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Predicting Drug–Membrane Interactions by HPLC: Structural Requirements of Chromatographic Surfaces

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Drug–membrane interactions have recently been studied by immobilized artificial membrane (IAM) chromatography (Pidgeon, C.; et al. *J. Med. Chem.* 1995, *38*, 590–595. Ong, S.; et al. *Anal. Chem.* 1995, *67*, 755–762), and the molecular recognition properties of IAM surfaces toward drug binding/partitioning appear to be remarkably close to the molecular recognition properties of fluid membranes. The structural requirements of chromatography surfaces to emulate biological partitioning are unknown. To begin to elucidate the surface structural requirements needed to predict drug partitioning into membranes, three bonded phases were prepared. The chromatography bonded phases were prepared by immobilizing (i) a single-chain analog containing the phosphocholine (PC) headgroup (IAM.PC.DD), (ii) a long-chain alcohol containing polar OH groups protruding from the surface (12-OH-silica), and (iii) a long-chain fatty acid containing OCH_3 groups protruding from the surface (12-MO-silica). The 12-OH-silica surface can be considered as an immobilized "octanol" phase with OH groups protruding from the surface and is therefore a solid phase model of octanol/water partitioning systems. As expected, improved capability of predicting solute–membrane interactions as found for the chromatographic surface containing the PC polar head-group because the PC headgroup is also found in natural cell membranes. For instance, the IAM.PC.DD column predicted drug partitioning into dimyristoylphosphatidylcholine liposomes ($r = 0.864$) better than 12-OH-silica ($r = 0.812$), and 12-MO-silica ($r = 0.817$). IAM. PC.DD columns also predicted intestinal drug absorption ($r = 0.788$) better than 12-OH-silica ($r = 0.590$) and 12-MO-silica ($r = 0.681$); reversed phase octadecylsilica (ODS) columns could not predict intestinal absorption ($r = 0.10$). Collectively, these results suggest that chromatographic surfaces containing interfacial polar groups, i.e., PC, OH, and OCH_3, model drug–membrane interactions, but surfaces lacking interfacial polar functional groups (e.g., ODS surface) are poor models. Most interestingly, drug partitioning into octanol/water systems does not correlate with drug binding to the immobilized octanol phase. However, drug partitioning into immobilized octanol correlates with drug partitioning into liposomes ($r = 0.812$).

Immobilized artificial membranes (IAMs) are solid phase membrane mimics whereby phospholipid molecules are covalently bonded to silica particles at a monolayer density.[3–5] Recently, we have developed IAMs as a novel method for rapidly predicting drug–membrane partitioning and drug–membrane transport by using IAMs as a stationary phase in high-performance liquid chromatography (HPLC) systems.[1,2]

The success of IAM chromatography in predicting drug–membrane interactions is based on the structural similarities between the immobilized ligand comprising IAMs and the phospholipids comprising membrane bilayers.[2] Most interestingly, the phospholipids found in cell membranes contain predominantly diacylated phosphocholine (PC) lipids, but all of the IAM surfaces shown in Chart 1 are prepared from PC ligands. One ligand is a PC diacylated ester, and the other ligands are single-chain PC analogs with and without the glycerol backbone. In spite of these structural differences, all of the IAM surfaces shown in Chart 1 give virtually identical results in predicting drug partitioning into fluid dimyristoylphosphatidylcholine (DMPC) liposomes.[2,6] Thus the glycerol backbone, the linkage between the glycerol backbone, the acyl chain linkage (ether linkage or ester linkage), and the number of acyl chains are not critical structural features of surfaces that are intended to model drug–membrane interactions. The most important structural components of IAMs are a phospholipid headgroup and a hydrocarbon chain.

A natural question arising from the above finding is whether surfaces prepared by immobilized ligands with polar groups other than PC protruding from the surface can be good membrane models. In particular, since the octanol/water partitioning system has previously been extensively used to predict drug–membrane interactions prior to IAM chromatography, we speculated that immobilized octanol may create a surface that is a good membrane

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(1) Pidgeon, C.; Ong, S.; Liu, H.; Qiu, X.; Pidgeon, M.; Dantzig, A.; Munroe, J.; Hornback, W. J.; Kasher, J. S.; Glunz, L.; Szczerba, T. *J. Med. Chem.* **1995**, *38*, 590–594.
(2) Ong, S.; Liu, H.; Qiu, X.; Bhat, G.; Pidgeon, C. *Anal. Chem.* **1995**, *67*, 755–762.
(3) Pidgeon, C. U.S. Patent 4,927,879, 1990.
(4) Pidgeon, C. U.S. Patent 4,931,498, 1990.
(5) Pidgeon, C.; Venkatarum, U. V. *Anal. Biochem.* **1989**, *176*, 36–47.
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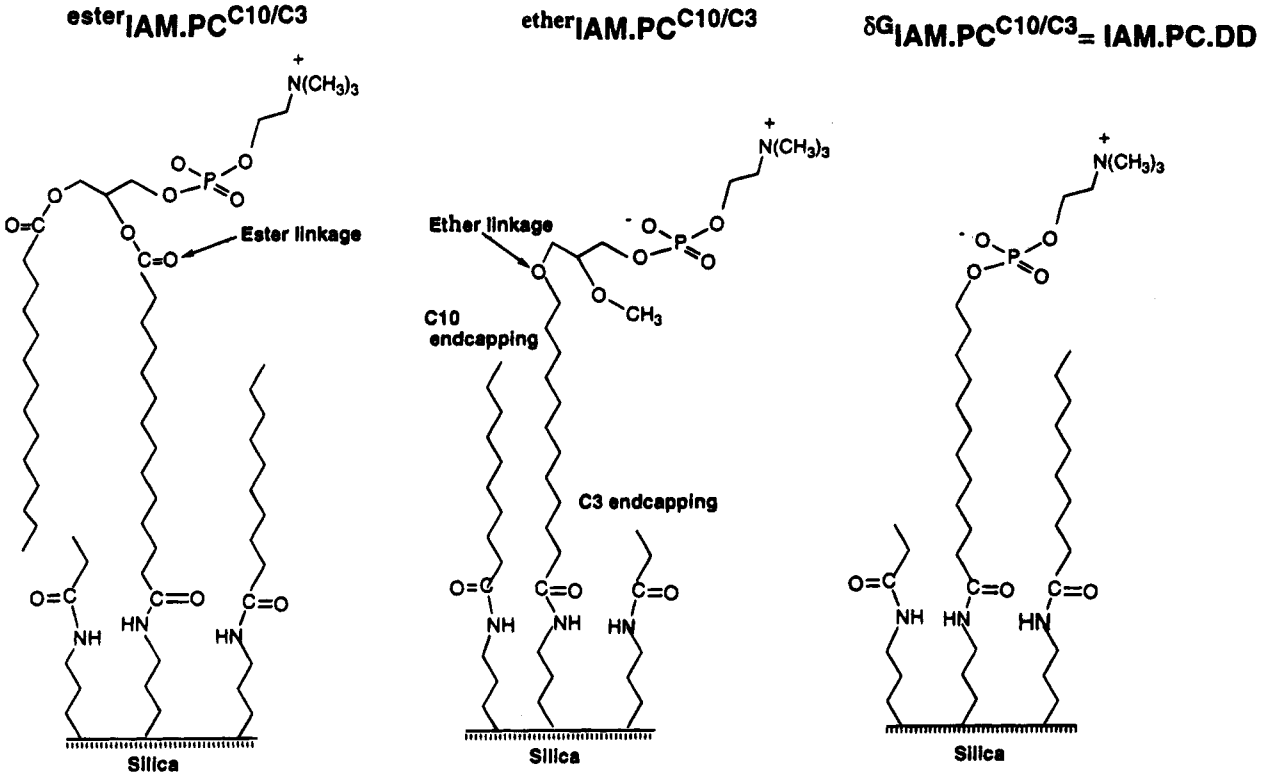
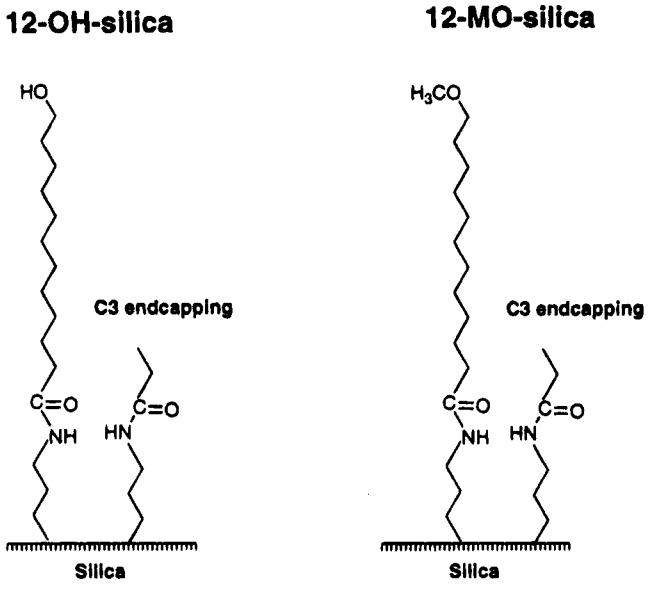
Chart 1. Structures of Three IAM.PC Bonded Phases



model. To address this question, we synthesized 12-hydroxydodecanoic silica propyl amide (denoted as 12-OH-silica) and evaluated the ability of this surface to predict drug partitioning into *n*-octanol/water phases and also to predict drug partitioning into fluid membranes. 12-OH-silica is effectively immobilized alcohol and can be considered as a solid phase model of the *n*-octanol/water partitioning system. 12-OH-silica contains both hydrogen bond donor and acceptor capabilities at the surface. To probe the effect of hydrogen bonding at the chromatographic interface, a surface lacking hydrogen bond donor capabilities was also prepared by immobilizing 12-methoxydodecanoic acid (12-MO) on silica propyl amine (SPA) to form 12-MO-silica. The general structures of 12-OH-silica and 12-MO-silica are shown in Chart 2 which shows that monolayers of OH groups form on the 12-OH-silica surface and monolayers of OCH_3 groups form on the 12-MO-silica surface.

Chart 2. Structures of 12-OH-silica and 12-MO-silica Bonded Phases

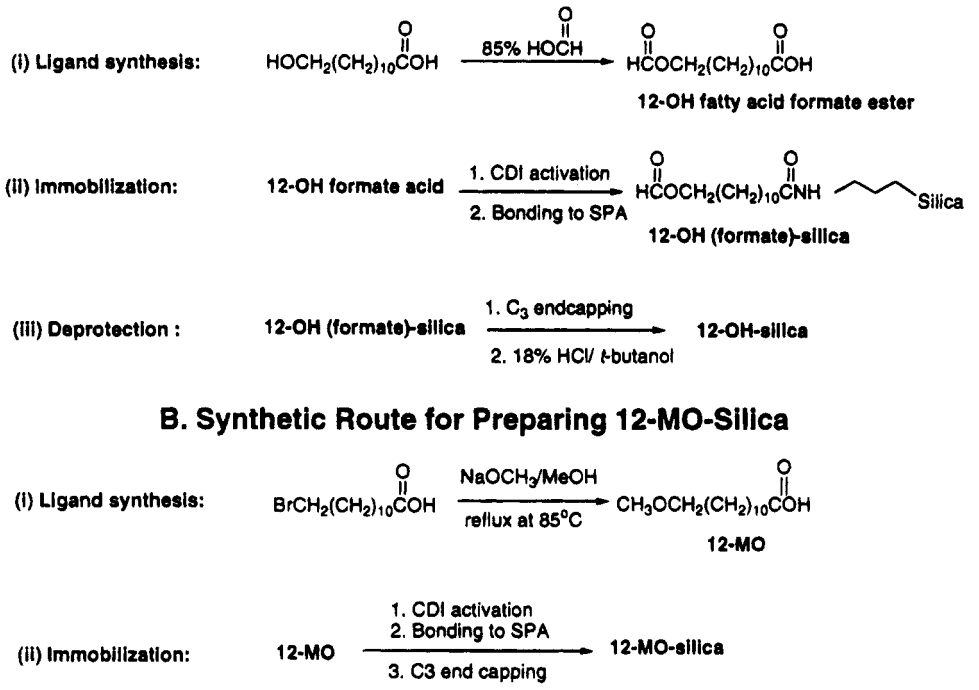


EXPERIMENTAL SECTION

Chemicals. 12-Bromododecanoic acid, 12-hydroxydodecanoic acid, sodium methoxide (25 wt % solution in methanol), tetrahydrofuran, alcohol-free chloroform, and propionic anhydride were purchased from Aldrich Chemical Co. (Milwaukee, WI). Tetrahydrofuran was dried by refluxing over sodium under nitrogen. Methanol was from Mallinckrodt Inc. (Paris, KY) and was distilled over CaH_2. The following chemicals were ordered from Sigma Chemical Co. (St. Louis, MO): xylometazoline, oxymetazoline, naphazoline, tetrahydrozoline, clonidine, propranolol hydrochloride, alprenolol, oxprenolol, metaprolol, pindolol, nadolol, atenolol, tramazoline, phosphate buffered saline (PBS) tablets, 1,1′-carbonyldiimidazole (CDI), salicylic acid, *m*-nitrobenzoic acid, acetylsalicylic acid, benzoic acid, phenol, acetanilide, theophylline, *p*-nitroaniline, antipyrine, *m*-nitroaniline, aniline, and *p*-toluidine. The imidazolidine derivatives ST608, ST475, ST476, ST603, STH2224, STH2100, ST585, ST600, ST606, and ST590 were kind gifts from Boehringer-Ingelheim Co. Hydrochloric acid and formic acid were from Fisher Scientific (Pittsburgh, PA). SPA chromatography particles were kindly provided by Regis Technologies Inc. (Monton Grove, IL). Chromatography columns were prepared by bonding synthetic ligands to the SPA. The SPA particles were 12 μm for preparing both 12-MO-silica and 12-OH-silica chromatography surfaces and 5 μm for preparing the IAM.PC.DD column.

Syntheses. (i) Synthesis of IAM.PC.DD. The single-chain PC ligand that lacks a glycerol backbone ($^{\delta G}$IAM.PC$^{C10/C3}$, shown in Chart 1) is commercially produced as an IAM.PC.DD column

Scheme 1. Syntheses of 12-OH-silica and 12-MO-silica Surfaces



(where "DD" stands for drug discovery), supplied by Regis Technologies Inc.

(ii) Synthesis of 12-OH-silica. A linear bifunctional lipid was required that contained an OH group on one end of the alkyl chain and a carboxyl group at the other end of the chain. Octanol is an eight-carbon primary alcohol with an ω-methyl that cannot be used to immobilize octanol with the orientation of the OH groups protruding from the surface. The eight-carbon bifunctional analog of octanol, ω-carboxyl-1-octanol, was not commercially available. Consequently, we used ω-carboxyl-1-undecanol as the bifunctional lipid. Although ω-carboxyl-1-undecanol contains four additional carbons compared to octanol, immobilization of the ω-carboxyl-1-undecanol causes orientation of the lipid such that the four additional carbons contribute only ~5–6 Å of hydrophobic thickness to the surface.

Scheme 1A shows the synthetic pathway for preparing 12-OH-silica. The ligand required for preparing this surface was ω-carboxyl-1-undecanoyl formate (12-OH fatty acid formate ester). To prepare this ligand, 10 mL of formic acid (88 wt %) and 2.0 g (9.24 mmol) of 12-hydroxydodecanoic acid were dissolved in 20 mL of *tert*-butyl alcohol and heated at 65 °C for 1.5 h. TLC on silica gel ($CHCl_3$:MeOH = 9:1) showed that there is only one spot (rf = 0.49) which differed from the starting material (rf = 0.31). A new peak at 1725 cm^{-1} was found in the IR spectrum of the reaction mixture, indicating that an ester bond was formed between formic acid and 12-hydroxydodecanoic acid. Flash chromatography was used to purify the product (12-OH fatty acid formate ester) with 400 mL of organic solvent ($CHCl_3$:MeOH = 9:1). Fractions containing the 12-OH fatty acid formate ester were pooled, rotary evaporated, and vacuum pumped to dryness, to give 1.969 g of 12-OH fatty acid formate ester (87.2% yield): ^1H NMR ($CDCl_3$) δ 8.80 (s, 1H), 4.14 (t, 2H), 2.31 (t, 2H), 1.66–1.62 (m, 4H), and 1.25 (br s, 14H).

The procedure for immobilization of the 12-OH fatty acid formate ester on SPA, followed by propionyl (C3) endcapping to form 12-HO-silica, was the same as that for the synthesis of IAMs described previously.[7,8] Briefly, 1.5 g of 12-OH fatty acid formate ester was activated by 0.50 g of CDI in chloroform and then bonded to 5.0 g of SPA, followed by C3 endcapping. The density of the immobilized ligand (i.e., 12-OH fatty acid formate ester) was 110 μmol/g of silica, measured using elemental analysis.[7] The formate group was deprotected on the surface as follows: 5.0 g of ω-propyl amide 1-undecanoyl formate silica (12-OH(formate)-silica) was suspended in 30 mL of *tert*-butyl alcohol, 30 mL of concentrated HCl (37%) was slowly added, and the mixture stirred mildly at 50 °C for 2 h. The suspension was filtered, and the particles were washed with 120 mL of methanol, 40 mL of chloroform, and 40 mL of acetone. The chromatography packing material was dried by vacuum pumping at 40 °C overnight.

(iii) Synthesis of 12-MO-silica. Scheme 1B shows the synthetic pathway for preparing 12-MO and the immobilization strategy used to tether 12-MO to SPA. 12-MO was synthesized as described.[9] The procedure for immobilization of 12-MO on SPA, followed by C3 endcapping to form 12-MO-silica, was the same as that for the synthesis of IAMs described previously.[7,8] The 12-MO-silica bonded phase contains the polar OCH_3 group protruding from a silica surface. Briefly, 1.0 g of 12-MO was activated by 0.70 g of CDI in THF and then bonded to 12.0 g of SPA, followed by C3 endcapping. The density of immobilized ligand (i.e., 12-MO) was 171 μmol/g of silica, by elemental analysis and FT-IR spectroscopy.[7]

Chromatography. All HPLC columns containing IAM stationary phases were packed at Regis Technologies Inc. The columns were (i) a 0.46 × 3.0 cm IAM.PC.DD column (5 μm), (ii) a 0.46 × 3.0 cm 12-OH-silica column (12 μm), and (iii) a 0.46 × 15 cm

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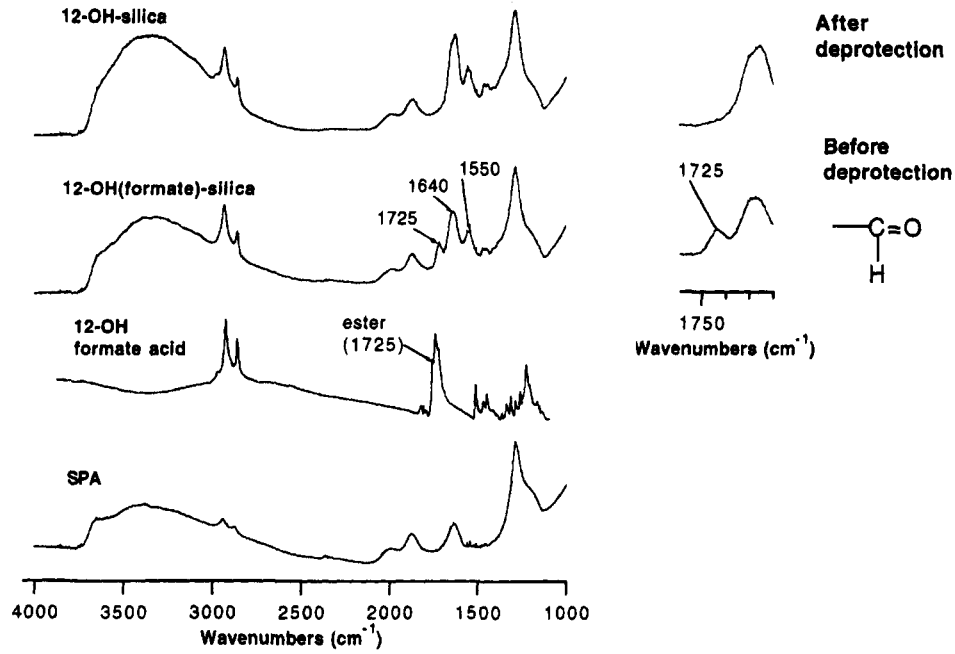

Figure 1. FT-IR spectra of SPA, 12-OH fatty acid formate ester, 12-OH(formate)-silica, and 12-OH-silica. The amide I band at 1640 cm^{-1} and the amide II band at 1550 cm^{-1} in the IR spectrum of 12-OH(formate)-silica indicate that the ligand (12-OH fatty acid formate ester) was covalently bonded to SPA. The characteristic absorptions of the formate group at 1725 cm^{-1} (C=O of ester) completely disappeared in the IR spectrum of 12-OH-silica. The insets below the spectra show the intensity of the ester carbonyl band at 1725 cm^{-1} before and after deprotection.

12-MO-silica column (12 μm). For all studies, the injection volume was ~15 μL of an aqueous solution of the drug (~0.1 $\mu g/\mu L$) dissolved in 0.01 M PBS buffered at pH 7.4 or the same buffer with pH adjusted to 5.4. The flow rate was 1or 2 mL/min, and solute detection was at 220 nm. Chromatograms were obtained using a Rainin HPLC pumping system equipped with a Knauer Model 87 detector and interfaced with a Macintosh computer. Rainin Dynamax software was used to record the chromatograms on the computer.

The retention times (t_r) of solute molecules on IAM chromatography columns were used to calculate the solute capacity factors (k'_{IAM}) using eq 1, where t_r is the retention time (in

$$k' = (t_r - t_0)/t_0 \quad (1)$$

minutes) of the test compound and t_0 corresponds to the column dead time or void volume. In our laboratory, solute retention times exhibit a day-to-day variation of less than 4%. The capacity factor, k', is linearly proportional to the equilibrium partition coefficient, K, of a solute that partitions between the stationary phase and the mobile phase,

$$k' = (V_s/V_m)K \quad (2)$$

where V_m is the total volume of solvent within the HPLC column and V_s is the volume of the IAM surface created by the immobilized ligands. The phase ratio V_s/V_m is constant for a given column. Furthermore, the membrane partitioning coefficient K_m is directly related to the drug permeability across the membrane by eq 3, where P_m is the permeability, D_m is the diffusion

$$P_m = D_m K_m / L \quad (3)$$

coefficient of the solute, and L is the thickness of the membrane. D_m is related to the molecular size and to a first approximation is inversely proportional to molecular weight. Thus, drug retention behavior on columns can be used to test the capability of predicting drug–membrane permeability since the k' measured by chromatography is linearly related to K_m.[1,2]

RESULTS AND DISCUSSION

All of the bonded phases used in this work, IAM.PC.DD, 12-OH-silica, and 12-MO-silica, contain a monolayer of immobilized lipid. IAM synthetic strategies are well established,[7,8] and the synthesis of the IAM.PC.DD column is available.[2] The synthesis of 12-MO-silica shown in Scheme 1 was straightforward because protection/deprotection strategies were unnecessary. However, the synthesis of the 12-OH-silica surface was more difficult because the free surface hydroxy groups required protection before the immobilization step in the preparation of the surface. The free OH's were protected with a formate group. The characteristic IR band of formate at 1725 cm^{-1} (Figure 1) was used to monitor the integrity of the formate group during (i) immobilization of 12-OH fatty acid formate ester to SPA, (ii) endcapping with C3 anhydride, and (iii) deprotection with acid to generate free surface OH groups. Figure 1 is a typical set of IR spectra which demonstrate our method of monitoring the bonding and deprotection. The intensity of the formate IR band did not change after endcapping (not shown), indicating that the formate protecting group was stable to the endcapping reaction conditions. Acid deprotection of the formate groups generated surface OH groups. The IR band intensity of the formate groups decreased ~90% in the IR spectra after deprotection (Figure 1, compare upper two spectra), which indicates that the deprotection reaction was >90% efficient.

Figure 2 shows the correlations between drug partitioning into DMPC liposomes[10–12] and drug partitioning into the three bonded

(10) Betageri, G. V.; Rogers, J. A. *Int. J. Pharm.* **1987**, *36*, 165–173.

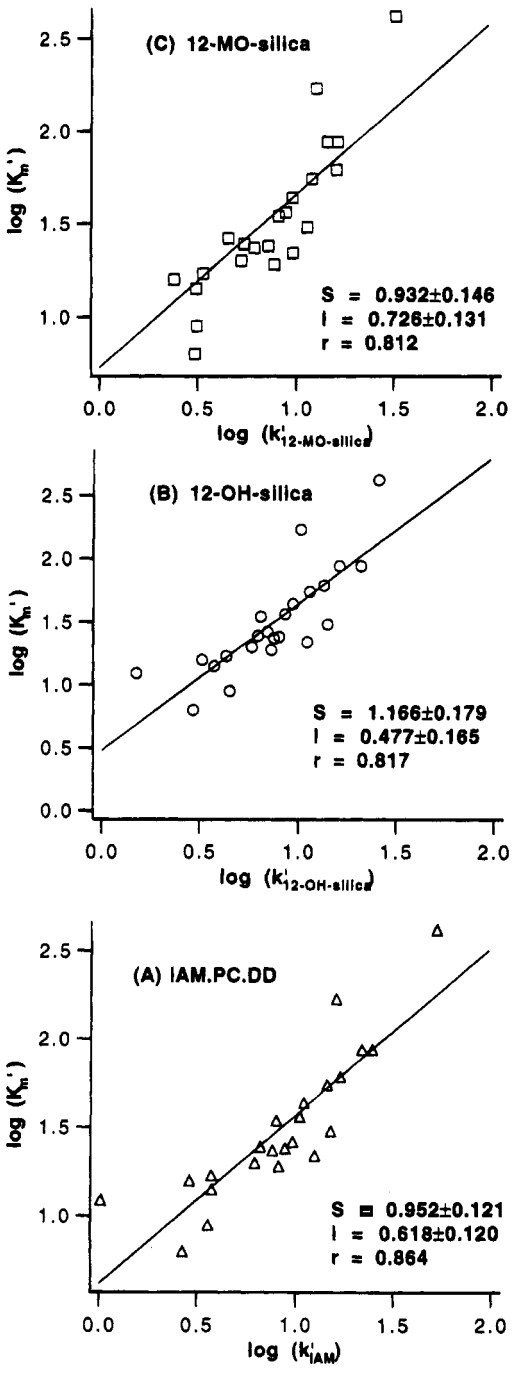

Figure 2. (A) Correlation of drug partitioning in IAM.PC.DD (log K'_{IAM}) and in liposome (log K_m) for 23 drugs including seven β-blockers, six imidazoline derivatives, and 10 imidazolidine derivatives. The liposome partition coefficients of these 23 drugs were measured using DMPC liposomes in 0.01 M PBS buffer (pH 7.4).[10-12] IAM capacity factors, K'_{IAM}, were measured on a 0.46 × 3.0 cm IAM.PC.DD column using a mobile phase of 0.01 M PBS (pH 7.4). The same correlation was done on a 0.46 × 3.0 cm 12-OH-silica column (B) and a 0.46 × 15 cm 12-MO-silica column (C).

phases: IAM.PC.DD, 12-OH-silica, and 12-MO-silica. The structures of the 23 solutes used for the partitioning studies are shown in Table 1. As shown in Figure 2, IAM.PC.DD columns predicted drug partitioning into DMPC liposomes (r = 0.886, Figure 2A) better than 12-OH-silica (r = 0.812 Figure 2B) and also better than 12-MO-silica (r = 0.817, Figure 2C). Similar results were found for comparing the abilities of these bonded phases to predict intestinal transport in in situ tissue models. For instance, the correlation between solute intestinal absorption and solute partitioning using IAM.PC.DD (r = 0.788, Figure 3A) was better than the correlation using the 12-OH-silica column (r = 0.590, Figure 3B), the 12-MO-silica column (r = 0.681, Figure 3C), and the *n*-octanol/water partitioning system. Most importantly, solute partitioning into nonpolar octadecylsilica (ODS) did not correlate with solute intestinal absorption (r = 0.10).[1]

The above results indicate that IAM.PC.DD better predicts solute–membrane interactions than 12-OH-silica and 12-MO-silica columns. This is because solute–IAM interactions involve not only hydrogen-bonding van der Waals interactions but also electrostatic interactions. Although 12-OH-silica and 12-MO-silica have hydrogen bond acceptors and donors, these surfaces cannot model electrostatic interactions common between molecules and membranes that occur during the partitioning process. IAM.PC.DD contains the zwitterionic PC headgroup and thus can model virtually all of the molecular interactions found in cell membranes during drug partitioning (Chart 3C). It was thus expected that the IAM.PC.DD phase would model the partitioning process because this phase contains the PC headgroup found in biological membranes. However, it was very surprising that 12-OH-silica and 12-MO-silica could model drug–membrane interactions better than ODS reversed phase columns. This suggests that polar groups on the chromatographic surface might be important for modeling the solute–membrane interaction for some compounds (Chart 3D).

12-OH-silica is effectively immobilized alcohol on silica surface, and a key question is whether drug partitioning into *n*-octanol/water systems correlates with drug partitioning into the 12-OH-silica bonded phase. Drug partitioning into 12-OH-silica is compared to drug partitioning into *n*-octanol/water systems[10-12] in Figure 4. Surprisingly, a poor correlation between drug partitioning into 12-OH-silica and drug partitioning into *n*-octanol/water was found for a total of 22 compounds studied (r = 0.297, Figure 4). Although it is known that in octanol/water systems, water-saturated *n*-octanol is tied up in a tetrahedral hydrogen-bonded complex (A_4W) that retains a high degree of hydrophobicity because of the four eight-carbon nonpolar chains surrounding the polar water center,[13] the *n*-octanol molecules in the water-saturated octanol phase are virtually randomly oriented (Chart 3A). In contrast, 12-OH-silica bonded phases have immobilized alcohol molecules arranged in a monolayer structure with OH groups protruding from the silica surface. It has been recognized that the ordered structure of the membrane bilayer is important in drug–membrane interactions. The 12-OH-silica surface is an ordered liquid containing a polar region and a nonpolar region (Chart 3B). Obviously, the physical–chemical properties of the immobilized alcohols on the 12-OH-silica surface should be different compared to the nonbonded randomly oriented octanol molecules in the *n*-octanol/water system. Therefore, drug–12-OH-silica interactions are not expected to be similar to the drug–octanol interactions in the octanol/water system, as shown in Figure 4.

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(13) Hansch, C.; Muri, R. M.; Fujita, T.; Maloney, P. P.; Geiger, F.; Streich, M. *J. Am. Chem. Soc.* **1963**, *85*, 2817–2824.
(14) De Young, L.; Dill, K. A. *Biochemistry* **1988**, *27*, 5281–5289.
(15) Schanker, L. S.; Tocco, D. J.; Brodie, B. B.; Hogben, C. A. M. *Pharmacol. Exp. Ther.* **1958**, *123*, 81–88.
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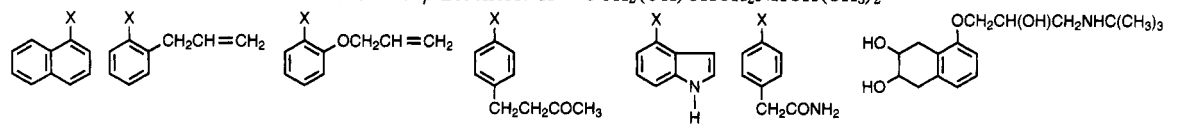
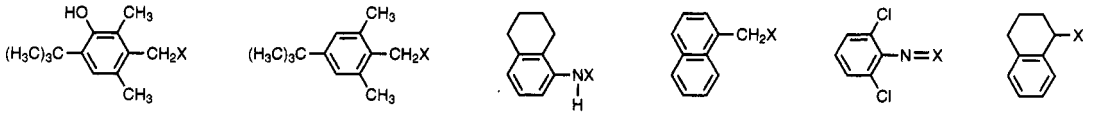
Table 1. Structures of Drugs Used To Compare Drug Partitioning into DMPC Liposomes with Drug Partitioning into Bonded Phases

Structure of β-Blockers: X = $OCH_2(OH)CHCH_2NHCH(CH_3)_2$



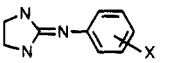
propranolol alpranolol oxprenolol metoprolol pindolol atenolol nadolol

Structure of Imidazoline Derivatives: X =



oxymetazoline xylometazoline tramazoline naphazoline clonidine tetryzoline

Structure of Imidazoline Derivatives:



STH2224 (X = 2,3-di-Br)
ST476 (X = 2,3-di-Cl)
ST608 (X = 2-Cl, 3-CH_3)
ST600 (X = 2-CH_3, 5-F)
STH2100 (X = 2-Cl, 3-Br)
ST585 (X = 2-CH_3, 5-Cl)
ST475 (X = 2,5-di-Cl)
ST606 (X = 2-CH_3, 3-Br)
ST590 (X = 2-CH_3, 4-Br)
ST603 (X = 2-Cl, 5-Br)

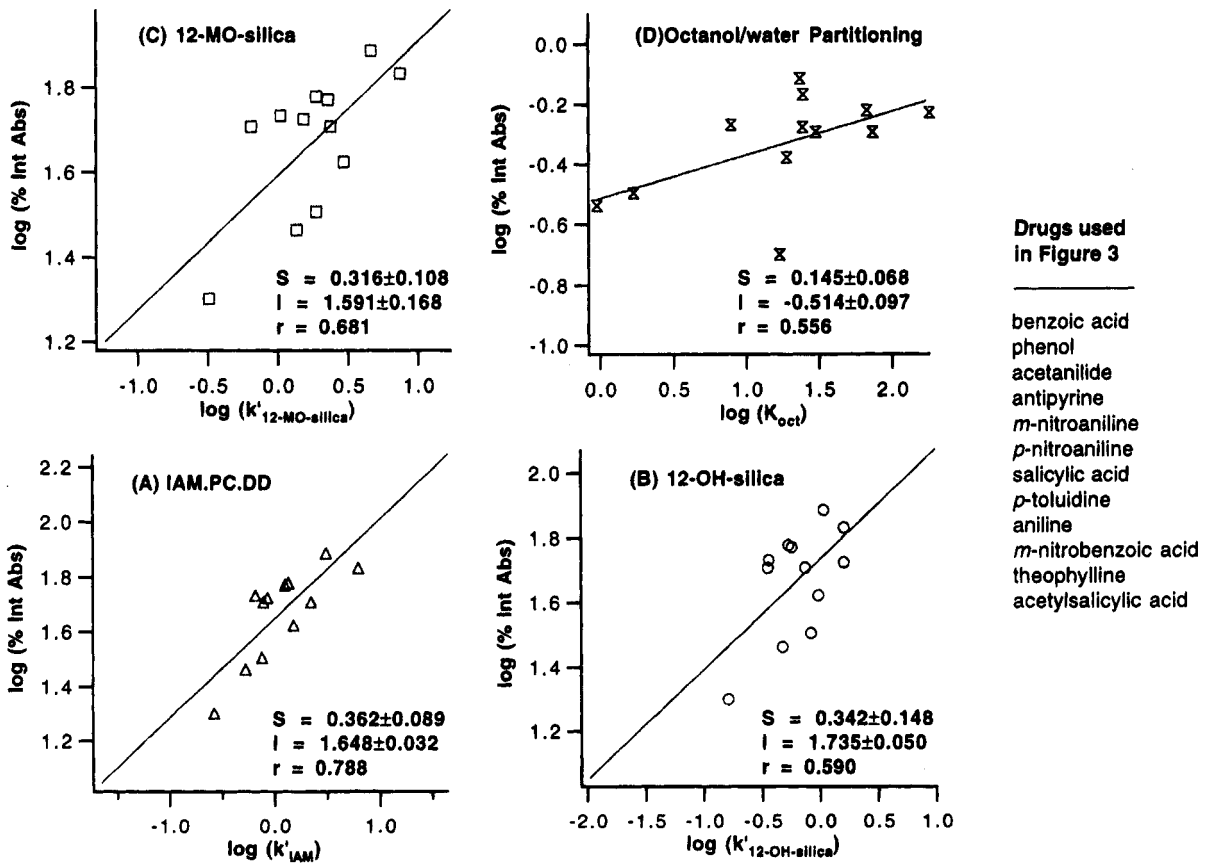
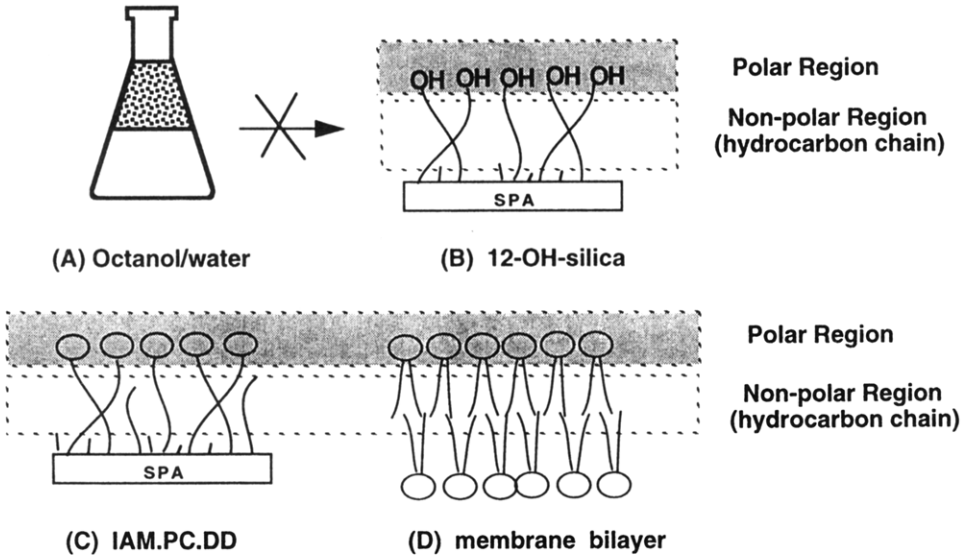


Figure 3. Correlation of rat intestinal drug absorption with drug partitioning into an IAM.PC.DD (log K'_{IAM}) column (A), a 12-OH-silica (log $K'_{12\text{-OH-silica}}$) column (B), a 12-MO-silica (log $K'_{12\text{-MO-silica}}$) column (C), and the *n*-octanol/water system (D). The intestinal absorption of the drugs from rat small intestine was measured by Schanker et al.,[15] and the effective pH at the surface of the intestinal epithelial is about 5.4. The partitioning coefficients of these compounds in *n*-octanol/water system were measured using distilled water and *n*-octanol supensions.[16] IAM capacity factors, K'_{IAM}, were measured on a 0.46 × 3.0 cm IAM.PC.DD column. $K'_{12\text{-OH-silica}}$ values were measured on a 0.46 × 3.0 cm 12-OH-silica column, and $K'_{12\text{-MO-silica}}$ values were measured on a 0.46 × 15 cm 12-MO-silica column. The mobile phase of 0.01 M PBS (pH 5.4) was used in the all of capacity factor measurements.

Chart 3[a]



[a] The solvent structure of octanol in the octanol/water partitioning system (A) is fundamentally different compared to the monolayer structure of 12-OH-silica (B). The interfacial polarity of 12-OH-silica is similar to that of IAM.PC.DD (C) and the membrane bilayer (D).

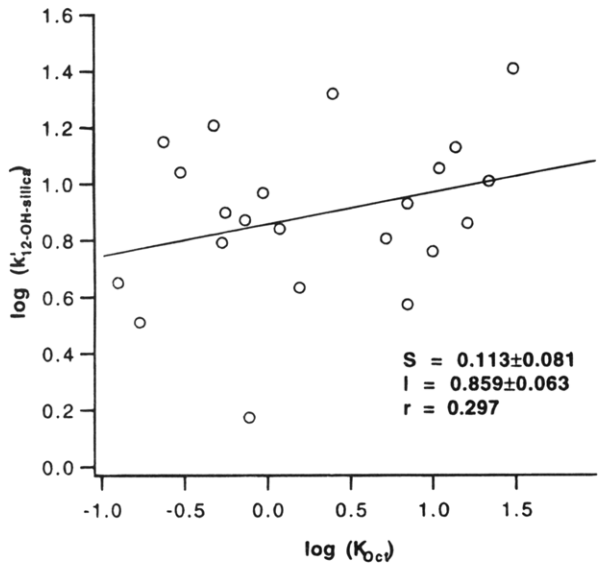


Figure 4. Comparison of drug partitioning in 12-OH-silica (log $K'_{12\text{-OH-silica}}$) and in the *n*-octanol/buffer phase for 22 drugs including six β-blockers, six imidazoline derivatives, and 10 imidazolidine derivatives. 12-OH-silica capacity factors, $K'_{12\text{-OH-silica}}$, were measured on a 0.46 × 3.0 cm 12-OH-silica column using a mobile phase of 0.01 M PBS (pH 7.4). The *n*-octanol/buffer partition coefficients of these drugs were measured elsewhere[10-12] using a 0.01 M PBS (pH 7.4) buffer.

We have previously postulated that drug–membrane interactions on IAM surfaces are a bulk phase property of the immobilized lipids. In other words, individual functional groups of the immobilized lipids are not critical for the membrane–drug interaction. This was based on the observation that IAM drug–membrane interactions did not depend on interfacial ester vs ethers, the number of fatty acid chains, etc. The molecule–membrane interaction was thus proposed to be a property of the structure of the interface. The importance of the bulk interfacial properties of the IAM surface in monitoring solute–membrane interaction was also demonstrated in this report. Thus the 12-OH-silica and the 12-MO-silica have polar interfacial properties and a ~15 Å hydrocarbon environment for the partitioning process, and both of these surfaces were able to model to some degree of success the partitioning process.

Critical experimental data supporting the importance of the polar interfacial region in membrane–solute interaction is found by comparing the data obtained with use of the *n*-octanol/water system to those from the 12-OH-silica system (Figure 4). The partitioning in the octanol/water system of the 22 drugs does not correlate with the partitioning into 12-OH-silica (r = 0.297). Similarly, drug partitioning into the octanol/water system does not correlate with drug partitioning into DMPC liposomes for the 23 drugs shown in Table 1 (r = 0.483).[2] However, drug partitioning into the immobilized alcohol phase gives a good prediction (r = 0.812). The fact that immobilized alcohol can mimic partially the solute–membrane interaction but nonimmobilized octanol cannot (i.e., *n*-octanol/water partitioning) strongly supports the idea that the interface created by the lipid molecules, not the individual lipid molecules themselves, controls the solute–membrane interactions.

CONCLUSION

In conclusion, an ordered monolayer of immobilized lipids containing both a polar and a nonpolar region is critical for a chromatographic surface to accurately monitor the interaction between solutes and biological membranes. Differences in the interfacial polar functional groups do not eliminate the ability of the surface to predict drug–membrane interactions, i.e., surface PC headgroups, surface OH groups, and surface OCH_3 groups all provide the interfacial properties necessary to predict drug–membrane interactions better than reversed phase C18 surfaces. However, when the interfacial polar region is comprised of the phospholipid headgroup found in biological membranes (i.e., not monolayers of OH groups or OCH_3 groups), further improved capabilities of predicting solute–membrane interactions are found for that surface. Thus IAM.PC.DD was a better *in vitro* screen for predicting drug–membrane interactions than 12-OH-silica or 12-MO-silica column.

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