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ADSORPTION BEHAVIOUR OF SEVERAL SUPPORTS IN REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY AS DEMONSTRATED BY THE DETERMINATION OF RELATIVE PARTITION COEFFICIENTS OF SOME 4-HYDROXYCOUMARIN DERIVATIVES

WILLEM F. VAN DER GIESEN* and LAMBERT H. M. JANSSEN
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, State University of Utrecht, Catharijnesingel 60, 3511 GH Utrecht (The Netherlands)
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SUMMARY

The adsorptive behaviour of four supports in reversed-phase thin-layer chromatography was investigated. Oleyl alcohol was used as the stationary phase and methanol–water mixtures as the mobile phase. A series of 4-hydroxycoumarins were used as test compounds. Kieselguhr and cellulose show adsorption. Kieselgel also is not a good support, probably because of its large specific surface area. Kieselguhr modified by silanization behaves as a support without adsorptive sites. The R_M values obtained with silanized Kieselguhr as the support correlate very well with the partition coefficients for the octanol–water system.

INTRODUCTION

It is sometimes very difficult or even impossible to determine partition coefficients by means of shake-flask experiments, but instead one can use reversed-phase chromatography. This is a good alternative technique, especially when the compounds are available in small amounts, when they contain impurities or when they are liable to decompose. Sometimes there is a discrepancy between the values of the partition coefficients obtained chromatographically and those obtained with shake-flask experiments. In such instances it has generally been concluded that in addition to a partitioning process there has been adsorption of the compounds on to the solid support[1–5]. However, it is not always possible to compare the results of the two methods and it is clear that the values obtained chromatographically may not always be reliable. Mirrlees *et al.*[6] assumed that if in the case of some test compounds (with known partition coefficients) it is only partitioning that causes retention in a reversed-phase high-performance liquid chromatographic (RP-HPLC) system, then this must also hold for every other compound in that system. However, we think this is rather speculative. Hulshoff and Perrin[7,8] developed a method for examining whether or not adsorption occurs in a reversed-phase thin-layer chromatographic (RP-TLC) system. Using oleyl alcohol as the stationary phase they found that Kieselguhr is a good

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support for phenothiazines and benzodiazepines. Experiments in our laboratory, however, have indicated that Kieselguhr is not an inert support for a series of 4-hydroxycoumarin derivatives. For this reason special attention has been given in this investigation to the choice of support in RP-TLC for some acidic compounds. A series of 4-hydroxycoumarin derivatives were used to demonstrate the influence of several supports.

THEORETICAL

Hulshoff and Perrin[7], using methanol–water mixtures as the mobile phase, derived the relationship between the partition coefficient and a number of chromatographic parameters in an RP-TLC system. They express this relationship as follows:

$$R_M = \log P + \log \phi + bC \tag{1}$$

where P is the partition coefficient of a neutral species in the stationary phase–water system, ϕ is the stationary phase/mobile phase phase-volume ratio, C is the methanol concentration (%, v/v) in the mobile phase and b is a constant depending on the compound and on the chromatographic system used. Of the two variables ϕ and C, one can be kept constant. At a given loading of the support the phase-volume ratio, ϕ, is a constant.

If at a constant loading the methanol concentration, C, in the mobile phase is varied and if the measured R_M values are plotted against C, then one expects to find a straight line with an intercept given by $\log P + \log \phi = R_{M_w}$, *i.e.*, R_M values with 100% water as solvent in the mobile phase. As ϕ is a constant for a given RP-TLC system, R_{M_w} is a measure of the log P value of a compound.

On the other hand, the composition of the mobile phase can be kept constant and the phase-volume ratio, ϕ, can be varied by using different amounts of stationary phase. If it is assumed that ϕ is a linear function of the amount of stationary phase, then

$$R_M = \log {}_sP + \log k + \log S \tag{2}$$

(see Appendix A for derivation), where ${}_sP$ is the partition coefficient if the mobile phase is not 100% water but is a mixture of water and methanol, k is a constant and S is the amount of stationary phase calculated as grams per gram of unloaded support. A graph of R_M against log S should be a straight line with a slope of unity. If the experimental slopes deviate from unity, this means that processes other than liquid–liquid partitioning are involved in the retention mechanism.

When retention is caused by both liquid–liquid partitioning and adsorption on to the solid support a relationship more complex than eqn. 2 must be used (see Appendix B for derivation):

$$R_M = \log {}_sP + \log k + \log S + \log (1 + K_A S^{-1} s_A d_s) \tag{3}$$

where K_A is the adsorption constant (cm), s_A is the specific surface area of the support (cm^2/g) and d_s is the density of the stationary phase (g/cm^3). If $K_A = 0$, then the

adsorption term in eqn. 3 is zero and eqn. 3 reduces to eqn. 2. In the case of adsorption, $K_A > 0$ and the relationship between R_M and log S is no longer linear.

In order to demonstrate the effect of adsorption as given in eqn. 3 some calculations were performed. In Fig. 1 log S + log $(1 + K_A S^{-1} s_A d_s)$ is plotted against log S. Some calculated curves are shown for some selected values of $K_A s_A d_s$. Curve 1 represents the situation where no adsorption occurs, whereas curves 2 and 3 were calculated using increasing values of K_A. It is clear that if very strong adsorption occurs a straight line with zero slope will be found, because then it will be possible to write eqn. 3 as

$$R_M = \log {}_sP + \log k + \log (K_A s_A d_s) \quad (4)$$

In Fig. 1 the region between the two dashed lines represents the usual loading range of the support. The important conclusion to be drawn from this figure is that the curves in this range can be considered as straight lines independent of the degree of adsorption. In other words, one should not look for deviations from linearity in order to detect adsorption phenomena; instead, one can use the slopes of the lines obtained by plotting R_M against log S to detect adsorption, as suggested by Fig. 1. The degree to which the slope of the line deviates from unity when R_M is plotted against log S is a measure of the adsorption.

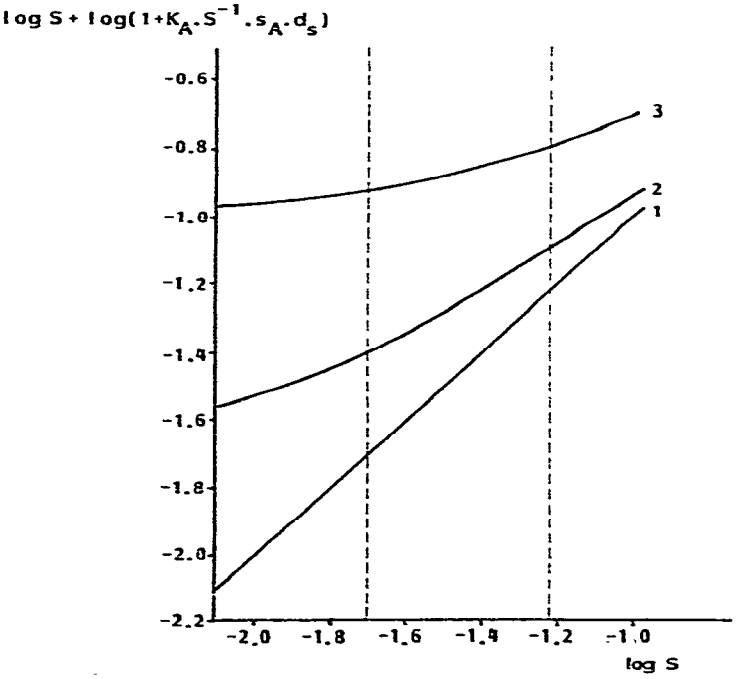

Fig. 1. Theoretical curves of log S + log $(1 + K_A S^{-1} s_A d_s)$ *versus* log S. The broken lines indicate the region of the usual loading range (S = 0.02–0.06). For curves 1, 2 and 3 $K_A s_A d_s$ = 0, 0.02 and 0.10, respectively. These values were chosen so as to obtain values for the slopes of the curves (considered as straight lines) between the broken lines of the same order of magnitude as found in this investigation. See also Table I.

More extended calculations were performed to demonstrate the points just discussed. In Table I it can be seen that in the usual loading range straight lines are indeed obtained, as shown by the values of the correlation coefficient, r. In Fig. 2 values of the slopes of the lines discussed above are given. This figure may be used to obtain the value of $K_A s_A d_s$ for a slope value found experimentally. It should be noted that this relationship is valid irrespective of the kind of support and the kind of stationary phase.

TABLE I

SLOPES OF THE THEORETICAL CURVES OF LOG S + LOG $(1 + K_A S^{-1} s_A d_s)$ *VERSUS* LOG S FOR SEVERAL VALUES OF $K_A s_A d_s$

The values of S used were 0.02, 0.03, 0.04, 0.05 and 0.06. The slopes of the curves in the second column were calculated from the curves given in Fig. 1; a linear behaviour is assumed over the range $S = 0.02$–0.06. This region is indicated by the broken lines in Fig. 1. The correlation coefficients, r, were obtained from a linear least-squares method.

$K_A s_A d_s$	*Slope*	*Correlation coefficient, r*
0.000	1.000	
0.001	0.971	1.000
0.003	0.917	1.000
0.005	0.870	1.000
0.010	0.771	0.999
0.020	0.630	0.998
0.030	0.534	0.997
0.040	0.463	0.996
0.050	0.410	0.996
0.060	0.368	0.995
0.070	0.333	0.994
0.080	0.305	0.994
0.090	0.281	0.993
0.100	0.260	0.993

In principle there may be another way of detecting adsorption[7]. Eqn. 3 can be rearranged to

$$\frac{1}{R_F} = {}_sPkK_A s_A d_s + 1 + {}_sPkS \tag{5}$$

A graph of R_F^{-1} against S will be a straight line. If the intercept with the R_F^{-1} axis is unity, then obviously K_A approaches zero. A correlation between the magnitude of the deviation from unity and the magnitude of adsorption is difficult to establish, because ${}_sP$ is one of the factors of the adsorption term of eqn. 5. For this reason we prefer to use eqn. 3 in order to check whether there has been adsorption on to the solid support in RP-TLC.

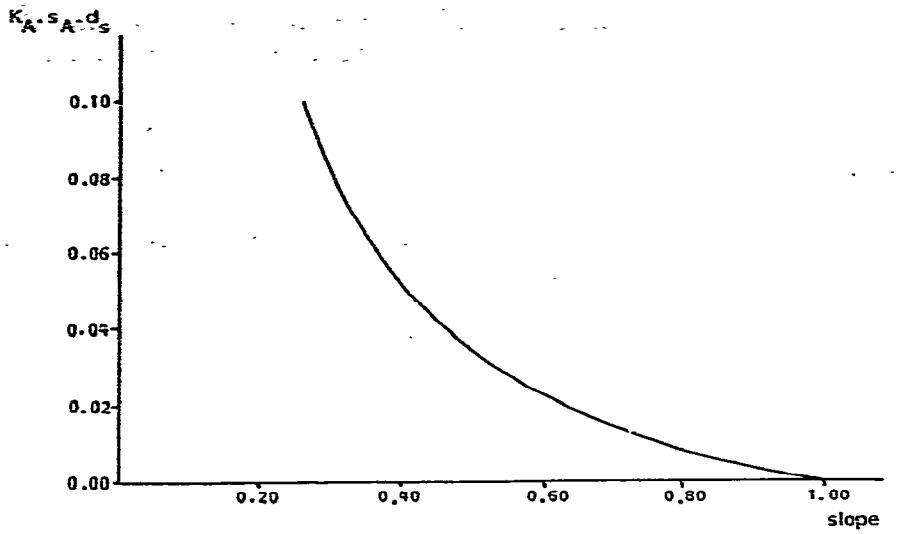

Fig. 2. $K_A s_A d_s$ as a function of the slope (s) using the numbers in Table I. This relationship can be best represented by a sixth degree polynomial function: $K_A s_A d_s = 2.3772\ s^6 - 10.1914\ s^5 + 18.1304\ s^4 - 17.3027\ s^3 + 9.5433\ s^2 - 3.0330\ s + 0.4762$. This figure permits the calculation of $K_A s_A d_s$ if the experimental value of s is known. An example of such a calculation is given in the text.

EXPERIMENTAL

Chemicals were of analytical-reagent grade unless specified otherwise. Demineralized water was used throughout.

Acenocoumarol (Sintrom; Ciba-Geigy, Arnhem, The Netherlands), coumetarol (Dicumoxane; ACF, Maarssen, The Netherlands), ethyl biscoumacetate (Tromexan; Ciba-Geigy) and phenprocoumon (Marcoumar; Hoffman-La Roche, Mijdrecht, The Netherlands) were gifts from the manufacturers.

4-Hydroxycoumarin (Merck, Darmstadt, G.F.R.), warfarin sodium (British Pharmacopoeia quality; ACF), methanol (Merck), 38% hydrochloric acid (Merck), cellulose MN 300 (Machery, Nagel & Co., Düren, G.F.R.), Kieselgel H (Merck), Kieselguhr MN (Machery, Nagel & Co.), dichloromethane (Baker, Deventer, The Netherlands) and chlorotrimethylsilane (Aldrich-Europe, Beerse, Belgium) were used as supplied.

Oleyl alcohol (Merck), containing 95% *cis*-9-octadecen-1-ol, was passed through a glass column (60 × 2.5 cm I.D.), the lower half of which was filled with basic aluminium oxide (Merck) and the upper half with charcoal pellets (Norit, Amsterdam, The Netherlands). The resulting product was colourless and odourless, the density at 25°C being 0.848 g/cm^3.

Silanization of Kieselguhr

Silanized Kieselguhr was prepared by adding 30 ml of chlorotrimethylsilane to a stirred suspension of 300 g of Kieselguhr MN in 2.5 l of dichloromethane at room temperature. Stirring was stopped after 1 h and the silanized Kieselguhr was allowed to settle. The yellow supernatant was decanted and the silanized Kieselguhr was

washed by multiple decantations with methanol until the supernatant was colourless. Finally, the silanized Kieselguhr was dried at 60°C. Three preparations yielded identical results.

Preparation of TLC plates

Using standard TLC equipment (Shandon Southern, Camberley, Great Britain) glass plates (20 × 20 cm) were covered with a 0.25-mm layer of a slurry consisting of a mixture of the support, stationary phase (oleyl alcohol) and ethanol (see Table II). A Warring blender was run at maximum speed for 1 min to homogenize the slurries. The values of the loading, S, ranged from 0.01 to 0.07. The plates were allowed to dry for 16 h at room temperature. The mobile phases consisted of solutions of hydrochloric acid in methanol–water mixtures. The methanol concentration in the mobile phase ranged from 20 to 70% (v/v); 0.1% solutions were made of 4-hydroxycoumarin, acenocoumarin, warfarin and phenprocoumon in methanol and 0.05% solutions were made of coumetarol and ethyl biscoumacetate in dichloromethane.

TABLE II

COMPOSITION OF THE SLURRIES USED TO PREPARE THE TLC PLATES

The amounts given are sufficient for five plates (20 × 20 cm). To each slurry oleyl alcohol was added in an amount dependent on the desired final loading S.

Support		*Solvent*	
Name	*Amount (g)*	*Amount (ml)*	*Ethanol concentration (%, v/v)*
Kieselguhr MN	20	85	70
Cellulose MN 300	15	80	95
Kieselgel H	20	75	90
Kieselguhr, silanized	19	55	90

Volumes of 1 μl of the solutions was spotted on the plates, in varying allocations, at 1.5-cm intervals along a line 2 cm from the lower edge of the plate. Each compound was spotted twice on the same plate. A migration of 10 cm on all plates was obtained by cutting the layer at 12 cm from the lower edge. After development, the spots were revealed by spraying with a freshly prepared mixture of equal volumes of 1% iron(III) chloride in 50% ethanol and 1% potassium hexacyanoferrate(III) in 50% ethanol[9]. This mixture gives blue spots on a yellow background.

The diagonal method described by Hulshoff and Perrin[7] was used to check whether or not the conditions had changed during development. If the plates are equilibrated for 2 h in a chromatographic chamber saturated with the vapour of the mobile phase before development, then one can be certain that the conditions do not alter during development. The temperature was maintained at 25°C.

RESULTS AND DISCUSSION

To examine the possible adsorption of some 4-hydroxycoumarin derivatives on to several supports, the compounds were chromatographed at constant mobile phase composition, but with varying amounts of stationary phase. The R_M values obtained were plotted against log S.

The results for Kieselguhr MN as support are shown in Fig. 3. A linear relationship is found. The values of the slopes of these lines are given in Table III. The slopes are smaller than unity; it is clear that adsorption on to the Kieselguhr plays a significant role in the retention process.

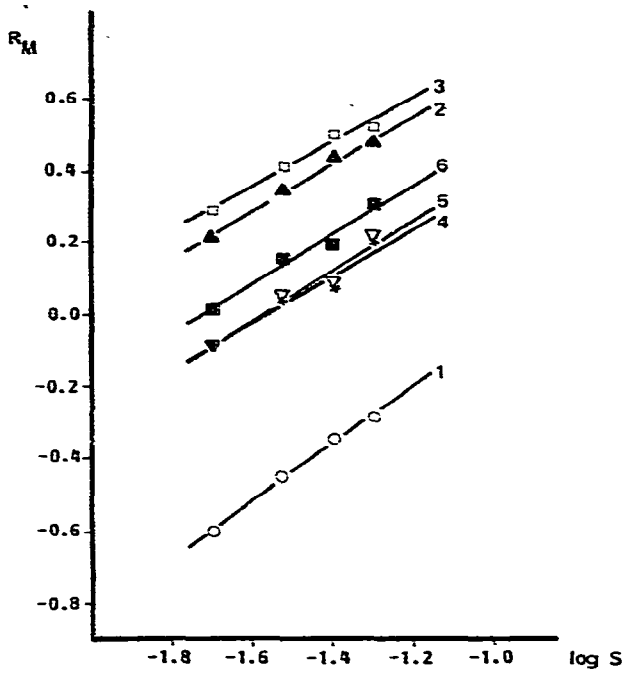

Fig. 3. R_M values of 4-hydroxycoumarins plotted *versus* log S using Kieselguhr MN as support. Every point is the mean of 6–8 determinations. The drawn lines were calculated using the least-squares method for linear regression. 1, 4-Hydroxycoumarin; 2, warfarin; 3, acenocoumarin; 4, phenprocoumon; 5, ethyl biscoumacetate; 6, coumetarol. Mobile phase: 1 *M* HCl in 30% (v/v) methanol for compounds 1–3 and 1 *M* HCl in 60% (v/v) methanol for compounds 4–6.

On the other hand, phenothiazines[7] and benzodiazepines[8] as representatives of lipophilic basic and neutral compounds, respectively, did not show adsorption on to Kieselguhr, provided that the loading was above a certain minimum value. Acids are good proton donors for hydrogen-bridge formation with silanol sites of the solid support; this might cause adsorption of the (acidic) 4-hydroxycoumarin derivatives on to Kieselguhr.

With the help of the calculated function given in the legend to Fig. 2, the K_A values of the several 4-hydroxycoumarin derivatives were calculated in the cases where Kieselguhr served as the support. For this calculation the value of the specific

TABLE III

SLOPES OF THE STRAIGHT LINES OF R_M *VERSUS* LOG S, AND THE ADSORPTION CONSTANT K_A FOR KIESELGUHR AS SUPPORT

The experimental slopes were obtained from Figs. 3–6. In the last column the value for the adsorption constant K_A is given, calculated as described in the text. Standard deviations are given in parentheses.

Compound	*Slope*				$K_A \cdot 10^7$ *(cm)*
	Kieselguhr	*Cellulose*	*Kieselgel*	*Silanized Kieselguhr*	
4-Hydroxycoumarin	0.80 (±0.03)	0.46 (±0.02)	0.89 (±0.13)	1.06 (±0.07)	2.4 (±0.4)
Warfarin	0.67 (±0.04)	0.51 (±0.02)	1.19 (±0.03)	1.00 (±0.10)	4.7 (±0.8)
Acenocoumarin	0.62 (±0.06)	0.43 (±0.02)	1.15 (±0.08)	1.02 (±0.11)	5.8 (±1.4)
Phenprocoumon	0.66 (±0.11)	0.69 (±0.03)	1.23 (±0.07)	1.05 (±0.10)	4.9 (±2.0)
Ethyl biscoumacetate	0.70 (±0.11)	0.41 (±0.01)	0.82 (±0.11)	1.09 (±0.10)	4.0 (±1.8)
Coumetarol	0.69 (±0.09)	0.49 (±0.02)	1.19 (±0.06)	1.12 (±0.07)	4.2 (±1.6)

surface area, s_A, of Kieselguhr was assumed to be $4.2 \cdot 10^4$ cm^2/g (ref. 10) and the density, d_s, of oleyl alcohol was taken to be 0.848 g/cm^3. The resulting K_A values are given in the last column in Table III. Asshauer and Halász[11] found K_A values of the same order of magnitude for the adsorption of some organic solvents on to Porasil A as support in a gas–liquid chromatographic system.

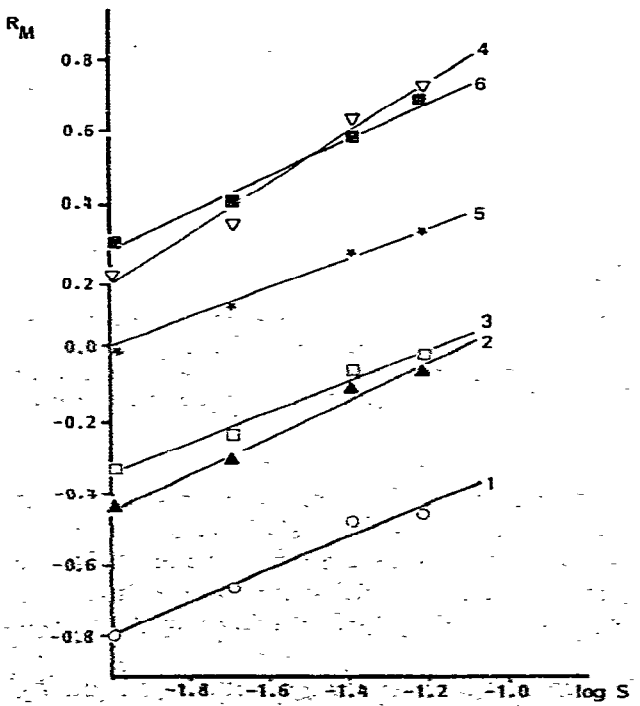

Fig. 4. R_M values of 4-hydroxycoumarins plotted *versus* log S using Cellulose MN 300 as support. Further details as in Fig. 3, except for the mobile phase, which was 0.1 *M* HCl in 45% (v/v) methanol.

The results for cellulose MN 300 are shown in Fig. 4. As with Kieselguhr, straight lines are obtained when R_M is plotted against log S. The slopes of the lines in Fig. 4, given in Table III, are smaller than those for Kieselguhr. It can be concluded that adsorption plays an important role here also. However, Bird and Marshall[12] found that cellulose had only some very weak adsorptive sites for penicillins.

The results for Kieselgel are shown in Fig. 5. The behaviour of Kieselgel is remarkable: straight lines with slopes smaller and larger than unity are obtained when R_M is plotted against log S (see also Table III). Obviously Kieselgel does not meet the requirements for an inert support. Other reports in the literature point to adsorption phenomena when Kieselgel is used. Kuchař *et al.*[13] found considerable adsorption on to Kieselgel G in an RP-TLC system for two series of aryl-aliphatic acids; nevertheless, they used the R_M values they obtained as substitutes for log P values. Biagi *et al.*[14], using octanol-impregnated Kieselgel G, found the relationship log $P_{oct} = 0.569$ $R_{M_w} + 1.354$ ($n = 39$, $r = 0.838$) between the partition coefficient in the system octanol-water and the R_{M_w} values for a series of benzodiazepines. The correlation coefficient is low and the coefficient 0.569 deviates considerably from unity (see eqn. 1); this suggests that partitioning was not the only process involved in the chromatographic system used.

The specific surface area of Kieselgel is so large that it is of considerable

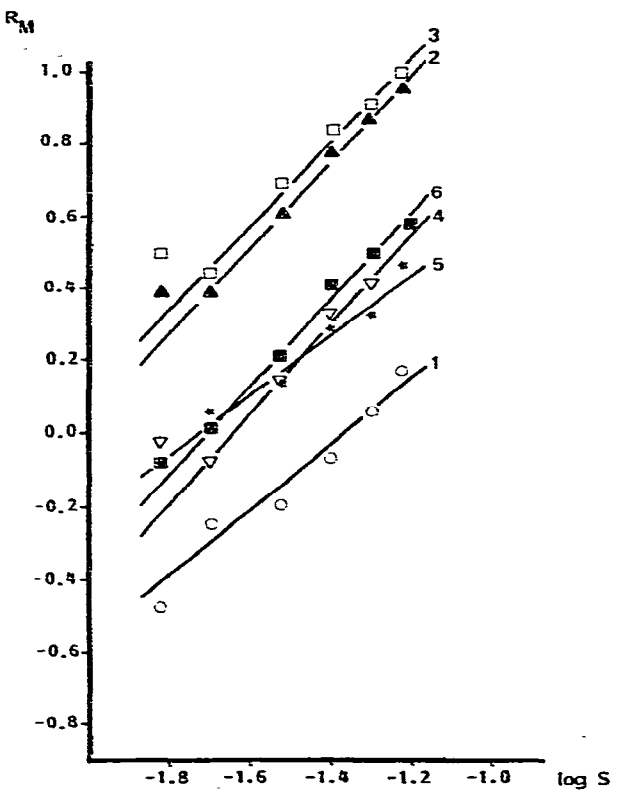

Fig. 5. R_M values of 4-hydroxycoumarins plotted *versus* log S using Kieselgel as support. Further details as in Fig. 3. The points measured at the lowest S value were excluded in the calculation of the drawn lines.

importance. Scott and Kucera[15] found that for some solvents, which had a mean molecular diameter of about 6.6 Å and covered a surface area of about 34.2 Å^2, on average $6.6 \cdot 10^{20}$ solvent molecules per gram of Kieselgel are needed to form a monomolecular layer. Assuming for oleyl alcohol a mean molecular diameter of 8 Å, which corresponds to a surface area of 50 Å^2, one finds that a mean number of $4.5 \cdot 10^{20}$ molecules or 0.20 g of oleyl alcohol is needed to form a monomolecular layer per gram of Kieselgel. It is reasonable to say that the amount of stationary phase required to form a monomolecular layer depends mainly on the surface area of the support. If values of 4.2 and 620 m^2/g are assumed[10,16] for the specific surface areas of Kieselguhr and Kieselgel, respectively, then it can be estimated that 0.0014 g of oleyl alcohol per gram of Kieselguhr is needed to form a monomolecular layer. As these numbers only give the order of magnitude, it is clear that with Kieselgel the usual amounts of oleyl alcohol in RP-TLC are certainly not enough to form a monomolecular layer, whereas with Kieselguhr a multimolecular layer is formed. It is very unlikely that a monomolecular layer will behave as a bulk phase, whereas it is very likely that a multimolecular layer will do so. This effect, combined with adsorption phenomena, is probably the reason for the strange behaviour of Kieselgel.

The results obtained so far urged us to seek a better support that showed no adsorptive properties. We found this support by blocking the active silanol sites of Kieselguhr MN with chlorotrimethylsilane. The method used to modify the Kieselguhr is in fact a slight modification of the method proposed by Little and co-workers[17,18]. They found that carbon tetrachloride is the best solvent in which to perform

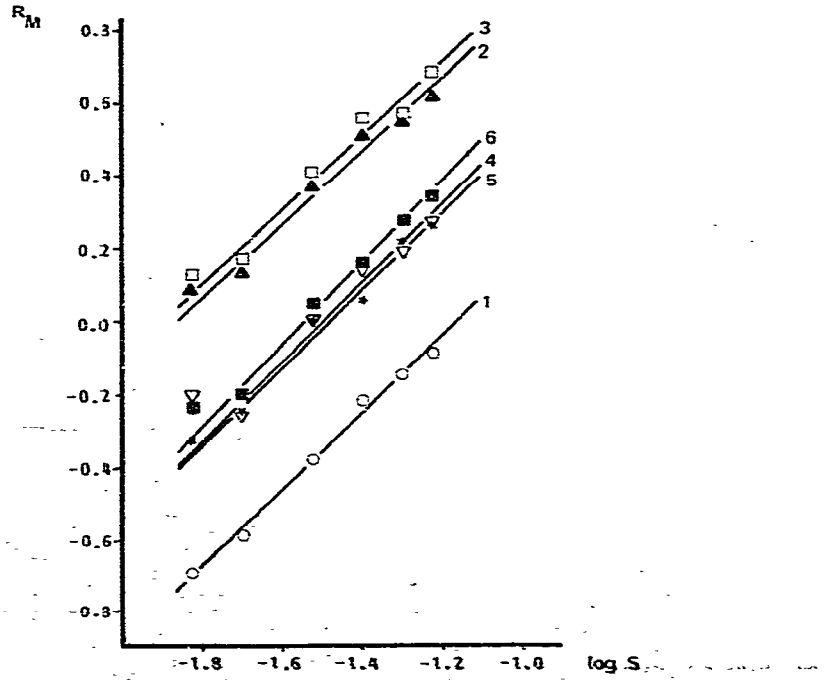

Fig. 6. R_M values of 4-hydroxycoumarins plotted *versus* log S using silanized Kieselguhr as support. Further details as in Fig. 3. The points measured at the lowest S value were excluded in the calculation of the drawn lines.

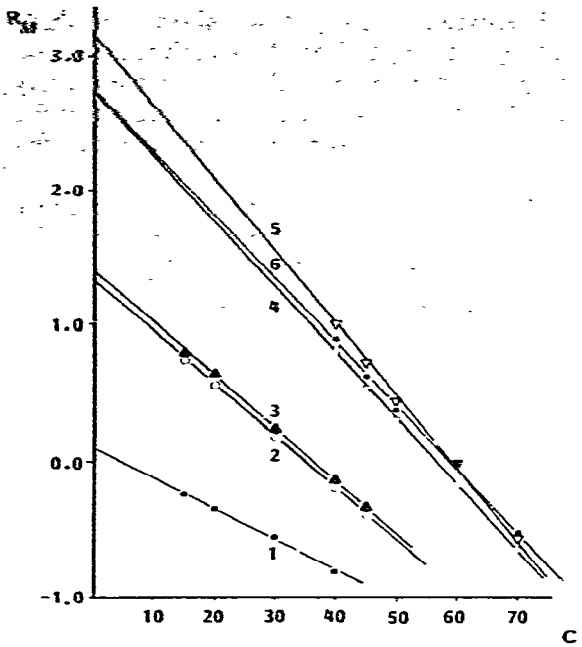

Fig. 7. Effect of the methanol concentration, C (%, v/v), in the mobile phase on the R_M values of 4-hydroxycoumarins. Stationary phase: oleyl alcohol (loading $S = 0.02$) on silanized Kieselguhr as support. Every point is the mean of 6–8 determinations. The drawn lines were calculated using the least-squares method for the linear regression. 1 = Hydroxycoumarin; 2 = warfarin; 3 = acenocoumarin; 4 = phenprocoumon; 5 = ethyl biscoumacetate; 6 = coumetarol.

the chemical reaction. However, we preferred to use dichloromethane as the solvent in order to achieve a better sedimentation rate in the decantation step. The method of silanizing the Kieselguhr is simple. The adherence of the silanized Kieselguhr to the glass plates is adequate and no decomposition of the support occurs even with the very acidic mobile phases we used in our investigations.

TABLE IV

SLOPES (b) AND INTERCEPTS (R_{M_0}) OF THE LINES WHEN R_M IS PLOTTED *VERSUS* THE METHANOL CONCENTRATION OF THE MOBILE PHASE, USING OLEYL ALCOHOL AS THE STATIONARY PHASE (LOADING $S = 0.02$) ADSORBED ON TO SILANIZED KIESELGUHR AS SUPPORT

Also given are the partition coefficients (octanol–water) obtained from shake-flask experiments (log P_{oct}). Standard deviations are given in parentheses.

Compound	b	R_{M_0}	*Correlation coefficient, r*	*Log* P_{oct}
4-Hydroxycoumarin	−2.24 (±0.06)	0.10 (±0.02)	0.9993	2.37
Warfarin	−3.77 (±0.03)	1.32 (±0.01)	0.9999	3.28
Acenocoumarin	−3.84 (±0.08)	1.38 (±0.03)	0.9993	3.24
Phenprocoumon	−4.83 (±0.09)	2.73 (±0.05)	0.9995	4.56
Ethyl biscoumacetate	−5.35 (±0.17)	3.15 (±0.09)	0.9984	4.93
Coumetarol	−4.67 (±0.13)	2.73 (±0.07)	0.9987	4.55

The results obtained with silanized Kieselguhr as support are shown in Fig. 6. Straight lines are found when R_M is plotted against log S. The slopes of the lines and the corresponding standard deviations are given in Table III. These slopes are unity within one standard deviation, so the results for this support are superior to those for any of the other supports discussed above. This proves clearly that silanized Kieselguhr behaves as a support without adsorptive sites.

Using silanized Kieselguhr as support and oleyl alcohol as stationary phase we determined R_M values with several methanol–water mixtures as mobile phases. For all 4-hydroxycoumarin derivatives straight lines are obtained when R_M is plotted against C (see Fig. 7). Extrapolating to $C = 0$ gives R_{M_0} values (see Table IV). Also given in Table IV are the slopes, b, of the lines in Fig. 7, together with the partition coefficients in the octanol–water system obtained from shake-flask experiments[19].

To calculate the partition coefficient, P_1, of a solute in one solvent system when the partition coefficient, P_2, of the same solute in another solvent system is known, a Collander-type equation can be used[20,21]:

$$\log P_1 = a \log P_2 + c \tag{6}$$

where a and c are constants characterizing the solvent systems involved. This type of equation is useful for many solvent systems in which the non-polar phase is varied and in which the polar phase is always water, particularly when the solutes are divided into proton donors, proton acceptors and neutral species[21].

As the R_{M_0} values in this investigation represent the partition coefficient in the oleyl alcohol–water system it should be possible to use a Collander-type equation to correlate log P values in a certain solvent system and R_{M_0} values in another solvent system. We found the following correlation*:

$$\log P_{oct} = 0.859\ (\pm 0.034) R_{M_0} + 2.187\ (\pm 0.074) \tag{7}$$
$$n = 6;\ r^2 = 0.994;\ s = 0.088;\ F = 644$$
$$0.787 < \text{slope}^{**} < 0.931;\ 2.030 < \text{intercept}^{**} < 2.344$$

The constant 2.187 in eqn. 7 is the sum of the constant c in eqn. 6 and the logarithm of the phase-volume ratio, ϕ. When the mobile phase is not pure water but a methanol–water mixture, log P_{oct} and R_M correlate well with each other. As an example, in eqn. 8 the correlation is given when the methanol concentration C in the mobile phase is 30% (v/v):

$$\log P_{oct} = 1.192\ (\pm 0.029) R_{M_{30}} + 3.025\ (\pm 0.030) \tag{8}$$
$$n = 6;\ r^2 = 0.998;\ s = 0.055;\ F = 1684$$
$$1.130 < \text{slope} < 1.254;\ 2.962 < \text{intercept} < 3.088$$

We conclude that a Collander-type equation can also be used when the organic phase is kept constant and the aqueous phase is changed. The Δlog P values in the solvent system stationary phase–water are equal to the ΔR_{M_0} values.

* The standard deviation is given in parentheses; n = number of compounds; r = correlation coefficient; s = standard error of estimate or $s_{y.x}$; F = value of the F-test of significance.

** 90% confidence interval.

However, these $\Delta\log P$ values are not equal to the ΔR_M values obtained using a certain methanol–water mixture as the mobile phase. This would only have been true if the coefficient b in eqn. 1 had been independent of the choice of the solute. It is clear from Fig. 7 and Table IV that b has a characteristic value for every compound. These findings for 4-hydroxycoumarins are similar to the findings for phenothiazines and benzodiazepines[7,8].

RP-TLC has some drawbacks compared with RP-HPLC: a large number of experiments are needed to obtain accurate R_M values, and detection of the spots can be a serious problem. One great advantage of RP-TLC, however, is the fact that it enables one to examine the adsorption on to the solid support for every solute, following the method described above in which various amounts of stationary phase are used. In RP-HPLC it is very difficult to vary the loading of the support in the column, and the loading is only possible within a very limited range[22].

APPENDIX A

Phase-volume ratio, ϕ, as a linear function of the loading, S

It is assumed here that the total pore volume of the support is filled with stationary and mobile phase. In other words, the pore volume of the support available for the mobile phase decreases with increasing amounts of stationary phase, according to the equation

$$V_m = V_p - V_s = V_p - d_s^{-1}S \tag{9}$$

where V_m and V_s (cm^3) are the volumes of the mobile and the stationary phase, respectively, V_p (cm^3) is the total pore volume of the support, d_s (g/cm^3) is the density of the stationary phase and S is the amount of stationary phase in grams per gram of unloaded support. The phase-volume ratio, ϕ, is given by V_s/V_m, or

$$\phi = \frac{d_s^{-1}S}{V_p - d_s^{-1}S} = \frac{S}{d_sV_p - S} \tag{10}$$

In our investigations the S values range from 0.01 to 0.06. V_p for Kieselguhr is 1.16 cm^3/g (ref. 10) and d_s for oleyl alcohol is 0.848 g/cm^3. Using these values, Table V can be compiled. It is clear from this table that the relative change of $(d_sV_p - S)^{-1}$ is small compared with the relative increase of S. The error is negligible when $(d_sV_p - S)^{-1}$ is considered to be constant. Therefore, the following relationship may be used:

$$\phi = kS \tag{11}$$

This relationship was assumed earlier by Hulshoff and Perrin[7].

TABLE V

DEPENDENCE OF $(d_sV_p - S)^{-1}$ ON S

S	$(d_sV_p - S)^{-1}$	S	$(d_sV_p - S)^{-1}$
0.01	1.0270	0.04	1.0597
0.02	1.0377	0.05	1.0710
0.03	1.0486	0.06	1.0826

APPENDIX B

Derivation of eqn. 3

The predominant mechanism of solute retention in liquid–liquid chromatography is partitioning between the mobile phase and the liquid stationary phase. The solute may also be retained by adsorption on to the solid support. Mathematically this can be represented by the equation

$$P_{obs} = \frac{\Delta V_s c_s + \Delta A c_A}{\Delta V_m c_m} \tag{12}$$

where P_{obs} is the equilibrium constant of a solute in a chromatographic system in which only liquid–liquid partitioning and adsorption on to the solid support take place, c_s and c_m (g/cm^3) are the concentrations of the solute in adjacent volumes of stationary phase (ΔV_s) and mobile phase (ΔV_m) and c_A (g/cm^2) is the concentration of the solute at the part of the surface area of the support (ΔA) adjacent to ΔV_s. Eqn. 12 can be rearranged to

$$P_{obs} = \frac{\Delta V_s}{\Delta V_m} \cdot \frac{c_s}{c_m} + \frac{\Delta A}{\Delta V_s} \cdot \frac{\Delta V_s}{\Delta V_m} \cdot \frac{c_A}{c_s} \cdot \frac{c_s}{c_m} \tag{13}$$

where V_s/V_m is the phase-volume ratio ϕ, c_s/c_m is the partition coefficient ${}_sP$ (where s indicates that a mixed solvent such as a methanol–water mixture is used as the mobile phase) and c_A/c_s is the adsorption coefficient K_A (cm). Substitution of these terms into eqn. 13 gives

$$P_{obs} = \phi \cdot {}_sP \left(1 + K_A \cdot \frac{\Delta A}{\Delta V_s}\right) \tag{14}$$

In TLC R_F can be defined as the ratio of the amount of the solute in the mobile phase and the total amount of solute:

$$R_F = \frac{\Delta V_m c_m}{\Delta V_m c_m + \Delta V_s c_s + \Delta A c_A} \tag{15}$$

Combining eqns. 12 and 15 one obtains the well known equation

$$P_{obs} = \frac{1}{R_F} - 1 = \text{antilog } R_M \tag{16}$$

Combining eqns. 11, 14 and 16 we obtain

$$R_M = \log {}_sP + \log k + \log S + \log\left(1 + K_A \cdot \frac{\Delta A}{\Delta V_s}\right) \tag{17}$$

$\Delta A/\Delta V_s$ can be replaced by $S^{-1}s_A d_s$, s_A (cm^2/g) is the specific surface area of the support and d_s (g/cm^3) is the density of the stationary phase. This results in

$$R_M = \log {}_sP + \log k + \log S + \log (1 + K_A S^{-1} s_A d_s) \tag{18}$$

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