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Simple coating of capillaries with anionic liposomes in capillary electrophoresis

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Abstract

A new and relatively simple method was developed for coating of capillaries in electrophoresis with liposomes. The liposomes, with a diameter of about 100 nm, are large unilamellar vesicles prepared by extrusion. The liposomes contained 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphatidylcholine (POPC) or POPC with different proportions of bovine brain phosphatidylserine (PS) and cholesterol. They formed a bilayer structure on the silica surface enabling the separation of neutral compounds. The effectiveness of the coating in separation was evaluated with use of uncharged steroids as model compounds. The coating was also studied by measuring the electroosmotic flow. The best results, taking into consideration both separation and stability, were achieved with anionic 80:20 mol% POPC/PS liposomes. In addition, the effect of coating conditions on the results was investigated. Among the buffers studied [*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), phosphate, tris(hydroxymethyl)aminomethane (Tris) and *N*-tris(hydroxymethyl)methylglycine (Tricine)], HEPES seemed to have a significant effect on the success of the coating. Successful separation of steroids was achieved only when HEPES buffer was used in the coating procedure and in the background electrolyte solution for the separation. With all other buffers the peaks of the model compounds overlapped.
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Keywords: Coated capillaries; Liposomes; Electroosmotic flow; Background electrolyte composition; Phospholipids; Hydroxyethylpiperazine-ethanesulfonic acid

1. Introduction

Liposomes, also called phospholipid vesicles, were first discovered by Bangham and Horne in 1962 [1]. Today, these vesicles fascinate scientists in many fields owing to their similarity to biological membranes. Phospholipids are one of the three major groups in biological membranes, along with glycolipids and cholesterol. The structure and characteristics (including surface-charge, size and lamellarity) of liposomes are easily modified by altering their phospholipid composition and the method of preparation [2]. This enables the tailoring of liposomes for specific purposes. The unique characteristics of liposomes derive mainly from the bilayer structure. Phospholipids spontaneously form bilayers rather than micelles in aqueous media as a result of the two attached fatty acyl chains in the structure. In bilayers the hydrophobic acyl chains of the phospholipid

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molecules face towards each other, while the hydrophilic head groups form the exterior of the bilayer. Phospholipids are of two types, glycerophospholipids and sphingophospholipids, which are derivatives of glycerol and sphingosine, respectively [3]. The most common glycerophospholipid is zwitterionic phosphatidylcholine (PC), which can be considered to constitute the backbone of cellular membranes. The net charge of liposomes based on PC can be modified, for example by adding negatively charged lipids such as phosphatidylserine (PS). Cholesterol is another important but uncharged component in biological membranes (Fig. 1).

Liposomes and their interactions with different analytes have been investigated by differential scanning calorimetry [4,5], nuclear magnetic resonance spectroscopy [6–8], X-ray diffraction [6], fluorescence measurements [4,5,9,10], monolayer studies [4], photon correlation spectroscopy [11] and electron spin resonance [6]. In addition, liquid chromatography (LC) has been widely used to examine the characteristics and behaviour of liposomes containing phospholipids [12–17]. In several instances, liposomes and phospholipids have been used as a stationary phase for LC in the separation of analytes such as proteins [12,13]. A phospholipid bilayer has also been applied in high-performance liquid chromatography (HPLC) as a noncovalent immobilized artificial membrane chromatographic (IAM) phase for the separation of peptides [15] and for interaction analysis [16,17].

Capillary electrophoretic (CE) studies related to liposomes are appearing in increasing numbers [18,19].The applicability of CE to liposome research has been investigated by Zhang et al. [20], Tsukagoshi et al. [21] and Roberts et al. [22]. For studies of liposome–analyte interactions, liposomes have been utilised as carriers [23–27] and as a coating material [28] in capillary electrophoresis. Cunliffe et al. [28] used zwitterionic 1,2-dilauroyl-*sn*-phosphatidylcholine (DLPC) liposomes as a capillary coating. After preconditioning, the capillary was coated by rinsing with a buffered liposome solution containing calcium. With this solution, 4 min was found to be adequate for coating; in the absence of calcium the time required for coating was prolonged to 75 min.

With liposomes now being introduced as coating material in both HPLC and CE techniques, characterisation of the coating procedure and the factors affecting the procedure have become important. The formation of liposome coating on different surfaces [29–31] has been studied by atomic force microscopy (AFM) [31–33]. These studies have clarified the progress of the coating formation and the effect of coating conditions on the final form of the coating. For example, whether supported phospholipid bilayers (SPLs) or supported vesicular

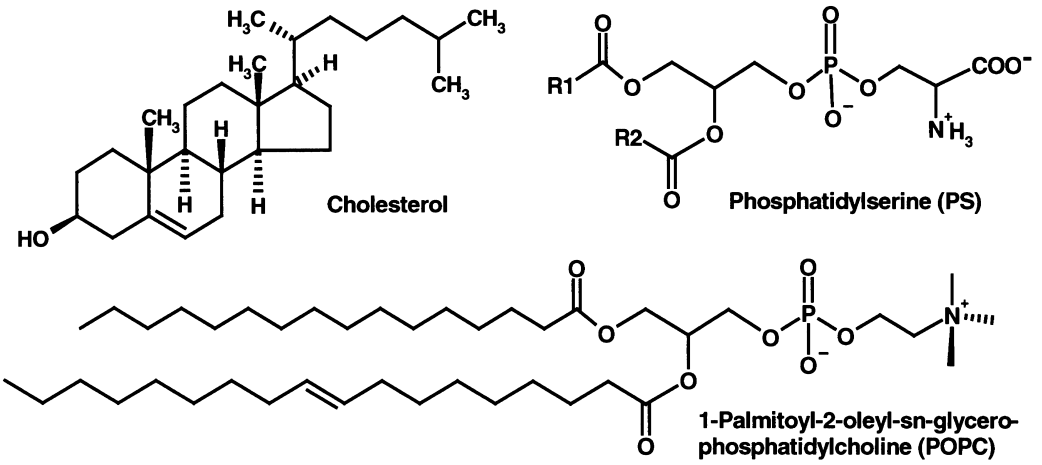

Fig. 1. Structures of phospholipids and cholesterol.

layers (SVLs) are formed has been shown to depend on the conditions during the coating procedure [32].

The usefulness of anionic liposomes as carriers of neutral analytes in electrokinetic capillary chromatography (ECC) has been demonstrated in our laboratory [23–25]. The effects of the buffer solution and the liposome composition on the separation were investigated. The results showed an improvement in the separation when the total lipid concentration and the amount of negative charges in the liposomes was increased. Hydrophobic analytes were shown to interact preferably with membranes in the fluid phase rather than with membranes in the gel phase, demonstrating the importance of the phase transition temperature of the lipid for the separation [24].

In the present study, we developed a method for applying liposomes as coating material for CE capillaries and evaluated the effectiveness of the coating by examining the interactions of steroids with the coated capillaries. The electroosmotic flow (EOF) and the separation of steroids were used in evaluating the quality of the coating. The steroids were five steroidal hormones. Aldosterone is a mineralocorticoid, testosterone and its precursor androstenedione are androgens, and progesterone and 17α-hydroxyprogesterone are progestants. These steroids are neutral under the conditions applied and thus the separation is based on hydrophobic interactions with the liposome coating. As well, the effect of various buffers on the coating process was investigated.

2. Experimental

2.1. Materials and equipment

N-(2-hydroxyethyl)piperazine-*N′*-(2-ethanesulfonic acid) (HEPES), *N*-tris(hydroxymethyl)-methylglycine (Tricine), tris(hydroxymethyl)-aminomethane (Tris), 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphatidylcholine (POPC), bovine brain phosphatidylserine (PS), cholesterol, 17α-hydroxyprogesterone, androstenedione and *d*-aldosterone were purchased from Sigma (St. Louis, MO, USA). Progesterone, testosterone, sodium dihydrogenphosphate and the pH solutions (4, 7 and 10) used for calibrating the pH meter were purchased from Merck (Darmstadt, Germany). Sodium hydroxide (1.0 *M*) and hydrochloric acid (1.0 *M*) were purchased from FF-Chemicals (Yli Ii, Finland), methanol from Mallinckrodt Baker (Deventer, The Netherlands) and acetone from Lab-Scan (Dublin, Ireland).

Uncoated fused-silica capillaries used for coating and in CE separations were from Composite Metal Services (Worcestershire, UK). Dimensions were 50 μm I.D.×375 μm O.D. unless otherwise mentioned. The length of the capillary to the detector was 51.5 cm with a total length of 60 cm. A Hewlett-Packard ^{3D}CE system (Agilent, Waldbronn, Germany) equipped with a diode array detector (detection at 200, 214, and 245 nm) was used for the electrophoretic measurements.

A Jenway 3030 pH meter (Jenway, Felsted, UK) and a MeterLab PHM220 pH meter (Radiometer, Copenhagen, Denmark) were used to adjust the pH of the electrolyte solutions. Distilled water was further purified with a Millipore water purification system (Millipore, Molsheim, France).

2.2. Methods

2.2.1. Liposome preparation

Appropriate amounts of the lipid stock solutions were mixed in chloroform to obtain the desired compositions. The resulting mixture was evaporated to dryness under a stream of nitrogen and traces of solvent were removed by evacuating under reduced pressure for 24 h. The lipid residues were hydrated in the indicated buffer at 60 °C to yield multilamellar vesicles (MLVs) with a lipid concentration of 4 m*M* and the vesicles were maintained at this temperature for 30 min with subsequent vigorous vortexing. The resulting dispersion was processed to large unilamellar vesicles (LUVs) by extrusion 19 times through Millipore (Bedford, MA, USA) 0.1-μm pore size polycarbonate filters using a Liposofast low pressure homogenizer (Avestin, Ottawa, Canada) essentially as described previously [23]. The prepared liposome solutions were stored in the refrigerator.

2.2.2. Buffer and sample preparation

The concentration of the separation buffer in the background electrolyte (BGE) solution was 40 m*M* and the pH value of the buffer was adjusted to 7.40 with 1.0 *M* sodium hydroxide (in the case of Tris

with 1.0 *M* hydrochloric acid) and filtered through 0.45-μm filters (Gelman Sciences, Ann Arbor, MI, USA). HEPES buffer was also used at concentrations of 10–150 m*M* as a preconditioning buffer, i.e. for rinsing of the capillary during the coating process. Neither the BGE solution nor the preconditioning buffer contained liposomes.

The steroid samples were prepared from stock solutions (1–2 mg/ml in methanol). Unless otherwise mentioned, the steroid concentrations in the injected sample were 20 μg/ml of aldosterone, androstenedione and testosterone and 50 μg/ml of 17α-hydroxyprogesterone and progesterone in methanol–40 m*M* buffer (10:90, v/v). The migration time of methanol was used as a marker for the EOF. All solutions were stored in the refrigerator.

2.2.3. Capillary coating

The fresh capillary was rinsed, with a pressure of 930–940 mbar, for 10 min with 0.5 *M* hydrochloric acid, for 15 min with water and for 5 min with the preconditioning buffer. The liposome solutions were diluted (3 m*M*) with buffer before capillary coating. Preconditioning buffer was always used as liposome solvent to ensure that the buffer conditions were the same during the whole coating process. Liposome coating was applied to the capillary inner surface as follows: the capillary was rinsed for 10 min with 3 m*M* liposome solution at 930–940 mbar and then it was left to stand with the liposome solution for 15 min. Because the liposome coating changes the surface charge of the inner wall of the capillary, the interactions of the liposomes with the capillary inner wall were determined by measuring the changes in the EOF.

2.2.4. Capillary electrophoretic separations

Unless otherwise mentioned, CE separation conditions were as follows: voltage 20 kV, temperature of the capillary cassette 25 °C and sample injection 5 s at 50 mbar. Before each injection the capillary was rinsed for 2 min with the BGE solution. During a long series of runs, the quality of the buffer used as BGE solution was ensured by changing the buffer vials after every sixth run.

3. Results and discussion

3.1. Liposome coating procedure

After the preconditioning with hydrochloric acid and water the capillary was flushed with preconditioning buffer for 5 min. Five min was selected as sufficient to stabilise the conditions in the capillary before the coating procedure was started. The effect of three different liposome rinsing times on the coating was studied: 5, 10, and 20 min. However, there was no significant difference between the mean electroosmotic mobilities using different rinsing times (the RSD was $<7\%$ as compared to each other), and 10 min rinsing time was selected as long enough to rinse the capillary thoroughly but short enough to keep the method rapid and not waste the liposome solution.

The formation of bilayers on surfaces in coating processes has been observed in studies with double chained surfactants [34] and lipids with two alkyl chains [28,32].However, the formation of the bilayer takes some time [33]. We studied the effect on the final coatings of waiting 5 min to 23 h before removing the liposome solution from the capillary. Based on the EOF of successive injections of methanol there seemed to be no difference between 5 and 15 min. The difference in the mean electroosmotic mobilities was less than 1.5%. The corresponding RSD values, as calculated for 36 runs, were 2.8 and 1.5%, respectively. When the capillary was kept filled with the liposome solution for 23 h the capillary became slightly unstable, as indicated by a fluctuation of the EOF (RSD of 4.5%). Thus, 15 min standing time was considered sufficient to achieve a stable coating, in agreement with previous studies [33].

We also studied the effect of voltage in the preconditionting step on the coating. Different voltages (10–30 kV) were applied to the capillary after the liposome flush. Application of voltage for 10 min had no significant effect on the stability of the coating and this was abandoned as time consuming. Some conclusions can nevertheless be drawn from the results. Although the stability of the coating was not affected, the EOF was faster when higher voltage was applied during the preconditioning step. Appli-

cation of voltage during coating thus seems to affect the stabilisation process, possibly because the nature of the coating is changed or some liposomes are lost.

The results obtained with the different liposomes were similar and the following method was chosen for further investigations of the coating stability and the interactions between the coating and steroids: 10 min 0.5 *M* HCl flush+15 min water flush+5 min 40 m*M* HEPES flush+10 min liposome solution flush+ 15 min stabilisation with the liposome solution in the capillary. A short flushing of the capillary with the liposome solution between runs, as in previous work involving liposome coating of silica capillary [28], was tested. However, there was no improvement in the stability of the coating. Rinsing instead with HEPES buffer resulted in a more stable coating and 2 min rinsing with HEPES between runs was made part of the procedure. The calcium ion acts as a strong fusogenic agent for bilayer formation and has played an important role in earlier liposome coating studies [28,32,35,36]. In our study, coatings were achieved in the absence of calcium and sodium was the cation in the background electrolyte solution.

3.2. Influence of buffer solution

The influence of different buffer solutions on the liposome coating was investigated. The results for steroid separations with 40 m*M* HEPES buffer at pH 7.4 were compared with the results obtained with phosphate, Tris and Tricine buffers at the same concentration and pH. The steroids were separated only when HEPES was used as preconditioning and separation buffer (Fig. 2a). In the case of phosphate, Tris and Tricine, only one intensive peak appeared containing both methanol and the steroids, indicating the simultaneous migration of all studied steroids (Fig. 2b–d).

Clearly, the POPC/PS liposomes interact significantly less with the capillary wall when phosphate, Tris or Tricine buffer is used. During a long series of runs with Tris buffer (36 repetitions), liposomes seemed to interact slightly with the capillary wall. In addition, when the EOFs in uncoated and liposome coated capillaries were compared for phosphate, Tris and Tricine buffers, a minor increase (1–7%) was observed in the EOF of liposome treated capillaries.

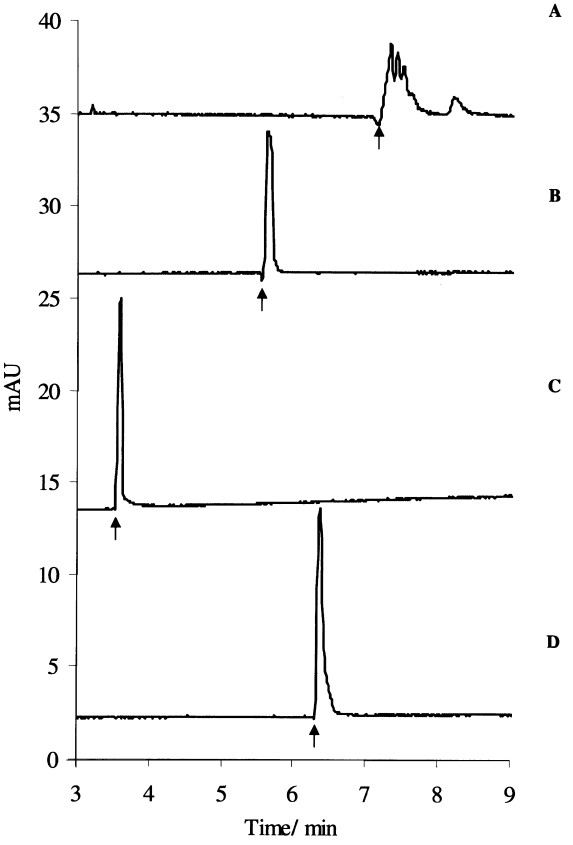

Fig. 2. Separation of steroids on liposome coated capillaries where four different buffer systems, (a) HEPES, (b) phosphate, (c) Tricine and (d) Tris, 40 m*M* at pH 7.4, were used during coating and separation processes. The liposome solution used for the capillary coating contained 3 m*M* 80:20 mol% POPC/PS in buffer. The sample contained 20 μg/ml of aldosterone, androstenedione and testosterone and 50 μg/ml of 17α-hydroxyprogesterone and progesterone in methanol–buffer (10:90, v/v). The sample was injected for 5 s at 50 mbar. Separation conditions: voltage 20 kV, detection 245 nm, temperature of capillary cassette 25 °C, fused-silica capillary of 60 cm (51.5 cm to the detector)× 50 μm I.D.×375 μm O.D. The capillary was rinsed with BGE solution for 2 min between runs. The EOF is marked with an arrow.

With HEPES the result was the opposite, i.e. the EOF was clearly slower (24%) with coated capillaries. From these results, it appears that some interactions of the liposomes with the capillary wall occur with all studied buffers, but the interactions are strongest when HEPES is used during the coating and separation steps. Thus, the liposomes seem to

provide an effective coating on the capillary inner wall only in the presence of HEPES.

The role of HEPES for the coating process is even more significant when it is remembered that the liposomes are anionic. In theory, this should prevent their interaction with the negatively charged capillary wall. However, Roberts et al. [22] observed a binding of negatively charged liposomes to the silica surface of the capillary. They studied the behaviour in CE of liposomes containing dimyristoylphosphatidylcholine (DMPC), dicetyl phosphate (DCP), 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanide [DiI-$C_{18}(C_5)$] and cholesterol (7:2:2:1, v/v) in 9.5 m*M* phosphate buffer, pH 7.4, and observed the adsorption of the liposomes onto the capillary wall during successive injections until the capillary was saturated with liposomes.

HEPES is one of the most popular biological buffers because of the evident absence of interactions with biological matrices. It has been demonstrated that zwitterionic buffers such as HEPES suppress capillary wall adsorption of the sample [37–39] and improve sample stacking [40]. In addition it has been shown that HEPES can act as a complexing reagent [41]. In a study by Messana et al. [42], on the other hand, it was demonstrated that the ion-paired complex between the negative form of HEPES and cationic nicotinamideadenine dinucleotide was sensitive to the applied electric field in CE. Thus, the conditions in CE can affect the characteristics of HEPES.

We also studied the effects of concentration and pH of the HEPES buffer on the liposome coating process. The effect of concentration was investigated by increasing the concentration of HEPES during the coating process from 10 to 150 m*M*, while the concentration in the BGE solution was kept constant (40 m*M*). The electroosmotic mobility, μ_{eo}, in the liposome coated capillary was measured using methanol as a neutral marker. Methanol has no or only weak interaction with the capillary wall. The calculated μ_{eo} values formed a curve with the minimum EOF at concentration range of 30–60 m*M*, showing that the HEPES concentration should be in this range during the coating procedure in order to achieve an effective liposome coating. Over the concentration range studied there was a 20% difference between the lowest and the highest electroosmotic mobility. The pH of HEPES during the coating procedure also affected the coating. When the pH of the preconditioning buffer was increased in the effective buffering range (6.8–8.2), the EOF in the coated capillary increased (20%) as well, indicating the influence of pH either on the coating stabilisation or on the nature of the coating.

3.3. Stability of the liposome coating

The stability of the coating was studied by following the repeatability of the EOF. When the silica surface, which is originally negatively charged, is coated with liposomes, the liposomes shield the negative charges and, hence, the EOF is suppressed.

The electroosmotic mobilities obtained for a large number of runs in an uncoated capillary, a capillary coated with 80:20 mol% POPC/PS and a capillary coated with 100 mol% POPC are seen in Fig. 3. There was a 2-min flush with BGE before each injection and the duration of each run was 12 min, except for POPC. In the case of POPC the running times decreased from 50 min (for runs 1–8) to 30 min (for runs 9–38). In the case of 80:20 mol% POPC/PS there are two curves, one for a capillary with 30 μm I.D. and one for a capillary with 50 μm I.D. The EOF in the 80:20 mol% POPC/PS coated capillaries was almost as stable as the EOF in the uncoated capillary. The EOF was more stable in the capillary with smaller I.D., probably due to the larger ratio of contact area of coating to capillary volume. The EOF in the POPC/PS coated capillaries increased slightly as the capillary was used. Probably some liposomes leaked out from the capillary or the nature of the coating was changed. Although the EOF was suppressed at the beginning in the case of POPC, it increased rapidly after a few runs. Presumably the adsorption of the liposomes onto the silica surface was not strong enough to withstand the applied electric field or the buffer flushing between the runs. Under these conditions, POPC liposomes alone cannot provide a stable liposome coating, but 80:20 mol% POPC/PS liposomes seemed to work very well.

The mean values of μ_{eo} for a large number of runs, n, and for different liposome coatings are summarised in Table 1. The total running time means the time from the coating procedure to the end

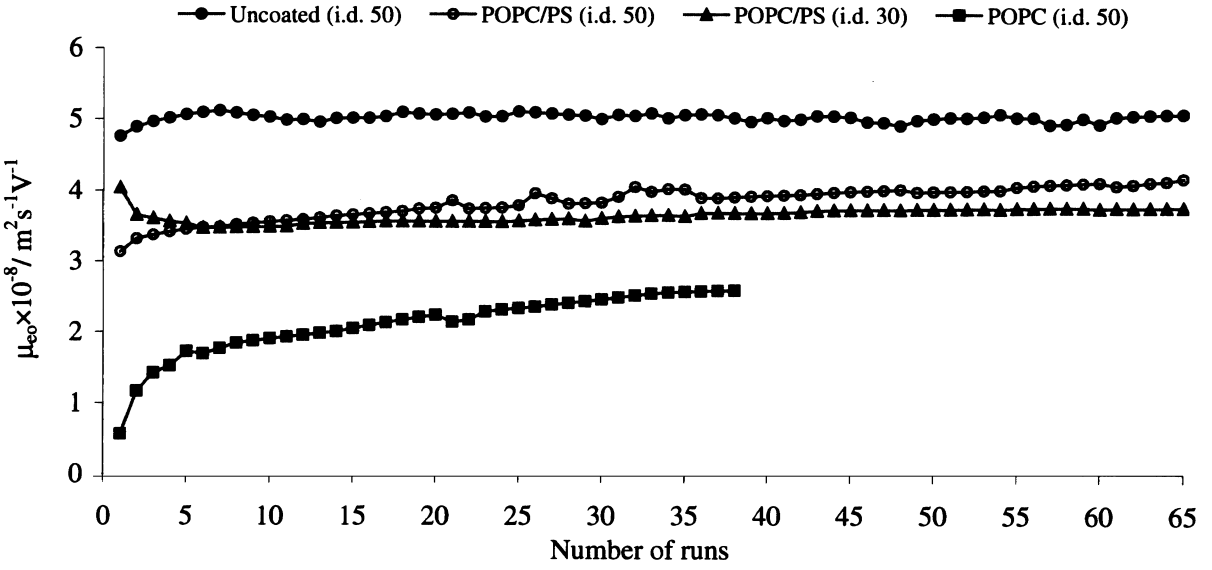

Fig. 3. Repeatability of the electroosmotic flow, μ_{eo}, in an uncoated capillary and in POPC and 80:20 mol% POPC/PS coated (30 and 50 μm I.D.) capillaries using 40 m*M* HEPES at pH 7.4 as preconditioning buffer and BGE solution. Methanol was used as a neutral marker for the μ_{eo} measurements. Running conditions: voltage 20 kV, injection 5 s at 50 mbar, detection 200 nm, temperature of capillary cassette 25 °C, length of the fused-silica capillary to the detector 51.5 cm and total length 60 cm.

of the last run. It includes the buffer flushes between the runs and the analysis time for each run. The repeatability was very good for the 30 μm I.D. 80:20 mol% POPC/PS coated capillary, as can be seen from the small RSD (%) value for such a long series of runs. The RSD (%) for the POPC coated capillary was poor. The reason for the poor performance is suggested in Fig. 3. The large RSD (%) values do not result from the constant fluctuation of EOF but rather from the systematic increase in EOF. A more stable coating was achieved when cholesterol was added to the POPC liposomes, but still the leakage of the coating was a major problem. Only when PS was added as well was a more or less stable coating achieved (see Table 1). However, it seemed that the liposomes containing POPC/PS/cholesterol could not completely coat the capillary because the separation of steroids was poor and the EOF, compared to that in the uncoated capillary, was not much suppressed. EOF also fluctuated in the uncoated capillary, and slowly increased as the capillary was used. For example, with a 60 cm (effective length

Table 1
Stability of the electroosmotic flow, μ_{eo}, in capillaries coated with different liposomes measured by RSD (%), for repetitions (*n*)

Capillary	$\mu_{eo}\times10^{-8}$ ($m^2s^{-1}V^{-1}$)	RSD (%)	*n*	Total time of running (min)	Stability	Separation of steroids
Uncoated	5.02	1.9	65	910	++	−
POPC 100 mol%	2.06	20.7	35	1240	−	++
POPC/PS 80:20 mol% (I.D. 30 μm)	3.64	2.8	65	1078	++	++
POPC/PS 80:20 mol% (I.D. 50 μm)	3.83	6.0	65	910	++	+
POPC/chol 80:20 mol%	2.28	18.9	35	961	−	++
POPC/chol 60:40 mol%	4.30	2.7	35	426	++	−
POPC/PS/chol 60:20:20 mol%	4.03	4.4	65	1105	++	−
POPC/PS/chol 40:20:40 mol%	3.90	2.6	65	1105	++	−

Explanations for the symbols describing the quality of capillaries (stability and separation of steroids): ++ good, + moderate, − poor.

51.5 cm)×50 μm I.D. uncoated capillary, 65 injections of methanol gave 1.94% RSD for the μ_{eo}.

Tests were made to determine if the liposome coating can withstand overnight storage in different solutions without losing its stability. When the capillary was stored in water, the coating was apparently dissolved, since the EOF measured the following day was almost as fast as with an uncoated capillary. When it was stored in the liposome solution, the coating evidently remained attached to the capillary wall, but the EOF fluctuated strongly. When the capillary was kept in 40 m*M* HEPES for ≈13 h, no significant loss of coating occurred as evidenced by the more or less stable EOF. The capillary was also stored in HEPES solution for ≈60 h and even after such a long time the coating was still stable and there was only a slight increase in the EOF (2%). Nevertheless, to ensure reliable data, a freshly coated capillary should be employed for each series of runs.

3.4. Separation of neutral compounds

The exploitation of molecule adsorption onto capillaries has been discussed in a review on capillary electrochromatography with physically and dynamically adsorbed stationary phases [43]. Hence, in addition to comparing the EOF in coated and uncoated capillaries, we studied the coating by separating uncharged steroids. The sample contained aldosterone, androstenedione, testosterone, 17α-hydroxyprogesterone and progesterone in methanol–buffer.

In an earlier study where liposomes were used as a carrier in ECC, the separation of steroids was poor with liposomes containing only POPC in BGE solution [24]. In our present study with liposome-coated capillaries, the separation of steroids was most successful when liposomes containing POPC alone or POPC and PS or cholesterol were used as coating. Baseline separation of the steroids was achieved when the capillary was coated with 100 mol% POPC, 80:20 mol% POPC/PS (I.D. 30 μm) or 80:20 mol% POPC/chol. When a capillary with I.D. of 50 μm was coated with 80:20 mol% POPC/PS, all steroids could be detected, but baseline separation was not achieved (Fig. 2a). With 60:0:40 mol%, 60:20:20 mol% and 40:20:40 mol% of POPC/PS/chol, only progesterone could be separated from the other steroids. However, the stability of capillaries coated with these liposomes having a negative charge was fairly good (RSD <4.4%).

Steroids were completely separated when the capillary was coated with POPC, but the coating was not stable and the migration times were long (>30 min). Addition of 20 mol% of PS improved the stability of the coating and the steroids were separated in less than 12 min. Cholesterol also stabilised the coating but not as much as PS. Even though the EOF fluctuated, the electrophoretic mobilities of the analytes were almost constant in successive separations with all coatings.

Figs. 2a and 4 show that the liposomes are, in fact, adsorbed onto the silica surface. The interactions of the steroids with the liposome coating were much stronger with the capillary of 30 μm I.D. rather than 50 μm I.D. (Fig. 5). To increase the injected amount of sample in the 30-μm I.D. capillary, the injection time was increased from 5 to 10 s and the sample concentration was doubled. As can be seen from Fig. 4, baseline separation is achieved for all five steroids with the 30 μm I.D. capillary coated with anionic 80:20 mol% POPC/PS.

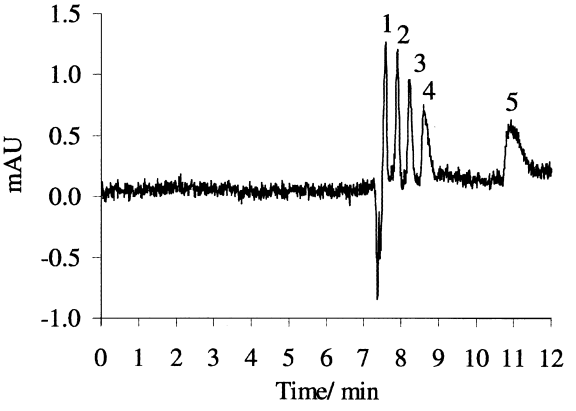

Fig. 4. Separation of steroids with a 80:20 mol% POPC/PS coated capillary (30 μm I.D.×375 μm O.D.). HEPES 40 m*M* at pH 7.4 was used as a preconditioning buffer and BGE solution. The sample contained 40 μg/ml of aldosterone (1), androstenedione (2) and testosterone (3) and 100 μg/ml of 17α-hydroxyprogesterone (4) and progesterone (5) in methanol–40 m*M* HEPES (20:80, v/v). Separation conditions: voltage 20 kV, injection 10 s at 50 mbar, detection 245 nm, temperature of capillary cassette 25 °C and fused-silica capillary with length 51.5 cm to the detector and total length 60 cm.

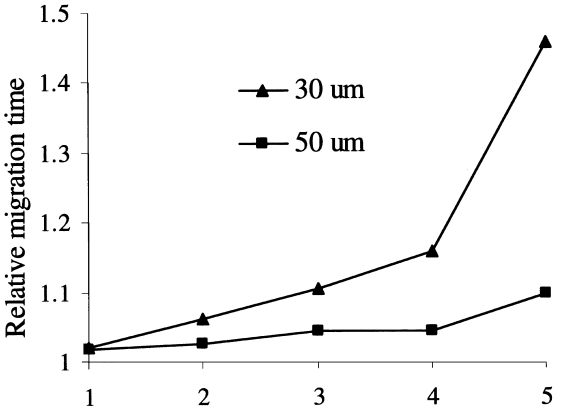

Fig. 5. Relative migration times (t_r/t_{eo}) of steroids using 30 and 50 μm capillaries. Experimental conditions for the separation with 30 μm capillary and those for 50 μm capillary as in Fig. 4. Numbering of compounds as in Fig. 4.

4. Conclusions

The liposome coating method that was developed is simple and rapid; it can be accomplished simply by rinsing a silica capillary with liposomes during the preconditioning step and allowing the capillary to stand, filled with the solution for 15 min. The liposome composition as well as the buffer system play an important role in the coating process. Among the liposomes tested, POPC/PS offered the best coating stability and a good separation of steroids, but only when HEPES was used as preconditioning and separation buffer. The POPC/PS coated capillaries remained stable even in a large number of successive runs. The stability of the coating and the separation of steroids in the capillaries coated with different liposomes are summarised in the last two columns of Table 1. The storage of the capillaries affected the liposome coating: only capillaries that were filled with HEPES buffer during storage seemed to maintain their properties. Since the compounds have different specific liposome–water partitioning coefficients [26,27], our results confirm that liposomes indeed coated the capillary. The narrower capillary of 30 μm I.D. improved the separation of steroids, probably as a consequence of larger ratio of liposome contact area to capillary volume. Thus, capillaries can usefully be coated with liposomes by the method described above, and the capillaries can be used to study the interactions between phospholipid bilayers and uncharged steroids.

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