



Liposomes: preparation and characterization with a special focus on the application of capillary electrophoresis

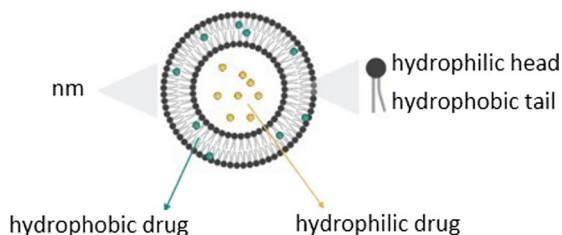
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Abstract

Liposomes are nowadays a matter of tremendous interest. Due to their amphiphilic character, various substances with different properties can be incorporated into them and they are especially suitable as a model system for controlled transport of bioactive substances and drugs to the final destination in the body; for example, COVID-19 vaccines use liposomes as a carrier of mRNA. Liposomes mimicking composition of various biological membranes can be prepared with a proper choice of the lipids used, which proved to be important tool in the early drug development. This review deals with commonly used methods for the preparation and characterization of liposomes which is essential for their later use. The alternative capillary electrophoresis methods for physico-chemical characterization such as determination of membrane permeability of liposome, its size and charge, and encapsulation efficiency are included. Two different layouts using liposomes to yield more efficient separation of various analytes are also presented, capillary electrochromatography, and liposomal electrokinetic chromatography.

Graphical abstract



Keywords Analytical method · Capillary zone electrophoresis · Liposomes · Nanoparticles

Introduction

Liposomes are small spherical vesicles formed by phospholipids and they have been greatly studied since their discovery in 1964, because they can be used to deliver large amounts of substances through the organism to the final destination [1–3]. Liposomes are formed by amphiphilic molecules of lipids that assemble in an aqueous environment

to form vesicles that encapsulate the aqueous phase at their center [4].

The main advantage of liposomes, as drug carriers, is their composition, which makes them biocompatible, biodegradable, non-toxic, and able to encapsulate both, hydrophilic and hydrophobic drugs [2]. Hydrophilic drugs are present in the aqueous compartment inside the liposome, while highly lipophilic drugs are trapped in the lipid bilayer. The encapsulation efficiency of lipophilic drugs is always higher compared to the hydrophilic ones, because lipophilic drugs are strongly repelled by the aqueous environment on both sides of the membrane and thus are very tightly incorporated into the hydrophobic part of the membrane [5].

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Liposomes are used as a transport system for many bioactive materials, such as cytostatics, proteins, peptides, enzymes, DNA, or vaccines [6]. By encapsulating the compound into a liposome, the drug is protected from early inactivation and from the enzymatic processes that commonly occur in the body. Encapsulation into liposomes also reduces the exposure of healthy tissues to the drug [2, 7].

Although liposomes can be used to transport a wide variety of substances, their use in practice is less common. The main reasons are their physico-chemical instability due to oxidation and hydrolysis of phospholipids, low solubility in aqueous solutions, short circulation time in the human body caused by rapid detection by the immune system, and leakage of encapsulated drugs [8].

Composition of liposomes

Naturally occurring phospholipids, such as glycerophospholipids, sphingolipids, polysaccharides, sterols, or synthetic lipids, which show higher stability, are most often used in the preparation of liposomes. The hydrophilic part consists of a phosphoric acid residue to which a small organic molecule can be bound. The molecule may be positively charged, negatively charged, or zwitterionic. Liposomes are most often prepared from zwitterionic phospholipids, for example, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), or from negatively charged phospholipids, such as phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidic acid (PA), and phosphatidylinositol (PI) [4, 9, 10].

Due to the structure of lipids, spontaneous membrane formation occurs in an aqueous medium. The polar parts of phospholipids interact with both external and internal aqueous media, while hydrophobic fatty acid chains interact with each other and their interaction with the aqueous medium is reduced [11, 12].

Classification of liposomes

Size and lamellarity

Liposomes are most often classified according to their size and the number of phospholipid bilayers within the liposome (lamellarity). Based on the number of lamellae, liposomes are divided into unilamellar (single phospholipid bilayer), multilamellar (multiple phospholipid bilayers, diameter greater than 0.5 μm), and multivesicular vesicles (smaller vesicles within the main vesicle, diameter greater than 1 μm). Liposomes with a single phospholipid bilayer are then divided into small unilamellar vesicles (SUVs) with a diameter of 20–200 nm, large unilamellar vesicles (LUVs)

with a diameter of 100–1000 nm, and giant unilamellar vesicles (GUVs) with a diameter greater than 1000 nm. Multilamellar vesicles (MLVs) usually contain 5–25 phospholipid bilayers and thus are more suitable for encapsulating hydrophobic drugs. On the other hand, LUVs are more suitable for encapsulating hydrophilic drugs, because they have only one phospholipid bilayer and a much larger volume of aqueous solution within the liposome than SUVs [4, 5, 10, 13, 14].

Composition and use

Based on their composition and use, liposomes can be divided into conventional, cationic, pH-sensitive, long-circulating liposomes, and immunoliposomes [1]. Conventional liposomes, also called the first-generation liposomes, are liposomes containing neutral or negatively charged phospholipids that are used in drug encapsulation studies or as model cell membranes. Their circulation time in the bloodstream is relatively short, because they accumulate in the cells of the immune system [1, 5, 10]. Cationic liposomes composed of positively charged phospholipids are suitable for the transport of negatively charged macromolecules, such as DNA, RNA, or oligonucleotides [1, 5]. pH-sensitive liposomes are destabilized in an acidic environment, which is used for rapid drug release and tumor treatment [15]. Long-circulating liposomes are used to prolong the circulation in the human body, for example by binding a polyethylene glycol (PEG) chain to the phospholipid molecule of liposome. The circulation time can be extended from a few minutes up to several hours, even days in some cases [5, 10]. Finally, immunoliposomes are liposomes, with monoclonal antibodies (or their fragments) bonded to their surface, and thus, they can be detected by cells that have a specific antigen on their surface. Most commonly, long-circulating liposomes are combined with immunoliposomes, where antibodies are bonded to a molecule of PEG [1, 16].

In vivo behavior

Liposomes can be further divided by parameters affecting their in vivo behavior. These parameters include the flowability of the phospholipid bilayer or the surface charge of the liposome. Lipids have a characteristic phase transition temperature T_c . Below this temperature, they are in the gel state, while above this temperature, they are in the fluid state. The flowability of the phospholipid bilayer can be thus affected by using lipids with different T_c values. If lipids with T_c below body temperature are used, the phospholipid bilayer is more fluid, leading to drug leakage from the liposome. On the other hand, liposomes composed of lipids with T_c higher than human body temperature are less fluid, and thus, the drug leakage is suppressed [10, 17].

Another important parameter influencing *in vivo* behavior of liposomes is the surface charge that may affect the interaction of liposomes with cell membranes. The mechanism of liposome transport into the cells is based on the adsorption to the cell surface and subsequent endocytosis. Negatively charged liposomes are degraded by endocytosis very quickly, while neutral liposomes do not interact with the cells and the drug is thus released extracellularly [17, 18].

Preparation of liposomes

There are many methods for preparing liposomes and the choice of an appropriate method depends on several factors, such as toxicity and concentration of the encapsulated drug, the type of solution used for dispersion of liposomes, size of the liposomes, preparation costs, and finally the encapsulation efficiency of the method [7, 19].

Lipid film hydration method

Hydration of a lipid film is one of the most widely used methods for the preparation of liposomes [20]. To facilitate the formation of the phospholipid bilayer, the temperature of the buffer solution and the temperature during the hydration should be higher than the T_c of the lipid with the highest T_c . However, this temperature should also be taken into consideration in terms of the possible degradation of active substances encapsulated into liposomes. The MLVs of various sizes are produced by intensive shaking and homogenous unilamellar vesicles of uniform size can be formed by sonication or extrusion [21, 22].

Ethanol or ether injection method

Another method of liposome preparation is the ethanol injection method, which is based on rapid injection of the lipid suspension into the aqueous phase. The advantages of this method include the simplicity of the operation and the possibility of preparing a large quantity of liposomes [20, 23, 24]. An alternative to the ethanol injection method is the ether injection method, where lipids are dissolved in diethyl ether and injected into the aqueous phase. Thanks to the higher solubility of lipids in ether, liposomes of higher lipid concentrations can be made using this method [2, 20, 23].

Emulsification method

Finally, liposomes can be prepared by the emulsification method; typically, a technique called reverse-phase evaporation is utilized. This method provides a higher encapsulation efficiency compared to the injection methods [20, 22, 25].

Stability of liposomes

The main difficulty when dealing with liposomes is their low chemical and physical stability. The chemical stability is affected by either oxidation or hydrolysis of phospholipids [26]. They can be protected from oxidation by the addition of antioxidants, by shortening the time of their exposure to the light, or by performing the preparation in a nitrogen or argon atmosphere [22].

The stability of liposomes can also be affected by their size, composition of the phospholipid bilayer, surface charge, or the method of their preparation. Smaller liposomes can pass more easily through the cell membranes; on the other hand, the encapsulation efficiency is decreased and surface energy increased, resulting in lower liposome stability. By the addition of cholesterol (Chol) into the phospholipid bilayer, the stability of the liposome increases due to lower membrane fluidity and lower risk of aggregation, and at the same time, the permeability of the membrane decreases which leads to lower drug leakage [8].

To ensure longer stability, the liposomes are stored in the form of a dry powder obtained by lyophilization, during which cryoprotective agents, such as sucrose, glucose, or trehalose, are added to the liposome preventing the leakage of the encapsulated drug. This method is used for thermolabile drugs which would be degraded by elevated temperature during the heat-drying method [2, 10].

Applications of liposomes

Nowadays liposomes are mainly used for the treatment of cancer, fungal infections, for the transport of analgesics, and in the production of viral vaccines. The first liposome-based product approved by the U.S. Food and Drug Administration in 1995 was Doxil[®], which contains encapsulated doxorubicin and is used to treat ovarian and breast cancer [27]. As a result of the COVID-19 pandemic, the research and development activities in the field of liposomes are now globally more intensive than ever. Both Pfizer/BioNTech and Moderna COVID-19 vaccines use cationic liposomes as mRNA carriers into cells, because cationic liposomes and negatively charged mRNA form a stable complex [28]. Liposomes in both vaccines are composed of synthetic cationic or ionizable lipids, distearoyl PC, and Chol. The PEG-2000 is bound to one of the lipids in both vaccines and is believed to be responsible for some allergic reactions, as it is known that human body can develop immunity against PEG [28–30].

Analysis and characterization of liposomes

To ensure the proper function of liposomes as carriers for bioactive substances, it is necessary to characterize their properties, such as size, lamellarity, surface charge, quantitative composition, and encapsulation efficiency [31]. Commonly used methods to analyze and characterize liposomes include ^{31}P nuclear magnetic resonance (NMR) [32] which is used for studying the membrane fluidity and thermotropic phase transitions, dynamic light scattering (DLS) [33], atomic force microscopy (AFM) [34], fluorescence spectroscopy [35], high-performance liquid chromatography (HPLC) [36], capillary electrophoresis (CE) [4], and others.

Size and lamellarity

Liposome size is mostly determined by transmission electron microscopy (TEM), DLS, AFM, and size-exclusion chromatography (SEC) [37]. A general disadvantage of the TEM method is that negative staining with osmium oxide or uranyl acetate changes the structure of the liposome [31]. Cryo-TEM represents an alternative that, unlike TEM, does not require the staining and fixation of the samples, which makes it more suitable for size characterization [38].

In the DLS method, the light of a laser beam is scattered depending on the size and shape of the liposomes. By analyzing the fluctuations in light intensity caused by the Brownian motion of liposome particles in solution, the diffusion coefficients can be obtained that are related to the hydrodynamic size of the liposomes [39].

Other methods for liposome size determination are SEC and high-performance SEC. By these methods, liposomes can be separated from free analytes and at the same time divided into groups based on their size [40]. The main disadvantage of SEC is the loss of lipids due to their adsorption in the gel. To overcome this problem, the gel can be saturated with lipids before the analysis using small, sonicated liposomes [40–42].

Encapsulation efficiency

Encapsulation efficiency is defined as the ratio of the amount of the encapsulated substance in the liposome to the total weighed substance [43]. To determine the encapsulation efficiency, the free drug is separated from the liposome-encapsulated one by dialysis, gel filtration, or centrifugation. However, these methods can cause liposome destruction and release of the encapsulated drug. Among them, dialysis is the most gentle, but also the most time-consuming and instrumentally challenging one [44, 45]. Gel filtration and centrifugation are thus used more often. After the separation

of the encapsulated and free drug, the membrane of the liposome is disrupted and the amount of released encapsulated substance is determined by fluorescence spectroscopy, enzymatic, or electrochemical methods [37].

Quantitative composition

Most of the methods used to determine the quantitative composition, such as the determination of total phosphate content or the amount of Chol in the membrane, are based on the formation of colored products, which can be then determined spectrophotometrically. A method called Bartlett decomposition is used to determine the total phosphate content where the phosphate is transformed to the inorganic form and creates a blue product after reaction with ammonium molybdate [37, 46].

The amount of Chol in liposomes can be determined by enzymatic methods. The hydrolysis of the cholesterol ester produces free Chol, which is then oxidized by cholesterol oxidase to hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and phenol creating a colored product [37].

Proliposomes

Attempts to overcome the above-mentioned disadvantages of liposomes (low physical and chemical stability, leakage of encapsulated substance, hydrolysis, oxidation, and aggregation) led in 1986 to the discovery of proliposomes. In contrast to liposomes, proliposomes exhibit high stability. They are dry, free-flowing particles formed by phospholipids, a porous powder, and the drug [47–49]. Upon contact with the aqueous phase or with body fluids, they form a suspension of liposomes. The phospholipids commonly used for proliposome preparation are phosphatidylcholine and phosphatidylglycerol. Proliposomes also contain water-soluble porous carriers such as sorbitol, mannitol, or microcrystalline cellulose [47].

Liposomes and capillary electrophoresis

Capillary electromigration methods are suitable for the study of liposomes, because they exhibit several advantageous features, such as low sample consumption, fast and efficient separations, and a high degree of automation [4, 50]. Capillary electrophoresis is a method suitable for characterizing the drug–liposome interactions, for determination of encapsulation efficiency, for studying the drug leakage from liposomes, as well as for liposomes characterization (e.g., size, surface charge, and permeability of the phospholipid membrane) [4].

Determination of membrane permeability

Permeability of liposome membrane is a key parameter that must be determined when studying liposomes to prevent undesired leakage of encapsulated substances during their circulation in the bloodstream [51]. Tsukagoshi et al. [52] analyzed liposomes composed of dipalmitoyl PC with encapsulated dyes, eosin Y and rhodamine B, by CE with chemiluminescence detection. The dependence of the liposome stability and its membrane permeability on the concentration of encapsulated buffer was monitored by the separation, detection, and quantitation of free and encapsulated dye. Liposomes were dispersed in 10 mM carbonate buffer (pH=9.0), while within them, eosin Y in carbonate or phosphate buffer of different concentrations was encapsulated. The peak area ratio of free eosin Y to encapsulated eosin Y was then calculated from obtained electropherograms. The higher the ratio, the greater was the permeability of the liposome membrane. The peak area ratio, and thus the permeability, was greater when the concentration of encapsulated carbonate buffer was lower than the concentration of carbonate buffer used to disperse the liposomes. The authors hypothesized that water was released from the liposome to equalize the concentration of carbonate buffer to maintain the osmotic balance. This resulted in shrinkage of liposome with subsequent release of the encapsulated dye.

Franzen et al. [53] used CE to study the effectiveness of oxaliplatin encapsulation and the drug leakage from the PEGylated liposome. The liposomes' samples were sonicated by a sonication probe to induce faster leakage of the drug and the electropherograms (Fig. 1) showed an increased peak of free oxaliplatin and decreased peak of encapsulated oxaliplatin with increasing sonication time. The authors assumed that the disappearance of the liposome peak was caused by the complete release of encapsulated oxaliplatin rather than the destruction of the liposome. Using DLS, they found that liposome particle size decreased with increasing sonication time.

Determination of size and charge

Although CE is not a widely used method for the determination of liposome size, Duffy et al. [54] used CE with laser-induced fluorescence detection (CE-LIF) to study the properties of liposomes, including their size. To eliminate the electroosmotic flow (EOF) and to reduce the adsorption of the liposomes to the capillary wall, the capillary was coated with poly(acryloylaminopropanol). Liposomes prepared from PC, PS, PE, and Chol were dispersed in 2.5 mM sodium tetraborate (pH=9.3) and the encapsulated substance was 10 μ M fluorescein. They were able to calculate the volume of solution captured in liposomes and thus calculate the radius of the liposome from corrected fluorescence

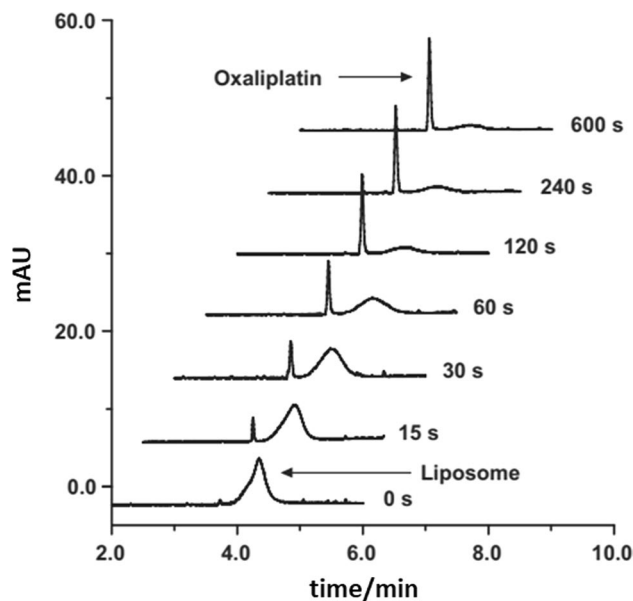


Fig. 1 Release of encapsulated oxaliplatin from PEGylated liposome with increasing sonication time. Reprinted and modified with permission from Ref. [51]

intensity, fluorescein concentration in the liposomes, and detector sensitivity.

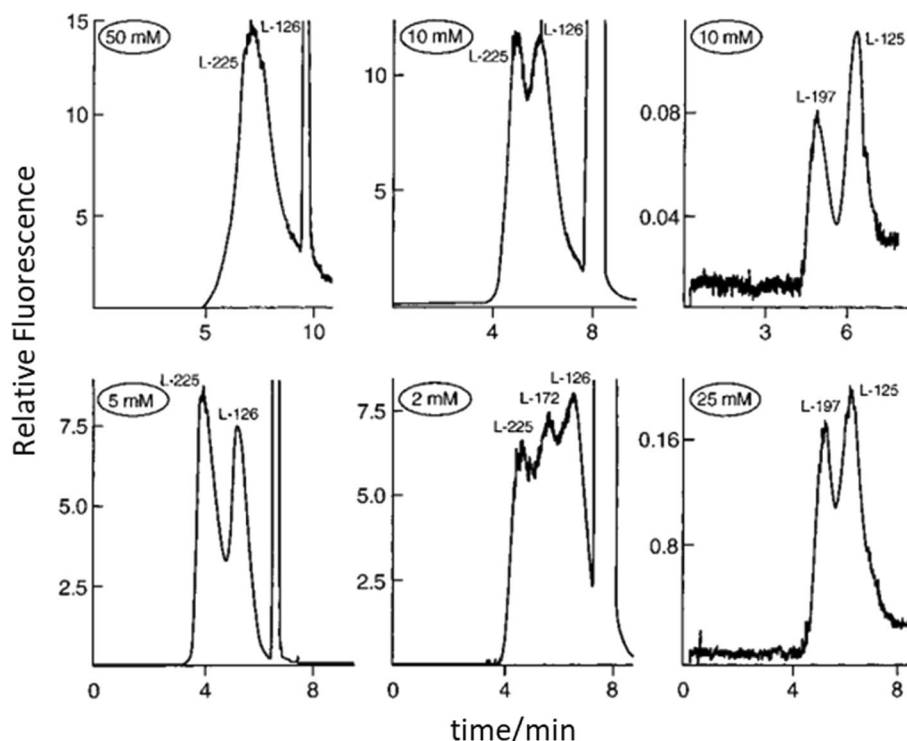
Radko et al. [55] measured mobilities of liposomes composed of PC/PG/Chol in various ratios by extrusion and non-extrusion methods leading to the formation of liposomes with the diameter range from 125 to 488 nm. Based on the measurements of free and encapsulated fluorescein in the capillary coated with 3% non-crosslinked polyacrylamide in the Tris-HCl background electrolyte (BGE) of the different ionic strength, they determined that the electrophoretic mobility is size-dependent (Fig. 2).

For determination of the liposome charge, Wiedmer et al. [56] used CE with UV/Vis detection at 200 nm to measure the electrophoretic mobility of liposomes and DLS to determine the liposome size. The liposomes were composed of palmitoyloleoyl (PO), PC, and PS in various molar ratios and they were dispersed in 50 mM 2-(*N*-cyclohexylamino) ethanesulfonic acid (pH=9.0). The charge was calculated from electrophoretic mobility, size of the liposome, and the known value of buffer viscosity. The results confirmed the presumption that the higher the content of negatively charged lipids in the liposome, the greater the charge of the liposome.

Determination of free and encapsulated drugs

Capillary electrophoresis can also be used to study the liposomes as a drug transport system and to determine the amount of free and encapsulated drug [4]. Liposomes are very often used as carriers for toxic drugs, and it is thus

Fig. 2 Electropherograms showing the size-dependent separation of liposomes varying in their size in 3% linear polyacrylamide gel matrix. Numbers in ovals show ionic strength of Tris–HCl background electrolyte. Reprinted and modified with permission from Ref. [53]



crucial to develop a method for simple and fast determination of encapsulated and free drugs, which could be toxic for the organism. Ansar et al. [57] used CE to separate free and encapsulated doxorubicin in 20 mM phosphate buffer (pH = 6.5) with 10% sucrose as a BGE. Free doxorubicin was determined from the calibration curve by measuring the sample with fluorescein used as an internal standard due to its high stability in phosphate buffer and high absorption coefficient. The total amount of doxorubicin was determined after its release from the liposome using Triton X-100 detergent for membrane disruption.

Another example of the determination of free and encapsulated substances is a study by Chen et al. [58]. They encapsulated oligonucleotides into large neutral or positively charged liposomes, because neutral liposomes do not migrate in the electric field and positively charged liposomes migrate very slowly in the opposite direction than negatively charged oligonucleotides. Free oligonucleotides could thus be separated from the encapsulated ones due to their migration to the anode. To determine total oligonucleotides, liposomes were disrupted by phenol–chloroform extraction, or by Triton X-100 detergent.

Nguyen et al. [59] developed a CE method coupled with MS detection that has a high potential in pharmaceutical quality control and in drug development. They were able to determine the encapsulation efficiency, the stability of liposomes, and the leakage of encapsulated drug from the liposome into the human plasma. The drug release in human plasma was measured before and after incubation of

liposome with encapsulated cisplatin (the amount of free and encapsulated cisplatin and total cisplatin were determined in one run), the stability of liposome was measured after incubation at 37 °C, and the triggered release of encapsulated drug into human plasma was measured after sonication.

Capillary electrochromatography and liposomal electrokinetic chromatography

Apart from being analyzed and characterized by CE, liposomes can also be used to facilitate separations. For this purpose, they can be used in two different layouts, capillary electrochromatography (CEC), or liposomal electrokinetic chromatography (LEKC). CEC combines the features of CE and HPLC. Liposomes are used to coat the capillary wall forming thus a stationary phase. By applying voltage, the EOF is generated and it carries the mobile phase with analytes through the capillary. The charged compounds are separated not only by their different electrophoretic mobilities but also due to their distribution between the mobile and the liposomal stationary phases [60–62]. To coat the capillary wall with liposomes, the capillary can be simply flushed by them, or the avidin–biotin technique can be used. The latter is based on the strong electrostatic interaction between biotin present on the surface of the liposome and the avidin protein, which is bound to the coated capillary wall (usually coated with agarose) [63]. However, the liposomes with biotin are relatively expensive and bound biotin changes the liposome characteristics. Additionally, the avidin–biotin

complex creates a new undesired place where analytes can interact and thus interfere with the drug–lipid interactions in the capillary. Another technique for coating of the capillary wall includes covalent attachment of liposomes after wall activation by 4-nitrophenyl chloroformate [64–66]. The main advantages of CEC over HPLC include higher separation efficiency, low sample consumption, and low analysis costs [61].

Godyń et al. [67] used CEC for studying the blood–brain barrier permeability of different compounds. Prior to the analysis of 25 drug compounds, the capillary was coated with POPC/PS (80:20 mol%) liposomes representing the natural composition of the phospholipid bilayer of the blood–brain barrier. They coated the capillary by flushing it 10 min with 0.5 M HCl, 15 min with water, and then 10 min with liposomes and based on the EOF marker measurements found out that the EOF in the coated capillary was suppressed. They compared the data from CEC to parallel artificial membrane permeability assay as a widely used method. Based on their research and *in vivo* data of the studied compounds, they concluded that CEC as a relatively fast and inexpensive method could be used as an alternative screening method in early drug development.

In the case of LEKC, the liposomes are added directly to BGE creating a pseudo-stationary phase [68]. This method is based on the same principle as micellar electrokinetic chromatography. The separation of the analytes occurs due to their distribution between the aqueous mobile phase and the liposomal pseudo-stationary phase [69, 70]. Nakamura et al. [71] successfully used the LEKC to separate hydrophobic neutral analytes, namely, biphenyl and naphthalene. Liposomes added to the BGE were composed of dimyristoyl PC and dimyristoyl PG in 10 mM Tris–HCl buffer with 50 mM NaCl (pH = 7.0). Without liposomes in the BGE, both analytes migrated together with EOF and thus were not separated. After the addition of the liposomes to the BGE, the analytes were baseline separated based on their distribution between the two phases.

Wiedmer et al. [72] used LEKC to separate neutral steroid hormones using BGE containing negatively charged liposomes composed of POPC, PS, and Chol of different ratios creating the pseudo-stationary phase. They used bare fused silica capillary despite acknowledging that some quantity of liposomes may be adsorbed to the capillary wall and thus act as a stationary phase, which results in a combination of LEKC and CEC techniques. They also used polyacrylamide coated capillary with suppressed EOF with the same BGEs to avoid liposome adsorption to the wall and faster separation of more hydrophobic steroids. The differences in the interactions between steroids and liposomes with increasing amount of cholesterol were observed.

The main asset of the LEKC is the possibility to simulate and study the interactions of drugs with cell

membranes, since liposomes are structurally very similar to them [73]. Lipophilicity plays a significant role when formulating a drug, allowing the drug to pass through the phospholipid bilayer of the membrane. The octanol–water partition coefficient P_{ow} is widely used to quantify the lipophilicity of drugs. However, this parameter is not suitable for the prediction of the drug–membrane interactions. Charged drugs interact with membranes due to both, the hydrophobic and electrostatic interactions. Their P_{ow} value can thus be rather low, but they can still relatively strongly interact with liposomes due to the electrostatic interactions. LEKC proved to be a suitable method for studying the complex interactions between drugs and cell membranes simulated by liposome membranes [74, 75]. Carozzino and Khaledi [73] investigated the influence of the type and concentration of the buffer, its ionic strength, and liposome composition on separation of neutral (phenol) and positively charged (drugs including tetracaine and lidocaine) compounds by LEKC using negatively charged liposomes. They found out that none of the parameters mentioned above affected the partition coefficients of the neutral analytes. On the other hand, the partition coefficients of the positively charged analytes decreased with increasing ionic strength, because the electrostatic interactions between analytes and the liposomes were screened by their interactions with the higher number of counter-ions in the solution. At the same time, the P_{ow} partition coefficient increased with the increasing content of negatively charged lipids present in liposomes, because the electrostatic interaction between positively charged liposomes and negatively charged liposomes increased. All liposomes used to study the effect of liposome composition were prepared with the same concentration of Chol (30% of total lipid concentration) and different combinations of other phospholipids such as dipalmitoyl PC, dipalmitoyl PG, dipalmitoyl PS, dipalmitoyl PE, phosphatidylinositol, and sphingomyelin.

Burns and Khaledi [68] developed a fast LEKC method for determination of liposome–water partition coefficient K_{lw} , which better corresponds with cell membranes, as the composition and structure of liposomes are closer to cell membranes than to octanol. The method utilizes the fact that LEKC retention factors calculated from migration times are directly proportional to liposome–water partition coefficient. Studying drug–membrane interactions by LEKC takes only few minutes in opposite to conventional methods, which can take up to days. To validate the method, the authors calculated K_{lw} values from two quantitative structure–partition relationship models and showed great agreement between the experimentally obtained and predicted values. One of the two models was based on the relationship between K_{lw} and P_{ow} and the other was the linear solvation energy relationship (LSER). The strong correlation between the K_{lw} values

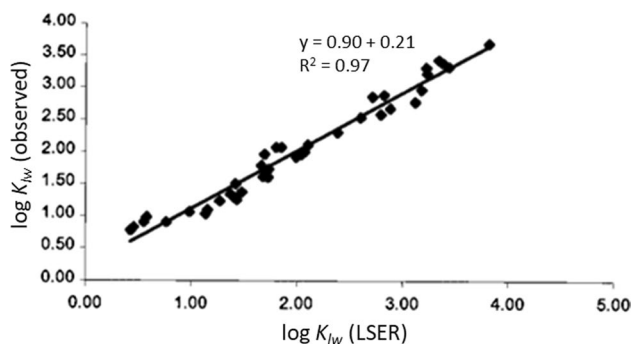


Fig. 3 Correlation between the K_{1w} values of uncharged aromatic compounds obtained from LEKC and predicted by LSER. Reprinted and modified with permission from Ref. [66]

for uncharged aromatic compounds obtained from LEKC measurements and from LSER prediction is shown in Fig. 3.

Ruokonen et al. [76] studied the effect of temperature (at 25, 37, and 42 °C) on distribution constants K_{1w} of local anesthetics by LEKC using liposomes of three different compositions as pseudo-stationary phase. One type of the liposomes was formed by lipids from human red blood cells for better correspondence with the natural composition of cell membranes. No correlation between temperature and distribution constant of anesthetics was found for any of the tested liposomes; however, the K_{1w} values slightly increased with elevated temperature in most cases. On the other hand, they determined that the K_{1w} values are dependent on the amount of Chol and charged lipids, and surprisingly, the data obtained when using LEKC with red blood cells liposomes showed up to three times lower K_{1w} constants than when using liposomes composed of POPC/POPG or POPC/POPG/Chol, indicating that selection of liposomes for LEKC plays a significant role when conclusions for the *in vivo* interactions of drugs with cell membranes are to be drawn.

Conclusion

The properties of liposomes, such as their composition, size, and preparation technology, determine their overall stability, their behavior in the body, as well as their utility as drug carriers. These parameters are crucial in the early drug development and quality control of new products. Capillary electrophoresis methods proved to be promising alternative to the commonly used ones. The main advantages of CE include the possibility of studying the liposome–drug interactions directly inside the separation capillary, which combined with the high degree of automation, several detection techniques available, and short analysis times, make CE a versatile tool for comprehensive analysis and characterization of liposomes. The low sample and buffer

consumption, along with the scarce use of organic solvents, results in low operational costs and environmental friendliness of CE methods. Based on the above-mentioned, further development and increasing number of CE applications can be expected, especially in the area of LEKC modeling of drug–cell membrane interactions and LEKC investigation of controlled drug release.

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