After conducting analysis of MG values under the new definition, and by experimental measurement, they were found to be constant values not affected by conditions of electrophoresis.

Acknowledgement

In concluding this paper, the anthors wish to express their gratitude to Prof. Y. Ôshima of Kyushu University for his generous guidance. Thanks are due to San-ei Chemical Manufacturing Co. Ltd. for kindly supplying the dyes used in this work.

factors, i. e., current, voltage, time of electrophoresis, dropped positions and length of filterpaper, while the dropped quantity was fixed at 25γ. The reason for fixing dropped quantity above the unit γ, is derived from the fact that variation of concentration and dropped volume are directly represented in different angles formed between the graph representing the relation of migration distance with dropped positions of the dyes and abcissa. As for the dyes belonging to group A MG values were obtained in consideration of concentration and dropped volume in adsorption analysis, the approvable variation of dropped quantity being within the range of 12.5~100 γ.

As it is seen from this table the MG value was in accordance with the above definition the constant values without being affected by any of the above electrophoresis conditions. However, these MG values in comparison with those values of amino acids[3,4] and serum proteins,[3,5] involve a somewhat expanded range of error. As the MG value is derived from the condition of the parallel relation indicated in the graph between migration distance and dropped positions of each migrant, deviation of the values is probably due to the fact that the dyes classified in each group, strictly speaking, do not bear parallel graphs. Among the dyes of group A, the MG of kiton blue could not be obtained as its R value was liable to the influence of dropped quantity. In regard of sample solutions containing dyes which carry different MG values when dropped quantity either increases or decreases, it is difficult to identify the dyes contained by means of MG value. In consideration of MG values of dyes belonging to group A, the MG is regarded to be applicable to a substance with a R value of 1.00 in adsorption analysis, within the renge where the R value remains at 1.00.

SUMMARY

MG values of dyes classified in groups by means of adsorption analysis with acetic acid solutions of 30% and 4% could be obtained in a horizontal type apparatus permitting evaporation.

(II)

	tartrazine	amaranth	napthol yellow S	alizarine red S	brom phenol blue
MG	1.00	0.94±0.03	0.87±0.03	0.75±0.03	0.68±0.03
	methyl orange	crystal violet	fuchsine	bismarck brown R	
MG	0.46±0.04	0.20±0.04	0.12±0.03	0	

(III)

	indigo carmine	orange II	uranine	methyl violet	auramine	methylen blue
MG	1.00	0.82±0.03	0.40±0.03	0.23±0.04	0.11±0.04	0

(IV)

	new coccine	orange G	naphthol yellow S	sunset yellow	pontacyl green BL
MG	1.00	0.72±0.04	0.65±0.04	0.42±0.05	0

(V)

	tartrazine	amaranth	kiton blue A	brom phenol blue
MG	1.00	0.79±0.05	0.17±0.03	0

(VI)

	indigo carmine	alizarine red S	methyl orange	malachite green
MG	1.00	0.84±0.02	0.52±0.05	0

Table I MG values of dyes

(I) A group a, b, c, d, e, f, g, h and i in the table is respectively quantitative combination of dyes in the following:

	a	b	c	d	e	f	g	h	i
new coccine	25	100	100	12.5	12.5	100	100	12.5	12.5
test-dye	25	100	12.5	100	12.5	100	12.5	100	12.5
malachite green	25	12.5	12.5	100	100	100	100	12.5	12.5

in 30% acetic salution

(II) B group dropped quantity: 25 γ, 6.5～13 V/cm, 0.20～0.38mA/cm, 13 hours, in 30% acetic acid solution

(III) C group dropped quantity: 25γ, 6.5～13V/cm, 0.19～0.38 mA/cm, 13 hours, in 30% acetic acid solution

(IV) B′ group dropped quantity: 25γ, 0.5～13V/cm, 0.12～0.26 mA/cm, 40 120 minutes, in 4% acetic acid solution

(V) C′ group dropped quantity: 25 γ, 6.5～13 V/cm, 0.12～0.29 mA/cm, 40 120 minutes, in 4% acetic acid solution

(VI) D′ group dropped quantity: 25 γ, 6.5～13 V/cm, 0.12～0.27 mA/cm, 40 120 minutes, in 4% acetic acid solution

dropped position: 8 cm toward the anode～8 cm toward the cathode from the center of filterpaper, length of filterpaper: 40 cm, 35 cm, Tôyô Filterpaper No. 50, in the horizontal type apparatus permitting evaptoration.

(I)

new coccine	orange G	sunset yellow	wool violet	pontacyl green	acdid blue VS	rohdamine	malachite green	time (hour)	voltage (V/cm)	current (mA/cm)	dropped position	combination of dyes	length of filterpaper (cm)
1.00	0.88	0.84	0.73	0.71	0.49	0.11	0	1	6.5	0.19	center	a	40
1.00	0.89	0.86	0.73	0.73	0.51	0.14	0	〃	〃	〃	〃	〃	〃
1.00	0.90	0.81	0.69	0.71	0.52	0.14	0	〃	〃	〃	〃	〃	〃
1.00	0.91	0.87	0.67	0.67	0.52	0.07	0	〃	〃	0.21	〃	b	〃
1.00	0.87	0.82	0.67	0.64	0.63	0.06	0	〃	〃	〃	〃	c	〃
1.00	0.95	0.91	0.79	0.79	0.64	0.18	0	〃	〃	〃	〃	d	〃
1.00	0.98	0.94	0.70	0.70	0.64	0.17	0	〃	〃	〃	〃	e	〃
1.00	0.94	0.83	0.69	0.67	0.50	0.11	0	2	〃	0.20	〃	a	〃
1.00	0.91	0.82	0.71	0.67	0.49	0.10	0	〃	〃	〃	〃	〃	〃
1.00	0.79	0.74	0.63	0.62	0.51	0.11	0	〃	〃	0.22	〃	b	〃
1.00	0.90	0.85	0.69	0.64	0.56	0.14	0	〃	〃	〃	〃	g	〃
1.00	0.90	0.85	0.68	0.65	0.55	0.10	0	〃	〃	〃	〃	h	〃
1.00	0.92	0.86	0.65	0.61	0.52	0.09	0	〃	〃	〃	〃	i	〃
1.00	0.92	0.86	0.70	0.64	0.56	0.09	0	1	13.0	0.40	center	a	40
1.00	0.92	0.87	0.70	0.64	0.53	0.09	0	〃	〃	〃	〃	〃	〃
1.00	0.94	0.86	0.70	0.64	0.52	0.09	0	〃	〃	〃	〃	〃	〃
1.00	0.91	0.86	0.71	0.65	0.54	0.10	0	〃	〃	〃	〃	〃	〃
1.00	0.93	0.87	0.70	0.67	0.53	0.10	0	〃	〃	〃	〃	〃	〃
1.00	0.90	0.83	0.69	0.61	0.56	0.20	0	〃	〃	〃	〃	b	〃
1.00	0.93	0.84	0.73	0.68	0.54	0.14	0	〃	〃	〃	〃	c	〃
1.00	0.98	0.94	0.83	0.78	0.70	0.15	0	〃	〃	〃	〃	d	〃
1.00	0.99	0.93	0.81	0.76	0.64	0.14	0	〃	〃	〃	〃	e	〃
1.00	0.87	0.79	0.69	0.68	0.58	0.18	0	〃	〃	〃	〃	f	〃
1.00	0.93	0.82	0.72	0.65	0.54	0.17	0	〃	〃	0.42	〃	g	〃
1.00	0.89	0.78	0.67	0.64	0.55	0.13	0	〃	〃	〃	〃	h	〃
1.00	0.91	0.84	0.71	0.65	0.52	0.19	0	〃	〃	〃	〃	i	〃
1.00	0.90	0.83	0.68	0.62	0.49	0.08	0	2.5	6.5	0.21	8 cm anodic side from the center	a	40
1.00	0.92	0.82	0.68	0.61	0.56	0.11	0	〃	〃	〃	〃	〃	〃
1.00	0.90	0.84	0.66	0.68	0.46	0.11	0	〃	〃	〃	〃	〃	〃
1.00	0.94	0.89	0.73	0.69	0.60	0.10	0	〃	〃	〃	8 cm cathodic side from the center	〃	〃
1.00	0.93	0.85	0.73	0.69	0.61	0.14	0	〃	〃	〃	〃	〃	〃
1.00	0.89	0.79	0.66	0.60	0.50	0.15	0	〃	〃	〃	8 cm anodic side from the center	b	〃
1.00	0.91	0.85	0.64	0.57	0.44	0.07	0	〃	〃	〃	〃	c	〃
1.00	0.88	0.82	0.67	0.59	0.53	0.18	0	〃	〃	〃	〃	d	〃
1.00	0.97	0.89	0.77	0.69	0.56	0.19	0	〃	〃	〃	〃	e	〃
1.00	0.93	0.85	0.77	0.71	0.62	0.08	0	〃	〃	〃	8 cm cathodic side from the center	f	〃
1.00	0.95	0.86	0.77	0.68	0.61	0.07	0	〃	〃	〃	〃	g	〃
1.00	0.92	0.84	0.83	0.65	0.57	0.09	0	〃	〃	〃	〃	h	〃
1.00	0.96	0.87	0.69	0.65	0.57	0.08	0	〃	〃	〃	〃	i	〃
1.00	0.92	0.85	0.69	0.63	0.50	0.10	0	3	6.5	0.20	center	a	35
1.00	0.91	0.85	0.68	0.63	0.51	0.10	0	〃	〃	〃	〃	〃	〃
1.00	0.93	0.80	0.70	0.64	0.50	0.09	0	〃	〃	〃	〃	〃	〃
1.00	0.91	0.85	0.68	0.64	0.51	0.14	0	〃	〃	〃	〃	〃	〃
1.00	0.92	0.85	0.70	0.64	0 51	0.11	0	〃	〃	〃	〃	〃	〃
1.00	0.94	0.85	0.73	0.66	0.54	0.08	0	〃	〃	0.22	〃	b	〃
1.00	0.91	0.85	0.68	0.60	0.49	0.06	0	〃	〃	〃	〃	c	〃
1.00	0.96	0.87	0.74	0.69	0.56	0.11	0	〃	〃	〃	〃	d	〃
1.00	0.95	0.88	0.71	0.65	0.55	0.25	0	〃	〃	〃	〃	e	〃
1.00	0.96	0.87	0.74	0.70	0.56	0.23	0	〃	〃	〃	〃	f	〃
1.00	0.89	0.83	0.64	0.60	0.50	0.11	0	〃	〃	0.23	〃	g	〃
1.00	0.91	0.85	0.71	0.67	0.56	0.10	0	〃	〃	〃	〃	h	〃
1.00	0.93	0.86	0.67	0.61	0.51	0.09	0	〃	〃	〃	〃	i	〃
1.00	0.88	0.82	0.67	0.61	0.50	0.09	0	3	6.5	0.19	center	a	40
1.00	0.83	0.83	0.68	0.62	0.51	0.10	0	〃	〃	〃	〃	〃	〃
1.00	0.90	0.84	0.69	0.63	0.52	0.11	0	〃	〃	0.21	〃	〃	〃
1.00	0.91	0.85	0.70	0.64	0.53	0.12	0	〃	〃	0.22	〃	〃	〃
1.00	0.92	0.86	0.71	0.65	0.54	0.13	0	〃	〃	〃	〃	〃	〃
1.00	0.85	0.79	0.65	0.61	0.52	0.09	0	〃	〃	0.19	〃	b	〃
1.00	0.92	0.85	0.69	0.63	0.52	0.08	0	〃	〃	0.20	〃	c	〃
1.00	0.96	0.90	0.78	0.73	0.62	0.14	0	〃	〃	〃	〃	d	〃
1.00	0.98	0.92	0.76	0.72	0.61	0.16	0	〃	〃	0.21	〃	e	〃
1.00	0.85	0.78	0.67	0.63	0.53	0.13	0	〃	〃	〃	〃	f	〃
1.00	0.92	0.85	0.68	0.63	0.53	0.09	0	〃	〃	0.22	〃	g	〃
1.00	0.91	0.81	0.67	0.62	0.53	0.09	0	〃	〃	〃	〃	h	〃
1.00	0.94	0.86	0.66	0.62	0.51	0.09	0	〃	〃	0.23	〃	i	〃
1.00	0.92±0.03	0.85±0.04	0.70±0.04	0.66±0.04	0.54±0.04	0.13±0.04	0						

(Continued on next page)

age, dropped positions and time of electrophoresis, provided the graph denoting the relation between dropped positions and migration distance among migrants is parallel to each other.

As previously reported,[6,7] the dyes which have R values close to each other in adsorption analysis have almost parallel graphs representing the relation between dropped positions and migration distance, thus making it possible to obtain MG values of dyes belonging to groups A, B and C[6] with 30 % acetic acid solution and those of groups B′, C′ and D′[6] with 4 % acetic acid solution, respectively. The definitions of MG values are listed as follows:

1) 30 % acetic acid solution;

i) group A

$$MG = \frac{\text{migration distance of another dye from malachite green}}{\text{migration distance of new coccine from malachite green}}$$

ii) group B

$$MG = \frac{\text{migration distance of another dye from bismarck brown R}}{\text{migration distance of tartrazine from bismarck brown R}}$$

iii) group C

$$MG = \frac{\text{migration distance of another dye from methylen blue}}{\text{migration distance of indigo carmine from methylen blue}}$$

2) 4 % acetic acid solution;

i) group B′

$$MG = \frac{\text{migration distance of another dye from pontacyl green BL}}{\text{migration distance of new coccine from pontacl green BL}}$$

ii) group C′

$$MG = \frac{\text{migration distance of another dye from brom phenol blue}}{\text{migration distance of tartrazine from brom phenol blue}}$$

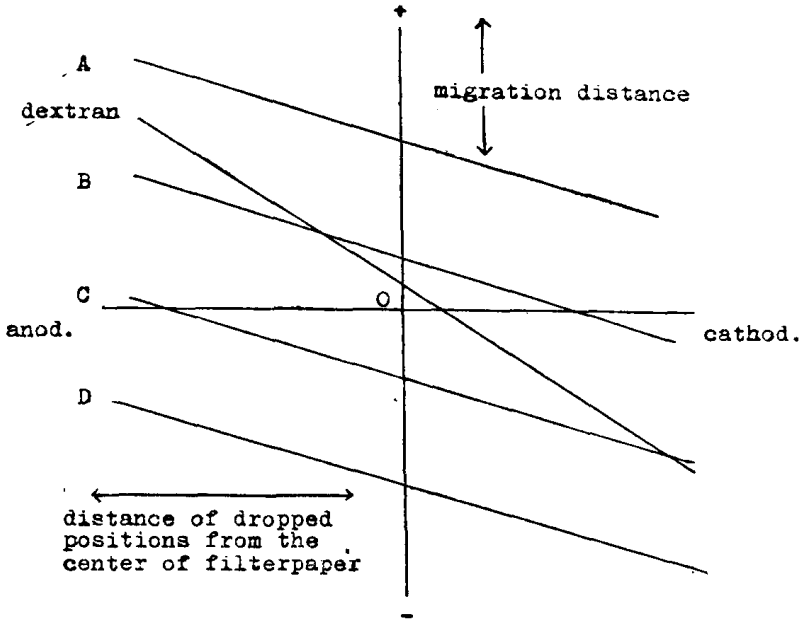
iii) group D′

$$MG = \frac{\text{migration distance of another dye from malachite green}}{\text{migration distance of indigo carmine from malachite green}}$$

As for the dyes belonging to groups other than A, the MG values in average, of 30 examples as well as their standard deviations are given in TABLE I. These involve five different

7) K. Sakamoto and K. Saito., *J. Agr. Chem. Soc. Jap.*, **32**, 390 (1958)

Fig. 1 The relationships between the migration distance of A (B, C, D and dextran) and dropped positions



Thus, MG values can be proved not to be influenced by the osmotic flow into filterpaper and the time of electrophoresis. That is, not to be affected by osmotic flow also means not to be influenced by dropped positions. Furthermore, when V_{0A}, V_{0B}, V_{0C} and V_{0D} respectively represent the net-initial velocity of A, B, C and D and V_{0el} represents the velocity of electro-osmosis, formulae (3—1) and (3—2) are represented as follows.

$$MG_B = \frac{V_{0B} - V_{0el} - V_{0A} + V_{0el}}{V_{0D} - V_{0el} - V_{0A} + V_{0el}} = \frac{V_{0B} - V_{0A}}{V_{0D} - V_{0A}} \quad \cdots\cdots(4—1)$$

$$MG_C = \frac{V_{0C} - V_{0el} - V_{0A} + V_{0el}}{V_{0D} - V_{0el} - V_{0A} + V_{0el}} = \frac{V_{0C} - V_{0A}}{V_{0D} - V_{0A}} \quad \cdots\cdots(4—2)$$

Thus it can be observed that MG values are not under the influence of electro-osmosis. And as V_{0el} is the function of current, $V_{0A(B,C,D)}$ having no relation with current, from formulae (4—1) and (4—2) it can be seen that the MG value is not under any influence of current. Furthermore in formulae (4—1) and (4—2), V_{0A}, V_{0B}, V_{0C} and V_{0D} containing voltage, (v, in common,) MG values being their quotients, are realized not to be under the cs influence of voltage. Consequently, as seen in FIG. 1, MG values are recognized to be constant values regardless of current, volt-

EXPERIMENT AND DISCUSSION

I Materials and method

Electrophoresis of 31 kinds of dyes was conducted with acetic acid solutions of 30 % and 4 % using the same apparatus as previously described in the experiment process.[6]

II MG values of dyes

The graph in FIG. 1 showing the relation of dropped positions of migrants A, B, C and D with migration distance is not parallel with that of dextran but, a parallel relation is maintained only among A, B, C and D, one another. Suppose V'_{0A}, V'_{0B}, V'_{0C} and V'_{0D} represent the apparent initial velocity of A, B, C and D respectively, and the apparent migration distance i. e., MD'_A, MD'_B, MD'_C and MD'_D shown after the time-length of t, then as each straight-line graph being parallel to each other, migration distance may be calculated by the following formulae:

$$\int_0^t V_{0A}\ (a'/a)^t\ dt = MD'_A \cdots\cdots (1—1)$$

$$\int_0^t V_{0B}\ (a'/a)^t\ dt = MD'_B \cdots\cdots (1—2)$$

$$\int_0^t V_{0C}\ (a'/a)^t\ dt = MD'_C \cdots\cdots (1—3)$$

$$\int_0^t V_{0D}\ (a'/a)^t\ dt = MD'_D \cdots\cdots (1—4)$$

Suppose MG_B and MG_C represent the MG value of B and C, respectively, the following formulae are obtained:

$$MG_B = \frac{MD'_B - MD'_A}{MD'_D - MD'_A} \cdots\cdots (2—1)$$

$$MG_C = \frac{MD'_C - MD'_A}{MD'_D - MD'_A} \cdots\cdots (2—2)$$

By substituting formulae (1—1), (1—2), (1—3) and (1—4) into the right-side of formulae (2—1), (2—2), MG_B and MG_C are represented by the following formulae:

$$MG_B = \frac{V'_{0B} - V'_{0A}}{V'_{0D} - V'_{0A}} \cdots\cdots (3—1)$$

$$MG_C = \frac{V'_{0C} - V'_{0A}}{V'_{0D} - V'_{0A}} \cdots\cdots (3—2)$$

on the Migration Distance in Paper-electrophoresis Part IX. On the MG Values of Dyes*

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Received July 22, 1958

MG values of dyes could be obtained in the horizontal-type apparatus permitting evaporation with 30% and 4% acetic acid solutions. In this experiment the fact that MG values are constant values not influenced by current, voltage, time of electrophoresis, dropped positions and length of filterpaper was both theoretically and experimentally proved, and conditions for regulating the applicable range of MG values were examined.

The authors reported that MG values of amino acids[1,2,3,4] and serum proteins[4,5] are constant regardless of current, voltage, time of electrophoresis, dropped position and length of filterpaper. In that occasion[3,4,5] it was found that for the establishment of MG value, a parallel relation between the graphs of dropped positions and migration distance of the migrants and the graph of dextran is required. However, when dyes were migrated in acetic acid solutions of 30% and 4%, the dyes which had parallel graphs with dextran were very small in number.[6] For this reason, further experiments of analysing these MG values in order to expand the application of these definitions were carried out.

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* This work is also presented in "*Bull. Agr. Chem. Soc. Jap.*, 33, No. 1 (1959)" and to be published at the Anual Meeting of the Agricultural Chemical Society of Japan, April, 1959.

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