DESIGN OF STERICALLY STABILIZED LIPOSOMES

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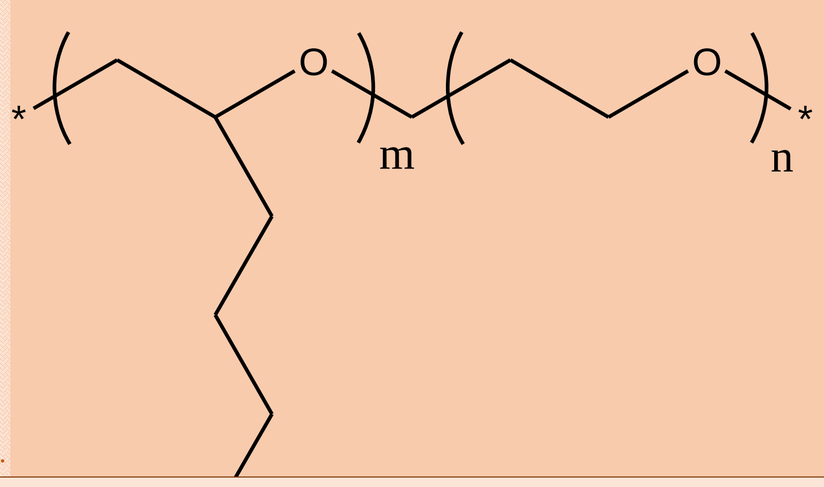
INTRODUCTION: Liposomes have been considered as almost universal carriers for drugs and diagnostic agents, as they being biodegradable non toxic and are able to accommodate both hydrophilic and hydrophobic agents. A major hurdle towards in vivo utilization of liposomes is their prompt by the cells of the RES. Typically, prolonged circulation of the liposomes is achieved using poly(ethylene glycol) (PEG) covalently connected to a lipid residue which is incorporated into the liposome bilayer. The polymer chains create a repulsive barrier around liposomes, which reduces the interactions with blood components and consequently increases the blood circulation time. At a certain critical content referred to as saturation limit, which depends on lipid composition and PEG-molecular weight, the PEG-lipids induce a transition from bilayers to a micellar phase.

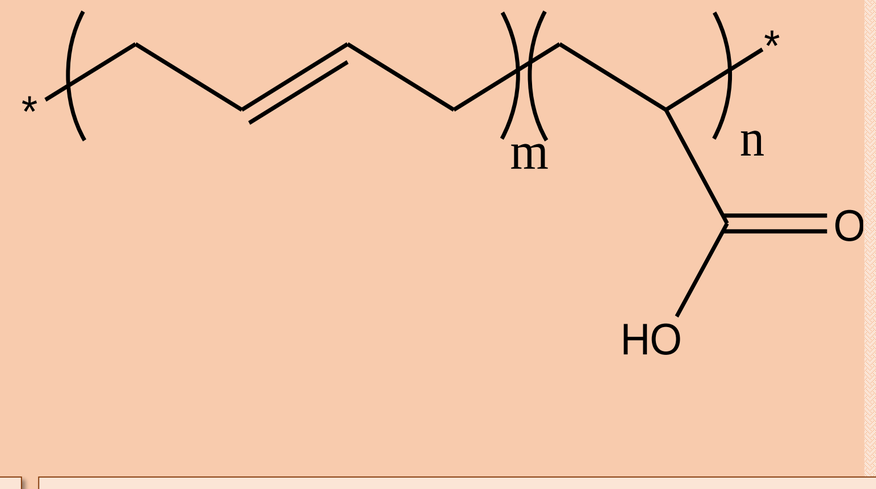
AIM: In this study we investigate the effects of the novel copolymers on the morphological properties and membrane integrity of lipid bilayers based on dipalmitoylphosphathidylcholine:cholesterol and pH-sensitive liposomes based on dioleoylphosphathidyl ethanol amine:cholestherylhemisuccinate liposomes. The copolymers were selected to differ in the structure and number of a hydrophobic anchors and the type and lenght of the hydrophilic chains. The chemical structures, composition and nominal molecular weight of the copolymers are given in Figure 1. To meet this objective we used dynamic light scattering, cryo-transmission electron microscopy and fluorescence spectroscopy.

Polyhexenyl-polyoxiethylen oxide



$EOHO_{206}$ Mw =14 000; PEO content(%) – 83
$EOHO_{208}$ Mw =7 800; PEO content (%) – 64

Polyisoprene-polyacrylic acid



PI-PAA
Mw = 12 000; PI content (%) - 36

Figure 1.

RESULTS AND DISCUSSION: The utilized method for praparation of liposomes is known to yield uniform, unilamelar vesicles with mean diameter of about 140 nm. The apparent particle diameters were found slightly depend on polymer/phospholipid and the copolymer composition. Within the all copolymer to phospholipid ratios studied, the size distributions were monomodal with a low polydispersity index (below 0.2) (see Fig. 2).

The liposome morphology was investigated by cryo-TEM. Within the seria of liposomes stabilized with non-ionic $EOHO_{208}$ and $EOHO_{206}$ polymers in all micrographs the predominant objects were intact, well separated and unilamellar liposomes. The first signs for liposomal destabilization (liposomal openings, bilayer fragments or discks) were observed at copolymer concentrations as high as 7.5 mol %. The fraction of disks observed at 7.5 mol % was higher for the copolymer $EOHO_{208}$. Within the serias of liposomes stabilized with pH-sensitive polymer PI-PAA the predominant objects in all images were liposomes but a micellar fraction co-exist with well separated and intact liposomes at copolymer concentration 5 mol % (Fig. 3).

A leakage assay using calcein was carried out in order to evaluate the membrane permeability of non pH-sensitive DPPC:CHOL and pH-sensitive DOPE:CHEMs liposomes stabilized with increasing amounts (2.5 – 7.5 mol%) of copolymer PI-PAA as a function of pH.The results obtained show that PI-PAA can induced pH-dependant calcein release from DPPC liposomes at low pH 4.5 and at the same time the tested polymer did not compromise the pH-sensitivity of DOPE liposomes (Fig. 4).

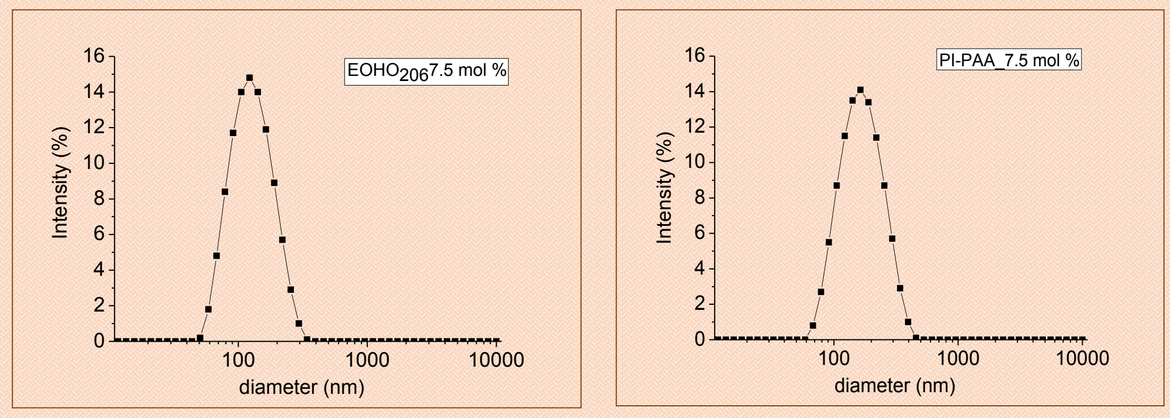


Figure 2. Size distribution of liposomes stabilized with 7.5 mol % of copolymers $EOHO_{208}$ (left) and PI-PAA (right)

Figure 3. Cryo-TEM images of DPPC:Chol liposomes stabilizied with 7.5mol % $EOHO_{206}$ (left) and 7.5 mol % PI-PAA (right). The arrow show liposomal opening (left) and micelles (right).

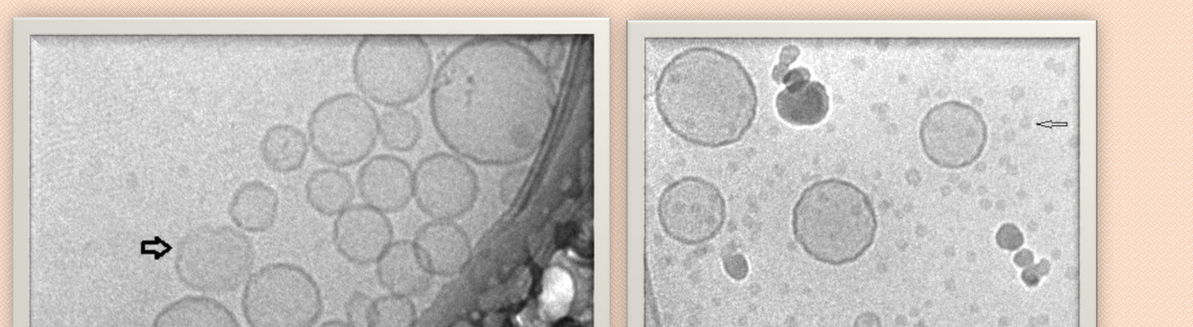


Figure 4. Spontaneous pH-dependent leakage at 37°C of calcein from DPPC:CHOL (left) and DOPE:CHEMs (right) liposomes Plain (squears) or stabilized with 2,5 (circles)or 5 mol% (triangles)PI-PAA.

CONCLUSION: This study shows that the utilized block copolymers can be considered as promising sterically stabilizing agents for the development of long circulating liposomes. An important advantage of the copolymer PI-PAA is that incorporation in the DOPE:CHEMs membrane does not deteriorate the pH-sensitivity of the formulation and even more the acid triggered calcein leakage was optimized. In addition, this polymer can induce pH-dependant release from non pH-sensitive DPPC liposomes wich make this polymer promissing candidate for preparation of second generation pH-sensitive liposomes.

ACKNOWLEDGEMENTS: The present study received financial support from the National Science Fund (Grand ДФНИ-501/25)