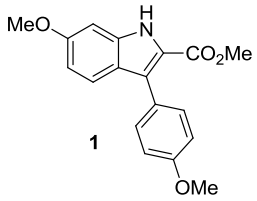
Nanoliposomes for encapsulation and delivery of the potential antitumoral methyl 6-methoxy-3-(4-methoxyphenyl)-1*H*-indole-2-carboxylate

Ana S. Abreu[a,b], Elisabete M. S. Castanheira[a], Maria-João R. P. Queiroz[b], Paula M. T. Ferreira[b]

[a]Centro de Física (CFUM) and [b]Centro de Química (CQ-UM), Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
anabreu@quimica.uminho.pt

Nanoliposomes are new technological developments for the encapsulation and delivery of bioactive agents. Because of their biocompatibility and biodegradability, along with their size, nanoliposomes have potential applications in a vast range of fields, including nanotherapy. Nanoliposomes are able to enhance the performance of bioactive agents by improving their bioavailability, *in vitro* and *in vivo* stability, as well as preventing their unwanted interactions with other molecules [1].
Nanoliposomes may contain, in addition to phospholipids, other molecules such as cholesterol (Ch) which is an important component of most natural membranes. The incorporation of Ch can increase stability by modulating the fluidity of the lipid bilayer preventing crystallization of the phospholipid acyl chains and providing steric hindrance to their movement.
Further advances in liposome research found that polyethylene glycol (PEG), which is inert in the body, allows longer circulatory life of the drug delivery system [2].

In this work, a potential antitumoral fluorescent indole derivative **1**, methyl 6-methoxy-3-(4-methoxyphenyl)-1*H*-indole-2-carboxylate (Figure 1), previously synthesized by us [3], has been encapsulated in different nanoliposome formulations, composed of egg-yolk phosphatidylcholine (Egg-PC), dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylglycerol (DPPG), distearoyl phosphatidylcholine (DSPC), with or without Ch and distearoyl phosphatidylethanolamine-(polyethylene glycol)2000 (DSPE-PEG).



1

Figure 1. Structure of methyl 6-methoxy-3-(4-methoxyphenyl)-1*H*-indole-2-carboxylate.

Compound **1** was evaluated for the *in vitro* cell growth inhibition on three human tumor cell lines, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and a melanoma cell line (A375-C5), after a continuous exposure of 48 h, exhibiting low GI_{50} values in the three tumor cell lines (Table 1).

Table 1. Values of compound **1** concentration needed for 50% of cell growth inhibition (GI_{50}).

	GI_{50} (μM)		
	MCF-7	**NCI-H460**	**A375-C5**
1	0.37 ± 0.02	0.33 ± 0.03	0.25 ± 0.02

Different formulations of nanoliposomes were prepared by injection of an ethanolic solution of the lipid mixture and compound **1** in an aqueous media under vigorous stirring, above the melting transition temperature of the lipids. The nanoliposomes prepared were then extruded (3 cycles) through a 100 nm polycarbonate filter. Hydrodynamic diameter, size distribution and zeta potential were measured by dynamic light scattering (DLS). These parameters were monitored in different days in order to determine the more stable formulations (Table 2). The results showed that nanoliposomes have sizes ≤ 120 nm and are generally monodisperse and stable after two weeks. Evaluation of the zeta potential of a nanoliposome preparation can help to predict the stability and *in vivo* fate of liposomes.

Table 2. Hydrodynamic diameter, polidispersity and zeta potential of several nanoliposomes.

	Mean Diameter (nm)	Polidispersity	Zeta potential (mV)
Egg PC/Ch/DPPG	103.5 ± 0.9	0.124 ± 0.009	-51.9 ± 5.84
2 weeks after	95.4 ± 0.53	0.138 ± 0.010	
DPPC/Ch/DSPE-PEG	115.4 ± 0.51	0.146 ± 0.008	-29.5 ± 1.16
1 week after	116.3 ± 1.62	0.154 ± 0.008	
2 weeks after	116.0 ± 0.77	0.152 ± 0.011	
DSPC/Ch/DSPE-PEG	119.8 ± 2.00	0.190 ± 0.012	-26.9 ± 3.66

Permeability studies of compound **1** between DPPC/DPPG liposomes (donor liposomes) and nitrobenzoxadiazole(NBD)-labelled DPPC/DPPG liposomes (acceptor liposomes) were performed using two different sizes of dialysis membranes (6-8 KDa and 12-14 KDa). For these experiments, three different fluorescent labelled lipids, bearing a NBD moiety in different positions of the phospholipids, NBD-PE (labelled at headgroup), NBD-C_6-HPC and NBD-C_{12}-HPC (labelled at fatty acid) were used (Figure 2 and 3). The transfer of compound **1** from DPPC/DPPG liposomes was monitored by FRET (Förster Resonance Energy Transfer) from compound **1** (energy donor) to the NBD-labelled lipids (energy acceptor).

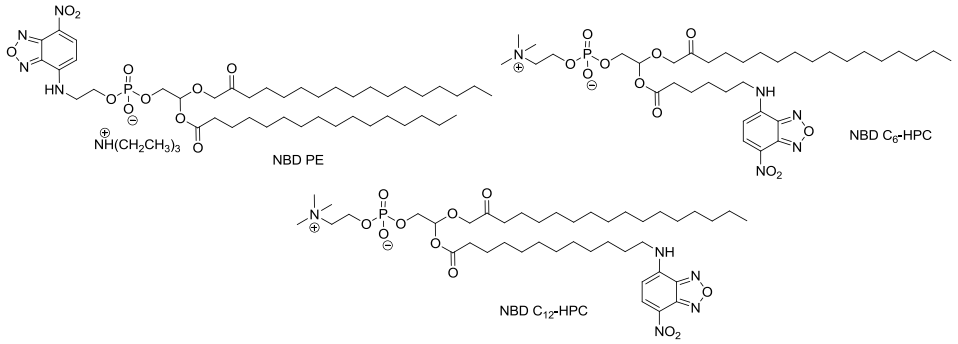


Figure 2. Chemical structure of the fluorescent-labelled lipids incorporated in nanoliposomes.

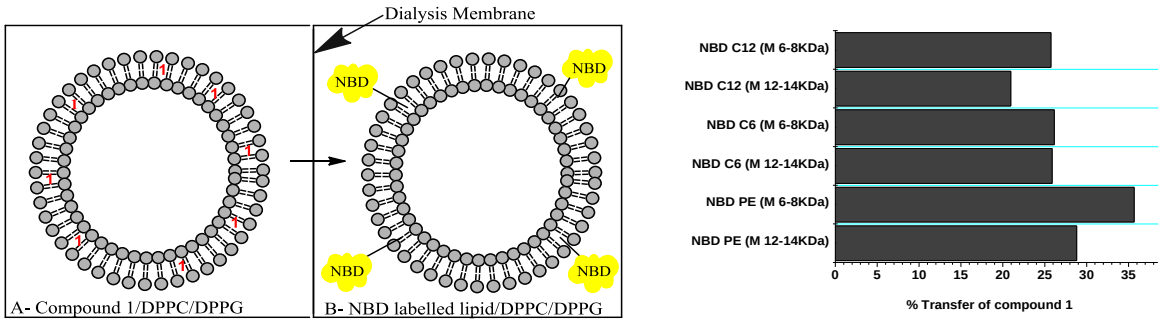


Figure 3. Schematic dialysis experiment and percentage of drug transfer through a dialysis membrane.

Comparing the energy transfer to the several NBD-labelled lipids (Figure 3, right side), it can be concluded that compound **1** locates mainly near the polar head groups of phospholipids in the acceptor liposomes. It is also observed that compound **1** transfer is more efficient for the smaller dialysis membranes (6-8 KDa).

References

[1] M.R. Mozafari, S.M. Mortazavi, Nanoliposomes: From Fundamentals to Recent Developments (2005) Trafford, printing in Victoria, BC Canada.
[2] T.L. Andresen, S.S. Jensen, K. Jorgensen, Progress in Lipid Research **44** (2005) 68-97.
[3] M.-J.R.P. Queiroz, A.S. Abreu, E.M.S. Castanheira, P.M.T. Ferreira, Tetrahedron **63** (2007) 2215-2222.

Acknowledgements

This work was funded by FCT-Portugal and FEDER through CFUM, CQ-UM, Project PTDC/QUI/81238/2006 (cofinanced by FCT and by program FEDER/COMPETE, ref. FCOMP-01-0124-FEDER-007467) and Post-doc. grant of A.S. Abreu (SFRH/BPD/24548/2005).