STUDIES ON THE THIN-LAYER CHROMATOGRAPHY OF SOME INDANOLS

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SUMMARY

Indanols have been chromatographed on thin layers of cellulose impregnated with ethyl oleate as the stationary phase, and aqueous ethanol as the mobile phase. The R_F values are more reproducible on layers prepared by incorporating the impregnant into the solvent used to slurry the support before coating the layers. Supports impregnated with the stationary phase by chromatographing the impregnant into the prepared layers are less reproducible. R_F values determined from chromatograms run in saturation chambers are more reproducible than those determined from chromatograms run in conventional tanks. Results obtained from precoated layers which were impregnated with the stationary phase by the chromatographic method are more reproducible than the results obtained from laboratory prepared plates which were impregnated with the stationary phase and chromatographed under comparable conditions.

Some effects of the molecular structure of the indanols on their chromatographic behaviour are discussed.

INTRODUCTION

While the chromatographic behaviour of a compound is dependent upon its molecular structure, the reproducibility of this behaviour is governed by the extra-molecular factors[1] of the system in which it is chromatographed.

In partition chromatography, the R_F value is related to the partition coefficient, α, by the equation[2]:

$$\alpha = \frac{A_M}{A_S}\left(\frac{1}{R_F} - 1\right) \tag{1}$$

where A_M/A_S is the ratio of the thickness of the mobile phase to that of the stationary phase.

In reversed phase systems therefore it is essential that the manner of introducing the impregnant into the support is one which will give a constant A_S value. In a previous paper[3], we reviewed the methods available for preparing reversed phase thin-layer chromatoplates and we concluded that the best reproducibility was obtained when the impregnant was incorporated into the solvent used to slurry the

support. When this method was used to impregnate cellulose with tri-*n*-butyl phosphate, the distribution of the impregnant on the chromatoplates was found to be reasonably constant[4,5].

A fairly recent development in thin-layer chromatography is the commercial availability of pre-coated layers. So far as the author is aware, no attempt has been made to use these layers in reversed phase systems. One obvious disadvantage in using such layers for reversed phase thin-layer chromatography is that they cannot be impregnated by the slurry process, but they must be impregnated by other methods[3].

In the work described here, pre-coated cellulose layers were impregnated by a chromatographic method. The R_F values obtained from these layers have been compared with those determined from laboratory coated layers, some of which were impregnated by the slurry method and some of which were impregnated by the chromatographic method. For each type of plate, results were obtained by developing the chromatograms in a saturation chamber and also in a conventional thin-layer chromatographic tank.

The compounds used in this investigation were substituted indanols.

Gas–liquid chromatography[7] has been used successfully for the separation of these compounds. GREEN *et al.*[8,9] reported the separation of 2-indanol[8] and 5-indanol[9] from other phenolic compounds but not from other indanols. The system ethyl oleate–aqueous ethanol, used by GREEN *et al.*[8] in the separation of phenols by reversed phase paper chromatography has been modified and used by the author and his co-worker in the reversed phase thin-layer separation of phenols[3,10–12]. Because of the observed migration of 2-indanol in the ethyl oleate–aqueous ethanol system on paper[2], it was decided to investigate the thin-layer chromatography of 15 substituted indanols using ethyl oleate as the stationary phase and aqueous ethanol as the mobile phase[3,10–12].

EXPERIMENTAL

Ethyl oleate (H and W reagent grade) was dissolved in diethyl ether to form a solution of 0.02 *M* concentration.

Aqueous ethanol (37.5%) was prepared as previously described.[3,10–12]

The preparation of the layers

(a) By the slurry method

Cellulose (MN 300) (15 g) was slurried with the ethyl oleate solution (70 ml) and the slurry was used to coat the plates.

(b) By the chromatographic method

(i) *Laboratory prepared layers*. Cellulose (MN 300) (15 g) was mixed with water (90 ml) to give a homogeneous slurry which was used to coat glass plates. The layers were allowed to air dry for 24 h. The dried layers were placed in a double saturation chamber[6] and the ethereal solution of the impregnant (40 ml) was added. This solution was allowed to rise up the layers. The layers were left in contact with the impregnant solution for half an hour longer than the time ($1\frac{1}{2}$ h) taken for the solvent front to reach the top of the plate.

(ii) *Pre-coated layers*. MN Polygram Cell 300 layers, from which a band of ad-

sorbent 1.5 cm in width had been removed from each of two parallel sides, were impregnated by the chromatographic method described above. It was found that the solvent front took 2 h to reach the top of the layers so that the layers were in contact with the impregnant solution for 2½ h.

The application of the compounds and the running of the chromatograms

Solutions of the indanols (1 µl of 0.25% w/v solutions in cyclohexane) were applied rapidly and simultaneously[6] to the layers. Some of the chromatograms were developed in our double saturation chamber[6]. The others were developed in a normal (Shandon) TLC tank. The eluent was added to both the saturation chamber and to the tank after the plates had been placed in position.

TABLE I

SYSTEMS INVESTIGATED AND KEY TO TABLES II AND III

System No.	*Substrate support*	*Method of impregnation*	*Manner of elution*
1	MN 300 Cellulose	Slurry	Saturation chamber
2	MN 300 Cellulose	Slurry	Tank
3	MN 300 Cellulose	Chromatography	Saturation chamber
4	MN 300 Cellulose	Chromatography	Tank
5	MN-Polygram Cell 300	Chromatography	Saturation chamber
6	MN-Polygram Cell 300	Chromatography	Tank

The eluent front was allowed to rise 13.5 ± 0.5 cm up the plates.

The indanols were identified as yellow spots on a purple background by spraying the chromatograms with the alkaline potassium permanganate we have used previously to identify phenols[13].

TABLE II

REPRODUCIBILITY OF R_F VALUES OF STANDARD COMPOUND (4-INDANOL)

	Data for system No.					
	1	*2*	*3*	*4*	*5*	*6*
Total number of R_F values determined	390	390	390	390	102	102
Mean R_F value	0.69	0.54	0.75	0.65	0.62	0.54
Reproducibility (R_F units)	±0.02	±0.05	±0.05	±0.07	±0.03	±0.04
Standard deviation ($\sigma \times 10^{-3}$)	0.91	2.87	2.87	3.16	1.81	1.92

RESULTS

These are quoted in Tables II and III.

DISCUSSION

(1) *The reproducibility of R_F values for 4-indanol*

The six systems studied are shown in Table I.

TABLE III

MEAN R_F VALUES OF SUBSTITUTED INDANOLS

Key	Indanol	R_F values in system No.					
		1	2	3	4	5	6
1	4-Indanol	0.69	0.54	0.75	0.65	0.62	0.54
2	1-Methyl-4-indanol	0.53	0.48	0.68	0.55	0.53	0.42
3	2-Methyl-4-indanol	0.50	0.46	0.66	0.55	0.54	0.41
4	5-Methyl-4-indanol	0.44	0.40	0.59	0.50	0.50	0.37
5	6-Methyl-4-indanol	0.51	0.46	0.64	0.54	0.55	0.42
6	7-Methyl-4-indanol	0.54	0.48	0.66	0.55	0.57	0.45
7	7-*tert.*-Butyl-4-indanol	0.22	0.18	0.36	0.26	0.33	0.16
8	5,7-Di-*tert.*-butyl-4-indanol	0.02	0.02	0.03	0.02	0.00	0.00
9	5-Indanol	0.65	0.57	0.74	0.65	0.67	0.53
10	1-Methyl-5-indanol	0.53	0.46	0.66	0.55	0.56	0.42
11	3-Methyl-5-indanol	0.54	0.48	0.67	0.56	0.56	0.43
12	4-Methyl-5-indanol	0.47	0.42	0.62	0.52	0.51	0.38
13	6-Methyl-5-indanol	0.46	0.41	0.61	0.50	0.50	0.37
14	7-Methyl-5-indanol	0.56	0.46	0.66	0.56	0.56	0.44
15	6-*tert.*-Butyl-5-indanol	0.06	0.06	0.14	0.09	0.11	0.04

From Table II, it can be seen that in systems where the chromatograms were run in saturation chambers (systems 1, 3, and 5), the R_F values are higher than the R_F values obtained for the corresponding layers which were run in conventional tanks (systems 2,4, and 6). This lowering of the R_F values under conditions of tank elution is to be expected because no precautions were taken to pre-equilibrate the tank atmosphere with the eluent vapours. Under these conditions, evaporation of the more volatile component of the mobile phase, ethanol, will occur both from the surface of the eluent in the tank and from the surface of the layer. Thus the eluent rising up the plate is richer in water than is the eluent originally placed in the tank. This view is supported by results from our previous work[3] when we showed that the R_F values of phenols chromatographed in the ethyl oleate–aqueous ethanol system were dependent upon the concentration of ethanol in the mobile phase; the higher the ethanol content of the mobile phase, the higher were the R_F values. In the saturation chamber, almost instantaneous saturation of the small dead volume of the saturation chamber occurs and hence there is little, if any, depletion of the more volatile component from the mobile phase. These results emphasise the need to saturate the atmosphere surrounding the chromatogram in order to minimise the loss of the more volatile components from the eluent system.

In addition to the lowering of the R_F values of the compound under conditions of tank elution, the table shows that the reproducibilities of its R_F values are also worse than those obtained for the corresponding layers which have been eluted in the saturation chamber. This is to be expected because the evaporation of ethanol from the surface of the layers will create a concentration gradient of the mobile phase over the layer, *i.e.* the A_M value of the CONSDEN[2] equation does not remain constant over the layer.

For laboratory prepared layers, the degree of the reproducibility of the R_F values also depended on the method used to impregnate the stationary phase into the supporting material. For the same elution technique, the slurry method of

impregnating the cellulose (systems 1 and 2) gives better reproducibility of R_F values than does the chromatographic method of impregnation (systems 3 and 4).

The reproducibilities of values obtained from the laboratory prepared layers are worse than the reproducibilities obtained from the precoated layers when both layers were impregnated by the chromatographic technique. It is possible that the longer contact between the support and the impregnant solution in the case of the precoated layers resulted in a more uniform penetration of the impregnant into the cellulose in the latter type of layer so that the A_S value is more constant than in the former type of layer. Alternatively, although the precoated layers were taken from two different boxes (the precoated layers being available in boxes of 25 layers), it is not known if the boxes were from the same or from different factory coated layers. Furthermore, the reproducibilities in the case of the laboratory prepared, chromatographically impregnated layers were based on layers obtained from not less than five different coated batches, while the values obtained from the precoated layers were determined from two different coated batches at the most. However, the R_F values determined from the laboratory prepared, chromatographically impregnated layers are higher than those obtained from the precoated layers chromatographed under the same conditions. This observation tends to support the argument put forward that the penetration is more uniform and that a greater concentration of the stationary phase is taken up by the precoated layers because we have previously shown that the R_F values of phenols decrease with an increase in the loading of the ethyl oleate substrate into cellulose[3].

So far, only the overall, *i.e.* the batch to batch, variations in the reproducibility of the R_F values have been considered. It is also possible to consider the variations which occur on a given layer, and, for systems 1–4, the variations which occur from layer to layer in the same coated batch. In system 1, the R_F values on individual layers were reproducible to $\pm$ 0.01 R_F units, the individual layer reproducibility for systems 2–6 was $\pm$ 0.02 R_F units. The reproducibilities of R_F values for layers from the same coated batches of laboratory prepared layers were $\pm$ 0.01 for system 1, $\pm$ 0.025 R_F units for systems 2 and 3, and $\pm$ 0.035 R_F units for system 4.

(2) *The chromatographic behaviour of the substituted indanols*

The R_F values shown in Table III are the mean of 6 determinations taken from layers bearing two spots of 4-indanol. The R_F value for the standard in each system was reproducible to within the limits shown in Table II. The R_F values for the individual indanols were also reproducible to within the limits observed for the standard for a given system.

The general behaviour of the indanols as a whole conforms to the behaviour of the standard in the different systems, *viz.*,

(i) The R_F values are higher in saturation chambers than they are on layers run in conventional tanks.

(ii) The R_F values are lower on laboratory coated, slurry impregnated layers than they are on laboratory coated, chromatographically impregnated layers.

(iii) The R_F values are higher on laboratory coated, chromatographically impregnated layers than they are on precoated layers impregnated in the same way.

Separation of 4-indanol from 5-indanol is possible in system 1 only.

The introduction of a methyl group into a parent indanol results in a lowering

of the R_F values. This lowering of the R_F values is greatest when the methyl group is in a position *ortho* to the hydroxyl group. This is to be expected from the behaviour of phenols[3].

Little or no separation of the methyl isomers, other than the separation of the *ortho* isomers from the non-*ortho* isomers, occurs, even when the methyl group is in the alicyclic ring rather than in the aromatic ring. The electronic effect of the methyl group in the alicyclic ring will be limited to the inductive release of electrons, while in the aromatic ring this inductive effect should be enhanced by the hyperconjugative release of electrons. Both of these in turn should affect the strength of the phenolic group and its degree of solvation by the mobile phase. However, the lack of chromatographically significant differences between the methyl isomers in which the methyl group is not *ortho* to the phenolic group suggests that electronic effects play but a small part in the chromatographic mechanism in this system, and that the molar volume of the methyl group, rather than its electronic effects, is the chromatographically significant parameter.

The presence of a bulky *tert.*-butyl group considerably reduces the R_F values, particularly when this group is in a position *ortho* to the hydroxyl group.

CONCLUSION

The reproducibility of R_F values in the reversed phase thin-layer chromatographic systems studied is shown to be best when the impregnant is dissolved in the solvent used to slurry the impregnant support, and the resultant chromatograms, loaded with the compounds to be separated, are eluted in a saturation chamber. Impregnation of the support by a chromatographic method, coupled with elution in a conventional tank, gives low reproducibility of R_F values. A reasonable degree of R_F value reproducibility is obtained when precoated layers which were impregnated by the chromatographic method are used.

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DISCUSSION

SHELLARD (to Dr. GRAHAM): Did you deliberately keep your tank unsaturated?

DE ZEEUW: What is the difference in procedure between the saturation chamber and tank?

GRAHAM: Firstly we must clarify terms. By saturation chambers I mean a sandwich type chamber developed by us at Salford. This does not contain any filter paper, so it is not a so-called over-saturated system. By tank I mean a glass vessel into the bottom of which is placed the eluent. Once again the system is not a so-called oversaturated one, *i.e.* the walls of the tank are not lined with filter papers to assist with the saturation of the tank atmosphere. Furthermore, in each case the dry spotted plate is placed into the saturation (or sandwich) chamber or tank before the eluent is added. Under such conditions evaporation of the alcohol from the binary mobile phase will undoubtedly take place. Thus the lower R_F values in the tank system must be accounted for mainly by the change in the composition of the mobile phase. That the composition of the mobile phase is altering is unquestionable because the reproducibility of R_F values alters, *i.e.* they are worse in the tanks. This indicates variations in the A_M value. In addition, the chemical interactions of the solutes with the mobile and stationary phases are also of importance, in particular it is the interaction between solute and mobile phase which is responsible for the removal of the compound from the stationary phase and hence its migration in the system. This in turn will be related to the composition of the mobile phase. Thus the changes in R_F values will be primarily related to the chemical interactions, and variations due to differences in flow rates will be secondary to these chemical interactions in a given system.