# Quaternary ammonium derivatives of cholesterol: polymerization and vesicle formation

**S. K. Abid* and D. C. Sherrington**
*Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, UK*
*(Received 28 August 1990; accepted 12 November 1990)*

The ability of nine amphiphilic quaternary ammonium derivatives of cholesterol to form small unilamellar vesicles in water has been investigated using the entrapment of [$^{14}$C]sucrose coupled with gel filtration. In all cases vesicles were detected and the proportion of [$^{14}$C]sucrose entrapped suggested that these were indeed of the small unilamellar type. In the case of one amphiphile with a pyridinium ion head-group, studies over a period of four weeks showed the vesicles to be very stable, although there was some evidence for fusion and growth of the lamellar structures. Five of the amphiphiles also contained a polymerizable methacrylate group. Photoinitiation of polymerization of these in aqueous dispersions using 2,2-dimethoxy-2-phenylacetophenone as the initiator yielded only oligomeric products with $DP_n \sim 5$–13. In contrast polymerization in isotropic benzene solutions using thermal fragmentation of azobisisobutyronitrile yielded polymers with $DP_n$ up to $\sim 180$. In aqueous solution the 'rigid' bilayer structures may impose some additional steric restriction to polymer growth, a restriction that is absent in benzene solutions.

**(Keywords: polymerization; cholesterol; quaternary ammonium derivatives; methacrylate derivatives; vesicle formation; [$^{14}$C]sucrose entrapment)**

## INTRODUCTION

For some time now liposomes and vesicles have displayed useful potential as small particulate (20–100 nm) drug delivery vehicles[1]. However, their inherent lack of storage stability is one major factor that has made their exploitation rather limited. The average lifetime of individual small vesicles is many orders of magnitude larger than that of surfactant micelles ($10^{-3}$–$10^{-6}$ s). However, vesicles do tend to aggregate and fuse irreversibly with time, whereas micelles are constantly re-forming. Vesicles are also rapidly destabilized or collapsed in the presence of electrolyte or cosolvents (e.g. alcohols), and they are also susceptible to extremes of temperature. It has been shown that more stabilized lamellar structures can be created by polymerization of appropriate bilayers[2] or by surface coating with pre-formed polymers[3,4].

By the early 1980s several groups had reported the successful synthesis of polymerizable amphiphiles, and their incorporation into and polymerization within liposomal or vesicular structures. Regen[5] described a cationic amphiphile with a methacrylate moiety at the terminus of one hydrophobic alkyl chain. In short order several amphiphilic diacetylenes were described[6–9], as well as amphiphiles containing conjugated diene residues[10,11] and additional methacryloyl derivatives[12,13]. In all these examples the hydrophobic character of each amphiphile was provided by two aliphatic hydrocarbon chains, mimicking phospholipid structures. Since then the area has been reviewed periodically[14,15] and possible areas of application have been investigated beyond drug encapsulation and delivery[16–19].

In parallel with the above efforts there have also been attempts to produce polymerizable phospholipid analogues employing methacrylate monomers without any long hydrophobic structural component, e.g. 2-(methacryloyloxy)ethyl-2-aminoethyl hydrogenphosphate[20], 2-(*p*-methacryloyloxybenzoyloxy)ethyl hydrogen phosphate[21] and 2-(methacryloyloxy)ethyl-2-(trimethylammonium)ethyl phosphate[22].

Natural cell membranes contain phospholipids as the major structural component. Cholesterol, however, also plays an important role in the structuring and function of cell membranes, and in particular adds to the rigidity of the membranes. In addition cholesterol and its esters are important neutral lipids[23–25]. These esters are of course well known thermotropic liquid crystals and not surprisingly there has been a great deal of interest in producing polymerizable monomers containing the cholesterol residue, as a route to side-chain thermotropic liquid-crystal polymers. Most work has exploited the terminal –OH group in order to attach a (meth)acrylate residue[26–28], but recently polymers with the cholesterol residue attached 'side on' as the side-chain have been reported[29]. The thermotropic liquid-crystal behaviour of 2-[2-(methacryloyloxy(ethyldimethylammonium]ethyl-5-β-cholesten-3-yl phosphate and 2-[6-methacryloyloxy)-hexyldimethylammonium]ethyl-5-β-cholesten-3-yl phosphate and polymers derived from these ionic cholesterol derivatives has also been described[30].

Despite the central role played by cholesterol in natural systems, there appears to have been little work on

* Permanent address: Department of Medical Biochemistry, Kufa College of Medicine, Kufa University, Kufa-Najaf, Iraq

0032-3861/92/010175-06

Ib $CHOL\text{-}OCCH_2N^{\oplus}(CH_3)_2CH_2CH_2OC(CH_3)C=CH_2Cl^{\ominus}$

IIb $CHOL\text{-}OCO(CH_2)_2N^{\oplus}(CH_3)_2CH_2CH_2OC(CH_3)C=CH_2Br^{\ominus}$

IIb $CHOL\text{-}OC(CH_2)_{10}N^{\oplus}(CH_3)_2CH_2CH_2OC(CH_3)C=CH_2Br^{\ominus}$

IVb $DHCHOL\text{-}OC(CH_2)_{10}N^{\oplus}(CH_3)_2CH_2CH_2OC(CH_3)C=CH_2Br^{\ominus}$

Vb $DHCHOL\text{-}OCCH_2N^{\oplus}(CH_3)_2CH_2CH_2OC(CH_3)C=CH_2Cl^{\ominus}$

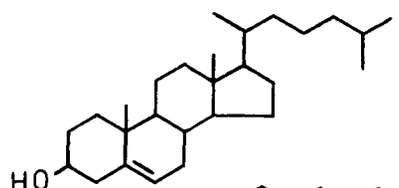
Ic $CHOL\text{-}OCCH_2\text{-}N^{\oplus}\text{(pyridinium)}\ Cl^{\ominus}$

IIc $CHOL\text{-}OCOCH_2CH_2\text{-}N^{\oplus}\text{(pyridinium)}\ Br^{\ominus}$

IIId $CHOL\text{-}OC(CH_2)_{10}N^{\oplus}(CH_3)_3Br^{\ominus}$

IVd $DHCHOL\text{-}OC(CH_2)_{10}N^{\oplus}(CH_3)_3Br^{\ominus}$

CHOL-OH =



DHCHOL-OH =

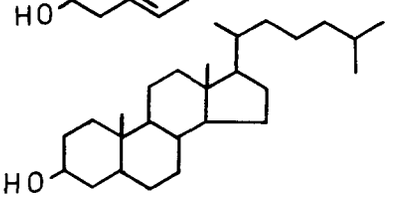


**Figure 1** Structure of amphiphiles

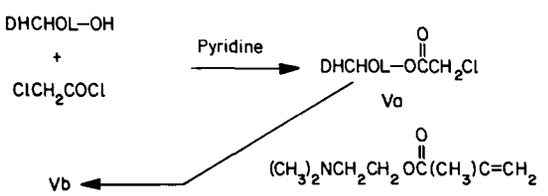



**Figure 2** Synthesis of methacrylate derivative, Vb

derivatizing this molecule with an ionic group such that the resultant species might form vesicular bilayers themselves, in the absence of phospholipids. We have already reported our initial synthetic work associated with this concept[31], following work directed similarly by Cho and Chung[32]. We now describe the synthesis of another monomer containing a dihydrocholesterol residue as the sole hydrophobe with a quaternary ammonium ion head-group, and the polymerization and vesicle formation of this and our previous molecules[31].

## EXPERIMENT AND RESULTS

### *Materials*

Dihydrocholesterol (DHCHOL–OH; Aldrich), dimethylaminoethyl methacrylate (DMAEMA; Aldrich), pyridine (Fisons), choroacetyl chloride (BDH), azobisisobutyronitrile (AIBN; Koch Light Laboratory), 2,2-dimethoxy-2-phenylacetophenone (DMPAP; Aldrich), 2,5-diphenyloxazole (PPO; Aldrich), 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP; Aldrich), Triton X-100 (Aldrich), Sephadex G50 (50–150 $\mu$m, Aldrich) and [$^{14}$C]sucrose (1 mCi, Amersham International) were used as supplied. The remaining materials were general-purpose laboratory reagents.

### *Structural analyses*

$^1$H n.m.r. spectra ($CDCl_3$) were recorded on a Perkin–Elmer R32 spectrometer at 90 MHz. Elemental microanalyses were carried out using a Carlo Erba 1106 instrument, and melting points were determined on a Gallenkamp digital melting-point apparatus. Polymer/oligomer molecular weights were measured by vapour-phase osmometry using an Hitachi 115 instrument.

### *Syntheses of amphiphiles*

The synthesis and structural characterization of amphiphiles I–IVb, Ic, IIc, IIId and IVd (*Figure 1*) have already been reported in detail[31]. Species Vb was prepared as shown in *Figure 2*, and the procedure was identical to that for IIb except that cholesterol was replaced by dihydrocholesterol. Analytical data for Vb and the choloromethyl intermediate Va are summarized in *Table 1*. (Note that compound numbers have been retained to facilitate cross-reference with ref. 31.)

### *Photochemically initiated polymerization of monomers I–Vb*

Monomer (0.5 g) and 2,2-dimethoxy-2-phenylacetophenone (DMPAP) (1% by weight of monomer) were

**Table 1** Analytical data for compounds Va and Vb

| Compound | Melting point (°C) | Elemental microanalytical data: Calculated C | H | Cl | N | Found C | H | Cl | N |
|---|---|---|---|---|---|---|---|---|---|
| Va | 182 | 74.9 | 10.5 | 7.7 | – | 76.0 | 10.9 | 7.7 | – |
| Vb | 204 | 71.4 | 10.3 | 5.7 | 2.3 | 71.7 | 10.5 | 5.9 | 2.1 |

| | $^1$H n.m.r. resonances in $CDCl_3$ |
|---|---|
| Va | 4.8–4.4 (br m, 1H, OC*H*); 4.1 (s, 2H, $ClCH_2$); 2.4–0.7 (br m, 46H, *DHCHOL–*) |
| Vb | 6.1, 5.7 (d, 2H, $C(CH_3{=}CH_2)$); 5.7 (s, 2H, $OCOCH_2\ N^+$); 4.7 (m, 3H, $OCH{+}OCH_2$); 4.4 (m, 2H, $CH_2N^+$); 3.7 (s, 6H, $(CH_3)_2N^+$); 1.9 (s, 3H, $CH_3C{=}CH_2$); 2.4–0.7 (br m, 46H, *DHCHOL–*) |

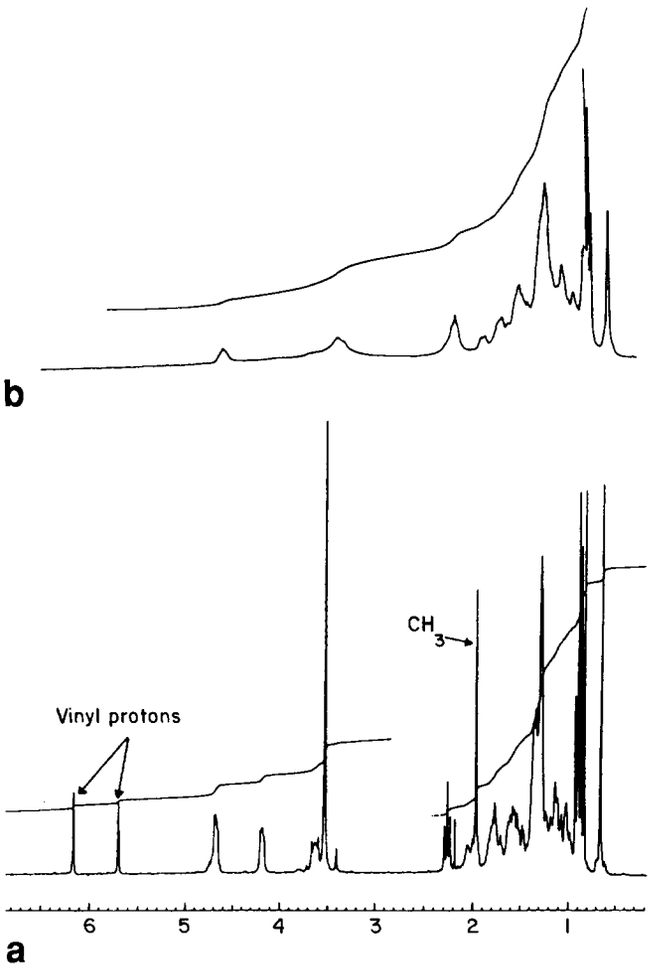



**Figure 3** $^1$H n.m.r. spectra (a) for monomer IVb and (b) for the corresponding oligomer produced by photoinitiated polymerization in water

introduced into a 25 ml volumetric flask and the solution made up to the mark with doubly distilled water. The mixture was vigorously hand shaken to disperse the components and a viscous emulsion formed. This was placed in front (50 cm) of a mercury ultra-violet lamp (200 W) and irradiated for 7 h. During this period a white precipitate gradually sedimented. The resulting mixture was transferred to a small round-bottomed flask and the product freeze-dried. The solid formed was washed with dry ether and dried at room temperature overnight in a vacuum oven. In each case the $^1$H n.m.r. spectrum in $CDCl_3$ confirmed the disappearance of the monomer vinyl group resonances and the methacryl methyl group, and showed typical broadening of the remaining ones, associated with polymerization. Although the conversion to polymer/oligomer could not be quantified with great accuracy, in each case the $^1$H n.m.r. spectrum suggested a conversion in excess of ~90%. *Figure 3* shows the spectrum obtained for the polymer/oligomer from IVb. Evaluation of molecular weights of products from I and III–Vb yielded values of 7250, 3700, 3000 and 7200 respectively. The product from IIb exhibited poor solubility in chloroform. These molecular weights correspond to average degrees of polymerization ($DP_n$) of ~12, 5, 4 and 13 respectively, so that the products are in effect only short oligomers in terms of their backbone structure.

*Thermally initiated polymerizations*

Monomer (0.5 g) and azobisisobutyronitrile (1% by weight of monomer) were dissolved in benzene (25 ml) and the mixture charged into a glass phial. This was degassed in the usual way on a vacuum line and the phial sealed with a gas torch. The phial was then placed in a water bath at 75°C for 15 h. The phial was opened and the benzene removed under vacuum at 40°C. The residue was washed with dry ether, then vacuum dried at room temperature to yield a bright white product. The $^1$H n.m.r. spectra of the products again indicated a high conversion to polymer/oligomer (probably >90%) and *Figure 4* shows the spectra for monomer IVb and the resultant polymer. Other analytical data for the polymers are summarized in *Table 2*. In these cases the polymer molecular weights were significantly higher and the vapour-phase osmometry data can be regarded only as a guide. The corresponding average degrees of polymerization ($DP_n$) for the polymers from I and III–Vb are ~65, 25, 25 and 178 respectively.

*Vesicle preparation*

The procedure adopted was similar to that described in the literature[33]. Each quaternary ammonium derivative of cholesterol and dihydrocholesterol (50 mg) was dissolved in chloroform (5 ml) in a 150 ml round-bottomed flask, and the solvent removed at 35°C under vacuum to leave a thin film on the wall of the flask. The

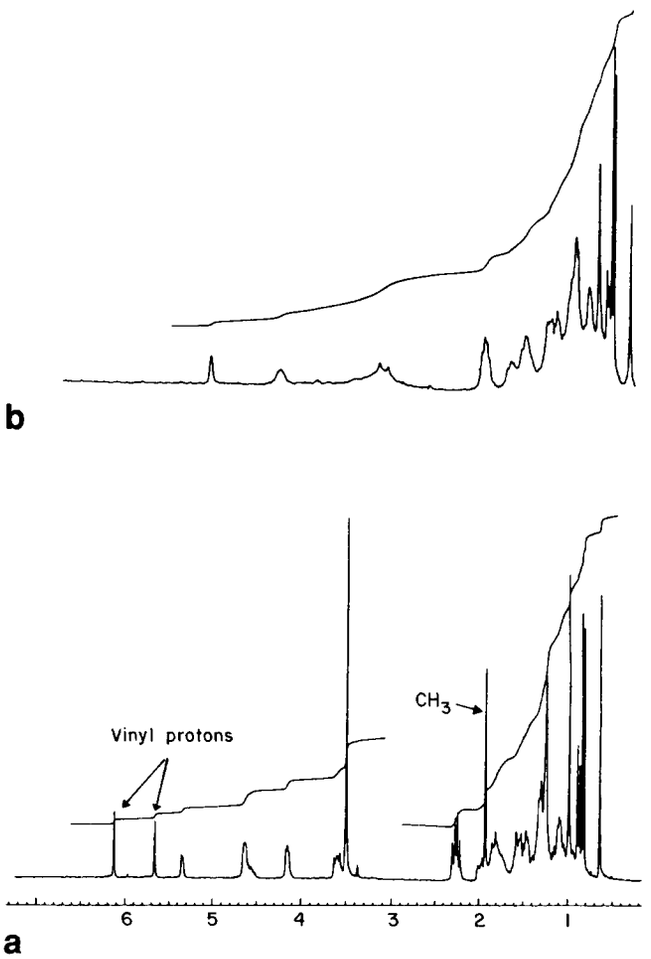



**Figure 4** $^1$H n.m.r. spectra (a) for monomer IIIb and (b) for the corresponding polymer produced in benzene by thermal initiation using AIBN

**Table 2** Analytical data for polymers prepared from I–Vb in benzene solutions

| Polymer/ monomer | Molecular weight | Microanalytical data (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Monomer | | | | Polymer | | | |
| | | C | H | Br | N | C | H | Br | N |
| Ib | ~40000 | 71.0 | 10.4 | 5.7[a] | 2.1 | 69.8 | 10.3 | 5.7[a] | 2.4 |
| IIb | –[b] | 64.1 | 9.2 | 11.7 | 1.8 | 63.0 | 9.0 | 12.0 | 2.0 |
| IIIb | ~20000 | 69.0 | 10.2 | 10.6 | 1.8 | 68.4 | 10.1 | 10.6 | 1.6 |
| IVb | ~20000 | 68.5 | 10.6 | 10.2 | 1.6 | 68.2 | 10.7 | 10.1 | 1.6 |
| Vb | ~100000 | 71.4 | 10.3 | 5.7[a] | 2.3 | 69.4 | 10.4 | 5.5[a] | 2.1 |

[a] Chlorine analysis
[b] Insufficiently soluble for *MW* determination

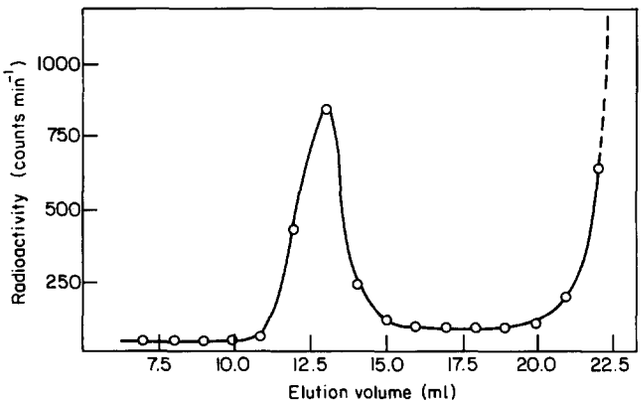



**Figure 5** Gel-filtration elution profile for [$^{14}$C]sucrose entrapped in vesicles of Ib

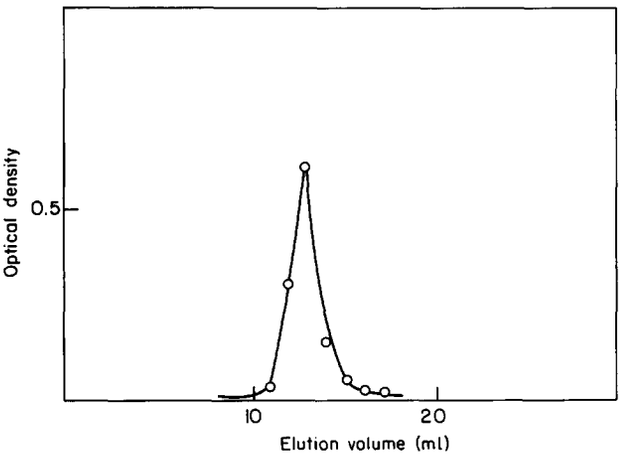



**Figure 6** Gel-filtration elution profile for vesicles of Ic detected by scattering of u.v. light

film was then dispersed in doubly distilled water (10 ml) at 70°C by shaking the flask intermittently by hand. Dispersion was completed by mechanical shaking for 1 h and sonication for two periods of 3 min each using an MSE 150 W sonicator with a 1 mm titanium probe.

*[$^{14}$C]Sucrose entrapment*

A sample of the vesicle solution was mixed with a solution of [$^{14}$C]sucrose (1 $\mu$Ci) and sonicated in a bath. After standing for 24 h at room temperature the solution was chromatographed on a Sephadex G50 column as follows. A sample of solution (0.5 ml) was applied to a Sephadex G50 column (1 × 40 cm) at room temperature. Doubly distilled water was used as the eluent. The flow rate was adjusted to ~1 ml min$^{-1}$ and the void volume of the column was ~11 ml (determined previously by running a solution of Blue Dextran 2000). Each eluent fraction (1 ml) was radio-assayed by adding a scintillation cocktail (10 ml, see below) and counting the [$^{14}$C]sucrose $\beta$-radiation activity.

*Scintillation counting*

The scintillation cocktail was prepared as recommended by Nuclear Enterprises NE as follows. 2,5-Diphenyloxazole (3 g) and 1,4-bis(5-phenyloxazol-2-yl)benzene (0.1 g) were dissolved in toluene (666 ml) and then mixed with Triton X (333 ml). The mixture was shaken and left for 2 days before being used.

Each 1 ml chromatographic fraction was collected in a 20 ml screw-cap glass vial and the scintillation cocktail (10 ml) added. The vial was closed and thoroughly shaken by hand. The radioactivity was counted for three periods of 5 min each in a Packard Tricarb Liquid Scintillation Spectrometer 3255, and the counts per minute plotted against the chromatographic elution fraction (ml). *Figure 5* shows the results for compound Ib.

*Ultra-violet absorption detection of vesicles*

As a control experiment an attempt was made to detect vesicular structures via their ability to scatter (and apparently absorb) u.v. light. A vesicle solution of Ic (0.5 ml) prepared as described earlier but without incorporation of any [$^{14}$C]sucrose was applied to a Sephadex G50 column (1 × 40 cm, 11 ml void volume). The column was eluted at room temperature with doubly distilled water at a flow rate of 1 ml min$^{-1}$. The absorption of each fraction (1 ml) was measured at 290 nm using a Perkin–Elmer Double Beam Spectrophotometer 124, and the optical density data plotted against elution fracton (ml). *Figure 6* shows the results for compound Ic.

## DISCUSSION

*Syntheses*

Compounds Va and Vb were synthetized successfully in good yields (77% and 97% respectively) following the precedent set by our earlier syntheses[31]. The spectral and analytical data (*Table 1*) were consistent with the assigned structures. The tendency for Vb to form a lyotropic liquid-crystal phase spontaneously on shaking with water paralleled the behaviour of Ib[31].

*Polymerizations*

*Aqueous solutions.* Monomers Ib, IIb and Vb formed clear viscous solutions in water while IIIb and IVb formed more opaque solutions. This probably reflects the presence of the larger hydrophobic unit in IIIb and IVb arising from the $(CH_2)_{10}$ spacer between the cholesterol or dihydrocholesterol group and the quaternary ammonium head-group. Initial attempts to induce polymerization of these liquid-crystal phases thermally using azobisisobutyronitrile (AIBN) as the free-radical source were unsuccessful, and it is not clear why this was so. The AIBN appeared to be solubilized satisfactorily but it might be that the radicals were generated and dissipated in the hydrophobic domains of the lamellar structure. The absolute bulkiness of the monomer molecules might also play a negative role in this respect. Despite this, however, oligomerization was achieved readily by photoinitiation as described earlier, to yield saturated molecules, and this was confirmed by $^1$H n.m.r. spectra (*Figure 3*). The degrees of polymerization of the products were ~4–13. Such low values might be due to radical transfer reactions but are more likely to be associated with some steric restriction in the lamellar structure. It was interesting to observe that, as polymerization took place, the viscosity of each solution decreased rather than increased, before eventually phase separation occurred. Presumably during this process the vesicular structures (see later) composed of monomers are gradually converted into vesicles composed of oligomers, and this may result in some net contraction of the bilayer lamellae. This in turn might reduce inter-lamellar interactions and allow a fall in the bulk viscosity.

*Benzene solutions.* In benzene all the monomers (I–Vb) appeared to form isotropic solutions and were readily polymerized to reasonably high-molecular-weight species using thermal fragmentation of AIBN (*Table 1*). Confirmation of polymerization was obtained from $^1$H n.m.r. spectra (*Figure 4*). However, the maximum degree of polymerization observed was only ~180. It seems therefore that in benzene solutions the steric effect on the propagation reaction is not so severe but still exists. Towards the end of polymerization these solutions also displayed some cloudiness and, indeed, tended to gel. However, the basis of this gelling was not clear. Certainly it is not crosslinking because the products can be solubilized in appropriate solvents, but rather it is from weaker intermolecular association involving the polymer molecules.

*Vesicle formation*

Thin films of each amphiphile formed from chloroform solutions were dispersed in water to form vesicular structures as described earlier. The dispersion was carried out above the estimated gel–liquid crystal phase transition temperature to produce solutions with an appearance similar to that of aqueous solutions of lecithin liposomes. The procedure adopted is reported to produce vesicles with hydrodynamic radii in the range 200–800 Å and with a relatively small size distribution. Such species are also believed to trap within their volume a discrete aqueous compartment[34–36].

In the present work such vesicle formation has been substantiated by the [$^{14}$C]sucrose entrapment data generated from gel filtration. [$^{14}$C]sucrose was chosen for the study because it does not interact with cationic vesicles and can be used at low ionic strength. The latter is required to avoid aggregation of cationic vesicles[33]. The gel filtration elution profile for amphiphile Ib (*Figure 5*) shows that radioactive sucrose entrapped within the aqueous internal compartment of the vesicle is eluted in fractions 11–15 ml, while free sucrose appears in fractions, 20 ml upwards, corresponding to the volume of total penetration of the column. The recovery of the vesicles in fractions 11–15 ml suggests that they are almost totally excluded from the pore volume of the column packing, since the interstitial (void) volume of the column is 11 ml. This is consistent with the porosity characteristics of Sephadex G50.

The results for all the amphiphiles studied show remarkable agreement with the picture presented for amphiphile Ib. In each case [$^{14}$C]sucrose entrapped in vesicles is eluted in the range 11–15 ml. The exact elution peak maximum and the shape of the peaks vary a little but these differences are not significant. Clearly in all cases discrete vesicle-encapsulated sucrose solution has been achieved. Furthermore, since the peaks appear at the same elution volume, the size of vesicles produced is very similar in each case and the integrated activity of the sucrose encapsulated implies that the internal aqueous volumes of each are also very similar. Almost certainly the species are small unilamellar vesicles previously reported to be produced under similar circumstances[33].

Confirmation that the species eluted in fractions 11–15 ml are indeed small discrete particulates was obtained by detection of their scattering of u.v. light. The elution profile for species Ic detected by u.v. absorption (*Figure 6*) shows remarkable coincidence with that from the [$^{14}$C]sucrose entrapment experiment with Ic.

Integrating the background radioactivity in those fractions lying between the vesicle fraction and the fractions containing free sucrose shows this to be only ~7–16% of the sucrose activity found within the vesicles. This background is low and probably arises from imperfect performance of the gel filtration column. More importantly, however, the ratio of vesicle-trapped to untrapped sucrose from amphiphiles IIb, Ic and IVd (as examples) is 0.24, 0.23 and 0.24 respectively. Such low values tend to confirm that the vesicles formed are indeed of the small unilamellar type.

*Stability of vesicles*

For amphiphile IIc with the pyridinium ion head-group, more extended experiments were carried out to assess the stability of vesicles entrapping [$^{14}$C]sucrose, by carrying out successive gel filtration separations on a given vesicle-containing solution over a period of four weeks. The sample was prepared in the standard way and the gel filtration procedure was carried out as previously. *Figure 7* shows the elution profiles initially and after 1–4 weeks. Clearly vesicle-entrapped [$^{14}$C] sucrose remains present over the full period and these vesicles are consistently eluted in fractions 11–15 ml. Although it is difficult to quantify the results, there is some suggestion that the breadth of the vesicle profile does broaden with time and that the proportion of sucrose entrapped probably rises. This suggests that there might be some fusion of unilamellar species to form larger, possibly multilamellar entities. However, the changes are small and the stability of the vesicles is really

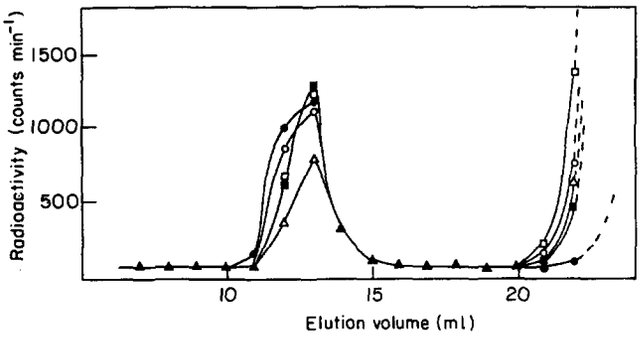



**Figure 7** Gel-filtration elution profiles for [$^{14}$C]sucrose entrapped in vesicles of IIc: (△) initially; (□) after 1 week; (○) after 2 weeks; (■) after 3 weeks; (●) after 4 weeks; (▲) data essentially conicidental. (Note that curves for 1 and 3 weeks between elution volumes ~10–15 ml are drawn as coinciding for simplicity)

rather remarkable. In the past a large amount of effort has gone into trying to produce polyvesicles in order to increase stability, and, indeed, polymerization of cholesterol-based amphiphiles does seem to achieve this[32]. However, the present results suggest that appropriate choice of a rigid hydrophobe and a head-group offers an alternative approach to achieving enhanced stability. Since incorporation of a single-chain cationic amphiphile is also reported to destroy these vesicular structures[37], a convenient release trigger is also retained.

## ACKNOWLEDGEMENT

We are grateful to Professor R. Marshall, University of Strathclyde, for the use of radiochemistry laboratory facilities.

## REFERENCES

1 Gregoriadis, G. (Ed.), 'Liposome Technology', CRC Press, Boca Raton, FL, 1984, Vols. I–III
2 Day, D., Hub, H. H. and Ringsdorf, H. *Isr. J. Chem.* 1979, **18**, 325
3 Kunitake, T. and Yamada, S. *Polym. Bull.* 1978, 1, 35
4 Iwamoto, K. and Sunamoto, J. *J. Biochem. (Tokyo)* 1982, **91**, 975
5 Regen, S. L., Czech, B. and Singh, A. *J. Am. Chem. Soc.* 1980, **102**, 6638
6 Johnson, D. S., Sanghera, S., Pons, M. and Chapman, D. *Biochim. Biophys. Acta* 1980, **602**, 57C
7 Hub, H. H., Hupfer, B., Koch, J. and Ringsdorf, H. *Angew. Chem., Int. Edn. Engl.* 1980, **19**, 938
8 O'Brien, D. F., Whiteside, T. H. and Klingbeil, R. T. *J. Polym. Sci., Polym. Lett.* 1981, **19**, 95
9 Lopez, E., O'Brien, D. F. and Whiteside, T. H. *J. Am. Chem. Soc.* 1982, **104**, 305
10 Gros, L., Ringsdorf, H. and Schupp, H. *Angew. Chem., Int. Edn. Engl.* 1981, **20**, 305
11 Hupfer, B., Ringsdorf, H. and Schupp, H. *Makromol. Chem.* 1981, **182**, 247
12 Akimoto, A., Dorn, K., Ringsdorf, H. and Schupp, H. *Angew. Chem., Int. Edn. Engl.* 1981, **20**, 90
13 Regen, L., Singh, A., Oehme, G. and Singh, M. *J. Am. Chem. Soc.* 1982, **104**, 791
14 Reed, W. F. and Guterman, L. R. *J. Radn. Coat.* 1986, April, 19 (*Chem. Abstr.* 1986, **105**, 24637a)
15 Ringsdorf, H., Schlarb, B. and Venzmer, J. *Angew. Chem., Int. Edn. Engl.* 1988, **27**, 113
16 Tundo, P., Kippenberger, D. J., Politi, M. J., Klahn, P. and Fendler, J. H. *J. Am. Chem. Soc.* 1982, **104**, 5352
17 Kurihara, K., Fendler, J. H., Ravet, I. and Nagy, J. B. *J. Mol. Catal.* 1986, **34**, 325
18 Murakami, Y., Kikuchi, J., Akiyoshi, K. and Imori, T. *J. Chem. Soc., Perkins Trans. 2* 1985, **12**, 1919
19 van Esch, J., Roks, M. F. M. and Nolte, R. J. M. *J. Am. Chem. Soc.* 1986, **108**, 6093
20 Nakai, S., Nakaya, T. and Imoto, M. *Makromol. Chem.* 1977, **178**, 2963
21 Nakai, S., Nakaya, T. and Imoto, M. *Makromol. Chem.* 1978, **179**, 2349
22 Umeda, T., Nakaya, T. and Imoto, M. *Makromol. Chem., Rapid Commun.* 1982, **3**, 457
23 Fairley, J. L. and Kilgour, G. L. 'Essentials of Biochemistry', Reinhold, New York, 1966, 2nd Edn., Ch. 4
24 Lehninger, A. L. 'Biochemistry', Worth, New York, 2nd Edn., 1975, Ch. 11
25 Mezer, D. E. 'Biochemistry, The Chemical Reactions of Living Cells', Academic Press, New York, 1977, Ch. 5
26 Toth, W. J. and Tobolsky, A. V. *J. Polym. Sci. (B)* 1970, **8**, 289
27 Tanaka, Y., Kabaya, S., Shimura, Y., Okada, A., Kurihara, Y. and Sakakibara, Y. *J. Polym. Sci. (B)* 1972, **10**, 261
28 Minezaki, S., Nakaya, J. and Imoto, M. *Makromol. Chem.* 1974, **175**, 3017
29 Leube, H. and Finkelmann, H. *Polym. Bull.* 1988, **20**, 53
30 Yasuzawa, M., Nakaya, T. and Imoto, M. *Makromol. Chem., Rapid Commun.* 1985, **6**, 721
31 Abid, S. K. and Sherrington, D. C. *Polym. Commun.* 1987, **28**, 16
32 Cho, I. and Chung, K. C. *Macromolecules* 1984, **17**, 2937
33 Dorn, K., Klingbiel, R. J., Specht, D. P., Thminski, P. N., Ringsdorf, H. R. and O'Brien, D. F. *J. Am. Chem. Soc.* 1984, **106**, 1627
34 Fendler, J. H. *Acc. Chem. Res.* 1980, **13**, 7
35 Lim, Y. Y. and Fendler, J. H. *J. Am. Chem. Soc.* 1979, **101**, 4023
36 Kano, K., Romero, A., Diermouni, B., Ache, H. J. and Fendler, J. H. *J. Am. Chem. Soc.* 1979, **101**, 4030
37 Cho, I. and Kim, G. C. *J. Mol. Catal.* 1988, **49**, L7