THIN-FILM ELECTROPHORESIS

PART I. THE ELECTROPHORETIC BEHAVIOUR OF COAL-TAR FOOD COLOURS ON PAPER AND THIN FILMS

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Over the last decade a large number of papers have dealt with the paper chromatographic behaviour of food colours. The introduction of *The Colouring Matter in Food Regulations*, 1957[1] resulted in attempts to standardise detection methods and this culminated in the publication, in 1960, of a monograph[2] on the separation and identification of the food colours permitted in the United Kingdom. While the majority of the papers published use R_F values as the means of identification, YANUKA *et al.*[3] maintain that it is difficult to obtain reproducible results for absolute R_F values. They have described an identification method based on a characteristic curve composed of eight spots, instead of on a single R_F value. The curve is obtained by simultaneously running eight chromatograms of the colour under investigation at eight different pH values in one single solvent system.

The separation and identification of food colours by thin-layer chromatographic techniques was first reported over ten years ago[4,5], but this and subsequent work[6–9] appears to have been confined to alumina.

With regard to electrophoretic studies on food colours, these have usually been carried out on paper[10–12], although the use of cellulose acetate membrane[12] and thin film supports[13] have recently been described.

The rapid progress in thin-film chromatography over the last few years has prompted the use of such materials as alumina, kieselguhr and silica gel as supporting adsorbents for electrophoretic studies of amines and amino acids[14,15], periodate and iodate[16], and phenols and phenol carboxylic acids[17]. Apart from a preliminary report[13], no other work appears to have been published on the electrophoretic behaviour of food colours on thin films.

Except for Oil Yellow GG, Oil Yellow XP, Naphthol Yellow S and Ponceau 3R all the permitted coal-tar food colours[1] have been subjected to electrophoresis in six different electrolytes of widely varying pH values on the thin-film materials, kieselguhr (Shandon), alumina G (Merck) and silica gel G (Merck), as well as on Whatman No. 1 filter paper.

EXPERIMENTAL

Preparations of electrolyte solutions

Acetic acid (N) solution, ammonium hydroxide (0.1 N) solution and the buffer solutions of pH 4.0, 6.0 and 8.0 were prepared in the manner previously described[10]. The buffer solution of pH 9.2 is a 0.05 M aqueous solution of borax.

Preparation of the colour solutions

Aqueous solutions(0.1 %w/v) of the appropriate powdered colour were used throughout.

Apparatus

The thin films were prepared from a slurry of the appropriate adsorbent in water (30 g adsorbent to 60 ml water) with a Shandon "Unoplan" Leveller and Spreader in the usual way and dried at 105°.

The Baird and Tatlock Constant Current/Constant Voltage Electrophoresis Apparatus was used for the experiments.

Procedure

For electrophoresis on thin films it was found convenient to score the dry film into strips by means of a scriber. The scored plate (20 cm × 17.5 cm) was then placed across the bridge of the horizontal electrophoresis tank and contact made between the film and each electrolyte compartment by means of a filter paper wick previously soaked with the electrolyte solution under study and with one edge resting along the full width of the plate of film. The lid was then placed on the electrophoresis tank and the film allowed to become saturated with electrolyte solution by means of capillary action through the wicks. Even though the electrolyte fronts normally took only 10 to 15 min. to meet, a period of one hour was allowed to elapse before applying the test solution. This time interval ensured that migration of the colours due to capillary rise of the electrolyte was minimal, as shown by preliminary experiments with colour spotted at various points on the film between the electrode compartments.

Despite the apparent delay of one hour in preparing plates, it was considered advantageous to use this procedure rather than to prepare the plate directly from a slurry of the thin-film adsorbent made from the electrolyte solution. In this alternative procedure, the film would have to be spread on one plate at a time.

A further variation in the procedure involving spraying of the thin-film plate with the appropriate electrolyte gave rise to non-reproducible results.

For electrophoresis on paper, strips of Whatman No. 1 filter paper, previously soaked in electrolyte solution, were placed across the glass ribbed support (at right angles to the ribs) provided with the Baird and Tatlock horizontal tank.

The colour solution was streaked on the cathode end of the support in each case and electrophoresis allowed to proceed for a timed period at a constant potential.

RESULTS

The colours were subjected to electrophoresis singly for a period of one hour at a constant potential of 200 V. The various mobilities were found to be reproducible and are summarised in Table I. The recorded mobilities represent migration towards the anode (positive electrode) in each case except when the mobility is preceded by a negative sign.

While the results for electrophoresis on Whatman No. 1 paper quoted in Table I are those obtained for the paper lying across the ribs of the glass bridge of the electrophoresis tank, it is interesting to note that different migration distances were obtained when the paper was allowed to lie on a smooth glass surface during electrophoresis.

TABLE I

MIGRATION DISTANCES (mm) OF COAL TAR FOOD DYES ON PAPER AND THIN-FILM SUPPORTS AFTER ELECTROPHORESIS AT 200 V FOR ONE HOUR

Dye	*Molecular weight*	*Number of ionisable sodium atoms*	*Support*	*Electrolyte solution*					
				N acetic acid	*4.0*	*6.0*	*8.0*	*9.2*	*0.1 N ammonia*
Ponceau 4R	604	3	P	47^{F}	$36^{T\&F}$	$28^{T\&F}$	16^{T}	41	79
			K	39	37	44	41	56	29
			A	28	44	53	46	53	32
			S	41	39	47	39	52	26^{F}
Amaranth	604	3	P	19^{F}	15	8	7	15	46^{T}
			K	35	32	46	42	58	31
			A	27^{T}	33^{T}	48^{T}	$37^{T\&F}$	$51^{S\,F}$	$19^{T\&F}$
			S	42	40	47	39	47	23^{F}
Fast Red E	502	2	P	11	9^{T}	5^{T}	3	4	21^{T}
			K	41	33	45	34	55	24
			A	$21^{T\&F}$	5	5	$26^{T\&F}$	$24^{T\&F}$	1
			S	37	38	42^{T}	32	47	$21^{SF,ST}$
Carmoisine	502	2	P	14	8	5^{ST}	3^{ST}	18	38^{T}
			K	34	32	38	34	53	29
			A	23^{T}	31^{T}	47^{T}	34	$42^{T\&F}$	27
			S	38	34	34	34	44	21^{F}
Black PN	866	4	P	6^{T}	2^{T}	1	1	4	21^{T}
			K	38	33	45	44	50	16
			A	1	4	1	2	3^{T}	0
			S	35	38	42^{T}	39	48	23^{SF}
Ponceau SX	480	2	P	21^{T}	12^{T}	7^{T}	4^{T}	26^{T}	59^{T}
			K	32	34	38	36	52	27
			A	25^{ST}	25^{ST}	43^{T}	37	$39^{T\&F}$	24^{ST}
			S	36	33	36	35	46	20^{F}
Ponceau MX	480	2	P	18^{T}	9^{ST}	5^{T}	2^{T}	18^{ST}	30^{T}
			K	39	32	42^{T}	35	51	21
			A	26^{ST}, 17^{ST}	22^{T}	$43^{T\&F}$	$28^{T\&F}$	$34^{T\&F}$	$21^{T\&F}$
			S	34	35	37^{T}	34	44	19^{SF}, ST

(continued on p. 353)

TABLE I (*continued*)

Dye	*Molecular weight*	*Number of ionisable sodium atoms*	*Support*	*Electrolyte solution*					
				N acetic acid	*4.0*	*6.0*	*8.0*	*9.2*	*0.1 N ammonia*
Orange G	452	2	P	44	30	26^{F}	14^{F}	42	67^{T}
			K	35	33	38	36	46	20
			A	33	43	52	34	47	27
			S	40	34	34	36	44	18^{F}
Orange RN	350	1	P	11	7	5^{T}	2	7	12
			K	25	23	26	27	40	12
			A	24	12^{T}	47^{F}, 32^{F}	20	$28^{T\ \&\ F}$	16^{T}
			S	23	21, 32^{faint}	19	25^{ST}	$24^{T\ \&\ F}$	10
Red 2G	509	2	P	22	21	13^{ST}	8	13	50^{T}
			K	34	27	32	31	46	19
			A	31	43	49	31	47	27
			S	33	31	30	23	40	15^{F}
Sunset Yellow FCF	452	2	P	22	17^{T}	17^{ST}	9	19	45^{T}
			K	35	30	35	37	52	30
			A	34	32^{T}	50^{ST}	36	51	28
			S	40	38	43	35	44	$18^{ST\ \&\ SF}$
Red 6B	566	2	P	13	10^{T}	4^{T}	1	10	34^{T}
			K	35	27	36^{ST}	32	49	18
			A	27	32	45	34	45	25^{SF}
			S	32	32^{ST}	34	33	42	$15^{ST\ \&\ SF}$
Red 10B	467	2	P	23^{T}	13	9^{ST}	5^{ST}	17	28
			K	45	31	41	30	49	20
			A	35	40	52	33	49	32
			S	36	34	39	33	43	20^{SF}
Chocolate Brown HT	652	2	P	$25^{T\ \&\ F}$	$30^{T\ \&\ F}$	$12^{T\ \&\ F}$	$10^{T\ \&\ F}$	$43^{T\ \&\ F}$	$65^{T\ \&\ F}$
			K	$21^{T\ \&\ F}$	$24^{T\ \&\ F}$	faded	18^{F}	$53^{T\ \&\ F}$	13^{F}
			A	0	0	0	0	0	0
			S	26^{T}	19^{F}	13^{F}	28^{F}	faded	$16^{T\ \&\ F}$

(continued on p. 354)

TABLE I (*continued*)

Dye	*Molecular weight*	*Number of ionisable sodium atoms*	*Support*	*Electrolyte solution*					
				N acetic acid	*4.0*	*6.0*	*8.0*	*9.2*	*0.1 N ammonia*
Yellow RFS	432	2	P	37	30	41ST	24	29	68
			K	42	30	42	29	50	23
			A	38	47	50	35	51	31
			S	41	39	43	29	47	18^{F}
Yellow RY	418	2	P	42	27	20ST	7^{T}	32	61^{T}
			K	40	36	44	40	54	29
			A	34	37	53	40	51	24
			S	42	40	45	39	51	28^{F}
Brown FK	520 / 328	2 / 1		Brown Yellow	Brown Yellow	Brown Yellow	Brown Yellow	Brown Yellow	Brown Yellow
			P	4 2	3 —	1 3	1 4	2 7	4 11
			K	12^{F} —4	3 —	22 —10	36 16	48 36	20 11
			A	1 14	14 25	47 41	36 28	40 30	27 24
			S	21^{T} 2	28^{F} 8	faded 6	29 21	41 25	19^{F} 11^{T} & F
Blue VRS	566	1	P	12	17	22ST	22^{T}	4	26
			K	4	7	6	—1	24	8
			A	21	18	26	12	25ST	16
			S	2	1	1	1	1	0
Violet BNP	720	1	P	12^{T}	12^{T}	8^{T}	4^{T}	14^{T}	19^{T}
			K	2	2	1	7	33^{T}	6^{T} & SF
			A	7^{T}	0	8^{T}	8^{T} & F	6ST	5^{T}
			S	3	1	1	0	1	1
Green S	576	1	P	18ST	19	5^{T}	11	25^{T}	42ST
			K	10	16	10	20^{T}	35^{F}	9
			A	24	8	32	24	24	18
			S	6	4	2	8	3	1
Red FB	583	2	P	0	0	0	0	0	1
			K	27	23^{T}	27^{T}	1	42	19
			A	0	0	1	1	2	2
			S	26^{T}	17^{T} & F	13^{T}	17^{T}	19^{T} & F	10^{T}

(*continued on p. 355*)

TABLE I (*continued*)

Dye	*Molecular weight*	*Number of ionisable sodium atoms*	*Support*	*Electrolyte solution*					
				N acetic acid	*4.0*	*6.0*	*8.0*	*9.2*	*0.1 N ammonia*
Yellow 2G	568	2	P	48	36	22	25	43	79
			K	32	30	31	34	54	25
			A	35	45	53	42	43	31
			S	38	32	36	34	41	19F
Tartrazine	534	3	P	32	38	25	14	40	76
			K	33	36	51	49	59	35
			A	39	42	49	49	52	35
			S	44	43	47	41	47	21ST & F
Indigo Carmine	466	2	P	43T	18ST	11ST	6T	21	36
			K	40	33	44	33	52	22
			A	34	26F	42F	35T & F	48	30F
			S	37	36	44	35	47	22ST
Chocolate Brown FB	558 / 516	1 / 1		Brown Yellow	Brown Yellow	Brown Yellow	Brown Yellow	Brown Yellow	Brown Yellow
			P	4 2	1 0	1 3	0 —	2 9	3 9
			K	15 —7	20 0	25 —10	36 20	43 33	20 8
			A	0 20	13 29	46 41	28 20	38 32	19 15
			S	0 —	7 —	4 —	32 24	40 25	13 —
Erythrosine BS	876	2	P	0	1	1	1	2	5
			K	0	0	0	30T	37	20
			A	0	10	38T & F	24T & F	26	20T
			S	0	6T & F	10T	19ST	16T	8ST & F

T = tailed; F = faded; ST = slightly tailed; SF = slightly faded; P = Whatman No. 1 paper; K = kieselguhr; A = alumina G; S = silicagel G.

Some examples of observed distances (in mm) under these different conditions using acetic acid (*N*) as electrolyte are, Tartrazine 32 and 47, Sunset Yellow FCF 22 and 31, Carmoisine 14 and 16, and Amaranth 19 and 31, the latter figure in each case being for the electrophoresis with the paper lying on the smooth glass surface.

Fig. 1 illustrates the application of kieselguhr as a supporting medium for the electrophoretic separation of food colours.

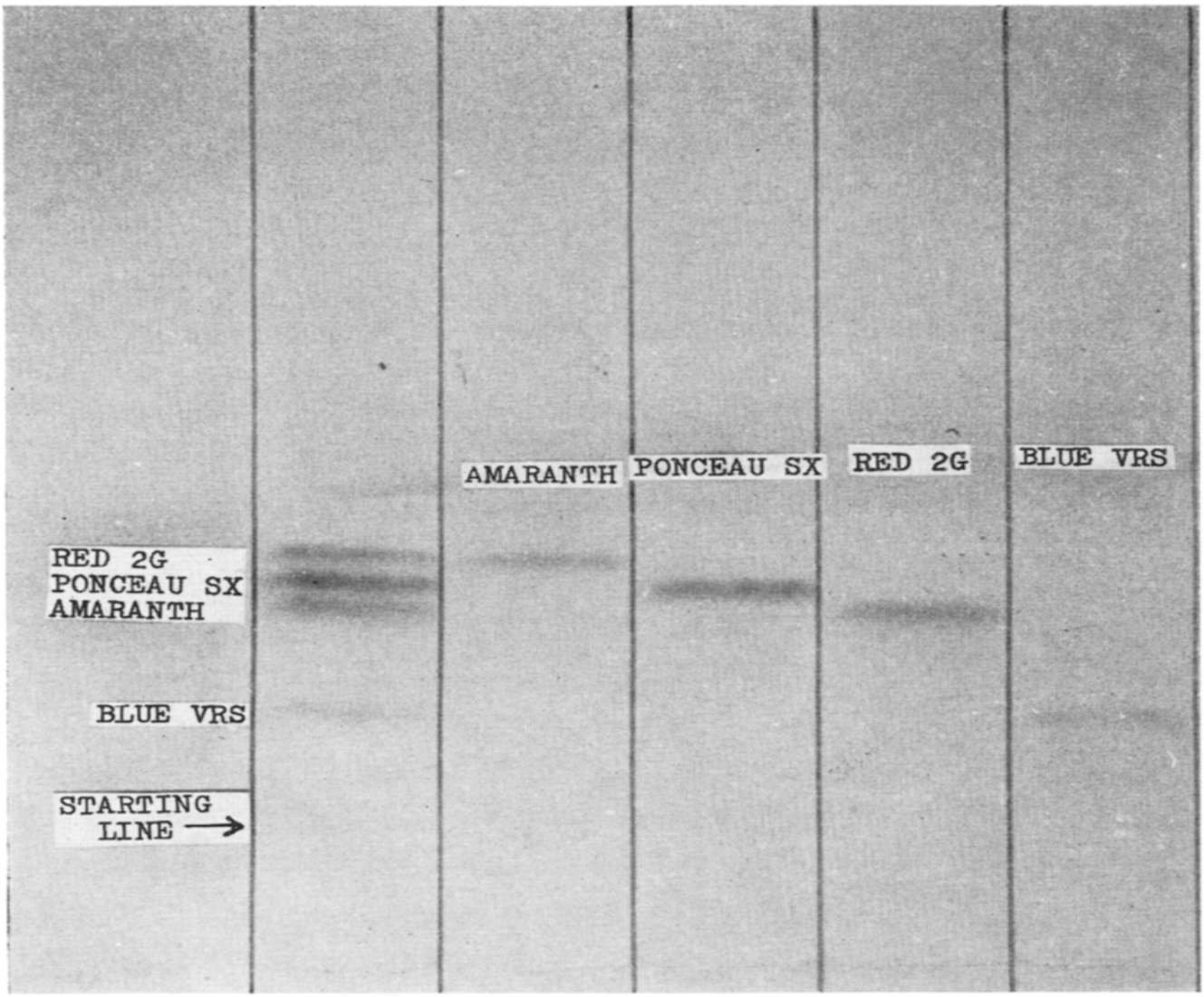

Fig. 1. Typical separation of food colours (45 min) on kieselguhr (pH = 9.2).

DISCUSSION

The mobility of a charged ion under the influence of an electric field depends upon a variety of factors. Some of these are characteristic of the environment, while others depend on the charge and structure of the ions under investigation.

In the present investigation the environmental factors have been varied with respect to the pH of the electrolyte and the supporting medium. In maintaining a constant voltage of 200 V throughout the series of experiments, it is appreciated that

varying the electrolyte and supporting medium results in current variation. Every precaution was taken to standardise the other environmental variables, as indicated by the procedural factors with regard to the ribbed glass bridge in the experiments using paper and the saturation of the thin film with electrolyte in the remaining experiments.

The different mobilities obtained for the paper lying across the ribbed glass plate compared with those obtained with the paper lying on a smooth glass surface may be attributed, at least partially, to what MORI AND KIMURA[18] define as "dark current", that is, the current flowing in areas other than the supporting medium. These authors have also shown that apparatus used as well as different grades of paper also affect mobility. In view of differences between various commercial electrophoresis units, especially tanks, it is difficult to make a comparison of results previously reported[10–12].

The nearest approach to thin-film electrophoresis of food colours so far reported is the study using cellulose acetate strips[12]. On this support of minimal adsorptive power, it was found that the mobility was greater for colours with the higher charge, while for colours of the same charge, the mobility decreased with increasing molecular weight[12]. These workers claim that the use of cellulose acetate eliminates tailing, but it has been found[12,19] that this support very readily permits the isolation of isomeric colours often present in the commercial products. For example, in a typical run, Erythrosine BS gives rise to as many as four bands[19]. While this may be advantageous in production control, it can be a source of difficulty to the analyst concerned only with identifying the major constituents present.

Even on a smooth non-adsorptive support such as cellulose acetate, two colours out of eleven studied did not comply with the expected migration pattern on the basis of charge and molecular weight[12]. The presence of phenolic hydroxyl groups in the molecule is suggested as one of the factors responsible for these discrepancies[12]. This might well be true, for attention has already been drawn to the fact that methyl groups in different positions in Ponceau MX can under certain conditions be responsible for causing the colour to separate into two bands on electrophoresis[10]. That such discrepancies readily occur is apparent by inspection of the mobilities quoted in Table I for Chocolate Brown FB and Brown FK.

It is clear, therefore, that great care must be taken in drawing conclusions from electrophoretic studies on such complex molecules as the food colours. However, some general trends are apparent and a comparison of the mobilities of certain groups and pairs of colours is interesting.

Generally speaking, the mobility is greater for these colours with the higher number of dissociable sodium ions. Two notable exceptions are Red FB and Black PN on both paper and alumina under all conditions of pH, although the mobility of Black PN in ammonia (0.1 *N*) on the paper support is appreciable.

Colours of the naphthyl-azo-naphthol derivatives have approximately the same mobilities as those of the phenyl-azo-naphthol group.

Migration distances for the middle pH ranges, that is, pH 4.0, 6.0 and 8.0 are rather less on the paper support than under more acid or alkaline conditions. This trend is not apparent for the thin film supports.

Owing to the different effects of substituents on the phenolic hydroxyl and amino groups in the molecule and often the different position occupied by these groups

themselves, care has to be taken over comparisons in the mobilities of the different colours and attention will be given to only a few cases from Table I.

The phenyl-azo-2-naphthol derivatives have similar migration distances to those of the phenyl-azo-1-naphthol group. With the former group, Orange G with its extra sulphonic acid group over Orange RN always possesses the higher mobility. The same is true of Sunset Yellow FCF. In this latter case, the extra sulphonic acid group is on the *p*-phenyl position as against the 8-naphthol position in Orange G, but this factor does not influence the migration velocity in the case of the thin-film supports. On the paper support, however, Sunset Yellow FCF shows a reduced mobility when compared with Orange G.

Of the phenyl-azo-1-naphthol derivatives, comparison may be made between Red 10B and Red 2G which are identical except for the 8-naphthol position. This carries an amino group in the former case and an acetamido group in Red 2G. As expected, the mobility is similar for all supports at all conditions of pH. There are, however, sufficient differences in mobilities in a few cases to permit separation of the two colours, that is, except where the support is alumina.

The tri-aryl methanol anhydride colours do not show any enthusiasm for migration on silica gel films, but kieselguhr and alumina show several conditions of pH for separating the three colours.

Except where the migration was minimal, that is, on alumina, the behaviour of Chocolate Brown HT was unsatisfactory on all supports and under all conditions of pH in that extensive tailing always occurred. The only conclusion that can be drawn from studies on this colour is that, except on alumina, its isolation by electrophoresis would be extremely difficult.

Finally, mention must be made about the quality of the electropherograms in relation to those obtained on paper and cellulose acetate strips. A strong criticism of paper electrophoresis of food colours as an analytical tool is the tailing that occurs during separation[10,12]. This difficulty is due to its strong adsorptive capacity and the characteristic is to some extent true of alumina. Resolution is much sharper for the other two supports, particularly for kieselguhr and tailing does not often occur. Of course, this is to be expected since kieselguhr has little adsorptive capacity, but a disappointing feature, as indeed with all the thin-film supports, is that the range of mobilities is not as great as that obtained on paper. However, the much sharper resolution more than compensates for this shortcoming (Fig. 1).

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SUMMARY

The electrophoretic behaviour of the coal-tar food colours permitted in the United Kingdom, except for Oil Yellow GG, Oil Yellow XP, Naphthol Yellow S and Ponceau 3R has been examined in six different electrolytes of widely differing pH values. The supports used consisted of Whatman No. 1 paper, kieselguhr, alumina and silica gel. The results, which illustrate the high degree of resolution obtained using thin film supports, are presented and discussed.

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