1. Introduction

Through recent years the prevalence of increasing amounts of estrogenic chemicals in the environment has become a major issue of concern. Compounds that elicit estrogenic activity have been linked to hormone related cancers and developmental abnormalities in both humans and wildlife. Lately, considerable attention has been focused on the development of rapid and inexpensive assays capable of detecting these compounds in aqueous matrices. In the framework of the development of a biosensor based on an indirect receptor assay we report the synthesis and characterization of 17-β-estradiol labeled liposomes filled with sulforhodamine B as a reporter dye.

2. Experimental

Materials and reagents

- All solvents were of HPLC-grade and were filtered before use.
- The estradiol-dipalmitoyl phosphatidyl ethanolamine (DPPE) conjugate was synthesized in-house. DPPC, DPPG, DPPE, sulforhodamine B (Sigma-Aldrich); cholesterol (UCB).

Type of instrumentation

- Whatman cyclopore, hydrophilic filters with pore diameter of 1.0 μm (Merck); Slide-A-Lyzer? 10K MWCO dialysis cassettes (Pierce Chemical Company).
- Rotavapor R (Büchi); Biofuge 15 ultracentrifuge (Heraeus Sepatech); PU 8740 UV/vis scanning spectrophotometer (ATI Unicam); PCS 100M (Malvern Instruments); Tri-Carb 1500 liquid scintillation analyzer (Packard).

3. Results and Discussion

Synthesis of DPPE-estradiol conjugate

The target compound (Figure 1) was prepared by classical condensation of the estradiol oxime carboxylic acid (0.128 mmol), with the amine, DPPE (0.130 mmol). Bicyclohexylcarbodiimide and N-hydroxysuccinimide were used as coupling agents (18 h, RT). The reaction mixture (7 mL) was diluted with 75 mL of water and extracted with ethyl acetate. Silicagel flash column chromatography yielded the target compound. The conjugate was dissolved in a mixture of chloroform/methanol (2:1). The final reaction product was characterised by Thin Layer Chromatography (TLC) and Mass Spectrometry (MS).

Synthesis of liposomes

For the synthesis of estradiol labeled liposomes the reverse-phase evaporation method [1,2] was used. This procedure includes:

- Addition of the aqueous sulforhodamine B solution to the mixture of the four lipids (DPPC:cholesterol:DPPG: estradiol-DPPE) dissolved in organic solvent;
- Evaporation of the organic solvent at 75 mbar at 45°C;
- Vortex mixing for 15 s;
- Evaporation of the residues of organic solvents at 95 mbar at 45°C;
- Extrusion ten times of the sample over a 1.0 μm filter;
- Dialysis of the resulting vesicles during 24 h to 25 mM NaCl in 10 mM Tris buffer at 4°C;
- Centrifugation for 10 min at 18200 x g.

Characterization of the liposomes by Dynamic Light Scattering (DLS)

The dynamic light scattering method gives an idea of the diameter of the formed liposomes together with the size distribution (Table 1).

<table>
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<tr>
<th>Liposome characteristics</th>
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<td>Mean diameter</td>
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<tr>
<td>Volume</td>
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<td>Liposome conc.</td>
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<td>Estradiol conc.</td>
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4. Conclusion

Using the reverse-phase evaporation technique uniformly sized vesicles were produced as demonstrated by the results of the dynamic light scattering analysis. The competition experiments performed showed the binding capacity of the conjugate DPPE-estradiol with the truncated hER, offering application potentials of the synthesized liposomes in the development of a screening test for xeno-estrogens in aqueous samples based on these liposomes.

5. References


6. Acknowledgements

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