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Review

Physico-chemical characterization of liposomes and drug substance–liposome interactions in pharmaceutics using capillary electrophoresis and electrokinetic chromatography☆

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ABSTRACT

Liposomes are self-assembled phospholipid vesicles and have numerous research and therapeutic applications. In the pharmaceutical and biomedical sciences liposomes find use as models of biological membranes, partitioning medium and as drug carriers. The present review addresses the use of capillary electrophoresis and liposome electrokinetic chromatography for the characterization of liposomes in a pharmaceutical context. Capillary electrophoretic techniques have been used for the measurement of electrophoretic mobility, which provides information on liposome surface charge, size and membrane permeability of liposomes. The use of liposome electrokinetic chromatography and capillary electrophoresis for determination of liposome/water partitioning and characterization of drug–liposome interactions is reviewed. A number of studies indicate that capillary electrophoresis may have a role in the characterization of liposome drug delivery systems, *e.g.*, for the investigation of encapsulation efficiency and drug leakage. The well-known characteristics of capillary electrophoresis, *i.e.*, low sample volume requirement, high separation efficiency in aqueous media without a stationary phase, minimal sample preparation, and a high degree of automation, makes it an attractive approach in liposome research.

Contents

1. Introduction

Since their discovery in the early sixties [1] liposomes have been subject to intense studies in the areas of pharmaceutical, biophysical and biological sciences. In relation to the pharmaceutical and biomedical sciences, liposomes have in particular found use as cell membrane models, drug delivery systems and as analytical tools [2–6]. Liposomes are characterized by a layered structure consisting of one or more bilayer(s) and thereby resemble cell membranes. The building blocks of liposomes are amphiphilic lipids, most commonly phospholipids, which in aqueous media self-assemble to form colloidal vesicles encapsulating a fraction of the aqueous phase [7]. The phospholipids are the major constituents of the membranes of living cells, the lipid composition varying between different cell types. Glycerophospholipids are the most frequently used naturally occurring phospholipids

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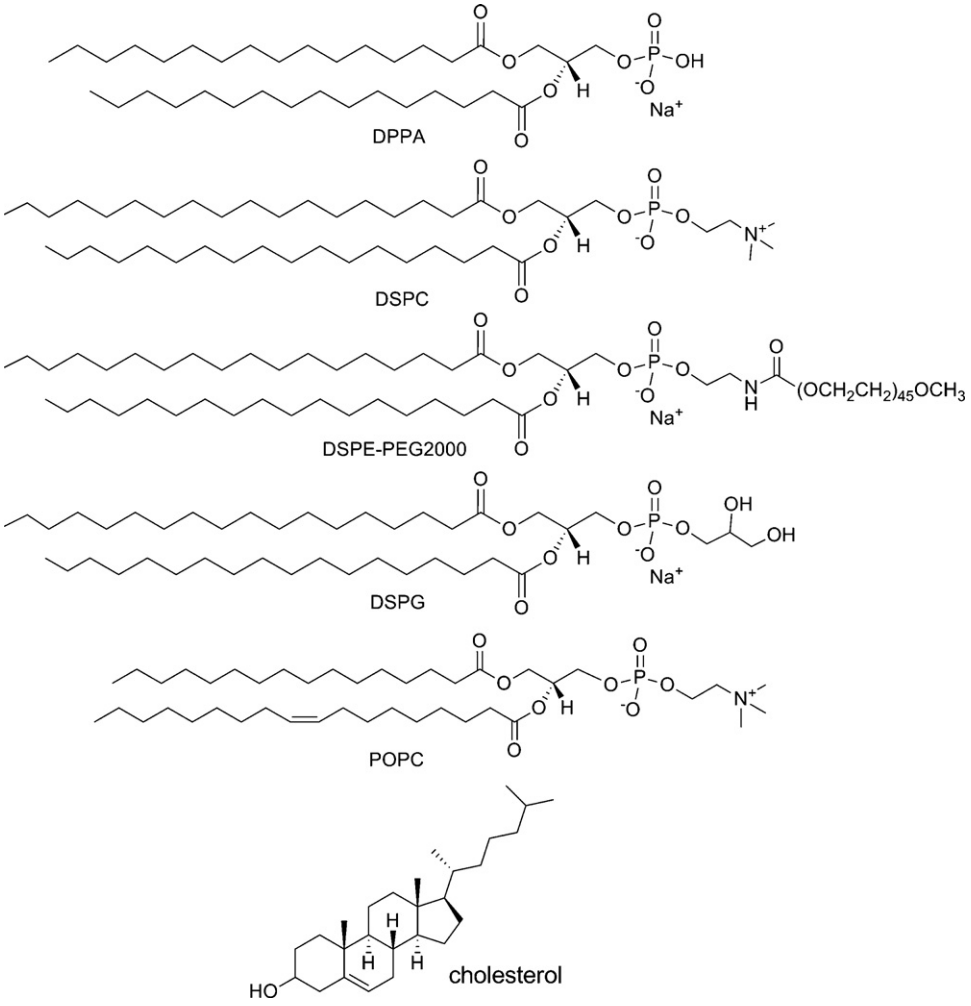

Fig. 1. Structures of selected lipids and cholesterol used in liposome preparation. DPPA, 1,2-dipalmitoyl-*sn*-glycero-3-phosphate monosodium salt; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DSPE-PEG2000, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] monosodium salt; DSPG, 1,2-dioctadecanoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) monosodium salt; POPC, 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine.

applied in liposome preparation. These amphiphilic molecules consist of a glycerol backbone, a hydrophobic region composed of fatty acid chains and a hydrophilic part containing a small organic entity. Other major naturally occurring membrane forming lipids encompass the sphingolipids, glycosphingolipids, and glycoglycerolipids. Cholesterol and proteins are additional major membrane constituents [8]. The hydrophilic head group differentiates the various glycerophospholipids. The head group defines the zwitterionic (at most pH ranges), cationic or anionic character of the hydrophilic region of the phospholipids. In eukaryote cells the main phospholipids are the zwitterionic phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE), the anionic phosphatidyl serine (PS), but also the zwitterionic sphingomyelin is present [9]. Less common phospholipids found in the eukaryote cell membranes are anionic phosphatidic acid (PA) and phosphatidylinositol (PI) [8]. The acyl chain typically varies with respect to the degree of saturation and chain length, the number of carbon atoms varying between 14 and 20. In addition to the naturally occurring phospholipids a number of synthetic or modified phospholipids are applied in liposome manufacturing. Structures of selected lipids and cholesterol used in liposome preparation are depicted in Fig. 1.

Classification of liposomes is frequently done according to their size and lamellarity. Primary liposome characteristics, in addition to the chemical composition, are size (25–5000 nm), the number of bilayer(s), bilayer fluidity, and the surface charge [10,11]. Small unilamellar vesicles (SUVs) have diameters in the range of 25–100 nm, large unilamellar vesicles (LUVs) ranges from 100 to 1000 nm and multilamellar vesicles (MLVs) are normally above 100 nm in diameter [9,11]. The bilayer thickness is reported to be ~4 nm for unilamellar dimyristoylphosphatidylcholine liposomes [12]; it is however to some extent dependent on the length of the acyl chain [13].

An adequate characterization of liposome preparations and formulations is necessary, in order to ensure that liposomes encompass the required and expected properties for their specific application. For instance, when comparing different batches of liposomal drug formulations intended for patient treatment, or comparing different literature studies involving liposomes, it is crucial to have a sufficient characterization of the sometimes complex liposomal systems. Liposome size, surface charge, lamellarity, phase transition temperature, phospholipid composition and concentration, physical and chemical stability, entrapped volume, degree of drug encapsulation and permeability are among the most relevant and commonly determined liposome characteristics. Some of these properties together with main analytical techniques for their determination are described in the following sections. However, the interested reader is referred to the following references for a more thorough treatment of the topics [2,3,10,14–16].