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# ELECTROANALYSIS OF FOOD COLOURING MATTERS

by

DEEPAK BHANOT, B.Sc(Hons), M.Sc, M.Tech.

A Doctoral Thesis

submitted in partial fulfilment of the requirements

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by DEEPAK BHANOT (1981)



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# DEDICATED WITH REVERENCE TO THE MEMORY OF KEDAR SWAMI BABAJI

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"When work is done as sacred work, unselfishly, with a peaceful mind, without lust or hate, with no desire for reward, then the work is pure".

THE BHAGAVAD GITA

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# SYNOPSIS

A systematic study has been made of the effect of tetraphenylphosphonium chloride on the D.P. polarographic peaks of the sixteen permitted synthetic food colouring matters. The D.C. half-wave potentials and D.P. peaks of fertrazine, Yellow 2G and Brilliant Blue FCF, are shifted to more negative potentials on the addition of TPPC. The mean limiting diffusion currents and peak currents of these and certain other synthetic colouring matters are altered significantly on its addition, the changes being greater in D.P. polarography. For both D.C. and D.P. polarograms the changes occur at TPPC concentrations up to 100  $\mu$ g ml<sup>-1</sup>; above this concentration half-wave and peak potentials and limiting diffusion and peak currents change very little in most instances. Other phosphonium and argonium compounds also shifted the potentials but to a lesser extent than TPPC.

The anodic and cathodic linear sweep voltammetry of the permitted synthetic food colours at glassy carbon and carbon paste electrodes has also been studied. Most colours give well-defined D.C. voltammetric peaks in pH2 Britton-Robinson buffer in the range 0.6-1.1V (vs S.C.E.) at a stationary glassy carbon electrode. The peak currents can be used to determine food colours in the range 2-100  $\mu$ g ml<sup>-1</sup> either directly or after extraction on polyamide columns. The method is applicable with good precision to single food colours and quality control applications are feasible with some combinations of colouring matters. Serious interference from other constituents has been observed in the determination of Amaranth in a blackcurrant health drink.

Linear sweep anodic peaks of synthetic food colouring matters at a stationary carbon paste electrode are generally sharper, better resolved from cut-off current and have a lower baseline current. All the food colours studied also gave cathodic peaks at a stationary glassy carbon electrode but there was fluctuation of peak potential and instability of peak shape with some colours. The cathodic peak potentials of the yellow food colours are particularly well separated. The more limited negative potential range

available with carbon paste restricted study to ten food colours.

The voltammetric waves of four food colours showed some reversibility when studied by cyclic voltammetry. The anodic peaks of Green S, Patent Blue V, and Brilliant Blue FCF, show small peaks on the reverse scan, and Indigo Carmine is unique in having a large peak on the reverse scan of the cathodic peak.

Data obtained in the static studies has been applied to the development of flow-injection methods for the determination of food colours. Carbon paste and glassy carbon electrodes used anodically in the wall-jet configuration have been shown to give good precision, rectilinear calibration graphs and low level detection (nanogramme range) for the determination of food colours by flow injection analysis.

#### CHAPTER 1

## INTRODUCTION TO VOLTAMMETRIC METHODS

Analytical chemistry with its spectrum of instrumental techniques has become one of the most important and stimulating applications of physical chemistry. Interpretation of results provided by analytical techniques have led to large scale developments and a better understanding of problems in chemistry, pharmacy, biology, medicine, materials and energy technology, environmental pollution, and the related fields of geology and oceanography.

Sensitivity, selectivity and reliability are the prime requirements of analytical techniques. The application range should be wide and the operation mode simple and rapid. For routine and control applications a reasonable balance between costs and output of analytical information is desirable. The adaptability of certain analytical techniques to automatic handling and data processing is becoming increasingly important.

The class of techniques that are used to study solution composition through current-potential relationships are termed electrochemical techniques. For quantitative and qualitative analysis the voltammetric techniques are by far the most important. In this chapter the theoretical basis of voltammetric techniques is briefly reviewed.

#### POLAROGRAPHY

#### Historical

Polarography invented by Jaroslav Heyrovsky in 1922 is a branch of voltammetry carried out using the dropping mercury electrode<sup>(1)</sup>. For many years most analytical applications of polarography were to the determination of metal ions and a few other inorganic species such as oxygen and nitrate ion; determinations of organic substances were much less common and gained ground only slowly.

The relative decline in the use of polarography in the decade 1950-60 can be attributed to three causes - basic problems inherent to the technique, lack of attention to instrument design, and poor training in electroanalytical chemistry. Florence(2)in an article points out that polarography reached its nadir around 1967. The environmental crisis had only just awakened the public conscience, and atomic absorption spectrophotometry was in a rapid state of development, and modern solid-state operational amplifier circuitry had not made its full impact on commercial polarographic instrumentation. During the past few years, however, a sudden increase in interest in electroanalytical techniques has become apparent. Ironically during the decade 1955-65, when the future use of polarography in trace analysis was being questioned, enormous advances in the theory and instrumentation of polarographic techniques were taking place simultaneously (3). Two significant developments were the introduction of differential pulse polarography and stripping techniques which extended the lower limit of detection to true trace analysis. Another factor that contributed to the decided increase in the importance of polarography is the growing realization of its applicability in organic analysis, especially in the pharmaceutical industry. Consequently industrial and other laboratories have begun to adopt the use of polarographic methods to complement other techniques.

#### **Principles**

Classical DC polarography 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 resulting current is plotted versus the applied potential. The DME consists of a narrow bore (0.06 - 0.08 mm i.d) capillary through which mercury flows and emerges in the form of droplets. Each droplet presents a completely new electrode surface. The high overpotential of hydrogen on mercury makes possible the electroreduction of many species at negative potentials unattainable on solid electrodes. The DME can be used up to -1.6V vs S.C.E.

in acidic solutions and up to -2.6V in basic or non-aqueous solutions. Its disadvantages include a limited positive potential range, oscillations arising due to a continuous changing surface of mercury, influence on drop size of the changes in interfacial tension at the mercury solution interface due to applied potential or chemical agents in the solution.

#### Capacitance and Faradaic Currents

The total observed current consists of a faradaic component arising as a result of transfer of electrons across the electrodesolution interface and the capacitance current, which flows because the electrode-solution interface exhibits capacitive characteristics.

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 constant over the lifespan of a single drop, the capacitance current in polarography is primarily the result of the change in electrode area. As the drop grows, new mercury surface is exposed to the solution, and the current must flow to charge this new surface to that potential dictated by the electrode potential and solution conditions. The charging current can be either anodic or cathodic 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, anions are adsorbed on the surface of the electrode, and the adsorption of these anions results in an excess of negative charge on the solution side of the interface. The negative charge  ${}_{\mathcal{C}}^{i}$  ) induces a positive charge on the electrode, therefore, anodic current must flow to charge the electrode-solution interface. As the applied potential becomes very negative, the quantity of )anions adsorbed also decreases. At the potential where there is no excess of anions (or cations) on the electrode surface no current is required to charge the double-layer. This is the potential of zero charge,  $E_{max}$ . At potentials more negative than  $E_{max}$ , a negative current is required to charge the double-layer.

The capacitance current,  $I_c$ , resulting from the growth of a mercury drop is directly proportional to the rate of change of drop area, dA/dt.

$$I_{c} = C_{d\ell} (E_{max} - E) dA/dt$$
(1.1)

where  $\mathsf{C}_{d\ell}$  is the differential capacity and E the potential of the electrode.

The change in drop area is very large at the beginning of a new drop; therefore  $I_c$  jumps at the beginning of the drop. As the drop grows, dA/dt decreases and  $I_c$  decreases. The variation of  $I_c$  with time is given by

$$(I_c)_t = 0.00569 C_{de} (E_{max} - E) m^{2/3} t^{-1/3}$$
 (1.2)

where  $(I_c)_t$  is the capacitance current after time t sec and m is the rate of mercury flow in mg.s<sup>-1</sup>.

The faradaic current, unlike the capacitance current increases with time unless complicating phenomenon such as adsorption are present. The mean limiting current, which is diffusion controlled, is given by the Ilkovic equation

 $i_d = 607 n CD^{\frac{1}{2}} m^{2/3} t^{1/6}$  (1.3)

where  $i_d$  is mean limiting diffusion current ( $\mu A$ ); n is the number of electrons involved in the reduction or oxidation; D the diffusion coefficient of electroactive species (cm<sup>2</sup> s<sup>-1</sup>); C is bulk concentration (m mol cm<sup>-3</sup>); m the rate of mercury flow rate (mg. s<sup>-1</sup>) and t the drop age (sec).

Changes of instantaneous current with time during the life of a single drop for  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  M solution are shown in Figure 1.1.



FIGURE 1.1 Instantaneous current with time during the life of a single drop at  $10^{-4}$ M,  $10^{-5}$ M, and  $10^{-6}$ M depolarizer concentrations.

Determination at levels below 10<sup>-5</sup>M becomes difficult as the charging current assumes significant proportions, and resolution between two waves becomes very poor in the presence of a relatively large concentration of a more easily reducible species.

A number of difficulties encountered in classical DC polarography have resulted in major advances and innovations in the analytical use of polarography<sup>(4)</sup>. Some of the limitations and drawbacks together with some suggestions are discussed below.

# Resolution, Wave Shape and Readout

Calculation of the mean-limiting current and the  $E_1$  value requires evaluation of the average current at a large number of potentials. Obtaining the required parameters from a d.c. polarogram can be time consuming compared with the usually simple readout of absorbance in UV-visible spectrophotometry. Furthermore, the ideal-shaped curve is all too infrequently obtained. Maxima and other distortions are often observed introducing the necessity of using semi-empirical or even arbitrary procedures for the evaluation of the waves.

Any approach improving the ease and convenience of readout should be useful. Obviously the production of peak-shaped curve with direct readout of peak current and peak potential as alter-

natives to measurement of i and  $E_1$  would be advantageous. Further the restriction placed by wave shape on resolution of two closely spaced reductions could be improved by a readout of two peaks referred to a common base-line. Most modern techniques of polarography have the advantage of considerably improved resolution over DC polarography.

#### Time taken to record a DC polarogram

Conventional DC polarography is necessarily relatively slow because the time taken to record a polarogram is restricted by the slow rate at which the potential must be scanned. If the scan rate is made too fast in DC polarography, insufficient data points are available to define accurately the polarogram, and the constant potential condition is violated. Typically 5-15 min. are required to record a polarogram with drop time between 2-10 sec. Any modern polarographic technique capable of reducing this time scale substantially is likely to be of considerable benefit.

#### Sensitivity and Charging Current

In conventional DC polarography with diffusion-controlled limiting currents and aqueous media, the charging current generally masks the faradaic current when the concentration of the electroactive species is in the  $10^{-6}$  to  $10^{-5}$  M region. However, even in the  $10^{-5}$  to  $10^{-4}$ M range, the magnitude of the charging current is significant, and curves with sloping base-lines rather than the idealized sigmoidal shaped curves are observed. Any technique discriminating against the charging current should increase the sensitivity of the determination and lower the limit of detection. In nonaqueous media the charging current may be somewhat higher, thereby raising the limit of detection in certain solvents.

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## Automation of Instrumentation and Readout

With the advent of digital electronics and minicomputers, etc. most instrumental methods of analysis are capable of a considerable degree of automation. Automation at all stages of operation, including sample preparation, experimental control, data acquisition, and interpretation are available as optional extras on the most recent commercially available instrumentation. Certain polarographic methods lend themselves more readily to automation than the DC technique.

#### Dependence of Drop Time on Potential

In conventional DC polarography, the dependence of drop time on potential presents difficulties, particularly at very negative potentials, where large changes in drop time occur. The potential dependence of drop time is frequently overlooked, but it is not always negligible. The use of mechanically controlled drop times, a feature of virtually all modern polarographic instrumentation obviates this difficulty.

#### PULSE POLAROGRAPHY

Pulse polarography was developed originally in England by G C Barker<sup>(5,6)</sup> as an extension of his work on square-wave polarography. As the name implies, the potential is applied periodically during short time intervals. Both the format for application of the pulse and current readout may be varied in several ways. Pulse polarography is an excellent example of the use of improved faradaic-to-charging current ratio and in addition has several important advantages over conventional DC polarography.

#### Principles

In pulse polarography only a single pulse is applied to the system per mercury drop, late in the drop life. Approximately 20-40 msec after the application of the pulse the charging current

has decayed to almost zero. Measurement of the faradaic current remaining after this time is then made and the polarogram is a plot of the faradaic current produced versus the applied potential.

In integral or normal pulse polarography, potential pulses of gradually increasing amplitude are applied to an electrode, starting from an initial potential where no faradaic current Figure 1.2 illustrates the potential waveform in normal flows. pulse polarography. The potential pulses are of approximately 60 msec duration. The pulses are applied at a fixed time in the life of the drop, so a constant electrode area is maintained. Current values are measured in the last 20 msec (16.7 msec in the case of PAR174). An electronic integration procedure is used, and hence, the name 'integral pulse polarography'. The output is the difference between the current measured over the selected time interval after the pulse duration and the current measured just prior to the pulse application when the electrode is at the initial potential. The limiting current of a normal pulse polarogram is diffusion controlled as is the case in conventional DC polarography. The NP polarograms for most organic compounds, however, give peakshaped curves, owing to their irreversible characteristics.

In derivative or differential pulse polarography, a normal DC voltage ramp is applied to the system. Near the end of the drop life, a small amplitude pulse (5-100 mV) is superimposed onto the ramp (Figure 1.3). The current is measured twice: once before applying the pulse and once during the last 16.7 msec of the pulse when the capacitance current has decayed. As the measured signal is the difference in current a peak-shaped curve is obtained with the peak maximum occurring near  $E_{\frac{1}{2}}$  if the pulse amplitude is sufficiently small.

# Theoretical and Analytical Implications

Theoretical treatments of pulse polarography include those of Barker and Gardner<sup>(7)</sup>, Los and co-workers<sup>(8-14)</sup> and Parry, Osteryoung



FIGURE 1.2

POTENTIAL WAVEFORM & RESULTING CURRENT FOR PULSE POLAROGRAPHY



FIGURE 1.3 DIFFERENTIAL PULSE EXCITATION WAVEFORM & RESULTING CURRENT - TIME BEHAVIOR

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and Oldham<sup>(15-21)</sup>. The best introduction to the subject seems to be the paper by Osteryoung and Hasebe<sup>(22)</sup>. 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 relationship to concentration of the electroactive substance.

# Current-Concentration Relationship Normal Pulse Mode

The normal pulse current on the diffusion plateau is given by the Cottrell equation (16):

 $i_{d} = n F A c \sqrt{\frac{D}{\pi t_{m}}}$ (1.4)

where A is the electrode area in  $cm^2$ , c the bulk concentration in mM, and t<sub>m</sub> the time in seconds, measured from pulse application, at which current is measured.

The Cottrell equation has some important features. First, the current is directly proportional to the concentration, and none of the constants should change appreciably in value due to change in matrix. Second, the current decreases as  $t_m^{-\frac{1}{2}}$  meaning thereby that the measurement time should be as small as possible in order to maximize the signal. On the other hand, the measurement time must be large enough so that the capacitive current is negligible.

The sensitivity of NPP with respect to DC polarography can be readily calculated by substituting the appropriate term for the growing drop in the Cottrell equation and including the factor  $(^{7}/_{3})^{\frac{1}{2}}$  of the Ilkovic equation

$$i_{\rm NPP} = 462 \ n \ D^2 \ c \ m^{2/3} \ t_d^{2/3} \ t_m^{-\frac{1}{2}}$$
 (1.5)

$$i_{DC} = (7/3)^{\frac{1}{2}} 462 \text{ n } D^{\frac{1}{2}} \text{ C } m^{2/3} t_{d}^{1/6}$$
 (1.6)

$$\therefore \frac{i_{NPP}}{i_{Dc}} = \left(\frac{3 t_{d}}{7 t_{m}}\right)^{\frac{1}{2}} \approx 3-6$$
 (1.7)

The NPP detection limits are 3-6 times lower than the DC values depending on capacitive background current as well as on sensitivity.

# Differential Pulse Mode

In the differential pulse case, we are measuring, not a constant current on a plateau, but rather the rate at which the current increases to the plateau value as the potential is changed.

The normal polarographic current-potential relation for a reversible reaction may be written as

$$E = E_{\frac{1}{2}} + \frac{RT}{nF} \ln \frac{i_d - i}{i}$$
(1.8)

 $\omega + t^{(i)}$  i the Differentiating equation (1.8) and substituting Cottrell equation for the limiting current, the expression

$$\Delta i = \frac{n^2 F^2}{RT} A C \Delta E \sqrt{\frac{D}{\pi t_m}} \frac{P}{(1+P)^2}$$
(1.9)

is obtained where  $\Delta i$  = differential pulse current;  $\Delta E$  = pulse amplitude; and P = exp (E -  $E_1$ )  $\frac{nF}{RT}$ . Maximising  $\Delta i$  with respect to E by differentiating and equating to zero leads to P = 1 for  $\Delta i = \Delta i_{max}$ . Thus

$$\Delta i_{\text{max}} = \frac{n^2 F^2}{4 RT} A C \Delta E \sqrt{\frac{D}{\pi t_m}}$$
(1.10)

Equation (1.10) is valid only for small amplitude case because a

differential method is being approximated by the derivative.

When large pulses are used the resulting equation is

$$\Delta i = n F A C \sqrt{\frac{D}{\pi t_m}} [(P_A \sigma^2 - P_A) / (\sigma + P_A \sigma^2 + P_A + P_A^2 \sigma)]$$
(1.11)

and

$$\Delta i_{\max} = n F A C \sqrt{\frac{D}{\pi t_m}} \left( \frac{\sigma - 1}{\sigma + 1} \right)$$
(1.12)

where:

$$P_{A} = \exp \frac{nF}{RT} \left[ \frac{E_{1} + E_{2}}{2} - E_{\frac{1}{2}} \right]; \quad \sigma = \exp \frac{nF}{RT} \left[ \frac{E_{2} - E_{1}}{2} \right]$$

 $E_2 - E_1 = \Delta E$ , the pulse amplitude;

- $E_2$  = potential at which current  $i_2$  is measured after the application of the pulse.
- $E_1$  = potential at which current  $i_1$  is measured in the absence of the pulse.

 $\Delta i_{max}$  = peak or maximum current.

If  $\frac{\Delta E}{2}$  becomes large with respect to  $\frac{RT}{nF}$ , then  $(\frac{\sigma-1}{\sigma+1})$  approaches unity and the equation for the maximum current becomes that for the Cottrell limiting current.

If the peak difference current in differential pulse is denoted by  $i_{DPP}$  and the diffusion current in normal pulse is  $i_{NPP}$  then equation (1.12) can be re-written as

$$i_{\text{DPP}} = i_{\text{NPP}} \left( \frac{\sigma - 1}{\sigma + 1} \right)$$
(1.13)

The ratio  $(\frac{\sigma-1}{\sigma+1})$  depends only on the number of electrons transferred and on pulse amplitude,  $\Delta E$ , and is always less than unity.

For small pulse amplitudes the DPP current increases roughly linearly with pulse amplitude, and therefore gives better sensitivity. However, as can be seen in Figure 1.4, at large pulse amplitudes the relative increase in current obtained by increasing  $\Delta E$  is less.



FIGURE 1.4 Variation of  $\left(\frac{\sigma-1}{\sigma+1}\right) = \frac{i_{DPP}}{i_{NPP}}$  with pulse amplitude for various values of n.(22)

In practice, the optimum pulse amplitudes to maximize sensitivity lie in the range 50-100 mV.

According to equation (1.9) for small amplitude pulses  $i_{DPP}$  increases linearly with  $n^2$ . This suggests the special usefulness of DPP analysis for electrochemical reactions involving large electron transfers.

The attractive analytical features of differential pulse current can, however, be offset by kinetic complications which cause the current to be less than that predicted by equation (1.13). If the electrochemical reaction rate of the substance sought is slow, even a small change or changes in matrix from sample to sample can cause major changes in reaction rates, and therefore major changes in the peak height-concentration ratio.

#### Resolution

The problem of resolution in the NPP case is more or less the same as resolution of DC polarographic waves. In the DPP case the peak half width  $W_1$  is defined as the width of the peak in millivolts at the point where the peak current is one-half its maximum height. The following expression has been derived (22).

 $W_{\frac{1}{2}} = 3.52 \frac{RT}{nF}$  (1.14)

For small amplitude pulses the peak width is independent of pulse amplitude and inversely proportional to the number of electrons transferred. This equation gives peak half-widths of 90.4, 45.2, and 30.1 mV for 1, 2, and 3 electrons, respectively. The equation for  $W_1$  at large pulse amplitudes depends on pulse amplitude and is more complicated.

$$W_{1} = \frac{RT}{nF} \ln \frac{(\sigma^{2} + 4\sigma + 1) + \sqrt{(\sigma^{2} + 4\sigma + 1)^{2} - 4\sigma^{2}}}{(\sigma^{2} + 4\sigma + 1) - \sqrt{(\sigma^{2} + 4\sigma + 1)^{2} - 4\sigma^{2}}}$$
(1.15)

The variation of  $W_1$  with  $\Delta E$  for various values of n is shown in Figure 1.5. It is worth noting that the peak is narrower, but broadens more rapidly with increasing  $\Delta E$ , for larger values of n. Also for all values of n the peak width approaches the pulse amplitude for large pulse amplitudes.

A compromise between resolution and sensitivity can be achieved by comparison of Figures 1.4 and 1.5. First, for pulse amplitudes above 100 mV the resolution worsens, but there is not much increase in sensitivity. Second, for pulse amplitudes below 20 mV the sensitivity increases rapidly with increasing amplitude, but the resolution is relatively insensitive to changes in pulse-



FIGURE 1.5 Variation of half-width,  $W_1$ , with pulse amplitude,  $\Delta E$  for various values of  $n^{2}(22)$ 

amplitude. Therefore generally the pulse amplitude is kept in the range 20-100 mV.

#### Peak Potential - Half-Wave Potential Relationship

The expression relating peak potential to half-wave potential is

$$E_{peak} = E_{\frac{1}{2}} - \Delta E/2$$
 (1.16)

For infinitely small pulses the peak potential will occur at the polarographic half-wave potential. As the pulse amplitude increases, however, the peak potential will be shifted in an anodic direction for a cathodic wave.

## Capacity Currents at the DME

In analytical applications of pulse techniques the residual capacity current at the DME has to be taken into account which results from an increase in electrode area with time. The current is proportional to  $t^{-1}/\frac{3}{7}$ , where t is drop time, so the capacity

current is decreased by working at longer drop times. The capacity current is relatively insensitive to pulse width while the faradaic current is relatively insensitive to drop time. Therefore, the faradaic current is always enhanced relative to the capacitive current by working at narrower pulse widths and longer drop times. Optimally potential scan rates used should not produce changes of more than a few millivolts during the life of each drop. Therefore longer drop times require slower scan rates and lengthened analysis times. The tendency is to work at the shortest drop times and fastest scan rates possible in order to minimize the time<sup>(4)</sup>.

In NPP each pulse has greater amplitude than the previous one and during the course of the scan the pulses become quite large. This results in steeply sloping base-line which is the main limitation on detection limit. In DPP, each pulse has the same amplitude (50-100 mV) which tends to decrease the problem of the capacitive background current. Although the faradaic response decreases with decreasing pulse amplitude, in the intermediate range of pulse amplitudes in some solutions the capacitive current will decrease more so that detection limits are actually improved by decreasing the pulse amplitude. The lower capacity current in the DPP mode permits detection limits about ten times lower than NPP.

A further problem with the capacitive background is that minute amounts of surfactants can produce peaks in DPP at the potentials where they are adsorbed on the electrode surface  $(^{23})$ . These peaks are often hard to distinguish from those due to electroactive substances and are likely to occur in natural samples. Amperometric titration with DPP end-point detection is often a suitable alternative which avoids the background problem. Alternatively the supporting electrolyte may be changed to change both the capacitive current characteristics and the peak position for the species of interest.

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# LINEAR SWEEP VOLTAMMETRY AND RELATED TECHNIQUES

Historically voltammetric studies at solid electrodes were carried out even before the present century and it was not until the 1920's that the dropping mercury electrode was initiated. Significant strides in solid electrode voltammetry in the 1950's were made with the introduction of modern electroanalytical techniques.

Solid electrode techniques have played an increasing part in the recent interest of the chemical industry in organic oxidation-reduction processes. Applications of solid electrodes appear in studies of organic semiconductors, the rates of mechanisms of photochemical and radical ion reactions and electrochemiluminescence. In studying the mechanisms of electrode reactions, the use of stationary electrodes with a cyclic potential scan makes it possible to investigate the products of the electrode reaction and detect electroactive intermediates. Furthermore, the time scale for the method can be varied over an extremely wide range, and both relatively slow and fairly rapid reactions can be studied with a single technique.

The utility and applications of solid electrode systems together with the relevant theory are amply reviewed by Adams<sup>(24)</sup>. A definitive treatment of linear sweep voltammetry is given by Nicholson and Shain<sup>(25)</sup>. This study surveys both 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

The diffusion problem to a plane electrode for a reversible reaction was first solved independently by Randles  $\binom{26}{}$  and Seveik  $\binom{27}{}$ . The equation for the peak current is given as

$$i_{\rm D} \approx 2.687 \times 10^5 \, {\rm n}^{3/2} \, {\rm A} \, {\rm D_0^2} \, {\rm C_0V}$$

(1.17)

where  $i_p$  is peak current ( $\mu A$ );  $D_0$  is diffusion coefficient (cm<sup>2</sup> sec<sup>-1</sup>),  $C_0$  is bulk concentration (m mole lit<sup>-1</sup>); and V is the rate of change of potential in volts. sec<sup>-1</sup>; A is electrode area (cm<sup>2</sup>)

The peak current is proportional to concentration, and it also depends on the rate at which potential changes.

Matsuda and Ayabe<sup>(28)</sup> examined the relations in peak voltammetry for reversible, quasi-reversible, and totally irreversible systems. For the reversible case

$$|E_{\rm p} - E_{\rm p/2}| = 0.057/{\rm n} \text{ volts}$$
 (1.18)

where  $E_p$  and  $E_{p/2}$  are the peak potential and the potential at which the current is one-half the peak value, respectively. Both  $E_p$  and  $E_{p/2}$  are independent of the voltage sweep rate. The peak polarogram of a reversible system is sharp, spanning a voltage range of roughly 0.12V for a one-electron system. The corresponding equations for the totally irreversible system are given by

$$i_p = 2.985 \times 10^5 n (\alpha n_a)^{\frac{1}{2}} A D_0^{\frac{1}{2}} C_0^{\sqrt{2}} V^{\frac{1}{2}}$$
 (1.19)

and

$$|E_p - E_{p/2}| = 0.048/a n_a \text{ volts}$$
 (1.20)

where  $\alpha$  is the electron transfer coefficient and  $n_a$  is the number of electrons involved in the rate determining step. As  $(\alpha \ n_a)$  decreases the peak polarograms become more spread out and the peaks tend to be rounded. This situation is frequently met in organic oxidations. The peak current is significantly less than the reversible situation, falling rapidly for decreasing  $(\alpha \ n_a)$ .  $E_p$  and  $E_{p/2}$  for irreversible processes vary with potential sweep rate. This differentiates reversible from irreversible pro-

cesses, but the variation is only about  $0.03/\alpha$  n volt per tenfold change in sweep rate.

The major practical problem facing 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 is, however, severely limited in the anodic range due to oxidation of mercury at potentials above +0.4V vs. S.C.E.

## PULSE VOLTAMMETRY AT STATIONARY ELECTRODES

Normal pulse polarography can be used to advantage with solid electrodes. With reversible systems reoxidation occurs during the time between pulses so that the reductant is fully regenerated. For such systems, the same curves can be obtained if the electrode is in stirred solution or in a quiet solution. Since the diffusion layer during pulses is thinner than the shear layer in a stirred solution, stirring has essentially no effect on the voltammogram. However, in an unstirred solution and for a sufficiently irreversible system where a resting potential cannot be chosen at which oxidation takes place, a net depletion will occur during the very short pulses which will not be eliminated during the much longer waiting period between the pulses. The paper by Oldham and Parry $^{(17)}$ describes the depletion effect from both a theoretical and experimental point of view. Both stirring and proper choice of resting potential can eliminate to some extent this effect in the application of normal pulse polarography to irreversible systems.

In the differential pulse voltammetric mode the pulse amplitudes are smaller than those in normal pulse mode and therefore the residual

current is smaller. Although a constant amplitude is used in DPV, the residual current is not linear over the working range for every electrode investigated by Dieker, van der Linden and Poppe<sup>(29)</sup>. Large differences were observed between electrodes made from different grades of Tokai glassy carbon. Gold and platinum are not recommended, owing to the extremely high residual currents. A detailed analytical evaluation of differential pulse voltammetry has been reported by Rifkin and Evans<sup>(30)</sup>, using a platinum electrode and computer based instrumentation. Less than  $10^{-6}$ M of reversibly oxidised species could be determined in acetonitrile and about  $10^{-6}$ M concentrations of irreversibly oxidised species in the same solvent.

At a stationary electrode the time delay between pulses need not be as long as at a DME where this parameter is tied to the drop time. However, if the delay approaches the time of the pulse width, transient disturbances resulting from pulse application will not have time to decay, and a decrease in sensitivity due to noise could result. On the other hand, long delays increase measurement time.

#### CYCLIC VOLTAMMETRY

Cyclic Voltammetry, first practised by Sevcik<sup>(27)</sup>, is unmatched in its ability to provide qualitative information about the steps in a redox reaction with only a modest expenditure of time and effort in the acquisition and interpretation of data. It is usually performed by varying the potential of the working electrode linearly with time until a switching potential is reached at which time the direction of potential sweep is reversed and the potential is reversed to the original value. The current-potential curve produced is called a cyclic voltammogram. The characteristic peaks are caused by the formation of a depletion layer in solution near the electrode. The position of peaks on the potential axis is related in a simple manner to the formal potential of the redox

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process, and the height and shape give information about the reactant concentration and the number of electrons in the half-reaction.

The cyclic voltammograms of reversible, quasi-reversible and irreversible processes are shown in Figure 1.6. A rapid charge-transfer process has the anodic and cathodic peaks almost coinciding. The separation is predicted by the relation between  $E_p$  and  $E_1$ 

$$E_p = E_{\frac{1}{2}} - 0.029/n V$$
 (1.21)

Thus  $E_{p,c}$  for a reduction is 0.029/n V more cathodic than  $E_1$ and  $E_{p,a}$  for the oxidation of the same system is 0.029/nV more anodic than  $E_1$ . The potential difference between the peaks for a reversible system will be

$$E_{p,a} - E_{p,c} \simeq 2(0.029/n) = \frac{0.058}{n}$$
 (1.22)

A quasi-reversible system shows a greater separation in  $E_p$ 's. The polarograms are more drawn out and the peaks more rounded.

For a very slow charge-transfer process, a complete separation of anodic and cathodic peak potentials is observed.



FIGURE 1.6 Cyclic polarograms for (a) reversible; (b) quasireversible; and (c) irreversible processes

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R S Nicholson used the separation in anodic and cathodic peak potentials to measure rate constants for electron transfer<sup>(31)</sup>. The theory was extended to uncomplicated multistep reversible and irreversible charge transfers by Pdcyn and Shain<sup>(32)</sup>.

Although CV has advantages for studying charge-transfer rates, it is most ideally suited for investigating the overall processes which may occur in a complex electrode reaction. The advantages are best illustrated by examples of organic oxidations at solid electrodes.

Tentative identification of new redox systems formed at the electrode often can be made by running the CV of suspected products and matching potentials. This is hardly unequivocal with complex organic compounds, and further physical evidence is necessary for positive identification. The voltammetric investigation of the oxidation of phenols and identification of the intermediates using CV is illustrated by Evans<sup>(33)</sup>.

# HYDRODYNAMIC VOLTAMMETRY

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One of the most important features of faradaic electrochemistry is the ease by which it can be adapted to monitor flowing streams. The mass transfer to the electrode surface occurs by forced convection rather than solely by diffusion, and currentpotential 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. The sensitivity of hydrodynamic electrochemistry under steady state conditions is impressive and often superior to far more sophisticated small amplitude pulse and sine wave techniques<sup>(34)</sup>. Presently hydrodynamic techniques have achieved some importance in laboratory and commercial electrosynthesis; liquid and gas chromatographic detection; and determination of redox enzyme activity, substrates, and co-factors.

Theoretical treatment of hydrodynamic voltammetry with various electrode configurations have been carried out by  $Levich^{(35)}$ . Levich was one of the pioneers in this area. The principles of current distribution and mass transport in flowing systems have
been reviewed by Newman<sup>(36)</sup> and more recently by Pungor et al<sup><math>(37)</sup>.</sup>

The equations for limiting currents for some geometries are given below:

Electrode Geometry	Limiting Current
Tubular	$i_{L} = 0.67 \text{ nF } \text{CD}^{2/3} \text{ L}^{1/3} \text{ V}^{1/3} \text{ R}^{-1/3}$
Fixed disc	$i_{L} = 0.78 \text{ nF } \text{CD}^{2/3} \text{ V}^{1/2} \text{ R}^{-1/2} \text{ v}^{-1/6}$
Conical	$i_L = 0.8 \text{ nF } \text{CD}^{2/3} \text{ L}^{-1/2} \text{ V}^{1/2} \text{ v}^{-1/6}$
Rotating disc	$i_{L} = 0.62 \text{ nF } \text{CD}^{2/3}  \text{v}^{-1/6}  \text{\omega}^{1/2}$

L = length, R = radius of disc,  $\omega = 2\pi N$  = angular velocity of disc.

The wall-jet is probably 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 onto a wall placed perpendicular to it and flows radially over the surface. Glauert<sup>(38)</sup> gave the first rigorous treatment of this phenomena. Later Yamada and Matsuda<sup>(39)</sup> defined the diffusion current in terms of various hydrodynamic parameters

 $i_d = 1.376 \text{ nF } CD^{2/3} v^{-5/12} V^{3/4} a^{-1/2} R^{3/4}$  (1.23)

where v = kinematic viscosity, stoke;

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

a = diameter of circular nozzle, mm;

R = radius of disc electrode, mm.

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 case the dependence is to the power 3/4 which is a distinct advantage over other cell geometries.

#### ELECTRODE MATERIALS

A number of electrode materials have been proposed for voltammetry and this subject is amply reviewed by  $Adams^{(24)}$ . None of the solid materials proposed is as good as the dropping mercury electrode, whose main advantage is that of a continually renewable surface. In this section greater emphasis is laid on the developments in electrode materials subsequent to Adam's review.

### Mercury Electrodes

The mercury electrode has the advantages which no solid electrode has but its range of applications is restricted on the anodic side. Further mercury electrodes have not found favour in monitoring flow streams because of the difficulty in maintaining drops in a flow stream and the resulting instability.

## Noble Metal Electrodes

The main emphasis has been on the platinum electrode, which has a large oxygen overpotential, but its use in the cathodic side is limited owing to its negligible hydrogen overpotential. It suffers from adsorption problems especially from hydrogen.

Gold has been used less extensively probably due to difficulties in sealing it in glass. It has a better cathodic operating range than platinum. In acid chloride media the anodic range is severely limited by oxidation of the metal to complex chlorides. Its anodic range is otherwise limited due to interference by surface oxide formation. Its advantage is that it does not absorb hydrogen.

In spite of the many problems associated with these materials there is often reference to tubular platinum and gold-mesh electrodes in the HDV field in literature.

## Carbon Electrodes

The developments in the chemistry of solid state carbon have contributed to the evaluation of different forms as electrode materials. The properties of polymeric carbons have been reviewed by Jenkins and Kawamura<sup>(40)</sup>. It is now possible to make different forms of carbon with desired properties. Some of the more useful forms which have a potential for application as electrode materials in voltammetry are reviewed.

#### Graphite

Graphite was the first carbon electrode to be studied. Although it has an anodic range of ca. +1.0V its cathodic range is limited and it suffers from problems of seepage of electrolyte and adsorption. Its utility can be extended by several different methods of fabrication (41).

## Wax Impregnated Graphite

The WIGE's show lower residual currents than the graphite rod electrodes but slight variations may result from impregnation procedures. Elving and Smith<sup>(42)</sup> pretreated the electrode surface with 0.003% solution of Triton X-100. This wetting agent assures a completely wetted surface and thereby increases reproducibility of surface area in test solution. This type of electrode has a range +1.2 to -1.2V vs. S.C.E. in both neutral and acidic media.

## Pyrolytic Graphite

The pyrolytic graphite electrode was introduced by Beilby et al<sup>(43)</sup> and Miller and Zittel<sup>(44)</sup>. The useful range is from +1.0 to -0.8V vs. S.C.E. in acidic chloride or nitrate media and a more restricted range in potassium chloride-hydrochloric acid media.

#### Polyethylene Graphite

This electrode is fabricated by mixing graphite with finely powdered polyethylene and heat sealing the mixture to the end of a rigid polyethylene tube $(^{45})$ . Preliminary investigations show the electrode to be as good as vitreous carbon and pyrolytic graphite.

## Silicone Rubber Based Graphite

Pungor et al<sup>(46)</sup> reported the use of the electrode in both static and flowing solutions. The operating range lies between -0.5 to +1.5V vs. S.C.E. Memory effects could result on account of surface coverage by a metal coating or by deposition of a non-conducting polymer.

#### Carbon Paste

Carbon paste electrodes are made by mixing powdered graphite with an organic liquid such as Nujol to a fairly thick consistency (47,48). The paste is then packed in a depression in a holder. A platinum or copper wire in the bottom of the depression makes electrical contact.

Carbon paste electrodes have a wide range of anodic and cathodic utility. Owing to an extremely low residual current the entire anodic range is available for study. In the cathodic range oxygen reduction leaves a residual wave which is difficult to get rid of. The carbon paste electrodes afford a renovation of the surface but use in HDV should be undertaken with due care so as not to disturb the surface.

#### Glassy Carbon

In 1962, starting from phenolic resins, Yamada and Sato<sup>(49)</sup> prepared a gas-impermeable carbon called glassy carbon. The glassy carbon has interesting physical properties in comparison with other carbons. Furthermore, it exhibits a much lower oxidation rate at elevated temperatures, suggesting a greater inertness to chemical attack than other types of carbons. This property, together with the very small pore-size makes glassy carbon as an attractive material for the preparation of inert electrodes.

Glassy carbon structure has been extensively studied by Jenkins and Kawamura<sup>(40)</sup>. They concluded that glassy carbon is made up from aromatic ribbon molecules which are oriented randomly and are tangled in a complicated manner. Ordinary low resolution transmission electron micrographs show the edges consist of a network arrangement of strings or microfibrils, the thickness of each being about 30 Å. Each string is considered to be a stack of graphite-like ribbon molecules. The thickness of each string is considered to  $L_c$ , determined by X-ray diffraction. The length of relatively straight parts of the molecules is estimated to be 100 Å, which is comparable to  $L_a$ calculated from X-ray diffraction studies.



FIGURE 1.7 Schematic structure model for glassy carbon.(40)

Orientation is random and fracture occurs at the interfibrillar boundary. No "loose ends" are apparent; this explains the charac-

teristic chemical inertness of glassy carbon. In the interior of the fragment the material retains its essential randomness and microfibril continuity.

Van Der Linden and Dieker<sup>(50)</sup> have recently reviewed the utility of glassy carbon as electrode material in electroanalytical chemistry. The glassy carbon electrode is suitable for use over the potential range from +1.2 to -0.8V vs S.C.E. in aqueous media. In basic media, the cathodic limit may be extended to about -1.6V vs. S.C.E. The use of freshly prepared 0.1M solution of tetrabutylammonium-hexafluorophosphate ( $Bu_4$  NPF<sub>6</sub>) in sulpholane has provided an extended anodic potential of +2.7V vs S.C.E. The cathodic range has been satisfactorily extended by plating in-situ a thin mercury film on to the glassy carbon surface.

# Carbon Fibres

Recently a report described the use of carbon fibres (8  $\mu$ m diameter), as voltammetric electrodes<sup>(51)</sup>. The surface of the fibre perpendicular to the fibre axis is ideal for voltammetric applications. Carbon fibres are formed by the high temperature pyrolysis of polymeric materials such as polyacrylonitrile or pitch, while plastically stretching the polymeric carbon material. The structure of these fibres appears to be similar to that of glassy carbon.

Electrochemical measurements with carbon fibre electrodes have been diverse in scope but limited in number. Potentialities exist for in-vivo measurements as damage to tissue is minimal. Dayton et al<sup>(52)</sup> discuss the advantages these microelectrodes have over conventional sized electrodes.

#### Reticulated Vitreous Carbon

The 1979 volume of Analytical Chemistry marked the opening of the age of RVC. It is a relatively impervious form of carbon (like glassy carbon) which is formed in a rigid three dimensional honeycomb-like structure and the matrix having 100 pores per inch appears to be most suitable for electrochemical studies. RVC has a

low microscopic surface area compared with conventional porous graphite; the matrix is rigid, extremely inexpensive (compared to glassy carbon), and it is quite easily machined and mounted in various cell designs. The RVC material is extremely useful for electroanalytical and electrosynthetic work<sup>(53)</sup>.

One of the great remaining problems of analytical electrochemistry is the fact that potential changes at carbon electrodes are accompanied by alteration of surface chemistry as well as traditional double-layer charging. The latter nonfaradaic event can be made to occur very rapidly, but changing the redox states of surface functional groups results in faradaic currents which can take minutes to settle down. Unfortunately, the faradaic currents can be orders of magnitude larger than the accompanying double-layer charging at even moderately fast potential scan rates. The result is that stationary electrode voltammetry at bare carbon is never very useful for trace analysis. The slow faradaic surface reactions makes it difficult to reproduce the surface state prior to initiating a scan.

## CHAPTER 2

# ADSORPTION ON THE DROPPING MERCURY ELECTRODE AND ITS INFLUENCE ON ELECTRODE PROCESSES

#### INTRODUCTION

Mairanovskii(54) has given an excellent treatment of adsorption at the dropping mercury electrode and the present discussion is largely based on this review.

The adsorption on a phase boundary for simple compounds is given by the Langmuir adsorption isotherm

$$\Gamma = \Gamma_{\infty} \frac{\beta \zeta}{1 + \beta \zeta}$$
(2.1)

where  $\Gamma$  is the number of moles of adsorbed compound on 1 cm<sup>2</sup> of mercury-solution boundary surface;  $\Gamma_{\infty}$  is the quantity of adsorbed compound if the surface is completely covered by a monomolecular layer; c is the concentration of the adsorbed compound in the bulk of solution (M); and  $\beta$  is the adsorption coefficient ( $m^{-1}$ ) given as

$$1000\beta = \delta e^{W/RT}$$
(2.2)

where  $\delta$  is the thickness of surface layer (adsorbed particles), W is the adsorption work that must be done to remove one mole of compound from the interface of mercury and solution.

The surface fraction  $\theta$  covered by adsorbed particles is

$$\theta = \frac{\Gamma}{\Gamma_{\infty}} = \frac{\beta \zeta}{\Gamma + \beta \zeta}$$

(2.3)

Langmuir's equation does not consider the interaction of adsorbed molecules. Frumkin<sup>(55)</sup> showed that particularly for large organic molecules containing polar groups, after a certain degree of coverage of the electrode surface by adsorbed molecules, their interaction promotes further adsorption, and the surface coverage increases faster than would be expected by the Langmuir equation (2.3). The equation derived by Frumkin known as Frumkin adsorption isotherm, is stated as

 $\beta c = \frac{\theta}{1-\theta} e^{-2r\theta}$ 

2.4

where r is the attraction factor.

The isotherm expresses the  $\theta = f(c)$  relationship. The isotherm has an S-shape; the rising part increases for larger values of r. At low degrees of coverage (low  $\theta$  values) the  $e^{-2r\theta}$  term is close to unity, and the Frumkin isotherm may be replaced by Henry's linear law (equation 2.5) or the Langmuir adsorption isotherm (equations 2.1 or 2.3).

$$\theta = \beta C$$
 (2.5

The Frumkin adsorption isotherm is not only valid for attraction of adsorbed particles, but also for their repulsion. In the latter case, the r factor has a negative value. Several authors (56-58)have proposed different isotherm equations based on different assumptions. Parsons (59) has given a short analysis of these equations. Methods of fitting isotherms to experimental results are discussed. An isotherm developed from the equation of state of a two-dimensional hard sphere gas is suggested. The predictions of this isotherm are compared with those of previous isotherms.

Gouy (60) found that the decrease of surface tension of mercury due to adsorption or organic molecules on the surface, compared

with the surface tension of solution not containing this compound, takes place only in certain potential ranges which includes the potential of zero charge of the electrode in the supporting electrolyte.

Maximum adsorption is usually observed at potentials close to the potential of zero charge of mercury, and, if the potential is shifted in either direction, the adsorption of organic molecules on the electrode decreases. Thus the electrocapillary curves for solutions containing organic compounds and those without them overlap at certain potentials.

The adsorption of organic molecules, which usually have larger dimensions than the water molecules and usually a much lower dielectric constant, causes a reduction of the capacity of the double layer. The double layer can be considered to be a condenser, one plate of which is formed by the charged mercury surface, the other by counter ions of opposite charge attracted from the solution by electrostatic forces. If the potential difference increases the exchange of adsorbed molecules by water molecules becomes energetically favoured and the capacity of the double layer increases. The quantity of organic material remaining in the adsorbed state at a given potential is thus determined by the energy balance, the energy required for the desorption of a part of the organic molecules, and the energy gained due to the increased capacity of the double layer.

In the adsorption of charged particles the potential of maximum adsorption  $(E_m)$  can be substantially different from the potential of zero charge in the solution not containing the adsorbing ions. When the adsorption of anions takes places, the  $E_m$  value shifts towards positive potentials from the zero charge potential in the original solution (free of adsorbing ions); when cations are adsorbed,  $E_m$  shifts to negative potentials.

#### FACTORS CONTROLLING THE EXTENT OF FILM FORMATION

Surface coverage as a function of time depends on the adsorption coefficient, the diffusion coefficient and the concentration of surface active material. The adsorption coefficient and the adsorption rate are potential dependent. The treatment in this section is based on the paper by Schmid and Reilley<sup>(61)</sup>.

#### Diffusion Controlled Film Formation

In the initial period of drop life, the amount of surface active material which has reached the surface by diffusion is insufficient to retard the electrode reaction. Later in the drop life sufficient surface active material reaches the electrode and is adsorbed on the expanding drop surface to produce a significant deceleration of the electrode reaction rate. The current thus decreases and eventually reaches zero when the drop is completely covered with the surface active material. The higher the concentration of the surface active material, the shorter is the time required to cover the drop. This is illustrated in Figure 2.1.



FIGURE 2.1 Current-time curves for a single drop  $(t \sim 6 \text{ sec})$  Concentration of surface active material is zero for a and increases from b to e (61).

The kinetics of film formation on a mercury drop have been derived with the help of Ilkovic equation taking diffusion as the rate controlling step<sup>(62)</sup>, this assumes that adsorption equilibrium is reached very rapidly and the adsorption coefficient is very large. The time v, at which the surface is completely covered, is given by

2.6

$$v = 1.82 \times 10^6 \frac{r_s^2}{DC^2}$$

where  $\Gamma_s$  is the number of moles. cm<sup>-2</sup> of mercury surface at surface saturation, D is the diffusion coefficient of the surface active agent, and C is the bulk concentration of surface active agent in moles. litre<sup>-1</sup>. Thus, the time required to cover the surface completely with adsorbed material on a fresh mercury drop is inversely proportional to the square of the bulk concentration of the surface active agent.

## Surface Coverage Limited by Adsorption Equilibrium

With weaker surface active substances thermodynamic adsorption equilibrium does not essentially allow complete coverage of the surface. The current-time curves for this case are shown in Figure 2.2.



FIGURE 2.2 Surface coverage controlled by adsorption equilibrium. Drop time  $\sim 6$  sec, a = zero conc., b  $\rightarrow$  e increasing concentration of surface active agent(61).

Practically from the instant of its formation the mercury drop will be partially covered with an equilibrium concentration of surface active material. The fraction of the surface covered is the same throughout the life of the drop and this fraction increases as the concentration of surface active material in the bulk phase increases. The curves correspond to various points in the Langmuir isotherm.

# ADSORPTION OF DEPOLARISER IN REVERSIBLE REDOX SYSTEM: ADSORPTION PREWAVES AND POST WAVES

During the polarographic investigation of certain reversible depolarisers small additional steps can be observed, independent of depolariser concentration, preceding the normal polarographic wave. Brdička and Knobloch<sup>(63)</sup> first observed such a wave on the polarograms of riboflavin. Brdička also found a similar wave on the polarogram of methylene blue<sup>(64)</sup> and assumed that the appearance of such waves is caused by adsorption phenomena.

If the adsorption of one of the components of the redox system is much larger than the adsorption of the other, then according to Brdička<sup>(65)</sup> the adsorption energy changes the potential at which the electrode process takes place. If the product of the electrode reaction is adsorbed, the liberated adsorption energy promotes the electron transfer in the forward electrochemical reaction. This means that the electrochemical process takes place at a more positive potential and on the polarogram an adsorption prewave appears. If the starting material is strongly adsorbed, then an adsorption postwave is observed.

At low concentrations of the depolariser in the solutions only a single wave appears on the polarograms at potentials where at higher concentrations the prewave is observed. The height of this prewave increases in proportion to the concentration of the compound that is being reduced. With increasing concentration of the depolariser the prewave height increases and reaches a limit. Usually, the main reduction wave begins to increase only after this

has occurred. The sum of limiting currents of both waves will be proportional to the concentration of compounds being reduced in the solution.

With increasing temperature the adsorptivity of materials decreases, therefore, at sufficiently high temperatures, the adsorption prewaves disappear. Furthermore, in presence of strongly adsorbed compounds that displace the depolariser from the electrode surface, a decrease of adsorption wave is observed.

# ADSORPTION OF COMPOUNDS NOT TAKING PART DIRECTLY IN THE ELECTRODE PROCESS

Usually, the adsorption of compounds not taking part directly in the electrochemical reactions decreases the rate of these reactions. Surface active substances that affect the electrode processes are usually classified according to their charge, as non-ionic, cationic or anionic. Common non-ionic adsorptive compounds are camphor, thymol, higher alcohols and higher hydrocarbons. Tetralkylammonium and Tribenzylammonium cations have been investigated as cationic substances. The anions of higher fatty acids and of sulphonic acids with long alkyl chains act as surface active anions. The effects of adsorption of these substances vary widely ranging from the shift of the polarographic wave, decrease of limiting current, appearance of minima, splitting of a wave to its complete elimination.

The effects of adsorption of non-reducible or non-oxidisable foreign substances on kinetic parameters for electrode reactions and limiting currents are discussed by several authors  $(^{66-68})$ . Various mathematical models have been proposed. These models explain some aspects satisfactorily but at times they are in conflict with experimental data.

Loshkarev and Kryuxova<sup>(69)</sup> explained the inhibition of electrode processes in the presence of adsorbed compounds by the difficulty in passage of the depolariser through the adsorbed layer to the electrode surface. They showed that the layer of

adsorbed material inhibits the electrode process of reduction of metal ions more if the atomic weight of the ion is smaller and its charge higher, or if the field intensity of the discharging ion is larger.

The potential interval in which the inhibiting effect of additives can be observed is determined by their adsorption region. When the desorption potential is reached the inhibiting effect ceases. In addition to the inhibiting effect, in the adsorption region of surface active compounds, the tangential movement of the mercury surface decreases. Therefore, the supplementary current caused by convection of the solution decreases. Beyond the desorption potential the tangential flow recovers resulting in increased current.

## CATION ADSORPTION

 $Frumkin^{(70)}$  has discussed the influence of cation adsorption on the kinetics of electrode processes. Earlier work had shown that the presence of cations in solution influenced the hydrogen overvoltage on mercury(71). This phenomenon influences the rate of anion or cation reduction. The slowing down of reactions with the participation of cations is specially great in the presence of organic cations, for instance  $N(C_4 H_9)_4^+$ , which are specifically adsorbed. The increase in the rate of anion electroreduction was explored for anions of different configurations. The reduction of flat configuration anions shows a much smaller sensitivity towards cations than the reduction of three-dimensional configuration anions. The half-wave potential of the anions is shifted to more positive values when the charge and concentration of the non-reducible cations in the solution increases and is explained by the formation of ion pairs which facilitate the approach of the anion to the " negatively charged electrode surface. The polarization of the anion by the electric field of the cation also enhances its reactivity. It follows that the rate determining step may be the electron transfer to the anions linked with the surface by cationic bridges as well as the formation of these bridges within the double layer,

which necessitates the penetration of the anion through its electric field.

The inhibiting effect of different compounds can depend on the nature of the inhibited electrochemical reaction. The relative inhibition effect often changes due to the specific interaction between the adsorbed compound and the depolariser. Furthermore, the relative inhibition changes because of the different adsorptivity of surface active compounds at different potentials. For adsorption of compounds of similar structure, their inhibiting effect on the same electrochemical process increases for compounds having a higher surface activity. As an example, the shift of the first reduction wave of the methyl ester of p-chlorophenylpropionic acid can be cited (72). This shift can be observed on replacement of a potassium chloride solution by tetrasubstituted ammonium salts. At a sufficiently high concentration of the tetrasubstituted salt, the first and second waves on the polarogram merge although in the absence of surface active agents the half-wave potentials of the two waves differ by about 0.5V. Increasing the chain length of the tetrasubstituted ammonium cation also increases the surface activity of the radical. This study raises serious doubts on the validity of the common practice of employing salts of these ions as supporting electrolytes.

Pietrzyk and Rogers<sup>(73)</sup> investigated the effect of dodecyltrimethylammonium chloride on the polarographic reduction of aromatic nitro compounds. The application of anodic and cathodic shifts to analysis of mixtures is discussed.

## CHAPTER 3

## ANALYTICAL CHEMISTRY OF SYNTHETIC FOOD COLOURS

#### INTRODUCTION

In recent years there has been a considerable increase in the use of additives in the food manufacturing industry. Additives are needed to preserve food, improve storage, or make wider distribution possible, to achieve some degree of uniformity for large scale production, to improve flavour, and to improve the texture and appearance of the product so as to make it more acceptable to the consumer. The use of food additives is governed by certain principles such as technological effectiveness, safetyin-use, and restriction of use to the practical minimum.

Food colours of both natural and synthetic origin are widely used in processed foods and play a major role in increasing their acceptability and attractiveness. Colours are added to foods for a number of reasons:

- To replace natural colours where they have been destroyed by heat processing and subsequent storage; or bleached out by use of preservatives; or are not light stable during prolonged storage.
- 2. To ensure uniformity of product from batch to batch, where raw materials of varying colour intensity have to be used.
- 3. To reinforce the colours of natural ingredients when these are too weak and the consumer associates stronger colour with food of a particular flavour.
- 4. To give an attractive appearance to foods which would otherwise look unattractive or unappetising.
- 5. Among other factors it is believed that the enjoyment of food depends on 'eye appeal'.

The use of food colourings or rather their omission can have

a significant effect on consumer demand as was evidenced in the case of a leading firm of retailers in the U.K. who deliberately left out synthetic colours in certain foods as a matter of policy. The consumer demand fell by about 50% as people became critical of the unattractive appearance of the products. The firm restored the colours at a lower level but it took about two years to regain previous sales levels.

The Colouring Matter in Food Regulations 1973, made under the Food and Drugs Act 1955, control the sale, consignment, delivery or importation of colouring matter for use as an ingredient in the preparation of foods and foods containing colouring matter in the U.K. The principal provisions of the present regulations are:

- a) the definitions of permitted colouring matter, diluent and permitted diluent;
- b) a prohibition of food having in it or on it any colouring matter other than a permitted one;
- c) general prohibition of the addition of any colouring matter, for purposes other than marking, to raw or unprocessed meat, game, poultry, fish, fruit or vegetables; or to tea (whether in leaf or essence form), coffee and coffee products, condensed and dried milk;
- d) the prohibition of the sale and advertisement for sale of any non-permitted colouring matter as an ingredient in food;
- e) the list of permitted colouring matters and both specific and general purity criteria for these colouring matters.
- f) the list of permitted diluents for colouring matters together with their general and, in some cases, specific purity criteria;
- g) the list of colouring matters permitted only in certain foods and the foods in which they may be present, and
- h) the labelling requirements of permitted colouring matters and permitted diluents, when sold as such.

The Committee  $(^{74})$  has indicated that it is not convinced that the presence of added colouring is of any benefit to foods specially prepared for infants and young children or that it affects their acceptance of the foods, though it may well, make them more attractive to those who buy them and so have an influence on their choice. The Committee has recommended therefore that the use of added colourings in infant foods be prohibited.

### PRESENT STATUS OF COLOURING MATTER REGULATIONS

(For structural formulae see pgs 61-64) The Regulations made in 1973 contained a permitted list of some 45 natural and artificial colours or groups of colours<sup>(75)</sup>. Eight of these colours, namely, Yellow 2G, Red 2G. Orange G, Orange RN, Brilliant Blue FCF, Brown FK, Chocolate Brown HT and Chocolate Brown FB were not included in the EEC Directive on Colouring Matters, but the U.K. was permitted to continue to authorise their use until the end of 1977. The colours Chocolate Brown FB and Orange G are to be deleted from the U.K. permitted list as a result of the sixth amendment of the EEC colour directive.

The committee on toxicity<sup>(74)</sup> has classified the colours into four groups. Those for which the available evidence suggests acceptability fall in Group A; those for which the available evidence suggests provisional acceptance subject to review within a specified period of further information (Group B); those for which available evidence is inadequate to express an opinion (Group E); and those for which no evidence on toxicity is available (Group F).

The committee recommends that only those colours classified in Groups A and B should be permitted in any revised regulations and that the use of Group B colours be further reviewed within five years. The following is the list of colours approved by the committee:

#### Group A

Brilliant Blue FCF, Indigo Carmine, Sunset Yellow FCF, Tartrazine.

#### Group B

Amaranth, Black PN, Brown FK, Carmoisine, Chocolate Brown HT, Erythrosine BS, Green S, Ponceau 4R, Quinoline Yellow, Red 2G and Yellow 2G.

Patent Blue V has been classified in Group E and though it was permitted by the 1973 regulations, the present committee recommends that its use should not be included in any revised permitted list.

In conclusion, the committee recognizes that colour plays a significant part in our enjoyment of food, as, indeed, it does in our enjoyment of many other facets of life. The addition of colouring matter affects neither the nutritional value nor the quality of a food. If the consumers are to continue to have an adequate and varied diet, attractively presented, the responsible use of colouring matter, the safety-in-use of which has been fully evaluated, still has a valid part to play in the food industry. It recommends therefore that the colouring matter in food should continue to be permitted.

#### ANALYTICAL CHEMISTRY

Walford, in a recent publication, has reviewed the developments in natural and synthetic colours<sup>(76)</sup>. The analytical chemistry of synthetic dyes is dealt with in texts by Venkataraman, Macek and Pearson<sup>(77-79)</sup>. Venkataraman discusses the analytical chemistry of the dyes under different application groups and under different analytical methods. Venkataraman's text is authoritative and Pearson deals with the subject in one section only. Both texts cover dye specification and intermediate analysis mainly.

#### THIN LAYER AND PAPER CHROMATOGRAPHIC DETERMINATION

The most common technique adopted in the identification of food colours is paper or thin layer chromatography. Spectrophotometric quantification can be applied after thin layer chromatographic separation. Absorption curves for twenty-eight water soluble permitted colours are given in the Food Regulations 1957. These were obtained using a Unicam SP500 spectrophotometer in acidic, alkaline and neutral buffer media<sup>(80)</sup>. Honkawa<sup>(81)</sup> determined food colour mixtures with a function generator and dual-wavelength spectrophotometer. Pearson<sup>(82)</sup> using paper chromatography gave R<sub>f</sub> values of colours included in the EEC directive with respect to 12 solvents. In another publication<sup>(83)</sup> he gave the R<sub>f</sub> values of water soluble colours permitted in U.K. and EEC after TLC separation on standardised plates coated with silica gel and using 10 solvent systems. Tewari et al<sup>(84)</sup> have applied thin-layer electrophoresis on silica gel-G plates to the separation and identification of 10 dyestuffs commonly used in liquors and beverages. The technique was found to be particularly useful for distinguishing fake from genuine samples.

Several TLC separations of water-soluble food dyes on silica gel layers have been published and the literature has been reviewed by Schweppe<sup>(85)</sup>. For TLC cellulose<sup>(86,87)</sup>, silica gel<sup>(88,89)</sup> and polyamide<sup>(90-92)</sup> have been used as adsorbents. As water soluble food dyes can be separated only with polar solvents, the use of several of these as eluting agents, particularly Dimethylsulphoxide, has been investigated for the separation of water-soluble dyes permitted in the six original EEC countries<sup>(93)</sup>. Separations of dyes on silica gel are usually not very good owing to trailing and streaking. DMSO improves the separations. Separation of water-soluble food dyes as their copper complexes removed trailing effect for three dyes.

Hoodless et al<sup>(94)</sup> discuss a thin layer chromatographic method for the separation and identification of 49 synthetic food colours.  $R_f$  and  $R_\chi$  (with respect to Orange G) values are tabulated and a scheme for the rapid identification of the components of a mixture of dyes is proposed by TLC on cellulose powder or silica gel G plates.

Gilhooley et al<sup>(95)</sup> developed a method of extraction of synthetic water-soluble food colours using polyamide columns. Quantitative determination of food colours was applied to jellies, jams, sweets, cakes, canned meats and sausages.

Takeshita et al<sup>(96)</sup> describe a method in which 20 water soluble acid dyes, isolated from food products by means of DEAE-sephadex, were detected by TLC using polyamide coated plates. This is a simple and rapid method for the detection of acid dyes in cellulosic and proteinaceous samples.

Lehmann et al<sup>(97)</sup> developed a method for the rapid detection of colouring matters in foods. In this method acid dyes from the substrate of foods are dissolved in ammonical alcohol, followed by acidification and adsorption of the dyes onto polyamide powder. Protein containing foods are treated with acetone to remove fat and water and to coagulate soluble protein. Elution through a chromatographic column filled with polyamide powder elutes the water soluble acid dyes. Since the colours are eluted at room temperature, they are not expected to undergo irreversible changes. The procedure does not have the disadvantages inherent in the wool dyeing technique<sup>(80)</sup>,

The particular advantage of the method is that it is less time consuming than other methods. Only a small amount of polyamide (0.5 - 1.0g) is required and it can be formed into a microchromatographic tube, Such columns do not 'run dry'. Moreover, the analyst can prepare polyamide powder from coarse commercially available material, and the used powder can be regenerated by washing with acetic acid and distilled water. However, Lehmann's method requires a large quantity of alkaline methanol and a concentration step before TLC. Difficulties may be expected when preparations contain compounds with phenolic hydroxyl groups. These are also adsorbed by polyamide and desorbed by the alkaline solution. Arrow-shaped spots are then present in chromatograms. Interferences of this sort can largely be suppressed by repeating the adsorption-desorption procedure.

Polyamide is able to form relatively stable hydrogen bonds with acidic and, in particular, phenolic groups. It possesses a high adsorption capacity and its surface can be modified at will. It is therefore more suitable than wool threads for adsorbing acid dyes from extremely dilute solutions. Another advantage is that

polyamide powder preferentially adsorbs the synthetic colouring matters from aqueous-alcoholic dye solutions which contain an excess of anthocyanins and other plant constituents and only afterwards fixes other adsorbable contaminants on any active centres still available.

Various other procedures have been suggested for the extraction and purification of food colours, including solvent extraction<sup>(98)</sup>, use of liquid ion exchangers<sup>(99)</sup>, and extraction with quinoline<sup>(100)</sup> and quaternary ammonium compounds<sup>(101,102)</sup>.

# HPLC DETERMINATION OF FOOD COLOURS

Qualitative techniques employed for the separation and analysis of dyes and dye components utilize the classical chromatographic methods of paper, thin layer and column chromatography. Quantitative analyses with these techniques, however, are usually either time consuming or prone to large deviation and errors. Gas chromatography has been successfully applied to some anthraquinone dyes and intermediates <sup>(103)</sup> but has a limited application to dyes in general because these compounds have high molecular weights, low vapour pressures and limited thermal stability at high temperatures.

The combined developments of small diameter porous packings, pumps capable of attaining high pressures (up to 5000 psi), and detectors with sufficient sensitivity makes high pressure liquid chromatography an attractive technique for carrying out rapid and precise quantitative analysis of dyes. Louis J Papa<sup>(104)</sup> has reviewed some of the applications of HPLC to analysis of classes of dyes.

Passarelli and Jacobs(105) analysed azo, anthraquinone and sulphonated dyes using HPLC, adsorption and ion exchange chromatographic methods with U.V. detection which gave precision and sensitivity equivalent to that obtained by gas chromatography using thermal conductivity detectors.

Knox and Laird<sup>(106)</sup> introduced a new form of high performance ion-pair chromatography in which a detergent (cetyltrimethylammonium bromide) is added at around 1% level to a propanolwater eluent. The column packing may either be a reversed-phase material (e.g. SAS silica) or a silica gel (e.g. Partisil). The method allows high resolution separations of a wide range of sulphonic acids and derived dyestuffs containing one to three  $-SO_3H$  groups. The degree of retention can be varied by changing cetrimide concentration, acidity of eluent, concentration and nature of additives.

In the reversed-phase system it is probable that cetrimidesulphonate ion-pairs are extracted from the water-rich eluent into an adsorbed layer rich in propanol and cetrimide. In the silica gel system ion pairs are probably present in the eluent phase and are adsorbed onto the silica gel surface with propanol acting as a protective agent for both ion pairs and surface. The new technique has been termed 'Soap Chromatography'. This technique is remarkably effective for the resolution of sulphonic acids of interest in the dyestuff industry. Separations can be carried out either on bonded reversed-phase materials or on adsorbents. The chromatographic efficiency is similar in both cases and equivalent in terms of plate height to that obtainable in high performance adsorption chromatography.

Ion-Pair High Performance Liquid Chromatography has been extensively reviewed by Tomlinson et al(107). The constitutional, environmental and operational factors which can effect solute retention in ion-pairing systems are discussed. A comprehensive list of pairing ions currently in use along with the phase systems and solutes examined.

HPLC has been used in a number of ways for dye analysis, mostly for the detection of impurities in single dyes (108,109)but also to a limited extent for the separation of dye mixtures. Anion-exchange columns have been used for this purpose and frequently gradient elution has been specified. Separation of seven permitted food dyes using a strong anion-exchange column

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was achieved using HPLC<sup>(110)</sup>. Chudy et al<sup>(111)</sup> investigated the chromatographic behaviour of food dyes in order to establish a scheme involving extraction of the dyes from the food matrix followed by qualitative and quantitative analysis of the extract by HPLC. Practically all common food dyes were separated on a SAS-Hypersil column except those for which both the HPLC peaks and spectral absorption overlap. For such dyes a prior separation is desirable. The limit of detection was 10  $\mu$ g dye per ml of solution used for injection. The HPLC procedure described can also be used to monitor the behaviour of dyes during various stages of food processing or to follow their degradation.

The use of enzymes as releasing agents for food colours from food constituents to ensure complete extraction has been proposed by Boley et al<sup>(112)</sup>. Samples of kippers, plain cake and sponge cake were analysed both with and without an enzymatic digestion. Results showed enhanced recovery of certain colours following enzymatic treatment. Further work has been done in this direction<sup>(113)</sup> and a method is proposed for the extraction, separation, identification and quantitative measurement of synthetic organic colouring materials in a wide range of foodstuffs. The colours were extracted with a liquid anion-exchange resin (Amberlite LA-2 in butan-1-01), and where irreversible binding of the colours to the food has occurred during processing, the colours were released by a preliminary enzyme digestion prior to extraction. The colours were then re-extracted from the resin phase into aqueous solution, followed by clean-up and concentration by column chromatography on polyamide. The final eluate was concentrated for examination by HPLC. For liquid foods and water soluble foods a simpler version of the method was employed. On the whole the method was found to have a limit of detection of  $1 \text{ mg kg}^{-1}$  and recovery values for most colours of 80% or better were achieved. The reproducibility was within 5% and further improvement is possible with a small fixedvolume loop injector. Practical difficulties arose with individual colours, such as, Brown FK, Erythrosine BS, Green S, Amaranth and Indigo Carmine but these were conveniently overcome by some modifications in the technique.

High-Pressure liquid chromatograms for the synthetic organic food colours are given in Appendix I of the colouring matter committee review<sup>(74)</sup>. The chromatograms are idealised in order to indicate the relative elution order of the intermediate organic substances using a defined separation system. A UV detector operating at 254 nm wavelength was used. The column packing is Pellicular strong anion exchange (SAX) and the solvent system is Primary - 0.01M sodium tetraborate, Secondary - 0.01M sodium tetraborate/sodium perchlorate. Alternate experimental conditions would result in variations in elution order and resolution.

# ELECTROANALYSIS OF FOOD COLOURS

Yoo <sup>(114)</sup>determined acid and basic dyes and food colours by amperometric titrations, potentiometry using ion selective electrodes, and differential pulse polarography. The electroanalytical determination of dyes is also reviewed.

Amaranth has been studied polarographically over a pH range of 2-10 in aqueous solution and was found to give a well-defined diffusion controlled wave suitable for quantitative work<sup>(115)</sup>. Irreversibility of reduction was indicated by the behaviour of half-wave potential to changes in pH and concentration. With pH a direct relationship of -0.08V per pH unit held between pH 2-7, while with concentration, the half-wave potential was found to apparently vary directly with the logarithm of concentration.

Two likely possibilities of reduction exist: (a) the azo group accepts two hydrogen atoms to form a hydrazo group, and (b) the azo group accepts four hydrogen atoms to yield the corresponding amines.

(a)



OR



The value of n calculated for the reaction was 4 from the diffusion coefficient obtained from conductance measurements, which corresponds to the rupture of the azo group to yield the corresponding amines. The reaction forms the basis of the quantitative determination of amaranth and similar azo colours by Ti (111) reduction<sup>(116)</sup>.

The polarographic reversibility of the Azobenzene-Hydrazobenzene system has been investigated by Nygård<sup>(117)</sup>. It is found that the concentration of the depolariser is an important factor in the reversibility of the system. Thus, the corresponding halfwave potentials for the oxidized and reduced forms at the same pH values approach one another at low concentrations. In strong acid and alkaline solutions, the half-wave potentials for both forms seem to be independent of depolariser concentration. Electrocapillary curves, current-time curves and the temperature dependence of the diffusion currents, all confirm that adsorption phenomena at the electrode-solution interface is responsible for the extraordinary polarographic behaviour of this system.

Issa et al<sup>(118)</sup> investigated the polarographic behaviour of 4-hydroxymonoazo compounds in Britton-Robinson buffers of varying pH. The reduction current in alkaline media was almost half of the limiting value in acid media, indicating consumption of four and two electrons, respectively. A general mechanism for the reduction in alkaline media is proposed:

The reduction of the - N = N - centre in acid solution proceeds in a way similar to that in alkaline solution. However, the reduction of the hydrazobenzene centre to amino compound would be slower and hence the rate determining step would be involved in this stage, i.e.

$$R - N - N - \overline{R} \xrightarrow{2H^+ + 2e^-} R - N \xrightarrow{H} H$$

As the pH of the medium increases, reduction to the amino compound slows down till it is completely inhibited in nearly neutral media (pH 6-7).

In an investigation of some para-substituted azobenzenes it was shown that electronaccepting substituents promote reduction to hydrazo derivatives whereas electron donors drive the reaction partially or totally to the corresponding amines (119).

Florence and co-workers (120-125) have contributed a number of publications on the study of azo compounds. They suggest that certain species form unstable hydrazo intermediates, and overall polarographic n values of 4 were obtained.

The food dye Green S is reduced at the dropping mercury electrode from 50% ethanolic solutions with the total consumption of two electrons (126). Polarograms follow theoretical predictions in the pH range 2.7-8.75. Carbonium ions of the triarylmethane type are reducible at the DME, and the parent species triarylmethane has been studied in various media. In liquid sulphur dioxide, the cation undergoes single-electron reduction to the radical which dimerises (127)

 $\phi_3 C^+ + e^- \Longrightarrow \phi_3 C^-$ 

(i)

$$2 \phi_3 C^* \rightarrow \text{Dimer}$$
 (ii)

In protonating solvents, a second reduction step is possible.

$$\phi_3 C^* + H^+ + e^- \rightarrow \phi_3 CH$$
 (iii)

Thus, in methanesulphonic acid<sup>(128)</sup> and sulphuric acid<sup>(129)</sup> media, this step is in competition with the dimerisation. The dye is reduced in a single wave up to pH 7.3, where splitting becomes discernible. At pH 8.75 two distinct waves become apparent whose half-wave potential exceeds 200 mV. The first wave appears to correspond to a reversible single-electron transfer but the second electron is taken up irreversibly in the subsequent wave. The second wave but not the first one is pH dependent. The first wave is ascribed to process (i) and the second to process (iii). Dimerisation of the free radical does not take place, as the difference between  $E_1$ 's for the waves does not change with depolariser concentration <sup>2</sup> (130). On decreasing the pH, the half-wave potential of the irreversible wave is displaced to more positive values resulting in a merger.

The following reduction scheme forms the foundation of a consistent theoretical interpretation of the polarographic behaviour of Green S in the pH range of interest.



Coulometric titration with electroanalytically generated Ti(111) has been applied to the determination of Tartrazine and Amaranth<sup>(131)</sup> and Munemori<sup>(132)</sup> described the coulometric titration of Indigo Carmine at pH5 with electroanlytically generated dithionite.

## CHAPTER 4

#### 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. It is capable of performing:

> DC Polarography Sampled DC Polarography Normal Pulse Polarography Differential Pulse Polarography Linear Potential Sweep Voltammetry Direct Stripping Voltammetry Differential Pulse Stripping Voltammetry Phase Sensitive AC Polarography (in conjunction with 174/50 Interface and Lockin Amplifier).

In the 174A a high compliance ( $\pm$  80V, 18 mA) potentiostat with high electrometer input impedance ( $10^{11}$  ohms) extends the usefulness of the differential pulse technique to include work in high resistance media such as non-aqueous solutions or aqueous solutions of low conductivity.

Any current that flows through the working electrode is converted to a voltage. The voltage is further amplified and applied 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 model 174A is supplied with a drop timer that precisely dislodges the mercury drop with minimal perturbation of the solu-

tion, allowing polarograms to be run with a variety of preselected drop times, often far more rapidly than is possible with naturally-falling mercury drops. It can also be used with the new Model 303 SMDE (Static Mercury Drop Electrode) and with stationary electrodes.

#### EA 1096 Detector Cell

The Metrohm EA 1096 Electrochemical Detector Cell made of polymethyl methacrylate-perspex or polychlorotrifluorethylene-Kel-F is eminently suitable for highly sensitive amperometric or voltammetric measurements with very small sample volumes, either in continuous-flow analysis or discontinuously with sample injection.

In accordance with the wall-jet principle, the liquid flows head-on against the working electrode. The cell body also carries a reference electrode and an auxiliary electrode. The effective cell volume is only about 1.3  $\mu$ , and the "dead volume" is also extremely small.

Any substance capable of being electrochemically oxidised or reduced at the surface of the working electrode can be determined. The voltammetric and amperometric methods for which the EA 1096 Detector cell has been developed are highly selective and their sensitivity extends to the nanogramme/picogramme region.

All interconnecting, feed and outlet pipes are made of flexible Teflon tubing whose dimensions correspond to those of the tubing used in liquid phase chromatography. For discontinuous working, the sample is flow-injected in a background electrolyte.

In all applications, the Detector cell is used either as an independent analytical system or as an electrochemical detector attached to a separating column in liquid phase chromatography.

The Detector cell with its mini-electrodes can be extended to form a Flow Injection Stand comprising a Detector cell, miniature electrodes, electrolyte bottle, septum injection block,

holder and flexible tubing. The glass bottle containing the supporting background electrolyte solution is connected directly to the reducing valve of an inert gas cylinder (taking care not to exceed maximum permissible pressure); by this simple expedient a steady flow of background electrolyte through the Detector cell is ensured.

## Mini Electrodes

The electrochemically active part of these electrodes is the sensor disc at the head end. Apart from the sensor discs, the following structural materials come in contact with the working solution: epoxide resin: cementing the sensor disk to the electrode stem; PCTFE (polychlorot ri fluorethylene/Kel-F). These electrode types are used either as working or auxiliary electrodes.

EA 286/1 glassy carbon electrode; EA 286/2 Platinum electrode; and EA 287 carbon paste electrode (it is supplied empty and the cavity is filled with carbon paste before use).

#### EA 442 Ag/Ag Cl Reference Electrode

The reference electrode is supplied in a dry state. Once in use the diaphragm is not allowed to dry out by keeping it stored in electrolyte solution. The electrolyte within the electrode is changed fairly frequently, as the pressurisation of the cell and temperature fluctuations cause samples to penetrate through the diaphragm.

#### CHEMICALS AND SAMPLES

#### Food Colours

The synthetic food colours were kindly provided by the Laboratory of the Government Chemist, London. Amaranth and Tartrazine were BDH grade chemicals and Sunset Yellow FCF and Green S were commercial samples provided by Beechams Ltd.

The colours were not further purified for the study. Table I lists the food colours investigated together with their structures and 1971 Colour Index classification numbers.

#### Phosphonium Compounds

Tetraphenylphosphonium chloride was obtained from Cambrian Chemicals, Croydon. The other Phosphonium compounds were obtained from Messrs Maybridge Research Chemicals, Cornwall; Albright and Wilson Ltd., Warley, West Midlands; Aldrich Chemical Co. Inc., Milwaukee, Wisconsin. Tetraphenylarsonium chloride was supplied by Schuchardt München, Germany.

### Samples

Samples of sweets, marmalades and jellies were purchased locally and these included: Chivers Tangy Orange Shred Marmalade, Bournville, Birmingham; Roses Lime Marmalade, Bournville, Birmingham; Quosh Strawberry Flavour Cordial, Beecham Products, Brentford, Middlesex; Bassetts Dolly Mixture, George Bassett and Co. Ltd., Sheffield and Rowntree's Jellies, Rowntree Mackintosh Ltd., York.

#### Britton-Robinson Buffer

Britton-Robinson buffer (pH 1.9; 0.04M in each constituent) 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 4.0M sodium hydroxide solution. All chemicals used were analytical grade reagents.

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### Nitrogen Gas Scrubbing Solution

The scrubber solution was prepared by boiling 2 gm of ammonium metavanadate (LR grade from Fisons Chemicals Ltd) with 25 ml of concentrated hydrochloric acid, diluting to 200 ml, and shaking with a few grams of heavily amalgamated zinc, adding a little more acid when precipitate or turbidity formed. The partly reduced solution was divided between two 250 ml gas washing bottles with coarse-porosity sintered glass gas-dispersion cylinders or glass bulbs with a number of holes. Each bottle contained 25 gm of heavily amalgamated zinc to rereduce the V(111) formed as oxygen was absorbed. The gas emerging from the second bottle was washed with water in a third one to remove any acid or scrubber solution carried over in the gas stream. The vanadium solutions tend to lose their violet colour and some precipitate formed after some time, but they were easily restored by adding a little hydrochloric acid.

	LIST	OF FOOD	COLOURING	MATTERS STUDIED
No	COMMERCIAL NAME	SE RIAL NO	1971 COLOUR INDEX	STRUCTURE
1	AMARANTH	E 123	16185	$Na_{0}S - O - N = N - O S_{0}Na$ $S_{0}Na$
2	CARMOISINE	E 122	14720	Na QS-O-N=N-OO SQNa
3	PONCE AU 4R	E 124	16255	QH NaQS-Q-N=N-Q NaQS-Q SO <sub>3</sub> Na
4	RED 2G	-	18050	HO NHEOCH <sub>3</sub> N=N-OO SOJNa Na O3S SOJNa

TABLE - I

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NaO ERYTHROSINE BS 45430 E 127 5 ΗQ SUNSET YELLOW FCF 15**985** E 110 6 Na0,S SO<sub>3</sub>Na 4 H0-0 YELLOW 2G 1 18 965 NaQS CH3 NaQS-O H0-0 TARTRAZINE. E 102 .1 914 0 8 sgNa

SQNa

— ( 00Na

0 ·(SONa)2 OUINOLINE YELLOW 47005 E 104 9 OT ¯o₃s ,oh E 142 44 090 GREEN S 10 NaOzs 0 NaOS 73015 E 132 INDIGO CARMINE 11 С н (HC2)N 1 PATENT BLUE 12 ۷ E 131 42051 .S0<sub>3</sub> HO · . . .

(SQNa) N(CH<sub>3</sub>)<sub>2</sub> NCH,

SO<sub>3</sub>Na N(CH) (a<sup>2+</sup>

 $\square 2$ 

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#### CHAPTER 5

# DIFFERENTIAL PULSE POLAROGRAPHIC STUDY OF FOOD COLOURING MATTERS

#### INTRODUCTION

Differential pulse polarography and pulse polarography have, to a large extent, supplanted conventional polarography in the recent electroanalytical literature<sup>(53)</sup>. This is attributed to the lower detection limits of the pulse techniques. DPP and PP are being widely employed for the determination of organic compounds and metal ions for which stripping voltammetry is unsuitable. In this section only a brief mention is made of the ever increasing applications in the determination of organic and drug compounds.

A large number of applications to the determination of drug compounds have been reported. Some typical examples are the determination of the following drugs: cardaic glycosides digoxin and digitoxin<sup>(133)</sup>; N-nitroso derivative of a tripeptide in pharmaceutical dosage form(134); glibornuride and trimethoprim in blood and urine samples (135, 136); antibiotics such as tetracyline hydrochloride, streptomycin and penicillin  $G^{(137,138)}$ ; sodium chromoglycate in urine<sup>(139)</sup>; penicillamine<sup>(140)</sup>; benzylpenicillenic acid<sup>(141)</sup>; cephalosphorins and their degradation products<sup>(142)</sup>; derivatives of dibenzodiazepines and dibenzyothiazepines<sup>(143)</sup>; 2-benzimidazolyl 2-pyridyl methyl sulphoxide in a pharmaceutical formulation (144); flurazepam and its major blood metabolites in the plasma of beagle dogs and rhesus monkeys<sup>(145)</sup>; hydrocortisone in pharmaceutical preparations<sup>(146)</sup>; corticosteroids in single component solutions, suspensions, ointments, creams, and single-component tablets (147-149); and methaqualone and some of its metabolites (150).

Numerous organic compounds have been determined by pulse techniques. Many examples of determinations of organic compounds of biological significance by pulse, differential pulse and other voltammetric methods are contained in the review by Smyth and Smyth<sup>(151)</sup> Typical methods for pesticides, carcinogens, and organic compounds are determinations of parathion, its major metabolites, and other nitro-containing pesticides (152); azomethine containing pesticides (153); linear alkylbenzene sulphonates in sewage (154); nucleic acids and proteins (155); doublehelical and thermally denatured DNA(156); vitamin B<sub>2</sub>, nicotonic acid, nicotinamide, ascorbic acid, and vitamins K<sub>1</sub> and K<sub>3</sub>(157); acrylamide monomer in polyacrylamide(158); carcinogenic nitrosamines(159); and aflatoxins in various foodstuffs(160).

#### APPLICATION TO FOOD COLOUR ANALYSIS

The established method of identifying food colours is thinlayer chromatography. This excellent method has the advantage of being inexpensive though quantification is difficult. Although relatively costly equipment is required, HPLC is now the nearest approach to an ideal method for the identification and determination of food colouring matters, combining efficient separations with precise quantification. Both TLC and HPLC methods, however, generally require separation of the colours from even simple food matrices before application to the plate or column.

The DC polarography of some food colours has already been discussed in Chapter 3. Differential pulse polarography has been applied to the analysis of single food colours and to mixtures present in soft drinks by Fogg and Yoo<sup>(161,162)</sup>. Procedures were given for the determination of Tartrazine-Sunset Yellow FCF, Tartrazine-Green S, Amaranth-Green S, and Chocolate Brown HT - Tartrazine-Green S mixtures in soft drinks without prior separation of the colouring matters from the samples. The differential pulse polarographic peaks obtained were sharp and afforded some measure of identification.

The ion-pair extraction method has been used in the determination of ionisable pharmaceutical compounds (163) and is now being adapted increasingly to paired-ion HPLC(164, 165). This ion-pair extraction approach was applied to food colourings by Fogg and Yoo and the acidic food colours Tartrazine and Sunset Yellow FCF

were extracted into chloroform from orange squash using tetraphenyl-phosphonium chloride. After evaporating the chloroform and dissolving the extract in pH9 Britton-Robinson buffer, two distinct polarographic waves were obtained. Subsequently the extraction step was found to be unnecessary; the addition of tetraphenylphosphonium chloride to orange squash buffered at pH9 altered the potential of tartrazine reduction. The direct differential pulse polarographic procedures for the determination of mixtures of colours were based on the separation of overlapping reduction waves by Tetraphenylphosphonium chloride addition.

#### EXPERIMENTAL

Polarographic measurements were made with a PAR 174A polarographic analyser. Three electrode operation was employed using a dropping mercury electrode, a platinum counter electrode and a saturated calomel reference electrode. Polarography was carried out at room temperature (approximately  $22^{\circ}$ C). Solutions for polarography were deoxygenated with nitrogen gas which had previously been passed through a vanadium(11) scrubber. D.C. polarography was carried out with a natural drop time (2.80s in Britton-Robinson buffer pH2 on open circuit), low pass filter 3 and a sweep rate of 5 mV s<sup>-1</sup>. DP polarography was carried out with a forced drop time of 1s, a scan rate of 5 mV s<sup>-1</sup> and a pulse height of 50 mV.

### EFFECT OF pH ON DP\_POLAROGRAMS

Solutions for polarography were prepared with Britton-Robinson buffer (pH 1.9) in 25 ml volumetric flasks. Aliquots of solutions of colouring matter and 10 ml of Britton-Robinson buffer were adjusted to the required pH with 2 or 4m sodium hydroxide solution, diluted to 25 ml in the calibrated flask so that the solution concentration was  $10^{-5}$ m. The solution was transferred to the polarographic cell and deoxygenated for 10 minutes. After recording the

polarogram, the pH was adjusted to different values directly in the polarographic cell, deoxygenating for 2-4 minutes at each pH value, and recording the polarogram in the DP mode.

#### Results and Discussion

The effect of pH on the DP peak potentials of the synthetic food colours is shown in Figure 5.1. The position of the peak, taken in conjunction with the colour of the colouring matter, might be used in routine determinations as tentative confirmation of the identity of the colouring matter. With experience even minor differences in peak shapes can sometimes be distinguished and used for identification purposes. Most colouring matters give excellent DP peaks over at least part of the pH range. The quality of the DP peak obtained for each colouring matter is indicated in Table I.

A closer look at Figure 5.1 suggests that in a mixture of Sunset Yellow FCF and Tartazine better peak resolution would be obtained at higher pH and between Yellow 2G and Tartazine at lower pH. Quinoline yellow is well separated from the other three colours at all pH values. Among the red colours peak resolution is greater at higher pH values and the same holds for green/blue colours. Black PN and Brown FK though not well resolved would give some degree of resolution at low pH values. The choice of pH in the final analysis would be governed by the quality of peaks obtained at respective pH values. The peaks at optimum pH values are compared with those at some other values for Quinoline Yellow and Patent Blue V, in Figure 5.2.

Qualitatively for some colours identification may be possible by visual observation of colour change with pH e.g. Patent Blue V is green at pH < 3 and changes to blue at higher pH values and the colour deepens with increasing pH. Erythrosine BS cannot be determined below pH4 because precipitation takes place. Green S, whose solution is light blue, deepens with increasing alkalinity.



FIGURE 5.1

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Effect of pH on D.P. peak potentials: 1 Sunset Yellow FCF; 2 Tartazine; 3 Yellow 2G; 4 Quinoline Yellow; 5 Carmoisine; 6 Amaranth; 7 Red 2G; 8 Ponceau 4R; 9 Erythrosine BS; 10 Green S; 11 Patent Blue V; 12 Brilliant Blue FCF; 13 Black PN; and 14 Brown FK.

# TABLE I

| ,                  |       |                                                        |                                                                                                                                       | Effect of TPPC (76                                        | u µg ml =1) on d.p. pola<br>colonting matter                                                                                                     | ography of L × 10-+ м                                                                                         |  |
|--------------------|-------|--------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|--|
| Coloaring matter   |       | Aprimum pri for<br>letermination in<br>absence of TPPC | Kemarks                                                                                                                               | Peak potential<br>(AE <sub>n</sub> quated)                | Peak current                                                                                                                                     | Remarks                                                                                                       |  |
| Fartrazine         | •••   | 78                                                     | No well defined peak                                                                                                                  | 220 mV at p11 4                                           | No change                                                                                                                                        | Exceptional improve-                                                                                          |  |
|                    |       |                                                        | pre-peak at pH >0                                                                                                                     | (new peak)<br>100 mV at pH 7                              | Slight enhancement                                                                                                                               | TPPC at pH 9                                                                                                  |  |
|                    |       |                                                        |                                                                                                                                       | 90 mV at pH 8<br>70 mV at pH 9                            | Significant enhance-<br>ment                                                                                                                     |                                                                                                               |  |
| Quinoline yellow   | ••    | 3                                                      | ip at pH 2 double that<br>at higher pH                                                                                                | 60 mV at pH 9                                             | Halved at pH 2                                                                                                                                   | Second wave appears<br>with TPPC                                                                              |  |
| Yellow 2G          | ••    | 3-6                                                    |                                                                                                                                       | 50 mV at pH 4<br>190 mV at pH 9                           |                                                                                                                                                  | Peak shape improved<br>at pH 9                                                                                |  |
| iunset Yellow FCF  | ••    | 8 or >6                                                | Peak splits at pH 8,<br>ip less at pH 3-5                                                                                             | U                                                         | Suppressed at all<br>pH values                                                                                                                   | Base line better<br>without TPPC                                                                              |  |
| larmoisine         | ••    | 2 or 7-8                                               | Second peak appears<br>at pH > 2                                                                                                      | Û                                                         | Suppressed at pH 4                                                                                                                               |                                                                                                               |  |
| Amarenth           |       | 2-6, 8-12                                              | ip greater at pH >11,<br>better base line at<br>high pH                                                                               | U                                                         | General suppression                                                                                                                              | *                                                                                                             |  |
| Ponceau 4R         |       | 7                                                      | No peak at pH <4.<br>Peaks at low pH small<br>and iroad, Distorted<br>base line at high pH                                            | Negligibla                                                | pH 8 peak present<br>(absent if no<br>TPPC). pH 7 peak<br>suppressed and<br>broader. pH 9<br>peak suppressed<br>but improved base<br>line        | TPPC improves base<br>line if suppression<br>twierated. Post-peak<br>appears at bigher<br>TPPC concentrations |  |
| ≧rythrosine BS     | ••    | 4, R.D                                                 | Precipitation at pH 2<br>at higher concentra-<br>tions. Ill-defined<br>peak at pH 5.5-7.5                                             | ~40 mV at pH 8.9<br>to more positive<br>potential         | pH 4 peak completely<br>suppressed. pH 6-<br>8 peak greatly<br>suppressed                                                                        |                                                                                                               |  |
| Red 2G             |       | 2-11                                                   | ip greatest at pH >8<br>and better base line                                                                                          | 30 mV at pH 4                                             | pH <7 peak, slight<br>enhancement. pH<br>9 peak, extensive<br>suppression                                                                        |                                                                                                               |  |
| Patent Blue V      | ••    | 2-3                                                    |                                                                                                                                       | · 0                                                       | Suppressed                                                                                                                                       |                                                                                                               |  |
| ndigo carmine      |       | 8-9                                                    |                                                                                                                                       | 0                                                         | Suppressed at low<br>TPPC concentration<br>but enhanced at<br>higher concentra-<br>tions                                                         | s                                                                                                             |  |
| Brilliant Blue FCP | ••    | 3                                                      | pH >8 peak ill-defined<br>and broad                                                                                                   | 220 mV at pH 3                                            | ip halved                                                                                                                                        | Peak at pH 9 now<br>well-defined                                                                              |  |
| ùreen S            | <br>- | 2-11                                                   | Better defined in acidic<br>solution and $i_p$<br>greater. $i_p$ decreases<br>as pH increases but<br>some increase again<br>at pH > 0 | 0 at pH <0.<br>$pH > 6$ , $E_p$<br>constant at<br>-0.68 V | General extensive<br>depression.<br>Reduced depression<br>at plf >0                                                                              |                                                                                                               |  |
| irown FK 🥠         | ••    | 10.6                                                   | Best shaped peak at<br>pH 10.6. Acidio<br>pH, broad peaks; PH<br>4-8, shoukders. ip<br>maximum at pH 7-8<br>but bad shape             | Q                                                         | Shoulders removed.<br>Slight enhancement<br>in acidic solution<br>but bad shape.<br>Depression in alkali<br>solution. pH 6-7<br>peak ill-defined | 10                                                                                                            |  |
| lhocolate Brown HT | ••    | 4-H                                                    | Double peak, <i>ip</i><br>constant                                                                                                    | 0                                                         | Complete suppression                                                                                                                             |                                                                                                               |  |
| Black PN           | ••    | 2, 10.5                                                | $E_{\rm p}$ constant at pH >8.<br>Peaks well defined at low and birk pH -10.                                                          | 0                                                         | Less affected at pH 2.<br>Greatly suppressed                                                                                                     |                                                                                                               |  |

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FIGURE 5.2 Optimum pH D.P. peaks for Quinoline Yellow and Patent Blue V compared with peaks at different pH values. Colouring matter concentration, 10<sup>-5</sup>M.

### EFFECT OF TETRAPHENYLPHOSPHONIUM CHLORIDE ON DP POLAROGRAMS.

The gross influence of tetraphenylphosphonium chloride on DP peaks was evaluated by taking 2.5 ml of  $10^{-4}$  M dye solution in a volumetric flask, adding 5 ml of 0.01. MTPPC (total concentration 750 µg ml<sup>-1</sup>) and diluting the volume to 25 ml with distilled water so that final dye concentration was  $10^{-5}$  M. The effect of increasing concentration of TPPC on the polarograms was studied by adding aliquots of 0.1M (37.4 mg.ml<sup>-1</sup>) solution using a 10 or 100 µl syringe at  $10^{-4}$  M dye concentration. Only brief deoxygenation ( $\sim$  2-4 min) was required after each addition of TPPC.

#### Results and Discussion

The effect of TPPC concentration on the peak potentials and peak currents of the DP peaks of the permitted food colours has been studied. In some cases changes in peak potential were observed at several pH values. The results are summarised in Table I. The effect of TPPC concentration on peak currents at selected pH values is shown in Figure 5.3.

For three permitted food colours (Tartrazine, Yellow 2G, and Brilliant Blue FCF) the value of the peak potential is affected significantly by the addition of TPPC. The effect on tartrazine at pH4 differs to some extent from that at pH9. At pH4 the peak potential of the initial peak varies very slightly on addition of TPPC, whereas the peak current decreases markedly with increasing TPPC concentration. For TPPC concentrations above 250  $\mu$ g ml<sup>-1</sup>, however, a second peak at a potential 170 mV more negative than the initial peak appears and this second peak increases in size and becomes the main peak.

At pH9 the peak potential of Yellow 2G is shifted 190 mV to a more negative potential at TPPC concentrations above 50  $\mu$ g ml<sup>-1</sup>, the peak current follows a similar pattern to that of Sunset Yellow FCF, passing through a peaked minimum at 50  $\mu$ g ml<sup>-1</sup> of TPPC.



FIGURE 5.3 Effect of TPPC concentration on D.P. peak currents, colouring matter concentration,  $10^{-4}M$ . Peak currents are shown relative to zero TPPC concentration.

The peak potential for  $2 \times 10^{-5}$ M Brilliant Blue FCF at pH3 is' displaced 220 mV to a more negative potential at TPPC concentrations above 50 µg ml<sup>-1</sup>, and i<sub>p</sub> passes through a peaked minimum, giving a steady value of about half the original at TPPC concentrations above 100 µg ml<sup>-1</sup>. With Brilliant Blue FCF, the effect of colouring matter concentration was studied. At the lower concentration of  $2 \times 10^{-6}$ M the effect of TPPC on E<sub>p</sub> and i<sub>p</sub> was essentially the same as at  $2 \times 10^{-5}$ M, whereas at  $2 \times 10^{-4}$ M the effect of TPPC on the main peak remained unchanged, but a minor peak of constant i<sub>p</sub> and E<sub>p</sub> was present at a potential 100 mV more positive than the main peak at all concentrations of `TPPC. With increasing TPPC concentration, i<sub>p</sub> for the main peak reached a peaked maximum and was then reduced to about 30% of its original value.

Although the addition of TPPC to the other permitted colouring matters had only a slight effect on their peak potentials, the effect on  $i_p$  was usually just as marked as for the other three dyes. The effect of TPPC concentration on the DP peak currents of several colouring matters, excluding those giving ill-defined peaks, is illustrated in Figure 5.3 and other details are given in Table I.

Figure 5.4 illustrates the change in peak potentials with increasing concentration of TPPC for the DP peaks of food colours at  $10^{-4}$ M, concentrations. It is seen that generally the potentials remain the same and unlike the changes in peak currents the change in potentials are smooth till the final values are attained. The final values of peak currents were normally reached around 200 µg ml<sup>-1</sup> TPPC concentration but the peak potentials reached steady values around 100 µg ml<sup>-1</sup> TPPC.

In some cases the peak shape was seen to get distorted due to general broadening or splitting with increasing amounts of TPPC. This was further confirmed by DC polarography as will be discussed later. The results for three colours, namely Chocolate Brown HT, Black PN and Quinoline Yellow have not been reported because of double peaks or broad peaks obtained even in the absence of TPPC. For Chocolate Brown HT at pH 4.0 the double peaks merge into one tailing "peak



FIGURE 5.4 Effect of TPPC concentration on D.P. peak potentials. Colouring matter concentration, 10<sup>-4</sup>M.

at TPPC concentrations above 500  $\mu$ g ml<sup>-1</sup>. At pH 9.0 the peak is ill-formed and becomes a double peak at TPPC concentration above 60  $\mu$ g ml<sup>-1</sup> and a triple peak above 100  $\mu$ g ml<sup>-1</sup>. In the absence of TPPC Quinoline Yellow, at pH 4.0, yields two welldefined peaks which distort till 40  $\mu$ g ml<sup>-1</sup> and then again the two peaks appear at TPPC > 60  $\mu$ g ml<sup>-1</sup>. Black PN shows two distorted peaks in absence of TPPC and results in two separate peaks at TPPC concentrations > 40  $\mu$ g ml<sup>-1</sup>.

The changes in peak currents and potentials observed on TPPC addition must be caused by the adsorption of tetraphenylphosphonium ion on the mercury surface  $\frac{f(Th)}{f(t)}$  means that the colouring matters are phenyl reduced through an adsorbed layer of tetraphosphonium ion. Plots of drop time against potential for Britton-Robinson buffer at pH4 and 9 containing TPPC show a marked decrease in drop time on the negative side of the electrocapillary maximum and confirms the adsorption. A limiting depression of the drop time is reached at around 100 µg ml<sup>-1</sup> TPPC, which presumably corresponds to complete coverage of the mercury surface.

# D.C, NORMAL PULSE AND DIFFERENTIAL PULSE POLAROGRAMS OF SELECTED COLOURS IN THE PRESENCE OF TPPC

A study has been made of the effect of TPPC on DC and normal pulse polarograms of the colouring matters. DC polarograms are not altered as markedly as are DP polarograms by the presence of TPPC, although the polarographic maxima of Yellow 2G and Red 2G are conveniently suppressed (Figure 5.5). The half-wave potentials seem to follow the shifts of d.p. peaks with increasing TPPC concentration and phenomena like peak splitting and broadening are reflected by the waves of the DC polarograms (Figure 5.6). Table II shows the type of wave and effect of TPPC addition on the DC polarograms of food colours at pH values selected where either there is improvement in wave shape height or potential shift is maximum. Figure 5.7 shows the changes in the limiting diffusion currents on increasing the concentration of TPPC in the



FIGURE 5.5 Effect of TPPC on the polarographic maxima of Yellow 2G and Red 2G at pH 9.0. Colour concentration, 10<sup>-4</sup>M.



FIGURE 5.6 Comparison of DP and DC polarograms to illustrate the effect of peak splitting/broadening on DC polarograms.

# TABLE 11

#### D.C. POLAROGRAPHY OF FOOD COLOURING MATTERS

| Colouring ma           | lter |    | թո     | Quality of d.c. wave in<br>absence of TPPC | Effect of TPPC (760 µg mi=*)<br>addition on limiting diffusion<br>corrent         | Kennarks                                                                                      |
|------------------------|------|----|--------|--------------------------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Tartiazine             | ••   |    | ษ      | Polarographic maximum                      | Wave suppressed even though<br>d.p. peak enhanced                                 | Maximum removed by 20 µg ml=1<br>of TPPC to give well defined<br>wave                         |
| Quinoline yellow       | ••   | •• | 4      | No maximum                                 | Some suppression                                                                  | Hi-defined wave reflecting d.p.<br>peaks                                                      |
| Yellow 2G 🛛            | ••   | •• | 5      | Polarographic maximum                      | Very little change                                                                | Maximum removed by 10 µg nd+)<br>of TPPC to give a welt defined<br>wave                       |
| Sunset Yellow FCF      |      |    | 0      | No maximum                                 | Some suppression                                                                  | Well defined wave                                                                             |
| Carmoisine             |      | •• | IJ     | Polarographic maximum                      | i <sub>d</sub> halved                                                             | Maximum removed by 20 µg ml+<br>of TPPC but wave not well<br>defined                          |
| Amaranth               |      | •• | U      | No maximum                                 | Very little change                                                                | Well defined wave                                                                             |
| Ponceau 4R 🕠           | ••   | •• | 3      | No maximum                                 | Very little change                                                                | Well defined wave; a second<br>wave appears at TPPC<br>concentrations >60 µg ml <sup>-1</sup> |
| Erythrosine <b>B</b> S | ••   | •• | 4      | Ill-defined wave                           | Almost totally suppressed (at<br>TPPC concentrations<br>>30 µg ml <sup>-1</sup> ) |                                                                                               |
| Keet 2G 🦷              | ••   | •• | U      | Polarographic maximum                      | is halved                                                                         | Maximum removed by 10µg ml=1<br>of TPPC to give a well defined<br>wave                        |
| Patent Blue V          | ••   |    | 4      | No maximum                                 | Very little change                                                                | Well defined wave                                                                             |
| Indigo carmine         | ••   | •• | 9      | No maximun                                 | ia doubled (precipitation at >600 µg ml <sup>-4</sup> of TPPC)                    |                                                                                               |
| Brilliant Blue FCF     | ••   | •• | 3      | Pokarographic maximum                      | ia doubled (at ≥10 µg ml=) of<br>TPPC)                                            | Maximum removed by 10 µg ml-><br>of TPPC to give well defined<br>wave                         |
| Green S                |      |    | 4      | No maximum                                 | Very little change                                                                | Well defined wave                                                                             |
| Brown FK               |      |    | 4      | No maximum                                 | Very little change                                                                | Reasonably well defined wave                                                                  |
| Chocolate Brown H      | r    | {  | 4<br>9 | Double wave<br>Ni-defined wave             | Slight suppression<br>Wave splits                                                 | Not ideat for analytical use                                                                  |
| Black PN               | ••   | •• | ν      | No maximum                                 | Slight suppression                                                                | Wave reflects characteristics of<br>twin d.p. pcaks                                           |

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FIGURE 5.7 Effect of TPPC concentration on DC limiting diffusion currents. Colouring matter concentration, 10<sup>-4</sup>M. Mean limiting diffusion currents are shown relative to zero TPPC concentration, 1 Indigo Carmine (pH 9); 2 Patent Blue V (pH4); 3 Amaranth (pH9); 4 Green S (pH4); 5 Sunset Yellow FCF (pH9); 6 Brown FK (pH4); and 7 Ponceau 4R (pH3). solution. Those colours which gave polarographic maxima in the absence of TPPC have not been included in Figure 5.7, but details are given in Table II. The DC and DP polarograms for all the colours at the pH values selected in Table II together with the polarograms at a TPPC concentration of 750  $\mu$ g ml<sup>-1</sup> are illustrated in Appendix.

The NP polarograms for the colours gave peak shaped waves and did not show much promise in analytical utility.

## DIFFERENTIAL PULSE POLAROGRAPHY OF COLOURS IN JAMS, JELLIES AND MARMALADES Experimental

About 10 g of the sample (peels carefully left out) was weighed and dissolved in water with slight heating. This volume was diluted to 50 ml in a volumetric flask. 10 ml of the diluted solution was taken in a 25 ml volumetric flask, 10 ml of Britton-Robinson buffer (pH 1.9) added and volume made up to the mark. The effect of TPPC was studied by adding 5 ml of 0.01M TPPC before making up the volume to 25 ml. Polarograms were run on the samples made up after deoxygenation for nearly 10 minutes. The pH was changed in the cell as in previous studies.

Ion-pair extraction with TPPC was also carried out on some samples, 10 ml of diluted solution was shaken with 5 ml of 0.01M TPPC and 5 ml of chloroform thrice and each time the lower chloroform layer collected. The chloroform was evaporated in a fume cupboard and the residue dissolved in 10 ml of Britton-Robinson buffer(pH 1.9). The solution was transferred to the 25 ml volumethad been ric flask diluted to 25 ml with distilled water after the pH adjusted to the required value. This 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(97).

#### Materials

Adsorbent - polyamide powder (MN SC6, Machery, Nagel & Co).
Michrochromatographic tubes - 15 x 150 mm with 3 x 100 mm exit
 tube in place of ground glass stopcock.
Methanolic sodium hydroxide - 1 g sodium hydroxide added to 1 litre
 70% methanol.
Methanolic acetic acid (1 + 1).
Cellulose powder coated TLC plates - cellulose layer 0.1 mm thick
 on aluminium sheet.
Solvent system - 2.5% (w/v) sodium citrate - 25% (v/v).

Ammonia - methanol (80 + 20 + 12).

#### Procedure

**Sample** (5 g) was warmed with a 50 ml distilled water and any insoluble material was removed by filtering through glass wool. After acidification with a few drops of acetic acid, 0.5 - 1 g of polyamide powder was added to extract the colour from the solution. The solution was agitated with a glass rod and the polyamide allowed to settle. A further 0.5 g of polyamide was then added and the solution re-agitated.

The polyamide sludge was transferred to a microchromatographic tube sealed with glass wool and the liquid allowed to drain. Elution was carried out with six 10 ml portions of hot water followed by 5 ml portions of acetone. During this procedure, powder in the column was agitated occasionally with a glass rod. The hot water removed sugars, acids, and flavouring matter, and acetone removed any basic dyes, water soluble carotenoids, and some anthocyanins. Polyamide retained all acidic synthetic dyes. The acid dyes were eluted with two 5 ml portions of warm methanolic sodium hydroxide. The pH of the eluate was adjusted to 5-6 with methanolic acetic acid (1 + 1); 10 ml of distilled water was added and 0.5 g polyamide added to re-adsorb the colouring matter. The polyamide was then transferred again to the microchromatographic tube. After the liquid was drained the column was washed with hot water until

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eluate had the pH of the water.- This "repeat procedure" ensured complete removal of interfering materials.

The dyes were desorbed with 10 ml methanolic sodium hydroxide, the eluate re-acidified with acetic acid and the solution concentrated to ca lml in a vacuum rotary evaporator. The concentrate was used for TLC.

#### Chiver's Orange Tangy Marmalade

Polarograms were run in the pH range 2-9. The peaks in the pH range 2-8 were rather broad. At pH9 no peak was obtained but simply a rising base-line was seen. In the presence of TPPC well defined peaks were seen at pH > 6 and a peak is also seen at pH 9.0. This is an analytically useful peak (Figure 5.8). Tartrazine was shown to give an enhancement of peak signal at pH 9.0 and TLC study also confirmed the presence of Tartrazine in the marmalade. Ion-pair extraction with TPPC also supported the results.

#### Rose's Lime Marmalade

Polarograms show a drop in base-line to the right of the peak in the low pH range, the base-line levels off in the neutral pH region and there is almost complete suppression of the peak at pH9. In the presence of TPPC the peaks are slightly raised but the drop in base-line at low pH values is still observed. The peaks show cathodic shifts, e.g. at pH4 the shift is 200 mV, at pH6 the shift is 150 mV.

The peak at pH6 gets narrower and slightly bigger. At pH9 in the presence of TPPC a well defined peak appears where only a rising base-line was seen in the absence of TPPC (Figure 5.9).

The sample was spotted against Tartrazine, Yellow 2G and Green S. The sample spot split into an inclined arrow-shaped yellow zone with a diffuse blue zone just above it i.e. the light green colour of the marmalade was due to the presence of two colours.







FIGURE 5.9 DPP peaks of Rose's Lime Marmalade solutions at pH6 and 9 with and without TPPC.

The yellow colour appears to be tartrazine (peak at pH6 without TPPC corresponds to tartrazine peak) confirmed by TLC. The other component could be Green S (small hump at -0.67V at pH 6.0 in the presence of TPPC could be due to Green S). The diffuse green spot roughly matches the Green S spot on the chromatogram.

#### Quosh Strawberry Flavour Cordial

The cordial was diluted 10-fold and 2.5 ml of the diluted solution was taken in a 25 ml volumetric flask, 10 ml of Britton-Robinson buffer added and the volume made up with distilled water. The DP peaks were sharp and the peak height is greatest in the pH range 5-7. Although the peaks get suppressed above pH8 the base-line is levelled. Presence of TPPC distorts peak shape up to pH6 and there is no significant improvement above this pH.

TLC confirmed the presence of carmoisine.

# Rowntree's Flavour Jellies

One cube of all the jellies (weighing  $\sim$  7.4 gm) was dissolved in 50 ml water. 2.5 ml of the solution was taken in a 25 ml volumetric flask, 10 ml of pH 1.9 Britton-Robinson buffer added and the volume made up to the mark with distilled water.

#### Orange Flavour Jelly

Broad peaks were obtained over the entire pH range. In the presence of TPPC suppression of peaks was observed. TLC examination indicated the presence of two colours. The yellow component gave an inverted arrow-shaped spot corresponding to tartrazine. The DP peak potentials of the jelly correspond to those of tartrazine at various pH values. The red component hardly moved from the base-line. On acidification the TLC eluate precipitated indicating that the red component could be Erythrosine BS.

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#### Strawberry Flavour Jelly

The DP polarograms give fairly well-defined peaks except in the pH range 6-8. The best peak appears at pH9. The potentials correspond to those for Amaranth or Carmoisine. In the presence of TPPC peak suppression and splitting start above pH6 and double peaks begin to appear. The presence of Carmoisine was confirmed by TLC examination.

#### Raspberry Flavour Jelly

Sharp DP peaks were observed over the pH range 2-9 but the peaks were better defined in the neutral pH range. The peak height is greatest at pH9 and the pre-peak which was present at pH4 also disappears at this pH. On TPPC addition no shifts in peak potentials were observed, but the peak at pH9 is suppressed. TLC examination confirmed the presence of Carmoisine.

#### Bassett's Dolly Mixture

The orange portion of the gum was carefully cut and DP polarograms were obtained with and without TPPC. The polarograms showed single peaks over the entire pH range. The peak at pH9 appeared at -0.62V in the absence of TPPC. In the presence of TPPC there was no shift in this peak but suppression was seen. This behaviour corresponds to that of pure Sunset Yellow FCF. The presence of Sunset Yellow FCF was confirmed by TLC. This example is a case where the presence of other excipients did not interfere in the polarographic determination of food colour.

# EFFECT OF OTHER PHOSPHONIUM AND ARSONIUM COMPOUNDS ON DP POLAROGRAMS

The success achieved with tetraphenylphosphonium chloride in the analysis of food colours when present singly or in a mixture called for a further investigation of the potentialities of other phosphonium and arsonium compounds as ion-pairing agents in food colour analysis. Seven other phosphonium compounds and tetraphenyl arsonium chloride were investigated. The Phosphonium and arsonium compounds are ingeneral toxic and their ingestion through skin can be harmful. Special precautions were taken in their handling and care was taken to avoid spillages.

Table III gives the peak potentials obtained for Tartrazine, Yellow 2G and Brilliant Blue FCF ( $1 \times 10^{-5}$ M solutions) at different pH values in the absence of any of these compounds. Tables IV, V and VI give the shifts and peak currents for the three colours due to the presence of the ion-pairing compounds. The shifts are in negative potential direction except when preceeded by a positive sign. The colours Tartrazine, Yellow 2G and Brilliant Blue FCF were selected for this study as they gave maximum shifts in the presence of tetraphenylphosphonium chloride.

For Brilliant Blue FCF large regular potential shifts are seen in acidic media due to the presence of these compounds. In neutral media the shifts decrease and positive potential shifts are seen in alkaline media. For Tartrazine the shifts are negligibly small in acidic media, increase in neutral media and again show identical shifts in alkaline media as in acidic media. For Yellow 2G shifts are large in acidic solutions, some decrease is seen in neutral solutions and again an increase in alkaline solutions. The Tartrazine peaks in the presence of most of the compounds show splitting in the pH range 4-6.

Tetraphenylphosphonium chloride appears to produce maximum shifts and its full potential in the analysis of food colours yet remains to be exploited.

However, in certain cases the other phosphonium or arsonium compounds could be used depending on individual merits and specific requirements.

## TABLE III

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Peak Potentials (E $_{\rm p}$ ) in Absence of Ion-Pairing Compounds at Colour Concentration 1 x  $10^{-5}\,\rm M$ 

| 1  | TARTRAZINE                      | YELLOW 2G                       | BRILLIANT BLUE FCF              |
|----|---------------------------------|---------------------------------|---------------------------------|
| рН | E <sub>p</sub> (volts vs S.C.E) | E <sub>p</sub> (volts vs S.C.E) | E <sub>p</sub> (volts vs S.C.E) |
| 2  | -0.170                          | -0.285                          | -0.500                          |
| 3  | -0.275                          | -0.370                          | -0.605                          |
| 4  | -0.370                          | -0.450                          | -0.690                          |
| 5  | -0.450                          | -0.510                          | -0.765                          |
| 6  | -0.530                          | -0.575                          | -0.840                          |
| 7  | -0.595                          | -0.645                          | -0.930                          |
| 8  | -0.650                          | -0.695                          | -1.070                          |
| 9  | -0.710                          | -0.745                          | -1.115                          |
|    |                                 |                                 |                                 |

# TABLE IV

PEAK CURRENTS  $i_p$  ( $\mu A$ ) AND POTENTIAL SHIFTS  $\Delta E_p$  (mV) FOR YELLOW 2G IN PRESENCE OF ION-PAIRING COMPOUNDS

| COMPOUND                                |                                    | 2          | 3          | 4          | 5        | <u>рн</u> 6 | 7          | 8          | 9           |
|-----------------------------------------|------------------------------------|------------|------------|------------|----------|-------------|------------|------------|-------------|
| Tetraphenylphosphonium                  | E (mV)                             | 95         | 100        | 50         | 50       | 45          | 55         | -          | 135         |
| chloride                                | ip (µA)                            | 0.92       | 1.0        | 1.17       | 1.4      | 1.7         | 1.9        | -          | 1.65        |
| Tri-n-butyl methyl                      | E (mV)                             | 95         | 100        | 45         | 15       | 10          | 25         | 60         | 100 /       |
| phosphonium iodide                      | ip (µA)                            | 1.2        | 1.3        | 1.88       | 2.0      | 1.5         | 1.6        | 1.75       | 1.7         |
| Triphenyl n-butyl                       | E (mV)                             | 105        | 110        | 70         | 70       | 65          | -          | 95         | 140         |
| phosphonium bromide                     | ip (µA)                            | 1.0        | 1.05       | 1.2        | 1.5      | 1.9         |            | 2.2        | 2.0         |
| Tri-n-butyl benzyl                      | E (mV)                             | 125        | 100        | 60         | 40       | 25          | 20         | 35         | 95          |
| phosphonium chloride                    | ip (uA)                            | 1.2        | 1.5        | 1.6        | 1.77     | 1.85        | 1.9        | 1.81       | 1.7         |
| Triphenyl methallyl                     | E (mV)                             | 95         | 120        | 85         | 60       | 50          | 55         | 105        | 140         |
| phosphonium chloride                    | ip (µA)                            | 0.87       | 1.09       | 1.32       | 1.6      | 1.85        | 2.1        | 2.08       | 1.81        |
| Methyl triphenyl<br>phosphonium bromide | E (mV)<br>i <sup>p</sup> (µA)<br>p | 80<br>1.27 | 60<br>1.38 | 10<br>1.45 | 0<br>1.4 | 15<br>1.45  | 25<br>1.92 | 65<br>2.11 | 105<br>1.85 |
| Triphenyl ethyl                         | E (mV)                             | 140        | -          | 95         | 70       | 55          | 55         | 85         | 130         |
| phosphonium iodide                      | ip (μA)                            | 1.25       | -          | 1.45       | 1.6      | 1.8         | 1.9        | 2.0        | 1.9         |
| Tetrabutyl phosphonium                  | E (mV)                             | 100        | 85         | 35         | 20       | 15          | 10         | 45         | 100         |
| chloride                                | ip (µA)                            | 1.22       | 1.45       | 1.5        | 1.6      | 1.88        | 1.98       | 1.98       | 1.98        |
| Tetraphenyl arsonium                    | E (mV)                             | 95         | 100        | 50         | 15       | +10         | +20        | 45         | 105         |
| chloride                                | ip (µA)                            | 0.8        | 0.53       | 1.5        | 1.85     | 2.0         | 1.75       | 1.75       | 1.8         |
| No ion-pairing<br>compound              | i <sub>p</sub> (µA)                | 1.4        | 1.5        | 1.58       | 1.55     | 1.65        | 1.7        | 1.8        | 1.78        |

# TABLE V

PEAK CURRENTS i (µA) AND POTENTIAL SHIFTS  $\Delta E_{p}$  (mV) FOR BRILLIANT BLUE FCF IN PRESENCE OF ION-PAIRING COMPOUNDS

| COMPOUND                   |                     | 2    | 3    | 4    | <u>рН</u> 5 | 6    | 7    | 8    | 9              |
|----------------------------|---------------------|------|------|------|-------------|------|------|------|----------------|
| Tetraphenylphoshonium      | E <sub>p</sub> (mV) | 250  | 240  | 215  | 185         | 140  | 65   | 70   | +115           |
| chloride                   | i <sup>p</sup> (μA) | 0.35 | 0.34 | 0.35 | 0.35        | 0.31 | 0.33 | 0.38 | 0.33           |
| Tri-n-butyl methyl         | E <sub>p</sub> (mV) | 165  | 145  | 140  | 145         | 150  | 110  | 10   | +55            |
| phosphonium iodide         | i <sup>p</sup> (μA) | 0.57 | 0.44 | 0.39 | 0.35        | 0.33 | 0.33 | 0.25 | 0.30           |
| Triphenyl n-butyl          | E(mV)               | 230  | 225  | 210  | 175         | 150  | 80   | -    | +95            |
| phosphonium bromide        | ip_(μA)             | 0.35 | 0.37 | 0.37 | 0.35        | 0.35 | 0.33 |      | 0.33           |
| Tri-n-butyl benzyl         | E <sub>p</sub> (mV) | 170  | 155  | 145  | 145         | 125  | 70   | +60  | +105           |
| phosphonium chloride       | ip (μA)             | 0.65 | 0.50 | 0.40 | 0.35        | 0.36 | 0.35 | 0.35 | 0.35           |
| Triphenylmethallyl         | E (mV)              | 230  | 220  | 210  | 175         | 140  | 70   | +60  | +105           |
| phosphonium chloride       | ip (μA)             | 0.38 | 0.35 | 0.36 | 0.35        | 0.35 | 0.35 | 0.35 | o • <b>3</b> 5 |
| Methyl triphenyl           | E_ (mV)             | 220  | 215  | 205  | 195         | 165  | 95   | +40  | +85            |
| phosphonium bromide        | ip (μA)             | 0.43 | 0.35 | 0.35 | 0.35        | 0.31 | 0.35 | 0.35 | 0.35           |
| Triphenyl ethyl            | E (mV)              | 210  | 210  | 210  | 195         | 160  | 95   | +40  | +85            |
| phosphonium iodide         | i <sup>p</sup> (µA) | 0.25 | 0.31 | 0.35 | 0.33        | 0.33 | 0.36 | 0.35 | 0.36           |
| Tetrabutyl phosphonium     | E (mV)              | 170  | 155  | 145  | 145         | 130  | 120  | +55  | +105           |
| chloride                   | ip (μA)             | 0.7  | 0.5  | 0.4  | 0.4         | 0.38 | 0.35 | 0.38 | 0.38           |
| Tetraphenyl arsonium       | E_ (mV)             | 240  | 225  | 195  | 165         | 135  | 60   | +75  | +115           |
| chloride                   | i <sup>p</sup> (μA) | 0.25 | 0.26 | 0.31 | 0.31        | 0.30 | 0.30 | 0.30 | 0.30           |
| No ion-pairing<br>compound | i <sub>p</sub> (µA) | 0.68 | 0.8  | 0.9  | 0.84        | 0.64 | 0.45 | 0.30 | 0.25           |

TABLE VI

PEAK CURRENT  $i_p$  ( $\mu A$ ) AND POTENTIAL SHIFTS  $\Delta E_p$  (mV) FOR TARTRAZINE IN PRESENCE OF ION-PAIRING COMPOUNDS

| COMPOUND                                 |                        | 2          | 3          | 4          | <u>р</u> Н<br>5 | - 6        | 7          | 8          | 9          |
|------------------------------------------|------------------------|------------|------------|------------|-----------------|------------|------------|------------|------------|
| Tetraphenyl                              | E_ (mV)                | -          | 85         | 205        | 170             | 120        | 105        | 90         | 75         |
| phosphonium chloride                     | ip (μA)                |            | 0.95       | 1.1        | 2.0             | 2.1        | 2.35       | 2.35       | 12.2       |
| Tri-n-butyl methyl<br>phosphonium iodide | E (mV)<br>ip (µA)      | -          | -          | -          | 90<br>-         | 50<br>0.95 | 45<br>1.65 | -<br>-     | 30<br>1,85 |
| .Triphenyl n-butyl                       | E <sub>p</sub> (mV)    | 0          | 70         | 150        | 110             | -          | 45         | 45         | 20         |
| phosphonium bromide                      | i <sub>p</sub> (μA)    | -          | 0.81       | 0.7        | 0.85            |            | 2.29       | 2.44       | 2.40       |
| Tri-n-butyl benzyl                       | E (mV)                 | 30         | 175        | 150        | 110             | 80         | 55         | 50         | 50         |
| phosphonium chloride                     | ip (µA)                | 1.85       | 0.93       | 1.42       | 1.48            | 1.65       | 1.9        | 1.95       | 1.85       |
| Triphenyl methallyl                      | E_ (mV)                | 60         | 65         | 130        | 80              | 50         | 40         | 30         | 25         |
| phosphonium chloride                     | ip (µA)                | 1.19       | 0.98       | 0.3        | 0.28            | 0.80       | 1.8        | 2.10       | 2.14       |
| Methyl triphenyl<br>phosphonium bromide  | E (mV)<br>ip (µA)<br>p | 10<br>0.83 | 55<br>1.08 | 50<br>1.25 | 20<br>1.38      | -          | 80<br>0.9  | 10<br>1.98 | 0<br>2.11  |
| Triphenyl ethyl<br>phosphonium iodide    | E_ (mV)<br>ip (μA)     |            | -          | -          | -               | 30<br>1.55 | 20<br>1.7  | 20<br>1.9  | 10<br>1.83 |
| Tetrabutyl phosphonium                   | E_ (mV)                | 0          | 185        | 130        | 100             | 70         | 45         | 50         | 35         |
| chloride                                 | ip (μA)                | 1.5        | 0.65       | 1.28       | 1.4             | 1.73       | 1.9        | 2.0        | 1.98       |
| Tetraphenyl arsonium                     | E_ (mV)                | 90         | 75         | 185        | 150             | 110        | 90         | 75         | 65         |
| chloride                                 | ip (µA)                | 0.88       | 0.625      | 1.5        | 1.8             | 2.0        | 2.4        | 2.48       | 2.35       |
| No ion-pairing<br>compound               | i <sub>p</sub> (⊬A)    | 1.0        | 1.02       | 1.1        | 1.4             | 1.6        | 1.75       | 1.875      | -          |

Note: In middle pH range with some compounds double peaks appear. Only main peak potentials and shifts are given.

#### CHAPTER 6

# VOLTAMMETRIC STUDIES AT STATIONARY GLASSY CARBON AND CARBON PASTE ELECTRODES IN STATIC SYSTEMS

#### INTRODUCTION

#### Glassy Carbon Electrode (G.C.E.)

The applications of glassy carbon electrode in electroanalytical chemistry have been reviewed recently by Van Der Linden and Dieker<sup>(50)</sup>. The topics covered include chemical and electrochemical aspects of the glassy carbon/electrolyte interface for both aqueous and non-aqueous electrolytes; analytical applications including voltammetry, stripping-voltammetry, amperometry, coulometry, potentiometry, chronopotentiometry; flow-through detectors, chemically modified electrodes; electrosynthesis and pretreatment techniques.

Glassy carbon electrodes were applied for the first time in electroanalytical chemistry by Zittel and Miller<sup>(166)</sup>. In comparison with other carbonaceous electrode materials both pyrolytic graphite and glassy carbon electrodes were found to have the largest available potential range. Alder et al (167) came to the same conclusion in their evaluation of a large number of electrode materials. For the anodic limit, defined as the potential at which the current becomes equal to one-half of the value of the peak current for the oxidation of  $10^{-3}$  M Fe (CN)<sub>6</sub><sup>4-</sup> (scan rate 300 mV s<sup>-1</sup>) they found the following values for glassy carbon: pH 1.0, + 1.3V vs S.C.E; pH 4.2, + 1.4V vs S.C.E; and pH 10.0, + 0.95V vs S.C.E. However, all these observations were based on voltammetric experiments in which the potential was changed relatively slowly. Dieker et al<sup>(29)</sup> showed that with the application of normal and differential pulse voltammetric modes much larger residual currents are observed because of chemical transformations of the surface. In this respect the carbon paste electrode was found to be preferable to the glassy carbon or the platinum electrode.

Although most attention has been paid to the applicability of glassy carbon electrodes in the positive potential range, it is important to note that rather negative potentials can be obtained even in aqueous electrolytes. Of the common mineral acids, nitric acid exhibits the most limited cathodic potential range, whereas hydrochloric acid is the most limited in the anodic range. For both these acids, the anion is probably reacting at the electrode. The widest usable potential span is obtained in phosphoric acid, whereas, sulphuric and perchloric acids show intermediate useful ranges<sup>(166)</sup>. In the negative potential region Weber and Volke<sup>(168)</sup> were able to reduce carbonyl group containing compounds in 50% (V/V) acetone-water mixtures at potentials as low as -2.0V vs S.C.E. The half-wave potentials were found to be only slightly more negative than those obtained at a dropping mercury electrode.

The voltammetric behaviour of several inorganic species has been studied since the pioneering work of Zittel and Miller<sup>(166)</sup> in 1965. In this section some important applications in the organic and biochemical fields are reviewed. The applications of G.C.E. as an electrochemical detector in HPLC will be discussed in the next chapter.

Fleet and Fouzder<sup>(169)</sup> have used a G.C.E. for the reduction of organolead compounds. In the case of differential pulse voltammetry the linear range of the calibration extends from  $2.5 \times 10^{-4}$  to  $10^{-7}$  M. This is an important application and could supplement other techniques in the determination of organometallic compounds in trace amounts in aquatic and natural environments. Jennings et al<sup>(170)</sup> have used linear cathodic sweep voltammetry for the determination of 2-ethylanthraquinone with a G.C.E.; the reduction of millimolar amounts in methanol, ethanol or propan-2-bl proceeds with a precision better than 2%.

The anodic voltammetric characteristics of 11 polynuclear aromatic hydrocarbons have been measured at stationary and rotated glassy carbon disc electrodes in sulpholane, and some of them in acetonitrile as well. The limit of detection with differential pulse voltammetry is about 5 x  $10^{-7}$  in sulpholane and 2 x  $10^{-8}$  m in acetonitrile<sup>(171)</sup>.

Chan and  $\operatorname{Fogg}^{(172)}$  developed voltammetric procedures for the determination of four analgesics, Ciramadol, Meptazinol, Dezocine and Pentazocine by linear sweep voltammetry at glassy carbon electrode. Although no cathodic or anodic waves were observed in aqueous media, a suitable anodic wave appeared in the ethanolic buffer (0.1M sodium acetate - 0.1M acetic acid in 98% ethanol). The calibration curves were found to be linear down to 10  $\mu$ g ml<sup>-1</sup>. Anti-inflammatory drugs Phenylbutazone and oxyphenbutazone have also been determined at the G.C.E. by Chan and  $Fogg^{(173)}$  by linear sweep and differential pulse methods. The proposed methods showed no interference from common tablet excipients such as lactose, microcrystalline cellulose, Amberlite IRP 88, magnesium stearate and starch. The effects of possible combinations with other drugs on the voltammograms were also studied. Of the five drugs studied only pentazocine interfered as it produces an anodic wave at +0.78V close to the oxidation peak of phenylbutazone. Rectilinear calibration plots were obtained down to the limit of detection of 0.5  $\mu$ g ml<sup>-1</sup>. Direct determination of phenylbutazone and oxyphenbutazone in biological fluids was impossible owing to serious interferences from a large number of compounds of both endogeneous and exogeneous origin. Prior separation is essential and an HPLC method with an electrochemical detector would prove valuable.

A differential pulse voltammetric determination of adrenaline at G.C.E. was reported by Ballantine and Woolfson<sup>(174)</sup>. Yao et al<sup>(175)</sup> investigated the voltammetric oxidation of adenine, adenosine, guanine and guanosine at the G.C.E. in aqueous solutions. In general, the nucleosides were oxidised at more positive potentials than their bases. The bases and their nucleosides were strongly adsorbed on the surface of glassy carbon at pH values around neutrality. At pH < 4 good linear relationships were obtained between the anodic peak current and the concentration. The differences between the peak potentials of each of the purine bases and their nucleosides were most pronounced at pH 2-4 so that simultaneous determination of purine bases and their nucleosides could be carried out in Britton-Robinson buffer in this pH range. Yao et al (176) also studied the anodic oxidation of deoxyribonucleic acid at the G.C.E.

Currents are allowed to reach steady state before measuring because of the low rate at which surface functional groups at rotated glassy carbon electrodes come into redox equilibrium.<sup>(177)</sup>. By doing so current transients are eliminated, and very low background currents may be achieved, permitting the quantitation of micromolar concentrations. Steady state voltammetry (SSV) was applied to the direct anodic oxidation of reduced nicotinamide adenine dinucleotide (NADH) in aqueous solution. When applied to NADH, the use of SSV prevents fouling of the glassy carbon surface. In addition, SSV gives a current limited plateau on the current-voltage curve for NADH, not normally observable with conventional scanning techniques.

Differential pulse voltammetry at the G.C.E. has been applied to the determination of ascorbic acid, pyridoxine and folic acid in a multivitamin preparation<sup>(178)</sup>. The individual vitamins all gave well-defined peaks in the anodic region with a linear response of peak current to concentration over the range  $10^{-6}$  to  $10^{-1}$ M. The water-soluble vitamins were extracted into aqueous solution and folic acid into dibasic potassium phosphate solution. Before determination of pyridoxine, the ascorbic acid peak was depressed by reaction with formaldehyde. Iron (11) present did not interfere with the analysis. The voltammetric method compared favourably in terms of accuracy and precision with official methods: it was found to be much simpler, with sample manipulation kept to a minimum.

#### Carbon Paste Electrode (C.P.E)

The useful potential range and the magnitude and reproducibility of residual currents in different media are the most important parameters governing the selection of an electrode material.

The need for a reproducible, easily renewable stationary electrode for the anodic region is great because the surface film formation and adsorption of reactants, intermediates, or products are all known to influence the reproducibility of peak current measurements. The use of carbon pastes (carbon or graphite intimately mixed with an organic liquid) as substitutes for noble metal electrodes offers several distinct advantages in solid electrode voltammetry. A new surface can be prepared in a few minutes before each run, simply by using a spatula. This method gives a variation coefficient of about 6% for the measurement of peak currents (179) but values as low as 2-3% (180)are attainable if care is exercised. The use of carbon paste electrodes was first reported by Adams in  $1958^{(181)}$ . The anodic limit for this electrode is about +1.3V vs. S.C.E in acid aqueous media, which is about the same as for pyrolytic graphite and glassy carbon.

An ideal pasting liquid would be completely water immiscible, of very low volatility and has no electroactive impurities. Among the liquids investigated were carbon tetrachloride, bromoform, benzene, mixed trimethyl- benzenes, ethylnaphthalene, bromonaphthalene and Nujol. The last two seem to present the best compromise of the desired characteristics.

Several varieties of carbon and graphite were evaluated. Nuchar, Norite and vegetable charcoal were found to be too gritty to give smooth pastes. Acheson Grade 38 powdered graphite and spectroscopic graphite (United Carbon Products) were ideally suited. Acetylene black, 50% compressed ("Shawingian Black") commonly employed as a battery mix was also found to be very satisfactory<sup>(179)</sup>. The terminology originally suggested placed CE (carbon electrode) followed by the type of pasting liquid, e.g. CE-BnP (carbon electrode-bromonaphthalene paste) and CE-NjP (carbon electrode-Nujol paste).

One of the most desirable characteristics of carbon paste electrodes is the extreme low level and flatness of the residual current over the entire useful potential range. The residual
currents encountered are much lower than those at noble metal electrodes where oxide formation and history effects are prevalent.

Starting with a fresh paste, no pretreatment is needed. In general, after a run, the electrode is simply washed with a stream of distilled water. In some cases only a slight stir in the solution sweeps away oxidation products before beginning the next run. On the other hand if film formation results it is wiser to fill the cavity with fresh paste after each run. Storage in an open beaker under distilled water is adequate for periods ranging from a week to a month.

The potentialities of carbon paste electrodes for cathodic reductions were investigated by Olson and Adams<sup>(182)</sup>. The electrode material shows a considerable hydrogen overpotential. Figure 6.1 shows current-voltage curves in a variety of supporting electrolytes to establish the usable potential range over which the CE-NjP could be used.





- A. 1M perchloric acid;
- B. 1M hydrochloric acid;
- C. 1M sodium acetate-acetic acid;
- D. 1M sodium perchlorate;
- E. 1M potassium chloride;
- F. 1M potassium nitrate;
- G. 1M ammonia-ammonium chloride;
- H. 1M sodium hydroxide

The greatest ranges are obtained in neutral and basic media. In general, the cathodic range extends from -1.2 to -1.4V vs. S.C.E., excluding strong acid media. This range is not as great as mercury but is a decided improvement over noble metals.

However, a relatively small but non-removable residual current is found on the cathodic side with  $CE-NjP^{(182)}$ . This is seen as a small wave between -0.4 and -0.6V. Attempts to remove this residual current completely were unsuccessful. It appears to be an oxygen wave, as suggested by the pH dependence and the fact that it decreases significantly on thorough deareation. It was reasoned that the wave was due to oxygen in the paste itself which could not be eliminated completely by solution deaeration. Accordingly, the Nujol was deaerated before mixing, the carbon was degassed and then an attempt was made to sorb nitrogen on the carbon at low temperatures. None of these treatments completely eliminated the residual current and it is believed that the mixing process which obviously is most conveniently done in the ordinary atmosphere would re-introduce oxygen in the paste. Fortunately, the residual wave has a very flat plateau and cathodic reductions could be carried out on top of this wave, i.e. using it as a baseline for peak current measurements.

Lindquist<sup>(183)</sup> found that by removing oxygen in a vacuum at high temperature and then blocking the surface of the carbon against further adsorption of oxygen, it was possible to improve the anodic potential range by 300-500 mV as compared with other graphite electrodes. In 0.1M sulphuric acid, the background was below 1  $\mu$ A up to a potential of about +1.7 volts vs. S.C.E. The cathodic range did not change as compared with a wax impregnated graphite electrode. The oxidation peaks obtained with this electrode for adenine are very reproducible even at 10<sup>-7</sup>M level.

The anodic oxidation of the triphenylmethane dyes, crystal violet, malachite green and brilliant green, has been studied at carbon paste and platinum electrodes and the oxidation pathways examined in acidic buffers (184, 185). The central carbon atom of triphenylmethane dyes is shown to be usually facile.

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Lindquist<sup>(186)</sup> investigated the Fe(W)/Fe(W)cyanide, quinonehydroquinone and phenol systems using seven carbon paste electrodes (i.e. seven different pasting liquids). The voltammograms were strongly influenced by the type of pasting liquid used. None of the electrodes had ideal properties. The electrodes which gave the most well-developed voltammograms had the smallest anodic potential ranges. The smallest differences between the electrodes were obtained on the system Fe(H)/Fe(H)cyanide, and the greatest on quinone-hydroquinone systems. In the case of phenol the half-peak potentials were rather independent of the type of paste, but the currents were strongly influenced by this factor.

The sequential oxidation of the amino group and the reduction of the nitro group in p-nitroaniline has been carried out due to the availability of a wide potential range for both anodic and cathodic reactions at the carbon paste electrode (187). The ratio of limiting currents,  $i_C/i_a$ , was close to 3. The nitro reduction was shown to be a 6-electron process using cyclic voltammetry. It was established that the amine oxidation is an overall 2-electron process. This novel determination of  $n_T$  using such a bi-functional molecule does not require a knowledge of diffusion coefficients and may be applicable to other solid electrode mechanism studies.

The voltammetric determination of ascorbic acid was carried out using a carbon paste electrode (188). The peak current was proportional to concentration of ascorbic acid in the range  $10^{-6}$ to  $10^{-3}$ M and the reproducibility was better than ±1%. The interference of chloride and sulphur compounds, substituted phenols, reductones, tin(11) and manganese(11) were evaluated. A method is proposed for determining ascorbic acid in the presence of excess of iron. Comparative titrimetric determination of ascorbic acid in some fruits, vegetables and beverages gave higher results than the voltammetric method.

Söderhjelm and Lindquist<sup>(189)</sup> developed a fast and simple method for the determination of pyridoxine in pharmaceutical preparations. The only interferences are from ascorbic acid and iron(11), both of which were removed by ion-exchange chromatography. The voltammetric method had a better relative standard deviation compared with a proposed colorimetric method employing 2,6-dichlorophenolindophenol.

Tocopherols were determined in vegetable oils, foods and pharmaceuticals by a newly developed carbon paste electrode prepared by mixing graphite powder containing 5% m/m ceresin wax with silicone oil MS 510 in the proportions 5:3 m/m to give a homogeneous paste (190). The samples were saponified and the unsaponifiable fraction was extracted and determined voltammetrically. No elaborate purification method was necessary as the substances that interfere with photometric procedures were electrochemically inactive in the potential range of operation. The peak current,  $i_p$ , was a linear function of concentration of tocopherol in the concentration range  $3 \times 10^{-6}$  to  $7 \times 10^{-4}$ m, with a 0.2M solution of sulphuric acid in 75% ethanol as solvent.

As the conducting graphite particles are mixed with a viscous organic liquid, the use of carbon paste electrode has been limited to aqueous solutions. This limitation, as applied to water-insoluble, electroactive organic compounds, was circumvented by dissolving these organics in the electrode itself<sup>(191,192)</sup>. A necessary requirement was that the organic compound (0) forms an ion along the path of electrolysis as

0 (insoluble) + ne<sup>-</sup>  $\rightarrow$  0<sup>n-</sup> (soluble) 0 (insoluble) - ne<sup>-</sup>  $\rightarrow$  0<sup>n+</sup> (soluble)

The behaviour of the system is very much analogous to the stripping of metal from a metal amalgam electrode to the metal in solution. The wave characteristics are similarly affected by adsorption on the electrode surface and by the extent of solubility of neutral

species in the electrode material. Ferrocene was most extensively used since the ferrocene-ferricinium couple behaved most reversibly and also exhibited desirable solubility properties.

A non-aqueous carbon paste electrode was reported in  $1965^{(193)}$ . The problem of preferential wetting of graphite by solvent was circumvented by the addition of a surface active agent (sodium lauryl sulphate) to the electrode material. The properties of this electrode material were studied in acetonitrile, nitromethane, and propylene carbonate. Background currents of less than 3  $\mu$ A were obtained from +1.1 to -0.7 volts vs. S.C.E. in acetonitrile less than 2  $\mu$ A from +1.0 to -0.7 volts in nitromethane and less than 3  $\mu$ A in the region +0.86 to -0.6 volts in propylene carbon-ate. These backgrounds are much lower and more reproducible than those obtained at platinum.

#### EXPERIMENTAL

# ANODIC VOLTAMMETRY AT A GLASSY CARBON ELECTRODE

Voltammetric behaviour of the food colours was studied in pH2 Britton-Robinson buffer, 0.01 and 0.1M sulphuric acid. The measurements were made with a PAR 174A polarographic analyser with the glassy carbon electrode replacing the dropping mercury electrode. The samples for voltammetry were prepared as described in the last chapter except that there was no necessity for deoxygenation before running the voltammograms. For studies on simulated soft drink samples, 30 ml of the basic uncoloured soft drink syrup and 20 ml of pH 1.9 Britton-Robinson buffer were pipetted directly into a dry Metrohm cell (EA 875-20)<sup>(161)</sup>; successive aliquots of concentrated solutions of colouring matters were added by means of a microsyringe of 10 or 100  $\mu$  capacity, and solutions stirred by means of a magnetic stirrer prior to scanning.

It was necessary to clean the glassy carbon electrode between scans to obtain reproducibility. A cleaning procedure similar to the one used by Chan and Fogg(172,173) was found to be satisfactory.

(v/v) ag-The electrode surface was washed first with 95% ethanol and then with chloroform or dichloromethane, then cleaned carefully with a non-abrasive tissue soaked in chloroform or dichloromethane and finally dried with a dry tissue. When not in use the electrode was stored clean in a boiling-tube fitted with a groundglass joint.

Experiments were carried out with the colouring matters to establish whether full voltammetric signal is obtained after they have been adsorbed on to a polyamide support (MN-Polyamid SC6-AC, Machery, Nagel and Co., Düren, W. Germany), and then removed again by extraction with methanolic sodium hydroxide solution<sup>(97)</sup>. Extractions were also made from soft drinks based on a lemonade syrup and the blackcurrent health-drink syrup. The eluting methanolic sodium hydroxide solution was acidified with methanol-acetic acid (1 + 1), v/v, evaporating to dryness and dissolving the residue in pH 2 Britton-Robinson buffer.

### Results and Discussion

Most permitted synthetic food colours in pH2 Britton-Robinson buffer give a well-defined DC voltammetric peak in the potential range +0.6 to +1.1V v.s. S.C.E. at a stationary glassy carbon electrode, except for Erythrosine BS which precipitates at this pH. Voltammetric waves were also obtained in 0.01 and 0.1M sulphuric acid. This media showed no improvement over voltammograms obtained using Britton-Robinson buffer and therefore the discussion is restricted to solutions made up in Britton-Robinson buffer.

The results of linear sweep voltammetry are summarised in Table I. Most of the colours give peaked waves, except, Patent Blue V and Brilliant Blue FCF, which give a narrow plateau suitable enough for quantitative analysis. The plateaux of Chocolate Brown HT and Yellow 2G are very close to the cut-off potential, that of Chocolate Brown HT, in particular, is not suitable for quantitative work. Erythrosine BS precipitates in pH2 buffer; a small peak is observed in pH range 5-9 and this is suitable for quantitative work.

#### TABLE I

#### VOLTAMMETRY OF FOOD COLOURING MATTERS AT A STATIONARY GLASSY CARBON ELECTRODE IN pH 2 BRITTON - ROBINSON BUFFER

|                    |     |      |                              | Linear-sweep voltan                    | metry                                        | Comparison with linear             | sweep voltammetry           |
|--------------------|-----|------|------------------------------|----------------------------------------|----------------------------------------------|------------------------------------|-----------------------------|
| Coloquing mat      | ler |      | Peak potential/<br>V         | Rectilinearity of<br>calibration graph | Quality of peak<br>(100 µg m) <sup>1</sup> } | Differential-pulse<br>voltannietry | Normal-pulse<br>voltammetry |
| Tartrazine         |     |      | Lus                          | Genns                                  | Smatt work                                   | Similar                            | Similar                     |
| Oninatine Yellow   |     |      | Ê.05                         | Fairly most                            | Small neak                                   | Not as good                        | Darkess                     |
| Sellow 2G          |     |      | 0.96                         | Not very grant                         | Small plateau                                | Not as cond                        | Underst                     |
| Sunset Yellow FCF* | ••  |      | (2,04)                       | tion                                   | Medium peak                                  | Larges peak                        | Small peak, no              |
| Campaisine         |     |      | 0.81                         | Great                                  | Medium peak                                  | Broad beak                         | Usedawa                     |
| Anaranth           |     |      | 11.88                        | Ganal                                  | Medium neak                                  | Similar                            | United                      |
| Ponceau d R+       |     | •••  | (1.)4(3                      | Gand                                   | Medium weak                                  | Langer neak                        | Hardwar                     |
| Erythrosme BS      |     | •••  | Precipitates a<br>at off 5-9 | t pH 2, small peak                     | and the second second                        | Slight improvement<br>at off 7     | Useless                     |
| Red 2011           |     |      | 28.0                         | Gant                                   | taree wak                                    | Similar                            | the loss                    |
| Patent Blue V      |     |      | 0.42                         | tional                                 | Platean                                      | Introved plateau                   | Userhoss                    |
| Indigo Carminet    |     | - 11 | 0.67                         | tional                                 | Large neak                                   | Smilar                             | la has                      |
| Brilliant Blue FCF | ••  |      | 6,90                         | Fairly good                            | Rising plateau                               | Similar, no<br>morevement          | Useless                     |
| Green St           |     |      | 66, 861                      | Genul                                  | Medium teak                                  | Similar                            | Useless                     |
| Hown FK            |     |      | 0.82                         | Good                                   | Snuth work/ulateau                           | Similar                            | Useless                     |
| Churdate Brown HT  |     | ••   | 0.80                         | 164                                    | Small rising of depart                       | No insprovement                    | No improvement              |
| Black PN           | ••  |      | 0.86                         | Good                                   | Small peak                                   | Similar                            | Usekss                      |

Good peaks also at pH 5.
† Good peaks also at pH 5, 7 and 9.
‡ The shape is slightly better at pH 5 and 7.



Effect of adsorption and of cleaning electrode on linear sweep voltammograms of Red 2G and tartazine at the G.C.E. FIGURE 6.2 The scan sequence is indicated on the voltammograms. Red 2G and tartrazine concentration = 40  $\mu g$  ml^1 (a) Red 2G, electrode not cleaned; (b) Red 2G, electrode cleaned; (c) tartazine, electrode not cleaned; and (d) tartrazine, electrode cleaned.

The effect of adsorption on subsequent scans when the electrode is not cleaned is illustrated in Figure 6.2 for Red 2G and Tartrazine, and is compared with results obtained with a properly cleaned electrode. The coefficient of variation (six determinations) at the lower level of 20  $\mu$ g ml<sup>-1</sup> was typically less than 2%.

In Figure 6.3 voltammograms obtained in producing calibration graphs for the direct determination of Sunset Yellow FCF and Tartrazine singly and in admixture in a lemonade syrupbased drink are shown. The concentration levels studied are in the range normally present in Beecham's sparkling orangeade drink. The calibration graphs are rectilinear but the peak height of each colouring matter is affected by the presence of the other colouring matter. This is clearly seen with the addition of Tartrazine to the Sunset Yellow FCF solution: the change in peak height for Sunset Yellow FCF is most marked at low concentrations of Tartrazine. In determining Sunset Yellow FCF in Sunset Yellow FCF-Tartrazine mixtures, calibration voltammograms must be run in the presence of Tartrazine, and vice versa.

Uncoloured soft drinks based on a blackcurrant health-drink syrup gave a large broad voltammetric peak at +0.64V vs. S.C.E. that was several orders of magnitude larger than would be expected for the small amount of natural colour present; this peak must have been caused by another constituent. Vitamin C gives an anodic peak at this potential and is the most probable cause of this interference. Attempts to separate the Amaranth from the interferent on a polyamide column were unsuccessful.

Voltammetric peaks at higher pH values are in general less well-defined than at pH2 as can be seen from Table II, which compares the peak potentials and quality of peaks for glassy carbon and carbon paste electrodes at pH values 2, 5, 7 and 9.

In view of the effect that the addition of tetraphenylphosphonium chloride has on the cathodic polarographic waves of food colouring matters, its effect on anodic voltammograms was studied. There was no effect on peak potentials, peak currents or the adsorp-



FIGURE 6.3 Linear sweep voltammetry of Sunset Yellow FCF and tartrazine in a lemonade based soft drink at the G.C.E. (a) Sunset Yellow: A to E, 0, 10, 20, 30 and 40  $\mu$ g ml<sup>-1</sup>, respectively; (b) tartrazine: A to E, 0, 10, 20, 30 and 40  $\mu$ g ml<sup>-1</sup> respectively; (c) Sunset Yellow: A to E, 0, 5, 10, 15 and 20  $\mu$ g ml<sup>-1</sup> respectively; tartrazine 20  $\mu$ g ml<sup>-1</sup> (d) tartrazine: A to E, 0,5,10, 15 and 20  $\mu$ g ml<sup>-1</sup> respectively; Sunset Yellow FCF 20  $\mu$ g ml<sup>-1</sup>.

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| TABLE II: | ANODIC PEAK POTENTIA! | S AT | CARBON PASTE | (CP) | AND GLASSY | CARBON (GC) | ELECTRODES | AT VARIOUS | pH VALUES |
|-----------|-----------------------|------|--------------|------|------------|-------------|------------|------------|-----------|
|           |                       |      |              | • •  |            |             |            |            |           |

|                       | pH 2.0   |                                                                      | pH 5.0           |                    | pH 7.0            |                   | pH 9.0            |                            |
|-----------------------|----------|----------------------------------------------------------------------|------------------|--------------------|-------------------|-------------------|-------------------|----------------------------|
|                       | GC       | CP                                                                   | GC               | СР                 | GC                | СР                | GC                | СР                         |
| Amaranth              | +0.84V** | +0.84V**                                                             | -                | +0.76V**           | -                 | +0.70V**          | +0.64V            | +0.64V*                    |
| Carmoisine            | +0.84V   | +0.82V**                                                             | -                | +0.70V*            | +0.64V            | +0.60V*           | +0.54V*           | +0.52V*                    |
| Red 2G                | +0.78V** | +0.72V**<br>+0.80V                                                   | +0.71V**         | +0.64V*<br>+0.70V  | +0.66V**          | +0.59V*<br>+0.64V | +0.61V*           | +0.54V*                    |
| Ponceau 4R            | +0.76V** | +0.74V*                                                              | +0.76V*          | +0.62V*            | +0.76V            | +0.56V*           | +0.60V            | +0.50V*                    |
| Erythrosine BS        | Preci    | pitates                                                              | +0.75V*          | . +0.72V*          | +0.75V**          | +0.75V*           | +0.76V*           | +0.78V*                    |
| Yellow 2G             | +1.02V*  | +0.96V**                                                             | -                | ·+0.90V*           | -                 | +0.76V*           | -                 | +0.70V*                    |
| Quinoline<br>Yellow   | +1.00V*  | +0.90V**                                                             | -                | +0.64V<br>+0.80V** | -                 | +0.73V**          | +0.76V            | +0.66V**                   |
| Tartrazine            | +1.04V** | +1.07V*                                                              | -                | +0.96V*            | -                 | +0.89V*           | -                 | +0.78V<br>+0.94V           |
| Sunset Yellow<br>FCF  | *0.84V** | +0.77V**                                                             | +0.78V**         | +0.70V**           | +0.76V            | +0.64V**          | +0.64V            | +0.56V*                    |
| Green S               | +0.80V*  | +0.78V**                                                             | +0.74V*          | +0.72V*            | +0.68V*           | +0.66V*           | +0.64V*           | +0.62V*                    |
| Patent Blue V         | +0.84V*  | +0.82V*                                                              | +0.80V           | +0.62V<br>+0.84V*  | +0.50V<br>+0.78V* | +0.52V<br>+0.77V* | +0.45V<br>+0.76V* | +0.48V<br>+ <b>D</b> .75V* |
| Brilliant Blue<br>FCF | +0.90V*  | +0.90V*                                                              | +0.62V<br>+0.90V | +0.60V<br>+0.92V*  | +0.56V*<br>+0.92V | +0.54V<br>+0.88V* | +0.56V*<br>+0.90V | +0.56V<br>+0.88V*          |
| Indigo Carmine        | +0.67V** | +0.62V**                                                             | +0.56V*          | +0.47V*            | +0.50V*           | +0.41V*           | +0.44V            | +0.36V*                    |
| Chocolate Brown<br>HT | ∿+0.80V  | +0.76V<br>+0.86V*                                                    | +0.70V*          | +0.74V*            | +0.70V*           | +0.70V*           | +0.62V            | +0.62V                     |
| Brown F <b>K</b>      | +0.80V*  | Peak dis <b>a</b> :-<br>pears on rep-<br>eated scans<br>+0.78-+0.84V | +0.64V*          | +0.76V*            | <b>+</b> 0.62V*   | +0.46V*           | +0.62V*           | +0.44V*                    |
| Black PN              | +0.84V*  | +0.83V**                                                             | -                | +0.74V**           | -                 | +0.68V**          |                   | +0.90V*                    |

\*\* Sharp peak

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\* Other analytically useful peaks - indeterminate

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tion characteristics of the glassy carbon electrode.

#### NORMAL PULSE AND DIFFERENTIAL PULSE VOLTAMMETRY

The results of normal and differential-pulse voltammetric studies on permitted food colours are compared with those of the linear sweep mode in Table I. A 50  $\mu$ g ml<sup>-1</sup> solution of each dye was used for all colours. A pulse amplitude of 50 mV and pulse delay time of 0.5 sec was used in the pulse modes.

Linear sweep voltammograms are generally peak-shaped owing to the diffusion characteristics at the stationary electrode. For differential-pulse voltammograms the peak appearance would normally be enhanced, giving improved resolution and ease of measuring peak currents. For most food colours, however, very little improvement was observed. Figure 6.4 illustrates the shape of voltammograms in the three modes for Sunset Yellow FCF, Ponceau 4R and Tartrazine. On repeated scans there is a drop in signal or distortion of peaks in all three modes except for Sunset Yellow FCF in the normal pulse mode.

In the normal pulse mode at a solid electrode, a plateaushaped voltammogram would be expected for a reversible electrode reaction and a slightly peak-shaped voltammogram for an irreversible electrode reaction<sup>(17)</sup>. Further, the decrease in wave or peak height between scans due to adsorption would be expected to be minimal<sup>(194)</sup>. In the normal pulse voltammetry of food colours, the oxidation wave generally appeared as a shoulder on a sharply rising base-line. In some instances the wave heights were not altered significantly over several scans, but nevertheless the waves were unsuitable for analytical purposes except for Sunset Yellow FCF, which gave a slightly peaked normal pulse voltammogram, the height of which remained constant for twelve consecutive scans.

#### CATHODIC VOLTAMMETRY AT GLASSY CARBON ELECTRODE

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Solutions for cathodic voltammetry were prepared as before but were deoxygenated for 10 minutes before running scans by passing nitrogen. The pH was adjusted to higher values directly in the



FIGURE 6.4

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4 DC, NP and DP voltammograms of Ponceau 4R, Sunset Yellow FCF and Tartrazine at the G.C.E. Colour concentration, 50  $\mu$ g ml<sup>-1</sup>; scan rate, 5 mV s<sup>-1</sup>.

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cell and only a brief deoxygenation was then required. During the period of potential scan an atmosphere of nitrogen gas was maintained over the surface of solution in the cell.

Linear sweep voltammetry was carried out at a sweep rate of 5 mV s<sup>-1</sup> with zero damping. Cyclic voltammetry was carried out at the same sweep rate, the scan direction simply being reversed at the end of the forward scan. Cleaning the glassy carbon electrode between scans was found to be necessary as in the case of the anodic studies.

The results of cathodic voltammetry of food colours with glassy carbon and carbon paste electrodes at pH values 2, 5, 7 and 9 are summarised in Table III. The peak potentials reported are for the initial peak. On successive scans at higher concentrations there were slight shifts in peak potential values. The potentials are compared with the half-wave potentials for the respective colours observed at the dropping mercury electrode.

Cathodic voltammetric peaks of food colours at pH2 at the stationary glassy carbon electrode are generally better resolved from the cut-off potential than are the corresponding anodic peaks. Selectivity is also greater, particularly for distinguishing between the permitted yellow colours.

Two food colours, namely, Chocolate Brown HT, and Brilliant Blue FCF, give unsatisfactory anodic peaks. The cathodic peak of Brilliant Blue FCF is an improvement over the anodic peak. The cathodic peak of Chocolate Brown HT, like its anodic peak, is best obtained in pH7 buffer: here again the cathodic peak is slightly better. The anodic and cathodic peaks for the two colours are compared in Figure 6.5.

The yellow colours are particularly well-separated cathodically: Sunset Yellow FCF (-0.15V), Tartrazine (-0.33V), Yellow 2G (-0.42V) and Quninoline Yellow (-0.81V). The corresponding peak potentials under anodic conditions are +0.90V, +1.08V, +0.96V and +1.05V.

The cathodic peaks of the red food colours at pH2 are close together as are the anodic peaks. Slight variations in peak charac-

(CP) TABLE III: CATHODIC VOLTAMMETRY OF FOOD COLOURS AT GLASSY CARBON (GC) AND CARBON PASTE ELECTRODES AND COMPARISON WITH HALF-WAVE POTENTIALS AT DROPPING MERCURY ELECTRODE (DME)

|                       |                          | рН 2.0                   |                           | рН 5                           | 5.0                       | рН 7.                                  | .0                        | рН 9                                    | ).0                       |
|-----------------------|--------------------------|--------------------------|---------------------------|--------------------------------|---------------------------|----------------------------------------|---------------------------|-----------------------------------------|---------------------------|
|                       | E <sub>p</sub> (V)<br>GC | E <sub>p</sub> (V)<br>CP | E <sub>1</sub> (V)<br>DME | E <sub>p</sub> (V)<br>GC       | E <sub>1</sub> (V)<br>DME | E <sub>p</sub> (V)<br>GC               | E <sub>1</sub> (V)<br>DME | E <sub>p</sub> (V)<br>GC                | E <sub>1</sub> (V)<br>DME |
| Tartrazine            | -0.33**                  | -0.36**                  | -0.17                     | -                              | -0.45                     | -0.56*                                 | -0.60                     | -0.52                                   | -0.71                     |
| Quinoline Yellow      | -0.81**                  |                          | -0.72                     | -0.50<br>(Rising<br>base-line) | -0.93                     | (Plateau)<br>-0.54*<br>(Rising Plateau | -1.08<br>(ب               | (Rising plate<br>-0.53*<br>(Broad hump) | eau)<br>-1.16             |
| Yellow 2G             | -0.42**                  |                          | -0.30                     | -0.70*                         | -0.48                     | -0.72                                  | -0.60                     | -0.48                                   | -0.73                     |
| Sunset Yellow FCF     | -0.15**                  | -0.08**                  | -0.10                     | -0.38<br>-0.62**               | -0.36                     | -0.55<br>-0.76**                       | -0.52                     | -0.84*                                  | -0.61                     |
| Carmoisine            | -0.14**                  | -0.09**                  | -0.09                     | -0.60*                         | -0.30                     | -0.62*                                 | -0.41                     | -0.41*                                  | -0.53                     |
| Amaranth              | -0.18**                  | -0.14**                  | -0.10                     | -0.76*                         | -0.30                     | -0.76*                                 | -0.44                     | -0.78*                                  | -0.55                     |
| Ponceau 4R            | -0.12**                  | -0.18                    | -                         | (Plateau)<br>-0.72*            | -0.54                     | -0.58*                                 | -0.71                     | -0.58                                   | -0.78                     |
| Erythrosine BS        | Pro                      | ecipitates               |                           | -0.76*                         | -0.65                     | -0.62*                                 | -0.83                     | -0.58*<br>(Plateau)                     | -1.03                     |
| Red 2G                | -0.22**                  | -0.20**                  | -0.25                     | -0.62**                        | -0.48                     | -0.75**                                | -0.64                     | -0.81**                                 | -0.74                     |
| Patent Blue V         | -0.37*                   | -0.39                    | -0.48                     | -0.66**                        | -0.70                     | -0.77**                                | -0.80                     | -0.80*                                  | -0.92                     |
| Indigo Carmine        | -0.08**<br>-0.48         | -0.09**                  | -                         | -0.44*<br>(Plateau)            | -                         | -0.49*                                 | -                         | -0.53**                                 | -0.40                     |
| Ərilliant Blue<br>FCF | -0.46*<br>(Plateau)      |                          | -0.51                     | -0.64*<br>-0.76*               | -0.76                     | -0.64*<br>(Broad hump)                 | -0.92                     | -0.64*<br>(Broad hump)                  | -1.10                     |
| Green S               | -0.43*                   |                          | -0.44                     | -0.63**                        | -0.62                     | -0.60                                  | -0.72                     | -0.68*                                  | -0.78                     |
| Brown FK              | -0.33**                  | -0.32**                  | -0.19                     | -0.52**                        | -0.34                     | -0.68*                                 | -0.52                     | -0.85*<br>(Plateau)                     | -0.60                     |
| Chocolate Brown<br>HT | -0.45<br>(Broad hump)    |                          | -0.02<br>-0.14            | -0.60                          | -0.22<br>-0.38            | -0.66*<br>(Plateau)                    | -0.38<br>-0.51            | -0.64*<br>(Plateau)                     | -0.52<br>-0.70            |
| Black PN              | -0.21*                   | -0.16                    | -0.11                     | -0.66*<br>(Plateau)            | -                         | -0.70                                  | -0.48                     | -0.50                                   | -0.66                     |

# TABLE III (continued):

Sharp peaks \*\*

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Not sharp but analytically useful \*

Maximum fluctuations to more negative potential:

- a)
- at GC: Yellow 2G (pH 2), 120 mV; carmoisine (pH 2), 80 mV; amaranth (pH 2 and 9), 220 and 80 mV. at CP; Tartrazine, 30 mV; Sunset Yellow, 40 mV; Carmoisine, 60 mV; Red 2G, 60 mV; Brown FK, 40 mV (all at pH 2). b)

The peak potentials quoted in the Table are for the first scan.



FIGURE 6.5 Comparison of anodic and cathodic peaks of Chocolate Brown HT (pH7) and Brilliant Blue FCF (pH2) at the G.C.E.

teristics such as the presence of the small post-peak observed for Red 2G, or the use of spiking with known food colours can aid identification. Adjusting the pH of solutions after measurement at pH2 can also be used. Thus at pH9 carmoisine gives a very small plateau at -0.41V, Ponceau 4R a rather broad peak at -0.58V, amaranth a broad peak at -0.78V and Red 2G a sharp peak at -0.81V. Erythrosine, which gives a good plateau at -0.58V at pH9, will already have been identified as it precipitates at pH2. The anodic and cathodic voltammograms of the red colours at pH9 are compared in Figure 6.6.

The blue food colours can be distinguished from each other by peak position (in the case of indigo carmine) and peak characteristics. Indigo carmine and Patent Blue V exhibit post peaks. Green S has a sharp peak at -0.43V, whilst Brilliant Blue FCF has a plateau at -0.46V. If the pH of the solutions are adjusted to 5, Brilliant Blue FCF exhibits two peaks of equal height at -0.64 and -0.76V, whereas Green S has a single sharp peak at -0.63V.

The peak potentials of the brown and black colouring matters are well separated and their peak characteristics differ.

## CYCLIC VOLTAMMETRY

Cyclic voltammograms of food colours can be useful in the identification of colours especially the range of blue colouring matters. Colours having an azo structure showed irreversibility i.e. lack of peak on reverse scan as is seen in Figure 6.7 for both anodic and cathodic initial scans. Some reversibility is observed in the oxidation of the triphenylmethane food colours Green S, Patent Blue V and Brilliant Blue FCF as they show a small peak on the reverse scan as shown in Figure 6.8. The indigoid food colour, is unique in showing a large peak on the reverse scan of the cathodic peak. The cyclic voltammograms for indigo carmine are shown in Figure 6.9.



FIGURE 6.6 Comparison of anodic and cathodic voltammograms of Red colours at pH 9.0 at the G.C.E.



FIGURE 6.7 Anodic and cathodic cyclic voltammograms of Red 2G and Ponceau 4R at pH 2.0 at the G.C.E. Colour concentration 50  $\mu$ g ml<sup>-1</sup>.

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FIGURE 6.8 Cyclic voltammograms of Triphenylmethane colours Patent Blue V, Green S and Brilliant Blue FCF at pH 2.0 at the G.C.E. Colour concentration 50  $\mu$ g ml<sup>-1</sup>

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## ANODIC VOLTAMMETRY AT CARBON PASTE ELECTRODE

The carbon paste was prepared by mixing 2-5 gm of spectroscopically standardised graphite powder, pellatable Grade I (Johnson Matthey Chemicals Ltd) with 1.0 ml of liquid paraffin (sp. gr. 0.850 - 0.865) in a mortar, and was stored in a sample tube. The electrode used EA 287 was supplied along with the Metrohm Flow detector cell EA 1096. The circular cavity of the electrode was filled with a Teflon spatula and pressed against a filter paper to give a smooth shiny surface. The excess paste was carefully removed from the sides with the teflon spatula and tissue paper. Every time a different colour was investigated the cavity was filled with fresh paste. When not in use the electrode was stored with its end dipping in distilled water.

The solutions were prepared and voltammograms run as before. The cleaning of the electrode surface was done simply by directing a stream of water from a wash bottle on the sides so as not to disturb the electrode face. The sides were wiped dry with tissue and the droplet on the end was carefully soaked. It was found that if proper care is not taken in washing the electrode signals were affected seriously.

Adsorption at the electrode lead to drop in signal height and in some cases wave distortion, with the exception of Patent Blue V, as is illustrated in Figure 6.10 which compares successive scans with and without cleaning the electrode surface for Green S, Ponceau 4R and Patent Blue V. It was found that the reproducibility of peak heights was quite good for Patent Blue V even without cleaning the electrode surface between scans.

The standard deviation for ten successive scans with intermittent cleaning, ranged from 0.01 to 0.6% and coefficient of variation from 1.2 - 11.4% as indicated in Table IV. Half of the colours give coefficient of variation values below 5% and only two, namely, Yellow 2G and Chocolate Brown HT give values greater than 10%. The coefficient of variation for Patent Blue V was quoted for scans without intermittent electrode cleaning. It was observed.



FIGURE 6.10

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Effect of adsorption and cleaning on linear sweep voltammograms of Green S, Ponceau 4R and Patent Blue V, at the C.P.E. Colour concentration, 50  $\mu g$  ml^1.

|                     | Without cleaning                   |                 | With cleaning                  |                       |
|---------------------|------------------------------------|-----------------|--------------------------------|-----------------------|
|                     | Loss of signal<br>after five scans | Mean<br>current | Coefficient of<br>variation ** | Rectili-<br>linearity |
|                     | (%)                                | μA**            | (%)                            |                       |
| Amaranth            | 41                                 | 3.74            | 3.1                            | Good                  |
| Carmoisine          | 47                                 | 3.23            | 3.2                            | Good                  |
| Red 2G              | 67<br>(peak splits)                | 2.94            | 6.8                            | Good                  |
| Ponceau 4R          | 53                                 | 1.47            | 4.2                            | Good                  |
| Yellow 2G           | 47                                 | 5.58            | 10.8                           | Good                  |
| Quinoline Yellow    | 61                                 | 1.66            | 3.3                            | Fair                  |
| Tartrazine          | 40                                 | 5.93            | 1.9                            | Fair                  |
| Sunset Yellow FCF   | 44                                 | 3.68            | 10.3                           | Fair                  |
| Green S             | 42<br>(peak splits)                | 2.42*           | 1.4                            | Fair                  |
| Patent Blue V       | <1                                 | 0.79*           | 2.2*                           | Good                  |
| Brilliant Blue FCF  | 15                                 | 0.86            | 1.2                            | Good                  |
| Indigo Carmine      | 63                                 | 3.32            | 7.1                            | Good                  |
| Chocolate Brown HT+ | 50                                 | 0.25            | 11.4                           | Fair                  |
| Brown FK++          | 29                                 | -               | -                              | Bad                   |
| Black PN            | 45                                 | 2.00            | 6.2                            | Bad                   |
| · .                 |                                    |                 |                                |                       |

TABLE IV: REPRODUCIBILITY OF ANODIC PEAK CURRENTS AT CARBON PASTE (pH2)

- \* without cleaning
- \*\* 10 determinations
- + peak ill-defined
- ++ peak shape distorts on repeated scans

that for Red 2G a small post peak is present and the signal becomes a double peak on successive scans without intermittent electrode cleaning. The second peak remains suppressed when the electrode surface is cleaned between scans. The distortion in the case of Green S has already been noted. The peaks of Brown FK get flatter and finally become unstable analytically on successive scans even with electrode cleaning.

The peaks obtained with the carbon paste electrode appear at more or less the same potential as for the glassy carbon electrode with only a few exceptions such as Carmoisine at pH 5.0; Ponceau 4R at pH7 and Sunset Yellow FCF at pH 2.0. The maximum separation between peak potentials at the two electrodes seems to be about 200 mV but at other pH values the peaks generally coincide.

Sharp peaks are obtained for Amaranth (pH 2-7), Quinoline Yellow (pH 2-9) and Black PN (pH 2-7) at the carbon paste electrode. No noticeable peaks were observed for the three colours in the quoted pH ranges at the glassy carbon electrode. For Carmoisine (pH 2-7), Ponceau 4R (pH 7-9), Tartrazine (pH 5-7), Yellow 2G (pH 5-9), Patent Blue V (pH 5), and Indigo Carmine (pH 9) the use of carbon paste electrode makes the peaks analytically useful as can be seen in Figure 6.11.

All the red colours, Yellow 2G, Patent Blue V and Indigo Carmine give rectilinear calibration plots in the concentration range 0-50  $\mu$ g ml<sup>-1</sup> in pH2 Britton-Robinson buffer. On the other hand Brilliant Blue FCF, Brown FK and Black PN give poor calibration plots.

The calibration of Sunset Yellow FCF and Tartrazine present as a mixture was carried out in a simulated uncoloured lemonade syrup in the concentration range 0-20  $\mu$ g ml<sup>-1</sup> as in the case of similar calibrations with the G.C.E. Rectilinear calibrations were obtained but as before the presence of one colour affected the peak height of the other as can be seen in Figure 6.12.





FIGURE 6.12 Linear sweep voltanmetry of Sunset Yellow FCF and Tartazine in a lemonade syrup based soft drink at the C.P.E. (a) Sunset Yellow FCF; 0, 5, 10, 15 and 20 μg ml<sup>-1</sup>; tartrazine 20 μg ml<sup>-1</sup>; (b) Tartrazine: 0, 5, 10, 15 and 20 μg ml<sup>-1</sup> respectively; Sunset Yellow FCF 20 μg ml<sup>-1</sup>.

In general, cyclic voltammograms at the carbon paste electrode showed near-irreversible oxidation with the notable exception of Patent Blue V (pH2), Green S (pH2), and Ponceau 4R (pH 2-5), which show corresponding peaks on reverse scan as can be seen in Figure 6.13. Otherssimply show small humps or just plain reverse scans. Comparable cyclic voltammograms were observed for Patent Blue V and Green S with the G.C.E. except Ponceau 4R which showed just a hump on reverse scan.

# CATHODIC VOLTAMMETRY AT CARBON PASTE ELECTRODE

With the carbon paste electrode scans in the cathodic potential range can be carried out with the added advantages mentioned before. The cathodic range is limited to around -0.4V vs. S.C.E. only with 1-Bromonaphthalene and liquid paraffin pastes. With 1-Bromonaphthalene the base-line keeps rising and the flat region is limited, but with liquid paraffin the base-line is flat till the cut-off potential. The mixing ratio of graphite:liquid paraffin used was the same as for anodic studies. Lower proportions of liquid paraffin resulted in shorter useful potential ranges. Higher proportions extended the range beyond -0.4V but during the period of scan the paste dissolves out of the cavity.

As the usable potential range with the carbon paste electrode is limited to -0.4V vs. S.C.E. in the cathodic direction the study was restricted to only ten of the synthetic food colours. The study was limited to pH2 solutions because at higher pH values the peaks would shift out of the useful cathodic range of the carbon paste electrode. The following ten colours were studied:

| Colour            | Peak Potential at G.C.E. in pH2 |
|-------------------|---------------------------------|
|                   | Burler (vs. S.C.E)              |
| Amaranth          | -0.18V                          |
| Carmoisine        | -0.14V                          |
| Ponceau 4R        | -0.12V                          |
| Red 2G            | -0.22V                          |
| Sunset Yellow FCF | ~0.15V                          |



Cyclic voltammograms of Patent Blue V, Green S and Ponceau 4R at pH2 at the C.P.E. FIGURE 6.13

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| Colour         | Peak Potential at G.C.E. in pH2<br>Buffer (vs. S.C.E) |
|----------------|-------------------------------------------------------|
| Tartrazine     | -0.33V                                                |
| Indigo Carmine | -0.08V                                                |
| Patent Blue V  | -0.37V                                                |
| Brown FK       | -0.33V                                                |
| Black PN       | -0.21V                                                |

The instrument operating conditions were the same as for cathodic voltammetry at the G.C.E. and the samples were deoxygenated as indicated earlier. Nitrogen was passed over the solution surface while scanning. The effect on peak shapes and potentials with changing pH could not be studied due to the restriction imposed on increasing the pH of the medium.

Table III compares the reduction potentials and the analytical usefulness of peaks at glassy carbon, carbon paste and the dropping mercury electrodes. In a majority of cases the peak shape and potentials were not very reproducible even on electrode surface cleaning with a stream of distilled water. Peaks were completely distorted on successive scans for Black PN and Patent Blue V and calibration voltammograms were ill-defined. The peaks of Carmoisine, Red 2G, Amaranth and Sunset Yellow FCF retained their original shape and potential on repeated scans without electrode surface being cleaned though an overall suppression was observed. For these very colours the peaks get broader or distorted and show cathodic shifts on successive scans when the electrode surface is washed with water. These phenomena were also accompanied with suppression of signals. It **could** be due possibly to surface alterations on account of the washing operation or adsorption of oxygen at the electrode face which results in distortions or potential shifts. Only Indigo Carmine, Brown FK and Tartrazine retained the original peak shape. Suppression in peak heights was observed for both Brown FK and Tartrazine with and without intermittent cleaning. Only Indigo carmine showed no suppression without cleaning but with cleaning drop in signals were noted.

Rectilinear calibration plots in the concentration range  $0-50 \ \mu g \ ml^{-1}$  were observed for Indigo Carmine, Sunset Yellow FCF

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and Tartrazine as shown in Figure 6.14. Slight cathodic shifts in peak potentials were observed for Tartrazine and Sunset Yellow FCF at higher concentration levels. The linearity of peak current and consistency of peak potential were seen for Indigo Carmine only. Rectilinearity was limited to lower concentration levels only for Carmoisine, Red 2G and Ponceau 4R. The peaks of Black PN, Patent Blue V and Amaranth were analytically useless.



FIGURE 6.14 Cathodic linear sweep voltammetric calibrations for Indigo carmine, Tartrazine and Sunset Yellow FCF at pH2 at the C.P.E. Colour concentration range 0-50 µg ml-!

## CHAPTER 7

# ELECTROCHEMICAL DETERMINATION OF FOOD COLOURS AT CARBON PASTE AND GLASSY CARBON ELECTRODES IN A FLOWING SYSTEM

#### INTRODUCTION

Flow-through techniques were introduced into general analytical practice slightly over twenty years ago and have since gained widespread use. On one hand, flow-through techniques can provide continuous information on the composition of a flowing solution (biological fluid, industrial effluent, eluent, etc) and on the other hand they are adaptable for routine sequential determinations.

Although most instrumental methods may be employed for following the concentration profile prevailing in the detector cell, optical detectors are most widely used. Consequently, most of the knowledge concerning flow-through methods and apparatus is related to applications of optical detectors. Lately there has been a parallel improvement of flow-through techniques in general and in the development of electroanalytical detectors.

Ion-selective and voltammetric electrodes are the main electroanalytical detectors used for carrying out analyses in flowing solutions; conductometric detection is mainly used for process stream analysis. Little<sup>(195)</sup> has assessed the utility and applications of electrodes based on a number of electroanalytical principles e.g. conductivity, permittivity, coulometry, etc. in liquid chromatography. Voltammetry is reported to have a wide application and some specificity. Very low detection limits are feasible ( $\sim 10^{-9}$ M). Detection in flowing systems has a number of advantages compared with static electrode systems, namely:

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

- b) The background current is decreased because at constant potential no current is needed to charge the double layer and the oxidation states of the functional groups in the carbon electrode surface are in equilibrium.
- c) Hydrodynamic voltammetry (HDV) is time independent, which permits steady state measurements. The need for high speed electronics is diminished.
- d) The residual charging current is independent on flow rate. Consequently, 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 is rarely met in practice. This advantage is gained, however, only if a high purity electrolyte is used.

Amperometric detection was first applied to column-chromatography about 30 years ago (196-198). Kemula(199) used the dropping mercury electrode for this purpose for many years, but the technique did not receive general acceptance because of the difficulties associated with application of a liquid detector in a flow-through detector(200). With the DME, high flow rates cause problems because drop formation is influenced by the flow and may not be uniform. Kissinger(201) has made remarkable contributions to the development of electrochemical detectors for chromatography. The applications of electrochemical detectors in HPLC have been compiled recently(202).

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<sup>(203,204)</sup>. A layer of mercury on a suitable substrate, mainly platinum, greatly extends the useful negative potential range<sup>(205-207)</sup>.

The application of electroanalytical detectors in liquid chromatography is restricted; only components reduced or oxidized within the available potential range of the measuring system can be determined. At the same time, electroanalytical detection sets

limitations on the separation technique and the composition of the mobile phase. Voltammetric detection can be made more selective by an appropriate choice of the potential of the working electrode or by using differential pulse mode of detection. In practice, the current is measured at a potential within the range of the convective limiting diffusion current.

The design criteria play an important role for the electrochemical detector in flow-through cells and some of the essential requisites are mentioned below:

- i) Geometric and hydrodynamic conditions should afford a rigorous mass-transfer analysis.
- ii) Detector volume should be as low as possible to avoid interference from other eluting components in liquid chromatographic applications.
- iii) Temperature sensitivity should be low so that thermostating is not necessary.
  - iv) Components of the cell should be easily dismantled to allow electrode replacement or polishing. The re-positioning of the electrodes is important as irreproducible location would affect the hydrodynamics of the system.
    - v) Flow velocity past the working electrode should be easily measured and controlled.
- vi) Detection limit should be low in order to compete with refractive index and UV detectors.
- vii) The detector should have a wide range of applications and few restrictions on solvents. Changes in solvent composition i.e. gradient elution should be possible.

- viii) The detector response should be instantaneous so that the detectable peak width is a minimum and the linear dynamic range should be large.
  - ix) The noise should be minimal. Gas-bubble noise at low level determinations is generally overcome by degassing the eluent.
    - x) The detector should be easy to use without the requirement of specialised knowledge.

#### APPLICATIONS OF ELECTROCHEMICAL DETECTION IN FLOW SYSTEMS

Liquid chromatography with amperometric detection provides significant improvements by combining the resolution of a chromatographic system with the sensitivity of electrochemical measurements. Electrochemical detection has been used extensively in the determination\_of\_electroactive\_species in\_biological fluids, industrial effluents, and in pollution\_monitoring. Some of\_the\_important applications are reviewed in this chapter.

King et al<sup>(208)</sup> determined commonly encountered phenolic antioxidants in a variety of commercial products using liquid chromatography with electrochemical detection. Sample extracts were chromatographed directly with few interferences on the reverse phase system. The typical linear range extended from  $10^{-11}$  to  $10^{-6}$  mole of injected analyte. Initial experiments were carried out on a carbon paste electrode. Typical minimum detectable quantities for the antioxidants were well below  $10^{-11}$  mole injected and the use of carbon paste electrodes was satisfactory. The glassy carbon working electrode was more widely applicable to parabens and Ionox-100. The minimum detectability of parabens was slightly greater due to the higher noise encountered ( $\sim 10^{-11}$  mole injected). Electrode filming problems were associated with parabens.

The electrochemical detection approach in liquid chromatography shows a great potential for the selective measurement of
trace organometallics in environmental samples. MacCrehan and  $Durst^{(209)}$  describe reductive electrochemical measurements for organomercury cations employing electrochemical detection. Previous work reports electrochemical reduction of organometallic compounds of mercury, tin, lead, arsenic and antimony at potentials below  $-1.1V^{(210,211)}$ . The limit of detection for methylmercury was 2 ng/gm or 40 pg and determinations were made in tunafish and shark meat. The gold amalgamated mercury electrode provided a negative potential range to about -1.2V for amperometric detection at pH 5.5 and gave reproducible results for an entire day's operation. The liquid chromatographic separation of methyl-, ethyl-, and phenyl-mercury was efficiently accomplished in a reverse-phase system by the formation of their neutral 2-mercapto-ethanol complexes.

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 constantpotential and pulse measuring techniques  $(^{212})$ . The analysis of nitrazepam, diazepam and chlordiazepoxide was used as a model system; the detectors were used in the reduction mode and the mobile phase was methanol-water (60:40) containing 0.05M ammonium acetate. The detection limit was found to depend strongly on reduction potential: at -0.93V vs. Ag/Ag Cl, 3 ng of nitrazepam could be detected, whereas at -1.30V the detection limit was 30 ng, owing to the high background current at this potential. A potential more negative than -1.1V was necessary for the detection of diazepam and chlordiazepoxide; at -1.30V the detection limit for these two compounds was 300 ng.

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 simply 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 only detects compounds whose half-wave potentials lie close to the initial potential chosen.

Wightman et al<sup>(213)</sup> modified the basal plane of pyrolytic graphite by anodic oxidation in citrate-acetate buffer for use as a detector for compounds separated by HPLC. The electrode performance compared very favourably with carbon paste electrodes for the detection of trace amounts of oxidisable organic compounds, and was comparable to reported mercury electrodes for the detection of reducible organic compounds. Mixtures of two amino acid derivatives, DN BSG and DN BSA, were analysed after derivatization with 2,4-dinitrobenzenesulfonyl chloride. The separation was relatively inefficient but the signals showed improvement with the modified basal plane. The electrode is superior to carbon paste as it is easier to prepare and exhibits slightly superior sensitivity. Unlike carbon paste, it is suitable for detection of reducible compounds at negative potentials.

Lewis and Johnson<sup>(200)</sup> examined the relative merits of DC, pulse and differential pulse amperometry at a flow-through glassy carbon electrode used in monitoring column chromatography eluents. A simple procedure is given for determining theophylline (1,3dimethylxanthine) in 20-100 µℓ of blood serum. Interference by 1,7-dimethylxanthine, a metabolite of caffeine, is compared for photometric and amperometric detection, and some advantages of combined photometric and amperometric detection are discussed. Theophylline and 1,7-dimethylxanthine could not be completely resolved under any of the chromatographic conditions investigated. The response of each of the three modes of electrochemical detection was compared to the photometric response at 254 nm. The values of  $E_1$  of the two compounds are very similar and complete resolution is not obtained for DC or pulse amperometric detection. Resolution of the mixture is likewise not possible by photometric detection, even with variable wavelength, because of the similarity of the absorption spectra of the two compounds. Of the techniques compared, the largest ratio of response was obtained for differential

pulse detection. The peak height for theophylline was about sevenfold that for 1,7-dimethylxanthine. There is significant merit in the simultaneous application of two detection systems in series for monitoring a chromatographic eluent. Advantages of a dual detection system include possible resolution of two components that are not separated by the chromatographic process, and a greater analytical accuracy because each sample component is detected twice.

An electrochemical detector for liquid chromatography was constructed based upon thin-layer cells with working electrodes prepared from a mixture of ceresin wax and graphite powder in a ratio of 1:1.3 by weight<sup>(214)</sup>. Catecholamines in blood plasma have been determined using both two electrode steady-state amperometry and simple thin-layer hydrodynamic amperometry. The four cateholamines  $\ell$ -dopa, norepinephrine, epinephrine and dopamine were separared on a strong cation exchange resin. The detection limit for epinephrine and norepinephrine standards, using the three electrode mode, was 0.2 to 0.3 pg or 2-3 parts per trillion with 100 µ $\ell$  injections. Both the three- and four-electrode detectors showed excellent dynamic response and sensitivity.

Chan and Fogg<sup>(215)</sup> used a Metrohm EA 1069/2 electrochemical detector cell together with the flow injection stand to determine the phenolic analgesic meptazinol. The system used a carrier stream of 0.05M sodium acetate - 0.1M acetic acid in 98% ethanol. Calibration graphs were rectilinear over the range 0.01 - 10  $\mu$ g ml<sup>-1</sup>. 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 internal reference electrode with silver chloride prevented large drifts in potential.

Sensitivity, selectivity, and wide dynamic range were all displayed by the dual electrochemical detector used for liquid chromatography<sup>(216)</sup>. Exhibiting a linear range of about 10<sup>5</sup>, it is, at the same time, capable of sensing sub-picomole quantities of certain species. For compounds which overlap chromatographically, but have different electrochemical formal potentials, the system

offers an instrumental separation. For routine investigations the system provides significant time reductions without causing proportionate increase in cost.

Several workers<sup>(195, 217-219)</sup> have made significant contributions in the design and development of electrochemical detectors for high performance liquid chromatography at the Imperial College, London. Applications are discussed with respect to real systems in environmental pollution monitoring and biomedical analysis.

### EXPERIMENTAL

### Apparatus and Reagents

Flow injection studies were carried out on the Metrohm EA 1096 electrochemical detector in conjunction with the flow injection stand (E 634). The glass bottle containing the supporting electrolyte (EA 1101) was connected to the loop injector and the detector by means of Teflon tubing (1.5 mm o.d/0.3 mm i.d). Flow of the supporting electrolyte, 0.01M sulphuric acid, was regulated by means of a constant pressure of 0.8 bar of nitrogen. The 0.01M sulphuric acid was degassed by means of a vacuum pump in order to avoid bubble formation in the detector cell. This was carried out at least once a day to ensure proper degassing. Injections were made by means of a Flarefit high pressure slide valve (loop capacity 70  $\mu$ 2) which is free of metal parts in contact with the eluent. For syringe injection a 10  $\mu$ 2 capacity glass syringe fitted with a stainless steel needle was used.

Difficulties were encountered with the blocking of the frit of the reference electrode provided with the flow cell and for this reason the flow cell was dismantled and used in a configuration similar to that of the PAR 310 mercury electrode detector cell. Thus of the three electrodes only the working electrode was inserted in the detector cell which was then partly immersed vertically in the electrolyte (0.01M sulphuric acid) contained in a beaker.

This beaker was kept in a larger beaker to prevent spillage of acid on the bench. Contact from the electrolyte to the reference electrode, as used for static work, was made by means of an agar bridge whereas the platinum counter electrode was placed dipping in the sulphuric acid. The sulphuric acid was replaced each time a different colour was studied.

The potential of the detector cell was kept fixed at +1.0V vs. S.C.E. except for Yellow 2G (+ 1.05V), Quinoline Yellow and Tartrazine (+ 1.10V) by means of the PAR 174A polarographic analyser.

A Tarkan 600 Y-t recorder operating at a chart speed of  $0.5 \text{ cm min}^{-1}$  was used for recording the detector response.

Metrohm glassy carbon (EA 286) and carbon paste (EA 287) electrodes were used as working electrodes. The glassy carbon working electrode was taken out of the cell and cleaned before injecting a different food colour solution by means of an alumina powder paste on tissue paper. The surface of the carbon paste electrode was renewed at such times.

Thirteen food colours were investigated in the present study and all thirteen gave reasonably good peaks at G.C.E. and C.P.E. in pH2 buffer under static electrode conditions.

The supporting electrolyte (0.01M sulphuric acid) was prepared from analytical grade reagent of sp. gr. 1.84 in a 5 litre volumetric flask and was transferred to the pressure bottle from time to time when needed. The same acid was used for the immersion of the detector cell in the beaker.

The details for making the carbon paste have already been given in Chapter 6.

### Procedure

A bubble-free flow of 0.01M sulphuric acid was obtained through the detector cell and the working electrode screwed in from the top

till it rested against the base. The positioning is a critical factor in order to ensure the same separation between the electrode face and the supporting electrolyte inlet. The cell was then clamped with its open ends immersed below the level of sulphuric acid in the beaker.

The appropriate connections were made by the lead from the PAR 174A polarograph and the constant potential applied to the cell. The recorder was switched on and when a stable base-line was achieved, sample injection was made with the constant volume injector.

The colour solutions were prepared in 0.01M sulphuric acid in order to avoid changes in electrolyte composition in the flow stream during injection. The response was observed over five injections at each level of concentration and calibrations were obtained for the food colours in the range 0.1 - 100 ppm. The amounts of food colours injected using the 70  $\mu$  loop were in the range 7-7000 ng.

## RESULTS AND DISCUSSION

Carbon paste and glassy carbon electrodes used anodically in the wall-jet configuration were shown to give good precision, rectilinear calibration graphs and low detection limits for the determination of food colours by flow injection analysis. The reproducibility and rectilinearity of response obtained on injecting solutions of food colours in the range 0.1-100 ppm into a stream of 0.01M sulphuric acid are summarised in Table I. Coefficients of variation are quoted in cases where the fall in signal appears to be insignificant otherwise an indication of the fall in signal over five injections is given. In general, the sensitivity was slightly higher for the carbon paste electrode, possibly due to lower backgrounds with this electrode; detection limits were not determined but in favourable cases clear peaks above baseline were obtained at 0.03 ppm (2.1 ng of injected colour). On the other hand, the precision at intermediate and lower concentrations was usually better with the glassy carbon

|                   |                        | GC Electrode                    |                                     | CP Electrode                    |                                     |
|-------------------|------------------------|---------------------------------|-------------------------------------|---------------------------------|-------------------------------------|
| Colour            | Concentration<br>(ppm) | Mean Current<br>(µA)            | Coefficient of<br>Variation*<br>(%) | Mean Current<br>(µA)            | Coefficient of<br>Variation*<br>(%) |
| Tartrazine        | 0.1                    | 0.0208                          | 6.3                                 | 0.0565                          | 20                                  |
|                   | 1                      | 0.277                           | 1.3                                 | 0.419                           | 13                                  |
|                   | 10                     | 2.62                            | 3.5                                 | 2.49                            | 10                                  |
|                   | 100                    | 6.85→5.90                       | 14% drop                            | 6.85→5.25                       | 23% drop                            |
| Quinoline Yellow  | 0.1<br>1<br>10<br>100  | -<br>0.067<br>1.0<br>5.19       | _<br>2.8<br><1<br>1.6               | 0.094<br>1.14<br>6.12           | -<br>4.8<br>6.7<br>3.7              |
| Yellow 2G         | 0.1                    | 0.0096                          | <1                                  | 0.0305                          | 3.7                                 |
|                   | 1                      | 0.078                           | 3.2                                 | 0.25                            | 4.8                                 |
|                   | 10                     | 0.45→0.30                       | 33% drop                            | 1.6→1.45                        | 9% drop                             |
|                   | 100                    | 2.00→1.40                       | 30% drop                            | 3.2                             | 3.5                                 |
| Sunset Yellow FCF | 0.1<br>1<br>10<br>100  | 0.0282<br>0.22<br>2.25<br>10.90 | 11<br><1<br><1<br><1<br><1          | 0.0088<br>0.134<br>1.56<br>8.08 | 12<br>1.2<br>1.8<br>3.0             |
| Carmoisine        | 0.1                    | 0.032                           | <1                                  | 0.027                           | 4.8                                 |
|                   | 1                      | 0.295                           | 1.7                                 | 0.230                           | 5.9                                 |
|                   | 10                     | 2.97                            | <1                                  | 2.55→2.20                       | 13.7% drop                          |
|                   | 100                    | 10.80                           | 1.2                                 | 9.31                            | 2.7                                 |
| Amaranth          | 0.1                    | 0.026                           | 8.6                                 | 0.0731                          | 8.4                                 |
|                   | 1                      | 0.260                           | 1.4                                 | 0.1896                          | 6.3                                 |
|                   | 10                     | 2.51                            | 1.2                                 | 2.05                            | 1.5                                 |
|                   | 100                    | -                               | -                                   | 5.96                            | <1                                  |

# TABLE I: FLOW INJECTION VOLTAMMETRIC DETERMINATION OF FOOD COLOURS

# TABLE I (contd)

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| Ponceau 4R         | 0.1                     | 0.005                                  | <1                    | 0.0598                      | 5.3                         |
|--------------------|-------------------------|----------------------------------------|-----------------------|-----------------------------|-----------------------------|
|                    | 1                       | 0.0712                                 | 1.5                   | 0.331                       | 3.0                         |
|                    | 10                      | 0.80                                   | <1                    | 3.02                        | 1.0                         |
|                    | 100                     | 7.25→6.60                              | 9% drop               | 8.3                         | 1.4                         |
| Red 2G             | 0.1                     | 0.0356                                 | 3.8                   | 0.0333                      | 7.9                         |
|                    | 1                       | 0.302                                  | <1                    | 0.245                       | 4.2                         |
|                    | 10                      | 2.65                                   | 1.1                   | 1.84                        | 12                          |
|                    | 100                     | 11.35→10.5                             | 7.5% drop             | 8.63                        | 1.0                         |
| Patent Blue V      | 0.1<br>1<br>10<br>100   | -<br>0.07<br>0.588<br>5.63             | -<br>13<br>2.8<br>2.8 | 0.09<br>0.856<br>6.97       | -<br><1<br>1.6<br>1.9       |
| Indigo Carmine     | 0.1                     | 0.031                                  | 7.2                   | 0.0212                      | 6.0                         |
|                    | 1                       | 0.235                                  | <1                    | 0.210                       | 4.8                         |
|                    | 10                      | 2.14                                   | 1.2                   | 2.21                        | 2.1                         |
|                    | 100                     | 17.3→16.6                              | 3.8% drop             | 8.62                        | 9.0                         |
| Brilliant Blue FCF | 0.1<br>1<br>10<br>100   | -<br>0.066<br>0.60<br>5.49             | 8.3<br><1<br>2.6      | 0.0806<br>0.67→0.58<br>3.28 | -<br>6.0<br>13% drop<br>2.0 |
| Green S            | 0.1                     | 0.033                                  | 8.8                   | 0.0432                      | 9.5                         |
|                    | 1                       | 0.158                                  | 1.4                   | 0.150                       | <1                          |
|                    | 10                      | 1.49                                   | 1.5                   | 1.47                        | 1.1                         |
|                    | 100                     | 11.20                                  | <1                    | 10.64                       | 1.0                         |
| Black PN           | 0.1 .<br>1<br>10<br>100 | 0.08<br>0.98<br>5.50 <del>.4</del> .25 | <1<br>4.8<br>22% drop | 0.1008<br>1.052<br>2.94     | -<br>5.4<br>5.6<br>3.7      |

\* For five determinations. If a systematic decrease in height was noticed, then the percentage decrease is noted instead.

electrode. For some colours at higher concentrations there was no significant fall in signal with carbon paste whereas larger drops are seen for glassy carbon electrodes.

Sequential injection of 95% Ethanol was attempted to improve further the reproducibility of peak response. The injected alcohol would clean the surface of the glassy carbon electrode. Ethanol (0.5 ml) was injected by means of a fourway Rheodyne valve after each sample injection. It was found that when alcohol reached the electrode face the signal became destabilised and did not attain the original value and subsequent sample injection produced no peak. Subsequently sequential injection of water was also tried but there was no improvement over results already reported in Table I.

Some of the earlier work on food colours was carried out by means of syringe injection but this resulted in erratic results which included generation of peaks for blank injections of 0.01M sulphuric acid through the septum. The blank peaks were further found to be of poor reproducibility.

Further care was exercised but the situation did not improve and it was then suspected that blank peaks could be arising due to uptake of metal from the stainless steel needle and metal plunger of the syringe. This was verified by making blank injections of 0.01M sulphuric acid (1-10  $\mu$ ) by means of a 10  $\mu$ glass syringe supplied by Scientific Glass Engineering, Australia having a stainless steel needle. The resulting signals showed an increase in size with volume injected but no linear relationship between volume injected was observed apparently due to different contact times between the sulphuric acid and the metal parts of the syringe. The same solution was taken up in a plastic syringe of volume 20 ml with a rubber plunger and injections made through 0.1 and 0.5 ml capacity loops using a four-way Rheodyne rotary valve. No signals were obtained (see Figure 7.1). Metal pick-up from the syringe needle was confirmed by cleaning the loops and plastic syringe several times with 0.01Msulphuric acid and making injection into the detector cell fitted with a carbon paste

electrode with 0.5 ml loop. No peaks were seen. When only 2-3 ml sulphuric acid was left in the plastic syringe the stainless steel needle was inserted and 10  $\mu$  volume solution withdrawn and released 10-15 times. Injection of the contaminated solution by means of the 0.5 ml loop yielded a small broad peak. The loop was changed to 0.1 ml capacity and the signal width was proportionately decreased.

The metal pick-up interference is not likely under static electrode conditions on account of the higher detection limits under such conditions. This was confirmed by first obtaining blanks for 0.01M sulphuric acid at a stationary carbon paste electrode in the Metrohm cell. The blanks obtained were flat baselines at 10, 5, 2 and 1  $\mu$ A current ranges. The dyes were normally determined in the 5-10  $\mu$ A range at 2-100  $\mu$ g ml<sup>-1</sup> concentration levels under static conditions. Subsequently the stainless steel needle was dipped in the acid and the solution pumped in and out 50 times, stirred with the needle to ensure sufficient contamination, and again the voltammograms run at  $1-10 \mu$ A current levels. No changes were observed in the base-lines as can be seen in Figure 7.2. The same solution was transferred to the plastic syringe with the rubber plunger and freshly prepared carbon paste electrode fitted into the Metrohm flow cell. Peaks were obtained at the 1  $\mu$ A current level with 0.1 ml loop and broader peaks were obtained when the loop capacity was changed to 0.5 ml.

It can be concluded that the use of a syringe with a stainless steel needle for the purpose of sample injection in acid solutions leads to metal uptake and causes a signal to appear on the recorder under hydrodynamic conditions. The sensitivity of the flow technique is low enough for the signal to be detected and for this reason a constant volume slide-valve with no metal parts in contact with the supporting electrolyte is recommended. However, for static electrode determinations, the use of a syringe with a stainless steel needle does not lead to any interference as the very nature of such determinations gives sensitivities not low enough to detect the interference.



FIGURE 7.1 Syringe vs loop injection of pure and needle contaminated 0.01M  $\rm H_2$  SO\_4 into the detector cell.



FIGURE 7.2 Blanks for 0.01M H<sub>2</sub> SO<sub>4</sub> (pure and needle contaminated) at a static carbon paste electrode at different current sensitivities.

# CONCLUSION

Most permitted synthetic food colours give analytically useful DC polarographic waves or differential pulse polarographic peaks over at least part of the pH range investigated. Tetraphenylphosphonium chloride has a pronounced effect on the mean limiting diffusion currents and peak potentials of some colours. The DC half-wave potentials and DP peaks of two pyroazole azo colouring matters, tartrazine and Yellow 2G, and of the triphenylmethane colour BrilliantBlue FCF, are shifted to more negative potentials on the addition of tetraphenylphosphonium chloride. For both DC and DP polarograms the changes occur at TPPC concentrations up to 100  $\mu$ g ml<sup>-1</sup>, above this concentration halfwave and peak potentials and limiting diffusion and peak currents change very little in most instances.

The effects of tetraphenylphosphonium chloride are more marked in DP polarography, although the DC polarographic maxima of Yellow 2G and Red 2G are conveniently suppressed by its addition. The DC half-wave potentials seem to follow the shifts of DP peaks and phenomena like peak splitting and broadening are reflected by the DC waves.

The differential pulse polarographic determination has been applied to the determination of food colours in samples of jams, jellies, and marmalades. The addition of tetraphenylphosphonium chloride proved particularly advantageous in the case of Chiver's orange tangy marmalade when a sharp peak was obtained at pH 9. In the absence of TPPC only a rising base-line was present.

The shifts in reduction potentials produced by other phosphonium compounds and tetraphenylarsonium chloride for tartrazine, Yellow 2G and Brilliant Blue FCF are not as significant as the shifts produced by tetraphenylphosphonium chloride.

Linear sweep voltammetry at a stationary electrode is a simpler technique than polarography at a dropping mercury electrode owing to the simpler electrode system and the absence of the need to deoxygenate solutions in the positive potential range. The glassy carbon electrode usually requires cleaning between scans

but this is a simple procedure and can be justified if an overall saving of time can be achieved. The surface of the carbon paste electrode is easily renewed or cleaned simply by washing in a stream of distilled water.

Most permitted food colours give well-defined DC voltammetric waves at different pH values at static glassy carbon and carbon paste electrodes. The peak currents have been used to determine the colours in the range 2-100  $\mu$ g ml<sup>-1</sup> either directly or after extraction on polyamide columns. The method is applicable with good precision to single food colours and quality control applications are feasible with some combinations of colouring matters.

The carbon paste electrode gives low-background currents and gives peak shaped signals for some colours at certain pH values when under similar conditions the glassy carbon electrode either shows no response or analytically useless waves. However, the reproducibility of results is slightly lower at the carbon paste electrode but rectilinear calibrations were observed for all the red colours and for Yellow 2G, Patent Blue V and Indigo carmine.

The determination of colours in simulated soft drink samples has proved to be a success generally but serious interference was encountered in the determination of amaranth in blackcurrant health drink. This shows that difficulties can arise from either constituents of foods and for new applications preliminary tests need to be carried out to establish the presence of interfering substances.

Cathodic linear sweep voltammetry has proved useful in resolving some colours, especially the yellow and red colours, which give closely spaced peaks under anodic conditions. A particularly useful application of the cathodic mode could be the identification of food colours in the presence of Vitamin C which interferes in the positive potential range.

The use of the carbon paste electrode in the negative potential range is limited to -0.4V and this restricted the study to ten food colours only. Rectilinear calibrations were obtained only for Indigo carmine, Sunset Yellow FCF and tartazine.

Cathodic voltammetry has a limited applicability and though

useful for qualitative identification little reliability can be placed on quantitative results.

A flow injection mode of operation employing electrochemical detection at a glassy carbon or carbon paste electrode has shown promise in the determination of food colours in the 7-7000 nanogrammes range. In general the sensitivity is slightly higher for the carbon paste electrode; detection limits were not determined but in favourable cases clear peaks above base-line were obtained at 0.03 ppm (2.1 ng of colour injected using a constant volume loop of 70  $\mu$ & volume). On the other hand the precision at intermediate and lower concentrations is usually better with the glassy carbon electrode. For some colours at higher concentrations there was no significant fall in signal with carbon paste electrode and good precision was obtained.

Metal ion interference at the high sensitivities achieved using flow injection analysis was encountered due to metal pickup by the sample solution in contact with the metal plunger and stainless steel needle of the glass syringe. It is suggested that when working with acidic or alkaline media a loop injector valve should be used with no metal parts in contact with the injected solution. This also enhances the precision as personal errors in syringe injection are eliminated. Further the samples must be made in the flowing electrolyte media so that when using loop injection no distortion of signal occurs due to differences in ionic concentrations of the sample solution and streaming electrolyte.

Studies are in progress in this laboratory to extend the flow injection procedure to the determination of food colours following extraction from food matrices and to apply voltammetric detection to their determination after high performance liquid chromatographic separation.

The voltammetric technique in general and differential pulse polarography in particular are sufficiently sensitive and selective to offer a means of identification and determination of the products of degradation of food colours. Work on heat and light degradation has been initiated in this laboratory and the findings have been largely based on the differential pulse polarographic method.

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## APPENDIX

The following figures depict the influence of Tetraphenyphosphonium chloride on the DC waves and DP peaks of the permitted synthetic food colours at  $10^{-4}$  M concentration. The solutions were prepared by diluting 2.5 ml of  $10^{-3}$  M colour solution to 25 ml with 10 ml pH 1.9 Britton-Robinson buffer, adjusting the pH to the desired value and making up the volume with distilled water. Aliquots of 0.1M (37.4 mg ml<sup>-1</sup>) TPPC solution were added using a 10- or 100 µ£ syringe.









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