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Small diamines as modifiers for phosphatidylcholine/phosphatidylserine coatings in capillary electrochromatography

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Abstract

Greater stability of liposome coatings and improved resolution of model steroids in capillary electrochromatography (CEC) were sought by adding small diamines (ethylenediamine, diaminopropane, bis-tris-propane, or *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid, HEPES)) to the liposome solution before coating of fused silica capillaries. The phospholipid coatings consisted of 1 mM of 8:2 mol% phosphatidylcholine (PC)/phosphatidylserine (PS) and 5 mM of modifier in buffer solutions (acetate, phosphate, or Tris) at pH 4.0–7.4. The coating was based on a published procedure, and five steroids were used as neutral model analytes in evaluation of the coating. The results showed that under optimal conditions, the small linear diamines increased the packing density of anionic phospholipids, leading to improved separations. In addition, the choice of buffer for the liposome coating and separation appeared to influence the performance of the coatings. While buffers with amino groups take part in the phospholipid bilayer formation, buffers like phosphate may even have negative effect on coating formation. The factors affecting phospholipid coatings with diamines as modifiers are clarified.

Keywords: Amines; Capillary electrochromatography; Capillary electrophoresis; Phospholipid coating

1. Introduction

The driving force in the formation of biomembranes from phospholipids is the hydrophobic effect and these membranes can be considered to be self-assembling systems. It has been demonstrated by both theory and practice that phospholipids arrange in a uniform bilayer assembly on planar substrates, and this is also assumed to happen when capillaries for electrophoresis are filled with a phospholipid vesicle solution [1–4]. In previous work, we developed a simple coating method for capillary electrochromatographic applications [5]. Wiedmer et al. [6] have recently published a review covering the immobilization of liposomes in chromatographic columns and in capillary electrophoresis capillaries.

Liposomes, which can be considered to mimic biological membranes, have become an attractive research topic during the past few decades. Numerous studies on interactions between biomembranes and analytes such as proteins and drug compounds have been carried out [7–13]. Phosphatidylcholine (PC) is the most common phospholipid in mammalian cells. Like many natural phospholipids, PC is a zwitterionic compound containing negative phosphate groups and also a tertiary amino group. Phosphatidylserine (PS) is an anionic phospholipid. In addition to a phosphate and a primary amino group, it has a carboxy group, which gives it negative net charge. Mixtures of these two phospholipids have been extensively used in studies where liposomes have been crafted to mimic cell membranes [7,12,14–16].

Considerable interest has been show in the structure of these biomembranes and the mechanism by which they are formed [1–4,17]. Although hydrophobic interactions are the main driving force in membrane assembly, calcium ions, among others, greatly affect the membrane formation by working as a fusogenic agent packing the phospholipids more tightly together [18,19]. Modification of PC/PS membranes with calcium in capillary electrochromatography (CEC) enables the separation of neutral steroids in Tris buffer,

Abbreviations: LUV, large unilamellar vesicle; PC, phosphatidylcholine; PS, phosphatidylserine

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whereas no separation is achieved without calcium [20]. The use of (*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) as buffer solution stabilizes the formation of the phospholipid membrane, but in this case too, calcium improves the separation efficiency of neutral analytes [20]. It has been proposed [5,20] that HEPES mediates the coating by interacting with the phospholipids and forming a complex that is capable of binding efficiently to the capillary surface. Furthermore, protonation of the amino groups, depending on the coating pH, is essential for the stability of the coating.

In liquid chromatography silicapropylamino particles have been used as solid supports for phospholipids, which are bound on propylamino molecules covalently, forming artifical membranes [21]. We can assume that amines play an important role in phospholipid membrane formation and that zwitterionic HEPES could be replaced with small amines. Small free amines have not earlier been used as membrane stabilizers or modifiers for phospholipid coatings in capillary electrochromatography. In this work, we investigate stabilizing effect of amines with primary, secondary, and tertiary amino groups on liposome coatings in CEC, and their effect on the separation of neutral model analytes.

2. Experimental

2.1. Materials

17α-Hydroxyprogesterone, androstenedione, bovine brain phosphatidylserine, *d*-aldosterone, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid), and tris(hydroxymethyl)aminomethane (Tris) were from Sigma (St Louis, Mo, USA). Progesterone, testosterone, 1,3-diaminopropane (DAP), sodium chloride, and pH solutions (4, 7, and 10) used for calibrating the pH meter were from Merck (Darmstadt, Germany). 1,2-Ethylenediamine (EDA) and *bis-tris*-propane (BTP) were from Fluka (Buchs, Switzerland). Chicken egg phosphatidylcholine was from Avanti Polar-Lipids (Alabaster, AL, USA); sodium hydroxide and hydrochloric acid (1.0 M) were from FF-Chemicals (Yli Ii, Finland). Methanol and sodium dihydrogenphosphate were from Mallinckrodt Baker (Deventer, The Netherlands). Acetone was from Lab-Scan (Dublin, Ireland) and chloroform from Rathburn (Walkerburn, UK).

2.2. Equipment

Fused-silica capillaries of 50 μm i.d. (375 μm o.d.), length to detector 51.5 cm, and total length 60 cm were from Composite Metal Services (Worcestershire, UK). A Hewlett Packard ^{3D}CE system (Agilent, Waldbronn, Germany) equipped with a diode array detector (detection at 200 and 245 nm) and an air cooling device for the capillary cassette was used for the CEC experiments.

A MeterLab PHM220 pH meter (Radiometer, Copenhagen, Denmark) was used to adjust the pH of the electrolyte solutions. Distilled water was further purified with a Millipore Water Purification System (Millipore S.A., Molsheim, France).

A nitrogen-evaporating unit (Pierce, Reacti-Therm Heating Module, Rockford, IL, USA) and a desiccator equipped with a vacuum pump (KNF Neuberger, Freiburg, Germany) were used to evaporate chloroform from the phospholipid mixture. A shaking water bath (SB-16 Techne, Duxford, UK) with a thermostat (HETO, Birkeröd, Denmark) was used to hydrate liposomes into the buffer solution, and a vortexer (REAX 2000, Heidolph, Germany) was used to accelerate hydration. Liposomes were extruded to large unilamellar vesicles (diameter 100 nm) with a LiposoFast-Basic extruder (Avestin, Ottawa, Canada).

2.3. Methods

2.3.1. Buffers and samples

Buffers were prepared by dissolving accurate amounts of buffering reagent (acetic acid, sodium dihydrogenphosphate, or Tris) in water and the pH was adjusted to 4.0–7.4 with 1 M sodium hydroxide or hydrochloric acid. The ionic strengths of the buffers were adjusted to 20 mM with sodium chloride (3.1–16.5 mM) except for dihydrogenphosphate (ionic strength 32.3 mM after pH adjustment). Before use, the buffers were filtered through 0.45 μm syringe filters (Gelman Sciences, Ann Arbor, MI, USA). The steroid samples were prepared from stock solutions (1–2 mg ml^{-1} in methanol). The steroid concentrations in the injected sample were 20 μg ml^{-1} of aldosterone, androstenedione, and testosterone, and 50 μg ml^{-1} of 17α-hydroxyprogesterone and progesterone in 10/90% v/v methanol/background electrolyte (BGE) solution. The migration time of methanol was used as a marker for the electroosmotic flow (EOF). All solutions were stored in a refrigerator.

2.3.2. Liposome preparation

Liposomes were prepared as follows: appropriate amounts of the lipid stock solutions in chloroform were mixed to obtain the desired composition of 80 mol% of PC and 20 mol% of PS. The mixture was evaporated to dryness under a stream of nitrogen, and traces of solvent were removed by evacuation under reduced pressure (8–100 mbar) overnight. The lipid residues were hydrated in the indicated buffer at 60 °C to yield multilamellar vesicles with a lipid concentration of 1 mM, and the vesicles were maintained at this temperature for 60 min with subsequent shaking. During hydration, the vesicle-containing solution was vortexed more intensely three or four times. The resulting dispersion was processed to large unilamellar vesicles by extrusion 19 times through Millipore (Bedford, MA, USA) 0.1 μm pore size polycarbonate filters using a LiposoFast extruder. The liposome solutions were stored in a refrigerator. Modifiers were added to the li-

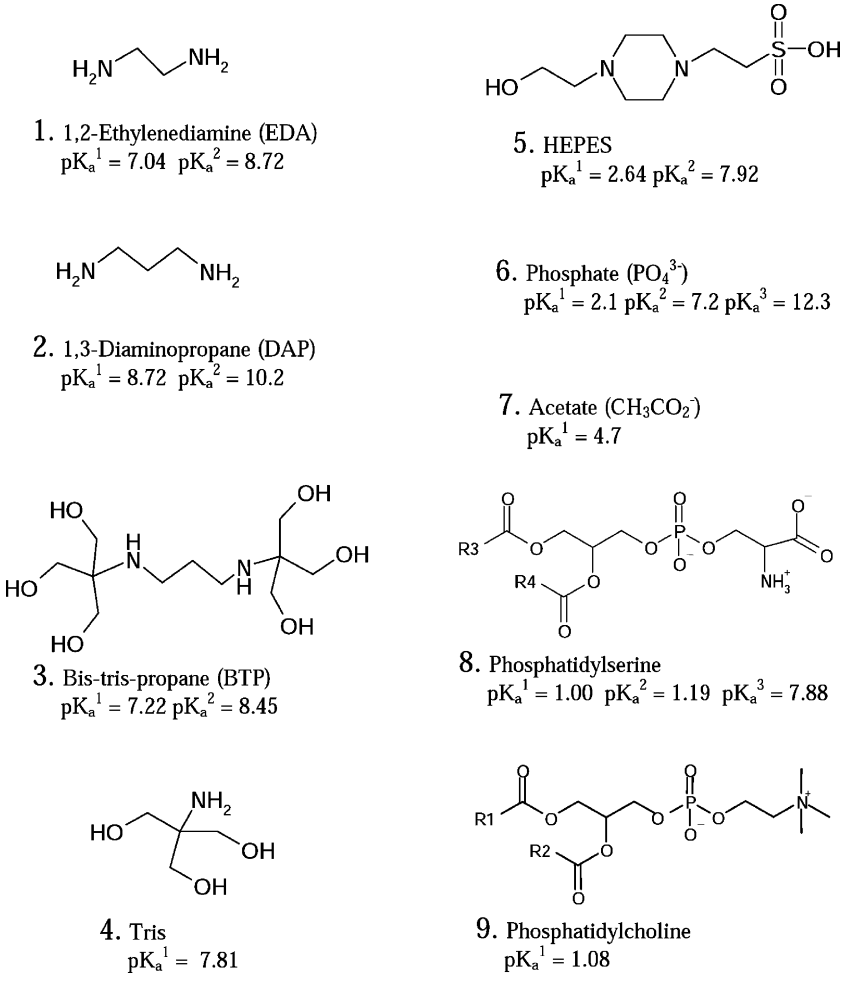

Fig. 1. Structures and pK_a values of diamine modifiers and buffers tested.

posome solutions before use. The structures and also pKa values calculated with Pallas 1.2 (CompuDrug Chemistry Ltd., Sedona, USA) are presented in Fig. 1.

2.3.3. Capillary coating

The fresh capillary was rinsed with a pressure of 930–940 mbar for 10 min with 0.5 M nitric acid and for 15 min with water or 5 mM modifier solution [5]. Phospholipid coating was applied to the capillary inner surface by rinsing the capillary for 10 min with 1 mM liposome solution at 930–940 mbar, and letting it stand filled with the liposome solution for 15 min. Finally the capillary was flushed for 5 min with BGE solution to remove unbound liposomes.

2.3.4. Capillary electrochromatographic separations

In CEC runs, the voltage was 20 kV and temperature of the capillary cassette 25 °C; injection was done hydrodynamically for 5 s at 50 mbar. Detection was carried out at 245 or 280 nm. Separations were repeated six times and before each injection the capillary was rinsed for 2 min with the BGE solution.

3. Results and discussion

Small diamines containing primary, secondary, or tertiary amino groups were investigated for their effect on phosphatidylcholine/phosphatidylserine bilayer. The diamines were 1,2-ethylenediamine, 1,3-diaminopropane, bis-tris-propane, and HEPES (see Fig. 1). The concentration range for the modifier experiments was set and the effect of preflushing the capillary with modifier before coating was studied with use of DAP as modifier. The effect of the four modifiers in different buffers and pH was then studied. Finally, possible coating mechanisms are considered.

Neutral steroids were used as model compounds and thus the separation mechanism is based on the distribution of analytes between the aqueous phase and the phospholipid membrane. Hydrophobic interactions govern the partition of analytes, and since analyte properties remain constant information on the membrane can be obtained. The data are mostly compared using retention factor k, which in the case of an electrically neutral analyte can be calculated directly from the migration time $k = (t_{migr} - t_{eo})/t_{eo}$. The retention factor

is a measure of the time the analyte resides in the stationary phase relative to the time it resides in the mobile phase.

3.1. Influence of modifier concentration and preflushing on coating performance

Effect of modifier concentration was tested with 0–5 mM DAP in Tris pH 7.4. Retention of progesterone on the phospholipid membrane increased with modifier concentrations until about 3 mM when it began to level off. A concentration of 5 mM was selected for the further experiments to ensure that the amount of modifier was effective.

As expected, neutral steroids could not be separated without the phospholipid coating (Fig. 2A). Likewise, a simple pre-flush of the capillary with modifier (5 mM DAP in water) before coating with liposome solution without modifier (in Tris pH 7.4) was not sufficient to obtain good separation (Fig. 2B). Similar separations to that depicted in Fig. 2B were obtained when the PC/PS coating was carried out without using any modifiers at any stage. However, preflushing of the capillary with DAP reduced the EOF slightly (by 11%) relative to the coating without preflush (see Table 1). This is a sign of interaction of DAP with the silica wall and the phospholipids. Good separation was achieved when modifier (5 mM) was added to the liposome coating solution (Fig. 2C). Evidently, DAP does not just act as binding agent between the silanol groups of the capillary and the liposomes but interacts with the liposome membrane. This result confirms what was shown earlier [20] that the Tris buffer alone does not work as amine modifier.

3.2. Effect of modifier and pH used in coating on the separation

Table 2 contains the retention factors of neutral analytes on the PC/PS coating as they vary with the buffer and modifiers added to the coating solution. The acetate buffer at pH 4.0 provided satisfactory separation with all four modifiers, but there

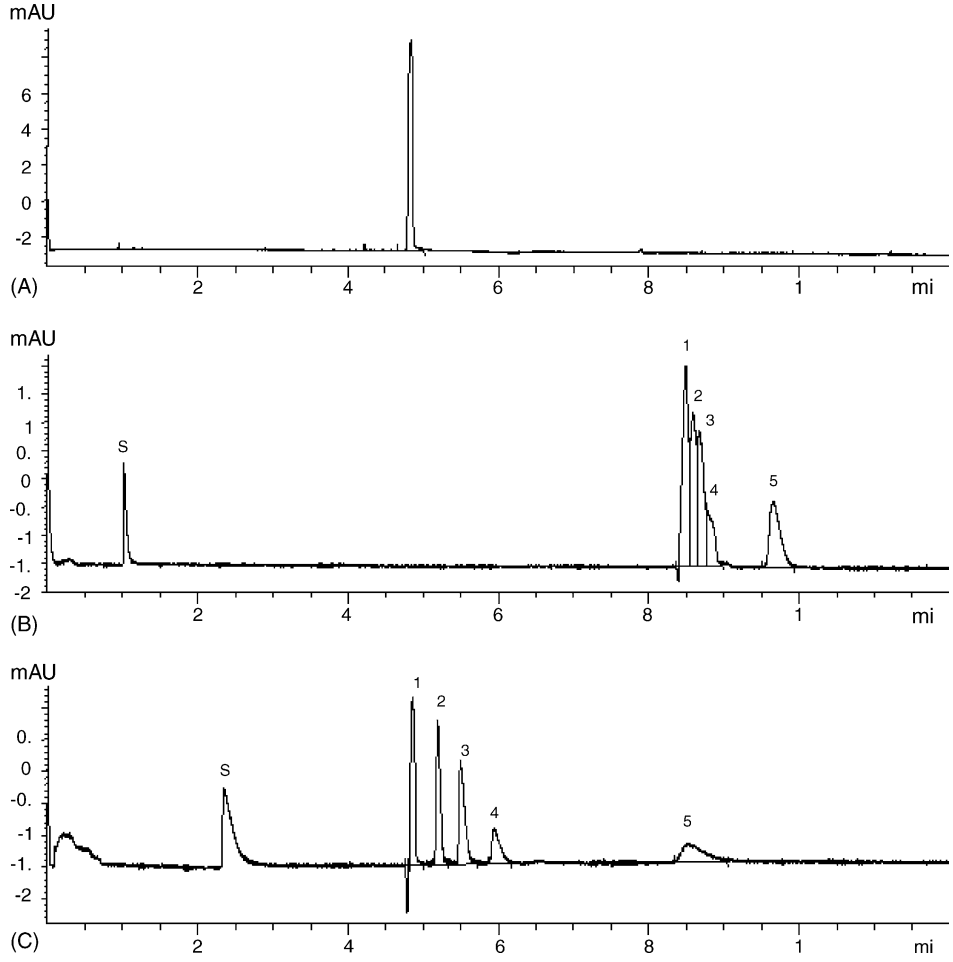

Fig. 2. Electropherograms obtained with Tris buffer having ionic strength of 20 mM and pH 7.4 and the effect of DAP on PC/PS coating. The sample contained 20 μg ml^{-1} of aldosterone (1), androstenedione (2), and testosterone (3), and 50 μg ml^{-1} of 17α-hydroxyprogesterone (4) and progesterone (5); injection 50 mbar for 5 s, run voltage 20 kV, capillary length 60 cm and length to the detector 51.5 cm. (A) No coating, (B) PC/PS coating using 10 min preflush with 5 mM DAP before coating with PC/PS 8/2 mol% 1 mM solution, and (C) Like B but with 5 mM of DAP added to the liposome solution. The migration order of the analytes is the same as in the list above aldosterone being the fastest and progesterone the slowest. In (B) and (C), the (S) indicates a system peak.

Table 1
Effect of modifier and buffer on electro-osmotic flow in PC/PS-coated capillaries

	EOF with different modifier (m^2/V s)/RSD (%)				
	No modifier	5 mM EDA	5 mM DAP	5 mM BTP	5 mM Hepes
Acetate pH 4	1.73E−08/18.78	1.92E−08/2.39	1.79E−08/8.91	1.68E−08/3.46	1.65E−08/14.36
Acetate pH 5	2.54E−08/7.80	3.11E−08/3.12	3.10E−08/3.02	2.85E−08/11.62	2.46E−08/10.80
Phosphate pH 7.4	3.57E−08/1.51	6.44E−08/0.87	4.95E−08/1.92	5.01E−08/0.27	3.92E−08/2.47
Tris pH 7.4	3.44E−08/0.19	4.69E−08/1.67	5.46E−08/0.77	3.47E−08/1.11	2.39E−08/7.11

Preflushing with 5mM DAP and coating with unmodified liposome in Tris pH 7.4 EOF 3.06E−08 m^2/V s (RSD 2.04%); EOF with uncoated capillary and pH 7.4 Tris 5.33E−8 m^2/V s.

was virtually no difference in resolution with different the modifiers. Even though the first four steroids were partly separated (resolution 0.8–1.3), only progesterone, which was the most hydrophobic, was baseline separated from other compounds (resolution ∼6). EOF was $1.7–1.9 \times 10^{-8}$ m^2/V s with all modifiers (Table 1). EOF was higher with acetate buffer at pH 5.0 ($2.5–3.1 \times 10^{-8}$ m^2/V s) than with the acetate buffer at pH 4.0 ($1.7–1.9 \times 10^{-8}$ m^2/V s) with similar deviation (RSD 3.0–11.6%). The retention of the analytes on the lipid membrane decreased with increase in the pH, showing that higher pH does not improve the separation capability of the coating. Resolutions remained low (0.5–1.0) for the first four compounds and, and even though 17-α-hydroxyprogesterone and progesterone were baseline separated, the resolution was lower at pH 5 (4.1–5.7) than at pH 4. If it is assumed that no changes occur in the membrane, the poorer separation may be explained by the increase in flow rate that higher pH induces.

Limited separations of the test analytes were achieved without a modifier as displayed in Table 2, with phosphate buffer at pH 7.4. No separation was obtained when EDA, DAP, or BTP was added as a modifier. However, the EOF clearly changed in response to the different amines, which means that they must have caused surface modifications in the capillary (EOF $\sim 3.6–6.4 \times 10^{-8}$ m^2/V s). Zwitterionic HEPES functioned to some extent as a modifier in the liposome coating system, enabling moderate separation. The phosphate ions of the buffer may form neutral amine adducts that cannot participate in the coating procedure. It must also be kept in mind that the phospholipids themselves have amino and phosphate functionalities. And, if the phosphate on the phospholipids participates in the coating procedure, the free buffer phosphate ions certainly compete with the ions involved in the coating mechanism. Clearly phosphate buffer is not suitable for this system.

Tris buffer solution at pH 7.4 with EDA or DAP added as coating modifier led to a good coating for the separation of the neutral steroids. The resolution achieved with EDA in Tris was better that that achieved with EDA in acetate buffer at pH 4 and 5. In fact, EDA in Tris

Table 2
Retention of test compounds on the PC/PS phospholipid membrane in various electrolytes with different modifiers added to the liposome solution

BGE	Test compound	Retention factor/RSD (%)				
		No modifier	EDA	DAP	BTP	HEPES
Acetate pH 4.0	Aldosterone	0.02/0.17	0.02/0.13	0.02/0.07	0.02/0.13	0.03/0.12
	Androsenedione	0.06/0.20	0.06/0.33	0.06/0.20	0.05/0.12	0.06/0.27
	Testosterone	0.13/0.25	0.10/0.53	0.09/0.35	0.07/0.19	0.10/0.47
	17-OH-progestrone	0.13/0.54	0.13/0.81	0.13/0.50	0.10/0.32	0.14/0.68
	Progestrone	0.47/1.73	0.48/2.15	0.45/1.43	0.35/0.79	0.50/2.37
Acetate pH 5.0	Aldosterone	0.02/0.16	0.02/0.05	0.02/0.04	0.02/0.02	0.02/0.03
	Androsenedione	0.04/0.12	0.03/0.11	0.05/0.96	0.05/0.15	0.05/0.21
	Testosterone	0.07/0.20	0.05/0.19	0.06/0.24	0.08/0.28	0.07/0.42
	17-OH-progestrone	0.10/0.23	0.08/0.33	0.10/0.47	0.12/0.47	0.13/6.11
	Progestrone	0.33/0.62	0.22/0.73	0.30/1.06	0.38/1.51	0.36/1.72
Tris pH 7.4	Aldosterone	0.01/0.30	0.01/0.03	0.01/0.03	0.01/0.08	0.02/0.05
	Androsenedione	0.03/0.29	0.08/0.09	0.08/0.13	0.01/0.12	0.05/1.54
	Testosterone	0.04/0.25	0.14/0.14	0.15/0.20	0.02/0.32	0.09/0.53
	17-OH-progestrone	0.05/0.30	0.22/3.16	0.24/0.37	0.03/0.40	0.14/0.91
	Progestrone	0.15/0.30	0.80/0.68	0.78/0.60	0.10/1.27	0.53/0.76
Phosphate pH 7.4	Aldosterone	0.02/0.03	0.00[a]	0.00[a]	0.00[a]	0.02/0.03
	Androsenedione	0.04/0.03	0.00[a]	0.00[a]	0.00[a]	0.05/0.02
	Testosterone	0.06/0.04	0.00[a]	0.00[a]	0.00[a]	0.08/0.03
	17-OH-progestrone	0.08/0.08	0.00[a]	0.00[a]	0.00[a]	0.12/0.06
	Progestrone	0.25/0.11	0.00[a]	0.00[a]	0.00[a]	0.40/0.25

[a] Only one apparent peak with insignificant retention (retention factor <0.02).

yielded the best results of all tested systems (resolution 3.1, 2.4, 2.2, and 8.5 for analyte pairs aldosterone–androstenedione, androstenedione–testosterone, testosterone–17-hydroxy-progesterone, and 17-hydroxyprogesterone–progesterone, respectively). With DAP, the resolution ranged from 2.6 to 7.4. HEPES too, acted as a coating modifier in Tris buffer at pH 7.4, but the resolutions for the test compounds remained in the range 1–6. The EOF was faster with the EDA- and DAP-modified systems ($\sim 4.7 \times 10^{-8}$ and $\sim 5.5 \times 10^{-8}$ m^2/V s, respectively) than with the coating without modifiers (EOF $\sim 3.4 \times 10^{-8}$ m^2/V s), while it was slower with HEPES as modifier ($\sim 2.5 \times 10^{-8}$ m^2/V s). The EOF in the uncoated capillary was 5.3×10^{-8} m^2/V s. These results show that HEPES decreased the phospholipid membrane charges on the silica wall. The pK_a values for HEPES's amino groups are about 2.6 and 7.9, which means that HEPES is only partially protonated in Tris at pH 7.4. Thus, HEPES is able to interact with the negatively charged capillary wall and/or with the liposomes, but clearly not in the same way as EDA or DAP. The stability of the coatings, measured as stability of EOF, was reasonable with all modifiers (RSD < 2%) except HEPES (RSD 7%).

3.3. Comparison of modifiers

EDA and DAP have linear structures with primary amino groups at the end of the chain. These amino groups are much more easily protonated than the amino groups of BTP and HEPES, which are secondary or tertiary. The pK_a values of BTP are in the same range as those of DAP, meaning that the protonation degree of these compounds should be on approximately the same level. However, BTP has a much greater steric barrier around its amine groups than do the small linear amines, the interactions between these amino groups and the liposomes and capillary wall can be expected to be considerably weaker therefore. The piperazine ring present in HEPES brings rigidity to the structure. Moreover, as the pH is increased, the protonation level of the amino groups in the piperazine ring is decreased.

3.4. EDA and DAP

At pH 5 or below, both EDA and DAP are almost completely protonated. At pH 7.4 DAP is slightly more protonated than EDA, and so is more positively charged. Positive charges are attracted to both the negatively charged silanol groups on the capillary wall and the negative charges of the PS (carboxy and phosphate groups) in the membrane. PC too, contains negatively charged phosphate able to interact with the protonated amines. There are several possible effects. As discussed above, short chained amines are able to link to the capillary surface, reducing the charge on the wall. This was seen in the lower EOF (11% lower) of the coated capillary when the capillary was flushed with 5 mM DAP and then coated with plain liposome solution than when DAP was included in the coating solution.

Positive charges of the amines can also act as anchors between the liposomes and the capillary wall. The modifiers were added to the liposome solutions after extrusion of liposomes, but since EDA and DAP are relatively small molecules and of positive charge, they probably penetrate relatively easily into the negatively charged lipid membrane [22]. If the amines do not fully penetrate into the membrane, their exposed positive ends can act as linkers between the phospholipids and the negatively charged capillary walls. This type of linking might enable similar fusogenic behavior to that observed when calcium is added to the phospholipid membranes [18,19]. The interactions between the amino groups and the phospholipids make the membrane structure more densely packed with increased overall surface charges and enhanced hydrophobicity. This interpretation is supported by the much improved retention of the model steroids to the membrane when EDA and DAP were employed as coating modifiers in Tris buffer at pH 7.4.

Relative to unmodified coating the electro-osmotic mobility in the capillary was noticeably increased, when modifiers in buffers at pH 5 or above were employed (Table 1). As the pH was increased, the amino groups of PS became partially deprotonated and this led to increase in the negative net charges contributed by PS in the lipid membrane. The strong interactions of the modifiers may also affect the packing density of the lipids in the membrane, making the structure tighter, with increased overall charge density explaining the increase in electroosmotic flow. The modifiers may also dehydrate the polar phospholipid headgroups and reduce the swelling of the membrane, rendering the structure more tightly packed [23–25]. EOF gradually weakened with repeated runs, however, indicating changes in the phospholipid membrane. It is also possible that the high electric fields that were applied somehow disturbed the coating. Evidently, the modifiers that were used do not prevent the phospholipid membrane from this kind of degradation. In addition, the phospholipid bilayers are dynamic structures, and thus some motion relative to the adjacent lipid monolayers can be expected.

3.4.1. BTP and HEPES

When BTP was used as a modifier, separation resolution remained on the same level as without modifier in the coating liposomes. Relative to the coating without modifier, neither pH nor buffer had any effect on the separation. With Tris buffer at pH 7.4 the EOF was on the same level when BTP was present as when no modifier was added, indicating that BTP does not take part in the coating.

Hautala et al. [26] have shown that the pH of HEPES, both in liposome coating solvent and in BGE solution, affects the liposome coating. pH played an important role also in this study, too, and a fairly good separation was obtained with HEPES as modifier and Tris buffer at pH 7.4. Surprisingly, in some degree HEPES also acted as modifier in phosphate

buffer, unlike the other diamines, which failed completely in phosphate buffer. This result supports the conclusion above that the phosphate buffer competes with the phosphate group of the phospholipids for the protonated amino groups of DAP and EDA. Evidently, too, small protonated diamines with primary amino groups and zwitterionic HEPES with only tertiary amines play different roles in phospholipid bilayer formation. Comparison of HEPES, DAP, and EDA also showed that somewhat better separations are achieved with DAP and EDA, but it must be added at the same time that the practical conditions for use of DAP and EDA are more limited.

4. Concluding remarks

Study was made of the effect of amines as modifiers on the phospholipid coating and on steroid separations in capillary electrochromatography. At suitable pH, the short linear diamines, here 1,2-ethylenediamine and 1,3-diaminopropane, had a profound effect on the phospholipid coating membranes consisting of phosphatidylcholine and phosphatidylserine. Positively charged amines may act as binding agents within the phospholipid monolayer, between bilayers, and between phospholipids and the negatively charged capillary wall. Hydrogen bonding between modifiers and phospholipids and electrostatic forces present in the system compact the phospholipid membrane coating by increasing the overall negative surface charge of the membrane, seen as increased electroosmotic flow. Modifier enhanced phospholipid coatings also gave better separations for neutral steroids, indicating increased hydrophobicity of the membrane.

It is notable that not only the pH but also the buffer itself has a profound impact on the success of the liposome coating. Buffers that contain amino functionality take also part in the liposome coating formation, improving the stability of the coating, whereas inorganic buffers such as phosphate may even prevent the coating formation. The degree of protonation of the amines in liposome solutions also plays an important role in the formation of phospholipid membrane coatings.

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