# Study of the release of a microencapsulated acid dye in polyamide dyeing using mixed cationic liposomes

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## ABSTRACT

The main objective of this work was to increase the retarding effect of the acid dye Telon® Blue RR (C.I. Acid Blue 62) release on polyamide fibres dyeing by encapsulation of the dye in liposomes as an alternative to synthetic auxiliaries, in order to reduce effluent pollution. The retarding effect achieved with the use of mixed cationic liposomes of dioctadecyldimethylammonium bromide (DODAB)/soybean lecithin (containing a 10% molar fraction of DODAB) was better in comparison with either pure soybean lecithin liposomes or synthetic auxiliaries. The retarding effect of liposomes on the dye release was analysed through changes in the absorption and fluorescence spectra of the acid dye at different conditions. The effect of temperature (in the range of 25 ºC - 70 ºC) on the spectroscopic behaviour of the dye in the absence and in presence of polyamide was also studied, in order to simulate the dyeing conditions. Exhaustion curves obtained in dyeing experiments showed that, below 45 ºC, the retarding effect of the mixed liposomes (lecithin/DODAB (9:1)) was similar to that of the auxiliaries, but better than the one of pure lecithin liposomes. At higher temperatures (above 45 ºC), the system lecithin/DODAB presents a better performance, achieving a higher final exhaustion level when compared with the commercial levelling agent without losing the smoothing effect of lecithin.

## INTRODUCTION

Liposomes are generally spherical structures (vesicles) constituted by a phospholipid bilayer that entraps an aqueous core (1). Depending on the nature of the lipids, different types of liposomes (multilamellar, unilamellar) ranging from very small (20 nm diameter) to very large vesicles (dozens of micrometers) can be formed. Due to their amphiphilicity, hydrophobic molecules can be trapped within the bilayer while hydrophilic molecules can be entrapped in the aqueous compartment (1-3). Physical-chemical properties such as permeability, phase transition temperature or stability depend on the phospholipids fatty acid composition (hydrocarbon chain length, degree of saturation as well as properties of the constituting polar head groups) (4,5).

In textile industry, there is a growing interest in the development of eco-friendly textile processing, with the use of naturally occurring materials (6-8). The non-uniformity that occurs in the dyeing process of polyamide, caused by the irregularities in the surface properties of the fibre, is reduced by the use of levelling agents. The leveling agents can promote both levelness and coverage of fibre irregularities, blocking the accessible sites in the fibre. These products reduce the initial rate of dye uptake and the extent of this retardation in the dyeing rate decreases with the increasing temperature of dyeing. Liposomes release the microencapsulated dye slowly, promoting a retarding effect, comparable with the one obtained with retarding agents, making them a good and eco-friendly alternative to commercial levelling products (8-11).

The retarding effect of liposomes at the first stage of dyeing process with hydrophobic dyes may be due to the higher affinity of the dyes to the liposomes in the bath in comparison to the fibres (9). Another factor can be the chemical structure of the dyes that may affect both the assembly properties of the dye-liposome system and the hydrophobic interactions with the hydrophobic regions within the fibre (11,12).

In previous work (9) we have studied the interaction of soybean lecithin liposomes with polyamide and cotton fibres and it was observed a retarding effect of these liposomes in dyeing assays (8-9). The effect of temperature, pH and surfactant addition on the control release of the dye was also described (13,14). The retarding effect is crucial to achieve level dyeing and is also present (although in less extent) when the dyeing assay is performed with non-encapsulated dyes but in the presence of liposomes (9).

The encapsulation efficiency of the liposomes depends on the chemical structure of the trapped agent (reactive or non-reactive dye), the type of media solution and the additives on the liposome formulation (11). Liposomes may be prepared using amphoteric, anionic or cationic amphiphilic molecules.

A number of synthetic cationic surfactants have been found to form stabilized vesicles and has been widely used in several biological applications such as gene delivery (16-19), antiseptic and disinfectant (20-22). The double-chain quaternary ammonium surfactant DODAB (dioctadecyldimethylammonium bromide) is a synthetic vesicles-forming lipid when dispersed in water above the gel to liquid-crystalline phase transition temperature ($T_m$) (23-25). Since $T_m$ of aqueous DODAB dispersions is between 44.8 and 45.5 °C, it is generally accepted that DODAB vesicles at room temperature are in the gel phase (23,24).

Commercial soybean lecithin, containing (%mol/mol) 22% phosphatidylcholine, 20% phosphatidylethanolamine, 20% phosphatidylinositol and 10% phosphatidic acid as main components, presents a very low $T_m$ ~ -20 ºC and forms large lamellar liposomes in aqueous media.

In this work, the potential usefulness of mixed cationic lecithin/DODAB liposomes for the encapsulation and dyeing with acid dyes is assessed for the case of

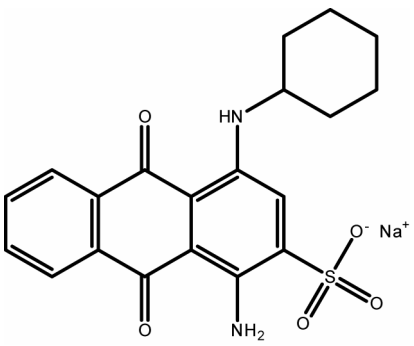
polyamide. The main objective is to increase the retarding effect of acid dye release and, consequently, improving the equalizing effect of the colour, without losing the smoothing effect of lecithin (9).

Dyeing assays were performed with the acid dye Telon® Blue RR (C.I. Acid Blue 62) encapsulated in mixed lecithin/DODAB liposomes and in pure lecithin liposomes, for comparison. To understand the interaction between liposomes and the encapsulated dye, fluorescence spectroscopy was used, due to its high sensitivity and dependence on the environment. As Telon® Blue RR dye is itself fluorescent, the use of external probes is avoided and information about lipid/dye interactions is obtained through the intrinsic fluorescence of the dye.

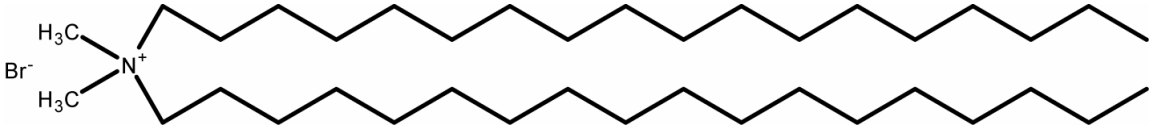
## MATERIALS AND METHODS

Commercial soybean lecithin, containing (%mol/mol) 22% phosphatidylcholine, 20% phosphatidylethanolamine, 20% phosphatidylinositol and 10% phosphatidic acid as main components, was supplied by Stern (Fort Wayne, Indiana, USA).

Acetic acid and sodium acetate were purchased from Sigma-Aldrich (St. Louis, Missouri, USA), while the synthetic lipid dioctadecyldimethylammonium bromide (DODAB) was obtained from Tokyo Kasei (Tokyo, Japan). The dye Telon® Blue RR (Acid Blue 62) sodium salt was purchased from DyStar (Frankfurt, Germany). Structures of the dye and DODAB are shown below.




**Telon® Blue RR dye (sodium salt)**




**DODAB**

## Liposome preparation with Telon® Blue RR (Acid Blue 62)

Liposomes were prepared according to the thin film hydration method. Briefly, lipids were dissolved in chloroform and dried under an argon stream. The lipid film was then hydrated with a buffered aqueous solution (pH = 5.5) containing the dye ($1.02\times10^{-5}$ M) and sonicated for 15 minutes at room temperature for lecithin vesicles and above 45 ºC (the melting temperature of DODAB) (23-25) for lecithin:DODAB mixed vesicles (containing 10% molar fraction of DODAB). The quantity of dye that was not encapsulated into the liposomes was removed by gel filtration chromatography through a Sephadex G-50 (from Sigma-Aldrich) column.

## Spectroscopic Measurements

All the solutions were prepared using spectroscopic grade solvents and ultrapure water (Milli-Q grade). Absorption spectra were recorded in a Shimadzu UV-3101PC UV-Vis-NIR spectrophotometer, equipped with a Peltier temperature controllable cuvette holder. Fluorescence measurements were performed using a Fluorolog 3 spectrofluorimeter, equipped with double monochromators in both excitation and emission and a temperature controllable cuvette holder.

In order to study the effect of temperature on the dye release, aliquots obtained from the chromatographic column, containing liposomes with encapsulated dye, were submitted to increasing temperatures (from 25 ºC to 70 ºC), to simulate the first part of

the dyeing process of polyamide. Telon® Blue RR emission was obtained with an excitation wavelength of 640 nm.

Spectroscopic measurements in the presence of polyamide were also performed, to a more realistic simulation of the dyeing conditions. For this purpose, a piece of polyamide was placed at one face of the fluorescence cuvette containing the solution with the encapsulated dye. The temperature was raised from 25 ºC to 70 ºC and absorption and fluorescence spectra of the remaining dye were measured at several temperatures.

## Dyeing

Dyeing experiments were performed in an Ahiba Turbo Color dyeing machine with a dye-bath ratio (ratio fibre/bath, w/v) of 1:50 in acetate buffer solution (pH=5.5). Dyeing was started at 40 ºC and the temperature was increased to 95 ºC (or 85 ºC in presence of liposomes) at a gradient of 0.8 ºC/min, keeping the dyebath at this temperature during 30 minutes.

The percentage of dyebath exhaustion was measured on a Shimadzu UV/Vis. spectrophotometer at $\lambda_{max}$ of absorption of the dye. The percentage of dyebath exhaustion (%*E*) was calculated according to the following equation:

$$\%E = \left( \frac{A_0 - A_F}{A_0} \right) \times 100$$

where $A_0$ and $A_F$ are the concentrations of the dyebath before and after dyeing, respectively.

## RESULTS AND DISCUSSION

### Telon® Blue RR in homogeneous media

In order to understand the dye behaviour in environments of different polarity, solutions of Telon® Blue RR in different solvents were performed. Telon Blue RR has a very low solubility in non-polar media. UV-Visible absorption and fluorescence emission spectra were recorded in ethanol and acetate buffer pH = 5.5 (Figure 1).

**(Figure 1)**

Absorption spectra are similar in ethanol and aqueous media, the latter presenting a very small red shift (2 nm). From the absorption spectra obtained at several dye concentrations, molar absorption coefficient values (ε) in ethanol and acetate buffer (pH=5.5) were determined through the Lambert-Beer law, $\varepsilon = 8.75\times10^{3}\ M^{-1}\ cm^{-1}$ at 634 nm for ethanol and $\varepsilon = 1.06\times10^{4}\ M^{-1}\ cm^{-1}$ at 636 nm in acetate buffer.

Fluorescence emission in ethanol and acetate buffer exhibit distinct spectral features (Figure 1). In aqueous media, the emission is large and structureless (with a maximum near 715 nm), while in ethanol a more structured spectrum with two bands appears (with maxima at ~680 nm and ~708 nm). For the same concentration, the dye is substantially more fluorescent in ethanol than in buffer solution. This different behaviour is essential to assess the dye environment when encapsulated in liposomes and the changes along the dyeing process of polyamide.

### Telon® Blue RR encapsulated in lecithin liposomes

The fluorescence emission of Telon®Blue RR encapsulated in lecithin liposomes was studied at different temperatures (Figure 2). At room temperature, the fluorescence emission spectra of the dye exhibits notable differences relative to the emission in pure

water (Figure 1), presenting a more structured band and a higher fluorescence quantum yield. This behaviour shows that the dye feels a less hydrated environment when encapsulated in lecithin liposomes.

**(Figure 2)**

An expected decrease of fluorescence intensity with increasing temperature is observed (~50% reduction between 25 ºC and 70 ºC) due to the increase of non-radiative deactivation pathways. However, a change in the emission spectral shape is observed, the spectra at higher temperatures being more similar to the one of free dye in buffer solution (spectral shape of free dye does not vary in this temperature range - *data not shown*). In fact, at lower temperatures, the fluorescence spectrum presents two bands (at ~685 nm and ~715 nm), and the spectral shape is similar to the one observed in ethanol.

This behaviour indicates that, at lower temperatures, the dye is in a less hydrated environment in lecithin liposomes, which can correspond to a location in the liposome membrane, probably near the polar head groups. With increasing temperature, the dye relocates to a more hydrated environment (Figure 2), as inferred from the emission spectral shape and position. Above 55 ºC, the emission spectrum is less structured with only one band and the maximum emission wavelength reaches the value in pure aqueous buffer ($\lambda_{max}$ ~ 716 nm). This behaviour shows that, at higher temperatures, the dye is released from the liposome membrane to the aqueous solution. The potential of lecithin liposomes on the dyeing of polyamide and cotton has already been proven for other dyes (9). These results with Telon® Blue RR dye are also promising for dyeing assays in real conditions (see below).

## Telon® Blue RR encapsulated in lecithin:DODAB liposomes

With the intent of improving the dyeing efficiency with acid dyes, a mixed liposome formulation with commercial soybean lecithin and a cationic synthetic lipid (DODAB) was optimized. The molar ratio 9:1 lecithin:DODAB revealed promising in preliminary polyamide dyeing assays. Therefore, the fluorescence emission of Telon® Blue RR in mixed lecithin/DODAB liposomes (9:1) was studied at several temperatures (Figure 3). As observed for lecithin liposomes, a 47% decrease in emission is detected in the range 25 ºC to 70 ºC. Until the melting temperature of DODAB (~ 45 ºC) (23,24), the fluorescence spectrum is clearly structured with two bands (more structured than the ones observed in lecithin at the same temperatures, e.g. 25 ºC and 35 ºC).

**(Figure 3)**

All spectra are narrower and more intense in lecithin/DODAB liposomes than in pure lecithin liposomes (Figures 2 and 3). Considering that the concentration of dye is the same, this behaviour shows that the dye is globally in a less hydrated environment in the mixed liposomes than in lecithin ones. Figure 4 shows a schematic representation of the interaction of the dye with the liposome components. This behaviour leads us to expect a higher retarding effect in dyeing with the mixed cationic liposomes, promoted by the electrostatic attraction between the positive charge of the cationic lipid and the anionic dye molecule.

**(Figure 4)**

**Telon® Blue RR encapsulated in lecithin liposomes in the presence of polyamide**

From UV/visible absorption measurements, it can be observed that the concentration of dye in solution decreases with increasing temperature (diminution of *ca.* 50% between 25 ºC and 70 ºC), as the dye is progressively being incorporated into the fibre (Figure 5). The effect is very small at low temperatures (≤ 35 ºC). The rising of the spectra at lower wavelengths is due to the characteristic light scattering effect caused by the presence of large structures. Previous work showed that the mean hydrodynamic radius ($R_H$) of soybean lecithin liposomes was near 800 nm (8).

**(Figure 5)**

A decrease in fluorescence intensity with increasing temperature is also observed (Figure 6), but in a higher extent (*ca.* 78%) than in the absence of polyamide (Figure 2).

**(Figure 6)**

The changes in spectral shape are similar to those previously observed in the absence of polyamide suggesting the relocation of the dye in a more hydrated medium at higher temperatures. This shows that, besides the effects previously observed (50% reduction in emission), there is an additional decrease in the dye fluorescence in the presence of polyamide. This behaviour is certainly due to the incorporation of Telon® Blue RR into the polyamide fibre that promotes further release of dye from the liposomes. The decrease in fluorescence emission is especially notable between 65 ºC and 70 ºC, showing that lecithin liposomes release the dye mainly at higher temperatures, which is confirmed by the exhaustion curves (discussed below).

## Telon® Blue RR encapsulated in lecithin/DODAB liposomes in the presence of polyamide

The absorption spectra of Telon® Blue RR encapsulated in lecithin/DODAB liposomes in the presence of polyamide are shown on Figure 7.

**(Figure 7)**

As observed for lecithin liposomes, the dye is progressively incorporated into polyamide but in a higher extent than for lecithin liposomes (in this case, a diminution in absorbance of 72% in the range 25-70 ºC is detected). It is observed that changes in absorbance are negligible at temperatures below 45 ºC, while a significant and progressive decrease is detected at temperatures ≥ 55 ºC. This behaviour shows that, not only the consumption of dye is larger when it is encapsulated in lecithin/DODAB liposomes, but also the retarding effect of these liposomes is more effective, when compared with neat lecithin liposomes. Another distinct feature is the much lower effect of light scattering in the absorption spectra, indicating that these mixed cationic liposomes are smaller than the lecithin ones. In fact, neat DODAB vesicles are smaller ($R_H \approx 544$ nm) (26) than the neat soybean lecithin liposomes ($R_H \approx 800$ nm) (8).

Figure 8 shows the effect of the temperature in the fluorescence of the dye in the presence of polyamide in solution. A decrease in fluorescence intensity of the dye with increasing temperature is observed in a higher extent (*ca.* 75%) than in absence of polyamide (Figure 3), due to the progressive incorporation of the dye into the fibre.

**(Figure 8)**

The behaviour is roughly similar to the one observed with lecithin liposomes, but some important differences are detected. The diminution in fluorescence is almost insignificant between 25 ºC and 35 ºC, showing that dye release practically does not

occur at these temperatures. The fluorescence spectrum remains slightly structured at 55 ºC, indicating that, in average, dye molecules that remain out of the fibre are in a less hydrated environment than in lecithin liposomes at the same temperature. Therefore, for dyeing experiments with these cationic liposomes, we expect a higher retarding effect at lower temperatures, but a higher exhaustion level at higher temperatures, as confirmed by the exhaustion curves obtained in dyeing assays.

## Exhaustion curves

Figure 9 compares the exhaustion curves for Telon® Blue RR free in solution, in the presence of auxiliaries, and when encapsulated in liposomes (lecithin and lecithin/DODAB).

**(Figure 9)**

It can be observed that the commercial auxiliaries have a higher retarding effect, but the exhaustion is not complete at the end of the dyeing process, 90% as compared to 95% obtained with lecithin/DODAB. There is a significant difference when comparing the quantity of free dye left in solution in the case of auxiliaries and of lecithin/DODAB (5%), with implications in both, the efficiency of dyeing and in removing the dye from the effluent. Liposomes have in fact a dye retention effect, lecithin/DODAB being more effective than the pure lecithin ones. Considering the final level of exhaustion, both types of liposomes have a similar performance, near to that obtained with the free dye.

## CONCLUSIONS

The acid dye Telon® Blue RR was encapsulated in liposomes, either composed of soybean lecithin or lecithin/DODAB mixtures containing 10% molar fraction of

DODAB. The effect of increasing temperature on the behaviour of the encapsulated dye was studied through UV-visible absorption and fluorescence emission. For this acid dye, the retarding effect of mixed cationic liposomes showed to be more effective than that of pure lecithin liposomes. Comparing with commercial auxiliaries, the retarding effect of the lecithin/DODAB liposomes in dyeing experiments was similar, but a higher final exhaustion level was achieved. This confirms the potential of the cationic lipid DODAB when used in mixed liposome formulations for dyeing applications.

## ACKNOWLEDGEMENTS

This work was funded by Project ECONATUR, Program SI I&DT - QREN, Agência de Inovação - Portugal, and by Foundation for the Science and Technology (FCT) through the financial support to Centro de Física (CFUM) of University of Minho.

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## FIGURES AND CAPTIONS

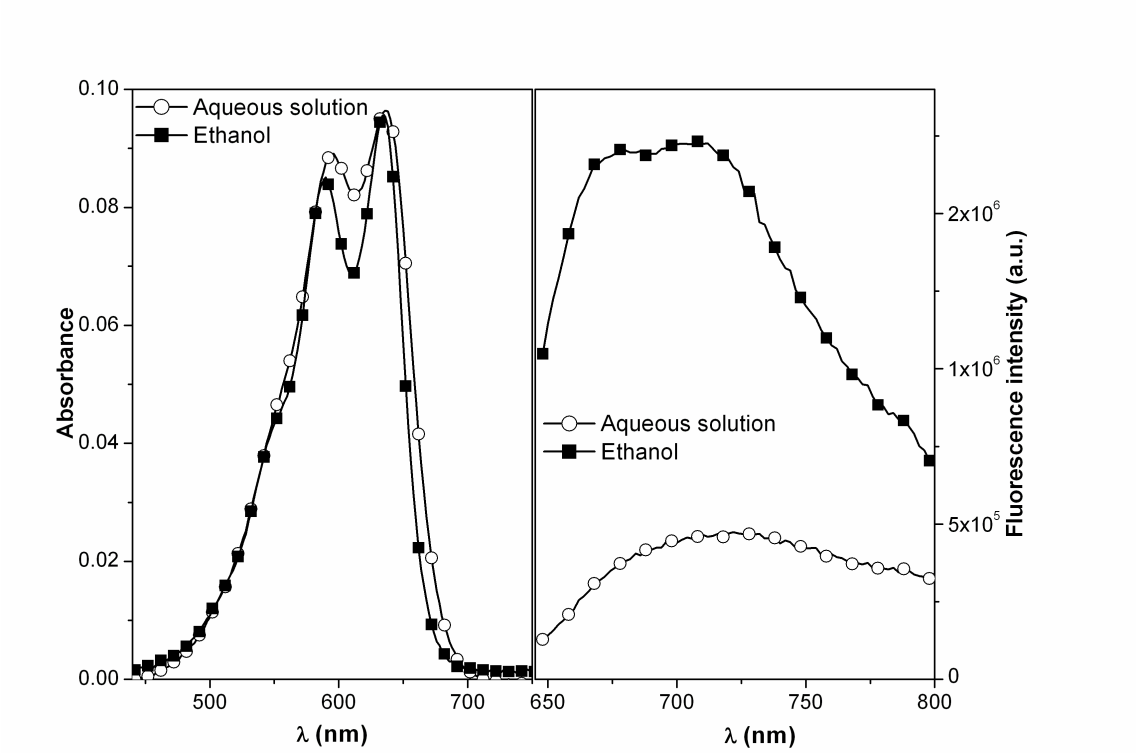


**Figure 1.** Absorption (left) and fluorescence emission (right) spectra of Telon® Blue RR dye ($1.0\times10^{-5}$ M) in aqueous acetate buffer (pH = 5.5) and in absolute ethanol.

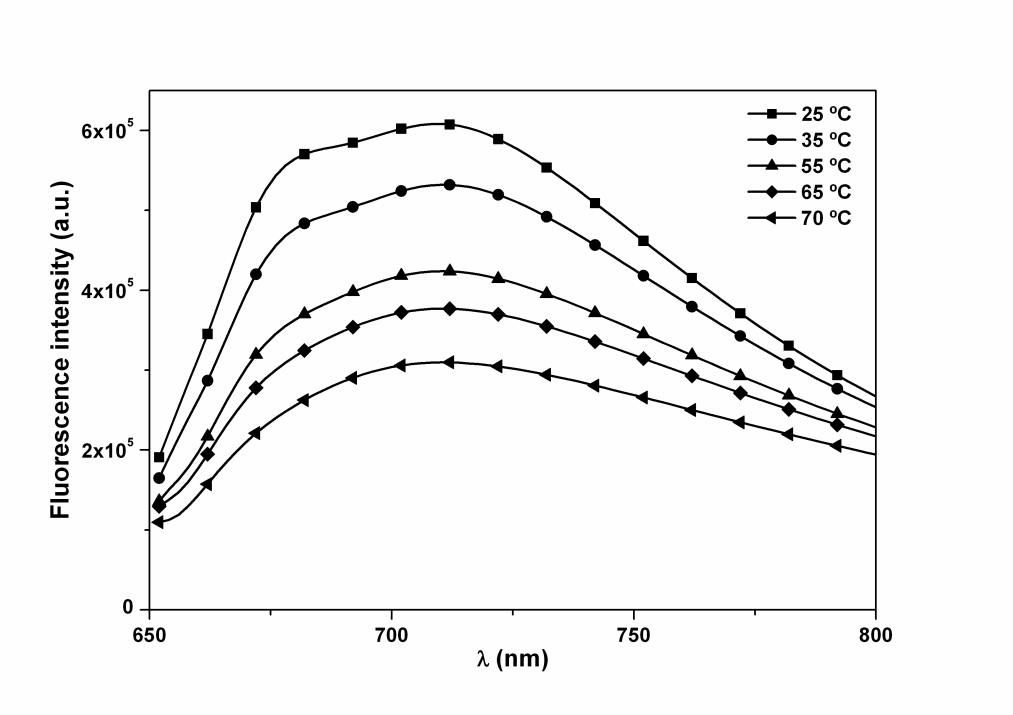


**Figure 2.** Fluorescence spectra of Telon® Blue RR ($2.2\times10^{-5}$ M) encapsulated in soybean lecithin liposomes (1.4 mM), with increasing temperature ($\lambda_{exc}$=640 nm).

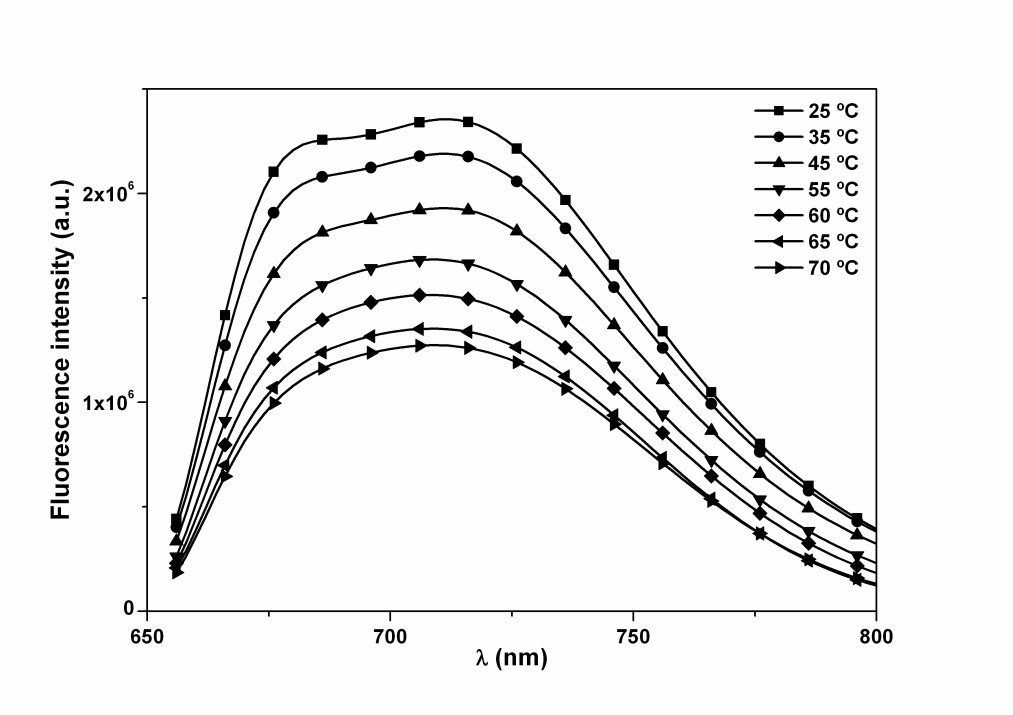


**Figure 3.** Fluorescence spectra of Telon® Blue RR ($2.2\times10^{-5}$ M) encapsulated in mixed soybean lecithin/DODAB liposomes (1.29 mM in lecithin), with increasing temperature ($\lambda_{exc}$=640 nm).

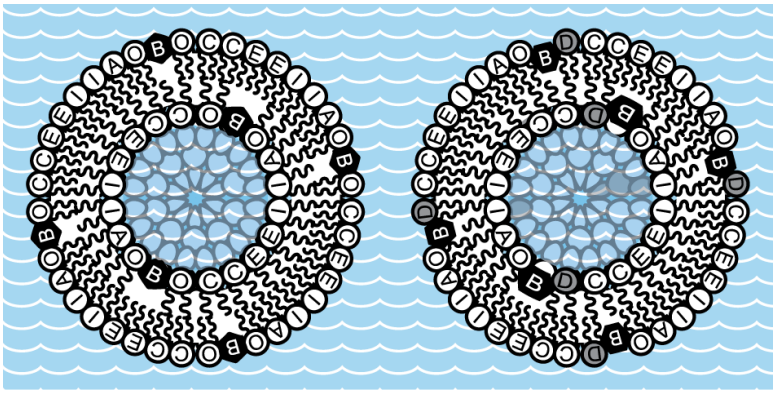

**Figure 4.** Proposed mechanism for the interaction of Telon® Blue RR acid dye (**B**, in black) with liposomes; left side: soybean lecithin liposomes; right side: DODAB:lecithin 1:9 (mol/mol) liposomes. Soybean lecithin components (in white): **C:** phosphatidylcholine, **E**: phosphatidylethanolamine, **I**: phosphatidylinositol, **A**: phosphatidic acid, **O:** other components; **D**: DODAB molecules (in gray).

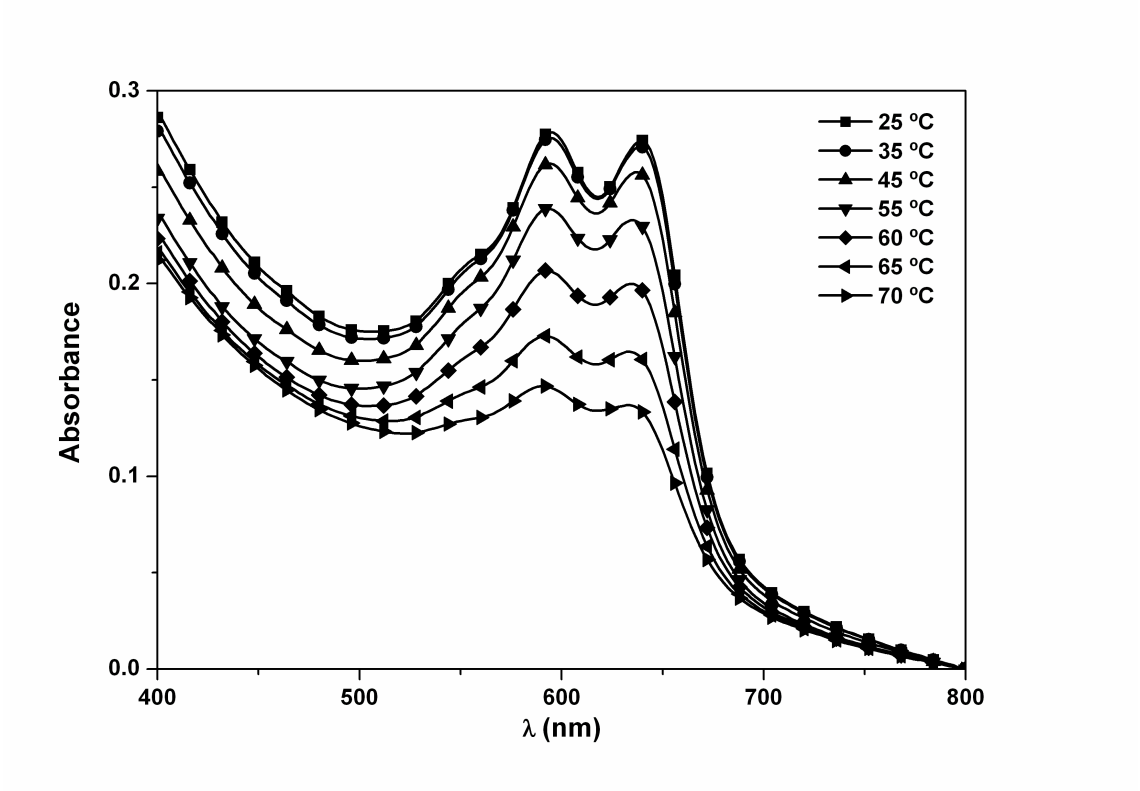


**Figure 5.** Absorption spectra of Telon® Blue RR ($3\times10^{-5}$ M) encapsulated in soybean lecithin liposomes (1.4 mM) in the presence of polyamide, with increasing temperature.

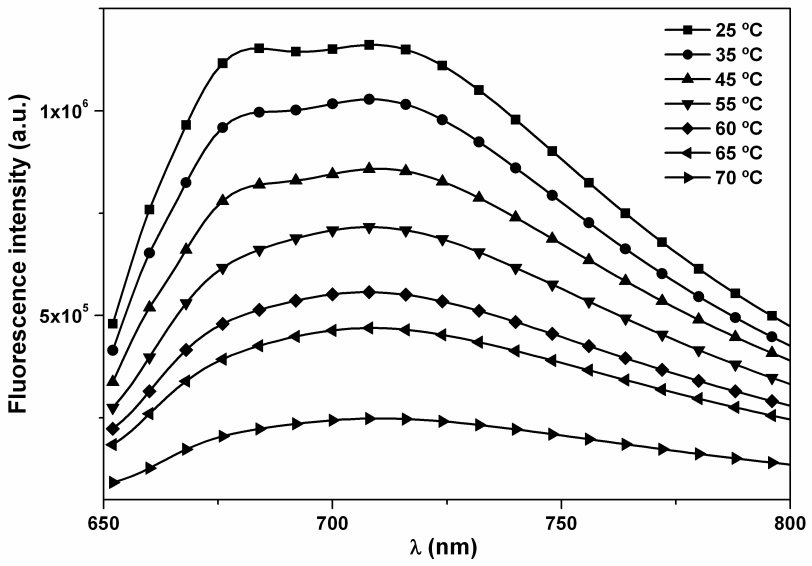


**Figure 6.** Fluorescence spectra of Telon® Blue RR ($3\times10^{-5}$ M) encapsulated in soybean lecithin liposomes (1.4 mM) in the presence of polyamide, with increasing temperature ($\lambda_{exc}$=640 nm).

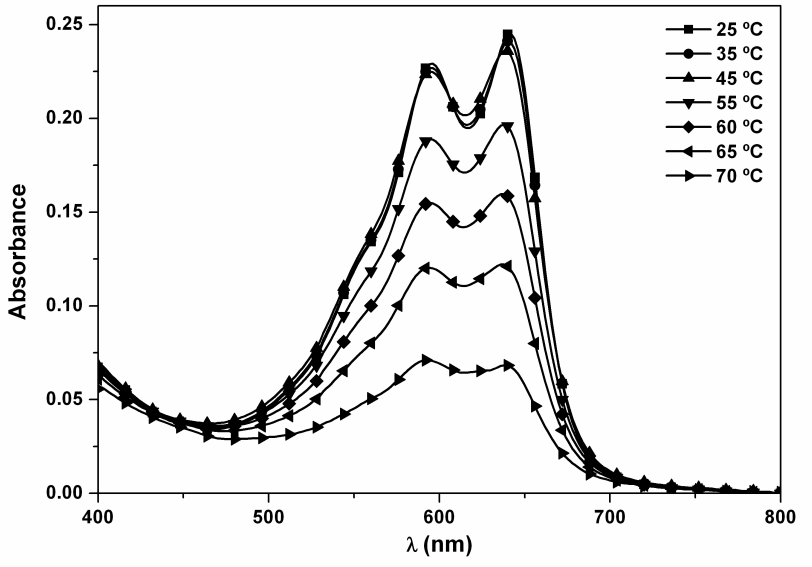


**Figure 7.** Absorption spectra of Telon® Blue RR ($3\times10^{-5}$ M) encapsulated in mixed lecithin/DODAB liposomes (1.29 mM in lecithin), in the presence of polyamide, with increasing temperature.

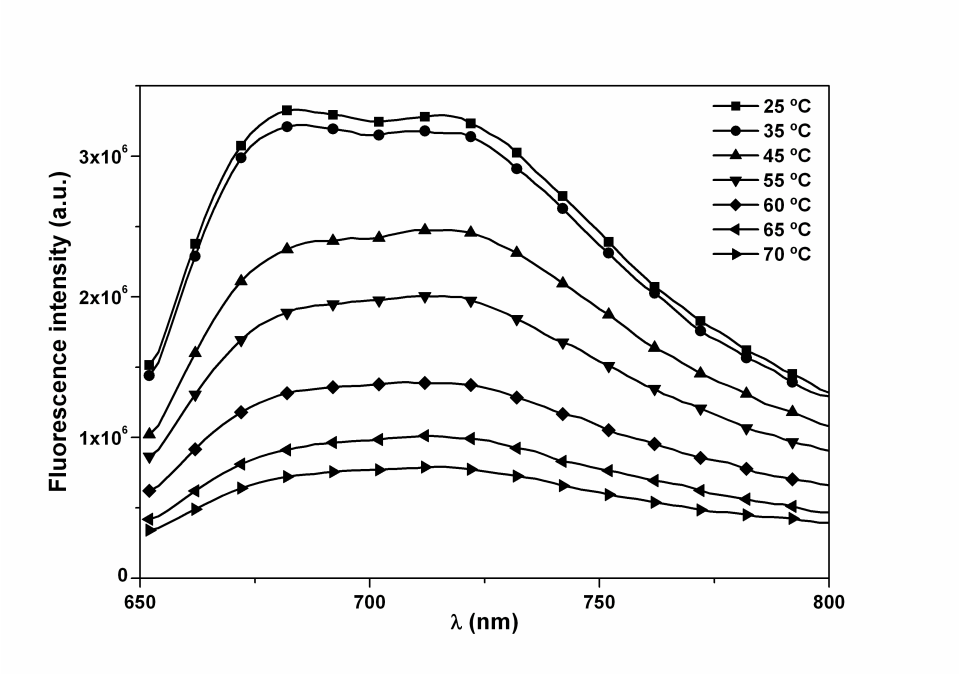


**Figure 8.** Fluorescence spectra of Telon® Blue RR ($3\times10^{-5}$ M) encapsulated in mixed lecithin/DODAB liposomes (1.29 mM in lecithin), in the presence of polyamide, with increasing temperature ($\lambda_{exc}$=640 nm).

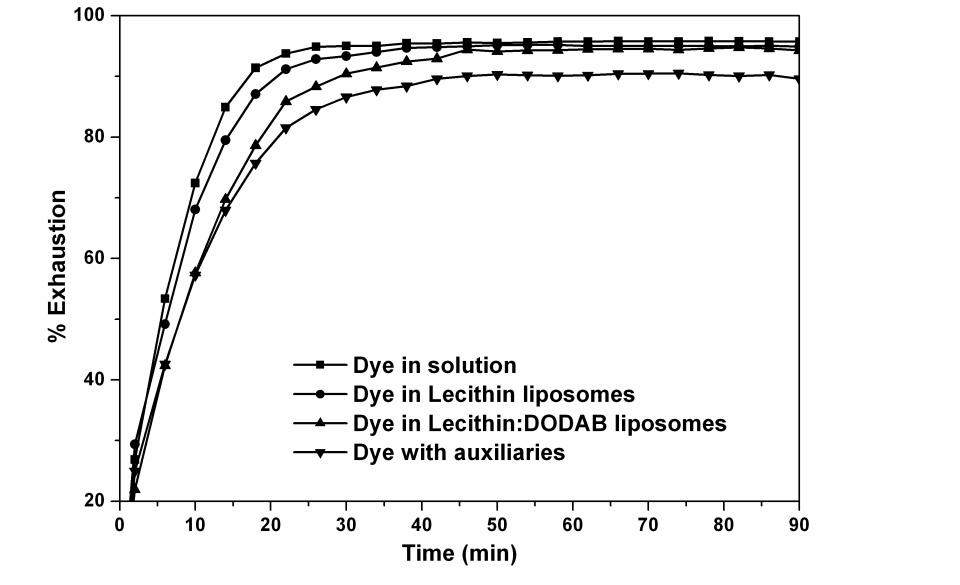


**Figure 9.** Exhaustion curves for the dyeing of polyamide with Telon® Blue RR in solution, either alone, with auxiliaries Sandogene CN/Sandogene NH (1% each), or when encapsulated in lecithin or lecithin/DODAB liposomes.