STUDIES RELATED TO THE DEGRADATION OF FOOD COLOURING MATTERS

by

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TO MY PARENTS

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CONTENTS

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Summary

A differential pulse polarographic method has been developed for the determination of ascorbic acid and food colours in the same solution. Aniline, sulphanilic acid and naphthionic acid were shown to be formed from particular food colours: visible spectrophotometric methods involving diazotization and coupling with N-1-naphthylethylene-diamine dihydrochloride were developed for monitoring their formation. The method is not subject to interference from the other amines formed from the cleavage of the azo bonds. Ammonia was shown to be present in the degraded solution and an indophenol method was developed for following its formation.

These methods have been used to monitor permitted food colouring matters and ascorbic acid during interaction in accelerated light and heat degradation studies in the presence and absence of EDTA. Heat degradation studies were also carried out in the absence of ascorbic acid.

EDTA was found not only to stabilise ascorbic acid but also to stabilise food colouring matters in the presence of ascorbic acid. Full yields of ammonia were obtained from the degradation of azo groups via sulphanilic acid and naphthionic acid during the light degradation studies. Interaction of food colours and ascorbic acid has also been shown to occur at room temperature in the dark and to yield ammonia and simple amines.

In the heat degradation studies (130°C) relatively low yields of amines and ammonia were obtained despite complete visible degradation of food colours. This indicated the formation of more complex nitrogen containing compounds.

EDTA was shown to stabilise acetamido groups from photolytic hydrolysis in several drug compounds as well as in the food colouring matters Red 2G and Black PN.

A rapid differential pulse polarographic method for the determination of food colours in boiled sweets directly without prior separation or extraction procedures has been developed.

A detector cell designed and constructed in our workshops and previously used with a glassy carbon electrode has been adapted very successfully for use with a sessile mercury drop electrode. The use of the system in flow injection analysis has been demonstrated by determining food colours in the reductive and oxidative modes. Ascorbic acid, sulphite and other species have also been determined. In the determination of ascorbic acid deoxygenation is not necessary.

VITAMIN C DETERMINATION

Vitamin C exists in food in two biologically available forms, L-ascorbic acid and its oxidation product dehydroascorbic acid. The latter undergoes mutarotation in aqueous solution - which is believed to be due to the opening of the lactone ring with the formation of 2, 3-dioxogulonic acid[1], a compound possessing no vitamin activity and present only in small amounts in food.

There are several methods available for the determination of vitamin C and they may be classified into the following main classes:-

1) Photometric methods
2) Biological methods
3) Oxidimetric methods
4) Polarographic and voltammetric methods
5) Chromatographic methods
6) Other methods.

Photometric methods

This method includes:- Nephelometric, Fluorimetric, Colorimetric, and Spectrophotometric methods as follows:-

a) Nephelometric method:

This method[2] depends on the ability of L-ascorbic acid to reduce silver nitrate to a colloidal silver solution which can be measured turbidimetrically. The interference from reducing substances and anions such as Cl^- and CN^- by giving turbidity with silver nitrate are the main limitations of this method.

b) Fluorimetric method:

Ascorbic acid can be determined indirectly by oxidation to dehydroascorbic acid which is then reacted with o-phenylenediamine forming a fluorescent quinoxaline which can be measured in a fluorimeter. This method shows excellent agreement when compared with the indophenol, N-bromosuccinimide and p-nitroaniline methods but substances having diketo groups such as pyruvic acid interfere with the development of L-ascorbic acid using this method.

c) Spectrophotometric methods:

In this method absorption of the solution containing L-ascorbic acid at 265 nm λ max is measured[4,5]. The maximum wavelength of absorption of L-ascorbic acid is pH[6,7] and temperature[8] dependent. Some workers[9-11] had found that L-ascorbic acid solution is largely destroyed when the solution is exposed to ultra violet irradiation and hence represents a great difficulty in determining the compound.

Recently[12] ascorbic acid was determined spectrophotometrically at 336 nm via a decrease in absorbance in 0.7 mM tetrachloro-p-benzoquinone (chloranil) in 80% acetone.

d) Colorimetric method:

(i) Indirect methods:

By the formation of hydrazones of dehydroascorbic acid. L-ascorbic acid must be oxidised to dehydroascorbic acid which is then reacted with the following reagents to develop a colour which can be measured colorimetrically:-

(1) p-Sulphophenyl hydrazine[13]. But this is not selective due to the interference from diketo-L-gulonic acid.

(2) 2, 4 - Dinitrophenylhydrazine:

This method was used for determination of dehydroascorbic acid and ascorbic acid by Herbert et al.[14] Roe & Keuther[15] used this method later for estimation of total ascorbic acid (dehydroascorbic acid and ascorbic acid) by measuring the red colour produced by the reaction in sulphuric acid at 510-540 nm[16-18]. Another degradation product of ascorbic acid,2,3 - diketogulonic acid,also reacts with the reagent[19].

ii) Direct methods:

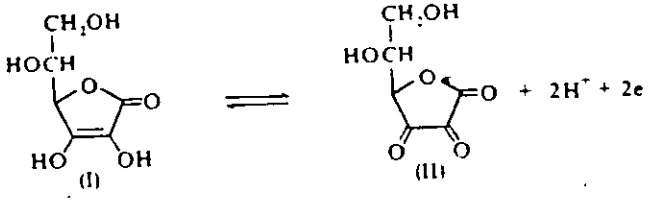
In this method many reagents react with L-ascorbic acid resulting in the forming a colour for which the intensity is concentration dependent. Examples of these are: Ammonium molybdate[20], Arsenotungstate[21], Arsenotungstomolybdate [22], Uranyl nitrate[23], Diazotized sulphanilamide[24], Diazotised p-nitro nitroaniline[25], ... etc. But these reagents cannot be considered specific for L-ascorbic acid.

Polarographic determinations:-

Ascorbic acid is one of the best known examples of the application of anodic polarography in pharmaceutical analysis and much information has appeared in the literature on the polarography of this compound and its related isomer erythrobic acid (isoascorbic or d-araboascorbic acid).

The polarographic technique was first successfully used by Kodicek and Wenig[26] and later other workers[27-34].

Brezina and Zuman[35] gave the details of conditions for the determination of the compound. They suggested that the analysis is best performed in buffer solutions from pH 2.0 - 7.0 to prevent chemical oxidation by traces of oxygen. (Ascorbic acid is readily oxidised and is widely used in pharmacy as an antioxidant to prevent the oxidation of other pharmacologically active compounds). A wide range of buffer systems has been employed. Further details of analytical methods for pharmaceutical preparations are provided by Heyrovosky and Zuman[36], who described the electrode process as the production of dehydroascorbic acid by oxidation.



The process is pH dependent, half wave potentials of +0.18V and -0.13V occurring at pH 1.8 and pH 9.0 respectively in Britton-Robinson-buffer. Dehydroascorbic acid is not reducible at the dropping mercury electrode[37]. The mechanistic aspects of the electro-oxidation of ascorbic acid using controlled potential techniques at the hanging mercury drop electrode by Perone et al.[39] obtained kinetic data on the electrode process. Malik and Singh[39] have developed an amperometric titration for vitamin C for analysing pharmaceuticals. The best titration was obtained at 0.2V in

Britton-Robinson buffer pH 5.2, potassium aquopentacyanoferrate(III) was used as titrant. Schubert and Roland[40] had determined L-ascorbic acid in oxalic acid at the dropping mercury electrode in acetate buffer. Interference was found at pH 5.5 from sulphydryl compounds. Later on other workers[41] have determined the compound in acetate buffer saturated with sodium oxalate at pH 5.5 using differential pulse polarography. Up to 25 ppm concentrations were studied and well-formed peaks obtained down to 0.2 ppm. The technique was also used to determine ascorbic acid in fruit juices and lemonades[42]. Another application[43] of DPP for determination of ascorbic acid was carried out indirectly by oxidation of ascorbic acid with a chloroform solution of iodine, the excess of which was then removed followed by polarographic measurement of the iodate formed after bromine oxidation of the resulting iodide. Several workers have determined vitamin C in various foods by polarographic oxidation. Kajita et al.[44] measured L-ascorbic acid in different foods. Interference were removed by extraction of complexes of o-phenylenediamine with chloroform before determination. Kajita and Senda[45] performed a simultaneous determination of L-ascorbic acid and erythrobic acid in alkaline extracts of canned foods. The two compounds give resolved waves at pH 9 to 11. Other workers[46] have determined L-ascorbic acid and L-dehydroascorbic acid in plant materials and preserved foods. Samples were diluted with 2% aqueous oxalic acid filtered and chromatographically separated from anthocyanins on a polyamide column prior to polarographic determination. Single sweep cathode ray polarography has been used to analyse ascorbic acid in a large range of dietary foods without prior separation. Mallela et al.[47] have estimated ascorbic acid amperometrically with

manganese (III) pyrophosphate and use of a rotating platinum electrode at 0.0V.

(3) Voltammetric study

In this technique the determination of ascorbic acid is carried out using a solid electrode instead of a dme. Cescon and Montalti[48] determined L-ascorbic acid in aerated aqueous solution at a vibrating platinum electrode. Lindquist[49] has determined ascorbic acid voltammetrically using a carbon paste electrode using cyclic and linear sweep voltametry. The half-wave potential was greater than at the dropping mercury electrode by about 0.2V. The peak current was proportional to ascorbic acid concentrations in the range $(10^{-6}$-$10^{-3})$M and the relative standard deviation was generally less than ± 1%. Chloride and sulphur compounds such as sulphides and thiols did not interfere and sulphite could be determined at the same time as the ascorbic acid. Although some substituted phenols interfered, these could be detected by reversing the direction of polarization. Reductones interfered but tin(II) and manganese(II) did not and a method was developed for determining ascorbic acid in the presence of an excess of iron. The method can be used for determination in fruits, vegetables and beverages. The peak height varied with scan rate and the peak potential varied giving a break corresponding to the pKa value of 4.5. The method was applied successfully to the determination of multivitamin tablet (Nutri-vitamin) which contains in addition to vitamin C, iron and pyridoxine. Mason et al.[50] have used a tubular carbon electrode to determine vitamin C in pharmaceutical dosage forms in the presence of other vitamins. The peak current was found to be proportional to

concentration in the range 10^{-6} - 10^{-3}M, and the relative standard deviation was less than 1%.

A graphite electrode was also used in the determination of vitamin C[51]. The oxidation of ascorbic acid at platinum electrodes was studied by Brezina et al.[52].

Oxidimetric determinations:

These methods of determination of ascorbic acid are based on the utilization of the oxidation-reduction behaviour of the vitamin and the titrant. Some of these methods are given below:-

a) Indophenol method:

In this method the reagent 2, 6-dichlorophenol-indophenol is used. It has been agreed that this blue dye provides the simplest and most rapid method for determining ascorbic acid. The method first used by Tillmans et al.[53-55] in 1928, involves acid extraction of the vitamin from the sample and titration of the extract with a standardised solution of the dye until a pink end point is reached. Due to the convenience of the method and the good correlation of the results obtained with biological methods of assay, the method has received intensive study to define the optimum conditions of assay and now the indophenol method has been adopted as an official method of determination of L-ascorbic acid in fruit and vegetable juices[56], biological materials[57] and in pharmaceutical preparations[58].

The main interference comes from sulphydryl compounds, phenols, sulphites and copper (I), iron (II) and tin (II), so the indophenol reactions must be carried out as quickly as

possible to eliminate the influence of slowly reducing substances[59]. The pH was important in the presence of certain organic compounds as it was reported that at about pH 4.0 glutathione, tannic acid, pyrogallol[60] and cysteine[61] reacted with the dye. However, the indophenol oxidation-reduction proved not to be specific for L-ascorbic acid because any substance that has a reduction potential lower than that of the dye could be a possible source of interference[59,62,63]. Modifications of the indophenol method were proposed for the vitamin determination in pharmaceutical preparations containing iron salts, allied substances[62] and sulphur dioxide[64]. Iron might be removed[65] or the estimation performed in the presence of metaphosphoric acid[66]. However, Chapman et al.[67] have reported that metaphosphoric acid in the presence of ferrous iron introduced a positive error in the titration and was pH dependent[68]. Other procedures were proposed whereby the reaction of the indophenol dye with L-ascorbic acid was rendered more selective[69,70].

b) Iodometric methods:

Iodometric determination of ascorbic acid is the official method in the United States Pharmacopeia XVIII (USP). In some iodometric methods[71] the titration was carried out with a standard iodine solution using starch as indicator. Other methods involved the use of potassium iodate as the oxidising agent for L-ascorbic acid determination in solutions[72], pharmaceutical preparations[73]. Very much later on[74,75] an iodometric method based on controlled potential colometry was developed.

c) Bromometric titration

The titration in this method is usually performed with potassium bromate[76] standard solution in 2.5 M hydrochloric acid medium

using ammonium phosphomolybdate as indicator. The bromometric method was found to be inaccurate for the determination of ascorbic acid in tablets[77].

d) Ferric chloride method:

In this method the iron is reduced by the action of L-ascorbic acid and is then determined colorimetrically by coupling either with a dipyridyl[78] or with a batho-phenanthroline[79].

e) Mercuric chloride:

This method is based on reduction of mercuric chloride to mercurous chloride by ascorbic acid[80], the method is specific. No interference from other substances present in black currant and other coloured fruit juice syrups was observed.

f) Indirect complexometric method:

A method was developed by Baczyk and Kachelska[81] in which ascorbic acid reduces cupric acetate. The product precipitate, which was equivalent to the amount of L-ascorbic acid present in the solution, was determined complexometrically with EDTA (disodium salt) using sodium salt of 2-(2-oxy-3, b-disulph-1, naphylazo) phenyl-arsenic acid (APANS) as indicator. However, this method is not selective because of the interference from other substances having a lower reduction potential than that of the reagent.

g) N-Bromo-succinimide method:

This method was developed by Barakat et al.[82]. The method is based on the property that N-Bromo-succinimide specifically reacted with L-ascorbic acid (1:1 stochiometry) without interference from its degradation products. Inorganic and organic sulphur-

containing compounds intereferred with the assay[83]. Later on other workers[84] improved this method by using acetone as complexing agent instead of formaldeyhde.

h) Methylene blue:

In this method first proposed by Martinin and Bonsignore[85,86], ascorbic acid is extracted with trichloro acetic acid solution and then titrated with a methylene blue standard solution in the presence of sodium citrate, bicarbonate and thiosulphate. Other workers[87] later found that this method is not accurate for the determination of the vitamin in hydrated vegetables.

i) Potassium hexacyanoferrate (III) in acid medium:

This method has been used to determine ascorbic acid successfully[88].

(4) Determination of ascorbic acid in biological fluids:-

An early method based on a biological assay used guinea pigs[89] as test animals. The amount of test material just sufficient to prevent scurvy was taken as an equivalent to 0.5-0.6 mg ascorbic acid. Besides this method, several other biological methods were developed. Harris et al.[57] described a simple curative method based on weight changes in guinea pigs during scurvy. A bio assay method based on the serum levels of alkaline phosphatase was reported[91]. General reviews of the biological methods are given by some workers[91,92]. The first degradation product of ascorbic acid, dehydroascorbic acid is biologically active. There are several methods of extraction of ascorbic acid from plant and animal products depending upon the product to be analysed[92]. After the proper extraction any one of the available methods is used for the determination of ascorbic acid. Very recently

Schenk et al.[93] developed a method for determination of brain ascorbate with ascorbate oxidase.

Chromatographic methods:

Chromatographic techniques provide a solution for the problem of interference encountered in the other technqiues. In this technique ascorbic acid could be separated and determined alone. The following chromatographic techniques have been widely used for quantitative and qualitative determination of ascorbic acid and various breakdown products both in model systems and in foods and other biological materials.

Column chromatography

An ion exchange resin, Zeo karb 215 has been used [94] in the assay of canned foods to separate L-ascorbic acid from ferrous iron in 0.25% oxalic acid solution. Column chromatography has been used for purification of the osazone formed in the reaction of dehydroascorbic acid with dinitro phenylhydrazine. Mapson[95] described the use of an alumina column for this purpose.

Thin-layer chromatography:

This technique proved to be useful because of the easy oxidation of ascorbic acid on the huge surface area of the absorbent layer[96]. Strohecker and Pies[97] used the technique to separate the osazone formed by the reaction of dehydroascorbic acid and 2,4-dinitro phenylhydrazine which was then eluted and measured colorimetrically.

Paper chromatography:

The separation of ascorbic acid from its oxidation products and related dienols by paper chromatography was tried and proved to be successful[98,99]. Another worker[100] has used paper

impregnated with ion exchange resins for separation of L-ascorbic acid. Shimi et al.[101] studied the zones strip technique for various compounds including ascorbic acid. They found that below a certain maximum concentration the length of the developed zone was proportional to the logarithm of the zone content.

Gas chromatography:

Ascorbic acid was determined as its trimethylsilylether derivative by gas chromatography[102].

Liquid chromatography with electrochemical detection:

A thin layer amperometric detector with a carbon paste electrode was used[103]. Improved sensitivity and selectivity was reported over classical titration and colorimetric redox procedures. This technique was applied to foodstuffs, pharmaceuticals and body fluids.

Electrometric methods:

Electrometric titration was used to overcome the possibilities of obscured end points in the 2,6 - dichlorophenol-indophenol visual titration. A considerable improvement was made using a platinum and mercury electrode pair[104]. Liebman et al.[105] successfully adopted in the titration the dead stop end point method, using polarised electrodes. By the use of electrodes of reduced surface area, improved precision was reported[106].

Determination of ascorbic acid using isotachophoresis

Isotachophoresis is an electrophoretic separation technique that allows quantification of the separate sample ions at nanomole levels by measuring the zone length formed in a capillary. This

technique has been used[107] to separate and quantitate a number of organic acids such as ascorbic acid, isoascorbic, citric, isocitric etc. in food.

Indirect potentiometric determination of ascorbic acid and other reductants using an iodide selective electrode:

In this method[108] the compounds can be determined under appropriate conditions by oxidation with a purified ethanolic solution of iodine and measurement of the iodide formed with an iodide selective electrode.

Determination of ascorbic acid plus dehydroascorbic acid:

The method developed by Roe and Kuther[15] is still widely used. In this procedure ascorbic acid is oxidised with active carbon to dehydroascorbic acid which is then condensed with 2, 4-dinitrophenyl-hydrazine at 37°C. The resultant osazone is dissolved in sulphuric acid giving a red colour which is measured spectrophotometrically at 510 to 540 nm. If 2,3-dioxogulonic acid is present it reacts and gives a great interference. Another method[3] is a fluorimetric procedure in which ascorbic acid is oxidised with active carbon to dehydroascorbic acid. The latter is then reacted with o-phenylenediamine to form a fluorescent quinoxaline compound. The selectivity of the method was improved by determining any fluorescence due to interfering substances after complexing dehydroascorbic acid with boric acid to prevent formation of the fluorescent vitamin compound. This method has been accepted by the Association of Official Analytical Chemists. Recently high performance liquid chromatography with spectrophotometric detection has been used to separate and quantitate ascorbic acid and dehydroascorbic acid[109]. A polarographic method for the

determination of ascorbic acid and dehydroascorbic acid in plant materials and preserved foods was developed[46].

Flow injection analysis:

Redox potential detection has been applied to the determination of reducing agents injected into a nonsegmented stream of cerium (IV) solution. Since ascorbic acid is readily oxidised by cerium (IV) it was selected as an example and solutions containing this compound alone were first analysed[110]. The working electrode used was a platinum or graphite electrode.

Ruzicka and Hansen [199] cite unpublished work by Ramsing in which a carbon paste thin layer electrode was used to determine ascorbic acid in the 0.4 to 10 mM concentration range.

Food Colour Analysis

(i) General Introduction

Previously in this laboratory a study was carried out on determining the extent of degradation of food colouring matters in light and heat[111]. For the light study an apparatus was used which provided a reasonably intense light environment, the light intensity being increased and made more uniform by the reflective inner wall of the apparatus. The temperature was substantially reduced around the sample solutions from 80°C to 30°C by the action of a draught generated by a fan at the bottom of the apparatus (fig 1).

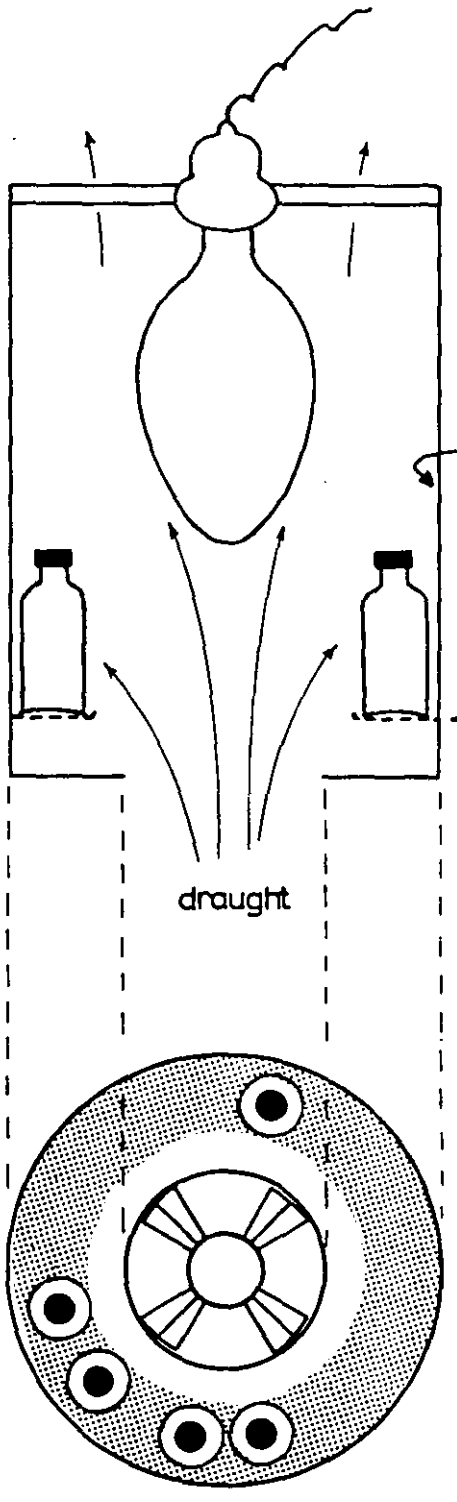
The apparatus contained a mesh shelf which raised the sample bottles above the base of the apparatus to allow a maximal air circulation around them and to position them suitably near the light source.

The heat degradation study was carried out using glass vials. The vials were sealed by a glass blower using a glass blower's torch, care being taken to prevent the wall thickness lessening during the sealing process by undue uneven flow of glass.

The solutions in the sealed vials were placed in the oven at preset 110°C or 130°C or 55°C. In the last case the solutions were held in autoclavable screw-top made from duran glass (neutral glass).

Decay curves were obtained using differential pulse polarography for both heat and light degraded solutions. Most of the thermal decay curves were found to be 1st order decay. In some cases it was found that more complex kinetics were involved in modifying this

Photodegradation apparatus



View from above showing placement of the sample bottles.

Scale 1 : 5

Fig. 1

simple picture. However the light degradation curves were found to be variable in shape depending on the specific colouring matter. For both heat and light decay curves it was found that the common practice of comparing stabilities on the basis of the ratio of the decay losses at a single time was not accurate,especially for some of the light degraded solutions.

Comparison of photostability taking account of the whole decay curve showed that amaranth, Black *PN*, Brilliant blue FCF, Patent Blue V, Red 2G, Patent Blue V, Sunset Yellow FCF and Tartrazine showed excellent stability, while those of carmoisine, Green S, Quinoline Yellow and Yellow 2G were found to be still very good.

Brown FK showed rapid initial fall-off, but the decay rate slowed, leaving the solution coloured pale brown even at high exposure times.

Of the rest ofthe colouring matters namely Chocolate Brown HT, and Ponceau 4R, the shape of the decay curves and overall decay periods, suggest that under normal lighting over intermediate storage periods,there might be little decay.

Indigo carmine and erythrosine showed a poor photostability. This has been reported previously in the literature.

In the case of thermal stability, Brilliant Blue FCF, Quinoline Yellow and Tartrazine were the most stable.

Erythrosine showed good stability in contrast to its photostability together with Sunset yellow FCF, Yellow 2G, Patent Blue V and Green S. Intermediate stability was found for Amaranth, Black PN, Chocolate Brown HT, Carmoisine and Brown FK. Ponceau 4R was degraded

rapidly but Indigo Carmine showed very poor stability as was the case in the light study. From the above study there is little evidence that the stability is related to the primary structure of the dyes. It may be possible that the Chocolate Brown HT, Black PN shown lower stability because both of them have two azo groups.

During the polarographic analysis new polarographic peaks appeared for some solutions as a result of thermal degradations. Among these were Indigo Carmine and Red 2G. The new peak in Red 2G solutions was identified by comparison with a standard as being due to Red 10B.

This was later confirmed by HPLC. The Red 2G peak was separated from the Red 10B by means of tetraphenylphosphonium chloride. This compound produced a shift in the $E_{\frac{1}{2}}$ values of respective colouring matters and hence increased the resolution of their polarographic peaks.

Thermally degraded solutions were separated by the HPLC technique using reversed phase ion-pair chromatography. Trials showed the superiority of a methanol/water/cetrimide eluent on an SAS-hypersil column over an isopropanol/water/acetic acid/cetrimide eluent on the same column. Phenol-4-sulphonic acid, sulphanilic acid, sodium naphthionate and sodium naphol-4-sulphonate were possible thermal decay products for a range of azo type colouring matters.

(ii) Analysis of Food Colouring Matters:-

Introduction

The practice of colouring food has been known since early times. Originally the colouring matters came from vegetable or animal sources.

The relation between the colour of the food and its flavour is very good. Usually when some people are presented with fruit jellies, for example where the colours are not consistent with the flavour e.g. an orange flavour but a green coloured jelly, their perception of the flavour almost will be determined by the colour of the jelly and not by the actual flavour.

The visual appearance of foods to which colour makes an important contribution is a very important factor in the consumer's evaluation of the product.

The reasons for the addition of food colours are to:

(1) Ensure the natural colour of foods which would otherwise have little or no colour e.g. fruit yogurt, ice cream or soft drinks.

(2) Ensure batch to batch uniformity of colour where raw materials of different source and varying colour intensity have been used.

(3) Restore or maintain the appearance of the processed foods where natural colours have been destroyed by processing (chemicals e.g. SO_2, heat or by storage (light)).

(4) Gives colour to processed foods which would otherwise be without colour such as boiled sweets. A great amount of the foods consumed by the developed countries of the world is subjected to some kind of processing or baking before reaching

the consumer and hence the addition of food colours is necessary.

The Food Additives Contaminants Committee (FACC) in their Interim Report on the review of the colouring matters in food regulations[112] (1973) have determined the levels of food colours consumed in the U.K.

Although the addition of food colours does not affect the nutritional value of the food it makes the food more attractive to the consumer. However, there is mounting evidence that some colours in use are unsafe,[112] and strong pressure has been exerted on the industry to use as small amount as possible of synthetic colours. Considerable attention has been directed towards the toxicological evaluation of food colours whether they are of synthetic or natural origin[113].

There is a big difference between countries on the colouring matters they permit. The E.E.C. countries' list - for instance - differs from that of the U.S.A. A notable difference is the dismissal of amaranth from the U.S.A. and Russian list of permitted colour while it still appears in the E.E.C. countries' list. When the U.K. joined the E.E.C. there were eight synthetic colours permitted in the U.K. which were absent from the E.E.C. list, while four colours banned in the U.K. were permitted in other E.E.C. countries[112].

Now both E.E.C. and the U.K. lists have been amended to rectify these discrepancies and to admit more recent toxicological data.

The U.K. amendments comprised:

(1) The colouring matter in food (amendment).
Regulations 1975 which deleted orange RN.

(2) The colouring matters in food (amendment).
Regulations 1976, which deleted five colouring matters.

The colours which may be added to food are natural, nature-identical (synthetic) or artificial and inorganic.

The natural colours originate from vegetables spices and insects. These colours tend to have low tinctorial powers when compared to the synthetic dyes, high cost in use, are unstable and have fastness deficiencies.

There is a limited range of synthetic (nature-identical) colours available. Synthetic dyes are available in a wide range of colours, they have high tinctorial power and at the dosage levels used are cheap. Some of them are unstable towards light, high temperature and to chemical agents such as acids or alkalis especially in the presence of metals or at elevated temperatures. The problem with artificial colours is that they are affected by reducing agents which can result in complete loss of colour. The presence of ascorbic acid and sulphur dioxide could therefore cause fading. It is usual to add these synthetic colours as late as possible in food processing. Food colour lakes are also used; they are prepared by precipitating the aluminium salt of the colour on to aluminium hydrate. Vegetable carbon, titanium oxide and iron oxides and hydroxide are used to colour foods.

Determination of food colouring by colorimetry

Naturally visible spectrophotometry (colorimetry) provides a useful method for determining food colours. A considerable effort has been made to develop colour scales that correlate well with visual grading or pigment concentration and to the development of models to predict product colour after processing[114]. Most fruits and vegetables have been subjected to an investigation of their

colour properties. Francis and Clydesdale[115] have reviewed the application of colorimetry to most of the food colours including tomatoes and tomato products, green vegetables, citrus products, potato products, cereal products, meat colour, sugar beers, and wine, tea and coffee. Kramer[116] has discussed the application of colorimetry to quality control.

Many studies relating pigment concentration to colour have been made in particular with reference to the use of synthetic dyes. The colour of any material is dependent on several factors, the concentration of pigments, the nature and particle size, and as a result of these factors light scattering also may be important[117-119]. The disadvantages of the light scattering is that it alters considerably the observed colour of the food and also it causes problems in the measurement of colour.

The Present Synthetic Colour Classes:

The synthetic food colours permitted in the E.E.C. countries and in the U.S.A. contain many different chromophore systems but always with an aromatic system incorporated in the food colour. The azo group is the most common chromophore the food colours being predominantly either red or yellow. Black, brown, green and blue dyes are based on triphenylmethane. The latter are brilliant colours with high tinctorial powers but they are unstable towards light.

Erythrosine with a xanthenechromophore system, Quinoline yellow with a quinophthalone structure and finally Indigo Carmine with an Indigoid group are also permitted. The structures are given in table (3).

The water solubility of the dyes is mainly because of the presence of sulphonic acid groups on the aromatic rings, the dyes being isolated as their sodium salts.

Synthetic food dyes are frequently known by trade names which do not give the actual chemical structure of the compound.

There are several classifications for the food colours in use. In the U.S.A. the permitted food colours are refered to as FD and C colours. The Colour Index (CI) Numbers of the Society of Dyers and Colourists and the American Association of Textile Chemists and Colourists are also used. Meanwhile in EEC countries in their directive of 23.10.62 (as amended) they allowed E reference number to be used[120].

Specification and purity of commercial food colours:

In most countries there are specifications of purity for each single permitted food colour not only a list of permitted colours. In the U.K. the specifications of purity prescribed in the current colouring matters in food regulations are for the most part those included in the E.E.C. Directive on Colouring Matters[120]. The FACC[112] in their recent report consider these specifications are far from adequate in that they contain no minimum limit for the total content of the dye, and only a little indication has been given to the nature and levels of subsidiary dyes, residues of starting materials intermediates or other contaminants. The FACC have recommended that colour specifications should normally consist of a minimum colour and maximum impurity levels as follows:

(a) The content of subsidiary dyes

(b) Sodium chloride and sulphate

(c) Unchanged intermediates

(d) Breakdown products

(e) Free aromatic amine

(f) Trace metal including arsenic, lead and in some cases cadmium, chromium and mercury

(g) Volatile matter

(h) Water insoluble matter, diethylether extractable.

The FDA already adopt similar specifications for food colours in the United States.

The analysis of synthetic colours is the subject of a monograph edited by Venkataraman[121]. Considerable attention has been given to the analysis of dyes for uncombined intermediates. Synthetic food colour reaction intermediates are highly ionic materials with several sulphonic acid groups per molecule.

HPLC is one of the techniques used to determine and to analyse highly polar components but the use of normal phase adsorption method is complicated by high solute retention coupled with poor peak shape and resolution. Reversed phase chromatography is also not very useful because of insufficient polar stationary phases for the dyes.

Strong anion exchange columns have been successfully used by many workers[122-127]. Another successful approach is ion chromatography in which small amounts of suitable salts are added to the mobile phase, the salts being chosen as to generate large ions of opposite charge to the molecule examined which can then form reversible ion pair complexes, a technique developed by Persson[128] and Eksborg[129]. A critical review of the use of this technique has been given by Tomlinson[130] Passarelli and Jacobs[131] analysed azo, anthraquinone and sulphonated dyes using HPLC adsoprtion, and ion exchange chromatographic methods with U.V. detection which gave precision and sensitivity equivalent to that of conductivity detectors.

Using tetrabutyl ammonium hydroxide, tetraethylammonium hydroxide and tridecylamine as the sources of counter ions Withmer et al.[134] analysed tartrazine and its intermediates by reversed phase liquid chromatography.

Knox and Laird[133] used a detergent (cetyltrimethylammonium hydroxide) as the counter ion for determining dye stuff intermediates. The column packing may either be a reversed-phase material e.g. SAS silica or silica gel, e.g. Partisil. When detergents such as cetrimide are used the technique is termed soap chromatography. Gas liquid chromatography coupled with mass spectrometry has been used to determine α and β naphthylamines in amaranth[134]. In general this technique is not widely used because the dyes themselves have high molecular weight, low vapour pressure and limited thermal stability.

Techniques for identification of food colours:

Usually the food colours are determined and identified after dissolution or extraction from solid samples.

Liquid samples can be determined by spectrophotometric techniques, Brown et al.[135] have suggested the use of Laser Raman Spectroscopy for identification of food colours in beverages, FD and C Red 40 was detected in cherry juice and cherry soda without prior purification. Recently differential pulse polarography has been used to identify and quantitively determine sunset yellow FcF, tartrazine, green S and amaranth in soft drinks[136]. The measurement of visible spectra could be used to determine aqueous extracts of food colour. The method has a serious problem due to formation of turbid solutions in most cases. Colouring matter in meat products can be determined by extracting the dyes into acidic, neutral or alkaline solution and recording the spectra[137].

Identification of the dyes was then done by comparing the recorded spectra with spectra of known dyes for bathochromic and hypochromic shifts with pH.

Techniques for separation of food colours

In most techniques involved when the extract contains a mixture of colour the components should be separated before any identification takes place. The techniques mostly used are paper chromatography[138,139] or thin layer chromatography[140-143] and to some extent ion-exchange chromatography[144] and more recently high performance liquid chromatography[145]. After the separation the colours may be identified by absorption spectra and by comparing their R_F values, etc in various solvent systems.

Hoodless et al.[143] using TLC devised a scheme for the rapid identification of 49 synthetic dyes. R_F and R_X values are tabulated, where R_X is the mobility relative of orange G.

Four cellulose coated TLC plates are spotted with the unknown colour mixtures, amaranth and orange G and then developed in four separate solvent systems. The positions of the unknown colours relative to the two standards are used to give an initial identification of the dyes.

Various TLC separations of water soluble food dyes on silica gel layers have been critically reviewed by Schweppe[146] for LTC Cellulose[148], silica gel[149-150] and polyamide[151-153] have been used as adsorbents. Gilhooley et al.[154][156], have developed a method of extraction of synthetic water-soluble food colours using polyamide columns.

But TLC and paper-chromatography are in general slow techniques and sometimes not accurate in the identification of the food colours.

HPLC therefore is more accurate and faster. The soap chromatography technique has been used successfully[145]. Most of the permitted food colour in U.K. and many other non-permitted colours could be separated using a single-column system. The system used in this procedure was 12 cm column packed with 4.6 μm SAS Hypersil eluted isocratically with iso-propanol:water:certimide:glacial acitic acid, 41:59:0.25:0.25 (v/v/w/v).

Extraction and purification of food colours:

The extraction and purification process are associated with each other. The extract should be purified from any possible interfering co-extractive materials. Several methods for the extraction and purification of food colours have been described, including extraction on wool from acidic solution[142], extraction with quinoline[155], column chromatography[156], use of liquid ion[157-158] exchanger, solvent extraction,[158], use quaternary ammonium compounds[159] and adsorption colours on to polyamide powders[160]. One of the most widely used methods is the wool-dyeing[142]. In this method usually white knitting wool is added to a hot acidic suspension of the food until the colour has been removed. After washing the wool with cold water the colour is stripped with an ammonia solution. The dyes are taken up very slowly by the wool so this technique is useful only for qualitative study. The AOAC method[158] uses a liquid anion-exchange resin to extract the dyes. The food is first finely ground with celite and one of the three aqueous phases. The mixture is then packed into a column, the fats and oil-soluble colours removed by elution with

the liquid anion-exchange resin in hexane and butanol. Eventually the colours are extracted from the resin into ether. The choice of aqueous phase used whilst grinding the food is important if maximum recovery of the colour is to be achieved. Three ways are described.

(1) HCl (0.1M) is used if aluminium lakes colours are present. This is normally indicated by the slow extraction of the colour.

(2) Using dilute acetic acid. This is suitable for most colours except FD and C Blue No 2 (Indigo Carmine) which fades in acidic medium and erythrosine which is not eluted quantitatively.

(3) pH (7.5) buffer for use with Indigo Carmine and Erythrosine.

Graichen[157] has found that the recovery of colours from biscuits and cakes containing more than 4 dyes was less than 90% and the use of an unsuitable aqueous phase when colours were present as aluminium lakes resulted in a substantial decrease in their recovery.

Another author[160] has devised a procedure for extracting and purifying synthetic colours from foods for acid dyes. The dyes are dissolved in ammonical alcohol, then after acidification adsorbed on to polyamide powder as a slurry which is then transferred into a column. The proteins, sugars etc are washed from the column and the dyes can be eluted with ammoniacal alcohol. This method was improved further by another worker[154]. The new method suggests defatting the dyes and then pouring the solution through a short polyamide column. The coextracted materials were washed away, and then the dyes eluted by methanol ammonia solution. Several solvent systems were used for elution. It was found that the more basic the solvent, the better the recovery was of the colour but with

more increase in the coextractive concentrations. The addition of surfactant, polyoxyethylene sorbitan, helped to prevent clogging of the polyamide column.

More recently Boley et al.[161] have proposed the use of enzymes as releasing agents for food colours from food constituents to ensure complete extraction. They used papin and a lipase and many samples of plain cakes, sponge cake and kippers were analysed. Results showed enhanced recovery of synthetic dyes following the enzymatic pre-digestion. Further work on this subject was carried out[162], and a method is described for the extraction, separation, identification and quantitative measurement of synthetic dyes. Synthetic dyes are well known for their ability to bind with proteins. Because of this property the proteins can be determined by their binding with the dyes. If the protein is hydrolysed however, the ability to bind the dyes with smaller protein molecules disappears.

Providing that the clear extracts contain only one single colour, it is possible to determine quantitatively the colour by measuring its UV-visible spectrum and comparing its absorbance at one wave length to a Beer's Law Calibration Curve for that wave length. If the extract contains more than one colour the case becomes difficult. However,if the colours are known it is possible to determine their concentration. Certainly this is a good method to determine concentrations of different colours directly from one spectrum without any need to go through time-consuming separation stages. The absorbance of a mixture of colours,if there is no interaction between them,is the sum of the absorbance of the individual colours in solution. Therefore,by considering the absorbance of a mixture

with x compounds at x different wavelengths to find a linear equation from which any unknown concentrations could be determined if the absorptivities at all wavelengths are known[163]. Other workers[164] have proposed a procedure for determining the concentration of synthetic dyes in a mixture, by curve-fitting the visible spectrum of the dye mixture with a predicted curve.

Natural colours:

The interest in the naturally occurring colouring matters has increased due to the fact that there is a great doubt about the safety of the present synthetic colours in use. These natural colours include flavonoids, cartenoids, porphorins, melanoidins, quinnoids, and betanines. Some examples of these pigments are permitted, but the stabilities of these colours generally are very low when compared with synthetic ones, also the natural colours are not necessarily safer than the synthetic colours.

It is therefore strongly recommended that more attention and greater understanding of the stability and fate of colours during the processing is required.

Legislation on colours:

From 1925 onwards, toxicological evidence mounted that some synthetic colours are possibly hazardous to health if used in food. Under the Public Health (preservatives etc. in food) Regulations 1925 some colours were banned from use in food due to their health hazards. These include compounds and lists of metals such as antimony, lead, mercury, zinc, etc. and five "coal-tar" colours such as picric acid, victoria yellow etc.

In 1954 the Food Standards Committee of the Ministry of Food produced a report[165] in which the committee recommended:

(1) Notification of the presence in foods of added colour should be given to the buyer.

(2) Published Health Regulations should be amended so as to permit a list of specified colours only.

(3) The permitted list should be reviewed within five years to examine any new information.

(4) Any colour-except for marking purposes should be banned from milk, or meat, game, fish, fruit and raw or unprocessed vegetables (in bread colours already banned). The ban also includes baby and infant foods.

In 1957 a permitted list of colours was proposed[166] and some specifications for the purity of the permitted colours were prescribed[167].

In 1973 a review of the regulations was published by the Food Additives and Contaminants Committee on behalf of the Ministry of Agriculture, Fisheries and Food (MAFF)[168]. In this review further restriction of the use of colours in food was recommended. The final report will indicate the kind and levels of further restriction. In the U.S.A. the first national law was passed in 1906, much later than the UK laws of 1860 and 1875. This delay was mainly due to conflict between federal authority and the assumed right of each state to conduct their own food and drug control laws[169]. The 1906 law listed seven synthetic colours for food use, two of which (Erythrosine, Indigo Carmine) remain on the permitted list until today.

In January 1963 the provisional listing was due to terminate to allow the necessary toxicological tests to be carried out. Since the sixties several colours have been deleted or restricted in their use.

The rest of the world countries followed either that in the U.K. or U.S.A. The countries that used to be part of the British Empire follow the U.K. system while the countries in the U.S.A's sphere of trade influence tended to follow the U.S.A. system. In Scandinavia, however, there has been action to ban the synthetic colours all together.

Toxicological data on food colours:

Over the past two decades the food colours were subjected to extensive evaluation by many experts[3]. The first review of the safety of food colours was carried out in the early sixties by the World Health Organisation and the Food and Agriculture Organisation[170], in which it was admitted that several food colours were consumed with little understanding of their effect on health. Some of the problems faced by experts in their evaluation of the colour is the instability of food colours. Many azo colours in the synthetic dyes are unstable under variable conditions including reduction and oxidation by another chemical agent. Storage and heating cause fading, leading to decolourisation and the formation of a coloured product whose toxicity is not understood[171].

Indigo carmine which is known to be unstable when stored in aqueous solution is readily converted to isatin 5-sulphonic acid and then to 5-sulphoanthranilic acid which is not clearly known from the toxicological point of view[172]. Erythrosine BS (tetraiodofluorescein) is regarded as adventitious source of iodine[173], and it has been suggested that

iodine supplementation in the diet may cause thyrotoxicosis, it is likely that this colour's use will be restricted. The situation is even worse in the natural colours because of poor stability, and their decomposition during the storage time may produce toxic impurities[174]. As far as the specification is concerned, the EEC specification for instance is far from satisfactory. It contains no minimum limit for total of dye, and gives little indication of the nature and levels of subsidiary dyes, residues of starting substrates, intermediates or other contaminants[2]. Other compounds such as Brown FK contain more than one component (it contains six compounds), and two of these components are believed to be mutagenic in an Ames type[175] assay system. As a precautionary measure the U.S.A. has banned orange B on the assumption that non-detectable amounts of β-naphthylamine which is carcinogenic is still associated with the commercial sample of this colour[176]. The case in the natural colour is the same. Many natural colours contain more than one, and in most cases many coloured together with non-coloured components. Furthermore the composition varies according to the source and the methods of preparation. Ponceau 3R is no longer permitted in EEC countries after it was reported that it caused cancer in Wistar rats. Of about 20 long-term studies on amaranth only two of them have indicated clearly the carcenogenic effect and these two have been described as inadequate by other experts[177]. This illustrates the difficulties facing the experts in their investigations. However the alternative colour to amaranth in the U.S.A. is Allura Red AC which on the evidence of long-term studies it appears that the possibility of a relationship between exposure to Allura Red and tumour acceleration in mice cannot be ruled out.

Sudan I (1-phenyl azo-2-naphtyol) often quoted to be carcinogenic in the mouse but not in the rat has *a close* structural analogy with sunset yellow FCF which is permitted in most countries. In cases of triphenylmethane dyes some restriction was imposed on their use by some health authorities in the sixties following alleged carcinogenicity of some tested compounds. This led to the removal of Brilliant Blue FCF from the EEC and U.K. lists. But later it was found that the concentration but not the dose of an injected compound determined the tissue response, and the dye was reintroduced.

Metabolic Data:

A major problem in animal feeding studies is the difficulty in extrapolating findings in animals to man and the variability of response of individuals makes the determination of the safe level of exposure for a carcinogen impossible. A great number of food colours are biodegraded in the body and its metabolic products are responsible for effects observed in animals. The best example is the three dyes orange G, orange RN, Red 10B which are not permitted together with Red 2G which has been considered acceptable for low daily intake.

A single metabolite, aniline (excreted largely as p-aminophenol) is mainly responsible for the disturbance of the integrity of red cells and effects may include methaemoglobinameia and the production of "Heinz bodies". Strangely enough the three compounds were excluded from the permitted list in the U.K. not because of its effects on the blood, but for other reasons. Orange RN for instance was banned for its toxic effect on the liver of a sensitive species.

Degradation of food colours:

There are several studies of food colours degradation in the

literature[178-193]. These studies were carried out in the presence or absence of chemical agents under light, storage or heat conditions. Another type of degradation study was the investigation of the stability of food colouring matters during residence in the body and the effects of metabolic degradation. The latter study is not the subject of this section, but a brief mention of the study will be provided.

The degradation studies were either quantative or qualative. Some references have identified the products, others have not. Most of the references dealt with stability towards light, storage or heat conditions. Fewer references discussed the degradation product from non azo colours such as Erythrosine, Indigo Carmine and Green S. Some have anticipated that the azo group will cleave yielding simple amines but no quantative determination has been undertaken together with time effect.

The heat degradation studies of thirteen food colours at pH 3.0 and pH 7.0 together with the effect of ascorbic acid has been studied by Nursten and Williams[178]. The heat study was carried out at 121°C for 20 min using (83-25) ppm, and the results were:

(1) Effect of ascorbic acid:

(a) At pH 7.0 in the presence of 1000 ppm, the loss was less than 5% for Amaranth, Erythrosine, Green S, Ponceau 4R, Sunset Yellow FcF, Tartrazine, Yellow 2G. The loss was (5.2%) Red 2G, (5.4%) Indigo Carmine, (11%) Brown FK, (18%) Chocolate Brown HT (λ max shift), (35%) Carmosine, and 90% Black PN.

(b) At pH 3.0 the loss was greater than 5% except for Green S,

Tartrazine, Yellow 2G.

(2) In absence of ascorbic acid:

(a) PH (7.0): The loss was less than 5% for Amaranth, Brown FK, Black PN, Carmosine, Erythrosine, Red 2G, Tartrazine and Sunset Yellow and more than 5% for the rest of the food colours.

(b) At pH (3.0): The results are the same but Green S, and Yellow 2G give a loss of less than 5%. Jouffery[179] has conducted a similar study into heat and light effects in aqueous solution. This is summarised in table (1) using (1 → 7 scale). See overleaf.

Table (1)

Food Colour	Degree of Stability			
	Light	Heat	Oxidation Media	Reduction Media
Indigo Carmine	Inadequate	-	Unstable	Unstable
Erythrosine	Poor	Good	Little stability	Very little
Amaranth	Moderate	Good	Limited Stability	Unstable
Black PN	Good	Good	Variable sensitivity	Variable
Carmosine	Good	Good	Stable	Stable
Quinoline Yellow	Good	Good	Slight modification	Stable
Patent Blue V	Good	Good	Turns green	Yellow
Sunset Yellow FcF	Good	Good	Very light	Unstable
Tartrazine	Good	Good	Doubtful	Discoloured
Ponceau 4R	Good	Good	Weak	Unstable

Summary of the degradation studies available is given in table (2).

Table (2) Effect of diffuse and natural light.

Ref No	The Author	Dyes Studied	Result
180	Helou	Amaranth	Poor
181,182	Goodhart & Peacock	Amaranth	Moderate
179,183	Jouffery & Spiess	Amaranth	Adequate
179,183	Jouffery & Spiess	Black PN	Good
180	Helou	Brilliant Blue	Poor but good at pH 2.0
184	Cooper	Brilliant Blue	Good
182	Peacock	Brilliant Blue	Moderate
179,183	Jouffery & Spiess	Carmosine	Moderate
179	Jouffery	Erythrosine	Poor
185	Jodlbaur & Tapper	Erythrosine	Develop Acidity
186	Evenson	Erythrosine	Unstable
179	Jouffery	Indigo Carmine	Unstable
179,183	Jouffery & Spiess	Patent Blue V	Good
179	Jouffery	Ponceau 4R	Good
179,183	Jouffery & Spiess	Quinoline Yellow	Good
187	Porter	Red 2G	Good
182, 179, 183	Peacock, Jouffery & Spiess	Sunset Yellow	Good
182, 179, 183	Peacock, Jouffery & Spiess.	Tartrazine	Good

Heat degradation

Ref No	Author	Dyes Studied	Result
179	Jouffery	Amaranth	Good
179	Jouffery	Black PN	Good
188	Swarts et al.	Brilliant Blue FcF	Unstable at high temp
178	Nursten & Williams	Chocolate Brown HT	Unstable
179	Jouffery	Erythrosine	Good
178	Nursten & Williams	Green S	Good
178	Nursten & Williams	Red 2G	Good
189	Nursten & Williams	Sunset Yellow FcF	Good
189	Luck	Tartrazine	Good

Storage Stability

Ref No	Author	Dyes Studied	Result
180	Helou	Amaranth	Average
190	Banerjee et al.	Erythrosine	Poor
180	Helou	Indigo Carmine	Poor at pH 2.0
180	Helou	Sunset Yellow	Good
180	Helou	Tartrazine	Good

Stability of food colours during residence in the body

Various workers[191-192] have found that in "animal experiments" reduction of the azo group of food colours, e.g. amaranth, by azo reductase enzyme in the liver, is of minor significance, and that microorganisms in the intestines, such as enterococci and coli bacteria, account for most of the reductive action. Koether[193] describes this for carmoisine, amaranth and ponceau 4R (etc) and mentions the presence of naphthionic acid as a degradation product. Amaranth appears to be almost completely reductively cleaved in the small intestine.

Flow Injection Analysis:

It had generally been thought that the air segmented continuous flow analysis technique, introduced in 1957 by Skeggs[194] was unchangeable and that the air segmentation was essential for this technique to be carried out. This was due to the usefulness of the air segmentation in promoting mixing of the sample with reagents and in limiting sample dispersion. The technique has some potential disadvantages.

1) Air bubbles may cause the stream to pulsate rather than flow regularly.
2) The air bubbles are introduced and then have to be removed prior to the determination.
3) Reagent consumption is generally higher than for discrete analysers.

Most of the early work on unsegmented flow systemswas done on electrochemical systems by Nagy et al.[195]. Based on this principle a new concept of continuous flow analysis was finally introduced by Ruzicka and Hansen[196] and later on by Stewart et al.[197,198]. Ruzicka and Hansen termed the technique Flow Injection Analysis (FIA).

The main modification was the use of a flow-induced sample dispersion as the sample in the carrier stream was pumped through narrow bore tubing. This mechanism was used to effect controlled mixing of the stream as opposed to the gross mixing of a mechanically stirred chamber. This reduces excessive sample dilution. Another advantage of using this technqiue is that the response curves do not reach the steady-state plateau observed in the air segmentation technique but have the form of very sharp peaks. There is no need to remove air bubbles, and there is no sample carry over.

The principles and applications of flow injection analysis have been described in detail by Ruzicka and Hansen[199-201] and Betteridge[202].

Basic Principles

Flow injection analysis is based on the injection of a sample into a continuously moving, non-segmented carrier stream, propelled by a peristaltic pump. The injected sample forms a well defined zone which is then mixed with the carrier stream and the reagent dispersion and diffusion controlled processes to form a species which can be determined in a flow through detector.

The concept of flow injection analysis can be explained from its three principles:-

1) Sample injection
2) Reproducible timing
3) Controlled dispersion.

Sample injection has to be achieved without disturbing the movement of the stream flow and in such a way that the precision of reproducible injection is high.

Originally this was done by using a syringe and a simple flap valve[196,203-204] but later these were replaced by a rotary valve[199] similar to that used in HPLC technique, and a multi injection valve. This resulted in a higher reproducibility of sample introduction, a higher sampling rate and a lower sample consumption. A highly reproducible timing of the sample in the system is required and this can be readily achieved in the absence of air as the stream is totally non-compressible. Since the sample does not pass through the pump

on its way to the detector, its path through the system is well defined and the dispersion of the sample zone and the resident time can be chosen to suit the requirements. The controlled dispersion of the sample zone which occurs during its passage through the system toward the detector results in a response curve which has a peak shape characteristic of flow injection system.

As the sample zone moves down stream it broadens and changes from its original symmetrical shape to a more gaussian form. By changing the parameters, such as the radius of the tube, the flow rate, the time of analysis and the length of the delay coil, the dispersion can be manipulated easily to suit the requirements of a particular analytical method so that the optimum reponse is obtained at minimum time and reagent.

According to Ruzicka and Hansen[199] the dispersion types fall into three categories, limited, medium and large.

In limited dispersion, the injected sample remains substantially undiluted by the surrounding carrier stream. This type of dispersion is used in electrochemical techniques. One example of the use of limited dispersion is in the potentiometric determination of calcium activity[205]. In medium dispersion, the commonest one, one or more reagents are mixed with the sample solution in order to form a coloured, fluorescent, electroactive or other product which can be detected by a flow through the detector[200,206].

The requirements of this type of dispersion are that the reactants must be mixed sufficiently and allowed enough time to react before reaching the detector. Because of the reproducibility of FIA, the reaction need not be completed but must react sufficiently

for the product(s) to be detected.

Originally the system used was very simple in that the reactant was injected into a reagent stream, given time to react and the products formed were detected. However systems have been developed since then which allow the sequential addition of reagents at different parts of the flow system without upsetting the flow[200]. Large dispersion can be used in two ways, either to obtain a suitable small dilution or to produce a concentration gradient which extends over a well defined period of time. The second use is in the extension of the length of the sample zone. An example of the use of large dispersion is in flow injection titrations[207-210].

Application of Voltammetric Detection in Flow Injection Analysis: Detection in flowing systems has a number of advantages compared with determination in static systems namely,

1) The current is increased because of increased mass transport to the electrode, the diffusion layer being thinner in flowing streams. The detection limit is ~10^{-8}M for single ion analysis in DC hydrodynamic voltammetry and about 10^{-9}M for hydrodynamic anodic stripping voltammetry.

2) The residual charging current is independent of flow rate, so the signal to noise ratio is increased by increasing the flow rate of the analyte, as long as mass transfer rates do not exceed charge transfer rates. This rarely happens in practice.

3) The background current is decreased because at constant potential no current is required to charge the double layer and the oxidation states of the functional groups in the

carbon electrode specimens are in equilibrium.

4) Hydrodynamic voltammetry is independent of time, which allows steady state measurements and so there is no need for expensive electronics. Several papers describing the use of FIA for the determination of various chemical species by different detection procedures have been published[211-222].

Different types of detectors have been used in automated analysis. These include calorimeters, electrochemical detectors, spectrophotometers and others.

Electrochemical detectors have been used extensively in HPLC [223-226], and could be adapted for flow analysis. Recently Ruzicka and Hansen[199] cite unpublished work by Ramsing in which a carbon-paste thin layer electrode was used to determine ascorbic acid in the range of 0.4 to 10 mM concentration range.

The theory and practical aspects of using ion selective electrodes and voltammetric detectors were examined by Pungor et al.[227]. The use of ion selective electrodes in combination with flow injection analysis has been reported by many workers[205,210,212,216]. A recent review by Betteridge which contains a comprehensive tabulation of detectors for flow injection analysis cites only one voltammetric approach and this involves anodic stripping[202].

The performance of a flow cell with interchangeable working electrodes made from glassy carbon, carbon paste and mercury is compared with commercially available cells using both constant potential and pulse measuring techniques[228]. Electrodes made from

glassy carbon were found to have a relatively large cathodic range, but needed a cumbersome polishing procedure, while carbon paste electrodes exhibited a particularly low background in the anodic region and a fresh electrode surface could be obtained by removing the top layer of the paste.

Constant potential amperometry was found to be preferable to normal pulse measurements. The latter technique gave high background currents when solid electrodes were used. The differential pulse technique was better in this respect, but this technique can be used only for detecting compounds having half-wave potentials close to the chosen inttial potential. The benefit of DC, pulse and differential pulse amperometry at a flow through glassy carbon electrode used in monitoring column chromatography eluents was examined by Lewis et al.[229].

Kemula[237] used the DME for amperometric detection in columnchromatography, but because of the difficulties associated with the application of a liquid detector in a flow-through detector the technique did not receive general acceptance. An example of the problems with DME is that at the high flow rates the drop formation is influenced by the flow and may not be uniform.

In view of the limited negative potential range of solid electrodes, continuous efforts are still being made to enable mercury electrodes to be used in flowing systems[231-232]. A layer of mercury on a suitable substance, such as platinum extends the useful negative potential[233-235] range.

Chan and Fogg[236] used a Metrohm EA 1069/2 electrochemical detector cell together with the Metrohm flow injection stand to

determine the phenolic analgesic meptazinol. The system used a carrier stream of 0.05 M sodium acetate, 0.01 acetic acid in 98% ethanol, and calibration graphs were rectilinear over the range 0.01 - 10 gml^{-1}. In order to improve the reliability of the technique it was necessary to modify some of the manufacturer's instructions, namely, the reference electrode was placed after the detector electrode in the flowing stream. Further a pre-saturation of the internal reference electrode with silver chloride prevented large drifts in potential.

Recently Fogg and Bhanot[237] have investigated the determination of food colours in a flowing system using both carbon paste and glassy carbon electrodes. Good precision, rectilinear calibration graphs and low limits of detection were obtained in this study. Procedures are also given very recently by Fogg and Bsebsu[238-339] for the flow injection voltammetric determination of phosphate, silicate arsenate and germanate by injection of β-heteropoly acids preformed in various aqueous, aqueous acetone, aqueous ethanolic reagents into eluents consisting of reagent blank silicate and phosphate can be determined at 10^{-7} and 10^{-6} M levels respectively. Arsenate has only been determined at the 10^{-5}M level, and the precise determination of germanate is difficult due to adsorption at the glassy carbon electrode. Also nitrite[240] can be determined by reduction at a glassy carbon electrode held at +0.3V (VS SCE) by flow injection voltammetry at concentrations above 1 x 10^{-6}M level and by direct injection of sample solution into an element 3.2M in HCl and 20% M/V in potassiumbromide

Principles of Polarography

Voltammetry is the branch of electroanalytical chemistry which deals with the effect of the potential of an electrode, in an electrolysis cell, on the current that flows through it. Polarography introduced by Heyrovsky[241] in 1922 is a branch of voltammetry carried out using a dropping mercury electrode (DME). This DME consists of a very fine-bore glass capillary. One end of the capillary is connected to a reservoir of mercury and the other end is immersed in the solution being investigated. Due to the hydrostatic pressure of the mercury column the mercury emerges as a succession of droplets about 3s interval.

Working with a DME provides many advantages:

(1) The electrode surface is renewable

(2) The mercury electrode has a high hydrogen overvoltage. This enables the use of the DME in the range up to -1.8V in acid and about -2.3V in basic solutions.

(3) As successive drops are born into solutions of identical composition and grow at the same rate and reach the same maximum size, each drop therefore duplicates the behaviour of the previous drop. This leads to high reproducibility of the currents from one drop to the next and independence of the previous history of the experiment.

(4) When compared with solid electrodes, the DME has the advantage that the increasing area of the electrode during the lifetime of a drop more than offsets the decreasing current observed with an electrode of fixed size and makes quantitative analysis much more practicable.

But the DME technique has some limitations such as

(a) Mercury is easily oxidised (above +0.4V) so the mercury cannot be used at potential more positive than +0.25V.

(b) Dissolved oxygen is reducible at the DME giving two waves ($E_{\frac{1}{2}}$ = 0.05V, -0.9V versus SCE). Oxygen therefore must be removed prior to any measurement. This is usually achieved by bubbling an inert gas (normally nitrogen) through the solution.

(c) The continuous variation of electrode area gives rise to significant currents (capacitance current) even in absence of electroactive species.

(d) Special care should be exercised in handling the mercury because of its toxicity.

Classical D.C. Polarography

The technique is used to investigate solution composition by reduction or oxidation of the electroactive species at a dropping mercury electrode (DME). A potential is applied between the DME and a reference electrode (such as a saturated calomel electrode), and the current is plotted versus the applied potential. Although classical D.C. polarography has been used widely for chemical analysis for both inorganic and organic compounds, it is not useful at concentrations below 10^{-5}M and resolution becomes very poor in the presence of a relatively large concentration of a more easily reducible species. This high determination level is due to the fact that the capacitance current (charging current) becomes larger than the faradaic current (proportional to the concentration) in solutions with lower concentrations than 10^{-6}M and hence the determination of such low concentrations is not possible. The capacitance current will result from either a

change in the potential of the electrode or a change in the size of the electrode. Since the potential remains virtually unchanged over the life span of a single drop, the capacitance current therefore mainly results from the change in electrode area. The charging current can be in either direction depending on the potential of the electrode with respect to the potential of zero charge. At potentials more positive than the point of zero charge negative capacitance current occurs, and positive ones at potentials more negative. Faradaic current arises as a result of the transfer of electrons across the electrode solution interface. In these processes, oxidation or reduction occurs, and because they obey Faraday's Law they are called faradaic. Changes of instantaneous current with time during the life of a single drop for (a) 10^{-4}M, (b) 10^{-5}M and (c) 10^{-6}M are schematically shown in figure (2).

The capacitance current (i_c) decays with drop age according to $t^{\frac{1}{3}}$, while the faradaic current (i_F) increases at $t^{1/6}$ as follows:

$$i_F = 607n\, D^{\frac{1}{2}} C m^{\frac{2}{3}} t^{1/6} \quad \text{(Ilkovic equation)} \qquad .....(1)$$

$$i_c = 0.0057\, C_{dl}\, (E_{max} - E)\, m^{\frac{2}{3}} t^{-\frac{1}{3}}$$

i = microamp, C = mMl^{-1}, m = mgs^{-1},

D ($cm^2\, S^{-1}$)

C_{dl} = differential capacity

E_{max} = potential at zero charge

E = potential of the electrode.

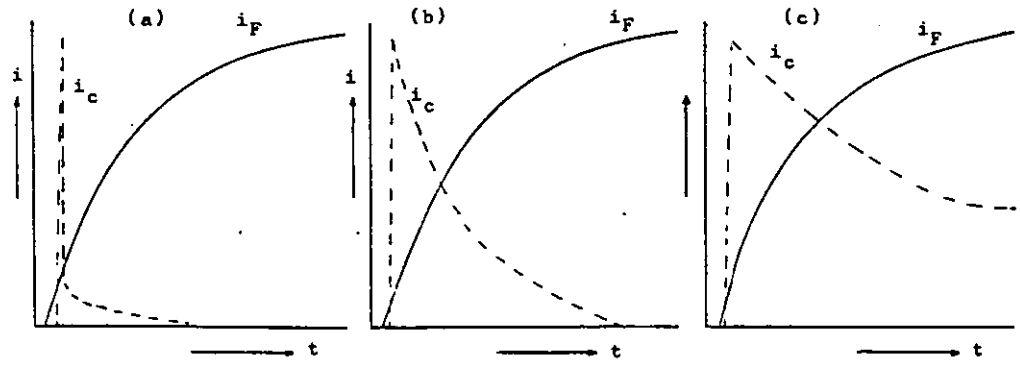


Figure 2. Effect of condenser current: Changes of instantaneous current with time during the life of a single drop. i_F faradaic current; i_c charging current. (a) 5×10^{-4}M; (b) 5×10^{-5}M; and (c) 5×10^{-6}M. (Schematic different current scales).

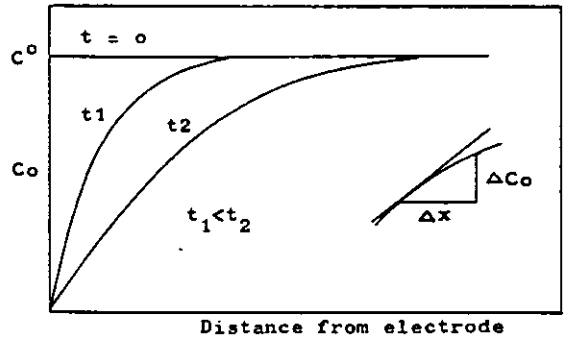


Figure 3. Variations of concentration at o, t_1 and t_2 sec after the beginning of electrolysis (left); definition of the gradient of concentration (right).

To overcome the capacitance current problem, development of both N. pulse and differential pulse becomes particularly important for trace analysis.

Pulse technique

The theoretical treatments of pulse polarography include those of Barker and Gardner[242] and other workers[243-248]. The best introduction to the subject seems to be the paper by Osteryoung and Coworker[249]. From the analytical point of view, the important theory of the pulse polarographic technique defines those parameters which affect the signal, the pulse polarographic current and its relation to concentration of the studied substance.

Theory of pulse polarography (NPP)

The technique was first introduced in England by G. Barker[250] to improve the sensitivity and signal/noise ratio by shortening the electrolysis time (to about 0.05S) and hence increasing the limiting diffusion current (Figure 3). This technique involves imposing a series of potential pulses of increasing amplitude to successive drop life times the potential time (E-t) curve, input, signal wave form and current response obtained are shown in Figure 4. The initially high capacitance current decays at a slower rate, and hence the faradaic current is sampled during the final 16.7 ms of the pulse duration. The limiting current (plateau) of a normal pulse polarogram is diffusion controlled as is the case in conventional d.c. polarography. The NPP for most organic compounds, however gives peak-shaped curves, owing to their irreversible characteristics. The normal pulse currenton the diffusion plateau is given by the Cottrell equation[251].

$$i_{N_{pp}} = nFAD^{\frac{1}{2}}C(\pi t_m)^{\frac{1}{2}}$$

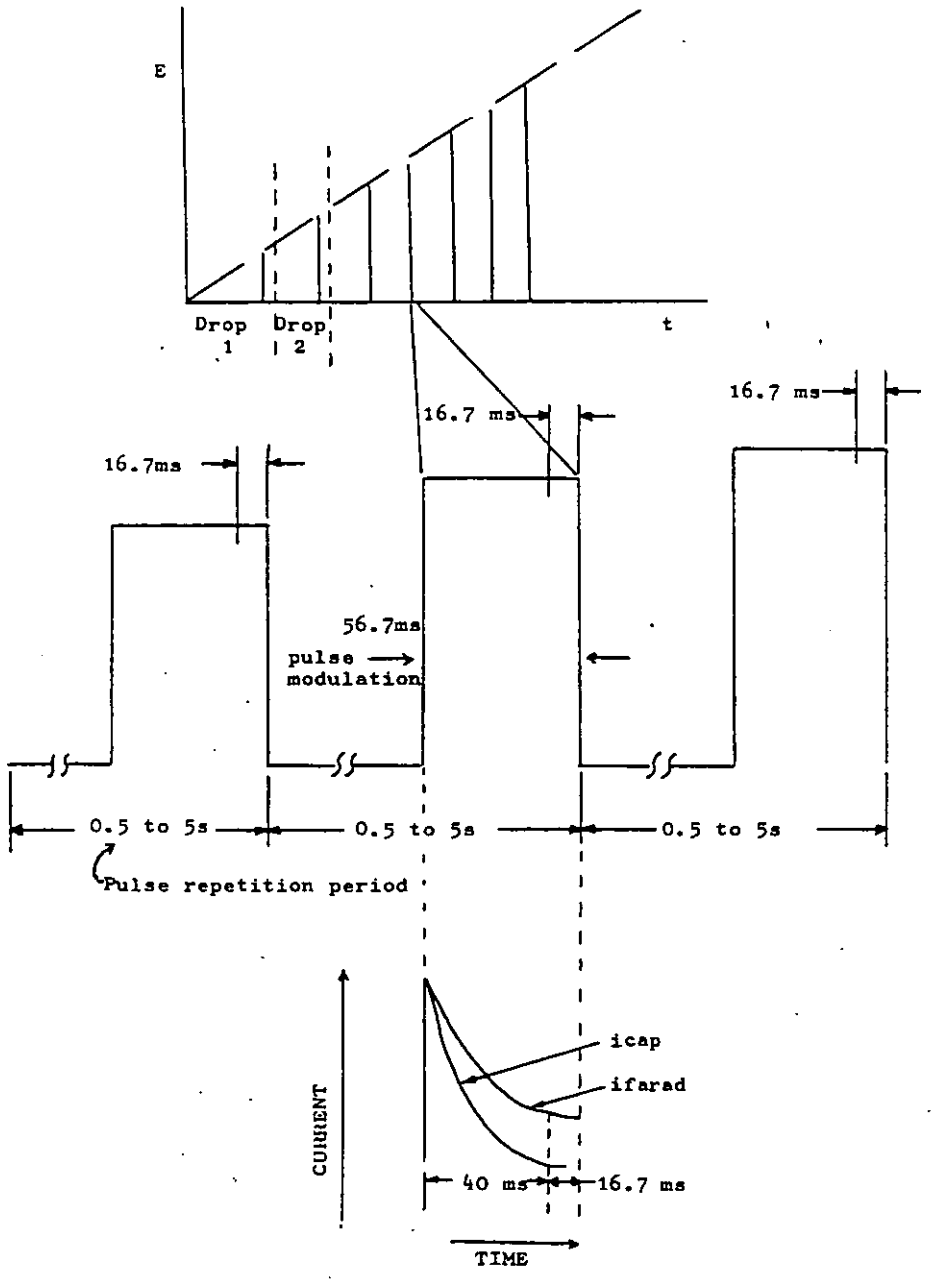


Figure 4. Potential waveform and resulting current for pulse polarography.

Where A is the electrode area (cm^2), c the bulk concentration in mM, t_m the time in seconds, measured from the pulse application at which the current is measured, n is the number of electrons transferred, F is the Faraday (96500 coulomb/equivalent) and D is the diffusion coefficient of the reacting species in cm^2/sec. The equation indicates that the current decays as a function of $t^{\frac{1}{2}}$. By sampling the current for a short period (last 16 ms of the pulse), when the capacitance current has decayed to a low value, a favourable measurement of the faradaic current over the capacitance current can be achieved.

The sensitivity of NPP with respect to d.c. polarography is given by

$$\frac{i_{NPP}}{i_{Dc}} = \left(\frac{3t_p}{7t_d}\right)^{\frac{1}{2}}$$

For reasonable values of t_d and t_p this ratio lies in the range 6-7[252]. This means an improvement in detection limit of 6-7 times for NPP over dc, depending on the capacitance current and the sensitivity. In NPP, the potential pulse is applied only for small fraction of the drop time (0.5-5S), only a small amount of material is deposited on the electrode. This implies that this technique at least in theory is less affected by problems of adsorption than is dc or DPP, and therefore is less sensitive to the exact matrix.

Differential pulse polarography

In differential pulse polarography, small amplitude pulses of fixed height (5-100 mV) of about 60 ms duration, superimposed on a conventional dc ramp voltage, are applied to the dme near the end of the drop life time. The current is sampled twice during the

life time of each drop before applying the pulse and the other during the last 16 ms of the pulse when the capacitance current has decayed. The measurement of current difference versus applied potential gives the differential pulse polarogram, a peak shape which is advantageous in quantitative measurement. The theoretical relationship between the peak current, i_p and pulse modulation amplitude DE, has been investigated by some workers[253]. The maximum peak current when the pulse modulation amplitude is less than the value of RT/nF is defined as:

$$i_p = \frac{n^2F^2AC}{4RT} \left(\frac{D}{\pi t}\right)^{\frac{1}{2}} DE$$

At small pulse amplitude the differential pulse current increases linearly with the pulse amplitude, and therefore an increase in DE results in DE results in a better sensitivity.

At larger pulse amplitude however the capacitance currents increase together with peak broadening which leads to poor resolution. In practice the maximum sensitivity is obtained using (50-100 mv) pulse amplitude. The peak half width in DPP will be given by the following equation[249].

$$W_{\frac{1}{2}} = \frac{3.5}{nf}, \quad f = \frac{F}{RT}$$

The peak width is independent of pulse amplitude at small pulse amplitudes but it is inversely proportional to the number of electrons transferred. At large pulse amplitudes, the peak broadens more rapidly with increasing DE, for larger values of n. The above model only applies to reversible systems, for irreversible systems i_p values are generally lower and peak widths broader than the reversible values.

The best possible resolution and excellent reproducibility of peak shape could be achieved by operating with scan rates of 2-5 mVs^{-1} and drop times of 1-2 seconds.

In general the faradaic current is smaller in DPP than in NPP even for a fast electrode reaction. The capacitance current is also smaller in DPP due to the use of small uniform pulse amplitude.

Figure (5) shows the pulse response to be larger than the differential pulse or dc response at relatively high concentrations (e.g. $10^{-4}M$). The situation is different at low levels where the favourable signal/noise ratio of DPP gives well-defined peaks where the detection limit is as much as ten times lower than NPP.

Differential pulse polarography has some limitations, in that the size of the peak currents (i_p) for many organic reductions is governed by the rate of electrochemical reaction at which the current increases when the potential pulse is applied and hence is sensitive to the exact matrix.

Any slight change in matrix composition results in alteration of the peak current. In this case a resort to other polarographic techniques may be required. Another problem is the nature of the capacitance current, which has the form of a differential capacitance curve. Adsorption of compounds (depolarizer, products of reduction, or inert cations) from the solution alters the double layer capacitance and will increase the capacitance at particular potential regions.

At high sensitivities the presence of trace amounts of a surface active compound can produce spurious peaks and/or increase the peak current. One way to avoid the capacitance current problem is to employ

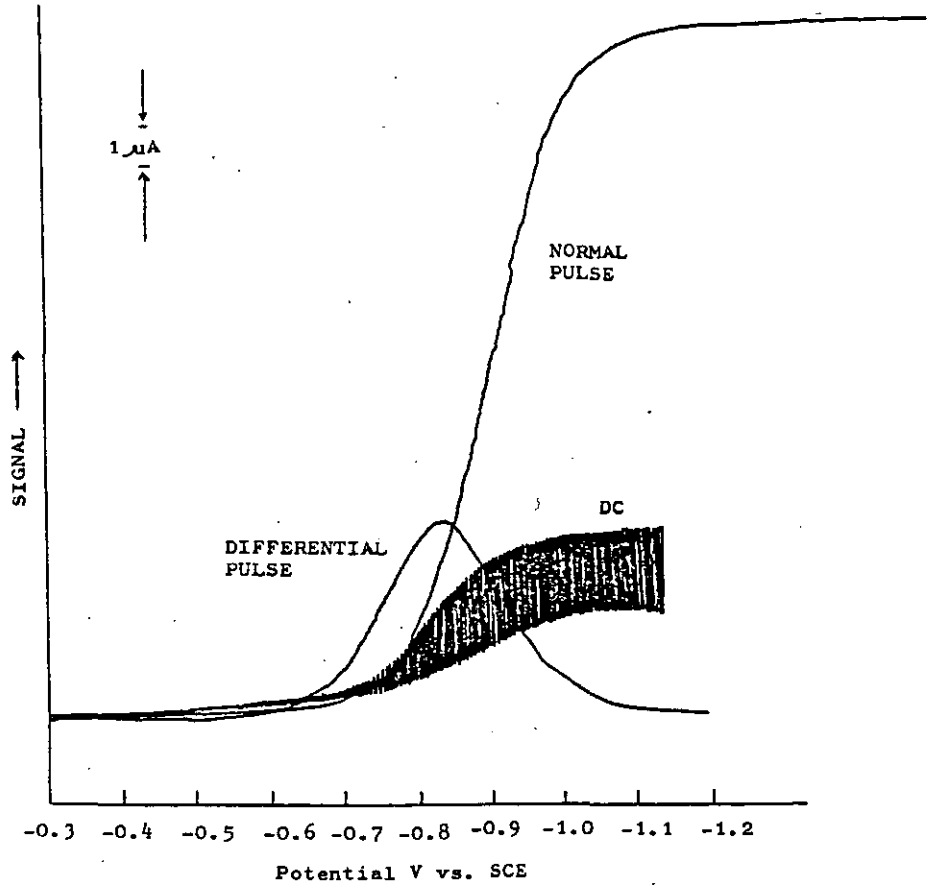


Figure 5. Comparison of dc and pulse polarography 2 x 10^{-4}M Cr (VI) in 1M NaOH; scan rate 2 mV/sec; Drop time 2 sec ; modulation amplitude (DPP) 100 mV.

amperometric titration with DPP end point detection. Change of supporting electrolyte may change the capacitance characteristics as well as the peak potential for the particular substance. This means that any surfactant used as supporting electrolyte should be examined carefully for adsorption effects.

Linear sweep voltammetry at solid electrodes:

Solid electrode techniques have been used extensively owing to the recent interest of the chemical industry in organic oxidation reduction processes. The utility and applications of solid electrode systems together with the relevant theory are discussed in detail by Adams[254]. Other workers[255] gave the definitive treatment of linear sweep voltammetry. This study includes single-sweep and cyclic voltammetry for simple systems and those with various chemical reactions coupled to reversible and irreversible charge transfers.

Single sweep peak voltammetry:

For linear diffusion to a plane electrode of area A cm^2, the peak current obtained for a reversible system is described by Randles[256] and Sevcik[257]. The equation for the peak current is given as:

$$i_p = 2.687 \times 10^{-5} n^{3/2} A D^{\frac{1}{2}} C V^{\frac{1}{2}}$$

where i_p is peak current (MA), Do is diffusion coefficient (cm^2 sec^{-1}), Co is bulk concentration (m mole lit^{-1}), and v is the rate of change of potential in volts. sec^{-1}, A is electrode area (cm^2).

The peak current is proportional to concentration, and it also depends on the rate at which potential changes. Increasing this rate decreases the thickness of the layer of solution that is depleted while the rising part of the wave is being scanned and therefore increases the peak height.

The major practical problem with solid electrode voltammetry is the maintenance of a uniform working electrode surface. Contamination can result due to adsorption of the products of electrode reaction or by surface film formation caused by the working electrode potential exceeding the anodic or cathodic limits for the solvent under investigation. Chemical cleaning and potential cycling have been successfully applied in the past but on the other hand the dropping mercury electrode has the advantage that every drop produces a fresh surface. The use of the DME, however, severely limits the anodic range due to the oxidation of mercury at potentials above +0.4V vs S.C.E.

Hydrodynamic voltammetry:

Presently hydrodynamic techniques have achieved some importance in laboratory and commercial electrosynthesis, liquid and gas chromatographic detection, determination of redox enzyme activity and flow-injection analysis.

Theoretical treatments of hydrodynamic voltammetry with various electrode configurations have been carried out by Levich and Co workers[258]. The principles of current distribution and mass transport in flowing systems have been reviewed by Newman[259] and more recently by Pungor et al.[260].

The double layer charging current at solid electrodes can be made to occur very rapidly but changing the redox state of surface fucntional groups results in faradic currents which can take some time to settle down. For this reason the stationary electrode in quiet solution is not useful for trace analysis. Mass transfer to the electrode surface occurs by forced convection rather than solely by diffusion, and current potential curves recorded under conditions of convective mass transfer are relatively insensitive to scan rates. The recording of voltammograms under these conditions is referred to as hydrodynamic voltammetry.

This technique has many advantages which includes the high sensitivity, which can be achieved with very small volumes and the ease of achieving steady state conditions at the electrode.

The Wall Jet Cell[261,262] is the most versatile design for a wide range of continuous monitoring applications. In this configuration the sample enters the cell through a fine nozzle and impinges on the planar glassy carbon electrode.

The term "wall-jet" was first used by Glaurt[262], who also gave the first rigorous treatment of this phenomena. Later other workers[263] defined diffusion current in terms of various hydrodynamic parameters:

$$i_d = 1.376nFCD^{2/3}\nu^{-5/12}V^{3/4}\alpha^{1/2}R^{3/4}$$

where ν = kinematic viscosity, stroke

V = volume of flow rate, mls^{-1}

α = diameter of circular nozzle, mm

R = radius of disc electrode, mm

n,F,C,D as stated before

The other symbols have the usual significance. For most other electrode geometries the limiting current depends on 1/3 - 1/2 power of flow rate, but in the wall-jet design the dependence is the power 3/4 which is a distinct advantage over other cell geometries.

GENERAL: INSTRUMENTATION, CHEMICALS AND SAMPLES

Instrumentation:

Model 174A Princeton Applied Research Polarographic Analyser

The model 174A employs modern integrated circuit technology to combine very high sensitivities and low noise with economy and ease of operation. The facilities provided by this instrument comprise:

(i) DC polarography

(ii) Sampled DC polarography

(iii) Normal pulse polarography

(iv) Differential pulse polarography

(v) Various voltammetric modes (sweep/stripping voltammetry).

(vi) Phase-sensitive AC polarography (requires 174/50 interface and lock in amplifier).

This equipment includes a high compliance potentiost at (±80V, 18MA), high electrometer input imedance (10^{11}ohms) which together extends the usefulness of the differential pulse technique to work over a wide range of solution conductivities. Current flowing through the working electrode is converted to a voltage. The polarograph amplifies the voltage further and applies it to the vertical or (Y) axis of the X-Y recorder output.

The "X" axis output is driven by a signal derived from the programmer scan potential generator. In current sampled DC and pulse techniques, the current is sampled during the last 16.7 ms of the timing period. The measured current value is stored in a memory and held until the next sample is taken. Thus the most recently sampled value is always applied to the "Y" axis output.

The instrument is provided with a droptimer that precisely dislodges the mercury drop with minimal perturbation of the solution, allowing polarograms to be run with a variety of preselected drop times, often far more rapidly than is possible with naturally falling mercury drops.

Light apparatus:

Sunlight is a notoriously irreproducible influence on degradation. Weather conditions, geographic location and a host of other posssible factors intervene. Consequently, the American drug industry has made use of "Fadeometers", specially designed chambers with critically controlled environments for assessing the light fastness of their products.

For the present series of experiments there was no need to use so sophisticated and costly an apparatus: Features such as controlled humidity (available for tablet experiments) and specimen rotators were found not to be necessary. Exact thermostating was also dispensed with due to little thermal degradation even well above the operating temperature of the constructed apparatus (about 10-12°C above ambient). The apparatus was set up as in Figure (1). A description of the apparatus has been given on page (17).

Spectrophotometer

SP8 - 100 (Pye Unicam Ltd, Cambridge): Simple scanning spectrophotometer (700-190 nm) with single output range, viz, 2.0 absorbance units full scale (AUFS).

Chemicals and Samples:

Vitamin C; Fisons analytical grade reagent

Food Colours

The synthetic food colours were kindly provided by Pointing Limited

and Williams (Hounslow) Limited.

These were all commercial grade compounds. Table (3) lists the food colours investigated together with their structures and 1971 Colour Index Classification numbers.

Phosphonium Compound:

Tetraphenylphosphoniumchloride was obtained from Cambrian Chemicals, Croydon. Analytical grade reagents.

Britton-Robinson buffer (pH 1.9-2.0, 0.04M in each constitutent) was prepared by dissolving 2.47 gm of boric acid, obtained from BDH Chemicals in 500 ml of distilled water containing 2.3 ml of glacial acetic acid (Fisons Chemicals Ltd) and then adding 2.7 ml of orthophosphoric acid (Fisons Chemicals Ltd) and diluting to 1 litre with distilled water. The pH of the buffer was adjusted as required by means of 2.0 or 4.0 M sodium hydroxide solution. All chemicals used were analytical grade reagents.

Acetate Buffer (1M):

Acetate buffer (pH 4.7) was prepared by diluting 62.5 ml of 1M acetic acid solution and 50 ml of 1M sodium acetate solution to 1ℓ and adjust. to pH 5.5 with 1M sodium hydroxide solution.

Nitrogen Gas Scrubbing Solution:

White spot nitrogen, as supplied by the British Oxygen Corporation virtually free of oxygen. To remove final traces or to act as as a safeguard, a Vanadium (II) scrubbing solution was used when deoxygenating samples for polarographic analysis. Approximately 2g ammonium metavanadate was boiled with 25 ml concentrated hydrochloric acid. The solution was diluted to 200 ml (green), and shaken

with a few grams of heavily amalgamated zinc (sky blue), more acid being added if any precipitate or turbidity formed.

This semi-reduced solution was then split between two 25 ml gas scrubbing bottles, whose inlet tubes were fitted with gas distribution devices, either sintered glass frits or multihole bulbs. Each bottle contained 25g heavily amalgamated zinc to re-reduce V(III) that formed in the presence of O_2. Prior to use nitrogen was bubbled through until the mid-blue coloration changed to violet. From time to time additional hydrochloric acid or amalgamated zinc was added to regenerate the solution, which was completely replaced every 9-12 months.

The two scrubbing bottles containing V(II) solution were used in series with a third containing water whose purpose was to limit carry over of acid fumes etc. Vanadium was preferred to chromium, despite the latter's greater efficiency, because of its greater ease of preparation and regeneration.

Other Reagents:

Sulphanilic and naphthionic acids were obtained from BDH Chemicals Ltd, Poole, England. Ethylenediaminetetraacetic acid (EDTA) disodium salt was also obtained from BDH as an analytical grade reagent.

Mercury

Trebly distilled mercury was supplied by Alexander Pickering & Co Ltd, Slough, England.

Development of a Polarographic Method for Monitoring Ascorbic Acid and Food Colours in the Same Solution

Ascorbic acid can be determined polarographically using the DC or DPP modes. Zuman[36] has reported the determination of ascorbic acid in acetate buffer at pH 4.5 using DC polarography. Ascorbic acid is oxidised at the mercury surface at a slightly positive potential. Other workers[41] have reported more recently the use of differential pulse polarography in determining ascorbic acid at pH 5.5 in acetate buffer in the presence of sodium oxalate. They reported that ascorbic acid was determined at +0.22V. A method similar to this latter method was adopted for determining ascorbic acid in the presence of food colours except that oxalate was excluded from the media because of its possible reduction of the food colours. Because food colours are reduced at negative potentials the following method was developed for the determination of food colours and ascorbic acid in the same solution.

Development of the method:

Preparation of acetate buffer pH (5.5): Dilute 62.5 ml of 1M acetic acid solution and 50 ml of 1M sodium acetate solution to 1ℓ and adjust to pH 5.5 with 1M sodium hydroxide solution.

Ascorbic acid solution: 0.005% m/v ascorbic acid:

Dissolve 0.1g of ascorbic acid in distilled water and dilute to 2ℓ in a calibrated flask. Transfer 1, 2, 4, 6, 8, 10, 12, 18, 20 and 24 mls by pipette into calibrated flasks add 20 mls of acetate buffer pH 5.5 to 100 ml by distilled water.

The polarographic method:

Transfer 30 ml of each solution prepared to a polarographic cell, deoxygnate the solution for 10 mins and obtain the anodic wave (DC) or the peak (DPP) between +0.1 V and -0.12V.

Three electrode operation was employed using a dropping mercury electrode, platinum counter electrode and a saturated calomel reference electrode. Differential pulse polarography was carried out with a forced drop time of 1s, a scan rate of 5 mVs^{-1} and a pulse height of 50 mV.

The peak potential was found to be pH dependent. At higher pH values the peak potential is shifted towards more negative potentials and vice versa. The peak potential was also found to be concentration dependent. At higher concentrations the peak potential shifts towards more positive potential. In more acidic solutions (>3.4) the anodic wave (DC) or peak (DPP) has a negligible slope and occurs too near the background current to be accurately measured. At higher pH values the ascorbic acid is too readily oxidised[264].

The relationship between the peak height and the concentration was found to be rectilinear, see Figure (6).

Polarographic method for determination of food colours

Dissolve 0.1g of an authentic food colour sample in water and dilute to 2ℓ in a calibrate flask. Transfer by pipette 1, 2, 3, 4, 10, 15 and 20 mls into a calibrated flask, add 20 ml of acetate buffer and dilute to 100 ml by water, transfer 30 ml of each solution to polarographic cell. Record the polarographic peaks (waves) for each food colour in the range between 0.0V and -1.2V. After deoxygenating for 10 mins. obtain a calibration curve for each one.

Mixture of ascorbic acid and food colour

Different concentrations of the mixture have been prepared and polarographed as above. The scan was started from +0.19V to -1.2V.

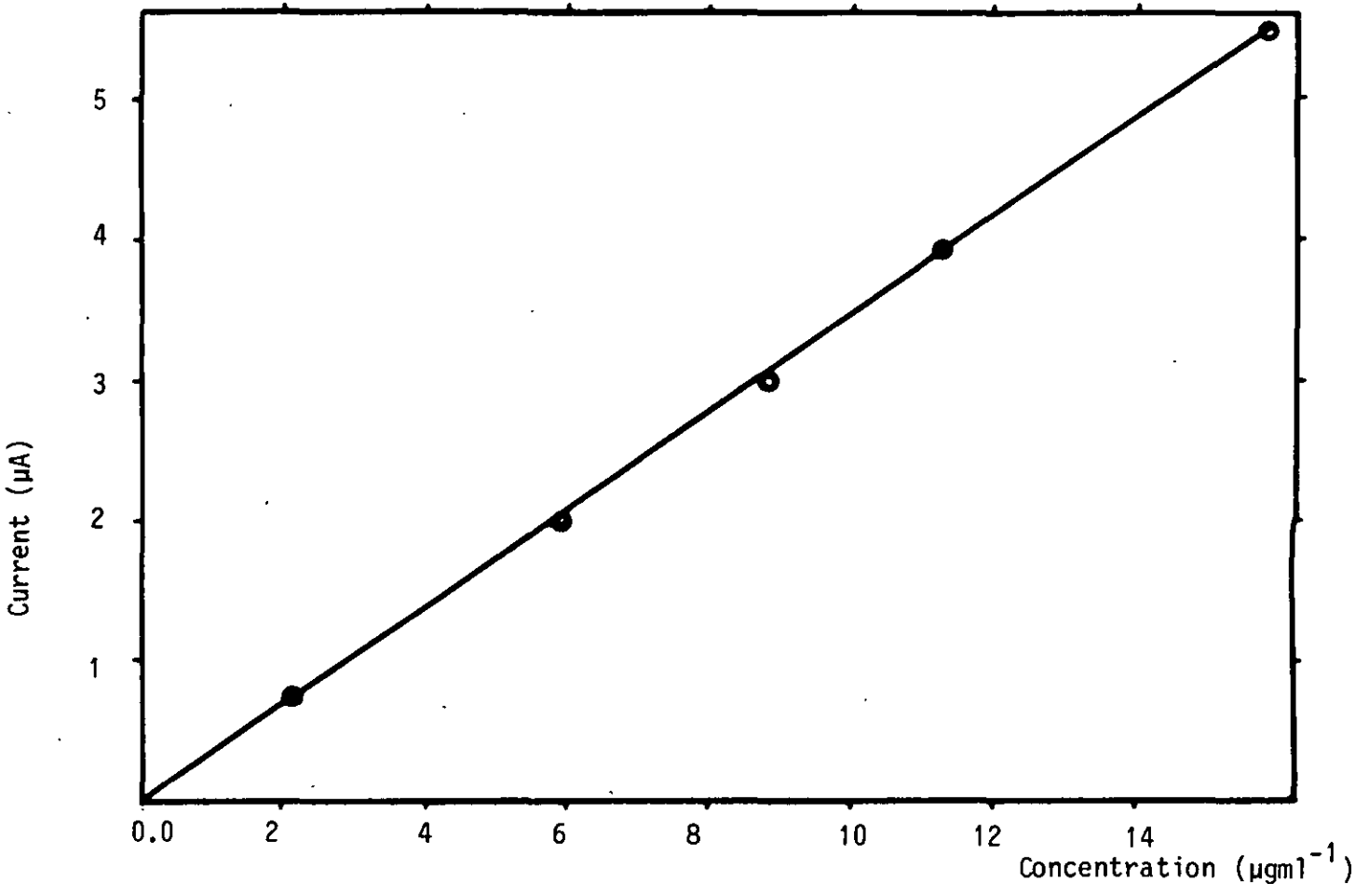


Figure 6. Calibration graph obtained for ascorbic acid by d.p.p.

Stability, storage of ascorbic acid

Ascorbic acid standard solutions are not very stable and require frequent (e.g. daily) standardisation. The decomposition of ascorbic acid solutions was examined thoroughly by Erdy and Bodor[265] In agreement with other authors[266] they concluded that decomposition is caused by two factors at least; the presence of oxygen in and over the solution and bacterial activity. Of these the former plays the main role. The decomposition is accelerated by the presence of heavy metal ions, especially copper[267]. It is very likely that while atmospheric oxygen is reduced first to hydrogen peroxide in solution, the latter together with copper ions take part in an induced chain reaction (similar types of which have been reviewed by Medalia[267]) resulting in the oxidation of ascorbic acid. Erdy and Bodor[265] attempted to use organic complexing agents to avoid the effect of heavy metal ions. They found that the addition of 0.5g of disodium ethylene-diaminetetraacetate (Na_2EDTA) per litre of 0.1M ascorbic acid solution reduces the decomposition of ascorbic acid even when stored in air to a reasonably low level. With 0.01M solutions the authors found that in 60 days 95% of ascorbic acid has been decomposed without (EDTA) but decomposition is less than 15% if a 0.5g/ℓ EDTA was added. Because of the interference from EDTA high concentration (0.5g/ℓ) in the titration, the authors reduced this amount to 0.1g per litre.

Use of EDTA to stabilize ascorbic acid:

Because of the ease of ascorbic acid oxidation in air EDTA was used to inhibit this oxidation. Different concentrations of EDTA then were added to a solution of 100 ppm ascorbic acid in the range of (0.0025% - 0.01%). But concentrations greater than (0.0050%) m/v were found to distort the polarographic wave. All results were obtained in the presence of 0.0025% m/v of EDTA.

The necessity of adding EDTA to inhibit oxidation of ascorbic acid by air during the storage period is illustrated by the figures given in table (4).

Preparation of solutions containing mixtures of ascorbic acid and food colour in the presence of EDTA: (0.0025% m/v).

In attempting to monitor the degradation of ascorbic acid and the food colour in a mixture containing the two compounds the following procedure was followed:

Transfer by pipette 12.5 ml of food colour stock solution (200 ppm), add 0.0125g of ethylenediaminetetra-acetic acid (disodium salt), and 0.05g of ascorbic acid and dilute to the mark in a 500 ml calibrated flask. The mixture prepared is 100 ppm in ascorbic acid and 5 ppm in food colour.

The solutions were de-oxygenated with nitrogen gas. 30 ml of the solution were transferred to a polarographic cell, deoxygenated and the polarograms obtained as described above. The remainder of the solution was then divided into two parts contained in tightly sealed, screw-capped, autoclavable bottles. One bottle was put in the light box and the other stored in the cupboard in the dark at room temperature. The interaction between the food colour and ascorbic acid was followed polarographically at different periods of time.

Results and discussion:

Ascorbic acid under the conditions used here gives a differential pulse polarographic peak at +0.06V. Figure (7) shows typical DC waves and differential pulse polarographic peaks of ascorbic acid. Ascorbic acid was determined by this oxidation wave at the same time as

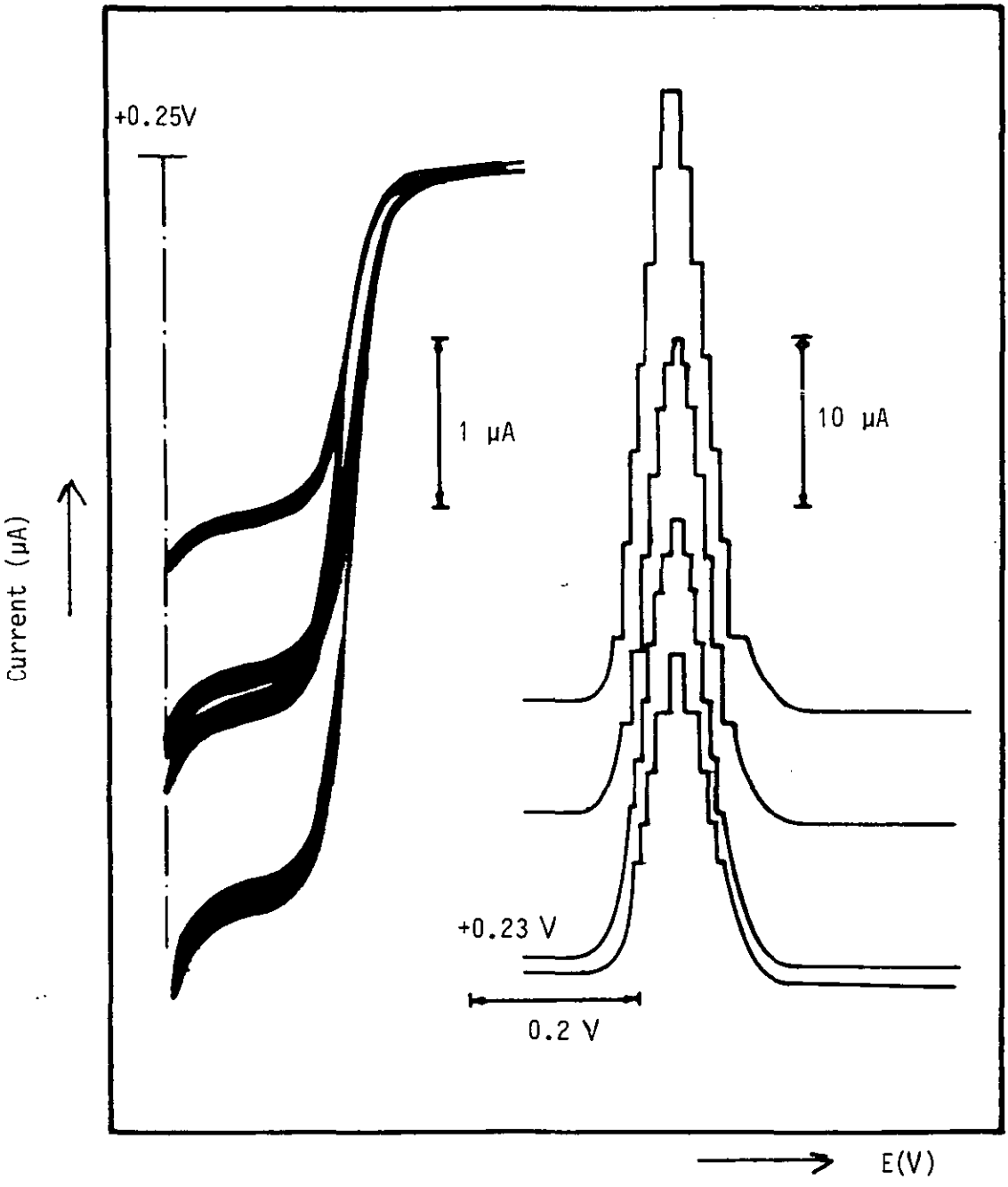


Figure 7. Typical DC and DPP polarograms obtained for vitamin C in acetate buffer pH 5.5. Concentration (100 ppm).

the food colours were determined by means of their reduction waves at more negative potentials. Examples of the polarograms obtained of the mixture of ascorbic acid and the food colours are shown in Figures (8-15). It should be noted that Linquist and his co-worker[41] measured the ascorbic acid peak at +0.22V (pH 5.5), which was found not to be the case here. The interaction of ascorbic acid and a food colour is illustrated by examples of some of the food colours in Figures (16 - 20) and appendix(1). Plots are shown for the degradation of ascorbic acid and a food colour separately and in the presence of each other. Clearly in a mixture both compounds degrade significantly more rapidly. In table (5) the time for loss of half of the food colours in the presence of 100 ppm of ascorbic acid is given together with approximate time for complete loss of 100 ppm ascorbic acid. The effect of addition of EDTA to the solutions degraded in the light is shown by the figures in Table (6).

The use of the ratio (100:5) ascorbic acid to food colour was clearly very useful in following the interaction of the two compounds over a wide range of time. This was also found to be useful in providing information on the formation of a new compound polarographically active at the surface of the mercury drop as it was the case for brown FK, black PN and tartrazine in the light degradation or during the storage period (see Figure 21).

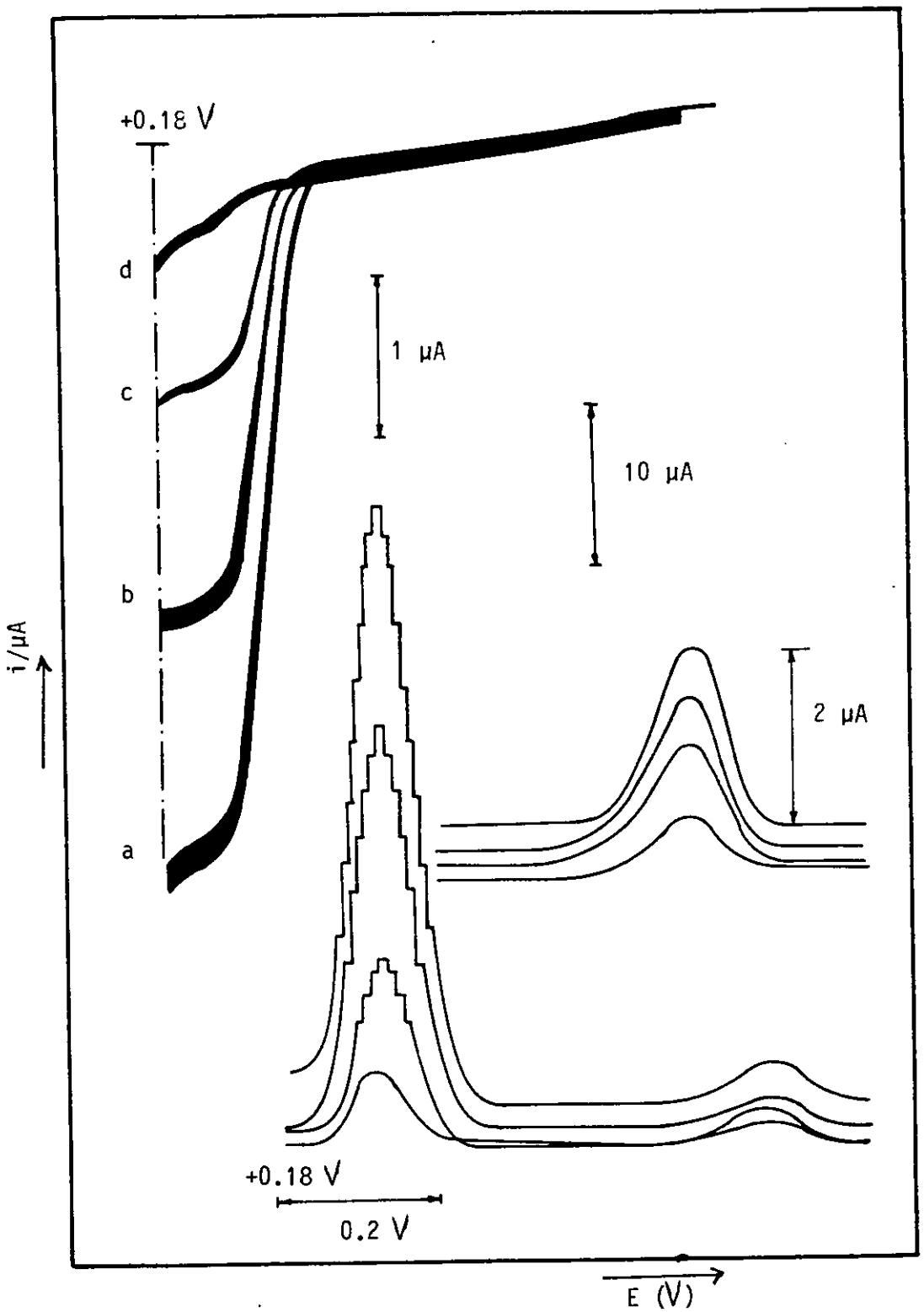


Figure 8a. Typical DC and DPP polarograms obtained for Sunset Yellow FCD and ascorbic acid in acetate buffer pH 5.5 during light degradation studies. A = Fresh B = 60h C = 105h D = 120h Initial Sunset Yellow FCF concentration = 5 ppm.

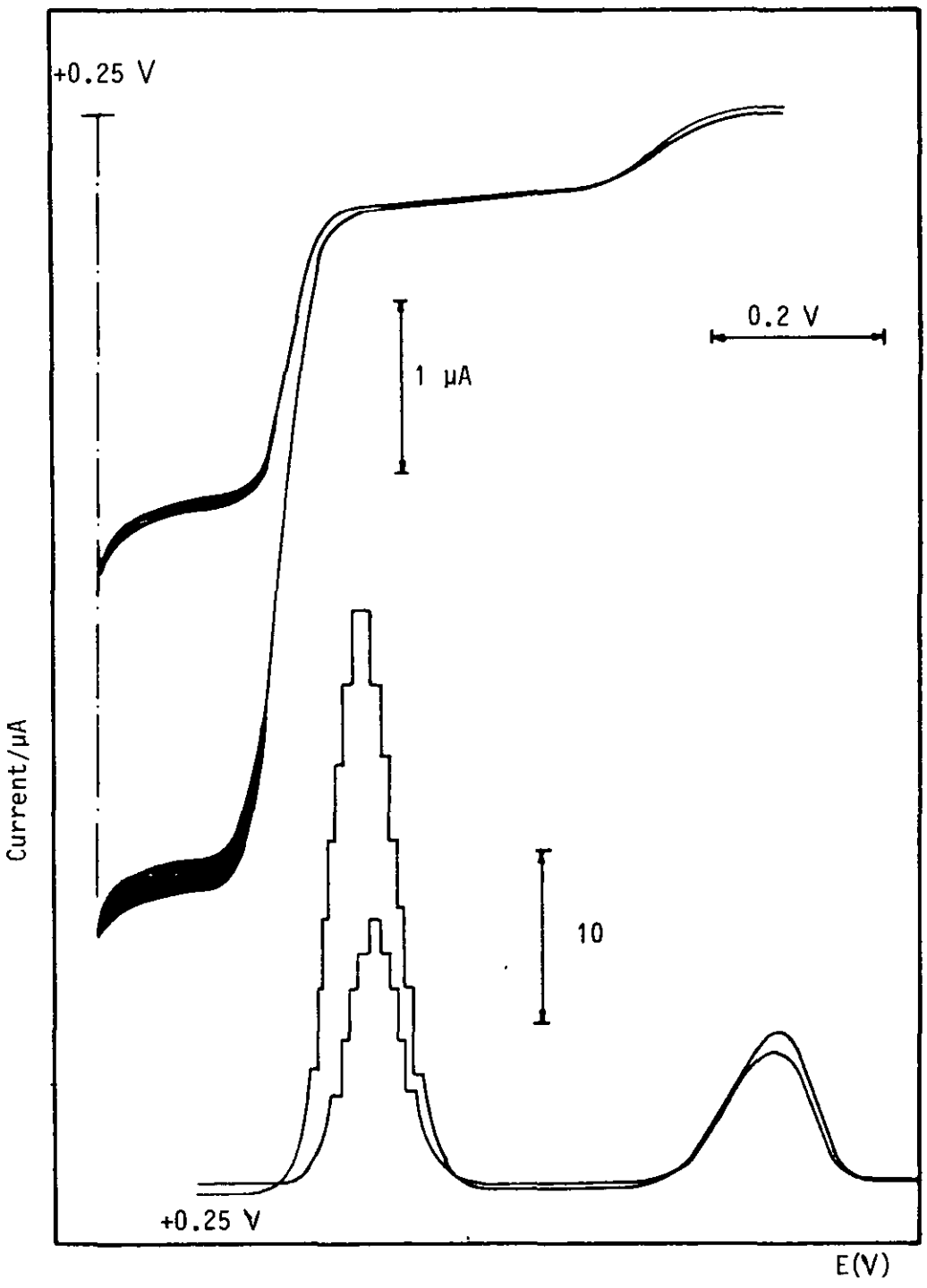


Figure 8b. Ascorbic Acid = Initial Concentration = (100 ppm)
EDTA concentration 25 ppm
Sunset Yellow FCF = 10 ppm
A = Fresh
B = 60 hrs.

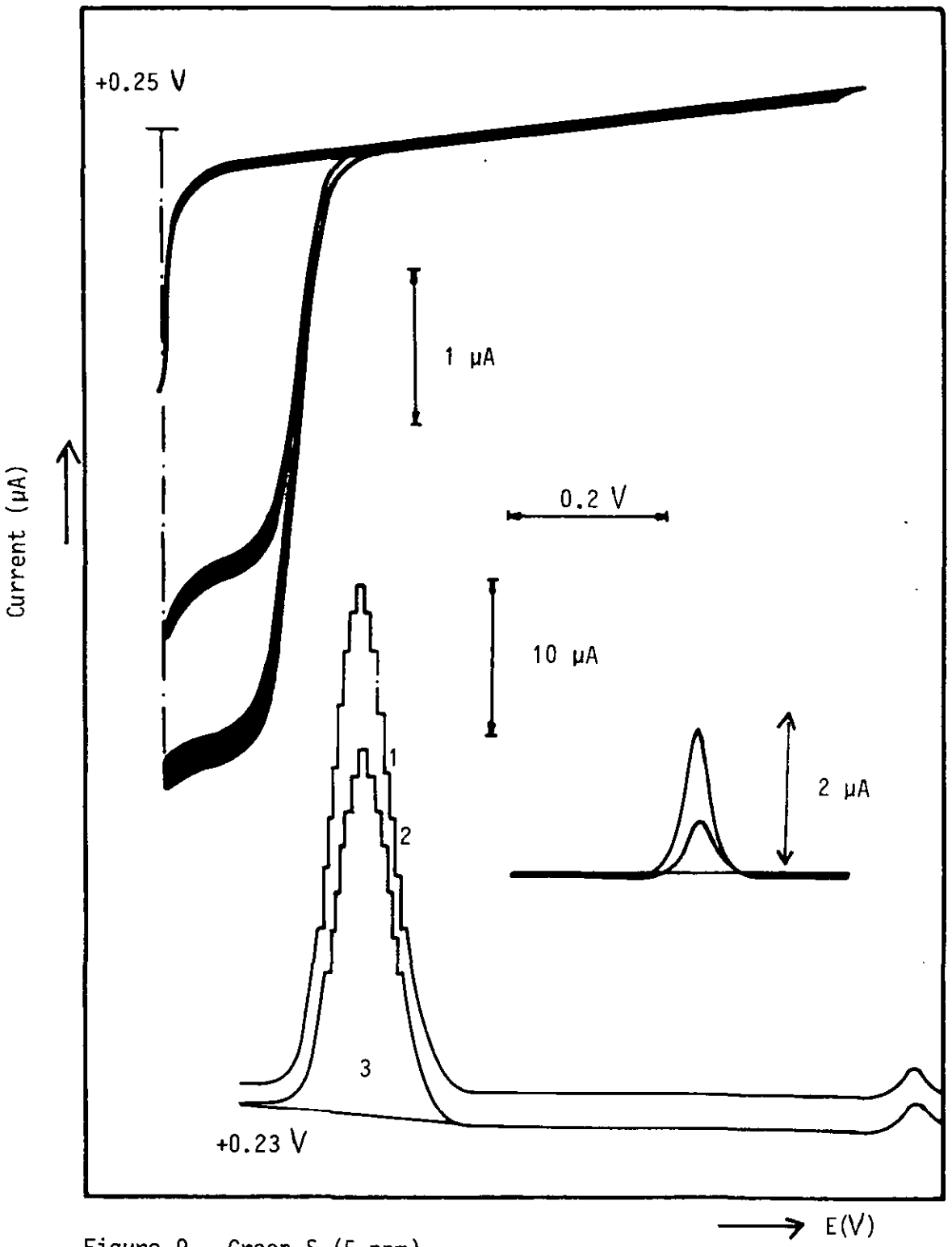


Figure 9. Green S (5 ppm)
(1) Fresh (2) 24 hrs (3) (70 hrs).

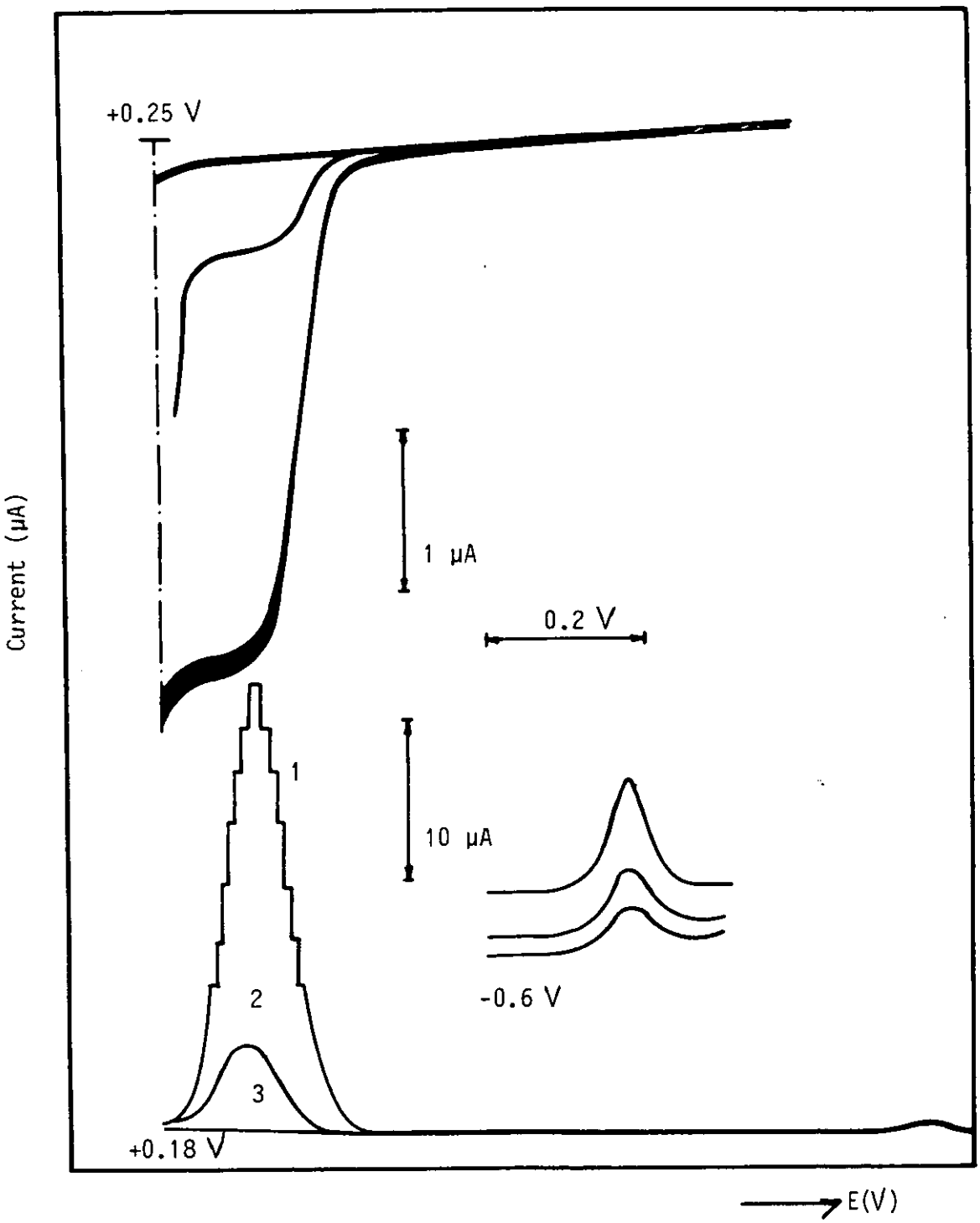


Figure 10. Brilliant Blue
1. Fresh 2. 40 hrs 3. 62 hrs

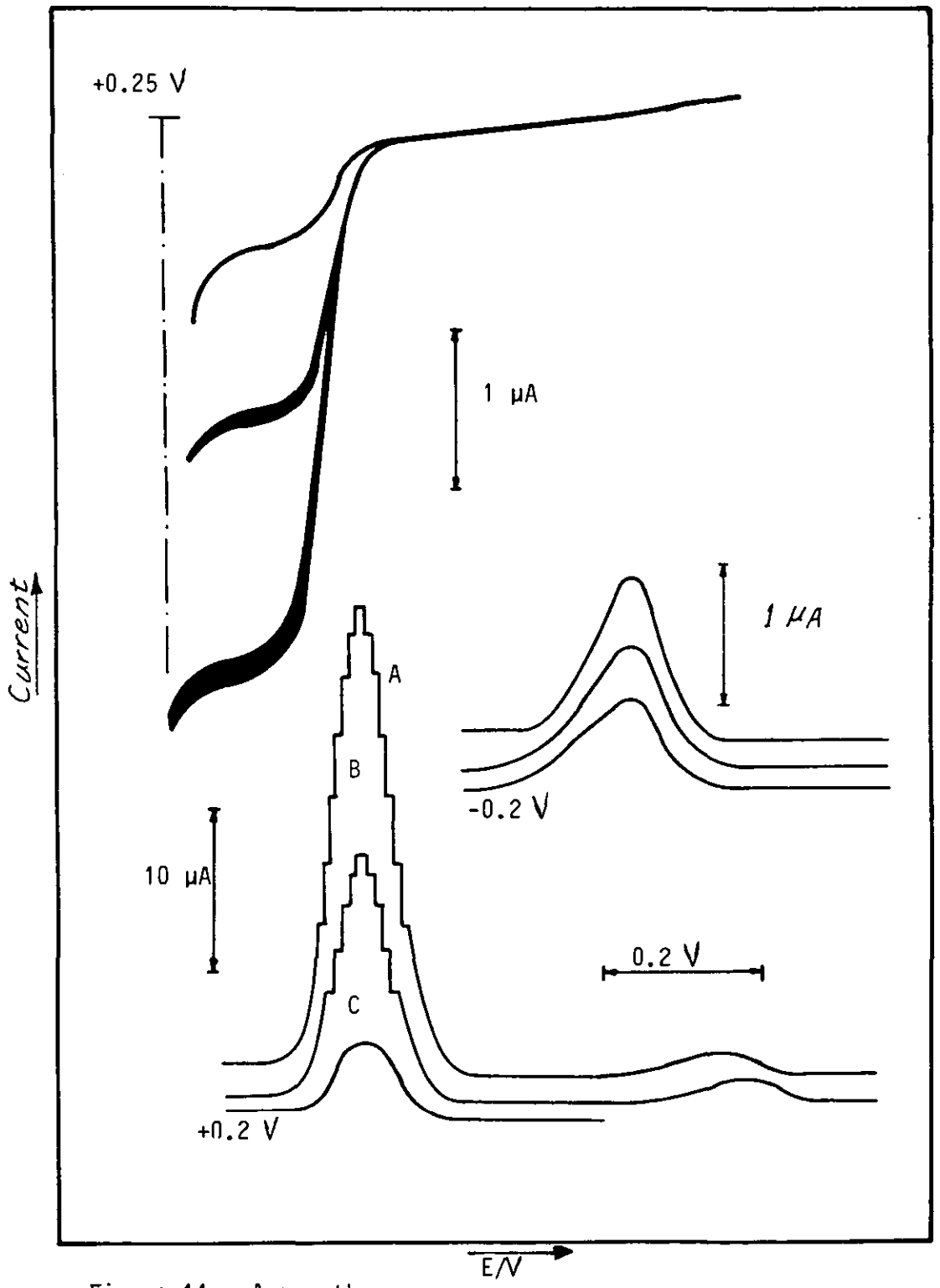


Figure 11. Amaranth
A = Fresh
B = 65 hrs
C = 115 hr

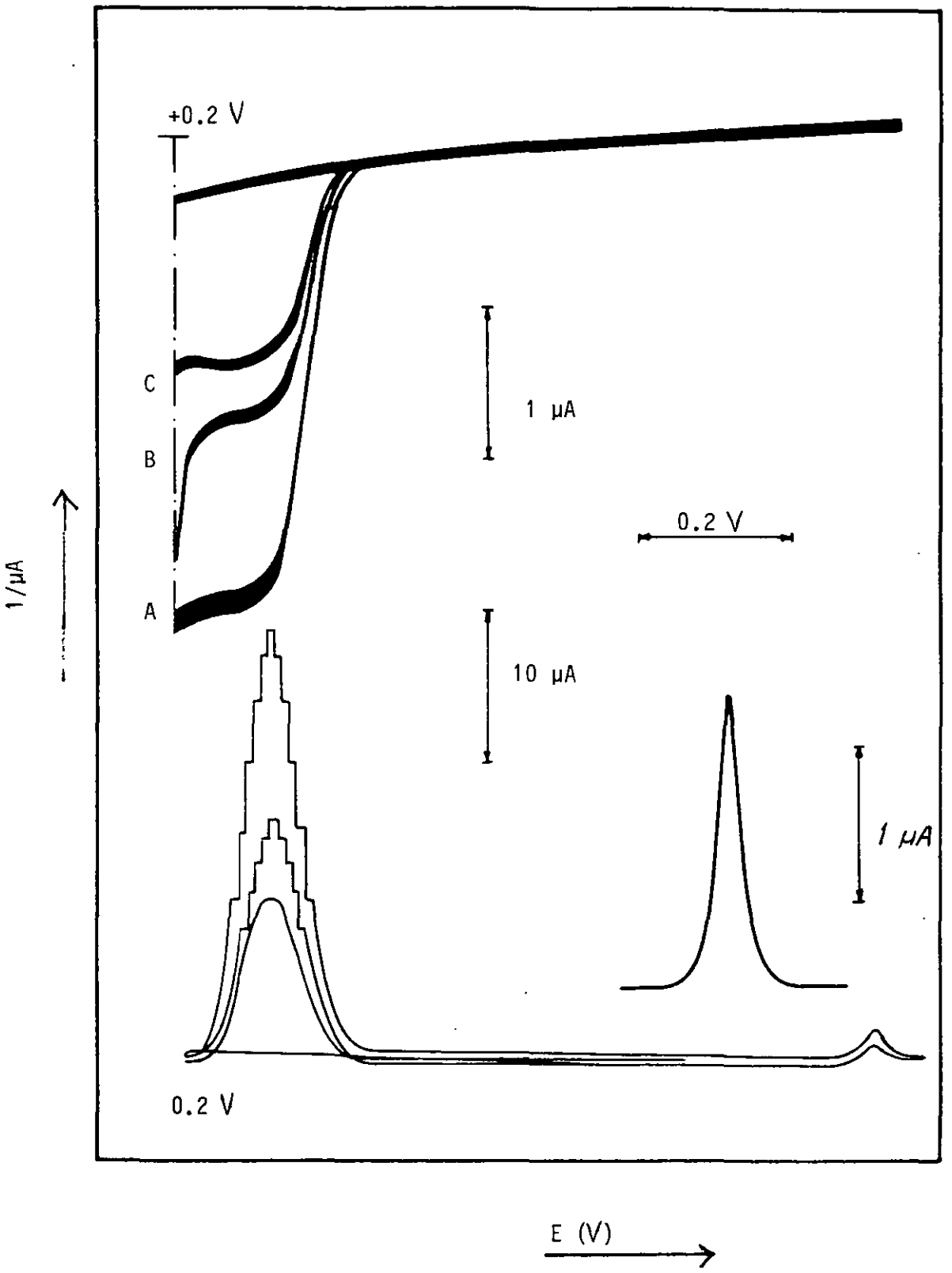


Figure 12. Patent Blue
A = Fresh B = 60h C = 80h

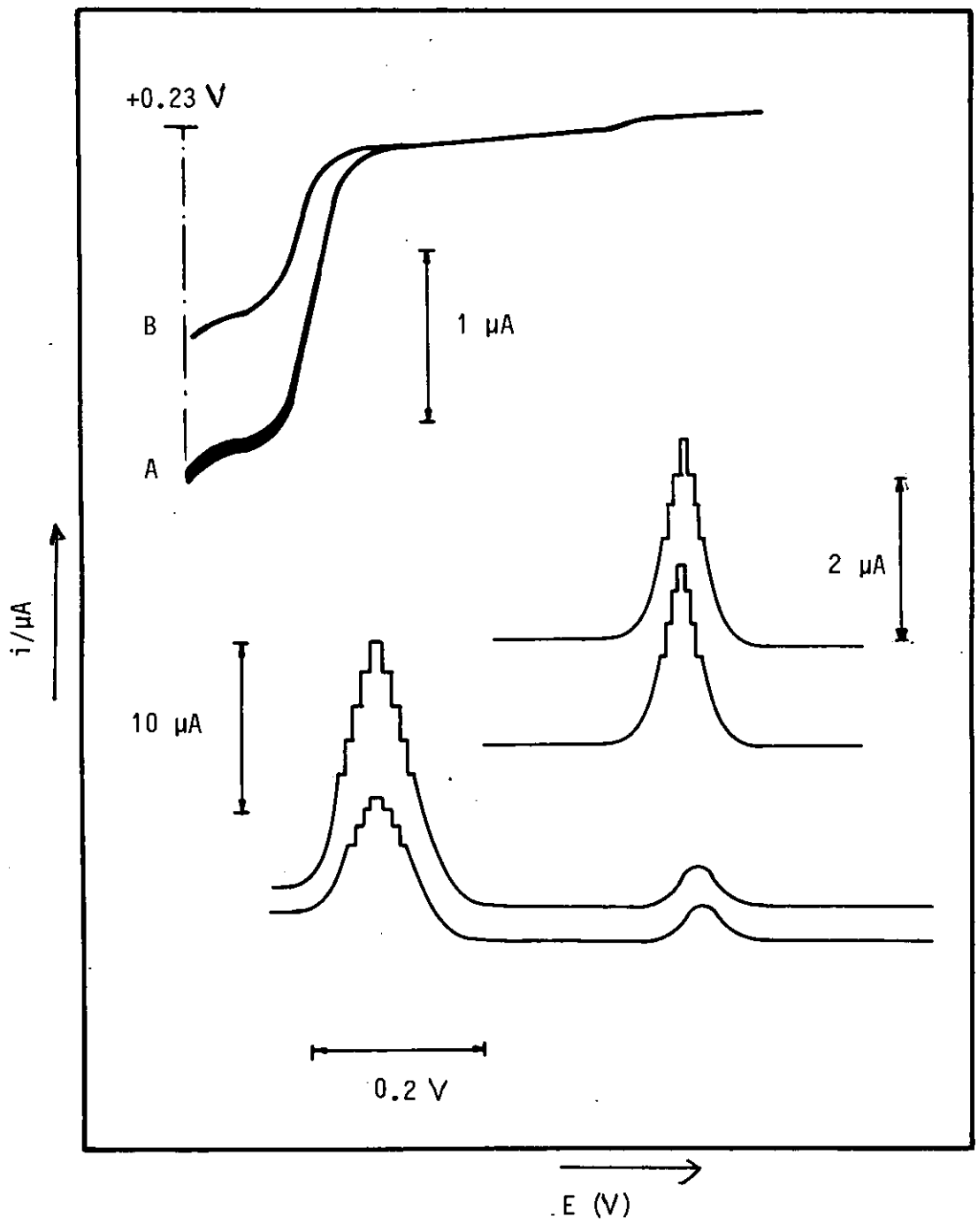


Figure 13. Carmoisine, no EDTA (A) Fresh (B) 5 hrs.

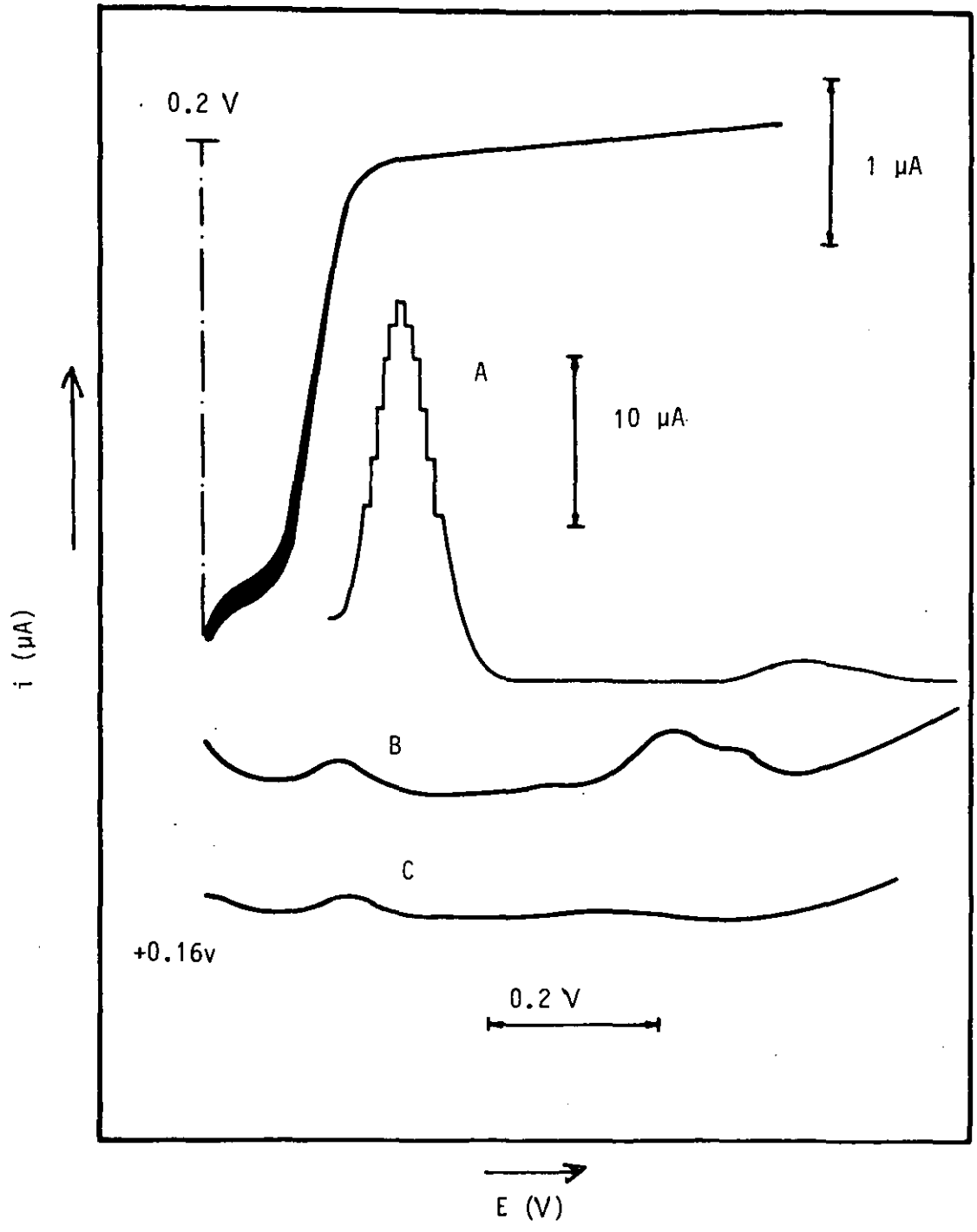


Figure 14. Brown FK, A = Fresh B = 24 h C = 60

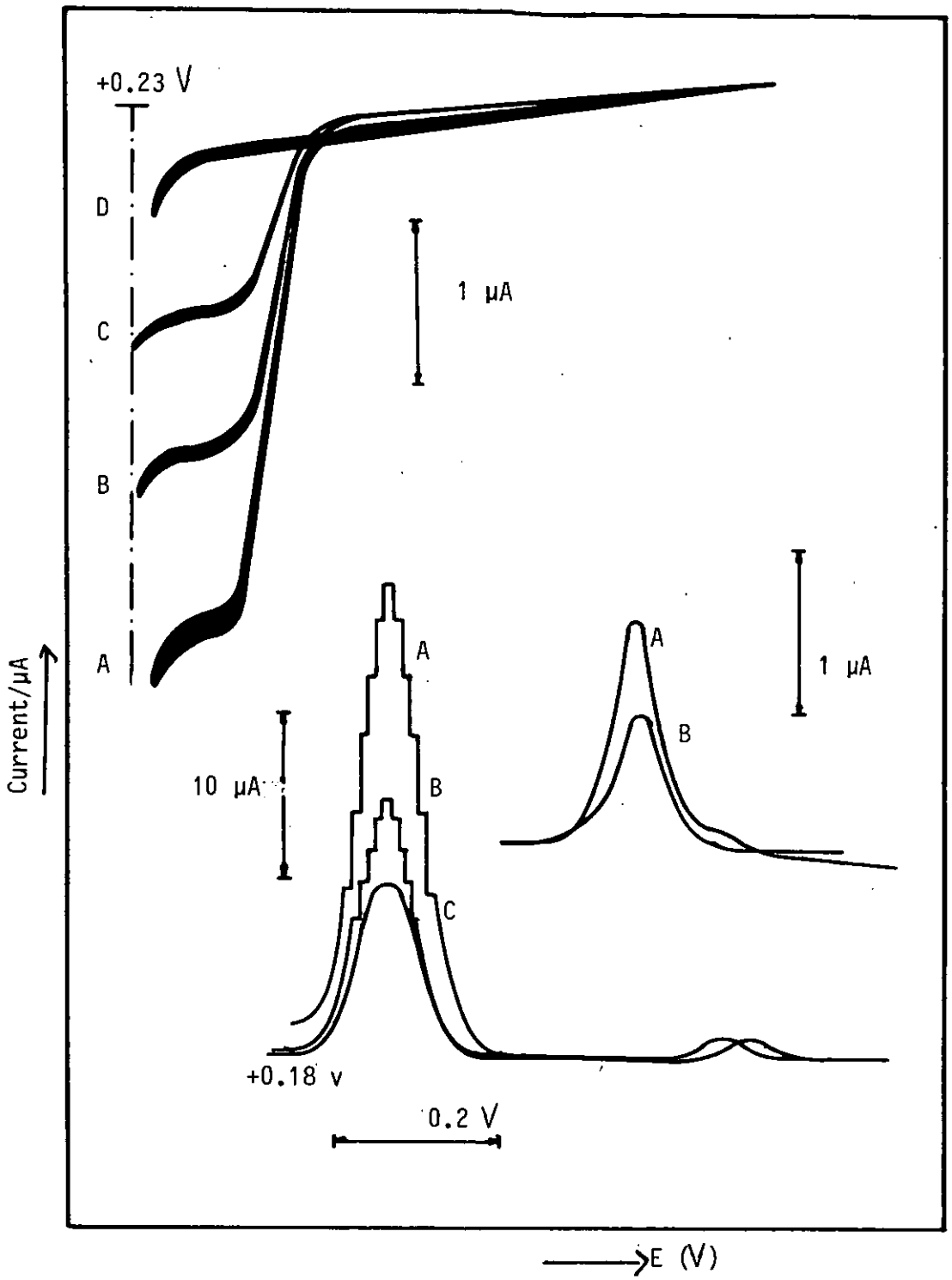


Figure 15. Black PN
A = Fresh B = 24 h C = 46h

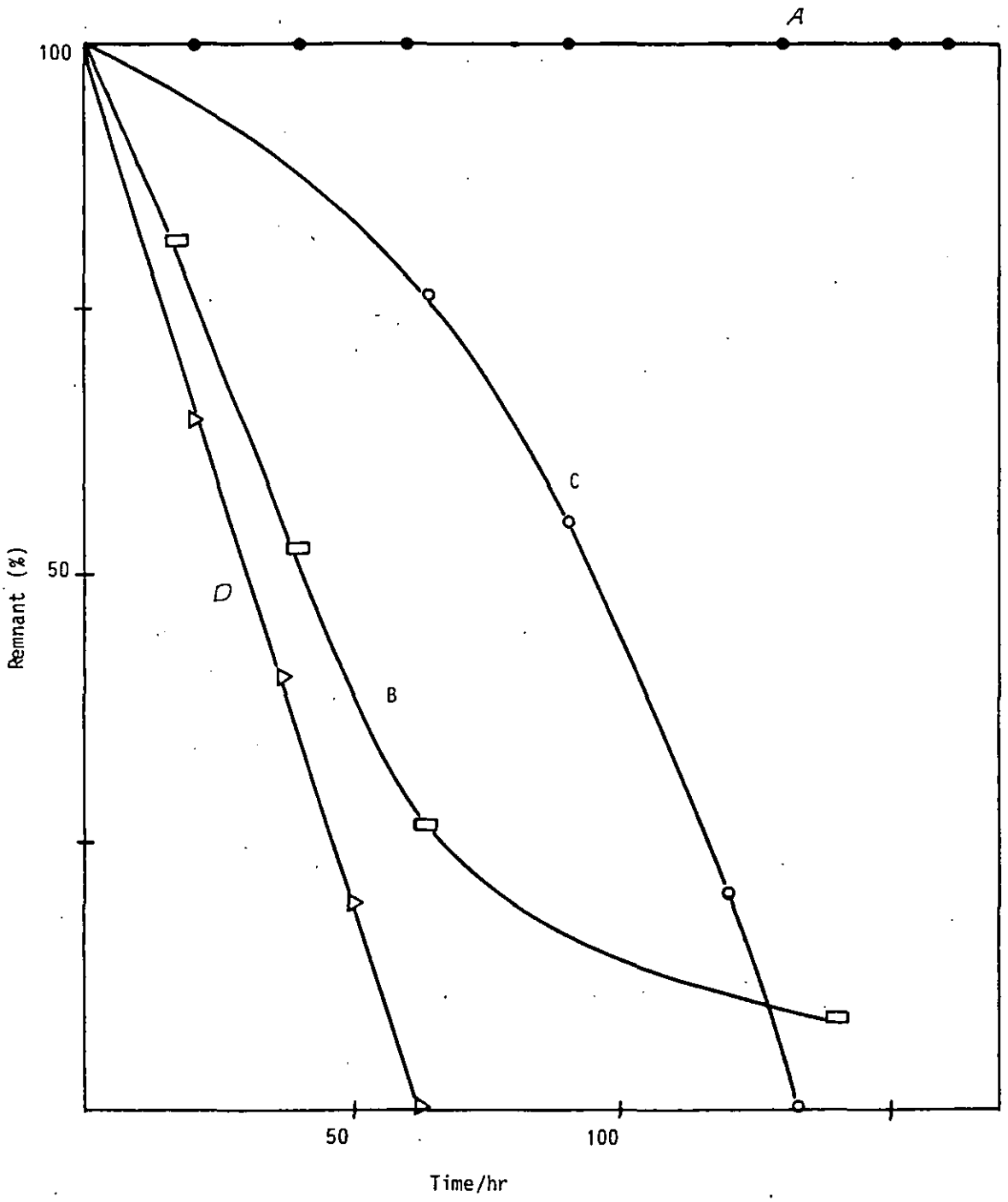


Figure 16. Light degradation in acetate buffer pH 5.5 of amaranth and ascorbic acid.
(A) Amaranth
(B) Amaranth in the presence of ascorbic acid
(C) Ascorbic acid alone
(D) Ascorbic acid in the presence of amaranth.

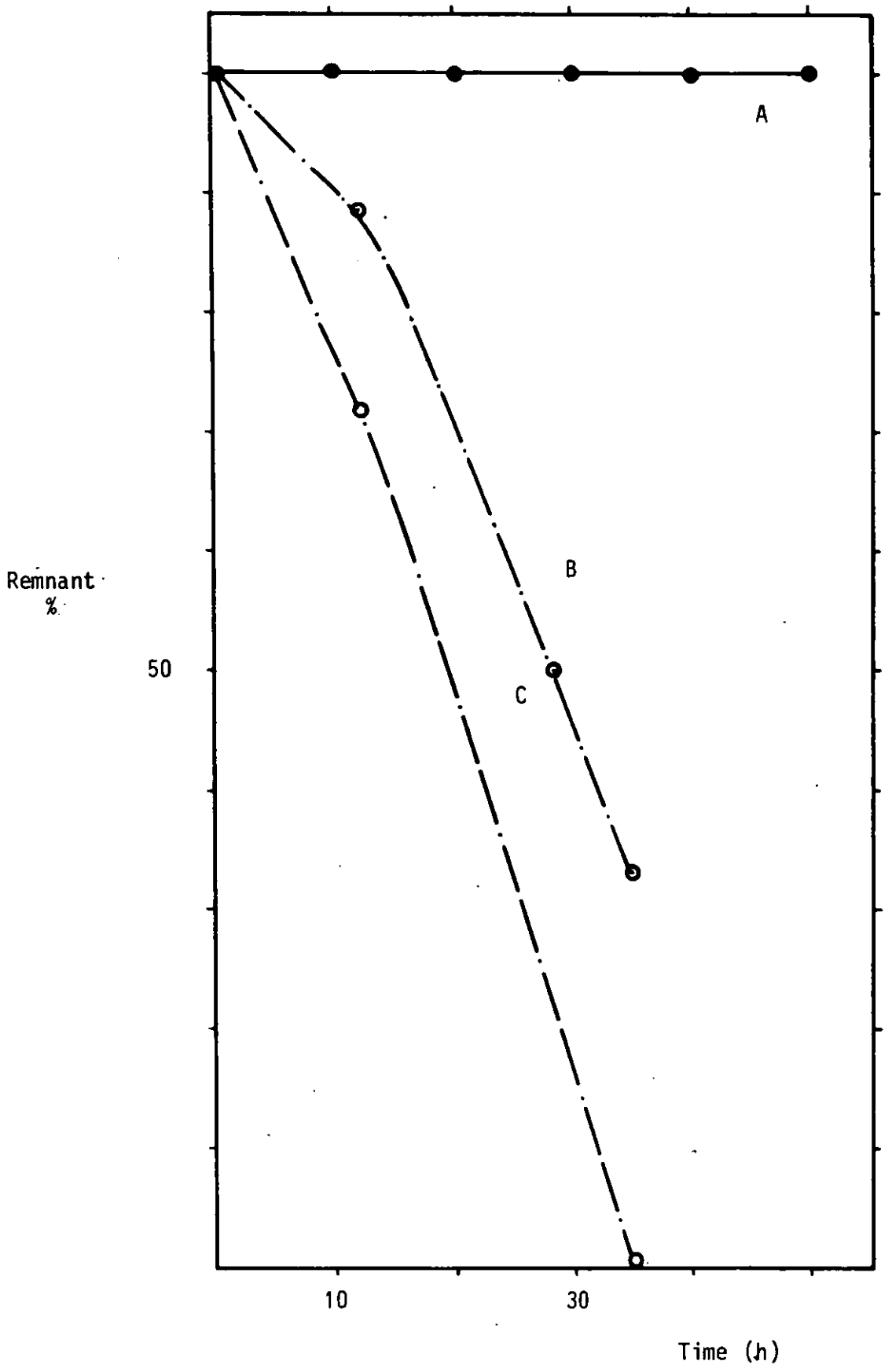


Figure 17. A = Green S B = Green S in presence of ascorbic acid, C = Ascorbic acid in presence of Green S

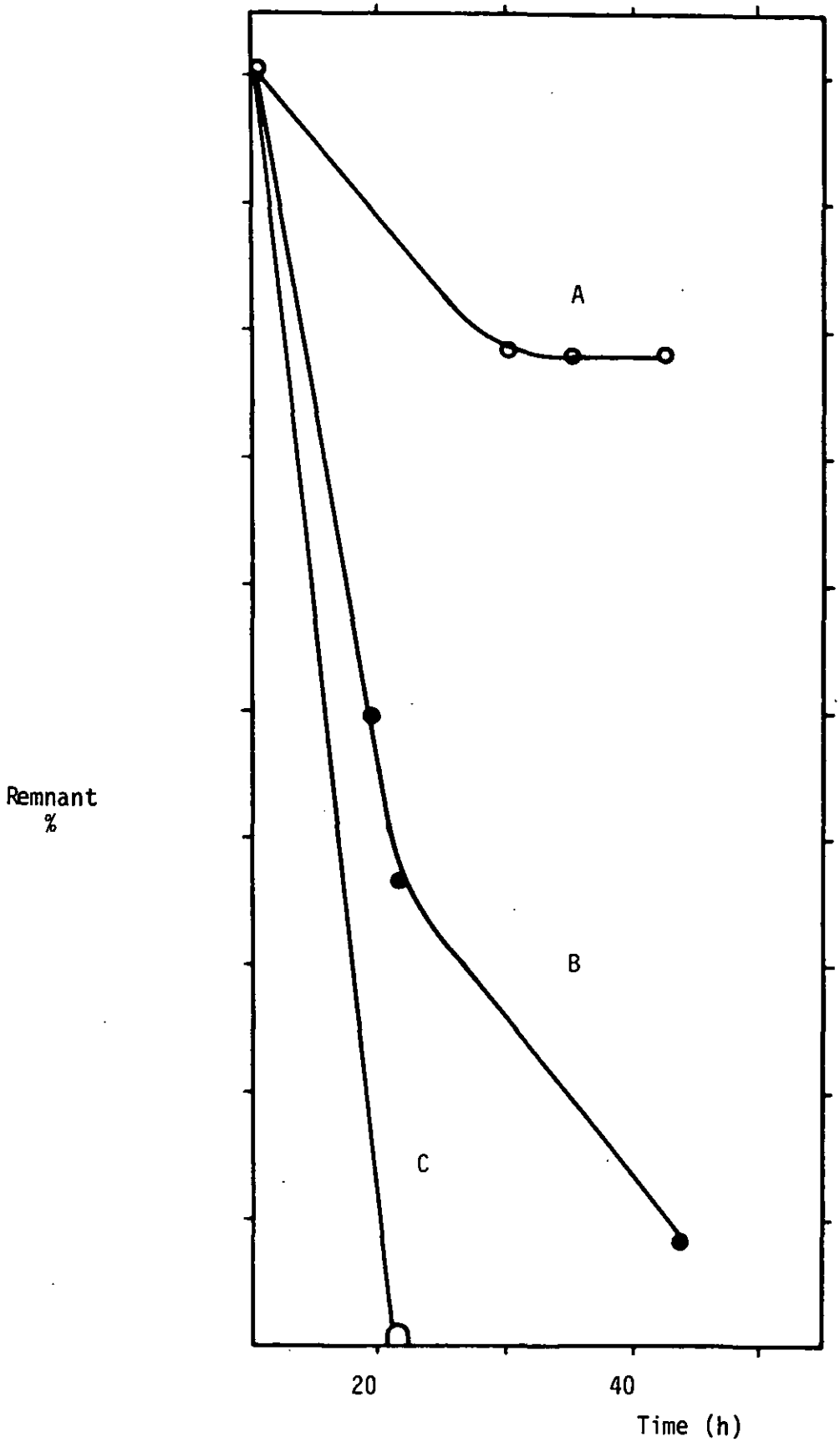


Figure 18. A = Brown FK B = Brown FK in presence of ascorbic acid, C = Ascorbic acid in presence of Brown FK

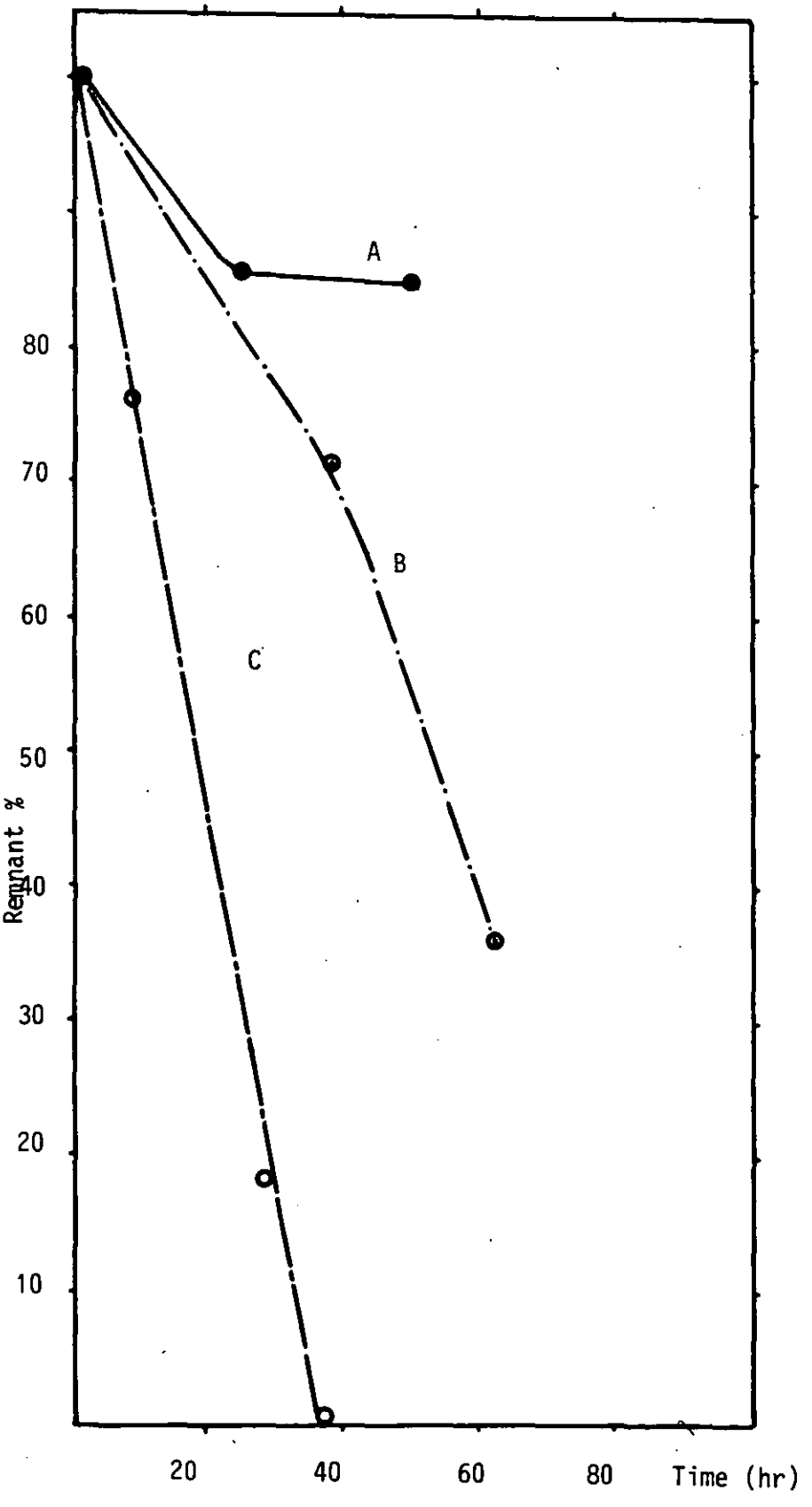


Figure 19. A = Chocolate Brown HT, B = Chocolate Brown HT in presence of ascorbic acid, C = Ascorbic acid in presence of Chocolate Brown HT.

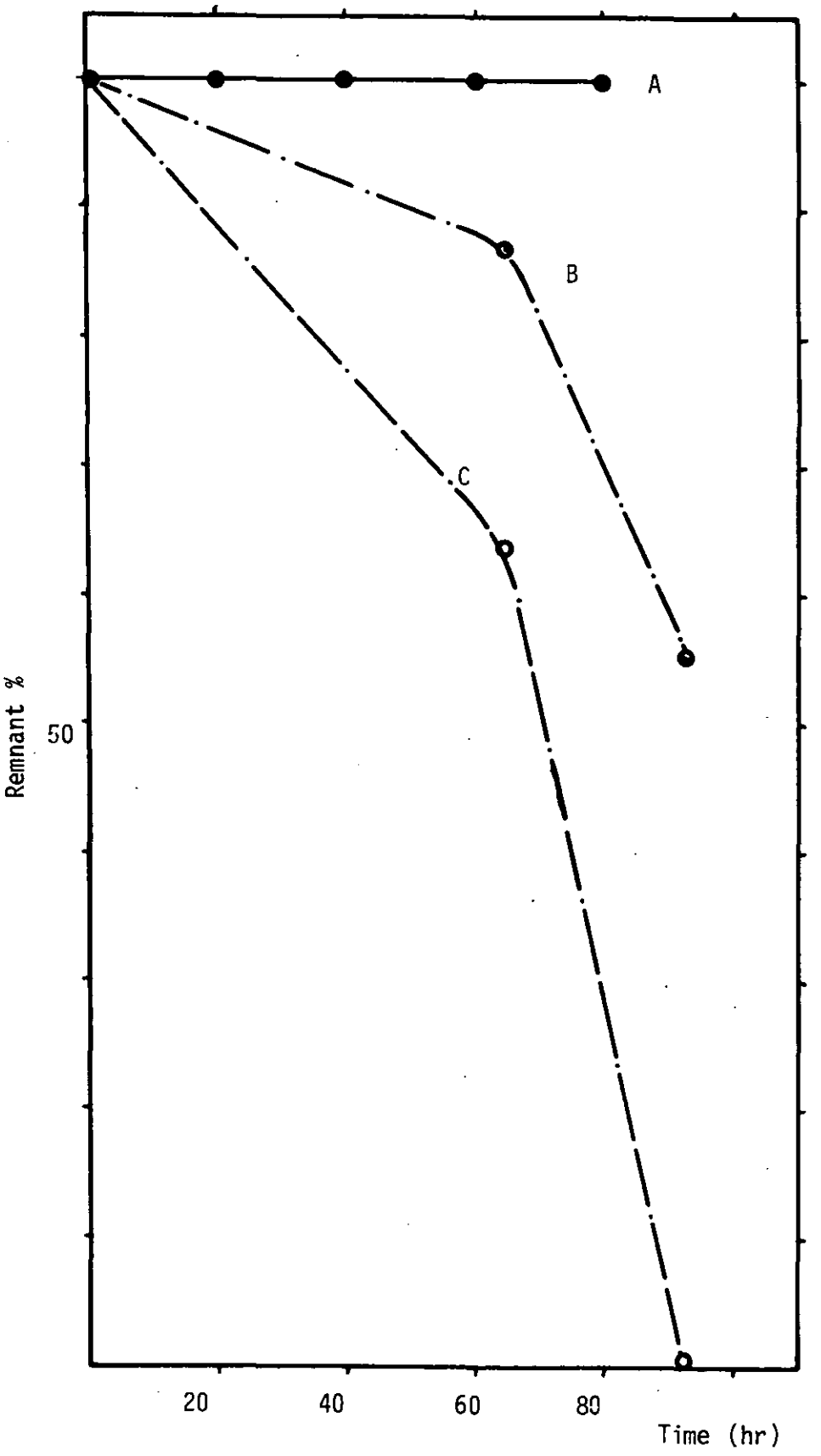


Figure 20. A = Red 2G, B = Red 2G in the presence of ascorbic acid, C = Ascorbic acid in presence of Red 2G

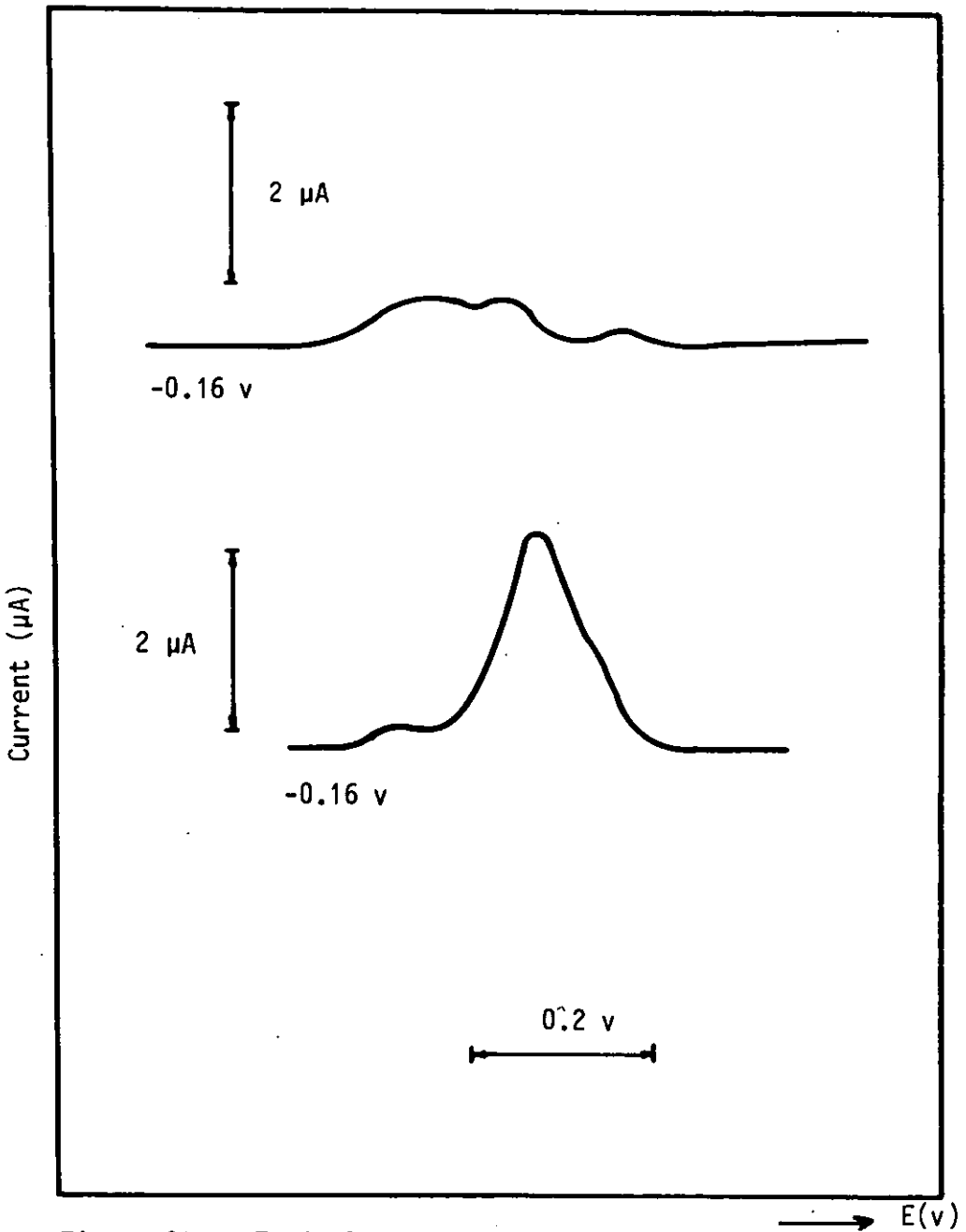


Figure 21a. Typical DP Polarograms obtained for the degradation product Brown FK during its degradation in the presence of ascorbic acid · concentration = 1000 ppm. Food colour initial concentration = 5 ppm.

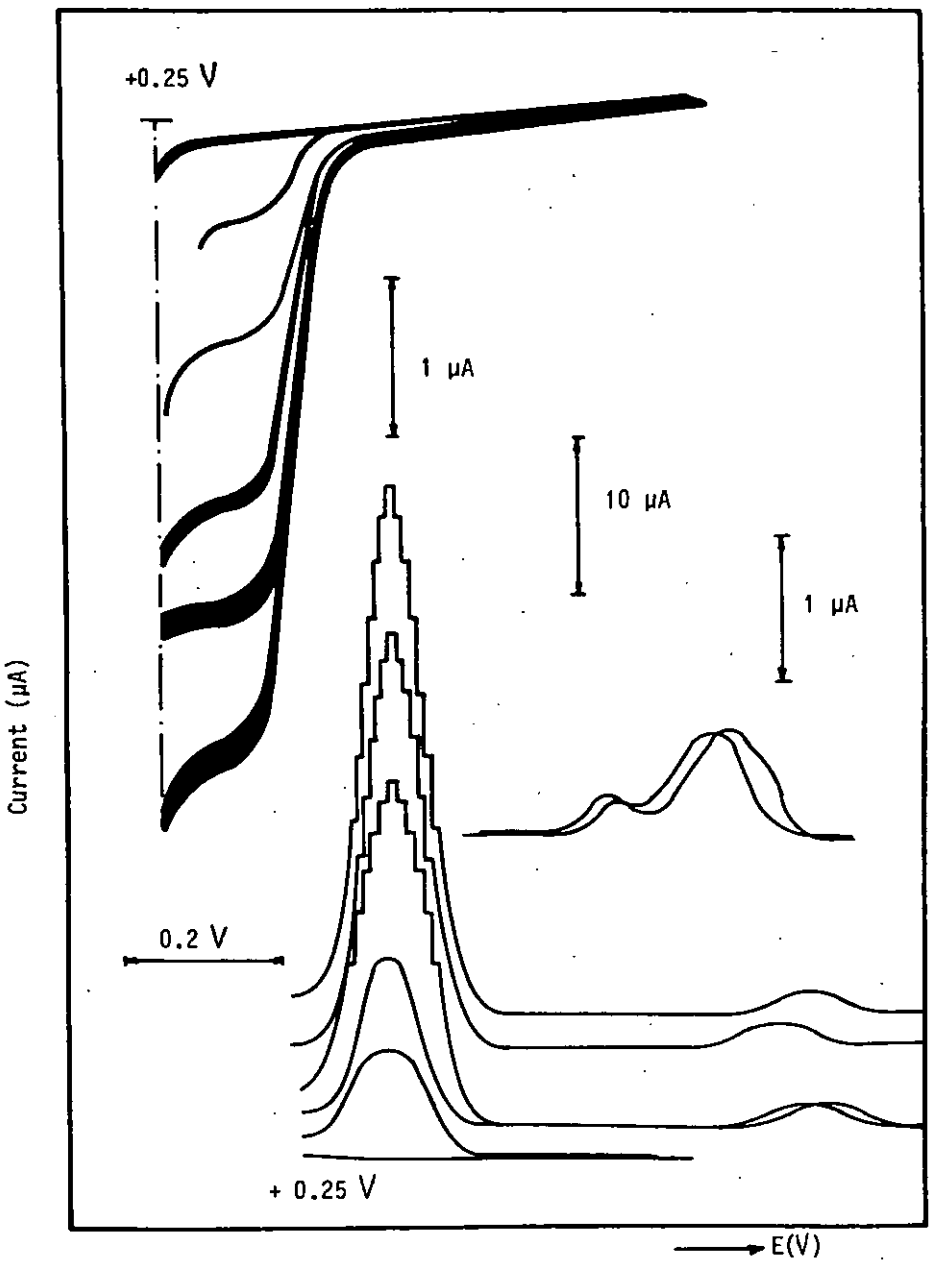
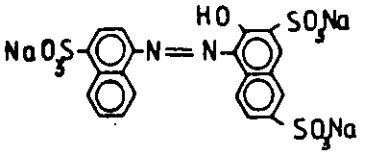
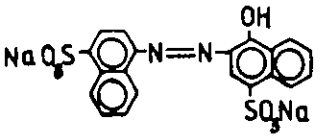
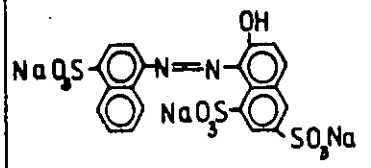
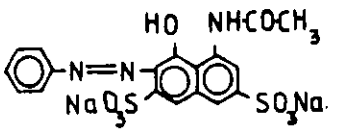
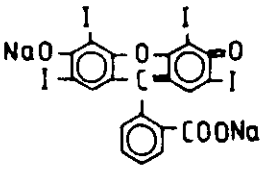
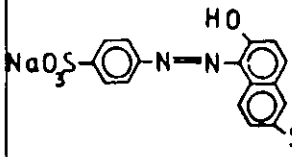
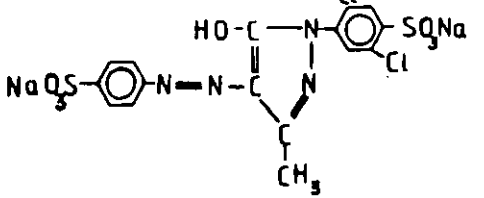
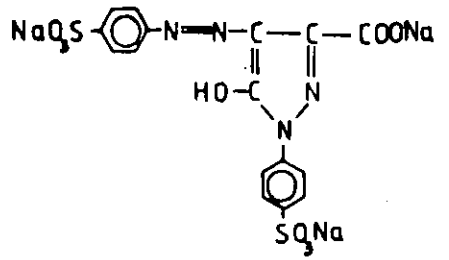


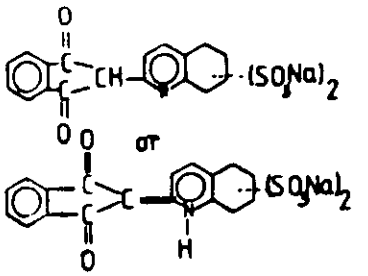
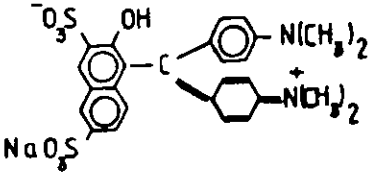
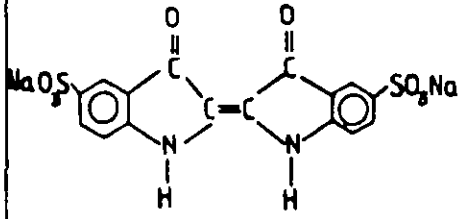
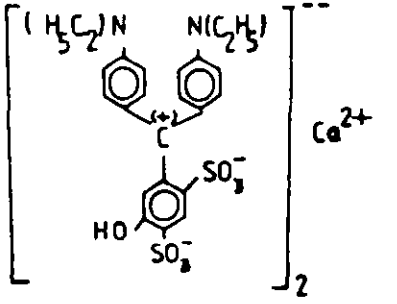
Figure 21b. Typical DP polarograms obtained for the degradation product of Tartrazine during its degradation in the presence of ascorbic acid (dark degradation studies). Ascorbic acid initial concentration = 1000 ppm. Food colour initial concentration = 5 ppm.

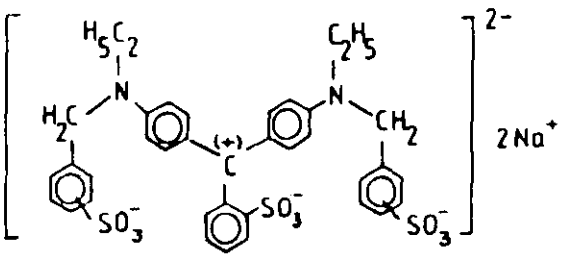
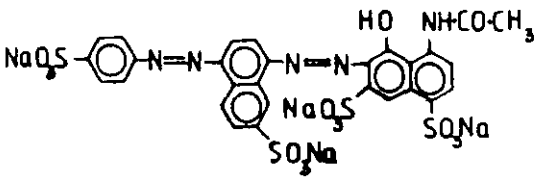
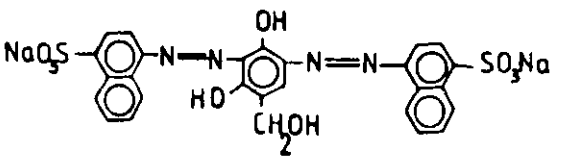
TABLE-3

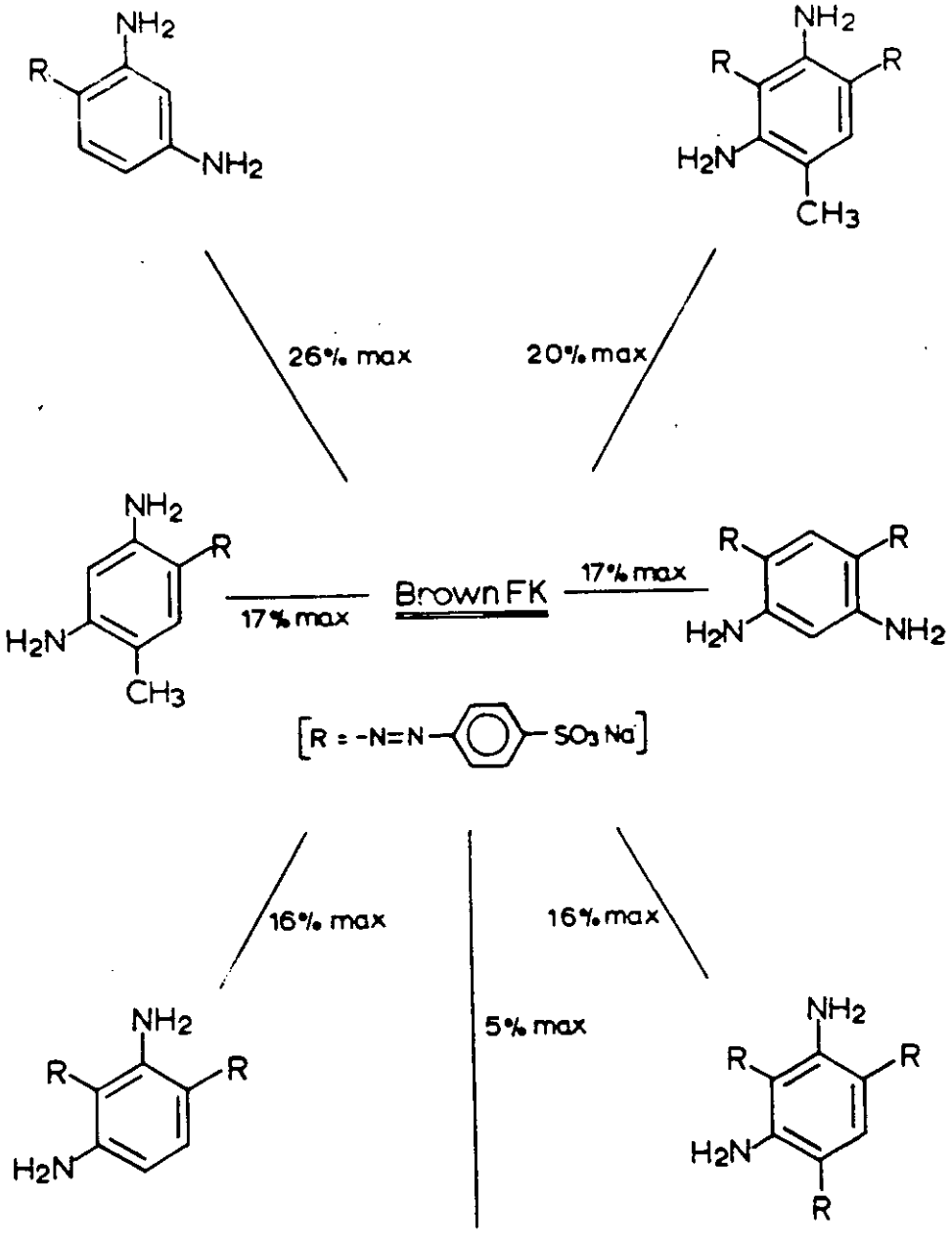
LIST OF FOOD COLOURING MATTERS STUDIED

No	COMMERCIAL NAME	SERIAL No	1971 COLOUR INDEX No	STRUCTURE
1	AMARANTH	E 123	16185	
2	CARMOISINE	E 122	14720	
3	PONCEAU 4R	E 124	16255	
4	RED 2G	—	18050	

5	ERYTHROSINE BS	E 127	45430	
6	SUNSET YELLOW FCF	E 110	15985	
7	YELLOW 2G	—	18965	
8	TARTRAZINE	E 102	19140	

9	QUINOLINE YELLOW	E 104	47005	
10	GREEN S	E 142	44090	
11	INDIGO CARMINE	E 132	73015	
12	PATENT BLUE V	E 131	42051	

13	BRILLIANT BLUE FCF	—	42090	
14	BLACK PN	E 151	28440	
15	CHOCOLATE BROWN HT	—	20285	
16	BROWN FK	—	—	MIXTURE OF COMPOUNDS



Subsidiary Dyes

Structures of the U.K.-permitted synthetic food colouring matters (cont'd).

Table (4)

Effect of EDTA in stabilising ascorbic acid (100 ppm) from oxidation by air in the dark.

a) Without EDTA

Time/day	0	1	2	3	4	5
Remnant of ascorbic acid % of original	100	55	42	37	15	0

b) With EDTA (0.0025%)

Time/day	0	4	30	50	75	82	90
Remnant of ascorbic acid % of original	100	94	44	23	7	3	1

Table (5)

Light degradation of food colours and ascorbic acid in a mixture:

Food colour	Approximate time for complete loss of 100 ppm of ascorbic acid (h)	Time for loss of half of food colour (5ppm) in presence of 100 ppm of ascorbic acid (h)
Brown FK	22	18
Ponceau 4R	42	30
Sunset Yellow FCF	90	36
Carmoisine	66	38
Tartrazine	66	42
Brilliant Blue	62	42
Amaranth	62	44
Yellow 2G	56	50
Patent Blue V	80	52
Chocolate Brown HT	38	52
Green S	70	55
Quinoline Yellow	62	62
Black PN	80	75
Red 2G	90	95

Table (6)

Effect of EDTA in stabilizing ascorbic acid (100) ppm from oxidation by air during light degradation.

a) Without EDTA

Time/h	0	2	10
Remnant of ascorbic aicd % of original	100	50.0	1.0

b) With 0.002% of EDTA

Time/hr	0	64	90	132
Remnant of ascorbic % of original acid	100	76	56	0

Differential Pulse Polarographic Monitoring of Permitted Synthetic Food Colouring Matters and Ascorbic Acid in Accelerated Light Degradation Studies and the Spectrophotometric Determination of Ammonia and Simpler Amines formed:

Introduction:

Ascorbic acid is added to certain soft drinks as a vitamin and/or as an antioxidant. Most soft drink preparations are coloured artificially with permitted food colouring matters, and problems can arise owing to interaction of some or all of the food colouring matter with ascorbic acid. This is particularly troublesome should the bottled soft drink be displayed, for example, in bright sunlight in a shop window or on a garage forecourt. The problem is exacerbated in countries which experience strong sunlight and this has to be taken into account when exporting soft drinks. Studies of the determination of food colouring matters and their degradation products are being undertaken in this laboratory. Differential pulse polarography has been used to determine mixtures of food colouring matters[268] and to monitor the degradation of individual food colouring matters in accelerated light and heat degradation studies[111]. The addition of tetraphenylphosphonium chloride, which alters the peak potentials of some food colours, is useful in effecting separation of overlapping polarographic peaks[269]. The use of voltammetry with a glassy carbon electrode has been assessed with static and flow-injection systems[237]. The formation of Red 10B from the permitted food colour Red 2G by heat degradation has been monitored polarographically[270].

The interaction of permitted food colouring matters with ascorbic acid during an accelerated light degradation experiment has been studied here.

Experimental Light Degradation Studies

Food colour solutions, 1000 ppm: Dissolve 0.1g of an authentic food colour sample in water and dilute to 100 mℓ in a calibrated flask.

Ascorbic acid/EDTA solution, 2% m/v ascorbic acid. Dissolve 2g of ascorbic acid and 0.05g of ethylenediaminetetraacetic acid (disodium salt) in water and dilute to 100 ml in a calibrated flask.

Ascorbic acid/EDTA solution, 0.2% m/v ascorbic acid. Prepare as above to contain the same amount of EDTA but only 0.2g of ascorbic acid.

Solutions for degradation. In the general study of the interaction of food colours and ascorbic acid concentrations of 5 and 100 ppm respectively were used. In attempting to determine the stoichiometry of the reaction between the food colours and ascorbic acid, solutions of 5 ppm in food colour and 1000 ppm of ascorbic acid were degraded in light. In later studies in which the amounts of small amines and ammonia were monitored solutions 10 ppm in food colour and 1000 ppm in ascorbic acid were degraded. In all cases 20 ml of acetate buffer (pH 5.5) was included in the preparation of each 100 ml of solution for degradation.

Polarographic determination of food colour and ascorbic acid in degraded solutions

The food colour contentration was determined by applying differential pulse polarography directly to the degraded solutions after deoxygenation. Ascorbic acid was determined similarly but using a ten-fold dilution with water before polarographing,for solutions with higher ascorbic acid concentrations.

Sepctrophotometric determination of amines

Sodium nitrite solution, 0.1% m/v

Hydrochloric acid, 1 + 1 $^{v}/_{v}$. Mix equal volumes of concentrated

hydrochloric acid solution and water.

Sulphamic acid solution, 0.5% m/v

Potassium bromide solution, 20% m/v

N-1-Naphthylethylenediamine dihydrochloride solution, 0.1% m/v

Standard sulphanilic acid solution, 1×10^{-4}M

Dissolve 0.433g of sulphanilic acid in water and dilute to 250 ml in a calibrated flask. Transfer by pipette 5 ml of this solution to a 500 ml calibrated flask and dilute to volume with water.

Standard Naphthionic acid solution, 1×10^{-4}M

Dissolve 0.618g of naphthionic acid in a little water and 2ml of 1M sodium hydroxide solution, and dilute to 250 ml with water in a calibrated flask. Transfer by pipette 5 ml of this solution to a 500 ml calibrated flask and dilute to volume with water.

Standard aniline solution, 1×10^{-4}M

Dissolve 0.465g of aniline in a little water and 2ml of 1 + 1 $^{v}/_{v}$ hydrochloric acid solution and dilute to 500 ml in a calibrated flask. Transfer by pipette 5 ml of this solution to a 500 ml calibrated flask and dilute to volume with water.

Potassium permanganate solution, approx. 0.01M

Dissolve 1.58g of potassium permanganate in 100 ml of water in a 250 ml beaker, cover with a clock glass, boil gently for 15-20 mins., allow to cool to room temperature, filter and collect the filtrate in a flask covered with aluminium foil. Store this solution in the dark. As required dilute 10 ml of this solution to 100 ml.

Procedure for destruction of excess ascorbic acid

Place an aliquot (10 ml) of degraded solution in a 50 ml conical flask, add a few drops (0.4-0.5 ml) of concentrated sulphuric acid

and warm slightly. Add 0.01M potassium permanganate solution dropwise until a faint pink tinge remains 10s after addition. Transfer the solution quantitatively to the calibrated flask to be used for determining amines.

Procedure for the determination of sulphanilic aicd and aniline

Transfer by pipette an aliquot of standard sulphanilic acid or aniline solution or degraded food colour solution (previously treated with permanganate if necessary) into a 100 ml calibrated flask, add 2 ml of 1 + 1 v/v hydrochloric acid solution and 2 ml of 0.1% m/v sodium nitrite solution, mix and allow to stand for 20 min. Add 2 ml of 0.5% m/ sulphamic acid solution, mix and allow to stand for 3 min. Add 2 ml of 0.1% m/v N-1-naphthylethlylenediamine dihydrochloride solution, dilute to volume, mix and allow to stand for 30 min. Coupling is only about 50 per cent complete after 30 min. for aniline under these conditions[271] although good reproducibility was obtained. Similar results were obtained after full coupling in 3-24 hours. Measure the absorbance of this solution at 545 nm.

Procedure for determination of naphthionic acid

Transfer an aliquot of standard naphthionic acid or sample solution to a 50-ml beaker, adjust the pH to 7.0 if necessary by addition of dilute hydrocholoric acid or sodium hydroxide solution, and add 2ml of 0.1% m/v sodium nitrite solution and 2.5 ml of 20% potassium bromide solution. Mix well and add carefully over a period of 5 min. to a cooled mixture of 3 ml of concentrated sulphuric acid and 3 ml of water. Allow the mixutre to stand for 3 min., add 2 ml of 0.5% m/v sulphamic acid solution, mix and allow to stand for 5 min. Add 3 ml of ethanol (96%), and after 3 min. transfer quantitatively to a 50-ml calibrated flask containing 4 ml of 0.1% m/v N-1-

naphthylethylenediamine dihydrochloride solution, dilute to volume and allow to stand for 30 min. Measure the absorbance of the solution at 550 nm.

Spectrophotometric determination of ammonia

Sodium hypochlorite solution

Dilute 10 ml of a commercial sodium hypochlorite solution (10-14% available chlorine) to 25 ml.

Alkaline phenol solution

Carefully dissolve 30g of phenol and 20g of sodium hydroxide in water and dilute to 100 ml.

Sodium nitroprusside solution, 0.1% m/v

Distillation of ammonia from degraded solutions

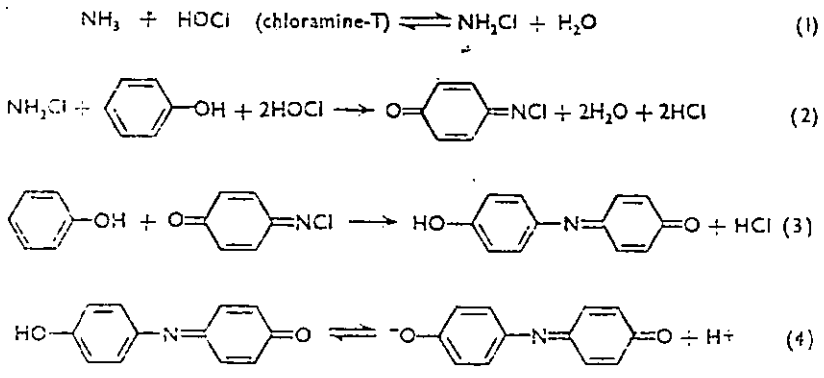
Transfer an aliquot (e.g. 25 ml) of degraded food colour solution to a 50-ml conical flask, add a few drops (0.4-0.5 ml) of concentrated sulphuric acid, warm slightly and add 0.01M potassium permanganate solution dropwise until a faint pink tinge remains for 20s. Transfer the solution quantitatively to an ammonia distillation flask and add 25 ml of 10% m/v sodium hydroxide solution. Distil the ammonia directly into 25 ml of 0.1M hydrochloric acid solution contained in a 50 ml conical flask rinsing the condenser with a little water into the flask after completion. Transfer the solution to a 50-ml calibrated flask and dilute to volume with water. Aliquots of this solution (e.g. 20 ml) were taken for the indophenol reaction: the pH of the aliquot was adjusted to be > 7 before adding the reagents.

Ammonia determination using indophenol:

(a) Introduction:

In 1859 Berthelot[272] found that ammonia in the presence of

hypochlorite and phenol gives an intense blue colour and modifications of this method have been used extensively in analysis. The sensitivity of the reaction is very dependent on the order of addition of reagents, on the pH, temperature, time and the addition of catalysts and on solvent extraction conditions. Other workers[273] proposed the following mechanism for the reaction in alkaline solution:



As low as 0.2 ppm of nitrogen has been detected as ammonia in a great variety of materials and the methods both manual and automatic are generally very sensitive[274].

The latest developments using nitroprusside catalyst are found to give a good conversion of NH_3 to the dye[275] although comparison of the results to methods involving the analysis of other compounds which also give the indophenol dye, the reactions are still non-quantitive. Rommers and Visser[276], who used chloramine T as the oxidant, suggested that variation in the results was due to the following side reaction:

The nitrogen trichloride decomposes giving HCl and N_2. Other workers[277] have found that chloramine T reacts with phenol which further affects the procedure. Recently[278] the possibility of using indophenol derivatization in the differential pulse polarographic (d.p.p.) determination of ammonia has been described. The polarographic method can be considered as an alternative, near the limit of the colorimetric method, in the presence of coloured intereference, or in turbid solutions.

(b) Procedure for spectrophotometric determination of ammonia

Transfer by pipette an aliquot of neutral or slightly alkaline standard ammonium chloride solution or sample solution to a 25-ml calibrated flask. Add by pipette, in turn with swirling and at 1-2 min intervals, 0.2 ml of sodium hypochloride solution, 0.5 ml of alkaline phenol solution and 0.2 ml of sodium nitroprusside solution. Heat the flask at 60-65°C in a water bath for 3-5 min. to form the indophenol blue. Cool, dilute to volume and measure the absorbance at 630 nm. or deoxygenate the solution and record the differential pulse polarogram between -0.22 and -0.44 V. Measure the peak height at -0.33V.

Results:

The interaction of ascorbic acid and food colours is illustrated by the figures in Table (7). The time taken for all the food colour to degrade in presence of 1000 ppm ascorbic acid is given. The food colours are listed in order of increasing stability under these conditions.

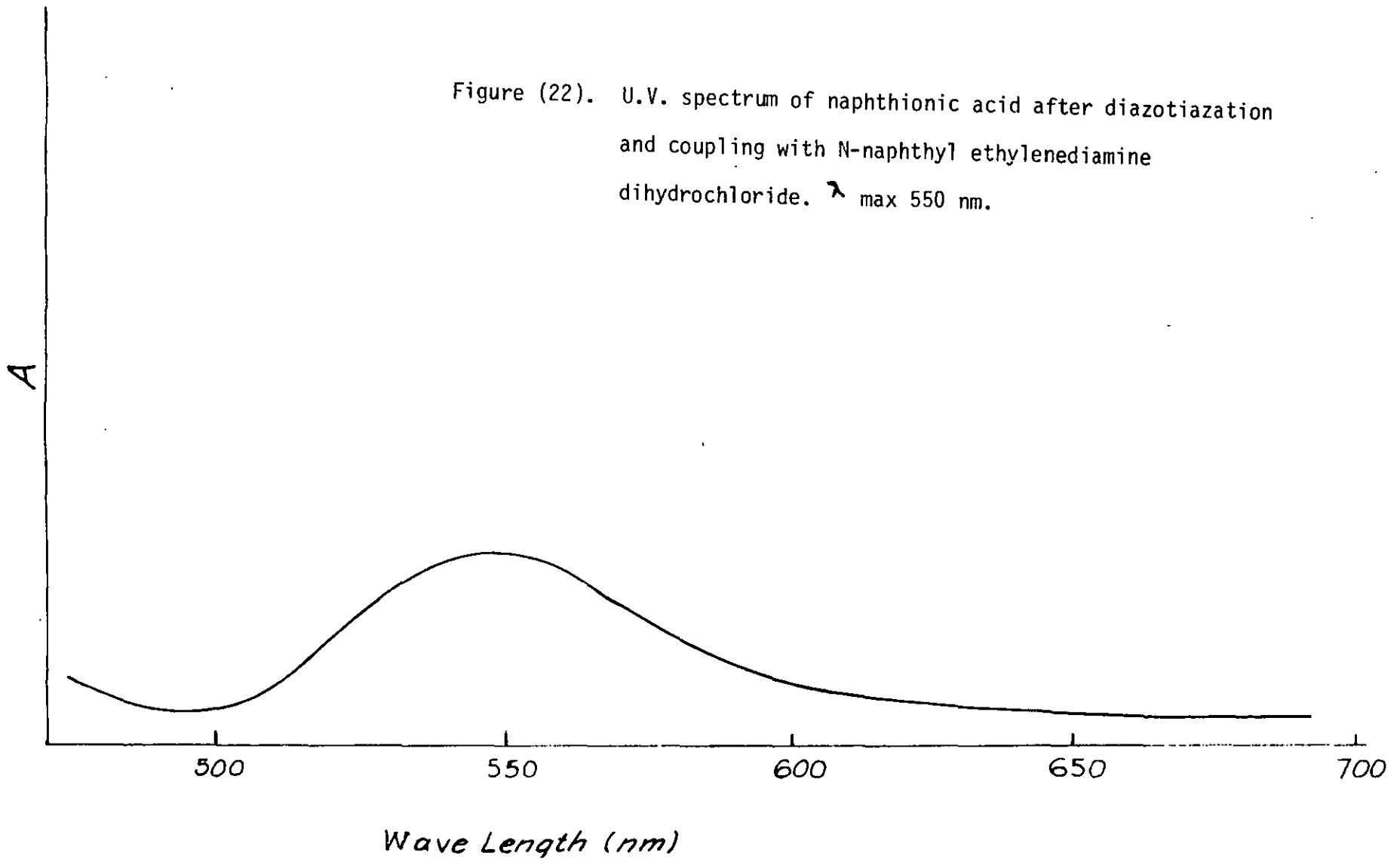
Effect of EDTA in stabilising ascorbic acid (1000 ppm) from oxidation by air in light degradation studies is seen from the figures given in Table (8).

As ascorbic acid is a reducing agent it was anticipated that reductive splitting of the azo group to form the corresponding amines might occur in the presence of intense light, and this was found to be the case. For this reason the feasibility of using spectrophotometric methods to detect and determine these amines was investigated.

The structures of the permitted synthetic food colouring matters containing azo bonds are given in Table 3. Clearly if reductive splitting occurs Red 2G should give aniline, Sunset Yellow FCF, Yellow 2G, Tartrazine, Black PN and Brown FK should give sulphanilic acid, and Amaranth, Carmosine, Ponceau 4R and Chocolate Brown HT should give naphthionic acid, in addition to larger amines. Aniline and sulphanilic acid are known to diazotise and couple with N-1-napthylethylenediamine in dilute hydrochloric acid solution, but naphthionic acid was found to require more concentrated acid conditions in order to react and a satisfactory procedure was developed for this. The maximum absorbance was found to be at 550 nm see Fig (22). The relationship between absorbance and time is shown in Fig. (23). Molar absorbitivity was found to be 29.5×10^4 $\ell\ mol^{-1} cm^{-1}$. Certain other large amines were tested and were found not to react under the conditions developed for the spectrophotometric determination of naphthionic acid and it is possible that there is little or no interference in these methods from the other amines formed during the degradation of the food colours shown in Figs.(8-15). Ascorbic acid was found to interefere with the spectrophotometric reactions and any ascorbic acid remaining in the degraded solution was destroyed carefully with potassium permanganate before determining the amine.

When these methods were applied to the degradation studies the

Figure (22). U.V. spectrum of naphthionic acid after diazotiazation and coupling with N-naphthyl ethylenediamine dihydrochloride. λ max 550 nm.



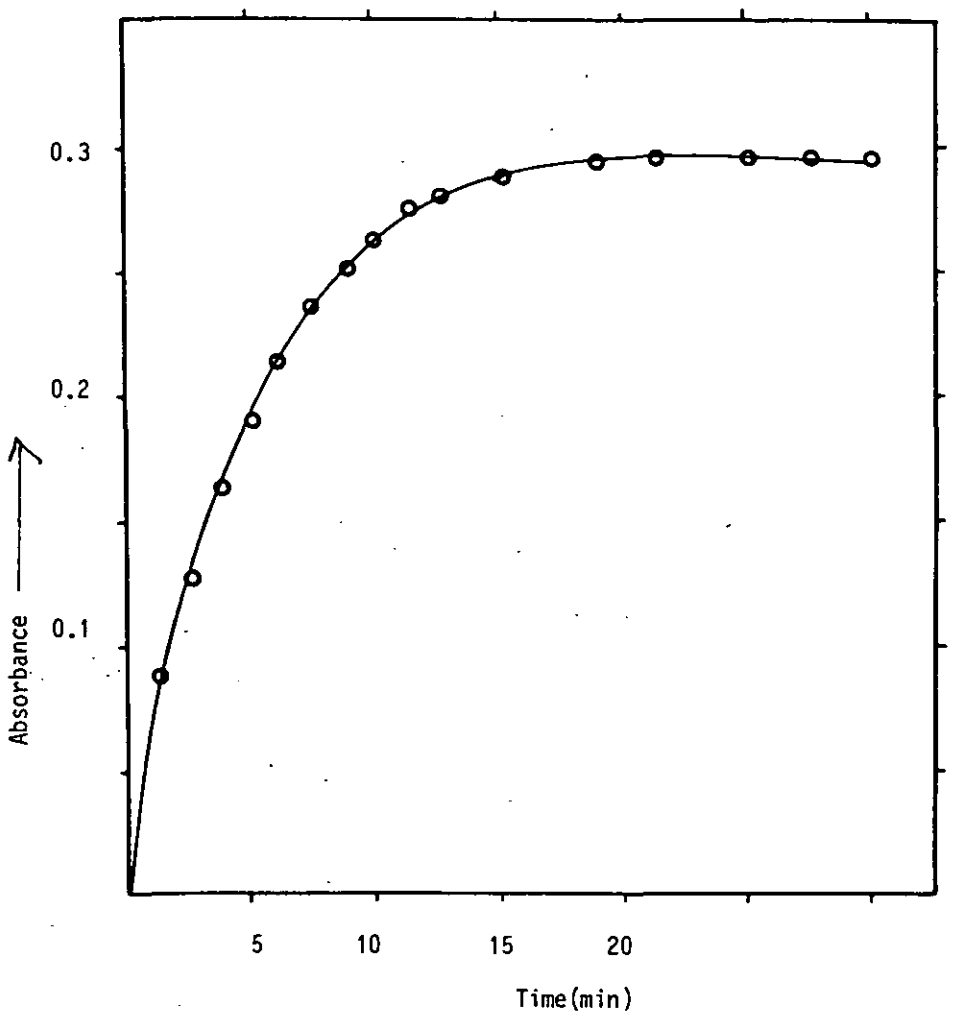


Figure 23. The dependence of absorbance of naphthionic acid derivative on time.

yields of the simpler amines, such as sulphanilic acid, were found to increase to almost quantitative amounts with increased time of degradation, but then to decrease markedly again. Subsequently aniline, sulphanilic acid and napthionic acid were shown to degrade under the conditions of the light degradation. Ammonia was shown to be formed and this was determined polarographically or spectrophotometrically using the indophenol method. In this study the ammonia was distilled before being determined as minor interferences were observed from other degradation products which underwent indophenol type reactions when the indophenol method was applied directly to the degraded solutions. An attempt to determine the stoichiometry of the reaction of food colour and ascorbic acid was made for those food colours which degraded rapidly in the presence of ascorbic acid. A typical degradation plot is shown in Fig. (24) and the actual polarograms shown in Fig. (25). The initial degradation of ascorbic acid ceased after food colour had disappeared visually. This amount of ascorbic acid corresponds to a stoichiometry of 1 to 4 for food colour to ascorbic acid for Chocolate Brown HT, Black PN and Brown FK. The other food colours gave ratios of 1 to 2. The following example illustrates the method of calculating the stoichiometric relationship.

A mixture of (5 ppm) Chocolate Brown HT and 1000 ppm ascorbic acid:

Note: ascorbic acid diluted 10 fold with water before polarographing. 100 ppm ascorbic acid gives 36 μA peak height.

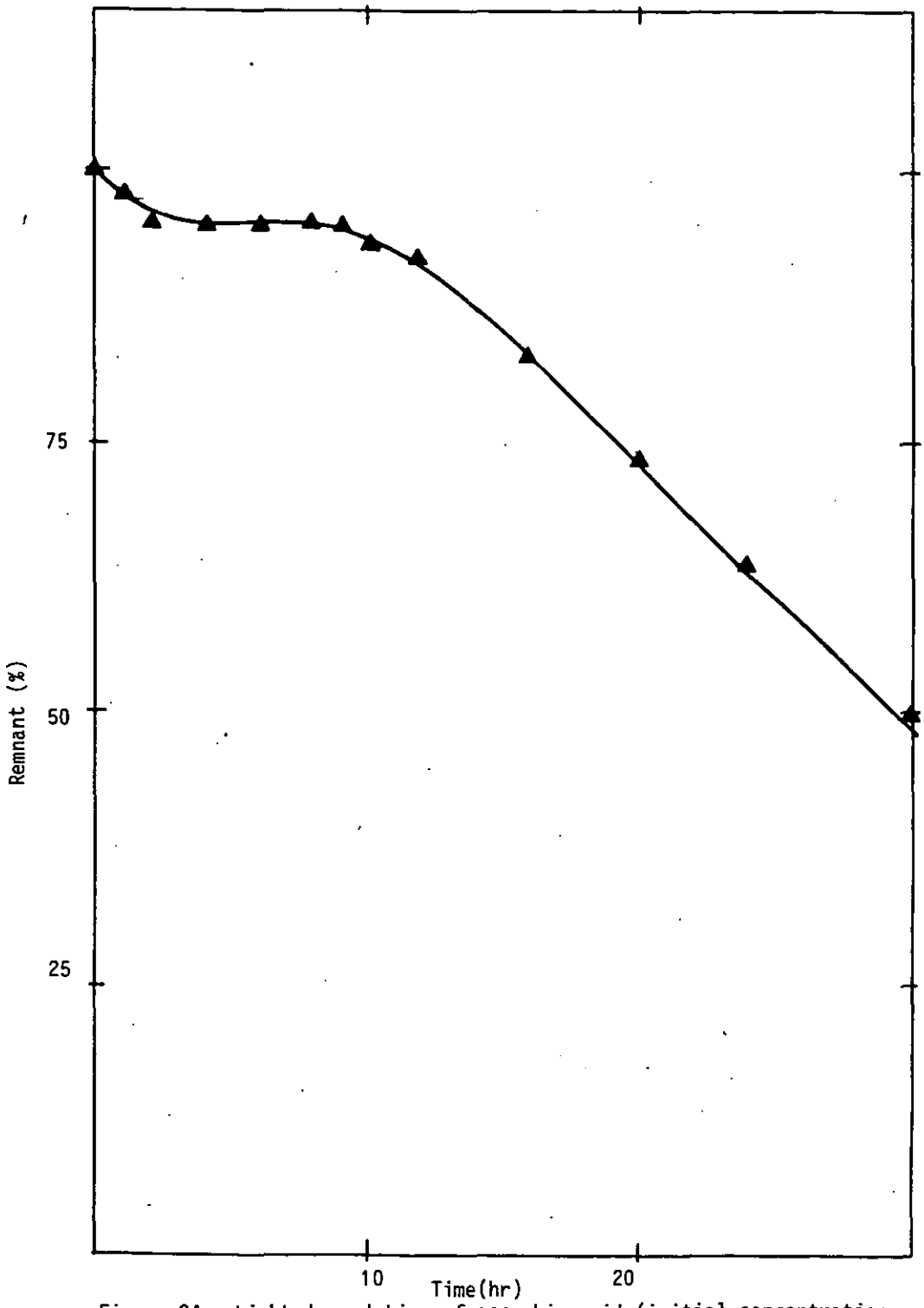


Figure 24. Light degradation of ascorbic acid (initial concentration = 1000 ppm) in the presence of Chocolate Brown HT initial concentration = 5 ppm, EDTA = 25 ppm. Solution became colourless after 2 hrs.

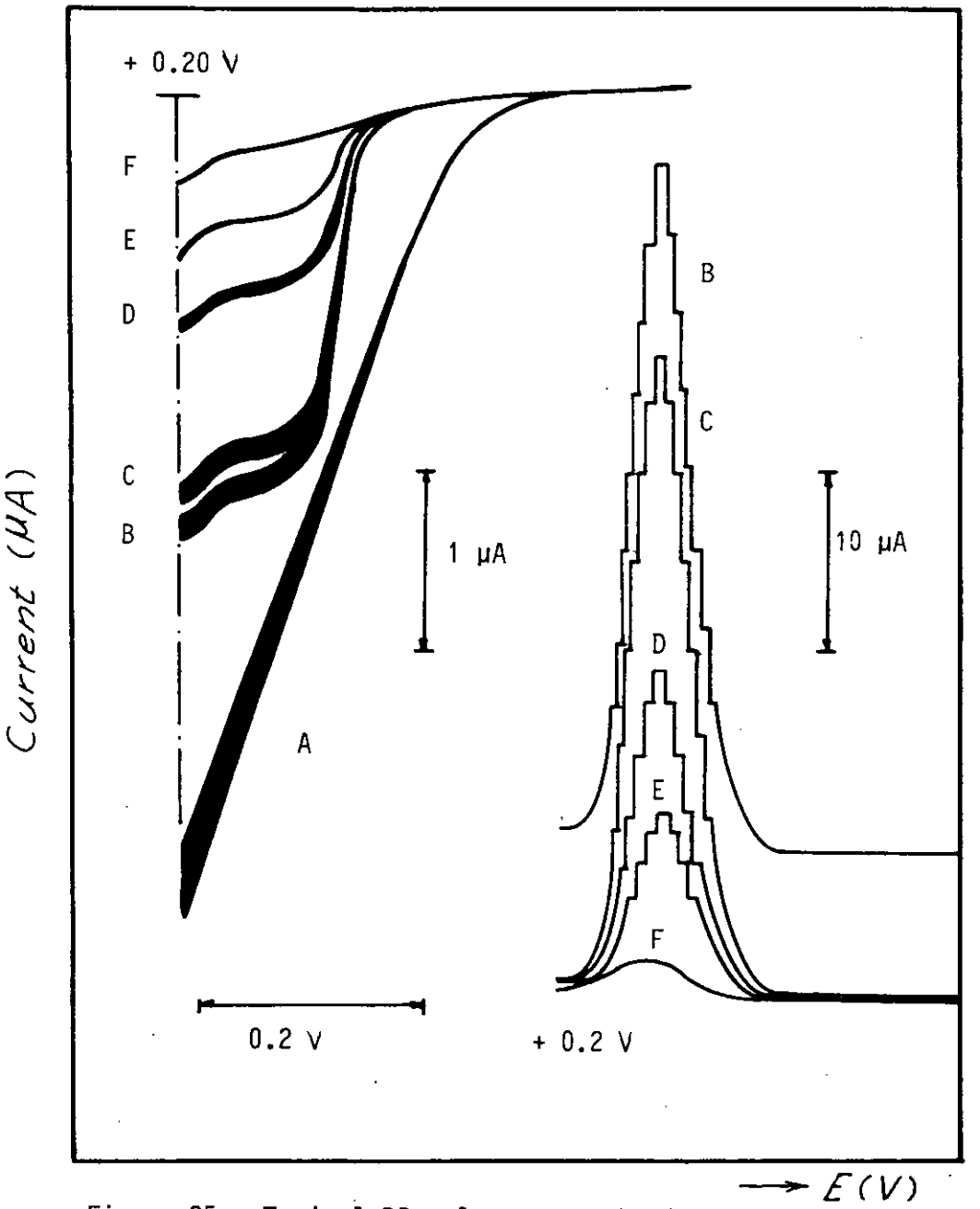


Figure 25. Typical DP polarograms obtained for ascorbic acid, initial concentration = 1000 ppm in the presence of Chocolate Brown HT initial concentration = 5 ppm. EDTA concentration = 25 ppm. Solution became colourless after 2 hrs. Light degradation.

(A) 1000 ppm
(B) 1000 ppm diluted to 100 ppm before polarography (Fresh solution)
(C) (2-8) hrs
(D) 30 hrs
(E) 50 hrs
(F) 74 hrs.

$\therefore$ $\frac{100}{36}$ = 2.77 ppm i.e. 2.77 ppm gives 1 μA

after the loss of 5 ppm food colour the remnant of ascorbic acid (100 ppm) is 33.4 μA.

$\therefore$ 36-33.4 = 2.6 μA used

2.6 x 2.77 = 7.22 ppm ascorbic acid

7.22 x 10 = 72.2 ppm of ascorbic acid reacted with 5 ppm food colour.

$\frac{72.2}{5}$ = 14.4 ppm ascorbic acid for each 1 ppm food colour.

The molar ratio is 3.7.

$\therefore$ $\frac{14.4}{3.7}$ = 3.90 = 1:4

1 mole of food colour reacted with 4 moles of ascorbic acid.

Some time after the disappearance of the food colour degradation of ascorbic acid accelerated again and the rate of degradation became more rapid than it would have been in the absence of the food colour degradation products. It may be significant that the time at which the rate of ascorbic acid degradation increases again (10 hours, see Fig. (26)) corresponds with that at which there is a marked increase in the production of ammonia. The yield of ammonia remains essentially constant during the period of ascorbic acid stability (2-10 hours).

Data on the degradation of sulphanilic acid, naphthionic acid and aniline in the presence and absence of ascorbic acid is given in Table (9). The percentage of amine remaining and the percentage yield of ammonia (based on the formation of one ammonia molecule per

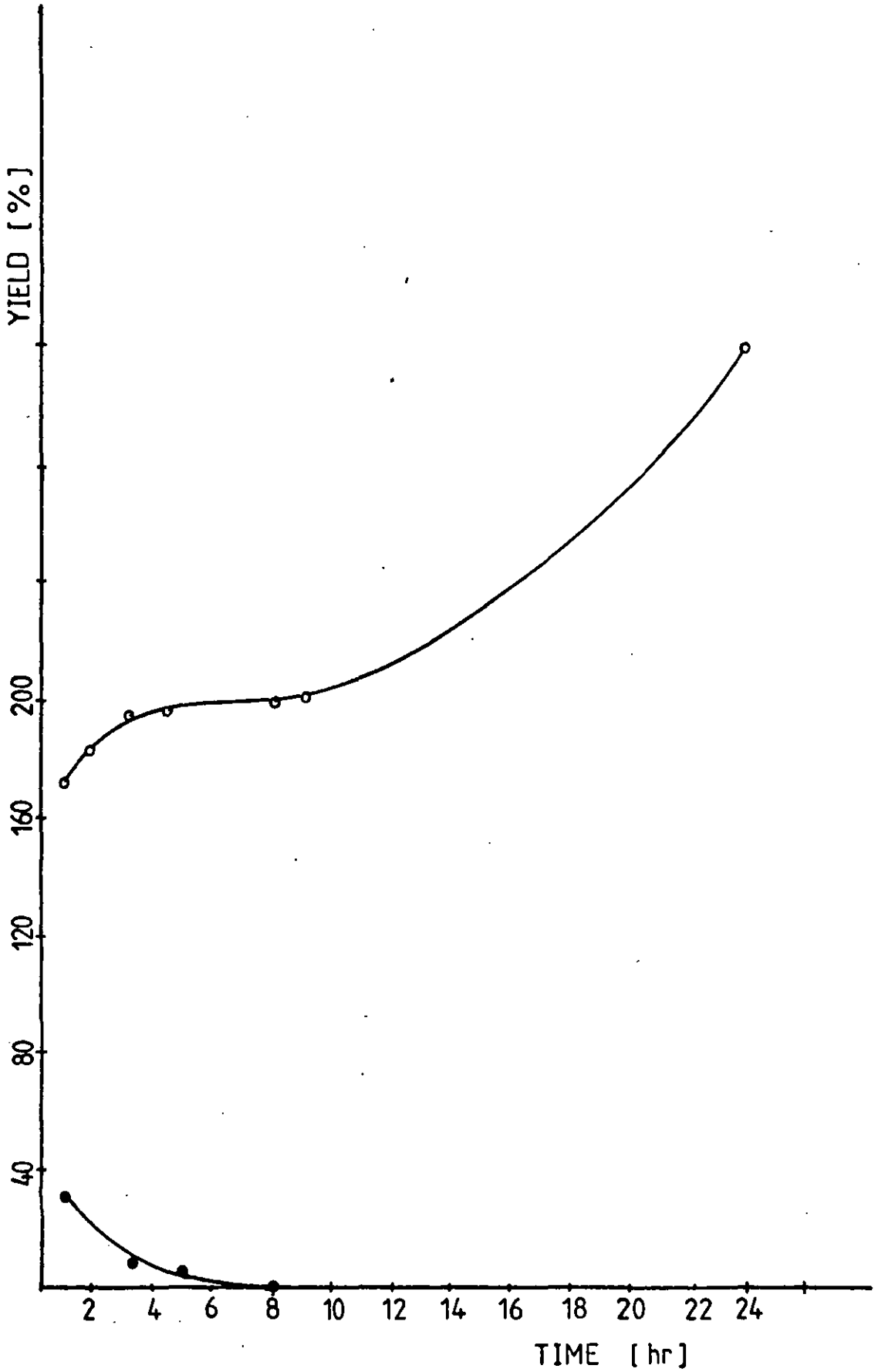


Figure 26. Light degradation of Chocolate Brown HT (5 ppm) in the presence of ascorbic acid (1000 ppm) showing the formation of ammonia.

amine molecule) are given. Napthionic acid clearly degrades rapidly giving a full yield of ammonia within 16 hours even in the absence of ascorbic acid: in its presence this time is halved. Sulphanilic acid is considerably more stable than is naphthionic acid, 15 days being required for complete loss in the presence of ascorbic acid and much longer in its absence. Aniline is much more stable than the other two amines although even for aniline 50% degradation to ammonia was observed after 14 days in the presence of ascorbic acid.

In Table (10) data is given concerning the formation of amine-aniline, sulphanilic acid or naphthionic acid and of ammonia during the degradation of the food colours in the presence of 1000 ppm of ascorbic acid. All the data was obtained after the complete loss of food colour from the solution, and after removal of any remnant ascorbic acid with potassium permanganate. Thus in all cases maximum formation of amine is seen as the first measurement time - usually after one additional hour of light treatment - and even by this time ammonia has been formed indicating that some of the amine has already degraded. Clearly ammonia can be formed from both amines formed by cleavage of the azo double bond and this is apparent in the results. Red 2G, Sunset Yellow FCF, Tartrazine, Carmoisine, Ponceau 4R and Amaranth, all of which contain a single azo group, give 200% yield of ammonia indicating that not only do the amines that have been determined - aniline, sulphanailic acid and naphthionic acid - degrade, but also those with more complicated structures formed from the part of the molecule on the other side of the azo bond. This is probably to be expected in view of the increasing ease of degradation from aniline to naphthionic

acid. Black PN and Chocolate Brown HT, which have two azo groups in their structures, give a yield of 400% of ammonia, typical polarograms of full yield of ammonia resulted from degradation of Sunset Yellow FCF and Chocolate Brown are shown in Figures (27,28). Brown FK, which is a mixture of related compounds (168) having 1-3 azo groups, gives a yield of 200%. No results have been given in Table (10) for Yellow 2G owing to the good light stability of this food colour (see Table (7)): the eventual yield of ammonia was confirmed as being 200 per cent.

DISCUSSION

The present study has provided information on the enhanced degradation of food colouring matters and ascorbic acid in the presence of each other during light degradation. The formation of aniline, sulphanilic acid and napthionic acid during these degradations has been demonstrated and their concentrations have been determined spectrophotometrically. These simple amines have been shown to degrade further giving ammonia. Naphthionic acid, in particular, degrades rapidly in this way even in the absence of ascorbic acid, although the degradation is even more rapid in its presence. Sulphanilic acid gives appreciable amounts of ammonia within a few hours in strong light in the presence of ascorbic acid, whereas ammonia is only observed after several days in the absence of ascorbic acid. Aniline is much stabler than is sulphanilic acid.

Quantitative yields of ammonia are obtained in all cases indicating that both nitrogen atoms in the azo linkages are converted to ammonia eventually. In the case of Red 2G and Black PN additional ammonia

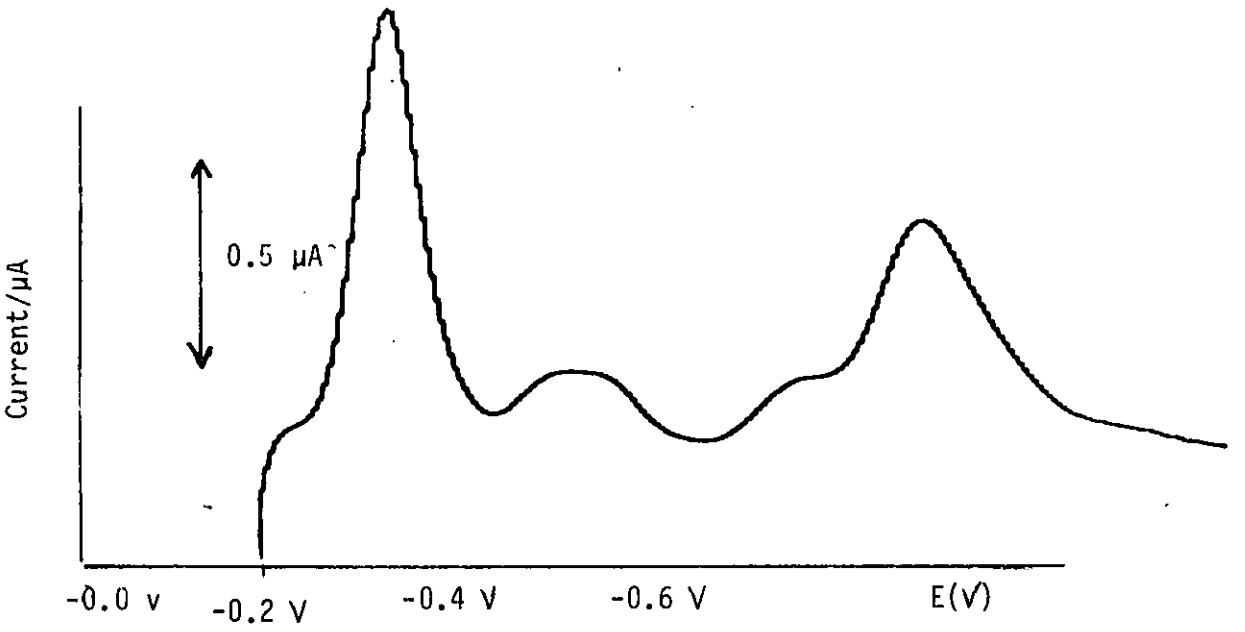
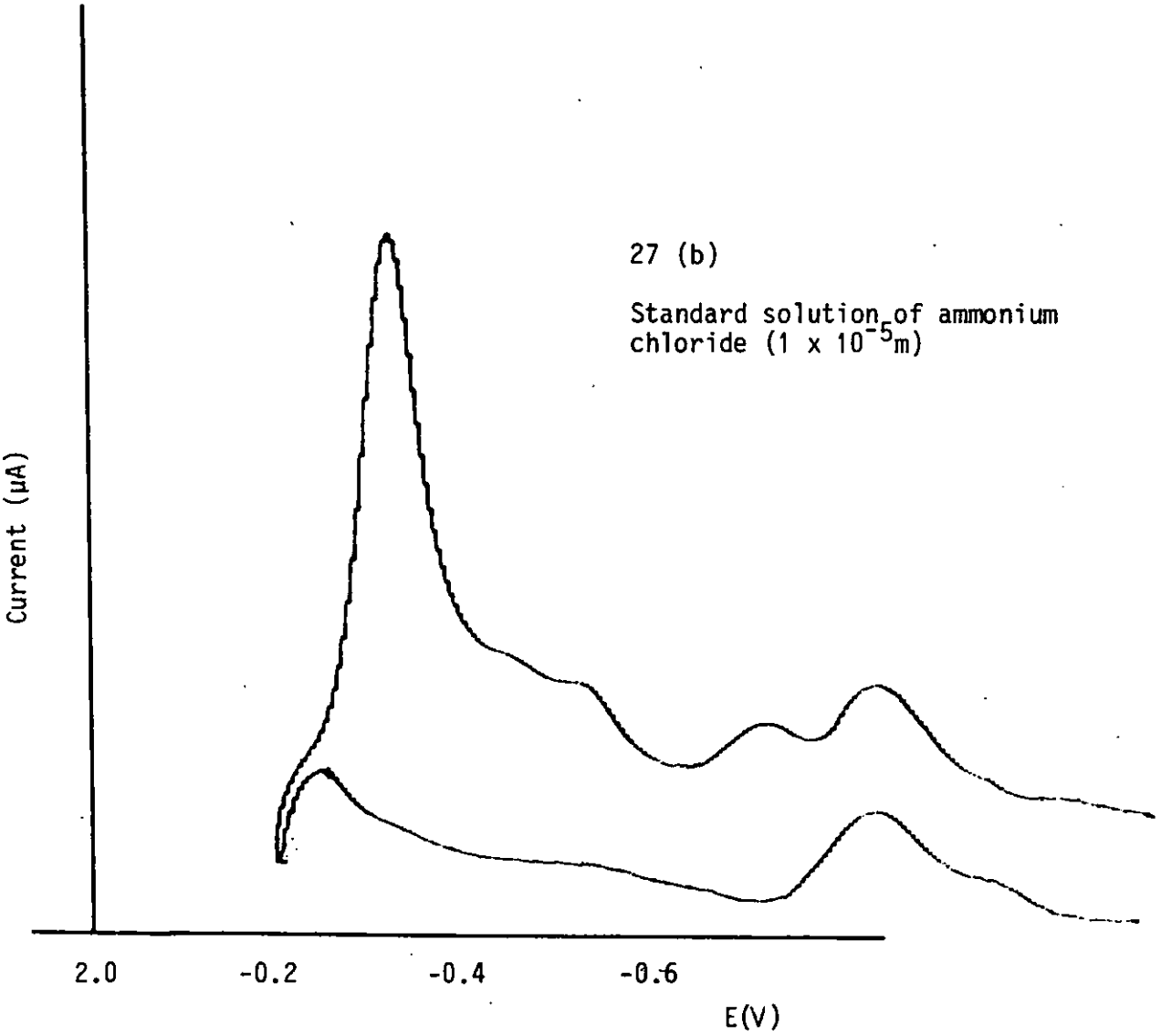


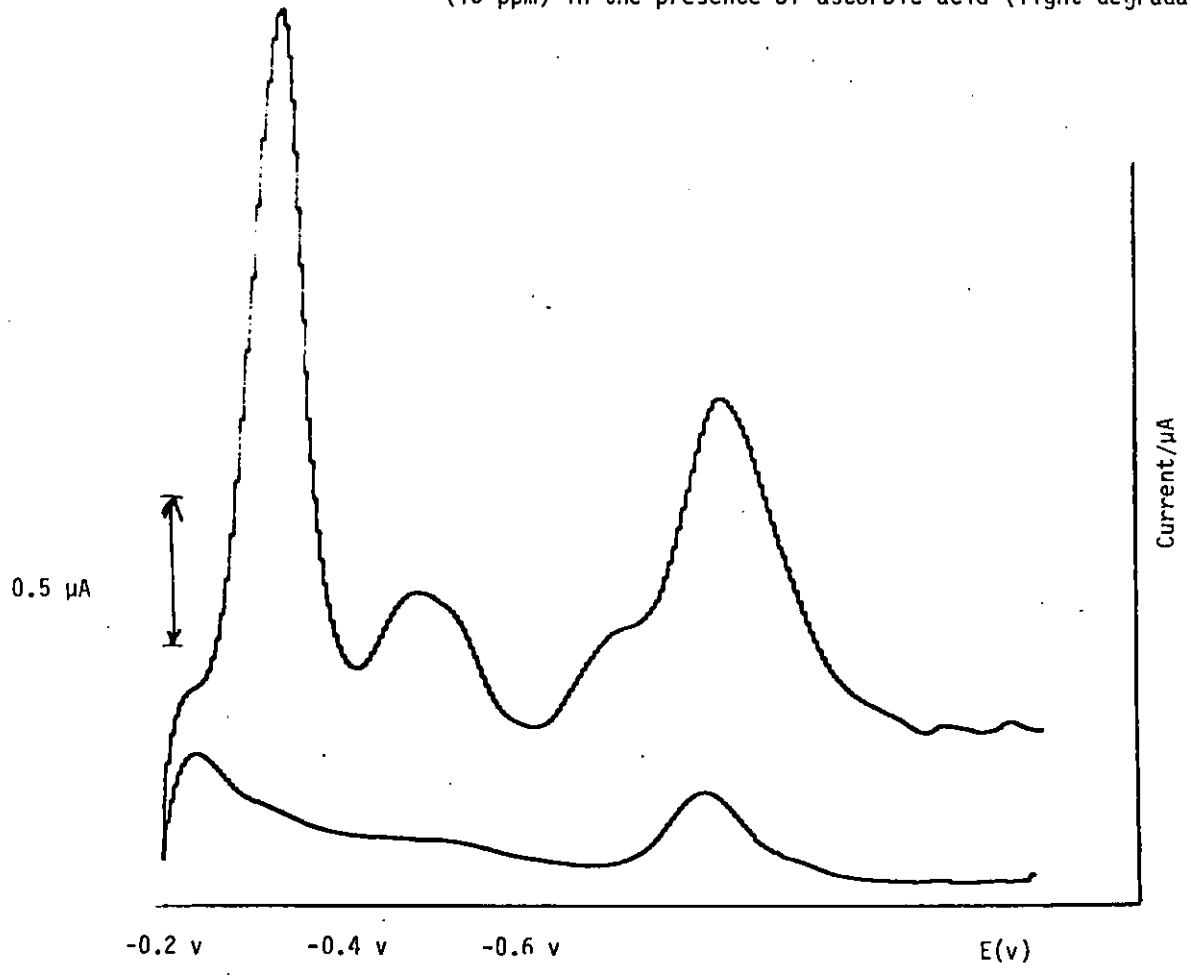
Figure 27. Typical DP polarograms obtained for ammonia formed from the degradation of Sunset Yellow FCF (10 ppm) in the presence of ascorbic acid (light degradation).



27 (b)

Standard solution of ammonium chloride (1×10^{-5}m)

Figure 28. Typical DP Polarogram obtained for Ammonia formed from the Degradation of Chocolate Brown HT (10 ppm) in the presence of ascorbic acid (light degradation).



might have been expected from photolysis of the acetamino groups in these molecules, but this was not observed. Brown FK gave a yield corresponding to the formation of two ammonia molecules per molecule: as Brown FK is reported to be a mixture of compounds containing from 1-3 azo groups and additional amino groups[168] a higher yield than this might have been expected. The light degradation technique might serve as a useful means of assessing samples of Brown FK.

Degradation in the present study was carried out in fairly intense light. The purpose of using these accelerated degradation conditions was to make the identification of products easier. When a complete list of products is obtained it will still be necessary to show that these are formed under less extreme and more normal food processing and storage conditions, and to study the extent to which any of them are formed.

Another worker[111] in this laboratory has investigated the light and heat degradation of food colours by HPLC technique, see page (19).

It should be noted that the pH at which the present studies were carried out (pH 5.5) was chosen as being convenient for the polarographic determination of ascorbic acid. Addition of EDTA was made so that the reaction of ascorbic acid with dissolved molecular oxygen, which is extremely rapid, could be disregarded. Clearly

EDTA is not normally present in foodstuffs. Further, soft drinks normally have a pH of 2.5-3, and other additives such as preservatives, anti-oxidants and sugars may also have significant effects on the stability of the food colours.

TABLE (7)

LIGHT DEGRADATION OF FOOD COLOURS (5ppm) AND ASCORBIC ACID (1000PPM) IN A MIXTURE

Food Colour	Time for loss of half of food colour in absence of ascorbic acid/h	Time for loss of all food colour in presence of 1000 ppm of ascorbic acid/h
Chocolate Brown HT	73	1
Carmoisine	**	2
Black PN	**	2
Brown FK	92	3
Ponceau 4R	80	12
Red 2G	**	23
Sunset Yellow FCF	**	24
Amaranth	**	24
Tartrazine	**	42
Green S	**	52
Brilliant Blue FCF	**	64
Patent Blue V	**	64
Yellow 2G	130	>96*
Quinoline Yellow	70	>96*

* Ascorbic acid degrades completely first

** > 2 weeks.

TABLE (8)

EFFECT OF EDTA IN STABILISING ASCORBIC ACID (1000 ppm) FROM OXIDATION BY AIR DURING LIGHT DEGRADATION

(a) Without EDTA

Time/h	0	5	10	20
Remnant of Ascorbic Acid % of Original	100	50.2	20.4	3.1

(b) With EDTA (0.0025%)

Time/h	0	20	45	65	90	130
Remnant of Ascorbic Acid % of Original	100	100	61.4	45.7	17.1	1.42

TABLE (9)

LIGHT DEGRADATION OF SULPHANILIC ACID, NAPHTHIONIC ACID AND ANILINE WITH AND WITHOUT ASCORBIC ACID PRESENT

(a) Naphthionic acid

Time/h	With ascorbic acid		Without ascorbic acid	
	Naphthionic acid remaining %	Molar yield of Ammonia remaining %	Naphthionic acid remaining %	Molar yield of Ammonia remaining %
0	100	10*	100	10*
1.5	27.2	84.1	62.2	48.4
5	4.2	98.6	24.7	85.4
7.5	1.5	100.1	24.7	86.1
12	0	100.6	4.9	96.6
16	0	100.4	0	100.2

* These results indicate that some ammonia is obtained on heating naphthionic acid with sodium hydroxide solution. The results in this column should be viewed accordingly.

(b) Sulphanilic acid

Time	With ascorbic acid		Without ascorbic acid	
	Sulphanilic acid remaining %	Molar yield of Ammonia remaining %	Sulphanilic acid remaining %	Molar yield of Ammonia remaining %
/hours				
1	92.2	9.8		
3	77.8	18.5		
5	73.8	23.1		
15	71.7	26.8		
20	70.8	28.3		
/days				
2	62.3	36.5		
4	49.3	45.6	100	0
8	27.4	67.4		
10	19.1	77.0	66.6	24.1
12	11.3	87.1		
15	0	95.7	66.0	24.1
21	-	-	61.5	29.3

(c) Aniline

Time/days	Aniline remaining %	Molar yield of Ammonia remaining %	Aniline remaining %	Molar yield of Ammonia remaining %
7	100	0	100	0
12	6.6	95.9	85.8	14.8
20	0	99.9	39.1	62.4
25	-	-	11.4	91.7

TABLE (10)

FORMATION OF SIMPLE AMINES AND AMMONIA ON LIGHT DEGRADATION OF AZO FOOD COLOURS IN THE PRESENCE OF ASCORBIC ACID

The amines and ammonia were not determined until all trace of the food colour had disappeared visibly. The time for this is given in parenthesis with the name of the food colour. The additional times of light treatment before measurement are given in the tables.

(a) Red 2G (23 hours)

Additional Time	Molar Yield of Aniline %	Molar Yield of Ammonia %
/h		
1	84.7	-
5	59.6	145
10	52.1	48
20	45.7	155
/d		
2	40.1	160
6	34.7	168
10	18.6	182
15	0	196

(b) Sunset Yellow FCF (24 hours)

Additional Time	Molar Yield of Sulphanilic Acid /%	Molar Yield of Ammonia /%
/h		
1	120.4	90.1
5	66.0	137
10	58.4	141
20	52.2	147
/d		
2	44.9	153
6	34.9	166
10	18.9	181
15	0	196

(c) Tartrazine (42 hours)

Additional Time	Molar Yield of Sulphanilic Acid /%	Molar Yield of Ammonia /%
/h		
5	67.3	138
10	58.3	148
20	51.4	149
/d		
2	44.9	153
6	34.8	158
10	18.6	181
15	0	196

(d) Black PN (2 hours)

Additional Time	Molar Yield of Sulphanilic Acid /%	Molar Yield of Ammonia /%
/h		
1	62.1	140
5	37.9	158
10	34.8	162
20	29.3	176
/d		
2	17.6	241
8	9.7	363
10	0	402

(e) Brown FK (3 hours)

Additional Time	Molar Yield of Sulphanilic Acid /%	Molar Yield of Ammonia /%
/h		
1	86.7	110
5	59.8	141
10	51.2	145
20	47.6	151
/d		
3	43.4	157
8	25.5	173
16	0	198

(f) Carmoisine (2 hours)

Additional Time/h	Molar Yield of Naphthionic Acid /%	Molar Yield Ammonia /%
1.5	26.3	183
5	3.7	199
7.5	1.4	199
24	0	201

(g) Ponceau 4R (12 hours)

Additional Time/h	Molar Yield of Naphthionic acid /%	Molar Yield of Ammonia /%
1.5	25.9	184
5	3.6	200
7.5	1.2	205
12	0	206
24	0	207

(h) Amaranth (24 hours)

Additional Time/h	Molar Yield of Naphthionic Acid /%	Molar Yield of Ammonia /%
1.5	24.8	186
5	3.3	199
7.5	1.4	203
12	0	203
24	0	204

(i) Chocolate Brown HT (1 hour)

Additional Time/h	Molar Yield of Naphthionic Acid /%	Molar Yield of Ammonia /%
1.5	25.6	182
5	3.8	199
7.5	1.0	201
12	0	221
24	0	399

FURTHER DIFFERENTIAL-PULSE POLAROGRAPHIC AND VISIBLE SPECTROPHOTOMETRIC STUDIES OF THE DEGRADATION OF PERMITTED SYNTHETIC FOOD COLOURING MATTERS WITH AND WITHOUT THE ADDITION OF ASCORBIC ACID: DEGRADATION IN THE DARK AND IN THE LIGHT WITHOUT THE STABILISING ACTION OF EDTA

Previously, differential-pulse polarography was used to monitor the interaction of permitted synthetic food colouring matters with ascorbic acid in accelerated light degradation studies. The loss of both colouring matter and ascorbic acid was shown to be accelerated when they were present together. Further, the complete conversion to ammonia of the nitrogen atoms in the azo group(s) of those food colouring matters that are azo compounds was demonstrated. The simple amine intermediates in these conversions, namely aniline, sulphanilic acid and naphthionic acid, were monitored by visible spectrophotometry using a diazotisation procedure. The rate of formation of ammonia from these amines by the action of light was shown to increase in the order aniline < sulphanilic acid < naphthionic acid. Naphthionic acid was particularly labile, as were other more complex amines formed in the degradation of the food colours. These degradation studies were performed in pH 5.5 acetate buffer solution, the ascorbic acid being protected from the oxidation by dissolved molecular oxygen by the addition of the disodium salt of ethylenediaminetetraacetic acid (EDTA).

In this section further studies of the degradation of food colouring matters with the addition of ascorbic acid are reported. In particular, the effect of omitting EDTA in the light degradation studies and the interaction of food colouring matters and ascorbic acid in the dark have been studied.

Experimental:

The food colouring matters and ascorbic acid were determined by differential-pulse polarography as previously described using a PAR 174A polarographic analyser (Princeton Applied Research) and a Gould H-2000 X-Y recorder. Spetrophotometric measurements were made with a Pye Unicam SP8-100 spectrophotometer.

The solutions were deoxygenated with nitrogen gas and were contained in tightly sealed autoclavable bottles. These bottles were also used in degradation studies carried out on solutions kept in the dark and these solutions were also deoxygenated.

Results:

Degradation of Food Colouring Matters and Ascorbic Acid in Accelerated Light Degradation Conditions Without EDTA Stabilisation

In this study the effect of omitting the EDTA from the accelerated light degradation study was investigated and the main degradation experiments were carried out in the presence of EDTA at high ascorbic acid concentrations. The results obtained are shown in Table (11) and should be compared with those given in Table (10) in the previous section. Results for Yellow 2G have been omitted in both instances as the ascorbic acid degrades before the food colouring matter. Clearly in some instances the presence of EDTA not only protects the ascorbic acid from interaction with oxygen but also stabilises the food colouring matters with respect to reaction with ascorbic acid. Thus the times for the complete loss of food colouring matter with and without EDTA are 23 and 10 h (Red 2G), 24 and 6 h (Sunset Yellow FCF), 12 and 4 h (Ponceau 4R) and 24 and 4 h (amaranth), respectively. The formation of the full yield of ammonia from the intermediate amines is also much faster in the absence of EDTA for Red 2G, Sunset Yellow FCF and Brown FK. The formation

of amines and ammonia from the food colouring matters in the absence of ascorbic acid has so far been followed only for Chocolate Brown HT and Brown FK, because the food colouring matters are considerably more stable with, or without, the presence of EDTA under these conditions. Chocolate Brown HT had degraded visibly after 21 d when a 400% yield of ammonia and no naphthionic acid were recorded. Brown FK disappeared visibly after 2 months when the yields of ammonia and sulphanilic acid were 180 and 22%,respectively.

An unusual effect of omitting the EDTA was observed for Red 2G and Black PN. In the presence of EDTA these two food colouring matters gave final molar yields of ammonia of 200 and 400%, respectively, indicating the complete conversion of one and two azo groups, respectively, to ammonia. In the absence of EDTA the yields of ammonia were 300 and 500%, respectively, which indicates that in the absence of EDTA the acetamido groups in these food colouring matters are being hydrolysed and converted to ammonia, whereas in the presence of EDTA they are not. In general the acetamido groups are stabilised in this way and this was confirmed by tests on solutions of acetanilide. After 2 d of light degradation, no ammonia was formed from acetanilide in the presence of EDTA, but a molar yield of 98.2% was obtained in its absence.

Degradation of Food Colouring Matters and Ascorbic Acid in the Dark at Room Temperature

As the food colouring matters degraded extremely rapidly under accelerated light conditions in the presence of ascorbic acid without EDTA, a study was made to determine whether any interaction could be observed in the dark in the absence of EDTA. The stabilising effect of EDTA on ascorbic acid in the dark is seen from the results given in Table (12) and the

degradation of naphthionic acid, sulphanilic acid and aniline in the dark in the presence of ascorbic acid but without EDTA is shown in Table (13). Formation of ammonia from all three amines over a period of several days is apparent, but a feature of the degradation of napthionic acid is that after the initial degradation period some of the amine remains intact. This might be expected as the ascorbic acid has degraded completely by this stage. On the other hand, the degradation of sulphanilic acid and aniline continued to some extent after the ascorbic acid had degraded. For naphthionic acid the further degradation of the amine on the addition of more ascorbic acid later in the degradation study was confirmed (Table (14)).

The food colouring matters were found generally to degrade completely within 3 weeks in the dark in the presence of high concentrations of ascorbic acid (Table 15); Black PN degraded rapidly within 1 d. The amounts of simple amine and ammonia formed are given in Table (14). The stability of some proportion of the simple amines formed in these degradations, and in particular of naphthionic acid, is clearly seen. This would be expected from the degradation results with the simple amines (Table C). The final yield of undegraded napthionic acid formed from carmoisine (47%), Ponceau 4R%) and amaranth 47%) are similar to the percentage remnant when naphthionic acid is treated similarly (41%), whereas the remaining yield from Chocolate Brown HT (84%) is considerably larger. This latter yield is consistent, however, as one molecule of Chocolate Brown HT gives two molecules of napthionic acid. The further degradation of the naphtionic acid on further addition of ascorbic acid was confirmed for carmoisine and Ponceau 4R (Table 14).

In previous heat degradation studies at intermediate temperatures (e.g.

80°C) deacetylation of the acetamido group of Red 2G was observed and Red 10B was formed[270]. In this study Red 10B was formed during the interaction of Red 2G and ascorbic acid in the dark (see figure 29). The hue of the degrading solution and the half-wave potential of the polarographic wave were found to be changed in the same way as in the heat study. Black PN, which also has an acetamido group, degrades rapidly in the dark in the presence of ascorbic acid to give a pale yellow solution (at pH 5.5). When this solution was acidified to pH 2, the solution became purple. The solution at pH 5.5 exhibits a new differential-pulse polarographic peak close to that of Black PN but the wave is more clearly defined at pH 2 (E_p = 0.00 V). See Fig. (30). Better separation of the differential-pulse polarographic peaks of Black PN and this product can be achieved by the addition of tetraphenylphosphonium chloride in an analogous manner to that with Red 2G and Red 10B[270]. This product, formed from Black PN, may be the deacetylated derivative of Black PN, but sulphanilic acid and ammonia are also present in these solutions, which indicates that the product could be a monoazo dye. Some degradation of the azo bond that yields sulphanilic acid has clearly occurred in the solution. The formation and subsequent degradation of the new product can be followed polarographically.

Solutions of the triphenylmethane food colours, Green S, Patent Blue V and Brilliant Blue FCF, turned green on interaction with ascorbic acid in the dark and new differential polarographic peaks were observed. At pH 5.5 the peak potentials for Green S, Patent Blue V and Brilliant Blue FCF are at -0.64, -0.73 and -0.80 V, respectively. The corresponding peaks for their products are at -0.40, -0.56 and 0.40 V, respectively. See figure (31).

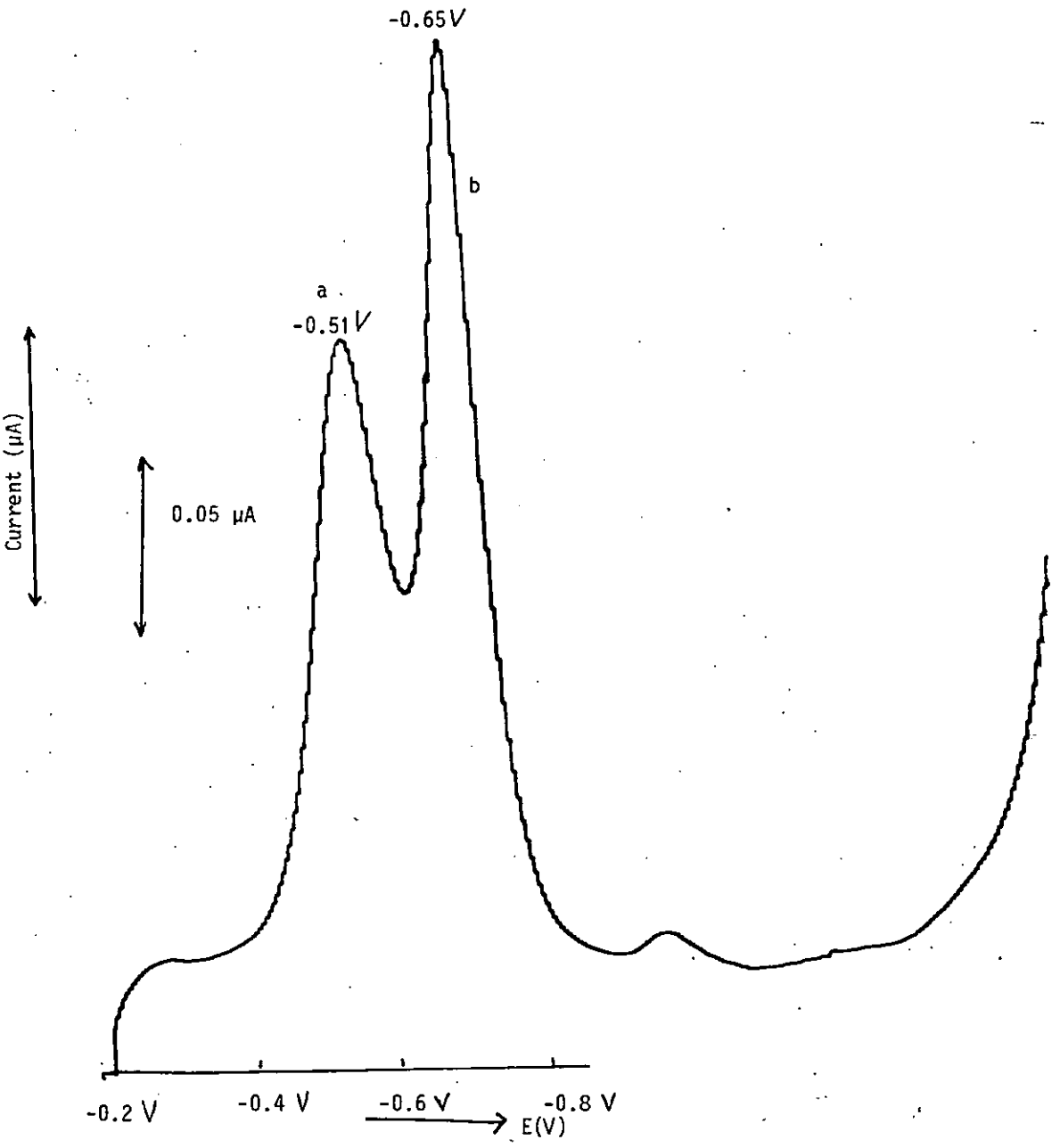


Figure 29. DP Polarogram obtained for Red 10 B formed from the degradation of Red 2G (5 ppm) during dark degradation studies in the presence of ascorbic acid and in the absence of EDTA (a) Red 2G (b) Red 10B

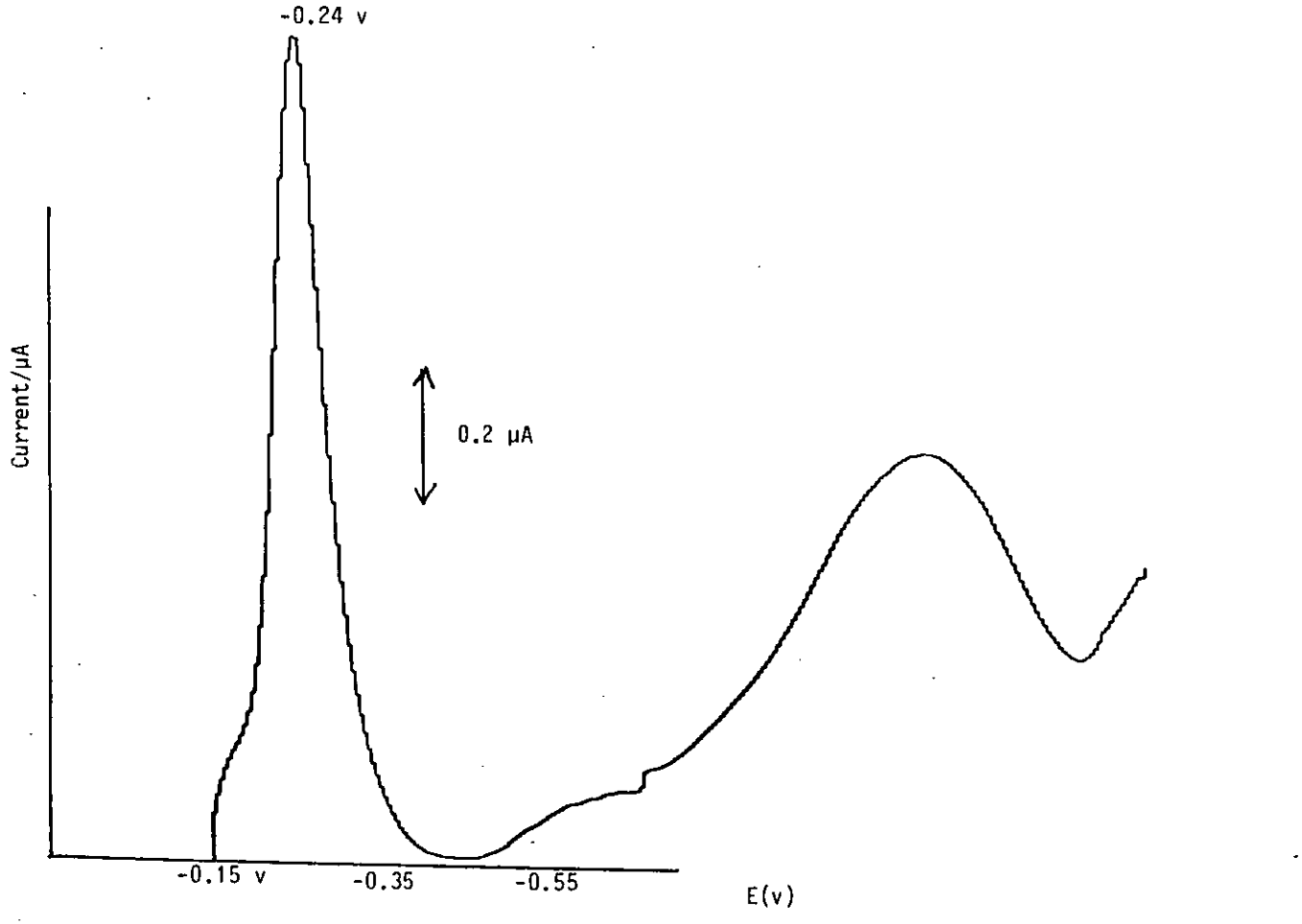


Figure 30. The New DP Polarographic Peak Obtained from the Degradation of Black PN (5 ppm) in the presence of ascorbic acid (1000 ppm) at pH 5.5 in acetate buffer (dark degradation studies).

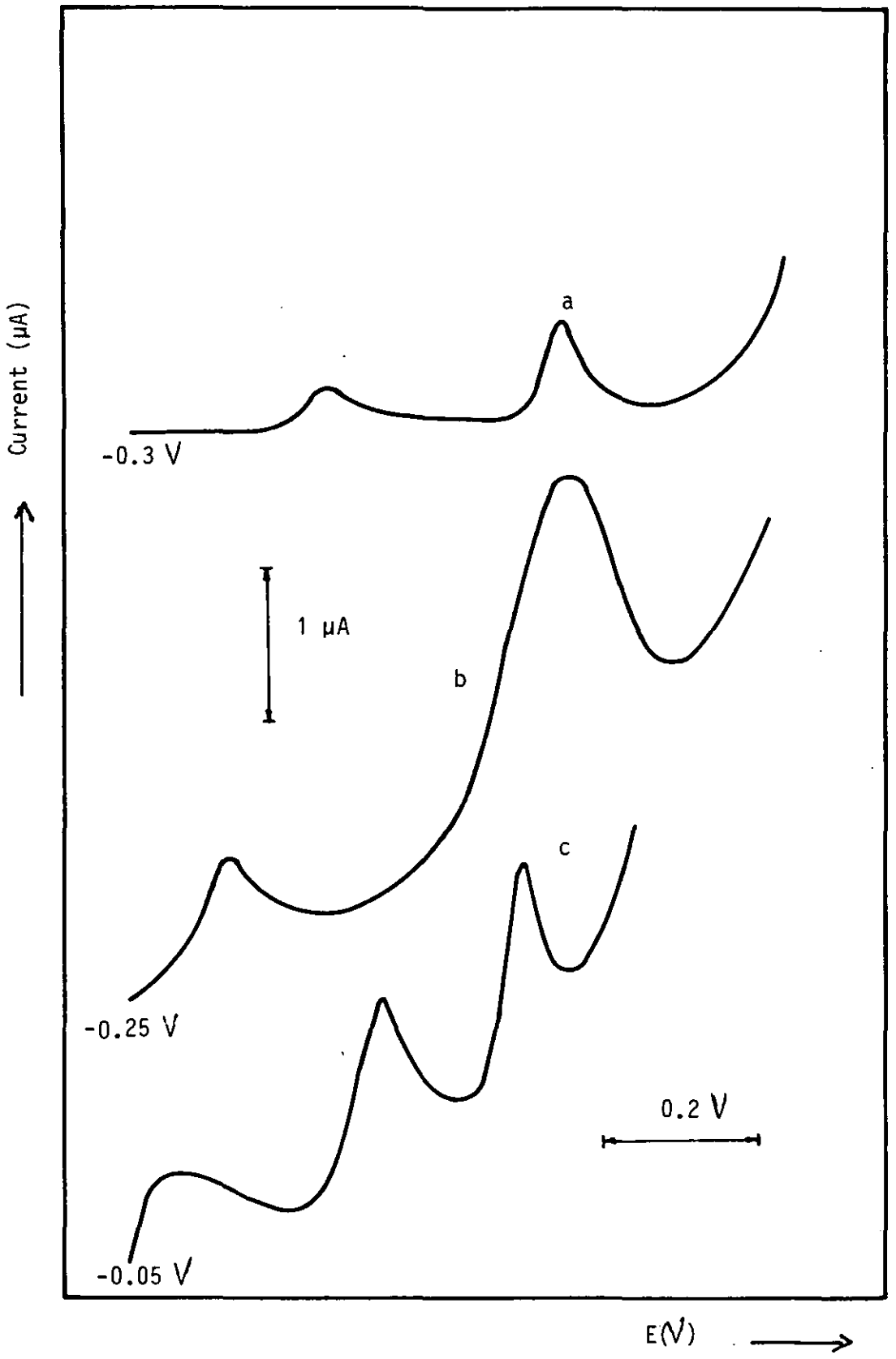


Figure 31. DP Polarograms obtained for the new compounds formed from the degradation of triphenylmethane compounds (5 ppm) in the presence of ascorbic acid (1000 ppm) in acetate buffer pH 5.5 (dark degradation studies)
(a) Patent Blue V
(b) Green S
(c) Brilliant Blue

The final observation made was that ascorbic acid solutions stored in the dark at pH 5.5 became yellow even in the absence of the food colouring matters. Further, a new cathodic polarographic wave appeared with a half-wave potential of -0.90 V and increased in size as the anodic wave of ascorbic acid at +0.05 V became smaller. The new compound gave an improved polarographic wave at pH 2 (Ep = -0.54 V). Neither the colour nor the new wave was observed in the accelerated light degradation studies. Figure (32) shows the polarographic peak of this compound at pH 2.0.

Discussion:

The previous study of the interaction of food colouring matters and ascorbic acid was carried out in the presence of EDTA. Ascorbic acid is very rapidly oxidised by dissolved molecular oxygen and EDTA was added to stabilise the ascorbic acid. This allowed the degradation of the food colouring matters in the presence of relatively low concentrations of ascorbic acid to be studied in order to obtain an indication of the stoicheiometry involved. EDTA is used extensively as an antioxidant synergist - stabilier,[279] and it is therefore not surprising that not only is the reaction of ascorbic acid with molecular oxygen inhibited but also its reaction with the food colouring matters. The degradation rate of food colouring matters with ascorbic acid has been shown here to be generally more rapid in the absence of EDTA and the results obtained under these conditions may be considered to be more relevant to actual food processing conditions, although other antioxidants are frequently present in foodstuffs. EDTA was also shown to protect the acetamido groups in Red 2G and Black PN from hydrolysis and the subsequent formation of ammonia. This effect seemed to have a wider application in the stabilisation of drugs, such as paracetamol, and studies on this have been carried out and the results are given in

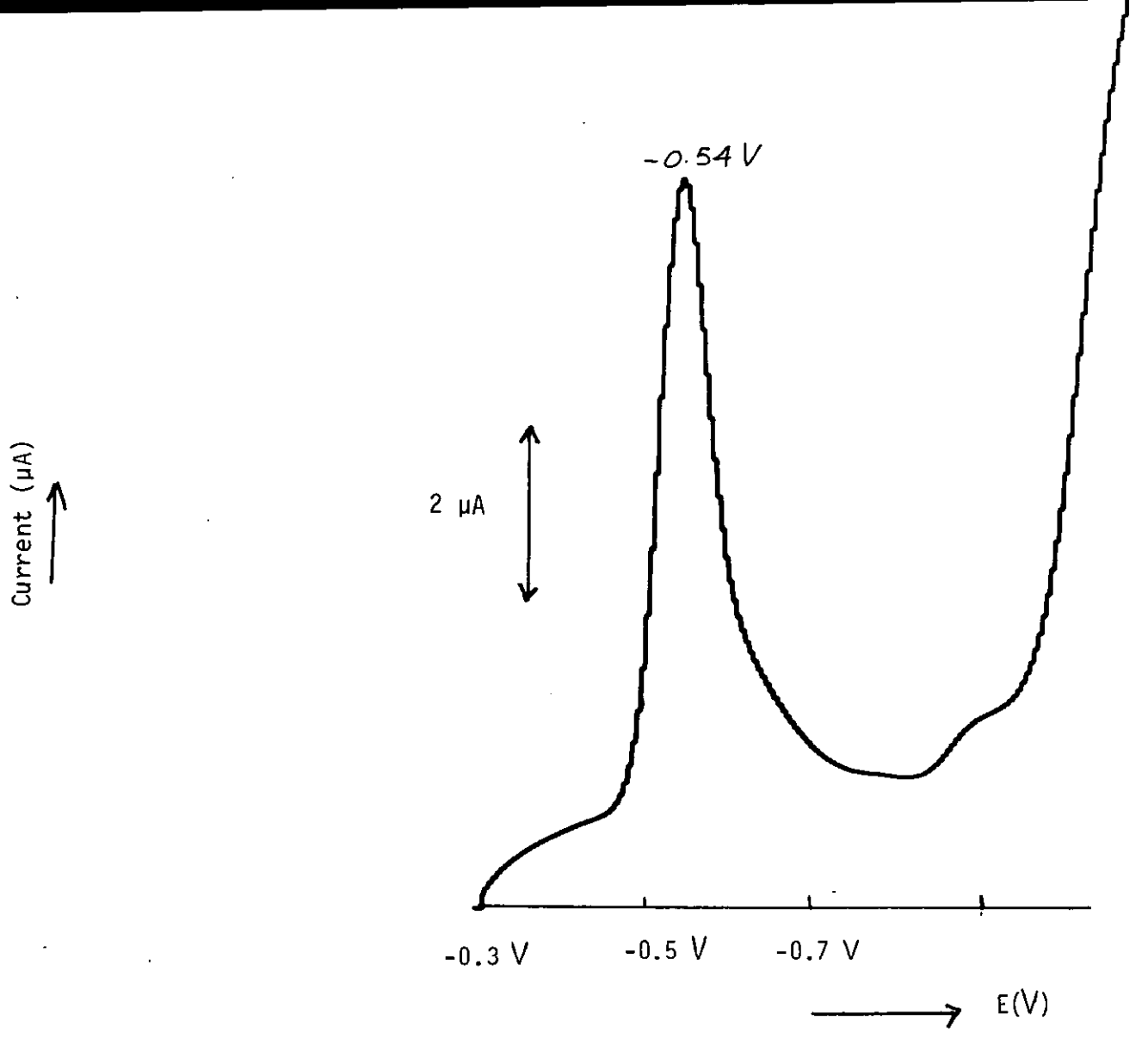


Figure 32. DP Polarographic Peak Obtained from the Degradation of Ascorbic Acid (1000 ppm) in acetate buffer pH 5.5 (40 days) dark degradation studies.

a later section.

The rate of degradation of food colouring matters in the dark in the presence of ascorbic acid was found to be faster than had been expected, but such degradation in blackcurrant health drinks, for example, must be well known in the soft drinks industry. A feature of these degradations in the dark under the conditions used here is that, as the ascorbic acid tends to degrade before the food colouring matter, simple amines such as naphthionic acid remain as final degradation products. For Red 2G a major degradation product is Red 10B and an intermediate coloured compound, possibly the deacetylated derivative, or a monoazo compound, is formed from Black PN. Black PN is particularly susceptible to interaction with ascorbic acid even in the dark. Coloured intermediates showing characteristic polarographic half-wave potentials were also observed for the triphenylmethane colours, Green S, Patent Blue V and Brilliant Blue FCF. The identity of the coloured product formed from ascorbic acid in the dark is not known. A possible product is 2,3 - diketogulonic acid but this has not been confirmed.

TABLE (11)

FORMATION OF SIMPLE AMINES AND AMMONIA ON LIGHT DEGRADATION OF AZO FOOD COLOURS IN THE PRESENCE OF ASCORBIC ACID BUT WITHOUT THE ADDITION OF EDTA

The amines and ammonia were not determined until all of the food colouring matter has disappeared visibly. The additional times of light treatment before measurement are given. All food colouring matter solutions were 10 ppm initially. The initial ascorbic acid concentration was 1000 ppm.

Food colour	Time for colour to disappear/h	Additional time/d	Amine formed	Molar yield of amine, %	Molar yield of ammonia, %
Red 2G	10	1	Aniline	98.5	160
		6		0	304
Sunset Yellow FCF	6	1	Sulphanilic acid	22.1	181
		6		7.9	195
Tartrazine	42	2	Sulphanilic acid	78.4	125
		6		62.5	140
		10		26.0	170
Black PN	4	1	Sulphanilic acid	43.3	310
		10		0	494
Brown FK	4	1	Sulphanilic acid	10.1	192
		6		7.6	192
Carmoisine	3	1	Naphthionic acid	0	200
Ponceau 4R	4	1	Naphthionic acid	0	200
Amaranth	4	1	Naphthionic acid	0	201
Chocolate Brown HT	3	1	Naphthionic acid	0	402

TABLE (12)

EFFECT OF EDTA IN STABILISING ASCORBIC ACID FROM OXIDATION BY AIR IN THE DARK

Results show remnant of ascorbic acid as a percentage of the original.

Conditions	Original concentration of ascorbic acid, p.p.m.	Time/d 0	1	2	3	4	5	16	22
Without EDTA	1000	100	-	-	-	-	47	13	0.7

		Time/d 0	4	30	36	50	75	82	90
With EDTA (0.0025%)	1000	100	-	55	30	4	-	-	-

TABLE (13)

DEGRADATION OF SULPHANILIC ACID, NAPHTHIONIC ACID AND ANILINE IN THE DARK IN THE PRESENCE OF ASCORBIC ACID WITHOUT EDTA

Amine	Time/d	Amine remaining %,	Molar yield of ammonia %
Naphthionic acid	0	100	12.6*
	20	41.1	70.5*
	70	41.1	70.9*
	77†	17.3	94.4*
	84†	12.2	99.7*
Sulphanilic acid	0	100	0
	10	76.9	25.3
	40	30.7	72.6
	70	20.2	81.8
	80	20.2	82.6
	150	13.4	88.9
Aniline	0	100	0
	10	100	0
	35	50.2	51.4
	50	34.8	67.2
	70	22.4	81.2
	80	22.4	81.5

* These results indicate that some ammonia is obtained on heating naphthionic acid with a sodium hydroxide solution.

† With a further addition of 2000 ppm of ascorbic acid at 70 d.

TABLE (14)

FORMATION OF SIMPLE AMINES AND AMMONIA ON DEGRADATION OF AZO FOOD COLOURING MATTERS IN THE DARK IN THE PRESENCE OF ASCORBIC ACID (1000 ppm) WITHOUT EDTA

The amines and ammonia were not determined untill all of the food colour had disappeared visibly.

Food colour	Time for colour to disappear/d	Additional time/d	Amine formed	Molar yield of amine, %	Molar yield of ammonia, %
Red 2G*					
Sunset Yellow FCF	23	1	Sulphanilic acid	13.1	190
		10		7.3	195
		30		7.2	196
Tartrazine	24	1	Sulphanilic acid	12.8	190
		10		7.8	194
		20		7.6	195
Black PN	1	1	Sulphanilic acid	41.2†	306
		15		22.4†	369
		30		12.1†	381
Brown FK	16	1	Sulphanilic acid	13.2	183
		5		7.5	191
		30		7.4	195
Carmoisine	18	5	Naphthionic acid	46.9	166
		10		46.9	167
		30		41.9	168
		50‡		11.8	200
Ponceau 4R	15	1	Naphthionic acid	44.8	165
		10		44.9	166
		20		44.9	166
		40§		11.8	200

continued .....

Food colour	Time for colour to disappear/d	Additional time/d	Amine formed	Molar yield of amine, %	Molar yield of ammonia, %
Amaranth	12	1	Naphthionic acid	46.7	162
		10		46.7	164
		20		41.7	162
Chocolate Brown HT	21	1	Naphthionic acid	83.6	330
		20		83.6	330
		50		83.6	330

* The colour changed to pink, which was found to be due to the formation of Red 10B. After 25 d 25.7% of the Red 2G remained and the yield of Red 10B was found to be 72.5%. Determination of aniline and ammonia were not made owing to the stability of the Red 10B formed.
† The purple degradation product was present in these solutions but was destroyed by the addition of the permanganate.
‡ With a further addition of 2000 ppm of ascorbic acid after 30 d.
§ With a further addition of 2000 ppm of ascorbic acid after 20 d.

TABLE (15)

DEGRADATION OF FOOD COLOURING MATTERS AND ASCORBIC ACID

IN ADMIXTURE IN THE DARK

Food colour	Approximate time for complete loss of 100 ppm of ascorbic acid*/d	Time for loss of half of food colour in the presence of 100 ppm of ascorbic acid*/d	Time for loss of all food colour in the presence of 1000 ppm of ascorbic acid†/d
Black PN	30	15	1
Amaranth	32	25	12
Ponceau 4R	60	14	15
Brown FK	40	15	16
Carmoisine	46	25	18
Chocolate Brown HT	40	17	21
Sunset Yellow FCF	56	44	23
Tartrazine	54	54	24
Red 2G	42	40	25‡
Green S	42	16	25
Indigo Carmine	44	45	25
Brilliant Blue FCF	35	32	30
Patent Blue V	60	>60	31
Erythrosine	45	13	>32§
Quinoline Yellow	57	>60	>32§
Yellow 2G	16	>60	>32§

* In the presence of 25 ppm of EDTA
† In the absence of EDTA
‡ Time for complete change of Red 2G to Red 10B and other products
§ Ascorbic acid degrades completely first.

FURTHER DIFFERENTIAL PULSE POLAROGRAPHIC AND VISIBLE SPECTROPHOTOMETRIC STUDIES OF THE DEGRADATION OF PERMITTED SYNTHETIC FOOD COLOURING MATTERS WITH AND WITHOUT THE ADDITION OF ASCORBIC ACID: ACCELERATED HEAT DEGRADATION STUDIES

Previously the permitted synthetic food colouring matters and ascorbic acid were determined by differential pulse polarography during accelerated light degradation studies on solutions containing both food colour and ascorbic acid. Simple amines, viz. aniline, sulphanilic acid and naphthionic acid, and ammonia were shown to be formed during the degradation when the food colour contained one or more azo groups. A full yield of ammonia was obtained eventually from the azo nitrogen atoms. In the absence of EDTA to stabilise the ascorbic acid from reaction with dissolved oxygen, the degradations were found generally to take place more rapidly, and further a full yield of ammonia was also obtained from the acetamido groups of Red 2G and Black PN, whereas no ammonia has been obtained from this source in the prescence of EDTA.

Food colours were also shown to react with ascorbic acid in the absence of EDTA in the dark at room temperature. As the ascorbic acid degraded in a similar time to that required for the food colour to be lost visibly, appreciable amounts of the simple amines remained undegraded. More recently the decay curves of the food colouring matters during accelerated heat and light degradation have been discussed[280].

In this study the yields of simple amines and ammonia formed on degrading the food colours containing azo groups alone and in admixture with either EDTA or ascorbic acid at 130°C in sealed vials have been determined, and the degradation has been monitored by

differential pulse polarography. The results show marked differences to those obtained previously for accelerated light degradation.

Experimental:

Visible spectrophotometric measurements were made with a Pye Unicam SP8-100 spectrophotometer. Ammonia and the simple amines (aniline, sulphanilic acid and naphthionic acid) were determined as previously using the indophenol reaction and diazotisation procedure respectively.

Accelerated heat degradation studies were carried out [280] in sealed round-bottomed glass vials of 30 ml capacity which were kept at 130°C in a Townson and Mercer model 8-250 Mark 2 oven. The vials were constructed by a glass blower from Pyrex borosilicate glass tubing of two diameters. The body of the tube (approximately 10 cm long) was constructed from 26 mm o.d. tubing (2cm thick) and the stem from 8 mm o.d. tubing (1.5 mm thick). The length of the stem was initially 18 cm so that the vial could be resealed several times after repeated sampling. It is important that the main body of the vial is not completely filled. In four years of extensive use the author had one vial explode in the oven for this reason. Particular attention to safety must be made when the tubes are being taken from the oven and adequate precautions such as the wearing of heavy duty gloves and screening should be taken. In this work the vials were sealed by a qualified glass blower. The use of a modern autoclave with its built-in safety features would not be as convenient, but might be considered as an alternative to the present system. This would allow the use of a wide range of autoclavable large capacity, screw-cap bottles. The use of suitable screw-cap micro-reaction

vessels (capacity < 5 ml) which are available from HPLC suppliers and which are reported to be autoclavable might be considered.

Degradations were carried out on food colour solutions in pH 5.5 acetate buffer prepared as described previously and using the amount of buffer indicated there.

Results:

EDTA was shown to stabilise ascorbic acid from heat degradation (Table 16). After 3 hours of heating a 100 ppm solution of ascorbic acid had completely degraded whereas 38% of the ascorbic acid remained when EDTA was present. The stability of 100 and 1000 ppm solutions of ascorbic acid containing EDTA were very similar in terms of the percentage remnant at each time interval, whereas when EDTA was omitted the 1000 ppm solution of ascorbic acid was considerably more stable than the 100 ppm solution.

EDTA was also shown to stabilise the food colouring matters from heat degradation both in the presence and absence of ascorbic acid. This is seen in Table (17) which gives the times of heating for complete visible loss of food colours from 10 and 100 ppm solutions in the presence and absence of EDTA. Significant increases in degradation times are apparent when EDTA is present.

The yields of simple amines and ammonia obtained when 10 ppm solutions in pH 5.5 buffer without the addition of either ascorbic acid or EDTA were heated at 130°C in sealed vials were determined (Table 18). Some of the food colours gave high yields of simple amines after the food colour had degraded visually, but further heating did not change this yield. Subsequently it was shown that aniline and sulphanilic

acid solutions could be heated for 40 days without loss: there was no loss from a 10 ppm naphthionic acid solution for 20 days but at 30 days only 44% remained (Table 19). Other food colours gave no simple amine and a full yield of ammonia. The yield of amine did not appear to be related particularly to the amine formed. Thus, Red 2G (which gives aniline), tartrazine, Brown FK and Black PN (which give sulphanilic acid) and amaranth (which give naphthionic acid) gave yields of 63, 55, 22, 26 and 54% of amine. Sunset Yellow and Yellow 2G (which give sulphanilic acid) and Chocolate Brown HT, Ponceau 4R and Carmosine (which give naphthionic acid) gave zero yields).

The effect of increasing the concentration of the food colour solution being degraded,was to decrease the percentage yield of simple amine in the case of Red 2G, tartrazine Ponceau 4R, Black PN and amaranth (Table 18). In the case of Brown FK the yield of sulphanilic acid was increased. In the cases of Chocolate Brown HT, Sunset Yellow FCF and Carmoisine, for which zero yield of amine had been obtained at 10 ppm, definite yields were obtained at higher concentrations.

The yields of ammonia given in Table (18) indicate that at 10 ppm more complex amines,if formed,are degraded rapidly to ammonia as the total nitrogen is accounted for in the simple amine and ammonia. At higher concentrations, however, the yields of ammonia are much lower and apparently the azo nitrogen atom which would yield the more complex amine is retained in an organic form.

At higher concentrations of Red 2G, Red 10B was observed to be formed as an intermediate during the degradation. At higher concentrations of Ponceau 4R a stable yellow colour was developed in the solution and a new dpp peak at -0.48V (pH 5.5) was found in place of that of Ponceau 4R at -0.65V.see fig. (33). New dpp peaks were also observed in degraded Black PN solutions see Fig. (34).

When 10 ppm solutions of the food colours which also contained 1000 ppm of ascorbic acid were heated at 130°C in sealed vials, degradation was very rapid.(Table 17), generally taking place in hours in contrast to the days taken without ascorbic acid. At this concentration most of the food colours gave an appreciable yield of simple amine at the time of disappearance of the colour, but the simple amine generally degraded to ammonia on further heating. Higher concentrations of food colour took slightly longer to disappear visually and the yield of simple amine that was formed was not decreased on further heating presumably owing to the prior degradation of ascorbic acid. The increased rate of degredation of both ascorbic acid and the food colours in admixture is illustrated in Fig. (35) for Brown FK.

Previously in the accelerated light degradation studies limited success was obtained in determining the stoichiometry of the reaction of ascorbic acid with the food colours. The difficulties experienced in determining these stoichiometries accurately arose from the rapid oxidation by air of the ascorbic acid even when stabilised with EDTA. In the present study 10 ppm solutions of several food colours degraded within 0.5-2 hours and it was possible to determine the stoichiometry of the reation more readily. A typical degradation curve for the ascorbic acid in these circumstances is given in Fig. (36) for Black PN. This is similar in form to that given for the light degradation study.

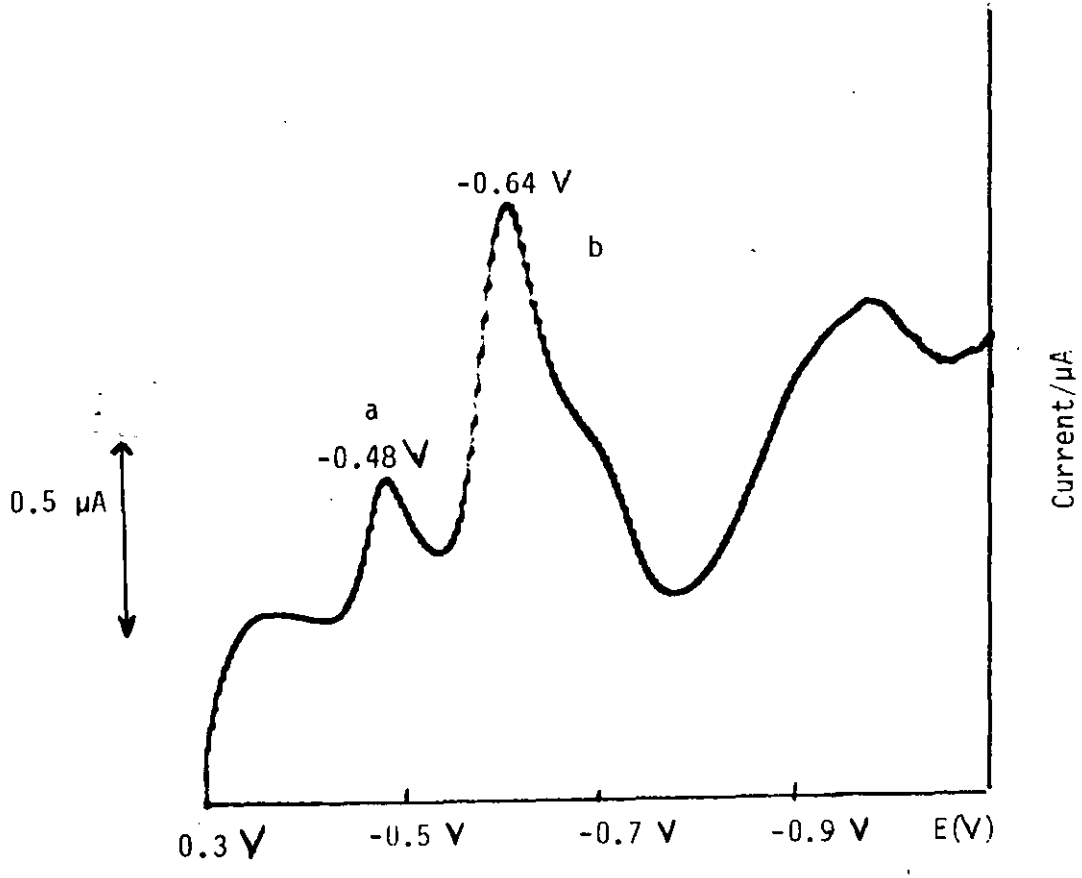


Figure 33. The new DP Polarographic Peak Obtained from the Heat Degradation of Ponceau 4R (40 ppm) (3 days). (a) The New Peak (b) Remnant of the Original.

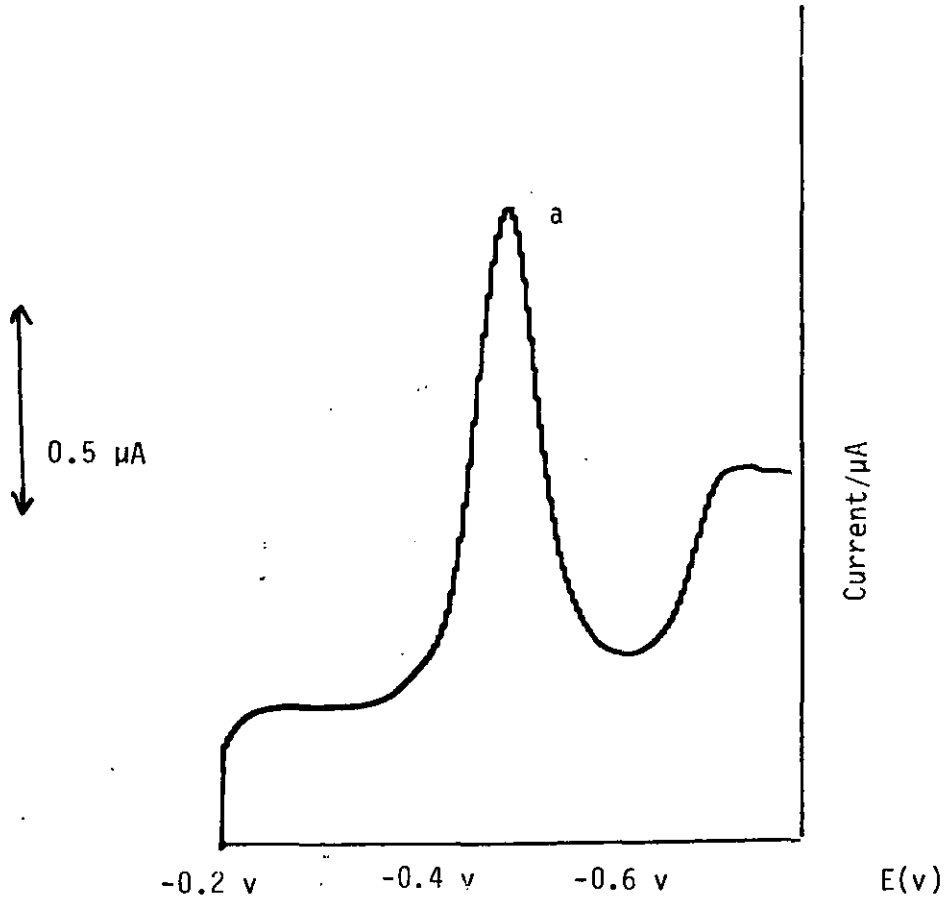


Figure 33. The new DP Polarographic Peak Obtained from the Heat Degradation of Ponceau 4R (40 ppm) (10 days).

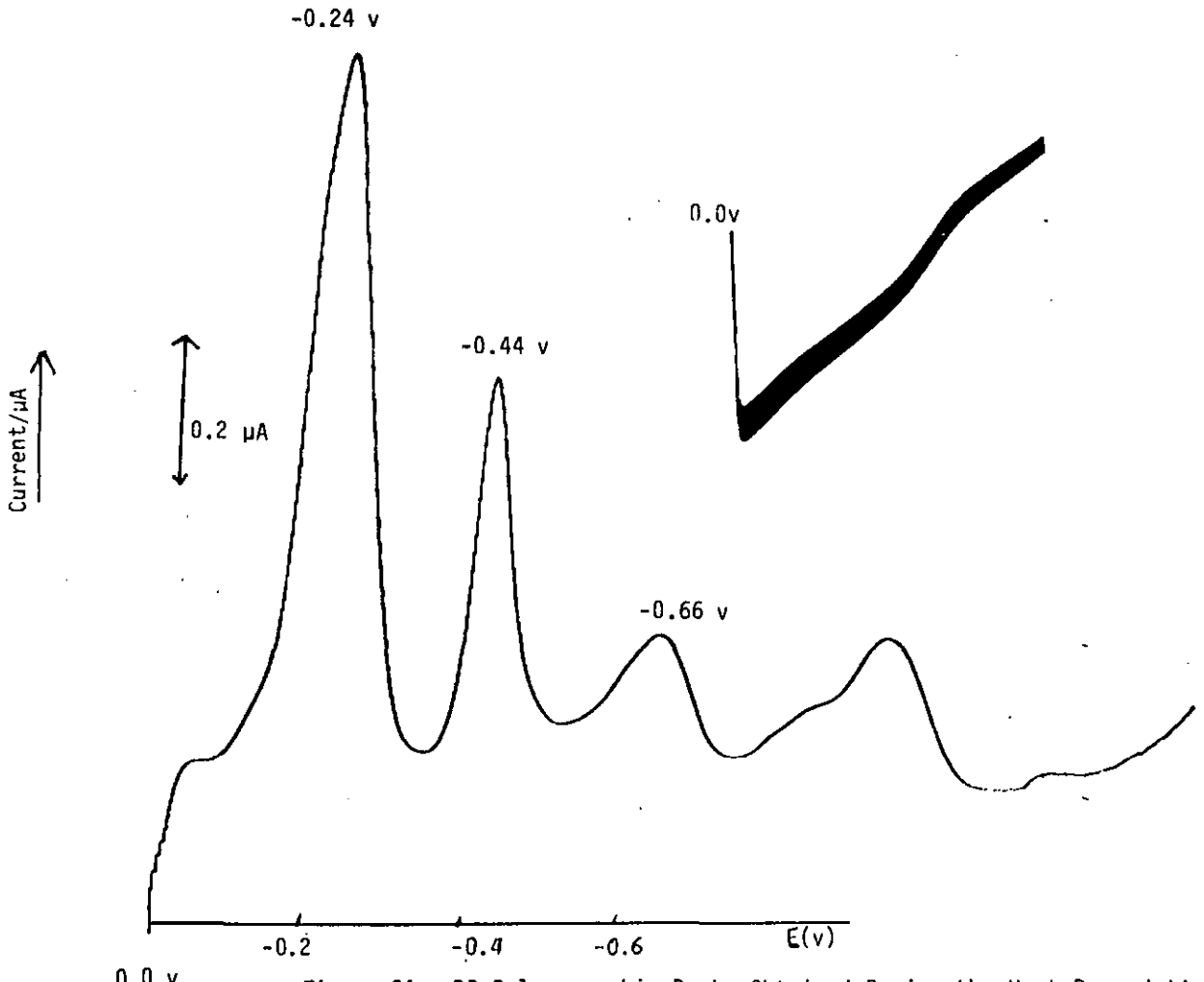


Figure 34. DP Polarographic Peaks Obtained During the Heat Degradation of Black PN (100 ppm) (7 days).

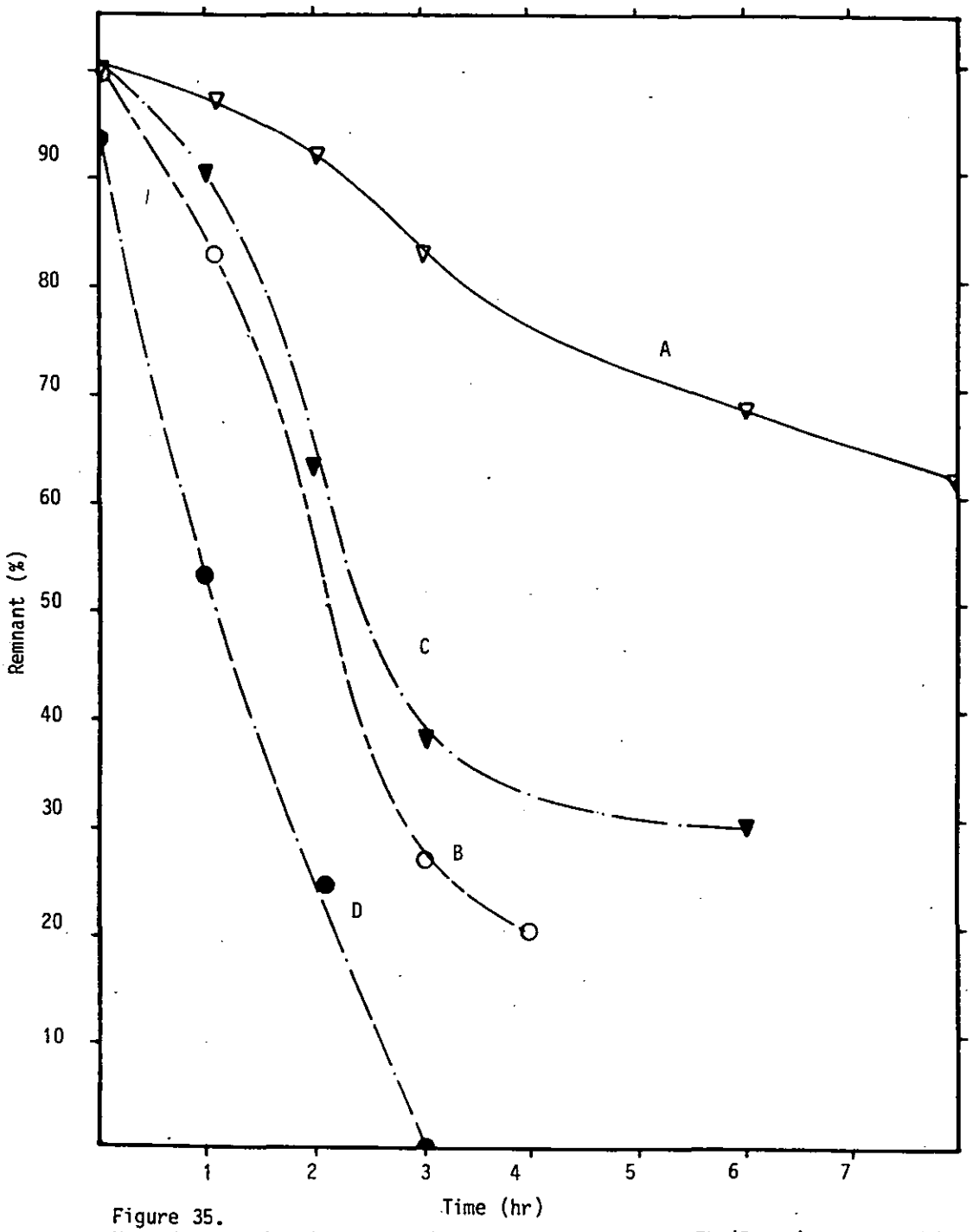


Figure 35.
Heat degradation in acetate buffer pH 5.5 of Brown FK (5 ppm) and ascorbic acid (1000 ppm) with EDTA 25 ppm. (A) Brown FK (B) Brown FK in the presence of ascorbic acid (C) Ascorbic acid alone (D) Ascorbic acid in the presence of Brown FK.

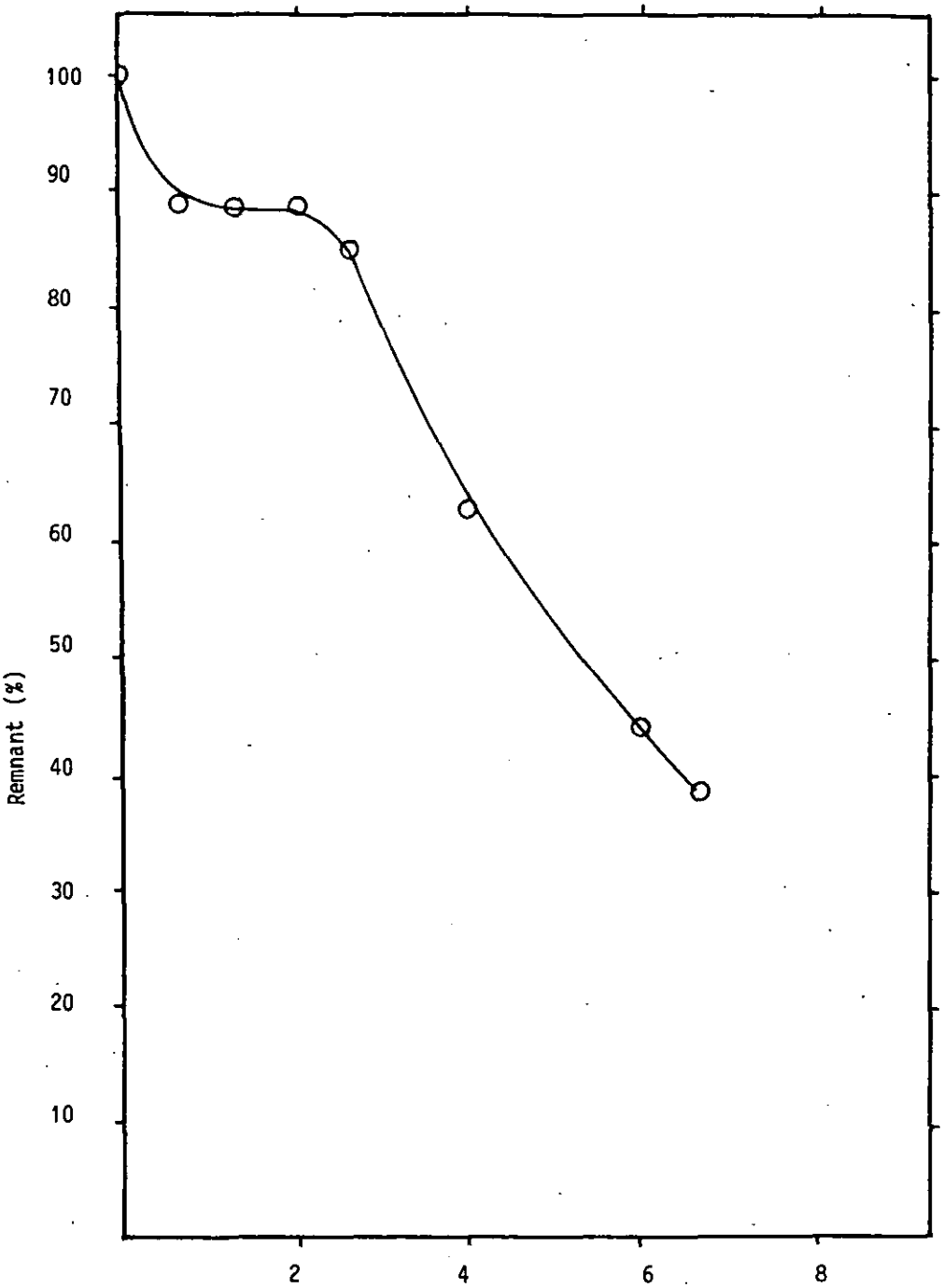


Figure 36. Heat degradation of Ascorbic Acid (initial concentration (1000 ppm) in the presence of Black PN (initial concentration 5 ppm) solution became colourless after 0.3 hr.

Thus, the concentration of ascorbic acid reached a plateau value when the food colour has disappeared visibly but later degraded more rapidly again. Previously this renewed degradation of the ascorbic acid was associated with the production of ammonia. In the present case most of the ammonia is formed within 20 min. mainly by degradation via the more complex amines but the renewed degradation of the ascorbic acid appears to be associated with the degradation of the the simple amines to ammonia (see Table 18). The molar ratio of ascorbic acid to food colour required up to the plateau region was found to be 2.3 (Sunset Yellow FCF, and Ponceau 4R), 2.4 (Carmoisine), 3.0 (Brown FK), and 3.9 (Chocolate Brown HT).

Ascorbic acid solutions (100 ppm) heated at 130°C in sealed vials were found to become yellow in colour, and the solution gave a new polarographic peak at -0.90 V (pH 5.5) which was better defined at pH 2 (-0.54V). The colour and the new polarographic peak were observed previously when ascorbic acid solutions were degraded in the dark at room temperature. In this heat study the change in colour occurred after 2-3 hours and it was also observed that the solution had developed a pronounced foaming ability. This latter property had not been observed in the room temperature studies. Ascorbic acid solutions of 100 ppm did not show these effects. Some food colouring matters, e.g. Chocolate Borwn HT, Amaranth and Brown FK showed some foaming properties in the absence of ascorbic acid but the effect was considerably less than that for ascorbic acid.

Discussion:

This study has provided information on the degradation of food colouring matters under extreme heat degradation conditions. Simple amines, such as aniline, sulphanilic acid and naphthionic acid, and also

ammonia, have been shown to be formed in various yields from those food colouring matters containing an azo group. At the 10 ppm level a stable finite yield of the simple amines is obtained from several food colouring matters. If higher yields of these amines are formed during the early stages of the degradation then some of the amine must be interacting with other products to give ammonia. EDTA was shown to inhibit the degradation of the food colouring matters, whereas conversely degradation was very rapid in the presence of ascorbic acid.

The degradation mechanism was found to be concentration dependent, as low yields of simple amines and ammonia were formed at higher concentrations of food colouring matter. The likelihood of biomolecular reactions would be expected to increase with increasing concentration of food colouring matter, and it would certainly appear that larger molecules, incorporating nitrogen atoms, are being formed at these higher concentrations of food colouring matter.

The eventual aim of the work being carried out in this laboratory on food colouring matters is to identify products that may be formed by their degradation during food processing and storage. The approach being taken is to use extreme conditions in which extensive degradation occurs in a relatively short period of time. This allows numerous experiment to be made and facilitates identification of possible products as these are formed in high yields. The conditions used are generally far more extreme than those occurring in normal processing of food products containing synthetic food colouring matters. Investigations would have to be made to see whether degradation products formed under extreme conditions were being formed under normal food processing

and storage conditions. In the case of heat processing, for example, normal practice would be to add the synthetic food colouring after the heat processing of the food in order to restore colour lost by degradation of the natural colours. Indeed the heat degradation of naturally occurring colours might be of as much toxicological interest as that of the synthetic food colours.

Preliminary HPLC studies have been carried out on heat and light degraded solutions of synthetic food colouring matters by another worker in this laboratory[281]. The results showed that phenol-4-sulphonic acid, sulphanilic acid, sodium naphthionate and sodium naphol-4-sulphanate were possible thermal decay products for a range of azo type colouring matters.

TABLE (16)

EFFECT OF EDTA IN STABILISING ASCORBIC ACID FROM OXIDATION BY AIR AT 130°C

Remnant of ascorbic acid /% of original	Original concentration of ascorbic acid /ppm	Time/h						
		0	1	2	3	5	22	45
without EDTA	100	100	31	9	0	-	-	-
	1000	100	95	-	-	27	5	0
with EDTA (25 ppm)	100	100	90	63	38	-	9	2
	1000	100	100	-	44	-	10	1

TABLE (17)

STABILITY OF FOOD COLOURS (10 and 100 ppm) AT 130°C

IN THE PRESENCE AND ABSENCE OF ASCORBIC ACID AND EDTA

Times for complete visible degradation of the food colour are given.

	No EDTA, No ascorbic acid	0.0025% EDTA, No ascorbic acid	No EDTA, 1000 ppm ascorbic acid	
Azo food colours	10 ppm	10 ppm	10 ppm	100 ppm
Black PN	0.5 d	6 d	0.3 h	6 h
Chocolate Brown HT	0.5 d	3 d	2 h	6 h
Ponceau 4R	0.5 d	4 d	1 h	6 h
Carmoisine	1 d	8 d	1 h	3 h
Amaranth	1 d	8 d	2 h	6.5 h
Brown FK	1 d	4.5 d	2h	5 h
Sunset Yellow FCF	1 d	18 d	2h	15 h
Tartrazine	7 d	26 d	23 h	34 h
Red 2G	8 d	19 d	2 h	11 h
Yellow 2G	28 d	>45 d	*	*
Non-azo colours				
Indigo Carmine	0.3 d	0.1 d	6 h	-
Green S	1.5 d	1.8 d	34 h	-
Patent Blue V	10 d	17 d	*	-
Brilliant Blue FCF	14 d	25 d	30 h	-
Quinoline Yellow	18 d	20 d	*	-
Erythrosine	38 d	12 d	3 h	-

* The ascorbic acid degraded first.

TABLE (18)

FORMATION OF SIMPLE AMINES AND AMMONIA ON HEAT DEGRADATION AT 130°C IN SEALED CONTAINERS OF AZO FOOD COLOURS IN THE ABSENCE AND PRESENCE (1000 ppm) OF ASCORBIC ACID

The amines and ammonia were not determined until all trace of the food colouring matter had disappeared visibly. No EDTA was added.

Food colouring matter	Concentration of Food Colour /ppm	In absence of ascorbic acid				With ascorbic acid			
		Time for complete degradation /d	Additional time/d	Molar Yield of amine/%	Molar Yield of ammonia/%	Time for complete degradation /h	Additional time/d	Molar Yield of amine/%	Molar Yield of ammonia/%
Red 2G	10	8	5-50	63.6*	129	2	0	98	148
							5	0	186
	50	11	5-50	54.5	121	-	-	-	-
	100	13	5-50	38.8	59	11	0	72	17
							2	26	41
Sunset Yellow FCF	10	14	1-50	0**	183	2	0	72.3	70.5
							5	0	102
	100	26	1-20	9.6	41	15	0-5	37	55
	200	48	1-30	8.2	40	-	-	-	-

(continued)

Food colouring matter	Concentration of Food Colour /ppm	In absence of ascorbic acid				With ascorbic acid			
		Time for complete degradation /d	Additional time/d	Molar Yield of amine/%	Molar Yield of ammonia/%	Time for complete degradation /h	Additional time/d	Molar Yield of amine/%	Molar Yield of ammonia/%
Tartrazine	10	7	5-50	55.2**	121	23	0	68	128
							5	0	154
	50	50	10-50	18.5	60.0	-	-	-	-
	100	-	-	-	-	34	0-5	26	67
Black PN	10	0.5	1-30	—25.8**	308	0.3	0	76	378
							5	0	424
	100	4	1-20	17.8	59	1	0-5	38	172
	200	6	1-20	17.6	58	-	-	-	-
Brown FK	10	1	1-20	22.1	166	2	0	80	120
							5	0	154
	100	14	1-30	46.8	76	5	0-2	48	95
Carmoisine	10	1	1	0***	202	1	0	33	82
							5	0	94
	40	4	1	0***	83	-	-	-	-
	100	6	1-20	8.3	42	3	0-5	21	26

(continued)

Food colouring matter	Concentration of Food Colour /ppm	In absence of ascorbic acid				With ascorbic acid			
		Time for complete degradation /d	Additional time/d	Molar Yield of amine/%	Molar Yield of ammonia/%	Time for complete degradation /h	Additional time/d	Molar Yield of amine/%	Molar Yield of ammonia/%
Ponceau 4R	10	0.5	1	0***	205	1	0	0	184
	40	+	+	+	+	-	-	-	-
	100	+	+	+	+	6	0-10	14	66
Amaranth	10	1	1-20	53.6	126	2	0	36	68
							5	0	91
	40	4	1-30	8.0***	81	-	-	-	-
	100	-	-	-	-	6.5	0	29	55
							2	9.4	65
	200	7	1-25	10.5	29	-	-	-	-
Chocolate Brown HT	10	0.5	1	0	394	2	0	83	173
							5	0	205
	40	2	1-25	18.0***	133	-	-	-	-
	100	4.5	1-25	17.4	126	6	0-2	31	52
	200	7	1-25	17.3	125	-	-	-	-
	500	12	1-25	12.2	57	-	-	-	-
Yellow 2G	10	28	1	0**	202	28++	0	0	191

* aniline ; ** sulphanilic acid ; *** naphthionic acid ; + A coloured product is (see text); the molar yield of ammonia after 2 additional days heating was 69 and 64% for the 40 and 100 ppm solutions respectively; ++ the ascorbic acid degraded first.

TABLE (19)

DEGRADATION STUDIES OF SULPHANILIC ACID, NAPHTHIONIC ACID AND ANILINE AT 130°C WITH AND WITHOUT ASCORBIC ACID BUT WITHOUT EDTA

Amine	Time/d	without ascorbic acid*		with 1000 ppm** ascorbic acid	
		Amine remaining /%	Molar yield of ammonia /%	Amine remaining /%	Molar yield of ammonia /%
Naphthionic acid	0	100	13+	100	0
	1	-	-	27	30
	2	-	-	0	35
	20	100	13+	-	-
	30	44	69	-	-
Sulphanilic acid	0	100	0	100	0
	1	-	-	45	27
	2	-	-	24	39
	20	100	0	-	-
	30	100	0	-	-
Aniline	0	100	0	100	0
	1	-	-	67	20
	2	-	-	16	27
	20	100	0	-	-
	30	100	0	-	-

* 10 ppm amine initially

** 40 ppm amine initially

\+ These results indicate that some ammonia is obtained on heating naphthionic acid with sodium hydroxide solution

DETERMINATION OF FOOD COLOURS IN BOILED SWEETS BY DIFFERENTIAL PULSE POLAROGRAPHY

Introduction:

A method was proposed for the determination of synthetic colours in foods using high performance liquid chromatography (HPLC) by Boley et al[162]. Although the method was found to be satifactory for most synthetic food colours, recoveries of Indigo Carmine were variable and as low as 15-30%. To overcome this problem a more rapid method was developed in which Indigo Carmine is largely protected from conditions likely to cause its degradation[282]. The sample is crushed, to facilitate rapid dissolution and then dissolved in water under an atmosphere of nitrogen. The colour is then simultaneously extracted from the sample and concentrated by passing the solution through a SEP-PAK C_{18} sample preparation cartridge, from which it is eluted with a minimum volume of methanol. The eluent is diluted to a known volume with water and then examined by HPLC. Other workers[283] have used HPLC for determination of synthetic dyes in gelatin containing sweets, following polyamide adsorption and ion-pair extraction with tri-n-octylamine. The gelatin must be eliminated first because it interferes with the normal ion-pair extraction of dyes with tri-n-octylamine into chloroform. Dyes are identified by thin layer chromatography and HPLC, and quantitated by HPLC or colorimetry.

Previously in this laboratory[284] differential pulse polarography was used to determine the colours in jams, jellies and marmalades. The effect of tetraphenylphosphonium chloride (TPPC) on polarograms was studied by adding 5 ml of 0.01M TPPC before making up the volume to 25 ml polarograms were run on the samples after deoxygenation. Ion-pair extraction with TPPC was also carried out on

some samples. The solution was then polarographed in the usual manner. The identity of some colours was further established by TLC studies following the method given by Lehmann et al.[160].

The following method is proposed here for the determination of food colours in boiled sweet directly by dissolving the sample in warm distilled water. The samples were buffered by Britton-Robinson buffer deoxygenated and polarographed. The proposed method is rapid and simple, and uses less reagent and chemicals.

The sweet samples were purchased locally.

Procedure:

Samples (1-4 boiled sweets) of 2-10 g were weighed and dissolved in 20 ml Britton-Robinson buffer of a particular pH. No heating was required, and the volume was made up to the mark in a 100 ml volumetric flask. For determination at lower concentrations the stock solution of 2g/100 ml was diluted further. In some cases where the boiled sweet contained more than one food colour for which the polarographic peaks were close to each other 5 ml of 0.01M. Triphenylphosphonium chloride (TPPC) was added to the solution before making the final volume to the mark. The samples were deoxygendated prior to polarographic determination.

Results and discussion:

The boiled sweets of a variety of colours were investigated. The food colours were identified as Tartrazine, Indigo Carmine, Sunset Yellow FCF and Erythrosine. Standard solutions of the above food colours were polarographed individually in order to establish the optimum conditions. Sunset Yellow FCF was found to give a well

defined peak at -0.2V in pH 2.0, Erythrosine at -1.0V in pH 9.0. Britton-Robinson buffer. No TPPC was added.

Ingigo Carmine and Tartrazine were determined at pH 9.0 in the presence of TPPC.

Calibration curves for the identified food colours in the samples studied are shown in the figures (37-41).

The orange colour was found to give two peaks very well separated, the first one is -0.2V at pH 2.0 and the other at the -1.3V at pH 9.0 which identified as Sunset Yellow FCF and Erythrosine. The yellow boiled sweets were found to contain Tartrazine and Erythrosine. The green sweets were found to contain larger amounts of Tartrazine and Indigo Carmine as well as Erythrosine. The violet coloured sweets were also studied and were found to contain Indigo Carmine and Erythrosine.

Table (20) shows the amount of mg/Kg of food colours found in the sweets.

The limit of determination ranged from (0.2 - 0.4) mg/Kg.

The advantage of this method:

The method is very simple, being comprised of fewer steps than other methods such as high performance liquid chromatography or TLC used previously in determination of food colours in sweets.

Gelatin which causes interference for example in paired-ion chromatography is not a problem in this method. Recovery was also determined by addition of the equivalent of 10% of solution of a boiled sweet which did not have the food colour under investigation to a standard solution of that synthetic food colour. No change was noticed in the shape or

Figure (37) D.P. polarograms obtained for Tartrazine found in green boiled sweets in the presence of TPPC.
Concentrations (1) 1% (2) 0.5% (3) 0.25% (4) 0.1%

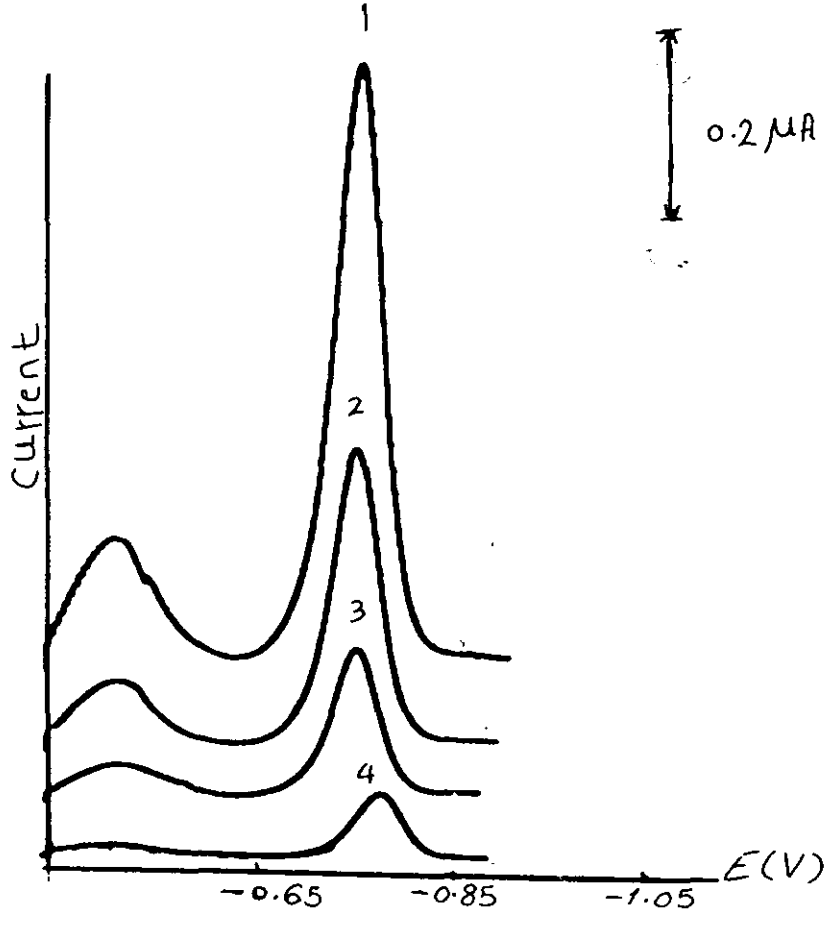


Figure (38) D.P. polarograms obtained for Tartrazine found in yellow boiled sweets in the presence of TPPC.

Concentrations (%) (1) 5 (2) 2 (3) 1 (4) 0.5

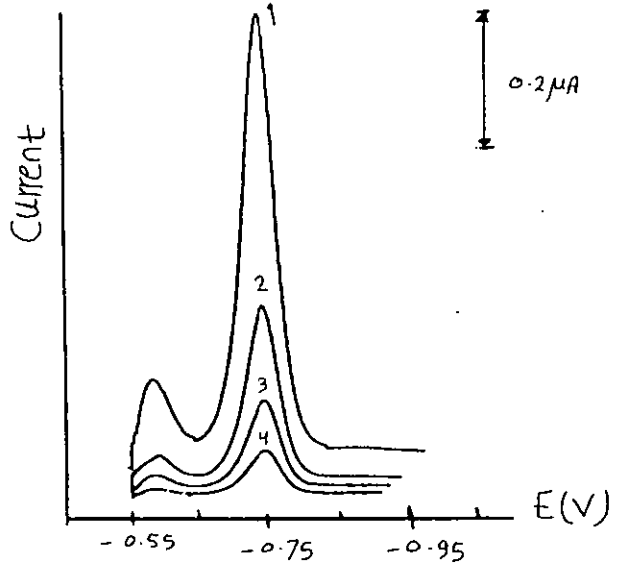


Figure (39) D.P. polarograms obtained for the food colours found in violet boiled sweets in the presence of TPPC.

Concentrations(%): A= 7.0 B=3.5 C= 1.75 D= 0.7

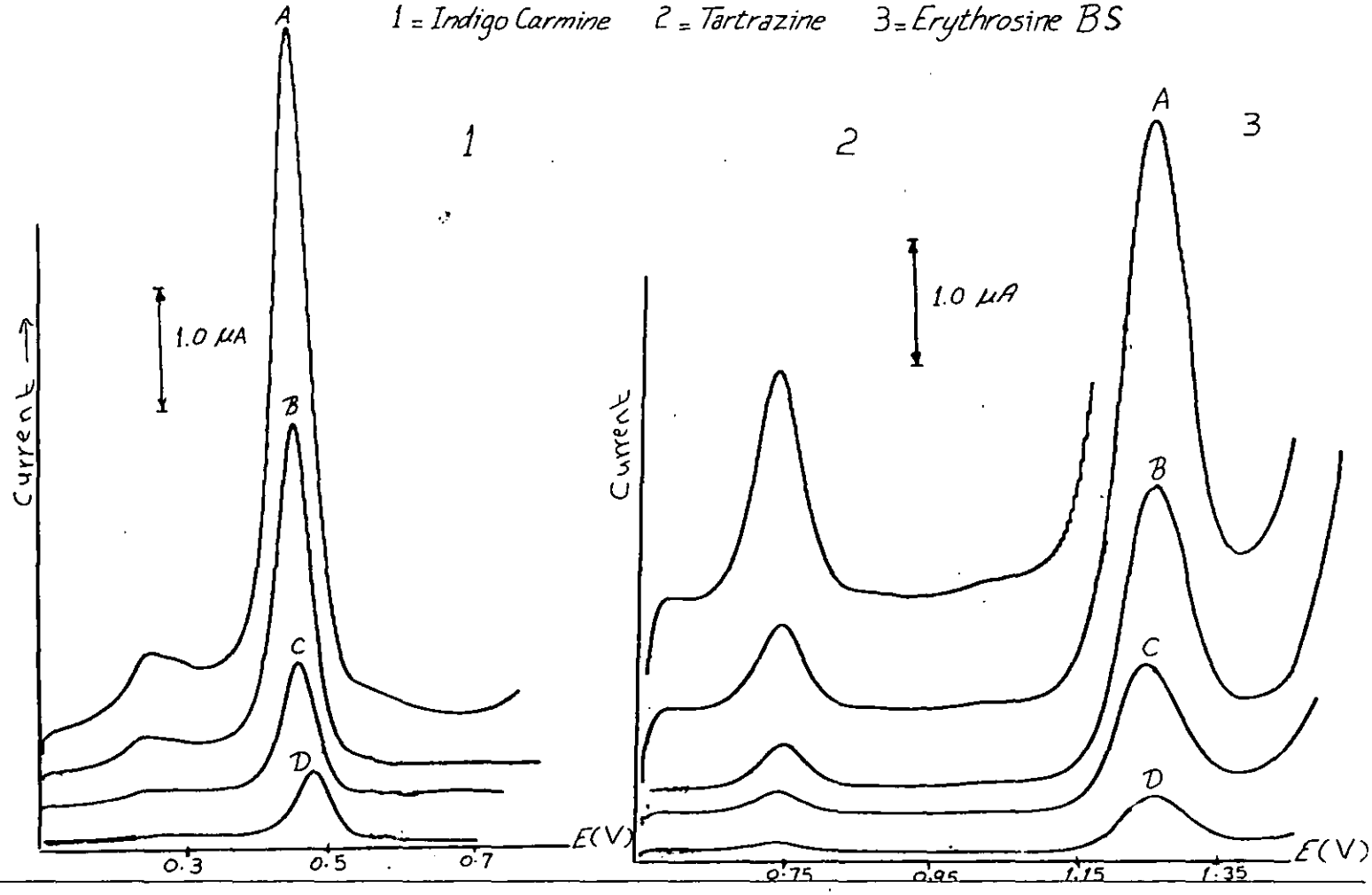


Figure (40) D.P. polarograms obtained for the food colours found in orange boiled sweets in the presence of TPPC.

Concentrations (%) A= 5 B= 2.5 C= 1.25 D= 0.5

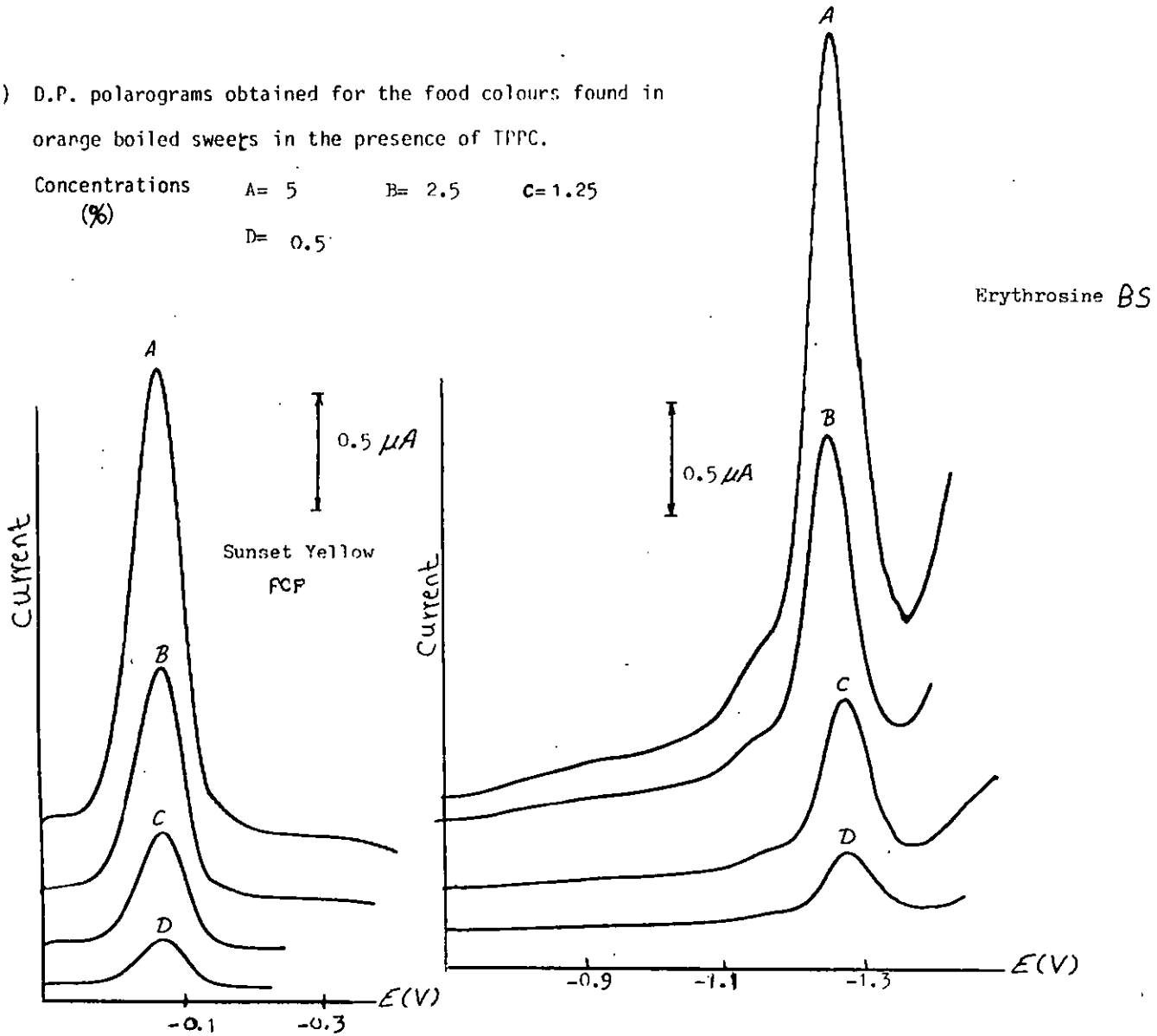
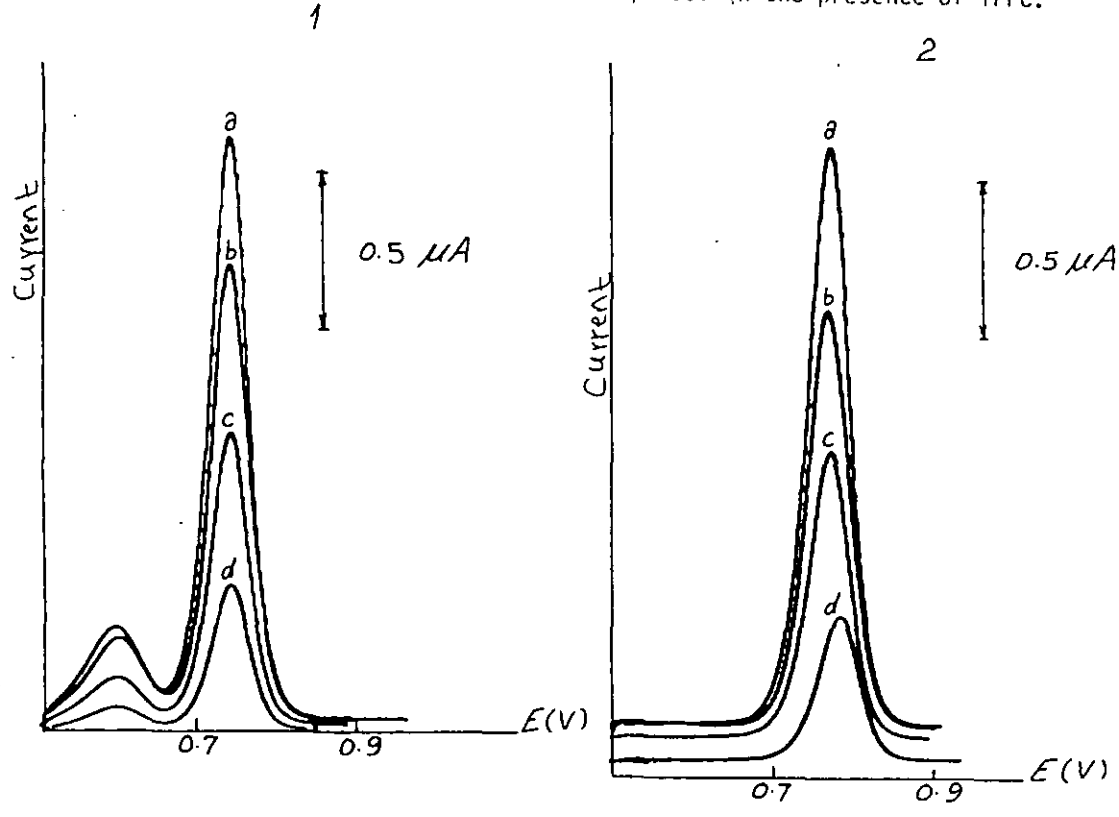


Figure (41) D.P. polarograms obtained for standard soloutions of Tartrazine (1) in the presence of 10% orange sweets (2) Tartrazine soloutions alone.
Concentrations (a) 4 ppm (b) 2 ppm (c) 1 ppm (d) 0.5 ppm pH 9.0 in the presence of TPTC.



height of the polarographic peak. A comparison of peaks obtained before and after the addition of the boiled sweets is demonstrated in Figure (42).

Figure (42) A comparison of peaks obtained before and after the addition of the boiled sweet, no TPPC pH 2.0.

concentrations

(1) 5% (2) 2.5% (3) 1.25% (4) 0.5% (5) 0.2%

A= in presence of 10% yellow sweets

B= Orange sweet alone

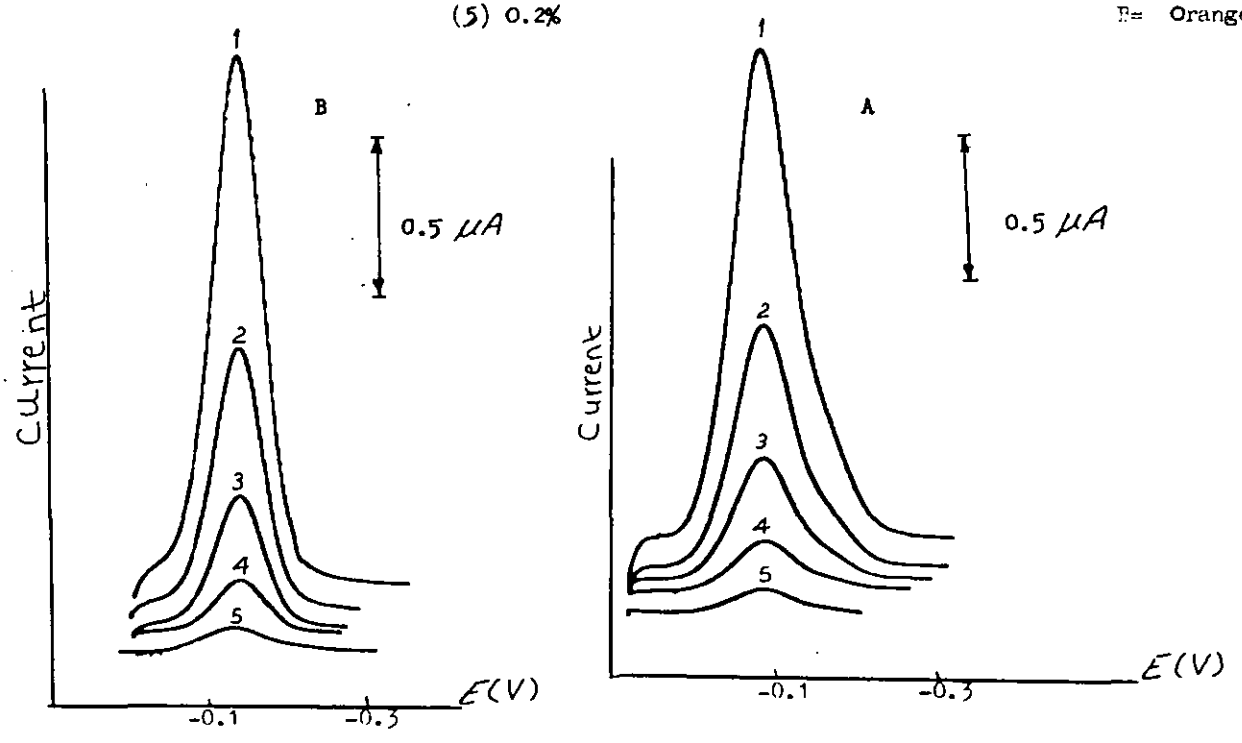


TABLE (20) THE AMOUNT OF FOOD COLOURS(mg/Kg)FOUND IN THE SWEETS.

Colour of sweet used	Synthetic colour found	mg/Kg
Orange	Sunset Yellow FCF	83.47
	Erythrosine	304.8
Violet	Tartrazine	25.4
	Erythrosine	77.1
	Indigo Carmine	1785.7
Green	Tartrazine	133.3
	Indigo Carmine	350.0
	Erythrosine	241.2
Yellow	Erythrosine	761.9
	Indigo Carmine	180.0

Effect of EDTA on the Stability of Acetamido Groups in Drug Compounds:

Introduction

The use of EDTA as a stabiliser for certain compounds has been reported in the literature. EDTA is a well known stabilizer for sulphite solutions.

The effect of EDTA and ascorbic acid on the kinetics of oxidation of an aqueous solution of isoprenalinehydrochloride has been investigated[285]. EDTA alone was effective at pH 2.8 but not at pH 7.0. As expected the addition of metal ions increased degradation. EDTA gave complete protection against added cupric ions at pH 2.8 but some degradation occurred at pH 7.0. In contrast, EDTA protected ascorbic acid against ferric ions at pH 2.8 but enhanced the degradation at pH 7.0. A similar finding was reported by Green et al.[286].

Activation by EDTA of nucleotide pyrophosphatase during preincubation of particulate enzyme preparations has been reported[287].

Other workers[288] have found that the antibiotic, antiviral, antitumor drugs chromomycin, mithramycin, and olivomycin afford DNA cleavage inhibition patterns in the presence of (methidicumpropyl-EDTA) iron (II) [MPE.Fe(II)].

In our investigation of the effect of EDTA on the light stability of food colours, in the presence of ascorbic acid, it was noticed that the acetamido groups in two food colours namely Black PN and Red 2G was protected from degradation. This led to an extention of the work to study the stability of drugs containing the acetamido group and this is the subje of this section. The study was carried out in the light and in the dark. The investigation was carried out in the presence and the absence of ascorbic acid.

Results and discussion:

The present study has provided information on the degradation of some drug compounds, in the presence or absence of EDTA. EDTA clearly stabilises the solutions of these compounds. (see tables 21-30)

The effect of ascorbic acid on the degradation, is apparant. The presence of 0.0075% of EDTA was found to be satisfactory in stabilizing these compounds.

Structures of compounds studied are given in table (31).

Dark study:

Acetanilide: Table (21) EFFECT OF EDTA IN STABILISING ACETAMIDO GROUP.

(1) In the absence of ascorbic acid

(a) Without EDTA

	Time (day)	Aniline formed (%)	Ammonia (%)
Concentration 0.19%	0	0.1*	0
	60	13.6	0
	120	22.1	-

(b) With EDTA (0.0075%)

Time (day)	Aniline formed (%)	Ammonia (%)
0	0.1*	0.0
30	2.5	0.0
60	2.5	0.0
90	2.5	0.0
120	2.5	0.0

* This is the amount of aniline found in the substance as impurity.

(2) In the presence of ascorbic acid (1000 ppm)

(a) With EDTA (0.0015%)

Concentration 20 ppm	Time (day)	Aniline formed (%)	Ammonia (%)
	0	0.1	-
	30	55.7	40.8
	40	55.7	40.9
	45	55.7	41.2

(b) With EDTA (0.0075%)

Concentration 20 ppm	Time (day)	Aniline formed (%)	Ammonia (%)
	0	0.1	0.0
	10	2.5	0.0
	30	4.1	0.0
	40	4.0	0.0
	60	4.1	0.0

(c) Without EDTA

Time (day)	Aniline formed (%)	Ammonia (%)
0	0.1	0.0
30	6.5	91.3
60	6.1	91.4

Light Study: Table (22) EFFECT OF EDTA IN STABILISING ACETAMIDO GROUP.

(1) In the presence of ascorbic acid (1000 ppm)

(a) With EDTA: (0.0075%)

	Time (hr)	Aniline formed (%)	Ammonia (%)
Concentration 25 ppm	0	0.1	0.1
	75	-	0.1
	96	-	0.1

(b) Without EDTA

Time (hr)	Aniline (%)	Ammonia (%)
0	0.1	
75	0.1	95.1

Paracetamol:

Dark Study: Table (23) EFFECT OF EDTA IN STABILISING ACETAMIDO GROUP.

(1) In the absence of ascorbic acid:

(a) Without EDTA:

	Time (day)	P-aminophenol formed (%)	Ammonia formed (%)
Concentration 0.19%	0	0.0	14.1*
	60	13.1	16.5
	120	19.8	14.9

(b) With EDTA (0.0075%)

	Time (day)	P-aminophenol formed (%)	Ammonia (%)
Concentration 0.19%	0	0.0	16.3*
	60	1.64	14.5
	120	1.65	14.4
	150	1.69	14.8

* These results indicate that some ammonia is obtained on heating paracetamol with sodium hydroxide solution.

(2) In the presence of ascorbic acid (1000 ppm)

(a) Without EDTA:

	Time (day)	P-aminophenol formed (%)	Ammonia (%)
Concentration 25 ppm	30	-	12.4
	60	-	15.1
	90	-	14.7

Light study: Table (24) EFFECT OF EDTA IN STABILISING ACETAMIDO GROUP.

In the presence of ascorbic acid (1000 ppm)

(a) Without EDTA

	Time (hr)	P-aminophenol formed (%)	Ammonia (%)
Concentration 25 ppm	0.0	-	17.1
	75	-	97.6
	96	-	97.6

(b) With EDTA (0.0075%)

	Time (hr)	P-aminophenol formed (%)	Ammonia (%)
Concentration 25 ppm	0.0	-	18.7
	75	-	16.7
	96	-	16.9

Lignocainehydrochloride

Light study: Table (25) EFFECT OF EDTA IN STABILISING ACETAMIDO GROUP.

(1) In the presence of ascorbic acid (1000 ppm)

(a) Without EDTA:

	Time (hr)	Ammonia formed (%)
Concentration 25 ppm	0.0	15.0*
	48	74.9
	120	5.3

(b) With EDTA (0.0075%)

	Time (hr)	Ammonia formed (%)
Concentration 25 ppm	0	15.8*
	48	17.4
	120	17.6

* These results indicate that some ammonia is obtained on heating liqnocainehydrochloride with sodium hydroxide solution.

Dark study: Table (26) EFFECT OF EDTA IN STABILISING ACETAMIDO GROUP.

In the presence of ascorbic acid (1000 ppm)

(a) With EDTA (0.0075%)

Concentration 25 ppm	Time (day)	Ammonia (%)
	0.0	15.1
	70	15.4
	30	13.9
	40	16.9

(b) Without EDTA

Concentration 25 ppm	Time (day)	Ammonia (%)
	0.0	15.9
	30	88.4

Sulphadiazine: Table (27) EFFECT OF EDTA IN STABILISING ACETAMIDO GROUP.

Light study:

(a) Without EDTA:

Concentration 25 ppm	Time (hr)	Ammonia formed (%)
	0.0	18.5
	24	27.9
	48	38.0
	96	14.2
	120	41.8
	240**	78.3

** With a further addition of 2000 ppm of ascorbic acid at 120h.

(b) With EDTA (0.0075%)

Concentration 25 ppm	Time (hr)	Ammonia formed (%)
	0.0	16.3
	24	18.5
	48	18.9
	96	18.8
	120	

Dark Study Table (28) EFFECT OF EDTA IN STABILISING ACETAMIDO GROUP.

In the presence of ascorbic acid (1000 ppm)

(a) With EDTA: (0.0075%)

Concentration 25 ppm	Time (day)	Ammonia (%)
	0.0	19.0
	10	18.9
	20	18.5
	30	19.3

(b) Without EDTA:

Concentration 25 ppm	Time (day)	Ammonia (%)
	0.0	18.5
	30	80.1

Succinylsulphathiazole:

Light study: Table (29) EFFECT OF EDTA IN STABILISING ACETAMIDO GROUP.

In the presence of ascorbic acid:

(a) Without EDTA:

Concentration 25 ppm	Time (hr)	Ammonia (%)
	0.0	13.4
	48	45.4
	96	45.6
	240**	90.1

** With a further addition of 2000 ppm of ascorbic acid at 96 h.

(b) With EDTA (0.0075%)

Concentration 25 ppm	Time (hr)	Ammonia formed (%)
	0.0	13.95
	48	13.9
	96	15.2

Dark study Table (30) EFFECT OF EDTA IN STABILISING ACETAMIDO GROUP.

In the presence of ascorbic acid:

(a) With EDTA (0.0075%)

Concentration 25 ppm	Time (day)	Ammonia (%)
	0.0	13.8
	30	14.1

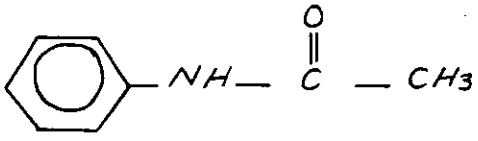
(b) Without EDTA

Concentration 25 ppm	Time (day)	Ammonia (%)
	0.0	14.3
	30	91.4

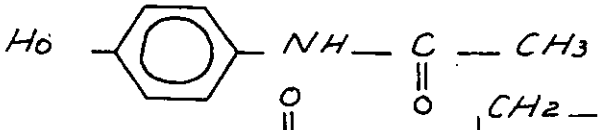
Table 31

Stucture of Compounds Studied

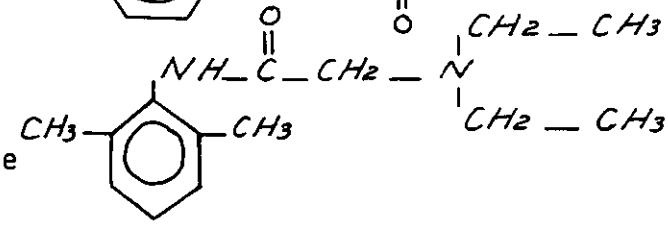
1) Acetanilide



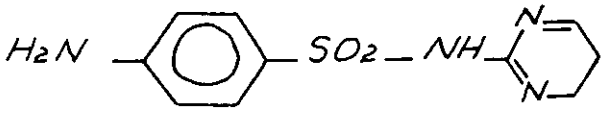
2) Paracetamol



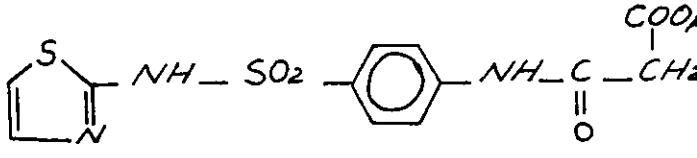
3) Lignocaine hydrochloride



4) Sulphadiazine



5) Succinyl sulphathiazole



A SIMPLE WALL-JET DETECTOR CELL FOR USE EITHER WITH A SOLID ELECTRODE OR WITH A SESSILE MERCURY DROP ELECTRODE AND AN ILLUSTRATION OF ITS USE IN THE OXIDATIVE AND REDUCTIVE FLOW INJECTION VOLTAMMETRIC DETERMINATION OF FOOD COLOURING MATTERS

Introduction:

Oxidative electrochemical detection with glassy carbon and carbon paste working electrodes has become widely used in high performance liquid chromatography. For general purposes UV detection which is simpler and more universal remains the detection method of choice and electrochemical detection tends to be used (i) for selectivity (usually with the electrochemical detector in series with a UV detector), (ii) at determined levels too low to be determined by UV detection, and (iii) at higher levels to determine compounds which have negligible UV absorption.

Reductive electrochemical detection at negative potentials generally requires the use of a mercury based electrode and the removal of oxygen from eluent and sample solution. Difficulties in removing final traces of oxygen, however, causes relatively high background currents and has retarded developments in using the reductive mode at negative potentials. Nevertheless, Lloyd[289] has shown recently that very low detection limits can be obtained in the determination of organic explosives by careful removal of oxygen from eluent and sample solution with nitrogen gas. Sample solutions were deoxygenated in a modified syringe before injection. Lloyd used a PAR 310 static mercury drop mode but modified the end of both the capillary and the eluent inlet device. He also found that it was necessary to deoxygenate the electrolyte in which the detector cell was placed. He claimed that the hanging mercury drop electrode was subject to the

contamination problems of the mercury film electrode.

Previously in this laboratory flow injection analytical procedures with voltammetric detection using a glassy carbon electrode have been developed for the determination of food colouring matters[237], phosphate and silicate as heteropolyacids[238-239], nitrite as nitrosyl bromide[240], aromatic amines by loss of nitrite signal after on-line diazotisation[290], and nitrate after reduction to nitrite[291] and for the on-line bromimetric determination of phenols, amines, aspirin and isoniazid[292]. Because of initial difficulties in using a Metrohm detector cell (EA 1096) caused by blocking of the reference frit the detector cell was used with the counter and reference electrodes removed and the detector cell partially immersed in a suitable electrolyte. Contact between the electrolyte and a platinum counter electrode and a potentiometric calomel electrode was made by means of salt bridges. This configuration is similar to the PAR 310 static mercury drop electrode detector cell in that although the working electrode is below the level of the electrolyte eluent only comes into contact with it when the pump is switched on. This present arrangement has proved to be convenient and trouble free. A detector cell which holds only the working electrode can clearly be constructed much more simply than conventional detector cells holding three electrodes, and several versions of such a detector have been designed and constructed in our workshops. The use of these home-built detectors was mentioned previously[239] and some later results were obtained using the final design[291-292].

The details of the construction of this detector cell have not been published previously mainly because it was felt that workers would prefer to purchase commercial detector cells and to use them conventionally

with the three electrodes inserted. Recently, however, our work has been redirected at reductive voltammetric detection at negative potentials, and the detector cell proved to be equally satisfactory when used in an inverted position on top of a glass U-tube holding a sessile mercury drop electrode.

The main purpose of this section is to describe the construction of the detector cell and the sessile mercury drop unit, and to illustrate their use in the reductive mode at negative potentials. The permitted synthetic food colouring matters were chosen for this first study. The oxidative determination of the food colouring matters at a glassy carbon electrode with the Metrohm detector cell has been reported previously[237]. Nevertheless oxidative signals for some of the food colours obtained using the home-built detector cell with a glassy carbon electrode are given here for comparison with the reductive signals.

EXPERIMENTAL

Construction of the detector cell and the sessile mercury drop electrode holder

Details of the construction of the detector cell from PTFE rod of 2.5 cm diameter are given in Fig. (43). In our workshops the turning and recessing necessary were carried out before the machining of the sides of the cell. The detector cell was designed to accomodate a Metrohm glassy carbon electrode (EA 286) which is cylindrical and of 7 mm outer diameter. The eluent inlet block was drilled as follows. The first 1 cm depth was drilled threaded (¼" x 28 threads per inch U.N.F.) and flat-bottomed for convenient butting of a flanged tube. The next 1 cm depth was drilled straight with 1 mm diameter, and the remaining small depth (approx 1 mm) was drilled with a fine No. 80 drill (0.35 mm).

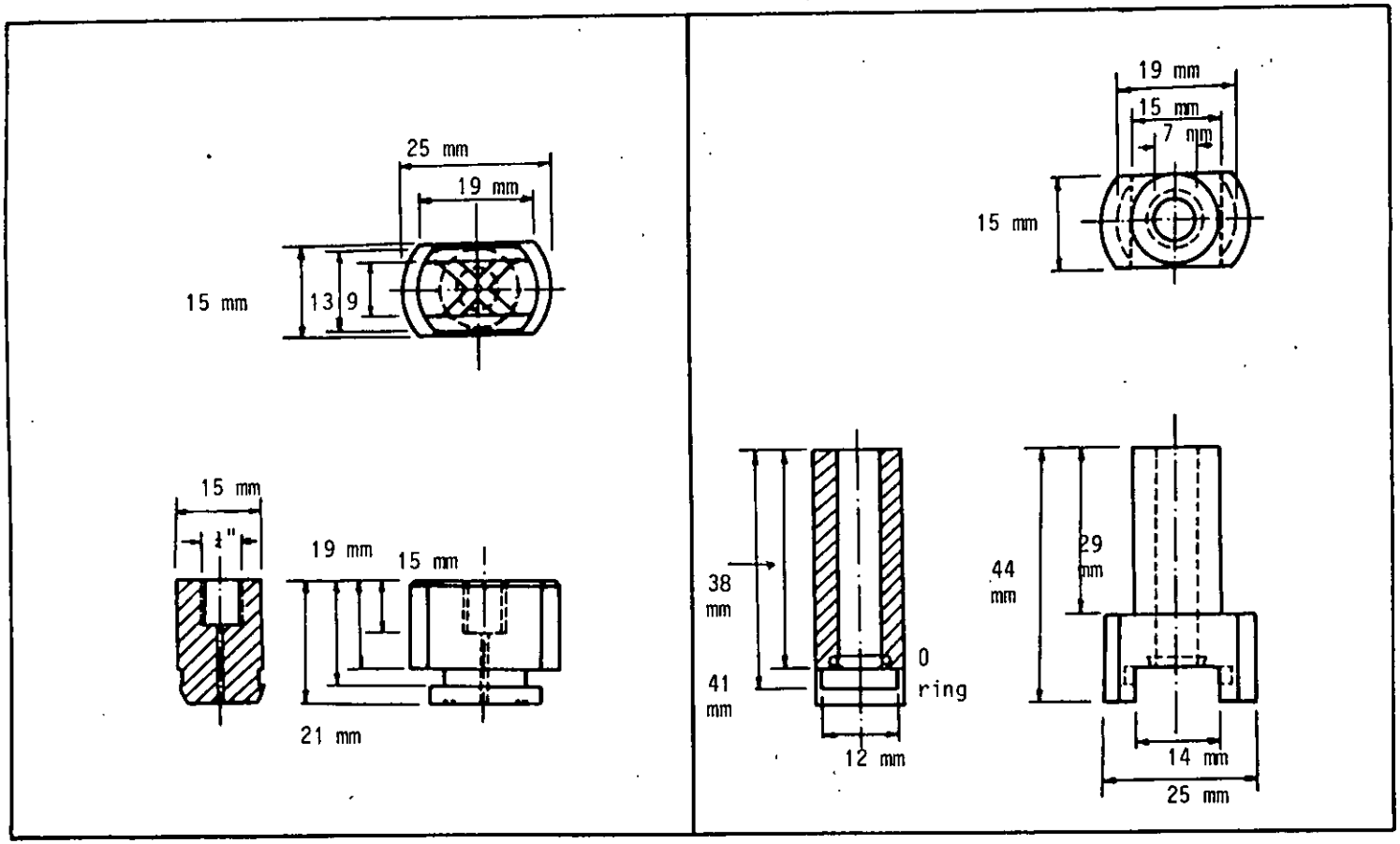


Figure 43.

Details for construction of detector cell (i) Part holding eluent entry port, (ii) part holding electrode.

The depth and width of the X- channel, which allows separation of the eluent exit port and the glassy carbon electrode, and allows escape of eluent into the bulk electrolyte, was 0.25 mm and 2.5 mm respectively. The o ring was 1.5 mm thick and 7 mm in internal diameter: the recess which holds it (1.4 mm deep and 1.9 mm wide) was set back 1.5 mm from the end.

Details of the sessile mercury drop electrode holder are given in Fig. (44). Two methods of construction were used. Initially the end of a length of platinum wire was beaded in a blow-pipe flame and then flattened to produce a platinum pin. The head of the pin was sealed firmly in place in the glass capillary tube (2 mm inner diameter, 7 mm outer diameter) by glassblowing techniques. The end of the platinum pin was then soldered to copper wire by tamping molten solder down the capillary tube. The construction of the U-tube was completed by normal glass blowing techniques. A more convenient method of construction (see Fig. 44(ii)) was as follows. A length of 2 mm i.d./7 mm o.d. capillary tubing was joined to a length of 8 mm o.d. medium walled tubing. A disc of platinum foil approximately 4 mm in diameter was lightly tacked on the end of capillary tubing sealing the bore. Another length of capillary tubing was butt-jointed over the platinum disc, care being taken to maintain the capillary bore. A U-bend was formed in the 8 mm tubing and the capillary tube was cut-back to approximately 2 mm from the platinum disc. To make electrical contact with the platinum disc small pieces of small diameter solder were passed down the 8 mm tube and shaken down the capillary to the platinum disc. A length of single-strand copper wire was inserted into the tube and pushed round the U-bend. The solder was heated until softening occurred and the wire was pushed down to ensure good connections subseqentially between the copper wire, the hardened solder and the

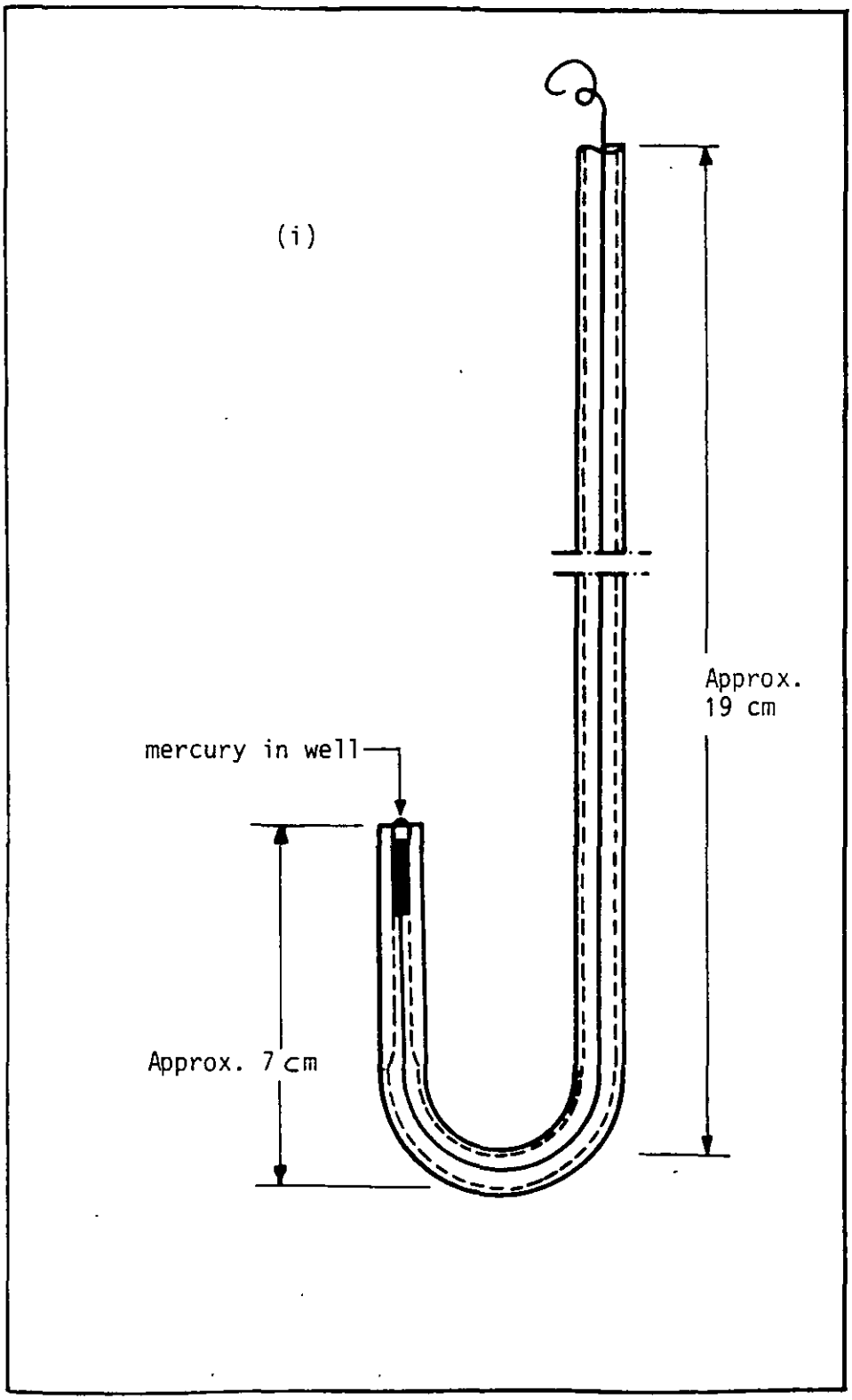


Figure 44. Sessile mercury drop electrode holder. (i) general construction (ii) detail of alternative construction (iii) well (filled with mercury). see over leaf

platinum disc.

In this work the well holding the mercury drop was of 2 mm diameter. Mercury was placed in the well whilst the platinum contact was still dry. An indication of the amount used in relation to the size of the well is given in Fig. 44(iii). The position of the mercury electrode in the detector cell is also shown. Experiments with different amounts of mercury soon indicate the optimum amount to use.

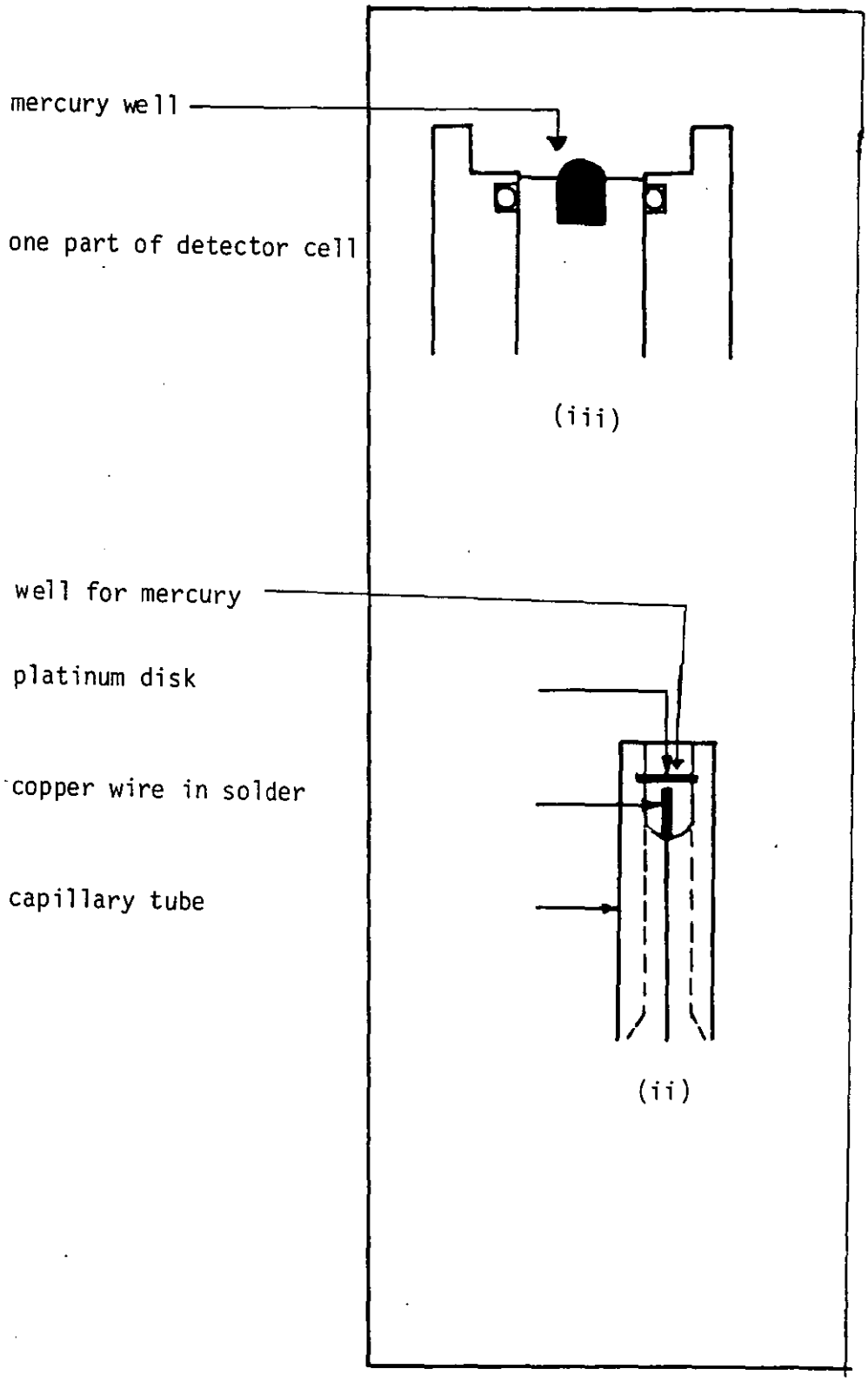

mercury well
one part of detector cell
(iii)
well for mercury
platinum disk
copper wire in solder
capillary tube
(ii)

Figure 44.

Determination of synthetic food colouring matters

Reagents

Britton-Robinson buffers (0.04M in each of the three buffer components)

Dissolve 2.47 g of boric acid in 500 ml of distilled water containing 2.3 ml of glacial acetic acid, then add 2.7 ml of orthophosphoric acid and dilute to 1 ℓ with water. This solution has a pH of approximately 2. Adjust amounts of the solution to the pH required by addition of 1M sodium hydroxide solution. Buffer solutions of pH>7 were deoxygenated with nitrogen and then treated with sodium sulphite heptahydrate to give a final concentration in the buffer of 0.16 g ℓ^{-1}.

Sulphuric acid solution, 0.01M.

Dilute 0.55 ml of concentrated sulphuric acid to 1 ℓ.

Standard food colouring matter solutions

Dissolve 0.100 g of food colour in water and dilute to 100 ml with water in a calibrated flask. These solutions contain 1000 ppm of food colour. Prepare more dilute solutions by successive ten-fold dilution of these solutions in 100 ml calibrated flasks adding 20 ml of 0.01M sulphuric acid solution or Britton-Robinson buffer of the appropriate pH at the final dilution stage. In the case of solutions prepared with Britton-Robinson buffers of pH>7, which contained sulphite, the final dilution was made with water previously deoxygenated with nitrogen gas. These solutions were deoxygenated briefly for about 2 minutes before being injected. Solutions prepared with Britton-Robinson buffers of pH<7 were deoxygenated with nitrogen gas for 15 minutes before injection.

Operation of the detector cell in the oxidative mode with a glassy carbon electrode

The cell was used partially immersed in 0.01M sulphuric acid solution as shown in Fig.45(a). The eluent (0.01M sulphuric acid solution) was presented from below the glassy carbon electrode at a rate of 10 ml min^{-1} by means of an Ismatec Mini-S peristaltic pump. A platinum counter electrode and a saturated calomel reference electrode were placed in the 0.01M sulphuric acid solution on opposite sides of the detector cell as close to the working electrode as possible. Injections (120 µl) of food colouring matter solutions in 0.01M sulphuric acid solution were made by means of a low-pressure Rheodyne valve (5020). The valve was connected to the detector cell with 1 m of 0.58 mm bore tubing. Neither eluent nor sample solution was deoxygenated or degassed. The potential of the glassy carbon electrode was controlled by means of a PAR 174A polarographic analyser (Princeton Applied Research); current peaks were recorded on a Linseis L650 recorder. A schematic diagram of the system is shown in Fig.(46).

Usually three injections were made of each solution tested.

Operation of the detector cell in the reductive mode with a sessile mercury drop detector

In this case determinations of the food colours were made initially in Britton-Robinson buffer solutions of pH previously found to be most suitable for their polarographic determination[284] but later all of them were determined at alkaline pH values so that sulphite could be used. The cell was used partially immersed in Britton-Robinson buffer of this pH. Eluent of the same composition was presented from above the mercury drop, as indicated in Fig. (45b) by means of the Ismatec Mini-S peristaltic pump at a rate of 6 ml min^{-1}. Counter and

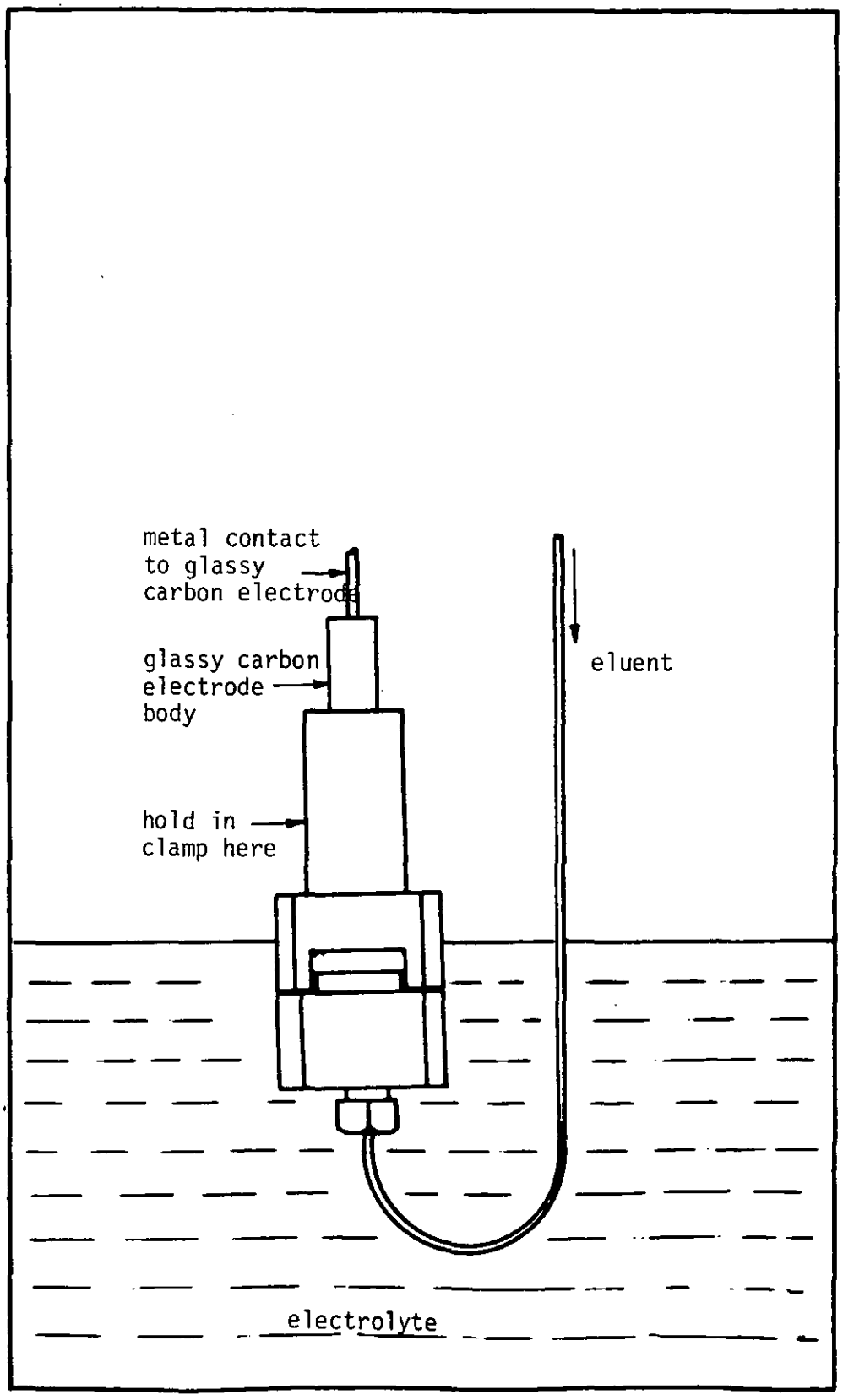


Figure 45a. Detector configuration (a) when used with a glassy carbon electrode, and (b) when used with a sessile mercury drop electrode. (see over leaf)

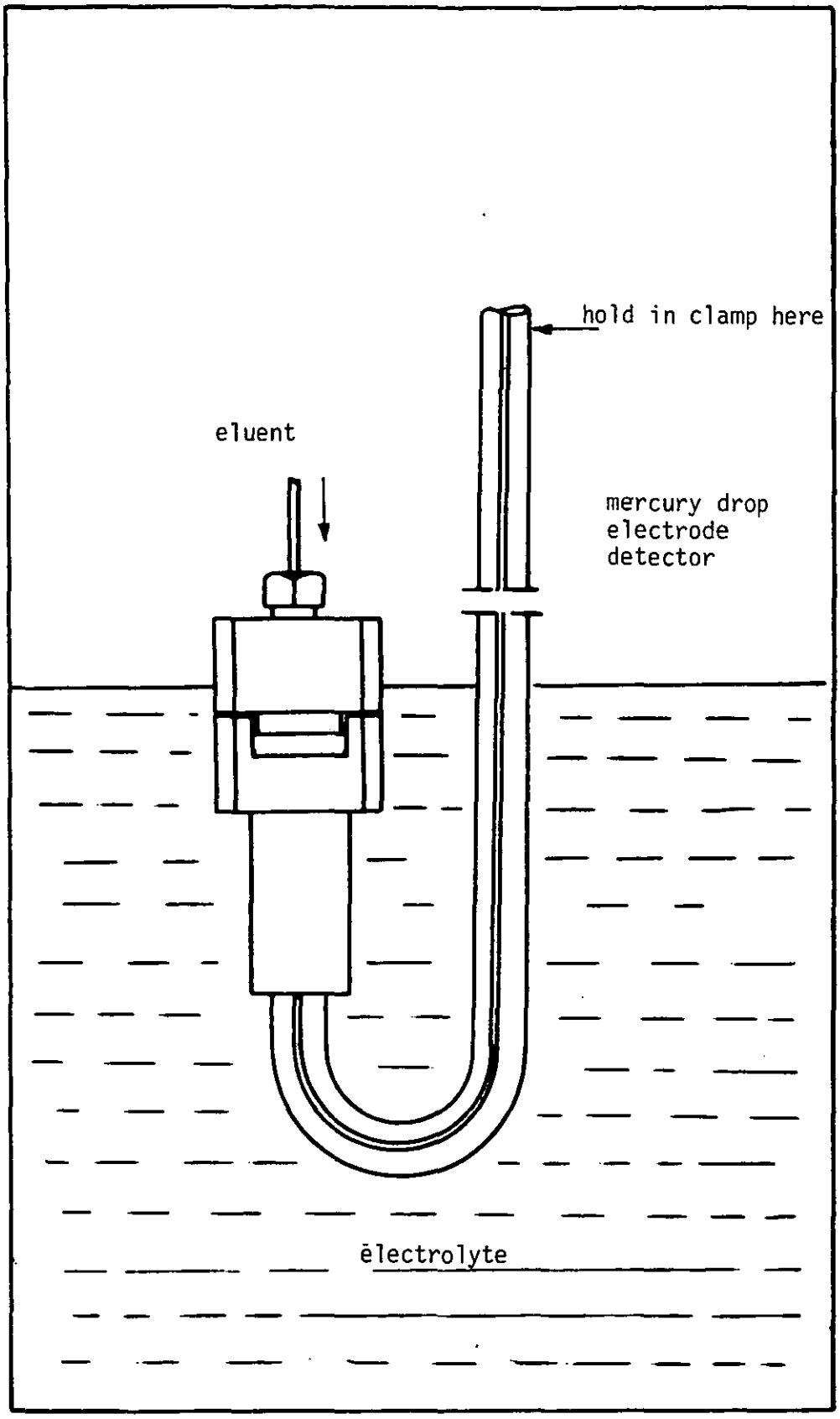

hold in clamp here
eluent
mercury drop
electrode
detector
electrolyte

Figure 45b.

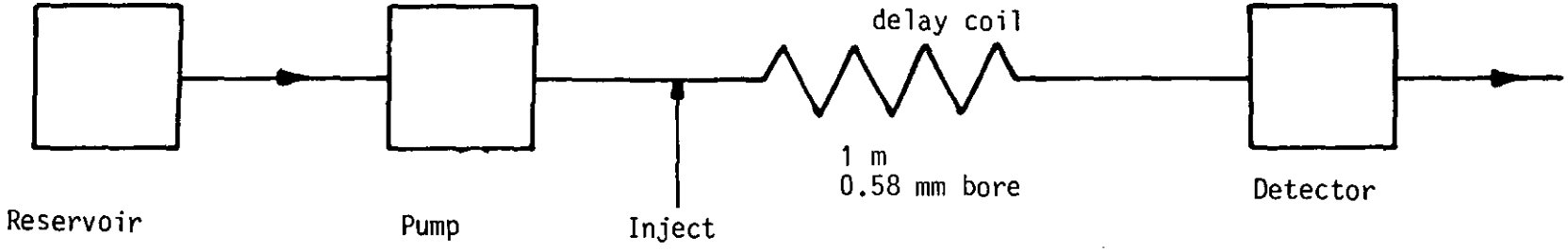


Figure 46 Schematic of flow injection system.

reference electrodes were placed as indicated above, and solutions (120 µl) of the food colouring matters in Britton-Robinson buffer were injected by means of the Rheodyne valve (5020). The length of the 0.58 mm bore tubing between the valve and detector was again 1 m.

Eluent contained in a 500 ml beaker covered as far as possible with a cling film was deoxygenated by means of nitrogen gas for 20 minutes before use and also during use. The beaker acts as the reservoir. Sample solutions were also deoxygenated. The addition of solid sodium sulphite to the eluent (pH>7) after the 20 minutes deoxygenation with nitrogen to give a sodium sulphite heptahydrate concentration of about 0.16 g ℓ^{-1} was found to lower the background current due to residual oxygen markedly and allowed the detection limit to be lowered an order of magnitude. High concentrations of sulphite reduce chemically many food colouring matters but no reaction was observed at the sulphite concentration used here even at the lowest concentrations of food colour measured. This was tested by means of differential pulse polarography. Note that the sample solutions of pH>7 were prepared using Britton-Robinson buffer containing sulphite.

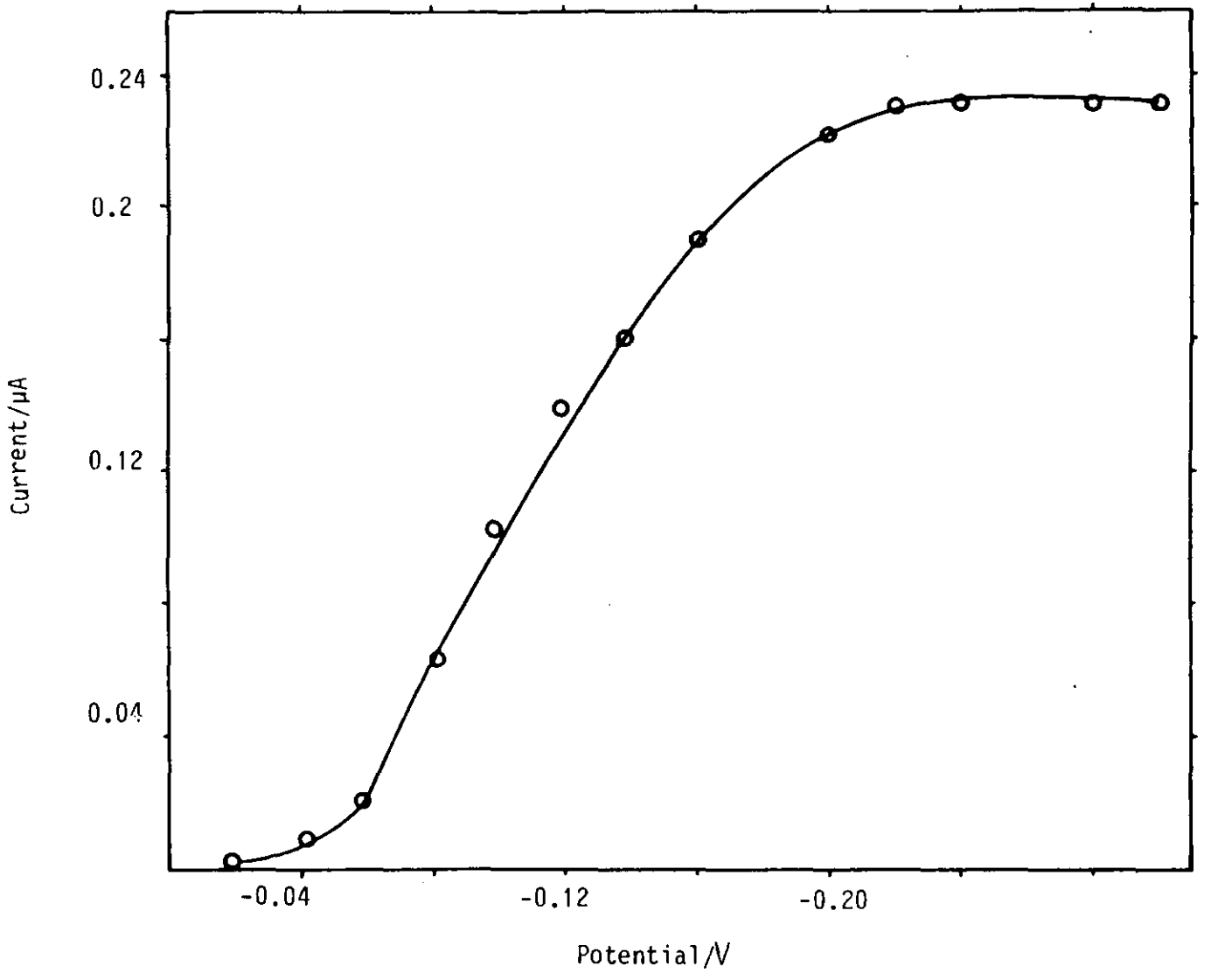
Usually three injections were made of each solution tested; the coefficient of variation of the signals obtained was typically <1%. When filling the sample loop it is important not to re-introduce air into the sample solution.

RESULTS

The main purpose of this work was to study the characteristics of the detector cell when used reductively with a sessile mercury drop electrode. In Table (32) the pH chosen for the determinations, the potentials at which the determinations were made and the current obtained

at levels of food colour from 01 - 100 ppm are given. Without the addition of sulphite to the eluent (pH<7) determinations could only be made down to the 1 ppm level (Table (32)). From the current levels obtained it is clear that the response is rectilinear from 0.1 to about 60 ppm: above this level there is a slight loss of signal. A typical hydrodynamic voltammogram obtained by making injections at different potentials is shown in Fig. (47). The plateau of each hydrodynamic voltammogram was found typically to begin at a potential about 150 mV more negative than the peak potential observed in differential pulse polarography. Signals obtained from Sunset Yellow FCF over the full range of concentrations studied here are given in Fig. (48): these are typical of those obtained with all the food colours.

Oxidative signals at a glassy carbon electrode were obtained for some of the permitted food colouring matters in 0.01M sulphuric acid solution. The results are compared in Table 1 with those obtained reductively at the sessile mercury drop electrode. The plateau of each hydrodynamic voltammogram began at a potential about 200 mV more positive than the peak potential obtained by linear sweep voltammetry (scan rate 5 mV s^{-1}) and this plateau potential became slightly more positive with increasing concentration of food colouring matter. Determinations were made at potentials sufficiently positive to be suitable at all concentrations. The results clearly show that the signals obtained at the sessile mercury drop electrode are rectilinear over a much wider concentration range than those obtained at a glassy carbon electrode. In Fig. (49) signals obtained for Sunset Yellow FCF at the 10 and 100 ppm level at the sessile mercury drop electrode and the glassy carbon electrode are compared. The loss of rectilinearity in the glassy carbon electrode signal is clearly seen.


0.24
0.2
0.12
0.04
Current/μA
-0.04
-0.12
-0.20
Potential/V

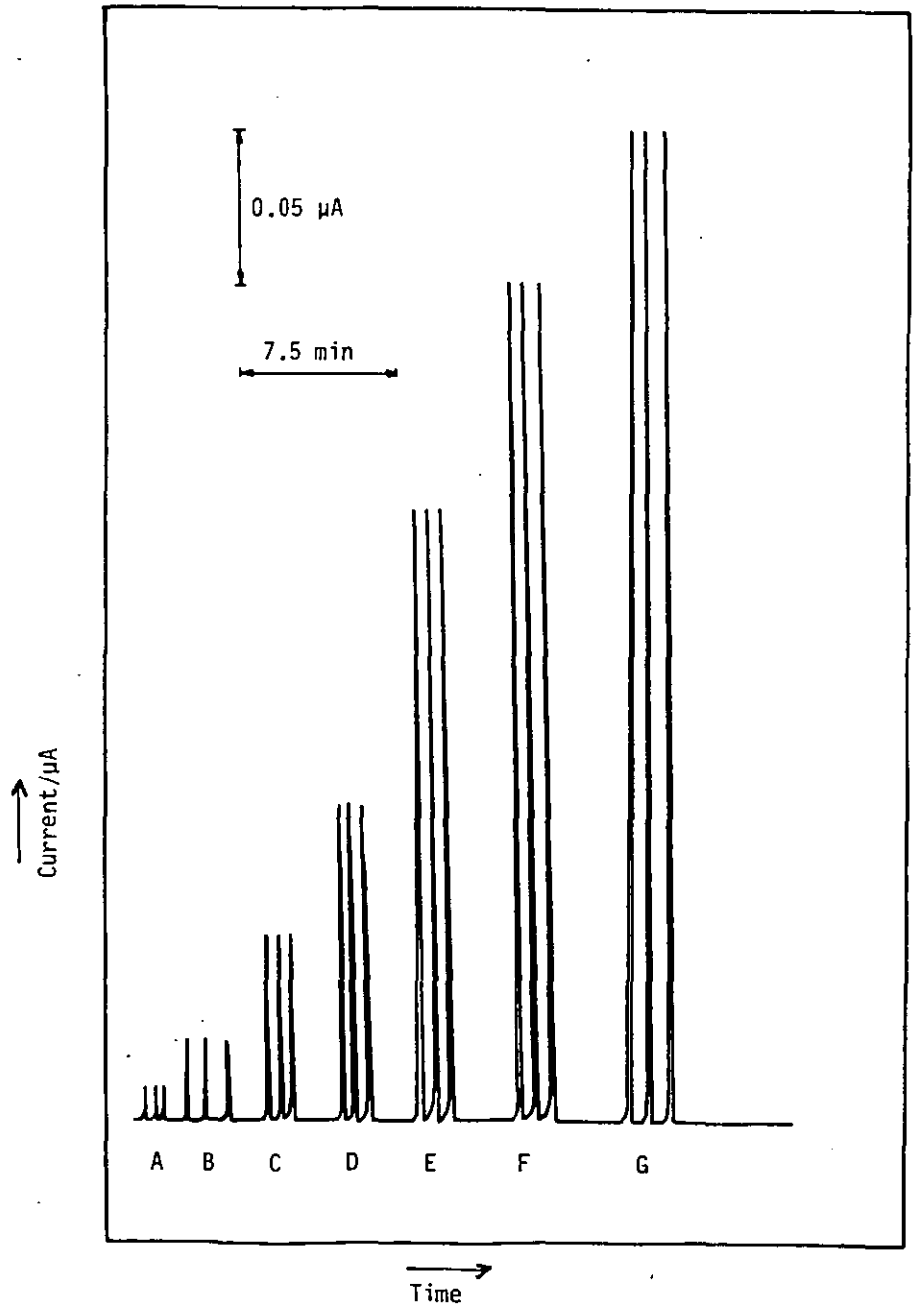


Figure 48. Signals obtained for constructing a calibration graph (pH9) for the determination of tartrazine at a sessile mercury drop electrode. Tartrazine concentration: A 0, B 0.25, C 0.8, D 1.5, E 3.1, F 4.2 and G 5.0 ppm.

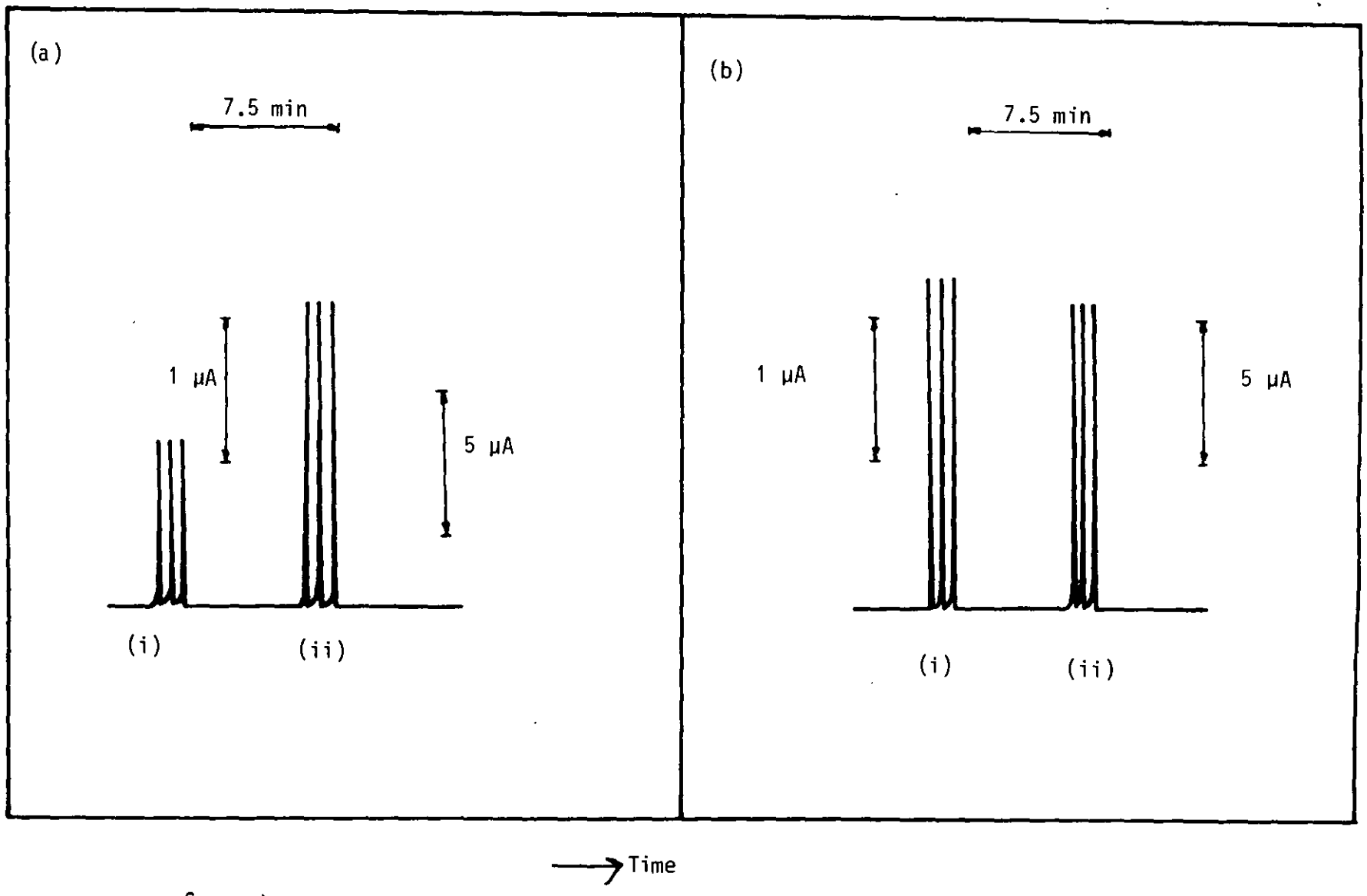


Figure 49. Comparison of signals for Sunset Yellow FCF at (a) sessile mercury drop (pH9) and (b) glassy carbon electrodes Sunset Yellow FCF concentrations: (i) 10, (ii) 100 ppm.

Study of the effect of injection volume, coil length and flow rate:

The peak height and the peak width in flow systems depend on the dispersion of the sample during its passage from injection to detector. The factors affecting dispersion such as injection volume, coil length, and flow rate were investigated.

The peak height was found to be proportional to the injection volume in the range (100-120 µl). Therefore the peak height and thus the sensitivity increased with increasing injection volume up to 120 µl. Above this volume no increase in the peak height occurred. The effect of coil length was also investigated and the peak height was found to decrease with increase in coil length above (1m), see Table (33).

Flow rate influences the characterisation of the peak shape by its effect on the thickness of the diffusion layer at the mercury electrode. The effect on peak height is shonw in Fig. (50).

The figure shows clearly that the peak width and the mean residence time decrease with increasing flow rate. The decrease in the peak current with the decrease in flow rate could be attributed to diminished rate of diffusion of the electroactive species to the electrode surface.

The combination of an injection volume of 120 µl with a coil length of 1m and a flow rate of 6 ml min^{-1} was found to be convenient for routine analysis using this stationery mercury electrode. The peak height was also found to be affected by the low pass filter, i.e. increase of the low pass filter value decreases the peak height up to 10%, but the noise decreases also.

Background current:

The dependence of the base line current on the working potential is shown in Figure (51) for Britton Robinson buffer mobile phase (pH<7)

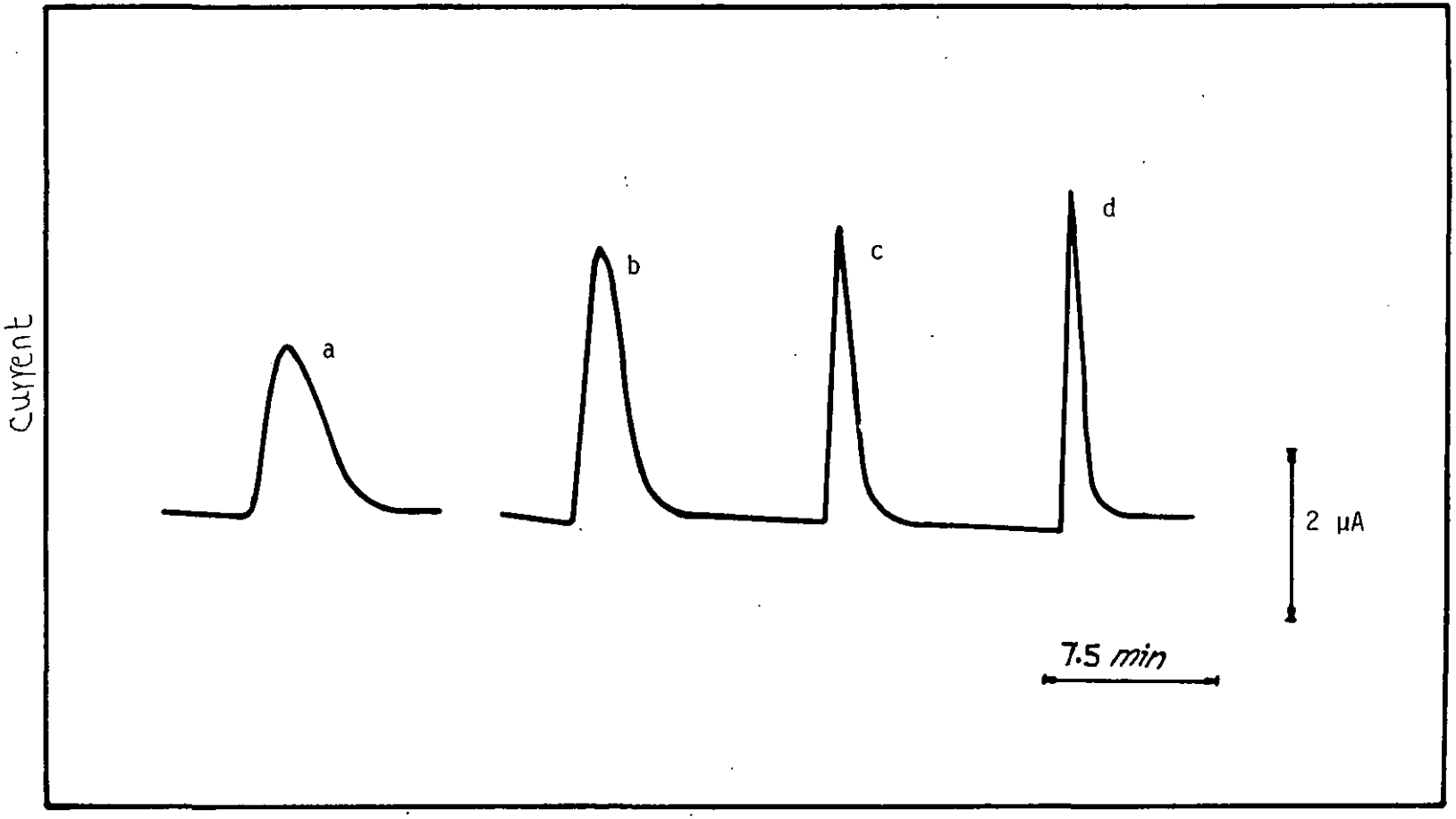


Figure 50. The dependence of the peak height on flow rate.
(a) 1 ml/min (b) 2 ml/min (c) 4.5 ml/min (d) ml/min 7

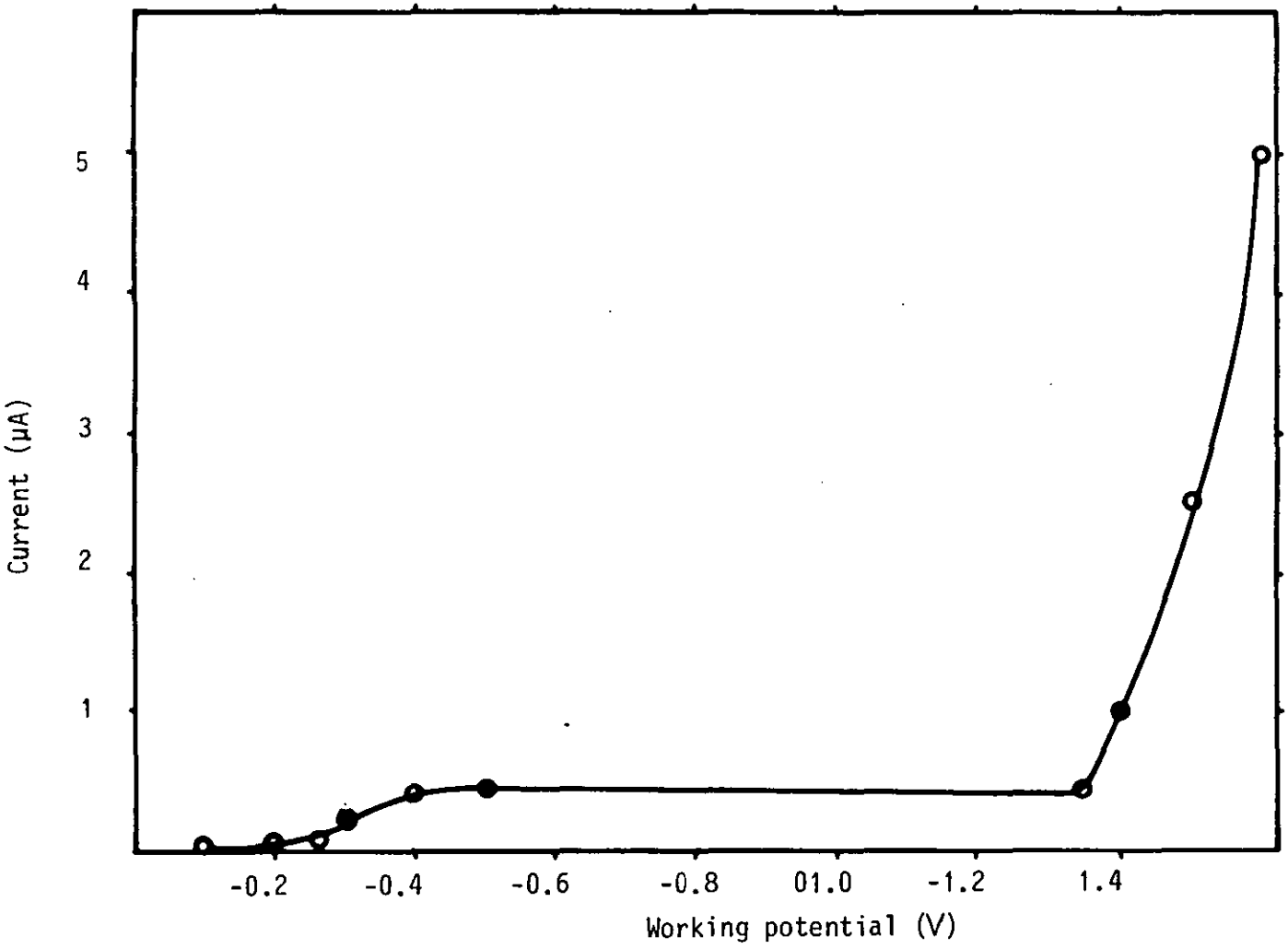


Figure 51. The relationship between the working potential and the background alkaline solution, sulphite concentration 0.16 g/l.

in the presence of sulphite. Clearly the base line between +0.2V and -1.2V is very flat and ideal for normal measurements.

However the relation between the background and the flow rate is shown in Table (34). It is clear that the background is flow rate dependent up to 5.5 ml min^{-1}: above that rate no significant dependence on the flow rate was observed. The stability of the flow rate had a great affect on the baseline noise. After the renewal of the electrode steady state conditions were usually re-established within a few minutes.

DISCUSSION

The construction of a simple voltammetric detector cell and its use in flow injection analysis have been described. The cell, which holds only the working electrode and the eluent inlet, is used partially immersed in an electrolyte in which conventional platinum counter and potentiometric calomel reference electrodes are also placed, as close to the working electrode as possible. The detector cell can be used equally conveniently with a solid electrode such as a glassy carbon electrode or with a sessile mercury drop electrode. The cell is positioned differently when used with two types of electrode: with the solid electrode the eluent is presented from below, whereas with the mercury drop electrode it is presented from above.

This configuration in which the detector cell is partially immersed in electrolyte is similar to that of the PAR 310 static mercury drop detector (Princeton Applied Research). The static mercury drop of the PAR 310 can be used in the hanging mercury drop mode or as a dropping mercury electrode. In the PAR 310 and the present cell the working electrode "sees" only the eluent emerging from the eluent inlet port,

despite being immersed in the electrolyte into which the eluent flows. Applications of the use of the PAR 310 detector cell in HPLC[293-294] and FIA[295] have been reported. Hanging mercury drop electrodes in general can be problematic and capillaries on the PAR 310 have been modified recently by the manufactureres in order to obtain more consistently good performance.

The present cell is simple and inexpensive to construct. A distinct advantage over many detector cells that hold all three electrodes separated by narrow eluent channels is that any air or gas bubbles that are formed cannot become trapped in the system. Air bubbles trapped at the reference electrode frit in the conventional cells are particularly problematic. Also when partly non-aqueous eluents are used there is a tendency in conventional detectors for solid potassium chloride to form and block small reference frits, and such frits have to be maintained carefully. Degassing of the eluent is not necessary with the present cell. The present cell is not as neat in use as is a totally enclosed cell because of the need to immerse it partially in electrolyte, and many chromatographers would consider this a distinct disadvantage.

Unsatisfactory design of voltammetric detector cells can give rise to problems associated with uncompensated resistance. This is caused by the resistance of the electrolyte between the reference and working electrodes being high because of the narrowness of the eluent channels. In a good design the reference electrode is placed as close as possible to the working electrode in order to lower the resistance. In some detectors the uncompensated resistance is lowered in a different way by placing relatively large working and counter electrodes opposite each other across a thin eluent stream. The large rectilinear range

obtained using the sessile mercury drop electrode in the present cell indicates that the uncompensated resistance in this cell is very small.

The interlocking mechanism allows the part of the cell holding the eluent inlet to be removed, thus revealing the mercury drop in situ in the other part of the cell. This allows easy filling of the cavity with mercury and easy renewal as required.

Sessile mercury drop and small mercury pool electrodes have been described previously for use with HPLC. Lund et al[228] have described an elegant device holding all three electrodes. This device can be used with a solid, carbon paste or mercury pool electrode and has negligible uncompensated resistance. Rabenstein and Saetre[297] have described two versions of a small mercury pool electrode. The simplest is constructed from a standard T connector used for HPLC. To one of the three channels is connected a short length of 0.8 mm bore Teflon tubing. This is filled with mercury and electrical contact is made by means of a platinum wire of similar diameter to the tube. The position of the mercury at the T junction, where it makes contact with the eluent, is adjusted by means of the platinum wire. With this design the counter and reference electrodes are downstream in the overflow beaker and the uncompensated resistance is high giving rise to non-rectilinear calibration graphs at higher concentrations. The mercury contact in the advanced version is similar but the reference electrode is incorporated in the cell block close to the working electrode and only the counter electrode is in the overflow beaker. This latter detector showed excellent rectilinearity for the determination of glutathione from 0.1 - 4 x 10^{-4}M. Janata and Ruzicka[298] have recently used a small mercury pool electrode in studying the

combination of flow injection analysis with voltammetry.

In this work the present detector has been used to carry out flow injection analysis in its simplest form in that the technique has been used simply to present electroactive compounds to the working electrode. The availability of such systems with a sessile mercury drop might renew interest in techniques related to polarography for use in quality control. These flow systems are self cleaning and samples can be injected in rapid succession, and although mercury - which is an excellent electrode material - is used, it is used in small and more controllable quantities than in polarography. There is also the possibility of using the full potentiality of the FIA system and carrying out on-line derivatisation. Alternatively derivatisation can be carried out before injection.

Table (32) Flow injection voltammetric determination of food colouring matters reductively at a sessile mercury drop electrode and oxidatively at a glassy carbon electrode.

The current signals in μA are given: the values of the blank have been subtracted. The blank signals (reductive mode) were typically 0.005 and 0.050 μA for eluents of pH>7 (sulphite added) and <7, respectively. The blank signal at glassy carbon (oxidative mode) was typically <0.005 μA.

			Reductive Mode					Oxidative Mode				
Colour	pH of determination	Potential of determination /V	Concentration /ppm	Current /μA			Mean Current /μA	Potential of determination /V	Current /μA			Mean Current /μA
Tartrazine	9.0	-0.85	0.1	0.012	0.012	0.012	0.012	+1.4	0.025	0.023	0.023	0.024
			1	0.127	0.126	0.126	0.126		0.291	0.293	0.293	0.292
			10	1.28	1.28	1.28	1.28		2.90	2.84	2.85	2.86
			100	12.5	12.5	12.5	12.5		7.60	7.10	6.80	*
Quinoline Yellow	9.0	-1.28	0.1	0.010	0.010	0.010	0.010					
			1	0.100	0.110	0.108	0.106					
			10	0.990	0.980	0.980	0.983					
			100	9.20	9.18	9.19	9.19					
Yellow 2G	7.5	-0.82	0.1	0.006	0.006	0.006	0.006	+1.32	0.010	0.010	0.010	0.010
			1	0.071	0.072	0.071	0.071		0.085	0.084	0.084	0.084
			10	0.720	0.721	0.720	0.720		0.52	0.39	0.34	*
			100	6.46	6.45	6.46	6.46		2.20	1.70	1.54	*
	2.0	-0.48	0.1	not measurable								
			1	0.074	0.075	0.074	0.074					
			10	0.723	0.730	0.730	0.728					
			100	6.44	6.44	6.44	6.44					
Sunset Yellow FCF	9.0	-0.78	0.1	0.010	0.010	0.010	0.010	+1.20	0.032	0.028	0.029	0.030
			1	0.100	0.100	0.102	0.010		0.245	0.247	0.248	0.247
			10	1.55	1.15	1.15	1.15		2.45	2.42	2.42	2.43
			100	10.6	10.6	10.6	10.6		10.9	10.8	10.8	10.8

(Table continued)

Colour	pH of determination /V	Potential of determination /V	Concentration /ppm	Current /μA			Mean Current /μA	Potential of determination /V	Current /μA			Mean Current /μA
	2.0	-0.22	0.1	not measurable								
			1	0.100	0.100	0.099	0.100					
			10	1.17	1.16	1.16	1.16					
			100	10.6	10.6	10.6	10.6					
Amaranth	9.0	-0.71	0.1	0.0131	0.0130	0.0130	0.0130					
			1	0.121	0.120	0.120	0.120					
			10	1.19	1.18	1.18	1.18					
			100	11.1	11.1	11.1	11.1					
Carmoisine	9.0	-0.70	0.05	0.009	0.009	0.009	0.009	+1.18	not measurable			
			0.1	0.017	0.017	0.017	0.017		0.035	0.034	0.036	0.035
			1	0.140	0.140	0.140	0.140		0.310	0.320	0.310	0.313
			10	1.45	1.45	1.45	1.45		3.12	3.14	3.14	3.13
			100	13.9	13.9	13.9	13.9		11.4	11.4	11.3	11.4
Indigo Carmine	9.0	-0.57	0.05	0.012	0.012	0.012	0.012					
			0.1	0.21	0.020	0.020	0.020					
			1	0.190	0.190	0.190	0.190					
			10	1.84	1.84	1.84	1.84					
			100	16.8	16.8	16.8	16.8					
Red 2G	9.0	-0.89	0.1	0.019	0.019	0.019	0.019					
			1	0.141	1.141	0.141	0.141					
			10	1.38	1.38	1.38	1.38					
			100	12.3	12.3	12.3	12.3					
Ponceau 4R	9.0	-0.94	0.1	0.009	0.009	0.009	0.009					
			1	0.102	0.100	0.101	0.010					
			10	0.980	0.990	0.980	0.983					
			100	9.20	9.20	9.20	9.20					

(Continued)

Colour	pH of determination	Potential of determination /V	Concentration ppm	Current /μA			Mean Current /μA	Potential of determination /V	Current /μA			Mean Current /μA
Patent Blue V	7.5	-1.01	0.1	0.008	0.008	0.008	0.008					
			1	0.080	0.080	0.080	0.080					
			10	0.800	0.800	0.800	0.800					
			100	7.10	7.10	7.10	7.10					
Brilliant Blue FCF	7.5	-1.16	0.1	0.007	0.07	0.007	0.007					
			1	0.060	0.060	0.060	0.060					
			10	0.600	0.600	0.600	0.600					
			100	5.30	5.30	5.30	5.30					
Green S	7.5	-0.93	0.1	0.012	0.012	0.012	0.012					
			1	0.112	0.100	0.110	0.111					
			10	1.094	1.094	1.094	1.094					
			100	10.1	10.0	10.1	10.1					
Erythrosine	9.0	-1.18	0.1	0.013	0.013	0.013	0.013					
			1	0.130	0.130	0.130	0.130					
			10	1.29	1.29	1.29	1.29					
			100	11.9	11.9	11.9	11.9					
Black PN	9.0	-0.83	0.1	0.008	0.008	0.008	0.008	+1.18				
			1	0.075	0.075	0.075	0.075		0.088	0.086	0.087	0.087
			10	0.740	0.740	0.740	0.740		0.106	0.106	0.105	0.106
			100	7.20	7.20	7.20	7.20		6.10	5.10	4.60	*

* Loss of signal owing to adsorption

Flow Rate Effect on Background

Table (33)

Flow rate ml/min	Background µA
1.0	0.01
1.5	0.015
2.0	0.02
3.0	0.025
5.5	0.03
8.5	0.032
11.5	0.034

pH (2.0) at -0.22 V no sulphite was added

Table (34)

Effect of Length Coil (m)

Peak Current µA	Length Coil (m)
2.25	0.20
2.20	0.50
2.2	1.0
1.8	1.6
1.8	2
1.5*	4

*Broad peak

A mobile phase 0.04 M Britton-Robinson buffer pH (2.0) at a flow rate of 6 ml/min^{-1} peak currents obtained by injection 120 µl Sunset Yellow FCF, peak potential -0.26V. Concentration 25 ppm current range 5 µA.

DETERMINATION OF L-ASCORBIC ACID (VITAMIN C) IN A FLOWING SYSTEM USING A SESSILE MERCURY DROP ELECTRODE

SUMMARY:

Ascorbic acid was found to give a very well defined DC hydrodynamic voltammetric plateau in the range +0.1 to +0.22V versus SCE at a sessile mercury drop electrode in pH 5.0-5.5 1m acetate buffer.

The current signals can be used to determine the vitamin in the range 0.01 - 100 $\mu g m l^{-1}$ directly, i.e. 1 ng - 10 µg. Deoxygenation was unnecessary with the use of this fixed positive potential.

Introduction:

Electrochemical methods of determining L-ascorbic acid have been reported recently by several workers. Ruzicka and Hansen[199] cite unpublished work by Ramsing in which a carbon paste thin layer electrode was used to determine ascorbic acid in the 0.4 to 10 mm concentration range. Another worker[110] has determined ascorbic acid using platinum or graphite electrode. Redox potential detection has been applied to the determination of reducing agents injected into a non-segmented stream of cerium (IV) solution. As ascorbic acid is readily oxidised by cerium (IV) it was selected as an example and solutions containing this compound alone were analysed first. The use of a reticulated vitreous carbon flow through electrode in flow injection analysis in both the amperometric and coulometric mode of operation has been reported[299]. The reduction of ferricyanide ion and the oxidation of ascorbic acid, L-Dopa and epinephrine were investigated. The detection limit of L-ascorbic acid was found to be a few tenths of a nanogram.

Experimental and Results:

The measurements were made as previously with a PAR 174 polarographic analyser. The detector cell was used as previously, partially immersed in acetate buffer pH 5.5.

Deoxygenation was not necessary in this determination. The potential of the mercury drop was held at +0.19V using a PAR 174 polarographic analyser. Current signals were monitored as before on a Linseis L650.

Solution of L-ascorbic acid: (0.1g%) containing 0.0025% EDTA

Accurately weigh out 0.1g of L-ascorbic acid. Dissolve in water in a 100 ml volumetric flask, add 0.0025g of EDTA, and dilute to the mark with distilled water. Transfer 5 ml of this solution to another 100 ml graduated flask, and dilute to volume. The final concentration will be 50 ppm. Aliquots of the latter solution were pipetted into a 100 ml volumetric flask, 20 ml of buffer was added, and the solution was diluted to the mark with distilled water.

For smaller concentrations (less than 1 ppm) further dilution of the standard solution of 50 ppm was necessary as follows:

Eight mls of the solution were diluted in a 100 ml volumetric flask to the mark with distilled water, the final concentration prepared was 16 ppm. Aliquotes of this solution were transferred into 100 ml volumetric flasks to prepare different concentrations. In the case of lower concentrations, (less than 0.1 ppm), the latter solution (16 ppm) was further diluted to 2 ppm in another 100 ml volumetric flask. Successive dilutions were made for the required concentration. As in the previous section, the combination of an injection volume of 100 µl with a coil length of 1 m and a flow rate

of 6 m/min^{-1} was found to be convenient for routine analysis using this electrode.

The current reached its baseline value about 20 seconds after an injection allowing a maximum sampling frequency of about 180 samples per hour.

The coefficient of variation for 5 measurements was generally less than 1%.

The background current:

Because the background current is almost negligible over the range of positive potential of the mercury drop electrode, the baseline was found to be very flat and the determination of vitamin C at +0.19V was found to be extremely simple. Furthermore the background current was not affected by increasing the flow rate here as it was noticed at relatively high negative potentials. In general at potentials more positive than zero potential there is no interference from oxygen which is the major cause of background current. This advantage can be utilized for the determination of any electroactive compound oxidised within this range. A typical hydrodynamic voltammetric polarogram obtained with vitamin C is shown in figure (52). From the figure the detection limit is found to be 0.01 ppm which indicates that the sensitivity of this method is very high.

A typical hydrodynamic voltammogram obtained by making injections at different potentials is shown in figure (53). The plateau of the voltammogram was found to begin at a potential about 100 mV more positive than the peak potential observed by differential pulse polarography.

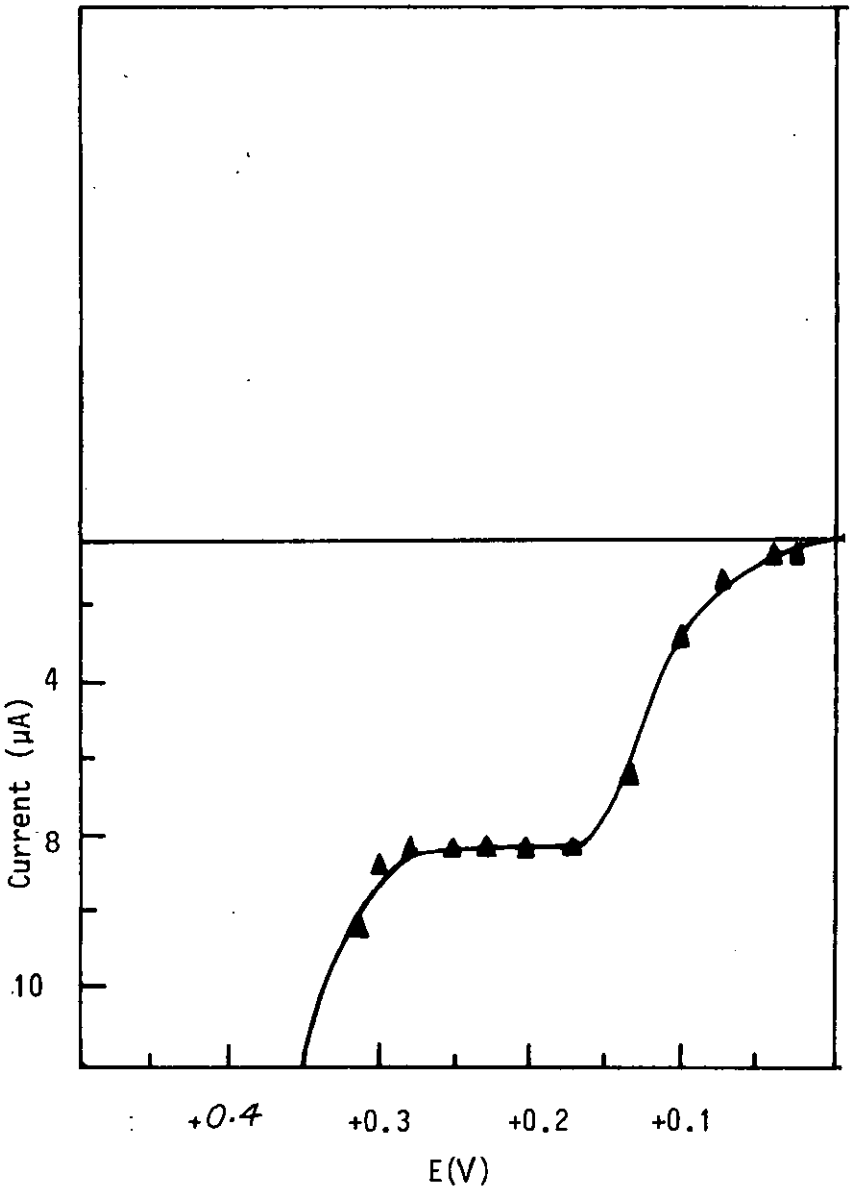


Figure 52. Hydrodynamic voltammogram obtained for ascorbic acid (40 ppm) in acetate buffer pH 5.5

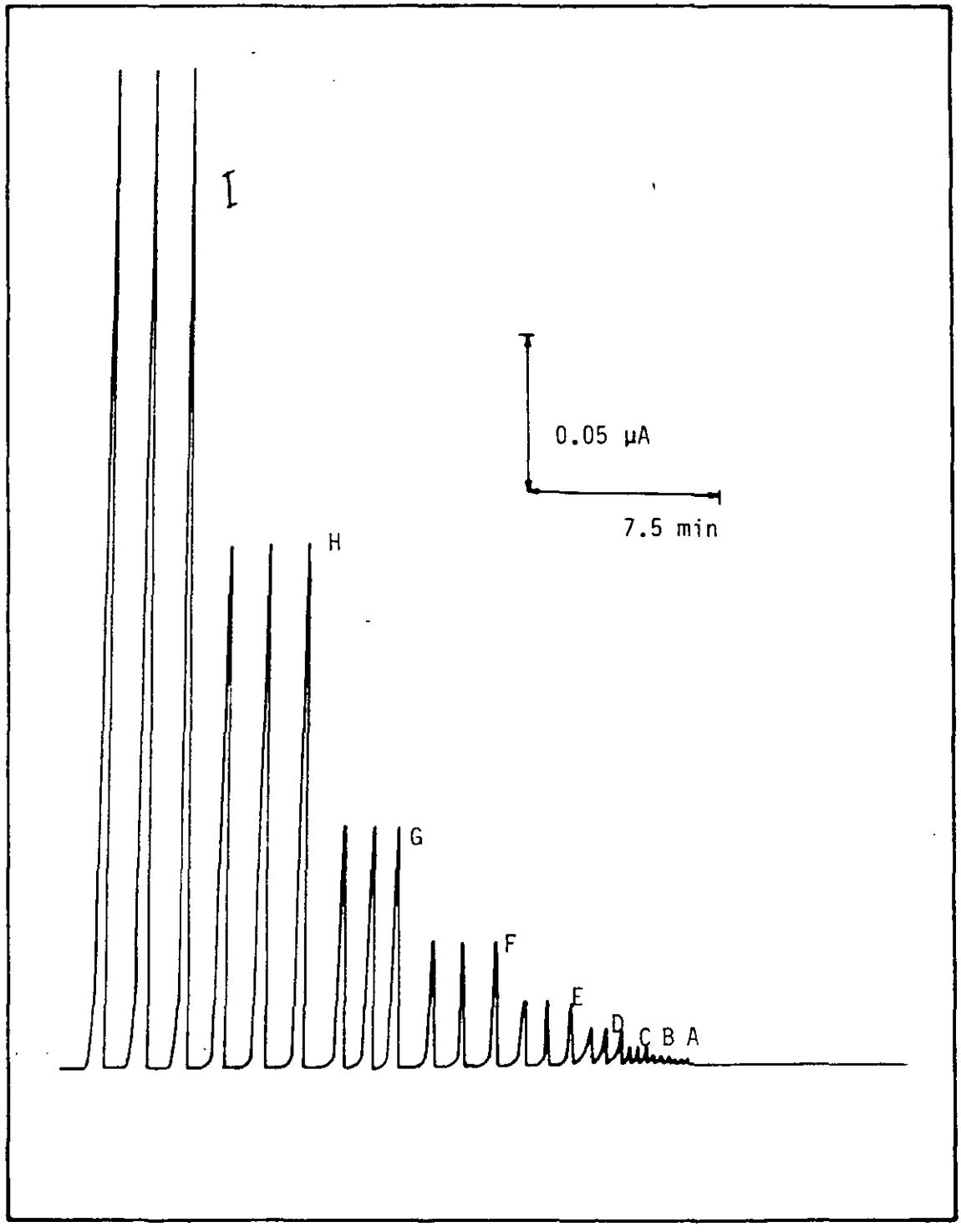


Figure 53. Typical Voltammetric Signals Obtained for Ascorbic Acid see Table (35).

Concentration(ppm).

A= Blank
B=0.01
C=0.025
D=0.05
E=0.1
F=0.2
G=0.4
H=0.8
I=1.6

Discussion:

Ascorbic acid down to the 5×10^{-8}M level can be determined very conveniently by flow injection voltammetry at a mercury drop electrode by direct injection of the sample. This low detection limit is possible because oxygen does not interfere at this potential. This method is very simple and rapid. The sampling rate is very high when compared with differential pulse polarographic determination in a static system.

TABLE (35)

Ascorbic acid determination at +0.19V in a flow system using mercury drop electrode.

Concentration (ppm)	Current (μA)
1.6	0.32
0.8	0.165
0.4	0.083
0.2	0.043
0.1	0.022
0.05	0.012
0.025	0.007
0.01	0.003
Blank	0.002

TABLE (36)

Determination of ascorbic acid at high concentration.

Concentration (ppm)	Current (μA)
4	0.831
20	4.17
50	10.42
100	20.80
200	38.92

Voltammetric determination of sulphite by use of a sessile mercury electrode in a flow injection system

The determination of sulphite in drinks such as fruit juices, is very important. Flow injection analysis using a sessile mercury drop as a detector, can be used for determining sulphite or dissolved sulphur dioxide in acidic solution. At concentrations in the range 1.0-50 $\mu g ml^{-1}$, the method has linear response, but at higher concentration a slight drop in the current signal was observed. The determination of lower concentrations was not possible due to the high background caused by diffusion of molecular oxygen from the atmosphere. Further work is needed to lower the detection limit. Ascorbic acid for instance could be used to remove the trace of oxygen from the system due to the ease of its oxidation by air.

Experimental:

The carrier stream was pumped continuously at a rate of 6 ml/min^{-1} by means of Ismatec Mini-S peristatic pump. Injections of 120 µl of sulphite solutions into 0.1 M nitric acid were made by means of a low pressure rheodyne valve (5020). The valve was connected to the detector cell wall used as previously with 1 m of 0.58 mm bore tubing. The detector cell was used as previously, partly immersed in 0.01 M sulphuric acid. The potential of the mercury drop was held at -0. 60V versus a saturated calomel electrode by means of a PAR 174A polarographic analyser. Current signals were monitored with a linseis L-650 y-t recorder. The flow injection system used is as previously.

Reagents:

Sulphite solutions:

A stock solution (1000 $\mu g m\ell^{-1}$ of SO_3^{-2}) was prepared by dissolving 1.0g of anhydrous sodium sulphite (BDH chemicals, analar grade) in 500 ml of deoxygenated water containing about 0.0025% EDTA. The volume was made up to 1l with deoxygenated water. Working solutions (50-100 $\mu g m\ell^{-1}$) were prepared by dilution of 5-10 ml of stock solution to 100 ml. Further solutions were prepared as required by dilution of the working solution. The final solution contains

Nitric acid (0.1M):

The stock solution 1M was prepared by diluting 31.5 ml of concentrated nitric acid (BDH chemicals) to 500 ml. The carrier solution (0.1M) for flow injection analysis was prepared by successive dilution of the stock solution.

0.01M H_2SO4:

Dilute 0.5 ml of concentrated sulphuric acid (BDH chemicals) with distilled water.

Procedure:

When a steady base line had been reached a 120 µl of sample was injected into the carrier stream.

The sample solutions were prepared by successive ten-fold dilution of these solutions in 100 ml calibrated flasks adding 10 ml of 0.1m nitric acid solution. The dilutions were made with water previously deoxygenated with nitrogen gas. These solutions were deoxygenated for 10 min before injection.

Result and discussion:

Sulphite sample solutions can be injected into an acidic eluent in a flow injection system and determined voltammetrically at a mercury drop electrode by the sulphur dioxide signal. A typical hydrodynamic voltammogram obtained by making injections at different potentials is shown in figure (54). The signals obtained were found to be rectilinear in the range 1-60 $\mu g m\ell^{-1}$. The coefficient of variation was found to be less than 1%.(5 measurements). The responses for 1-10 $\mu g m\ell^{-1}$ of sulphite are shown in Figure (55). This method is clearly very simple and rapid. Only two reagents are required Further work is needed to lower the limit of detection possible using L-ascorbic acid or any other reducing agent which can react with the trace of oxygen in the system. The molecular oxygen dissolved through the eluent and the sample is the major cause of high background.

Figure 54. Hydrodynamic Voltammogram Obtained for Sulphite (100 ppm) in dilute acid.

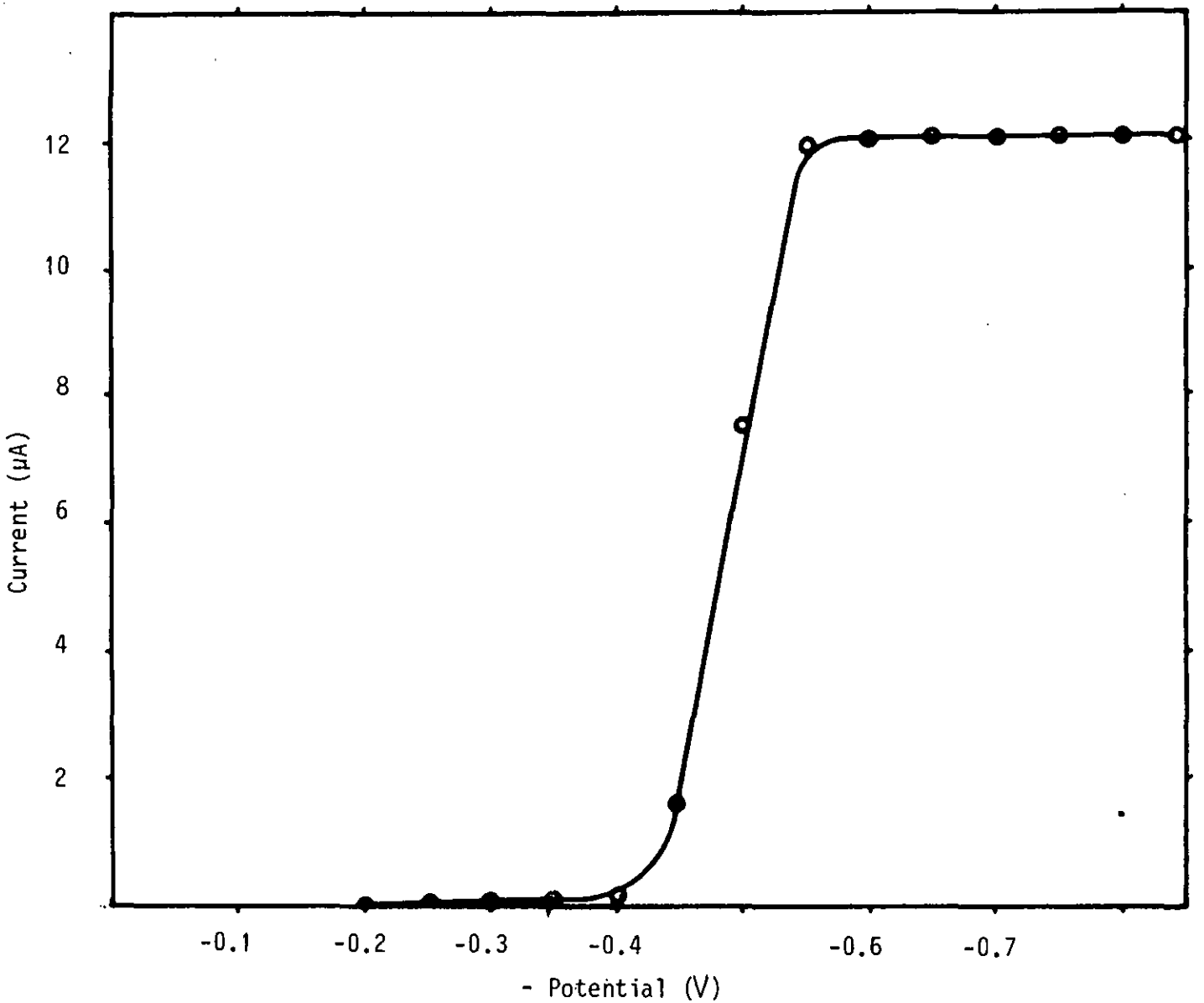
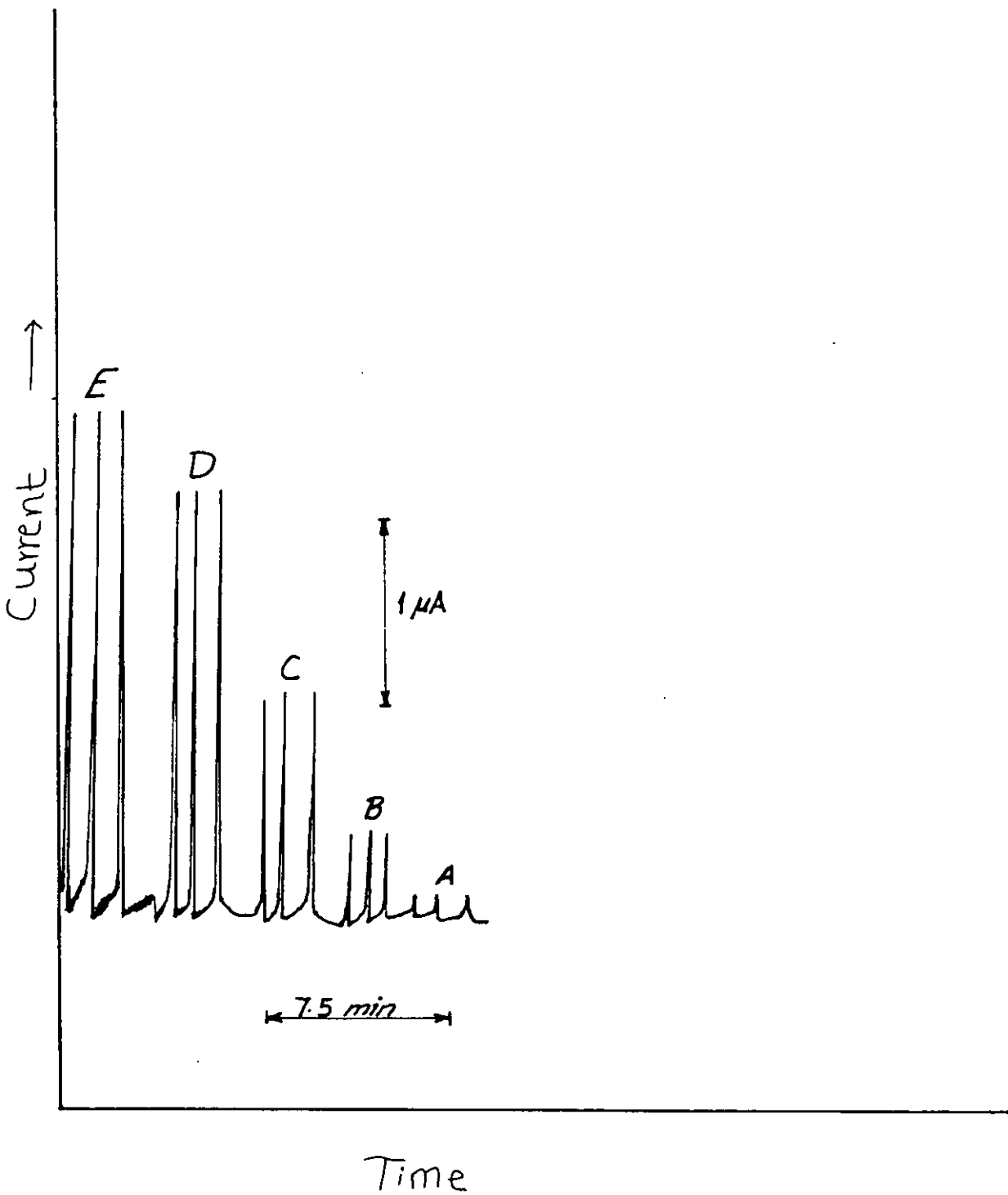


Figure (55). Typical current signals obtained for (1-10) ppm of sulphite using flow injection system.

Concentration A 0, B 1, C 4, D 8, E 10 ppm.



GENERAL DISCUSSION:

Permitted food colouring matters and ascorbic acid were determined by differential pulse polarography to monitor their interaction during light degradation studies at pH 5.5 in acetate buffer containing EDTA. The formation of aniline, sulphanilic acid and naphthionic acid during these degradations has been demonstrated and their concentrations have been determined spectrophotometrically. These simple amines have been shown to degrade further to give ammonia which was determined spectrophotometrically as indophenol.

Certain other large amines were tested and were found not to react under the conditions developed for the spectrophotometric determination of naphthionic acid and it is possible that there is little or no interference in these methods from the other amines formed during the degradation of the food colours.

Ascorbic acid was found to interfere with the reaction used to produce the spectrophotometric derivatives and any ascorbic acid remaining in the degraded solution was destroyed carefully with potassium permanganate before determining the amine. When these methods were applied to the degradation studies the yields of the simpler amines, such as sulphanilic acid, were found to increase to almost complete formation.

An attempt to determine the stoicheiometry of the reaction of food colour and ascorbic acid was made for those food colours that degraded rapidly in the presence of ascorbic acid. Limited success was achieved due to the length of time necessary for the complete loss of most of the food colours.

In general, solutions of food colouring matters have been shown by differential-pulse polarography and visible spectrophotometry to degrade faster under accelerated light degradation conditions at pH 5.5 in the presence of ascorbic acid when EDTA is absent. A full yield of ammonia from azo nitrogen atoms is obtained, and for Red 2G and Black PN the acetamido nitrogen atoms are also converted into ammonia in the absence of EDTA. Degradation of food colouring matters in the dark, in the presence of ascorbic acid but in the absence of EDTA, has been shown to occur extensively over a period of several days. The relatively high concentration of ascorbic acid used degraded in a time similar to that required for the disappearance of colour of the food colouring matters and for this reason the simple amines formed, particularly naphthionic acid, remained in appreciable yield. Red 2G was observed to degrade by deacetylation to Red 10B, which is relatively stable to attack by ascorbic acid. Black PN also was observed to degrade initially to a coloured product, which could be either the deacylated derivative of Black PN or a mono azo compound.

Ascorbic acid solutions stored in the dark became yellow and the unidentified product gave a polarographic reduction wave at -0.90V (versus S.C.E.) at pH 5.5.

Differential pulse polarography (dpp) has been used to monitor solutions at pH 5.5 in acetate buffer of permitted synthetic food colouring matters containing azo groups during accelerated heat degradation studies carried out at 130°C in sealed vials. Complete visible loss was effected i.e. 0.5 to 28 days depending on the food colour.

The yields of simple amines—aniline, sulphanilic acid and naphthionic acid - were determined by visisble spectrophotometry. The relatively high yields of aniline and sulphanilic acid obtained after complete visible loss of food colour were independent of further heating time: the stability of these two amines under the experimental conditions was varified separately. The ammonia formed in these experiments was determined by visible spectrophotometry and clearly is not formed from the simple amines which are stable. The molar yields of simple amines and ammonia from the food colour solutions were concentration dependent: in general the molar yields were lower at higher concentration presumably owing to the formation of stable nitrogen compounds of higher molecular weight. Red 10B was observed as an intermediate in the degradation of Red 2G at higher concentrations, and a stable yellow compound was formed in the degradation of Ponceau 4R also at higher concentrations.

The length of time required for complete visible loss of food colour was increased considerably by the addition of EDTA, whereas in the presence of 1000 ppm of ascorbic acid without the addition of EDTA degradation occurred within a few hours. In the latter case relatively high yields of simple amines were again observed but the amines were degraded further to ammonia by ascorbic acid.

At higher concentrations of the food colours degradation of ascorbic acid was complete before that of the food colour and the simple amines formed remained undegraded. The ascorbic acid was determined by dpp and by this means the stoichiometry of the reaction of ascorbic acid with the food colours was found to be 2.3, 2.3, 2.4, 3.0 and 3.9 for Sunset Yellow FCF, Ponceau 4R, Carmoisine, Brown FK and Chocolate Brown HT respectively.

The differential pulse polarographic determination has been applied to the determination of food colours in boiled sweets. No prior separation or extraction procedures were necessary.

The construction of a simple wall-jet detector cell which can be use either with a solid electrode or with a sessile mercury drop electrode is described. The detector is used partly immersed in electrolyte solution to give contact with a counter and a reference electrode. Use of the cell is illustrated by the determination of permitted synthetic food colouring matters at the 0.1 $\mu g\ ml^{-1}$ level with both electrodes. Without the addition of sulphite to the eluent (pH<7) determinations could only be made down to the 1 ppm level. Ascorbic acid was found to give a very well defined DC hydrodynamic voltammetric plateau in the range +0.1 to +0.22V versus SCE at a sessile mercury drop electrode in pH 5.0 - 5.5 1m acetate buffer in a flowing system. The current signals can be used to determine the vitamin in the range 0.01 - 100 $\mu g ml^{-1}$, i.e. 1ng-10µg. Deoxygenation was unnecessary with the use of this fixed slightly positive potential.

Flow injection analysis using a sessile mercury drop as a detector has been applied to the determination of sulphite or dissolved sulphur dioxide in acidic solution. At concentrations in the range 1.0 - 50 $\mu g ml^{-1}$, the method has linear response, but at higher concentration a slight drop in the current signal was observed.

Ammonia and simple amines have been shown to be produced in the degradation of permitted food colouring matters. In the case of heat degradation evidence has been given that some nitrogen remains in an organic form in the degraded solutions. Further work is required

to identify them and other organic degradation products. Preliminary HPLC studies on the degradation products have been carried out by Whetstone (PhD thesis, LUT, 1982) in this laboratory and this gives some indications of the large molecules of product formed.

The sessile mercury drop detector has been shown to be very satisfactory for use with FIA. Further studies are required for work at potential where oxygen does not interfere, and in applying the detector to HPLC studies.

APPENDIX (I)

Light degradation in acetate buffer pH (5.5) of some of the food colours and ascorbic acid.

Initial food colour concentration = 5 ppm

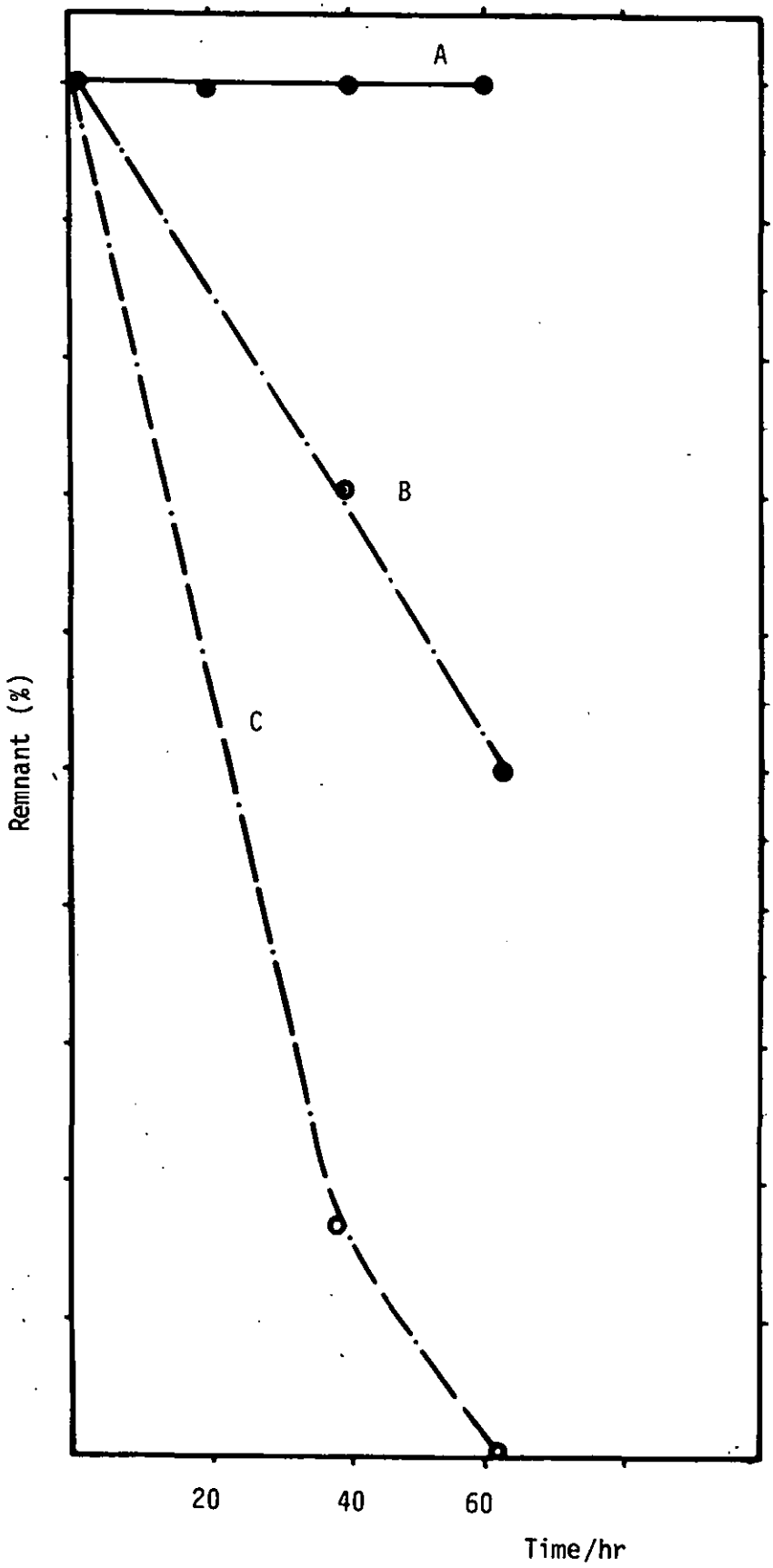
Initial ascorbic acid concentration = 100 ppm

A = The food colour alone

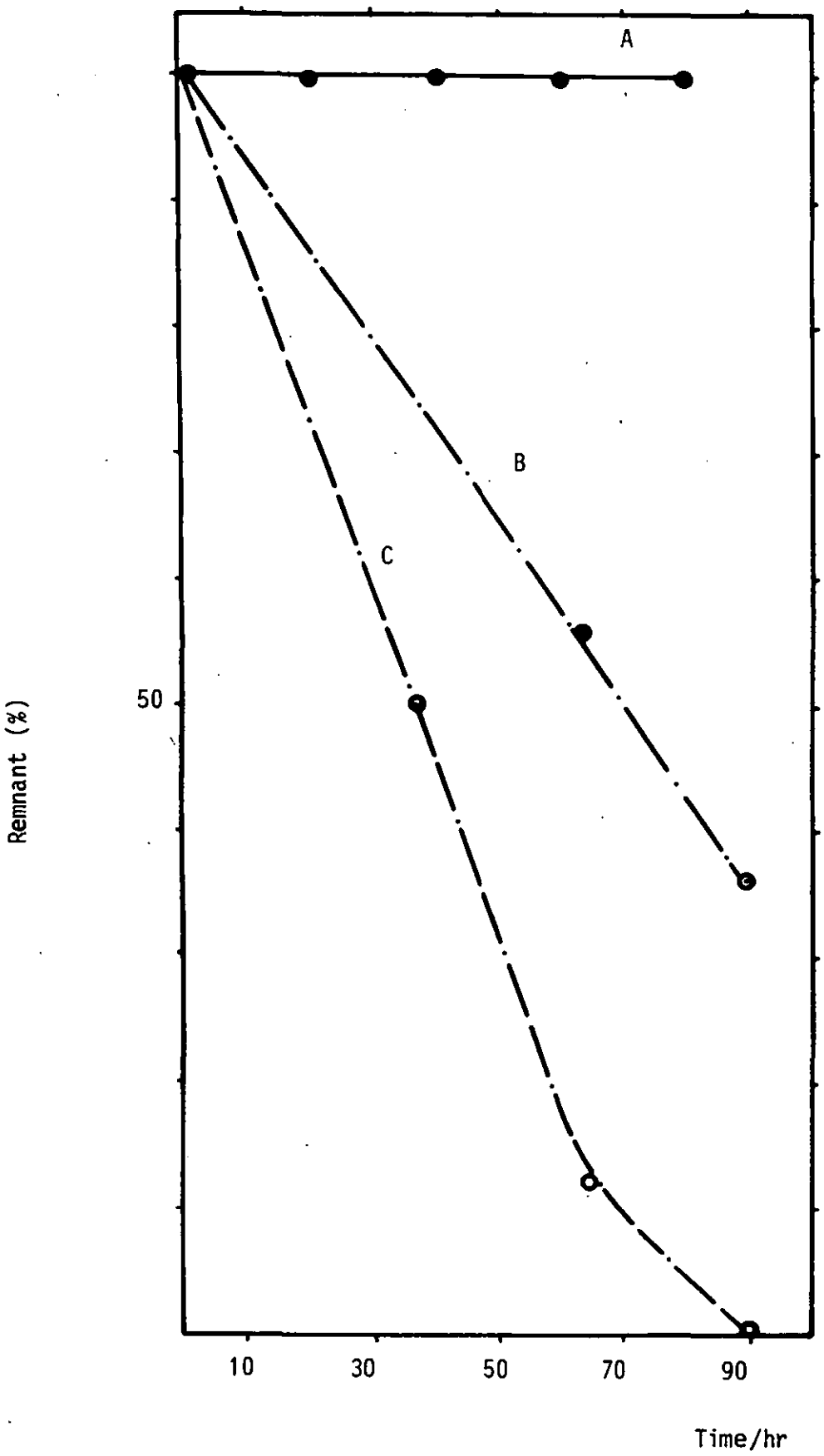
B = The food colour in the presence of ascorbic acid

C = Ascorbic acid in the presence of food colour.

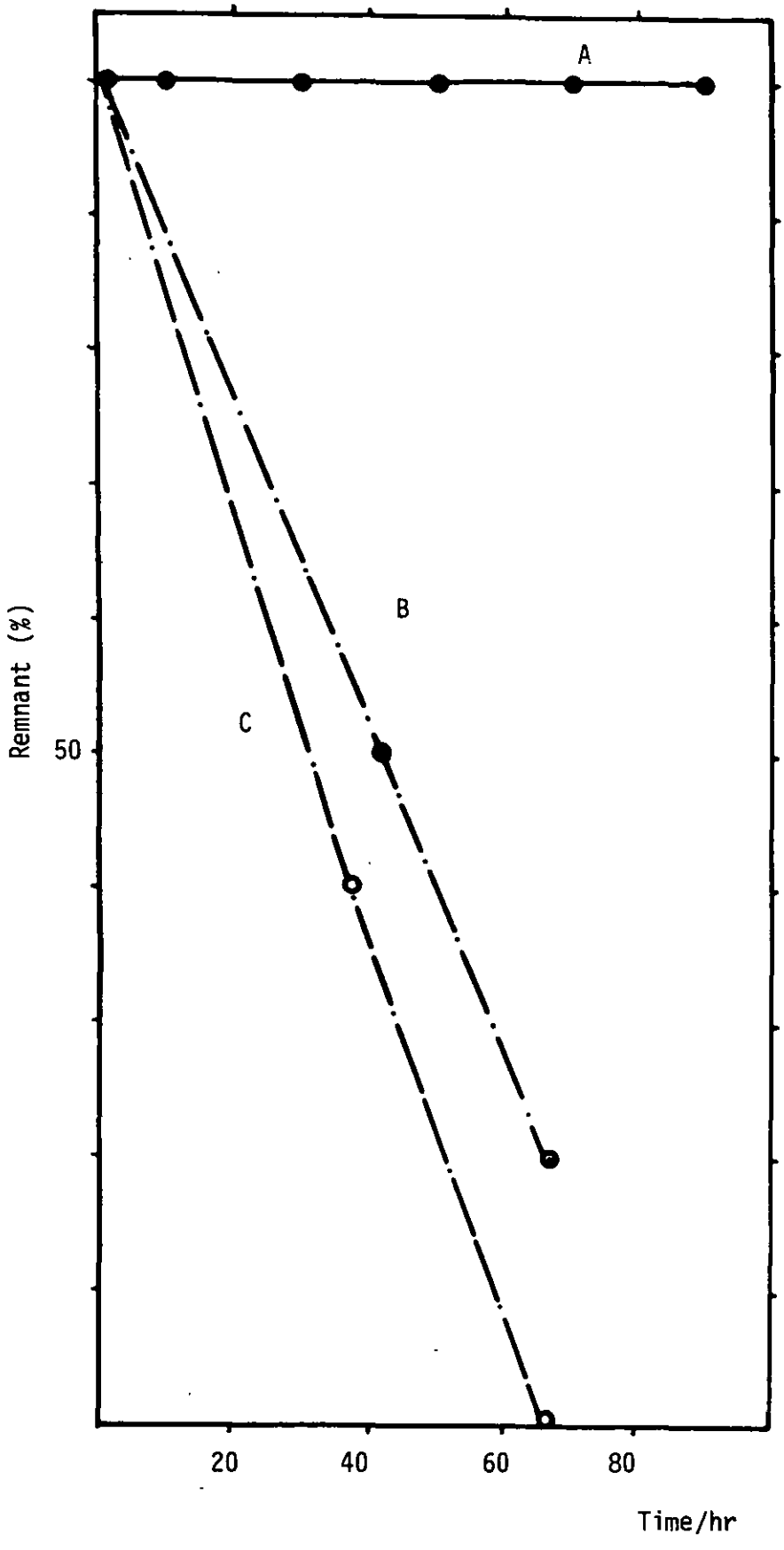
Quinoline Yellow



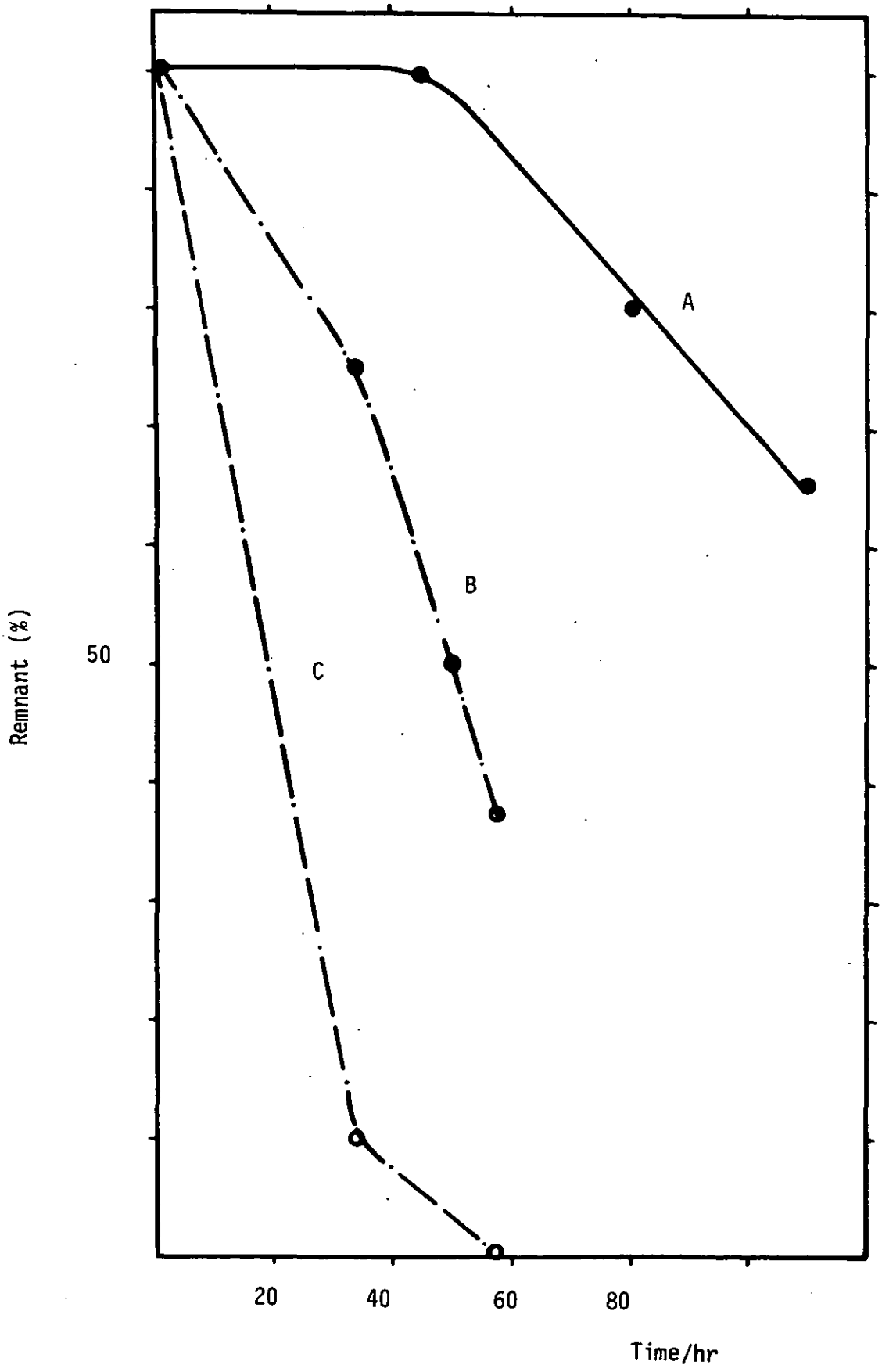
Sunset Yellow FCF

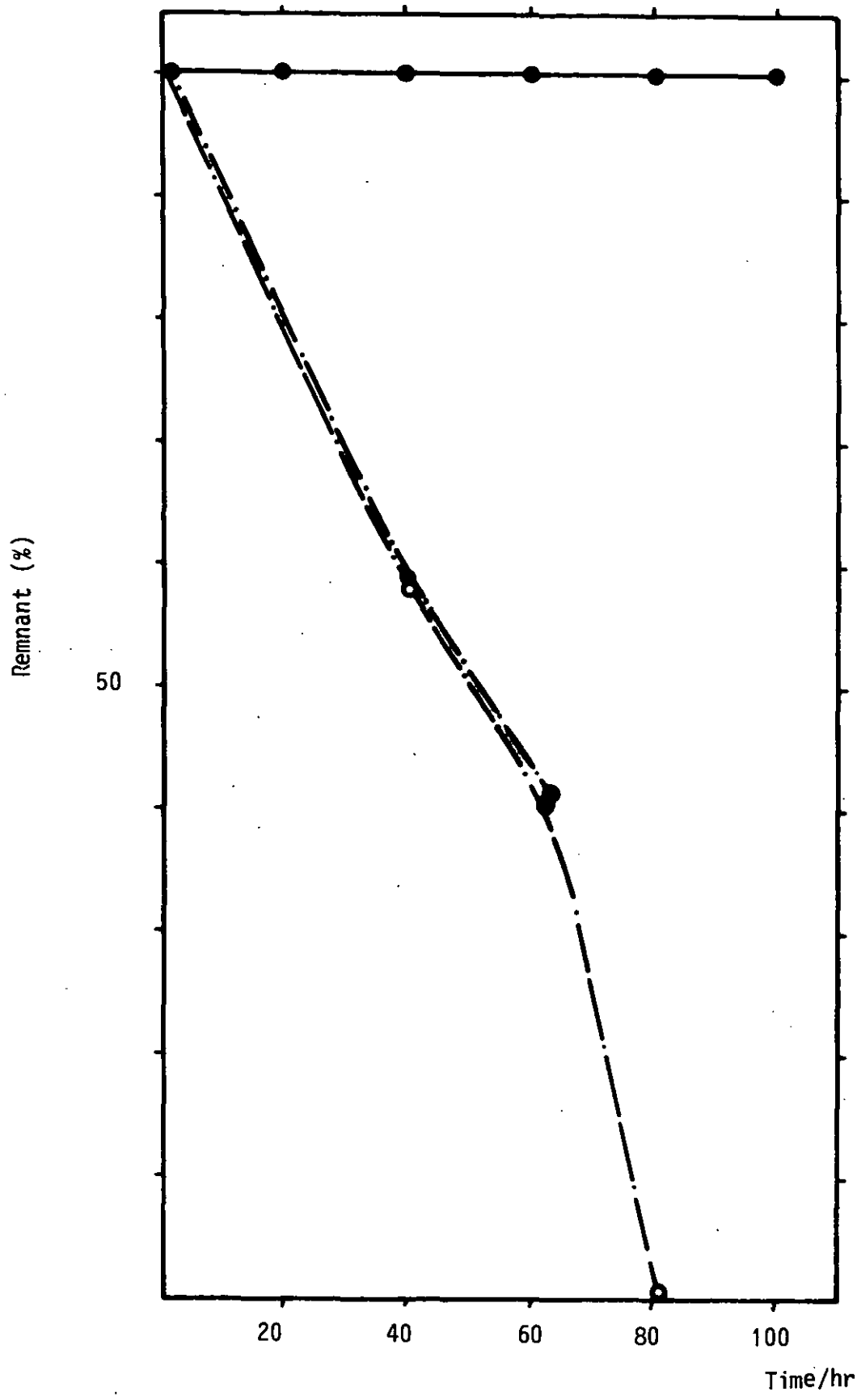


Tartrazine

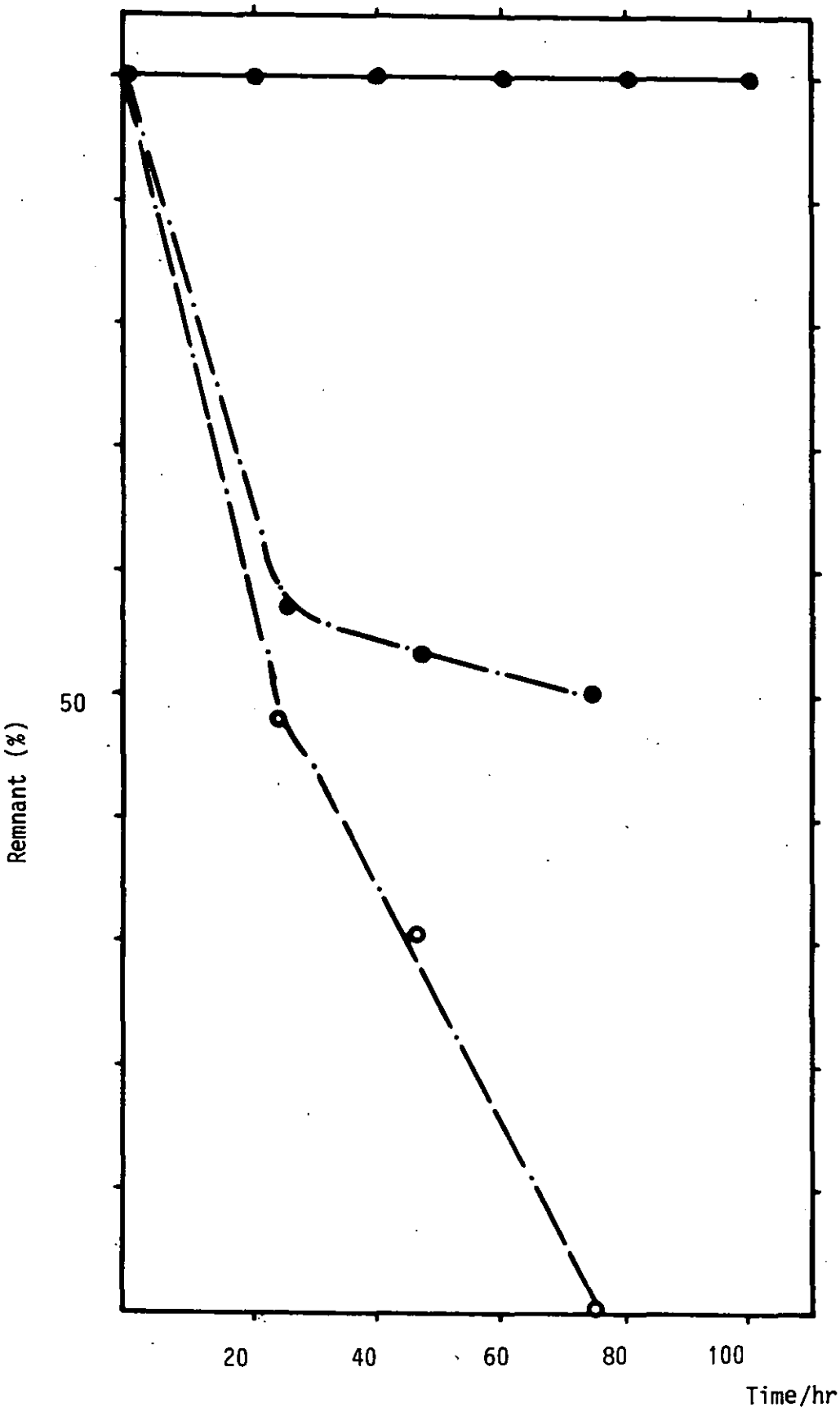


Yellow 2G

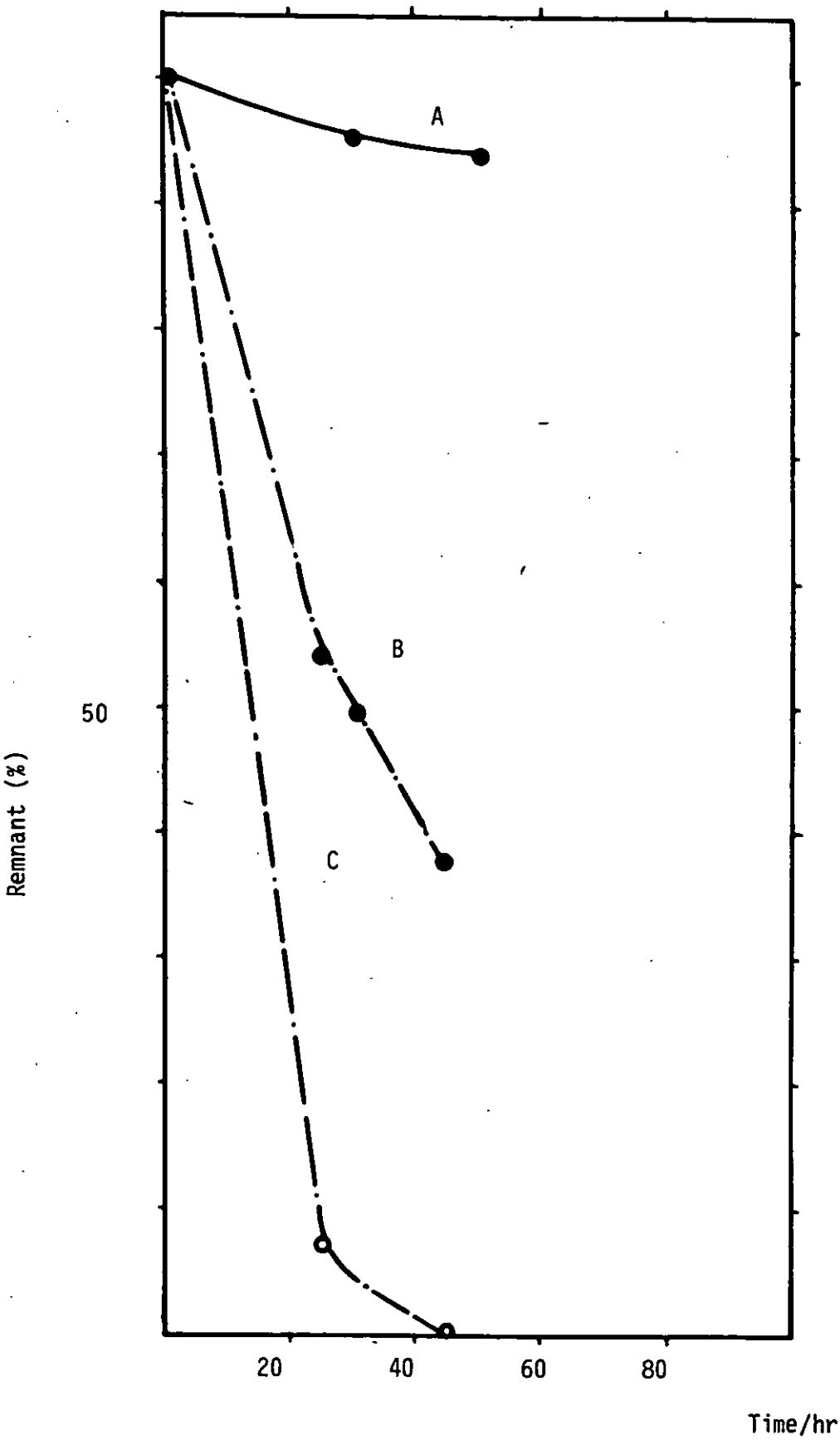


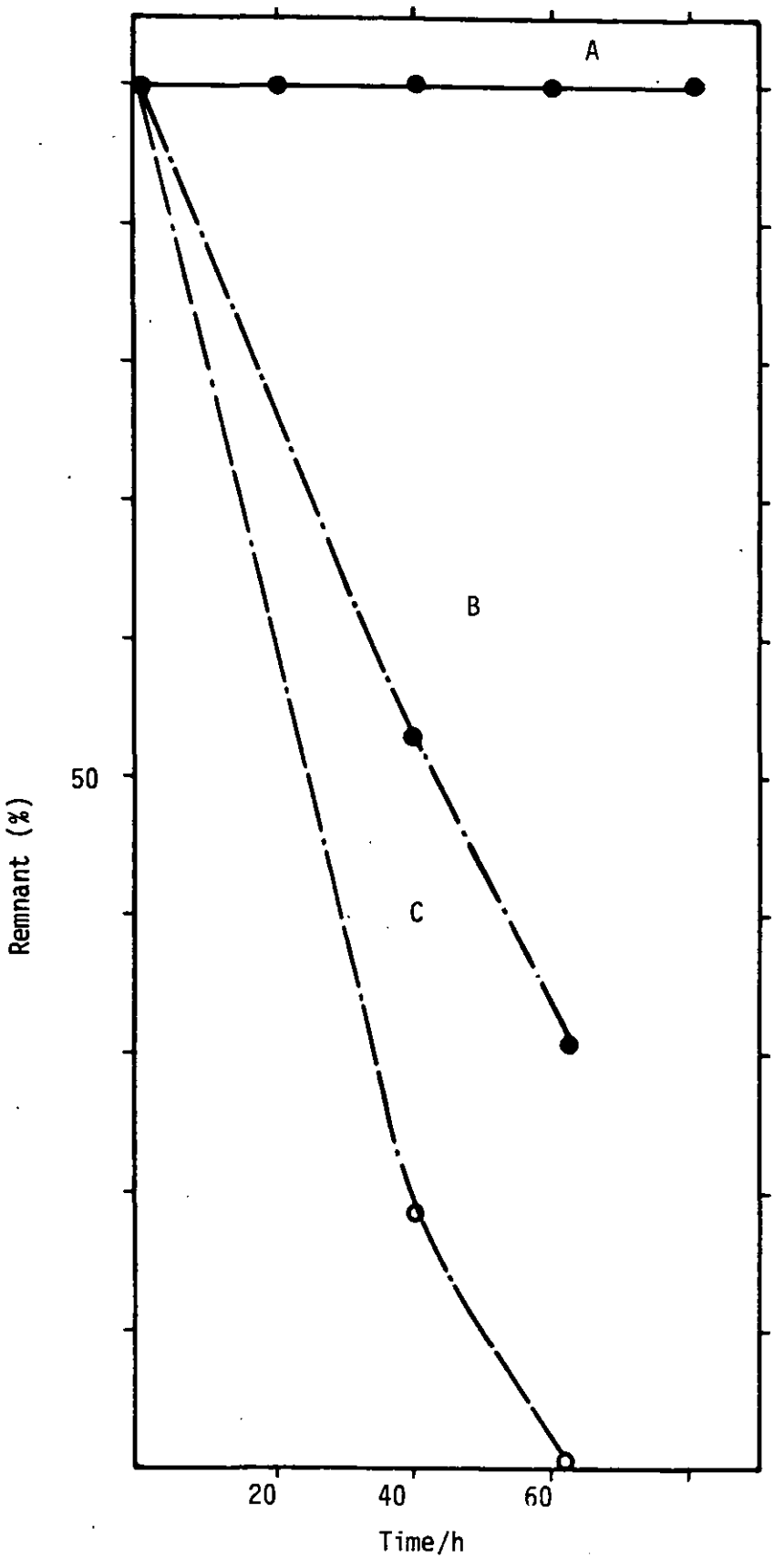

Patent Blue (V)
Remnant (%)
50
20
40
60
80
100
Time/hr

Black PN

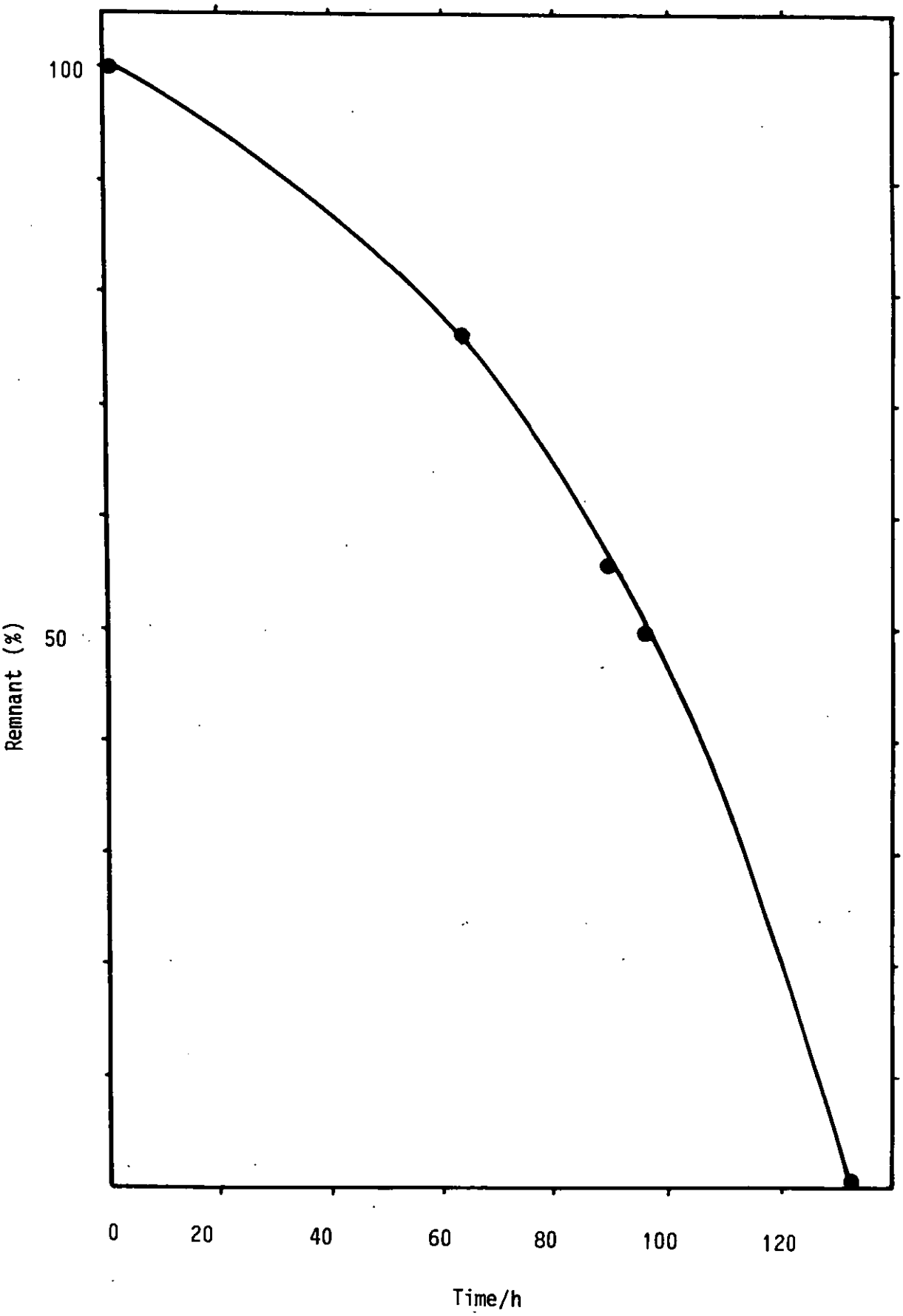


Ponceau 4R




A
B
C
50
Remnant (%)
20
40
60
Time/h

Ascorbic Acid



APPENDIX (II)

Degradation in the dark in acetate buffer pH (5.5) of some of the food colours and ascorbic acid.

Initial concentration of the food colour = 5 ppm.

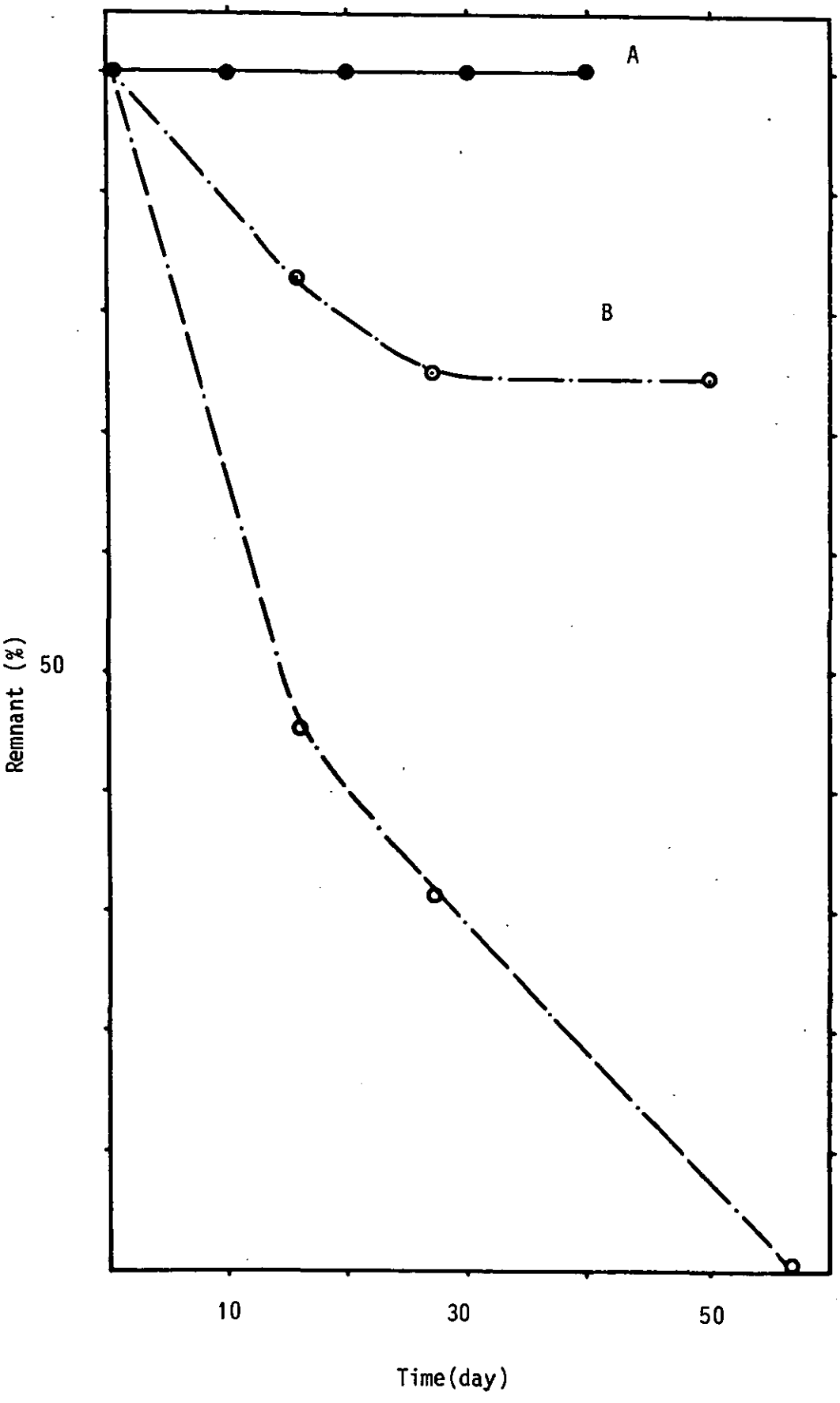
Initial concentration of ascorbic acid = 100 ppm.

A = The food colour alone

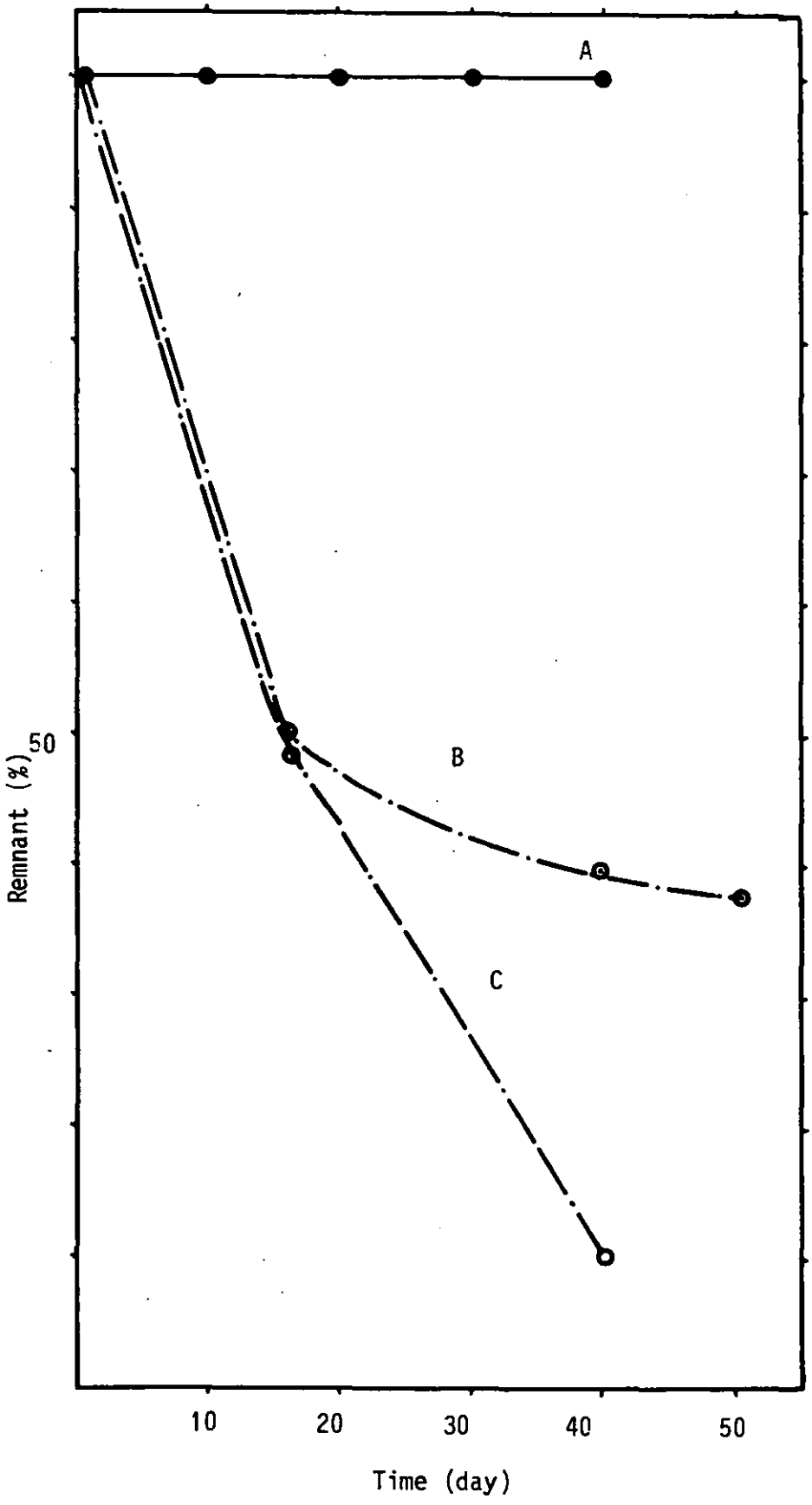
B = The food colour in the presence of ascorbic acid

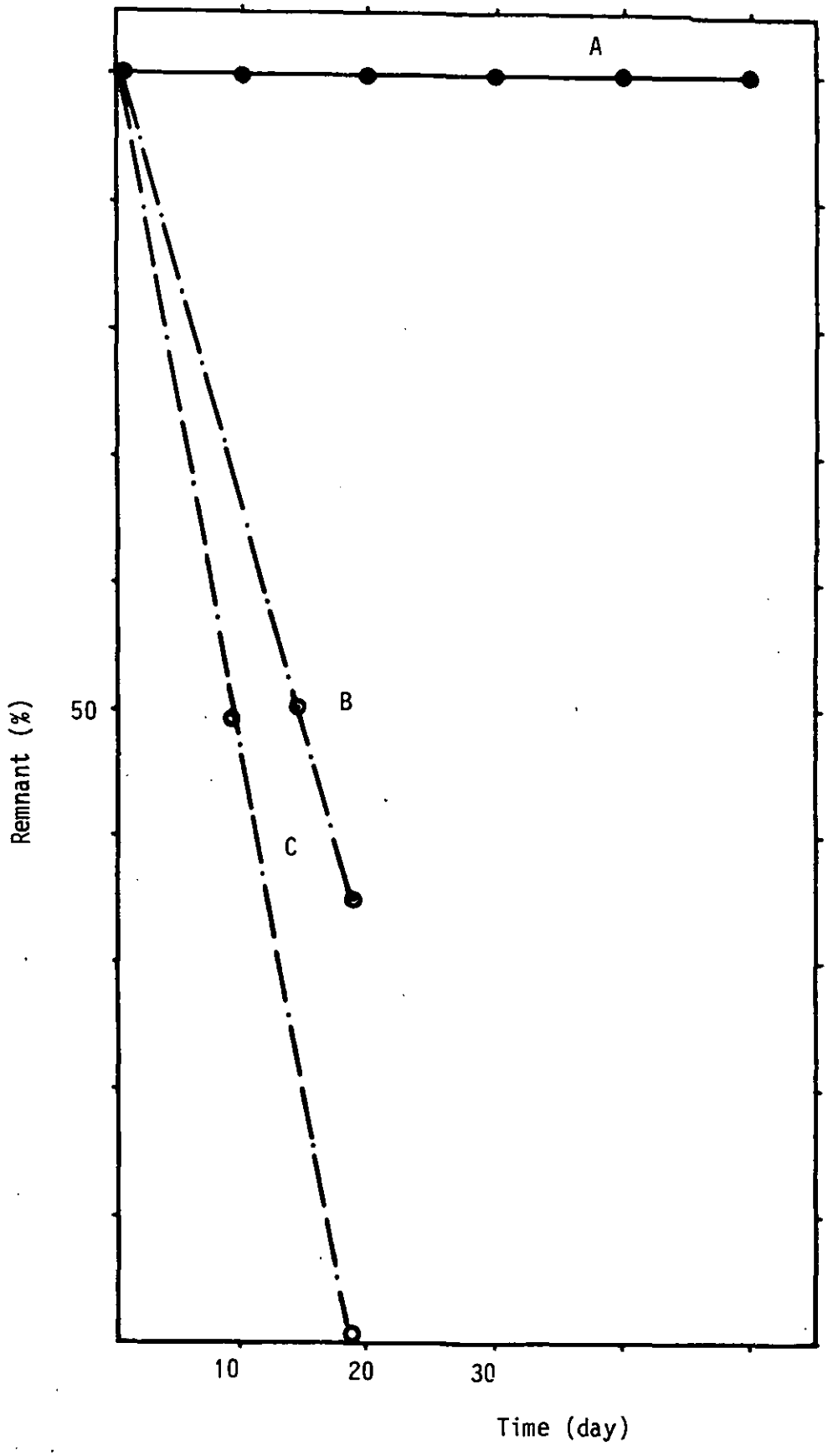
C = Ascorbic acid in the presence of food colour.

Quinoline Yellow

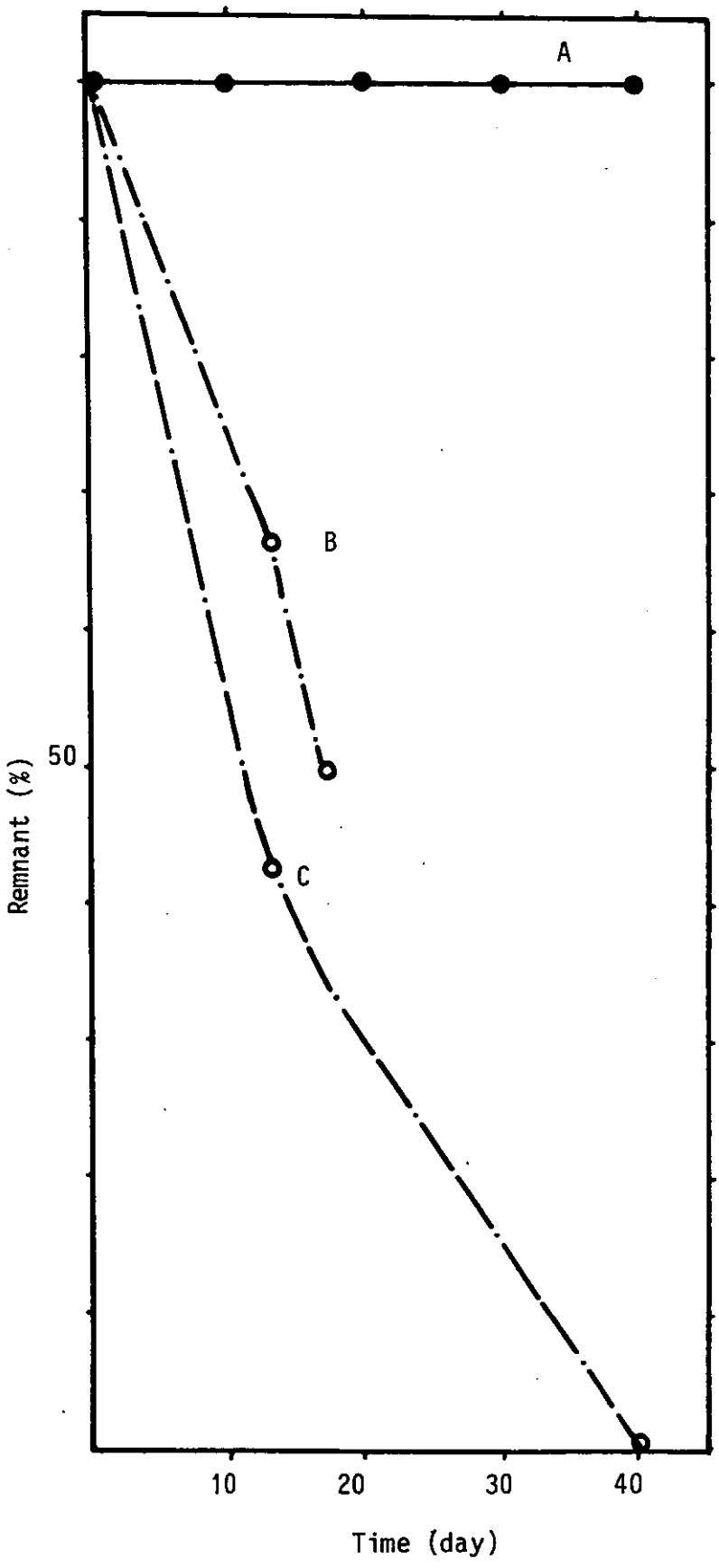


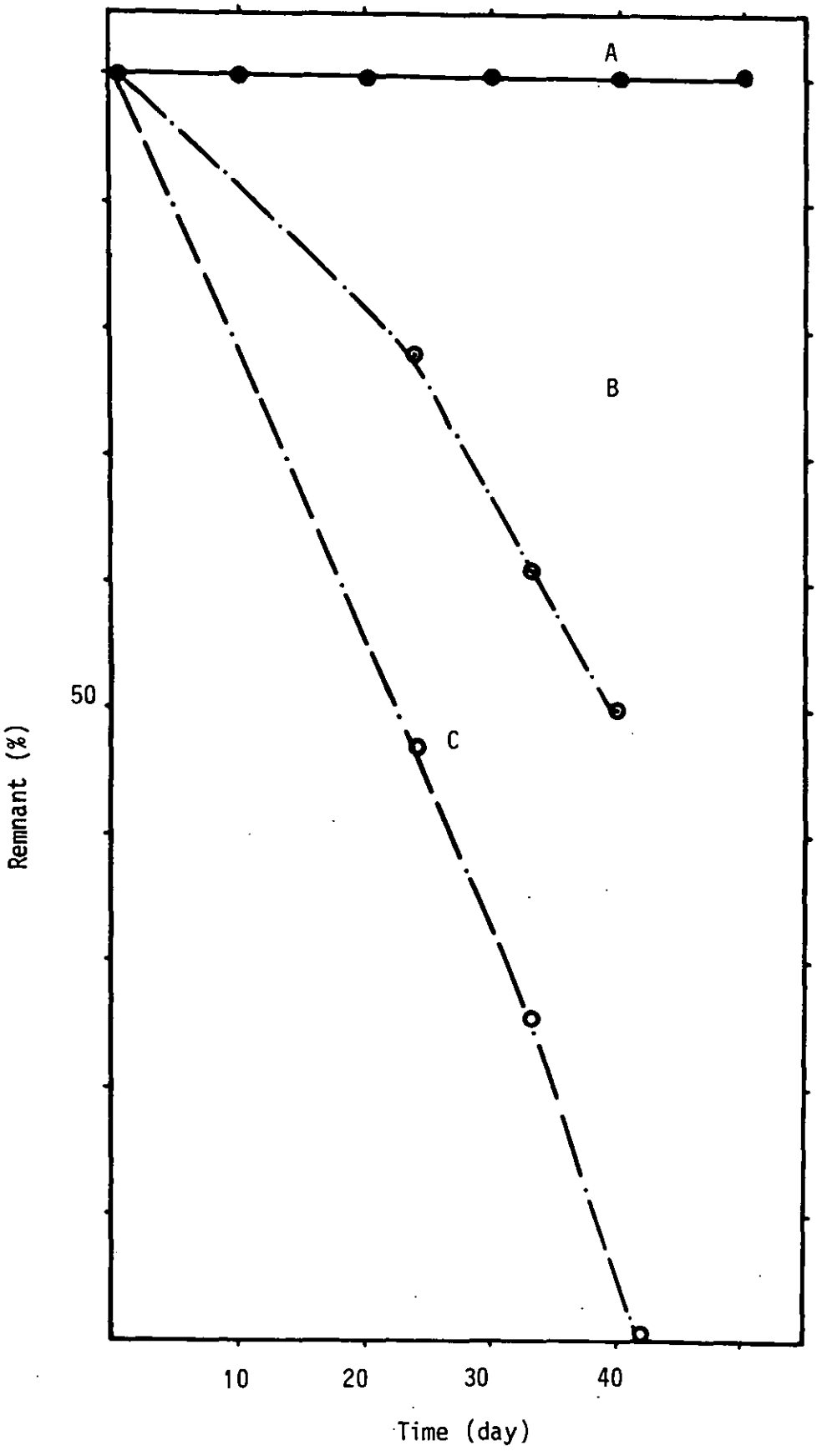
Green S



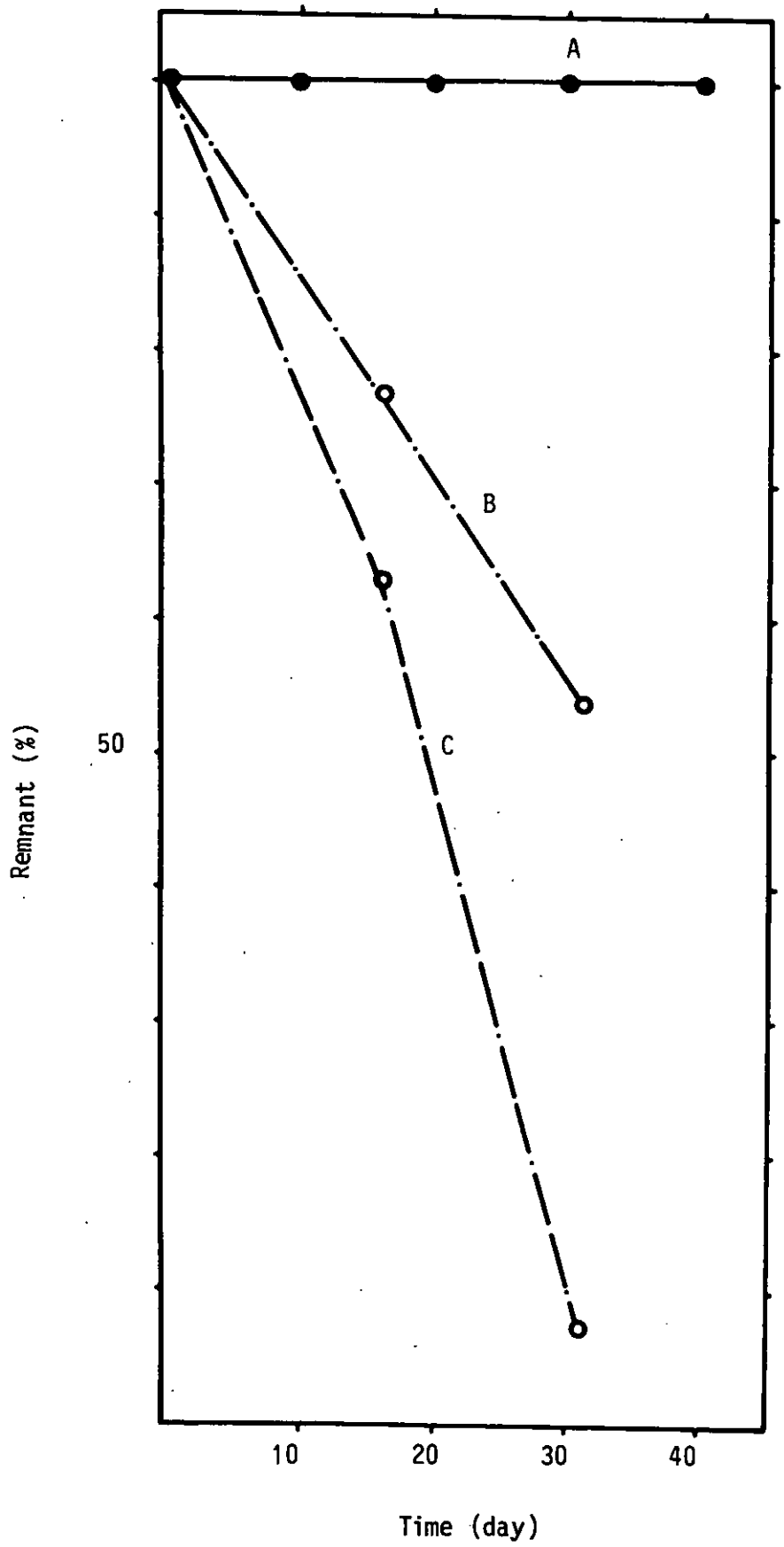

A
B
C
50
Remnant (%)
10
20
30
Time (day)

Chocolate Brown HT

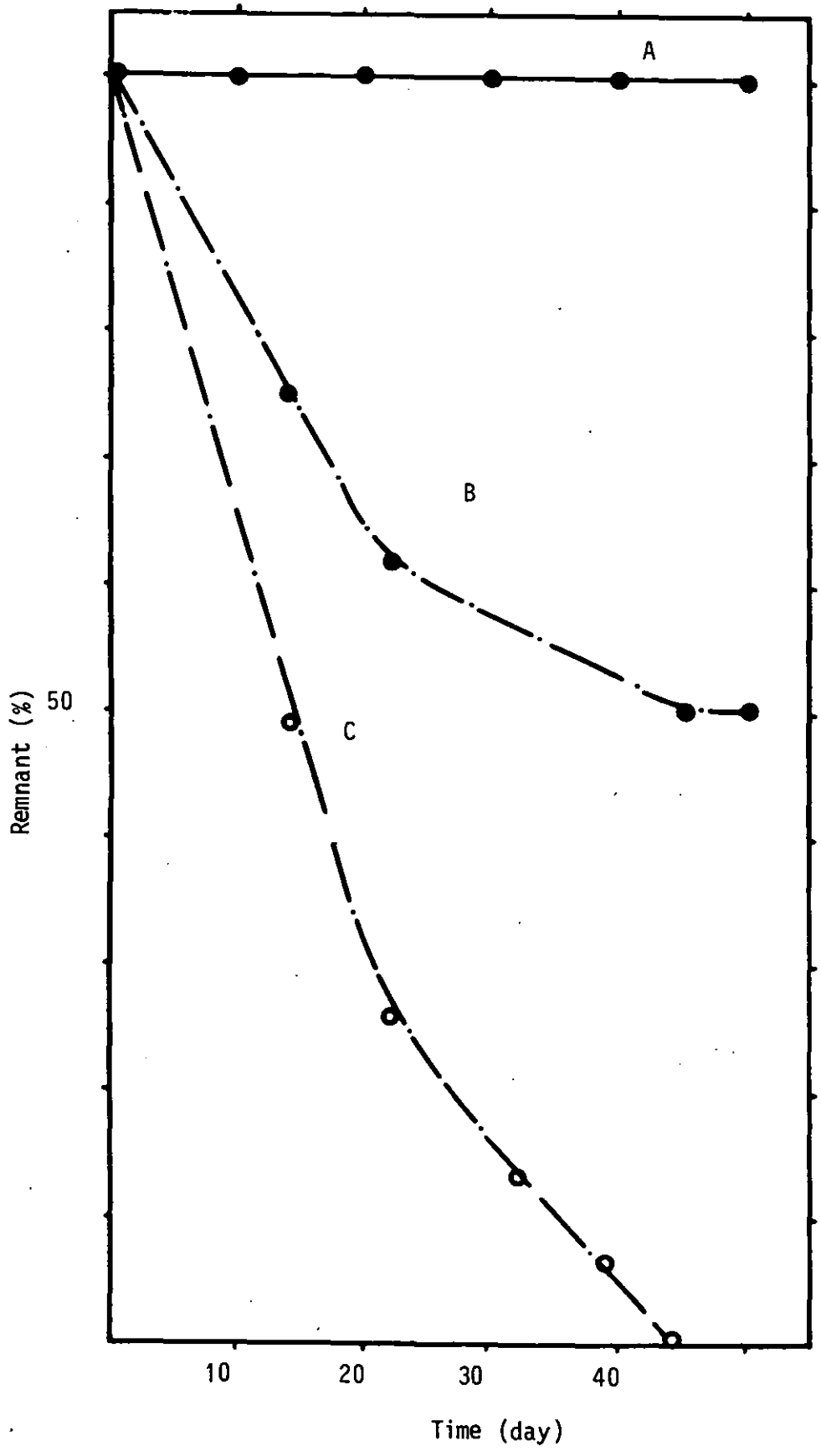



A
B
C
50
Remnant (%)
10
20
30
40
Time (day)

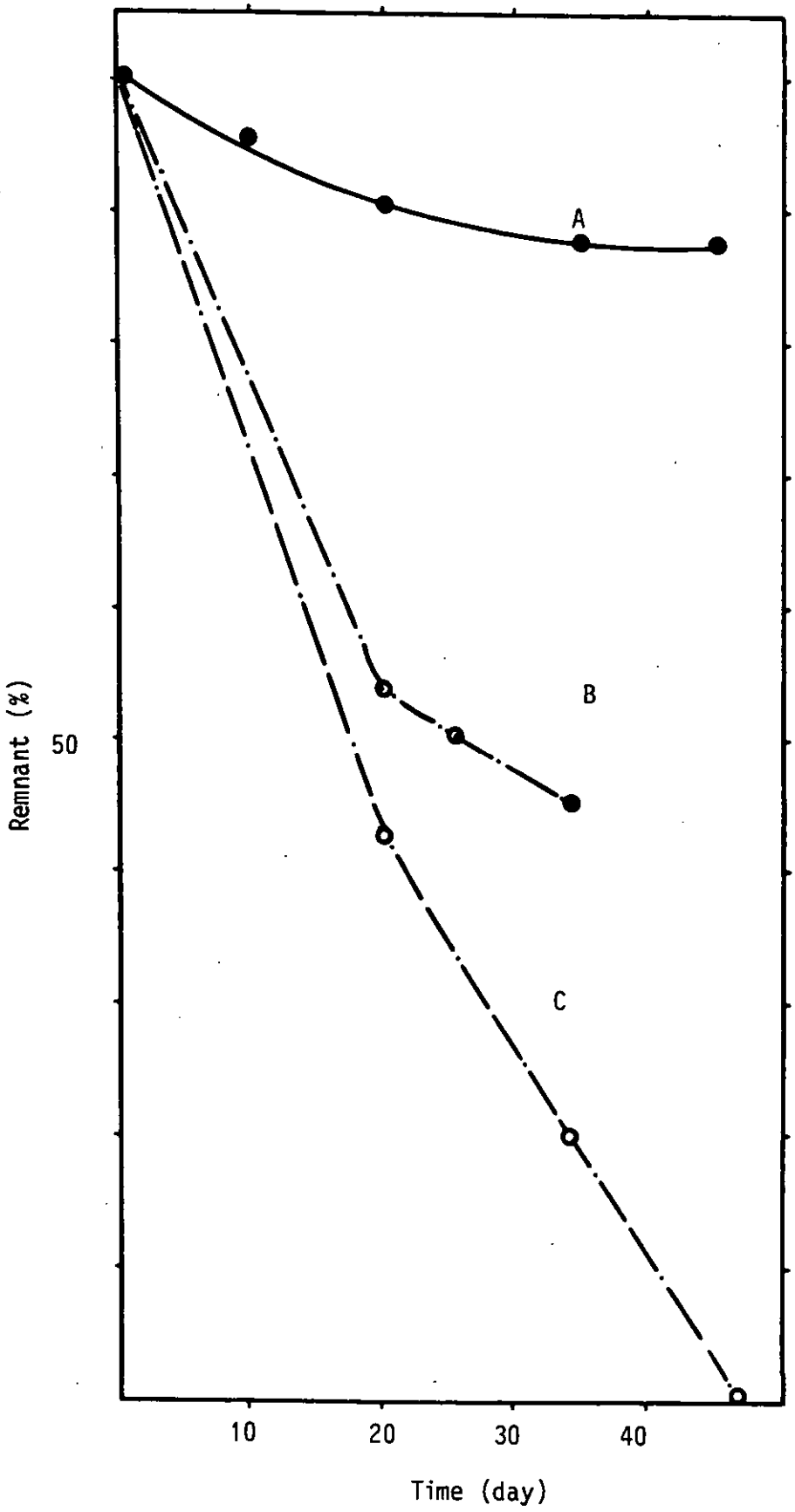
Brilliant Blue FCF

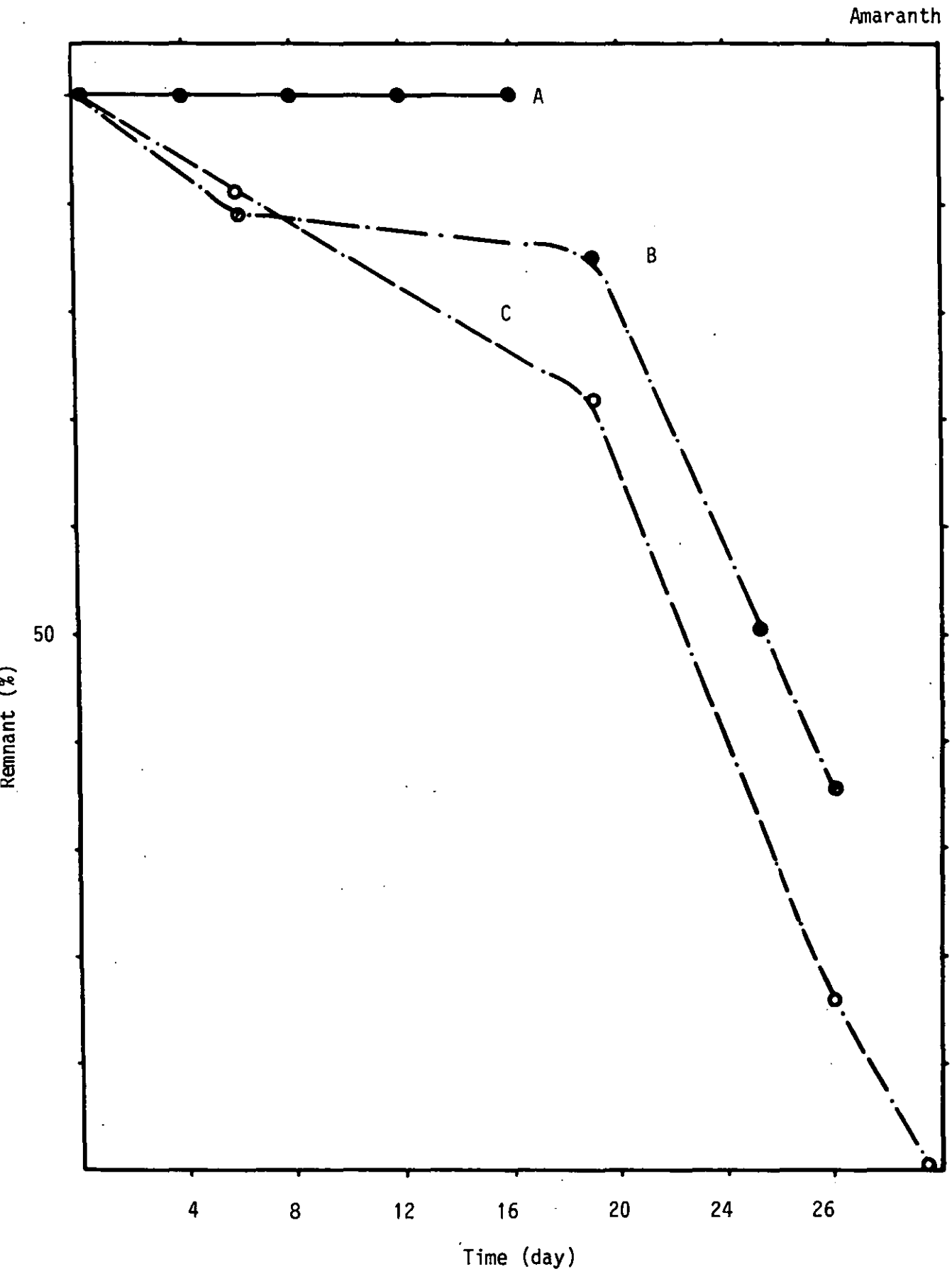


Indigo Carmine

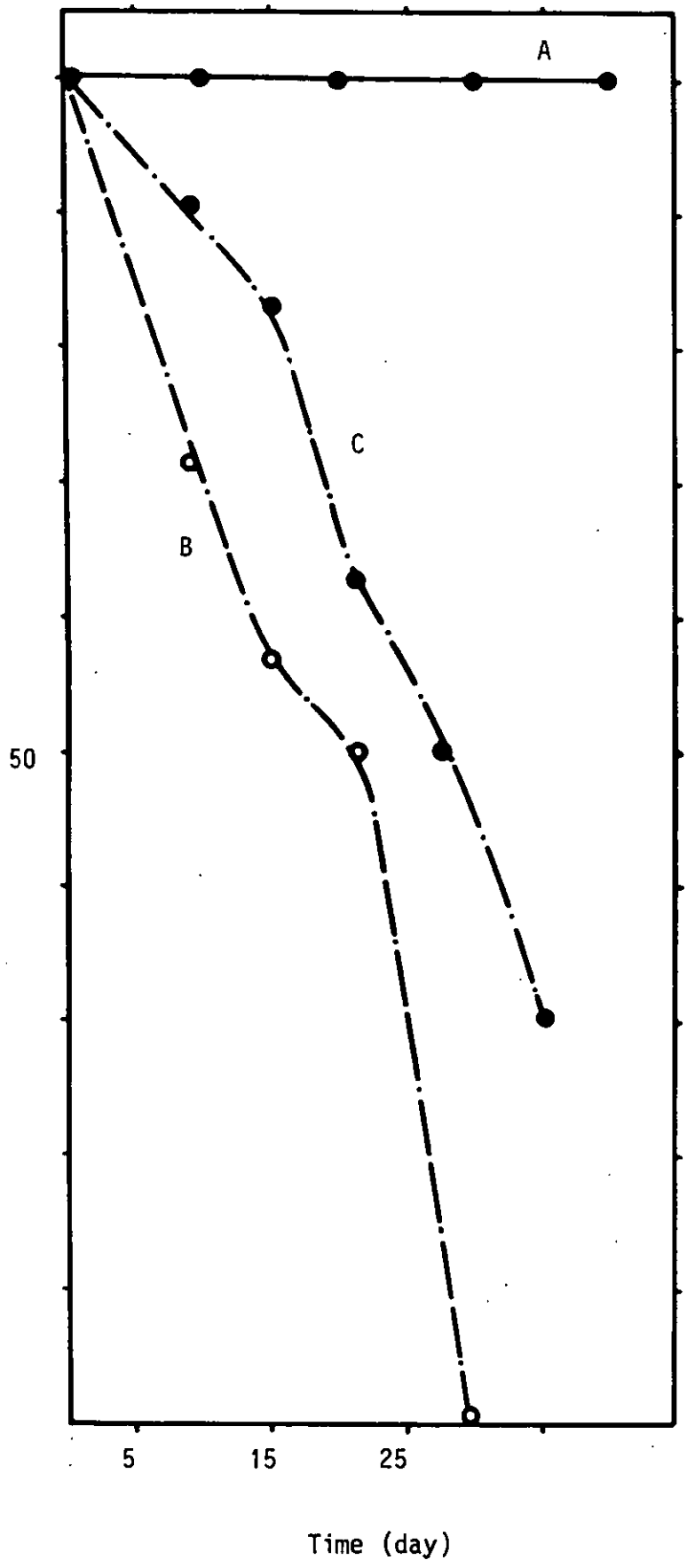


Carmoisine

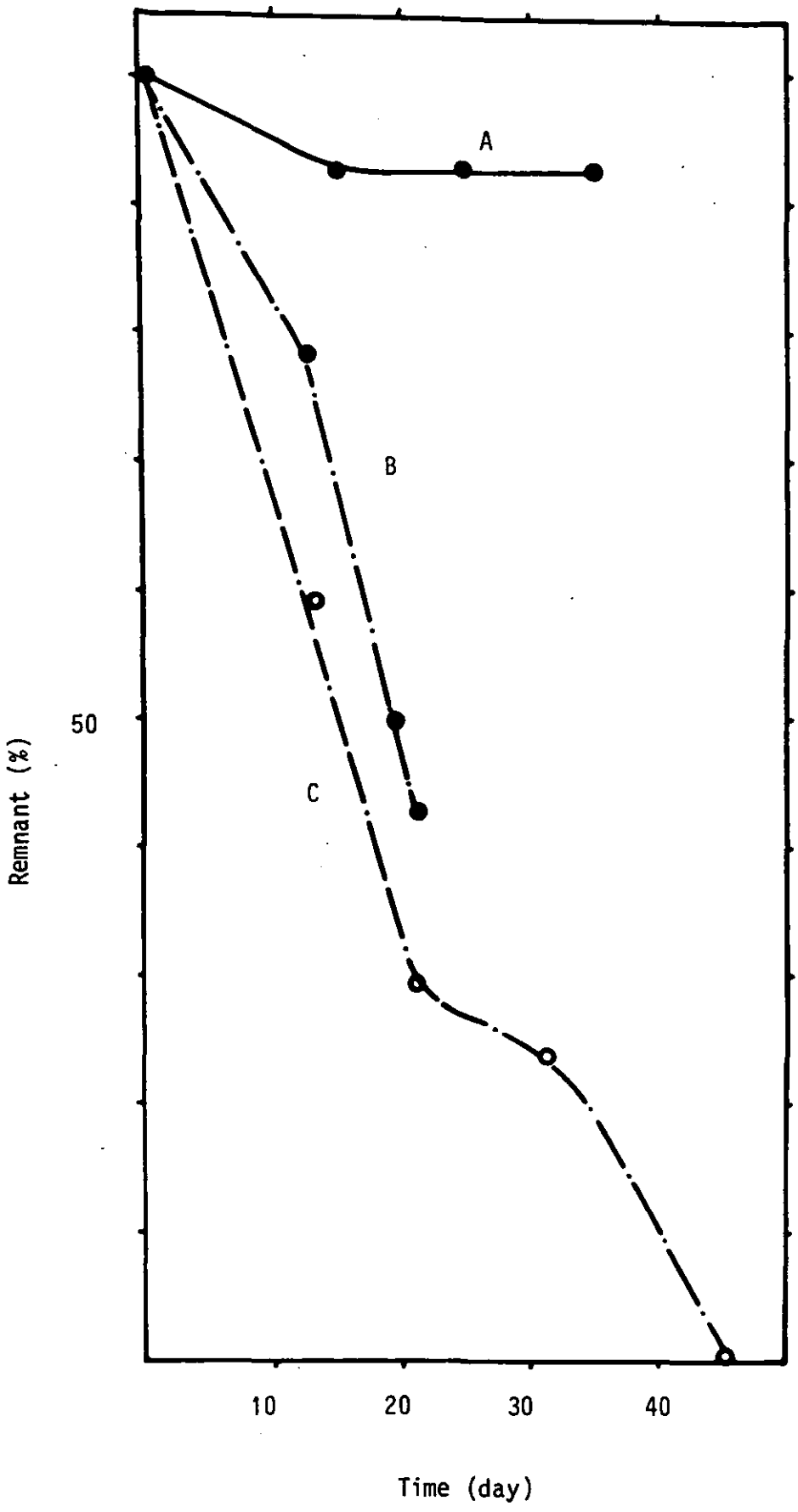



Amaranth
A
B
C
50
Remnant (%)
4
8
12
16
20
24
26
Time (day)

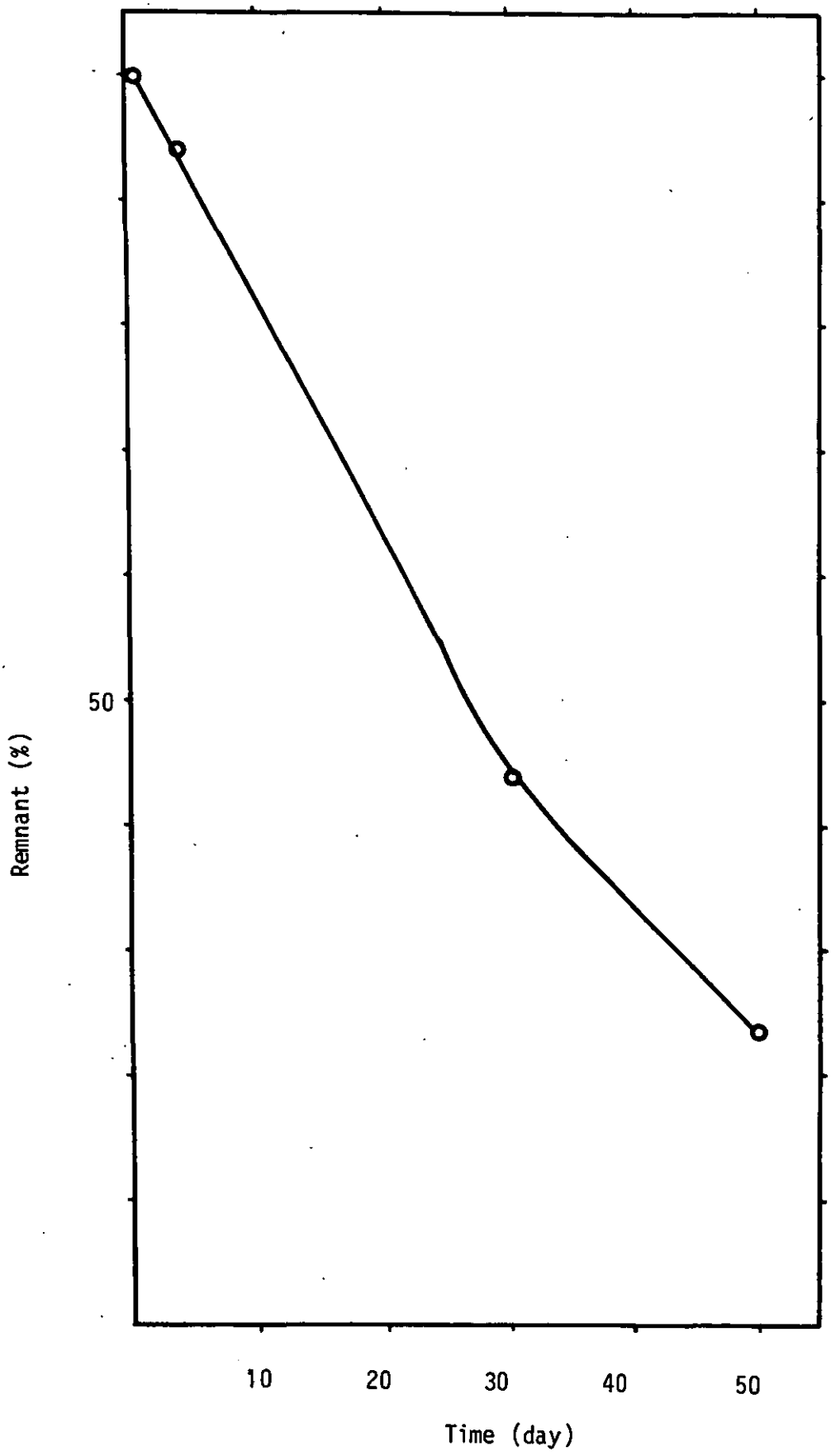
Black PN



Erythrosine BS

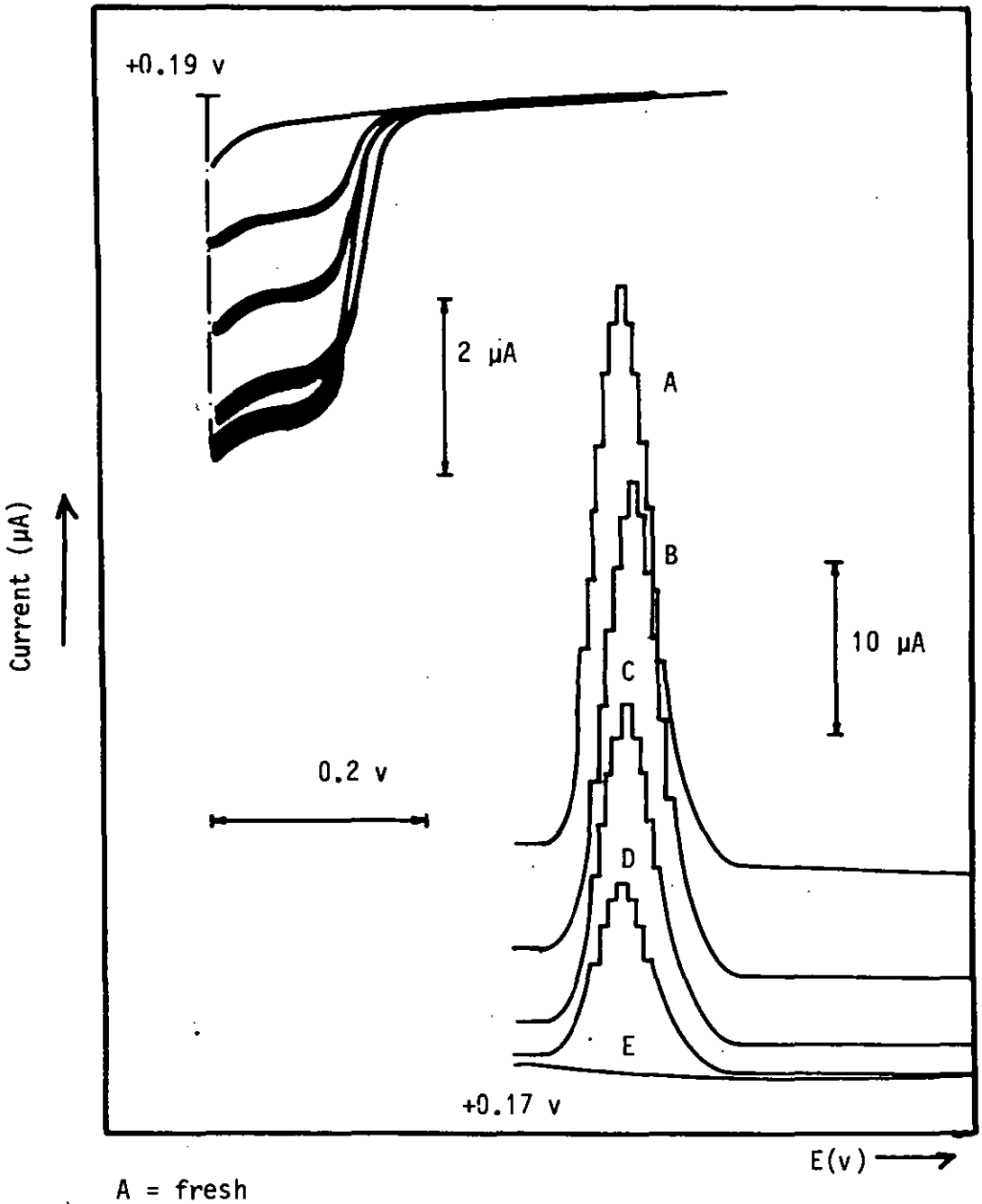


Plot for the degradation in the dark of ascorbic acid



APPENDIX (III)

DPP peaks obtained for the light degradation of ascorbic acid (initial concentration = 1000 ppm) in the presence of food colour (initial concentration = 5 ppm). EDTA concentration = 25 ppm. The second polarogram was obtained just when the solutin became colourless.



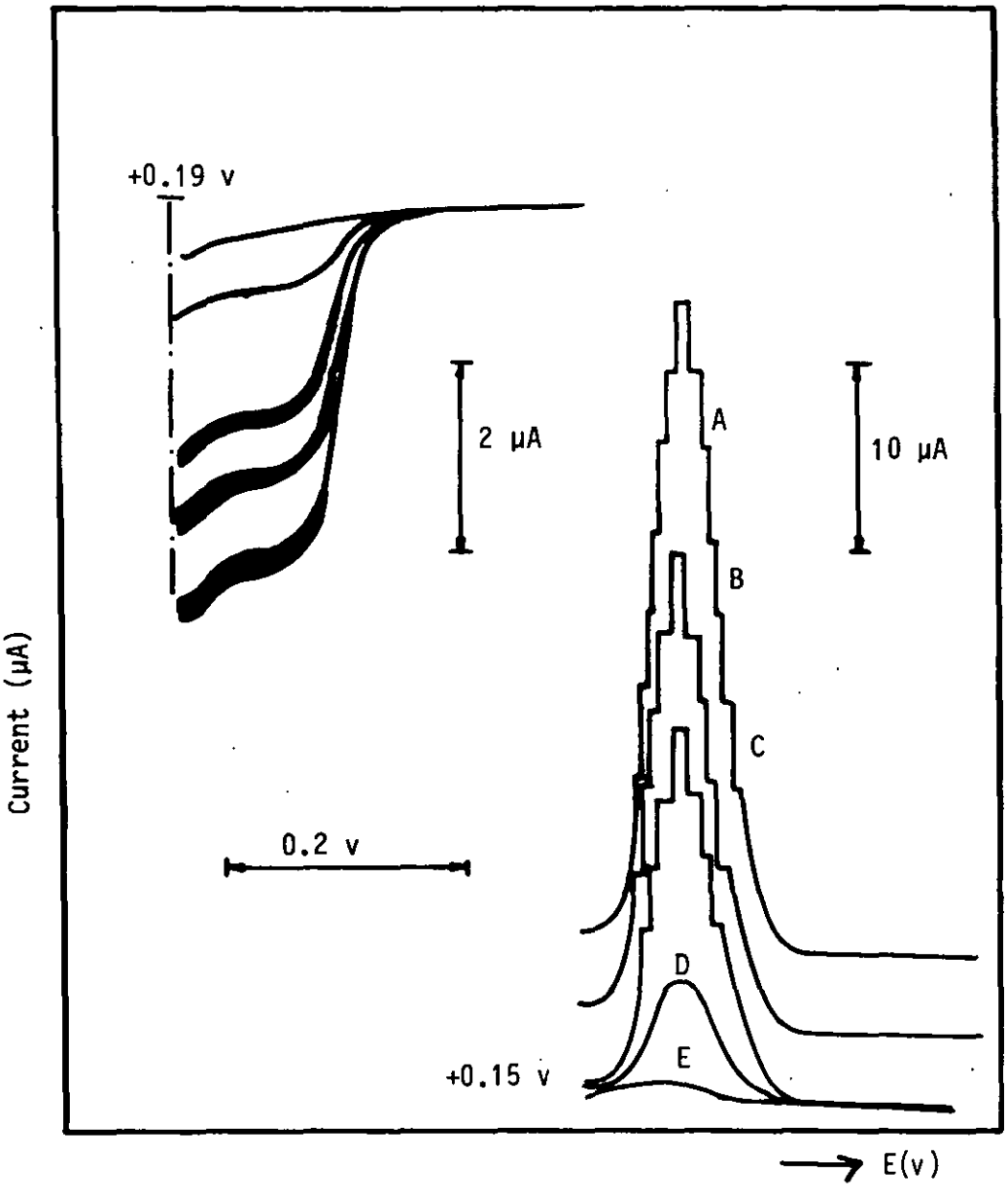
A = fresh

B = 42 hrs

C = 68

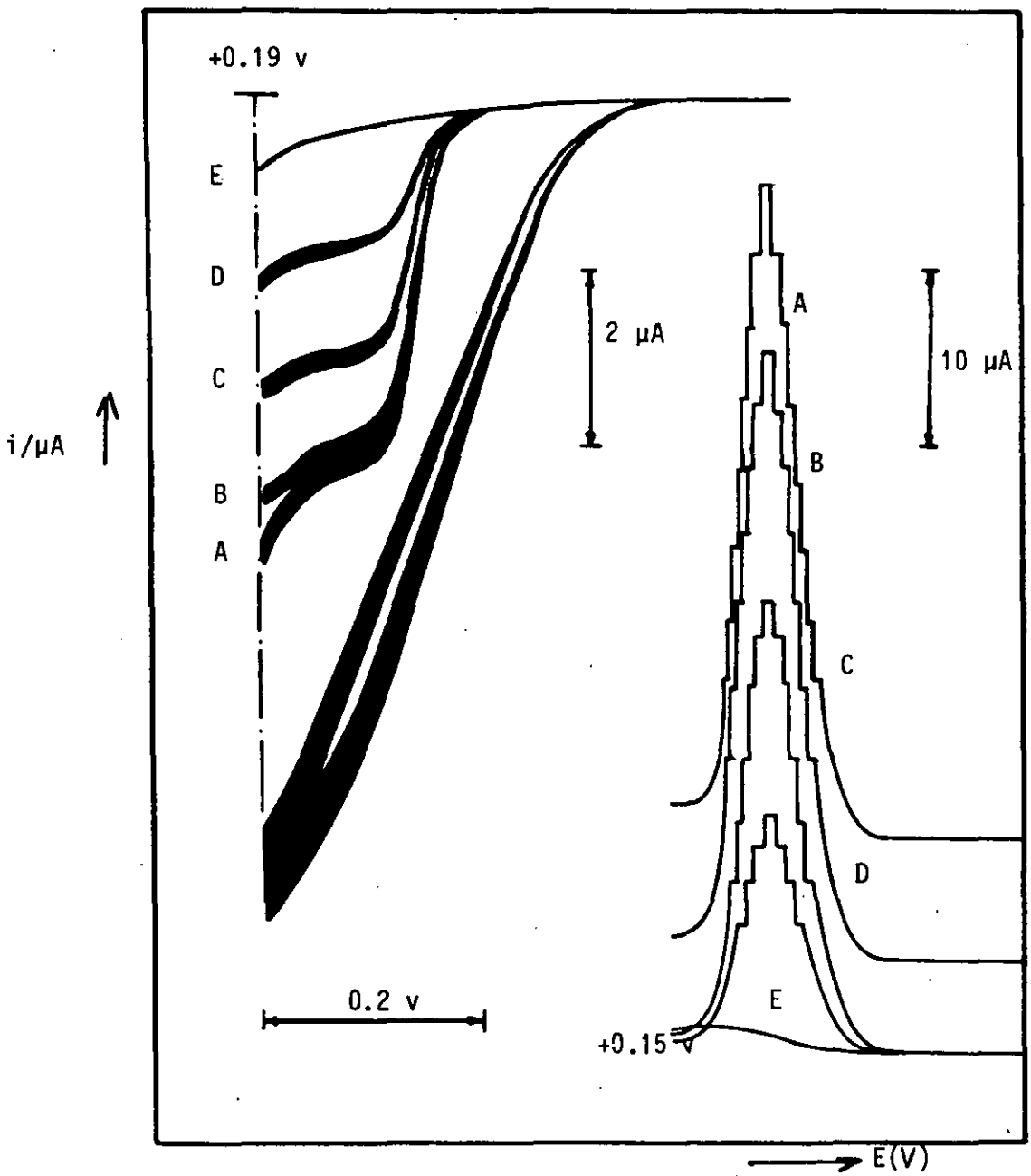
D = 94

E = 140

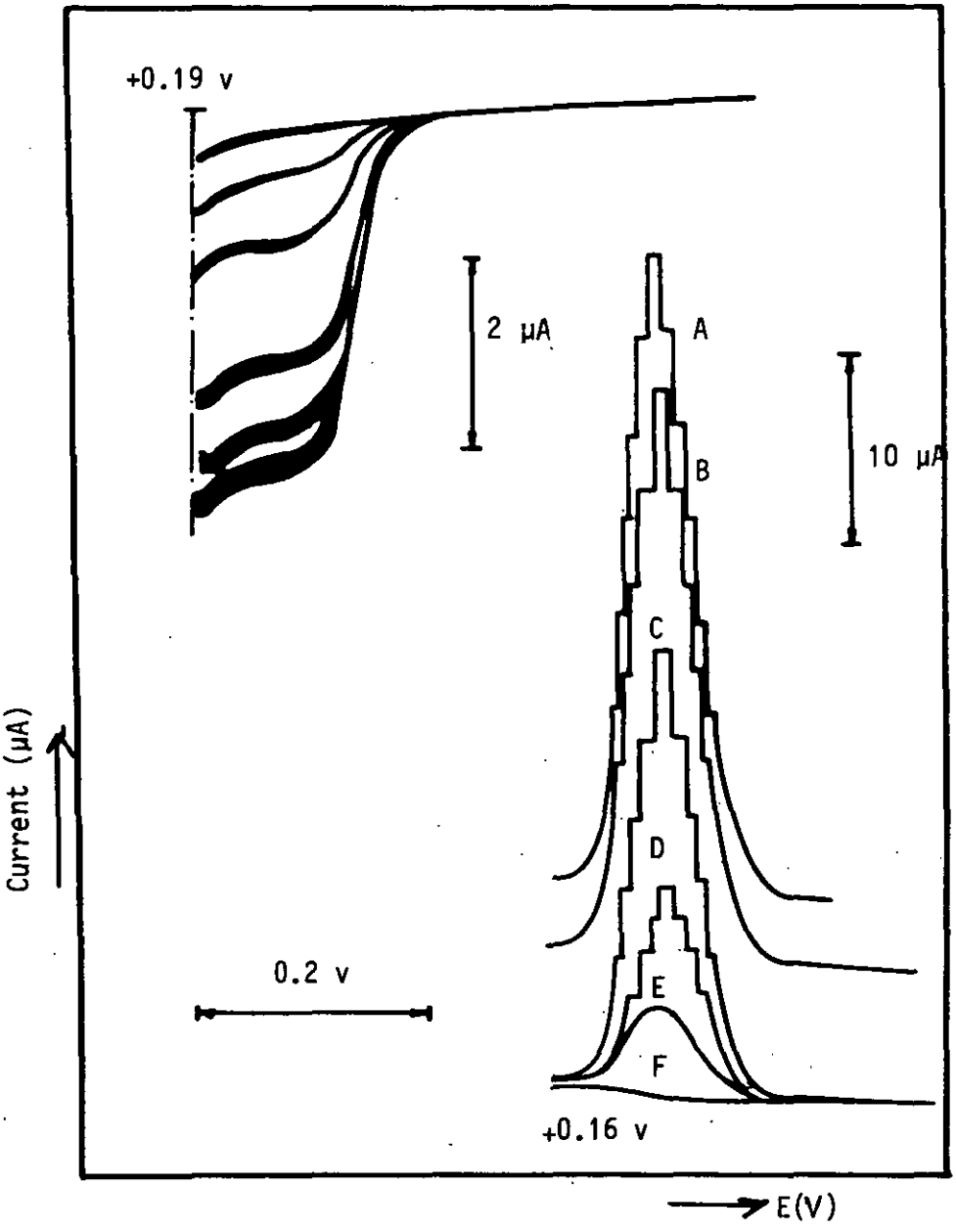


A = Fresh B = 53 hrs C = 73 D = 120 E = 130

Black PN

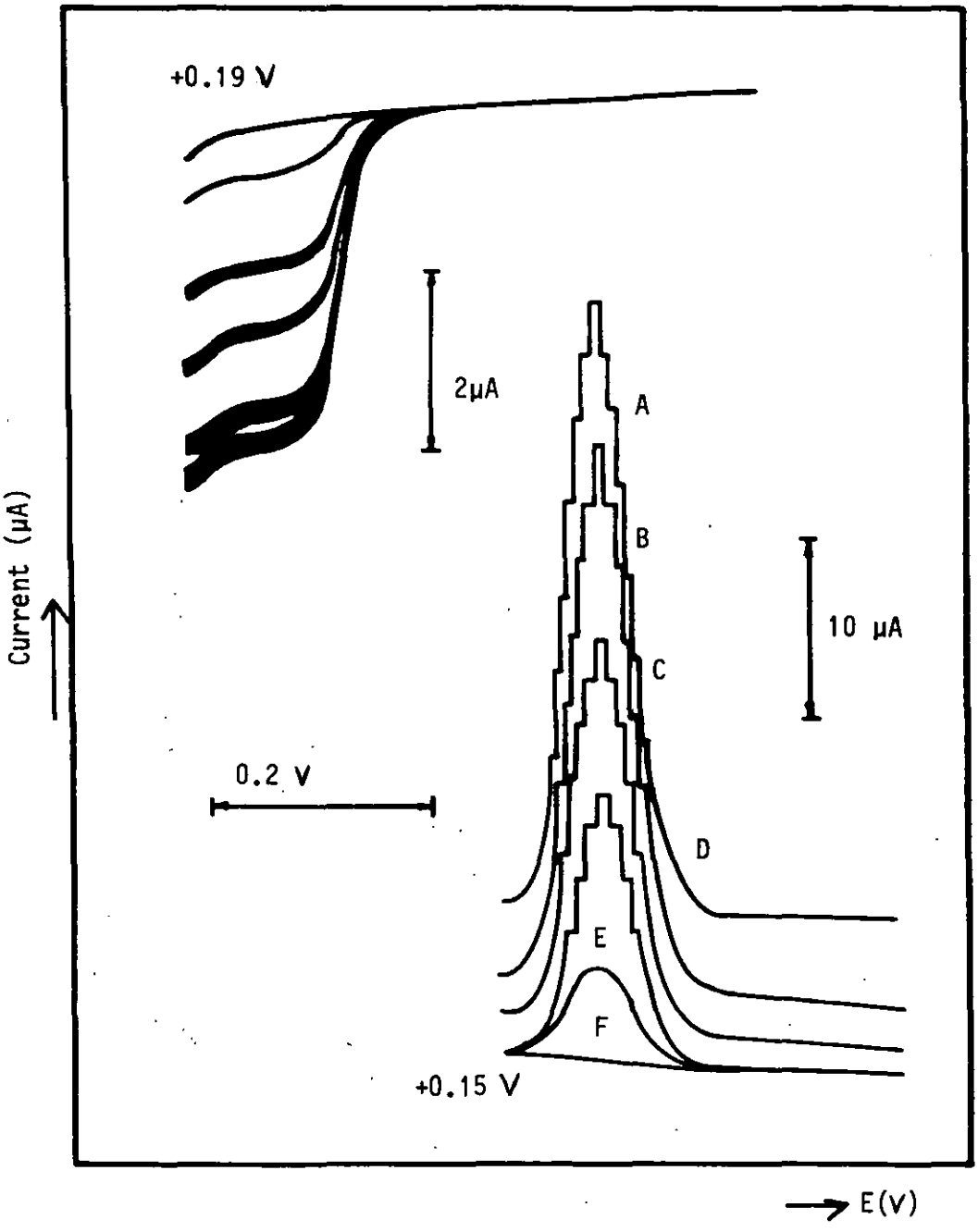


A = fresh B = 2-8h C = 25 hrs D = 44 hrs C = 88



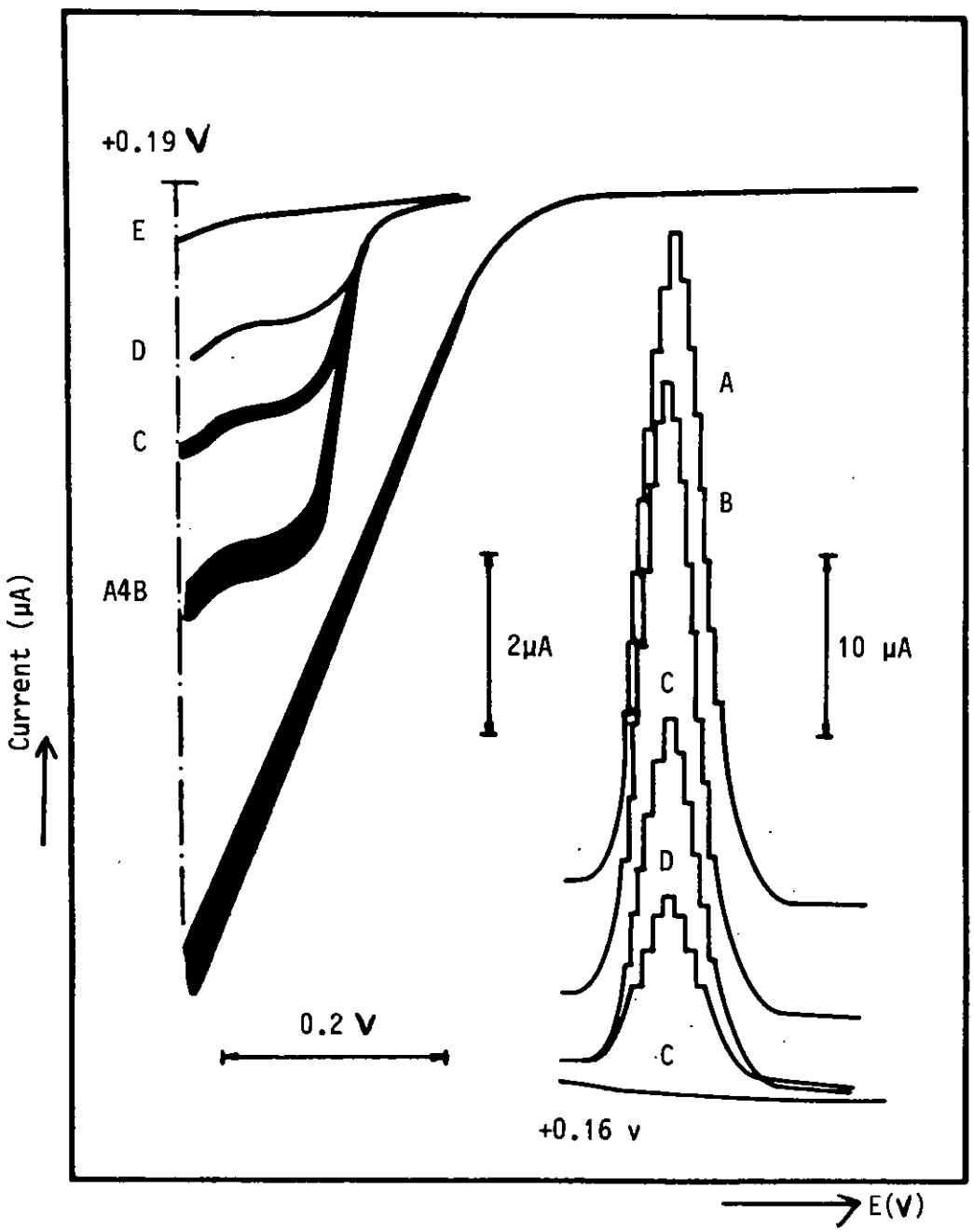
A = fresh B = 23 hrs C = 46 hrs D = 75 hrs

E = 94 hrs F = 120 hrs



A = fresh B = 24 hr C = 47 hr D = 75 hrs E = 95 hrs

F = 120 hrs



A = fresh B = 4-9 hrs C = 28 hrs D = 6 hrs E = 85 hrs

REFERENCES

1. A.A. Christie, "Vitamins," Ed. M. Stein
Churchill Livingstone, Edinburgh and London 1971, p. 150.

2. V.A. Bunakov and S.V. Debol'skaya, Uch Zap.
Pyatigoriskii Gos. Farmatseut. Inst., 5, 209 (1961)
cf; C.A. 59; 10458.

3. M.J. Deutsch and C.E. Weeks, J.A.O.A.C., 48, 1248 (1965).

4. S.A. Schou and J. Morch, "Report on the Stability of Pharmaceutical Preparations" Copenhagen 1959. Arc print from: Arch. Pharm. Chemi., 66 (1959).

5. R Tardif, J. Pharm. Sci., 50, 693 (1961).

6. T.J. Macek, Am. J. Pharm., 132, 433 (1960).

7. A. Szepey, Acta. Pharm. Hung., 36, 280 (1966).

8. H. Jonaitis and B. Kriciuniene, Lietuvostsr Aukstosios Mokyklos, 2, 409 (1962) cf., C.A. 59: 13485.

9. S. Guinand and S. Vodar, Compt. rend., 213, 526 (1941).

10. S. Guiand, Compt. rend., 213, 1003 (1941).

11. M. Vocher and Y. Lortie, Compt. rend., 213, 736 (1941).

12. N.K. Pandey, Anal. Chem., 54, 793 (1982).

13. J. Baraud, Bull. Soc. Chim., France, 837 (1951).

14. R.W. Herbert, E.L. Hirst, E.G.V. Percival, R.J.W. Renolds and F. Smith, J. Chem. Soc., 1270 (1933).

15. J.H. Roe and C.A. Kuether, Science 95, 77 (1942).

16. J.H. Roe, M.B. Mills, M.J. Oesterling, and C.M. Damron, J. Biological Chem., 179, 201 (1948).

17. J.H. Roe and M.J. Oesterling, J. Biological Chem., 152, 511 (1944).

18. K.K.G. Nair, C.P. Tewari and P.S. Krishnan Indian. J. Chem., 1, 131 (1963).

19. M.B. Mills, C.M. Damron and J.H. Roe, Anal. Chem., 21, 707 (1949).

20. F.D. Snell and C.T. Snell, "Colorimetric Methods of Analysis" D. Van. Nostard Company, Inc., New York, 3rd Edn., Vol III (1953) p.361, 363, 364.

21. E.E. Vonesch and A.L. Remezzano, An. Farm. Bioquim, B. Aires 11, 70 (1940).

22. E.E. Vonesch, An. Farm. Bioquim. B. Aires 14, 48 (1943).

23. M.Z. Barakat, N. Badran and S.K. Shehab, J. Pharm. Pharmac., 4, 46 (1951).

24. J.V. Scudi and H.D. Ratish, Ind. Engng. Chem. Analyt. Edn., 10, 420 (1938).

25. C.E. Weeks and M.J. Deutsch, J.A.O.A.C., 48, 1245 (1965).

26. E. Kodicek and K. Wenig, Nature, 142, 35 (1938).

27. J.L. Ciminera and P.W. Wilcox, J. Am. Pharmac. Asso., Scientific Edn., 35, 363 (1946).

28. W.S. Gillam, Ind. and Enging. Chem. Anal. Edn., 17(4), 217 (1945).

29. D. Cozzi, Ann. Chim. Applicata, 29, 439 (1939).

30. M. Dezelic, J. Grujic-Vasic and R. Popovic, Technika, 19, 2087, (1964) cf., C.A. 65, 15163e.

31. C. Ratzkowski and J. Korol, Canad. Inst. Food. Sci. Technol. J., 10, 215 (1977).

32. R. Bredicka and P. Zuman, Coll. Czech Chem. Commum., 15, 766 (1950).

33. I.M. Kolthoff and J.Lingane, "Polarography" Interscience Publishers, New York, Vol. II (1952).

34. J.E. Page and J.G. Waller, Analyst, 71, 65 (1946).

35. M. Brezina and P. Zuman, "Polarography in Medicine, Biochemistry and Pharmacy" Interscience, Chichester and New York 1958.

36. J. Heyrovsky, J. and P. Zuman, "Practical Polarography" p.102. Academic Press, London (1968).

37. B.R. Hajratwala, Aust. J. Pharm. Sci., NS3, 33 (1979).

38. S. P. Perone and W.J. Kretlow, W.J., Anal. Chem., 38, 1760 (1966).

39. W.V. Malik and K.L. Singh, Indian. J. Technol., 6 (11) 344 (1968).

40. E. Schubert and U. Ronald, Dienahrug, 12, (7), 715 (1968).

41. J. Linquist and S.M. Farroha, Analyst, 100, 377 (1975).

42. G. Sontag and K.G. Kainz, Mikrochim. Acta., 1, 175 (1978).

43. D. Amin, Micro. Chem. J. 28(2) 174 (1983).

44. T. Kajita, Y. Yamamoto and M. Senda, Bunseki Kagaku, 22(8), 1051 (1973).

45. T. Kajita and M. Senda, Bunseki Kagaku, 19(3), 330 (1970).
AA, 21, 3767 (1971)

46. K. Grundova, J. Davidek, J. Velisek and G. Janicek, Lebensm-Wiss. Technol. 6(1), 11 (1973).

47. S.P. Mallela and B.L. Khandelwal, Microchimica Acta, II, 245 (1977).

48. P. Cescon and M. Montalti, Annali. Chim., 60(1), 63 (1970).

49. J. Lindquist, Analyst, 100, 339 (1975).

50. W.D. Mason, T.D. Gardner and J.T. Stewart, J. Pharm. Sci., 61 (8), 1301 (1972).

51. I.G. Volova, M.A. Kuznetsova and Kh.Z. Brainina, Ref Zh.-Khim., Abstr No 11R11.

52. M. Brezina, J. Koryta, T. Loucka, D. Marsikova and J. Pradac, J. Electroanal. Chem.-Interfacial. Electrochem., 40 (1), 13 (1972).

53. J. Tillmans, P. Hirsch and Reinshgen, Z. Lebensmittelunters,u.-F., 56, 272 (1928).

54. J. Tillmans, P. Hirsch and W. Hirsch, Z. Lebensmittelunters,u.-Forsch., 63, 1 (1932).

55. J. Tillmans, P. Hirsch. and Jackish, Z. Lebensmittelunters,u.-F, 63, 241 (1932).

56. Official Methods of Analysis, 9th Edn. Assoc. Agric. Chemists, Washington, D.C. 1960.

57. L. Harris and M. Oliver, Bio. Chem., J., 36. 155 (1942).

58. W.L. Hall and M.J. Deutsch, J.A.O.A.C., 48, 1236 (1965).

59. O.A. Bessey and C.G. King, K. Biol. Chem., 103, 687 (1933).

60. H.J. Halder and M.J. Erwine, Biol. Chem., 2, 954, (1963).

61. C. Goh, J. Ferment. Technol., 28, 117 (1950).
cf., Biol. Abst., 26, 543 (1952).

62. L.W. Mapson, J. Soc. Chem. Ind., 62, 223 (1943).

63. L.W. Mapson and S.M. Partridge, Nature, 164, 479 (1949).

64. L.F. Levy, Nature, 152, 599 (1943).

65. J.H. Singer and M.N. Milner, Analyst, 68, 272 (1943).

66. O. Gawron and R. Berg, Ind. Engg. Chem. Analyt., Edn. 16, 757 (1944).

67. D.G. Chapman, O. Rochon and J.A. Campell, Anal. Chem., 23, 111 (1951).

68. E. Winter, Z. Anal. Chem., 134, 401 (1952).

69. G.A. Snow and S.S. Zilva, Bio Chem. J., 37, 630 (1943).

70. R. Stroheker and Fr. Matt. Z. Anal. Chem., 133, 342 (1951).

71. Egyptian Pharmacopoeia (1953).

72. British Pharmacopoeia (1963). HMSO, London.

73. S.K. Gungtly, Indian. J. Pharm, 10, 3 (1948).

74. R. Karlsson, Talanta, 22, 989 (1975).

75. L.E. Edholm, Talanta, 23, 709 (1976).

76. R. Ripan and G. Tautu, Studia Univ. Babesbalyai, S'er. Chem., 2, 107 (1964) cf., C.A. 64:21.

77. M. Rozynska, Z. Nasierowska, J. Suffezynski and Z. Wojeik, Acta. Pol. Pharm., 22, 21 (1965).

78. H.Y. Yuan, Bull. Inst. Chem. Acad. Sin., 10, 33 (1965).

79. L. S'. Vann, Clin. Chem., 11, 979 (1965).

80. L. KumTatt and P.C. Leong, Analyst, 89, 674 (1964).

81. S. Baczyk and O. Kachelska, Mikrochem. Ichnonal. Acta, [5], 873 (1964).

82. M. Barakat, M.F.M.E.L. Wahab and M.M.El. Sadr, Anal. Chem. 27, 536 (1955).

83. C.E. Weeks, R.L. Imblum and M.J. Deutsch, "FDA Experience in Vitamin C Assays", presented at the symposium on vitamin, 78th annual meeting of the Association of Official Agricultural Chemists, October 19-22 (1964) Washington.

84. M. Sarwar, Z. Iqbal and S. Zaidi, Microchimica Acta, 11, 699 (1975).

85. E. Martini and A. Bonsignore, Boll. Soc. Ital. Biol. Sper., 9, 388 (1934).

86. Ibid, Biochem. Z., 273, 170 (1934).

87. T. Sabalitschka and A. Priem., Z. Physiol. Chem., 270, 194 (1941).

88. G. Sitarama[Sastry] and G. Gopala[Rao], Talanta, 19, 212 (1972).

89. H.C. Sherman, V.K. Lamer and H.L. Campbell, J. Am. Chem. Soc., 44, 165 (1922).

90. B. S. and H. Shwachman, J. Biol. Chem., 151, 439. (1943).

91. K.H. Coward, Biological Standardization of the Vitamins, 2nd Edn, P.93, Paris, Bailliere et al.

92. M. Oliver, The Victims, Ed. W.H. Sabrell and R.S. Harris, Vol. 1 2nd Edn. p.338-344, New York, Academic Press.

93. J.O. Schenk, E. Miller and R.N. Adams, Anal. Chem. 54, 1452 (1982).

94. T.L. Parkinson, Chemy. Ind., 17 (1952).

95. L.W. Mapson, Bio-Chem. J., 80, 459 (1961).

96. R. Strohecker and H.M. Henning, vitamin bestimmungen. Verlag. Chemie, G.M.B.H. Woinheim/Bergstr p.232 (1963).

97. R. Strohercker and H. Pies, Z. Lebensmittel Unters, u. Forsch., 118, 394 (1962).

98. J. Herrman and W. Andrae, Nahrung, 7, 243 (1963).

99. W. Feldheim and M. Czerny, Z. Anal. Chem, 162, 193 (1958).

100. D.V. Myrhe and F. Smith, J. Org. Chem., 23, 1229 (1958).

101. I.R. Shimi, M.S. Nour. El. Dein and G.M. Imam, Analyst, 86, 535, (1961).

102. M. Vecchi and K. Kaiser, J. Chromatogr., 26, 22 (1967).

103. L.A. Pachla and P.T. Kissinger, Anal. Chem, 48, 364 (1976).

104. L.J. Harris, L.W. Mapson and Y.L. Wang, Biochemical, (8), 36, 183 (1942).

105. H. Liebmannand A.D. Ayres, Analyst 70, 411 (1945).

106. R.C. Curtis, Analyst, 83, 54 (1958).

107. A. Baldesten, S.G. Hjalmarsson and G. Neumann, Fr. Z Anal. Chem. 290, 148 (1978).

108. R. Christova, M. Ivanova and M. Novkirishka, Anal. Chim. Acta, 85, 301 (1976).

109. R.C. Rose and D.L. Nahrwold, Anal. Biochem., 114, 140 (1981).

110. Bo karlberg and S. Thelander, Analyst, 103, 1154 (1978).

111. M.R. Whetstone, Ph.D Thesis, Loughborough University of Technology, 1982.

112. Food Additives and Contaminants Committee.
Interim Report on the Review of the Colouring Matters in Food Regulations, FAC/REP/29. Ministry of Agriculture, Fisheries and Food, HMSO, London, 1979.

113. J.J.P. Drake, Developments in Food Colours - 1, J. Walford (Ed.)., Applied Science Publishers, London, 1979.

114. B.A. Eagerman, F.M. Clydesdale and F.J. Francis, J. Food Sci., 38, 1056 (1973).

(115) F.J. Francis and F.M. Clydesdale, Food Colorimetry, Avi Publishing Co., Inc. Conn. U.S.A. (1975).

(116) A. Kramer, Food Technol., 30[10]62 (1976).

(117) F.M. Clydesdale, Food Technol., 26[7]45 (1972).

(118) A.C. Little and G. Mackinnely, Food Technol., 23, 25, (1969).

(119) J.N. Yeatman, Food Technol., 30[11]50 (1976).

(120) E.E.C. Council Directive of 23/10/1962, Official. J. European Communities, No. 115, 11/11/62, p.2645/62.

(121) K. Venkatarman (Ed.). The Analytical Chemistry of Synthetic Dyes, J. Wiley and Sons, Inc. London (1977).

(122) M. Singh, J. Ass. Offic. Analyt. Chemists, 57, 219 (1974).

(123) J.E. Bailey and E.A. Cox, J. Ass. Offic. Analyt. Chemists, 58, 609 (1975).

(124) J.E. Bailey and E.A. Cox, J. Ass. Offic. Analyt. Chemists, 60, 5 (1976).

(125) M. Singh, J. Ass. Offic. Analyt. Chemists, 60, 173 (1977).

(126) D.M. Marmion, J. Ass. Offic. Analyt. Chemists, 60, 168 (1977).

(127) M. Singh, J. Ass. Offic. Analyt. Chemists, 60, 1067 (1977).

(128) B.A. Persson, Acta. Pharm. Succica, 5, 343, (1968).

(129) S. Eksborg and G. Schill, Anal. Chem., 45 2092 (1973).

(130) E. Tomlinson, T.M. Jefferies and C.M. Riley, J. Chromatog., 159, 315 (1978).

(131) R.J. Passarelli and E.S. Jacobs, J. Chromatogr. Sci., 13, 153 (1975).

(132) D.P. Wittmer, N.O. Nuessle and W.G. Haney, Anal. Chem., 47, 1422 (1975).

(133) J.H. Knox and G.R. Laird, J. Chromatog., 122, 17 (1976).

(134) B. Stavrić, R. Klassen and W. Miles, J. Ass. Offic. Analyt. Chemists, 62, 1020 (1979).

(135) C.W. Brown and P.F. Lynch, J.Fd-Sci., 41, 123 (1976).

(136) A.G. Fogg and K.S. Yoo, Analyst, 104, 723 (1979).

(137) J.E. McNeal, J. Ass. Offic. Analyt. Chemists, 59, 570 (1976).

(138) D. Pearson and R. Walker, J. Assoc. Publ. Analysts, 3, 45 (1965).

(139) D. Pearson, J. Assoc. Publ. Analysts, 11, 127 (1973).

(140) G.J. Dickes, J. Assoc. Publ. Analysts, 3,49 (1965).

(141) W.B. Chapman and D.Oakland, J. Assoc. Publ. Analysts, 6, 124 (1968).

(142) The Association of Public Analysts. Separation and Identification of Food Colours Permitted by the Colouring Matter in Food Regulations, London 1960.

(143) R.A. Hoodless, K.G. Pitman, T.E. Stewart, J. Thomson and J.E. Arnold, J. Chromatog., 54, 393 (1971).

(144) L. Lepri, P.G. Desideri and V. Coas, J. Chromatog., 161, 279 (1978).

(145) J. Chudy, N.T. Crosby and I. Patel, J. Chromatog., 154, 306 (1978).

(146) Schweppe, in "Thin-Layer Chromatography - a Laboratory Handbook", E. Stahl (Ed.), Springer-Verlag, Berlin, 1969 pp.624-626.

(147) P. Wollenweber, J. Chromatogr., 7, 557 (1962).

(148) M.H. Penner, J. Pharm. Sci., 57, 2132 (1968).

(149) J.F. Barrett and A.J. Ryan, Nature, 199, 372 (1963).

(150) H. Kamikura, J. Food Hyg. Soc. (Japan), 7, 338 (1966).

(151) J. Davide K and E David Kova, Z. Lebensm, Unters. Forsch., 131, 99 (1966).

(152) K.T. Wang, Nature, 213, 212 (1967).

(153) J. Davidek and E. Davidkova, J. Chromatog., 26, 529 (1967).

(154) R.A. Gilhooley, R.A. Hoodless, K.G. Pitman and J. Thomson, J. Chromatog., 72, 325 (1972).

(155) M. Mottier and M. Potterat, Anal. Chim. Acta, 13, 46 (1955).

(156) Y. Yanuka, Y. Shalon, E. Weissenberg and I. Nir-Grosfeld, Analyst, 88, 872 (1963).

(157) C. Graichen, J. Ass. Offic. Analyt. Chemists, 58, 278 (1975).

(158) Association of Official Analytical Chemists, Official Methods of Analysis 12th Edn, Washington, DC, U.S.A. 1975.

(159) B. Drevon and J. Laur, Ann. Fals. Frandes, 52, 155 (1959).

(160) G. Lehmann, P. Collet, H.G. Hahn and N.R.F. Ashworth, J. Ass. Offic. Analyt. Chemists, 53, 1182 (1970).

(161) N.P. Boley, N.T. Crosby and P. Roper, Analyst, 104, 472 (1979).

(162) N.P. Boley, N.G. Bunton, N.T. Crosby, A.E. Johnson, P. Roper and L. Somers, Analyst, 105, 589 (1980).

(163). G.W. Ewing, Instrumental Methods of Chemical Analysis, McGraw-Hill, London (1969).

(164) I. Saguy, S. Mizrahiand T.J. Koppelman, J. Fd. Sci., 43, 121 (1978).

(165) Ministry of Food Standards Committee Report on Colouring Matters, HMSO, London, 1954, p4.

(166) T.W. Raven. The Use and Stability of Artificial Colouring Matters in Canned Fruit and Vegetables, Research leaflet No. 7, The Fruit and Vegetable Canning and Quick Freezing Research Association, Chipping Camden, Glucoce., 1962.

(167) J. Walford. "Developments in Food Colours - 1", Applied Science Publishers, London, 1980.

(168) Ministry of Agriculture Fisheries and Food FACC Interim Report on the Review of the Colouring Matter in Food Regulations, HMSO, London, 1973.

(169) Ministry of Agriculture Fisheries and Food. Food Quality and Safety: A Century of Progress, HMSO, London 1976.

(170) Specifications for identity and purity and toxicological evaluation of food colours, WHO/Food Add./66.25, FAO Nutrition Meeting Report Series No. 388, 1964.

(171) K.S. Khan and I.C. Munor, CRC Crit. Rev. Toxicol., 6, 81 (1979).

(172) J.J.P. Drake, Toxicology, 5, 3 (1975).

(173) R.L. Voight, F.A. Brown and J.J. Clin. Endocrin. Met., 34, 747 (1972).

(174) K. Kojima, "The toxicological assessment of natural food colorants" in "chemical toxicology of food.", Elsevier, 1978.

(175) J.P. Brown, G.W. Roehm and R.J. Brown, Mutation Res., 56, 249 (1978).

(176) Federal Register, 43, (192), 45611 (1978).

(177) Joint FAO/WHO Expert Committee on Food Additives, 19th Report, 1975, World Health Organisation, Technical Report Series 576.

(178) H.E. Nursten and G. Williams, Chem. Ind. London, 50, 1798 (1969).

(179) C. Jouffrey, R. Sci. Tech. Pharm., 2(3), 117 (1973).

(180) J.H. Helu, Rev. Farm. Bioquim. Univ. Sao Paulo, 13 (1), 1 (1975).

(181) F.W. Goodhart, H.A. Lieberman, D.S. Mody and F.C. Ninger, J. Pharm. Sci, 56, 63 (1967).

(182) W.H. Peacock, "The Application Properties of the Certified "Coal Tar", Colorants", Technical Bulletin No. 715, Calco Chemical Division, American Cyanamid Co. (1944).

(183) E. Spiess, Rev. Techn. Pharm. SUTIP, 10, 4015 (1965).

(184) J.R. Cooper, Diss. Abstr. Int. B., 33, 4421 (1973).

(185) A. Jodlbauer and V. Tappeiner, Deut. Archiv. Klin. Med., 82, 520 (1944).

(186) O.L. Evenson, J. Assoc. Offic. Anal. Chem., Nov., 773 (1939).

(187) J.J. Porter, Text. Res. J., 735 (1973).

(188) C.J. Swartz, L. Lachman, T. Urbanyi, S. Weinstein and J. Cooper, J. Pharm. Sci., 51 (4), 326 (1962).

(189) H. Luck, Z. Lebensmittel-Untersuch. U-Forsch, 126, 193 (1965).

(190) S.K. Banerjee, T.V. Matthew, A.H. Mukherjee and S.N. Mitra, Res. Ind. (New Delhi), 5, 18 (1970).

(191) M. Ikeda and T. Uesugi, Biochem. Pharmacol., 22, 2743 (1973).

(192) J.L. Radomski and T.J. Mellinger, J. Pharmac. Exp. Ther., 136, 259 (1962).

(193) B. Koether, Desch. Apotheker-Ztg. 101, 742 (1961).

194. L.T. Skeggs, Amer. J. Clin. Pathol., 28, 311 (1957).

195. G.Z. Nagy, Z. Feher , E. Pungor, Anal. Chim. Acta, 52, 47 (1970).

196. J. Ruzicka, E.H. Hansen, Anal. Chim. Acta., 78, 145 (1975).

197. J.W.B. Stewart, J. Ruzicka, H. Bergamin Filho and E.A.G. Zagatto, Anal. Chim. Acta, 81, 371 (1976.

198. J.W.B. Stewart, J. Ruzicka, Anal. Chim. Acta, 82, 137 (1976).

199. J. Ruzicka, E.H. Hansen, Anal. Chim. Acta, 99, 37 (1978).

200. J. Ruzicka, E.H. Hansen, Anal. Chim. Acta, 114, 19 (1980).

201. J. Ruzicka, E.H. Hansen, Chem. Tech., 91, 12, 757 (1979).

202. D. Betteridge, Anal. Chem., 50, [9] 832A (1978).

203. J. Ruzicka, J.W.B. Stewart, Anal. Chim. Acta, 79, 79 (1975).

204. J. Ruzicka, J.W.B. Stewart, E.A.G. Zagatto, Anal. Chim. Acta, 81, 387 (1976).

205. E.H. Hansen, J. Ruzicka and A.K. Ghose, Anal. Chim. Acta, 100, 151 (1978).

206. H. Poppe, Anal. Chim. Acta., 114, 59 (1980).

207. H.L. Pardue and B. Fields, Anal. Chim. Acta., 124, 39 (1981).

208. H.L. Pardue and B. Fields, Anal. Chim. Acta, 124, 65, (1981).

209. H.F.R. Reijnders, J.J. Van Staden, G.H.B. Eelderink and B. Griepink, Anal. Chim. Acta., 114, 235 (1980).

210. J. Ruzicka, E. Hansen and E.A.G. Zagatto, Anal. Chim. Acta., 88, 1 (1977).

211. E.H. Hansen, J. Ruzicka and B. Rietz, Anal. Chim. Acta, 89, 241 (1977).

212. E.H. Hansen, A.K. Ghose and J. Ruzicka, Analyst, 102, 705 (1977).

213. F.J. Krúg, J. Ruzicka and E.H. Hansen, Analyst, 104, 47 (1979).

214. J. Ruzicka, E.H. Hansen and H. Mosbaek, Anal. Chim. Acta., 92, 235 (1977).

215. J. Ruzicka, E.H. Hansen, H. Mosbaek and F.J. Krug, Anal. Chem., 49, 1858 (1977).

216. E.H. Hansen, F.J. Krúg, A.K. Ghose and J. Ruzicka, Analyst, 102, 714 (1977).

217. D. Betteridge and J. Ruzicka, Talnta, 23, 409 (1976).

218. L. Anderson, Anal. Chim. Acta, 110, 123 (1979).

219. Y. Hirai, N. Yoza and S. Ohashi, Anal. Chim. Acta, 115, 269 (1980).

220. L. Sodek, J. Ruzicka and J.W.B. Stewart, Anal. Chim. Acta, 97, 327 (1978).

221. J.A. Lown and D.C. Johnson, Anal. Chim. Acta, 116, 41, (1980).

222. B.O. Karlberg and S. Thelander, Analyst, 103, 1154, (1978).

223. E. Pungor, Z.S. Feher and G. Nagy, Anal. Chim. Acta, 51, 417 (1970).

224. Z.S. Feher and E. Pungor, Anal. Chim. Acta, 71, 425 (1974).

225. Z.S. Feher, G. Nagy, K. Toth and E. Pungor, Analyst, 99, 699 (1974).

226. P.T. Kissinger, Anal. Chem., 49, 447A.

227. E. Pungor, Z.S. Feher, G. Nagy, K. Toth, G. Horvai and M. Gratzl, Anal. Chim. Acta, 109, 1 (1979).

228. W. Lund, M. Hannisdal and T. Greibrokk, J. Chromat, 173, 249 (1979).

229. E.C. Lewis and D.C. Johnson, Clin. Chem., 24/10, 1711 (1978).

230. W. Kemula, Pure. Appl. Chem., 25, 763 (1971).

231. H.B. Hanekamp, P. Bos, U.A. Th. Brinkman and R.W. Frei., Fresenius. Z. Anal. Chem., 297, 404 (1979).

232. H.B. Hanekamp. P. Bos, and R.W. Frei, J. Chromatogr., 186, 489 (1979).

233. R. Stillman and T.S.Ma, Microchimica. Acta, 491 (1973).

234. L. Michel and A. Zatka, Anal. Chim. Acta., 105, 109 (1979).

235. R.C. Butcha and L.J. Papa, J. Chromatogr. Sci., 114, 213 (1976).

236. H.K. Chan and A.G. Fogg, Anal. Chim. Acta., 111, 281 (1979).

237. A.G. Fogg and D. Bhanot, Analyst, 106, 883 (1981).

238. A.G. Fogg and N.K. Bsebsu, Analyst, 106, 1288 (1981).

239. A.G. Fogg and N.K. Bsebsu, Analyst, 107, 566 (1982).

240. A.G. Fogg, N.K. Bsebsu and M.A. Abdalla, Analyst, 107, 1040 (1982).

(241) J. Heyrovsky, Chem. Listy., 16, 256 (1922).

(242) G.C. Barker and A.W. Gardner, Z. Anal. Chem., 173, 79 (1960).

(243) A.A.M. Brinkman and J.M. Los, J. Electroanal. Chem., 7, 171 (1964).

(244) A.W. Fonds, A.A. M. Brinkman and J.M. Los, J. Electroanal. Chem., 14, 43 (1967).

(245) A.A.M. Brinkman and J.M. Los, J. Electroanal. Chem., 14, 269 (1967).

(246) A.A.M. Brinkman and J.M. Los, J. Electroanal. Chem., 14, 285 (1967).

(247) A.W. Fonds, J.L. Molenaar and J.M. Los, J. Electroanal. Chem., 22, 229 (1969).

(248) A.W. Fonds and J.M. Los, J. Electroanal. Chem., 36, 479 (1972).

(249) J. Osteryoung and K. Hasebe, Rev. Polarogra, (Japan), 22, 1 (1976).

(250) G.C. Barker, Proc. Congr. of Modern Anal. Chem. in Ind., St. Andrews, P.199, 1957.

(251) F.G. Cottrell, Z. Physik. Chem., 42, 385 (1902).

(252) A.M. Bond in "Modern Polarographic Methods in Analytical Chemistry" (1980), Marcel Dekker, New York and Basel.

(253) E.P. Parry and R.A. Osteryoung, Anal. Chem., 37, 1634 (1965).

(254) R.N. Adams, "Electrochemistry at solid electrodes", Marcel Dekker Inc., New York, 1969.

(255) R.S. Nicholson and I. Shain, Anal. Chem., 36, 706 (1964).

(256) J.E.B. Randles, Trans. Faraday Soc. 44, 327 (1948).

(257) A. Sevcik, Coll, Czech. Chem. Communs., 13, 349 (1948).

(258) V.G. Levich, "Physico Chemical Hydrodynamics", Prentice-Hall, Englewood Cliffs, No. 5, (1962).

(259) J. Newman, in "Electroanalytical Chemistry", (A.J. Bonded), Vol. 6, p.187, Marcel Dekker, New York, (1973).

(260) E. Pungor, Z. Feher and M. Varadi, CRC. Crit. Rev. Anal. Chem., 97, 9(2), (1980).

(261) B. Fleet and C.J. Little, J. Chromatog. Sci., 12, 747 (1979).

(262) M.B. Glaurt, J. Fluid. Mech., 1, 625 (1956).

(263) J. Yamada and H. Matsuda, J. Electroanal. Chem., 44, 189 (1973).

(264) R. Strohecher and H.M. Henning, "Vitamin Assay-Tested Methods" Verlagchemie, GmbH, Weinheim/Bergstr 1966.

(265) L. Erdey and E. Bodor, Z.Anal. Chem., 136, 109 (1952).

(266) V.K.La mer, H.L. Campbell and H.C. Sherman, J. Am. Chem. Soc., 44, 172 (1922).

(267) A.L. Medalia, Anal. Chem., 27, 1678 (1955).

(268) A.G. Fogg and K.S. Yoo, Analyst, 104, 1087 (1979).

(269) A.G. Fogg and D. Bhanot, Analyst, 105, 234 (1980).

(270) A.G. Fogg and M.R. Whetstone, Analyst, 107, 455, (1982).

(271) G. Norwitz and P.N. Kellher, Anal. Chem., 53, 1238 (1981).

(272) M.P. Berthelot, Rep. Chim. Appl., 284 (1859).

(273) C.W. Gehrke, J.S. Killingley and L.S. Wall:, J.A.O.A.C., 55, 467 (1972).

(274) J.P. Riley, Anal. Chim. Acta., 9, 575 (1953).

(275) L.T. Mann, Anal. Chem., 35, 2179 (1963).

(276) P.J. Rommers and J. Visser, Analyst, 94, 653, (1969).

(277) J.A. Tetlow and A.L. Wilson, Analyst, 89, 453 (1964).

(278) A.G. Fogg and Y.Z. Ahmed, Anal. Chim. Acta., 101, 211 (1978).

(279) "Kirk-Othmer Encylcopaedia of Chemical Technology" Third Edition, Volume Eleven, John Willey, New York, 1980.

(280) A.G. Fogg and M.R. Whetstone, in preparation.

(281) A.G. Fogg and M.R. Whetstone, in preparation.

(282) N.P. Boley, N.T. Crosby, P Roper and L Somers, Analyst, 106, 710 (1981).

(283) M.A. Puttemans, L. Dryon and D.L. Massart, J. Assoc. Off. Anal. Chem., 66, 1040 (1983).

(284) D. Bhanot, Ph.D Thesis, Loughborough University of Technology, 1981.

(285) J.A. Clements, K. Hasson, G. Smith, J. Pharm. Pharmac., 32, 50P, (1980).

(286) S. Green et. al., J. Biol. Chem., 220, 237 (1956).

(287) T.Y. Lau and D.M. Carlson, J. Biol. Chem., 256, 7142, (1981).

(288) M.W. Van Dyke and P.B. Dervan, Biochem., 22, 2373, (1983).

(289) J.B.F. Lloyd, J. Chromatog., 256, 323, (1983).

(290) A.G. Fogg, N.K. Bsebsu and M.A. Abdalla, Analyst, 107, 1462 (1982).

(291) A.G. Fogg, A.Y. Chamsi and M.A. Abdalla, Analyst, 108, 464, (1983).

(292) A.G. Fogg, M.A. Ali and M.A. Abdalla, Analyst, 108, 840 (1983).

(293) M.R. Hackman and M.A. Brooks, J. Chromat., 222, 179 (1981).

(294) J.J. Vanderlee, H.B.J. Vanderlee-Rijsbergen, U.R. Tjaden

and W.P. VanBennekom, Anal. Chim. Acta, 149, 29, (1983).

(295) R.C. Scothorst and G. DenBoef, Anal. Chim. Acta., 153, 133, (1983).

(29) D.L. Rabenstein and R. Saetre, Anal. Chem., 49, 1036 (1977).

(29) J. Janata and J. Ruzicka, Anal. Chim. Acta., 139,105 (1982).

(29) A.N. Strohl and D.J. Curran, Anal. Chem., 51, 1045 (1979).