Aggregation of Steroidal Lariat Ethers: The First Example of Nonionic Liposomes (Niosomes) formed from Neutral Crown Ether Compounds

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N-(3-Dihydrocholesteryloxycarbonylmethyl)-(**1**) and *N*-(3-cholesteryloxycarbonylmethyl)-1-aza-4,7,10,13-tetraoxacyclopentadecane (**2**) aggregate in water to form the first examples of niosomes based on amphiphiles having an uncomplexed crown ether residue as the head group.

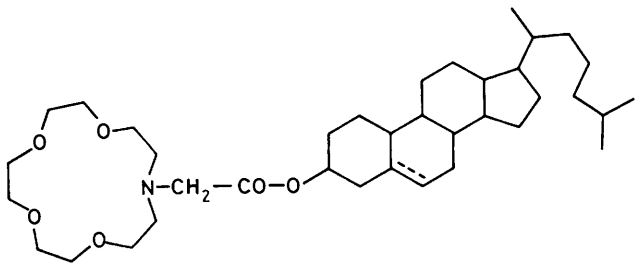
Vesicle formation is of considerable current interest because such structures may serve as membrane models,[1] in chemical reactivity studies,[2] or as vehicles for drug delivery.[3] Most known vesicles are highly fluid systems having charged head groups. Addition of cholesterol to vesicles often dramatically reduces the membrane fluidity.[4—6]

The nonionic liposomes or niosomes are not as well known[7—9] and are often based on polyethylene glycol derivatives. Crown ethers have been used as head groups, but have invariably yielded micelles rather than vesicles in aqueous solution.[10,11] To our knowledge, the few reports of vesicle formation from crown ether-based amphiphiles have always involved charged systems, resulting from cation complexation.[12]

The syntheses and cation binding properties of the steroidal aza-crown ether compounds (**1**) and (**2**) and the *X*-ray crystal structure of the carbamate analogue of the latter were recently reported.[13]

When either (**1**) or (**2**) (1—5 mM) was melted and then dispersed in deionized water using a Branson Cell Disruptor (Model 185, 40 W power, 10 °C) the turbidity of the dispersion decreased until a plateau was reached after 3—6 min. Solutions were centrifuged and then filtered through Nucleopore polycarbonate membranes of 0.2 μm porosity. The pH of the dispersion was unchanged (*ca.* 8.5) indicating that there was no protonation during the process. The resulting preparations are highly opalescent and exhibit the characteristic bluish colour of other vesicle suspensions, such as lecithin. One important property of the resulting solutions is their high stability after weeks at ambient temperature and exposure to light.

These niosome suspensions have been characterized using dynamic laser light scattering and electron microscopy. A Coulter Model N4-SD instrument was used and the data were analysed with the 'size distribution processor' (SDP) program. All solutions were 2 mM. Niosome diameters were also



(1) cholestanyl
(2) cholesteryl

Table 1. Niosome size determination by light scattering and electron microscopy.

	Laser scattering		Electron microscopy	
Compound	Diameter /nm	% Vesicles in range	Diameter /nm	Average
(1)	34 ± 13	97	28—33	30
(1)	168 ± 49	3		
(2)	35 ± 11	98	22—44	33
(2)	168 ± 47	2		

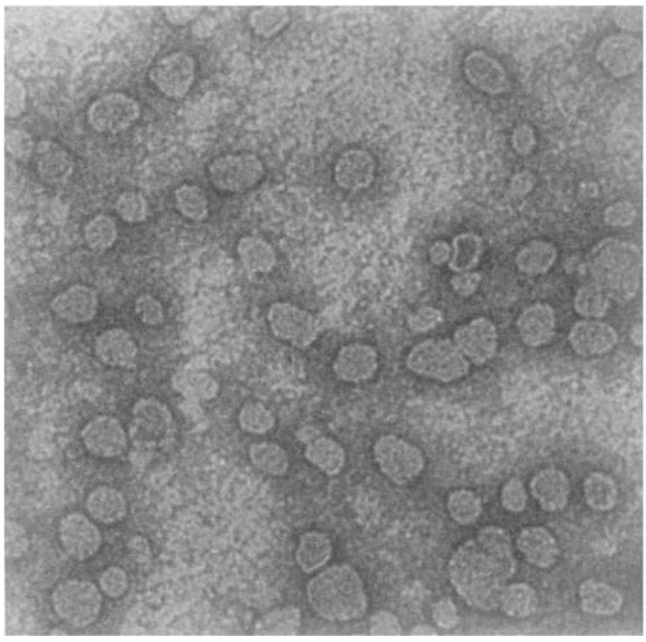


Figure 1. Electromicrograph (178 070×) of vesicles formed from compound (1); 1 cm corresponds to 56 nm.

determined by transmission electron microscopy (t.e.m.) following fixation with OsO_4 for 1 h and staining with uranyl acetate for 1 min. A typical electron micrograph is shown in Figure 1 for vesicles formed from (2). Size data are summarized in Table 1. Note the excellent agreement between these independently determined sizes.

The effect of Na^+ and K^+ cations (as the chlorides) on the aggregation of (1) was assessed by incremental addition of up to 25 mol. equiv. of NaCl or 50 equiv. of KCl. The salts were added both during the vesicle formation and to the preformed vesicle preparations. In all cases, the size range observed by laser light scattering ranged from 35 to 41 nm and these values were all within experimental error of each other. Thus, niosomes of (1) are not subject to osmotic shrinkage as observed in the polyethylene glycol-based cases.[9]

Further physical characterization was accomplished by determination of volume entrapment. Niosomes of (1) were prepared in the presence of 0.1 mM Methylene Blue. An aliquot was dialysed for 48 h against pure water to remove the extravesicular dye. Another aliquot was left intact. After disruption of niosomes with Triton X-100 (1% final conc.) both solutions were analysed for their peak absorbance at $\lambda_{max.}$ 664 nm. The observed volume entrapment was 3.9% with a standard deviation of 0.31. This value corresponds to an encapsulation efficiency of 7.8 $dm^3\ mol^{-1}$ of lipid, and is in excellent agreement with values reported by Deamer and Uster[14] for unilamellar liposomes of 30—40 nm diameter.

We have demonstrated that lariat ethers derived from cholesterol or dihydrocholesterol give the first examples of neutral vesicles formed from crown ether-based systems. Indeed, to our knowledge, this is only the second example of niosome formation from a pure, single substance. Preliminary studies using e.s.r. probes indicate that these niosome membrane systems are very rigid but this observation should be considered preliminary. It is interesting that the corresponding 18-membered ring systems behave differently, apparently aggregating into micelles rather than niosomes. The latter systems are under study and will be reported separately in due course.

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