

### **Acknowledgement**

In concluding this paper, the authors would like to express their cordial appreciation to prof. Y. Ôshima of Kyushu University for his constant guidance. Thanks are also due to San-ei Chemical Manufacturing Co. Ltd. for kindly supplying the dyes for this work.

(vi) alizarine red S, sulfonic acid salt with hydroxyl groups belonging to anthraquinon dyes:

These dyes migrate toward the anode.

(vii) The following amine dyes do not belong to group A or group A' in the adsorption analysis and are displaced toward the cathode when they are dropped on the central point, consequently making it impossible to find the accurate migration direction, but are further separated toward the cathode than dyes of (i)–(vi)

- a) triphenylmethane dyes: malachite green, rohdamine B, fuchsine, methyl violet, crystal violet
- b) diphenyl methane dyes: auramine
- c) thiazine dyes: methylen blue
- d) azine dyes: neutral red
- e) azo dyes: chrysoidine ES, bismarck brown R

(viii) All of the following dyes are not displaced from the original point on filterpaper.

- a) dis-azo dyes: congo red
- b) azo dyes: yellow AB
- c) triphenylmethane dyes: rose bengale B

Thus systematic migration resulted to be in accordance with the chemical structure and the dissociation state of these dyes in the above solutions is acknowledged.

## SUMMARY

Migration distance of 31 kinds of dyes in the horizontal type apparatus permitting evaporation with acetic acid solutions of 30% and 4% was measured. At the same time, adsorption analysis with the above acetic acid solutions disclosed the existance of a close relation between the extent of influence of osmotic flow into filterpaper due to the evaporation surface and the displacement ratio (R value) in adsorption analysis with the same filterpaper. In consideration with these facts migration directions of dyes were examined in the above solution.

ped on a given point of filterpaper—even on the central point in the horizontal type apparatus evaporation. Such being the case, in an apparatus of this type the dyes which enable correction of displacement of the original point with dextran should be limited to those whose straight line graphs between the axes of migration distance and dropped positions are parallel with graphs provided by dextran. However in consideration of the influence of electro-osmosis, even in case the substance dropped on the central point of filterpaper is displaced a little toward the anode, it can be considered to be actually migrated.

The results obtained after allowing each of the above dyes to migrate in acetic acid of 30 % and 4 % each are classified by their series and radicals as follows:

(i) acid blue VS, pontacyl green BL, wool violet 4 BN which are sulfonic acid salts belonging to triphenylmethane dyes:

All migrate toward the anode.

brom phenol blue, kiton blue A, sulfonic acids with hydroxyl group belonging to triphenylmethane dyes:

These also migrate toward the anode.

(ii) eosine which is phenolate with carboxyl group belonging to triphenylmethane dyes; uranine, with hydroxyl groups belonging to triphenylmethane dyes:

Either of them migrate though slightly toward the anode.

(iii) new cocchine, orange G, tartrazine, sunset yellow, amaranth, orange II, ponceau R, which are sulfonic acid salts with hydroxyl groups belonging to azo dyes:

Each migrate toward the anode.

methyl orange, tertiary amine and sulfonic acid salt belonging to azo dyes:

These migrate toward the anode.

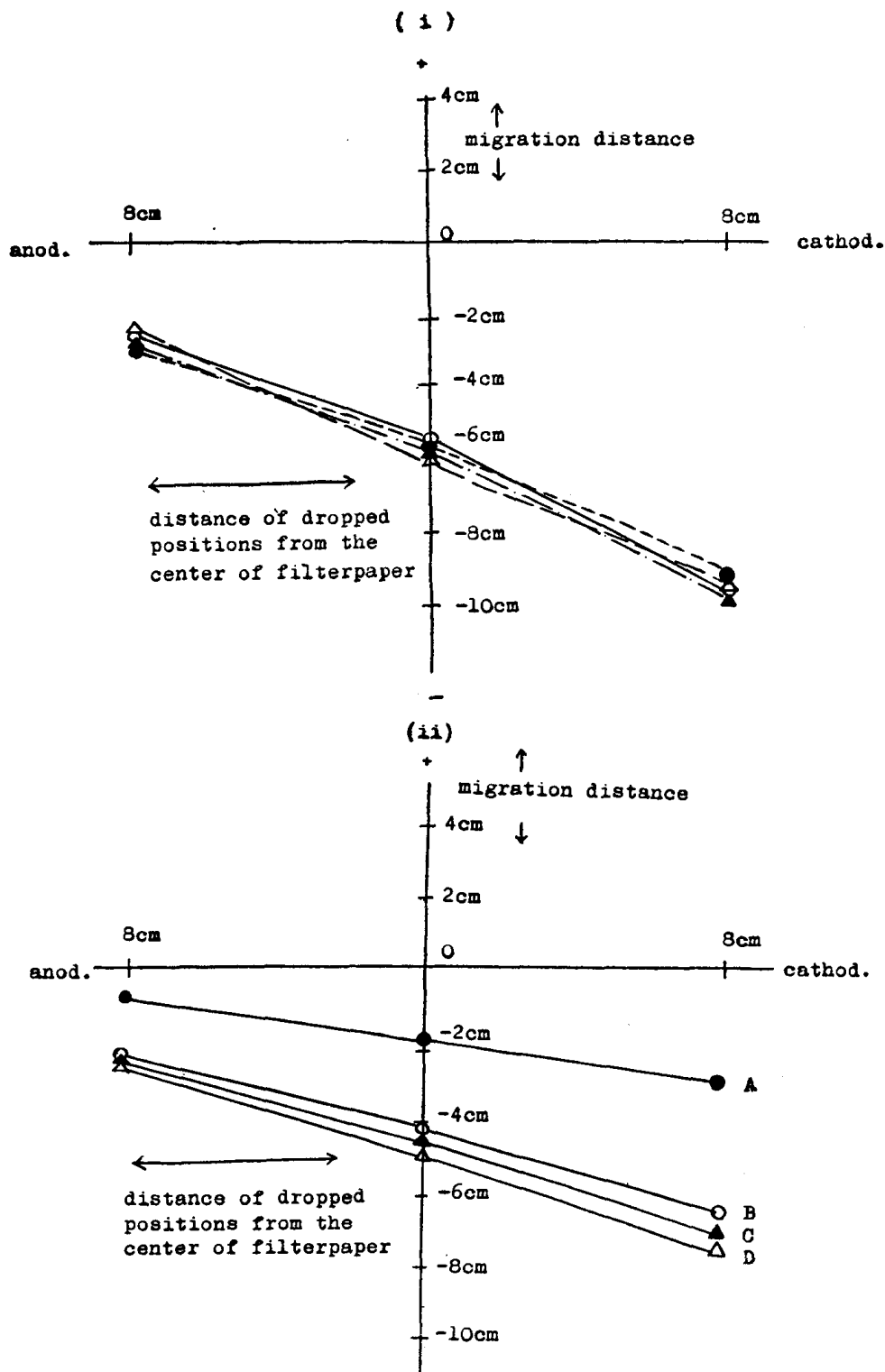
(iv) indigo carmine, sulfonic acid salt belonging to indigo dyes:

These migrate toward the anode.

(v) naphthol yellow S, sulfonic acid belonging to nitro dyes:

These migrate toward the anode.

Fig. 4 The influence of dropped quantity on the graphs showing the relation between migration distance and dropped positions



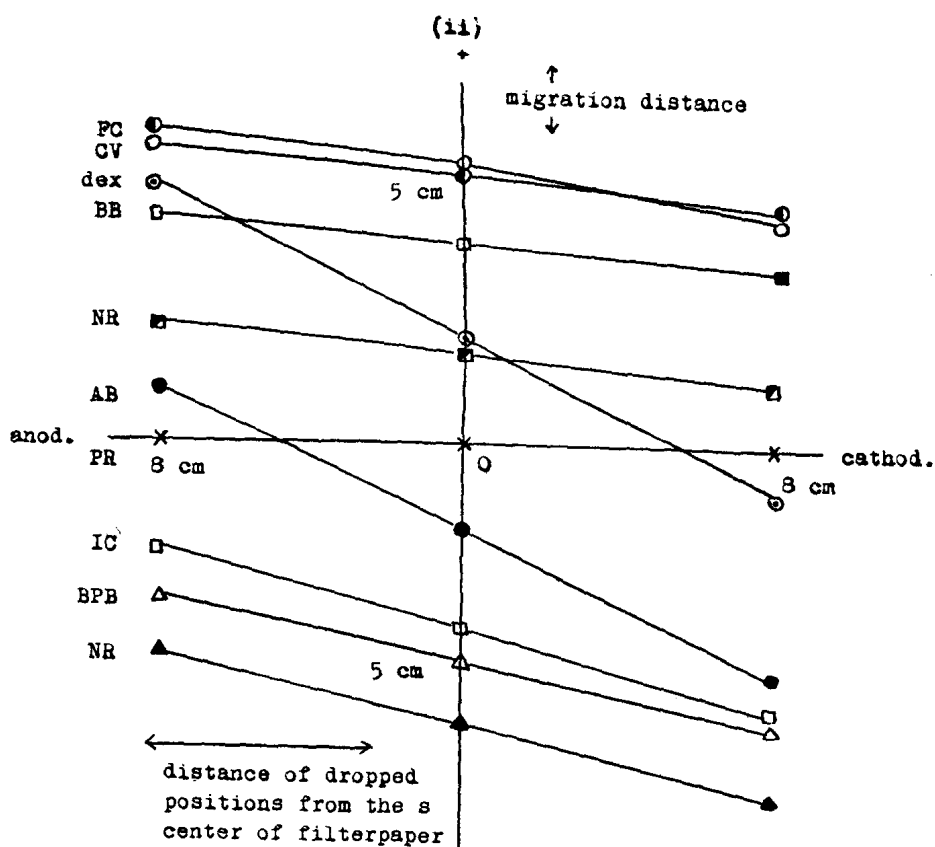
(i) orange G      (ii) wool violet 4 BN

The concentration and dropped volume of dye solutions are as follows

A : 2.5 r ( 0.1 %, 1/400 cc)      B : 12.5 r ( 0.5 %, 1/400 cc)

C : 25 r ( 1 %, 1/400 cc)      D : 100 r ( 1 %, 1/100 cc)

6.5 V/cm, 0.22 mA/cm, 3 hours, in 30 % acetic acid solution, length of filterpaper : 40 cm, in the horizontal type apparatus permitting evaporation



i) in 30% acetic acid solution

AB: acid blue VS (group A)

IC: indigo carmine (group C)

EO: eosine B (group E)

dex: dextran

0.21 mA/cm, 6.5 V/cm, 3 hours

NY: naphthol yellow S (group B)

NR: neutral red (group D)

RB: rose bengal B (group F)

ii) in 4% acetic acid solution

AB: acid blue VS (group A')

BPB: brom phenol blue (group C')

FC: fuchsine (group E')

CV: crystal violet (group G')

PR: ponceau R (group I')

0.13 mA/cm, 6.5 V/cm, 3 hours

NY: new coccine (group B')

IC: indigo carmine (group D')

BB: bismark brown R (group F')

NR: neutral red (group H')

dex: dextran

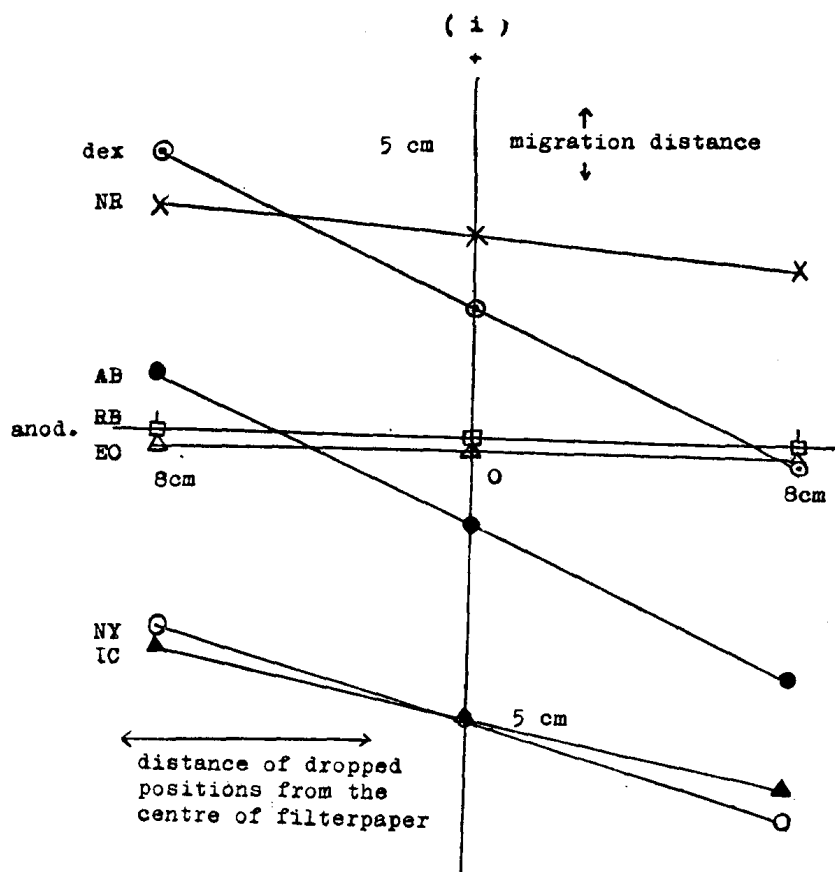
dropped quantity: 25  $\gamma$ , length of filterpaper: 40 cm

in the horizontal type apparatus permitting evaporation

As it is obvious from the figures, varieties of R values in adsorption analysis are represented in terms of varieties of angles each formed by straight line graphs with abscissa. As each dye is under influence of osmotic flow into filterpaper to a different extent, it is misleading to realize facts judging migration velocity and the direction of each dye and its electrification from the position separated after migration, when any given dye is drop-

in 40, 80 and 120 minutes, respectively. As reported previously it is seen the straight line graphs<sup>(1,3,8)</sup> plotted between the axes representing migration distance and dropped positions were entirely not parallel, and, it is especially significant that dyes having lines parallel with dextran are limited in their number, where the dyes having the same R value, as dextran, 1.00 in adsorption analysis belong to. In order to avoid complexity, test results will be given in FIG. 3 selecting one kind of dye from each group already classified by adsorption analysis with acetic acid solutions. On the other hand, the authors, having recognized that variation of concentration and dropped volume of dye solutions causes change of R values in the adsorption analysis, applied electrophoresis dropping each dye on the above-mentioned point of the filterpaper, varying concentration and dropped volume. The results obtained from some dyes are shown in FIG. 4.

Fig. 3 The relationship between migration distance and dropped positions



## ( II )

concentration (%)		0.1	0.5	1.0	1.0	1.0	group
dropped volume (cc)		1/400	1/400	1/800	1/100	1/400	
dye and amino acid	acid blue VS	0.35	0.98	0.97	1.00	0.98	A'
	wool violet 4 BN	0.41	0.70	0.79	0.99	0.96	A'
	orange G	0.80	0.90	0.90	0.90	0.90	B'
	new cocchine	0.79	0.89	0.87	0.93	0.88	B'
	pontacyl green BL	0.61	0.73	0.81	0.91	0.86	B'
	naphthol yellow S	0.67	0.73	0.71	0.85	0.83	B'
	sunset yellow	0.54	0.59	0.72	0.96	0.79	B'
	tartrazine	0.45	0.68	0.68	0.86	0.75	C'
	kiton blue A	0.36	0.65	0.63	0.81	0.74	C'
	amaranth	0.41	0.66	0.67	0.81	0.74	C'
	brom phenol blue	0.61	0.69	0.68	0.76	0.72	C'
	indigo carmine	0.32	0.57	0.57	0.74	0.67	D'
	alizarine red S	0.46	0.57	0.58	0.75	0.66	D'
	malachite green	0.53	0.58	0.52	0.72	0.64	D'
	methyl orange	0.62	0.60	0.59	0.60	0.61	D'
	rohdamine B	0.39	0.58	0.53	0.69	0.58	E'
	auramine	0.40	0.53	0.50	0.60	0.57	E'
	fuchshine	0.36	0.46	0.43	0.62	0.53	E'
	methylen blue	0.14	0.38	0.30	0.59	0.41	F'
	orange II	0.72	0.75	0.39	0.36	0.41	F'
	bismarck brown R	0.23	0.40	0.37	0.48	0.40	F'
	chrysoidine ES	0.19	0.36	0.31	0.55	0.37	F'
	methyl violet	0.10	0.35	0.28	0.46	0.32	G'
	crystal violet	0.08	0.35	0.26	0.34	0.29	G'
	uranine	0.33	0.27	0.28	0.21	0.26	H'
	neutral red	0.05	0.12	0.10	0.20	0.16	H'
	ponseau R	0.03	0.04	0.03	0.07	0.04	H'
	eosine B	0	0	0	0	0	I'
	congo red	0	0	0	0	0	I'
	rose bengale B	0	0	0	0	0	I'
	yellow AB	0	0	0	0	0	I'
	glycine	1.00	1.00	1.00	1.00	1.00	
	histidine	1.00	1.00	1.00	1.00	1.00	
	lysine	1.00	1.00	1.00	1.00	1.00	
	arginine	1.00	1.00	1.00	1.00	1.00	
	glutamic acid a	1.00	1.00	1.00	1.00	1.00	
	spartic acid	1.00	1.00	1.00	1.00	1.00	

Table I The influence of concentration and dropped volume on the R of dyes and amino acids in adsorption analysis

(I) in 30% acetic acid solution

(II) in 4% acetic acid solution

Tôyô Filterpaper No. 50, 25°~29°C, 66~85% R. H.

## (2) Migration distance of dyes

In acetic acid solutions of 30% and 4% respectively, each of the groups mentioned above was dropped on the center and at a point, 8 cm distant from both the anode and cathode side of the filterpaper, shown in FIG. 1 and the migration distance measured

( I )

concentration (%)		0.1	0.5	1.0	1.0	1.0	group
dropped volume (cc)		1/400	1/400	1/800	1/100	1/400	
dye and amino acid	acid blue VS	1.00	1.00	1.00	1.00	1.00	A
	orange G	1.00	1.00	1.00	1.00	1.00	A
	rohdamine B	0.96	1.00	1.00	1.00	1.00	A
	pontacyl green BL	0.96	1.00	1.00	1.00	1.00	A
	new cocchine	0.96	1.00	1.00	1.00	1.00	A
	malachite green	0.92	1.00	1.00	1.00	1.00	A
	sunset yellow	0.91	1.00	1.00	1.00	1.00	A
	wool violet 4 BN	0.87	1.00	1.00	1.00	1.00	A
	kiton blue A	0.80	0.91	0.93	1.00	1.00	A
	brom phenol blue	0.93	0.94	0.95	1.00	0.98	B
	naphthol yellow S	0.89	0.93	0.95	0.97	0.97	B
	amaranth	0.88	0.90	0.94	0.95	0.95	B
	chrystal violet	0.86	0.95	0.90	0.95	0.95	B
	tartrazine	0.91	0.94	0.94	0.88	0.94	B
	methyl orange	0.92	0.92	0.92	0.92	0.92	B
	fuchsine	0.88	0.92	0.89	0.95	0.92	B
	bismarck brown R	0.58	0.89	0.87	0.94	0.91	B
	alizarine red S	0.87	0.90	0.90	0.89	0.90	B
	indigo carmine	0.85	0.85	0.82	0.97	0.89	C
	methyl violet	0.67	0.79	0.80	0.98	0.86	C
	auramine	0.83	0.84	0.83	0.87	0.85	C
	methylen blue	0.67	0.79	0.80	0.98	0.85	C
	orange II	0.98	0.90	0.65	0.77	0.82	C
	uranine	0.79	0.80	0.79	0.80	0.81	C
	chrysoidine ES	0.70	0.71	0.71	0.88	0.77	D
	neutral red	0.48	0.55	0.51	0.69	0.59	D
	ponseau R	0.33	0.35	0.35	0.45	0.40	E
	eosine B	0.18	0.20	0.22	0.23	0.23	E
	congo red	0	0	0	0	0	F
	rose bengale	0	0	0	0	0	F
	yellow AB	0	0	0	0	0	F
	glycine	1.00	1.00	1.00	1.00	1.00	
	histidine	1.00	1.00	1.00	1.00	1.00	
	arginine	1.00	1.00	1.00	1.00	1.00	
	lysine	1.00	1.00	1.00	1.00	1.00	
	glutamic acid	1.00	1.00	1.00	1.00	1.00	
	aspartic acid	1.00	1.00	1.00	1.00	1.00	

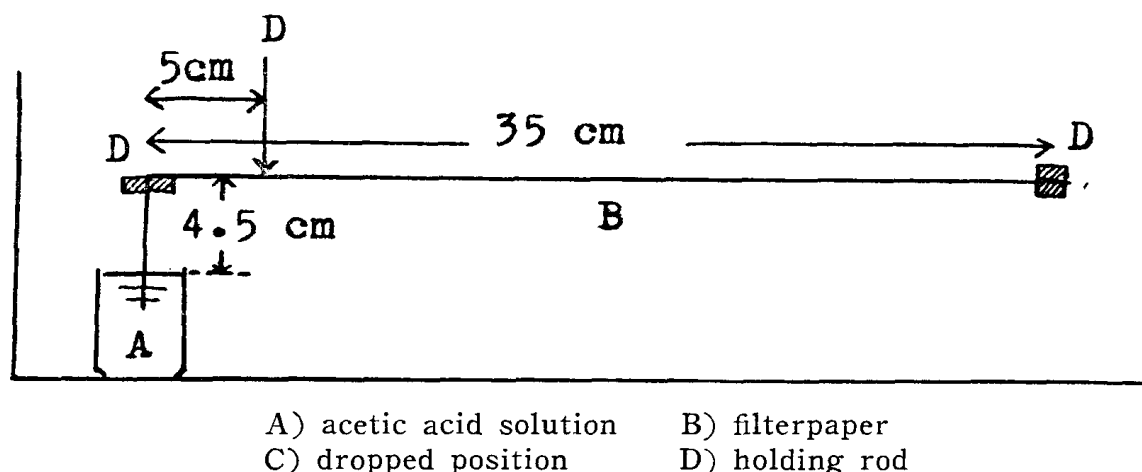
(continued on next page)

is to be seen between both of them which has considerable influence upon MG values. This relation will be described in detail later on.

Here in TABLE I, in order to obtain MG values dyes are classified into groups for convenience viz, in case of 30 % acetic acid solution, group A, B, C, D, E and F, while in case of 4 % acetic acid solution, group A', B', C', D', E', F', G', H', and I', according to their R values.



Fig. 2 Apparatus of adsorption analysis



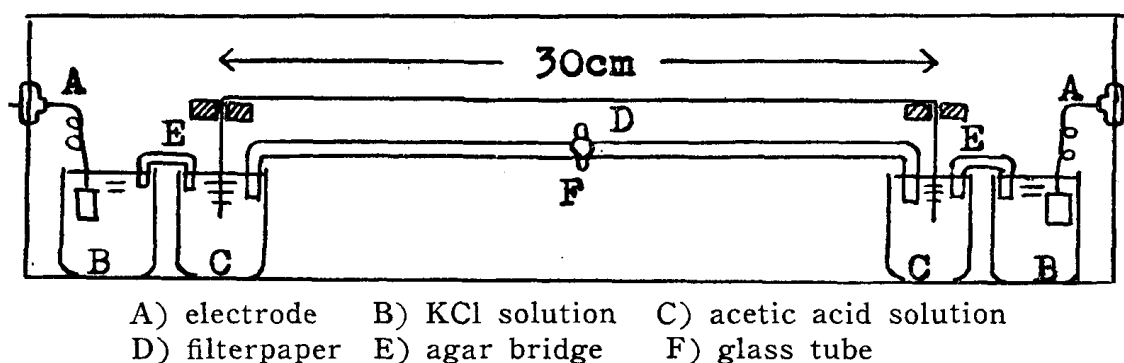
The difference in concentration of dye solutions and their volume to be dropped were examined in order to obtain a variation of  $R$  values. Concretely, to obtain  $R$  values in the former test, a portion of  $1/400$  cc of each dye solution of 1 %, 0.5 % and 0.1 % respectively, was examined and in the latter test, portions of  $1/100$  cc,  $1/400$  cc and  $1/800$  cc of 1 % dye solution were examined respectively. In this case the  $R$  values of dextran, glycine, histidine, arginine, lysine, glutamic acid and aspartic acid were also obtained. As it is obvious from the table, when 30 % acetic acid solution was used  $R$  values for various dyes were roughly above 0.7, regardless of the differences of concentration and dropped volume, whereas when 4 % acetic acid solution was used,  $R$  values in general, showed a decrease along with the decrement of concentration and dropped volume. However in both cases  $R$  values of amino acids resulted to be 1.00, as displacement proceeded with the soaked up front. Hence no particular relation could be observed between  $R$  values and the chemical structures. Varieties of  $R$  values of dyes thus obtained are considered to have been caused by the difference in adsorption to filterpaper of these dyes.

$R$  values obtained from adsorption analysis are the values resulting from soaking up the acetic acid solution into dried filterpaper which is not to be considered as an osmotic flow into filterpaper in paper-electrophoresis; however, a very close relation

and aspartic acid were used.

(2) The apparatus was of the horizontal-type permitting evaporation as shown in FIG.1. For part D in the figure Tôyô Filterpaper No. 50,  $2 \times 40$  cm and  $2 \times 35$  cm, and for part C 30 % and 4 % acetic acid solution were used respectively. Twenty minutes after the beginning of current application, the test solution was dropped on the filterpaper and subsequently subjected to electrophoresis under a constant voltage, and the current measured at an interval of 10 minutes, the arithmetical mean being assumed as the average current. Then after judging the center of deep colored specks as the positions of the dyes, migration distance was measured.

Fig. 1 Apparatus



## II. Results and discussion

### (1) Adsorption analysis

As reported previously,<sup>(1,3,8)</sup> when the substance migrates in the horizontal type apparatus permitting evaporation, evaporation due to Joule's heat increases and migration velocity is conspicuously influenced by the osmotic flow into filterpaper. As a means of observing the influence of this osmotic flow, the adsorption analysis<sup>(8)</sup> was conducted with filterpaper in the apparatus shown in FIG. 2.

R values, displacement ratios, calculated out according to the following definition are shown in TABLE I.

$$R = \frac{\text{displacement of substance from its dropped position}}{\text{distance of the soaked up front of acetic acid solution from dropped position of substance}}$$

on several scores of dyes in citrate buffer,<sup>(8)</sup> the graphs of which were not all found to be parallel to each other.

Experimental results of migration distance of these dyes placed in 30 % and 4 % acetic acid solutions are presented in this paper.

## EXPERIMENT AND DISCUSSION

### I. Materials and method

(1) Aqueous solutions 0.1, 0.5 and 1.0 % of the following 31 different dyes were used.

(i) triphenylmethane dyes

kiton blue A, pontacyl green BL, acid blue VS, wool violet 4 BN, brom phenol blue, rohdamine B, malachite green, uranine, fuchsine, fuchsine, methyl violet, crystal violet, eosine B, rose bengal B

(ii) diphenylmethane dyes

auramine

(iii) dis-azo dyes

congo red, bismarck brown R

(iv) azo dyes

new cocchine, tartrazine, orange G, sunset yellow, methyl orange, amaranth, chrysoidine ES, orange II, ponceau R, yellow AB

(v) nitro dygs

naphthol yellow S

(vi) anthraquinon dyes

alizarin red S

(vil) indigo dyes

indigo carmine

(viii) azine dyes

neutral red

(ix) thiazine dyes

methylen blue

In addition, a 2 % aqueous solution of dextran and 1.0, 0.5 and 0.1 % solutions of glycine, histidine, arginine, lysine, glutamic acid

# On the Migration Distance in Paper-electrophoresis Part VIII. Studies on the Migration Distance of Dyes<sup>\*(2)</sup>

By Kiyoshi SAKAMOTO, Kinuko SAITO and Ayako NAGATA

*Laboratory of Nutrition, Faculty of Junior Course,  
Kagoshima Prefectural University*

Received July 22, 1958

The migration distance of several scores of dyes in 30% and 4% acetic acid solutions in a horizontal-type apparatus permitting evaporation was measured. In these occasions, an extremely relationship was found to exist between the degree of influence of osmotic flow into filterpaper due to evaporation from the paper surface and the displacement ratios obtained from adsorption analysis with the same filterpaper.

The authors have already reported that amino acids<sup>(1,2)</sup> and serum proteins<sup>(3)</sup> show a decrease in migration velocities causing a logarithmic decrement in the horizontal-type apparatus permitting evaporation and simultaneously found that the relative distance ratios of these substances, named MG values,<sup>(4,5,6,7)</sup> are constant values regardless of voltage, current, time of electrophoresis, dropped positions and length of filterpaper. Further in the case of amino acids<sup>(1,4)</sup> and serum proteins,<sup>(3)</sup> it was found that parallell straight-line graphs could be plotted between migration distance and dropped positions, and the MG value was proved to assume a constant value regardless of the above-conditions by means of analysing the above graphs.<sup>(5,6)</sup> However, this fact was not the case with all of the other substances for throughout the experiments

---

\* This work is also presented in "Bull. Agr. Chem. Soc. Jap., No. 1 (1958)" and to be published at the Annual Meeting of the Agricultural Chemical Society of Japan, April, 1959.

- 1) K. Sakamoto and K. Saito., *J. Agr. Chem. Soc. Jap.*, **32**, 181 (1958)
- 2) K. Sakamoto and K. Saito., *J. Agr. Chem. Soc. Jap.*, **32**, 186 (1958)
- 3) K. Sakamoto and K. Saito., *J. Agr. Chem. Soc. Jap.*, **32**, 275 (1958)
- 4) K. Sakamoto and K. Tateoka., *J. Agr. Chem. Soc. Jap.*, **30**, 463 (1956)
- 5) K. Sakamoto and K. Tateoka., *Bull. Agr. Chem. Soc. Jap.*, **20**, 98 (1956)
- 6) K. Sakamoto and K. Saito., *J. Agr. Chem. Soc. Jap.*, **23**, 279 (1958)
- 7) K. Sakamoto and K. Saito., *Bull. Agr. Chem. Soc. Jap.*, **22**, 55 (1958)
- 8) K. Sakamoto and K. Saito., *J. Agr. Chem. Soc. Jap.*, **32**, 390 (1958)