*Special Issue Article*

# Achiral Dye/Surfactant Heteroaggregates for Chiral Sensing of Phosphocholines

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*ABSTRACT* An investigation, based on absorption and circular dichroism spectroscopy, was carried out on assemblies formed in water upon the interaction of heteroaggregates, composed of dyes (Congo Red or Evans Blue) and cetyltrimethylammonium bromide (CTAB), with four enantiopure phopshocholines (DMPC, DPPC, DOPC, and POPC) characterized by the same polar head and different hydrophobic tails. The results show that the nature of the lipid as well as the concentration ratios influence sensitively the absorption and chiroptical properties of the supramolecular structure. Intriguingly, the transfer of chirality from the lipid to the assembly may be triggered or not, depending on the nature of the lipid hydrophobic chain. These findings confirm the fundamental role of hydrophobic interactions in the transcription of chirality from molecules to complex architectures. *Chirality 00:000–000, 2015*. 

*KEY WORDS:* dye; heteroaggregate; chiral lipid; chiral architecture; circular dichroism; chiral information

Chiral homogeneity is a pervasive feature of Nature at all levels of complexity; in fact, Nature has a preference for one-handedness, from the molecular level to complex systems. Typical examples concern the hops plant, whose twining is always left-handed, the spiral shell of snails which, according to the species, shows a preferential handedness in spiraling, and the male of the marsh fiddler, whose left claw is always bigger than the right one. At the molecular level, the basic bricks of life, amino acids and sugars, are homochiral, as well as the biopolymers they form. The homochirality observed at the various levels of complexity raises questions about the mechanisms that determined it. Questions about when, where, and how molecules of a certain handedness might have overcome the others have stimulated the interest of scientists since the time of Pasteur.[1–11] The chiral homogeneity of Nature brings about also questions on the possible correlation between the chiral homogeneity observed at different levels of complexity. Although many investigations clearly evidenced a connection between chirality at the molecular scale and macroscopic chirality,[12] a clear description of the phenomena and of the interactions involved in the transfer of chirality between systems at different levels of complexity is still missing. Several studies suggested that hydrogen-bond, coulombic, and π-π interactions, and hydrophobic interactions as well, are involved in the propagation process responsible for the chiral homogeneity of complex biological systems.[13–27] In particular, the role of hydrophobic interactions in the transcription of chirality has recently emerged in investigations of dye/surfactant heteroaggregates, which are valuable models for studying the propagation of chirality at the molecular level.[28–31] A combined approach involving absorption and circular dichroism (CD) experiments and computational investigations allowed obtaining information on the contribution of the different interactions involved in the transfer of chirality in these systems. Such studies clearly highlighted that the expression of chirality is the result of a fine balance between electrostatic and hydrophobic interaction.[32]

Here we report on an absorption and CD spectroscopy study aimed at investigating the transfer of chirality from enantiopure phopsholipids to architectures they form with achiral heteroaggregates composed of dyes and the surfactant cetyltrimethylammonium bromide (CTAB), at a concentration of CTAB below its *cmc*. The dyes and phospholipids for this investigation were chosen among the molecules reported in Chart 1.

## MATERIALS AND METHODS

DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcoline), DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine), and POPC (2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine) were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification (purity >99%).

Congo Red (CR), Evans Blue (EB) and CTAB were purchased from Sigma Aldrich (St. Louis, MO).

Solvents were purchased from Sigma Aldrich and were of the highest grade available.

### *Stock Solutions*

Stock solutions of DMPC, DOPC, DPPC, and POPC were prepared in methanol (2.0 mM), stock solutions of CTAB, CR, and EB were prepared in water MilliQ (Millipore, Bedford, MA; 1.0 mM).

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Received for publication 28 July 2015; Accepted 28 September 2015
DOI: 10.1002/chir.22547
Published online in Wiley Online Library (wileyonlinelibrary.com).

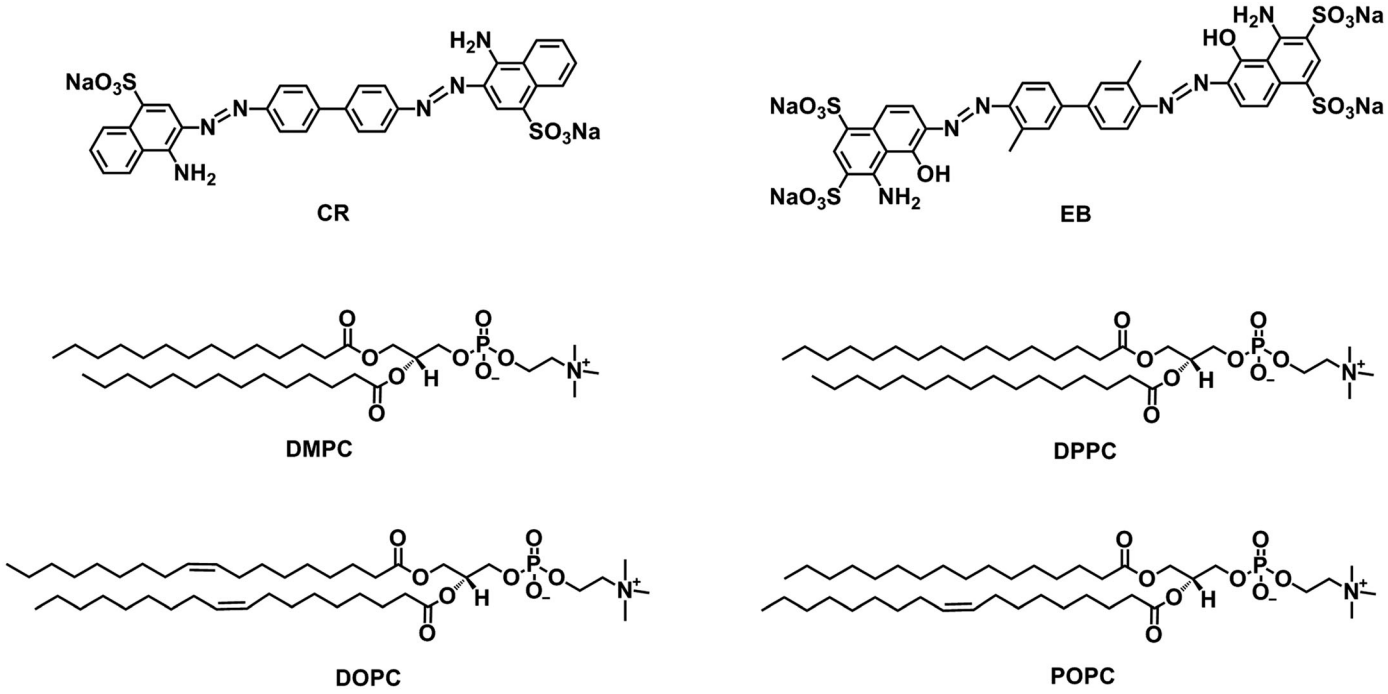


Chart 1

### *Preparation of Samples*

Samples for absorption and CD spectroscopy were prepared in water by adding the proper amount of the dye (CR or EB) stock solution to the appropriate volume of a CTAB aqueous solution to obtain a 20 μM concentration of the dye and 20, 40, 60 μM CTAB. A proper amount of a lipid methanolic solution was added to the resulting solution to obtain a concentration of lipid equal to that of CTAB (20, 40, 60 μM).

### *Absorption and CD Spectroscopy*

Absorption spectra were recorded on a Cary 300 UV–vis double beam spectrophotometer, equipped with a Peltier device for temperature control, using a quartz cuvette of 1.0 cm pathlength. CD spectra were recorded on a Jasco spectropolarimeter J-715 equipped with a Peltier device for temperature control (1.0 cm path length quartz cuvette). Measurements were run in the 800–250 nm spectral range in the case of EB, and in the 700–250 spectral range in the case of CR. CD spectra are the average of 16 scans obtained with an instrument scanning speed of 100 nm/min, response time of 1 s, and resolution of 1 nm.

CD and UV spectra were recorded at 25°C. Samples were analyzed soon after preparation and after heating for 2 h at 50°C.

DMPC, DOPC, POPC, DPPC, and CTAB are transparent in the investigated spectral region, thus the observed absorption and CD bands are due exclusively to the dye.

## RESULTS AND DISCUSSION

It is known that the interaction between ionic surfactants and dyes of opposite charge brings about the formation of aggregates whose features change considerably according to the experimental conditions.

In particular, in premicellar condition, i.e., below the *cmc* of the surfactant, such interactions give rise to dye-surfactant heteroaggregates of definite stoichiometry, whose formation can be evidenced by absorption spectroscopy in the UV–vis range.[28,29]

In previous studies we investigated the transcription of chirality in systems composed of an anionic dye and a chiral cationic surfactant below its *cmc*. In such systems, depending on the length of its hydrophobic chain, the chiral surfactant either stabilizes a chiral conformer of the dye or a chiral topological arrangement of different molecules of dye, as previously shown by combining CD spectroscopy and molecular dynamics simulations.[32]

Given the role of hydrophobic interactions in the transcription of chirality evidenced by our previous investigations,[31,32] the present work aimed at exploring if chiral lipids characterized by different hydrophobic chains might give rise to chiral induction phenomena in the supramolecular structure they form with achiral heteroaggregates composed of an anionic dye and CTAB. The results indicated that, depending on the hydrophobic region of the lipid and on the nature of the dye, only certain lipids are able to induce bands in the dye CD spectrum, thus confirming the central role of hydrophobic interactions in the transcription of chirality from molecules to complex systems.

Different phosphocholines were added as a methanolic solution to an aqueous solution of heteroaggregates composed of 20 μM dye (CR or EB) and CTAB, the latter at different concentrations (20, 40, or 60 μM), to obtain CTAB/lipid in a 1:1 ratio.

The resulting samples were analyzed at 25°C both by UV–vis absorption and CD spectroscopy experiments, soon after preparation. Heating was then carried out in order to allow the sample to reach the equilibrium condition. Samples were heated for 2 h at 50°C and afterwards CD and absorption spectra were recorded again at 25°C, after cooling. Effects evidenced in the CD and UV–vis spectra recorded soon after sample preparation were under kinetic control; these results were then compared with those recorded after equilibration.

The formation of the heteroaggregates upon mixing CR and CTAB in pure water was clearly evidenced by the change in the UV–vis spectra of the samples recorded soon after preparation (Fig. 1A). In fact, a general hypochromic effect was observed at all the ratios considered. Moreover, the band centered at about 500 nm showed a hypsochromic

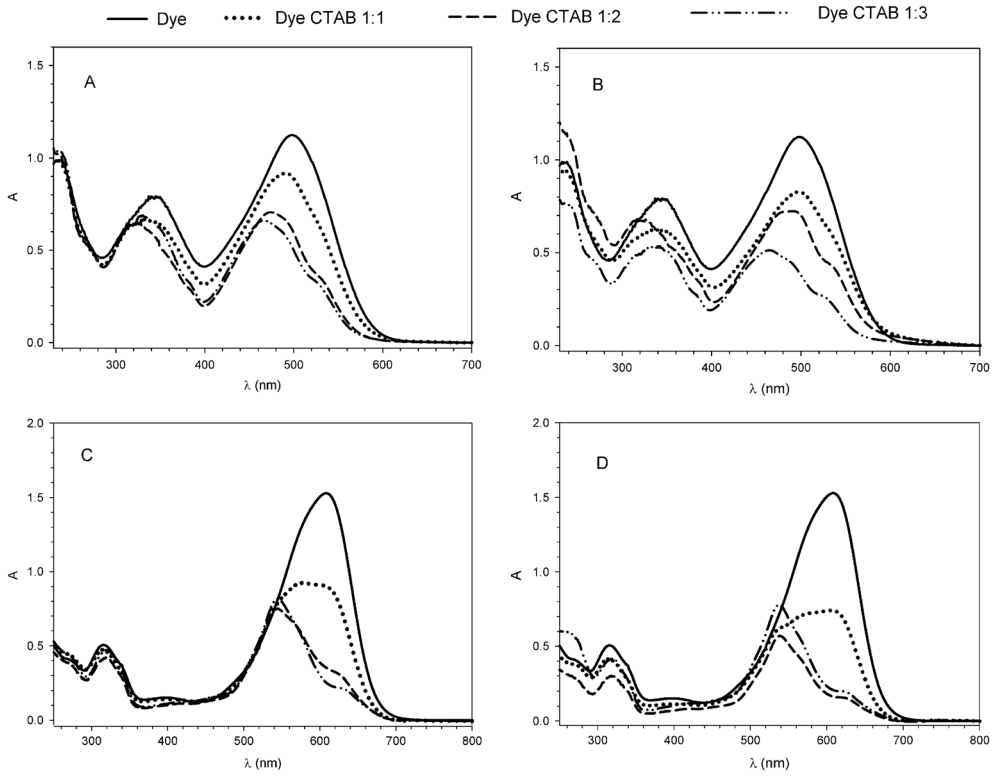


**Fig. 1.** Absorption spectra of the heteroaggregates dye/CTAB at different ratios (**A** and **B** for CR, **C** and **D** for EB), recorded at 25°C. Sample were analyzed soon after sample preparation (**A** and **C**), then they were heated at 50°C for 2 h and the spectra recorded again after cooling (**B** and **D**). Absorption spectra of a 20 μM aqueous solution of dye in the presence of 20 μM (dot line), 40 μM (dashed line), and 60 μM (dashed-dot line) CTAB. The solid line in **A-D** is the absorption spectrum of a 20 μM aqueous solution of dye.

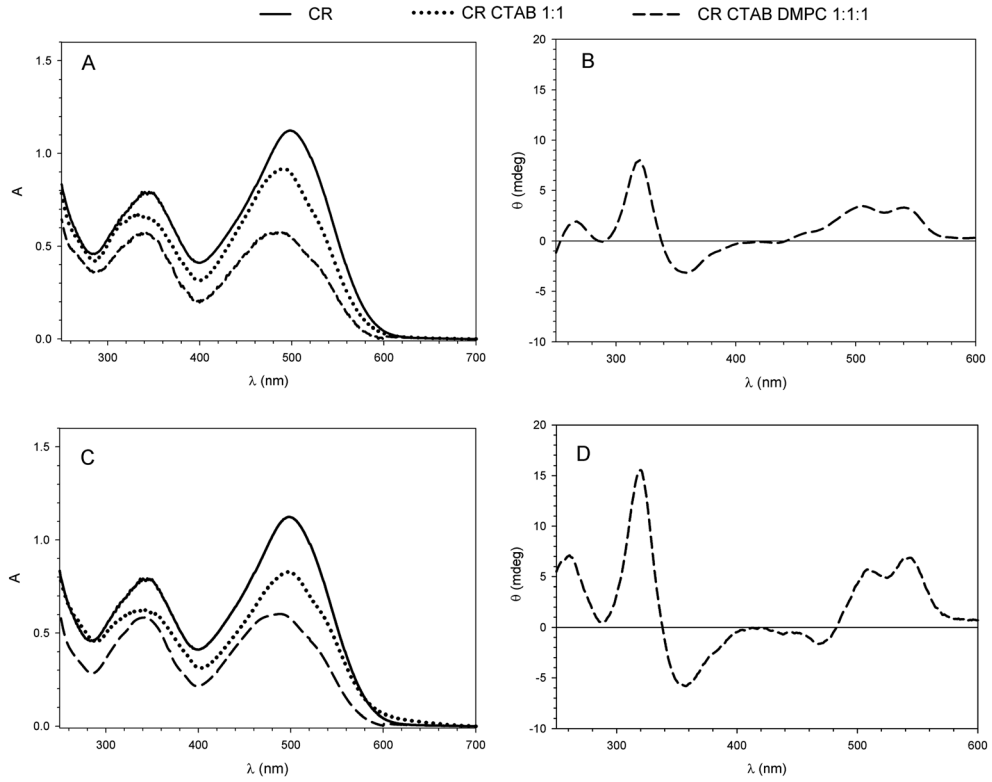


**Fig. 2.** Absorption (**A** and **C**) and CD (**B** and **D**) spectra of: a 20 μM aqueous solution of CR (solid line); an aqueous solution of 20 μM CR in the presence of 20 μM CTAB (dotted line); an aqueous solution of 20 μM CR in the presence of 20 μM CTAB and 20 μM DMPC (dashed line). All the spectra were recorded at 25°C, soon after sample preparation (**A** and **B**) and after heating at 50°C (**C** and **D**).

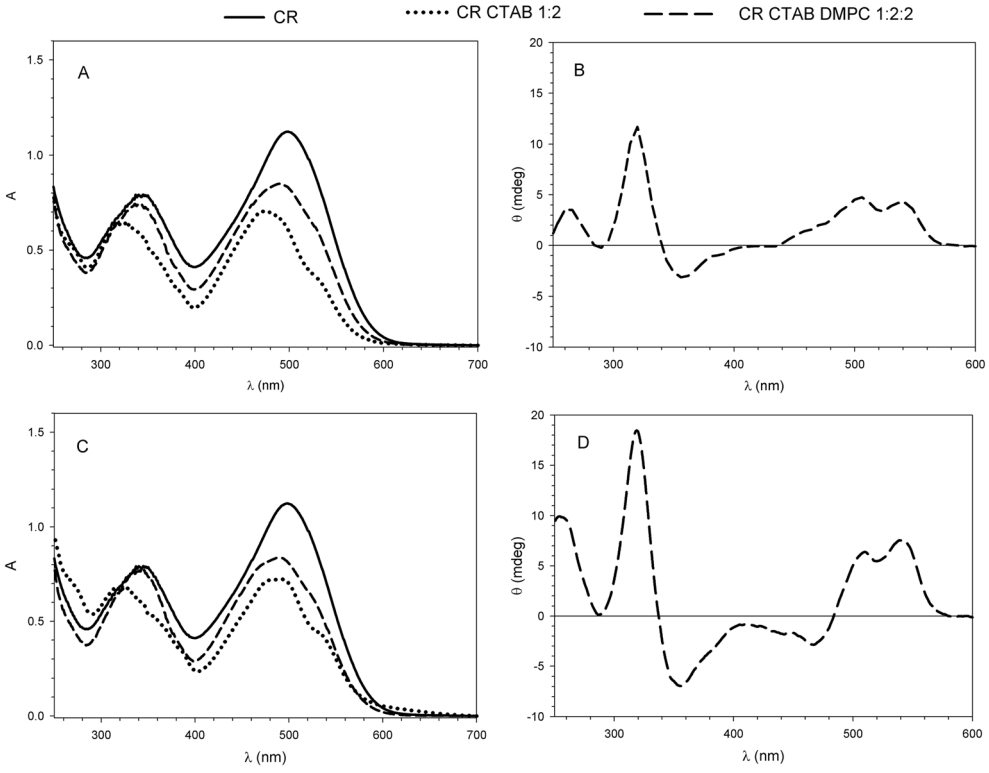


**Fig. 3.** Absorption (**A** and **C**) and CD (**B** and **D**) spectra of: a 20 μM aqueous solution of CR (solid line); an aqueous solution of 20 μM CR in the presence of 40 μM CTAB (dotted line); an aqueous solution of 20 μM CR in the presence of 40 μM CTAB and 40 μM DMPC (dashed line). All the spectra were recorded at 25°C, soon after sample preparation (**A** and **B**) and after heating at 50°C (**C** and **D**).

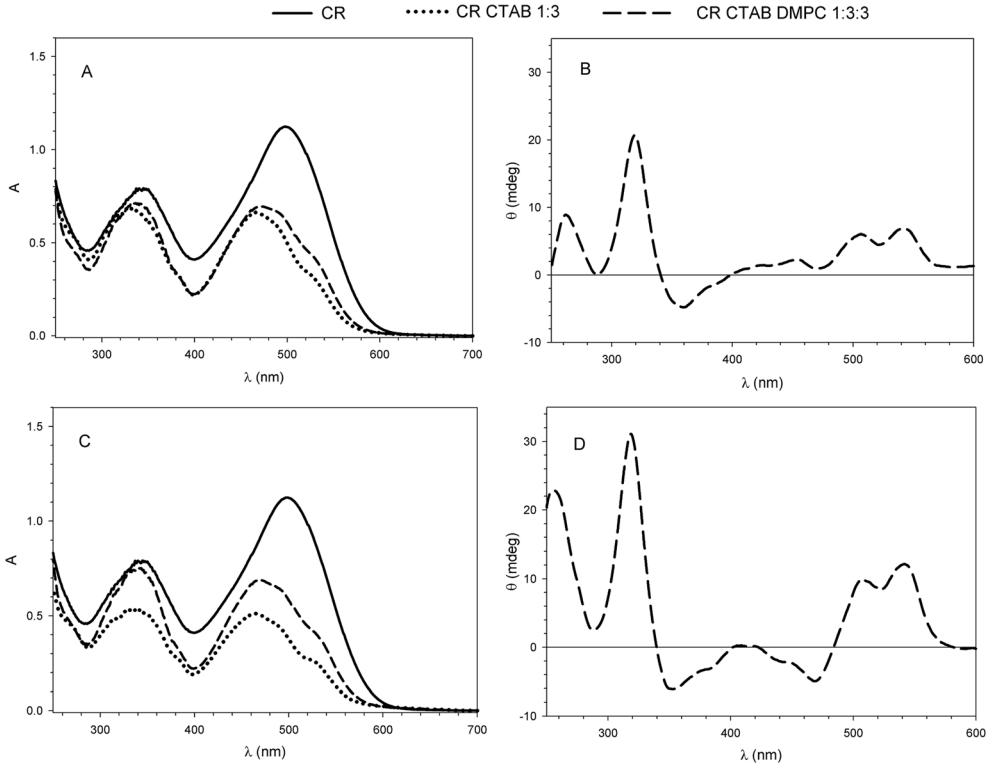


**Fig. 4.** Absorption (**A** and **C**) and CD (**B** and **D**) spectra of: a 20 μM aqueous solution of CR (solid line); an aqueous solution of 20 μM CR in the presence of 60 μM CTAB (dotted line); an aqueous solution of 20 μM CR in the presence of 60 μM CTAB and 60 μM DMPC (dashed line). All the spectra were recorded at 25°C, soon after sample preparation (**A** and **B**) and after heating at 50°C (**C** and **D**).

effect in addition to the appearance of a shoulder at all the molecular ratios considered. The absorption spectra did not change substantially after heating, except for the spectrum of the sample at a CR/CTAB 1:3 ratio, where the relevant hypochromic effect is due to the occurrence of flocculation. (Fig. 1B).

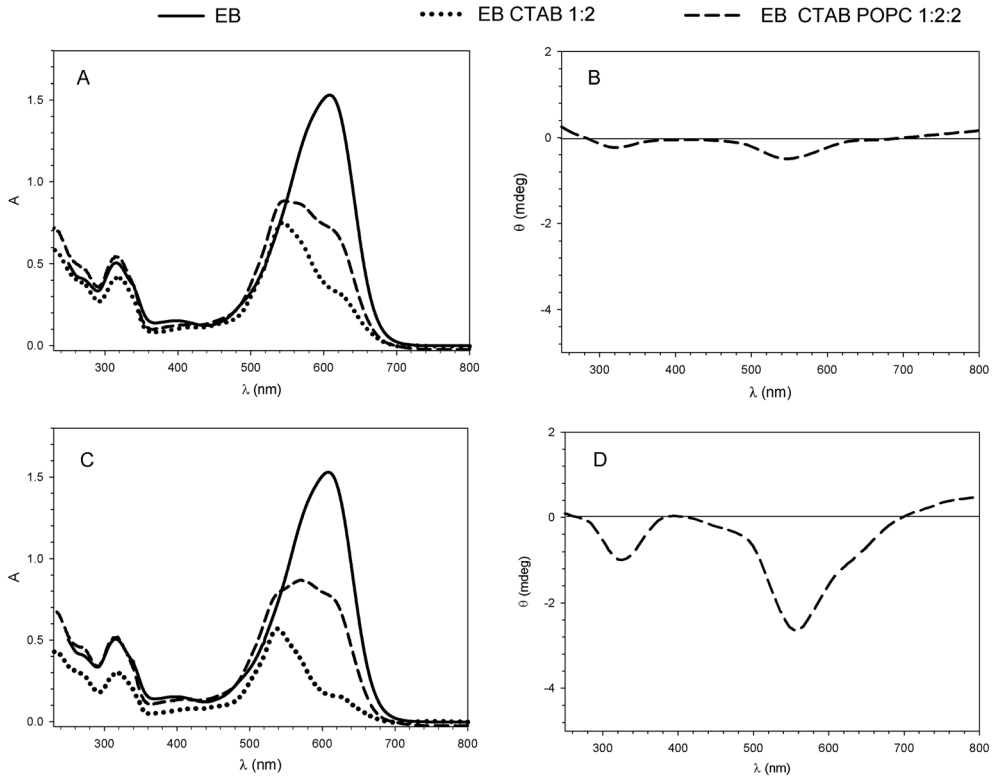


**Fig. 5.** Spectroscopic features of the heteroaggregates of EB/CTAB 1:2 and EB/CTAB/POPC 1:2:2. Absorption spectra of a 20 μM aqueous solution of EB in the presence of 40 μM CTAB (dotted line) and in the presence of 40 μM CTAB and 40 μM POPC (dashed line) before (**A**) and after (**C**) heating at 50°C. CD spectrum of a 20 μM aqueous solution of EB in the presence of 40 μM CTAB and 40 μM POPC before (**B**) and after (**D**) heating at 50°C. The solid line in **A** and **C** is the absorption spectrum of a 20 μM aqueous solution of EB. All the spectra were recorded at 25°C.

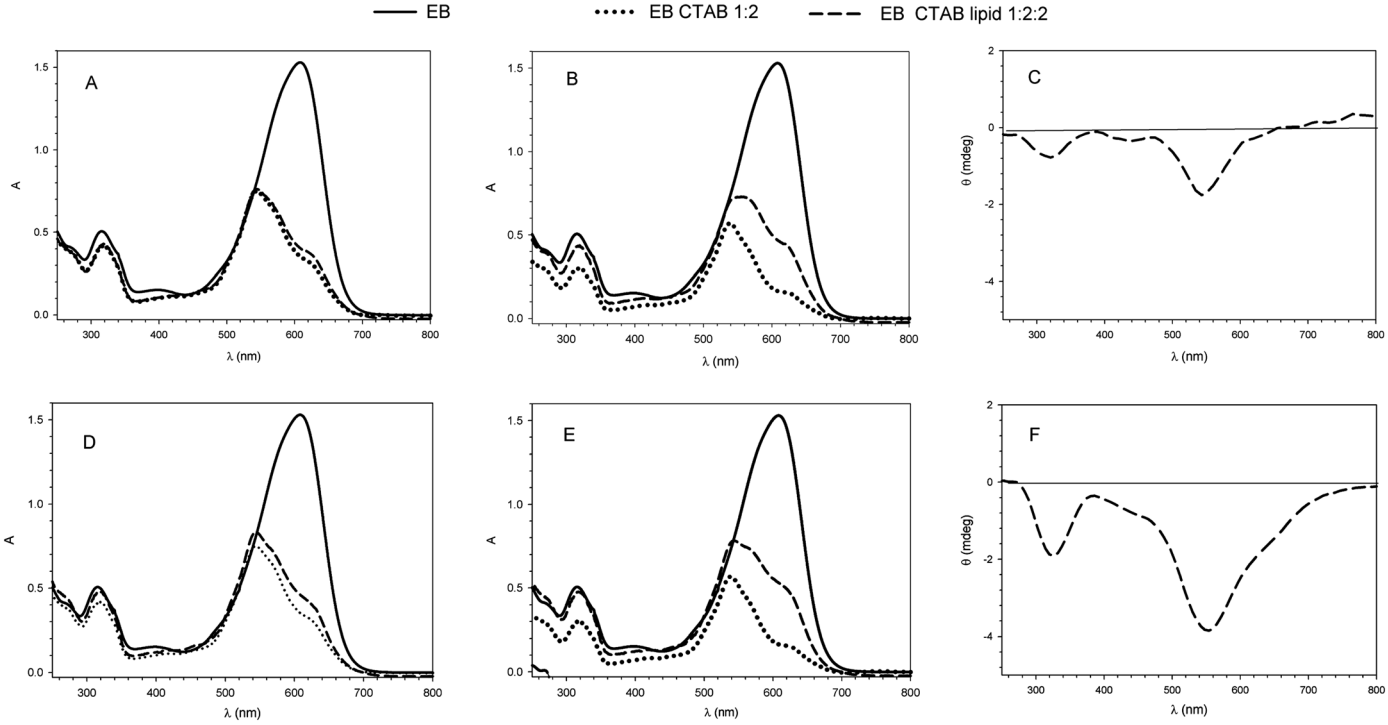


**Fig. 6.** Spectroscopic features of heteroaggregates EB/CTAB/lipid at the 1:2:2 ratio before and after heating at 50°C. Absorption spectra of a 20 μM aqueous solution of EB in the presence of 40 μM CTAB (dotted line) and in the presence of 40 μM CTAB and 40 μM DPPC (dashed line) before (**A**) and after heating (**B**). Absorption spectra of a 20 μM aqueous solution of EB in the presence of 40 μM CTAB (dotted line) and in the presence of 40 μM CTAB and 40 μM DOPC (dashed line) before (**D**) and after heating (**E**). CD spectrum of a 20 μM aqueous solution of EB in the presence of: 40 μM CTAB and 40 μM DPPC after heating (**C**); 40 μM CTAB and 40 μM DOPC after heating (**F**). The solid line in **A, B, D** and **E** is the absorption spectrum of a 20 μM aqueous solution of EB. All the spectra were recorded at 25°C.

Analogously, the formation of the heteroaggregate upon mixing EB and CTAB was clearly evidenced by a general hypochromic effect in the UV–vis spectra of the dye recorded soon after preparation (Fig. 1C). Furthermore, the appearance of a shoulder and a substantial blue shift of the band centered at ~610 nm were observed for samples at the ratio 1:2:2 and 1:3:3.

The addition of the lipids to the preformed heteroaggregates induced further changes in the absorption spectra of CR and EB, thus indicating the formation of a new assembly formed by the three components; however a CD spectrum was generated only by the addition of DMPC to CR/CTAB system and of DPPC, DOPC, and POPC to the EB/CTAB system. We will discuss only the results (absorption and CD spectra) of experiments where the addition of lipid induced the appearance of a CD band. All the other absorption spectra are available as Supporting Information (SI).

The addition of DMPC to CR/CTAB heteroaggregates (Figs. 2–4) induced a sharp hypochromic effect in the systems at the 1:1:1 ratio, a further weak blue shift in the UV–vis spectrum, and the appearance of a positive band in the CD spectrum in all the CR/CTAB/DMPC samples, whose intensity depends on the CR/CTAB/DMPC ratio (Figs. 2B, 3B and 4B). Heating of samples induced an increase of CD band intensity (Figs. 2D–4D).

The addition of phosphocholines to the EB/CTAB systems did not induce further changes in the UV–vis spectra of the dye, except for POPC samples (Figs. 5A,C and S4–6), and heating induced precipitation in all the samples at the 1:3:3 ratio (Fig. S6B).

EB samples showed the presence of CD bands in many cases, thus revealing the transfer of chirality from the lipid to the assembly. However, all the observed CD bands were of very modest intensity. In the case of POPC, a negative CD band was observed at the 1:2:2 ratio (Fig. 5B) whose intensity increased after heating (Fig. 5D). On the other hand, the appearance of a negative CD band was induced by the addition of DOPC and DPPC to the 1:2 EB/CTAB system (EB/CTAB/lipid 1:2:2) only after heating (Fig. 6C,F).

As expected, the transcription of lipid chirality to the dye was observed only upon the addition of the lipid to dye/CTAB heteroaggregates. In fact, dye/lipid systems (in the absence of surfactant) are CD silent, although their absorption spectra show in some cases a consistent hypochromic effect (Fig. S7-8), confirming the interaction of the lipid with the dye.

Therefore, it is clear that the chiral information of the lipid is transferred to the heteroaggregate as a whole as evidenced by the CD spectrum of the dye embedded in the supramolecular architecture. Regarding the origin of the observed CD spectra, in this kind of systems the occurrence of CD bands in the region where the dye absorbs can derive from two main phenomena: 1) the deracemization of the biphenyl moiety of dye, i.e., an imbalance in the 1:1 equilibrium ratio of the interconverting enantiomers of the dye biphenyl residue, 2) a preferential handedness in a chiral arrangement of different dye molecules, giving rise to exciton coupling. To ascertain the nature of the phenomena responsible for the occurrence of the CD bands, theoretical investigations are required.

The effect of heating in all the investigated samples was modest in the case of absorption spectra, whereas it was somewhat more evident, in some cases, in the CD spectra. These observations suggest that the systems formed under kinetic control are not radically different from those under thermodynamic control. Probably, in the case where a CD band was observed, the assembly formed under kinetic control undergoes a rearrangement upon heating that does not alter dramatically its morphology, but rather the mutual disposition of the components inside it and/or the conformation of the dye, resulting in the appearance of a CD band or in an increasing of the CD band intensity.

Dye-surfactant heteroaggregates are interesting systems that have been investigated in particular for the development of dye technology. However, information about their morphology and dimensions are not reported in the literature, to the best of our knowledge. On the basis of our investigations we suggest that dye-surfactant heteroaggregates, formed under premicellar conditions, might feature an extremely extended rod-like structure.

Upon addition of lipid to the preformed heteroaggregates, we envisage an evolution of the system towards two possible novel architectures. The novel architecture could retain a rod-like structure in which the added lipid is intercalated.

As an alternative, since the considered lipids exhibit a critical aggregative concentration far below the concentration of the investigated samples, the novel assembly could display a vesicular structure. In this case, upon addition of the lipid, the original rod-like heteroaggregate could decompose in small clusters which could be embedded in the vesicle.

Independently from the kind of morphology of the aggregates, it is evident that the lipid inside the structure is able in some cases to give rise to chiral induction phenomena

The above-described results clearly indicate that aggregates composed of dye, CTAB and lipid give rise to absorption and CD spectra that are markedly influenced by the nature of the system (type of lipid, concentration ratio). Further, the CD experiments showed on the one hand that CR/CTAB heteroaggregates are capable of discriminating DMPC with respect to other phosphocholines characterized by a different hydrophobic portion. In fact, DMPC is the only phosphocholine, among those analyzed in this work, capable of inducing the appearance of a band in the CD spectrum of CR/CTAB heteroaggregates. On the other hand, the EB/CTAB heteroaggregates investigated in the same concentration conditions showed a modest recognition capability of the lipids as the observed CD spectra of the different samples were similar and of modest intensity.

The formation and organization of dye/surfactant heteroaggregates are controlled by the balance between electrostatic and hydrophobic interactions. In the comparison of CR/CTAB and EB/CTAB systems, the electrostatic interactions might have a role more relevant in the latter case due to four versus two sulfonate groups of the dye. The modest contribution of the hydrophobic interaction to the organization of the assemblies could account for the modest recognition capability of EB/CTAB heteroaggregates toward phosphocholines characterized by different hydrophobic portions.

## CONCLUSION

The results described in this work evidenced that aggregates composed of dye, CTAB, and lipids are suitable systems for studying the transfer of chirality at different levels of complexity.

The absorption and CD investigation of achiral heteroaggregates in the presence of enantiopure phosphocholines

evidenced that the transfer of chirality from the lipid to the assembly is strictly dependent on the hydrophobic portion of the lipid, on the structure of the dye, and on the concentration ratios.

Once again it was highlighted that in some cases small variations in the hydrophobic portions of the chiral molecule may dramatically affect the occurrence and nature of chiral induction phenomena.

These preliminary observations suggest that dye/surfactant heteroaggregates could be used to develop sensors based on the concept of differential sensing for the detection of lipids and hydrophobic species. The screening of many dye/surfactant heteroaggregates could lead to the development of an electronic tongue for hydrophobic species. In fact, according to the concept of differential sensing, each dye/surfactant heteroaggregate will not necessarily recognize with high specificity a given hydrophobic species, but its response (optical and chiroptical changes) will be complementary to those of other heteroaggregates and the combination of all responses will generate a specific univocal response for each species.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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