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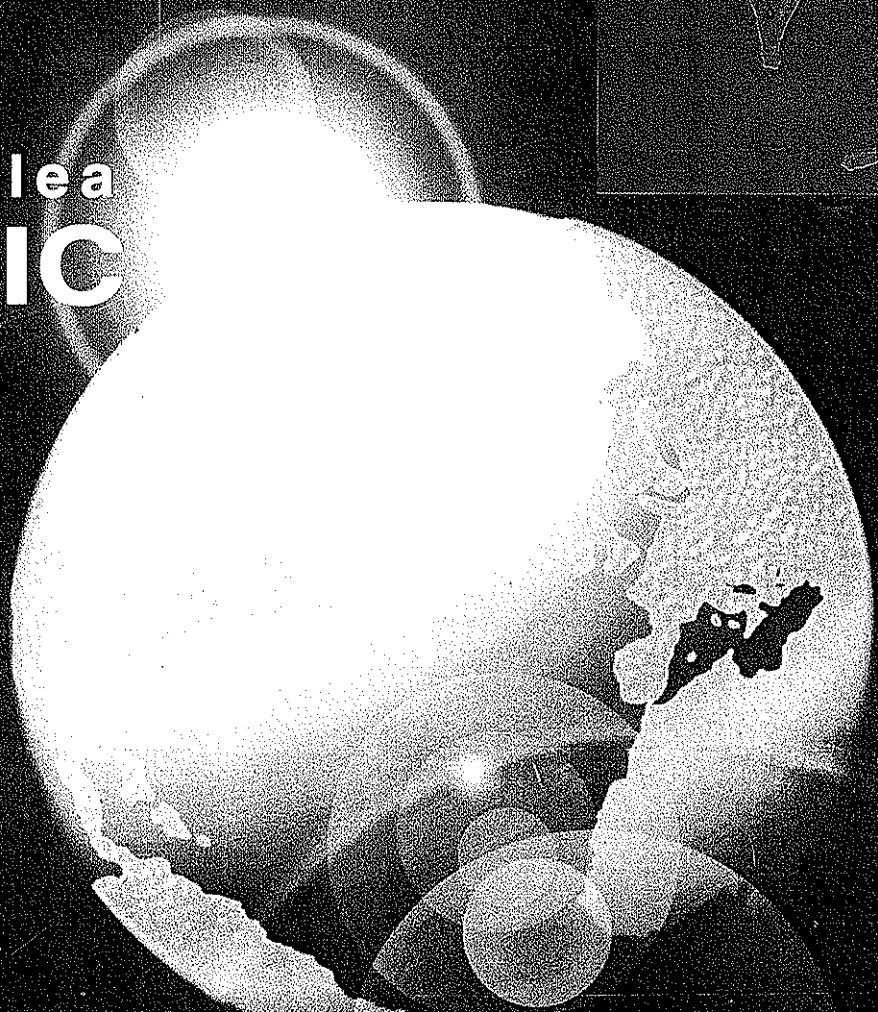

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Phosphatidylcholine/cholesterol liposomes as vehicles for anthraquinone disperse dyes in wool dyeing

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Summary

Studies on the use of liposomes of defined size (400nm) containing increasing amounts of cholesterol (CH) as carriers of anthraquinone disperse dyes to wool fibres are described. We investigated multilamellar lipid vesicles (MLV) made up egg phosphatidylcholine (PC) and containing the anthraquinonic disperse dye Oracetblau 2R, (C.I. Disperse Violet 1) at different PC:CH relative concentrations. We assessed physical stability by measuring the mean vesicle size of liposome suspensions after preparation and during the dyeing process. Kinetic aspects involving dye adsorption and bonding on untreated wool samples by means of multilamellar vesicles at different PC:CH molar ratios were also investigated.
This process led to the controlled exhaustion of dye in wool samples, which was directly dependent on the liposome lipid concentration. Increasing amounts of CH in bilayers resulted in a decrease in the dye exhaustion although improving the total amounts of the dye bonded to the wool fibres. This potential application also improved the dispersing efficiency of these systems with respect to the use of conventional dispersing agents. The optimum application of these systems both in the dye exhaustion and in the total amounts of bonded dye on untreated wool samples was directly correlated with the dye/lipid weight ratio for the maximum level of dye encapsulation efficiency.

Keywords: *Wool; multilamellar vesicles; phosfatidylcholine; cholesterol; liposomes as dispersing agents; anthraquinone disperse dyes; dye exhaustion kinetics; dye bonding.*

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Resumen "Vesículas multilamelares mixtas como agentes dispersantes de colorantes antraquinónicos en la tintura de la lana"

Se estudia la aplicación de liposomas de tamaño de vesícula definido (400 nm) conteniendo cantidades crecientes de colesterol (CH) como carriers de colorantes dispersos de tipo antraquinònico en la tintura de la lana. Se utilizaron vesículas multilamelares (MLV) constituidas por fosfatidilcolina de huevo (PC) y que contenían el colorante disperso de tipo antraquinónico Oracetblau 2R, (C.I. Disperse Violet 1) a diferentes concentraciones relativas PC:CH. Se estudió la estabilidad física de dichos liposomes midiendo el tamaño medio de las vesículas después de su preparación y durante el proceso de tintura. Se investigaron asimismo aspectos cinéticos relacionados con la adsorción y fijación de colorante sobre muestras de lana no pretratada utilizando vesículas multilamelares a diferentes relaciones molares PC:CH. Este proceso conduce a un agotamiento controlado del colorante sobre la lana, que está directamente relacionado con la concentración de fosfolípido en los liposomas. Cantidades crecientes de CH en las bicapas promueven una disminución en el agotamiento del colorante aunque mejoran la cantidad total de colorante fijado a las fibras de lana. Esta aplicación mejora asimismo la eficacia dispersante de estos sistemas con respecto al uso de agentes dispersantes convencionales. La aplicación óptima de estos sistemas tanto en el agotamiento de colorante como en la cantidad total de colorante fijado sobre lana no pretratada está directamente relacionada con la relación en peso de colorante/lípido correspondiente al nivel máximo de eficacia de encapsulación del sistema.

Palabras clave: *Lana; vesículas multilamelares; fosfatidilcolina; colesterol; liposomas como agentes dispersantes; colorantes dispersos antraquinónicos; cinéticas de agotamiento de colorantes; fijación de colorantes.*

Introduction

Over the last decade, a number of investigations have been carried out using diverse vehicles capable of reducing the degradative effect in wool fibres during conventional dyeing. Thus, the technology of microencapsulation has given rise to a number of innovations employing the basic principles of targeting, slow release and protection of this sensitive fibre (1). Nevertheless, some technological problems related to the staining of wool with disperse dyes at high temperatures still exist especially in the dyeing of wool/polyester blends (2). The selection of the appropriate disperse dyes and the use of suitable carriers are considered to be very important factors in preventing this effect (3,4).

Merino wool fibres contain about 1% by weight of lipids, the cholesterol (CH) being one of the main components (5-7). These lipids build the hydrophobic barrier of the Cell Membrane Complex (CMC) and are structured as the two lipid bilayers similar to those found in membranes of keratinised stratum corneum of the skin which are capable of forming multiple bilayer structures (8). Dyeing and diffusion properties of fibre, in particular, 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 (intercelullar 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: the similarity between the bilayer structuration of the CMC and that of the liposomes, the important role played by the CMC in the processing of chemicals into the fibres and the role of the hydrophobic interactions in the structural organization of wool. Thus, liposomes made with pure phosphatidylcholine 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 both the regularity and the homogeneity of these oxidative treatments minimizing wool degradation and enhancing the subsequent treatments in wool processing. 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 paper, we studied the effects of including CH in lipid bilayers to obtain improved applications in wool dyeing with disperse dyes. To this end, we describe work on the physical stability of multilamellar liposomes (MLV) containing the anthraquinone disperse dye Oracetblau 2R at different PC:CH molar ratios the dye concentration remaining constant. The application of these structures in dyeing of 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 2/28) yarns were used. Samples were Soxhlet extracted for 2 hours with methylene chloride and rinsed with water purified by the Milli-Ro system (Millipore) and dried at room temperature.

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

The disperse dye Oracetblau 2R, (Merck, C.I. 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-anisidide sulphonate solution (Lissapol LS)) (15).

The nonionic surfactant Triton X-100 (octylphenol with ten units of ethylene oxide and active matter of 100%) was specially prepared by Tenneco S.A. (Barcelona, Spain).

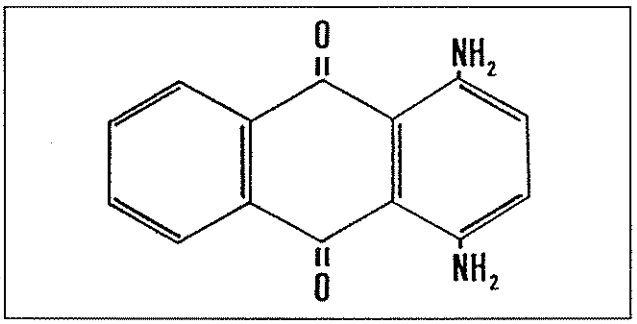

Figure 1. Chemical structure of the Oracetblau 2R dye (C.I. Disperse Violet 1).

Polycarbonate membranes of 400 nm and 800 nm, and membrane holders used for liposome extrusion were purchased from Nucleopore (Pleasanton, CA).

Preparation of MLV liposomes

Multilamellar vesicles of a defined size (400 nm), and different lipid concentrations (from 1.25 mmol/l to 3.0 mmol/l), containing diverse concentrations of CH (PC:CH molar ratios from 9.5:0.5 to 8.0:2.0), the dye concentration remaining constant (1.0 mmol/l), were prepared following a method described by Bangham (16).

ces and hydrogen bonds) was extracted with pure ethanol at 25°C for 60 min (12). Subsequent extractions with ammonia solution (0.5% at 60°C for 15 min) stripped the dye diffused inside the fibre and bonded ionically (15).

Results and discussion

Dispersion efficiency of liposomes

The variation of the total amounts of dispersed dye via MLV liposomes at different bilayer compositions (PC:CH ranging from 9.5:0.5 to 8.0:2.0 molar ratios), versus bilayer lipid concentration is indicated in Figure 3. A linear dependence can be established in all cases. The weight ratio dye/lipid (K) corresponded to the slope of the straight lines obtained. The K values obtained for each bilayer composition and the regression coefficients for each straight line are given in Table I. The K values decreased as the CH concentration in bilayers increased reaching the lowest value for 8.0:2.0 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 9.5:0.5 molar ratio) reduced by more than 140 times the lipid concentration needed to disperse the same amount of dye with respect to the use conventional dispersing agent. This proportion was of about 117 and 105 times for the PC:CH molar ratios 9.0:1.0 and 8.0:2.0 respectively.

The increase in the dye dispersion efficiency may results in the improvement in both the costs of the process (given the similar cost of the conventional dispersing agent and that of the commercially available lipids used) and the concentration of dye in the dyebath.

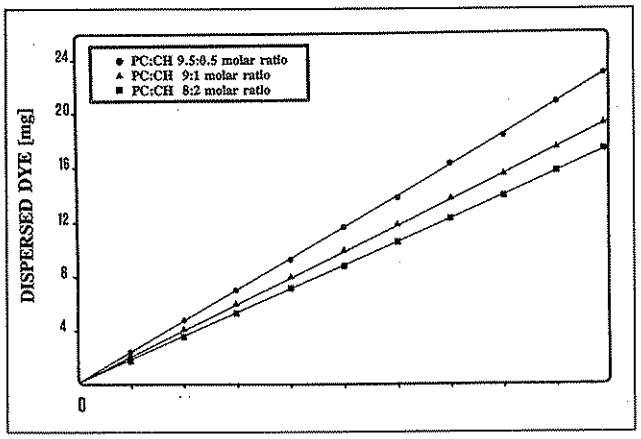

Figure 3. Maximum amounts of dispersed dye (mg) versus lipid concentration (mg) for three levels of CH in bilayers [PC:CH molar ratios 9.5:0.5 (●), 9.0:1.0 (▲), and 8.0:2.0 (■)].

Stability of liposome suspensions

The possible aggregation of liposomes during dyeing was monitored by measuring the variations in the mean vesicle size and polydispersity indexes of these suspensions using a quasi-elastic light scattering method (23). The results obtained for liposome suspensions (lipid concentration 2.0 mmol/l) at different bilayer compositions (PC:CH molar ratios from 9.5:0.5 to 8.0:2.0) and using the K ratios for the maximum encapsulation efficiency of each system (Table I) are given in Figure 4.

PC:CH molar ratio	Weight Ratio K	Regression Coefficients r^2
9.5:0.5	0.24	0.994
9.0:1.0	0.20	0.993
8.0:2.0	0.18	0.992

Table I. Weight dye/lipid ratios (K) corresponding to the maximum dispersion efficiency of MLV liposomes containing increasing concentrations of CH in bilayers. The regression coefficients of the straight lines are also given.

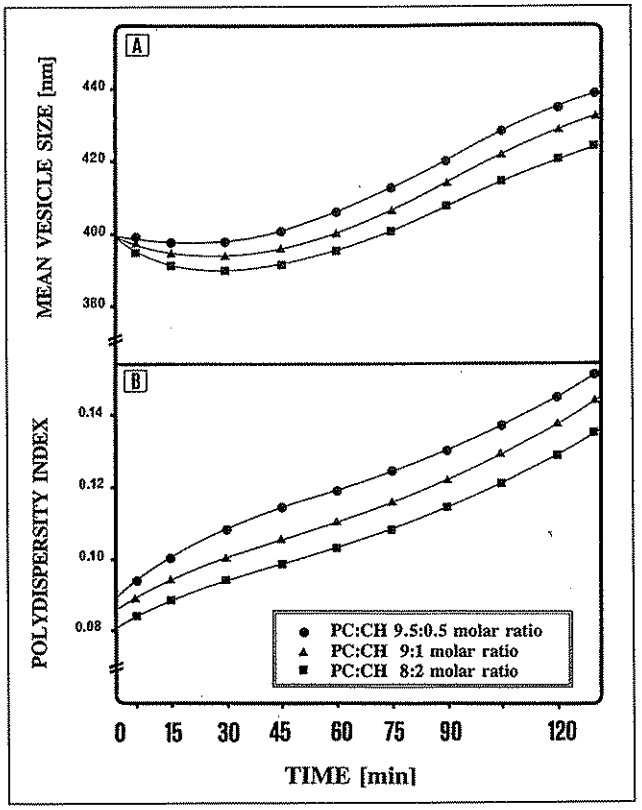

Figure 4. Mean vesicle size and polydispersity of MLV liposomes at different lipid compositions [PC:CH molar ratios 9.5:0.5 (●), 9.0:1.0 (▲), and 8.0:2.0 (■)] and total lipid concentration 2 mmol/l, during the dyeing process.

There was a small decrease in the particle size distribution in the initial stage of dyeing with a marked increase in the polydispersity indexes. After 45 min, both parameters increased progressively until reaching values of about 425-438 nm and 0.13-0.15 respectively after treatment. Increasing amounts of CH in bilayers enhanced the stability of these structures with respect to the aggregation, reducing the variations in both the mean particle size and the polydispersity indexes during dyeing. This behaviour is in

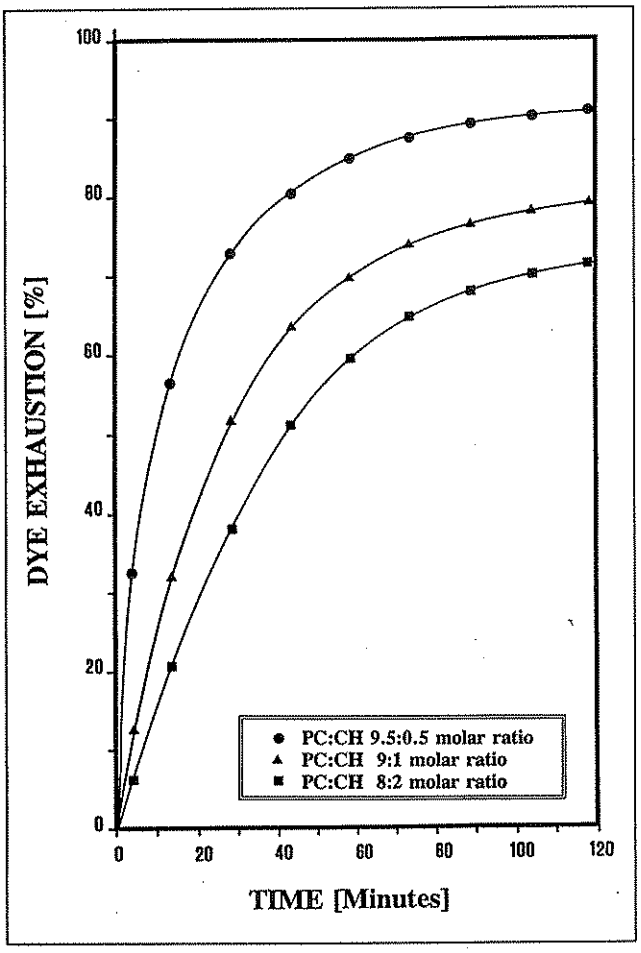

Figure 6. Exhaustion kinetics of Oracetblau 2R dye on untreated wool samples in dyeing via MLV liposomes at different PC:CH molar ratios [PC:CH molar ratios 9.5:0.5 (●), 9.0:1.0 (▲), and 8.0:2.0 (■)] using in all cases the lipid concentration for to the maximum dye exhaustion, the dye concentration remaining constant (1.0 mmol/l).

where C_b is the relative amount of bonded dye (%), C_a is the amount of absorbed dye (mg dye per g wool) and C_e is the total amount of extracted dye (mg dye per g wool).

In general, the higher the lipid concentration and the CH relative concentration in bilayers, the higher the percentages of bonded dye, except for the 9.5:0.5 PC:CH molar ratio, where the maximum percentage of bonded dye was obtained at the K ratio corresponding to the maximum dye exhaustion and ,in consequence, for the maximum encapsulation efficiency of the system.

Plotting the amounts of bonded dye on wool samples, given as a difference between the amounts of adsorbed dye and total dye extracted dye, versus lipid concentration for three levels of CH in bilayers graphs of Figure 7 are obtained. It may be seen that the curves showed the maximum amount of bonded dye (points a, b and c) for the K ratios corresponding the maximum levels of both the dye exhaustion and the encapsulation efficiency of each system (1.75, 2.25 and 2.50 mmol/l for the PC:CH molar ratios, 9.5:0.5, 9.0:1.0 and 8.0:2.0 respectively). It is interesting to note that the greater amounts of bonded dye were obtained for the maximum CH concentration in bilayers. This finding emphasizes the role played by the CH in improving the dye-fibre bonding forces and justify the incorporation of this component in liposomes to obtain improved applications in wool dyeing.

In order to determine the conventional colour fastness of dyed samples via liposomes the TM193 IWS Test (corresponding to the ISO 105: CO6 : 1978 and to the UNE 40-120-81) was carried out. It is interesting to note that the samples which showed the greater amounts of bonded dye (Figure 7) also showed the optimum level of wash fastness (> = 4). Thus, in

Lipid Conc	PC:CH molar ratio 9.5:0.5				
	Adsorbed dye	Extracted dye		Bonded dye	TM193 IWS
		A	B		
1.25	14.561	5.181	0.018	64.29	1-2
1.75	16.600	4.740	0.014	71.36	3-4
2.25	15.491	4.802	0.011	68.94	3-4
2.75	13.807	4.331	0.009	68.57	3-4

A mg dye/g wool fibre extracted by pure alcohol (12)
B mg dye/g wool fibre extracted by ammonia (15)

Table II. Amounts of adsorbed dye (mg dye/g wool), extracted dye (mg dye/g wool) and bonded dye (%) on wool samples after dyeing via MLV liposomes at different lipid concentrations and PC:CH molar ratios. The values of the colour fastness test TM 193 IWS (International Wool Secretariat) are also indicated (Tables II, III and IV correspond to the PC:CH molar ratios 9.5:0.5, 9.0:1.0 and 8.0:2.0 respectively).

Lipid Conc	PC:CH molar ratio 8.0:2.0 Adsorbed dye	Extracted dye		Bonded dye	TM193 IWS
			B		
1.25	10.412	2.825	0.010	72.77	3-4
			0.008		
1.75	11.687	1.756	0.008	84.90	4
			0.008		
2.25	12.905	0.581	0.007	95.44	>=4
			0.005		
2.75	13.190	0.364	0.003	97.21	>=4
			0.003		

A mg dye/g wool fibre extracted by pure alcohol (12)
B mg dye/g wool fibre extracted by ammonia (15)

Table IV. Amounts of adsorbed dye (mg dye/g wool), extracted dye (mg dye/g wool) and bonded dye (%) on wool samples after dyeing via MLV liposomes at different lipid concentrations and PC:CH molar ratios. The values of the colour fastness test TM 193 IWS (International Wool Secretariat) are also indicated (Tables II, III and IV correspond to the PC:CH molar ratios 9.5:0.5, 9.0:1.0 and 8.0:2.0 respectively).

ging from 1.25 mM to 3.0 mM, and for up to 24 hours following preparation.

The maximum percentages of dye exhaustion and the maximum amounts of bonded dye to untreated wool samples for each PC:CH dye/liposome system were correlated with the weight dye/lipid ratio (K) corresponding to the maximum encapsulation efficiency of the system. It is interesting to note that increasing amounts of CH in bilayers resulted in a decrease of the dye exhaustion on untreated wool samples although with an improvement in the total amounts of bonded dye.

The samples which showed the greater amounts of bonded dye also presented the optimum wash fastness level (> = 4) (TM193 IWS Test, corresponding to the ISO 105: CO6 : 1978 and to the UNE 40-120-81). These samples also attained a good level in the light fastness test (> = 6) (ISO/R, 105 (V), part 2ª, corresponding to the UNE 40-187-73).

The optimum application for these dye/liposome systems was reached for the maximum concentration of CH in bilayers and using the lipid concentration corresponding to the maximum dispersion efficiency of the system. As a consequence, the presence of CH in bilayers plays an important role in modulating the dyeing kinetics and improving the dye-fibre bonding forces during wool dyeing.

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