CHAPTER IV

RESULTS AND DISCUSSION

Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G are the food dyes studied. Even the dyes used are of food grade, their purities were examined by paper chromatographic and spectrophotometric techniques before their polarographic studies.

4.1 Purities of the dyes

4.1.1 Paper chromatography

Three solvent systems were used for testing purities of the dyes. There are solvent system I, 2% NaCl in 50% ethanol; solvent system II, the mixture of 2-methyl propan-1-ol, ethanol and water in the ratio 1:2:1, repectively; and solvent system III, 2.5% NaCl aqueous solution. The paper chromatogram of each dye in every solvent system studied showed a well-defined spot as illustrated in Figure 4 which is the paper chromatogram of Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G by the solvent system III. The R_f value of each dye in every solvent system was determined and compared with the literature one as shown in Table 1. These R_f values are slightly different from the literature values (32) owing to the water content in the paper used, concentration and pH-value of the dye solution, and the temperature of the chromatographic chamber.

4.1.2 Spectrophotometry

The ultraviolet-visible spectra of Amaranth, Ponceau 4R,

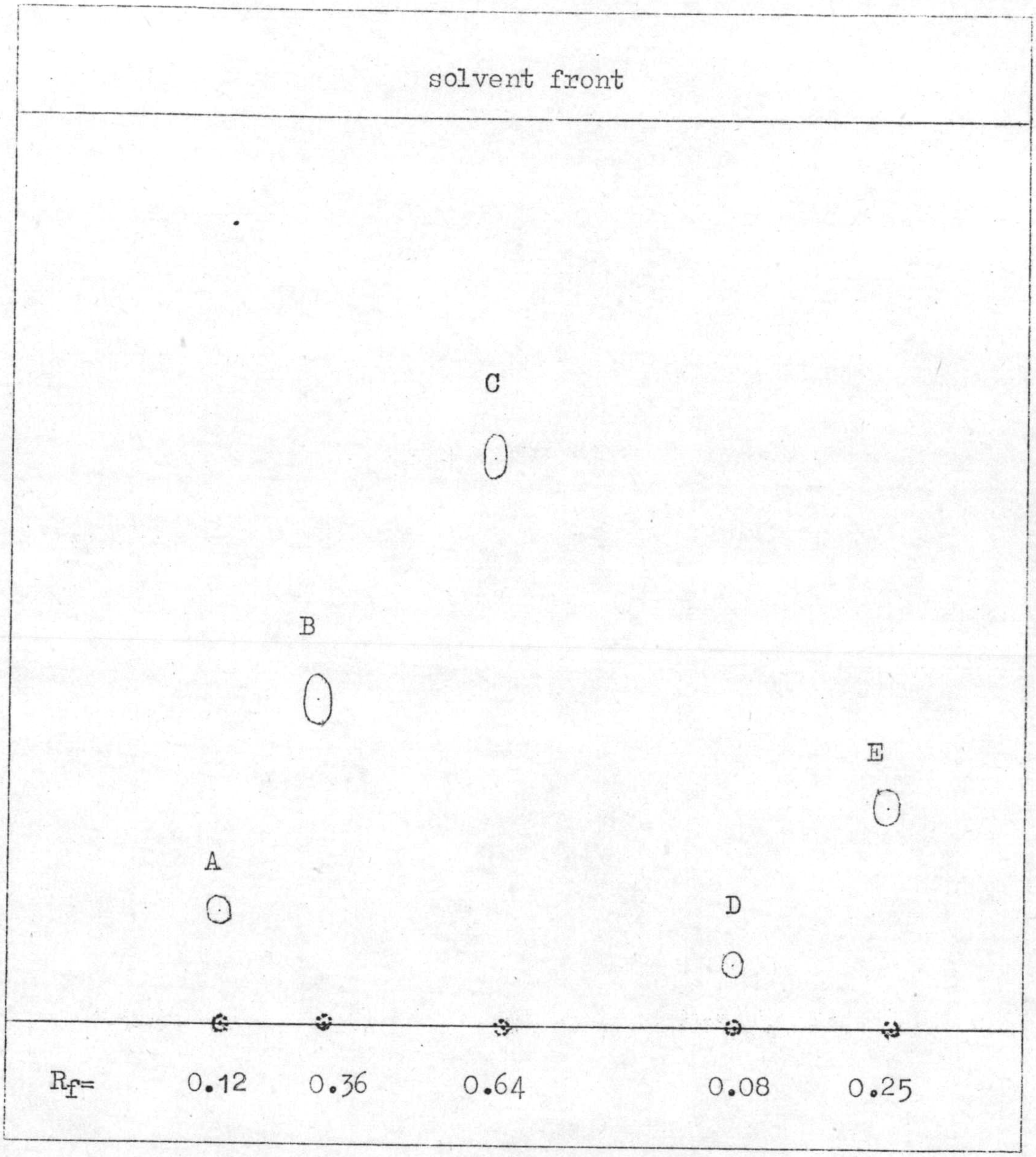


Figure 4 Paper chromatogram of the following dyes by the solvent system III; A) Amaranth, B) Ponceau 4R, C) Orange G, D) Orange RN, and E) Sunset Yellow FCF

Table 1 R_f values of Amaranth, Ponceau 4R, Orange G, Orange RN and Sunset Yellow FCF by the solvent systems I, II and III

Dye	solvent system I		solvent system II		solvent system III	
	R_f	$R_f^{(32)}$	R_f	$R_f^{(32)}$	R_f	$R_f^{(32)}$
Amaranth	0.30	0.27	0.24	0.29	0.12	0.15
Ponceau 4R	0.56	0.51	0.27	0.32	0.36	0.39
Orange G	0.74	0.78	0.53	0.51	0.64	0.61
Orange RN	0.82	0.84	0.83	0.80	0.08	0.08
Sunset Yellow FCF	0.69	0.72	0.40	0.46	0.25	0.29

(32) Pearson, D. The Chemical Analysis of Foods. 6th, ed. London: J & A Churchill, 1970. p 60-61

Orange G, Orange RN and Sunset Yellow FCF in 0.1 M HCl and 0.1 M NaOH solutions were performed and compared to the ones obtained from the literature (33), as shown in Figure 5A-5E. The spectrum of each dye studied indicated an insignificant difference from the literature one. The wavelengths at the maximum absorption of the dyes (λ max) in an acidic solution were measured and their molar absorptivities were calculated as listed in Table 2. The molar absorptivity of each dye was found in the order of 10^4 (see Table 2) which indicated the strong absorption of the dye in the visible range.

Thus, evidences from paper chromatographic and spectrophotometric analyses of Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G indicated that the purities of these dyes are high enough for polarographic studies.

4.2 Polarographic behavior

Polarographic studies of Amaranth, Ponceau 4R, Sunset yellow FCF, Orange RN and Orange G were performed in the common supporting electrolytes such as 0.1 M KCl, 0.1 M KNO_3 and 0.1 M $(C_2H_5)_4NCl$. The pH of the dye solution was controlled by using McIlvaine buffer for pH 2.0-7.2 and Michaelis borate buffer for pH 8.0-12.3 as described in Chapter 3.

4.2.1 Effect of pH on the polarographic wave

The polarographic waves of most organic compounds are pH dependence. Thus, a variation of pH of the test solution was investigated. The pH of the test solution recorded in this polarographic study is the final pH of the test solution.

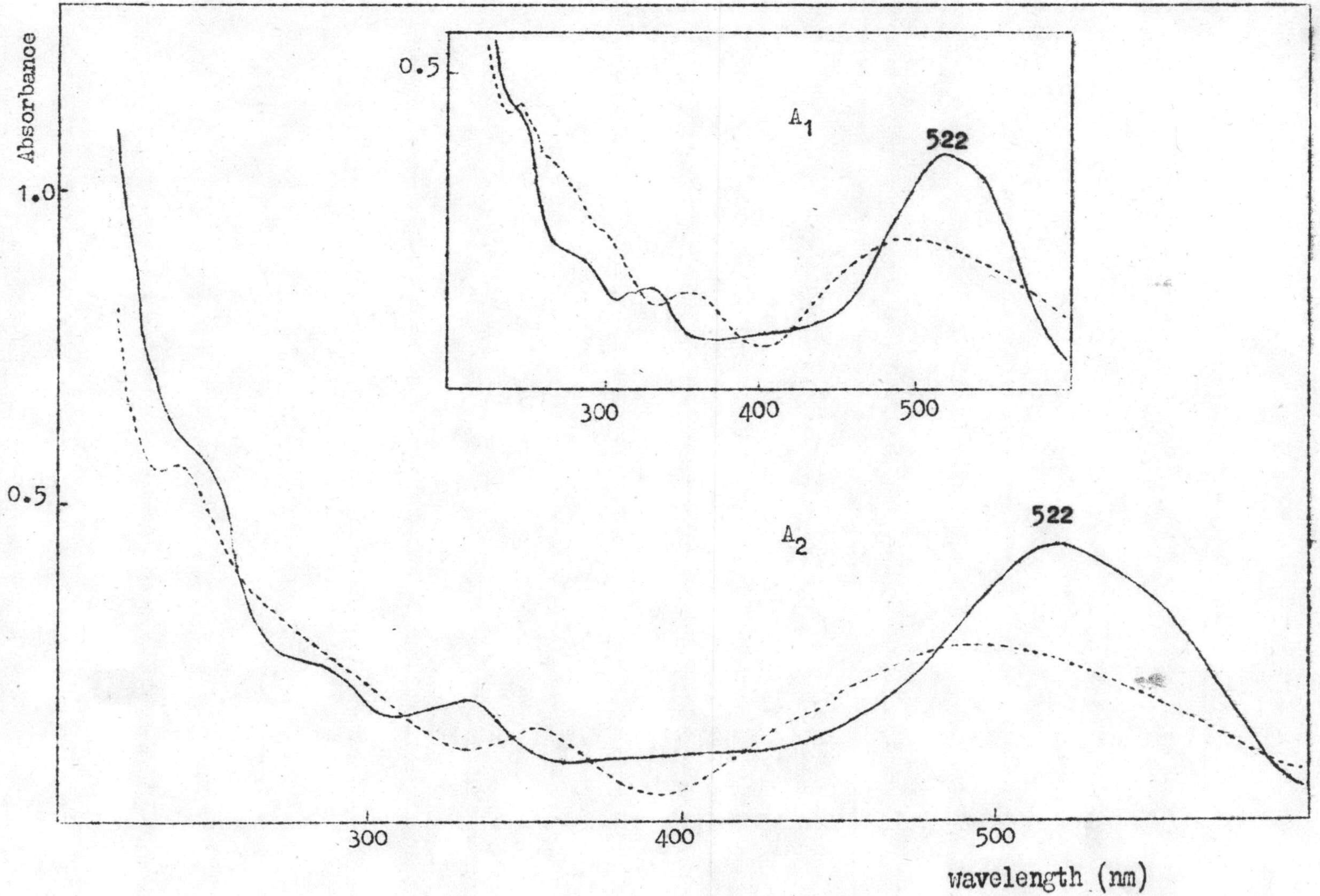


Figure 5A Comparison of UV-visible spectra of Amaranth between A_1) literature(33) and A_2) experiment ;_____ in 0.1M HCl and .....in 0.1M NaOH

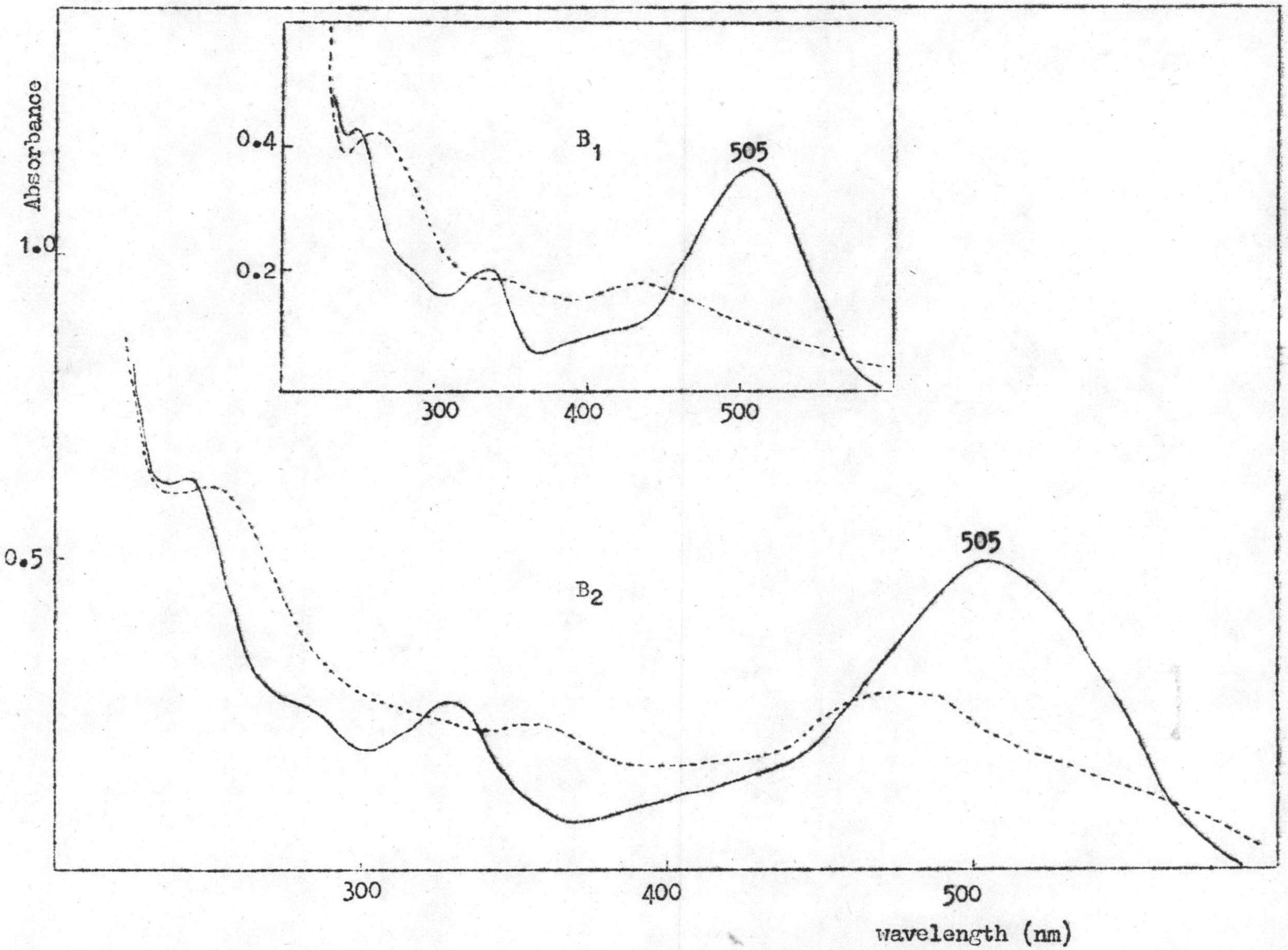


Figure 5B Comparison of UV-visible spectra of Ponceau 4R between B$_1$) literature(33) and B$_2$) experiment ; ______in 0.1M HCl and ......in 0.1M NaOH

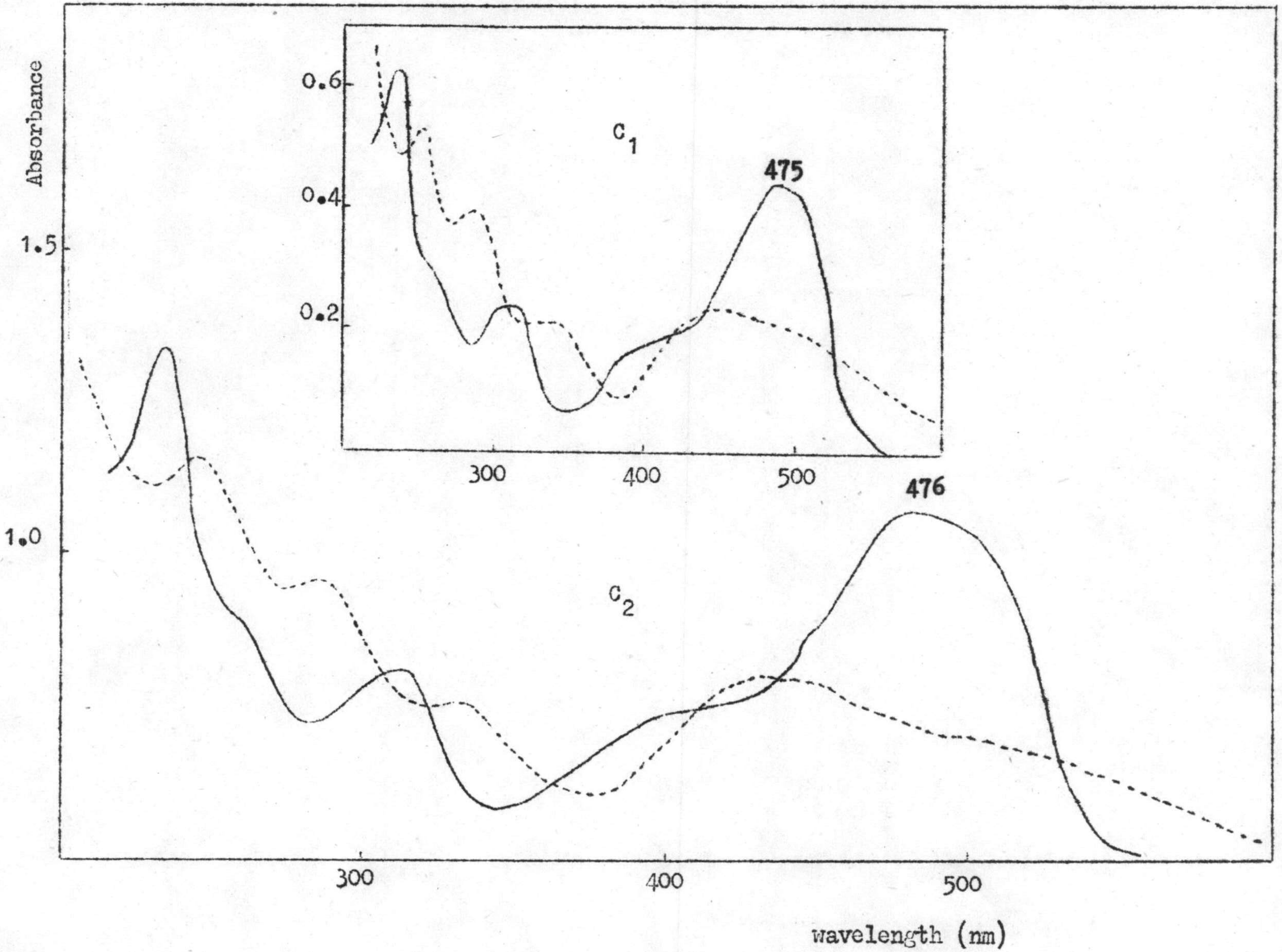


Figure 5C Comparison of UV-visible spectra of Sunset Yellow FCF between C_1) literature(33) and C_2) experiment; ____ in 0.1M HCl and ....in 0.1M NaOH

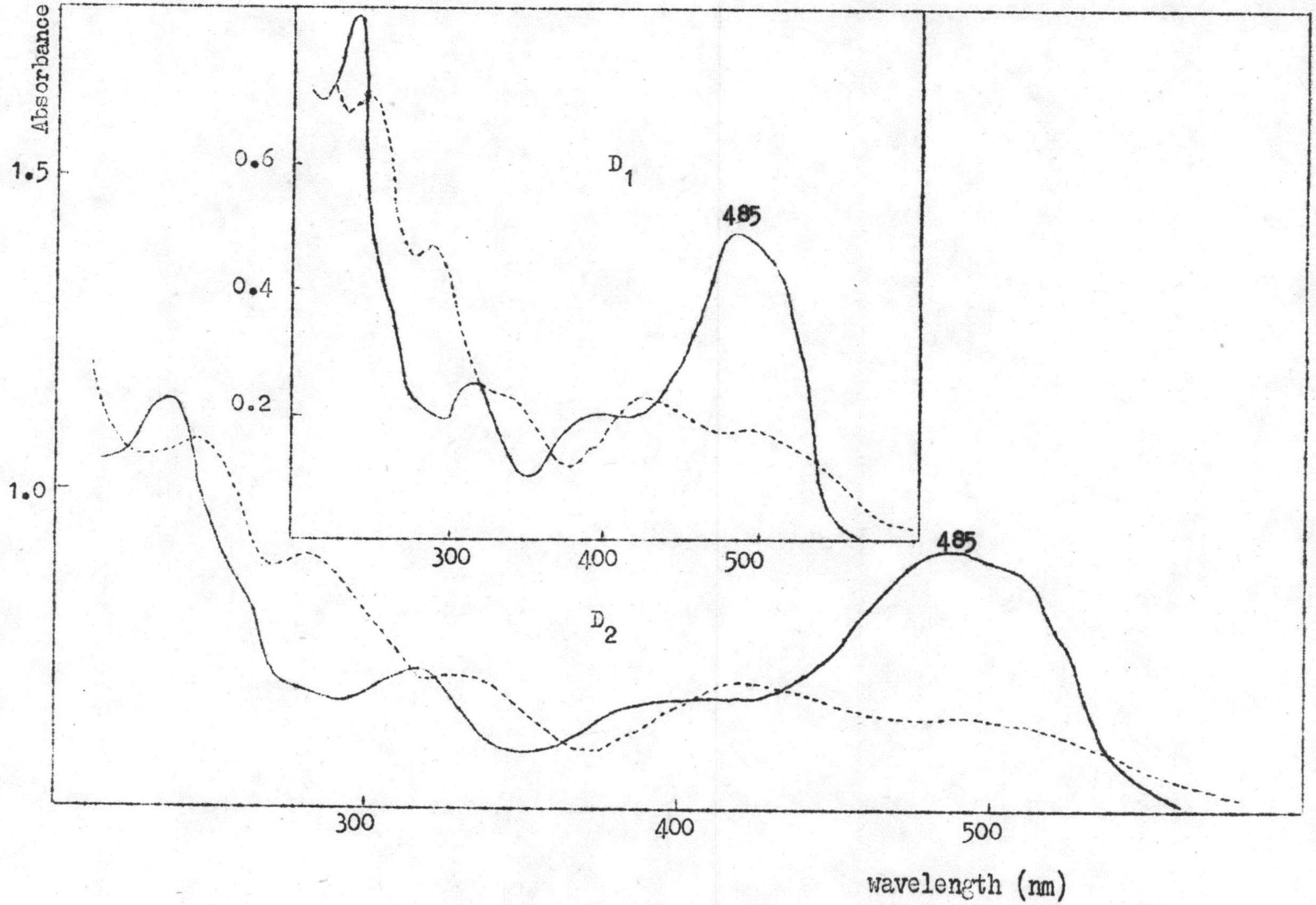


Figure 5D Comparison of UV-visible spectra of Orange RN between D_1) literature(33) and D_2) experiment ; ______in 0.1M HCl and .....in 0.1M NaOH

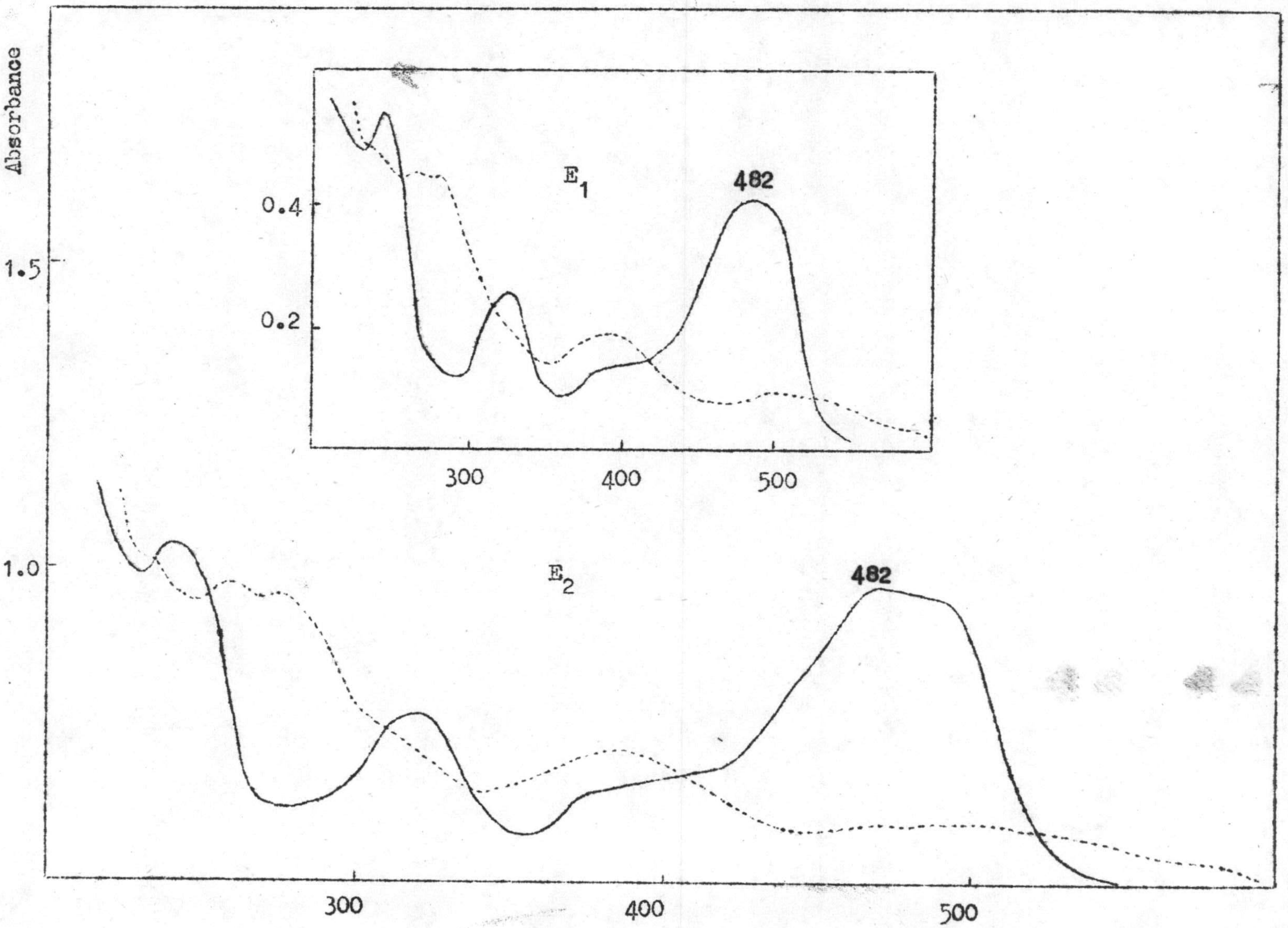


Figure 5E Comparison of UV-visible spectra of Orange G between E_1) literature(33) and E_2) experiment; ____ in 0.1M HCl and .....in 0.1M NaOH

Table 2 Absorption characteristics of dyes in the visible region

Dye	λ max (nm)	molar absorptivity ε (calculated)
Amaranth	522 in acid solution	16,400
Ponceau 4R	505 in acid solution	20,000
Orange G	476 in acid solution	21,800
Orange RN	485 in acid solution	16,200
Sunset Yellow FCF	482 in acid solution	22,400

4.2.1.1 Amaranth

The concentration of Amaranth understudied in every electrolyte at any pH is 5.0 x 10^{-4}M. The polarogram of Amaranth in 0.1 M $(C_2H_5)_4NCl$, 0.1 M KCl or 0.1 M KNO_3 at any pH in the range of 1-12 showed a single polarographic wave (see Figures 6-8). The wave is ill-defined as the pH of the dye solution in every electrolyte studied is lower than 4.0 and the wave becomes well-defined as pH is higher than 4.0. The effects of pH on the polarographic waves are demonstrated in Figure 6 for Amaranth in 0.1 $M(C_2H_5)_4NCl$, Figure 7 for Amaranth in 0.1 M KCl and Figure 8 for Amaranth in 0.1 M KNO_3. Data for explaining this effect are also listed in Tables 3, 4 and 5, respectively. For every electrolyte, as the pH increases its half wave potential shifts to more negative potential. The plot of the half wave potential versus pH of the solution showed a linearity and a slope of -0.060 was obtained (see Figure 9). The maximum diffusion currents were obtained at pH 4.00 for Amaranth in 0.1 $M(C_2H_5)_4NCl$, pH 5.10 for Amaranth in 0.1 M KCl, pH 5.20 for Amaranth in 0.1 M KNO_3. At other pH the diffusion currents seemed to be independent of pH of the solution (see Figure 10).

4.2.1.2 Ponceau 4R

The concentration of Ponceau 4R understudied in every electrolyte at any pH is 1.0 x 10^{-4} M. The polarogram of Ponceau 4R in 0.1 $M(C_2H_5)_4NCl$, 0.1 M KCl or 0.1 M KNO_3 at any pH in the range of 1-12 showed a single polarographic wave (see Figures 11-13). The wave is a well-defined wave at every pH in the electrolytes studied. The effects of pH on the polarographic waves are demonstrated in Figure 11 for Ponceau 4R in 0.1 $M(C_2H_5)_4NCl$, Figure 12 for Ponceau 4R

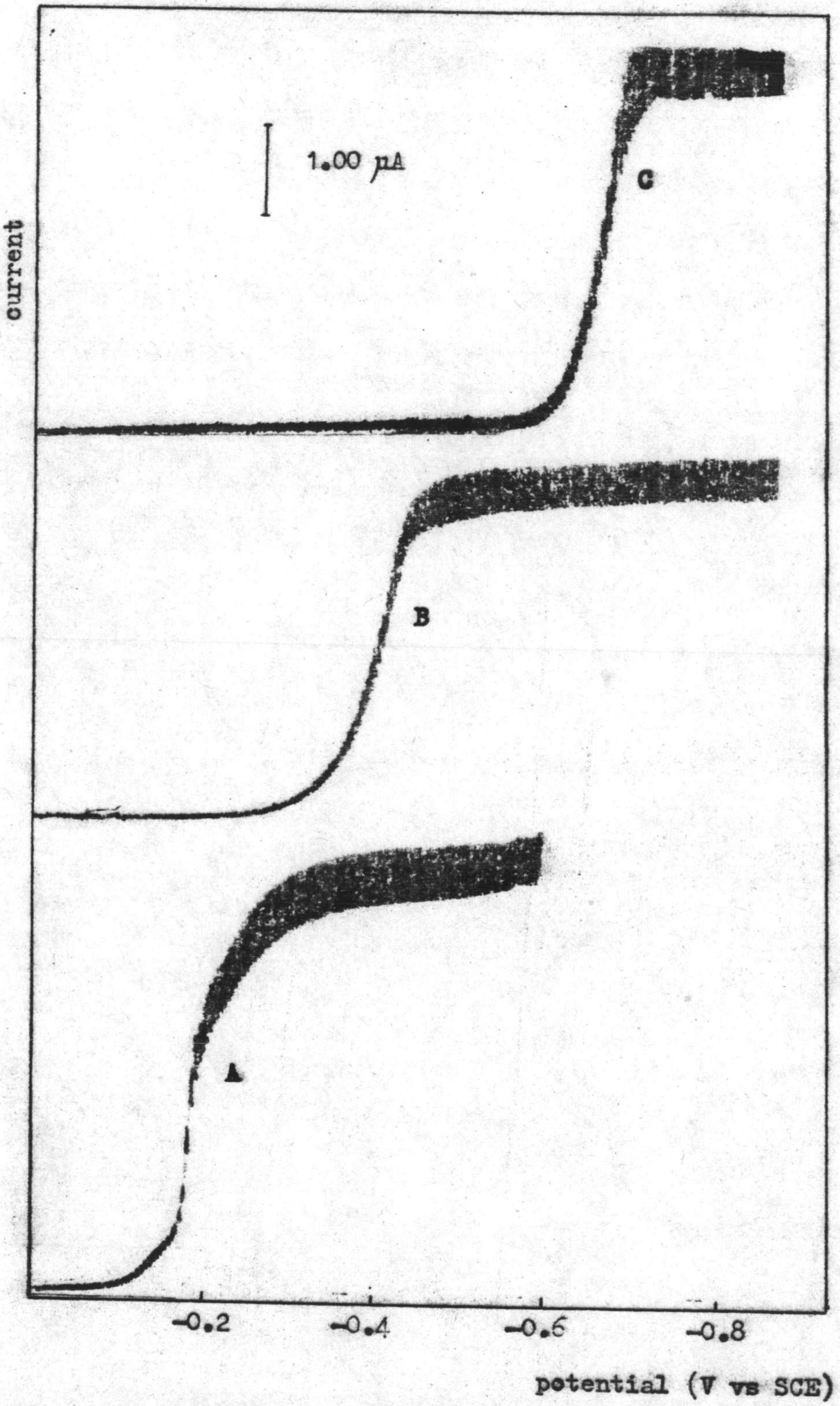


Figure 6 The polarograms of Amaranth in 0.1M $(C_2H_5)_4NCl$ at pH A) 2.50 , B) 5.00 and C) 8.25

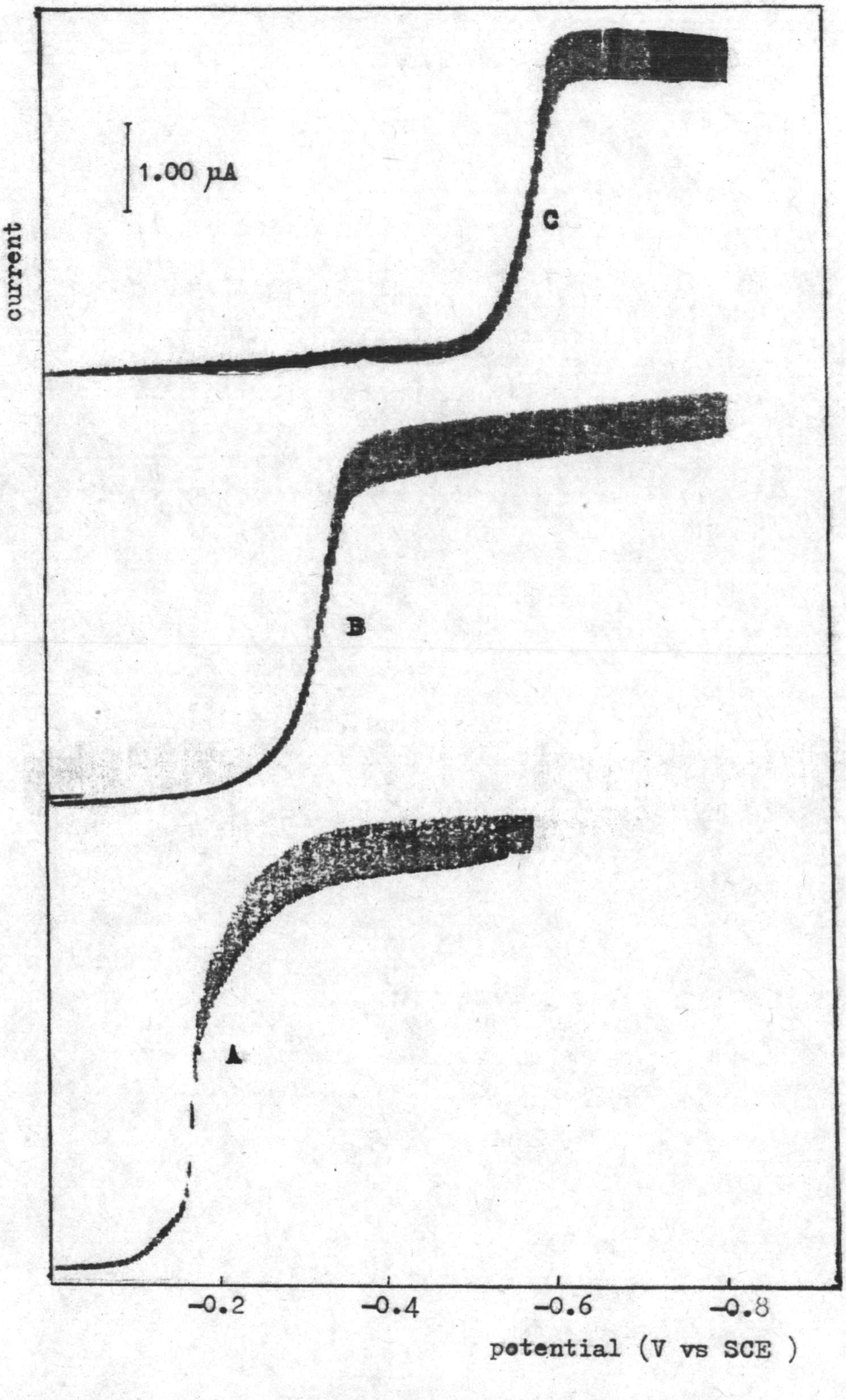


Figure 7 The polarograms of Amaranth in 0.1M KCl at pH
A) 2.50, B) 5.10, C) 8.15

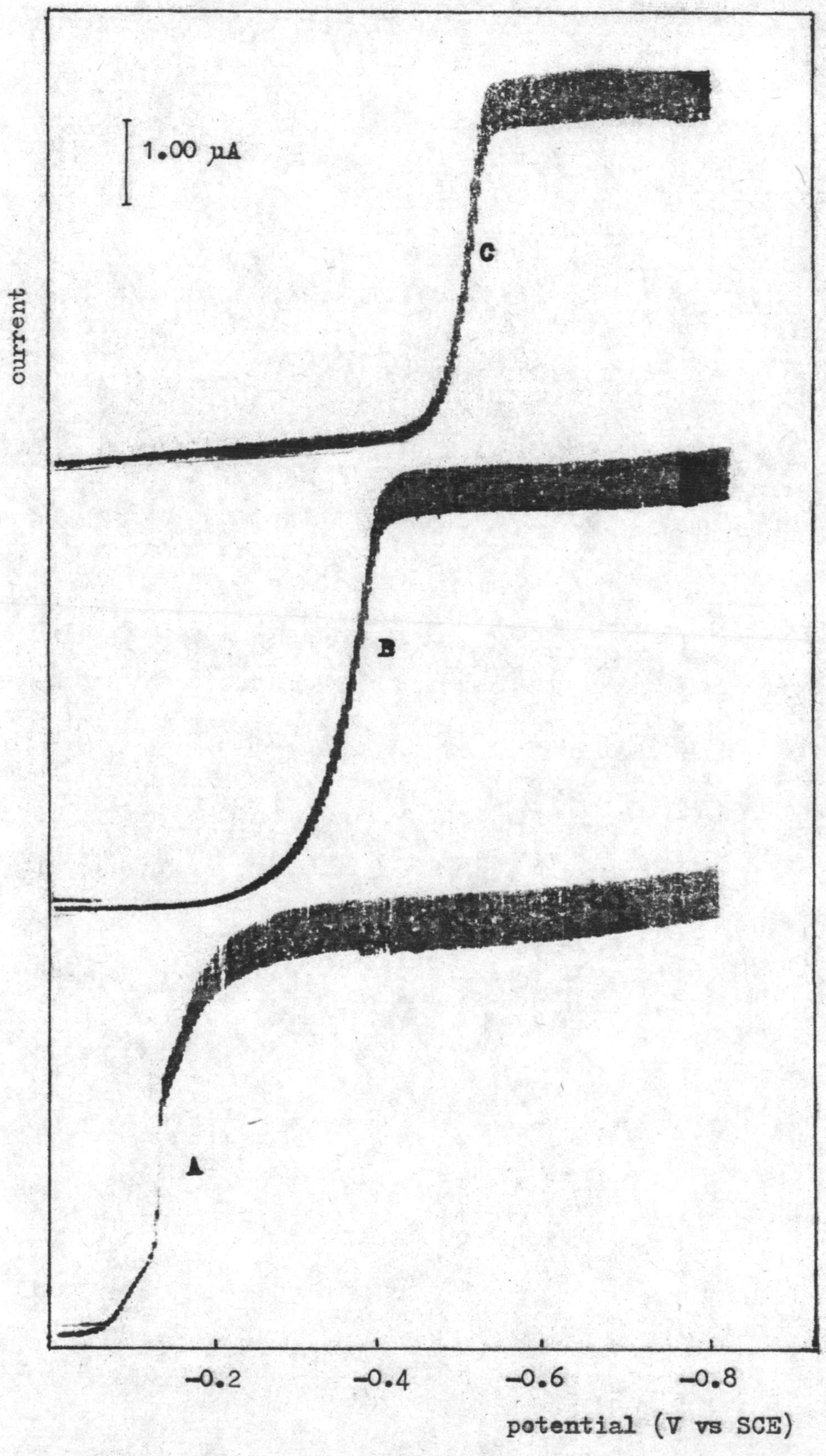


Figure 8 The polarograms of Amaranth in 0.1M KNO_3 at pH

Table 3 Effect of pH on the half wave potential of Amaranth in 0.1 M $(C_2H_5)_4NCl$

pH	$E_{\frac{1}{2}}$(V)	i_d^a(μA)	Remarks
1.40	b	b	ill-defined wave
2.50	b	b	ill-defined wave
3.00	b	b	ill-defined wave
3.50	b	b	ill-defined wave
4.00	-0.258	3.83	well-defined wave
5.00	-0.330	3.36	well-defined wave
6.08	-0.410	3.13	well-defined wave
7.20	-0.480	3.40	well-defined wave
8.25	-0.540	3.20	well-defined wave
9.30	-0.590	3.40	well-defined wave
10.20	-0.640	3.20	well-defined wave
12.00	-0.780	3.20	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

[b] cannot be measured accurately

Table 4 Effect of pH on the **half wave potential of Amaranth** in 0.1 M KCl

pH	$E_{\frac{1}{2}}$(V)	i_d^a(μA)	Remarks
1.40	b	b	ill-defined wave
2.50	b	b	ill-defined wave
3.00	b	b	ill-defined wave
3.50	b	b	ill-defined wave
4.00	-0.265	3.69	well-defined wave
5.10	-0.350	3.91	well-defined wave
5.95	-0.410	3.20	well-defined wave
7.25	-0.480	3.36	well-defined wave
8.15	-0.530	3.59	well-defined wave
9.30	-0.590	3.13	well-defined wave
10.50	-0.680	3.20	well-defined wave
12.00	-0.765	3.13	well-defined wave

[a] mercury height = 65.0 cm; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

[b] cannot be measured accurately

Table 5 Effect of pH on the half wave potential of Amaranth in 0.1 M KNO_3

pH	$E_{\frac{1}{2}}$(V)	i_d^a(μA)	Remarks
1.40	b	b	ill-defined wave
2.50	b	b	ill-defined wave
3.00	b	b	ill-defined wave
3.50	b	b	ill-defined wave
3.88	-0.258	3.45	well-defined wave
4.20	-0.285	3.69	well-defined wave
4.75	-0.312	3.88	well-defined wave
5.20	-0.348	3.98	well-defined wave
6.05	-0.405	3.55	well-defined wave
7.15	-0.470	3.75	well-defined wave
8.30	-0.540	3.52	well-defined wave
9.30	-0.585	3.14	well-defined wave
10.30	-0.660	3.36	well-defined wave
11.90	-0.770	3.20	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}} = 0.61$

[b] cannot be measured accurately

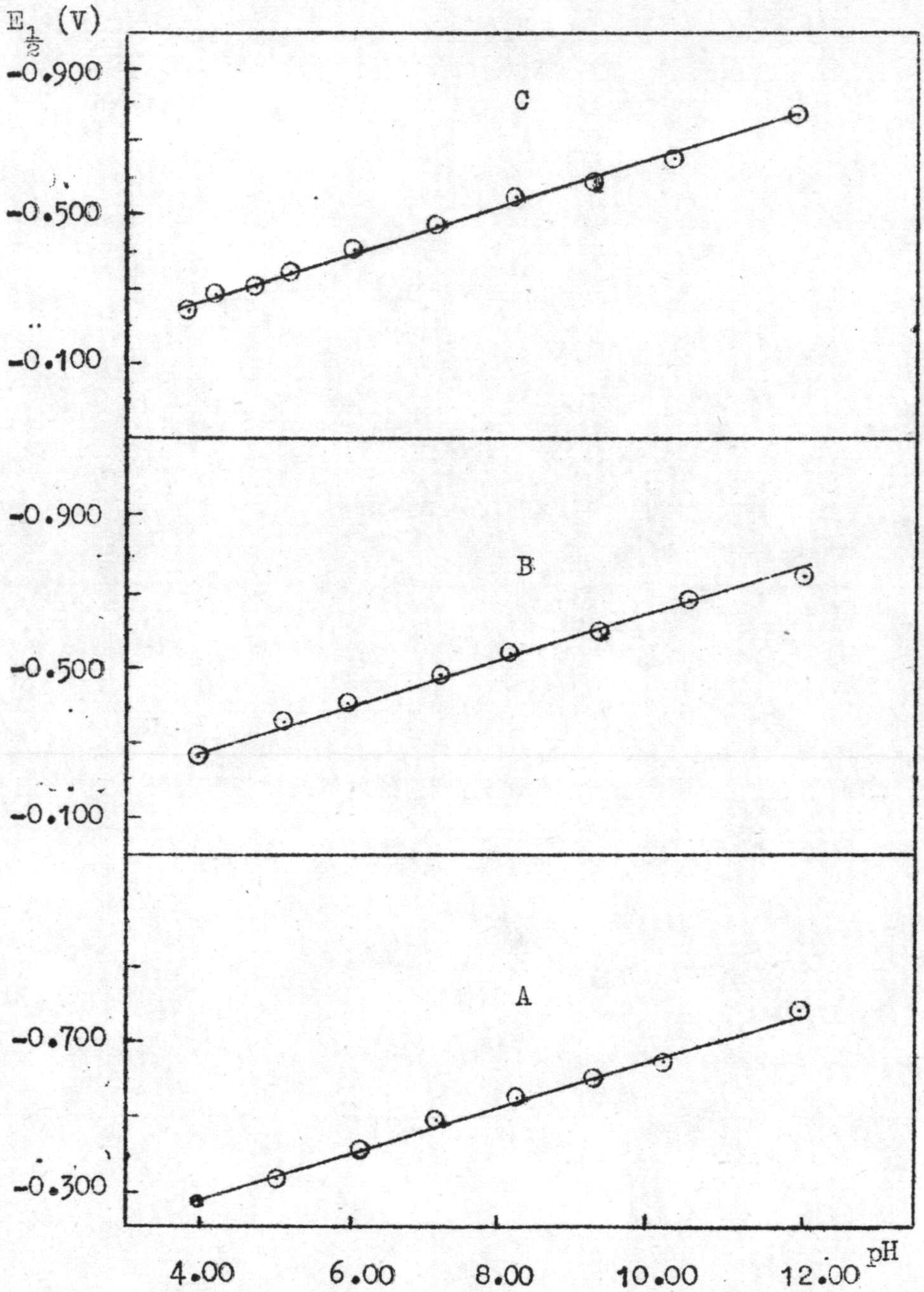


Figure 9 The effects of pH on the half wave potentials of Amaranth in A) 0.1M $(C_2H_5)_4NCl$, B) 0.1M KCl and C) 0.1M KNO_3

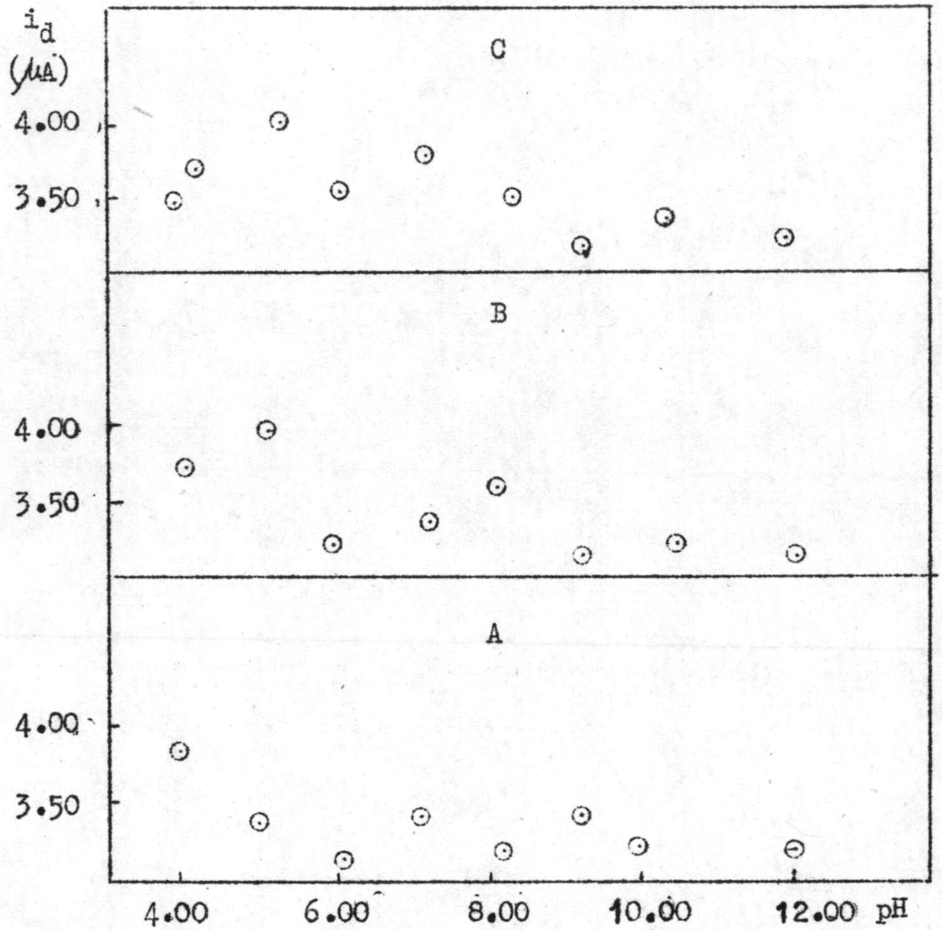


Figure 10 The effects of pH on the diffusion currents of Amaranth in A) 0.1M $(C_2H_5)_4NCl$, B) 0.1M KCl and C) 0.1M KNO_3

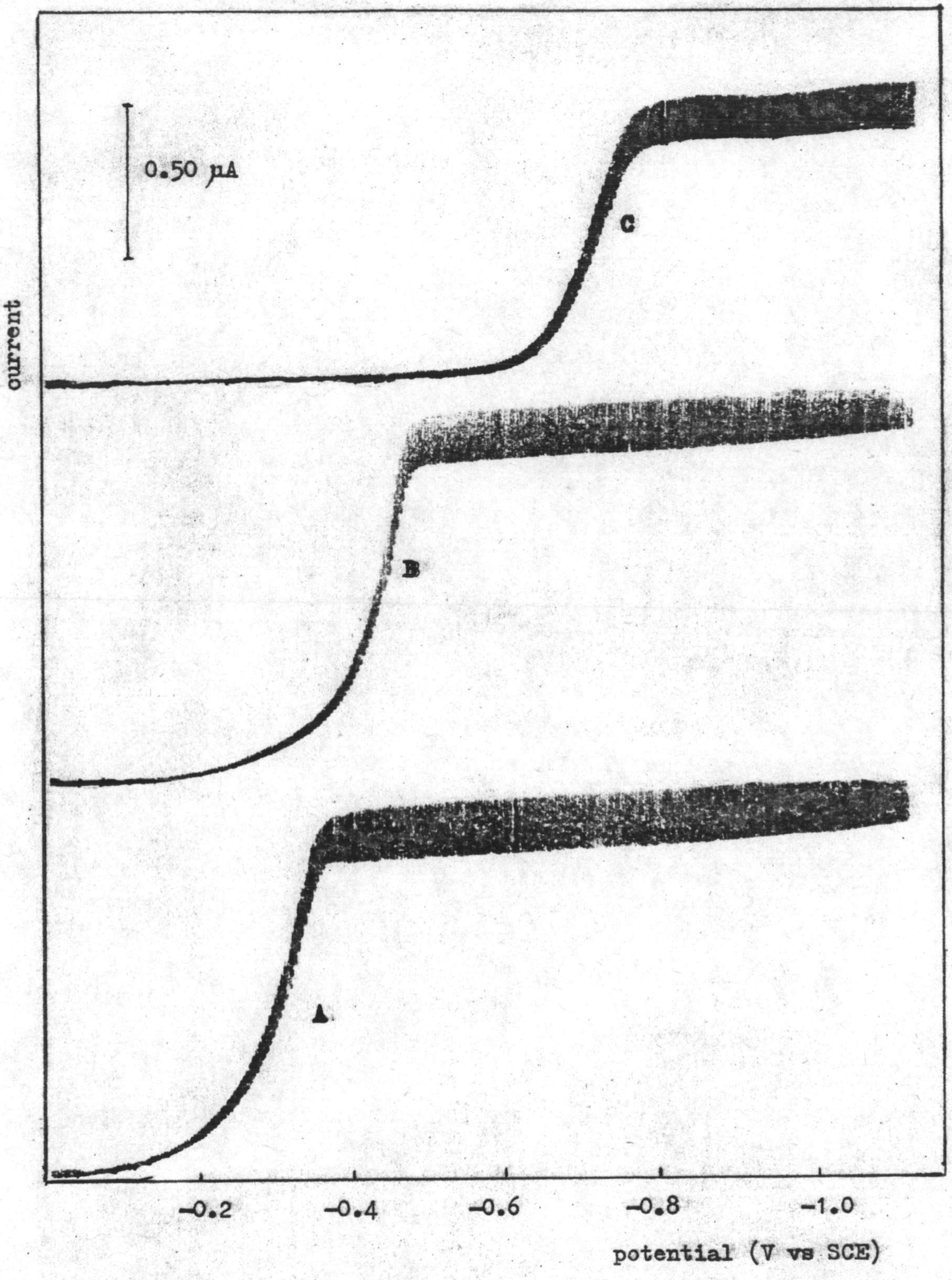


Figure 11 The polarograms of Ponceau 4R in 0.1M $(C_2H_5)_4NCl$ at pH A) 2.35, B) 3.60, C) 7.50

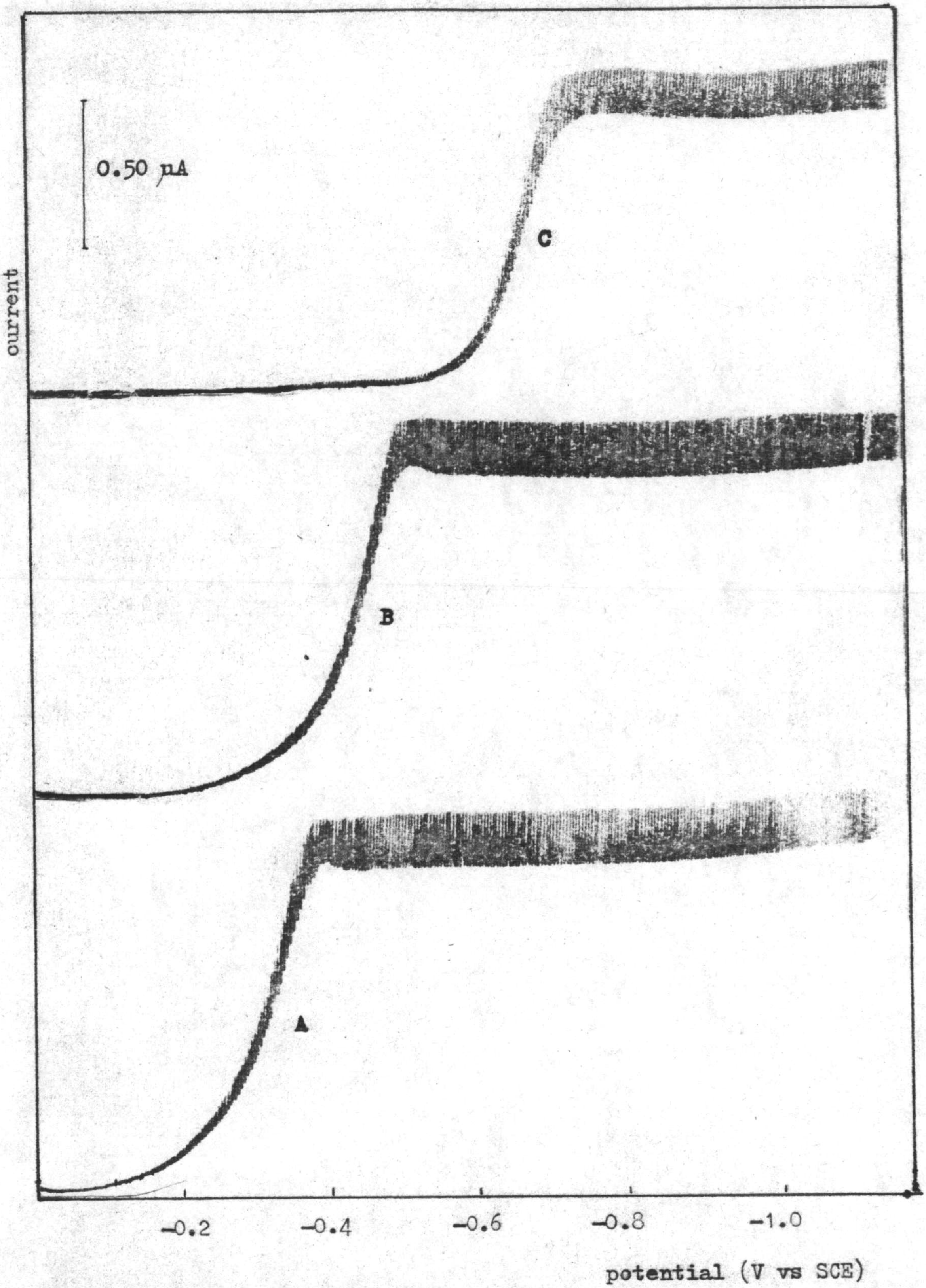


Figure 12 The polarograms of Ponceau 4R in 0.1M KCl at pH A) 2.45, B) 3.60, C) 7.21

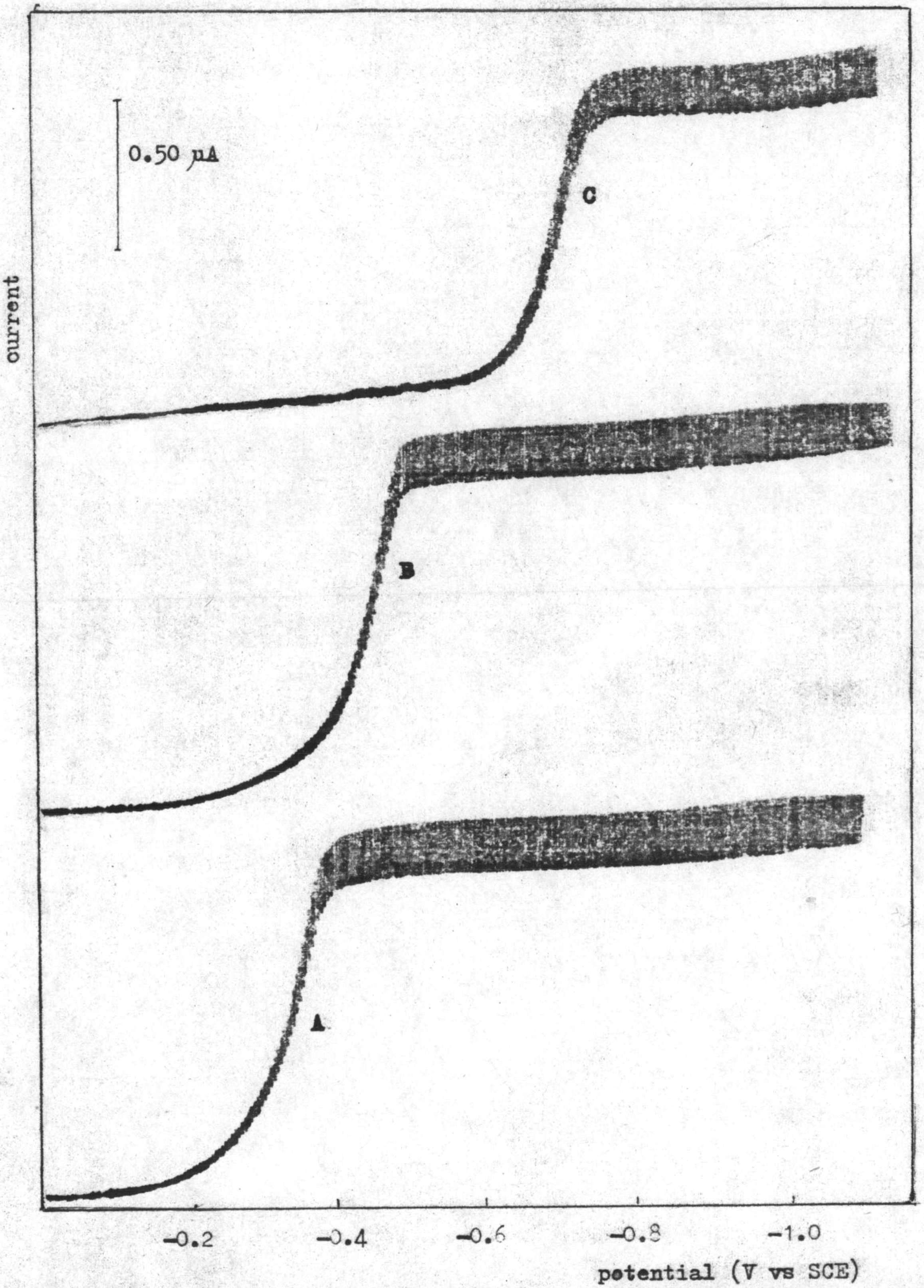


Figure 13 The polarograms of Ponceau 4R in 0.1M KNO_3 at pH A) 2.50, B) 3.60, C) 7.12

in 0.1 M KCl and Figure 13 for Ponceau 4R in 0.1 M KNO_3. Data for explaining this effect are also listed in Tables 6, 7 and 8, respectively. For every electrolyte, as the pH of the solution studied increases its half wave potential shifts to more negative potential. The shift of the half wave potentials at pH lower than 3.6 is more than the shift of the half wave potentials at pH higher than 3.6 . The plot of the half wave potential versus pH of the solution showed linearities of two sections (see Figure 14). The first section at pH 1.4-3.6, showed a slope of -0.105 in 0.1 M $(C_2H_5)_4NCl$ or 0.1 M KCl and a slope of -0.110 in 0.1 M KNO_3. The second section at pH4.0-12.0, showed a slope of -0.060 in 0.1 M $(C_2H_5)_4NCl$, -0.057 in 0.1 M KCl and -0.058 in 0.1 M KNO_3. The intersection points of these lines were found at pH 3.60 in 0.1 M $(C_2H_5)_4NCl$; 0.1 M KCl and in 0.1 M KNO_3. These pH are the pKa of Ponceau 4R (16,18). The maximum diffusion currents were obtained at pH 3.55 for Ponceau 4R in $(C_2H_5)_4NCl$, pH 3.10 for Ponceau 4R in KCl and pH 2.50 for Ponceau 4R in KNO_3. At other pH the diffusion current seemed to be independent of pH (see Figure 15).

4.2.1.3 Sunset Yellow FCF

The concentration of Sunset Yellow FCF understudied in every electrolyte at any pH is 5.0×10^{-4}M. The polarogram of Sunset Yellow FCF in 0.1 M $(C_2H_5)_4NCl$, 0.1 M KCl or 0.1 M KNO_3 at any pH in the range of 1-12 showed a single polarographic wave (see Figures 16-18). The wave is ill-defined as the pH of the dye solution in every electrolyte studied is lower than 3.5 and the wave becomes well-defined as pH is higher than 3.5. The effects of pH on the polarographic waves are demonstrated in Figure 16 for Sunset Yellow FCF

Table 6 Effect of pH on the half wave potential of Ponceau 4R in 0.1 M $(C_2H_5)_4NCl$

pH	$E_{\frac{1}{2}}$(V)	i_d^a(μA)
1.40	-0.165	0.967
1.95	-0.230	0.977
2.35	-0.305	0.938
3.05	-0.375	0.977
3.60	-0.410	1.035
3.90	-0.445	0.938
4.50	-0.485	0.938
5.15	-0.515	0.953
6.20	-0.565	0.918
7.50	-0.645	0.918
8.40	-0.710	0.953
9.30	-0.755	0.938
10.30	-0.815	0.905
11.95	-0.915	0.905

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

Table 7 Effect of pH on the half wave potential of Ponceau 4R in 0.1 M KCl

pH	$E_{\frac{1}{2}}$(V)	i_d^a (μA)
1.45	-0.180	0.977
2.00	-0.240	0.996
2.40	-0.290	0.957
3.10	-0.350	1.054
3.40	-0.410	1.015
3.60	-0.420	1.015
4.20	-0.470	0.915
5.18	-0.510	0.957
6.05	-0.565	0.996
7.21	-0.635	0.937
8.45	-0.675	0.937
9.40	-0.735	0.957
10.30	-0.770	0.915
11.95	-0.805	0.908

[a] mercury height 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}} = 0.61$

Table 8 Effect of pH on the half wave potential of Ponceau 4R in 0.1 M KNO_3

pH	$E_{\frac{1}{2}}(V)$	$i_d^a(\mu A)$
1.45	-0.140	0.976
2.00	-0.285	0.957
2.50	-0.320	1.030
3.05	-0.355	0.976
3.38	-0.410	0.996
3.60	-0.455	0.918
4.30	-0.470	0.918
5.15	-0.505	0.957
6.12	-0.560	0.977
7.12	-0.680	0.918
8.25	-0.702	0.923
9.30	-0.720	0.908
10.15	-0.760	0.923
12.00	-0.880	0.923

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

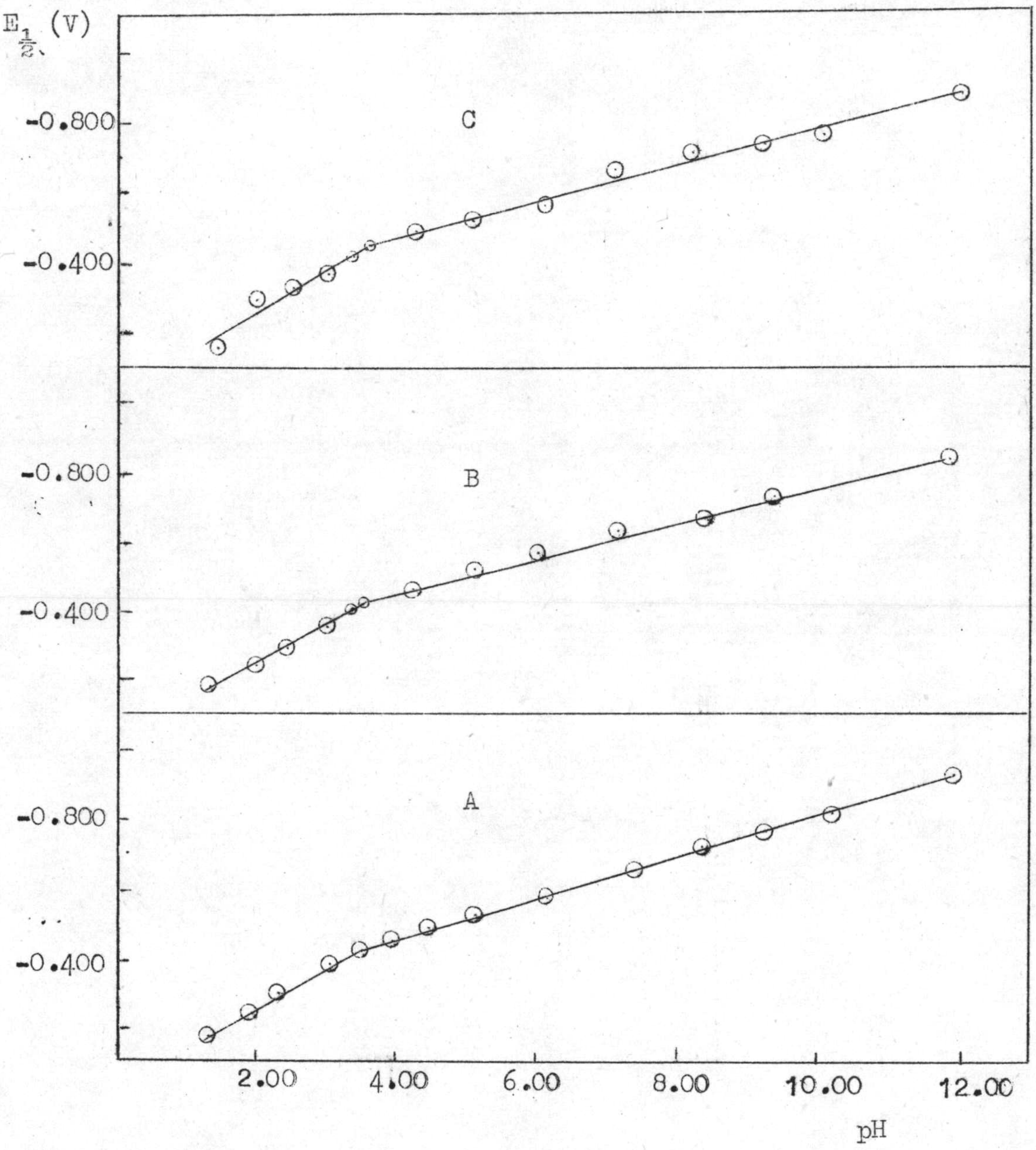


Figure 14 The effects of pH on the half wave potentials of Ponceau 4R in A) 0.1M $(C_2H_5)_4NCl$, B) 0.1M KCl and C) 0.1M KNO_3

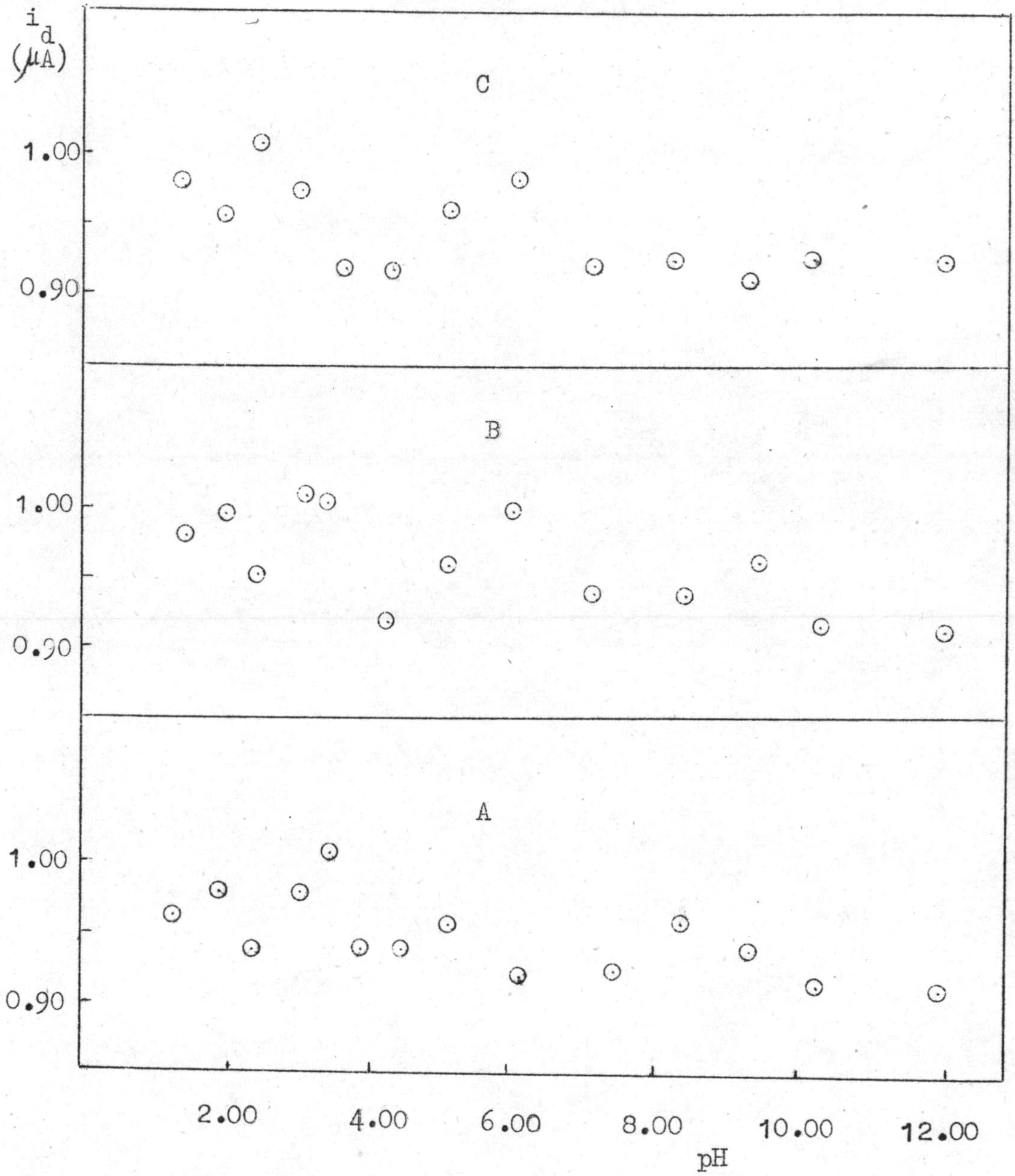


Figure 15 The effects of pH on the diffusion currents of Ponceau 4R in A) 0.1M $(C_2H_5)_4NCl$, B) 0.1M KCl and C) 0.1M KNO_3

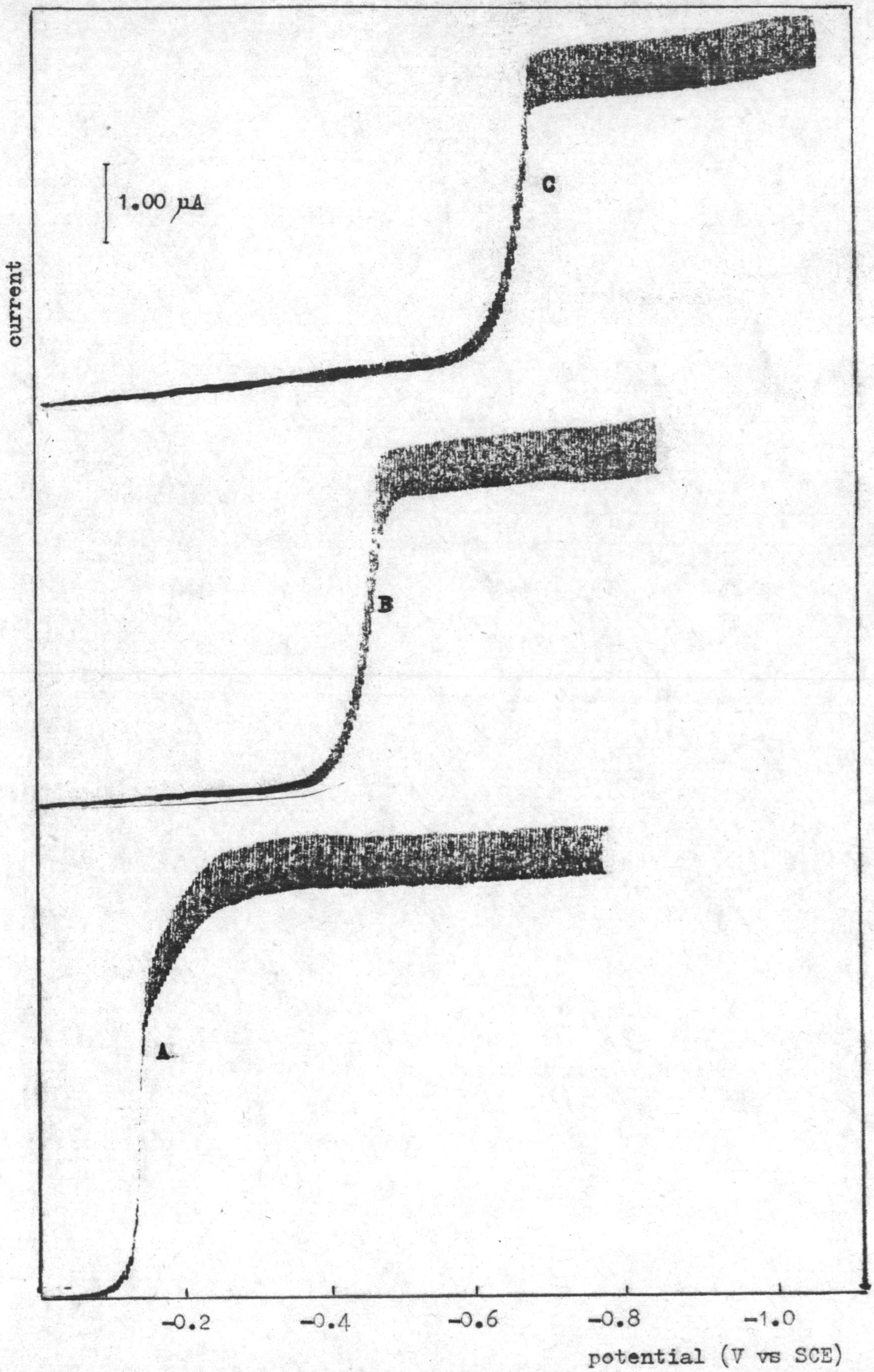


Figure 16 The polarograms of Sunset Yellow FCF in 0.1M $(C_2H_5)_4NCl$ at pH A) 2.50 , B) 6.10 and C) 9.20

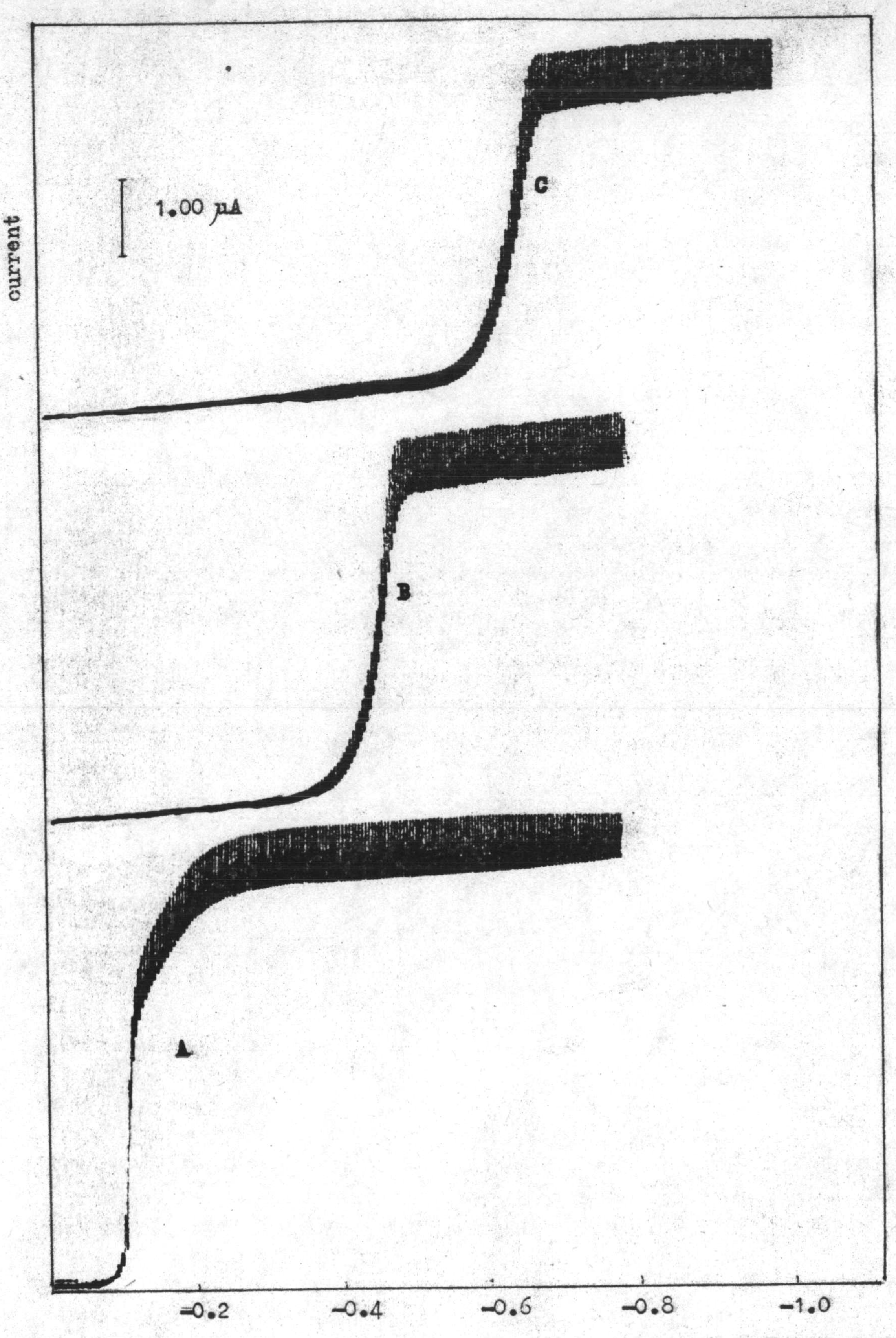


Figure 17 The polarograms of Sunset Yellow FCF in 0.1M KCl at pH A) 2.50, B) 6.05 and C) 9.05

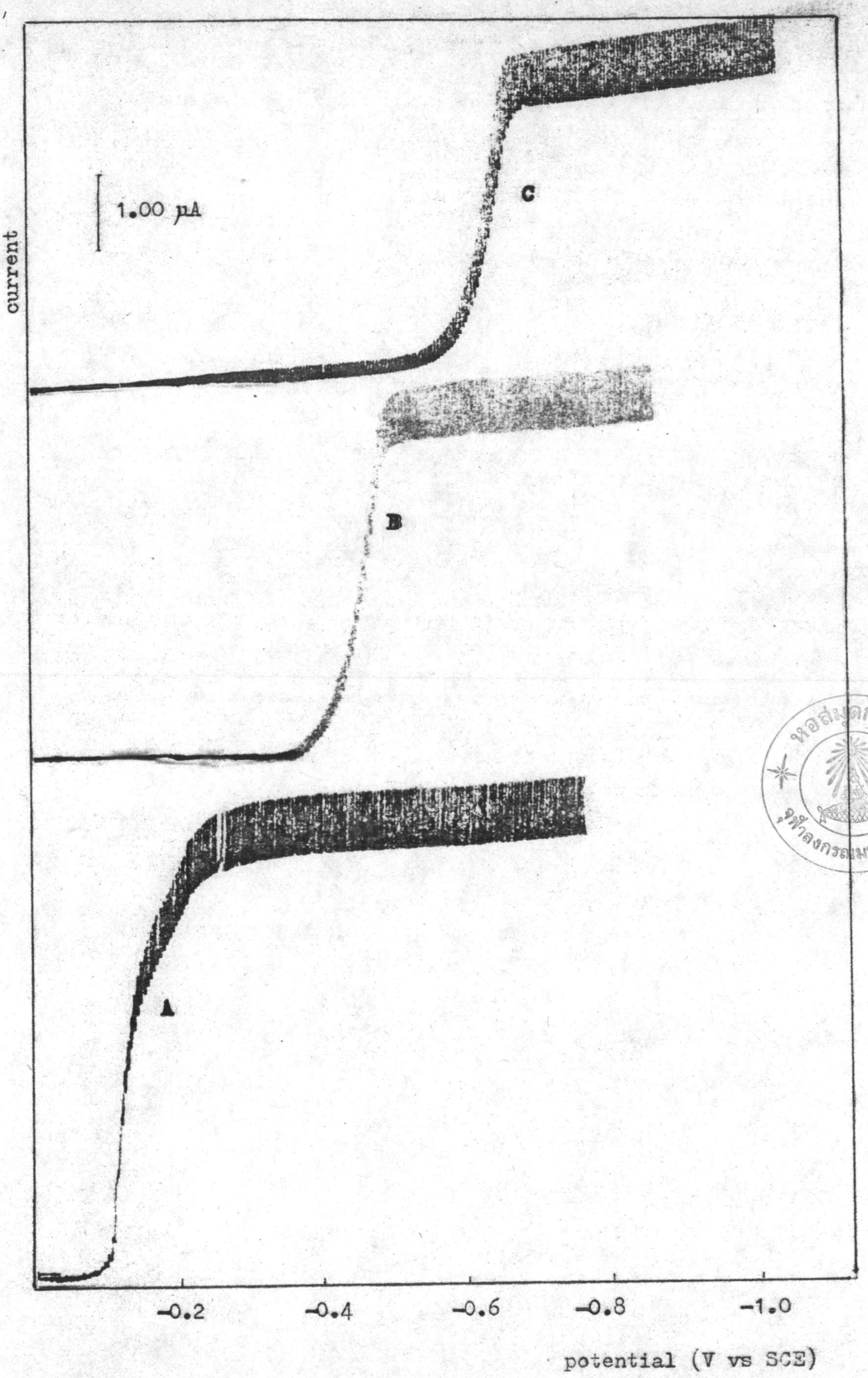


Figure 18 The polarograms of Sunset Yellow FCF in 0.1M KNO_3 at pH A) 2.50, B) 6.00 and C) 9.40

in 0.1 M $(C_2H_5)_4NCl$, Figure 17 for Sunset Yellow FCF in 0.1 M KCl and Figure 18 for Sunset Yellow FCF in 0.1 M KNO_3. Data For explaining this effect are also listed in Tables 9, 10 and 11, respectively. For every electrolyte studied, as the pH of the dye solution increases its half wave potential shifts to more negative potential. The shift of the half wave potential at pH lower than 5.2 is more than the shift of the half wave potential at pH higher than 5.2. The plot of the half wave potential versus pH of the solution showed linearities of two sections (see Figure 19). The first sections at pH 3.5-5.2, provided the slopes of -0.095 in 0.1 M $(C_2H_5)_4NCl$ and -0.100 in 0.1 M KCl and 0.1 M KNO_3. The second sections at pH5.2-12.0, indicated the slopes of -0.060 in 0.1 M$(C_2H_5)_4NCl$, 0.1 M KCl and 0.1 M KNO_3. The intersection points of these lines were found at pH 5.20 (see Figure 19). Thus, the pKa of Sunset Yellow FCF is 5.20 (16,18). The maximum diffusion currents of Sunset Yellow FCF were obtained at pH 4.85 in 0.1 M $(C_2H_5)_4NCl$, 4.25 in 0.1 M KCl and 5.15 in 0.1 M KNO_3. At other pH values the diffusion currents seemed to be independent of pH (see Figure 20).

4.2.1.4 Orange RN

The concentration of Orange RN understudied in every electrolyte at any pH is 4.0×10^{-4} M. The polarogram of Orange RN in 0.1 M $(C_2H_5)_4NCl$, 0.1 M KCl or 0.1 M KNO_3 at any pH in the range of 1-12 showed a single polarographic wave (see Figures 21-23). The wave is well defined in every electrolyte and every pH studied except pH 1.4. The effects of pH on the polarographic waves are demonstrated in Figure 21 for Orange RN in 0.1 M $(C_2H_5)_4NCl$, Figure 22 for Orange RN in 0.1 M KCl and Figure 23 for Orange RN in 0.1 M KNO_3. Data for

Table 9 Effect of pH on the half wave potential of Sunset Yellow FCF in 0.1 M $(C_2H_5)_4NCl$

pH	$E_{\frac{1}{2}}(V)$	$i_d^a(\mu A)$	Remarks
1.40	b	b	ill-defined wave
2.50	b	b	ill-defined wave
3.50	-0.220	4.31	well-defined wave
4.40	-0.305	4.16	well-defined wave
4.85	-0.350	4.45	well-defined wave
5.25	-0.380	4.16	well-defined wave
5.55	-0.410	3.91	well-defined wave
6.10	-0.448	3.91	well-defined wave
6.75	-0.495	3.83	well-defined wave
7.55	-0.520	3.91	well-defined wave
8.30	-0.600	3.71	well-defined wave
9.20	-0.640	3.42	well-defined wave
10.30	-0.715	3.83	well-defined wave
11.80	-0.825	3.83	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}} = 0.61$

[b] cannot be measured accurately

Table 10 Effect of pH on the half wave potential of Sunset Yellow FCF in 0.1 M KCl

pH	$E_{\frac{1}{2}}$(V)	i_d^a(μA)	Remarks
1.40	b	b	ill-defined wave
2.50	b	b	ill-defined wave
3.60	-0.245	4.47	well-defined wave
4.25	-0.295	4.78	well-defined wave
4.65	-0.345	4.08	well-defined wave
5.18	-0.385	4.45	well-defined wave
5.60	-0.410	4.14	well-defined wave
6.05	-0.440	4.14	well-defined wave
6.90	-0.510	4.41	well-defined wave
7.50	-0.535	4.22	well-defined wave
8.20	-0.600	4.06	well-defined wave
9.05	-0.632	4.22	well-defined wave
10.30	-0.710	4.22	well-defined wave
12.00	-0.835	4.06	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

[b] cannot be measured accurately

Table 11. Effect of pH on the half wave potential of Sunset Yellow FCF in 0.1 M KNO_3

pH	$E_{\frac{1}{2}}$(V)	i_d^a(A)	Remarks
1.50	b	b	ill-defined wave
2.50	b	b	ill-defined wave
3.55	-0.222	4.53	well-defined wave
4.30	-0.300	4.39	well-defined wave
4.65	-0.335	4.39	well-defined wave
5.15	-0.380	4.69	well-defined wave
5.50	-0.400	4.53	well-defined wave
6.00	-0.445	4.41	well-defined wave
6.80	-0.480	4.30	well-defined wave
7.30	-0.525	4.22	well-defined wave
8.50	-0.600	4.14	well-defined wave
9.40	-0.645	4.14	well-defined wave
10.30	-0.695	4.08	well-defined wave
12.00	-0.825	4.14	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

[b] cannot be measured accurately

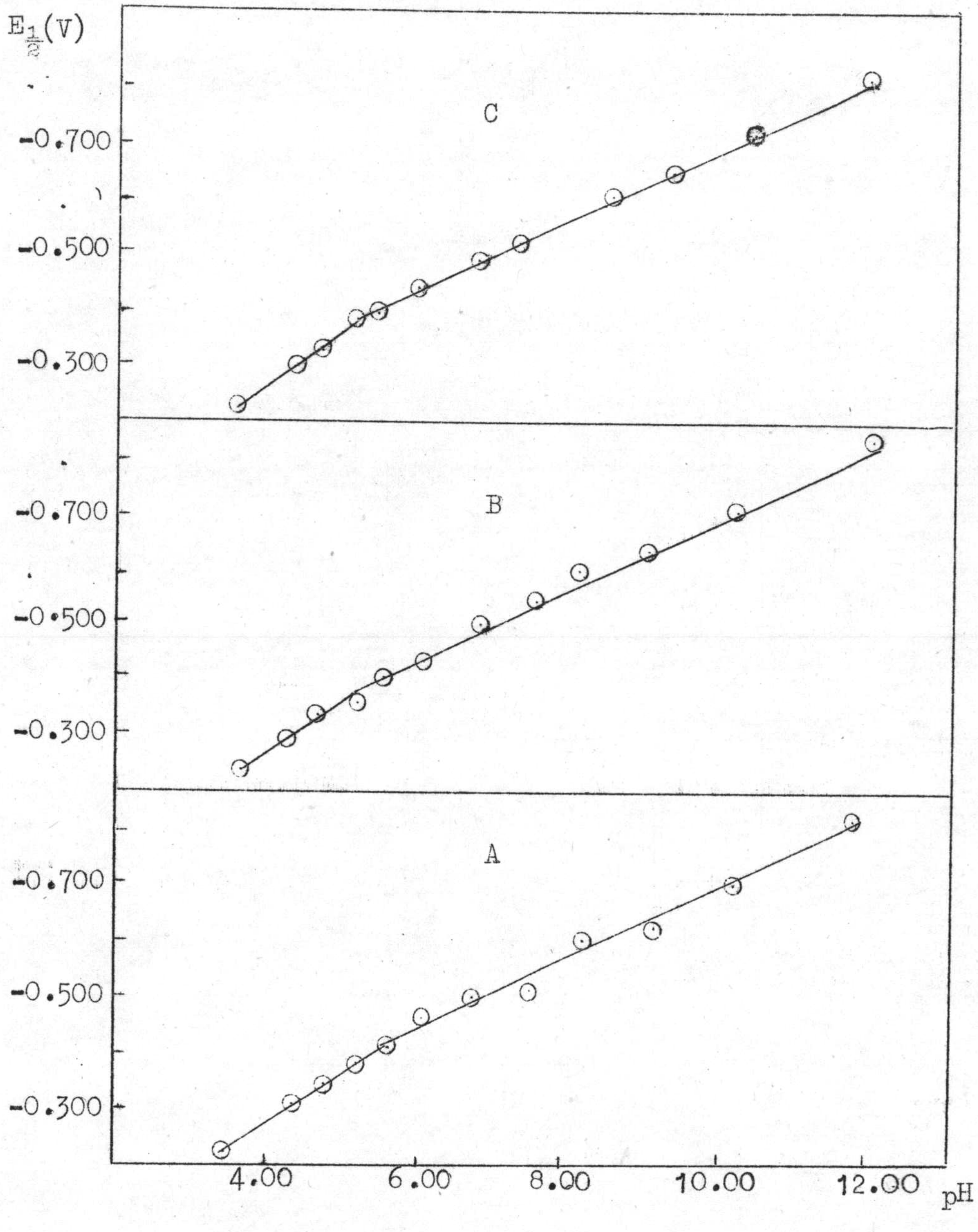


Figure 19 The effects of pH on the half wave potentials of Sunset Yellow FCF in A) 0.1M $(C_2H_5)_4NCl$, B) 0.1M KCl and C) 0.1M KNO_3

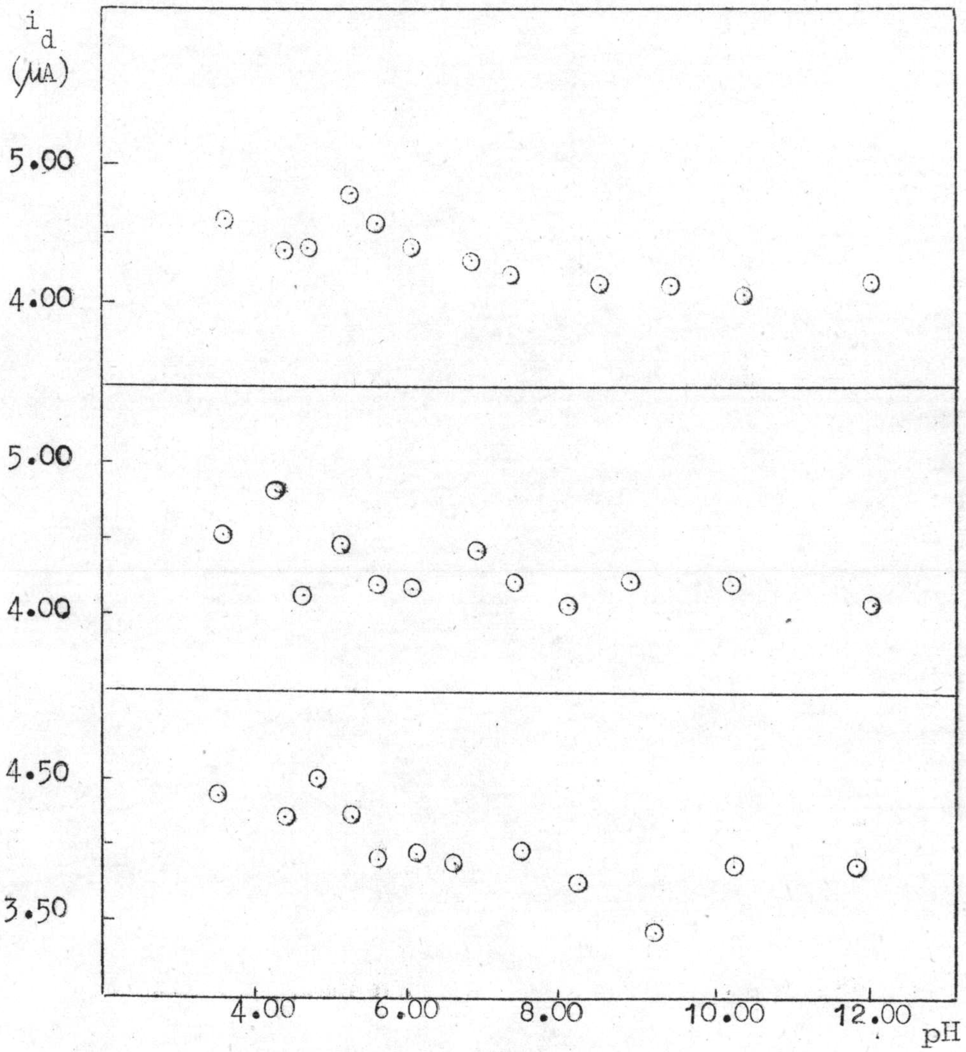


Figure 20 The effects of pH on the diffusion currents of Sunset yellow FCF in A) 0.1M $(C_2H_5)_4NCl$, B) 0.1M KCl and C) 0.1M KNO_3

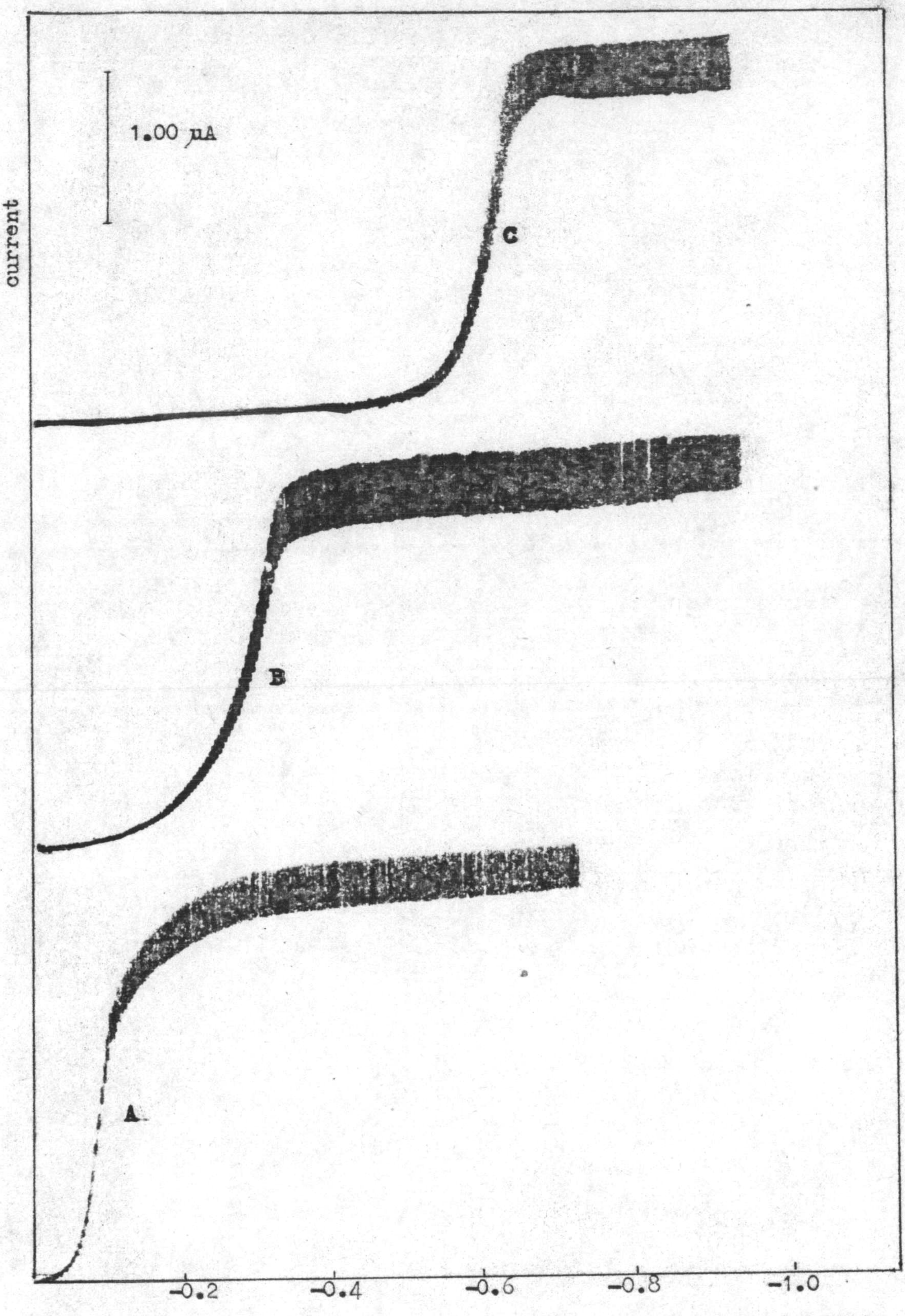


Figure 21 The polarograms of Orange RN in 0.1M $(C_2H_5)_4NCl$ at pH A) 1.40 , B) 4.10 and C) 8.20

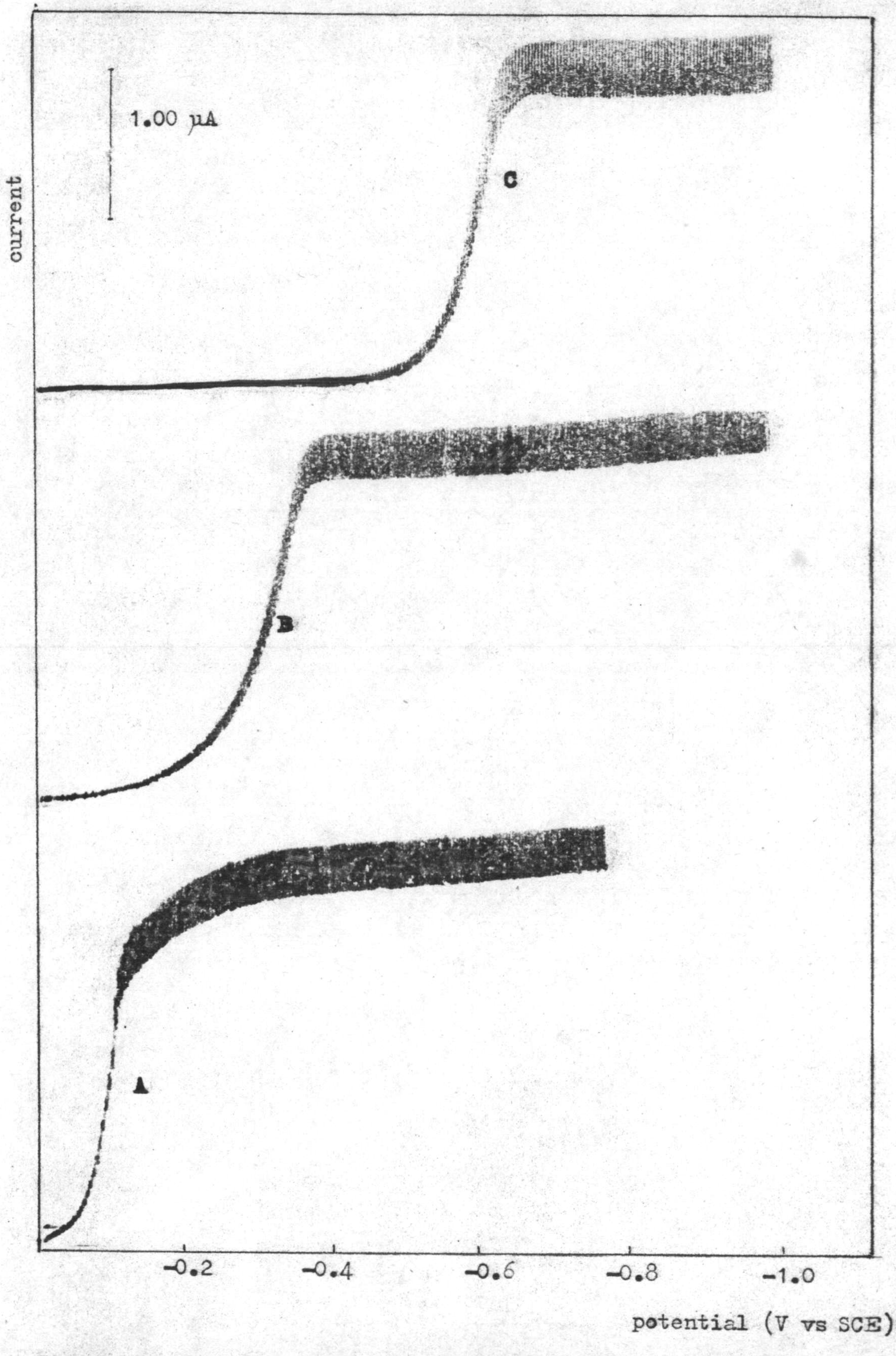


Figure 22 The polarograms of Orange RN in 0.1M KCl at pH A) 1.40, B) 4.02 and C) 8.20

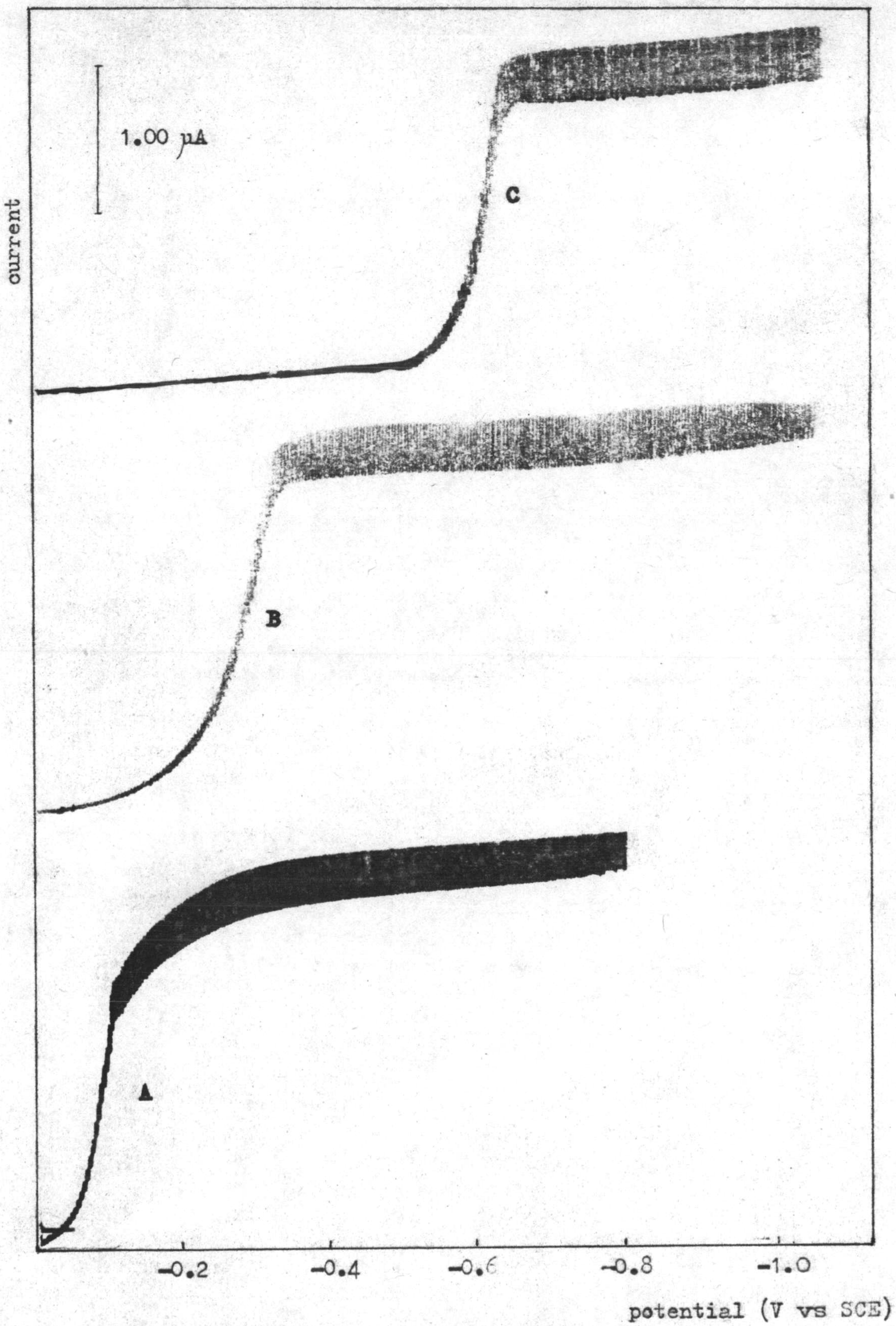


Figure 23 The polarograms of Orange RN in 0.1M KNO_3 at pH

A) 1.40,, B) 4.00 and C) 8.20

illustrating this effect are also listed in Tables 12,13 and 14, respectively. For every electrolyte studied, as the pH of the dye solution increases its half wave potential shifts to more negative potential. The shift of the half wave potentials at pH lower than 5.5 is more than the shift of the half wave potential at pH higher than 5.5. These resulted in two linear sections appeared in the plot of the half wave potential versus pH of the solution (see Figure 24). The first sections at pH 2.4-5.5, provided the slopes of -0.100 in 0.1 M $(C_2H_5)_4NCl$ and -0.090 in 0.1 M KCl or 0.1 M KNO_3. The second sections at pH5.5-12.0, indicated the slopes of -0.060 in 0.1 M $(C_2H_5)_4NCl$ and -0.058 in 0.1 M KCl and 0.1 M KNO_3. The intersection points of these lines were found at pH 5.50 (see Figure 24). Thus, the pKa of Orange RN in 0.1 M $(C_2H_5)_4NCl$, in 0.1 M KCl or 0.1 M KNO_3 is 5.50 (16,18). The maximum diffusion currents of Orange RN were obtained at pH 4.10 in 0.1 M $(C_2H_5)_4NCl$, 5.20 in 0.1 M KCl and 4.00 in 0.1 M KNO_3. At other pH values the diffusion currents seemed to be independent (see Figure 25).

4.2.1.5 Orange G

The concentration of Orange G understudied in every electrolyte at any pH is 1.0×10^{-4}M. The polarogram of Orange G in 0.1 M $(C_2H_5)_4NCl$, 0.1 M KCl or 0.1 M KNO_3 at any pH in the range of 1-12 showed a single polarographic wave except at pH 3.6-4.5 two polarographic wave were obtained. In every electrolyte studied the first reduction wave appeared at the pH range of 1.4-4.5 and the second wave occurred at pH higher than 3.5. The first wave is ill-defined as the pH of the dye solution in every electrolyte

Table 12 Effect of pH on the half wave potential of Orange RN in 0.1 M $(C_2H_5)_4NCl$

pH	$E_{\frac{1}{2}}$(V)	i_d^a (μA)	Remarks
1.40	b	b	ill-defined wave
2.40	-0.132	1.91	well-defined wave
3.25	-0.220	1.84	well-defined wave
4.10	-0.290	2.02	well-defined wave
5.10	-0.385	1.78	well-defined wave
5.50	-0.450	1.80	well-defined wave
6.50	-0.485	1.76	well-defined wave
7.20	-0.525	1.68	well-defined wave
8.20	-0.600	1.84	well-defined wave
9.30	-0.655	1.84	well-defined wave
10.50	-0.740	1.76	well-defined wave
12.00	-0.805	1.72	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}} = 0.61$

[b] cannot be measured accurately

Table 13 Effect of pH on the half wave potential of Orange RN in 0.1 M KCl

pH	$E_{\frac{1}{2}}$(V)	i_d^a(μA)	Remarks
1.40	b	b	ill-defined wave
2.45	-0.130	2.02	well-defined wave
3.20	-0.220	2.13	well-defined wave
4.02	-0.280	2.02	well-defined wave
5.20	-0.420	2.25	well-defined wave
5.50	-0.450	1.95	well-defined wave
6.20	-0.490	1.91	well-defined wave
7.20	-0.560	1.72	well-defined wave
8.20	-0.615	1.95	well-defined wave
9.20	-0.655	1.91	well-defined wave
10.30	-0.720	1.88	well-defined wave
12.00	-0.810	1.75	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

[b] cannot be measured accurately

Table 14 Effect of pH on the half wave potential of Orange RN in 0.1 M KNO_3

pH	$E_{\frac{1}{2}}$(V)	i_d^a(μA)	Remarks
1.40	b	b	ill-defined wave
2.45	-0.130	2.12	well-defined wave
3.20	-0.225	2.08	well-defined wave
4.00	-0.290	2.23	well-defined wave
5.20	-0.418	2.08	well-defined wave
5.50	-0.450	1.91	well-defined wave
6.20	-0.495	1.91	well-defined wave
7.20	-0.555	1.88	well-defined wave
8.20	-0.610	1.72	well-defined wave
9.30	-0.660	1.88	well-defined wave
10.30	-0.725	1.88	well-defined wave
12.00	-0.810	1.72	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

[b] cannot be measured accurately

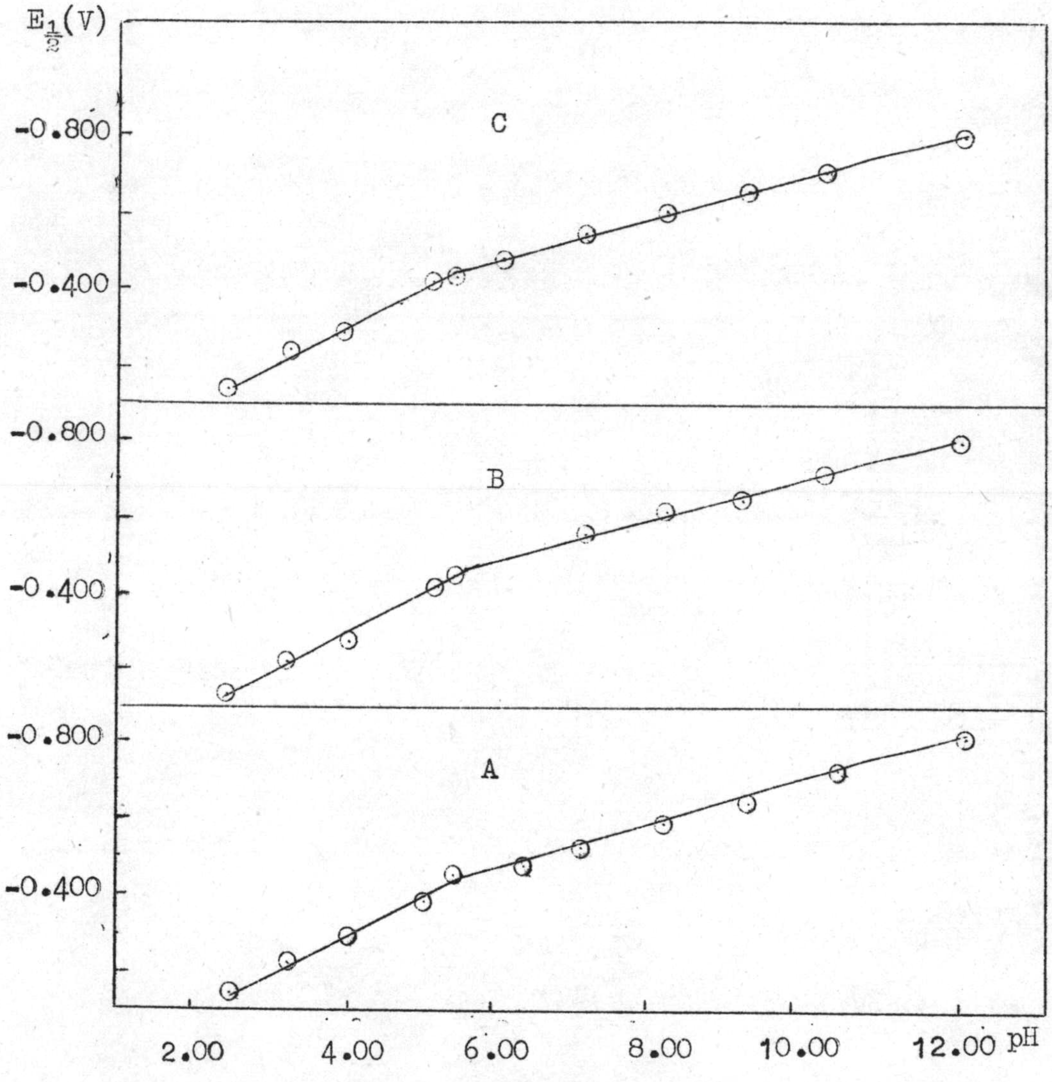


Figure 24 The effects of pH on the half wave potentials of Orange RN in A) 0.1M $(C_2H_5)_4NCl$, B) 0.1M KCl and C) 0.1M KNO_3

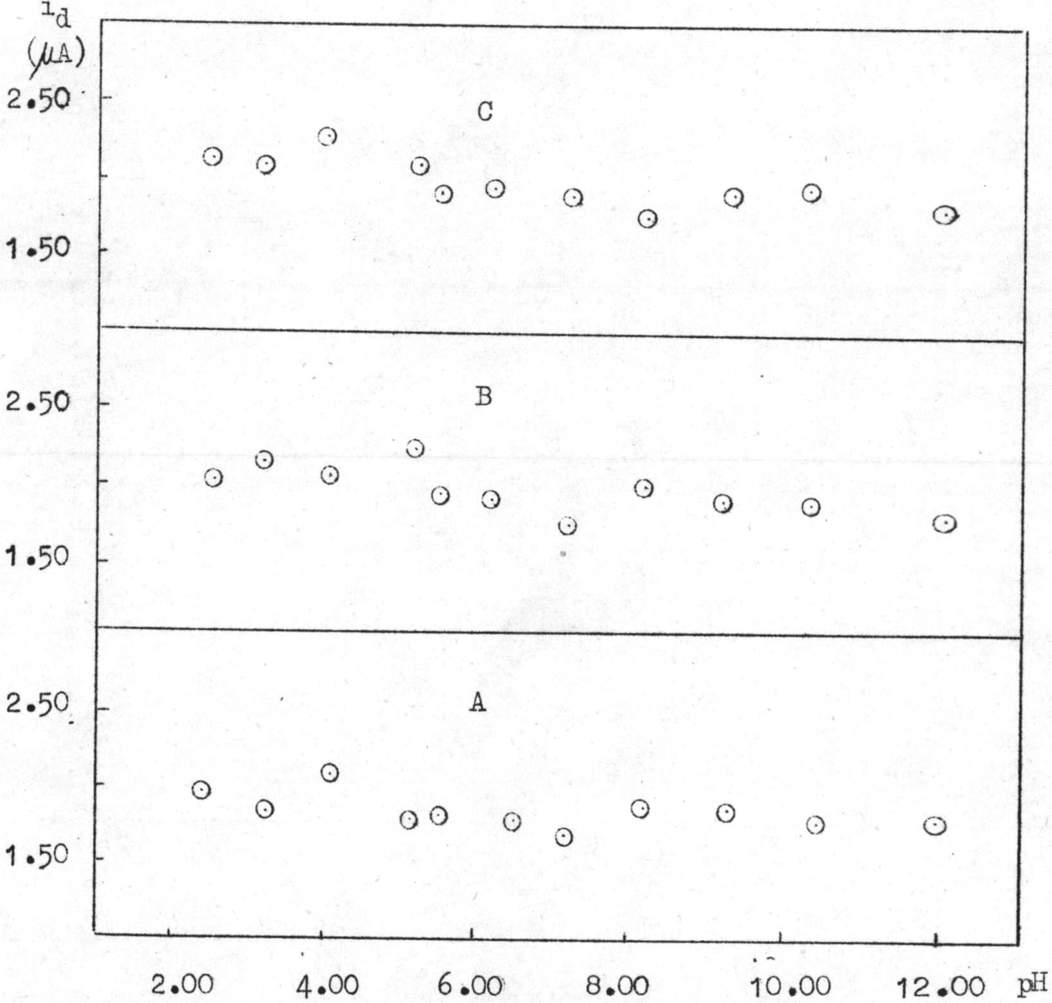


Figure 25 The effects of pH on the diffusions currents of Orange RN in A) 0.1 M $(C_2H_5)_4NCl$, B) 0.1M KCl and C) 0.1M KNO_3

studied is lower than 2.5 and the wave becomes well-defined as pH is higher than 3.5. The effects of pH on the polarographic waves are demonstrated in Figure 26 for Orange G in 0.1 M $(C_2H_5)_4NCl$, Figure 27 for Orange G in 0.1 M KCl and Figure 28 for Orange G in 0.1 M KNO_3. Data for explaining this effect are also listed in Tables 15, 16 and 17 respectively. As the pH of the dye solution increases the half wave potential of the first or the second wave shifts to more negative potential. The plot of the half wave potential of either the first wave or the second wave versus pH of the solution showed a linearity. The first wave provided the same value of slope, -0.065, in every electrolyte at pH 3.5-4.5. The second wave provided the slopes of -0.058 in 0.1 M $(C_2H_5)_4NCl$ and -0.057 in 0.1 M KCl and 0.1 KNO_3 (see Figure 29). As the pH increases, the diffusion current of the first wave decreases and this wave disappears at pH 4.70 and higher. The diffusion current of the second wave increases as the pH of the solution increases to pH 6.10. As the pH is higher than 6.10 the diffusion currents of the second wave in 0.1 M KCl and 0.1 M KNO_3 seemed to be independent of pH but in 0.1 M $(C_2H_5)_4NCl$ the diffusion current of the second wave decreases as the pH of the dye solution increases (see Figure 30).

4.2.2 Reversibility

The reversibilities of Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G were tested from the polarograms of well defined waves. For a reversible wave, a plot of the working electrode potential against log i/i_d-i gives a straight line of slope, $\left|2.303\ RT/nF\right|$ V or $\left|\frac{60}{n}\right|$ mV at 30°C. If the wave is irreversible the slope is larger

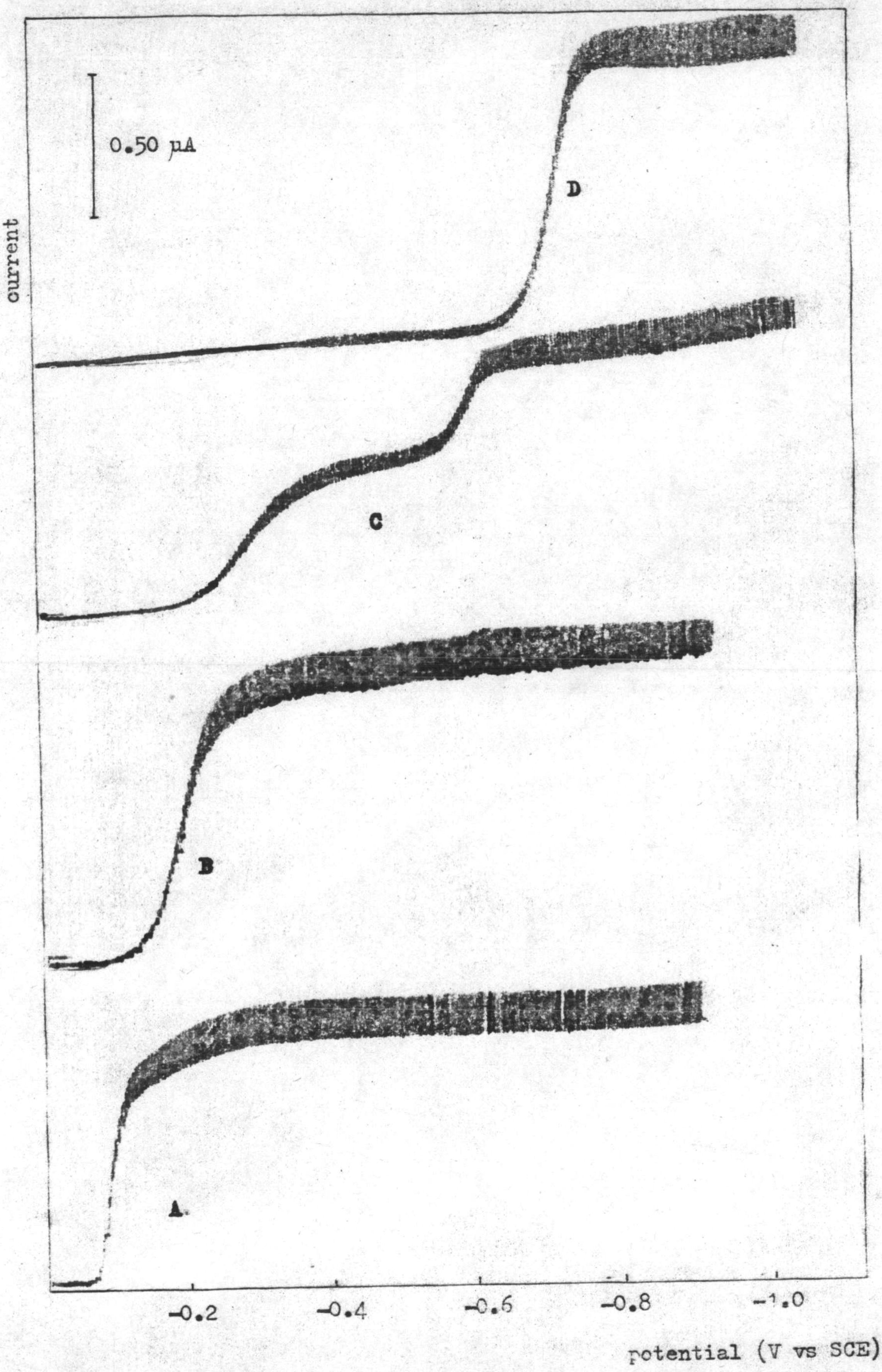


Figure 26 The polarograms of Orange G in 0.1M $(C_2H_5)_4$ NCl at pH

A) 2.50 , B) 3.50 , C) 4.50 and D) 7.30

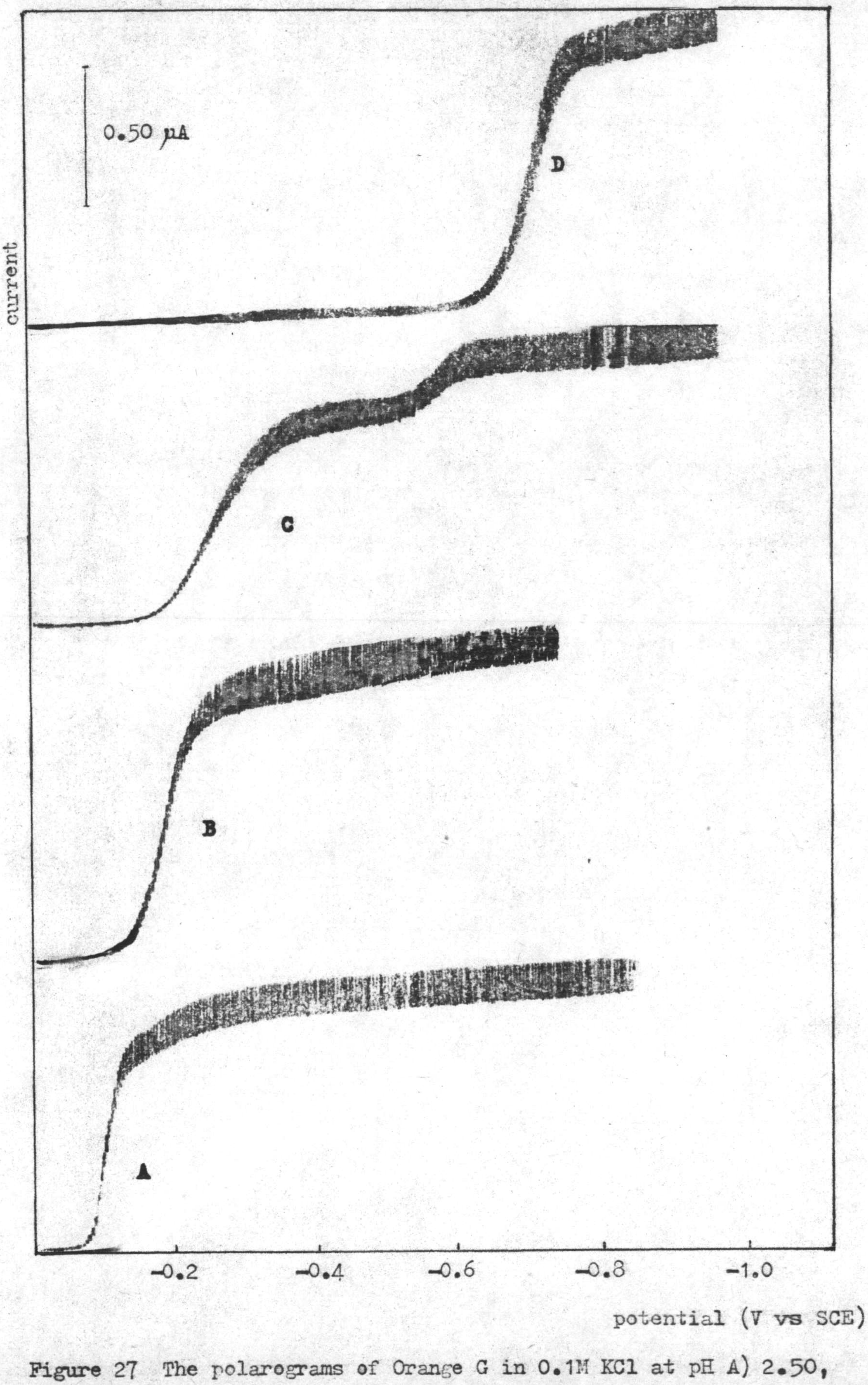


Figure 27 The polarograms of Orange G in 0.1M KCl at pH A) 2.50, B) 3.50, C) 4.05 and D) 7.20

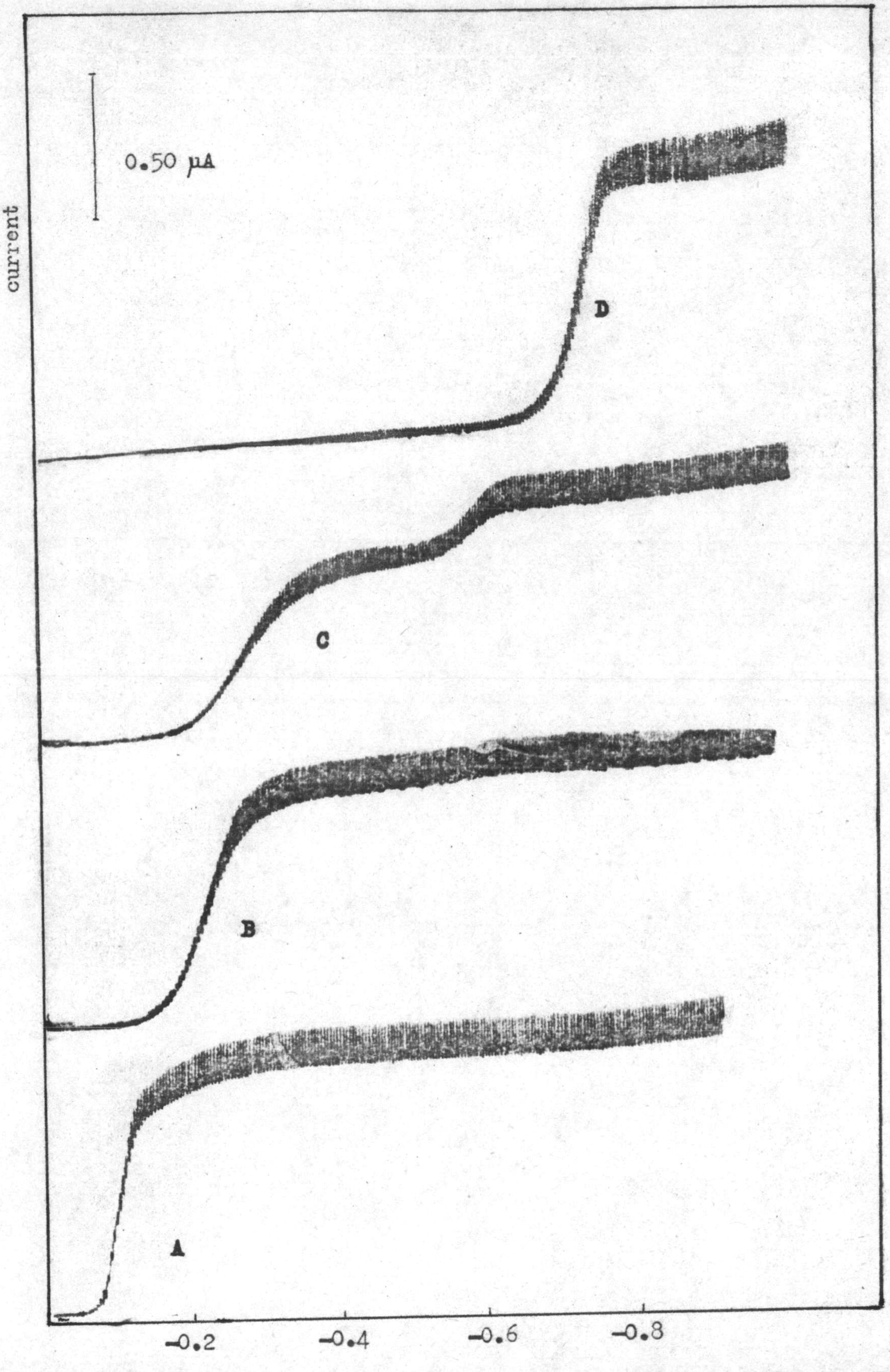


Figure 28 The polarograms of Orange G in 0.1M KNO_3 at pH A) 2.50 B) 3.50, C)4.05 and **D**) 7.20

Table 15 Effect of pH on the half wave potential of Orange G in 0.1 M $(C_2H_5)_4NCl$

pH	$E_{\frac{1}{2},1}$ (V)	$i_{d,1}^{a}$ (μA)	$E_{\frac{1}{2},2}$ (V)	$i_{d,2}^{a}$ (μA)	Remarks
1.40	b	b	-	-	ill-defined wave
2.45	b	b	-	-	ill-defined wave
3.50	-0.185	0.857	-	-	well-defined wave
3.60	-0.200	0.664	-0.550	0.176	well-defined wave
4.05	-0.240	0.625	-0.570	0.186	well-defined wave
4.50	-0.280	0.462	-0.580	0.360	well-defined wave
4.70	-	-	-0.605	0.710	well-defined wave
5.05	-	-	-0.615	0.727	well-defined wave
5.20	-	-	-0.620	0.738	well-defined wave
5.70	-	-	-0.630	0.742	well-defined wave
6.20	-	-	-0.675	0.781	well-defined wave
7.30	-	-	-0.715	0.779	well-defined wave
8.30	-	-	-0.775	0.779	well-defined wave
9.02	-	-	-0.810	0.732	well-defined wave
10.30	-	-	-0.870	0.722	well-defined wave
11.85	-	-	-0.960	0.722	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

[b] cannot be measured accurately

Table 16 Effect of pH on the half wave potential of Orange G in 0.1 M KCl

pH	$E_{\frac{1}{2},1}$ (V)	$i^{a}_{d,1}$ (μA)	$E_{\frac{1}{2},2}$ (V)	$i^{a}_{d,2}$ (μA)	Remarks
1.40	b	b	-	-	ill-defined wave
2.50	b	b	-	-	ill-defined wave
3.50	-0.200	0.836	-	-	well-defined wave
3.60	-0.220	0.664	-0.570	0.160	well-defined wave
4.05	-0.255	0.520	-0.580	0.201	well-defined wave
4.50	-0.280	0.380	-0.590	0.440	well-defined wave
4.70	-	-	-0.605	0.730	well-defined wave
5.00	-	-	-0.630	0.737	well-defined wave
5.20	-	-	-0.635	0.768	well-defined wave
6.10	-	-	-0.670	0.781	well-defined wave
7.20	-	-	-0.725	0.781	well-defined wave
8.20	-	-	-0.780	0.790	well-defined wave
9.05	-	-	-0.810	0.781	well-defined wave
10.30	-	-	-0.870	0.782	well-defined wave
11.85	-	-	-0.965	0.790	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

[b] cannot be measured accurately

Table 17 Effect of pH on the half wave potential of Orange G in 0.1 M KNO_3

pH	$E_{\frac{1}{2},1}$ (V)	$i_{d,1}^{a}$ (μA)	$E_{\frac{1}{2},2}$ (V)	$i_{d,2}^{a}$ (μA)	Remarks
1.40	b	b	-	-	ill-defined wave
2.50	b	b	-	-	ill-defined wave
3.50	-0.210	0.801	-	-	well-defined wave
3.60	-0.230	0.664	-0.570	0.176	well-defined wave
4.05	-0.270	0.508	-0.590	0.215	well-defined wave
4.50	-0.280	0.380	-0.595	0.440	well-defined wave
4.70	-	-	-0.600	0.730	well-defined wave
5.00	-	-	-0.630	0.732	well-defined wave
5.20	-	-	-0.635	0.745	well-defined wave
6.10	-	-	-0.675	0.781	well-defined wave
7.20	-	-	-0.720	0.790	well-defined wave
8.25	-	-	-0.785	0.764	well-defined wave
9.00	-	-	-0.810	0.781	well-defined wave
10.30	-	-	-0.865	0.766	well-defined wave
11.85	-	-	-0.965	0.781	well-defined wave

[a] mercury height = 65.0 cm ; $m^{\frac{2}{3}}t^{\frac{1}{6}}$ = 0.61

[b] cannot be measured accurately

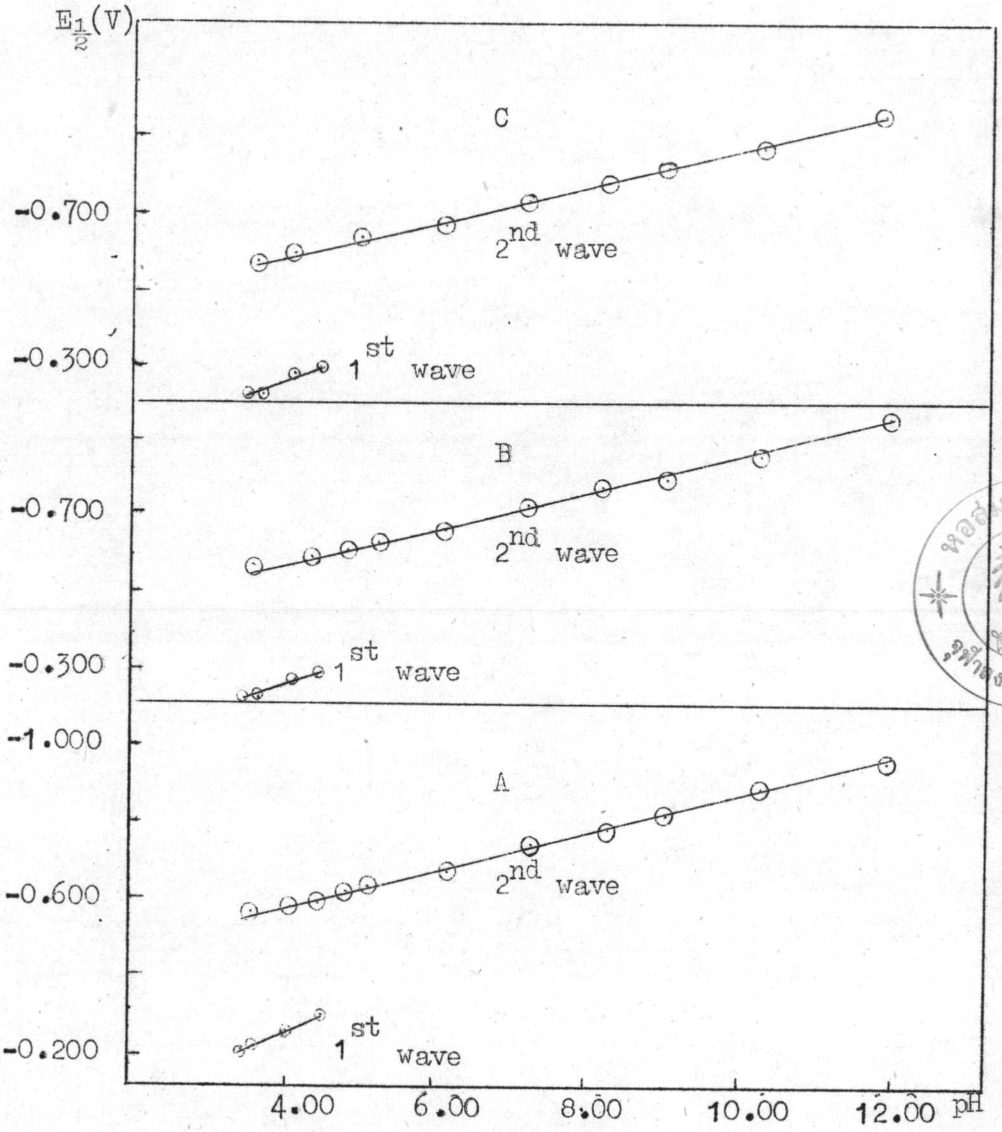


Figure 29 The effects of pH on the half wave potentials of Orange G in A) 0.1M $(C_2H_5)_4NCl$, B) 0.1M KCl and C) 0.1M KNO_3

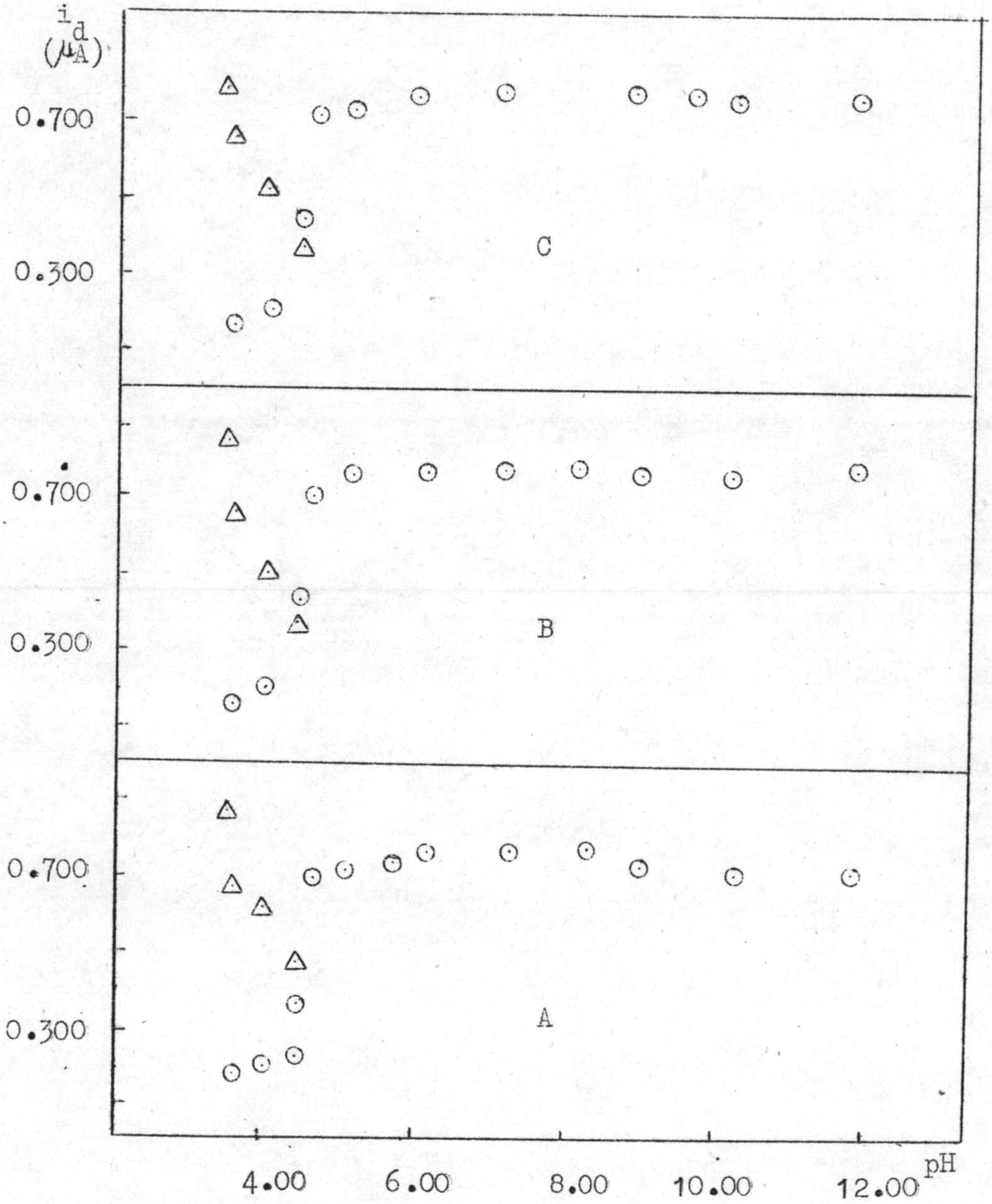


Figure 30 The effects of pH on the diffusion currents of Orange G in A) 0.1M $(C_2H_5)_4NCl$, B) 0.1M KCl, and C) 0.1M KNO_3. △ for the first wave, ⊙ for the second wave

than $\left|\frac{60}{n}\right|$ mV since the wave is more drawn out. Another simpler method, although less exact, is to determine the $E_{\frac{1}{4}} - E_{\frac{3}{4}}$ value for the wave. For a reversible wave the $\left|E_{\frac{1}{4}} - E_{\frac{3}{4}}\right|$ value equals to $\frac{57}{n}$ mV at 30°C and for an irreversible wave, the $\left|E_{\frac{1}{4}} - E_{\frac{3}{4}}\right|$ value is greater than $\frac{57}{n}$ mV. Results of these tests are listed in Table 18 for Amaranth, Table 19 for Ponceau 4R, Table 20 for Sunset Yellow FCF, Table 21 for Orange RN and Table 22 for Orange G.

In every electrolyte studied, Amaranth solution at the pH **providing** a well-defined wave yielded a reversible process (see Table 18). Ponceau 4R in every electrolyte examined at the pH 1.4-3.6 provided an irreversible wave and at the pH higher than 4, a reversible wave was obtained (see Table 19). Sunset Yellow FCF at pH 3.5-5.2 in every electrolyte investigated provided an irreversible wave and at the pH higher than 5.5 a reversible wave was obtained (see Table 20). Orange RN at the pH 2.4-5.2 in every electrolyte examined yielded an irreversible wave and at the pH higher than 5.5 a reversible wave was resulted (see Table 21). For Orange G in every electrolyte studied at the pH 3.5-4.5, the first wave was irreversible and the second wave observed between pH4.5-12.0 was a reversible wave (see Table 22).

From this study, it can be seen that the electrode reactions of Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G were not affected by the supporting electrolytes: $(C_2H_5)_4NCl$, KCl and KNO_3, but they were controlled by the pH of solutions.

4.2.3 Electron transferred and Proton transported values

Numbers of electron transferred and proton transported values of the dyes reported in this study were determined from the

Table 18 Tests for reversibilities of Amaranth in various electrolytes

pH	Slope of $-E_{de}$ vs log i/i_d-i	$E_{\frac{1}{4}} - E_{\frac{3}{4}}$	Electrode Reaction
in 0.1 M $(C_2H_5)_4NCl$			
4.00	0.030	0.028	reversible
5.00	0.030	0.028	
6.08	0.030	0.028	
7.20	0.030	0.028	
8.25	0.030	0.028	
9.30	0.030	0.028	
10.20	0.030	0.028	
12.00	0.030	0.028	
in 0.1 M KCl			
4.00	0.030	0.028	reversible
5.10	0.030	0.028	
5.95	0.030	0.028	
7.25	0.030	0.028	
8.10	0.030	0.028	
9.30	0.030	0.028	
10.50	0.030	0.028	
12.00	0.030	0.028	
in 0.1 M KNO_3			
3.88	0.030	0.028	reversible
4.20	0.030	0.028	
4.75	0.030	0.028	
5.20	0.030	0.028	
6.05	0.030	0.028	
7.15	0.030	0.028	
8.30	0.030	0.028	
9.30	0.030	0.028	
10.30	0.030	0.028	
11.90	0.030	0.028	

Table 19 Tests for reversibilities of Ponceau 4R in various electrolytes

pH	Slope of $-E_{de}$ vs log i/i_d-i	$E_{\frac{1}{4}} - E_{\frac{3}{4}}$	Electrode Reaction
in 0.1 M $(C_2H_5)_4NCl$			
1.40	0.045	0.043	irreversible
1.95	0.045	0.043	
2.35	0.045	0.043	
3.05	0.045	0.043	
3.60	0.045	0.043	
3.90	0.030	0.028	reversible
4.50	0.030	0.028	
5.15	0.030	0.028	
6.20	0.030	0.028	
7.50	0.030	0.028	
8.40	0.030	0.028	
9.30	0.030	0.028	
10.30	0.030	0.028	
11.95	0.030	0.028	
in 0.1 M KCL			
1.45	0.045	0.043	irreversible
2.00	0.045	0.043	
2.40	0.045	0.043	
3.10	0.045	0.043	
3.40	0.045	0.043	
3.60	0.045	0.043	
4.20	0.030	0.028	reversible
5.18	0.030	0.028	
6.05	0.030	0.028	
7.21	0.030	0.028	
8.45	0.030	0.028	
9.40	0.030	0.028	
10.30	0.030	0.028	
11.95	0.030	0.028	

Table 19 (continued)

pH	Slope of $-E_{de}$ vs log i/i_d-i	$E_{\frac{1}{4}} - E_{\frac{3}{4}}$	Electrode Reaction
in 0.1 M KNO_3			
1.45	0.045	0.043	irreversible
2.00	0.045	0.043	
2.50	0.045	0.043	
3.05	0.045	0.043	
3.38	0.045	0.043	
3.65	0.045	0.043	
4.30	0.030	0.028	reversible
5.15	0.030	0.028	
6.12	0.030	0.028	
7.12	0.030	0.028	
8.25	0.030	0.028	
9.30	0.030	0.028	
10.15	0.030	0.028	
12.00	0.030	0.028	

Table 20 Tests for reversibilities of Sunset Yellow FCF in various electrolytes

pH	Slope of $-E_{de}$ vs log $\frac{i}{i_d - i}$	$E_{\frac{1}{4}} - E_{\frac{3}{4}}$	Electrode Reaction
in 0.1 M $(C_2H_5)_4NCl$			
3.50	0.045	0.043	irreversible
4.40	0.045	0.043	
4.85	0.045	0.043	
5.25	0.045	0.043	
5.55	0.030	0.028	reversible
6.10	0.030	0.028	
6.75	0.030	0.028	
7.55	0.030	0.028	
8.30	0.030	0.028	
9.20	0.030	0.028	
10.30	0.030	0.028	
11.80	0.030	0.028	
in 0.1 M KCl			
3.60	0.045	0.043	irreversible
4.25	0.045	0.043	
4.65	0.045	0.043	
5.18	0.045	0.043	
5.60	0.030	0.028	reversible
6.05	0.030	0.028	

Table 20 (continued)

pH	Slope of $-E_{de}$ vs log $\frac{i}{i_d - i}$	$E_{\frac{1}{4}} - E_{\frac{3}{4}}$	Electrode Reaction
<u>in 0.1 M KCl</u>			
6.90	0.030	0.028	reversible
7.50	0.030	0.028	
8.20	0.030	0.028	
9.05	0.030	0.028	
10.30	0.030	0.028	
12.00	0.030	0.028	
<u>in 0.1 M KNO_3</u>			
3.55	0.045	0.043	irreversible
4.30	0.045	0.043	
4.65	0.045	0.043	
5.15	0.045	0.043	
5.50	0.030	0.028	reversible
6.00	0.030	0.028	
6.80	0.030	0.028	
7.30	0.030	0.028	
8.50	0.030	0.028	
9.40	0.030	0.028	
10.30	0.030	0.028	
12.00	0.030	0.028	

Table 21 Tests for reversibilities of Orange RN in various electrolytes

pH	Slope of $-E_{de}$ vs log i/i_d-i	$E_{\frac{1}{4}} - E_{\frac{3}{4}}$	Electrode Reaction
in 0.1 M $(C_2H_5)_4NCl$			
2.40	0.040	0.037	irreversible
3.25	0.040	0.037	
4.10	0.040	0.037	
5.10	0.040	0.037	
5.50	0.030	0.028	reversible
6.50	0.030	0.028	
7.20	0.030	0.028	
8.20	0.030	0.028	
9.30	0.030	0.028	
10.50	0.030	0.028	
12.00	0.030	0.028	
in 0.1 M KCl			
2.45	0.040	0.037	irreversible
3.20	0.040	0.037	
4.02	0.040	0.037	
5.20	0.040	0.037	
5.50	0.030	0.028	reversible
6.20	0.030	0.028	
7.20	0.030	0.028	
8.20	0.030	0.028	
9.20	0.030	0.028	
10.30	0.030	0.028	
12.00	0.030	0.028	
in 0.1 M KNO_3			
2.45	0.040	0.037	irreversible
3.20	0.040	0.037	
4.00	0.040	0.037	
5.20	0.040	0.037	
5.50	0.030	0.028	reversible
6.20	0.030	0.028	
7.20	0.030	0.028	
8.20	0.030	0.028	
9.30	0.030	0.028	
10.30	0.030	0.028	
12.00	0.030	0.028	

Table 22 Tests for reversibilities of Orange G in various electrolytes

pH	1 st reduction wave			2 nd reduction wave		
	Slope of $-E_{de}$ vs log i/i_d-i	$E_{\frac{1}{4}} - E_{\frac{3}{4}}$	Electrode Reaction	Slope of $-E_{de}$ vs log i/i_d-i	$E_{\frac{1}{4}} - E_{\frac{3}{4}}$	Electrode Reaction
in 0.1 M $(C_2H_5)_4NCl$						
3.50	0.045	0.042	irreversible	-	-	-
3.60	0.045	0.042		a	a	a
4.05	0.045	0.042		a	a	a
4.50	0.045	0.042		0.030	0.028	reversible
4.70	-	-	-	0.030	0.028	
5.05	-	-	-	0.030	0.028	
5.15	-	-	-	0.030	0.028	
5.70	-	-	-	0.030	0.028	
6.20	-	-	-	0.030	0.028	
7.30	-	-	-	0.030	0.028	
8.30	-	-	-	0.030	0.028	
9.02	-	-	-	0.030	0.028	
10.30	-	-	-	0.030	0.028	
11.85	-	-	-	0.030	0.028	
in 0.1 M KCl						
3.50	0.045	0.042	irreversible	-	-	-
3.60	0.045	0.042		a	a	a
4.05	0.045	0.042		a	a	a
4.50	0.045	0.042		0.030	0.028	reversible
4.70	-	-	-	0.030	0.028	
5.00	-	-	-	0.030	0.028	
5.20	-	-	-	0.030	0.028	

Table 22 (continued)

pH	1 st reduction wave			2 nd reduction wave		
	Slope of $-E_{de}$ vs log i/i_d-i	$E_{\frac{1}{4}} - E_{\frac{3}{4}}$	Electrode Reaction	Slope of $-E_{de}$ vs log i/i_d-i	$E_{\frac{1}{4}} - E_{\frac{3}{4}}$	Electrode Reaction
<u>in 0.1 M KCl</u>						
6.10	-	-	-	0.030	0.028	reversible
7.20	-	-	-	0.030	0.028	
8.20	-	-	-	0.030	0.028	
9.05	-	-	-	0.030	0.028	
10.30	-	-	-	0.030	0.028	
11.85	-	-	-	0.030	0.028	
<u>in 0.1 M KNO_3</u>						
3.50	0.045	0.042	irreversible	-	-	-
3.60	0.045	0.042		a	a	a
4.05	0.045	0.042		a	a	a
4.50	0.045	0.042		0.030	0.028	reversible
4.70	-	-	-	0.030	0.028	
5.00	-	-	-	0.030	0.028	
5.20	-	-	-	0.030	0.028	
6.10	-	-	-	0.030	0.028	
7.20	-	-	-	0.030	0.028	
8.25	-	-	-	0.030	0.028	
9.00	-	-	-	0.030	0.028	
10.30	-	-	-	0.030	0.028	
11.85	-	-	-	0.030	0.028	

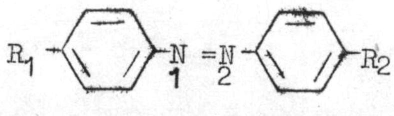
[a] The wave was too small to measure accurately

reversible waves only. Since the data from the present study were insufficient to calculate the electron transferred coefficients (α) of the irreversible electrode reactions, the number of electron transferred for the irreversible wave was not able to determine. As a result, the proton transported value of the irreversible wave could not be determined. Numbers of electron transferred of reversible waves were determined from slopes of the plots of the electrode potential against log i/i_d-i and proton transported values were calculated by equation 11 (see page 13). Tables 23-27 indicated that numbers of electron transferred for Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G are 2 as well as numbers of proton consumed by these dyes are also 2.

Therefore, the polarographic reductions of Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G in $(C_2H_5)_4NCl$, KCl and KNO_3 at pH about 5-12 involved 2 electrons and 2 protons.

4.2.4 Mechanism of reduction

For azo compounds the polarographic reductions take place at -N=N- giving hydrazo derivatives (16). Such a reduction would depend on the electron density at nitrogen-nitrogen double bond which in turn is a function of substituents. Otho or para hydroxyl group on the dye structure exerts a pull on the electron of -N=N- making N_1 slightly more negative than N_2



where $R_1 = SO_3H$ and $R_2 = OH$

Table 23 Numbers of electron transferred and proton transported for Amaranth in various electrolytes

pH	n	m	pH	n	m	pH	n	m
in 0.1 M $(C_2H_5)_4NCl$			in 0.1 M KCl			in 0.1 M KNO_3		
4.00	2.0	2.0	4.00	2.0	2.0	3.88	2.0	2.0
5.00	2.0	2.0	5.10	2.0	2.0	4.20	2.0	2.0
6.08	2.0	2.0	5.95	2.0	2.0	4.75	2.0	2.0
7.20	2.0	2.0	7.25	2.0	2.0	5.20	2.0	2.0
8.25	2.0	2.0	8.10	2.0	2.0	6.05	2.0	2.0
9.30	2.0	2.0	9.30	2.0	2.0	7.15	2.0	2.0
10.20	2.0	2.0	10.50	2.0	2.0	8.30	2.0	2.0
12.00	2.0	2.0	12.00	2.0	2.0	9.30	2.0	2.0
						10.30	2.0	2.0
						11.90	2.0	2.0

Table 24 Numbers of electron transferred and proton transported for Ponceau 4R in various eletrolytes

pH	n	m	pH	n	m	pH	n	m
in 0.1 M $(C_2H_5)_4NCl$			in 0.1 M KCl			in 0.1 M KNO_3		
3.90	2.0	2.0	4.20	2.0	2.0	4.30	2.0	2.0
4.50	2.0	2.0	5.18	2.0	2.0	5.15	2.0	2.0
5.15	2.0	2.0	6.05	2.0	2.0	6.12	2.0	2.0
6.20	2.0	2.0	7.21	2.0	2.0	7.12	2.0	2.0
7.50	2.0	2.0	8.45	2.0	2.0	8.25	2.0	2.0
8.40	2.0	2.0	9.40	2.0	2.0	9.30	2.0	2.0
9.30	2.0	2,0	10.30	2.0	2.0	10.15	2.0	2.0
10.30	2.0	2.0	11.95	2.0	2.0	12.00	2.0	2.0
11.95	2.0	2.0						

Table 25 Numbers of electron transferred and proton transported for Sunset Yellow FCF in various electrolytes

pH	n	m	pH	n	m	pH	n	m
in 0.1 M $(C_2H_5)_4NCl$			in 0.1 M KCl			in 0.1 M KNO_3		
5.55	2.0	2.0	5.60	2.0	2.0	5.50	2.0	2.0
6.10	2.0	2.0	6.05	2.0	2.0	6.00	2.0	2.0
6.75	2.0	2.0	6.90	2.0	2.0	6.80	2.0	2.0
7.55	2.0	2.0	7.50	2.0	2.0	7.30	2.0	2.0
8.30	2.0	2.0	8.20	2.0	2.0	8.50	2.0	2.0
9.20	2.0	2.0	9.05	2.0	2.0	9.40	2.0	2.0
10.30	2.0	2.0	10.30	2.0	2.0	10.30	2.0	2.0
11.80	2.0	2.0	12.00	2.0	2.0	12.00	2.0	2.0

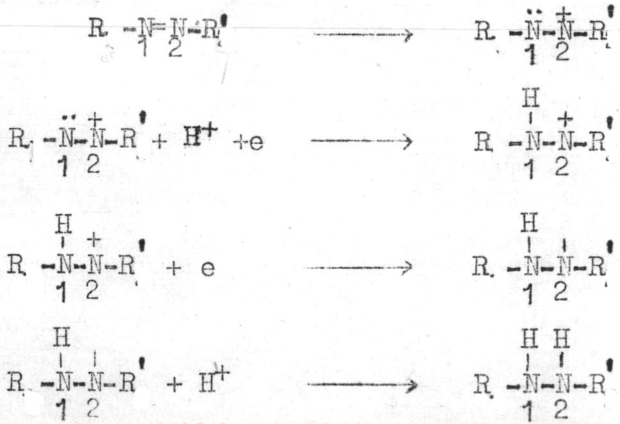
Table 26 Numbers of electron transferred and proton transported for Orange RN in various electrolytes

pH	n	m	pH	n	m	pH	n	m
in 0.1 M $(C_2H_5)_4NCl$			in 0.1 M KCl			in 0.1 M KNO_3		
5.50	2.0	2.0	5.50	2.0	2.0	5.50	2.0	2.0
6.50	2.0	2.0	6.20	2.0	2.0	6.20	2.0	2.0
7.20	2.0	2.0	7.20	2.0	2.0	7.20	2.0	2.0
8.20	2.0	2.0	8.20	2.0	2.0	8.20	2.0	2.0
9.30	2.0	2.0	9.20	2.0	2.0	9.30	2.0	2.0
10.50	2.0	2.0	10.30	2.0	2.0	10.30	2.0	2.0
12.00	2.0	2.0	12.00	2.0	2.0	12.00	2.0	2.0

Table 27 Numbers of electron transferred and proton transported for Orange G in various electrolytes

2 nd reduction wave			2 nd reduction wave			2 nd reduction wave		
pH	n	m	pH	n	m	pH	n	m
in 0.1 M $(C_2H_5)_4NCl$			in 0.1 M KCl			in 0.1 M KNO_3		
4.50	2.0	2.0	4.50	2.0	2.0	4.50	2.0	2.0
4.70	2.0	2.0	4.70	2.0	2.0	4.70	2.0	2.0
5.05	2.0	2.0	5.00	2.0	2.0	5.00	2.0	2.0
5.15	2.0	2.0	5.20	2.0	2.0	5.20	2.0	2.0
5.70	2.0	2.0	6.10	2.0	2.0	6.10	2.0	2.0
6.20	2.0	2.0	7.20	2.0	2.0	7.20	2.0	2.0
7.30	2.0	2.0	8.20	2.0	2.0	8.25	2.0	2.0
8.30	2.0	2.0	9.05	2.0	2.0	9.00	2.0	2.0
9.02	2.0	2.0	10.30	2.0	2.0	10.30	2.0	2.0
10.30	2.0	2.0	11.85	2.0	2.0	11.85	2.0	2.0
11.85	2.0	2.0						

The general scheme was proposed as follows (16)



$$R-\underset{1}{N}=\underset{2}{N}-R' \longrightarrow R-\underset{1}{\ddot{N}}-\underset{2}{\overset{+}{N}}-R'$$

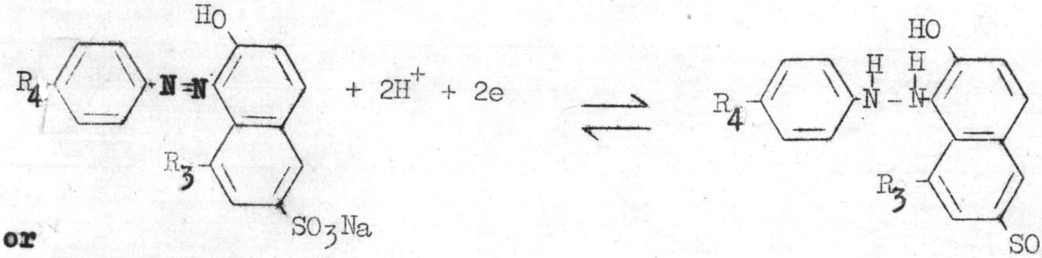
$$R-\underset{1}{\ddot{N}}-\underset{2}{\overset{+}{N}}-R' + H^+ + e \longrightarrow R-\underset{1}{\overset{H}{N}}-\underset{2}{\overset{+}{N}}-R'$$

$$R-\underset{1}{\overset{H}{N}}-\underset{2}{\overset{+}{N}}-R' + e \longrightarrow R-\underset{1}{\overset{H}{N}}-\underset{2}{N}-R'$$

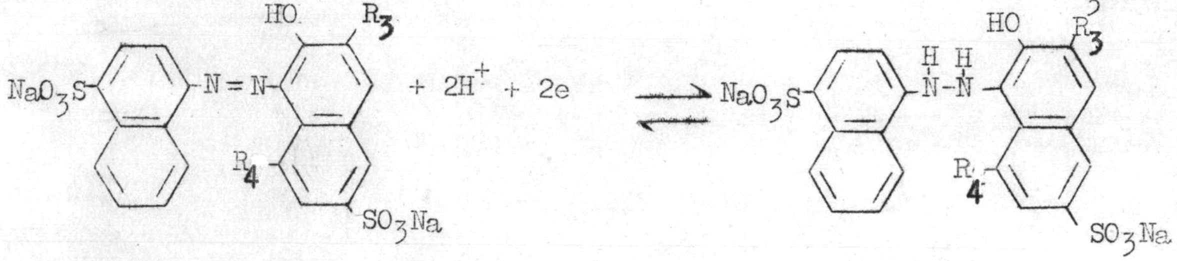
$$R-\underset{1}{\overset{H}{N}}-\underset{2}{N}-R' + H^+ \longrightarrow R-\underset{1}{\overset{H}{N}}-\underset{2}{\overset{H}{N}}-R'$$

(R and R' = alkyl or aryl group)

Therefore, the mechanism for reductions of the dyes studied which are also the azo compounds should be:



or



where R_3 and R_4 are H or SO_3Na

4.2.5 Sensitivity

From the previous study (4.2.1), the well-defined polarographic waves of Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G in 0.1M $(C_2H_5)_4NCl$, 0.1M KCl and 0.1 M KNO_3 were obtained in the pH **about** 3-12. The diffusion currents of these waves seemed to be independent of pH in the solutions with pH higher than 6. Thus, the dye solution at pH about 7 was taken to study. The concentrations of Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G studied were in the range 10^{-4}M and as low as possible to detect. The relationships between the concentrations and the diffusion currents of Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G are listed in Table 28. They all provided the linear relationships in the range 10^{-6}-10^{-4}M. (see Figures 31 A-31 E). Limits of detection for Amaranth, Ponceau 4R, Sunset Yellow FCF and Orange G were found to be 4.0 x 10^{-6}M as well as for Orange RN was found to be 3.0 x 10^{-6}M.

4.3 Food colors in some beverages

The common color shades used in beverages are red, orange , yellow and green. There are red, orange, yellow and green shades for Fanta. Green Spot and Bireley's are orange shades. Only orange and red shades of beverages were selected to study for analyses of Amaranth, Ponceau 4R, Sunset Yellow FCF, Orange RN and Orange G.

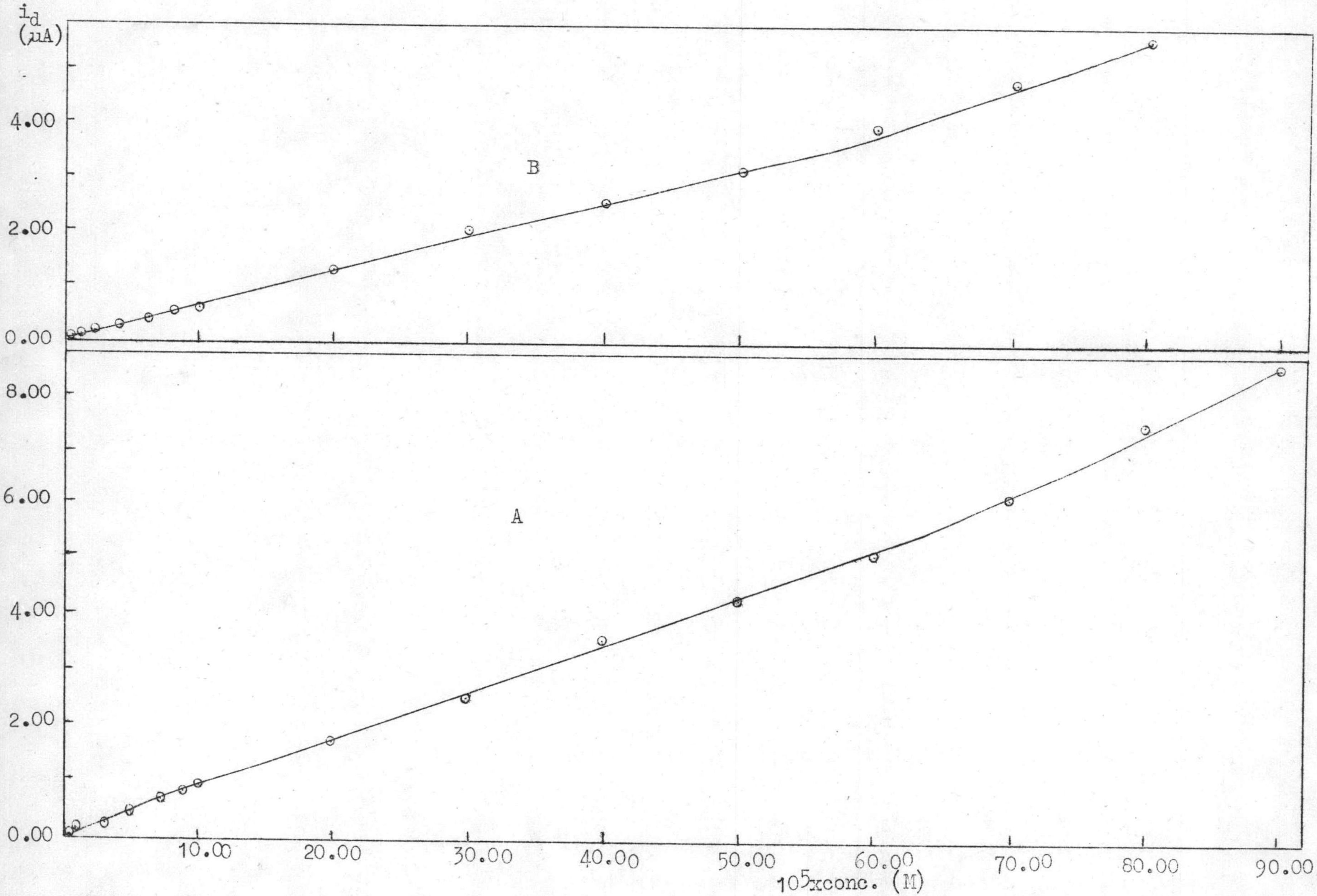
4.3.1 Identification of dyes

Color additives in Bireley's, Fanta (red and orange) and Green Spot were identified by paper chromatographic and visible spectrophotometric methods as mentioned in 3.3.1 and 3.3.2 , respectively. Their R_f values which were determined and shown in Table 29 were identified by comparison to the R_f values of Amaranth, Ponceau 4R, Orange G, Orange RN and Sunset Yellow FCF in the same developing solvent system. The R_f values of red color in Fanta were signigicant difference from the R_f values of Amaranth and Ponceau 4R

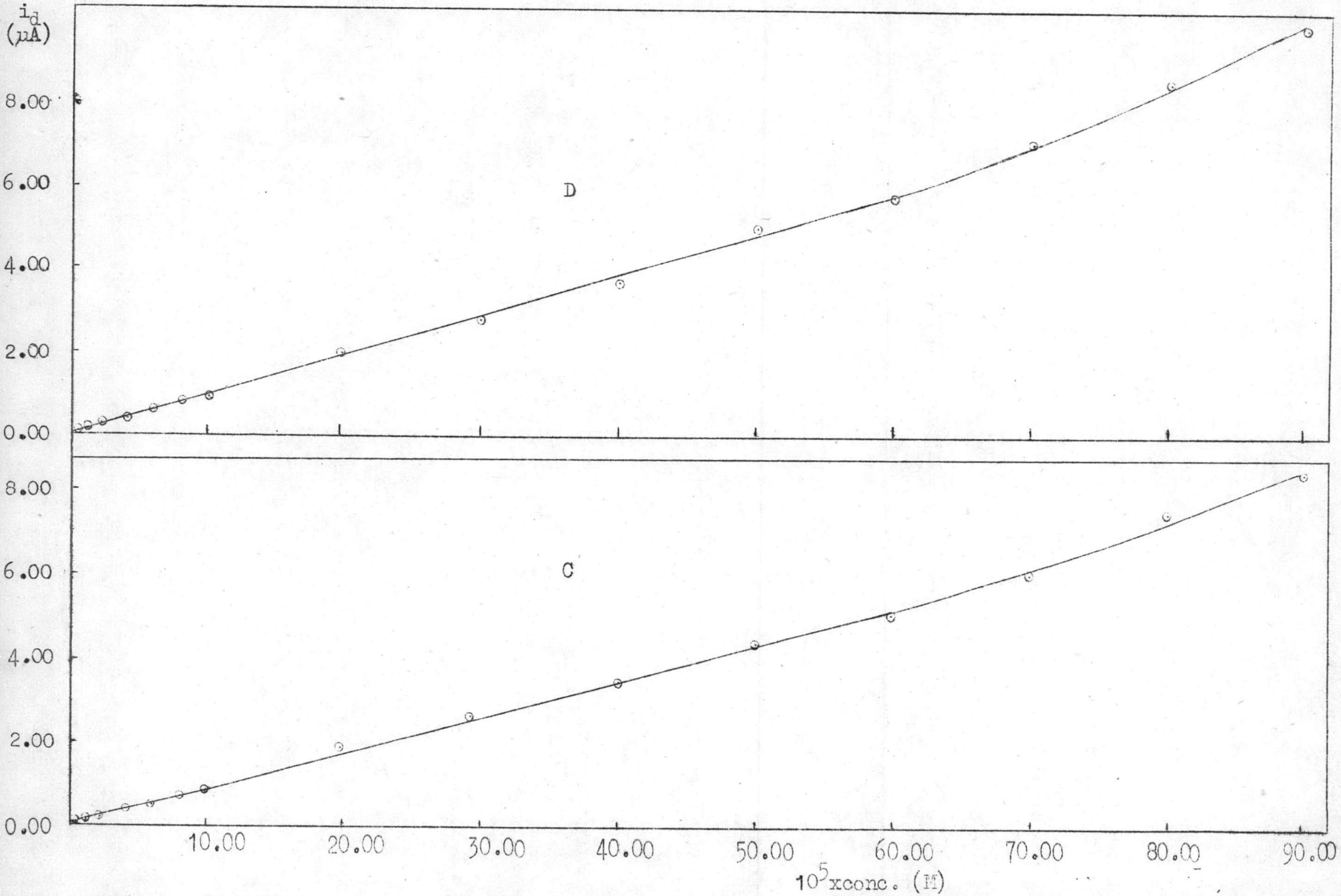
Table 28 The relationships between the concentrations and the diffusion currents of Amaranth and Ponceau 4R in 0.1 M $(C_2H_5)_4NCl$ pH 7.05 ; Sunset Yellow FCF, Orange RN and Orange G in 0.1 M $(C_2H_5)_4NCl$ pH 7.15

10^5 x conc.(M)	i_d(µA)	10^5 x conc.(M)	i_d(µA)	10^5 x conc.(M)	i_d(uA)	10^5 x conc.(M)	i_d(µA)	10^5 x con.(M)	i_d(µA)
Amaranth		Ponceau 4R		Sunset Yellow FCF		Orange RN		Orange G	
0.30	a	0.30	a	0.30	a	0.20	a	0.30	a
0.40	0.025	0.40	0.028	0.40	0.028	0.30	0.030	0.40	0.025
0.60	0.045	0.60	0.043	0.60	0.052	0.40	0.040	0.60	0.052
1.00	0.094	1.00	0.062	1.00	0.094	0.60	0.056	1.00	0.085
3.00	0.250	2.00	0.148	2.00	0.202	1.00	0.095	2.00	0.200
5.00	0.430	4.00	0.223	4.00	0.375	2.00	0.195	4.00	0.368
7.00	0.595	6.00	0.375	6.00	0.552	4.00	0.359	6.00	0.565
9.00	0.772	8.00	0.512	8.00	0.705	6.00	0.531	8.00	0.742
10.00	0.852	10.00	0.600	10.00	0.900	8.00	0.746	10.00	0.940
20.00	1.630	20.00	1.250	20.00	1.880	10.00	0.899	20.00	2.100
30.00	2.500	30.00	2.000	30.00	2.730	20.00	1.980	30.00	2.850
40.00	3.520	40.00	2.650	40.00	3.440	30.00	2.820	40.00	3.950
50.00	4.250	50.00	3.180	50.00	4.300	40.00	3.700	50.00	5.060
60.00	5.040	60.00	3.970	60.00	5.080	50.00	4.910	60.00	6.100
70.00	6.150	70.00	4.850	70.00	5.940	60.00	5.750	70.00	7.850
80.00	7.560	80.00	5.620	80.00	7.410	70.00	6.980	80.00	9.030
90.00	8.600			90.00	8.450	80.00	8.600		
						90.00	9.980		

[a] The wave was too small to measure accurately



Figures 31A-31B The relationships between the concentrations and the diffusion currents of Amaranth (A) and Ponceau 4R (B) in 0.1M $(C_2H_5)_4NCl$ pH 7.05 .



Figures 31C-31D The relationships between the concentrations and the diffusion currents of Sunset Yellow FCF **(C)** and Orange RN **(D)** in 0.1M $(C_2H_5)_4NCl$ pH 7.15.

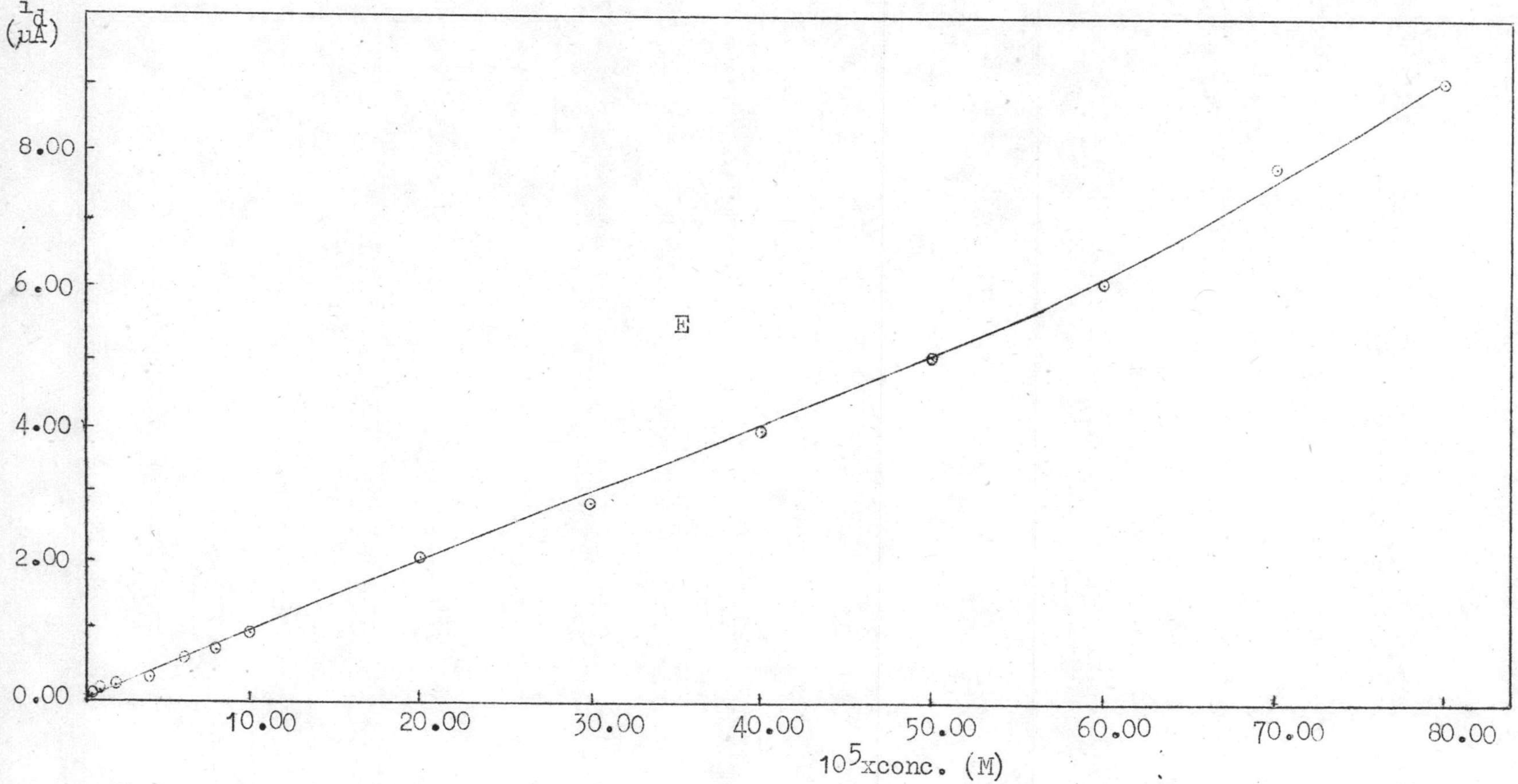


Figure 31E The relationship between the concentration and the diffusion current of Orange G in 0.1M $(C_2H_5)_4NCl$ pH 7.15

Table 29 R_f values of the red and orange colors in some beverages

Beverage	solvent system I	solvent system II	solvent system III
Fanta (red)	0.45 (0.27, Amaranth 0.51, Ponceau 4R)	0.52 (0.29, Amaranth 0.32, Ponceau 4R)	0.04 (0.15, Amaranth 0.39, Ponceau 4R)
Bireley's	0.69 (0.69, Sunset Yellow FCF)	0.39 (0.40, Sunset Yellow FCF)	0.25 (0.25, Sunset Yellow FCF)
Fanta (orange)	0.68 (0.69, Sunset Yellow FCF)	0.40 (0.40, Sunset Yellow FCF)	0.25 (0.25, Sunset Yellow FCF)
Green Spot	0.68 (0.69, Sunset Yellow FCF)	0.40 (0.40, Sunset Yellow FCF)	0.24 (0.25, Sunset Yellow FCF)

Data in the brackets are the experimental R_f values of the dyes from Table 1 in all three solvent systems. The R_f values of orange colors in Bireley's, Fanta and Green Spot were about the same as the R_f values of Sunset Yellow FCF in the three solvent systems. The differences were due to the composition of the solutions: the beverage solution contained substances other than standard dye solution, for example, sugar and preservative substances.

The comparisons of the maximum absorption wavelengths either between the red dye in Fanta and Amaranth or this red dye and Ponceau 4R also indicated the difference (see Table 30). The comparisons of the maximum absorption wavelengths of the orange dye in Bireley's, Fanta and Green Spot to the maximum absorption wavelength of Sunset Yellow FCF are insignificant.

Therefore, the red color in Fanta is not Amaranth or Ponceau 4R but the orange color in Bireley's, Fanta and Green Spot is Sunset Yellow FCF.

4.3.2 Determination of dyes in the beverages

Concentration of the dye additive was graphically determined by standard addition method: a series of the standard dye solution of concentration 0-36.20 mg/dm^3 was added to the beverage and the polarographic analysis of the dye in the beverage was performed in 0.1 M $(C_2H_5)_4NCl$ at pH 7.05. Since the red color of Fanta is not Amaranth or Ponceau 4R (see 4.3.1), the determination of the red color was not performed. The orange color of Bireley's, Fanta and Green Spot was determined for Sunset Yellow FCF. Three samples of each beverage, one week difference in buying, were analyzed and three trials were performed for each sample. Graphical determinations of Sunset Yellow FCF in Bireley's, Fanta and Green Spot are illustrated in Figures 32,33 and 34,

Table 30 The maximum absorption wavelengths of the dyes in some beverages in acid solution

Beverage	λ max (nm)
Fanta (red)	515 (522 for Amaranth 505 for Ponceau 4R)
Bireley's	482 (482 for Sunset Yellow FCF)
Fanta (orange)	481 (482 for Sunset Yellow FCF)
Green Spot	482 (482 for Sunset Yellow FCF)

Data in the brackets are the λmax of the dyes from Table 2

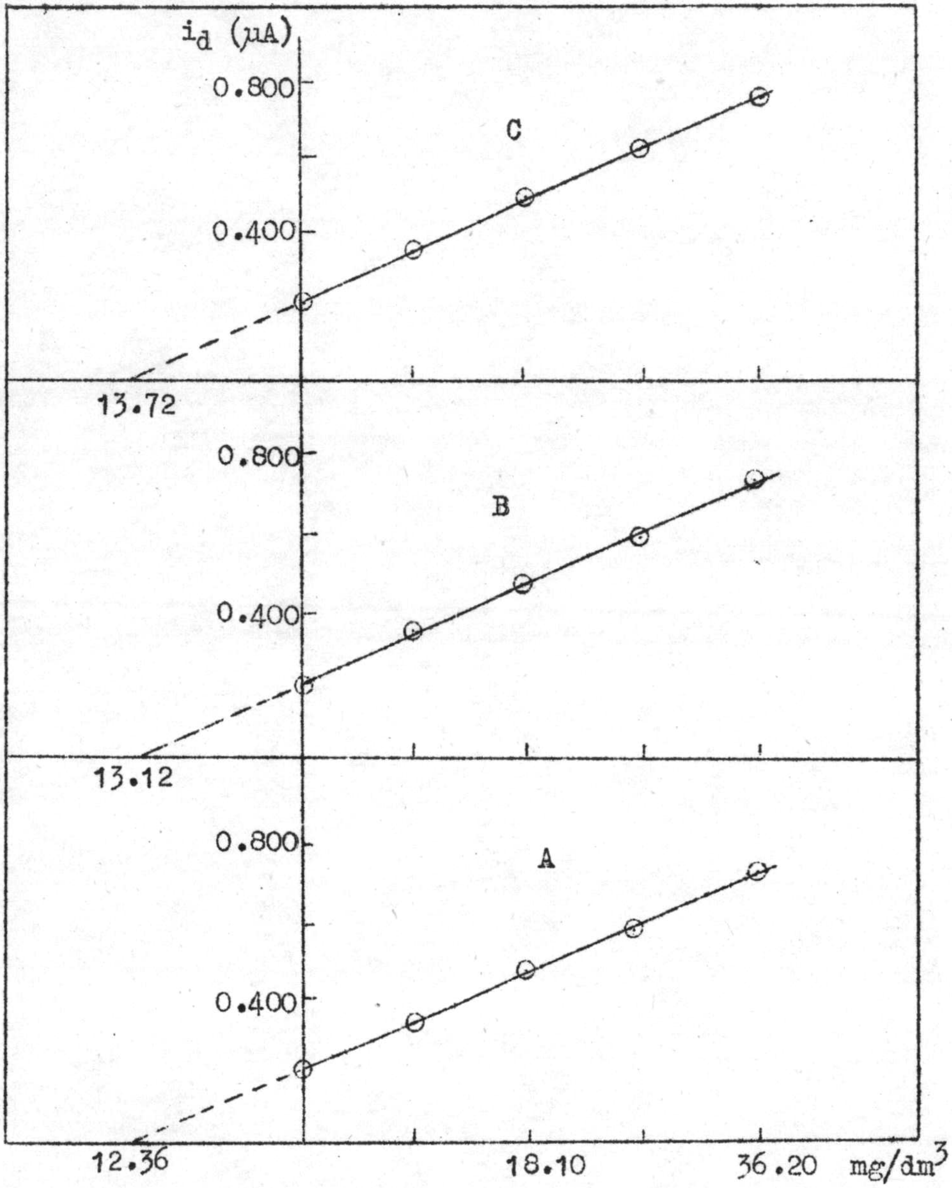


Figure 32 Graphical determinations of Sunset Yellow FCF **in** **Bireley's** A) bottle A, B) bottle B and C) bottle C

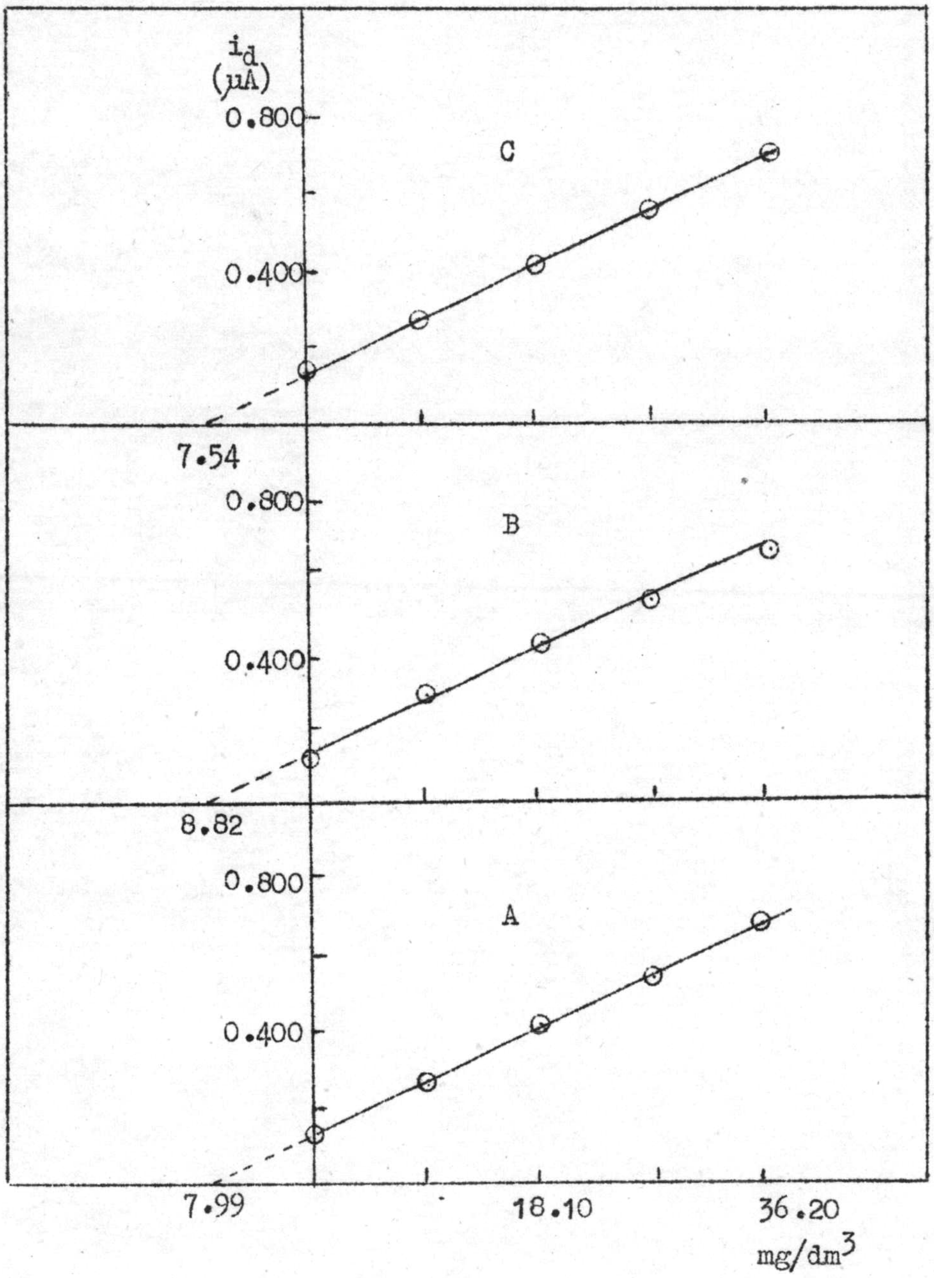


Figure 33 Graphical determinations of Sunset Yellow FCF in Fanta A) bottle A, B) bottle B and C) bottle C

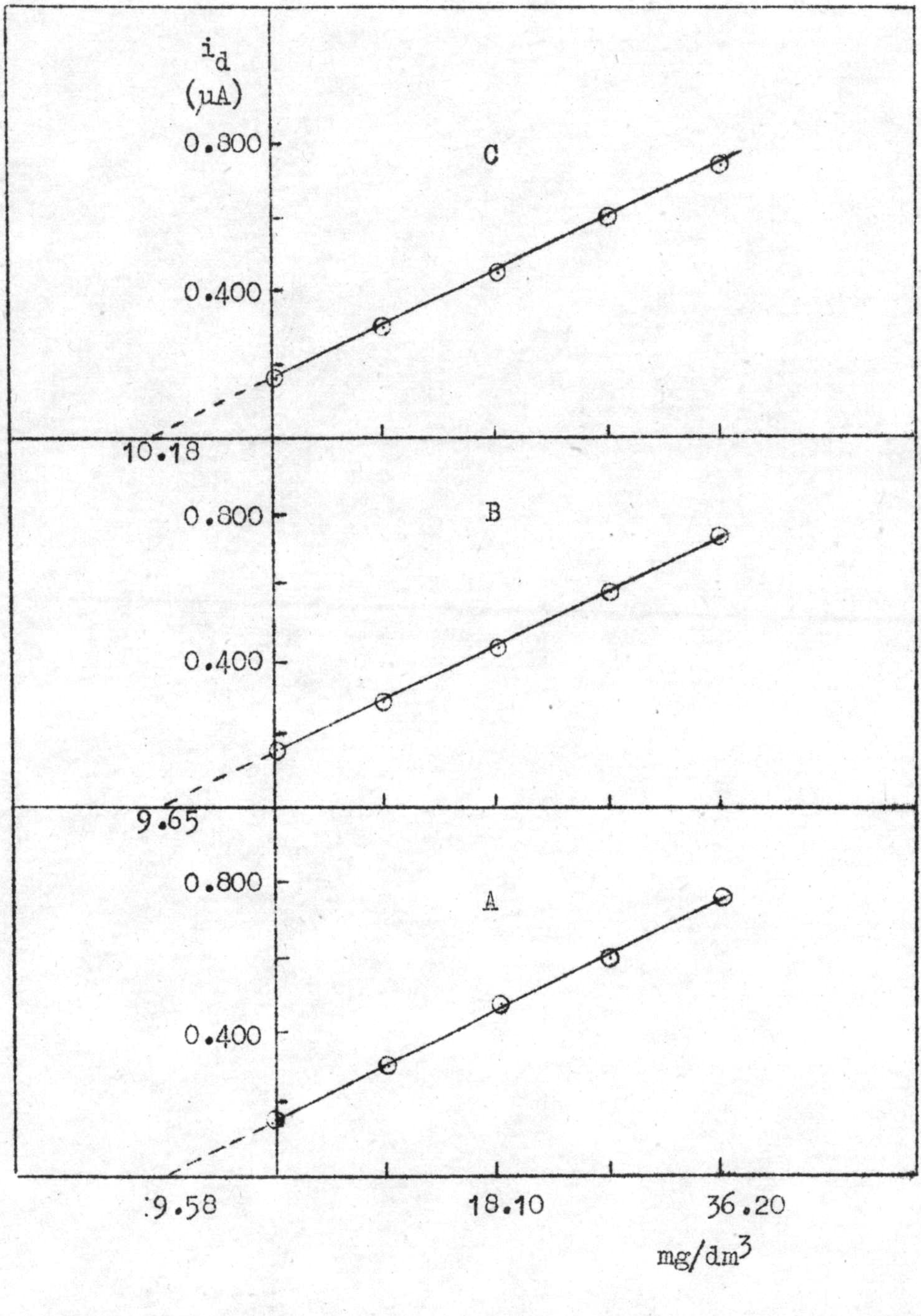


Figure 34 Graphical determinations of Sunset Yellow FCF in Green Spot A) bottle A, B) bottle B and C) bottle C

respectively. The results of these determinations are listed in Table 31. From this table, contents of Sunset Yellow FCF in Fanta was found to be the lowest, 7.54-8.82 mg/dm^3 and that in Bireley's was found to be the highest, 12.36-13.72 mg/dm^3. The contents of Sunset Yellow FCF in these three bottles of the same beverage were found to be insignificant difference. Thus, the manufacturers have controlled the amount of the dye additive in the beverages. The acceptable limit for Sunset Yellow FCF is 0.5 mg/kg body weight (1).

Table 31 Results of the determinations of Sunset Yellow FCF in some beverages

Sample	content* (mg/dm^3)
Bireley's (A)	12.36 ± 0.27
Bireley's (B)	13.12 ± 0.45
Bireley's (C)	13.72 ± 0.26
Fanta (A)	7.99 ± 0.53
Fanta (B)	8.82 ± 0.23
Fanta (C)	7.54 ± 0.26
Green Spot (A)	9.58 ± 0.57
Green Spot (B)	9.65 ± 0.68
Green Spot (C)	10.18 ± 0.59

*average ± average deviation of 3 trials