

**PEG-STABILIZED BILAYER DISKS: FUNDAMENTALS AND
PHARMACEUTICAL/BIOTECHNICAL APPLICATIONS OF A NOVEL
MODEL MEMBRANE**

KATARINA EDWARDS*, EMMA JOHANSSON and ANNA LUNDQUIST

Department of Physical Chemistry, Uppsala University, Box 579,
SE-751 23 Uppsala, Sweden

Open membrane structures, best described as bilayer disks, may be found in mixtures of phospholipids and various micelle-forming components. In many cases the disks are merely short-lived intermediates formed during a phase- or structural transition. Examples are the circular, or sometimes more irregular membrane patches often observed during surfactant mediated solubilization of liposomes [Silvander, M. *et al.* **J. Colloid Interface Sci.** 179 (1996) 104]. In other cases the disks appear to be in thermodynamic equilibrium. Stable disk-shaped objects, commonly termed “bicelles”, may for instance be found at certain compositions in mixtures of the long-chain phospholipid dimyristoylphosphatidylcholine (DMPC) and the short-chain phospholipid dihexanoylphosphatidylcholine (DHPC) [van Dam, L. *et al.* **Biochim. Biophys. Acta** 1664 (2004) 241]. The disks formed in the DMPC/DHPC-system are rather small, with diameters of 20 nm or less, and form only within a limited temperature interval. Large discs, with diameters of 200 nm or more, have, on the other hand, been discovered in dilute aqueous solutions containing a mixture of phospholipids, cholesterol and polyethyleneglycol-lipids (PEG-lipids) [Edwards, K. *et al.* **Biophys. J.** 73 (1997) 258, Johansson, E. *et al.* **Biophys. Chem.** 113 (2005) 183]. PEG-lipids are routinely used in order to prolong the blood circulation time of liposomes used for drug delivery applications. It is well established that upon inclusion of sufficiently high concentrations of PEG-lipids a transition from bilayer to micellar phase occurs. Further, systematic studies of the phase behaviour and aggregate structure in various lipid/PEG-lipid systems have revealed that either cylindrical or disk-shaped micelles may form as the bilayer saturation concentration is exceeded [Edwards, K. *et al.* **Biophys. J.** 73 (1997) 258]. The cylindrical micelles, which often adapt a long worm-like structure, are observed when PEG-lipids are mixed with the unsaturated phospholipid egg-phosphatidylcholine (EPC). Disk-shaped structures appear, on the other hand, in mixtures of PEG-lipids and saturated lipid analogues like DSPC and DPPC. Importantly, upon inclusion of 40 mol% cholesterol in the lipid mixtures the PEG-lipids induce formation of disk-shaped structures in all the investigated systems. The size of the disks is critically dependent on the PEG-lipid concentration. Large disks, perhaps better described as circular membrane patches, are found at PEG-lipid concentrations just above the bilayer saturation limit. Investigations based on a combination of dynamic light scattering and cryo-TEM [Johansson, M. and Edwards, K. **Biophys. J.** 85 (2003)

* E-mail: katarina.edwards@fki.uu.se

3839] show that the disks are well described by an ideal disk model assuming partial component segregation. More precisely, available data strongly suggest that the PEG-lipids accumulate at the highly curved rim of the disks while the phospholipids, and cholesterol, reside in the bulk of the bilayer aggregates. The PEG-stabilized disks show excellent long term stability and their size and structure remains unaltered in the temperature range between 25 – 37°C.

The open structure and good stability of the PEG-stabilized disks opens up for several interesting applications. One rather obvious, and potentially important, application of the disks is their use as model membranes. Very promising results have recently been obtained in studies where PEG-stabilized disks were employed as alternatives to liposomes in drug partition studies [Johansson, E. *et al. Biophys. Chem.* 113 (2005) 183]. Due to their structural similarity with biological membranes phospholipid liposomes have been extensively used as model membranes and during the last ten years several liposome-based methods for the determination of drug partitioning have been developed and tested. The results of these studies indicate the good potential of phospholipid liposomes to serve as models for biological membranes in partition studies. A number of problems have been identified, however, that need to be solved in order to improve the performance and ease of handling of the liposome-based techniques. First, the self-closed and often multilamellar nature of conventional liposomes may complicate the evaluation of experimental data obtained in studies of drug-membrane interactions. Second, since phospholipid liposomes do not represent thermodynamically stable but merely kinetically trapped structures they tend to aggregate and fuse with time. By substituting the liposomes with PEG-stabilized disks both these problems may be avoided.

In addition to their use in drug partition studies we believe that the sterically stabilized disks may find other important biochemical, biotechnical and pharmaceutical applications. In particular, the potential use of the disks as carriers for protein-, peptide-, and hydrophobic drugs deserves attention.