each of these transformations with a graphic method[4] as $H=55.42$ are brought together for ready comparison in Fig. 1.

Each deviation from the regression line is logit $D_0^2=37.8\left(=50.3\times\frac{3}{4}\right)$, probit $D_0^2=81.0$, and angle $D_0^2=138.7$(d.f.$=4$), and then each value of F_0 compared with the error term$=47.08$ is 0.92, 1.97, and 3.36, respectively. As angle F_0 is more than $F^4_{195}\fallingdotseq 2.447$, the angular transformation is rejected significantly. To conclude, it is apparent that a logistic transformation might be the fittest. The final decision must be given after many experiments have been performed for a competitive inhibition by atropine or non-competitive one by papaverine-like substances.

We wish to expess our thanks to Prof. H. Kumagai and Prof. Y. Ito of University of Tokyo for guidance and help in the course of this work.

Summary

1) A concentration-action relationship of acetylcholine was demonstrated using isolated intestines of 20 mice.

2) For mean responses of six acetylcholine concentrations was fitted a logistic sigmoid curve by the method of maximum likelihood.

3) The maximum likelihood estimate of the mean slope was 1.146, which was proved not to be significantly different from the theoretical value of one.

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85. **Kiyoshi Futaki**: Circular Paper Chromatography. Studies on a Factor that Influences Rf and Determination of Rr Values of Photographic Developing Agents.

(*Research Section, Kyoto Factory, Mitsubishi Paper Mills Ltd.**)

Rutter[1,2] has described a modified chromatographic technique employing circular filter paper and called attention to its advantages such as speed and sharpness of separation, simplicity and compactness of apparatus, reproducibility, and control of rate of solvent flow.

Rosebeek[3] developed another technique of circular paper chromatography in which he employed a filter paper cone immersed in the eluant and just touching the center of horizontally supported filter paper.

Lüderitz and Westphal[4] extended Rutter's technique by applying discrete spots of material in a circle about the center of a paper rather than as a single spot at the origin, so that many different substances could be compared.

Rao and Giri,[5] employing Lüderitz and Westphal's technique, reported the factors that influence Rf significantly. According to them, these factors are the distance moved by the solvent and distance of the initial spot from the center of filter paper.

* Nagaoka-Cho, Otokuni-gun, Kyoto-fu (二木 清).

1) L. Rutter: Analyst, **75**, 37(1950).
2) L. Rutter: Nature, **161**, 435(1948).
3) S. Rosebeek: Chem. Weekblad, **46**, 813(1950).
4) O. Lüderitz, O. Westphal: Z. Naturforsch., **7b**, 136(1952).
5) T. Rao, K.V. Giri: J. Indian. Inst. Sci., **35A**, 77(1953).

In these reports, a clear relationship between the distance of the initial spot from the center and its Rf values was not described. In the present series of experiments, studies on such relationship and determination of Rr values of photographic developing agents were taken up.

Experimental

Reagents and Apparatus—1) Filter Paper : Toyo Roshi No. 50, 19 cm. in diameter.

2) Developing Solvents : The solvents used in all these runs were the top layers obtained after shaking BuOH·AcOH·H_2O in volume ratio of 4:1:5 or benzene·AcOH·H_2O in 2:2:1.

3) Sample Solutions : Photographic developing agent (1-phenylpyrazolidin-3-one, catechol, hydroquinone, *p*-diethylaminoaniline sulfate, pyrogallol, *p*-N-methylaminophenol sulfate, N-(*p*-hydroxyphenyl)glycine, 2,4-diaminophenol sulfate, and *p*-phenylenediamine) was each dissolved into 0.5% MeOH or hydrous MeOH solution.

4) Spray reagent : Ammoniacal $AgNO_3$ (mixture of equal volumes of 0.1*N* $AgNO_3$ and 5*N* NH_4OH).

5) Apparatus : Inner Petrie dish which contains the irrigating solvent (18 cm. in outside diameter and 3 cm. high). Two larger Petrie dishes which enclose the former dish—bottom (22 cm. in outside diameter and 3.5 cm. high), cover (22.5 cm. in inside diameter and 3.3 cm. high).

Procedure (A)[6]—The center of a circular sheet of the filter paper was marked with a pencil. A radius was drawn lightly from the center of the paper to the edge.

A rectangular wick (4×35 mm.) was marked off along this radius. The sample solution (2.5 μL.) was applied to the center of the paper. After the spot had dried, the paper was cut along 3 sides of this rectangle, leaving the center intact, its length was adjusted to 25 mm., and the rectangular piece was bent so that it went through the central point (Fig. 1).

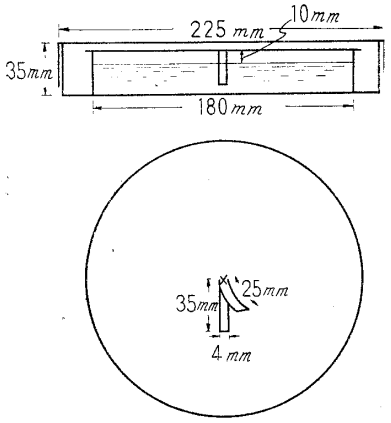

Fig. 1.

Apparatus for Procedure A

The developing solvent was placed in the inner Petrie dish. The spotted filter paper was then placed on top of the inner dish with the paper wick suspended into the center of the developing solvent. The level of the solvent was adjusted so that the distance between the liquid surface and horizontal plane of the paper was exactly 1 cm. This was covered with the largest Petrie dish.

When the solvent front moved a radial distance of about 8 cm., the chromatogram was removed and dried. Ammoniacal $AgNO_3$ solution was sprayed on the paper to detect the samples.

Throughout the developments the temperature was kept at 25°±1°.

Determination of Rf Values—Three diameters were drawn, so that the circle was divided into approximately 60°-angles. On each radius the center of each colored band was marked off carefully and the Rf (i.e. Rr[7]) was calculated along each radius.

$$Rr = \frac{\text{distance of center of each band from center of circle}}{\text{distance of solvent front from center of circle}}$$

Procedure (B)[3]—The center of a circular sheet of the filter paper was marked. Six points were marked with a pencil at the distance of 10, 15, 20, 25, 30, and 35 mm. from the center, so that each radial angle through the points made 60°. A hole (about 1 mm. in diameter) was then pierced at the center. 2.5 μL. of the sample solution was applied to the marked points around the center of the

6) cf. A. Saifer, I. Oreskes : Anal. Chem., **25**, 1539(1953).

7) In this paper, Rr represents Rf value of the case in which the sample is applied at the center of the filter paper. cf. R. H. Müller : Anal. Chem., **26**, 953(1954).

paper. After the spots had dried, the paper was laid across the inner Petrie dish containing the irrigating solvent, just touching the center hole of the paper on top of the filter paper cone immersed in the irrigating solvent. The distance between the liquid surface and horizontal plane of the paper was adjusted to 1 cm. (Fig. 2).

Then the paper was developed, dried and sprayed as mentioned in Procedure A.

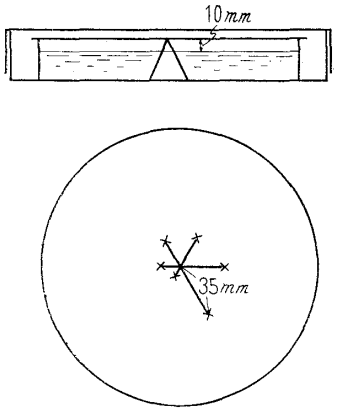

Fig. 2.

Apparatus for Procedure B

Determination of Rf Values—The Rf was calculated according to the following definition :

$$\mathrm{Rf} = \frac{\text{distance of center of each band from initial spot}}{\text{distance of solvent front from initial spot}}$$

Results and Discussion

The experimental results are given in Tables I and II, and Figs. 3~11, where c is the distance of initial spot from the center of the paper, b is the distance of the solvent front from the initial spot, and a is the distance of the sample that moved from the initial spot (Fig. 12). Rf is represented by a/b and Rr represents Rf of the case in which the sample solution is applied at the center of the paper (i.e. $c=0$). Solid lines in Figs. 3~11 represent the values obtained with butanol-acetic acid-water as the solvent and broken lines represent the values obtained with benzene-acetic acid-water as the solvent.

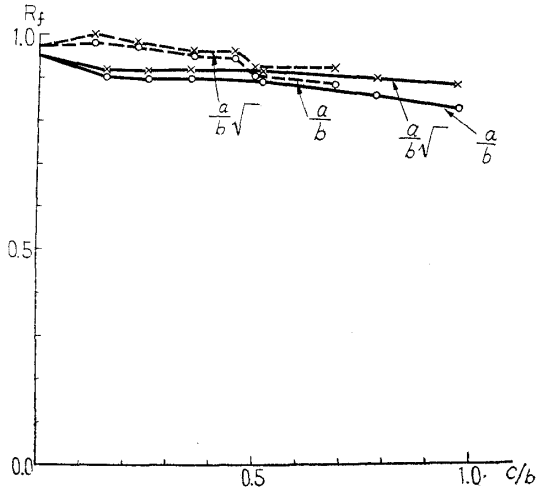

Fig. 3. 1-Phenylpyrazolidin-3-one

——— with BuOH·AcOH·H_2O

---------- with PhH·AcOH·H_2O

The lower line of each group represents observed Rf $\left(\rightarrow \frac{a}{b}\right)$, and the upper line represents Rr calculated from equation (1) $\left(\rightarrow \frac{a}{b}\sqrt{\ }\right)$

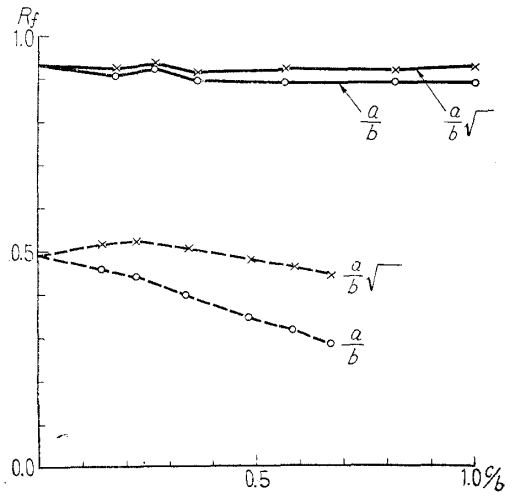

Fig. 4. Catechol

——— with BuOH·AcOH·H_2O

---------- with PhH·AcOH·H_2O

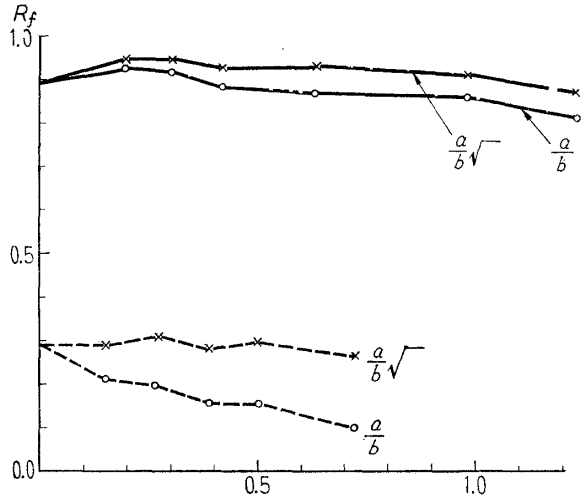

Fig. 5. Hydroquinone
——— with BuOH·AcOH·H_2O
---------- with PhH·AcOH·H_2O

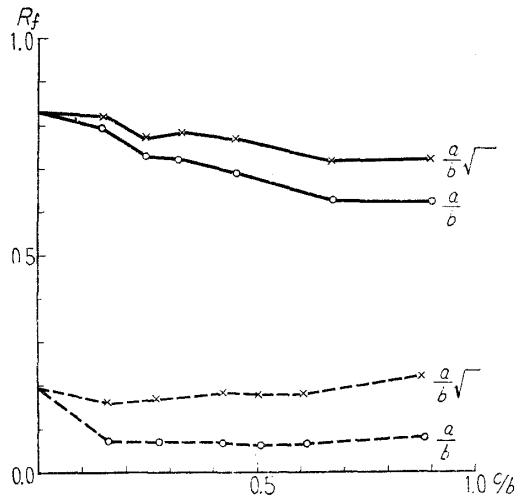

Fig. 6. *p*-Diethylaminoaniline Sulfate
——— with BuOH·AcOH·H_2O
---------- with PhH·AcOH·H_2O

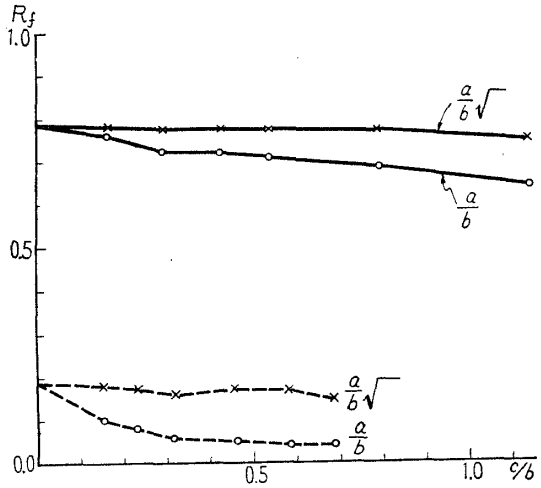

Fig. 7. Pyrogallol
——— with BuOH·AcOH·H_2O
---------- with PhH·AcOH·H_2O

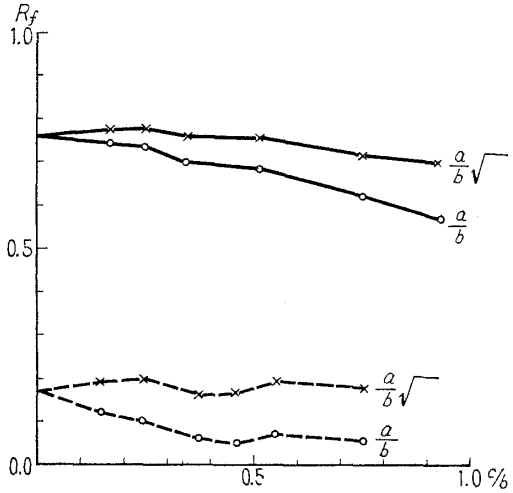

Fig. 8. *p*-N-Methylaminophenol Sulfate
——— with BuOH·AcOH·H_2O
---------- with PhH·AcOH·H_2O

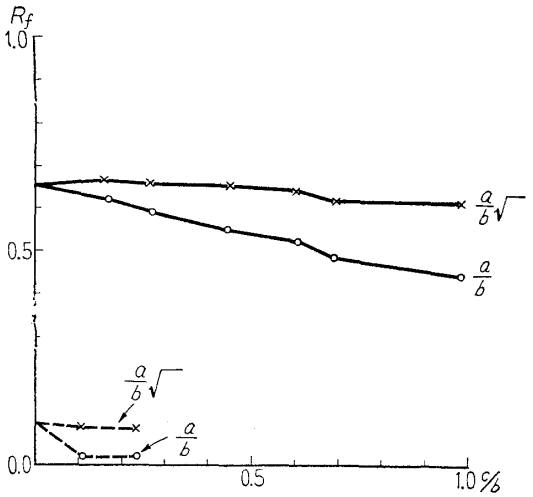

Fig. 9. N-(*p*-Hydroxyphenyl)glycine
——— with BuOH·AcOH·H_2O
---------- with PhH·AcOH·H_2O

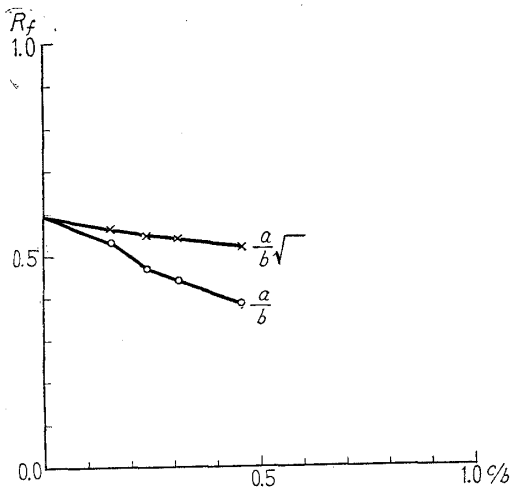

Fig. 10. 2.4-Diaminophenol Sulfate
——— with BuOH·AcOH·H_2O
---------- with PhH·AcOH·H_2O

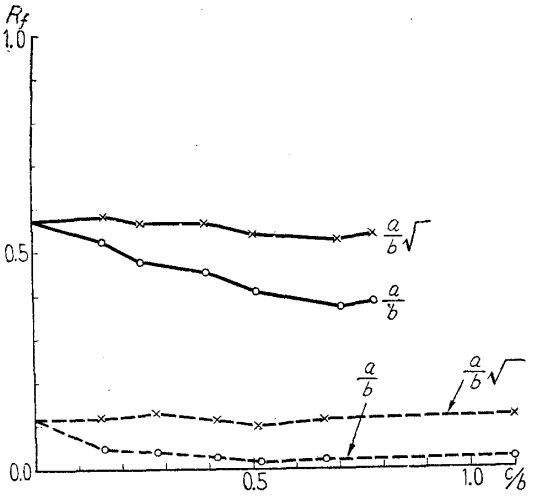

Fig. 11. *p*-Phenylenediamine
———— with BuOH·AcOH·H_2O
---------- with PhH·AcOH·H_2O

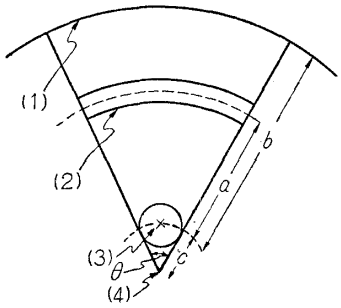

Fig. 12. A schema of Paper Chromatogram
(1) Solvent front
(2) Position of sample developed
(3) Initially spotted position of sample
(4) Center of filter paper

TABLE I. Rr Values of Photographic Developing Agents

Compound	Rr values (25°±1°C)	
	BuOH·AcOH·H_2O (4:1:5)	PhH·AcOH·H_2O (2:2:1)
1-Phenylpyrazolidin-3-one	.95	.96
Catechol	.93	.49
Hydroquinone	.89	.29
p-Diethylaminoanilne sulfate	.83	.20
Pyrogallol	.78	.19
p-(N-Methylamino)phenol sulfate	.75	.17
N-(*p*-Hydroxyphenyl)glycine	.65	.10
2,4-Diaminophenol sulfate	.59	.00
p-Phenylenediamine	.57	.11

As shown in Table II and Figs. 3~11, $a/b(=\mathrm{Rf})$ is markedly influenced by c/b; the larger the ratio of c/b, the smaller the observed values. Remember that Rr value is constant if temperature and some other conditions are held constant. If Rf value can be estimated from observed Rf value, it is convenient for the analysis by this kind of chromatography. To study the relationship between Rr and Rf values, it would be advisable to discuss the schematic model which is illustrated in Fig. 12.

The sample solution is spotted at a distance c from the center of the filter paper to form a small circle. After development, the distance of solvent front from the initial spot becomes b and the distance moved by the sample which forms the arc-band is a. Both edges of the arc are situated on the two lines which contact the circle of initial spot through the center of the paper. The angle between these two contact lines makes θ radian. Then,

$$\frac{\text{moved area of sample}}{\text{area of solvent from initial spot}} \fallingdotseq \frac{\frac{1}{2}\theta\{(a+c)^2-c^2\}}{\frac{1}{2}\theta\{(b+c)^2-c^2\}} = \left(\frac{a}{b}\right)^2 \cdot \frac{1+2c/a}{1+2c/b}$$

$$= (\mathrm{Rf})^2 \cdot \frac{1+2c/a}{1+2c/b}$$

If the sample solution is spotted at the center of the paper (i.e., $c=0$), and if a and b in this case are expressed as a_0 and b_0, then

TABLE II. Observed Rf Values of Photographic Developing Agents
(Applied around the center of the paper)

Compound	BuOH·AcOH·H_2O				PhH·AcOH·H_2O			
	c	*a*	*b*	Rf	*c*	*a*	*b*	Rf
1-Phenylpyrazolidin-3-one	10	55.3	61.0	.912	10	72.5	73.5	.993
	15	53.5	59.0	.908	15	64.3	66.0	.975
	20	53.0	58.0	.914	20	53.6	56.0	.957
	25	44.0	48.5	.907	25	52.4	55.0	.953
	30	34.0	38.0	.883	30	55.0	61.0	.915
	35	31.5	36.5	.863	35	45.5	51.3	.886
Catechol	10	54.2	59.6	.910	10	32.5	70.0	.464
	15	53.0	56.9	.932	15	30.0	68.0	.441
	20	50.0	55.0	.902	20	23.5	59.0	.400
	25	40.5	45.2	.897	25	18.0	51.3	.351
	30	32.5	36.2	.898	30	16.3	51.3	.317
	35	31.5	35.0	.900	35	15.4	53.0	.291
Hydroquinone	10	47.5	50.8	.935	10	13.7	65.3	.210
	15	44.7	48.5	.922	15	11.4	56.6	.201
	20	43.0	48.0	.892	20	8.0	51.5	.155
	25	35.0	40.0	.875	25	7.5	50.0	.150
	30	26.5	30.5	.868	30	4.0	41.3	.097
	35	23.5	28.5	.825	35	3.5	34.8	.100
p-Diethylaminoaniline sulfate	10	51.3	64.7	.794	10	5.5	65.7	.084
	15	44.5	61.0	.730	15	4.2	54.8	.077
	20	44.9	61.4	.732	20	3.5	48.4	.072
	25	38.1	55.0	.692	25	3.0	49.5	.061
	30	27.3	43.8	.623	30	3.0	49.5	.061
	35	24.0	38.8	.620	35	3.0	39.5	.076
Pyrogallol	10	45.0	59.3	.759	10	7.0	67.0	.104
	15	37.0	50.4	.734	15	5.5	67.5	.082
	20	34.4	47.5	.724	20	4.0	64.0	.062
	25	33.5	47.0	.713	25	3.0	54.0	.056
	30	26.5	38.0	.698	30	2.5	51.0	.049
	35	20.0	30.5	.655	35	2.3	51.0	.045
p-N-Methylaminophenol sulfate	10	45.5	61.5	.740	10	9.3	74.0	.125
	15	46.0	62.5	.737	15	6.5	63.3	.102
	20	41.5	59.3	.700	20	3.5	55.0	.063
	25	33.0	48.5	.682	25	3.2	55.3	.058
	30	24.5	40.0	.621	30	4.0	55.0	.073
	35	21.5	37.7	.572	35	2.8	47.0	.056
N-(*p*-Hydroxyphenyl)glycine	10	39.0	62.5	.625	10	1.8	67.2	.027
	15	32.0	54.0	.593	15	1.5	66.5	.023
	20	25.0	45.0	.555				
	25	21.5	41.0	.525				
	30	21.5	43.5	.494				
	35	15.8	35.5	.446				
2,4-Diaminophenol sulfate	10	34.5	65.0	.532	10	0	—	0
	15	30.3	64.3	.471	15	0	—	0
	20	28.8	64.5	.447	20	0	—	0
	25	20.6	52.5	.391	25	0	—	0
p-Phenylenediamine	10	32.0	60.5	.529	10	3.5	65.0	.054
	15	30.0	61.5	.488	15	2.5	54.3	.046
	20	27.0	59.0	.457	20	1.5	49.0	.031
	25	19.5	47.7	.409	25	1.0	49.0	.020
	30	15.9	42.2	.377	30	1.0	45.0	.022
	35	17.0	44.5	.382	35	1.0	35.3	.028

$$\frac{\text{moved area of sample}}{\text{area of solvent from center of circle}} \fallingdotseq \frac{\pi(a_0)^2}{\pi(b_0)^2} = \left(\frac{a_0}{b_0}\right)^2 = (\mathrm{Rr})^2$$

Now, it would not be unnatural to suppose that the ratio of $\frac{\text{moved area of sample}}{\text{area of solvent from initial spot}}$ is constant if some conditions are held equal. According to this hypothesis, the above two ratios should be equal. Then

$$(\mathrm{Rr})^2 = \left(\frac{a}{b}\right)^2 \frac{1+2c/a}{1+2c/b}$$

$$\mathrm{Rr} = \frac{a}{b}\sqrt{\frac{1+2c/a}{1+2c/b}} \quad \cdots\cdots(1)$$

or $$\mathrm{Rr} = \mathrm{Rf}\sqrt{\frac{1+2c/a}{1+2c/b}} \quad \cdots\cdots(1')$$

if $c=0$ and $\mathrm{Rr}=\mathrm{Rf}$

Using the observed Rf values (Table II), each Rr value was estimated by this equation (1) and plotted in Figs. 3~11 where the estimated Rr values are represented by the upper lines of each couple.

The estimated Rr values were in fairly good agreement with observed ones.

Summary

1) Rr values of several photographic developing agents were determined (Table I).

2) When sample is applied around the center of the filter paper, Rr value is estimated by the following equation :

$$\mathrm{Rr} = \mathrm{Rf}\sqrt{\frac{1+2c/a}{1+2c/b}}$$

where Rr represents Rf value of the case in which the sample is applied at the center of the paper, c is the distance of initial spot from the center of the paper, b is the distance of the solvent front from the initial spot, and a is the distance moved by the sample from the initial spot.

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