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Journal of Chromatography A, 800 (1998) 161–169

JOURNAL OF CHROMATOGRAPHY A

Evaluation of the immobilized artificial membrane phosphatidylcholine Drug discovery column for high-performance liquid chromatographic screening of drug–membrane interactions

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Received 18 August 1997; received in revised form 14 October 1997; accepted 7 November 1997

Abstract

Chromatographic retention factors (k') of a series of eight β-adrenoceptor antagonist compounds (β-adrenolytic drugs) were determined employing an immobilized artificial membrane column (IAM.PC.DD). The influence of mobile phase pH, ionic strength, and organic modifier composition was studied in order to examine column performance. After the IAM.PC.DD columns were exposed to approximately 7000 column volumes of a 0.01 *M* PBS mobile phase, five out of six columns tested showed significant peak broadening and decreased k' values indicative of premature column failure. The data suggested that the immobilized phospholipids stationary phase was removed by the 0.01 *M* PBS mobile phase. The β-adrenolytic drug's $\log k'_{IAM}$ values obtained with an IAM.PC.DD column were compared to an ^{ester}IAM.PC.MG column for predicting drug membrane interactions. For the linear regression analysis between $\log k'_{IAM}$ and the logarithm of the *n*-octanol–water partition coefficients ($r_{IAM.PC.DD}=0.8710$ vs. $r_{IAM.PC.MG}=0.9538$), the C_{18} HPLC retention factors ($r_{IAM.PC.DD}=0.8408$ vs. $r_{IAM.PC.MG}=0.9380$), the liposome partition coefficients ($r_{IAM.PC.DD}=0.8887$ vs. $r_{IAM.PC.MG}=0.9187$), and various pharmacokinetic parameters, significantly better correlations were obtained with the ^{ester}IAM.PC.MG column than the IAM.PC.DD column. © 1998 Elsevier Science B.V.

Keywords: β-Blockers; Immobilized artificial membranes; Mobile phase composition; Stationary phases, LC; Phospholipid stationary phase

1. Introduction

Combinatorial chemistry and high through-put receptor-drug screening techniques are widely adopted by most pharmaceutical companies to identify new compound leads. Once a new compound lead is identified, it is typically subjected to in vitro secondary structure activity studies. These processes allow the facile selection of compounds with the greatest potencies for the receptor under investigation. Of course compound selection from these in vitro screens does not mean that the compound will be active in vivo, since many other factors such as membrane transport, metabolism and toxicity play an important role. It is well recognized that the compound selection process would be greatly enhanced if in vitro screens were available to predict biologically

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0021-9673/98/$19.00
PII S0021-9673(97)01143-6

relevant in vivo membrane transport, metabolism and toxicity properties.

Several in vitro physicochemical screens have been developed over the years to predict passive transport through cell membranes including octanol–water partitioning [1,2], potentiometric-based octanol–water partitioning [3,4], liposome partitioning [5,6], retention factors in both reversed-phase chromatography [7,8], in microemulsion electrokinetic chromatography [9,10] and in immobilized artificial membrane (IAM) chromatography [11,12]. All of these techniques have shown some limited success in their ability to correlate physicochemical properties with drug membrane transport. It was noted that IAM chromatography offers several important advantages over other physicochemical models [11,12]. IAMs are solid phase membrane mimetics that are prepared by covalently immobilizing monolayers of cell membrane phospholipids to silica particles at high molecular surface densities. Thus, IAMs are used as a chromatographic stationary phase to mimic the lipid environment of a fluid cell membrane. The analyte retention (capacity) factors on IAM chromatographic columns have been shown to correlate with analyte equilibrium partition coefficients measured in fluid liposome systems [11], to predict drug permeability through Caco-2 cells [12], to predict salt–membrane interactions [13], and used for quantitative structure activity relationships [14]. From the above studies, clearly the IAM method is attractive for characterizing drug–membrane interactions since data collection/analyses are fast and large-volume automated screening of compounds is possible.

Currently, there are four commercially available IAM surfaces that have been used to evaluate drug–membrane passive transport properties: ^{ester}IAM.PC.MG [15], ^{ester}IAM.PC$^{C_{10}/C_3}$ [11], ^{ether}IAM.PC$^{C_{10}/C_3}$ [11,12], and $^{\delta G}$IAM.PC$^{C_{10}/C_3}$ (also denoted as IAM.PC.DD where DD represents Drug Discovery) [11]. The PC indicates that the headgroup used for the stationary phase is phosphatidylcholine; the major phospholipid found in cell membranes. The superscript ''ester'' denotes an ester linkage between the two acyl chains and the glycerol backbone of the PC molecule. The superscript ''ether'' denotes an ether linkage between the alkyl groups tethered to the PC molecules. The superscript ''δG'' denotes the deletion of the glycerol backbone from the PC molecule. The superscript ''C_{10}/C_3'' indicates that the unreacted propylamines moieties on the silica surface were end-capped with a mixture of C_{10} and C_3 acyl groups. The MG indicates that the silica surface was end-capped with methylglycolate. To clarify the above nomenclature, the IAM columns are simply classified as single-chain type (^{ether}IAM.PC$^{C_{10}/C_3}$ and IAM.PC.DD) and double-chain type (^{ester}IAM.PC.MG and ^{ester}IAM.PCPC$^{C_{10}/C_3}$).

In this study, we have evaluated the performance of the single-chain IAM.PC.DD stationary phase. The influence of different experimental conditions on the IAM chromatographic performance and column longevity were examined. The effects of pH, the ionic strength and the mobile phase composition on retention factors were investigated. Recently, the effect of temperature on retention factors obtained on an IAM.PC.DD column was investigated [16]. This topic will therefore not be discussed. The single-chain IAM.PC.DD column is compared to the double-chain ^{ester}IAM.PC.MG column [15] for predicting drug–membrane interactions for eight β-adrenoceptor antagonist compounds (β-adrenolytic drugs). The β-adrenolytic drugs were chosen as model compounds since they have a common polar moiety and substituents of broadly varying lipophilicity (hydrophobicity).

2. Experimental

2.1. Chromatographic system

Chromatograms were obtained primarily using a Rainin (Woburn, MA, USA) HPLC pumping system equipped with a Rainin Dynamax UV-DI1 detector and a model AI1 autosampler. The system was interfaced with a Macintosh computer in which Rainin Dynamax software was used to record and process the chromatograms. In some cases, a HP 1090 HPLC (Hewlett-Packard, CA, USA) was utilized. Analytical HPLC IAM.PC.DD columns (Regis, Morton Grove, IL, USA) were 10 cm×4.6 mm I.D. with a particle size of 5 μm and a pore size of 300 Å. For all studies, the injection volume was 20 μl of a 0.2 m*M* analyte solution prepared in 0.01 *M* PBS (Dulbecco's phosphate-buffered saline,

which contains 2.7 m*M* KCl, 1.5 m*M* KH_2PO_4, 137 m*M* NaCl, and 8.1 m*M* $Na_2HPO_4 \cdot 7H_2O$; Sigma, St. Louis, MO, USA). The mobile phase was 0.01 *M* PBS adjusted with NaOH or HCl to vary the pH. An isocratic eluent was used at a flow rate of 1 ml/min. The analyte detection was at 220 and 254 nm and the temperature of the experiment was 22±2°C. Dimethylsulfoxide (DMSO) was used as the void (t_0) marker and *m*-nitroaniline was used as an internal reference analyte.

2.2. Chemicals

The following chemicals were purchased from Sigma or Aldrich Chemical Co. (Milwaukee, WI, USA) and used without further purification: dimethylsulfoxide, *m*-nitroaniline, acebutolol hydrochloride, alprenolol hydrochloride, atenolol, metoprolol tartrate salt, oxprenolol hydrochloride, pindolol, propranolol hydrochloride, timolol maleate salt, and *p*-toluidine. Other reagents were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.3. Method

The IAM.PC.DD columns were conditioned by passing 50 ml of 0.01 *M* PBS buffer through the column prior to analyses. The injection-to-injection reproducibility was tested by making repetitive injections of *p*-toluidine. For example, the average retention time (t_r) for *p*-toluidine was 3.51_1 min and the void time (t_0) for DMSO was 1.45_9 min in a 0.01 PBS buffer at pH 7.0. The average retention factor [$k' = (t_r - t_0)/t_0$] of *p*-toluidine was 1.40_6 min with a percentage relative standard deviation of approximately 1% ($n = 10$). All columns were similarly tested and showed good initial injection-to-injection performance. We followed the manufacturer's recommendation for column conditioning and typically columns were used immediately and not stored for any extended period of time. The IAM.PC.DD columns were from two different lots.

3. Results and discussion

3.1. IAM.PC.DD column-to-column reproducibility

The retention factors (k') of eight β-adrenolytic drugs and *m*-nitroaniline were determined with six new IAM.PC.DD columns. After approximately 500 ml of 0.01 *M* PBS (pH 7.0) mobile phase was passed through the columns, two of the columns produced peaks that eventually split into two indicative of a possible void at the head of the column (data not shown). These columns were discarded. The remaining four columns were utilized to determine k' column-to-column reproducibility (Table 1). While many of the compounds had acceptable k' column-to-column variations (3–5%), *m*-nitroaniline and

Table 1
Retention (capacity) factor (k') column-to-column reproducibility[a]

Drugs	k' column 1	k' column 2	k' column 3	k' column 4	Ave. k'	S.D.	R.S.D. (%)
m-Nitroaniline	3.47_6	3.34_3	2.74_7	3.44_8	3.25_4	0.34_3	11
Acebutolol	4.94_9	4.85_3	4.78_4	5.16_7	4.93_8	0.16_7	3
Alprenolol	9.96_7	8.13_2	7.66_7	9.06_7	8.70_8	1.02_1	12
Atenolol	0.52_7	0.50_4	0.51_9	0.49_0	0.51_0	0.01_6	3
Metoprolol	2.14_1	1.98_2	2.02_8	1.89_1	2.01_1	0.10_4	5
Oxprenolol	3.55_2	3.26_7	3.18_9	3.45_7	3.36_6	0.16_7	5
Pindolol	7.18_8	7.12_5	6.40_1	7.11_7	6.95_8	0.37_2	5
Propranolol	29.27_9	28.21_4	26.64_8	29.07_9	28.30_5	1.19_8	4
Timolol	1.52_9	1.49_3	1.47_9	1.52_2	1.50_6	0.02_4	2

The following conditions were used: the injection volume was 20 μl of an analyte aqueous solution (0.2 m*M*) in 0.01 *M* PBS at pH 7.0 (buffered with HCl); the isocratic eluent was used at a flow-rate of 1 ml/min; the analyte detection was at 220 and 254 nm; the temperature of the experiment was 22±2°C; the sample contained 1% DMSO which was used as the void ($t_0 = 1.480$ min) marker. R.S.D. (%) [=(S.D./Ave)·100] denotes the percentage relative standard deviation.

[a] Retention (capacity) factors measured with HPLC IAM.PC.DD columns (10 cm×4.6 mm I.D. with a particle size of 5 μm and a pore size of 300 Å).

alprenolol had variations greater than 10%. When k' column-to-column values exceed 10%, these columns are typically rejected in our laboratory. It was noted that column 3 produced k' values for *m*-nitroaniline and alprenolol that were significantly smaller than the other columns and thus, produced most of the observed k' column-to- column variation. If the loading capacity of immobilized phospholipid on the silane stationary phase was less for column 3 as compared to the other columns, this would explain some of the variation in the k' values. Removal of some immobilized phospholipid by the mobile phase is another explanation for the variation in the k' values. To validate the latter idea, column 1 was subjected to 7200 column volumes of 0.01 *M* PBS eluent at pH 7.0 and the k' values were recorded. The k' values of *m*-nitroaniline and pindolol as a function of column volumes are plotted in Fig. 1. The k' values of *m*-nitroaniline and pindolol at 50 column volumes were 3.47_6 and 7.18_8, respectively. At 7200 column volumes, the k' values of *m*-nitroaniline and pindolol had decreased to 2.31_7 and 5.67_5, respectively. It was noted that significant deterioration of column 1 was evident by peak broadening and decreased k' values after only 4000 column volumes. This premature column failure was observed for the remaining columns although column 4 had significantly better column longevity than the others. These results suggested that the IAM.PC.DD columns were susceptible to either silica support dissolution or some immobilized phospholipid was removed by the mobile phase. A recent study of C_{18} columns with mobile phases above pH 8 suggested that premature column failure were influenced by the type and purity of silica support and by the nature of the silane stationary phase [17].

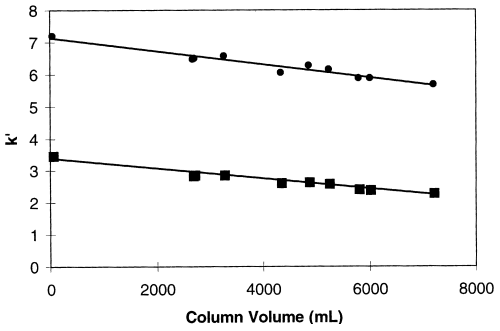

Fig. 1. The retention (capacity) factors (k') of pindolol (●) and *m*-nitroaniline (■) were determined as a function of column volume. Column 1 was used and the HPLC conditions were the same as denoted in Table 1. This decrease in k' as a function of column volume was observed for all the β-adrenolytic drugs.

3.2. Mobile phase pH and ionic strength effects

The k' values on IAM.PC.DD column 4 were determined for eight β-adrenolytic drugs and *m*-nitroaniline between pH 7.4 and 3.5. This IAM.PC.DD column exhibited good analyte peak shape over the above pH range with no loss in performance. Mobile phases with pH values greater than pH 7.5 and less than pH 2.5 were not used as recommended by the manufacturer. In Figs. 2 and 3, the retention factors as a function of pH are presented. The k' values for all the β-adrenolytic drugs smoothly decreased by approximately a factor of 2 with minimal changes in separation selectivity as the mobile phase was changed from pH 7.4 to 3.5. The k' value for *m*-nitroaniline decreased from approximately 3.6 to 2.9 over the same pH range.

In IAM.PC.DD chromatography, the retention of the β-adrenolytic drugs and *m*-nitroaniline are the results of two primary processes: (a) the interaction of the analyte with the charged headgroup of the stationary phase (adsorption), and/or (b) the distribution of the analyte between the aqueous mobile

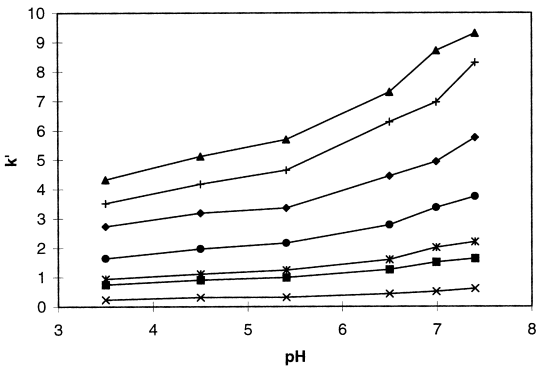

Fig. 2. The retention (capacity) factors (k') of alprenolol (▲), pindolol (+), acebutolol (♦), oxprenolol (●), metoprolol (★), timolol (■) and atenolol (x) were determined as a function of pH. Column 4 was used and the HPLC conditions were the same as denoted in Table 1. The pH of the mobile phase was adjusted with HCl.

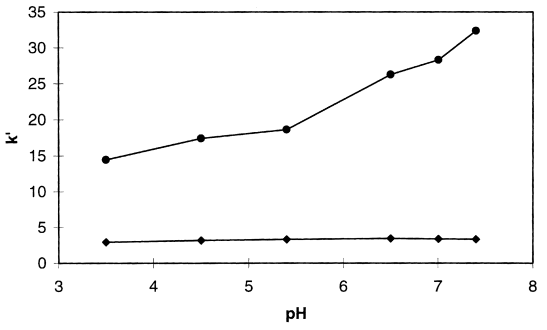

Fig. 3. The retention (capacity) factors (k') of propranolol (●) and *m*-nitroaniline (♦) were determined as a function of pH. Column 4 was used and the HPLC conditions were the same as denoted in Table 1. The pH of the mobile phase was adjusted with HCl.

phase and the organic stationary phase (partitioning). In Fig. 4, the retention factors as a function of mobile phase molarity are shown for acebutolol, oxprenolol, *m*-nitroaniline and timolol. An increase of buffer molarity produced a decrease in k' between 5–30% for all the β-adrenolytic drugs investigated while the k' value for *m*-nitroaniline was practically insensitive to the change. The occurrence of ion-pairing in the retention mechanism of the β-adrenolytic drugs can be excluded since the change in the k' values were not proportional to the concentration of the counter-ions (i.e. Cl^- or Na^+) in the mobile phase [18]. It is also clear that the observed relationship between the retention factors of the analyte and the pH of the mobile phase depends upon the nature

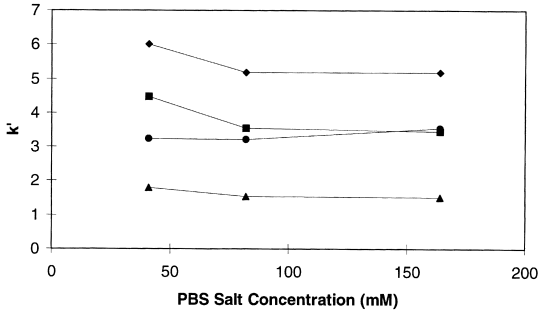

Fig. 4. The retention (capacity) factors (k') of acebutolol (♦), oxprenolol (■), *m*-nitroaniline (●) and timolol (▲) were determined as a function of PBS salt concentration (Dulbecco's phosphate-buffered saline, which contains 2.7 m*M* KCl, 1.5 m*M* KH_2PO_4, 137 m*M* NaCl, and 8.1 m*M* $Na_2HPO_4 \cdot 7H_2O$). Column 4 was used and the HPLC conditions were the same as denoted in Table 1. The pH of the mobile phase was 7.0.

of the analyte and the IAM.PC.DD stationary phase. Since all the β-adrenolytic drugs have pK_a values between 9.8 and 8.8 [8,19], only the protonated species are present in the bulk mobile phase when the pH of the mobile phase is between pH 7.4 and 3.5. It is noteworthy to mention that the pK_a value of 8.8 reported in Ref. [8] for pindolol is significantly different from the value of 9.70 reported in Ref. [19]. The pK_a value of *m*-nitroaniline is approximately 2.5 [20] and thus, only the neutral form is present over the same pH range. The IAM.PC.DD column has a neutral zwitterionic phosphatidylcholine stationary phase composed of a quaternary methylamine-phosphate headgroup. The IAM surface is not expected to change when the pH of the mobile phase is varied from pH 7.4 to 3.4 since the phosphate moiety of the phosphatidylcholine headgroup has a p$K_a < 1.5$ [21]. These observations indicate that the retention behavior of the β-adrenolytic drugs and *m*-nitroaniline are dominated by a partitioning mechanism instead of an adsorption mechanism. The effect of the pH of the mobile phase on the retention factors suggests that the β-adrenolytic drugs experience a decrease in mobile phase strength at low pH and therefore a decease in k'. Since *m*-nitroaniline is neutral, a change in solvent strength has appreciably less effect on the k' value.

3.3. Mobile phase organic modifier effects on k′

To obtain information on how k' is affected with changes in mobile phase organic composition, various acetonitrile fractions were added to the aqueous mobile phase (0.01 *M* PBS). A linear relationship between $\log k'$ and the percentage of acetonitrile (10–40% ACN) modifier in the mobile phase was observed for all the β-adrenolytic drugs and *m*-nitroaniline (Fig. 5). Apparently the *m*-nitroaniline k' plot intersects the lines relative to the β-adrenolytic drugs since it is neutral and the β-adrenolytic drugs are positively charged. The linear regression parameters of $\log k'$ vs. % ACN are reported in Table 2. The slopes (a) of the β-adrenolytic drugs are all similar with the intercepts corresponding to their $\log k'$ values at 0% acetonitrile ($\log k'_W$). It is interesting that the extrapolated $\log k'_W$ when compared to the $\log k'$ (Ave.) values determined in 0.01 *M* PBS (Table 1) showed deviation anywhere from

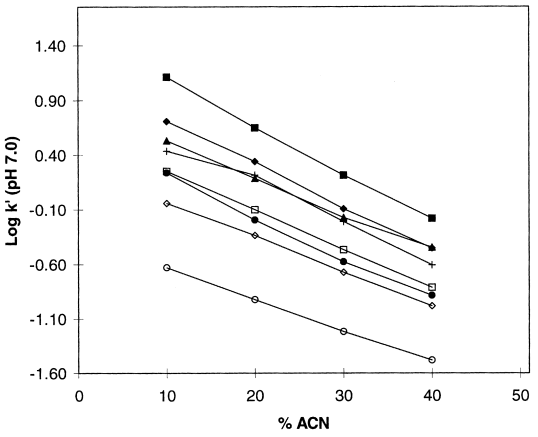

Fig. 5. Plots of the logarithm of the retention (capacity) factors (k') as a function of percentage of acetonitrile (ACN) in the mobile phase. The following compounds were used: propranolol (■), alprenolol (♦), pindolol (▲), acebutolol (●), oxprenolol (□), *m*-nitroaniline (+), metoprolol (◇), timolol (△) and atenolol (○). Column 4 was used and the HPLC conditions were the same as denoted in Table 1.

0–40%. This type of error in k' should be accounted for whenever k' is determined by a linear extrapolation at zero percent acetonitrile method. The IAM.PC.DD column 4 had good peak shape over the above acetonitrile range with no loss in performance.

The influence of different percentages of acetonitrile of the mobile phase on the chromatographic behavior of nine 4-phenyldihydropyridine calcium-channel adrenolytic drugs has been examined utilizing a ^{ester}IAM.PC.MG column [15]. The results are similar to ours in that a linear relationship was found between $\log k'$ and the fraction of acetonitrile modifier used in the mobile phase.

3.4. Log k′ (IAM) correlations to n-octanol–water partition coefficients and C_{18} HPLC retention factors

A linear relationship between the logarithm of the retention factors ($\log k'_{\text{IAM.PC.DD}}$) for the β-adrenolytic drugs determined on a single-chain IAM.PC.DD column (Table 1; Ave. values) at pH 7.0 and the logarithm of the partition coefficients ($\log k'_{\text{SF}}$) determined by an *n*-octanol–buffer shake-flask method is described in Eq. (1):

$$\text{Log } K'_{\text{SF}} = 1.448(\pm 0.334) \log k'_{IAM.PC.DD} - 1.301(\pm 0.255) \quad (1)$$

$n = 8$; $r = 0.8710$; $s = 0.47$.

Note that the values for K'_{SF}, which were originally reported at pH 7.4, have been adjusted to pH 7.0 following the procedure outlined in Ref. [8]. The *n* denotes the number of β-adrenolytic drugs considered to derive Eq. (1), *r* is the correlation coefficient, *s* is the standard error of the estimate and the number in the parentheses account for standard error of the regression coefficients. A linear relationship between $\log k'_{\text{IAM.PC.DD}}$ and $\log K'_{\text{HPLC}}$ determined by an *n*-octanol–buffer reversed-phase HPLC system utilizing a C_{18} column is described in Eq. (2). Again note that the K'_{HPLC} values have been adjusted to pH 7.0 to account for ionization differences [8].

Table 2
Relationship between the logarithm of the retention (capacity) factors (k') and the percent of acetonitrile (ϕ) in the mobile phase (see Fig. 5)[a]

Drug	*r*	*a*	$\log k'_{\text{W}}$ ($\phi = 0$)	$\log k'_{\text{IAM.PC.DD}}$ (Table 1)	% Diff
m-Nitroaniline	0.992	−0.0356	0.849	0.512	40
Acebutolol	0.995	−0.0374	0.578	0.694	20
Alprenolol	0.999	−0.0392	1.107	0.940	15
Atenolol	0.999	−0.0284	−0.356	−0.292	18
Metoprolol	0.999	−0.0314	0.276	0.303	10
Oxprenolol	1.000	−0.0358	0.612	0.527	14
Pindolol	0.996	−0.0327	0.841	0.842	0
Propranolol	0.999	−0.0432	1.527	1.452	5
Timolol	0.999	−0.0282	0.184	0.178	3

$\text{Log } k'_{\text{IAM.PC.DD}}$ (pH 7.0) $= a\phi + \log k'_{\text{W}}$.
[a] *r* is the correlation coefficient, *a* is the slope and $\log k'_{\text{W}}$ is the intercept.

$$\text{Log}K'_{\text{HPLC}} = 1.278(\pm 0.336)\log k'_{\text{IAM.PC.DD}} - 0.930(\pm 0.257) \quad (2)$$

$n = 8$; $r = 0.8408$; $s = 0.47$.

The reasonable correlations produced in Eqs. (1) and (2) suggest that the retention behavior of the β-adrenolytic drugs on an IAM.PC.DD column is dominated by a partitioning mechanism and thus, represents a hydrophobicity measurement.

Recently, Kaliszan et al. [22] reported a linear relationship between the logarithm of $k'_{\text{IAM.PC.MG}}$ for 10 β-adrenolytic drugs determined on a double-chain $^{\text{ester}}$IAM.PC.MG column at pH 7.0 and the $\log K'_{\text{SF}}$ and the $\log K'_{\text{HPLC}}$ parameters utilized in Eqs. (1) and (2). We have recalculated these equations utilizing only the eight β-adrenolytic drugs from this study and their corresponding $\log k'_{\text{IAM.PC.MG}}$ values with

$$\text{Log}\,K'_{\text{SF}} = 1.860(\pm 0.239)\log k'_{\text{IAM.PC.MG}} - 1.398(\pm 0.158) \quad (3)$$

$n = 8$; $r = 0.9538$; $s = 0.29$.

and

$$\text{Log}\,K'_{\text{HPLC}} = 1.672(\pm 0.252)\log k'_{\text{IAM.PC.MG}} - 1.032(\pm 0.166) \quad (4)$$

$n = 8$; $r = 0.9380$; $s = 0.30$.

It is interesting to note that significantly better correlations were obtained with the $^{\text{ester}}$IAM.PC.MG column. While it is not completely understood why the IAM.PC.DD columns give poorer correlations when compared to the $^{\text{ester}}$IAM.PC.MG columns, some of the variations are due to differences in the mobile phase between the two studies and/or the differences in the stationary phases (i.e. single-chain type vs. double-chain type). The mobile phase utilized by Kaliszan et al. [22] was 10% (v/v) acetonitrile in 0.1 *M* sodium phosphate buffer at pH 7.0 while our mobile phase was 0.01 *M* PBS at pH 7.0. However, a similar correlation between $\log k'_{\text{IAM.PC.DD}}$ and $\log K'_{\text{SF}}$ (or $\log K'_{\text{HPLC}}$) is found in Eqs. (1) and (2) when we use $\log k'_{\text{IAM.PC.DD}}$ data collected with a mobile phase composed of 10% (v/v) acetonitrile in 0.01 *M* PBS at pH 7.0. This suggests that differences between the mobile phases are not a significant factor. The $^{\text{ester}}$IAM.PC.MG column is composed of a double-chain PC ligand end-capped with methylglycolate while the IAM.PC.DD column is composed of a single-chain PC ligand that lacks a glycerol backbone and is end-capped with a mixture of C_{10} and C_3 acyl groups [15]. There is a good correlation ($r = 0.9898$) between k' values obtained on the IAM.PC.DD column [10% (v/v) acetonitrile in 0.01 *M* PBS at pH 7.0] and k' values obtained on the $^{\text{ester}}$IAM.PC.MG column [10% (v/v) acetonitrile in 0.1 *M* sodium phosphate buffer at pH 7.0] as described in Eq. (5):

$$k'_{\text{IAM.PC.MG}} = 1.488(\pm 0.088)k'_{\text{IAM.PC.DD}} + 0.170(\pm 0.434) \quad (5)$$

$n = 8$; $r = 0.9898$; $s = 0.91$.

A slope of 1.488 and an intercept of 0.170 indicates that the double-chain $^{\text{ester}}$IAM.PC.MG column had a ca. 1.5-fold higher k' value compared to the single-chain IAM.PC.DD column. The higher retention of the β-adrenolytic drugs on the $^{\text{ester}}$IAM.PC.MG suggests these compounds are probably embedded deeper into the $^{\text{ester}}$IAM.PC.MG stationary phase as compared to the IAM.PC.DD bonded phase and may account for the correlation differences.

3.5. Log k′ (IAM) correlations to various pharmacokinetic parameters

Kaliszan et al. [22] reported the linear regression analysis between $\log k'_{\text{IAM.PC.MG}}$ determined on a double-chain $^{\text{ester}}$IAM.PC.MG column, $\log K'_{\text{SF}}$ and $\log K'_{\text{HPLC}}$ and a number of pharmacokinetic parameters ($\log X$). The β-adrenolytic drugs were utilized in this study. When the statistical parameters *n*, *r* and *s* were compared between these equations, it was determined that the hydrophobicity measured on an $^{\text{ester}}$IAM.PC.MG column provided a more significant correlation for the various pharmacokinetic parameters than was determined by the *n*-octanol–buffer shake-flask method ($\log K'_{\text{SF}}$) or by the *n*-octanol–buffer reversed-phase HPLC method. For comparison purposes, the statistical parameters *n*, *r* and *s* are

Table 3
Comparison of regression equations (*n*: number of data points; *r*: correlation coefficients; *s*: standard error) relating pharmacokinetic parameters data (*X*) for eight β-adrenoceptor blocking compounds to retention factors (k') obtained utilizing an ^{ester}IAM.PC.MG [22] and IAM.PC.DD column (Table 2)[a]

$\log k'_{IAM.PC.MG}$				$\log k'_{IAM.PC.DD}$		
$\log X$	*n*	*r*	*s*	*n*	*r*	*s*
$\log r_T$	7 (9)	0.842 (0.834)	0.305 (0.444)	7	0.810	0.332
$\log f_b$	8 (13)	0.775 (0.829)	0.377 (0.367)	8	0.744	0.399
$\log r_a$	6	0.952	0.149	6	0.932	0.176
$\log K_{BC}$	8 (10)	0.865 (0.896)	0.202 (0.180)	8	0.894	0.181
$\log V_{uss}$	7 (9)	0.876 (0.858)	0.247 (0.233)	7	0.847	0.274
$\log K_p$	8 (10)	0.830 (0.870)	0.514 (0.447)	8	0.751	0.609
$\log r$	8 (9)	0.812 (0.822)	0.588 (0.575)	8	0.672	0.746
$\log B_{\%}$	6 (8)	0.801 (0.800)	0.320 (0.274)	6	0.776	0.337

$\log X = a \log k'_{IAM} + b$.

[a] The *n* denotes the number of β-adrenolytic drugs considered to derive the equation; *r* is the correlation coefficient; *s* is the standard error of the estimate; *a* denotes the slope while *b* denotes the intercept (data not shown). The numbers in the parentheses are from Kaliszan et al. [22]. The $\log k'$ values obtained on the ^{ester}IAM.PC.MG column utilized a mobile phase with 10% (v/v) acetonitrile in 0.1 *M* sodium phosphate buffer at pH 7.0. The $\log k'$ values obtained on the IAM.PC.DD column utilized a mobile phase with 10% (v/v) acetonitirle in 0.01 *M* PBS at pH 7.0. Log *X* represents the logarithm of the pharmacokinetic parameters: $\log r_T$, the ratio of fraction of drug bound and unbound to tissue; $\log f_b$, the fraction of drug bound in plasma; $\log r_a$, the ratio of the fraction of drug bound and unbound to albumin; $\log K_{BC}$, the true red cell partition coefficients; $\log V_{uss}$, the steady-state volume of distribution referenced to the unbound drug in plasma, $\log K_p$, the partition coefficient of drug between plasma protein and plasma water; $\log r$, the ratio of the fraction of drug nonrenally and renally eliminated; $\log B_{\%}$, the percentage binding of drug to serum proteins. All pharmacokinetic data from Hinderling et al. [8].

reproduced (Table 3, numbers in parentheses) for the linear relationship between $\log k'_{IAM.PC.MG}$ and the various $\log X$ parameters. We have recalculated these equations utilizing $\log k'_{IAM.PC.MG}$ and $\log k'_{IAM.PC.DD}$ with the same type and number of β-adrenolytic drugs in order to compare the statistical parameters *n*, *r* and *s*. In all cases except for one ($\log K_{BC}$), the statistics of the linear regression analysis between $\log k'_{IAM.PC.MG}$ and $\log X$ were superior to those provided by $\log k'_{IAM.PC.DD}$.

3.6. Log k′ (IAM) correlations to a liposome model

Rogers et al. [6,23] has determined the partition coefficients (K'_m) of a series of β-adrenolytic drugs in buffered dimyristoylphosphatidylcholine (DMPC) vesicles (liposomes). A K'_m value for timolol was not determined in this study. These DMPC liposome systems are suitable membrane models for distribution studies; however, if many compounds are under evaluation the technique is experimentally laborious. A linear relationship between $\log k'_{IAM.PC.DD}$ for 7 β-adrenolytic drugs determined on a single-chain IAM.PC.DD column [10% (v/v) acetonitirle in 0.01 *M* PBS at pH 7.0] and the logarithm of the DMPC liposome partition coefficients ($\log K'_m$) is described in Eq. (6):

$$\log K'_m = 0.994(\pm 0.362) \log k'_{IAM.PC.DD} + 0.796(\pm 0.216) \quad (6)$$

$n = 7$; $r = 0.7751$; $s = 0.48$.

Note that the values for K'_m, which were originally determined at pH 7.4 in a phosphate buffer solution, have been corrected to pH 7.0 using published values of the pK_a of each β-adrenolytic drug following the procedure outlined in Ref. [22]. A linear relationship between $\log k'_{IAM.PC.MG}$ and $\log K'_m$ is described in Eq. (7), again, note that the K'_m values have been corrected to pH 7.0 to account for ionization differences [22]:

$$\log K'_m = 1.123(\pm 0.409) \log k'_{IAM.PC.MG} + 0.513(\pm 0.284) \quad (7)$$

$n = 7$; $r = 0.7758$; $s = 0.48$.

In this example, both $\log k'_{IAM.PC.DD}$ and

$\log k'_{\text{IAM.PC.MG}}$ correlated poorly with $\log K'_{\text{m}}$. It is interesting that acebutolol does not correlate as well as the others. When acebutolol is removed, the IAM.PC.DD column produced a correlation $r = 0.8887$ while the $^{\text{ester}}$IAM.PC.MG column produced a superior correlation of 0.9187. Rogers et al. [6,23] also found that acebutolol correlated worse than the other β-adrenolytic drugs when they investigated the correlation between $\log K_{\text{SF}}$ (shake-flask method) vs. $\log K_{\text{m}}$. These results suggest that the K_{m} value for acebutolol may be in error.

4. Conclusions

The performance of the IAM.PC.DD column was evaluated utilizing eight β-adrenoceptor antagonist compounds (β-adrenolytic drugs). Premature column failure occurred for 5 out of 6 columns tested. These results suggested that the IAM.PC.DD columns were susceptible to either silica support dissolution or that some immobilized phospholipid stationary phase was removed by the 0.01 *M* PBS mobile phase. The quality of the IAM.PC.DD column may have improved in the last two years since these columns were purchased. The IAM.PC.DD column was compared to the $^{\text{ester}}$IAM.PC.MG column for predicting drug–membrane interactions for several β-adrenolytic drugs. For the linear regression analysis between $\log k'_{\text{IAM}}$ and the logarithm of the *n*-octanol–water partition coefficients, the C_{18} HPLC retention factors, the liposome partition coefficients and various pharmacokinetic parameters investigated, significantly better correlations were obtained with the $^{\text{ester}}$IAM.PC.MG column. The results suggested that the β-adrenolytic drugs were partitioning deeper into the $^{\text{ester}}$IAM.PC.MG stationary phase as compared to the IAM.PC.DD stationary phase. Since partitioning is a prerequisite for the β-adrenolytic drugs to transport across membrane barriers, the greater partitioning into the $^{\text{ester}}$IAM.PC.MG stationary phase may account for its superior correlation to physicochemical and bioactivity data.

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