

REPRODUCIBLE  $R_F$  VALUES IN THIN-LAYER ADSORPTION CHROMATOGRAPHY

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As commonly practised the technique of thin-layer adsorption chromatography is likely to give very variable results. For this reason many users hesitate to quote  $R_F$  values as they would in the case of paper chromatography. Though it is not essential for  $R_F$  values to be constant, there are many occasions when reproducibility is a distinct advantage.

The factors affecting  $R_F$  values in a given system have been investigated by many workers. TRUTER<sup>1</sup>, STAHL<sup>2</sup> and RANDEATH<sup>3</sup> have reviewed most of this work. Further investigations have been recently reported<sup>4-9</sup>. These studies, however, fail to account for a number of variations in  $R_F$  values experienced in this laboratory. The subject has therefore been re-examined, in order to develop a practical method of obtaining reproducible  $R_F$  values.

TABLE I

AZO COMPOUNDS USED IN THE INVESTIGATION

<i>Abbreviation</i>	<i>Compound</i>
AB	<i>trans</i> -Azobenzene
DAB	<i>p</i> -Dimethylamino-azobenzene*
S <sub>3</sub>	Sudan III (British Drug Houses Ltd., London)
AAB	<i>p</i> -Amino-azobenzene
HAB	<i>p</i> -Hydroxy-azobenzene

\* Also known as Dimethyl Yellow and as Butter Yellow.

This whole investigation has been carried out almost exclusively with one adsorbent-solute-solvent system; the adsorbent chosen was Kieselgel G (Merck\*), since a standard product in common use was desirable; the solute was a mixture of well defined coloured azo-compounds (see Table I) and the solvent was benzene, which is readily available in a high state of purity. Since the work was begun, HÖNEGGER<sup>4,5</sup> has published results with a very similar system, with which some comparisons can be made.

\* Unless otherwise stated, all results were obtained with one batch (No. T 61123).

## PRINCIPAL FACTORS STUDIED

Three factors especially have been investigated by the author; these are:

(A) The activity of the adsorbent at the time of development and the effect on activity of the relative humidity of the atmosphere.

(B) The pre-adsorption of solvent vapour by the adsorbent before the liquid phase reaches it during development of the chromatogram.

(C) The variation in the ratio of the liquid to solid phase in the direction of the development.

There has been very little systematic study of these three factors reported in the literature. Recent studies by GEISS *et al.*<sup>7,10</sup> on the effect of relative humidity on alumina-coated plates was not apparently extended to silica gel, but it was sufficient to show that the factor could be important where water-free solvent systems are used, as other workers<sup>4,11</sup> have pointed out.

(A) *Adsorbent activity and relative humidity*

It is known that the activity of silica gel, like that of alumina, depends very much on its content of loosely bound water<sup>11</sup>. In column chromatography a certain proportion of water is added to fully activated silica gel to obtain adsorbent of the required activity; this is clearly not practical in thin-layer chromatography.

It is not often realised that much of the activity, resulting from heating of a silica gel chromatoplate at 10°, is lost if subsequent spotting is carried out in the normal laboratory atmosphere.

We found that more than half the total amount of moisture adsorbed at equilibrium (in an atmosphere of about 50% relative humidity) was taken up within about three minutes, and that even breathing on a plate during the spotting process could markedly affect  $R_F$  values. The effect of relative humidity was therefore carefully examined in this laboratory as a result of these initial findings.

With fully active plates we found that "tailing" of spots was considerable; this "tailing" is said by KLEIN<sup>12</sup> to be due to an increase in "site energy band width" with increase in activity of the silica. KLEIN found the chromatographic resolving power (on columns) to be optimum at a certain intermediate activity, and that the best way of achieving such activity was by equilibration of the gel with an atmosphere of a definite relative humidity; his findings are applicable to thin-layer chromatography.

With plates equilibrated at humidities much above 60% we observed that "tailing" again became noticeable; this could be due to the amount of water adsorbed being sufficient to fill many of the fine pores and thus to reduce seriously the available surface area of the gel. Over the range 15–58% humidity no significant variation in tailing was noticed in the particular system studied; on the other hand, there were considerable variations in both  $R_F$  values and relative  $R_F$  values (see Table II).

We have shown that strict control of the relative humidity, and hence of activity, greatly improves the reproducibility of  $R_F$  values. This control is achieved by equilibration of the chromatoplate with an atmosphere of constant relative humidity immediately before development in an S-chamber\*. The relative humidities above certain saturated salt solutions<sup>16</sup> are sufficiently constant.

\* This was described by E. STAHL<sup>2</sup>. Simple versions have recently been described by DAVIES<sup>13</sup>, WASICKY<sup>14</sup> and JÄNCHEN<sup>15</sup>.

The results in Table II show how  $R_F$  values in benzene vary with the relative humidity. It is clear that precise control of humidity is necessary, if  $R_F$  values are to be reproducible to within  $\pm 0.01$  units.

As the activity of the silica gel decreases the separating power decreases (*i.e.*  $R_F$  values tend to become less different from one another). Hence relative  $R_F$  values should also show smaller differences from one another: see Table II. It is concluded that,

TABLE II

VARIATION OF  $R_F$  AND  $R_x$  VALUES WITH RELATIVE HUMIDITY

Substances, azo-compounds as in Table I; Kieselgel G (Merck), *ca.* 200  $\mu$ ; "AR" benzene, dried over  $\text{CaSO}_4$ ; S-chamber; 10.0 cm; "overrun" 15 min; temperature of humidity chambers = 20°; temperature during developments =  $22 \pm 2^\circ$ . The above results are in most cases the mean of duplicate experiments.

Relative humidity <sup>16</sup>	Salt solution	$R_F$ Values					$R_x$ Values*				
		AB	DAB	S <sub>3</sub>	AAB	HAB	AB	DAB	S <sub>3</sub>	AAB	HAB
0 %	—	0.81	0-0.12	0-0.05	0.05	0.04	—	100	—	—	—
15 %	LiCl · H <sub>2</sub> O	0.90	0.47	0.25	0.13	0.07	191	100	53	27	15
32 %	CaCl <sub>2</sub> · 6H <sub>2</sub> O	0.94	0.62	0.37	0.19	0.09	150	100	59	30	14
52 %	Na <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> · 2H <sub>2</sub> O	0.97	0.74	0.57	0.26	0.10	131	100	77	35	13
58 %	NaBr · 2H <sub>2</sub> O	0.974	0.777	0.632	0.329	0.121	125	100	81.0	42.2	15.6
78 %	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> · 5H <sub>2</sub> O	1.00	0.94	0.88	0.58	0.24	106	100	93	61	25
93 %	Na <sub>2</sub> SO <sub>4</sub> · 10H <sub>2</sub> O	1.00	1.00	1.00	1.00	0.90	100	100	100	100	90
100 %	H <sub>2</sub> O	1.00	1.00	1.00	1.00	1.00	100	100	100	100	100

\*  $R_x$  values =  $R_F$  values relative to DAB = 100.

unless the activity is kept constant, there is little advantage in using "markers", provided that the solvent front can be accurately located.

The rate at which the layer of silica gel reaches equilibrium with the moisture of the ambient atmosphere will depend on its thickness, as HONEGGER<sup>5</sup> has pointed out. No systematic study of the time required has yet been carried out. However, HONEGGER's figures suggest that 72 h are necessary for 3 mm layers; for normal 0.25 mm layers our results indicate that 16 h are quite sufficient. The equilibration of plates in constant humidity chambers can be undertaken before spotting (the method is described in the experimental section).

(B) *Pre-adsorption of solvent vapour by adsorbent*

It was found in this laboratory that the amount of benzene adsorbed by silica gel from air saturated with benzene vapour is a considerable proportion of that required to "wet" the gel. It follows that, if a chromatoplate is equilibrated with a saturated benzene atmosphere before development in liquid benzene, then  $R_F$  values should be lower than normal. This is because less mobile phase actually travels up the plate to wet it, part of the necessary benzene being already in the pores of the gel.

When chromatoplates were left in a benzene saturated atmosphere before development, in the manner described by WOLLISH *et al.*<sup>17</sup> and by TRUTER<sup>1</sup>,  $R_F$  values were found to be fairly reproducible, but were all lower than with development in an S-chamber (see Table III). It was also observed that the  $R_F$  values under the two conditions of development bore a nearly constant ratio to one another. The small

TABLE III

EFFECT OF PRE-EQUILIBRATION IN SOLVENT VAPOUR ON  $R_F$  VALUESSubstances as in Table I; conditions as for Table II; but relative humidity *ca.* 47%.

Compound	On pre-equilibrated plates*		Normal plates in S-chamber**		Ratio of $R_F$ values in the 2 systems§
	$R_F$ values	$R_x$ values***	$R_F$ values	$R_x$ values	
DAB	0.500	100	0.712	100	0.702
S <sub>3</sub>	0.363	72	0.509	71	0.673
AAB	0.170	34	0.258	36	0.659
HAB	0.065	13	0.099	14	0.656

\* Mean of 4 experiments; see experimental section for description of the method.

\*\* These  $R_F$  values are those estimated from values in Table II for silica gel of the same activity. The assumption is made that, if  $R_x$  values are the same, the activity of the silica is the same in the two systems.

\*\*\* See Table II.

§ The ratios of HONEGGER's<sup>5</sup> results in his "SN" and "SK" tanks on 250 $\mu$  layers of the same adsorbent (dried in air 72 h) are similar, 0.708 for Butter Yellow, 0.667 for Sudan Red G and 0.632 for Indophenol. His "SK" tank was similar to the "SN" (normal S-chamber) except that the tank was lined with filter paper soaked in benzene beforehand.

decrease in this ratio with decreasing  $R_F$  suggests that a little pre-adsorption of solvent vapour occurs in the S-chamber. With the solvent front moving more slowly as it rises up the plate, there will be more time in the higher regions of the layer for solvent vapour to diffuse ahead of the solvent front. Such a picture fits the observed results in Table III.

With an S-chamber the layer is not initially placed in contact with an atmosphere partly or wholly saturated with solvent vapour; moreover, the volume of the atmosphere in such a chamber is not large in comparison with the volume of adsorbent. Such conditions result in pre-adsorption of solvent vapour being at a minimum. Consequently, in this S-chamber  $R_F$  values are not only higher, but are also more reproducible and more nearly "absolute" than those obtained in ordinary tanks.

The adsorption of benzene vapour by a  $\sim 0.3$  mm layer of silica gel under normal chromatographic conditions was measured (see experimental section). Approximately 50% of the total benzene adsorbed at equilibrium was taken up within 30 min, which is of the same order of duration as a normal chromatogram. It is concluded that the average chromatogram in a simple rectangular tank is developed under conditions under which a variable amount of solvent vapour is being adsorbed by the gel layer. It is most probable that the upper surface of a layer will adsorb faster than the under surface and this fact could help in explaining some conflicting results in the literature with regard to the effect of layer thickness on  $R_F$  values.

### (C) Ratio of liquid to solid phase on chromatoplates

It is normal practice to allow the solvent to ascend the chromatoplate freely and to remove the plate from the developing chamber after a certain time and before the solvent front reaches the top of the adsorbent layer. When a plate, during development in a hydrocarbon, is viewed by transmitted light it will be seen that the transparency falls off towards the solvent front. This effect has been shown to be due to a decreasing

ratio of liquid to solid phase. It is equally apparent with horizontal as with vertical development.

A developed chromatogram was left in the solvent after the front had reached the top of the adsorbent film; two things were observed. Firstly, the transparency soon became uniform and, secondly,  $R_F$  values increased a little (see Table IV). The increase of  $R_F$  did not continue once the solvent distribution had become uniform over the plate, unless solvent was allowed to evaporate from the top of the plate. This way of leaving the plate in the solvent after the solvent front has reached the top of the layer will be referred to as "overrunning"; thus it might be said, for instance, that a given chromatogram was "overrun" 15 min.

TABLE IV

EFFECT OF "OVERRUNNING" ON  $R_F$  VALUES

Substances as in Table I; same conditions as for Table II, but plates equilibrated 24 h over saturated NaBr·2H<sub>2</sub>O at 20° before use.

Technique	Solvent front (cm)	Total time (min)	$R_F$ values					$R_x$ values*				
			AB	DAB	S <sub>3</sub>	AAB	HAB	AB	DAB	S <sub>3</sub>	AABHAB	
Normal	10.0	33	0.95	0.72	0.57	0.29	0.11	131	100	79	40	14
Overrun 15 min	10.0	47	0.975	0.775	0.625	0.325	0.11	125	100	80.5	42	15
Normal	13.1	47	0.95	0.71	0.57	0.30	0.10	134	100	79.5	42	14
Overrun 15 min	13.1	69	0.96	0.76	0.61	0.31	0.11	128	100	81	41	15

\* See Table II.

This "overrunning" technique has the following advantages:

(a)  $R_F$  values across the plate become a little more constant; this makes comparisons with control substances more reliable.

(b)  $R_F$  values in single component solvents become independent of the distance from origin to solvent front (see Table IV).

(c) The distance from origin to solvent front is accurately pre-determined. This is important with mixed solvents where frontal analysis of the solvent occurs, resulting in a concentration gradient up the plate. (BRENNER *et al.*<sup>9</sup> have pointed out that, for reproducible  $R_F$  values to be obtained under these conditions, the ratio

$$\frac{\text{Distance from "immersion line" to origin}}{\text{Distance from "immersion line" to solvent front}}$$

must be constant.)

(d) The  $R_F$  values become more accurately comparable with retention volumes on columns. This follows from the constancy of the ratio of liquid to solid phase up the plate and consequently of the term  $A_L/A_S$  in the classical MARTIN AND SYNGE equations.

The time necessary for the ratio of liquid to solid phase to become constant may vary with solvent and distance of travel. (15 min were found to be enough for benzene moving 10 cm). The overrunning must not be extended unnecessarily, since diffusion

of spots continues after the solvent has ceased advancing. A general disadvantage is seen in the fact that the time of development is increased with little, if any, gain in resolution.

This overrunning technique has been incorporated in the general chromatographic procedure employed in this work (see experimental section).

#### OTHER FACTORS AFFECTING $R_F$ VALUES

In the light of the three main factors discussed above other better known factors were briefly examined:

##### *Liquid-vapour equilibrium in solvent chamber*

It is clear from the published literature<sup>2, 4, 9, 15</sup> why this is an important factor. Very good equilibrium is necessary, especially with volatile solvents, and with the S-chamber this is rapidly obtained only where it is needed, *i.e.* in the immediate neighbourhood of the liquid phase on the adsorbent. Because of this and because of the minimum pre-adsorption of solvent vapour, as explained earlier, this type of developing vessel has been used throughout this work, except where it is stated otherwise.

##### *Position of origin and distance of travel of solvent front*

This has been included in the discussion of factor C above (see also Table IV).

##### *Temperature*

The  $R_F$  values at 11° were found to be lower than at 22° (see Table V). It was also noticed that the activity, as measured by relative  $R_F$  values, was a little greater at the lower temperature. The results suggest that control of temperature is necessary within a range of about two degrees, in the case of benzene.

TABLE V

#### EFFECT OF TEMPERATURE ON $R_F$ VALUES

Substances as in Table I; same conditions as for Table II, except for temperature; 2 experiments at each temperature.

Temperature	$R_F$ values					$R_x$ values*				
	AB	DAB	S <sub>3</sub>	AAB	HAB	AB	DAB	S <sub>3</sub>	AAB	HAB
21.5°	0.975	0.77	0.62	0.325	0.12	126	100	80	41.5	15
10.5°	0.975	0.745	0.57	0.275	0.10	130	100	76	36.5	13
$\Delta R_F/\Delta t$	0.000	0.0023	0.0045	0.0045	0.0018	—	—	—	—	—

\* See Table II.

##### *Layer thickness*

This factor has been extensively studied by others. A small increase in  $R_F$  with increase of layer thickness over the range 0.25 to 1 mm was noted by PATAKI AND KELLER<sup>8</sup>, but confirmed by HONEGGER<sup>5</sup> under certain conditions only. In the light of our present studies it seems that HONEGGER's results in the normal S-chamber ("SN" tank) on plates exposed to the atmosphere for 72 h before use are the most reliable; under these

particular conditions no variation of  $R_F$  with layer thickness over the range 0.25 to 3 mm was reported. We have found no variation over the range 0.1–0.5 mm in the few tests we have done here.

*Amount of material spotted onto plate*

No variation of  $R_F$  with amount of material applied was observed for up to only 10  $\mu\text{g}$  per component. With appreciably higher loads  $R_F$  values were found to increase a little.

*Angle of plane of plate with vertical*

The two angles, 10° and 90° to the vertical, were studied. With benzene the results were found to be identical. The time of development was also very similar in each case.

*Nature of adsorbent*

The structure and uniformity of silica gel will depend upon the method of preparation. The results quoted here, except those in Table VI, were all on one batch of Merck silica gel G. It was therefore pertinent to see if different batches of this adsorbent would

TABLE VI

RESULTS WITH DIFFERENT BATCHES OF KIESELGEL G. (MERCK)

Substances as in Table I; conditions as for Table II, but relative humidity = 58% in each case (24 h over saturated  $\text{NaBr} \cdot 2\text{H}_2\text{O}$ ); temperature =  $22 \pm 1^\circ$ . Results for batch T 61123 taken from Table VII. Results for batches T 63272 and 387435 were the averages from 2 experiments.

	T 61123		T 63272		387435	
	$R_F$	$R_x$	$R_F$	$R_x$	$R_F$	$R_x$
AB	0.974	124.7	0.980	130.5	0.985	123.5
DAB	0.777	100	0.750	100	0.795	100
S <sub>3</sub>	0.632	81.0	0.590	78	0.665	83
AAB	0.329	42.2	0.315	41.5	0.355	44.5
HAB	0.121	15.6	0.125	16.5	0.135	16.5
Time for 10 cm solvent travel*	33 min		20 min		18 min	

\* Without "overrunning" time.

give similar results. Three different batches were examined by the general procedure described here. Fairly small differences in  $R_F$  and relative  $R_F$  values were observed (see Table VI). A large variation in time of development was, however, noted; this is due to particle size differences. Thus it is clear that the manufacture of silica gel for thin layer chromatography must be carefully standardised, before  $R_F$  values can be quoted with real confidence.

STANDARD DEVIATION RESULTS

Table VII gives the results of  $R_F$  measurements on 10 chromatograms on one batch of adsorbent. The general procedure described in the experimental section was used; this procedure is designed to minimise the effect of all the variable factors discussed in this report.

TABLE VII

REPRODUCIBILITY OF  $R_F$  VALUES IN 10 EXPERIMENTS

Substances as in Table I; same conditions as for Table II, but relative humidity = 58% in each case; temperature =  $22 \pm 2^\circ$ .

	<i>AB</i>	<i>DAB</i>	<i>S<sub>3</sub></i>	<i>AAB</i>	<i>HAB</i>
Mean $R_F$ value	0.974	0.777	0.632	0.329	0.121
Maximum value	0.98	0.79	0.65	0.35	0.14
Minimum value	0.96	0.76	0.62	0.30	0.10
Mid range	0.970	0.775	0.635	0.325	0.120
Standard deviation	0.006	0.010	0.010	0.016	0.013
Mean $R_c$ value*	124.7	100	81.0	42.2	15.6

\* See Table II.

The standard deviation (estimated from the range) lay within the limits 0.006 (for  $R_F = 0.97$ ) and 0.016 (for  $R_F = 0.33$ ). It was greater for spots of intermediate  $R_F$  value. The temperature variation ( $\pm 2^\circ$ ) will account for some of the deviation.

## MULTIPLE DEVELOPMENT

Table VIII gives the results of an experiment on double development in one direction. The theoretical  $R_F$  values in column 3 were obtained from those in column 1 (1st development) using the equation of STARKA AND HAMPL<sup>18</sup>:

$$R_{F_2} = 2 R_{F_1} - R_{F_1}^2.$$

The general procedure (see experimental section) was used, and between developments the plate was dried in an oven, and then replaced in the constant humidity tank for 24 h. There was good agreement between experiment and theory.

TABLE VIII

## MULTIPLE DEVELOPMENT IN A SINGLE SOLVENT

Substances as in Table I; conditions as for Table II, but relative humidity = 58% in each case (24 h over saturated  $\text{NaBr} \cdot 2\text{H}_2\text{O}$ ). One experiment.

Substance	$R_F$ value		
	1st development	2nd development	Calculated
AB	0.97	1.00	0.999
DAB	0.78	0.95	0.950
<i>S<sub>3</sub></i>	0.62	0.86	0.855
AAB	0.32	0.54	0.537
HAB	0.12	0.22	0.221

## PREDICTION OF RETENTION VOLUMES ON COLUMNS

When it is necessary to separate macro amounts of material, it is clearly useful to be able to scale up thin-layer chromatograms on to columns. The prediction of retention



volumes on columns, from thin-layer results, will in the first place require accurate  $R_F$  values. These values must not only be reproducible, but must also be obtained under conditions that strictly correspond with those in column chromatography. The procedure described here should make it possible to obtain the absolute  $R_F$  values required.

A study of the exact relationship between thin-layer  $R_F$  values and retention volumes on columns of similar adsorbent is now being undertaken here.

#### EXPERIMENTAL

A simple method of obtaining plates of reproducible activity was designed. The use of the S-chamber is essential in order to minimise changes of activity of the layer during the development of the chromatogram (*e.g.* by gain by the layer of moisture from the atmosphere of the tank or vice versa). The use of the S-chamber also minimises pre-adsorption of solvent vapour by the layer. Great care must be taken to keep the activity of the plate unchanged after removal of the plate from the constant humidity chamber.

#### *General procedure for obtaining reproducible $R_F$ values*

The plates were coated with a layer of Kieselgel G (Merck) approx. 200  $\mu$  thick, using a mechanical spreader (Desaga) in the manner described by STAHL<sup>2</sup>. The plates were left to dry in a clean moderately dry atmosphere overnight or longer, and were then prepared for use in the S-chamber by removal of margins of the layer from three sides. The margins were of such width that the three spacing strips of the S-chamber\* were separated by several millimeters from the edges of the adsorbent layer. A transverse channel several millimeters wide was also cut from the layer to prevent the solvent front moving beyond this position. Such plates were then left for at least 15 h in a constant humidity vessel maintained at 20°. This vessel was a normal solvent tank (about 7 × 23 × 23 cm), lined with thick filter paper and containing about 50 ml of a saturated aqueous solution of sodium bromide (various saturated salt solutions<sup>16</sup> may be used to obtain different relative humidities, but particularly satisfactory results were obtained with NaBr, which gives 58 % relative humidity, see Table II). Several short lengths of glass tubing in the bottom of the vessel served to keep the plates above the level of the salt solution. For spotting on of the substances a plate was removed from the constant humidity chamber and all adsorbent above the origin immediately covered by a clean glass plate in close contact with the layer. When spotting was complete, the temporary glass cover was quickly replaced by the S-chamber cover plate and the chromatogram immediately developed in the solvent concerned (preferably at 20 ± 1°). The time the solvent reached the top of the layer was noted and the plate left for a further 15 min in the solvent. After development in this way the plate was laid flat and the coverplate removed to allow the solvent to evaporate evenly from the layer.

\* The S-chamber used throughout this work was similar to that of DAVIES<sup>14</sup>, but, in place of a single piece of glass rod as spacer, three strips of glass, approx. 1.5 × 10 mm in cross-section, were employed. These were stuck to a 20 × 20 cm piece of glass by means of epoxy-resin and ground flat to give a good seal on three sides, when placed over the coated plate.

### *Pre-equilibration of a plate in solvent vapour*

The plate was prepared, spotted and placed in an ordinary tank fitted with a good lid, through which passed the stem of a tap funnel reaching the bottom centre of the tank (see Fig. 1). The tank was lined with thick filter paper and about 25 ml of solvent placed in it: the plate was kept above the level of the solvent by means of a small platform. After 24 h about 100 ml more solvent were added from the funnel to develop the chromatogram without disturbing the equilibrium.

### *Method of horizontal development*

The general procedure, as described above, was used, but development was in a horizontal tank similar to that described by BRENNER *et al.*<sup>19</sup> Great care was taken to reduce solvent vapour loss to a minimum.

### *Measurement of adsorption of benzene vapour by silica gel*

The inside of a wide-neck 100 ml flask was coated with a layer of Kieselgel G *ca.* 0.3 mm thick. The gel was activated 1 h at 110° and weighed *in situ*. The coated flask was placed inside a tank with a saturated benzene atmosphere for a specified time, then re-stoppered and re-weighed. The uptake of benzene was found to reach a maximum of 0.595 ml per g at 20° after about 24 h. This figure is slightly greater than that

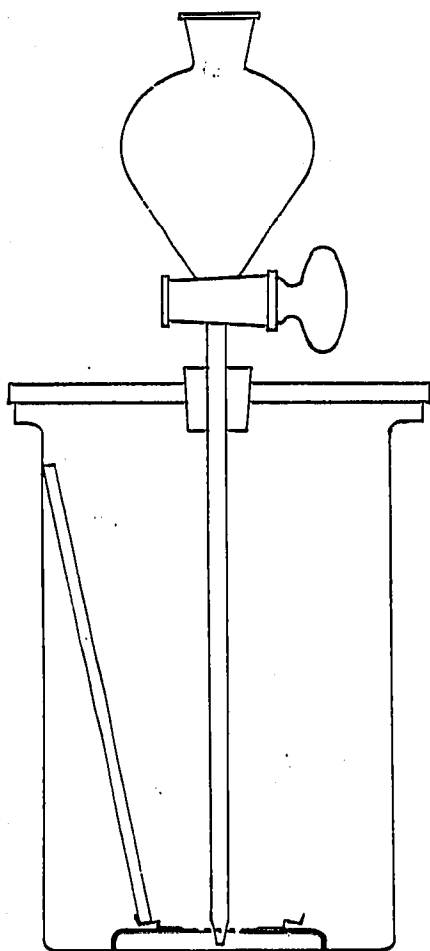


Fig. 1. Tank for pre-equilibration of plate in solvent vapour before development.

for the "total pore volume", 0.54 ml per g, obtained by the direct titration method of MOTT LAU AND FISHER<sup>20</sup>. The small difference could be due to more complete filling of the narrower pores in this vapour adsorption method.

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## SUMMARY

The factors affecting the reproducibility of  $R_F$  values in adsorption chromatography on thin layers of a given silica gel have been examined. It has been shown that the important factors are method of development and adsorbent activity, which is controlled by ambient relative humidity. Distance of solvent travel and temperature have a moderate effect. Layer thickness and angle of plane of plates appear to have little effect.

A procedure is described for obtaining reproducible  $R_F$  values and "absolute"  $R_F$  values.

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