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MULTILAMELLAR LIPOSOMES INCLUDING CHOLESTEROL AS CARRIERS OF AZOBENZENE DISPERSE DYES IN WOOL DYEING

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SYNOPSIS

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

INTRODUCTION

Many amphiphilic compounds have been used in the dyeing process as dispersing agents of water-insoluble disperse dyes. However, few studies have been performed on the mechanisms of these interactions [1, 2] and on the application of these systems on keratinic structures [3, 4]. The application of the azobenzene disperse dyes has increased considerably with the appearance of the synthetic fibers, some of which, such as polyesters are much more hydrophobic than the first man-made fiber on which these dyes were applied (cèlulose acetate) and therefore very resistant to conventional water-soluble dyes [5].

The dyeing of wool is undoubtedly a fibre degradative process, since it is usually carried out in water at the boil for periods of 1-8 hours under a variety of pH conditions, ranging from acidic (pH 1.8) to neutral (pH 7). The damaging effect at low pH is mainly due to amide bond splitting, whereas above pH 3, the damage is increasingly associated with disulphide bond hydrolysis. If dyeing is performed on the alkaline side, the damaging process involves both disulphide and amide bond cleavage [6].

Over the last decade, a number of investigations have been carried out using different carriers capable of reducing the degradative effect brought about in

conventional wool dyeing. Thus, the technology of microencapsulation has given rise to a number of innovations utilizing the basic principles of targeting, slow release and protection of this sensitive fiber [7]. 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 [8, 9]. The selection of the disperse dyes with low intrinsic saturation values for wool, low aggregation properties, rapid dyeing rates and good exhaustion properties on polyester, and the use of suitable carriers are considered to be very important factors in preventing this effect [10].

Merino wool fibers contain about 1% by weight of lipids, the cholesterol (CH) being one of the main components [11-13]. 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 [14]. Dyeing and diffusion properties of fiber, in particular, are believed to be governed by the lipid structure of the intercellular spaces that might act as "solvents" for hydrophobic textile chemicals [15]. TEM studies also provide 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) [16].

The ability of phospholipids to solubilize small amphiphile molecules such as dyes and drugs plays a major role in pharmaceutical applications [17] and biological staining [18]. The potential use of liposomes as carriers in wool finishing is based on (i) the similarity existing between the bilayer structure of the CMC and that of the liposomes, (ii) the important role played by the CMC in the processing of chemicals into the fibers and (iii) the relevant importance of the hydrophobic interactions in the structural organization of wool. In this sense, liposomes made with pure phosphatidylcholine or containing lipids present in the CMC such as cholesterol, have been used as vehicles for aqueous chlorine solutions in wool chlorination processes [19, 20]. These applications result in an improvement in both the regularity and the homogeneity of these oxidative treatments minimizing wool degradation and facilitating the subsequent treatments in wool processing. Likewise, the use of liposomes as carriers in commercial dyeing of untreated wool using milling acid dyes and disperse dyes has been reported [21, 22].

In the present paper, given that CH is one of the main components of wool's internal lipids, we studied the effects of including this component in lipid bilayers to obtain improved applications in wool dyeing with azobenzene disperse dyes. To this end, we describe work on the physical stability of multilamellar liposomes containing the azoic disperse dye C.I. Disperse Orange 1 at different PC:CH molar ratios, the dye concentration remaining constant. The application of these structures in thedyeing of untreated wool samples has also been examined, focusing on the kinetic aspects of dye adsorption and the dye-fiber bonding forces on wool fibers.

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.The azobenzene disperse dye (C.I. Disperse Orange 1) was supplied by Sigma Chemical Co. (St. Louis MO) and shown to be pure by

thin-layer chromatography (TLC).Phosphatidylcholine was purified from egg lecithin (Merck, Darmstadt, Germany) according to the method of Singleton (23) and shown to be pure by thin-layer chromatography (TLC). Cholesterol was purchased from Sigma Chemical Co. (St. Louis, MO). Lipids were stored in chloroform under nitrogen at -20°C until use.
Polycarbonate membranes of 400 nm and 800 nm, and membrane holders used for liposome extrusion were purchased from Nucleopore (Pleasanton, CA).
The nonionic surfactant Triton X-100 (octylphenol with 10 units of ethylene oxide and active matter of 100%) was supplied by Tenneco S.A. (Barcelona, Spain).

Preparation of Multilamellar Vesicle Liposomes (MLV)

Multilamellar vesicle liposomes of a defined size (400 nm) at different lipid concentrations (from 1.25 mM to 3.0 mM), varying lipid composition (PC:CH molar ratios from 9.5:0.5 to 8.0:2.0), and with a constant dye concentration (1.0 mM) were prepared following a method described by Bangham (24). Liposome suspensions were extruded through 800 and 400 nm polycarbonate membranes to obtain an uniform size distribution (25). After preparation the resulting liposome suspensions were left to equilibrate for 15 minutes and immediately applied in the wool dyeing processes.

Dyeing Procedure

Wool knitted samples were treated with MLV liposome suspensions freshly prepared at different PC:CH molar ratios (from 9.5:0.5 to 8:2) in the range of lipid concentrations from 1.25 mM to 3.0 mM, the dye concentration remaining constant (1.0 mM which corresponded to 1.90 % o.w.f.). The dye was applied with 5% o.w.f. sodium sulphate, acetic acid at pH 5.5 and liquor ratio 60:1. Dyeing was started at 50°C and the temperature was raised by 0.9 °C/ min to 90°C. Dyeing was continued for 120 min. Thereafter, samples were rinsed with water for 10 min and dried at room temperature. Laboratory dyeing was carried out in a Multi-Mat dyeing machine (Renigal, Spain).
Dyebath exhaustion was determined by spectrophotometry using a Shimadzu UV-265FW spectrophotometer. Liposome aliquots (0.5 ml) were periodically added to quartz cuvettes filled with 2 ml of aqueous solution of Triton X-100 (2% w/v), supplemented with sodium sulphate (5%). The interaction between Triton X-100 and liposomes resulted in a complete solubilization of lipid vesicles via mixed micelles formation [26], turning the liposome suspensions into a clear solution. Figure 1 shows the effect of the cleavage of liposomes by Triton X-100 on the absorption spectra of the dye (PC:CH molar ratios (from 9.5:0.5 to 8:0). It may be seen that the λ_{max} of the dye used in this study does not change in presence of increasing amounts of CH in the lipid/surfactant mixed micelles.

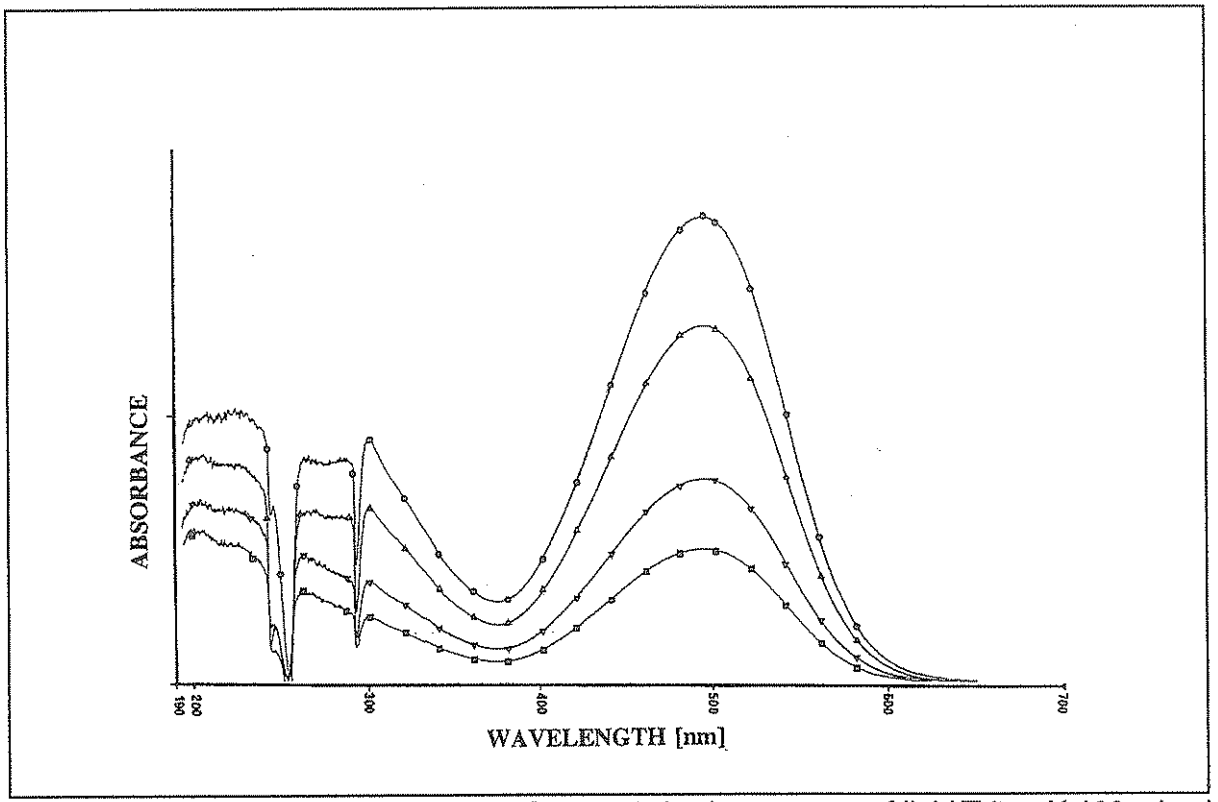

Fig. 1 - Adsorption spectra of Disperse Orange 1 dye in presence of lipid/Triton X-100 mixed micelles at different bilayer lipid compositions (PC:CH molar ratios 9.5:0.5 (●), 9:1 (▲), 8.5:1.5 (▼), and 8:2 (■).

Encapsulation Efficiency and Vesicle Size Distribution of Liposome Vesicles

The maximum amounts of dye dispersed with the MLV liposomes is defined as the weight ratio between the dispersed dye and the phospholipid in liposomes, K, and was determined spectrophotometrically. After preparation, liposome suspensions were left to equilibrate for 12 hours. Afterwards, vesicle suspensions were spun at 5000 r.p.m. at 25° for 15 min 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 addition of Triton X-100 [26].
Mean vesicle size and polydispersity of the liposome preparations were determined by a Photon correlator spectrometer (Malvern Autosizer 4700c PS/MV).

Bilayer Lipid Composition and Dye Extraction

The liposome concentration was determined using the Iatroscan MK-5 TLC-FID analyser. This is a useful method to quantify lipid mixtures even then are forming liposomes in water solutions (27,28).
The dye bonded superficially to the fibers by non polar forces was extracted with pure ethanol at 25°C for 60 min (21). Subsequent extractions with ammonia solution (0.5% at 60°C for 15 min) stripped the dye diffused inside the fiber and bonded ionically [5]. The concentration of extracted dye was evaluated in all cases by spectophotometry.

RESULTS AND DISCUSSION

Encapsulation Efficiency of MLV Liposomes

The dye encapsulation efficiency of MLV liposomes is defined as the amounts of dye dispersed in the aqueous media. This parameter is directly proportional to the lipid concentration of bilayers and gives light on the ability of lipid bilayer in both to disperse the dye and to carry this dye to the wool fiber. As above discussed, this parameter is defined in the present work as the K ratio (weight ratio between the dispersed dye and the phospholipid in liposomes). In order to determine this parameter a systematic study of the amounts of dispersed dye via MLV liposomes was carried out. The variation of the total amounts of dispersed dye at different lipid compositions (PC:CH from 9.5:0.5 to 8:2 molar ratios), versus bilayer lipid concentration is shown in Figure 2.

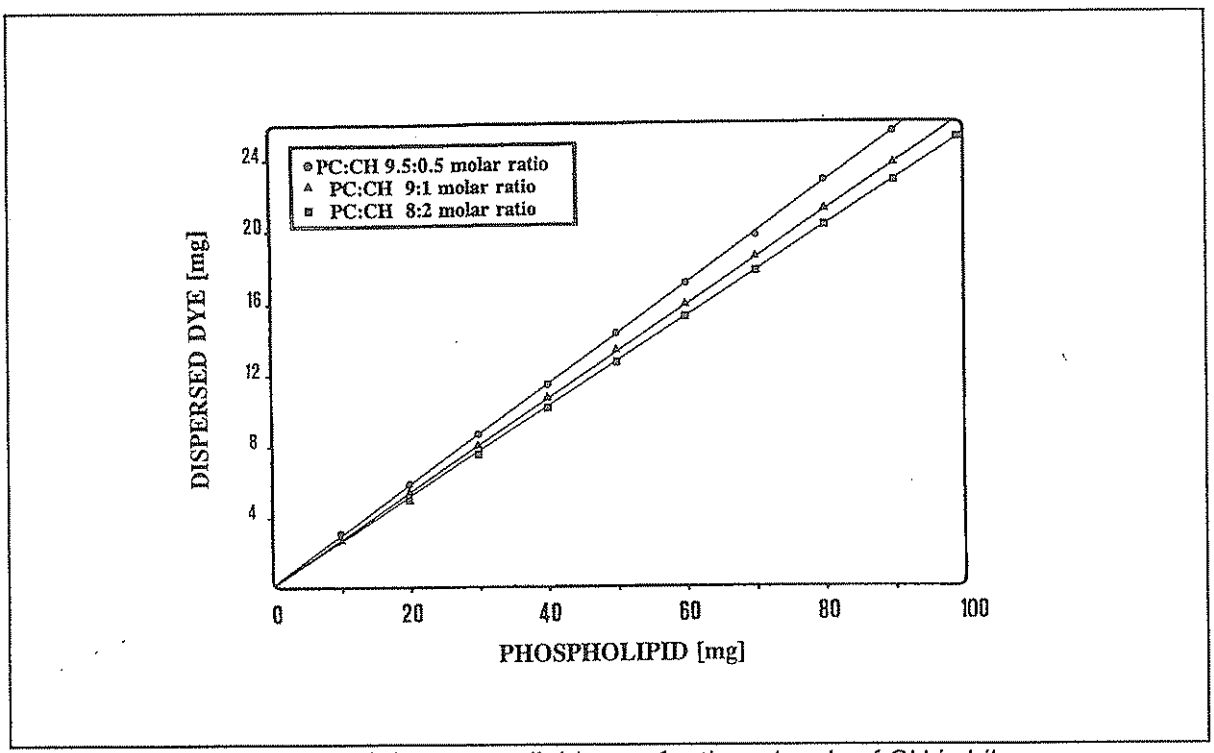

Fig. 2 - Maximum dispersed dye versus lipid conc for three levels of CH in bilayers.

A linear dependence between both parameters is observed in all cases. The weight ratio dye/lipid (K) corresponds to the slope of the straight lines obtained. Table I shows the K values and the regression coefficients for the straight lines obtained. The confidence intervals at 99% probability levels for each weight ratio estimated by linear regression have been also indicated. From these confiance intervals, it may be assumed that the K values are significantly different between them at 1% level.

Table I - Weight ratios for the maximum encapsulation efficiency for liposomes with increasing concentration of CH.

PC:CH molar ratio	Weight Ratio K Confidence interval at 99% PL	Determination Coefficients r^2
9.5:0.5	0.29 ± 0.06	0.994
9.0:1.0	0.27 ± 0.002	0.993
8.0:2.0	0.26 ± 0.005	0.992

It is noteworthy that K values decreased as the CH in bilayers increased reaching the lowest value for the bilayer composition PC:CH 8:2 molar ratio (K = 0.26). Given that 41 mg of this dye needs 1% of surface-active assistant per liter of water to be dispersed (sodium oleyl-p-anisidide sulphonate solution), the use of MLV liposomes resulted in a clear improvement in the dispersion efficiency with respect to the use of this conventional dispersing agents, reducing in more than 60 times the lipid concentration needed to obtain the same dispersing efficiency [5].

Stability of Liposome Suspensions

The possible aggregation of liposomes during dyeing was monitored by measuring the variations in the size distribution of these suspensions using a quasi-elastic light scattering method [29]. The results obtained for each lipid composition are given in Table II.

Table II - Mean vesicle size distribution and polydispersity of MLV liposomes at different lipid compositions (PC:CH molar ratios from 9.5:0.5 to 8:2 and total lipid concentration 2.0 mmol) during the dyeing process.

Time min	Mean vesicle size			Polydispersity Index		
	PC:CH 9.5:0.5	PC:CH 9:1	PC:CH 8:2	PC:CH 9.5:0.5	PC:CH 9:1	PC:CH 8:2
10	400	399	395	0.094	0.084	0.086
30	402	395	392	0.123	0.114	0.102
45	410	400	398	0.130	0.118	0.106
60	418	416	405	0.132	0.121	0.110
75	422	418	409	0.134	0.122	0.114
90	425	419	411	0.142	0.129	0.122
105	431	423	415	0.144	0.134	0.128
120	435	429	417	0.150	0.141	0.130

There is a small increase in the size of vesicles during the dyeing process, the polydispersity indexes remaining below 0.15 after treatment. Increasing amounts of CH in liposomes enhance the stability of these systems with respect to the aggregation, reducing both the mean particle size values and the polydispersity indexes during the dyeing. This behaviour is in agreement with the results reported by Scherphof et al. [30] in studies on liposome stability.

Dyeing Kinetics

We carried out kinetic studies on the dye exhaustion of the dye/liposomes systems on untreated wool samples varying both the bilayer composition (PC:CH molar ratios ranging from 9.5:0.5 to 8:2) and the lipid concentration (from 1.25 mmol to 3.0 mmol), the dye concentration remaining always constant (1.0 mmol, which corresponded to 1.90% o.w.f.). The results obtained for the PC:CH molar ratio 9:1 are plotted in Figure 3. The dye exhaustion increased as the lipid bilayer concentration rose reaching a maximum for 1.75 mmol lipid concentration (final dye exhaustion 82,7 %). From this concentration, increasing amounts of lipids in bilayers resulted in a decrease in the dye exhaustion with a minimum for 3.0 mmol (63.2%).

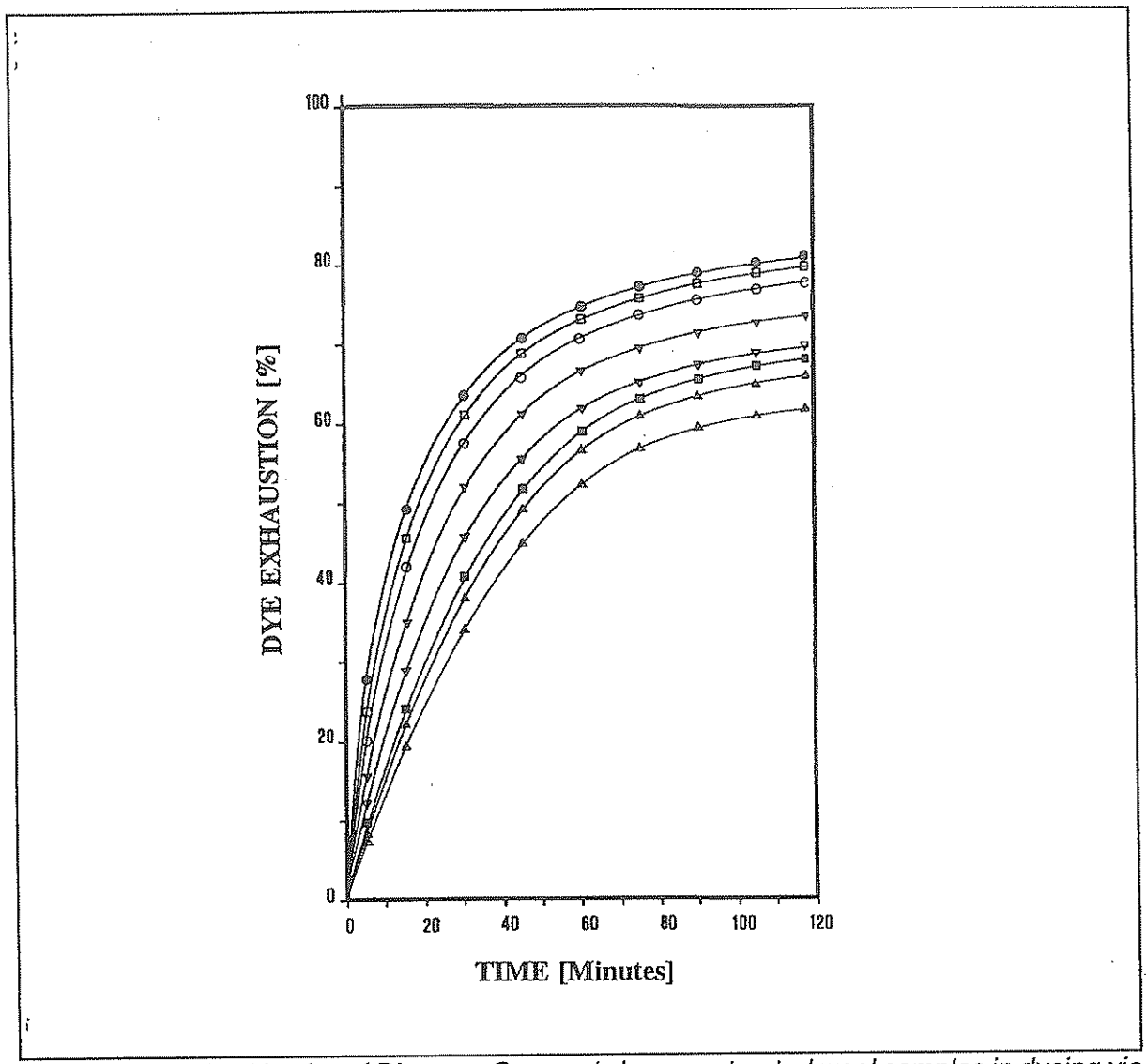

Fig 3 - Exhaustion kinetics of Disperse Orange 1 dye on untreated wool samples in dyeing via liposomes at different lipid concentrations (mmol) 1.25 (■), 1.50 (❑), 1.75 (●), 2.0 (○), 2.25 (▼), 2.50 (▽), 2.75 (s), 3.0 (△), and lipid composition PC:CH 9:1, the dye concentration remaining constant (1.0 mmol).

Similar behaviour was obtained for the different CH concentrations studied albeit the maximum dye exhaustion was achieved at different lipid concentrations (1.50 mmol for PC:CH 9.5:0.5 molar ratio and 1.75 mmol for 9:1 and 8:2 respectively). Increasing amounts of CH in bilayers resulted in a slight decrease of the maximum dye exhaustion in wool samples (results not shown).The weight ratio dye/lipid (K) for the highest dye exhaustion was always similar to that for the highest encapsulation efficiency.

Optical microscope observations of samples dyed in absence of liposomes or using MLV liposomes reveals that the use of liposomes resulted in more regular distribution of dye in the surface of fibers (6). This improvement was especially noticeable when using the dye/lipid molar ratio for to the maximum dye exhaustion for each lipid composition investigated. Increasing amounts of CH in bilayers also resulted in an enhancement in the distribution of dye in wool samples. These observations are in agreement with those reported for wool samples dyed with azo disperse dyes using unilamellar liposomes in absence of CH (3).

Influence of Liposome Composition on the Bonding of Dyes on Wool

In order to find out whether lipid bilayers (containing increasing amounts of CH) as carriers of dye caused changes to dye-fiber bonding forces after dyeing, extractions by pure ethanol (21) and ammonia (5) were performed on dyed samples. The results of dye extractions are given in Tables III, IV and V (corresponding to the PC:CH molar ratios 9.5:0.5, 9:1 and 8:2 respectively).

TABLES III, IV and V.

Amounts of adsorbed dye (mg dye/g wool), extracted dye (mg dye/g wool) and bonded dye (mg dye/g wool and %) in wool samples after dyeing via LMV liposomes at different lipid concentrations and different PC:CH molar ratios

Table III

		PC:CH molar ratio 9.5:0.5			
Lipid Conc	**Adsorbed dye**	**Extracted dye**		**Bonded Dye**	
(mmol)	(mg dye/g wool)	A	B	(mg dye/g wool)	%
1.25	15.47	3.97	0.01	11.48	74.24
1.50	16.94	3.84	0.01	13.08	77.24
1.75	16.44	3.78	0.01	12.65	76.93
2.00	15.85	3.73	0.01	12.11	76.39
2.25	15.26	3.69	0.01	11.55	75.71
2.50	14.61	3.65	–	10.95	74.95
2.75	13.94	3.61	–	10.32	74.04
3.0	13.38	3.57	–	9.81	73.27

A mg dye/g wool fiber extracted by pure alcohol [21]
B mg dye/g wool fiber extracted by ammonia [25]

Table IV

		PC:CH molar ratio 9:1			
Lipid Conc	**Adsorbed dye**	**Extracted dye**		**Bonded Dye**	
(mmol)	(mg dye/g wool)	A	B	(mg dye/g wool)	%
1.25	13.19	2.87	0.01	10.31	78.13
1.50	15.51	2.72	0.01	12.77	82.34
1.75	15.79	2.66	0.01	13.12	83.08
2.00	14.95	2.61	0.01	12.33	82.47
2.25	14.26	2.60	0.01	11.65	81.70
2.50	13.56	2.60	–	10.95	80.75
2.75	12.79	2.55	–	10.24	80.03
3.0	12.07	2.49	–	9.57	79.27

Table V

Lipid Conc	Adsorbed dye	PC:CH molar ratio 9:1 Extracted dye		Bonded Dye	
(mmol)	(mg dye/g wool)	A	B	(mg dye/g wool)	%
1.25	11.63	2.02	0.01	9.60	82.53
1.50	14.42	1.90	0.01	12.50	86.69
1.75	15.12	1.83	0.01	13.28	87.82
2.00	14.09	1.75	0.01	12.33	87.49
2.25	12.98	1.69	–	11.28	86.88
2.50	12.07	1.61	–	10.45	86.61
2.75	10.92	1.50	–	9.41	86.20
3.0	9.95	1.42	–	8.52	85.66

A mg dye/g wool fiber extracted by pure alcohol [21]
B mg dye/g wool fiber extracted by ammonia [25]

In general terms, pure ethanol extracted, in all cases, larger amounts of dye than ammonia solutions. This could be attributed to the high solubility of this dye in pure ethanol as well as to the presence of dye superficially bonded on the fibers by non-polar forces. However, the very small amounts of dye extracted via ammonia with respect to the lipid and dye concentrations of liposomes could be attributed to the important contribution of the non-polar forces in the dye-fiber bonds.
Furthermore, the extraction of the dye via ethanol was inversely related to the lipid concentration in bilayers and to the amounts of CH concentration present in these structures. However, the ammonia extractions showed in all cases similar low values.
Comparison of the amounts of extracted dye in wool samples dyed via liposomes in presence or not of CH [3] confirms that the presence of CH in bilayers decreased the amounts of extracted dye via ethanol.
Tables III-V also show the amount of dye bonded in wool fibers given as a difference between the amounts of the adsorbed dye and total extracted dye and the total percentages of bonded dye on wool samples. These percentages can be also expressed by the following equation:

$$C_b = (C_a - C_e)/C_a.100$$

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). It is noteworthy that the maximum percentages of bonded dye were obtained for each lipid composition at the dye/lipid weight ratios for the maximum dye exhaustion and, in consequence, for the maximum encapsulation efficiency of the systems.(1.50 mmol for PC:CH 9.5:0.5 molar ratio and 1.75 mmol for PC:CH molar ratios 9:1 and 8:2). The highest percentages were obtained for the maximum CH concentration in bilayers (Table V).
In general, two main factors may be considered to explain the role played by the CH in the exhaustion of dyes on the fiber and the subsequent bonding into the keratin structures: the effect caused by this component in reducing the

permeabilty of liposomes and enhancing their stability to the aggregation (30), and the presence of CH in the hydrophobic barrier of the Cell Membrane Complex, (structured as the two lipid bilayers similar to those found in liposomes) which governs the diffusion of dyes into the fiber.

CONCLUSIONS

The main contribution of this paper is the development of a new method to apply azobenzene disperse dyes to wool samples via liposomes MLV including CH in bilayers at controlled temperature (90°C). This process is suitable for improving the dye bonding in wool fibers in the absence of the dispersing agents normally used for this dye (5). This technological innovation simplifies the method currently used in the application of this dye, drastically reducing the lipid concentration needed to disperse the dye with respect to concentration of the conventional dispersing agent. The presence of increasing amounts of CH in bilayers, plays an important role in modulating the dyeing kinetics and in enhancing the dye-fiber bonding forces in the dyed wool samples.

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