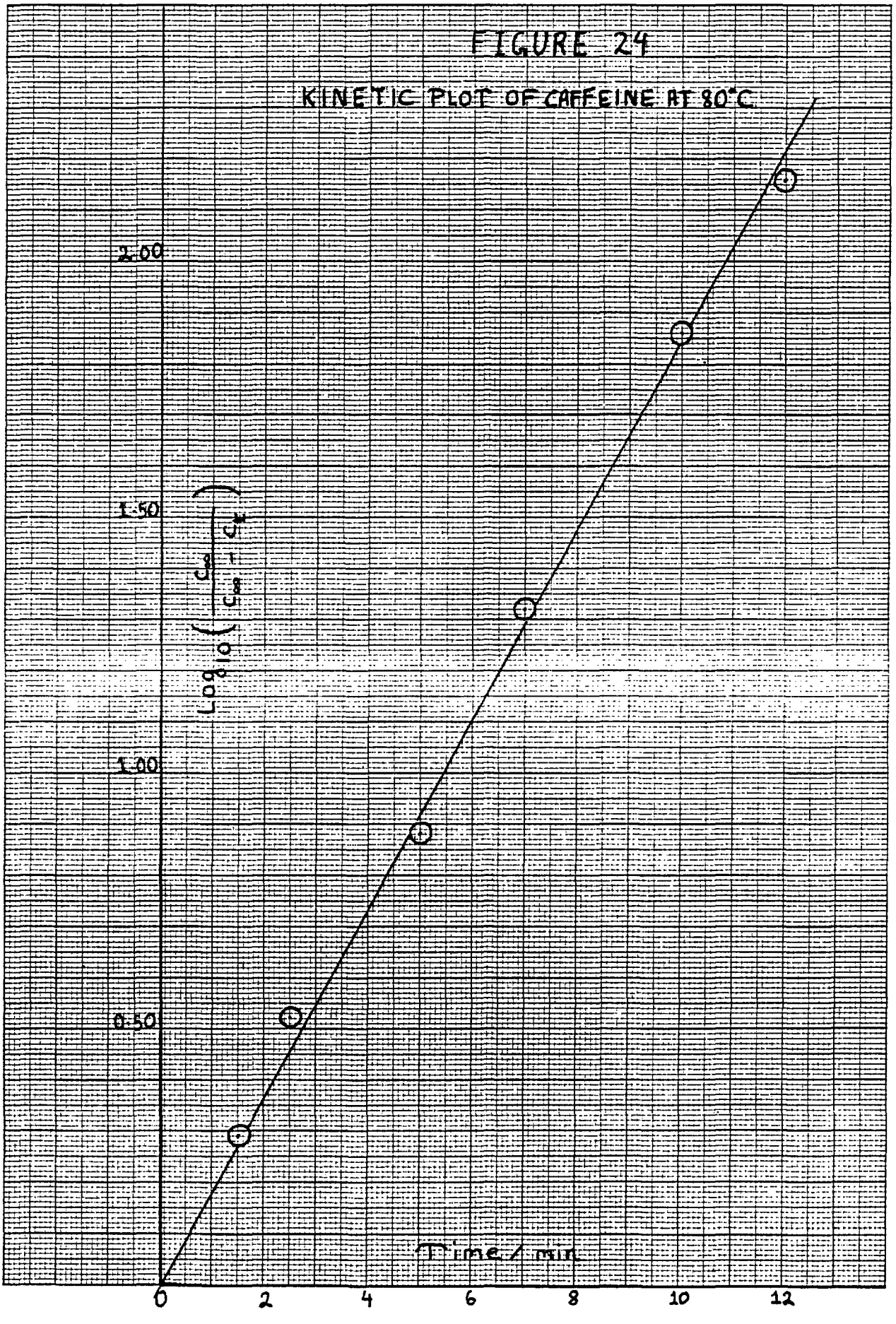


FIGURE 25

KINETIC PLOT OF CAFFEINE AT 95°C

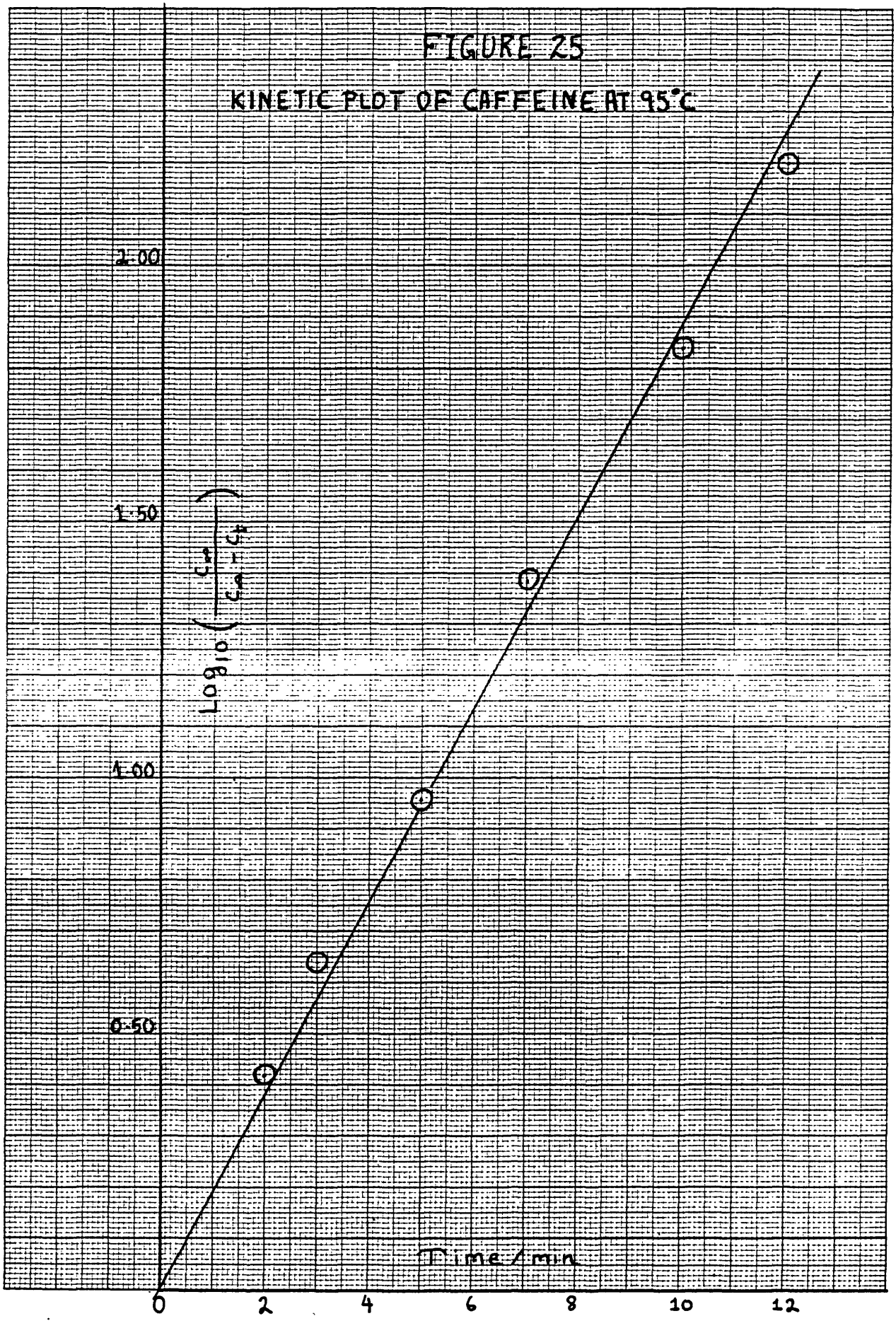


FIGURE 26

SPECTRUM OF PURE CAFFEINE SOLUTION

Concentration of caffeine = 0.003% in distilled water

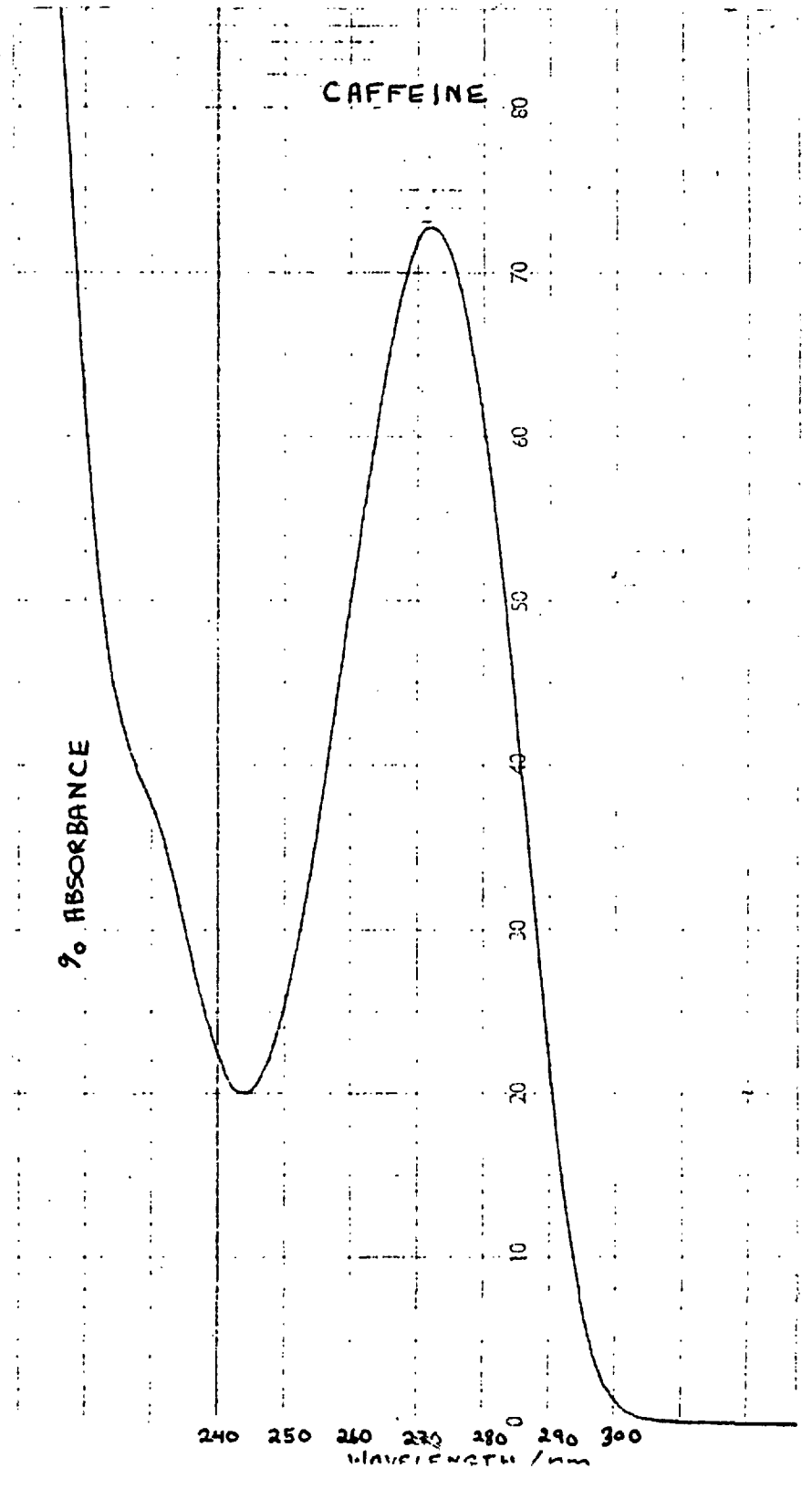


FIGURE 27

SPECTRUM OF TEA SAMPLE (4g of Koonsong B.P. Tea/200 ml Distilled Water) CONTAINING CAFFEINE

Infusion Temperature = 95°C.

Infusion Time = 20min.

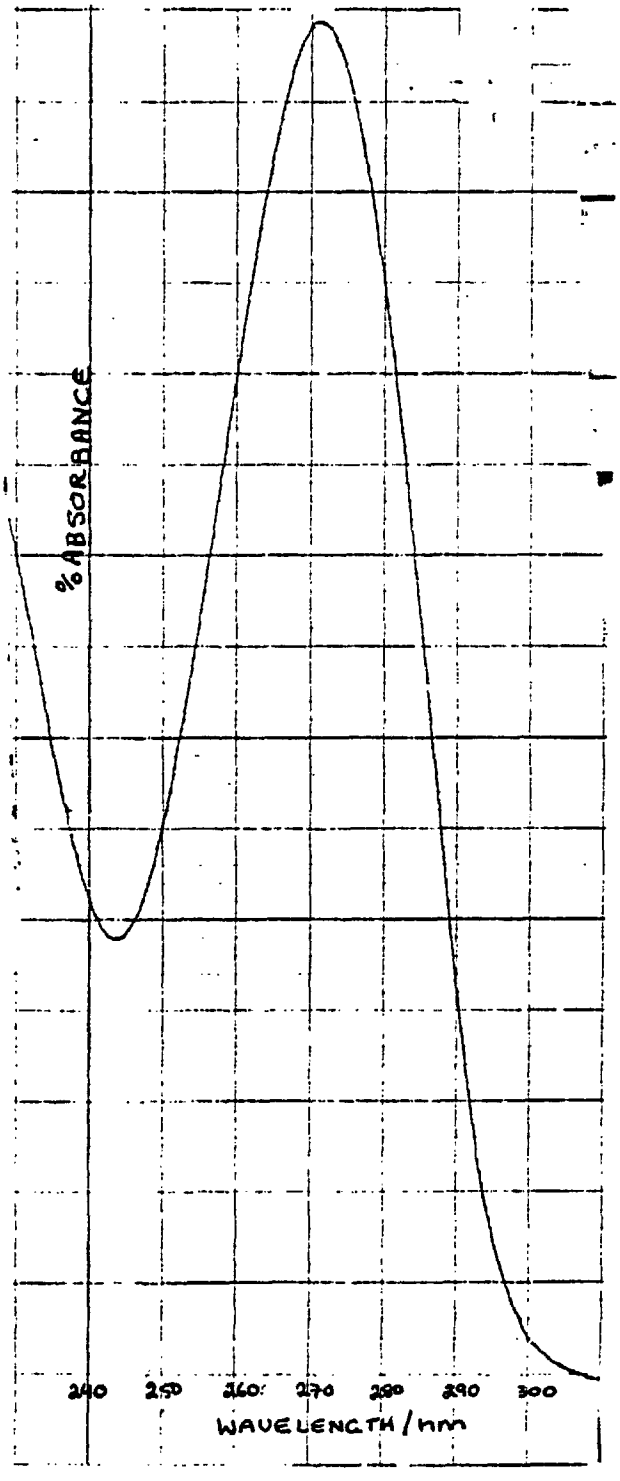


FIGURE 28

SPECTRUM OF PURE THEOBROMINE

Concentration of theobromine = 0.003%

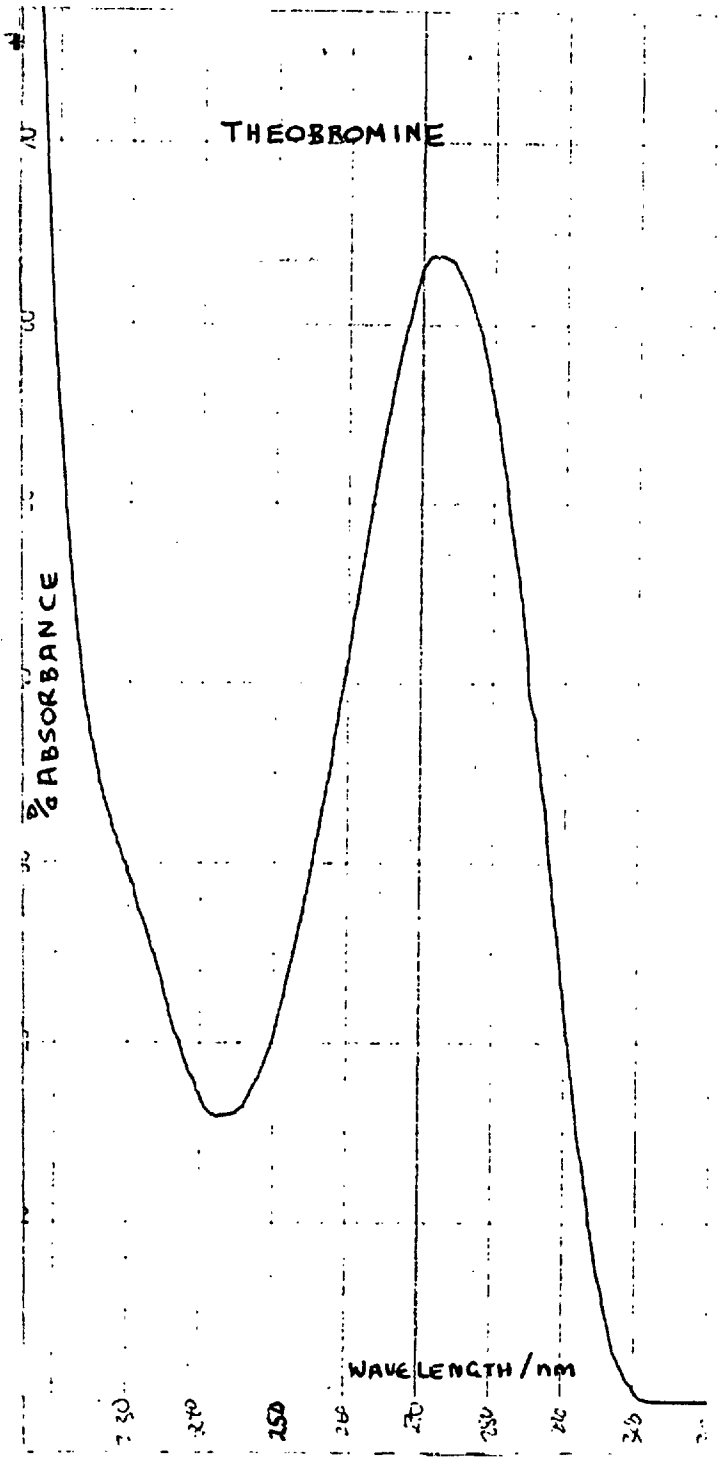
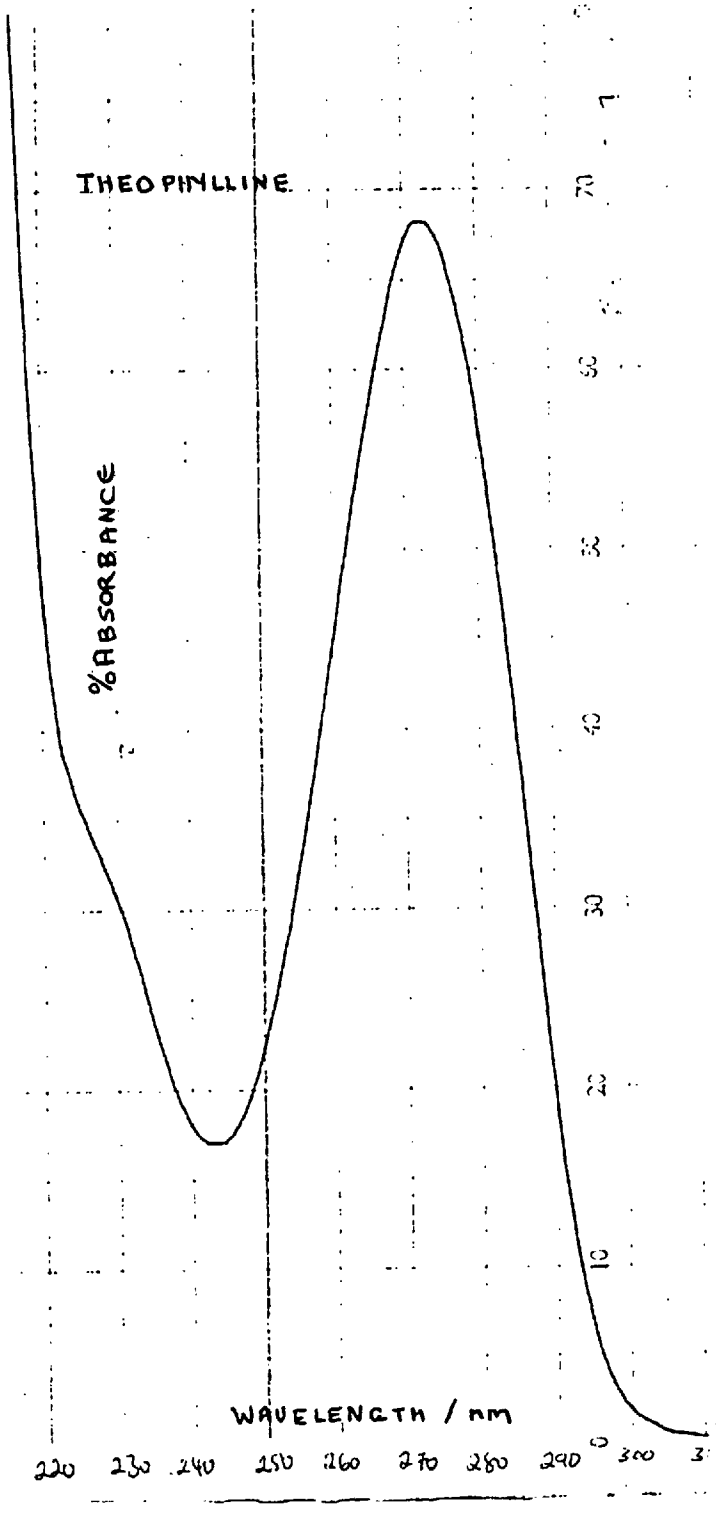


FIGURE 29

SPECTRUM OF PURE THEOPHYLLINE

Concentration of theophylline = 0.003%



DISCUSSION

I.0 THEAFLAVINS AND THEARUBIGINS

The value of f_2 for the calculation of %TR in the solution was taken as (0.02/0.733) in the present research, whereas Roberts and Smith put $f_2 = [(0.02/0.733) \times (375/9)]$. The factor 375/9 is a concentration factor (page 27) and because in the present research varying concentrations were used f_2 was taken only as (0.02/0.733), a constant for all the calculations of %TR in the solution. This value is arbitrary due to the incomplete knowledge of the chemistry and degree of hydration of the thearubigin complex. The concentration used by Roberts and Smith (i.e. 4.8g tea/200ml) was tried in one experiment at 95°C and here again f_2 for %TR was taken as (0.02/0.733) [see Table (5)]. %TR was found to be 0.323, which fitted on the straight line plot of I/%TR versus I/W for thearubigins at 95°C. The value of x_o (wt%) in the tea leaf for thearubigins was found to be I8.0 for Koonsong B.P. tea at 94°C which agrees well with the value of %TR in the tea leaf of I6.7 for Assam B.P. as found by Roberts and Smith method (I5).

In the case of theaflavins; the molar concentrations were determined in solution in the present research instead of the %TF as found by the Roberts and Smith method [cf. Chapter I, section I.I (c)]. x_o in wt% for theaflavins was found to be 0.I59 for Koonsong B.P. tea at 94°C as compared to the value of I.45 for Assam B.P. tea by the Roberts and Smith method. As the thearubigin content of a tea is always considerably higher than the theaflavin content, these results appear to be reasonable. Also diffe-

rent grades of tea have different theaflavin and thearubigin content as found in the present research with Assam B.C.P. and Koonsong B.P. tea.

ERRORS

Due to the experimental error of about ±3% in c(TF) and %TR in solution, there are uncertainties in the slopes and intercepts of the linear plots of I/c (or I/%TR) versus I/W for both theaflavins and thearubigins at all temperatures. This affects the derived quantities x_o, K and E. The equilibrium and kinetic parameters for theaflavins and thearubigins at 25°C are subject to further errors due to the formation of tea cream as mentioned earlier.

THE RATE OF EXTRACTION, ACTIVATION ENERGIES AND ENTHALPY CHANGES

From the results it appears that the rate of extraction of thearubigins is faster in most cases than that of theaflavins. For both the temperatures (79.5°C and 94°C) k'_I and k'_o for thearubigins are approx. 12% and 87% respectively higher than the corresponding parameters for theaflavins, k_oA at 79.5°C is about 40% higher and k_oA at 94.5°C is about 20% higher for thearubigins.

The effect on the rates of raising the temperature can be conveniently expressed by the Arrhenius equation:

$$k = A \exp(-E_A/RT) \qquad (a)$$

where k = rate constant

A = frequency (pre-exponential) factor

R = molar gas constant

T = absolute temperature

E_A = activation energy

Upon taking logarithms of equation (a):

$$\log_{10} k = \log_{10} A - E_A/2.303RT \qquad (b)$$

E_A can be evaluated from the slope of a linear plot of $\log_{10} k$ versus I/T. Alternatively E_A can be evaluated by taking the specific rates (k_1 and k_2) at temperatures T_1 and T_2 and inserting them in equation (b); if the two resulting expressions are subtracted from one another, then:

$$\log_{10}(k_2/k_1) = (E_A/2.303R)(T_2 - T_1)/T_1T_2$$

This is the equation which is used in the present research.

The activation energies for the infusion of theaflavins are almost all positive which is to be expected. The value for k_{-1} appears to be negative, but this is likely to be due to the experimental error. An uncertainty of ±3% in c leads to uncertainties in the derived quantities k'_{-1} and k_oAV/W which leads to a major percentage uncertainty in their small difference which equals k_{-1} [cf. equation (10a)]. The activation energies for the k_{-1} process are even more uncertain. For example, if 3% is added to the value of k_oA at 94°C this becomes 7.50×10^{-3} min^{-1} which changes the activation energy for k_oA to -0.70 KJ mol^{-1} and the activation energy for k_oAV/W to -0.50 KJ mol^{-1}. The apparent negative E_A values in Table (51) for thearubigins are therefore spurious. Both sets of activation energies (i.e. for TF and TR) are small because the rates of TF and TR do not increase very much on raising the temperature from 79.5°C to 94°C.

The enthalpy change of the reactions can be determined by the similar Van't Hoff equation:

$$\ln(k_2/k_1) = (\Delta H/R)(T_2 - T_1)/T_1T_2$$

where K_1, K_2 = partition coefficients at T_1 and T_2, respectively

ΔH = enthalpy change of the reaction

If the reaction is endothermic (absorption of heat), ΔH is positive and k increases with T, whereas if the reaction is exothermic (liberation of heat) ΔH is negative and k decreases with T. The enthalpy change for the infusion of theaflavins is positive which shows that the infusion process involves absorption of heat. For the infusion of thearubigins, however, ΔH is negative. This indicates that by raising the temperature one can extract more TF but less TR.

I.I CAFFEINE

The substance that is left after the chloroform extraction of caffeine and which shows some absorbance in the 273 nm region could possibly be due to theobromine and theophylline (Figs. 28 and 29). These materials are known to absorb in this region.

The wt% of caffeine in Koonsong B.P. tea was found to be 4.42 at 80°C and 4.58 at 94°C. These values agree well with Lehmann and Moran's results. For example they found the caffeine content of Twinings Lapsang Souchong tea and Windsor Castle Orange Pekoe tea to be 3.81% and 4.52% respectively at 90°C. Thus it can be said that the present results are reasonable as tea may contain upto 4% of caffeine in leaf (19).

The activation energies for the infusion of caffeine are all positive and small. Only for the k_{-I} step E is negative, and here experimental uncertainty (±3%) in the concentration value can account for this discrepancy. For example, if 3% is added to the k_{-I} value and 3% is subtracted from the value of k_oAV/W then k_{-I} would become 4.25×10^{-2} min^{-I}, 2.5 times the value reported in Table (52), which would make $E(k_{-I})$ positive. The activation energies are small because the rate of extraction of caffeine does not rise very much between 80°C and 94.5°C. This suggests that caffeine and the polyphenols could be extracted almost as quickly at 80°C as at 95°C or 100°C, and this has important implications for the manufacture of instant tea.

The enthalpy for caffeine infusion was found to be positive, so that the extraction of caffeine infusion involves absorption

of heat. Thus the total amount of caffeine that can be extracted does increase with rising temperature, another point to be borne in mind for instant tea manufacture.

Experiments with various stirring speeds appeared to show that the kinetics of the infusion of tea into distilled water are partly diffusion controlled. The method used here was simple and crude and hence from these results one could not arrive at a definite conclusion. Further research in this area should be carried out with horizontal rotating discs of tea leaves where more is known of the hydrodynamics. Diffusion control would also be consistent with the low activation energies obtained. According to the Stokes - Einstein equation:

$$D \propto T/\eta$$

where D = diffusion coefficient

T = absolute temperature

η = viscosity

η_1 at 353°K (T_I) = 0.3554 cpoise

η_2 at 367.5°K (T_2) = 0.2985 cpoise

$$\therefore D_I/D_2 = (T_2/\eta_2)/(T_I/\eta_1) = 1.24$$

The variation of the diffusion coefficient with temperature may be expressed by an Arrhenius - type equation:

$$\ln (D_2/D_I) = \frac{E_D}{R} \frac{(T_2 - T_I)}{T_I T_2}$$

where E_D = activation energy (for diffusion)

Hence E_D = 16.5 KJ mol^{-1}

This is of the same order of magnitude as the activation energies in Tables (50), (51) and (52).

TABLE 50

ACTIVATION ENERGIES AND ENTHALPY FOR INFUSION OF THEAFLAVINS

Activation energies are calculated from the rate constants for the infusion of theaflavins at 79.5°C and 94°C obtained in Tables (25) and (27). Enthalpy is calculated from the partition coefficients obtained in Tables (29) and (30).

$E(k'_{-I})$ /KJ mol^{-I}	$E(k'_o)$ /KJ mol^{-I}	$E(k_oA)$ /KJ mol^{-I}	$\underline{E(k_oAV)}_W$ /KJ mol^{-I}	$E(k_{-I})$ /KJ mol^{-I}	ΔH° /KJ mol^{-I}
+3.29	+8.89	+I7.08	+I7.08	-25.75	+42.9

TABLE 5I

ACTIVATION ENERGIES AND ENTHALPY FOR INFUSION OF THEARUBIGINS

Activation energies are calculated from the rate constants for the infusion of thearubigins at 79.5°C and 94°C obtained in Tables (26) and (28). Enthalpy is calculated from the partition coefficients obtained in Tables (29) and (30).

$E(k'_{-I})$ /KJ mol^{-I}	$E(k'_o)$ /KJ mol^{-I}	$E(k_oA)$ /KJ mol^{-I}	$\underline{E(k_oAV)}_W$ /KJ mol^{-I}	$E(k_{-I})$ /KJ mol^{-I}	ΔH° /KJ mol^{-I}
+4.58	+I0.99	-I.4I	-I.4I	+37.3	-39.I6

TABLE 52

ACTIVATION ENERGIES AND ENTHALPY FOR INFUSION OF CAFFEINE

Activation energies are calculated from the rate constants for the infusion of caffeine at 80°C and 94.5°C obtained in Tables (40) and (42). Enthalpy is calculated from the partition coefficients obtained in Tables (39) and (4I).

$E(k'_{-I})$ /KJ mol^{-I}	$E(k'_{o})$ /KJ mol^{-I}	$E(k_{o}A)$ /KJ mol^{-I}	$E(k_{o}AV_{W})$ /KJ mol^{-I}	$E(k_{-I})$ /KJ mol^{-I}	ΔH^{o} /KJ mol^{-I}
+I.55	+6.86	+4.30	+4.30	-46.0	+43.3

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