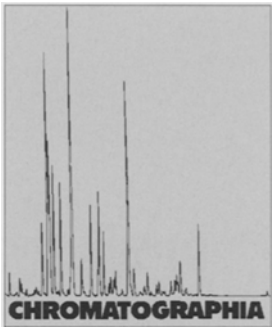
Biomembrane Lipids as Components of Chromatographic Phases: Comparative Chromatography on Coated and Bonded Phases



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Key Words

Column liquid chromatography
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Summary

Preparation of biomembrane lipid based stationary phases has been achieved by recycling 1 mM solutions of the appropriate lipid (soybean lecithin phosphatidylcholine, SLPC; phosphatidylcholine, sphingomyelin, phosphatidylethanolamine or phosphatidylserine) in methanol: water (80:20 *v/v*) through reversed-phase (C8) HPLC columns for 18 hours at 0.25 mL min^{-1}. The chromatographic characteristics (retention, peak symmetry and reproducibility and phase stability) have been assessed and compared with two commercially available bonded Immobilized Artificial Membrane (IAM) phases (IAM.PC.MG and IAM.PC.DD) by examination of the retention properties of a range of structurally diverse analytes (n = 119). The application of the SLPC phase for prediction of analyte lipophilicity (log *P*octanol/water) is shown to be comparable to the IAM.PC.MG and superior to the IAM.PC.DD bonded phases. Cross-phase comparison of analyte retention characteristics on four different lipid phases indicate that such phases may provide a rapid evaluation of analyte-lipid interactions. The dynamic coating methodology is economically viable for the small laboratory, rapid and reproducible, resulting in phase surfaces which are stable over longer periods of time than those of the commercially available bonded phases.

Introduction

In order for a drug to undergo absorption and be transported to its site of action, it must not only be soluble in aqueous media but also possess the ability to penetrate, and cross, lipid barriers. It is now a century since the pioneering studies of Overton and Meyer indicated a relationship between biological activity and oil/water partition coefficient [1]. As a result of these classical, and subsequent, investigations, it is accepted that drug hydrophobicity, or lipophilicity, is a critical property in medicinal chemistry in terms of both drug action and disposition. The terms hydrophobicity and lipophilicity are frequently used interchangeably. However, lipophilicity, as a result of the extensive contributions of Hansch and co-workers, is defined in terms of the octanol/water partition coefficient or more commonly log P [2–7], and is the term most frequently used by medicinal chemists, whereas hydrophobicity is more commonly used in chromatography.

Partition coefficients are traditionally determined by the shake-flask method [3]. However, as a result of the different experimental methods employed, published values for a single compound can vary considerably in the literature [8]. In addition, the shake-flask method is time consuming and tedious, requiring relatively large amounts of pure compound and rigorous conditions, particularly for the evaluation of compounds with log P values below approximately two or above four. There is therefore considerable interest in the development of more efficient methodologies for prediction of such data.

One of the most extensively investigated techniques for this purpose has been high-performance liquid chromatography (HPLC), and numerous stationary phases have been developed and assessed for their utility in predicting organic/aqueous partitioning data (e. g. octanol/water). Examples of such phases evaluated include silica surfaces coated with octanol [9–11], glyceryl coated controlled-pore glass [12], alkyl-bonded octadecyl (C18) and octyl (C8) phases [13–18], a polystyrene-divinylbenzene co-polymer phase [19], a series of silica phases [20], and phenyl and octadecyl modified silica gel [21]. Although many of these phases have been relatively successful as tools for prediction of drug

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partitioning between octanol and water, they have often not been so in the prediction of drug partitioning through biological membranes. This is due to the fact that octanol/water systems, which many of the phases were modelled upon or intended to mimic, are organised bulk phases with a uniform structure, and as such differ significantly to membrane systems which are interfacial phases with varying degrees of chain disorder in the hydrocarbon core of the bilayer, depending on the nature and density of lipids present [22].

It is therefore clear that if the advantages of the chromatographic approach for the determination of hydrophobicity, e. g. speed, efficiency, decreased organic solvent consumption and reproducibility, are to be exploited for the prediction of drug partitioning through biological membrane systems, the phases prepared must closely resemble the membrane systems in question. For this reason one of the most interesting approaches outlined above, developed in an attempt to predict octanol/water partitioning, was that by Miyake and co-workers [23], who used dipalmitoyl phosphatidylcholine-coated silica as a chromatographic stationary phase. Although the 'mode of coating' of the silica surface was unknown, the authors postulated that adsorption via bonding interactions between the polar lipid headgroups and the surface silanols may occur, and as such hypothesized that the hydrophobic alkyl lipid chains were protruding away from the silica surface [23]. This in fact would account for the good correlations obtained between log *P* and analyte retention [23]. The concept of utilising membrane forming lipids as stationary phases was subsequently further developed and commercialized by Pidgeon and co-workers when they introduced the so-called 'Immobilized Artificial Membrane' (IAM) phases [24].

IAM phases consist of phospholipid monolayers, e. g. phosphatidylcholine (PC) covalently bonded, via one of the lipid alkyl chains, to an aminopropyl silica support. In essence, the phases resemble half a membrane lipid bilayer with the phospholipids immobilized on the column surface so that the polar lipid headgroups protrude away from the silica surface providing the primary analyte-phase contact/interaction site [24]. In view of the structural similarity to biological membranes such phases are predicted to provide more realistic mimics of biological processes than a conventional reversed phase system. The phases developed thus far includes a range of phospholipids, e. g. phosphatidylethanolamine and phosphatidylserine [25, 26] with various end-capping reagents employed in order to decrease analyte interaction with residual propylamine moieties on the silica surface [27].

We have adopted an alternative approach to the application of biomembrane lipids as components of chromatographic stationary phases [28]. A phase prepared by dynamically coating soybean lecithin phosphatidylcholine onto a reversed-phase (C8) column was used for the prediction of log *P* octanol/water data of a large and structurally diverse compound library comprising acids, bases and neutral compounds, with a variety of pharmacological activities and physicochemical properties (e. g. log *P* range –0.52 to 7.63). Here we extend these initial observations by reporting on the stability, reproducibility of preparation and chromatographic properties of these phases in comparison to two commercially available bonded IAM phases, the IAM.PC.MG (a double acyl chain phosphatidylcholine lipid phase containing an ester linkage with the residual amines end-capped with methylglycolate) and the IAM.PC.DD (a single acyl chain phosphatidylcholine lipid phase with no glycerol backbone, the residual amines being end-capped with a propanoyl moiety) [27]. In addition, we have extended the dynamic coating methodology by utilising the four most commonly found biomembrane lipids [26], namely phosphatidylserine, phosphatidylethanolamine, sphingomyelin and an alternative source of phosphatidylcholine.

Experimental

Reagents and Chemicals

The following compounds and drugs were purchased from the companies indicated: Sigma (Poole, UK): caffeine, fenoterol, ketoprofen, acetanilide, metoprolol, 2-naphthol, haloperidol, benzophenone, biphenyl, flurbiprofen, phenobarbitone, chlorpheniramine, carbamazepine, acetylsalicylic acid, valproic acid, ranitidine, verapamil, thioridazine, carprofen, indomethacin, tolfenamic acid, promazine, chlorpromazine, trifluoperazine, quinidine, sulfamethoxazole, dipyridamole, ibuprofen, melphalan, nadolol, chlorambucil, theophylline, captopril, azathioprine, trimethoprim, amoxicillin, ephedrine, megesterol acetate; Aldrich (Poole, UK): 1-nitronaphthalene, paracetamol, 1-naphthoic acid, salicylic acid, sodium phosphate (dibasic, heptahydrate), HPLC grade acetonitrile, acetone, and methanol; Astra (Watford, UK): lignocaine; Fisons (Loughborough, UK): phenol, and B.D.H. Lab Chemicals Division (Poole, UK): naphthalene and sodium nitrite. Testosterone, propranolol, flufenamic acid, tiaprofenic acid, antipyrine, codeine phosphate, diphenhydramine, pyrilamine, hydroxyzine, desipramine were available in house (Department of Pharmacy, King's College London, UK). Soybean lecithin (min 95% pure: containing lysophatidylcholine maximum 3%), other phospholipids maximum 1%, moisture/oil content maximum 3% (Epikuron, Lucas Meyer & Co, Germany) was kindly supplied by Dr.M.J. Lawrence (Department of Pharmacy, King's College London, UK). Plant L-α phosphatidylcholine, plant L-α phosphatidylethanolamine, brain L-α phosphatidylserine (sodium salt) and brain L-α sphingomyelin, were obtained from Avanti Polar Lipids (Alabaster, USA). Cimetidine, 4-chloroaniline, 4-nitroaniline, 3-methyl-4-nitroanisole, 6-thioguanine, risperidone, paroxetine, diazepam, phenytoin, sulpiride, fluoxetine, clomipramine, imipramine, despiramine, moclobemide, chlordiazepoxide, mianserin and halofantrine as well as compounds coded 1–47 in Table I (consisting of confidential research compounds) were available in-house (SmithKline Beecham (SB) Pharmaceuticals, Harlow, UK).

Instrumentation

Reverse phase-high performance liquid chromatography (RP-HPLC) was carried out using the following systems:

1) Hewlett-Packard HP1050 (Stockport, UK) consisting of a solvent delivery system, degasser and UV detector (operated at 254 nm) all connected to a Gilson 231 (Middleton, USA) autosampler fitted with a 20 μL loop. Data collection and integration were performed using a Waters 860 data acquisition system (Milford, USA).
2) Waters 510 pump linked to an LDC detector and LDC Cl-4000 integrator (Stone, Staffs, UK). Samples were injected on column via a Rheodyne injec-

Table I. Logarithm of analyte capacity factors determined on Immobilized Artificial Membrane and coated biomembrane lipid phases.

COMPOUND	log P	log k						
		IAM/MG[a]	IAM/DD[b]	SLPC[c]	PC[d]	SM[d]	PS[d]	PE[d]
Acetanilide	1.16	−0.29	0.12	−0.16				
Acetylsalicylic acid	1.19	−1.15	−1.17	−2.08	−1.78	−1.16	−1.42	−0.96
Amoxicillin	0.33*		−1.00	−0.07				
Antipyrine	0.38	−0.62	−0.04	0.23	−0.41	−0.52	−0.47	−0.50
Azathioprine	0.10	−0.68		−1.24	−0.41	−0.54	−0.72	−0.49
Benzophenone	3.40	0.89	0.83	1.05				
Biphenyl	4.10			1.45				
Buspirone	3.43*				0.02	−0.62	0.04	−0.16
Caffeine	0.07	−1.30	−0.45	−0.76	−0.59	−1.21	−0.57	−0.85
Captopril	0.34		−1.17		−1.39		−1.35	−0.86
Carbamazepine	2.45	0.35	1.21	0.24				
Carprofen	3.93*	0.70	1.32	−0.35	−0.90	−0.23	−0.79	−0.85
Chlorambucil	1.70		−0.53	−0.32	−0.54	−0.59	−0.70	−0.49
Chlordiazepoxide	2.44				−0.02	−0.29	−0.13	−0.26
4-Chloroaniline	1.83	0.34	0.67	0.34				
Chlorpheniramine	3.39	1.12		0.32	−2.03	−1.04	−1.35	−1.79
Chlorpromazine	5.35		1.45	1.01	0.87	0.98	1.11	1.05
Cimetidine	0.40	−0.46	0.10	−0.66	−0.54	−1.17	−0.41	−0.77
Clomipramine	5.19				1.08	0.92	1.23	1.08
Codeine phosphate	0.27*	0.20	0.50	0.06	−0.15	−0.57	−0.09	−0.30
Desipramine	4.90	1.58		0.58	0.89	1.12	1.11	0.97
Diazepam	2.80				0.12	−0.33	0.14	0.00
Diphenhydramine	3.27	1.06	0.72	0.57				
Dipyridamole	2.13*			0.40				
Ephedrine	1.18		−0.07	−0.13				
Fenoterol	0.83*	0.39	0.97	−0.53				
Flufenamic acid	5.25	0.70	0.19	0.13				
Fluoxetine	4.05*				0.79	0.96	1.07	0.82
Flupentixol	4.51				0.54	0.44	0.70	0.51
Flurbiprofen	4.16	0.03	1.11	−0.51	−0.79	−0.79	−0.79	−1.25
Haloperidol	3.36		0.40	0.95	0.36	−0.28	0.46	0.18
Hydroxyzine	4.16*		0.82	0.92				
Ibuprofen	3.50	−0.08	1.33	−0.35				
Imipramine	4.80				0.96	1.02	1.12	0.98
Indomethacin	4.27	0.32	−0.16	−0.24	−0.87	−0.65	−0.81	−0.94
Ketoprofen	3.12	−0.46	0.70	−0.76	−2.09	−0.56	−1.22	−1.18
Lignocaine	2.26	0.40	−0.10	0.73				
Megesterol	3.90			1.11				
Melphalan	−0.52*		−0.45		−0.46	−0.45	−0.43	−0.44
3-Methyl-4-nitroanisole	2.32	0.13	1.06	0.76				
Metoprolol	1.88	0.26	0.33	−0.13				
Mianserin	3.90*				0.52	0.75	0.55	0.57
Moclobemide	2.13*				−0.28	−0.97	−0.40	−0.72
Nadolol	0.71	0.02	0.17	−0.29	−0.40	−1.23	−0.64	−0.75
Naphthalene	3.35			1.20				
1–Naphthoic acid	3.13	−0.84	0.05	−0.55	−0.80	−0.88		−0.92
2–Naphthol	2.80	0.89	1.42	0.71				
4–Nitroaniline	1.39	0.21	0.66	0.13	−0.42	−1.08	−0.44	−0.84
1–Nitronaphthalene	3.19		−0.08	0.91	−0.40	−0.55	−0.41	−0.48
Oxazepam	2.10		−0.10					
Paracetamol	0.51	−0.62	−0.14	−0.62	−1.80	−0.57		
Paroxetine	3.40*				0.71	0.89	1.13	0.79
Phenobarbitone	1.47	−0.29	0.14	−0.12	−0.41	−0.50	−0.48	−0.50
Phenol	1.49	0.00	0.31	0.06	−0.41	−0.51	−0.44	−0.50
Phenytoin	2.47				−0.20	−0.55	−0.23	−0.30
Promazine	4.55	1.73		0.90	0.90	1.06	1.14	1.09
Propranolol	3.56	1.17	1.30	0.38				
Pyrilamine	3.27	0.91	1.32	0.34				
Quinidine	3.44		0.99	0.59				
Ranitidine	0.27	−0.10	0.01	−0.34	−0.36	−1.42	−0.24	−0.43
Risperidone					−0.09	−0.33	−0.01	−0.28
Salicylic acid	2.26	−1.15	−0.50	−1.31				−0.86
Sulfamethoxazole	0.89	−1.15	−0.40	−0.97	−0.87	−1.03	−0.97	−0.89
Sulpiride	1.31*				−0.30	−0.36	−0.13	−0.37
Testosterone	3.32	0.13	1.25	0.52				
Theophylline	−0.02	−0.99	−0.48	−0.94	−0.74	−0.57	−0.39	−0.41
6-Thioguanine	−0.07	−0.76	−0.27	−1.13				
Thioridazine	5.90*		0.96	1.35				
Tiaprofenic acid	2.51	−0.46	0.60	−1.04	−0.82	−0.97	−0.92	−1.00
Tolfenamic acid	5.70*	0.79		0.52	−0.84	−0.11	−0.54	−0.47
Trifluoperazine	5.03				1.17	1.08	1.24	1.16
Trimethoprim	0.91	−0.29	0.52	−0.39	−0.29	−0.84	−0.45	−0.55

Table I. Continuation.

COMPOUND	log P	log k						
		IAM/MG[a]	IAM/DD[b]	SLPC[c]	PC[d]	SM[d]	PS[d]	PE[d]
Valproic acid	2.75		−0.21	−1.27	−1.29	−1.52	−1.17	−1.81
Verapamil	3.79	1.20	0.88	1.04	0.48	−0.56	−0.18	−0.23
1	4.42	1.62	0.28	0.80				
2	2.78	−0.56	0.37	−1.13	−0.96	−1.35	−2.09	−1.11
3	3.08			0.88				
4	1.59	0.31	0.31	0.33				
5	2.83	0.67	−0.44	0.73				
6	0.80	0.06	0.49	−0.55	−0.83	−0.93	−0.16	−0.44
7	0.16	0.14	−0.57	0.37				
8	2.84	0.22		−0.02				
9	2.58	0.34	0.28	−0.23	−0.22	−0.87	−0.46	−0.55
10	2.33	0.61	0.50	0.17	−0.41	−0.50	−0.15	−0.27
11	4.57				0.35	0.31	0.38	0.31
12	3.59	0.95	1.19	0.22	−0.39	−0.26	−0.24	−0.27
13	3.10	1.27	−0.25	0.83				
14	2.64	1.12	1.23	0.64	−0.16	0.01	0.04	−0.07
15	3.97	1.00	1.29	0.95				
16	2.78	0.29	0.45	0.32				
17	5.41	1.82	0.84	0.82				
18	0.93	0.20	0.32	−0.54	−0.41	−0.60	−0.05	−0.19
19	2.34	0.98	−0.12	0.10				
20				1.02	0.74	0.57	0.72	0.70
21	4.52	0.95	1.35	0.79				
22	4.25	1.03	1.37	0.34	−0.17	−0.11	0.48	0.18
23	4.05	1.07		0.79	−0.33	−0.36	0.10	−0.01
24	2.86	0.78		0.37				
25	3.30	0.81	0.19	0.49	−0.63	−0.69	0.25	−0.10
26	4.60	1.21	1.59	0.74	−0.29	−0.24	0.45	0.12
27	7.63			1.43	1.36	1.21	1.46	1.37
28	2.85	0.82	0.39	0.27	−1.13	−0.46	−0.21	−0.39
29	3.28	0.88	1.16	0.51				
30	3.69	0.94	1.40	0.60				
31	1.19	−0.32	0.54	−0.44	−0.38	0.80	−0.22	−0.44
32	3.71	1.00		0.46	−0.75	−0.72	−0.13	−0.25
33	2.73	0.64	0.81	0.02				
34	3.90		0.81	0.77	−0.18	0.02	0.11	0.03
35	5.23	1.16	1.30	1.06				
36	3.15	0.87		0.41				
37	3.40	1.23		1.22				
38	2.73		1.47	0.27				
39	5.44			1.25				
40	5.65			1.43				
41	5.75			1.18				
42	6.08			1.24				
43	4.66	1.33		0.86				
44	5.84			1.47				
45	5.84		1.23	1.44				

[a] Mobile phase: acetonitrile: phosphate buffer (35 mM, pH 7.4), (20:80 *v/v*), flow rate 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm, and IAM/MG refers to the IAM.PC.MG phase.

[b] Mobile phase: acetonitrile: phosphate buffer (35 mM, pH 7.4), (10:90 *v/v*), flow rate 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm, and IAM/DD refers to the IAM.PC.DD phase.

[c] Mobile phase: acetonitrile: phosphate buffer (35 mM, pH 7.4), (40:60 *v/v*), flow rate 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm, and SLPC refers to the soybean lecithin phosphatidylcholine phase.

[d] Mobile phase: acetonitrile: phosphate buffer (35 mM, pH 7.4), (60:40 *v/v*), flow rate 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm, and PC, PS, SM and PE refer to the phosphatidylcholine, phosphatidylserine, sphingomyelin and phosphatidylethanolamine phases respectively.

* Literature log *P* octanol/water value obtained by calculation [29].

tor (Cotati, USA) fitted with a 20 µL loop.

3) Waters 510 pump linked to a Spark Holland Promis Autosampler (P.Dekeyseyrant, Netherlands) fitted with a Rheodyne valve and 20 µL loop and connected to a Phillips 530 UV detector (Luton, UK) and LDC Cl-4000 integrator.

UV spectra were recorded using a double beam Kontron UviKon 860 scanning UV Spectrophotometer in 1 cm path length cells and spectra plotted using a Kontron chart recorder (Zurich, Switzerland).

Sources of HPLC Columns

The RP-HPLC stationary phase and guard columns utilised for the dynamic coating experiments were obtained from Hichrom (Reading, UK), and consisted of a Hypersil C8, MOS-1 (10 cm × 4.6 mm i. d., 100 A, 5 µm) column with matching guard column (1 cm × 4.6 mm i. d., 5 µm,).

Two commercially available IAM phases were used, IAM.PC.MG and IAM.PC.DD (3 cm × 4.6 mm i. d., 5 μm) from Regis Technologies (Illinois, USA) with Hichrom Hypersil C8 guard cartridges (1 cm × 4.6 mm i. d., 5 μm, MOS-1, packing material) as described above.

Dynamic Coating Methodology

For studies utilising soybean lecithin phosphatidylcholine (SLPC), the lipid was dissolved in a solution of methanol:water (80:20 *v/v*) at a concentration of 1 mM. This solution was recycled through the HPLC guard column and column for a period of 18 hours at a flow rate of 0.25 mL min^{-1} at ambient temperature. Coating of the C8 phases using the alternative membrane lipids, i. e. phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC) and sphingomyelin (SM) was carried out as described above. UV spectra of the coating solutions were recorded before and after the recycling period in the region 180–400 nm against a reference cell containing the mobile phase. On examination of the UV spectra and chromatograms following an injection of a standard compound test mixture (see below), on the C8 phase both prior to and post the dynamic coating treatment an assessment was made as to whether or not coating had occurred. Solutions of the coating lipids were prepared throughout the investigations and utilised to regenerate the phase surfaces after each period of use, this was carried out by recycling a 1 mM solution of the appropriate lipid through the phase either overnight or between experiments for a minimum period of 12 hours. Coated phases were stored in water when not in use.

Chromatographic Methodology

For chromatographic analyses, UV detection was carried out at 254 nm, the injection volume was 20 μL, and the flow rate varied between 0.5–2.0 mL min^{-1} depending on the mobile phase composition. For chromatographic analyses utilising the C8 stationary phase and the dynamically coated phases the mobile phases consisted of acetonitrile : sodium phosphate buffer (35 mM, pH 7.4) 60:40, 50:50, 40:60, 20:80 (*v/v*) and 100% sodium phosphate buffer (35 mM, pH 7.4). All coated phases were flushed well with HPLC grade water before and after use (for a minimum period of one hour) and stored in water when not in use. For studies utilising the IAM.PC.DD and IAM.PC.MG phases, the mobile phases consisted of acetonitrile:sodium phosphate buffer (35 mM, pH 7.4) 10:90, 15:85, 20:80 (*v/v*) and 100% sodium phosphate buffer (35 mM, pH 7.4). These columns were flushed with water after use and stored in acetone. All mobile phases prepared were filtered under vacuum using a Sartorius (Gottingen, Germany) filtration system with 0.2 mm Whatman nylon membrane filters (Maidstone, Kent), and degassed with helium (BOC, Guildford, UK) prior to use.

Analytes were prepared at approximate concentrations of between 0.5–1.0 mg mL^{-1} in filtered mobile phase components and sonicated until completely dissolved. Sodium nitrite (approximate concentration 1.0 mg mL^{-1}) was used as a completely unretained marker in all analyses. For all studies requiring capacity factors to be calculated, each analyte was injected in triplicate and the mean of all three retention times taken for conversion to a log capacity factor.

Assessment of Lipid Leaching

In order to quantitatively assess SLPC leaching from the dynamically coated C8 phase under typical chromatographic conditions between surface regeneration, experiments were performed in which waste eluent from a mobile phase containing 60% *v/v* acetonitrile:phosphate buffer (35 mM, pH 7.4) was collected over a five hour period. The solution was subsequently analysed by UV spectroscopy and the amount of SLPC determined.

Log *P* octanol / water

Measured log *P* octanol/water partition coefficient values used in these studies were determined by the shake-flask method obtained from in house data available at SmithKline Beecham, Harlow and the literature [29]. In cases where these were not available in literature, the calculated values are listed and are depicted in Table I by an asterisk.

Treatment of the Data

Statistical treatment of the data was carried out using ordinary least squares regression performed by relating single independent variables to the dependent variable of interest, Log *P*. In all analyses the logarithm of the calculated capacity factor was utilised. The maximum number of compounds having complete data for the model fitted was used in each regression. In all regressions, residual plots were examined to identify outliers and plots of Cook's distance to access influential compounds together with the usual checks for equality of variances and normality of the data were carried out [30, 31]. Statistical analyses and graphical presentations were performed using STATISTICA for Windows V5.1 produced by Statsoft Inc.

Equations are presented in the format shown in Eq. (1):

$$\log P = m(\pm \text{SEM}) \log k + c(\pm \text{SEM}) \quad (1)$$

Where m is the slope of the line obtained, SEM the standard error of the mean for each coefficient of the variable, and c the intercept of the line. Also included with each equation are relevant statistical parameters *n*, the number of compounds used to derive the relationship; *r*, the correlation coefficient; r^2, the square of the correlation coefficient *r* representing the proportion of the variation in the values which can be explained by the regression line; *F*, given by the ratio of the squares of standard deviations, and *p*, the *F* probability distribution which determines if the two sample data sets have different degrees of diversity.

Results and Discussion

Evaluation of Soybean Lecithin Phosphatidylcholine Dynamically Coated Phases

Following recycling of an aqueous-methanolic solution of soybean lecithin phosphatidylcholine (SLPC) (1 mM), through a C8 stationary phase for 18 hours, the quantity of lipid coated on to the phase was estimated to be approximately 35 mg by UV analysis of the coating solution both prior to and post coating [28]. The chromatographic properties of the coated phase were then evaluated by examination of the retention characteristics of a range of compounds, including acidic, basic and neutral analytes, selected from the compound library (Table I). Comparison of the capacity factors, obtained using the same stationary phase both before and

after coating and a mobile phase of acetonitrile:phosphate buffer (35 mM, pH 7.4) (40:60 *v/v*), indicated an increase in analyte retention for all compounds with no change in elution order. The properties of the coated phase were routinely monitored throughout the course of these investigations using a standard test mixture of analytes consisting of acetanilide, 2-naphthol, naphthalene, biphenyl and benzophenone, selected on the basis of the log *P* octanol/water range (1.2–4.1), and retention time on the coated phase (elution within 30 min). Comparison of the chromatograms obtained using this mixture on a C8 phase both prior to and post coating indicated no deterioration in analyte peak symmetry in terms of fronting or tailing following coating (Figure 1). During the initial investigation of the coated phases, after each day of use the SLPC coating solution was recycled through the stationary phase overnight (~16 hr) at a low flow rate (0.25 mL min^{-1}) in order to re-condition the phase surface. After re-conditioning, the test mixture was re-analysed and analyte capacity factors and peak symmetry determined. However, following further evaluation of the phases (see below), this repeated coating was deemed unnecessary. In fact after periodical examination of the test mixture to ascertain phase stability throughout the course of the investigations, the column surface was deemed to require regeneration if the observed decrease in capacity factors were 10% or greater, of the value obtained following the initial phase coating. If the regeneration was unsuccessful, i. e. restoration of the original retention characteristics was not observed, the phase was discarded. Capacity factors obtained from duplicate injections of the analyte test mixture onto one of the coated phases over five consecutive days of constant column use are presented in Table II. The results obtained, indicate that the stability of the coated surface under typical run conditions was acceptable in terms of reproducible chromatography, the coefficients of variation of the capacity factors being < 3% for all analytes. In addition, the stability of one coated phase was examined at six and twelve months after the initial coating timepoint. The results obtained, (Figure 2), again indicate phase stability under the described conditions of use and storage.

Assessment of lipid leaching under typical chromatographic conditions using a mobile phase of acetonitrile:phosphate buffer (35 mM, pH 7.4) (60:40 *v/v*), was carried out by UV examination of waste eluent collected over five hours. The results obtained indicated that the leaching of SLPC over this time period was < 0.7% of the material initially coated. Examination of the retention characteristics of the analyte standard test mixture prior to and following the leaching experiment indicated no changes in analyte elution, capacity factors, or peak symmetry.

Reproducibility of phase preparation was examined by dynamically coating five 10 cm C8 columns with SLPC, followed by examination of the chromatographic characteristics of each phase using the standard analyte test mixture. The coating methodology proved to be highly reproducible with minimal variation in analyte retention being observed between the five phases (Table III).

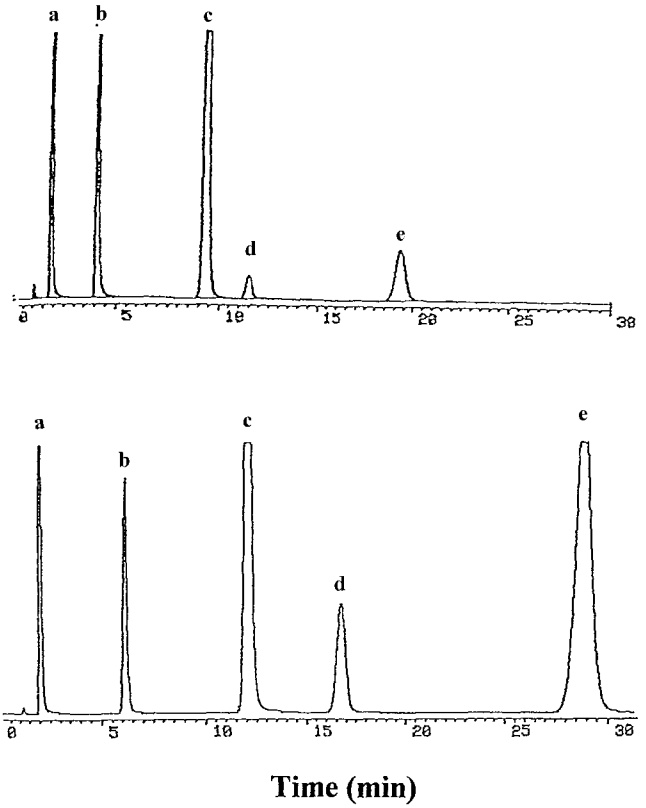

Figure 1. Typical chromatograms of the standard analyte testmix on the C8 stationary phase both prior (1) and post (2) coating with soybean lecithin phosphatidylcholine. Analyte elution: acetanilide (a), 2-naphthol (b), benzophenone (c), naphthalene (d), biphenyl (e). Chromatographic conditions: mobile phase acetonitrile:phosphate buffer (35 mM, pH 7.4) (40:60 *v/v*), at a flow rate of 1.0 mL min^{-1}, UV detection λ=254 nm.

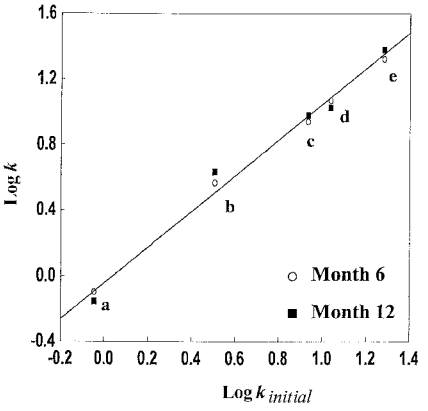

Figure 2. Reproducibility between the capacity factors of the standard test mixture analytes determined on a coated soybean lecithin phosphatidylcholine phase at six and twelve months. Analyte elution: acetanilide (a), 2–naphthol (b), benzophenone (c), naphthalene (d), biphenyl (e). Chromatographic conditions: mobile phase acetonitrile:phosphate buffer (35 mM, pH 7.4) (60:40 *v/v*), at a flow rate of 1.0 mL min^{-1}, UV detection λ=254 nm.

Comparison Between the IAM and Dynamically Coated Phases

In order to compare the dynamically coated phases with commercially available bonded phases, both the IAM.PC.MG and IAM.PC.DD phases were examined. Compounds were analysed on the IAM phases with various mobile phase compositions as indicated above (see Experimental).

Table II. Stability of a coated soybean lecithin phosphatidylcholine phase under typical mobile phase conditions and replicate injections over five days of the standard analyte test mixture components.
Chromatographic conditions: mobile phase acetonitrile:phosphate buffer (35 mM, pH 7.4) (60:40 *v/v*), at a flow rate of 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm. Where k = capacity factor, SD = standard deviation, and CV = coefficient of variation, n = 5.

Analyte	Capacity factor (k)	
	mean ± SD	CV (%)
acetanilide	0.83 ± 0.02	2.61
2-naphthol	3.58 ± 0.08	2.33
benzophenone	8.82 ± 0.12	1.41
naphthalene	11.05 ± 0.12	1.08
biphenyl	21.19 ± 0.21	1.00

Table III. Reproducibility of phase preparation: comparison of the capacity factors for the standard analyte test mixture components on five dynamically coated soybean lecithin phosphatidylcholine phases.
Chromatographic conditions: mobile phase acetonitrile:phosphate buffer (35 mM, pH 7.4) (40:60 *v/v*), at a flow rate of 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm. Where k = capacity factor, SD = standard deviation, and CV = coefficient of variation, $n = 5$.

Analyte	Capacity factor (k)	
	mean ± SD	CV (%)
acetanilide	1.43 ± 0.04	2.80
2-naphthol	5.23 ± 0.09	1.73
benzophenone	11.23 ± 0.16	1.40
naphthalene	15.66 ± 0.14	0.87
biphenyl	28.29 ± 0.13	0.47

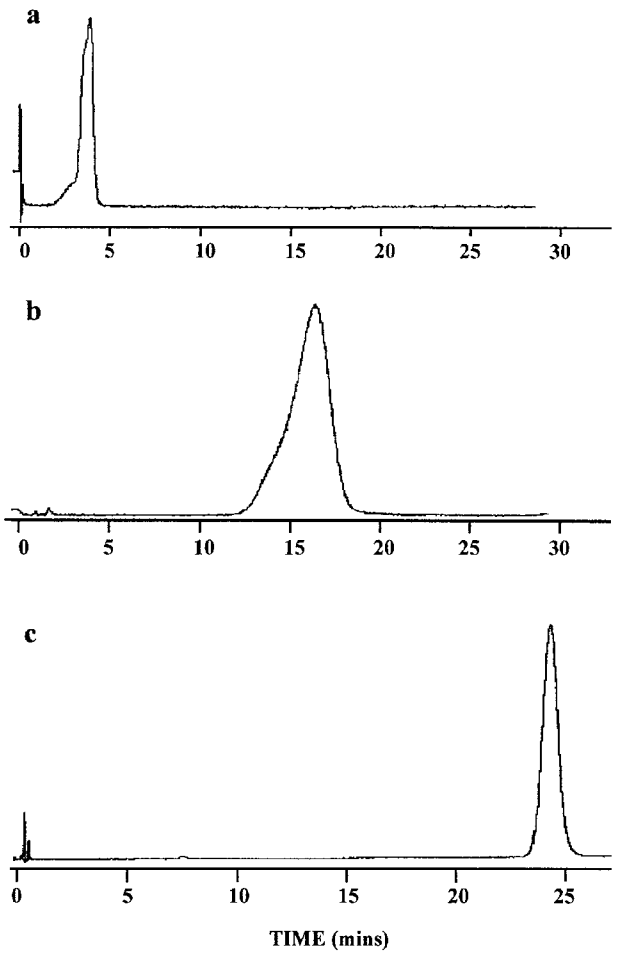

Figure 3. Chromatograms obtained for 3-methyl-4-nitroanisole on an IAM.PC.DD phase **(a)**, IAM.PC.MG phase **(b)** and a coated soybean lecithin phosphatidylcholine phase **(c)**. Chromatographic conditions: **(a)** and **(b)**: mobile phase acetonitrile:phosphate buffer (35 mM, pH 7.4) (10:90 *v/v*), at a flow rate of 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm, and **(c)**: mobile phase acetonitrile: phosphate buffer (35 mM, pH 7.4) (20:80 *v/v*), at a flow rate of 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm.

Peak symmetry achievable with these phases under the conditions outlined was much poorer than that normally seen for the dynamically coated RP-HPLC phases. For example, three chromatograms obtained following the analysis of 3-methyl-4-nitroanisole on the IAM.PC.MG, IAM.PC.DD and SLPC coated phases are presented in Figure 3. In addition, the lifetime of the IAM columns was significantly shorter than that usually seen for the coated reversed-phase columns, the chromatography deteriorating after 2–3 months of use with both peak fronting and tailing increasing markedly during this time. Similar observations regarding the stability of IAM phases have been reported [32] and these are thought to be associated with stripping of the PC moiety from the stationary phase resulting in increased interactions of analytes with aliphatic chains bound to the aminopropyl silica surface [32].

The stability of the IAM.PC phases has been investigated by perfusion of the columns with a variety of different mobile phases. For example, it has been reported that perfusion of non-end-capped columns with two litres of a citric acid buffer causes approximately 2% of the initially immobilized lipid to leach from the surface [27]. In contrast, the dynamically coated phase remained stable throughout the course of these investigations (see above), in addition, these phases could be regenerated as often as required.

In addition to the comparison of phase stability and peak symmetry, analyte retention characteristics were compared between the IAM.PC and dynamically coated SLPC phases where the log of analyte capacity factors obtained on the dynamically coated phase were compared to those obtained via the IAM.PC phases. The results obtained indicated poor correlations between the IAM.PC.DD phase and the coated phase ($r^2 = 0.12$). The relationship between the IAM.PC.MG phase and dynamically coated phase however, was considerably better, with $r^2 = 0.61$.

Taken together, these data indicate that a lipid based phase which shows comparable chromatography and increased stability relative to a commercially available bonded phase may be obtained by dynamically coating SLPC on to a C8 stationary phase.

Comparison between the Coated SLPC and Bonded IAM.PC Phases for Log *P* Prediction

Following the initial assessment of the dynamically coated SLPC phase in terms of chromatographic performance and stability, the retention characteristics of the compound library were analysed (Table I). Solutions of each analyte were injected

onto the dynamically coated phase and capacity factors determined using sodium nitrite as a totally unretained marker. Ideally, in order to successfully mimic a shake-flask octanol/water partition experiment, chromatographic retention data should yield information on analyte partition between hydrophobic and hydrophilic phases. Frequently however, mobile phases employed consist of an aqueous phase with varying amounts of organic modifier to ensure reasonable/practical retention times. For this reason, analyte capacity factors were determined using a range of mobile phase compositions and capacity factors back extrapolated to yield values for 100% aqueous phase [18, 33, 34]. Example extrapolations to aqueous for four compounds analysed at three different mobile phase compositions on the dynamically coated SLPC phase are shown in Figure 4.

Analyte log P octanol/water values were plotted against the corresponding logarithms of the capacity factors obtained at each mobile phase composition (including the extrapolated values for zero organic modifier), and the relationships statistically evaluated. Previously, we [28] and others [35], have noted in data analyses of this type that when carboxylic acids are treated separately to basic amines and neutral compounds, improved relationships between log capacity factors and log P octanol/water are observed. This difference is thought to arise due to significant partitioning of the charged forms of some amines into phospholipid phases which does not occur with carboxylate ions [36] and may be associated with energetic differences between protonated amine and carboxylate ion interactions with the negatively charged phosphate and positively charged choline moieties of the lipid head groups respectively [36]. As a result of the above we examined the relationships between lipophilicity and analyte retention following division of the data into two subsets. Eqs (2) and (3) describe the relationships between log P octanol/water and log capacity factors for basic/neutral and acidic compounds respectively, determined using the SLPC column with a mobile phase containing 40% acetonitrile (see also Figure 5).

$$\text{Log } P = 2.36(\pm 0.16) \log k + 1.92(\pm 0.12) \quad (n = 85, r = 0.85, r^2 = 0.73, F(1,83) = 223.3, p < 0.0001) \quad (2)$$

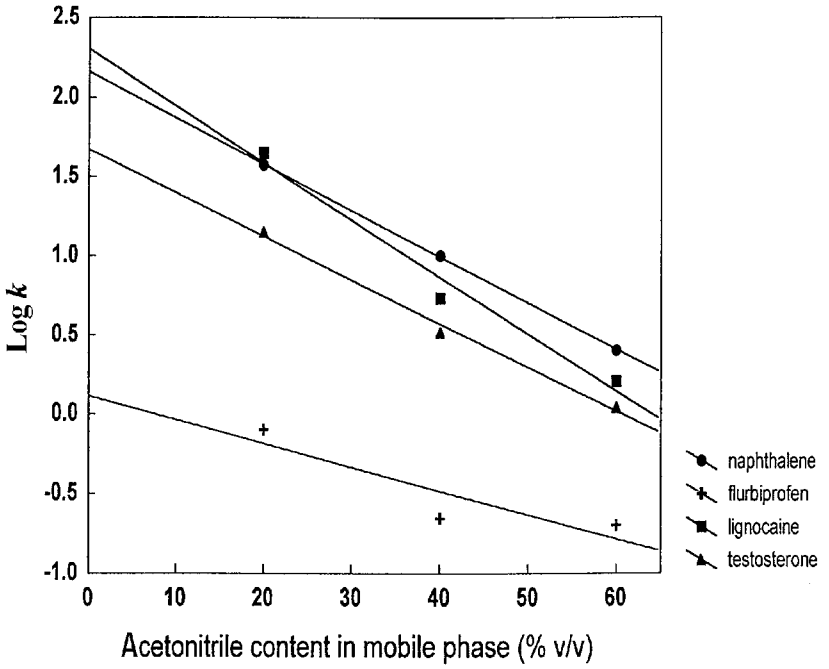

Figure 4. Example extrapolations to aqueous mobile phase for four compounds chromatographed under three mobile phase conditions on the coated soybean lecithin phosphatidylcholine phase. Equations for each analyte described below are in the form $\log k_w = m\psi + c$ are:
(ψ being the acetonitrile content in the mobile phase):
Naphthalene: $\log k_w = -0.029\,\psi + 2.162$ ($r = 0.99$); Lignocaine: $\log k_w = -0.036\,\psi + 2.310$ ($r = 0.99$)
Testosterone: $\log k_w = -0.028\,\psi + 1.674$ ($r = 0.99$); Flurbiprofen: $\log k_w = -0.063\,\psi + 2.199$ ($r = 0.99$)
Chromatographic conditions: mobile phase acetonitrile:phosphate buffer (35 mM, pH 7.4) (60:40; 40:60 or 20:80 v/v), at a flow rate of 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm.

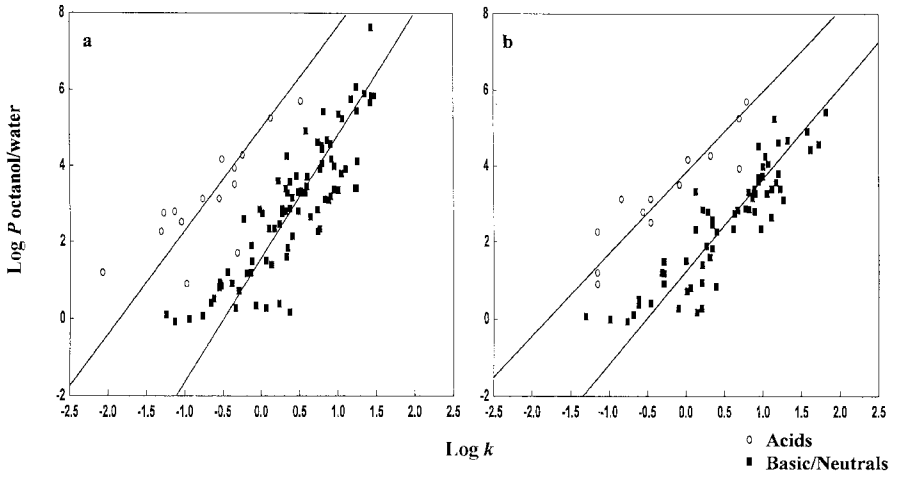

Figure 5. Correlation of capacity factors determined on a coated soybean lecithin phosphatidylcholine phase (a) and an IAM.PC.MG phase (b), with log P octanol/water for the compound series in Table I. Individual plots presented for acidic and basic/neutral analytes in each case. Chromatographic conditions: (a): mobile phase acetonitrile:phosphate buffer (35 mM, pH 7.4) (40:60 v/v), at a flow rate of 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm and (b): mobile phase acetonitrile:phosphate buffer (35 mM, pH 7.4) (20:80 v/v), at a flow rate of 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm.

$$\text{Log } P = 1.70(\pm 0.36) \log k + 4.30(\pm 0.33) \quad (n = 15, r = 0.79, r^2 = 0.63, F(1,13) = 22.3\ p < 0.0004) \quad (3)$$

The corresponding relationships for the extrapolated log k_w values are presented in Eqs (4) and (5).

$$\text{Log } P = 1.54(\pm 0.13) \log k_w + 0.38(\pm 0.25) \quad (n = 86, r = 0.78, r^2 = 0.62, F(1,84) = 134.8, p < 0.0001) \quad (4)$$

$$\text{Log } P = 1.36(\pm 0.28) \log k_w + 1.00(\pm 0.47) \quad (n = 16, r = 0.79, r^2 = 0.62, F(1,14) = 23.1, p < 0.0003) \quad (5)$$

It is of interest to note that using the extrapolated values (Eqs (4) and (5)) the differences between the correlation equations for basic / neutral and acidic analytes, both in terms of the gradients of the lines and the statistics of the relationships, are

not as great as those observed using the 40% acetonitrile organic modifier. Comparison of Eqs (2) and (3) with (4) and (5) indicates no advantages, in terms of the statistical evaluation of the relationships, in using the extrapolated log k_w values in comparison to those obtained using the 40% acetonitrile mobile phase. In addition, from a practical viewpoint we recommend that 40% organic component in the mobile phase be utilised as the most convenient condition, where analyte retention is neither excessively short for the polar compounds (as seen as seen at 60%), or long as seen at 20% organic for the more hydrophobic analytes [28].

In order to compare the coated to the two bonded IAM phases, a similar analysis was carried out using the same compound library. In the case of the bonded phases, a mobile phase of acetonitrile: phosphate buffer (35 mM; pH 7.4) was used with the content of organic modifier reaching a maximum of 20% by volume. These investigations were in general less successful than those using the dynamically coated SLPC phase as a result of the poorer chromatography, e. g. poor peak symmetry obtained with the bonded phases. As a result, the number of analytes used in the statistical analysis are reduced. However, the number, and structural diversity of analytes for which satisfactory chromatographic parameters (Table I) could be obtained was sufficient for a comparison between the coated and bonded phases to be made.

The 'best' correlations, between log P octanol/water and log k for both the bonded IAM phases, in terms of the statistical parameters, were obtained with mobile phases containing 20 and 10% acetonitrile by volume in phosphate buffer (35 mM; pH 7.4) for the IAM.PC.MG ($r^2 = 0.54$, $n = 76$) and IAM.PC.DD ($r^2 = 0.39$, $n = 80$) respectively. Separation of the analytes into basic/neutral and acidic compound subsets resulted in marked improvements in terms of the statistics of the relationships for the IAM.PC.MG phase, which are described by Eqs (6) and (7) for basic/neutral and acidic compounds respectively (Figure 5):

$$\text{Log } P = 1.94(\pm 0.12) \log k + 1.48(\pm 0.11) \quad (n = 63, r = 0.90, r^2 = 0.80, F(1,61) = 248.2, p < 0.0001) \quad (6)$$

$$\text{Log } P = 1.79(\pm 0.24) \log k + 3.74(\pm 0.17) \quad (n = 13, r = 0.92, r^2 = 0.84, F(1,11) = 58.0, p < 0.0003) \quad (7)$$

In the case of the IAM.PC.DD phase only relatively modest improvements were observed, Eqs (8) and (9) representing data obtained for basic/neutral and acidic analytes respectively:

$$\text{Log } P = 1.70(\pm 0.24) \log k + 1.56(\pm 0.20) \quad (n = 64, r = 0.67, r^2 = 0.44, F(1,62) = 49.2, p < 0.0001) \quad (8)$$

$$\text{Log } P = 1.31(\pm 0.39) \log k + 2.49(\pm 0.30) \quad (n = 16, r = 0.67, r^2 = 0.45, F(1,14) = 11.4, p < 0.0005) \quad (9)$$

Comparison of the data obtained for log P prediction using the dynamically coated SLPC phase with that obtained using the bonded IAM phases indicates, for the compound library used, that the coated phase produces data comparable to that obtained using the IAM.PC.MG phase and superior to that produced using the IAM.PC.DD phase.

It is also of interest to note that the relationship between log k values determined on the two bonded IAM phases yielded poor correlation coefficients indicating that the differences in phase surface have a significant effect on analyte-stationary phase interaction.

Investigation of Dynamic Phase Coating Methodology for Alternative Biomembrane Lipids

Following the development and examination of stationary phases coated with soybean lecithin (SLPC), additional investigations were carried out using alternative membrane lipids in order to establish the applicability of the approach. The additional lipids utilised were phosphatidylethanolamine (PE), sphingomyelin (SM) and phosphatidylserine (PS), and an additional source of phosphatidylcholine (PC) from a plant extract.

Dynamic coating and subsequent assessment of the above lipids was carried out in duplicate in a similar manner to that described previously for the soybean lecithin phase. The coating methodology was successfully duplicated for each lipid and good reproducibility obtained as determined by comparison of analyte capacity factors between duplicate phases. Examination of the retention characteristics of a more restricted compound set (n = 65), including acidic, basic and neutral compounds over the log P range of –0.52 to 7.63, on all four phases indicated the analyte elution order to be essentially unchanged across the phases with peak symmetry unaffected by coating.

The interaction between an analyte and a coated, or bonded, phase depends upon both polar and nonpolar interactions with the polar lipid head groups and their nonpolar hydrophobic chains. In theory, the higher the correlation for analyte retention between phases, then the greater the similarity between phases in terms of the analyte-lipid interaction. Examination of between phase analyte retention relationships should therefore enable phase/lipid categorisation and in turn limit the number of lipids required for evaluation of membrane partition phenomena. For example, a correlation of analyte retention data between two phases yielding a slope of approximately unity, with a minimal intercept value and a good correlation coefficient would indicate that the retention mechanisms, and hence analyte-lipid interactions, between the phases are essentially identical. Conversely, relationships yielding slopes other than unity, with poorer correlation coefficients would indicate that the analyte-lipid interaction between the phases differs and therefore the data derived provides additional information.

A cross-phase correlation analysis was carried out using the data derived from an examination of the compound set on the four lipid phases prepared. The slopes of the lines ranged between 0.75 and 0.92 for the SM versus PC and SM versus PE phase relationships respectively, and the correlation coefficients (r) ranged between 0.77 and 0.95 for the SM versus PC and PE versus PS phases respectively. In order to establish if the previously observed differentiation of analytes into basic/neutral and acidic subsets would influence the relationships, further correlations were carried out using two analyte subsets. Examination of the acidic compound subset (n = 13 to 15 analytes depending upon the correlation) yielded very poor relationships. Relationships for the basic/neutral analyte subset (n = 48 or 49) however, were considerably better, and improved compared to the total analyte group analysis with slopes ranging between 0.89 and 1.15 with correlation coefficients between 0.83 and 0.98 (Table IV).

The above data are in agreement with the observations presented previously regarding the differential interactions between acidic analytes and lipid phases in comparison to the basic/neutral com-

pounds. From the poor statistics and variable slopes of the correlations obtained in the cross-phase analysis, it would appear that acidic analytes interact differently with each lipid and in the particular case of the SM and PC phases, the derived relationship only accounted for 9% of the observed variability. There is also evidence in the literature that analyte structure is also of significance. Barbato et al. [37], using an IAM phase, have demonstrated differential relationships between lipophilicity and analyte retention for a series of arylalkyl and aromatic acid derivative nonsteroidal anti-inflammatory drugs. These authors [37] postulated that electronic interactions during the partitioning process influenced the lipophilic interaction. Thus, both the structure of the analyte and nature of the lipid headgroup may influence the interaction. This is obviously an area requiring systematic investigation.

The relationships presented in Table IV of the cross-phase correlations for the basic/neutral analyte subset yielded slopes approximating to unity, with relatively small intercepts and acceptable correlation coefficients. However, cursory examination of the cross correlation plots appeared to indicate greater variability in the data for the faster eluting, i. e. more polar analytes, in comparison to the later eluting compounds (Figure 6). In order to examine this hypothesis, the analyte sets for each correlation were divided at the median value into two subsets and the sum of squares of the residual values for each analyte for both the upper and lower portions of the curves compared. Using this approach the ratio of the sum of squares of the residuals of the upper and lower portions of the relationships should be 1, <1 or >1 if the data are uniformly scattered about the line, or greater variability is seen at the lower or upper ends of the line respectively. In each case the observed ratio was <1 indicating a reduced variability of the data the greater the retention (the ratios are presented in Table IV). Hence, the more polar the analyte, i. e. faster eluting, the greater the variability in the cross-phase correlation data, whereas the more lipophilic, slower eluting compounds, yielded considerably reduced variability. As the nonpolar hydrophobic analyte-phase interactions become more significant in analyte retention, then greater similarity between phases would be expected irrespective of the nature of the polar lipid head group. In contrast, for more polar analytes in which interac-

Table IV. Between phase correlation analysis for the retention of a series of basic and neutral analytes on four coated biomembrane lipid phases.

Phase Comparison[a]	Equation	Statistical Parameters				Ratio RSS[b]
		r	r^2	F	n	
PE v PS	$\log k_{PE} = 0.99 \log k_{PS} - 0.15$	0.98	0.96	1146	49	0.60
SM v PE	$\log k_{SM} = 1.15 \log k_{PE} - 0.19$	0.96	0.92	524	48	0.59
SM v PS	$\log k_{SM} = 1.11 \log k_{PS} - 0.35$	0.92	0.85	259	48	0.50
PE v PC	$\log k_{PE} = 0.92 \log k_{PC} - 0.002$	0.90	0.81	200	49	0.70
PS v PC	$\log k_{PS} = 0.89 \log k_{PC} + 0.15$	0.88	0.77	156	49	0.56
SM v PC	$\log k_{SM} = 0.93 \log k_{PC} - 0.16$	0.83	0.69	103	49	0.29

[a] PC, PS, SM and PE refer to the phosphatidylcholine, phosphatidylserine, sphingomyelin and phosphatidylethanolamine phases respectively.

[b] Ratio of the sum of squares of the residuals of the data of the upper and lower portions of the relationships. The division between the analytes was made at the median value for each relationship.

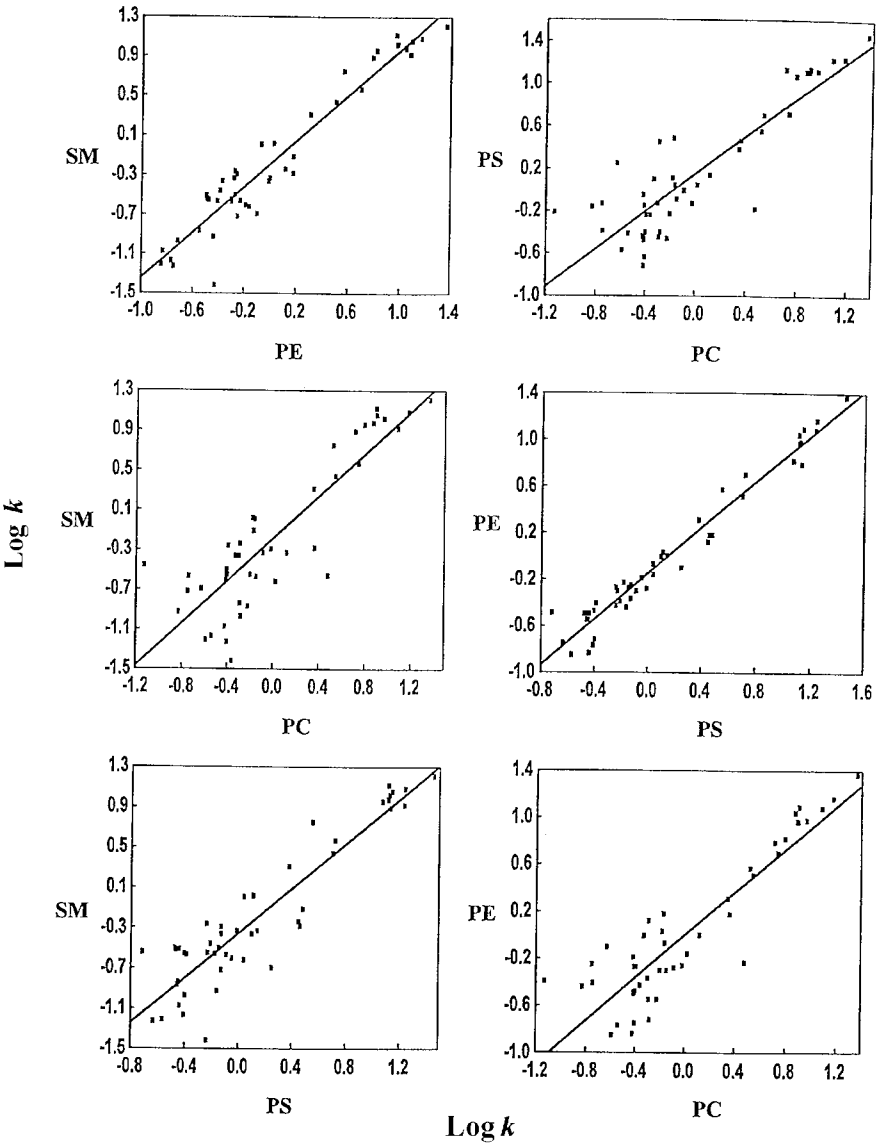

Figure 6. Between phase correlation for analyte logarithm capacity factors determined on four coated biomembrane lipid phases. Where PC, PS, SM and PE refer to the phosphatidylcholine, phosphatidylserine, sphingomyelin and phosphatidylethanolamine phases respectively. Chromatographic conditions: mobile phase acetonitrile:phosphate buffer (35 mM, pH 7.4) (60:40 *v/v*), at a flow rate of 1.0 mL min^{-1}, UV detection $\lambda = 254$ nm.

tions with the lipid head group are of greater significance during partitioning, then greater variability between phases would be expected. This view is supported by the greater variability of the data in the cross-phase correlation relationships with the more polar, rapidly eluting analytes observed in the present investigation.

The above investigation illustrates the possible variability associated with analyte-lipid interactions and also the potential of this chromatographic approach to rapidly examine such interactions. The determination of analyte retention data using a multiple phase approach may also provide an insight into the relative significance of the polar versus nonpolar, hydrophobic, interactions in partitioning for a particular analyte or series of analytes.

Conclusions

Dynamic coating of soybean lecithin phosphatidylcholine onto reversed-phase (C8) HPLC stationary phases has been shown to produce a phase comparable to the commercially available IAM bonded phases by an examination of the retention characteristics of a large and structurally diverse compound library. The methodology has also been used to prepare four other biomembrane lipid phases using PE, PS, SM and PC. Preparation of the coated phases is reproducible as shown by comparison of the chromatographic properties of a range of analytes determined on five columns individually coated with SLPC. The stability of the phases on extended use, their facile regeneration, together with the ability to rapidly and cost effectively prepare a variety of phases render the dynamic coating approach attractive. In contrast, the performance of the commercially available IAM phases was disappointing in terms of column stability, peak reproducibility and symmetry. In view of these problems, the dynamically coated phases offer distinct advantages.

Evaluation of the coated SLPC phase and the bonded IAM phases for prediction of analyte lipophilicity (log *P*) indicated that the coated phase produced data comparable to the IAM.PC.MG and superior to that produced using the IAM.PC.DD phase. Correlation analyses of analyte retention between phases coated with different lipids indicate that such phases may also provide useful methodology for the rapid comparison of analyte-lipid interactions.

In conclusion, dynamic coating of biomembrane lipids onto reversed-phase HPLC columns is a facile, cost effective process for the rapid preparation of a variety of phases, producing hydrophobicity data similar to that obtained with the commercially available IAM.PC.MG phase. Having shown the applicability of the coated phases for chromatographic analysis, it was deemed appropriate to examine the surface characteristics of the phases in order to provide some insight into the nature of the interaction between the hydrophobic surface and the coated lipid. Details of these investigations will be presented elsewhere [38].

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Note from authors

Since the submission of this manuscript we have become aware of the paper by Krause et al. who used a C_{18} stationary phase coated with dimyristoylphosphatidylcholine to examine peptide-lipid interactions. The attention of interested readers is drawn to this utility of dynamically coating reversed phase HPLC stationary phases with biomembrane lipids.

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