Original article

Phosphatidylethanolamine functionalized biomimetic monolith for immobilized artificial membrane chromatography

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ARTICLE INFO

ABSTRACT

In this research, a new phospholipid based monolith was fabricated by in situ co-polymerization of 1-dodecanoyl-2-(11-methacrylamidoundecanoyl)-sn-glycero-3-phosphoethanolamine and ethylene dimethacrylate to mimick bio-membrane environment. Excellent physicochemical properties of this novel monolith that were achieved included column efficiency, stability, and permeability. Moreover, the biomimetic monolith showed outstanding separation capability for a series of intact proteins and small molecules. In particular, it exhibited good potential as an alternative to the commercial immobilized artificial membrane (IAM) column (IAM.PC.DD2) for studying drug-membrane interactions. This study not only enriched the types of IAM stationary phases, but also provided a simple model for the prediction of phosphatidylethanolamine related properties of drug candidates.

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1. Introduction

Immobilized artificial membranes (IAMs) are a type of chromatographic stationary phase developed by covalently immobilizing membrane phospholipids on the surface of solid supports [1–3]. They have already been successfully employed to purify membrane protein, screen the inhibitor of receptors, and predict drug-membrane interactions and/or membrane permeability of drug candidates [4,5]. To date, numerous studies have demonstrated that there are good correlations between the IAM retention of drugs and their pharmacokinetic properties, including unbound volume of distribution [6], human oral absorption [7], and blood-brain uptake [8]. The first reported IAM column (i.e., IAM.PC) was fabricated via immobilizing monolayer of phosphatidylcholine (PC) analogue onto propylamine-silica supports by Pidgeon and Venkataram in 1989 [9]. So far, different silica-based IAM columns containing either single or double alkyl chains PC analogues are commercially available, such as IAM.PC.MG, IAM.PC.DD2, and IAM.PC.DD [2]. Although these PC-containing IAM columns have gained great success in mimicking the complex lipid environment of biological membranes [10–12], some disadvantages still cannot be neglected. Firstly, all these commercial IAM columns only mimic PC, but not other type of phospholipids [9,13]. Secondly, some residual amino groups and unreacted silanol groups still exist on the endcapped IAM surfaces, which affects the selectivity, stability and lifetime of the silica-based IAM columns [14]. Hence, it is meaningful to prepare novel phospholipid based stationary phases with better biomimetic property and stability [15,16].

Monolithic materials have attracted much attention and have been well applied in various fields, such as sample pretreatment, chromatographic separation, biomaterials, and simulation of biological processes due to their easy fabrication, wide functional diversity, high permeability, and good chemical stability [17–22]. For example, some single-chain PC or double-chains PC based

monoliths were successfully fabricated for the prediction of drug-membrane interactions [23,24]. Good correlations were achieved between the retentions of tested drugs on these PC based monoliths and the commercial IAM column (IAM.PC.DD2). Furthermore, mixed single-alkyl chain phospholipids (PC and phosphatidylserine (PS)) based monolith were also developed for assessing drug-induced phospholipidosis (DIPLD) risk [25]. The DIPLD potency of 79 tested pharmaceutical compounds measured on the mixed phospholipids based monolith are highly correlated with those obtained by other reported in vitro or vivo assays. Although PC is the most abundant phospholipid occurring in virtually all cell membranes, the other types of phospholipids (phosphatidylethanolamine (PE), phosphatidylglycerol (PG), PS, and phosphatidic acid (PA)) are also important in the function and structure of cell membrane. Among them, PE is a major phospholipid that makes up 20%–50% of total phospholipids in mammalian membranes [26]. Besides serving as a crucial component in membrane architecture, PE also has numerous biological functions such as forming essential intermediate structure in membrane fusion/fission, stabilizing membrane proteins in their suitable conformation [27]. In eukaryotic cell membranes, the main component of the outer leaflet is PC, whereas the inner leaflet mainly comprises PE. Phospholipid bilayers formed by these two phospholipids provide simple models for the inner and outer leaflets, respectively [28]. Inspired by the success of the PC-containing IAMs, the development of the PE-containing IAMs is also interesting. Nevertheless, only Ong et al. [29] developed a PE based IAM chromatography packing material by immobilizing single chain PE on propylamine silica so far. The proposed preparation process is time-consuming, laborious, and expensive, which limits its further application in bioanalysis.

In this research, a novel PE analogue 1-dodecanoyl-2-(11-methacrylamidoundecanoyl)-*sn*-glycero-3-phosphoethanolamine (MDSPE) was prepared and used to fabricate PE based monolith. For the purpose of achieving satisfactory chromatographic performance, the composition of the polymerization solution was systematically optimized. Micro-HPLC, scanning electron microscopy (SEM) and ζ-potential experiments were carried out to characterize the resultant monolith. Moreover, a series of drugs and intact proteins were chosen to evaluate its separation ability. Finally, the predictive ability of the MDSPE based monolith for drug-membrane interactions was evaluated and compared with that of the commercial IAM.PC.DD2 column and previously reported poly(1-dodecanoyl-2-(11-methacrylamid-oundecanoyl)-*sn*-glycero-3-phosphocholine (MDSPC)-*co*-ethylene dimethacrylate (EDMA)) monolith [24].

2. Materials and methods

2.1. Materials

Rnase A, insulin, cytochrome c (Cyt c), and the other analytes selected for studying drug-membrane interaction were supplied by Sigma-Aldrich (St. Louis, MO, USA) or provided by other labs. Methanol (MeOH) and deionized water were filtered with a 0.22-μm membrane filter before usage. The monomer MDSPE was synthesized in our lab according to Paltauf and Hermetter [30] with minor modification. The MS and NMR spectra of MDSPE are shown in Fig. S1. EDMA, acetonitrile (ACN), 2,2′-azobisisobutyronitrile (AIBN), MeOH, 3-(trimethoxysilyl)-propyl methacrylate (γ-MAPS), tetrahydrofuran (THF), naphthalene, ammonium acetate, and acetic acid were obtained from Aladdin (Shanghai, China). All the apparatus and chromatographic conditions used in this research are listed in the Supplementary data.

2.2. Preparation of the MDSPE based monolith

For anchoring bulk polymeric bed, fused-silica capillaries were first pretreated using γ-MAPS [31]. The MDSPE based monolith (poly(MDSPE-*co*-EDMA)) was then fabricated by a one-step approach (Fig. 1). According to the preparation protocol, the functional monomer (MDSPE), crosslinker (EDMA), porogens (MeOH and THF), and initiator (AIBN, 1% $m/m_{total\ monomers}$) were mixed and degassed for 10 min using an ultrasonic bath. The homogeneous solution was introduced into the pretreated fused-silica capillary. GC septa were used to seal both ends of the capillary. Subsequently, the capillary was incubated in 60 °C water bath for 12 h. To remove unreacted compounds and solvents, the resultant monolith was rinsed with HPLC-grade MeOH for 30 min. A 2–3 mm monolithic column was cut for morphology experiments. The double-chains PC based monolith (poly(MDSPC-*co*-EDMA)) was also prepared according to our previous work [24].

2.3. Calculations

The column permeability (K, m^2) was calculated according to the following equation [32]:

$$K = \left(\frac{uL}{\Delta P}\right) \times \eta$$

where u (m/s) represents the fluid velocity, L (m) represents the monolithic column length, η (Pa·s) represents the fluid viscosity, and ΔP (Pa) represents the column pressure drop.

3. Results and discussion

3.1. Fabrication of the MDSPE based monolith

To dissolve the monomer MDSPE and crosslinker EDMA, MeOH/THF mixture was selected as the porogens for fabricating the MDSPE based monolith. The polymerization-mixture composition could obviously affect the crucial properties of the proposed monolith, such as the column efficiency, permeability, efficiency, and morphology. Therefore, the amount of the crosslinker (EDMA), MeOH, and THF was systematically optimized.

The porogens weight content in the polymerization solution is a key factor that affects the backpressure and column efficiency of the proposed monolith. As shown in Table 1, the column backpressure of C1, C2, and C3 varied from 7.2 to 1.5 MPa with an increase in the proportion of the porogens from 67% (C1) to 77%
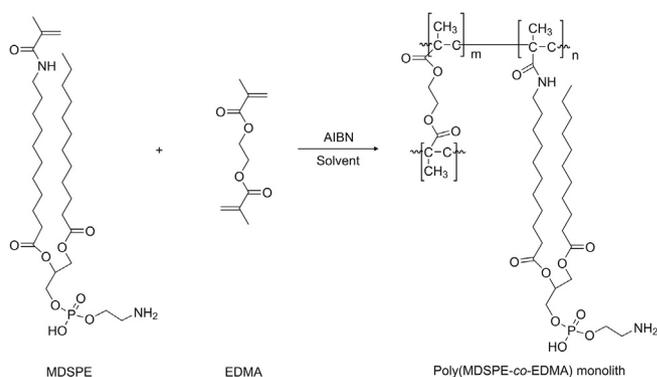
Fig. 1. Preparation of the poly(1-dodecanoyl-2-(11-methacrylamidoundecanoyl)-*sn*-glycero-3-phosphoethanolamine (MDSPE)-*co*-ethylene dimethacrylate (EDMA)) monolith by in-situ copolymerization method. AIBN: 2,2′-azobisisobutyronitrile.

Table 1

Composition of the polymerization mixtures used for the preparation of the poly(1-dodecanoyl-2-(11-methacrylamidoundecanoyl)-*sn*-glycero-3-phosphoethanolamine (MDSPE)-*co*-ethylene dimethacrylate (EDMA)) monolith.

Column	Monomers (%, *m/m*)		Porogens (%, *m/m*)		Monomers:porogens (%, *m/m*)		Back-pressure (MPa)	Column efficiency (theoretical plates/m)
	MDSPE	EDMA	MeOH	Tetrahydrofuran	Monomers	Porogens		
C1	59	41	51	49	33	67	7.2	20000
C2	59	41	51	49	28	72	5.2	32000
C3	59	41	51	49	23	77	1.5	11000
C4	64	36	51	49	28	72	5.4	24000
C5	54	46	51	49	28	72	4.0	26000
C6	59	41	46	54	28	72	10.2	12000
C7	**59**	**41**	**56**	**44**	**28**	**72**	**1.0**	**30000**
C8	59	41	61	39	28	72	0.3	28000
C9	59	41	66	34	28	72	0.1	8000

Conditions: column dimensions: 120 mm × 100 μm i.d.; mobile phase: H_2O:acetonitrile (ACN) (60:40, *V/V*); UV detection wavelength: 214 nm; flow rate: 400 nL/min; injection volume: 20 nL; sample: anisole.

(C3). The best column efficiency was obtained on the column C2 (3.2×10^4 theoretical plates/m). Considering the column backpressure and efficiency, the composition for column C2 was selected for the following experiments. The amount of the crosslinker has an important effect on the formation of the polymer monolith. When the EDMA proportion in the monomer mixture ranged from 36% (C4) to 46% (C5), no significant influence on the column backpressure was observed. The highest column efficiency under a suitable backpressure was still obtained on column C2 with 41% (*m/m*) of EDMA. The composition of the porogens could significantly affect the permeability of the resultant monolith. When the MeOH content was increased from 46% (C6) to 66% (C9), the backpressure significantly decreased from 10.2 to 0.1 MPa. A suitable column efficiency (3.0×10^4 theoretical plates/m) and backpressure (1.0 MPa) were obtained on the column C7 with 56% (*m/m*) MeOH in the porogens. Thus, the polymerization conditions for the column C7 was finally employed for all further experiments. SEM images also showed that of the optimal MDSPE based monolith possessed a uniform porous structure and the bulk polymer was tightly attached to the fused-silica capillary (Fig. 2).

3.2. Column permeability and stability

The permeability and mechanical stability of the MDSPE based monolith were evaluated by pumping various mobile phases (ACN, MeOH, and H_2O) under different linear flow rates. Solvent polarity and fluid viscosity were cited from the previous study [33]. Good linearies between linear velocity and column backpressure were observed on the column C7 with all the three solvents. The *K* values for the column C7 were calculated to be 4.91×10^{-14}, 4.35×10^{-14}, and 3.25×10^{-14} m^2 using ACN, MeOH, and H_2O as mobile phase, respectively (Table 2) [33]. Excellent mechanical stability of the column C7 can be confirmed by the good linearity between the

column backpressure and velocity up to 13 MPa of back pressure (figure not shown). These results also demonstrated that the proposed monolith did not shrink, swell, or deform under various mobile phases. Good permeability and mechanical stability of this monolith will benefit its applications in bioanalysis and bioseparation.

3.3. Retention mechanism and reproducibility

The retention of analyte on the MDSPE based monolith is mainly governed by hydrophobic interactions due to the long alkyl chains of MDSPE. For better understanding the retention mechanism, a test mixture of toluene and thiourea was selected. As shown in Fig. S2, the retention time of toluene obviously decreased when the ACN content increased from 40% to 90% in the mobile phase. However, the retention time for thiourea was observed to remain constant as the ACN proportion increased from 40% to 90% and then slightly increased with further increase of ACN proportion to 95%. These typical RPLC retention behavior indicated that the hydrophobic interactions contributed significantly to retention on the MDSPE based monolith when the ACN content was below 90%. In addition, the ζ-potential values of the proposed monolith were also tested using 50 mM ammonium acetate at pH 6.0, 7.4, and 8.0. The obtained ζ-potential values were −0.3, −1.5, and −2.1, respectively. Therefore, the weak electrostatic interaction might also contribute to the retention of charged compounds on this IAM monolith.

Finally, the reproducibility of the monolith was investigated by calculating the relative standard deviations (RSDs) for the retention time of two analytes (toluene and thiourea). H_2O:ACN (60:40, *V/V*) was selected as the sample solvent and mobile phase. As listed in Table 3, the reproducibility of run-to-run (*n*=10), day-to-day (*n*=3), batch-to-batch (*n*=3), and column-to-column (*n*=3) were all acceptable with RSDs≤5.3%. Based on these experiments, it could be concluded that the proposed MDSPE based monolith exhibited
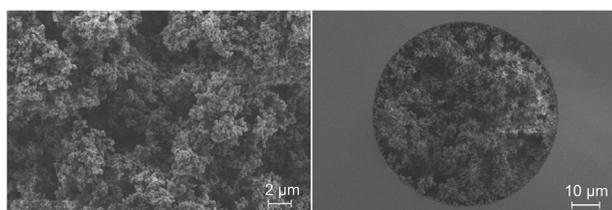
Fig. 2. Scanning electron microscopy images of column C7.

Table 2

Permeability of the poly(MDSPE-*co*-EDMA) monolithic column [33].

Mobile phase	Relative polarity	Viscosity ($\times 10^3$, Pa·s)	Permeability (K, $\times 10^{-14}$, m^2)
ACN	0.460	0.369	4.91
MeOH	0.762	0.544	4.35
H_2O	1.000	0.890	3.25

Table 3
Reproducibility of retention time on the poly(MDSPE-*co*-EDMA) monolith.

Reproducibility	RSD$_{Toluene}$(%)	RSD$_{Thiourea}$(%)
Run-to-run ($n=10$)	1.3	1.7
Day-to-day ($n=3$)	1.5	2.5
Column-to-column ($n=3$)	0.6	1.9
Batch-to-batch ($n=3$)	3.0	5.3

Experimental conditions: column dimensions: 150 mm × 100 μm i.d.; mobile phase: H$_2$O:ACN (60:40, *V/V*); UV detection wavelength: 214 nm; flow rate: 400 nL/min; injection volume: 20 nL; sample: toluene, thiourea. RSD: relative standard deviations.

good column efficiency, high permeability and stability, and satisfactory reproducibility for further micro-HPLC applications.

3.4. Separation of intact proteins and small molecule drugs

To evaluate the applicability of the PE functionalized monolith, three intact proteins (RNAse A, insulin, Cyt c) were first separated. As can be seen in Fig. 3, baseline separation was achieved within 15 min. In addition, a mixture of eight basic drugs was employed to further explore its separation ability. Fig. 4 shows that baseline separation and acceptable peak shape were obtained within 18 min for all drugs. All these results demonstrated that this PE functionalized monolith had good potential for the separation of both large and small molecules.

3.5. Prediction of drug-membrane interactions

IAM columns have been successfully applied to mimick drug-membrane interactions and predict related physiological properties, such as blood-brain barrier penetration and intestinal permeability [2]. The similarity between drug retention behavior on the IAM columns and its membrane adsorption processes allows developing suitable correlation models to estimate certain biopartitioning properties. Chromatographic hydrophobicity index (CHI) IAM values measured on the IAM columns are regarded as a reliable parameter to characterize the interactions between drugs and IAM stationary phase. For the determination of CHI IAM values of drugs, a series of standard compounds whose CHI IAM values have already been reported [34] were selected. A calibration curve (CHI IAM = 7.09 × t_R − 49.90) was established by plotting their reported CHI IAM values against the obtained retention time (t_R)
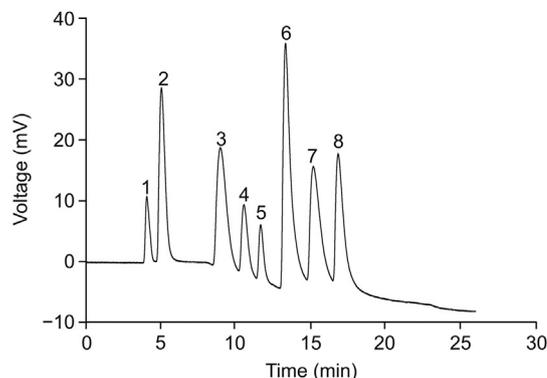
Fig. 4. Separation of drugs on the poly(MDSPE-*co*-EDMA) monolith. Experimental conditions: column dimension: 170 mm × 100 μm i.d.; mobile phase: H$_2$O containing 50 mM ammonium acetate (pH 7.4) (A), ACN (B); gradient: 0 min, 10% B; 15.0 min, 100% B; 18.0 min, 100% B; 18.5 min, 10% B; 26.0 min, 10% B; detection wavelength: 214 nm; flow rate: 600 nL/min; injection volume: 20 nL; samples: (1) 6-hydroxydopamine; (2) sulfanilamide; (3) hydrochlorothiazide; (4) lidocaine; (5) ketoconazde; (6) tioconazole; (7) amodiaquine; and (8) amiodarone.

values on the PE based monolith (Table S1). To assess the prediction ability of the monolith, 70 drugs with diverse structure were tested. Their log *P* (octanol-water partition coefficient) values and CHI IAM$_{7.4}$ values on the three different IAM columns are listed in Table 4. Despite the structural diversity of the investigated drugs, the CHI IAM$_{7.4}$ values obtained on the MDSPE based monolith were highly correlated to their log *P* values (Spearman's rho = 0.88, Fig. S3). This result indicated that hydrophobic interaction was still the determinant on the PE functionalized monolith, which is in accord with previous studies [35,36]. Statistical comparative study between CHI IAM$_{7.4}$ values on the MDSPE based monolith and IAM.PC.DD2 column was also performed. A significant Spearman's correlation of 0.95 (Fig. 5A) was obtained between the CHI IAM$_{7.4}$ $_{(MDSPE)}$ and CHI IAM$_{7.4}$ $_{(PC.DD2)}$ values, which indicated similar retention mechanisms on these two IAM columns. In our previous work, the MDSPC based monolith [24] and MDPA based monolith [31] were also fabricated for different applications. PA is a kind of acidic phospholipid, and therefore the MDPA based monolith is not compared here. Interestingly, this Spearman's correlation value was better than that between the retention of the MDSPC based monolith and IAM.PC.DD2 column (0.76) (Fig. S4). The reason is still not clear.

PC and PE, the two most abundant phospholipids in cell membranes, comprise a glycerol backbone esterified with phosphoric acid and two long acyl chains. The phosphate group is combined with choline in PC, whereas it is covalently bound to ethanolamine in PE [37]. Both of them play predominant roles in drug membrane transport. For better insights into the effect of the phospholipid type in predicting drug-membrane interaction, a comparative study between the MDSPC based monolith and MDSPE based monolith was also carried out. Apart from hydrophobic interaction, it has been reported that electrostatic interaction could contribute to the retention of charged analytes on the PC functionalized columns [11]. Two monomers (i.e., MDSPC and MDSPE) have very similar molecular structure, differing only in their polar head-groups. The phosphate anions of the head-groups, located close to the hydrophobic core of the monoliths, strengthen the interaction of the positively charged compounds with the IAM stationary phase. In contrast, the positively charged trimethylamine (MDSPC) or amino (MDSPE) groups, being exposed to the solvent at the outer terminal of the IAM surface, contribute to an electrostatic attraction with anions. It is worth noting that the MDSPC could be

Fig. 3. Separation of proteins on the poly(MDSPE-*co*-EDMA) monolith. Experimental conditions: column dimensions: 240 mm × 100 μm i.d.; UV detection wavelength: 280 nm; mobile phase: 0.1% trifluoroacetic acid (TFA) in H$_2$O (A), 0.1% TFA in acetonitrile (ACN) (B); gradient: 0 min, 25% B; 20 min, 90% B; 20.5 min, 25% B; 25 min, 25% B; flow rate: 1200 nL/min. Cyt c: cytochrome c.

Table 4
Selected drugs and their log P, chromatographic hydrophobicity index (CHI) immobilized artificial membrane (IAM)$_{7.4 \text{ (MDSPE)}}$, CHI IAM$_{7.4 \text{ (MDSPC)}}$, CHI IAM$_{7.4 \text{ (PC.DD2)}}$ values.

Compounds	log P [a]	CHI IAM$_{7.4 \text{ (MDSPE)}}$ [b]	CHI IAM$_{7.4 \text{ (MDSPC)}}$ [c]	CHI IAM$_{7.4 \text{ (PC.DD2)}}$ [d]
Amiodarone	7.20	70.50	51.17	73.81
Amitriptyline	4.92	53.64	24.80	47.19
Carbamazepine	2.77	20.59	19.63	22.18
Chlorpromazine	5.41	61.26	31.79	56.04
Clozapine	3.23	47.78	32.14	45.90
Diclofenac	4.51	23.01	27.51	34.48
Diltiazem	2.80	35.79	18.39	31.69
Fluoxetine	4.05	55.20	28.05	48.76
Hydrocortisone	1.61	17.96	18.31	21.88
Lidocaine	2.44	24.91	15.12	29.51
Metolazone	2.50	28.09	21.08	18.16
Nitrendipine	2.88	36.16	36.76	38.80
Oxprenolol	2.10	38.03	−11.44	29.17
Phenoxybenzamine	4.70	55.57	45.35	52.09
Pindolol	1.75	40.45	−7.79	31.62
Promethazine	4.81	22.62	28.22	22.15
Terbutaline	0.90	16.96	−26.30	18.19
Theophylline	−0.02	−19.38	−29.78	−11.88
Thioridazine	5.90	65.23	33.56	58.03
Tioconazole	4.40	46.36	42.81	49.75
Verapamil	5.23	39.82	22.80	37.49
4-hydroxycoumarin	1.01	−20.68	−27.30	−1.98
6-hydroxydopamine	/	−20.97	−33.41	−34.97
Acetazolamide	−0.26	−16.89	−19.48	−7.76
Amodiaquine	3.70	59.11	32.53	48.82
Atropine	1.83	20.18	−27.23	25.06
Auramine O	/	54.66	40.82	42.94
Caffeine	−0.07	−18.59	−29.24	−13.20
Cimetidine	0.40	−11.28	−26.56	9.38
Clofibrate	3.30	40.13	38.30	40.39
Dibucaine	4.40	48.22	22.30	53.73
2,6-pyridinedicarboxylic acid	−0.44	−21.22	−32.61	−24.77
Disopyramide	2.58	20.62	−26.91	24.72
Hydroxyzine	3.43	42.69	26.41	39.85
Indapamide	2.52	30.37	30.83	26.75
Isoniazid	−0.70	−20.39	−31.43	−20.48
Menadione	2.20	32.32	31.74	27.33
Methotrexate	−1.85	−21.36	−31.37	−10.27
Ofloxacin	−0.39	−20.95	−32.71	−24.00
Procaine	2.14	24.00	18.23	10.10
Quinine	3.44	47.65	12.35	41.35
Rifampin	2.70	24.57	22.37	29.24
Sotalol	−0.40	14.45	−28.95	11.48
Sulfanilamide	−0.62	−13.37	−18.11	−17.94
Tert-butylhydroquinone	2.91	32.01	35.12	34.89
Trimipramine	4.20	51.77	25.90	47.54
Amlodipine maleate	/	55.45	24.71	51.73
Ketoconazole	4.35	32.97	28.85	36.39
Citalopram hydrobromide	/	44.10	13.19	40.04
Tobramycin	−5.80	−20.97	−33.73	−23.93
Amikacin	−3.20	−21.46	−33.67	−24.06
Imipramine	4.80	51.90	21.66	46.70
Carbamazepine	2.77	20.52	20.35	25.31
Mianserin	3.83	47.77	34.05	47.39
Desipramine·HCl	/	56.08	22.73	50.79
Clomipramine	5.19	57.16	29.34	53.57
Chlorphentermine	2.60	13.18	7.07	41.24
Nortriptyline	3.90	55.18	23.23	52.90
Imipramine	4.80	56.08	19.84	46.70
Desipramine	4.90	55.30	23.68	48.26
Clomipramine	5.19	57.47	29.04	51.30
Oxazepam	2.24	27.53	28.46	33.60
Pentobarbital sodium	/	19.80	21.33	24.38
Hexobarbital	1.98	20.20	19.42	20.20
Domperidone	3.90	42.33	26.69	41.24
Clonidine	1.59	31.20	−8.27	29.01
Prochlorperazine	4.88	54.86	31.53	57.05
Theobromine	−0.78	−15.12	−33.33	−12.84
Benzene	/	28.21	25.92	24.54
Toluene	2.73	34.56	32.94	33.26

Experimental conditions: [a] log P values were from DrugBank database; [b] the conditions for poly(MDSPE-*co*-EDMA) monolith, see Fig. 4; [c] poly(MDSPC-*co*-EDMA) column dimension (130 mm × 100 μm); mobile phase: 50 mM ammonium acetate (pH 7.4) buffer (A), ACN (B); gradient: 0 min, 10% B; 15.0 min, 100% B; 18.0 min, 100% B; 18.5 min, 10% B; 26.0 min, 10% B; detection wavelength: 214 nm or 254 nm; flow rate: 600 nL/min; injection volume: 20 nL; [d] Regis IAM.PC.DD2 column dimension (100 mm × 4.6 mm i.d., 10 μm); mobile phase: 50 mM ammonium acetate (pH 7.4) buffer (A); ACN (B); gradient: 0 min, 0% B; 6.0 min, 100% B; 6.5 min, 100% B; 7.0 min, 0% B; 9.0 min, 0% B; detection wavelength: 214 nm; flow rate: 1.0 mL/min; injection volume: 20 μL. MDSPC: 1-dodecanoyl-2-(11-methacrylamidoundecanoyl)-*sn*-glycero-3-phosphocholine; PC: phosphatidylcholine.
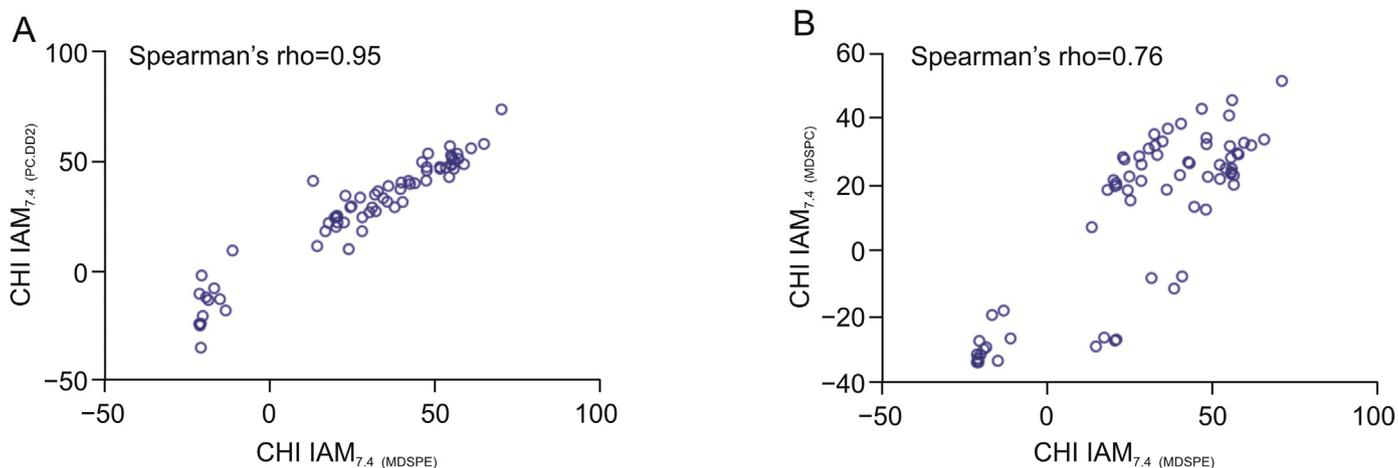
Fig. 5. (A) Spearman's correlations between chromatographic hydrophobicity index (CHI) immobilized artificial membrane (IAM)$_{7.4(PC.DD2)}$ values and CHI IAM$_{7.4(MDSPE)}$ values. (B) Spearman's correlations between CHI IAM$_{7.4\ (MDSPE)}$ values and CHI IAM$_{7.4\ (MDSPC)}$ values. MDSPC: 1-dodecanoyl-2-(11-methacrylamidoundecanoyl)-*sn*-glycero-3-phosphocholine.

synthesized through methylation on the amino group of MDSPE [38]. A Spearman's correlation of 0.76 (Fig. 5B) was observed between the CHI IAM$_{7.4\ (MDSPE)}$ and CHI IAM$_{7.4\ (MDSPC)}$ values of 70 drugs, indicating that the methylation on the amino group could affect the retention on IAM columns. If the 70 drugs are classified into three groups, i.e., neutral compounds (18), basic compounds (42) and acidic compounds (10), different Spearman's correlations, 0.98, 0.80, and 0.76 for neutral, basic, and acidic drugs, respectively, were observed (Fig. S5). This could be explained by the similar hydrophobic interactions on these two monoliths and the different electrostatic interactions due to different charge state of trimethylamine nitrogen (MDSPC) and amino nitrogen (MDSPE) at pH 7.4.

4. Conclusions

In this work, a novel phospholipid functionalized monolith was fabricated using MDSPE as the monomer, EDMA as the crosslinker, MeOH and THF as the porogenic solvents, and AIBN as the initiator. Satisfactory column permeability and efficiency were obtained after systematically optimizing the composition of the polymerization mixture. A series of intact proteins and basic drugs were baseline separated on this novel monolith. Statistical comparative studies between the PE functionalized monolith and those PC-containing IAM stationary phases (MDSPC based monolith and commercial IAM.PC.DD2 silica column) indicated that this novel biomimetic monolith has a great potential for studying drug-membrane interactions. The proposed method for constructing PE modified surface can be useful for mimicking a PE-containing environment, where PE plays an essential role. The monomer MDSPE can be further explored in surface modification of nanoparticles for drug delivery, rapid screening of drug candidates, and protein purification.

CRediT author statement

Peijie Zhu: Formal analysis, Investigation, Writing - Original draft preparation; **Weijia Chen:** Methodology, Writing - Reviewing and Editing; **Qiqin Wang:** Software, Validation, Resources; **Huihui Wu:** Software, Data curation; **Meng Ruan:** Investigation; **Hongwu Wang:** Visualization, Supervision; **Zhengjin Jiang:** Conceptualization, Methodology, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2021.09.002.

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