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Textilveredlung im Wandel der Zeit die Visionen - die Realität

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Verein der Österreichischen Textilchemiker und Coloristen (VÖTC)

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der Vereine der Textilchemiker und Coloristen

Barcelona, 8th January 1996
Dr. Alfonso De la Maza
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Dear Dr. De la Maza,

I am glad to inform you that the Technical Committee has accepted your Paper entitled "Use of Liposomes in Wool Dyeing" to be presented in the 17 IFVTCC Congress, which will be celebrated in Wien the 5-7 June of 1996 .

Yours Sincerely,

L. Schmidt
Scientific Committee

USE OF LIPOSOMES IN WOOL DYEING

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SUMMARY

The use of liposomes as carriers of commercially available milling acid dyes and disperse dyes in wool dyeing has been reported to improve fixation, homogeneity and smoothness at laboratory level (1-3).

These advantages together with additional ecological profits when these presumable biodegradable lipid vesicles are applied instead of either surfactants or levelling agents, brought us to optimize this process at industrial level.

An optimization of a wool dyeing process with yellow Lanaset 2R has been performed using a Box and Behnken design (4). The influence of the temperature and concentration of dye and liposome on wool dyeing process and properties were studied. Time influence was found out performing some additional replicates at three different times in the center of the experiment.

The different dyeing experiments were carried out using industrial conditions at a pilot plant level, having in mind mainly two aspects: to apply lower temperature and to avoid the use of conventional dyeing auxiliaries. After this first approach to the optimum, a second series of experiments to obtain a close range for the definitive experimental parameters has also been performed. At present, a similar strategy applied to dyeing of wool/polyester blends is under way.

The results obtained indicate the influence of the liposome concentration in the improvement of the dyeing kinetics with good envisagement for industrial application. In particular, with some specific liposome concentrations it is possible to reduce the dyeing temperature without using dyeing auxiliaries. The additional ecological benefit obtained by the use of lipidic vesicles can be explored in order to implement the application of liposomes at industrial level.

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Use of Liposomes for Wool Dyeing

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Keywords

Liposome, Dyeing, Phosphatidylcholine, Cholesterol, Vesicles, Milling acid dyes, Encapsulation.

Abstract

A new method of wool dyeing by means of some large unilamellar and multilamellar phosphatidylcholine (PC) liposome systems (LUV and MLV) may be considered suitable for modulating the dye exhaustion of two commercial milling acid dyes, Polar Red B (CI Acid Red 249) and Polar Blue G (CI Acid Blue 90), thereby increasing the dye bonding in untreated wool fibers. LUV liposomes result in a greater decrease in dye exhaustion compared with the use of MLV liposomes. This inhibition is also directly dependent on the cholesterol (CH) concentration in bilayers, specially for the initial and final stages of dyeing.

Additionally, the exclusive use of liposomes with only the encapsulated dye results in changes in dyeing kinetics. Thus, although initially the dye exhaustion is inhibited, the final dye exhaustion rises with the CH concentration in bilayers for PC: CH molar ratio 8.5 : 1.5. The use of LUV liposomes results in a greater increase in dye bonding compared with the use of MLV liposomes. The exclusive application of encapsulated dye enhances the percentage of bonded dye in all cases. The lipid concentration resulting in a maximum amount of bonded dye is 1.0 mM. The presence of CH in the bilayers always improves the dye-fiber bonding (maximum bonding efficacy for PC:CH molar ratio 8:2).

1. Introduction

Wool is probably the most reactive of all textile fibers towards dyestuffs of many different types since it belongs to the class of natural proteins containing a variety of functional groups. The wool fiber can be regarded as an assembly of cuticle and cortical cells held together by the "Cell Membrane Complex" (CMC) which forms the only continuous phase in the keratin [1,2].

The CMC plays an important role in the adhesion between cells of both the cuticle and cortex in the keratinised fiber, in the transport of dyestuffs and processing of chemicals into the fiber and in determining its surface properties [3].

The wool dyeing process is undoubtedly fiber degradative since it is usually carried out in water at the boil for periods of 1-3 hours under a variety of pH conditions with values ranging from acidic to neutral. 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.

The ability of phospholipids to solubilize small amphiphile molecules such as dyes and drugs plays a major role in pharmaceutical applications [4] and biological staining [5]. The use of liposomes as vehicles for different reagents to wool fibers could be founded on the relevant importance of the hydrophobic interactions in the structural organization of wool, the overall stability of this protein composite and on the lipidic nature of the CMC.

Under Transmission Electron Microscopy (TEM), the CMC between cells appears as two parallel non-staining layers referred to as the β -layers (formed by lipids), separated by a densely stained layer called the δ -layer which is built basically of proteins (Figure 1a). 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 [2]. TEM studies provide 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) [6] (Figure 1b).

Figure 1a) Transmission Electron Microscope microphotograph of transverse section of merino wool fiber showing several cortical cells separated by Cell Membrane Complex (CMC)(arrowheads) comprising the β -layers (white arrows) and δ -layer (black arrow).

The fact that the bilayer structuration of CMC is similar to that of the liposomes and the important role played by the CMC in the processing of chemicals into the fibers have led to possible applications of liposomes as carriers in wool finishing [7,8].

Polar dyes are commercial "milling acid dyes" whose main characteristic is that they diffuse much more slowly than typical levelling acid dyes, thereby exhibiting higher wet fastness. However, their migration and coverage properties are inferior, and the addition of levelling agents is normally required [9]. It is therefore possible that liposomes could be used as carriers for such dyes.

Figure 1b Scheme of the morphological structure of a wool fiber and possible vias of dye diffusion.

In this work we shall describe studies involving the use of phosphatidylcholine liposomes including increasing amounts of cholesterol as carriers of two structurally different milling acid dyes to untreated fibers in wool dyeing. The physico-chemical stability of some large unilamellar and multilamellar vesicle suspensions (LUV and MLV) was studied during the dyeing process. We also examined the application of these structures to the dyeing of untreated wool, with special emphasis on the kinetic aspects of dye adsorption and dye-fiber bonding forces on wool fibers.

2. Experimental

A. 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.

Two commercially available Ciba-Geigy milling acid dyes were selected (Polar Red B, CI Acid Red 249 and Polar Blue G, CI Acid Blue 90). The selection criterion employed was to choose structurally different dyes belonging to the milling acid groups; the method of application on wool fabrics was similar in both cases.

Phosphatidylcholine (PC) was purified from egg lecithin (Merck) according to the method of Singleton [10] and shown to be pure by thin-layer chromatography (TLC). Cholesterol (CH) was purchased from Sigma Chemical Co. (St. Louis, MO).

Triton X-100 (octylphenol ethoxylated with 10 units of ethylene oxide and an active matter of 100%) was supplied by Tenneco, S.A. (Spain).

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

B. Preparation of Liposomes Containing Dyes

The liposomes used for these studies were large unilamellar vesicles (LUV) and multilamellar vesicles (MLV) of a variety of lipid compositions. The conditions of each preparation are outlined below.

1. LUV

Large unilamellar vesicle suspensions of a defined size (400 nm) at different lipid concentrations (from 0.5 to 4.0 mM) containing the milling acid dyes Polar Blue G or Polar Red B were prepared by reverse phase evaporation under nitrogen atmosphere as described by Paternostre and Rigaud [11,12]. This procedure was essentially derived from the general procedure of Szoka and Papahadjopoulos [13]. After preparation the resulting liposome suspensions containing dyestuff were left to equilibrate for 15 minutes and immediately applied to the wool dyeing processes [14].

2. MLV

Multilamellar vesicle liposomes of a defined size (400 nm) containing the milling acid dyes Polar Red B or Polar Blue G in the same range of lipid concentrations and also including CH in the same molar ratios were prepared following a method described by Bangham [15]. The resulting milky suspensions were vortexed for 5 minutes and sonicated for 15 minutes at 30°C and 75 W (Labsonic 1510 B. Braun). Likewise, the vesicle suspensions were sequentially extruded through 800 and 400 polycarbonate membranes to obtain a uniform size distribution.

C. Characterization and stability of liposome suspensions

Encapsulation Efficiency

The percentages of encapsulated dye in liposomes (expressed in % volume) were determined using a spectrophotometric method. After preparation, liposome suspensions were cleared of unencapsulated dye by separation through Sephadex G-50 medium resin (Pharmacia Sweden) column chromatography [16] and then the concentration of entrapped dye was evaluated by spectrophotometry after the solubilization of the supernatant lipid bilayers by addition of Triton X-100 [17].

Physical Stability

The aggregation state of the vesicles was estimated as a measure of the physical stability of the liposome suspensions. This was done by monitoring the variation of the mean vesicle size distribution of liposome suspensions as a function of time. This parameter together with the polydispersity indexes of the liposome suspension was determined using a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV). Vesicle size distributions were made by particle number measurements. Samples were adjusted to the appropriate concentration range with a dye bath solution. Measurements were made at 25°C with a detection angle of 90°.

D. Dyeing Procedure

Wool knitted samples (1g) were treated with LUV or MLV liposome suspensions freshly prepared containing the milling acid dyes either Polar Blue G or Polar Red B, at different PC:CH molar ratios (from 10:0 to 8:2); the lipid concentration of bilayers ranging from 0.5 to 4.0 mM. The dye was applied at 1% (o.w.f.) with a 5% sodium sulphate solution, acetic acid to pH 5.5 and liquor ratio of 60:1.

Dyeing was initiated at 50°C and the temperature was raised by 0.9 °C/min to 90°C. Dyeing was continued for 120 min. Next, 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).

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 of aqueous solution of Triton X-100 (10 g/l), supplemented with sodium sulphate (5%) and acetic acid at pH 5.5. The interaction between the nonionic surfactant Triton X-100 and liposome structures resulted in a solubilization of lipid vesicles via mixed micelle formation, turning the liposome suspensions into clear solutions.

The wavelength of maximum absorption of each dye used in this research does not change in the presence or absence of the phospholipid-surfactant mixed micelles. A similar effect was observed when using liposomes containing CH in the bilayer in the range of PC:CH molar ratios from 10:0 to 8:2.

E. Dye Extractions of Samples

After the dyeing process, the superficial dye bonded to the fibers by nonpolar forces (hydrophobic interactions, van der Waals forces and hydrogen bonds) was extracted with pure ethanol at 25°C for 60 min [14]. Subsequent extractions with ammonia solution (0.5% at 60°C for 15 minutes) stripped the dye diffused inside the fiber and bonded ionically [18].

3. Results and Discussion

A. Stability of Liposome Suspensions

LUV and MLV liposome suspensions containing the Polar Blue G or Polar Red B dyes were treated under the same conditions as in the dyeing process but in the absence of wool and using the same lipid bilayer concentrations at different lipid compositions (PC:CH molar ratios from 10:0 to 8:2). There was a small decrease in the particle size distribution in the initial stage of the dyeing process in all cases. After 30 minutes, vesicle size increased slightly reaching maximum growth after approximately 100 minutes of dyeing (vesicle size about 430–435 nm), the polydispersity indexes remaining below 0.15 after treatment in all cases. Increasing amounts of CH in liposomes enhanced the stability of these systems with respect to the aggregation, reducing both the mean particle size distribution and the polydispersity indexes during dyeing. This behaviour is in agreement with the results reported by Scherphof et al. in studies on liposome stability [19]. The chemical structure of dye and the type of vesicles used do not seem to affect the physical stability of the dye-liposome system. Furthermore, the mean vesicle size distribution and the polydispersity index were maintained at around 400 nm and below 0.24 respectively for more than 24 hours.

As a consequence, the LUV and MLV liposome suspensions including the studied milling acid dyes were physically stable during the dyeing process for the lipid concentration range and lipid compositions studied.

B. Encapsulation Efficiency

The relative concentrations of encapsulated dye in LUV and MLV liposomes for both the acid dyes investigated at different lipid compositions (PC:CH molar ratios 10:0 and 8:2) were plotted versus the bilayer lipid concentration. The results obtained are indicated in Figure 2.

Figure 2 Percentages of encapsulation efficiency of LUV and MLV liposome suspensions containing Polar Blue G (graphs A and B) and Polar Red B (graphs C and D), versus bilayer lipid concentrations for PC:CH molar ratios 10:0 and 8:2.

The encapsulation efficiency increased in direct proportion to the lipid concentration of liposomes for both lipid structures studied. However, we were unable to find any relationship between the dye encapsulation percentages and the chemical structure of the dyes. Increasing amounts of CH in the bilayers slightly decreased the percentages of dye encapsulated in all cases. The higher encapsulation efficiency of the LUV suspensions (maximum value of about 25 % for 4.0 mM lipid concentration) could be explained by the unicompartamental architecture of these species whose vesicles show a higher internal volume [20].

C. Dyeing Kinetics

We made kinetic studies of dye exhaustion on untreated wool samples for both dyes studied via LUV and MLV liposomes, the PC concentration ranging from 0.5 to 4.0 mM. The results obtained are plotted in Figure 3.

Figure 3 Exhaustion kinetics of Polar Blue G (graphs A and B) and Polar Red B (graphs C and D) dyes on untreated wool samples in dyeing via LUV and MLV liposomes (at different PC concentration) versus time.

As can be seen, the use of liposomes as carriers in wool dyeing resulted in an inhibition of dye exhaustion for both liposome structures studied, this effect being slightly greater for LUV suspensions. This behaviour appeared to be closely connected with the lipid concentration of the bilayers. The maximum inhibition was reached for 4.0 mM and remained constant throughout the dyeing process [21].

The maximum percentages of dye exhaustion after dyeing were obtained for Polar Red B (around 80% for LUV liposomes), whereas the Polar Blue G reached lower values in all cases (around 75% also for LUV liposomes). These differences could be attributed to the chemical structure of dyes which probably induces different dye-phospholipid interactions. These interactions may also affect the subsequent action of these dye/liposome systems with the wool fibers, especially in the migration of dye from the solution to the wool surface and in the subsequent adsorption of these systems on the fiber surface.

As cholesterol is one of the main components of the internal lipids of wool¹, we studied the effect caused by including this component in lipid bilayers on wool dyeing. Accordingly, we investigated the dye exhaustion kinetics also for both dyes via LUV or MLV liposomes containing increasing amounts of CH in bilayers, the total lipid concentration remaining constant (4.0 mM). The results obtained for Polar Blue G dye via LUV liposomes are given in Figure 4. In general, the marked inhibition in the dyeing exhaustion with respect to dyeing in the absence of liposomes is attributable to the use of the highest lipid bilayer concentration which, as discussed above, led to a maximum inhibitory effect. With regard to the influence of the molecular composition of liposomes, this inhibition was directly connected to the presence of CH in bilayers; i.e., the greater the CH concentration in the bilayers, the higher the inhibition in dye exhaustion, especially for the initial and final stages of the dyeing process [22]. The use of MLV liposomes led to similar dyeing exhaustion curves albeit with a slight diminution in their inhibitory capacity of dye exhaustion. Similar dyeing behaviour was obtained using the Polar Red B, the dye chemical structure playing a role similar to the one when using liposomes without CH.

Figure 4 Exhaustion kinetics of Polar Blue G dye on untreated wool samples in dyeing via LUV liposomes the bilayer lipid concentration remaining constant (4.0 mM) and varying the PC:CH molar ratios from 10:0 to 8:2.

Bearing in mind the dyeing properties of the milling acid dyes applied in a low acid dyebath, dye-liposome interaction could be attributed to the ionic affinity between the polar groups of both lipids and dyes as well as to the hydrophobic interactions between nonpolar moieties of those structures. These interactions could affect the dye molecules entrapped in vesicles and also those present in the surrounding aqueous medium. Thus, the interaction of dye molecules with wool fibers can be modulated by the presence of these new (dye-liposome) complexes, affecting especially the migration and adsorption of dyes on the wool surface and the dye-fiber bonding mechanisms based on electrostatic bonds between dye anions and basic group charges of the keratin structure.

D. Influence of Liposomes on Bonding of Dyes on Wool

In order to find out whether liposomes as dye carriers brought about changes to dye-fibre bonding forces after dyeing, successive extractions by pure ethanol and ammonia were performed on dyed samples via LUV or MLV liposomes for both dyes and in the range of the lipid concentration studied. The results of these trials are

given in Tables I (Polar Blue G) and II (Polar Red B dye). In general, the amounts of extracted dye via ammonia were clearly higher than those extracted using pure ethanol.

Table I. Amounts of extracted dye (mg dye/g wool) by pure ethanol and ammonia on dyed samples via LUV and MLV liposomes for Polar Blue G dye, the bilayers lipid concentration ranging from 0.5 mM to 4.0 mM and for the PC:CH molar ratios 10:0, 9:1 and 8:2.

A = extractions with pure ethanol

B = extractions with ammonia

Polar Blue G					
PC:CH Molar Ratio	Lipid Conc. mM	LUV		MLV	
		Extracted Dye mg dye/g wool		Extracted Dye mg dye/g wool	
		A	B	A	B
10:0	4.0	0.012	1.198	0.022	1.798
	3.0	0.018	1.503	0.024	2.016
	2.0	0.022	1.906	0.025	2.200
	1.0	0.034	2.084	0.040	2.400
	0.5	0.049	2.983	0.048	3.020
	0	0.217	3.608	0.217	3.608
9:1	4.0	0.010	0.879	0.016	1.494
	3.0	0.014	1.194	0.020	1.708
	2.0	0.018	1.593	0.021	1.898
	1.0	0.027	1.762	0.033	2.104
	0.5	0.034	2.654	0.040	2.714
	0	0.003	0.729	0.014	1.299
8:2	4.0	0.006	1.008	0.017	1.502
	3.0	0.009	1.287	0.018	1.678
	2.0	0.018	1.584	0.027	1.911
	1.0	0.023	2.453	0.036	2.512
	0.5	0.003	0.729	0.014	1.299
	0	0.006	1.008	0.017	1.502

Table II. Amounts of extracted dye (mg dye/g wool) by pure ethanol and ammonia on dyed samples via LUV and MLV liposomes for Polar Red B dye, the lipid bilayer concentration ranging from 0.5 mM to 4.0 mM and for the PC:CH molar ratios 10:0, 9:1 and 8:2.

Polar Red B					
PC:CH Molar Ratio	Lipid Conc. mM	LUV		MLV	
		Extracted Dye mg dye/g wool		Extracted Dye mg dye/g wool	
		A	B	A	B
10:0	4.0	0.040	1.480	0.052	2.073
	3.0	0.061	1.780	0.073	2.374
	2.0	0.080	2.080	0.094	2.617
	1.0	0.100	2.180	0.126	2.902
	0.5	0.140	2.800	0.157	3.123
	0	0.200	3.508	0.200	3.508
9:1	4.0	0.038	1.189	0.043	1.764
	3.0	0.056	1.492	0.068	2.083
	2.0	0.073	1.784	0.084	2.301
	1.0	0.089	1.893	0.112	2.599
	0.5	0.123	2.491	0.136	2.831
	0	0.028	0.993	0.034	1.571
8:2	4.0	0.046	1.302	0.057	1.881
	3.0	0.063	1.604	0.074	2.216
	2.0	0.082	1.721	0.103	2.402
	1.0	0.117	2.246	0.121	2.601
	0.5	0.028	0.993	0.034	1.571
	0	0.046	1.302	0.057	1.881

Ethanol extractions of dyed samples via liposomes showed, in general, smaller values than those obtained from samples dyed without liposomes (using the levelling agent recommended by the manufacturer). The amounts of extracted dye were inversely correlated with the lipid concentration of liposomes in all cases. Furthermore, increasing amounts of CH in bilayers led to a progressive diminution in the amounts of extracted dye regardless of the type of liposome used. The use of unilamellar or multilamellar bilayer structures does not seem to affect the total amount of extracted dye. Similar tendencies were observed in subsequent extractions on the same sample using ammonia.

The overall evidence confirms the role of the CH in liposomes as effective carriers for bonding of milling acid dyes on wool fibers. This effect may be attributed to the fact that the CH affects the spontaneous release of encapsulated solutes and decreases the tendency of the vesicles to aggregate once they are formed. Likewise, the presence of CH in the CMC, may explain the role of this compound in the diffusion of the dye from the surface towards the center of the fiber and in the improvement of the dye-fiber bonding forces in wool dyeing.

Given that pure ethanol extractions remove the dye superficially bonded to the fibers by nonpolar forces, the use of liposomes in dyeing must lead to a decrease in such a dye. Furthermore, the decreasing amounts of dye removed with ammonia with respect to the lipid concentration of liposomes (particularly in the presence of increased amounts of CH) could be attributed to the increasing contribution of the nonpolar forces in the dye-fiber bonds. These interactions, especially those that are hydrophobic in nature, may play an important role in the dye-fiber bonding inside the fiber.

The total percentage of dye bonded to the wool fibers can be expressed by the equation (1):

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

where C_b is the amount of dye bonded (%), C_a is the amount of dye adsorbed (mg per g wool) and C_e is the amount of dye extracted (mg per g wool). These percentages for both dyes studied using LUV and MLV liposomes (lipid composition PC:CH from 10:0 to 8:2 molar ratios) for different bilayer lipid concentrations are given in Table III.

Table III. Percentages of total bonded dye for Polar Blue G and Polar Red B dyes, the lipid bilayer concentration ranging from 0.5 mM to 4.0 mM and for the PC:CH molar ratios 10:0, 9:1 and 8:2.

PC:CH Molar Ratio	Lipid Conc. mM	Polar Blue G		Polar Red B	
		Total Bonded dye %	Total Bonded dye %	Total Bonded dye %	Total Bonded dye %
		LUV	MLV	LUV	MLV
10:0	4.0	84.13	77.25	81.00	75.90
	3.0	81.51	75.43	78.36	73.34
	2.0	78.08	73.82	76.00	71.55
	1.0	77.21	72.88	75.95	69.38
	0.5	68.06	67.70	68.81	7.20
	0	62.16	62.16	62.92	62.92
9:1	4.0	87.99	81.91	84.35	79.01
	3.0	84.97	80.08	81.41	76.07
	2.0	81.29	78.68	78.92	74.40
	1.0	80.42	77.29	78.73	72.02
	0.5	71.88	70.14	72.65	69.78
8:2	4.0	89.90	83.84	86.50	80.91
	3.0	87.03	82.05	83.46	77.82
	2.0	84.65	80.77	80.88	74.94
	1.0	82.23	79.10	80.57	73.87
	0.5	73.88	73.51	75.04	71.70

The percentages of total bonded dye were directly correlated with the lipid concentration in bilayers reaching maximum values for 4.0 mM (81-84% for LUV and 76-77% for MLV in the absence of CH) and falling to values of about 62% for dyed samples in the absence of liposomes.

The use of LUV liposomes resulted in higher percentages of total bonded dye compared with the levels found with MLV liposomes regardless of the lipid concentration used in all cases. It is noteworthy that the presence of CH in liposomes increased the percentage of total bonded dye in wool in all cases i.e., the higher the CH proportion in bilayers, the greater the relative concentration of dye bonded to the fiber.

Figure 5 shows the amounts of bonded dye in wool fibers given as the difference between the amounts of adsorbed dye after dyeing and total extracted dye (Tables I and II) for both dyes studied using LUV or MLV liposomes, versus lipid concentration (lipid compositions (PC:CH 10:0 and 8:2 molar ratio).

We obtained a maximum amount of bonded dye when lipid concentration was 1.0 mM for both dyes investigated. The presence of increasing amounts of CH in the bilayers led to a rise in the total amount of bonded dye. This phenomenon was also observed when using unilamellar vesicle structures in all cases.

Figure 5 Amounts of bonded dye in wool fibers for Polar Blue G (graphs A and B) and Polar Red B (graphs C and D) via LUV and MLV liposomes versus lipid concentration (PC:CH 10:0 and 8:2 molar ratio). The results are given as a difference between the amounts of adsorbed dye and total extracted dye.

F. Dyeing Studies Using Only the Encapsulated Dye

Given that the main part of dye molecules included in liposome suspensions are placed in the aqueous medium which surrounds the vesicles (maximum encapsulation efficiency of LUV liposomes of about 25%), we carried out a series of experiments based on the liposome dyebath exhaustion using only the dye encapsulated into

the liposomic structures. To this end, wool samples were treated with LUV or MLV liposomes which contained the milling acid dyes after freeing the unencapsulated dye by separation through Sephadex G-50 resin. The PC:CH molar ratios varied from 10:0 to 8:2 and the total lipid concentration remained constant (4.0 mM).

Figure 6 shows the exhaustion kinetic of the Polar Blue G dye by means of LUV liposomes, the PC:CH molar ratios ranging from 10:0 to 8:2. Dye exhaustion kinetics showed different tendencies depending on the CH concentrations present in the bilayers. Thus, the initial dyeing phase showed an increasing inhibition of dye exhaustion, which reached the maximum for the PC:CH molar ratio 8.5:1.5. But increasing amounts of CH in the bilayers (8.0:2.0 molar ratio) reduced this inhibitory effect. However, the final dye exhaustion appeared to be directly correlated with the CH present in bilayers, reaching the highest exhaustion for the PC:CH molar ratio 8.5:1.5. Once more, exceeding CH concentrations resulted in decreased dye exhaustions. This behaviour was observed for both dyes regardless of the type of liposome used. The improved final dye exhaustion obtained using exclusively the encapsulated dye for the increased CH liposomes may confirm the role of the cell membrane complex (CMC) in the transport of dyestuffs given that the CMC presents a bilayer structuration (including CH Table I) which is similar to that of the liposomes.

Figure 6 Exhaustion kinetic of Polar Blue G dye in dyeing by means of LUV liposomes using only the encapsulated dye, the PC:CH molar ratio in bilayers ranging from 10:0 to 8:2.

A comparison of the dye exhaustion percentages obtained with complete liposomes or using only the encapsulated dye (Figures 4 and 6) reveals that, in both cases, initial dye exhaustion follows similar tendencies except for the lipid molar ratio PC:CH 8:2. However, the most striking result is that the final dyeing exhaustions show an opposite tendency depending on the kind of application used. Thus, in the former case the presence of increasing amounts of CH resulted in the final inhibition of dye exhaustion, whereas in the latter case this presence led to an increase in a final dye exhaustion on wool samples.

These findings emphasize the role of CH in liposomes as effective carriers for transport and bonding of dyes in wool and the importance of the location of the dye molecules in liposome suspensions i.e., in the lipid bilayers or in the surrounding aqueous medium. The opposite tendency also stresses the role of lipid

bilayers as carriers during the subsequent steps of the dyeing process: migration of dyes from the solution to the wool surface, adsorption on the fiber surface, diffusion of the dye from the surface towards the center of the fiber, and electrostatic bonding of the dye molecules in the keratin substrate.

Table IV shows the percentages of total bonded dye (calculated following equation 1), for both dyes studied using LUV or MLV liposomes (4.0 mM lipid concentration) the PC:CH molar ratios ranging from 10:0 to 8:2. An overall comparison of Tables III and IV shows that the exclusive use of the encapsulated dye results in increased dye bonded percentages with respect to those obtained using the complete liposomes. These findings emphasize the importance of the lipidic pathway involved in the use of liposomes as suitable carriers in the transport and bonding of dyestuff to hydrophobic domains.

Table IV. Percentages of total bonded dye for the Polar Blue G and the Polar Red B dyes using only the encapsulated dye, the lipid bilayer concentration remaining constant (4.0 mM) and for the PC:CH molar ratios 10:0, 9:1 and 8:2.

PC:CH Molar Ratio	Polar Blue G		Polar Red B	
	Total Bonded dye %		Total Bonded Dye %	
	LUV	MLV	LUV	MLV
10:0	94.82	92.93	91.80	89.89
9.5:0.5	96.13	94.90	93.36	91.23
9.0:1.0	97.51	95.68	94.48	92.58
8.5:1.5	97.96	95.77	95.07	92.78
8.0:2.0	97.30	95.03	95.55	92.64

Increasing amounts of CH in the bilayers also raised the total percentage of the bonded dye. As observed in Table IV the use of Polar Blue G dye resulted in higher percentages of bonded dye regardless of both the type of liposome used and the CH concentration present in bilayers.

In the light of our results we shall consider two major aspects related to the dyeing of wool fabrics via liposomes with milling acid dyes: Kinetics and bonding of dyes into the fiber.

Depending on both PC concentration and relative concentration PC:CH a variable inhibition of dye exhaustion during dyeing can be obtained. In fact, the function of levelling agents in a dyeing process is based on their regulatory action leading to an equilibrium between vehiculization of the dye to the fiber and the appropriate bonding in the keratinic structure. The special characteristics of liposome bilayers could encourage this levelling function; moreover, dye delivery by liposomes could be a versatile strategy for modulating the dyeing process in order to obtain a progressive dye exhaustion on the fiber with a suitable and durable dye bonding on the active sites of the keratinic structure. We postulate that the dye vehiculization by liposomes is as important as the lipidic pathways existing in the CMC realms.

The behaviour of liposomes as a delivery system of dye molecules present only in the intra-liposomic domain or in the whole space (liposome inside and outside) is clearly different. In the first case, the inhibition of the dye exhaustion observed during the initial steps of dyeing could be attributed to a certain delay on the part of the dye molecules since they must first cross through the lipid bilayer before interacting with the fiber. Nevertheless, the final exhaustion and bonding onto the fiber could be encouraged because of the physicochemical similarity of the lipid structure of CMC and the phospholipid bilayer of liposomes. The improved final bonding of dye molecules to wool fibers could be explained by this mechanism. We think that two key concepts emerge from this review: dyeing modulation (in a kinetic and dye bonding sense) and biomimetic

strategies (based on the similarity of lipid structuration of liposomes and wool CMC). Bearing both these concepts in mind, it may be possible to optimize dyeing experimental conditions by also improving fastness of wool dyed samples.

4. Conclusions

From our findings, we conclude that a new method of wool dyeing by means of LUV or MLV liposomes may be considered suitable for modulating the dye exhaustion of two commercial milling acid dyes, Polar Red B (CI Acid Red 249) and Polar Blue G (CI Acid Blue 90), in a low acid dyebath, thereby increasing the dye bonding in untreated wool fibers.

LUV and MLV liposome suspensions at pH 5.5 are physico-chemically stable during the dyeing process for both dyes studied, the bilayer lipid concentration ranging from 0.5 to 4.0 mM. The presence of increasing amounts of CH in liposomes improves the stability of these structures for both particle size distribution and polydispersity index.

The inhibition of dye exhaustion on untreated wool fabrics is directly dependent on both the liposome lipid concentration and the structure of liposomes. The use of LUV liposome structures results in a greater decrease in dye exhaustion compared with the use of MLV liposomes. This inhibition is also directly dependent on the CH concentration in bilayers, especially for the initial and final stages of dyeing. The exclusive use of the encapsulated dye results in changes in the dyeing kinetics. Thus, although initially the dye exhaustion is inhibited, the final dye exhaustion rises with the CH concentration in bilayers, the maximum effect being achieved for PC:CH molar ratio 8.5:1.5. The control of dye exhaustion allows modulation of dye adsorption on wool fibers, thereby improving both regularity and homogeneity of the dye adsorption and bonding on untreated wool fibers. Given that this control is especially important in the initial dyeing stages, (where excessive dye adsorption leads to staining irregularities), the bilayer lipid concentration and particularly the PC:CH ratio may act as a specific levelling mechanism for each dye.

As for the bonding of dyes in wool fibers, despite the aforementioned inhibition of dye exhaustion, the increasing concentrations of lipids in the bilayers enhance the percentage of bonded dye into the fibers. This tendency is also observed when the CH concentration in bilayers is increased. The use of LUV liposomes results in a greater increase in dye bonding compared with the use of MLV liposomes, regardless of the chemical dye structure. The exclusive application of encapsulated dye enhances the % of bonded dye in all cases.

The lipid concentration resulting in a maximum amount of bonded dye is 1.0 mM in all cases. Given that the presence of CH in the bilayers always improves the dye-fiber bonding, the maximum bonding efficacy is achieved at this lipid concentration for PC:CH molar ratio 8:2.

In this work, the importance of the role played by liposomes in wool dyeing using two different milling acid dyes has been demonstrated taking into account both the dyeing modulation and the strategic biomimetic aspects. It is not unreasonable to predict that an optimization of experimental conditions of dyeing using liposomes could be obtained thereby improving additionally fastness of wool dyed samples. Some other liposome strategies could be applied to wool processing taking into consideration the special lipo-keratinic structure of wool fibers, in particular the existence of lipids in the Cell Membrane Complex.

Independently of the validity of the results obtained in our work, it is important to demonstrate the real industrial interest of wool dyeing process using liposomes. Till now, the preliminary results obtained by our group at industrial level are very promising in order to reduce dyeing temperature without using conventional dyeing auxiliaries. In fact optimization studies of the wool dyeing process using liposomes have been performed, both for single dyes and dye combinations. However, one of the main problems for the liposome applications is the price of raw materials (lipids) and liposome

manufacturing. At present different qualities of liposomes are considered in order to obtain the better technological benefit at a lower cost. We hope that in a very near future we will be able to present a new wool dyeing process using industrial liposomes that could be commercially applied.

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