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COMPARISON OF VARIOUS NON-POLAR STATIONARY PHASES USED FOR ASSESSING LIPOPHILICITY

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SUMMARY

Two non-polar stationary phases, *i.e.*, a novel octadecylpolyvinyl (ODP) packing and a poly(styrene–divinylbenzene) (PLRP-S) gel, were compared with a standard phase (octadecylsilane, ODS) for their usefulness in determining lipophilicity. Various monosubstituted benzenes and neuroleptic drugs were used as solutes. The usefulness of the polystyrene divinylbenzene phase was limited by physical problems, long retention times and capacity factors which did not appear to express the same partitioning behaviour as in an octanol–water system. In contrast, the ODP phase proved very interesting. Like the ODS phase, it showed high selectivity and fast elution, and yielded retention data which reflect a partitioning behaviour comparable to that seen in an octanol–water system. It also proved superior to the ODS phase in that it did not require the addition of a masking agent.

INTRODUCTION

Since the pioneering work of Meyer[1] and Overton[2] on the narcosis of tadpoles, lipophilicity has become a major parameter in quantitative structure–activity relationships[3–6]. Although several calculation methods have been used to determine lipophilicity[7–9], its experimental determination remains crucial. A number of methods have been used to measure lipophilicity but only two are in common use today, namely the shake-flask method and reversed-phase high-performance liquid chromatography[10] (RP-HPLC). In the former method, the biphasic *n*-octanol–water system has been chosen as a standard owing to some analogies with biological systems[11]. The equilibrium of a solute between an organic and an aqueous phase defines the partition coefficient (expressed as log *P*). Time consumption, large errors caused by small impurities with strong chromophores and limitations to log *P* values in the range -2 to 4 are the main practical disadvantages of the shake-flask method. For extensive details on these limitations and shortcomings, the reader is referred to a review by Dearden and Bresnen[12].

In recent years, RP-HPLC has proved to be a successful alternative for assessing

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lipophilicity of drugs and other compounds. Alkylsilane-bonded phases, particularly the octadecyl type, are the non-polar stationary phases most frequently used. They are prepared by reacting the silanol groups of silica gel with organochloro- or organo-alkoxysilanes[13]. However, a high proportion of silanol groups (up to 50%) remain unreacted owing to steric hindrance[14]. During the chromatographic process, such residual silanol groups can interact with basic solutes by means of so-called silanophilic interactions, resulting in severe peak tailing and a decreased number of theoretical plates[15]. These phenomena severely disturb the partitioning behaviour[16,17]. The addition of a masking agent, usually an amine modifier, attenuates the silanophilic interactions without always suppressing them. Triethylamine, *n*-decylamine and N,N-dimethyloctylamine are masking agents in common use[16–21].

Other types of bonded phases have been proposed for the RP-HPLC determination of lipophilicity, *e.g.*, phenyl-modified silica gels, which were suggested to be superior to the ODS phase for measuring the partition coefficients of aromatic compounds[22]. Despite these efforts, the octadecylsilane (ODS) stationary phase is still considered to be the most convenient[23], and is indeed the most frequently used.

In order to avoid the silanophilic interactions, a poly(styrene–divinylbenzene) packing (PLRP-S) was used to assess the lipophilicity of 30 aromatic acids, yielding values which were well correlated with partition coefficients calculated according to Rekker's method[24]. The hydrophobic matrix of the PLRP-S is formed by a network of linear chains of polstyrene interconnected with divinylbenzene molecules[25–27]. Steric exclusion, which is claimed to govern the mechanism of retention[28], in fact appears restricted to relatively large molecules; this implies that retention of solutes of ordinary size should be based solely on partitioning. A poly(styrene–divinyl) stationary phase has the advantage of being stable over a broad pH range, allowing the determination of capacity factors and dissociation constants of compounds with comparatively high pK_a values (above 7)[29,30]. On the other hand, PLRP-S columns have a comparatively small number of theoretical plates and undergo considerably shrinking and swelling, which shorten their life.

A rigid macropourous polyacrylamide-based packing having a chemically bonded octadecyl phase has recently been developed. According to Dawkins *et al.*[31], this stationary phase, like ODS and unlike PLRP-S, may be viewed as a hydrophobic layer attached to a rigid hydrophilic support. This material is considered to be a direct replacement for C_{18} silica packings with the additional advantage of providing long-term physical and chemical stability over a wide pH range (1–13).

Recently, an octadecylpolyvinyl copolymer packing (ODP) became commercially available. The ODP gel is obtained by reaction of stearoyl chloride with the hydroxyl group of a vinyl alcohol copolymer gel[32,33]. It is claimed to offer the advantages of both the ODS and the polystyrene phases without their respective disadvantages, in other words, to afford high resolution, a large number of theoretical plates, expanded applicability with efficient separation of basic substances, stability over a wide eluent pH range and absence of silanophilic interactions, swelling and shrinkage. This phase is considered to be very stable chemically but physically weaker than silica gels.

To the best of our knowledge, the above-mentioned phases have never been objectively compared for their ability to assess lipophilicity. This study was therefore undertaken with the aim of comparing ODS, PLRP-S and ODP stationary phases for

their ability to measure lipophilicity. A correlation between retention data and octanol–water partition coefficients was established using a wide range of solutes of known lipophilicity. In addition, the ODS packing was used with and without a masking agent (*n*-decylamine).

EXPERIMENTAL

Chemicals

Monosubstituted benzenes (28 compounds) of the best available purity were obtained from Fluka (Buchs, Switzerland). Samples of neuroleptic drugs of pharmaceutical purity were kindly donated by various pharmaceutical companies. Analytical-reagent grade methanol and 3-morpholinopropanesulphonic acid (MPS) were obtained from Merck (Darmstadt, F.R.G.).

Chromatographic equipment

A Siemens S101 chromatograph equipped with an Orlita DMP-AE 10.4 pump was used. The detector was a Uvikon 740 LC (Kontron) operating at 254 nm. A Hewlett-Packard integrator was used for peak registration and calculation of retention times.

Columns

The ODS column (25 cm × 4 mm I.D.) was prepacked with LiChrosorb RP-18, particle size 10 μm (Knauer, Berlin, F.R.G.). The PLRP-S column (15 cm × 4.6 mm I.D.) was filled with macroporous, rigid spherical particles (5 μm) made of poly(styrene–divinylbenzene). The ODP column (15 cm × 6 mm I.D.) was prepacked with the octadecyl copolymer gel, particle size 5 μm (Asahi Chemicals, Kawasaki, Japan).

Mobile phase preparation

The mobile phases were prepared volumetrically from combinations of methanol and aqueous MPS buffer (0.02 *M*, pH 7.4) in the range 10–90%. All solutions were purified by filtration using a Milli-Q system (Millipore). All measurements were performed at room temperature (21 $\pm$ 1°C). The flow-rate was 1.5 ml/min for the ODS column and 0.8 ml/min for the PLRP-S column; for the ODP column it was 1.5 ml/min with the monosubstituted benzenes and 1.3 ml/min with the neuroleptic drugs. Isocratic capacity factors, k_i, were defined as

$$k_i = (t_R - t_0)/t_0 \qquad (1)$$

where t_R is the retention time of the solute and t_0 is the column dead time determined using methanol as the non-retained compound.

RESULTS AND DISCUSSION

In agreement with previous results[34], the increase in log k_i values with decreasing methanol concentration in the eluent was linear with the ODS packing. Indeed, the straight lines of log k_i *versus* methanol concentration display r^2 values ranging from

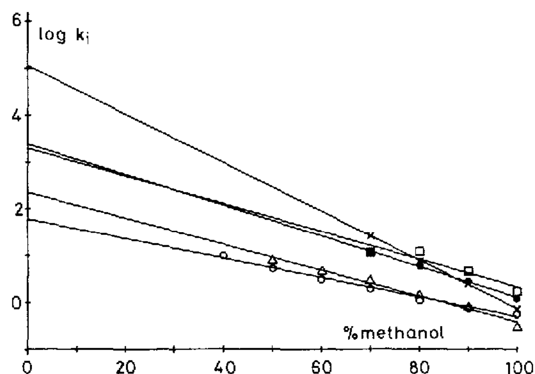

Fig. 1. Linear relationship between the isocratic capacity factors and the percentage of methanol in the mobile phase for five typical monosubstituted benzenes using the PLRP-S column. ×, Trifluoromethylbenzene; ●, N-methylaniline; □, chlorobenzene; △, aniline; ○, methyl phenyl sulphoxide.

0.9846 to 0.9983 without added decylamine, and from 0.9879 to 0.9996 in the presence of decylamine. Figs. 1 and 2 show that a linear increase in log k_i values is also observed with the PLRP-S and ODP phases, with r^2 values ranging from 0.9570 to 0.9995 with the former and from 0.9644 to 0.9986 with the latter phase. The log k_i values could thus be extrapolated linearily to 100% water content, yielding the log k_w values reported in Table I for the three stationary phases. Partition coefficients in the *n*-octanol–water system are also reported.

ODS stationary phase

The relationship between *n*-octanol–water partition coefficients (log *P* values) and the extrapolated capacity factors (log k_w values) determined using the ODS packing wih (log k_w[ODS/d] values) and without (log k_w[ODS] values) a masking agent are reported in eqns. 2 and 3, respectively.

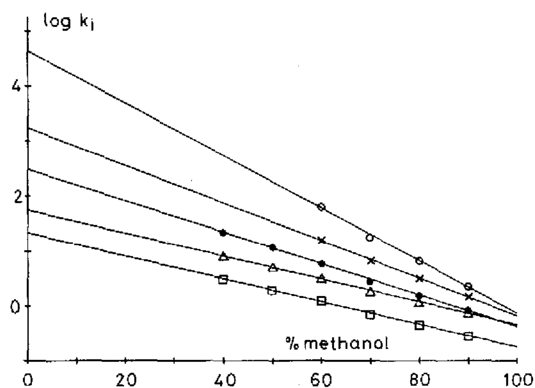

Fig. 2. Linear relationship between the isocratic capacity factors and the percentage of methanol in the mobile phase for five typical monosubstituted benzenes using the ODP column. ○, Biphenyl; ×, thioanisole; ●, methyl benzoate; △, benzaldehyde; □, benzyl alcohol.

$$\log P = 1.07(\pm 0.08) \log k_w[\text{ODS/d}] - 0.124(\pm 0.176) \quad (2)$$
$$n = 28; \quad r = 0.982; \quad s = 0.185; \quad F = 721$$

$$\log P = 1.21(\pm 0.12) \log k_w[\text{ODS}] - 0.587(\pm 0.270) \quad (3)$$
$$n = 28; \quad r = 0.971; \quad s = 0.236; \quad F = 430$$

The good relationship between these log k_w values and log P values[35] confirms previous findings[34,36]. As expected with neutral solutes, the use of *n*-decylamine only slightly improved the correlation. Indeed, the relationship would not be so good in the absence of a masking agent had basic compounds been included in the study (see later). Further, several workers have claimed that the polar character of silanol groups is essential for a non-polar stationary phase to mimic the properties of *n*-octanol by adsorbing both water and methanol molecules[37,38], as the stationary phase is viewed as a dynamic system made of silica substrate, linked organic chains and associated solvent molecules[39–41]. This reasoning ignores the abnormal behaviour of sulphonyl-containing solutes, which may interact very strongly with silanol groups and yield misleading log k_w values[34]. An additional shortcoming is that protonated masking agents must be avoided when investigating ionized acidic compounds (none of which was incorporated in the present study) giving either to ion-pair formation or to an ion-exchange mechanism[42].

PLRP-S stationary phase

The PLRP-S phase was characterized by a comparatively small number of theoretical plates and suffered from excessive shrinkage and swelling. The flow-rate was limited to 0.8 ml/min and the pressure increased dramatically at the column inlet. Retention times were inconveniently long, and only eluents with high methanol proportions could be used. Thus, no more than five compounds could be investigated with eluents containing down to 40% methanol (see Fig. 1). A dense structure and small particle size (5 μm) render PLRP-S an extremely compact stationary phase, and a pressure superior to 300 bar would be necessary to reach a flow-rate of 1.5 ml/min. It can be observed in Table I that the PLRP-S phase yields retention times that are significantly longer than those obtained with the other stationary phases. This may be related to problems of mass transfer due to the highly compact stationary phase.

The extrapolated capacity factors (log k_w[PLRP-S]) obtained with the PLRP-S stationary phase gave the following relationship with log P values:

$$\log P = 0.771(\pm 0.107) \log k_w[\text{PLRP-S}] - 0.708(\pm 0.391) \quad (4)$$
$$n = 28; \quad r = 0.946; \quad s = 0.321; \quad F = 221$$

This is a relatively unsatisfactory correlation, which can perhaps be partly explained by the technical problems mentioned above. An additional explanation may be that the PLRP-S phase does not possess polar groups such as the silanol groups in the ODS phase. As a consequence, the partitioning process will be limited to a hydrophobic mechanism, whereas lipophilicity is the sum of polar and hydrophobic contributions[43].

TABLE I

CAPACITY FACTORS EXTRAPOLATED TO 100% WATER OF 28 MONOSUBSTITUTED BENZENES DETERMINED WITH VARIOUS REVERSED-PHASE COLUMNS

No.	Compound	Log k_w[ODS/d][a]	Log k_w[ODS][b]	Log k_w[PLRP-S][c]	Log k_w[ODP][d]	Log P[e]
1	Aniline	0.95	1.13	2.34	1.46	0.90
2	Phenol	1.29	1.23	2.35	1.81	1.46
3	Benzaldehyde	1.54	1.67	3.03	1.74	1.45
4	Acetanilide	1.17	1.45	2.11	1.52	1.16
5	Benzamide	0.81	1.04	1.55	1.24	0.64
6	Benzyl alcohol	1.05	1.32	2.11	1.33	1.10
7	2-Phenylethanol	1.43	1.73	2.69	1.93	1.36
8	Benzonitrile	1.45	1.85	3.33	2.35	1.56
9	Benzenesulphonamide	0.78	0.90	1.61	1.12	0.31
10	Nitrobenzene	1.70	2.00	3.62	2.62	1.85
11	N-Methylaniline	1.51	1.74	3.38	2.26	1.66
12	N,N-Dimethylaniline	2.28	2.36	4.02	2.87	2.31
13	Phenyl acetate	1.57	2.04	3.28	2.20	1.49
14	Methyl benzoate	2.15	2.26	3.81	2.48	2.12
15	Thioanisole	2.72	2.71	4.76	3.23	2.74
16	Methyl phenyl sulphoxide	0.76	1.33	1.77	0.68	0.55
17	Methyl phenyl sulphone	0.89	1.29	2.20	1.18	0.49
18	Anisole	2.01	2.20	3.59	2.46	2.11
19	Benzene	1.91	2.08	3.88	2.40	2.13
20	Fluorobenzene	2.07	2.18	4.09	2.93	2.27
21	Chlorobenzene	2.72	2.75	4.54	3.25	2.84
22	Bromobenzene	2.89	2.89	4.76	3.63	2.99
23	Iodobenzene	3.14	3.16	5.06	3.89	3.25
24	Toluene	2.62	2.62	4.54	3.25	2.73
25	Trifluoromethylbenzene	3.11	3.11	3.16	3.68	2.79
26	Biphenyl	3.92	3.88	5.99	4.63	4.09
27	Benzophenone	3.03	3.11	4.58	3.71	3.18
28	Naphthalene	3.29	3.22	5.15	3.94	3.30

[a] Log k_w[ODS/d] is the lipophilic index extrapolated linearly to 100% water using the ODS column, with *n*-decylamine as a masking agent.
[b] Log k_w[ODS] is the lipophilic index extrapolated linearly to 100% water using the ODS column without any masking agent.
[c] Log k_w[PLRP-S] is the lipophilic index extrapolated linearly to 100% water using the PLRP-S column.
[d] Log k_w[ODP] is the lipophilic index extrapolated linearly to 100% water using the ODP column.
[e] Log *P* is the logarithm of the *n*-octanol–water partition coefficient (data from Hansch and Leo[35]).

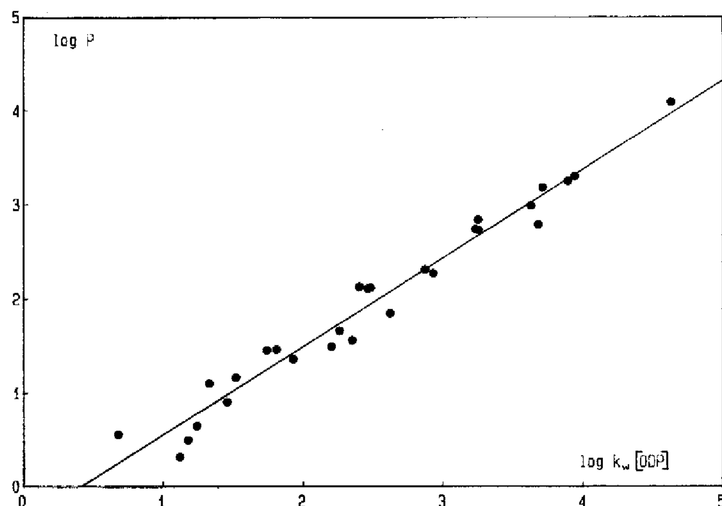

Fig. 3. Relationship between log k_w values of 28 monosubstituted benzenes (determined with the ODP phase) and partition coefficients (log P values) in the n-octanol–water system[29].

ODP stationary phase

The vinyl alcohol copolymer gel is very polar and is surface-modified with hydrophobic octadecyl groups[33]. Adsorption of both water and methanol can therefore be expected, resulting in a stationary phase that is viewed as being of dynamic nature[41,44]. Flow-rates of 1.3 and 1.5 ml/min were easily reached without too much pressure increase at the column inlet, and were chosen to obtain log k_w[ODP] values that were higher than log k_w[ODS] but lower than log k_w[PLRP-S] values (Table I). For thirteen compounds, log k_i values could be measured with eluents down to 40% methanol (Fig. 2).

The relationship between n-octanol–water partition coefficients and log k_w[ODP] values (Fig. 3) is given by the equation

$$\log P = 0.930(\pm 0.080) \log k_w[\text{ODP}] - 0.347(\pm 0.215) \quad (5)$$
$$n = 28; \quad r = 0.978; \quad s = 0.206; \quad F = 573$$

The correlation is as good as that obtained with the ODS phase (eqn. 3). Many advantages are therefore associated with the ODP phase. To assess its usefulness further, it was examined using a number of drugs the lipophilicity of which had previously been determined with the ODS column in the presence of n-decylamine[18].

Comparison of the lipophilicity of neuroleptics determined using the ODP and ODS phases

The ODS stationary phase has proved to be ineffective without a masking agent when studying basic compounds such as neuroleptics[18]. Although necessary in such instances, a masking agent has the disadvantages of introducing an additional variable into a standardized protocol.

TABLE II

LIPOPHILIC INDICES OF TWELVE NEUROLEPTIC DRUGS USING THE ODP AND ODS STATIONARY PHASES

Compound	*Log k_w[ODP]*	*Log k_w[ODS/d]*
Benperidol	3.84	3.65
Chlorpromazine	4.37	3.36
Clozapine	3.82	2.99
Flupentixol	5.21	4.33
Fluphenazine	4.87	4.31
Haloperidol	3.95	3.11
Mezilamine	2.83	2.64
Pipamperone	2.58	2.38
Sulpiride	1.53	0.61
Tefludazine	4.72	4.16
Thioridazine	4.82	3.90
Zetidoline	2.54	2.15

The ODP phase does not require a masking agent. In order to verify that the ODP phase is indeed capable of yielding a true measure of lipophilicity for basic compounds, we measured the log k_w[ODP] values of twelve neuroleptics and compared the results with log k_w[ODS/d] values (Table II):

$$\log k_w[\mathrm{ODP}] = 1.02(\pm 0.21) \log k_w[\mathrm{ODS/d}] + 0.567(\pm 0.676) \quad (6)$$
$$n = 12; \quad r = 0.962; \quad s = 0.331; \quad F = 122$$

Eqn. 6 indicates a reasonably good correlation, as also shown in Fig. 4. A better correlation was not to be expected as the two lipophilic phases are not totally identical

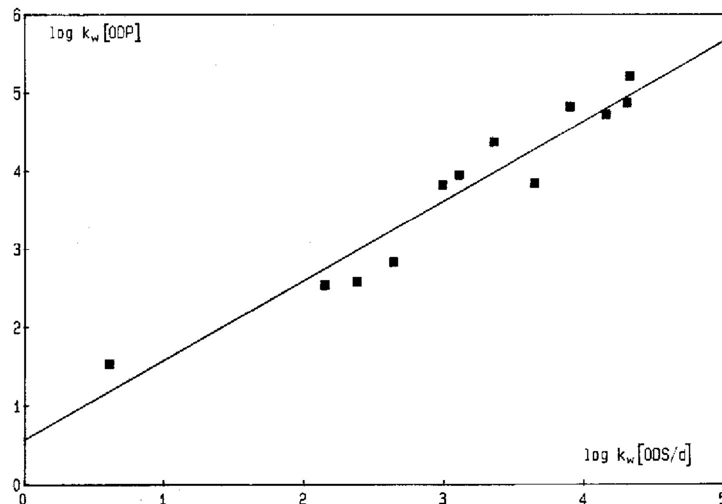

Fig. 4. Relationship between log k_w values of 12 neuroleptics determined with the ODP and ODS columns.

in terms of interactions between solute and stationary phase owing to the presence of a masking agent in one system only. Hence the log k_w values obtained with the two phases cannot be identical because the two sets of underlying intermolecular forces that they express are not identical. In this context, an open question concerns the compared hydrogen-bonding ability of the two phases towards solute molecules. The ODS phase is a strong hydrogen-bond donor and also a hydrogen-bond acceptor; the ODP phase, on the other hand, is a good hydrogen-bond acceptor, but its proportion of free hydroxyl groups and hence its hydrogen-bond donating capacity appear unknown at present and warrant specific studies. These questions, however, do not affect our conclusion that the ODP column is a valuable tool for determining the lipophilicity of basic drugs without the need to add a masking agent. A number of perturbative interactions can thus be avoided.

CONCLUSION

The ODS stationary phase is a selective and useful tool for assessing lipophilicity, and indeed is used extensively. However, its useable pH range is narrow, which restricts its applicability. In addition, the ODS phase can give rise to undesirable silanophilic interactions with ionized and basic compounds, thus necessitating a masking agent which imposes restrictions of its own.

The PLRP-S phase avoids silanophilic interactions and expands the usable pH range. However, of the three phases investigated, PLRP-S proved the least satisfactory for assessing lipophilicity owing to physical restrictions and because its retention mechanism differs from that of the two other phases. Indeed, its mechanism is based purely on hydrophobic interactions with the solute, whereas lipophilicity implies a combination of hydrophobic and electrostatic (polar) interactions[43,45].

The ODP packing indeed appears to combine the advantages of both the ODS and the PLRP-S phases without the disadvantages of either, *i.e.*, sharp resolution with a large number of theoretical plates, efficient separation of basic compounds without the need for a masking agent, stability over a wide pH range, reduced swelling and shrinkage and the possibility of having a reasonable flow-rate without undesirable pressure increases at the column inlet. The ODP stationary phase therefore offers a promising alternative to the ODS packing.

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