New Environmentally Friendly Liquid Dyes for Protein Supports

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Innovation in the field of dyeing protein supports such as leather and furs has shown continuous development because of environmental issues and demand for new products of higher dyeing performances. The most known alternative to powder dyes are liquid dyes showing high surface dyeing capacity and protein support affinity with no conditioning salts. The paper deals with the possibility of preparation and application of a new class of liquid dye-liposome dyes and their advantages.

Keywords: liposome, liquid dye, dyestuffs, protein support, liposome preparation

The use of the liposome for non-medical purposes is a recent approach and raises a large interest for fundamental research in view of their applicability in various fields.

Liposomes are formed by amphiphiles such as didodecylmethylammonium bromide [1], polyoxyethylene [2] hydrogenated castor oil ether [3] and lecithin [4]. However, lecithin liposomes are superior to other amphiphiles from the viewpoint of safety and bioadaptability for applications in the medical, cosmetic and food industries.

Many efforts to improve the properties of liposome preparations have been made, especially the vesicle size, stability and encapsulation efficiency which are still major problems. So far, a number of different methods for preparation of liposomes have been developed successfully. These methods can be classified for convenience into three categories based on dispersion technology [5]: (1) mechanical dispersion methods, for example, shaking or vortexing, sonication [6] and the use of a French press; (2) detergent-solubilization dispersion methods [7] including solubilization of lecithin with sodium chloride or octylglucoside; (3) solvent dispersion methods, such as ethanol injection, ether infusion and reverse-phase evaporation (REV) [8,9]. Liposomes prepared by the REV method are known to have a higher encapsulation efficiency for water-soluble agents than those prepared by other methods, but they usually have a diameter exceeding 200 nm [10]. The active loading, namely the remote loading of agents into preformed liposome by the use of special gradients across the liposome membranes, such as pH gradients [11] and ammonium salt gradients [12] can result in rather high encapsulation efficiency.

Liposome characteristics and properties are dependent on their composition, mono- or multilamellar structure, specific size and stability. Factors affecting adversely the liposome are aggregation induced by Van der Waals interactions that occurs frequently for large liposome without ionic charges and vesicle fusion. This affects mainly the small size liposomes, monolamellar (<40 nm), at temperatures close to the critical value. In this paper, the formulations, compositions and dyeing properties for collagen and keratin supports made up of some new liposome having embedded water soluble dyes are presented. There are known few applications of auxiliaries with liposome or encapsulated structures for leather processing [13-16]. The improved affinity of dyeing liposomes for different treated protein supports, in comparison with the same powder dyestuffs, was assessed by reflex spectroscopy, recognized method in other field of applications [17], but relatively new for leather and furs.

Experimental part
Materials and methods

The selected dyes for further testing were commercially available samples, as powders: i) Acid Blue 90 (CI 42655) - tri-phenyl-methane class dye; ii) Acid Red 249 – azoic class dye and iii) Acid Yellow – unknown structure. All available samples, as powders: i) Acid Blue 90 (CI 42655) - tri-phenyl-methane class dye; ii) Acid Red 249 – azoic class dye and iii) Acid Yellow – unknown structure. All selected dyes present good diffusion properties and wet resistance.

Lecithin was selected as a phospholipid; lecithin is a natural, non-toxic and commercially available emulsifying agent. Our product presented the following properties: fluid paste presenting a yellowish-brown color and a typical vegetable flavor, with a phospholipid content (non-soluble in acetone) of min. 62%. The protein supports were the bovine leather (wet blue, vegetable and synthetically retanned) and woolen sheepskins prepared for dyeing.

Microdispersion consisted in application of shared forces to the compound, stirring at 2000-10000 rot/min using: DRAIS lab-scale pearl mill (φ = 3mm) and Homomixer UltraTurrax model T18 (IKA Works, USA), and exposure to sonic vibrations in a lab-scale Ultrasonic Processor model UP100 (100W, 30KHz) (dr. Hielscher Gmbh).

The concentration of dyes in different stage of liposome structures preparation was accomplished by using a TLC and VIS spectroscopy on SPECORD M40 and M42 spectrometers (Karl Zeiss JENA).

Evidencing and morphostructural characterization was realized by:

a) TEM using a JEOL JEM 1010 with MegaView III CCD camera, at a 0.3 nm resolution and a magnifying of 600.000 x and photographic magnification up to 5.000.000x.
b) Optical microscopy using an OlympusBX51 with a video camera, dark background, DIC and fluorescence. Other apparatuses used during this study were: vacuum evaporator JEE 4C (10⁻⁴), automatic system for sample processing, mini-centrifuges, ultracentrifuge, thermostats and critical point dryer (Oxford Instruments).

Bovine leather and woolen sheepskin dyed samples were obtained by using usual formulations for powder dye testing [18] on symmetric topographic pairs of leather or skin in a Dr. Wacker apparatus. The study of liposome dyes affinity was carried out by using wet blue, vegetable and synthetically retanned leathers and by assessment of the influence of fatting on color changing.

Color analyses for leather and woolen sheepskins dyed with the same levels of powder dyes and liquid liposome dyes were carried out by using diffuse reflection device ILN 472 (UV-VIS-NIR 570, JASCO, Japan) and CIELAB software for color data processing.

Results and discussions
Preparation of liquid liposome dyes
Synthesis of liquid dyes in liposome formulation was performed by combining some physico-mechanical dispersion processes applied on dye-lecithin microdispersions, in the following order: emulsifying of lecithin in water, microencapsulation I (grinding of a dye-lecithin mixture in a ball mill at 2000 rot/min), microencapsulation II (grinding at 6000 rot/min) and solution ultrasonicating in order to lower the size of the resulted liposomes.

The selected complex process for liposome preparation was based on high revolution mechanical dispersion (6000-10000 rot/min), ultrasonic vibration, and high pressure filtering. The emulsifying agent is a natural compound (lecithin) extracted from soy beans. The main variables that must be taken into account in liposome synthesis are the following: molar ratio among the lipid components, suspended lipid concentration and suspension volume. In our work, we selected the liposome preparation methods with no organic solvents or detergents in order to obtain liquid, ecological dyes.

Determination of liposome dye particle sizes
The mean particle size was obtained on paired samples for each dye; the second sample (P) contained a double amount of lecithin.

Table 1 shows the samples obtained after Microencapsulation II (UltraTurrax) while table 2 presents the samples after encapsulation III (ultrasounds). One can see that no significant differences are revealed between the paired samples. At the same time the liposome-dyes population obtained by ultrasonicating is more uniform and clean. Most of the differences are linked to the associations among the liposome due to their instability over time and to chemical structure of each involved dye. Based on these observations, TEM and optical microscopy appear to be the most suitable methods for characterization.

Morphostructural characterization of liposome dyes
The morphostructural characterization of liposomal vesicles was performed through the negative staining method, which is frequently used in determination of liposome size for medical use. The dyes do not interact chemically with the biologic reagent but will create a dark background while the biologic material remains transparent.

In our case, for the characterization of the liposome dye solution, an adapted TEM technique was used. This consisted in removal of the dark background leading to the transparent liposome. This way the dye encapsulated in the liposomes might be easily revealed, mainly in the isolated ones.

![TEM images of liposome dye particles](image)

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The leather and woollen sheepskin dyeing with liposome dyes

The main advantages of liposome dyes in comparison with the same dyestuffs in powder form were assessed by dyeing leather and wool supports. The liposome dyes were assessed by using the classical technology for powder dyes in leather and woolen sheepskins wet finishing. The influence of greasy matters and synthans, anionic chemical auxiliaries, which compete with anionic dyestuffs were evaluated by dyeing chrome tanned (wet blue) leather and retanned (crust) leather.

The color depth in blue liposome dyed wet blue leather and in wet blue fatty leather is higher than in the similar dyeing with powder dyes (fig. 3). Variant S11bis, liposome dye, has showed the best performances. The performances of blue liposome dye (variant S11bis) are higher than with powder dye in the range of 0.25-1.5% concentrations on wet blue bovine leather (fig. 4).

The affinity of blue liposome dyes for retanned bovine leather support is higher than for the same dyeing with powder dyes (fig. 5), this could be an important economical advantage, according to the general opinion [19] that the dye’s performances capacity depend more heavily on the chemical nature of the dye. The colour depth of wool in...
woolen sheepskins dyed with red liposome dyes (fig. 6) and blue liposome dyes (variant S11A1, fig. 7) is higher than in similar dyeing with powder dyes.

Evaluation of dyeing fastness of blue liposome dye has revealed improvement of main characteristics in comparison with the same dyes on powder form (table 3).

**Conclusions**

The liposome dyes for protein supports are innovative products which could be obtained by encapsulated of dyestuffs in lecithin vesicle, using microdispersion methods, without conditioning additives, such as inorganic salts, or dyeing auxiliary materials with pollutant potential.

We established that the best method for dyeing liposome synthesis, which assures the obtaining of uniform shape of liposomes is based on mechanical dispersion, ultrasound vibration and high pressure filtration. The selection of emulsifier agent took into consideration the use of a natural material, the lecithin, ecological alternative for the use of solvents or tensides.

By using TEM and optical microscopy we established the correlation between the morphology of the liposome dyes, their stability and method of obtaining. The obtained dyeing liposomes have an uniform composition, elliptic or spheric shape and particle size in the range of 300-900 nm, depending on dyestuff type.

The use of liposome dyes in comparison with powder form for protein supports, such as leather and woolskin, has revealed improvement of dyeing performances in term of colour intensity assessed by using diffuse reflection method; affinity for protein supports, checked by dyeing experiments and dyed protein support fastness evaluated according to standardized methods.

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**Table 3**

<table>
<thead>
<tr>
<th>Fastness to perspiration, mark 1-5</th>
<th>BLUE dyeing on chrome tanned leather support</th>
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<td>0,5% powder</td>
<td>3-4</td>
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<tr>
<td>0,5% liposome</td>
<td>1/1</td>
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<tr>
<td>1% powder</td>
<td>1/1</td>
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<tr>
<td>1%</td>
<td>4-5</td>
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**Fastness to water spotting, mark 1-5**

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**Table 3**

DYEING FASTNESS OF THE DYEING LIPOSOME ON DIFFERENT LEATHER SUPPORTS IN COMPARISON WITH THE SAME BLUE POWDER DYES

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