Phosphatidylcholine/cholesterol liposomes as vehicles for anthraquinone disperse dyes in wool dyeing

A de la Maza, J L Parra, L Coderch and P Bosch

Centro de Investigación y Desarrollo, Consejo Superior de Investigaciones Científicas, Calle Jorge Girona 18-26, 08034 Barcelona, Spain

The use of liposomes containing increasing amounts of cholesterol as carriers for anthraquinone disperse dyes in the dyeing of wool fibres is described. Multilamellar lipid vesicles of egg phosphatidylcholine and containing CI Disperse Violet 1 at different phosphatidylcholine-cholesterol relative concentrations were used. Controlled exhaustion of dye on the wool was achieved, which was directly dependent on the liposome lipid concentration. Increasing the amount of cholesterol in the bilayers resulted in a decrease in dyebath exhaustion while improving the total amount of dye bonded to the wool fibres. The dye's dispersion efficiency was also improved, compared with the use of conventional dispersing agents. The optimum application of these systems, both in terms of dyebath exhaustion and total amount of dye bonded to the wool samples, was directly correlated with the dye/lipid weight ratio for the maximum level of dye encapsulation efficiency.

INTRODUCTION

Over the last decade several investigations have been carried out into ways of reducing the degradation of wool fibres during conventional dyeing. The technology of microencapsulation has been one method adopted, in which the targeted slow release of protective agents can be introduced into the dyeing process [1]. Nevertheless, some technological difficulties related to the staining of wool with disperse dyes at high temperatures still exist, especially in the dyeing of wool/polyester blends [2]. Selecting appropriate disperse dyes and using suitable carriers are considered to be very important factors in preventing this problem [3,4].

Merino wool fibres naturally contain 1% by weight of lipids, cholesterol (CH) being one of the main components [5-7]. These lipids form the hydrophobic barrier of the cell membrane complex (CMC). Their structure is similar to that of the two lipid bilayers found in membranes of the keratinised stratum corneum of skin, which are capable of forming multiple bilayer structures [8]. The dyeing and diffusion properties of fibres are believed to be governed by the lipid structure of the intercellular spaces that might act as 'solvents' for hydrophobic textile chemicals [9]. There is also evidence that dyes do, in fact, preferentially diffuse along easily swollen regions such as the CMC (intercellular diffusion) rather than through the cuticle cells (transcellular diffusion) [10].

The possible application of liposomes as carriers in wool finishing is based on three main factors:

- (a) The similarity between the bilayer structure of the CMC and that of the liposomes
- (b) The important role played by the CMC in the processing of chemicals into the fibres
- (c) The role of the hydrophobic interactions in the structural organisation of wool.

Thus liposomes made with pure phosphatidylcholine (PC) and containing lipids present in the CMC such as cholesterol have been used as vehicles for aqueous chlorine solutions in wool chlorination processes [11]. These applications result in an improvement in the homogeneity of these oxidative treatments, minimising wool degradation and enhancing the efficiency of subsequent treatments. Along these lines, the use of liposomes as carriers of commercially available milling acid dyes and disperse dyes in wool dyeing has been reported [12,13].

In the present work we studied the effects of including CH in lipid bilayers to obtain improved application in wool dyeing with disperse dyes. The physical stability of multilamellar liposomes (MLV) containing the anthraquinone dye CI Disperse Violet 1 at different PC:CH molar ratios was investigated, the dye concentration remaining constant. The application of these structures in dyeing untreated wool samples has also been examined, focusing on the kinetic aspects of dye adsorption and the dye-fibre bonding forces on wool fibres.

EXPERIMENTAL

Materials

Botany wool fabrics knitted from R64/2 tex (count 2128) yarns were used. Samples were Soxhlet extracted for 2 h with methylene chloride and rinsed with water purified by the Milli-Ro system (Millipore) and dried at room temperature.

Phosphatidylcholine was purified from egg lecithin (Merck) according to the method of Singleton [14], and shown to be pure by thin layer chromatography (TLC). Cholesterol was purchased from Sigma Chemical Co. (St. Louis, USA). Lipids were stored in chloroform under nitrogen at -20°C until used.

The disperse dye Oracethblau 2R, (Merck, CI Disperse Violet 1) was used; its chemical structure is given in Figure 1. This dye was selected as being a typical anthraquinone disperse dye, sparingly soluble in water (0.3 mg/l at 25°C) and needing a high concentration of surface-active agent to be dispersed (17 mg/l in 1% sodium oleyl-p-aniside sulphate solution (Lissapol LS, ICI)) [15].

Figure 1 Chemical structure of the CI Disperse Violet 1

The nonionic surfactant Triton X-100 (octylphenol with ten units of ethylene oxide, 100% active matter) was specially prepared by Tenneco SA (Barcelona, Spain). Polycarbonate membranes of 400 and 800 nm, and membrane holders used for liposome extrusion, were purchased from Nucleopore (Pleasanton, USA).

Preparation of MLV liposomes

Multilamellar vesicles of a defined size (400 nm), and different lipid concentrations (1.25–3.0 mmol/l), containing different concentrations of CH (PC:CH molar ratios from 19:1 to 4:1) were prepared following a method described by Bangham [16]. The dye concentration was held constant at 1.0 mmol/l.

A film was formed by removing the organic solvent from a solution of egg PC/CH/dye in chloroform/methanol (2:1) by rotary evaporation in a nitrogen atmosphere and low vacuum 47 kPa (350 mm Hg). An aqueous phase containing 5% sodium sulphate and acetic acid (pH 5.5) was added to the film formed. The solutions were then swirled to remove the lipid from the walls of the flask and to disperse large lipid/dye aggregates; glass beads were added to facilitate dispersion. The resulting milky suspension were vortexed for 5 min and sonicated for 15 min at 30°C and 75 W (Labsonic 1510 B, Braun). Liposome suspensions were extruded through 800 and 400 nm polycarbonate membranes to obtain a uniform size distribution [17]. After preparation the resulting liposome suspensions were left to equilibrate for 15 min and immediately used in wool dyeing.

Dyeing procedure

Knitted wool samples were heated in freshly prepared MLV liposome suspensions under the conditions mentioned above. The dye was applied at a liquor ratio of 60:1 along with 5% o.w.f. anhydrous sodium sulphate; acetic acid was added to adjust the pH to 5.5. Dyeing in a Multi-Mat (Renigal) dyeing machine was started at 50°C and the temperature was raised by 0.9 degC/min to 90°C and dyeing continued for 120 min. Samples were then rinsed with water for 10 min and dried at room temperature.

Dye bath exhaustion was determined using a Shimadzu UV-265FW spectrophotometer. Liposome aliquots (0.5 ml) were periodically added to quartz cuvettes filled with 2 ml aqueous solution of Triton X-100 (20 g/l), supplemented with sodium sulphate (5%). The interaction between the nonionic surfactant Triton X-100 and liposome structures resulted in solubilisation of the lipid vesicles via the formation of mixed micelles [18,19], turning the liposome suspension into a clear solution.

Figure 2 shows the effect of the cleavage of liposome vesicles by Triton X-100 on the absorption spectra of the dye at different PC:CH molar ratios. It can be seen that the $\lambda_{\max}$ value of the dye used in this study did not change in the presence of increasing amounts of CH in the lipid/surfactant mixed micelles.

Vesicle size distribution, dispersion efficiency and aggregation

Mean vesicle size and the polydispersity of the liposome preparations were determined by a Photon correlator spectrometer (Malvern Autosizer 4700c PS/MV). Vesicle size distribution was studied by particle number measurement. Samples were adjusted to the appropriate concentration range. The measurements were made at 25°C with a detection angle of 90°.

The maximum amounts of dye dispersed with the MLV liposomes was defined as the weight ratio between the dispersed dye and the lipid in the liposomes, K , and was determined spectrophotometrically. After preparation, liposome suspensions were left to equilibrate for 12 h. Vesicle suspensions were then spun at 5000 rev./min for 15 min at 25°C in order to remove the unencapsulated dye. Finally the concentration of dispersed dye was evaluated by spectrophotometry after the destruction of the supernatant lipid bilayers by adding Triton X-100 [18,19].

The aggregation state of the vesicles was estimated to provide a measure of the physical stability of the liposome suspensions. This was done by monitoring the variation in vesicle size distribution of the liposome suspensions as a function of time.

Figure 2 Absorption spectra of CI Disperse Violet 1 in the presence of lipid/Triton X-100 mixed micelles at different bilayer lipid compositions

Bilayer lipid composition

The lipid composition of different liposome vesicles studied was determined using an Iatroscan MK-5 TLC-FID analyser. Coupling TLC to an automated detection system based on flame ionisation detection is a recent innovation which has considerably improved the sensitivity of TLC and allows quantitative estimation of separated materials. This method has been used to quantify many kinds of lipids from different sources [20]. This procedure was chosen in the present study to quantify lipid mixtures, even when they were forming liposomes in aqueous solutions, on the basis of previous experience of lipid analysis of keratinised tissues such as wool [21,22].

Dye extraction

The dye superficially bonded to the fibres by non-polar forces (hydrophobic interactions, van der Waals forces and hydrogen bonds) was extracted with pure ethanol for 60 min at 25°C [12]. Subsequent extractions with ammonia solution (0.5% for 15 min at 60°C) stripped dye that had diffused into the fibre and bonded ionically [15].

RESULTS AND DISCUSSION

Dispersion efficiency of liposomes

The variation in the total amount of dye dispersed in the presence of MLV liposomes at different bilayer compositions as a function of bilayer lipid concentration is indicated in Figure 3. A linear dependence was established in all cases. The slope of the straight lines obtained represents the weight ratio between dye and lipid, K . The K values for each bilayer composition and the regression coefficients for each straight line are given in Table 1. The K values decreased as the CH concentration in the bilayers increased, reaching a minimum value at the 4:1 PC:CH molar ratio (K 0.18). The use of PC:CH liposomes always resulted in a large increase in dye dispersion efficiency, compared with that of the conventional dispersing agent normally used for this dye [13,15]. Thus the optimum application of these systems (PC:CH 19:1 molar ratio) reduced the lipid concentration needed to disperse the same amount of dye by more than 140 times. At PC:CH molar ratios of 9:1 and 4:1 this reduction was about 117 and 105 times respectively. In practical dyeing this improvement could be reflected in lower process costs and lower dye concentrations.

Stability of liposome suspensions

The possible aggregation of liposomes during dyeing was monitored by measuring the variations in the mean vesicle size and polydispersity indices of these suspensions using a quasi-elastic light scattering method [23]. The results obtained for liposome suspensions (lipid concentration 20 mmol/l) at different bilayer compositions and using the K ratios for the maximum encapsulation efficiency of each system (Table 1) are given in Figure 4.

There was a small decrease in the mean particle size

during the initial stage of dyeing, with a marked increase in polydispersity index. After 45 min both parameters increased progressively, reaching values of about 425–438 nm and 0.13–0.15 respectively after treatment. Increasing

Figure 3 Maximum amounts of dispersed dye as a function of lipid concentration for three levels of CH in bilayers

Table 1 Weight dyelipid ratios (K) and regression coefficients of straight lines corresponding to the maximum dispersion efficiency of MLV liposomes

PC:CH molar ratio	K	r^2
19:1	0.24	0.994
9:1	0.20	0.993
4:1	0.18	0.992

Figure 4 Mean vesicle size and polydispersity of MLV liposomes at different lipid compositions and 2 mmol/l total lipid concentration during the dyeing process (for key see Figure 3)

amounts of CH in the bilayers enhanced the stability of these structures with respect to aggregation, reducing the variations in both the mean particle size and the polydispersity index during dyeing. This behaviour is in agreement with the results reported by Scherphof et al. in studies on liposome stability [24]. Furthermore, the mean vesicle size was maintained at around 400 nm and the polydispersity index below 0.24 for more than 24 h after preparation.

Dyeing kinetics

Kinetic studies of dyebath exhaustion on untreated wool samples in the presence of MLV liposomes were carried out, varying both the bilayer composition and the total lipid concentration, at constant dye concentration. The results obtained for the PC:CH molar ratio 19:1 are plotted in Figure 5. The exhaustion increased as the bilayer lipid concentration rose, reaching a maximum for 1.75 mmol/l. Above this concentration, increasing amounts of lipids in bilayers retarded the rate of dyeing (minimum exhaustion for 3.0 mmol was 69.1%). A similar pattern in dyeing rates was observed at all the PC:CH molar ratios studied, although the lipid concentration needed to achieve the maximum exhaustion rose as the CH concentration in the bilayers increased. Thus, whereas the liposomes formed at the PC:CH molar ratio of 9:1 gave a maximum exhaustion at 2.25 mmol/l (79.2%), those formed by 4:1 needed 2.50 mmol/l lipid concentration to achieve this maximum (73.5%) (results not plotted). It is interesting to note that the weight ratio *K* for the maximum exhaustion was always similar to that for the maximum encapsulation efficiency.

Figure 6 shows the exhaustion of various dye/liposome systems on wool samples at different lipid bilayer compositions, in all cases using the lipid concentration to achieve maximum dyebath exhaustion. It is noteworthy that increasing amounts of CH in bilayers resulted in a decrease in exhaustion on wool throughout the dyeing process. This effect was considered an important factor in

Figure 5 Exhaustion rates of CI Disperse Violet 1 on untreated wool samples during dyeing in the presence of MLV liposomes at different bilayer lipid concentrations

Figure 6 Exhaustion rates of CI Disperse Violet 1 on untreated wool samples during dyeing in the presence of MLV liposomes at different PC:CH molar ratios (for key see Figure 3)

controlling the dyeing kinetics and improving the regularity of dye distribution in the wool fibres. Optical microscope observations of dyed samples provided evidence of a regular dye distribution throughout the fibres, this improvement being especially marked for the lipid concentrations corresponding to the maximum exhaustion. Furthermore, increasing amounts of CH in bilayers also resulted in an enhancement in dye distribution.

Influence of lipid bilayer composition on dye bonding
in order to determine whether, as dye carriers, the lipid bilayers (containing increasing amounts of CH) caused changes to dye-fibre bonding forces after dyeing, dye was extracted from the samples using pure ethanol [12] and ammonia [15]. The results obtained for different concentrations of CH in the liposomes are given in Tables 2-4. In general ethanol extracted larger amounts of dye than did the ammonia solutions. This was because of the high solubility of the dye in ethanol and the presence of dye superficially bonded to the fibres by non-polar forces. However, the fact that very small amounts of dye were extracted via ammonia could be attributed to the important contribution of the non-polar forces in the dye-fibre bonding. These interactions, especially those that are hydrophobic in nature, may play an important role in dye-fibre bonding inside the fibre. Furthermore, as the lipid bilayer concentration increased, the amounts of dye extracted from the samples using ethanol decreased. The amounts of extracted dye were, for each lipid concentration, inversely proportional to the CH concentration present in the bilayers. Ammonia extractions showed very low values in all cases.

Tables 2-4 also show the total amount of bonded dye on the wool samples, as determined by Eqn 1:

$$C_b = \frac{C_a - C_e}{C_a} \times 100 \quad (1)$$

where C_b is the relative amount of bonded dye (%), C_a is the

amount of absorbed dye (mg per g wool) and C_e is the total amount of extracted dye (mg per g wool).

In general, the higher the lipid concentration and the relative concentration of CH in the bilayers, the higher the amount of bonded dye. An exception was observed at the 19:1 PC:CH molar ratio, where the maximum amount of bonded dye was obtained at the K ratio corresponding to the maximum exhaustion.

Figure 7 was obtained by plotting the amounts of bonded dye on the wool samples (expressed as differences between the amounts of adsorbed dye and total dye extracted) versus lipid concentration for three levels of CH in the bilayers. The maximum amount of bonded dye (points A, B and C) occurred at K ratios corresponding to the maximum levels of both the dye bath exhaustion and

Table 2 Amounts of dye adsorbed by, extracted from and bonded to wool after dyeing in the presence of MLV liposomes at different lipid concentrations and a PC:CH molar ratio of 19:1

Dye concn (mg per g wooi)					
Lipid concn (mmol/l)	Adsorbed	Extracted ^a		Amount of dye bonded (%)	TM 193
		A	B		
1.25	14.6	5.18	0.018	64.3	2
1.50	15.8	5.18	0.016	67.2	2-3
1.75	16.6	4.74	0.014	71.4	3-4
2.00	16.2	4.95	0.010	69.4	4
2.25	15.5	4.80	0.011	68.9	3-4
2.50	14.6	4.54	0.009	68.9	3-4
2.75	13.8	4.33	0.009	68.6	3-4
3.00	13.0	4.14	0.009	68.1	3-4

a A Extracted by ethanol [12]
B Extracted by ammonia [15]

Table 3 Amounts of dye adsorbed by, extracted from and bonded to wool after dyeing in the presence of MLV liposomes at different lipid concentrations and a PC:CH molar ratio of 9:1

Dye concn (mg per g wool)					
Lipid concn (mmol/l)	Adsorbed	Extracted ^a		Amount of dye bonded (%)	TM 193
		A	B		
1.25	12.5	4.23	0.014	66.2	2-3
1.50	13.1	3.67	0.013	71.9	3-4
1.75	13.8	3.27	0.013	76.2	3-4
2.00	14.4	2.75	0.012	80.8	4
2.25	14.9	2.53	0.011	83.0	4 ^b
2.50	13.5	1.83	0.009	86.4	4 ^b
2.75	12.3	1.16	0.008	90.5	4 ^b
3.0	11.2	0.75	0.006	93.2	4 ^b

a For key see Table 2
b Or higher

Table 4 Amounts of dye adsorbed by, extracted from and bonded to wool after dyeing in the presence of MLV liposomes at different lipid concentrations and a PC:CH molar ratio of 4:1

Lipid concn (mmol/l)	Dye concn (mg per g wooi)				Amount of dye bonded (%)	TM 193
	Adsorbed	Extracted ^a				
		A	B			
1.25	10.4	2.83	0.010	72.8	3-4	
1.50	11.0	2.26	0.009	79.5	3-4	
1.75	11.7	1.76	0.008	84.9	4	
2.00	12.3	1.15	0.008	90.6	4 ^b	
2.25	12.9	0.581	0.007	95.4	4 ^b	
2.50	13.9	0.396	0.005	97.1	4 ^b	
2.75	13.2	0.364	0.003	97.2	4 ^b	
3.0	12.3	0.337	0.003	97.2	4 ^b	

a For key see Table 2
b Or higher

Figure 7 Amounts of bonded dye in wool fibres versus lipid concentration in liposomes for different levels of CH in bilayers (for key see Figure 3 and text)

encapsulation efficiency of each system (1.75, 2.25 and 2.50 mmol/l for the PC:CH molar ratios 19:1, 9:1 and 4:1 respectively). It was interesting to note that the greatest amounts of bonded dye were obtained at the maximum CH concentration in the bilayers. This finding emphasises the role played by the CH in improving the dye-fibre bonding forces and justifies the incorporation of this component in the liposomes to obtain improved application in wool dyeing.

The wash fastness of dyed samples in the presence of liposomes was determined using the IWS TM 193 test method (corresponding to ISO 105:C06 (1978) and UNE 40-120-81). The samples having the higher concentrations of bonded dye (Figure 7) also showed better levels of fastness (4 or higher). Thus, in general, the higher the amount of CH in the bilayers, the better the colour fastness of the dyed samples. The samples that showed the best wash fastness results also attained a good level of xenon arc light fastness (6 or higher) (ISO/R 105 (V) Part 2a, corresponding to UNE

40-187-73). The colour fastness results for the dyed samples are given in Tables 24.

Two principal factors seem to explain the important role played by CH in the exhaustion of dyes onto wool and the subsequent bonding into the keratin structures:

- (a) The effect caused by CH in reducing the permeability of liposomes and enhancing their stability to aggregation [24]
- (b) The presence of CH in the hydrophobic barrier of the CMC (structurally similar to the two lipid bilayers similar found in liposomes), which governs the diffusion of dyes into the fibre.

The first factor could be correlated with the decrease in dyebath exhaustion caused by CH present in the bilayers, and the second factor is related to the improvement in the dye bonding forces in the dyeing process.

CONCLUSIONS

The use of phosphatidylcholine-cholesterol multilamellar liposomes as vehicles for the anthraquinone dye CI Disperse Violet I in wool dyeing can be useful for:

- (a) Controlling dye exhaustion on untreated wool samples
- (b) Improving the dye-fibre bonding forces
- (c) Improving dye distribution on the wool fibres.

The dispersion efficiency of MLV liposomes with respect to this dye is inversely proportional to the concentration of CH in the bilayers, the minimum being obtained for the PC:CH molar ratio 4:1 (weight ratio dye/lipid $K = 0.18$) and the maximum for the PC:CH molar ratio 19:1 ($K = 0.24$) in the present work. However, the use of PC:CH MLV liposomes resulted in a clear improvement in the dye dispersion efficiency compared with conventional dispersin.

All the liposomes studied were stable during the dyeing process at pH 5.5. The amounts of dye exhausted and dye bonded to untreated wool samples for each PC:CH dye/liposome system were directly proportional to K , corresponding to the maximum encapsulation efficiency of the system. Increasing the amount of CH in the bilayers

resulted in a decrease in dyebath exhaustion on untreated wool samples and an increase in the total amount of dye bonded. The samples with higher amounts of bonded dye also gave better wash fastness results, and these samples also showed good light fastness performance.

* * *

This work was supported by funds from Dirección General de Investigación Científica y Técnica (DGICYT), Spain, Proyecto No. PB91-0065). The authors acknowledge the expert technical assistance of Mr G Knorr.

REFERENCES

1. G Nelson, *Rev. Prog. Col.*, **21** (1991) 72.
2. J Wang and H Asnes, *J.S.D.C.*, **107** (1991) 274.
3. J Wang and H Asnes, *J.S.D.C.*, **107** (1991) 314.
4. P R Brady, *Rev. Prog. Col.*, **22** (1992) 58.
5. L Coderch and C Soriano, *Tex. Res. J.*, **63** (1993) 369.
6. J Hemling and H Zahn, *Proc. 7th int. Wool Text. Res. Conf., Tokyo*, Vol. 1 (1985) 181.
7. D E Rivett, *Wool Sci. Rev.*, **67** (1991) 1.
8. D T Downing, *J. Lipid Res.*, **33** (1992) 301.
9. J D Leeder, *Wool Sci. Rev.*, **63** (1986) 3.
10. J D Leeder et al., *Proc. 7th Int. Wool Text. Res. Conf., Tokyo*, Vol. 5 (1985) 99.
11. A de la Maza and J L P m, *Text. Res. J.*, **63** (1993) 44.
12. A de la Maza et al., *J.S.D.C.*, **108** (1992) 540.
13. A de la Maza and J L Parra, *J.S.D.C.*, **110** (1994) 64.
14. W S Singleton et al., *J. Am. Oil Chem. Soc.*, **42** (1965) 53.
15. E R Trotman, *Dyeing and chemical technology of textile fibres*, 6th Edn (High Wycombe, UK: Charles Griffin, 1984) chapter 12.
16. A D Bangham, M M Standish and J C Watking, *J. Mol. Biol.*, **13** (1965) 238.
17. F Szoka et al., *Biochim. Biophys. Acta*, **601** (1980) 559.
18. A Helenius and K Simons, *Biochim. Biophys. Acta*, **415** (1975) 29.
19. D Lichtenberg, J Robson and E A Dennis, *Biochim. Biophys. Acta*, **737** (1983) 285.
20. R G Ackman, C A McLead and A K Banerjee, *J. Planar Chromatog.*, **3** (1990) 450.
21. L Coderch and C Soriano, *Text. Res. J.*, **63** (1993) 369.
22. L Coderch et al., *Text. Res. J.*, **62** (1992) 704.
23. C S Chong and K Colbow, *Biochim. Biophys. Acta*, **426** (1976) 260.
24. G L Scherphof, J Damen and J Wilschut in *Liposome technology*, Vol. 3, Ed. G Gregoriadis (Boca Raton, FL: CRC Press, 1986) chapter 14.