

Preparation and characterization of mixed monolayers and Langmuir–Blodgett films of merocyanine 540/octadecylamine mixture

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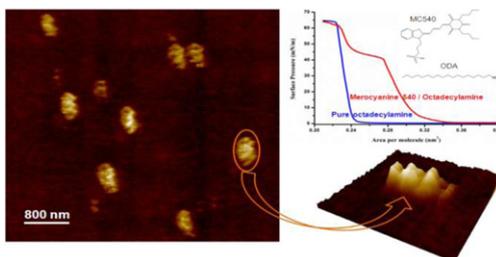
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HIGHLIGHTS

- ▶ Langmuir–Blodgett films of merocyanine 540/octadecylamine mixture were fabricated.
- ▶ Photophysical properties of merocyanine 540 were followed as spectroscopic.
- ▶ H-aggregate formation of merocyanine 540 at solid and liquid phases was characterized.
- ▶ Surface morphology of thin film was examined by using atomic force microscopy.

GRAPHICAL ABSTRACT

Langmuir–Blodgett films (LB) of merocyanine 540 (MC540) mixed with amphiphilic octadecylamine (ODA) on glass substrate are fabricated by co-spreading method. The formation of stable LB film of MC540 mixed with ODA on water subphase is checked by surface pressure–area (π -A) isotherm.



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ABSTRACT

Mixed monolayer of merocyanine 540 (MC540) dye and octadecylamine (ODA) at the air/water interface has been prepared using the co-spreading method. The pressure–area (π -A) isotherm studies revealed that the mixtures of MC540/ODA at a different ratio formed a stable monolayer at the air/water interface and these floating layers were easily transferred onto hydrophilic substrates as the Y-type Langmuir–Blodgett (LB) film. The specific area per molecule in the π -A isotherm of the mixed monolayer of MC540/ODA was larger than that of the pristine ODA. The area can also get increased up to 40 mN/m surface pressure by the increase of the dye concentration in the mixtures. The LB films of MC540/ODA mixture were formed at 30 mN/m surface pressure by transferring the mixed monolayer at the air/water interface on hydrophilic glass substrate via vertical dip-coating as mono- and multilayer films. The photophysical properties of MC540 in chloroform and LB films have been investigated using the absorption, steady-state and time-resolved fluorescence spectroscopy techniques. H-aggregate formation of MC540 both in chloroform and LB film were concluded from the spectroscopic results. The morphology of the one-layer mixed LB film of MC540/ODA on the glass substrate has been characterized by AFM (atomic force microscopy). The non-contact mode AFM image of the one-layer mixed LB film showed that the film surface consisted of MC540/ODA nanoclusters. In conclusion, our results contribute to understanding the strong interaction between MC540 and ODA at the air/water interface, and show the effects of some parameters on the mixed LB films of MC540.

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1. Introduction

Thin film of functional dye molecules has a significant role in the design of ultrafast, miniaturized, optoelectronic and photonic devices [1]. There are several techniques for the fabrication of thin films of functional dye molecules, such as spin-coating [2],

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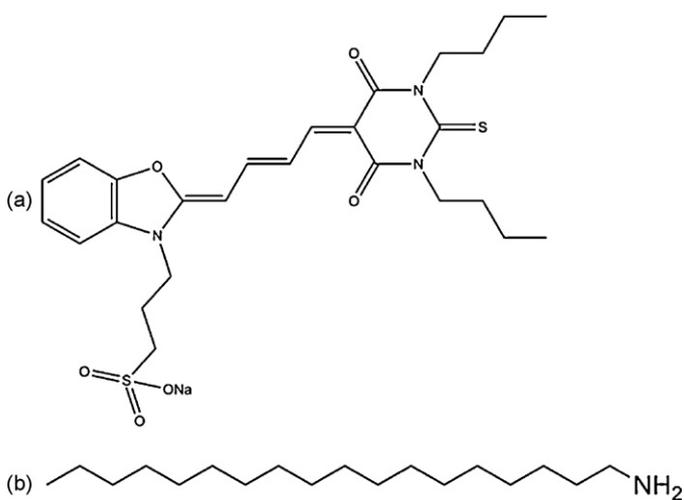


Fig. 1. Chemical structures of merocyanine 540 (MC540) and octadecylamine (ODA).

layer-by-layer [3], Langmuir–Blodgett (LB) techniques [4,5] etc. Among these, the LB technique is a unique method that provides flexibility in controlling the spatial distribution and the orientation of the dye molecules in the film matrix [4–6]. In the LB technique, the molecular architecture and thickness can be precisely controlled by monitoring certain parameters such as the pH of the subphase, barrier speed, dipping speed, molar composition, temperature, and the surface pressure of lifting of the LB film [7]. Therefore, the LB method has been widely used to fabricate thin films of a large variety of organic molecules. Amphiphilic organic molecules and numerous polyaromatic hydrocarbon derivatives have been extensively used for LB film applications because they form a superb monolayer at the air/water interface [8]. In contrast, nonamphiphilic and water-soluble organic molecules have presented limited use in LB film studies because nonamphiphilic molecules tend to form microcrystals at the air/water interface and water-soluble molecules move into the water subphase during the evaporation of the volatile solvent [9]. Therefore, nonamphiphilic and water-soluble molecules are difficult to transfer onto solid substrates for the purpose of the generation of high-quality LB films. This problem can be overcome by doping nonamphiphilic or water-soluble molecules with amphiphilic molecules such as fatty acids, octadecylamine and some phospholipids, which form excellent monolayers at the air/water interface [9–14]. The mixture containing water-soluble molecules (or nonamphiphilic molecules) and amphiphilic molecules usually results in the formation of a stable floating layer that could potentially be easily transferred onto a solid surface [15]. In the LB matrix, the water-soluble molecules can be embedded within the doped amphiphilic molecules or formed a water-insoluble complex with them. In contrast, nonamphiphilic molecules tend to form a sandwich-type structure as a result of squeezing between head groups of the amphiphilic molecules and water subphase [13–15].

Dye molecules have attracted a great deal of attention in the area of electronics and optoelectronics due to their potentially low cost and ease of designing at the molecular level [16]. The most important requirement for the use of functional dye molecules in the technological applications is the preparation of their thin films [17]. The thin film of dyes with the desired optical and morphological properties can be fabricated by the LB film technique, easily [4,5]. One of these dye molecules is merocyanine 540 (MC540), whose molecular system shows an extended conjugation that is responsible for its absorption at the longer wavelength in the visible spectrum (Fig. 1) [18]. MC540 is an anionic lipophilic

polymethine dye that is used as the fluorescent probe for studying biological membranes and sensitizers for photodynamic therapy [9–21]. The photophysical properties of the dye strongly depend on changes in environmental factors such as polarity, viscosity and temperature [18]. For example, the fluorescence quantum yield of MC540 in an aqueous solution is low due to its ability to form non-fluorescent aggregates [21]. MC540 molecules also have a tendency to bind micelles, liposomes or vesicles [22]. Therefore, their non-fluorescent aggregates dissociate into fluorescent monomers in the presence of a medium containing micelles or vesicles [21]. Although there are numerous reports on the LB film structures of amphiphilic merocyanine dyes [23], there is currently no study regarding the LB and the mixed LB films of MC540 doped with ODA. Therefore, it would be interesting to understand photophysical properties of MC540 in LB matrix and the role of interaction between the dye and ODA for biomedical application.

Herein we report the preparation of the mixed monolayer of MC540 with amphiphilic ODA at the air/water interface and the photophysical properties of the dye in an LB film. The spectroscopic properties of MC540 in chloroform and LB film were determined by using absorption and fluorescence (steady-state and time-resolved) spectroscopy techniques. The surface morphology of the one-layer mixed LB films has been characterized using the non-contact mode AFM (atomic force microscopy). Our results contribute to the understanding of the strong interaction between MC540 and ODA at the air/water interface, where the concentrations of MC540 can modify the film. Additionally, this study supports noteworthy findings related to the nanostructure and optical properties of MC540 dye at solid surface.

2. Experimental

2.1. Materials

MC540, octadecylamine (ODA), and chloroform were purchased from Sigma–Aldrich.

2.2. Methods

A commercially available LB trough (KSV, Minithrough system) was used for the deposition of mono- and multi-layer LB films. In LB film experiments, pure deionized water used for the subphase was obtained from a KrosClinic (model: KRS-R-75). The pH of the subphase was 6.3 and the temperature was 22 °C. The preparation of LB films of MC540/ODA mixture was outlined in the Supporting Information.

2.3. Instrumentation

The absorption spectra were recorded on a PerkinElmer (Model Lambda 35) spectrophotometer at room temperature. The absorption spectra of the dyes in chloroform were taken in a quartz cuvette with a dimension of 0.5 cm × 1.0 cm. Steady-state fluorescence spectra were taken with a Shimadzu RF-5301 PC Spectrofluorophotometer. Fluorescence decays for the lifetime measurements and the emission spectra were carried out with a Laser Strobe Model TM-3 lifetime fluorometer from Photon Technology International. The details of this method have been given elsewhere [15]. All measurements relating to fluorescence studies have been recorded by using a 0.5 cm × 1.0 cm fluorescence quartz cuvette. AFM image of the LB film was performed in air using Nanomagnetics instruments obtained from Ankara, Turkey. The image was acquired in a non-contact mode.

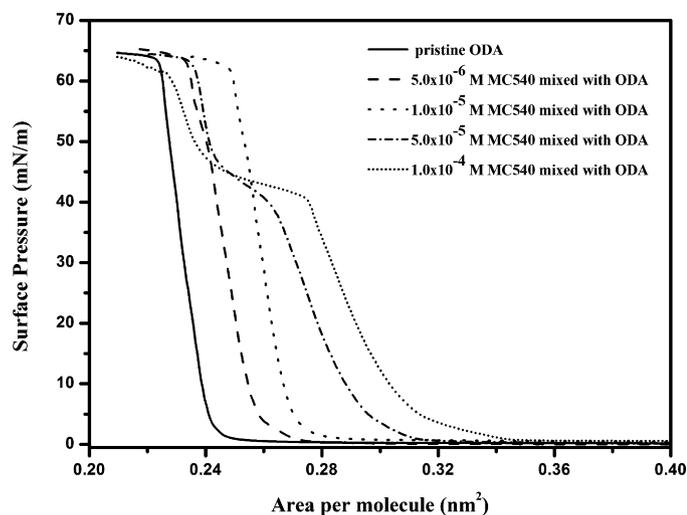


Fig. 2. Surface pressure–area (π -A) isotherms of pristine ODA and the mixture of MC540/ODA at different ratio.

3. Results and discussion

3.1. Surface pressure–area (π -A) isotherm of the mixtures of MC540/ODA

The formation of the stable and floating layers of both pristine ODA and MC540/ODA mixtures at the air/water interface was confirmed by using the surface pressure–area (π -A) isotherms. When 35 μ l of pristine ODA in chloroform (0.5 mg/ml was used for all experiments and kept constant) was spread and compressed on the pure water subphase, the obtained π -A isotherm showed a small liquid-phase region before it reached the close-packed solid phase [24]. The specific area per molecule for pristine ODA was calculated as ~ 0.24 nm², which is comparable with the value given in literature [11]. Following this, the same π -A isotherm study was performed for pristine MC540. In this instance, the surface pressure did not rise sufficiently to be applied to a high-quality LB film when pristine MC540 monolayer at the air/water interface was compressed at a slow rate. Additionally, fluorescence studies indicated that a section of MC540 molecules penetrated into the subphase during the evaporation of volatile solvents during the compression process (Fig. S1). Therefore, pristine MC540 molecules do not form a self-supporting monolayer at the air/water interface because the dye does not have long enough alkyl chains, which prevent submergence of the dye into subphase. In order to overcome this problem, it was anticipated that dye molecules mixed with any amphiphilic molecules could be incorporated in LB films via the acknowledged co-spreading method [10]. This procedure has been applied in many studies including nonamphiphilic and water-soluble molecules [9–15]. In this regard, the performed π -A isotherm studies verified that the MC540/ODA mixture formed highly stable and floating layers at the air/water interface. The ODA-shaped π -A isotherms were obtained by the mixtures of MC540/ODA at different ratios in which the MC540 concentration was altered from 5.0×10^{-5} M to 1.0×10^{-4} M. Fig. 2 shows the π -A isotherms of pristine ODA and the mixtures of MC540/ODA at a different ratio at 22 °C. The π -A isotherms of the mixtures showed an expansion in comparison to that of pristine ODA, and the degree of this expansion increased by increasing the level of MC540 in the mixture, which is clearly shown in Fig. 2 [11]. In other words, the specific area per molecule obtained from the π -A isotherm of the mixture containing 1.0×10^{-4} M MC540 is greater than those of the others containing a lower level of MC540 (Fig. 2). The expansion indicates that MC540 molecules are

retained among ODA molecules at the air/water interface, where ODA molecules can act as a supporting matrix. The shape of π -A isotherms of pristine ODA and MC540/ODA mixtures at low dye loading (5.0×10^{-6} M and 1.0×10^{-5} M) are very similar to each other, with the exception of the specific area per molecule. At the higher dye loadings (5.0×10^{-5} M and 1.0×10^{-4} M), the π -A isotherms showed a plateau region starting at surface pressures of 40 mN/m. The flat plateau becomes more apparent by the increase of the dye concentration in the mixture. Such a plateau in the π -A isotherms has been observed in some molecules and frequently interpreted in connection with phase transition, which is generated by effective changes in orientation and the arrangements of molecules at monolayer and molecular aggregation [25]. Since the plateau formation in the π -A isotherm is only observed at high dye loadings, the MC540 concentration in the mixture is considered for the molecular arrangement in the LB matrix as a driving force. The arrangement of MC540 dye molecules at a higher surface pressure bring about the plateau formation due to the fact that dye molecules at high loadings form aggregate structures that are arranged in a side-by-side and tail-to-tail conformation of dye molecules. Additionally, this plateau corresponds to a two-dimensional to three-dimensional (2D-to-3D) phase transition of MC540 molecules in ODA matrix. The absorption properties of MC540 in LB film revealed that the compression of mixture at the air/water interface for the higher surface pressures induced the plateau formation due to molecular aggregation of MC540 in ODA matrix, which is illuminated by the UV-vis absorption study (Fig. S2). As a result of spectroscopic study, the molecular aggregation of MC540 that took place at the higher surface pressure was determined by the increase in the absorbance of the MC540 aggregate band. The expansions or changes in the π -A isotherm were followed by the molecular area at several surface pressures when ODA was mixed with MC540 at different ratios. The molecular packing of a mixed monolayer could be drawn from the plot of the area per molecule (nm²) versus the concentrations of MC540 (Fig. S3). The surface area was increased by the increase in the ratio of MC540/ODA under 40 mN/m surface pressures (Fig. S3). In contrast, the surface area at higher pressures was increased up to 1.0×10^{-5} M and then decreased with the increase of the MC540 concentration. In this case, it is also possible that the dye molecules remain underneath the head groups of the ODA monolayer, compressing the barriers or some of the dyes that are submerged into the water subphase, as well as previous explanations related to the alteration of the π -A isotherm. The possibility of a particle of MC540 penetrating into the subphase was followed by a fluorescence study. The results of fluorescence study proved that MC540 molecules at the air/water interface did not pass into the water subphase. Additionally, the surface area at the lower dye concentration was sharply increased, while the change of the surface area at the higher dye concentration level was gradual (Fig. S3). This observation implies that the molecular aggregation of MC540 molecules in the ODA matrix take place at the higher concentration levels. Consequently, the strong electrostatic interaction between cationic amino groups of ODA and anionic MC540 molecules prevent the escape of the dye molecules into the water subphase. The strong interaction enables MC540 molecules to stand at the air/water interface. This result gives a remarkable contribution to the dye molecules binding to the model membrane systems.

3.2. Photophysical properties of MC540 in chloroform and LB film

3.2.1. Absorption spectroscopy

The absorption properties of dye in chloroform were initially investigated in a wide range of concentration (1.0×10^{-6} M– 1.0×10^{-4} M) for the determination of the molecular behavior of MC540 dye in the LB matrix. Fig. 3 shows the normalized

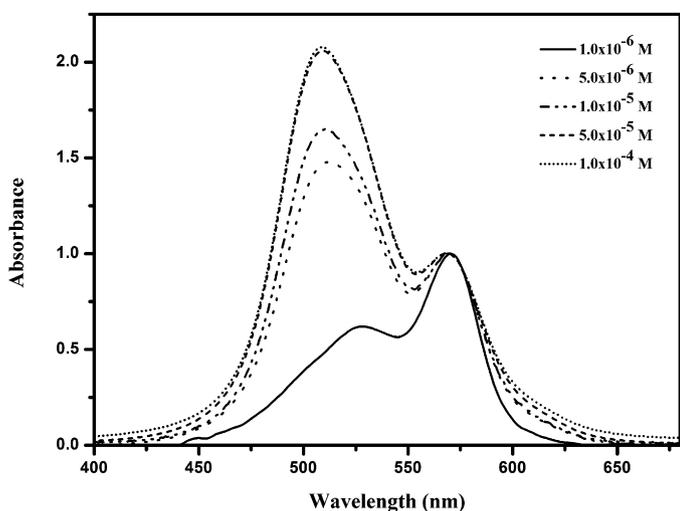


Fig. 3. Normalized absorption spectra of MC540 in chloroform.

absorption spectra of MC540 with respect to the monomer maximum in chloroform at different concentrations. MC540 dye in chloroform has two absorption bands at low concentration levels (Fig. 3). The intense band at 570 nm belongs to MC540 monomers in chloroform. This band maximum in chloroform was reported to be 568 nm and 572 nm [18,26]. The other band at ~ 528 nm related to H-dimer formation of MC540. The equilibrium between the maximum absorbance values of two bands in chloroform was changed by increasing the dye concentration levels. The absorbance of the monomer band decreased and that of the H-dimer increased with the shift of the band maximum when the dye concentration was increased to 1.0×10^{-4} M. In the meantime, the H-dimer band maximum of MC540 in chloroform shifted to the blue region, which appeared at 512 nm for 1.0×10^{-5} M and at 509 nm for 1.0×10^{-4} M. Additionally, the H-dimer band was broader compared to that in the lower dye concentrations. The absorption band observed at the blue region reveals the different molecular behavior of MC540 molecules in the concentrated solution. There are two absorption bands in the dimer spectrum of MC540 in chloroform as readily seen in Fig. 3. This is illuminated by the second derivative and deconvolution of the absorption spectrum of MC540 in chloroform [27]. These methods are important for the determination of the absolute maxima of absorption bands, which are particularly used for the characterization of overlapping absorption bands due to their intense aggregation [28]. The second derivative spectra of MC540 in chloroform depending on the dye concentrations (Fig. S4) demonstrated that there were two bands observed at 571 nm and 528 nm at 1.0×10^{-6} M dye concentration, which were attributed to monomer and H-dimer, respectively. A new band appeared in the blue region with increasing dye concentration compared to that in the diluted dye concentration. The maximum of this band was observed at 504 nm when the other band maxima were constant. The absorption band maxima of MC540 in chloroform were confirmed by the deconvolution spectrum of MC540 at 1.0×10^{-4} M dye concentration (Fig. S5). The deconvolution spectrum composed of three absorption bands which are located at 503 nm, 530 nm and 570 nm. The band positioning in the deconvolution spectrum are compatible with those in the second derivatives analysis of MC540 absorption spectra in chloroform. According to the spectral analysis of MC540 in chloroform, the band at ~ 528 nm and ~ 504 nm attributed to H-dimer and higher aggregates (H-aggregates) while the absorption band at 571 nm belonging to MC540 monomers. H-aggregate formation of MC540 is well-known in the presence of a cationic surfactant in nonpolar solvents [29]. Additionally, the formation of the band observed at 504 nm in chloroform contributes

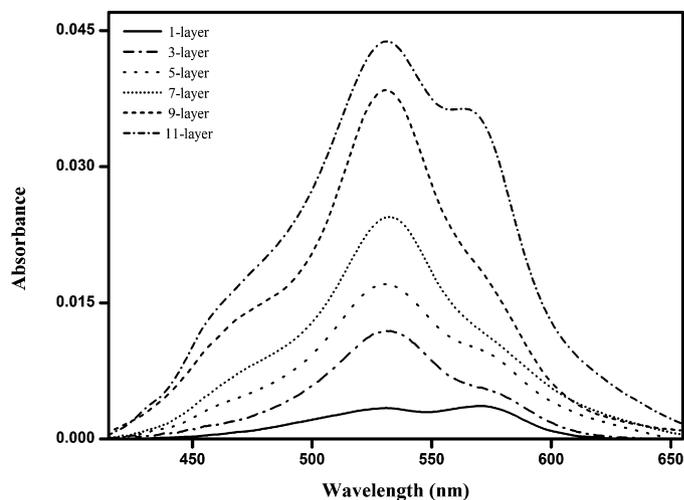


Fig. 4. Absorption spectra of mixed LB films of MC540/ODA.

color change of the dye solution (Fig. S6). Therefore, the spectroscopic characterization of the H-aggregate formation of MC540 in a solution is important for the biomedical applications.

Absorption characteristics of MC540 in the LB film were investigated by the preparation of mono- and multilayer films. For this purpose, the mixed LB films of MC540/ODA at a certain (1.0×10^{-4} M) dye concentration were fabricated at 30 mN/m surface pressure. Fig. 4 shows the absorption spectra of mono and multilayer LB films of MC540/ODA mixture. It can be seen from Fig. 4 that there are two intense absorption bands aroused at 530 nm and 571 nm in the absorption spectrum of the one-layer mixed LB film of MC540/ODA. The absorption band at 530 nm revealed the H-dimer structure of MC540, while the absorption band at 571 nm attributed to monomeric form of the dye in LB film. The small red-shift observed at the absorption band maxima of the H-dimer and monomer in the case of the mixed monolayer, with respect to those in chloroform stems from the organized aggregation of MC540 in LB film. The number of MC540/ODA layers transferred onto glass substrate was increased to 11. It was concluded upon the careful examination of Fig. 4 that absorption characteristics of MC540 in LB film were drastically affected by the mixed layers being transferred onto glass surfaces. This observation for the absorption property of MC540 implies that the interaction between the transferred layers takes place and this also triggers the molecular arrangement of the dye. It can also be concluded from Fig. 4 that the increase in the number of transferred layers decreased the intensity of the monomer band at 571 nm when it increased H-dimer band observed at 530 nm in LB films. Additionally, the multilayer LB film of MC540/ODA resulted in the formation of a new absorption shoulder of approximately at 470 nm. The new absorption band implies the formation of higher aggregates of MC540 in LB film known as H-aggregate. The clear absorption maximum of this band was confirmed by the deconvolution of the total absorption spectrum. Fig. 5 demonstrated the deconvolution absorption spectrum of the nine-layer mixed LB film of MC540/ODA. The analysis result exposed the presence of three absorption bands in the absorption spectrum of nine-layer LB film, which was located at 476 nm, 532 nm and 575 nm. In the multilayer LB films, the increase in the dye aggregation is due to the interaction of dye molecules in one monolayer with ODA and MC540 molecules of another monolayer. As results of the second derivative and deconvolution spectra of MC540 in different media, the increase in the degree of aggregation caused a blue-shift in aggregate band maximum of the dye. Spectroscopic differences and band splitting observed in aggregated system are explained according

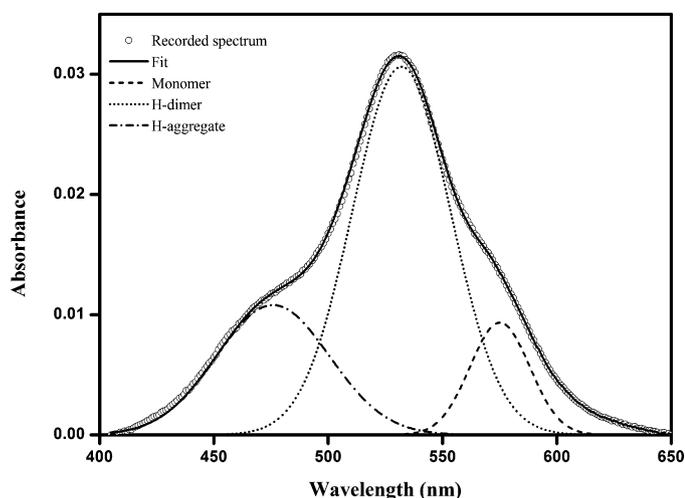


Fig. 5. Deconvolution of absorption spectra of nine-layer MC540/ODA LB film.

to the exciton theory based on monomer dipole–dipole interaction in the aggregates [30,31]. Strong electronic coupling between the dye molecules in aggregate units causes the formation of H-aggregates, J-aggregates and dimers. In the case of the H-aggregate, the absorption band maximum arises at the blue region compared to the monomer band and they decrease fluorescence properties of dye molecules. In contrast to the H-aggregate, J-aggregates have a red-shift and narrow absorption band with respect to the monomer and they enhance the fluorescence intensity of the dyes. The information related to spectroscopic and photophysical properties of aggregate structures is available in literature [15,30,31].

3.2.2. Fluorescence spectroscopy

In order to determine fluorescence properties of MC540 in chloroform and LB film, steady-state fluorescence spectra of the samples were studied at the 536 nm excitation wavelength. The presence of intense non-fluorescent H-aggregation of MC540 in chloroform drastically influences the fluorescent properties of the dye. Therefore, the characterization of fluorescence properties of dye in chloroform and LB film are essential for technological applications. It was reported that the fluorescence maximum of MC540 in chloroform was formed at 589 ± 1 nm [18,26]. The fluorescence properties of MC540 in a wide concentration range from 1.0×10^{-6} M to 1.0×10^{-4} M were examined. Fig. 6 shows the fluorescence spectra of MC540 in chloroform. One intense fluorescence

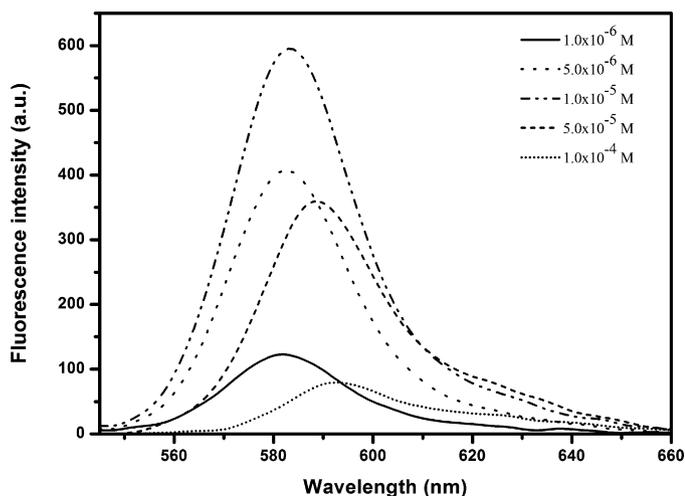


Fig. 6. Fluorescence spectra of MC540 at different concentration in chloroform.

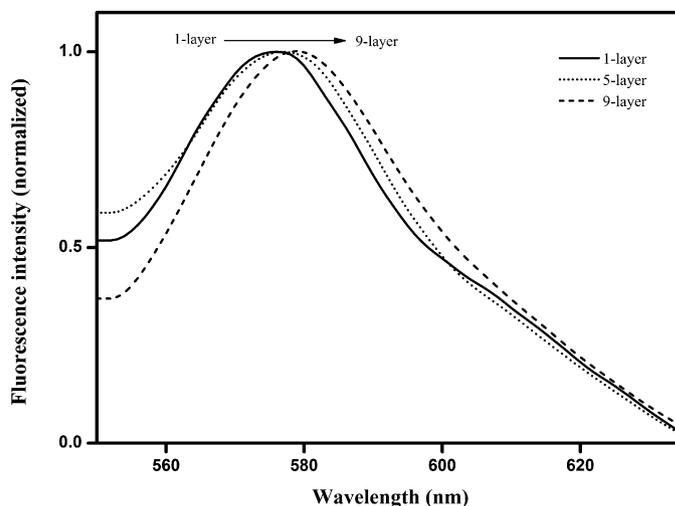


Fig. 7. Normalized fluorescence spectra of mixed LB films of MC540/ODA.

band at 583 nm was observed at dilute dye concentration which is attributed to the monomeric form of MC540 molecules in chloroform. The fluorescence maximum and intensity of MC540 in chloroform was altered with an increase in the dye concentration. For example, the fluorescence maxima were observed at 588 nm for 5.0×10^{-5} M and 593 nm for 1.0×10^{-4} M and the fluorescence intensity was strongly quenched at concentrations greater than 1.0×10^{-5} M. The fluorescence quenching and red-shift in the fluorescence spectrum of MC540 depending on the dye concentration is due to the intense aggregation and changing polarity [21,28,29,32]. Additionally, the fluorescence quenching is attributed to the reabsorption effect, which can be observed in the concentrated dye solution [33]. Although the H-aggregate of MC540 is observed at concentrations greater than 5.0×10^{-6} M (Fig. 3), the increase in the fluorescence intensity of MC540 can be explained by increasing the amount of fluorescent MC540 monomers (Fig. S7). In contrast, the strong quenching observed at 1.0×10^{-4} M of MC540 in particular indicates the presence of the reabsorption process as well as intense aggregation.

The normalized fluorescence spectra of mixed LB films of MC540/ODA were previously presented in Fig. 7. As shown in Fig. 7, the fluorescence band maximum of one-layer mixed LB film was observed at 576 nm. The fluorescence band ascribed to monomeric MC540 in the LB film. The fluorescence band maximum of MC540 in the LB film is blue-shifted in comparison to that in chloroform. The blue-shift can be related to the vibrational energy levels in the ground state of the MC540 molecules by varying the local environment around the dye molecules [28]. The retention of the dye molecules in the LB matrix supports this phenomenon. The relatively higher rigidity of the dye molecules provided by the local environment around MC540 molecules constricts the freedom of rotation. The significant differences in the fluorescence spectrum of MC540 in the LB film were not observed by an increase in the number of layers on the substrate and there were small differences in the intensities of the fluorescence spectra. Additionally, the fluorescence band maximum was red-shifted from 576 nm to 578 nm with increase in the number of layer. The small red-shift in the fluorescence band maximum can be ascribed to molecular aggregation because increasing the number of layers enhances aggregation of MC540 in the LB film (Fig. 4).

3.2.3. Time-resolved fluorescence spectroscopy

To determine the fluorescence lifetime of MC540 in chloroform and the one-layer LB film, fluorescence decay spectrum of the samples were recorded upon excitation at 536 nm. The

Table 1
The spectroscopic data and fluorescence lifetime values of MC540 in chloroform and LB film.

[MC540]	$\lambda_{\text{abs.max.}}$ (nm)	$\lambda_{\text{fluo.max.}}$ (nm)	τ_1 (ns)	τ_2 (ns)	χ^2
In chloroform					
1.0×10^{-6} M	571 ^a /528 ^b /–	583	1.20	–	1.00
1.0×10^{-5} M	571 ^a /528 ^b /504 ^c	588	1.40	–	0.95
1.0×10^{-4} M	571 ^a /528 ^b /504 ^c	593	1.60	–	1.10
In LB film					
One-layer	571 ^a /531 ^b /–	576	0.50	2.67	1.10
Nine-layer	571 ^a /531 ^b /476 ^c	578	–	–	–

^a Monomer.

^b H-dimer.

^c H-aggregate.

fluorescence lifetime values were calculated by using specific fit-software of PTI (Photon Technology International). The fluorescence decay spectra of MC540 with exponential fit in chloroform were presented in Fig. 8. The exponential analyses of the fluorescence decays of MC540 in chloroform were fitted to the single-exponential decays with the acceptable statistical χ^2 value. The obtained single-exponential decay indicates homogeneous environment around the dye molecules. As a result of decay analysis, the lifetime value of diluted MC540 (1.0×10^{-6} M) in chloroform was 1.20 ns. The lifetime of MC540 in chloroform was dependent on the increase in dye concentration and the lifetimes at 1.0×10^{-5} M and 1.0×10^{-4} M dye concentration were found to be 1.40 ns and 1.60 ns, respectively. The value of the lifetime for 1.0×10^{-4} M MC540 in chloroform was markedly greater than the lifetime observed for MC540 at a diluted concentration. The increase in the lifetime of dye molecules is explained by the presence of the reabsorption processes while non-fluorescent H-aggregate was strongly observed [34]. If there was no reabsorption process in the system, it would be observed as a decrease in the fluorescence lifetime of concentrated MC540 compared to that of the diluted one due to the fact that H-aggregates and H-dimer quench fluorescence intensity and decrease fluorescence lifetime due to their fast internal conversion process, in which the radiative transition is forbidden [28].

In the LB film, the fluorescence decay of MC540 was found to be bi-exponential (Fig. 9). The bi-exponential decay was interpreted by the inhomogeneous distribution of the dye molecules and the fact that the probe encounters different environments due to diffusion within its lifetime. According to the bi-exponential analysis of the fluorescence decays, fluorescence lifetime values of MC540 in the LB film were calculated as $\tau_1 = 0.50$ ns and $\tau_2 = 2.67$ ns. The long lifetime component was assigned to free MC540 monomers

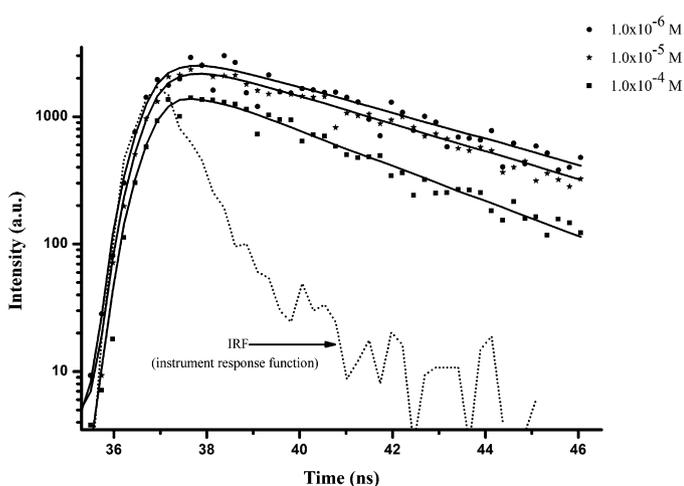


Fig. 8. Fluorescence decay spectra of MC540 with exponential fits in chloroform.

in the LB film when short lifetimes resulted from different orientation of the dye monomers. Additionally, the short lifetime might be due to the excitation energy transferred to the non-fluorescent aggregates resulting in a decrease in fluorescence lifetime [14,28]. Comparison of fluorescence lifetimes of MC540 molecules in chloroform and LB film reveals that the fluorescence lifetime in the LB is increased due to a more rigid environment for the dye molecules in the LB matrix. The spectroscopic results and fluorescence lifetimes of MC540 in different media were summarized in Table 1.

3.3. AFM observations of one-layer LB film of MC540/ODA

AFM is a useful technique for gaining information on surface morphology of the thin films, especially for the flat LB films [34]. One-layer of the mixed LB film of MC540/ODA was transferred onto hydrophilic glass surface to take its AFM images. Fig. 10 showed the AFM image of Y-type mixed LB film of MC540/ODA with phase image and 3D images. AFM image depicted that the MC540/ODA nanoclusters were formed in LB film. These nanoclusters are nearly the same sizes and have uniform distribution on the surface. The dimension (width \times length \times height) of the nanoclusters were determined as an average value of 300 nm \times 500 nm \times 10 nm for MC540/ODA mixture by examination of AFM images (Fig. S4). Additionally, the phase and 3D AFM images (Fig. 10 and Fig. S8) reveal that the stable nanoclusters are actually formed by agglomeration of several smaller nanoclusters whose dimension is 300 nm \times 150 nm \times 10 nm. Since the morphology of LB film depends on the film materials, the non amphiphilic dopant molecules affect the monolayer shape of amphiphilic molecules such as ODA, fatty acid and phospholipids which may form big

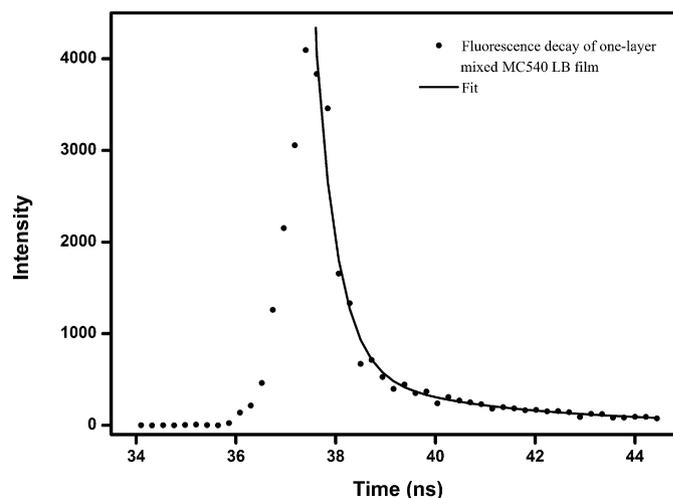


Fig. 9. Fluorescence decay spectrum of one-layer mixed LB film of MC540/ODA with exponential fits.

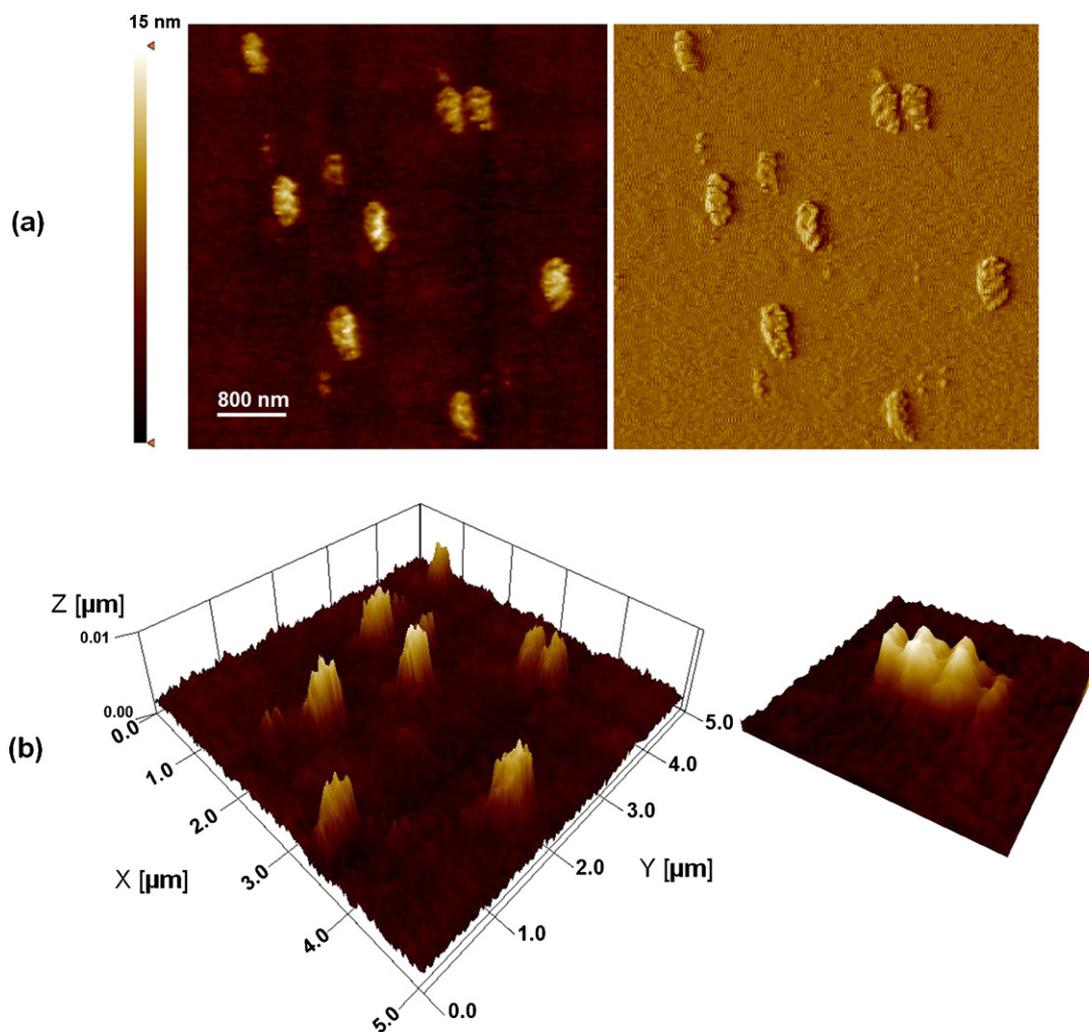


Fig. 10. AFM image ($5.0 \mu\text{m} \times 5.0 \mu\text{m}$) of one-layer mixed LB film of MC540/ODA (a) and 3D-AFM images (b).

clusters at air/water interface. In this regard, it is reported that the different domain morphologies have been observed in the mixed LB film of methylene blue (MB) mixed with dimyristoyl-phosphatidic acid (DMPA) as a function of MB surface density [12]. AFM study has demonstrated the crystalline domain structures of *N,N'*-bis (2,6-dimethylphenyl)-3,4,9,10-perylenetetracarboxylic diimide (DMPI) mixed with stearic acid (SA) in the mixed LB film [35]. In addition, we have recently showed the mixed monolayer of pyronin dyes/SA mixture in LB film has a surface morphologies consisting of nanoclusters [15]. It is concluded that the morphologic findings of MC540/ODA-LB film are compatible with the explanations in the surface pressure–area (π - A) isotherms studies and the spectroscopic results. The formation of the nanoclusters is attributed to self-assembled dye molecules in ODA matrix and the complex formation of dye molecules interacted with ODA.

4. Conclusions

This study shows that mixed monolayer of MC540/ODA can be readily prepared on pure water subphase while MC540 cannot merely form. The formation of the mixed monolayer of MC540/ODA at the air/water interface was confirmed by π - A isotherm studies. The strong interaction between MC540 and ODA form a water-insoluble complex and produce the floating layers at the air/water interface. The specific area per molecule obtained from π - A isotherms revealed that the MC540 dye molecules retained among

the arranged ODA molecules at the air/water interface. Additionally, the plateau formation at high surface pressure was observed due to molecular aggregation in the higher dye concentrations at the mixed monolayer. Optical properties of MC540 in chloroform and LB film were studied spectroscopically and the probe environment affected the photophysical properties of MC540. H-aggregates of MC540 in chloroform were observed by increasing the dye concentration, while the monomeric dye in diluted dye concentration was predominant. The intense H-type aggregation and reabsorption processes observed in chloroform induced the strong quenching in the fluorescence intensity of MC540 as well as the strong red-shifting of fluorescence maximum of the dye. In the LB film, the increase in the number of layers brought about forming the molecular aggregation structure of MC540. The absorption spectra of MC540/ODA demonstrated that the monomer and H-dimer formation of MC540 were available in one-layer mixed LB film when H-aggregates of the dye were formed in the multilayer mixed LB film. Our results concluded that the aggregation of MC540 is easily controlled by the number of layers. The molecular organization based on the number of transferred layers caused to the small changing in the intensity and maximum of fluorescence spectrum of MC540 in the LB film. According to time-resolved studies, the fluorescence decay spectra of LB film were bi-exponential when the decays in chloroform indicated compliance with mono-exponential kinetic. Additionally, MC540 molecules embedded in ODA matrix increased the fluorescence lifetime. The AFM image of one-layer

mixed LB film showed a surface covered with nanoclusters which are close to equal size and have uniform distribution. Consequently, this study is an excellent example related to the quality of mixed monolayer applications of the functional dye molecules, which are incapable of forming a monolayer by themselves at the air/water interface. Our results contribute to the understanding of the strong interaction between MC540 and ODA at the air/water interface, where the amounts of MC540 can modify the film properties and reveal how to build the various optical properties of MC540 thin films.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.colsurfa.2012.08.067>.

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