Photochemistry and Photobiology, 2014, 90: 517–521

Energy Harvesting Capability of Lipid-Merocyanine Macromolecules: A New Design and Performance Model Development

Lobat Tayebi[1,2], Masoud Mozafari[1], Rita El-khouri[3], Parvaneh Rouhani[4] and Daryoosh Vashaee*[4]

[1]School of Material Science and Engineering, Helmerich Advanced Technology Research Center, Oklahoma State University, Tulsa, OK
[2]School of Chemical Engineering, Oklahoma State University, Stillwater, OK
[3]Department of Chemistry, University of California, Davis, CA
[4]School of Electrical and Computer Engineering, Helmerich Advanced Technology Research Center, Oklahoma State University, Tulsa, OK

Received 4 September 2013, accepted 16 October 2013, DOI: 10.1111/php.12193

ABSTRACT

Light induced *cis/trans* isomerization in the family of merocyanine (MC) dyes offers a recyclable proton pumping ability which can potentially be used in hybrid bio-electronic devices. In this article, a hexadecyl MC dye is embedded in lipid molecules to make a macromolecular configuration of a lipid/hexadecyl MC membrane. Lipid molecules play a critical role in stabilizing the dye in a membrane structure for practical use in energy devices. In this study, we first examined the proton pumping characteristic of the lipid/hexadecyl MC membrane in a conventional photoelectrochemical cell. Next, a major modification in the cell was introduced by eliminating I_2/I-electrolyte which resulted in a two-fold increase in the open circuit voltage compared with that of the conventional cell. In addition, the charging time in the new cell was reduced approximately four orders of magnitude. This research demonstrated that the newly designed lipid- MC cell can act as a promising bioelectronic device based on the green energy of photoinduced MC dye proton pumping.

INTRODUCTION

An essential ingredient of biological information processing and energy transduction is proton motive force (pmf). Several essential photoinduced biological procedures and dye probed lipid macromolecules work based on photoisomerization proton pumping and electron transfer mechanisms (1–4). Therefore, creation of artificial stable systems that allow a light driven *trans*-membrane proton pump to be interfaced to an electrically active support is highly desirable.

Photo induced functional incorporation of merocyanine (MC) dyes embedded in lipid biopolymers promises a novel class of proton pumps. Such proton pumps open new opportunities in developing novel bio-inspired devices. Although most dyes are only sensitive to light, MC dyes belong to the family of both thermo- and photosensitive molecules. They can cycle between *cis* and *trans* isomeric states *via* protonation and deprotonation reactions induced by heat or light (5–7).

It has been demonstrated that the generation of thermal- and photodriven pmf results in *trans*-membrane proton pumping across a macromolecular thin-film doped with MC dyes (7). A mechanism was suggested based on vectorial proton pumping conducted by light-induced cycle of reversible conformational transitions (5). Vectorial proton pumping proceeds *via* conformational transitions in the dye molecule, facilitated by an acidic proton source. Specifically, the researchers showed photovoltage generation across membrane-like thin films consisting of oxidized cholesterol and MC in an acidic solution (7–9).

Different MC dyes are distinguished by their *N*-alkyl group substituents. Their chemical structures have a specific symmetry that causes variations in their binding and crystal structure followed by differences in the isomerization temperature, reaction mode, and the enthalpies involved (10).

In this manuscript, first we reproduced the conventional hexadecyl MC photoelectrochemical cell previously reported by Dutta and Nandy (8) to reiterate the proton pumping capability of the hexadecyl MC dye. Furthermore, we designed a new photoelectrochemical cell with hexadecyl MC dye embedded in lipid molecules in which both the current density and the open circuit voltage improved significantly in comparison to the conventional hexadecyl MC photoelectrochemical cell. Although there were isolated reports on the application of MC dyes as a proton pump membrane to generate photovoltage about two–three decades ago, to our knowledge, the effort was not pursued further. Our studies lend further support to the feasibility of using optical and thermal energy to generate pmf across a macromolecular membrane to design a new high-performance class of MC photoelectrochemical cell.

MATERIALS AND METHODS

Hexadecyl MC (HMC) dye was synthesized in our laboratory according to the previously reported method by Minch and Shah (11).

The resulting material was analyzed by X-ray diffraction measurement (XRD) using Bruker AXS D8 diffractometer. This instrument worked with voltage and current settings of 40 kV and 40 mA, respectively and used Cu-$K\alpha$ radiation (1.5405 Å). For qualitative analysis, XRD diagrams were recorded in the interval $15° \leq 2\theta \leq 55°$.

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy (600 MHz Bruker) was used to structurally characterize the synthesized hexadecyl MC dye.

---

*Corresponding author email: Daryoosh.vashaee@okstate.edu (Daryoosh Vashaee)

To investigate the absorption spectra of hexadecyl MC, the spectrophotometer Nanodrop 2000 was used. The solvent was acetone and the concentration of hexadecyl MC in acetone was 10^{-5} M.

Cholesterol (95%) was purchased from Alfa Aesar. The oxidized cholesterol was produced by heat and UV oxidation of cholesterol and recrystallization from *n*-octane. Pure graphite (99.999%) in appropriate rod shape was purchased from VWR international to use as electrodes. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipid. Thin glass filter (membrane) with 1 μm pores in average diameter was purchased from VWR international.

RESULTS

The ^{1}H NMR data reveal the presence of the hexadecyl MC product (Fig. 1A). Described by Morley *et al.* (12), this compound exists as a resonance hybrid with the zwitterionic form being the predominate structure.

Figure 1B shows the XRD plot of the synthesized HMC dye at room temperature. All the distinct diffraction peaks at 16.59°, 20.77°, 23° and 29.03° were very prominent and referred to the crystalline phase of HMC, which is in agreement with the reported data by Das *et al.* (9). According to the synthesis procedure, the cooling process of the final reaction mixture yielded blue-red crystals which were recrystallized three times from hot water. Herein, the average crystallite size was determined from the half-width of diffraction major peaks using the Debye–Scherrer's formula (7):

$$D = k\lambda/\beta\cos\theta \tag{1}$$

where D is the crystallite diameter, k is a constant (shape factor, about 0.9), λ is the X-ray wavelength (1.5405 Å as mentioned before), β is the full width at half maximum (FWHM) of the diffraction line, and θ is the diffraction angle. The FWHM was corrected for the instrument broadening and the average crystallite size was estimated approximately 39.4 nm.

We also examined the absorption spectra of our synthesized hexadecyl MC. Generally, MC dyes show different absorption spectra based on their solvents. Patnaik and Sahu (13) reported that a wide range of solvents such as water, acetone, benzene, toluene, ethanol, methanol, benzyl alcohol, acetonitrile, etc. show different absorption spectra; and there is always a shift in the main peaks of MC at 567 nm due to the change in transition energy and polarity. The main peaks of MC dye in those solvents that keep the polarity of the dye are in a range of 380–400 nm and 560–620 nm (7,13–17). It was also reported that hexadecyl MC with acetonitrile as solvent has absorption peaks in 375 and 580 nm (18).

To investigate the absorption spectra of hexadecyl MC, we used acetone as the solvent. The concentration of hexadecyl MC in acetone was 10^{-5} M. As Fig. 1C shows, hexadecyl MC in acetone has two absorption peaks at 394 and 507 nm.

To examine the proton pumping characteristic of hexadecyl MC dye, two different photoelectrochemical cells were designed. The first cell was previously introduced by Dutta and Nandy (8). We made a similar configuration as shown in Fig. 2A. It was composed of two L-shaped glass tubes connected with standard joints. A porous glass filter (membrane) with pores of 1 μm average diameter was mounted in the middle separating the liquid in the two tubes.

One of the tubes was filled with 0.1 M HCl and the other one was filled with saturated I_2/I^- solution in 0.1 mM concentration. Also, two graphite electrodes were placed symmetrically one in each tube. Here, HCl and I_2/I^- solution act as proton source and charge acceptor, respectively. The new configuration of photoelectrochemical cell is schematically shown in Fig. 2B. As depicted in this figure, the cell is composed of two graphite electrodes. However, instead of two different electrolytes, both electrodes are immersed in HCl solution, and a glass micro-pored filter is attached to the cathode.

The mixture of hexadecyl MC dye, POPC and oxidized cholesterol (mole ratio ~2:7:13) was prepared and used in both cells. Hexadecyl MC dye and POPC were dissolved in chloroform, and cholesterol was dissolved in *n*-decane. To prepare the mixture, hexadecyl MC and POPC were first mixed in appropriate molar ratio. Then, the solution was air-dried for 1 h and kept under vacuum for 3 h to make sure that no chloroform was trapped in the mixture. The oxidized cholesterol in n-decane was

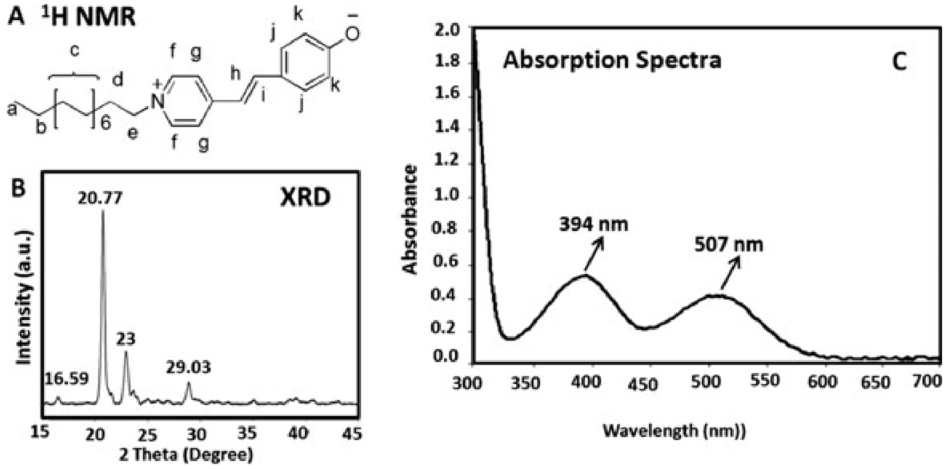

Figure 1. **(A)** Structural Characterization of the synthesized hexadecyl MC by ^{1}H NMR (DMSO, 600 MHz): a = 0.81–0.83 (t, 3H), b, c = 1.20–1.24 (m, 26H), d = 1.73–1.75 (m, 2H), e = 4.07–4.08 (t, 2H), k = 6.03 (d, 2H, J = 9 Hz), h = 6.41 (d, 1H, J = 15 Hz), j = 7.25 (br s, 2H), g = 7.43 (s, 2H), i = 7.62 (d, 1H, J = 15 Hz), f = 8.13 (d, 2H, J = 7.2 Hz). **(B)** XRD diagram of the synthesized hexadecyl MC. The diffraction peaks at 16.59°, 20.77°, 23° and 29.03° are referred to the crystalline phase of hexadecyl MC. The average crystallite size is approximately 39.4 nm. **(C)** The absorption spectra of hexadecyl MC in aceton. Two absorption peaks appeared at 394 and 507 nm. They are spectra of the equilibrium isomers of the material in our experimental condition. All the experiments in panels A, B and C were performed at room temperature.

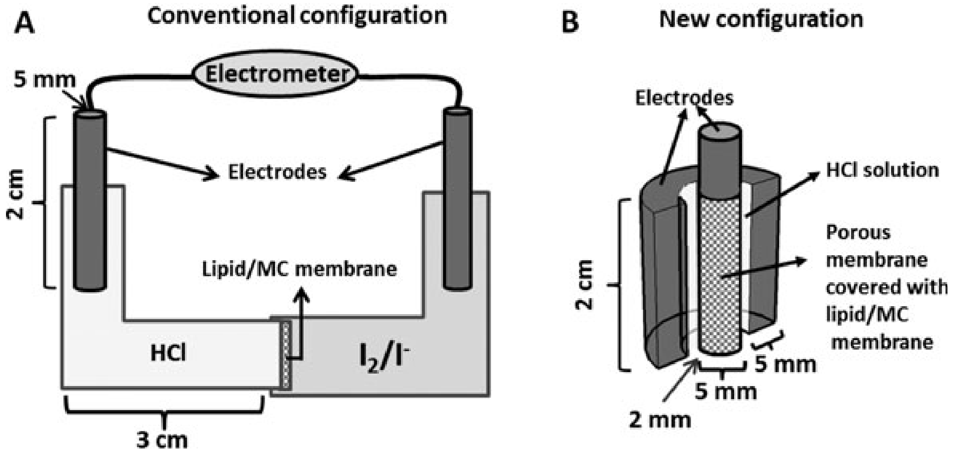

Figure 2. Cross-sectional schematic of the cells, **(A)** the conventional cell (7), and **(B)** the newly designed MC photoelectrochemical cell.

added to this mixture to reach the desired molar ratio of hexadecyl MC dye:POPC:oxidized cholesterol ~2:7:3. For simplicity, we call this mixture lipid/hexadecyl MC. For complete mixing, the mixture of lipid/hexadecyl MC was sonicated for ~15–20 min. The appropriate concentration of the lipid/hexadecyl MC mixture in *n*-decane was approximately 3 mM.

In the first cell, the lipid/hexadecyl MC mixture was brushed on the micropored filter. The filter was mounted between the two L-shaped tubes to hold a stable lipid/hexadecyl MC membrane (19). Although the membrane was micro-porous, the continuous coating of lipid/hexadecyl MC macromolecules successfully prevented mixing of the two solutions of hydrochloride acid and I_2/I-electrolyte.

The main difference between this cell and that of Ref. (8) was that we embedded the dye and the oxidized cholesterol in the POPC lipid molecules.

For the second cell, as shown in Fig. 2B, the micro-pored filter which was attached to the cathode was brushed by the lipid/hexadecyl MC mixture to form a stable membrane. The open circuit voltage between the two electrodes of each cell was measured upon illumination by visible light provided by a 60 W incandescent light bulb.

The temperature inside the photoelectrochemical cells after prolonged exposure to visible light was 24°C (equal to the room temperature).

DISCUSSION

The hexadecyl MC dye can exist in both polar and non-polar configurations. Only the polar configuration has proton pumping ability. The role of the oxidized cholesterol and the lipid membrane is to increase the concentration of the polar MC to enhance the proton transfer. Figure 3A shows the polar and non-polar configurations of hexadecyl MC dye. In the polar form, the rings of dye are benzenoid but in the non-polar form both rings are *p*-quinoidal (20,21). In the environment containing components of oxidized cholesterol, the N^+ terminal is available for binding because of the electron-donating character of the cholesterol (8). Hence, most of the dyes are in the polar form.

The *cis/trans* isomerization for the proton pumping action of the MC dye is suggested as shown in Fig. 2b. MC dye has a stilbene-like resonance structure. *Cis/trans* isomerization is well known for such configurations (5,22). However, in the specific molecules of our dye, the *trans* structure cannot be transformed to the *cis* one directly by photoelectrochemical process. The isomerization has to go through the steps shown in Fig. 2b. First

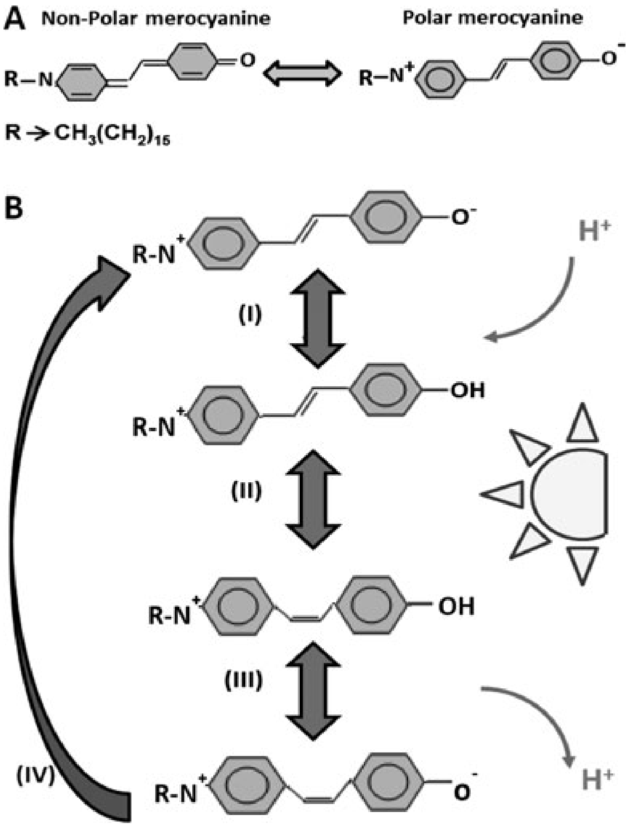

Figure 3. **(A)** Structure of the synthesized merocyanine dyes. At the presence of oxidized cholesterol and lipid molecules more fraction of the dyes are in polar form, which enhances the proton pump function of the membrane. **(B)** The complete cycle of the proton transfer mechanism in merocyanine dye: (I) Trans isomer, M_{Trans}, (II) protonated dye molecule H^+M_{Trans} forms, (III) H^+M_{Trans} transforms to H^+M_{cis}, (IV) H^+M_{cis} deprotonated to M_{cis} and reverts to M_{trans} (*i.e. I*). The procedure occurs thermally and/or photochemically. This model was first introduced in Ref. (5).

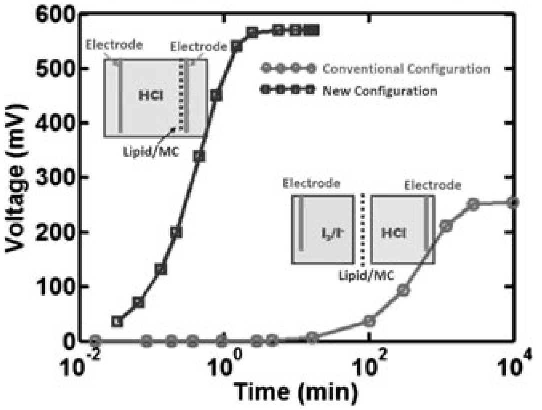

Figure 4. Capacitor charging curves of the conventional and newly designed MC photoelectrochemical cells.

the *trans* structure is protonated. As the resonance structure is stabilized by having a proton at the oxygen end of the MC in polar configuration, the protonated structure has a more stilbene-like characteristic (5,6). Then, H^+M_{cis} is obtained by photoelectrochemical process on H^+M_{trans}. H^+M_{cis} is not stable and easily loses its proton. The *cis* structure is returned to the *trans* configuration and the loop repeats (5).

This unique feature of MC *cis/trans* isomerization enables unidirectional proton transport; hence, the generation of a tappable pmf. In both cells, the pumping action of the MC dyes transfers H^+ ions from one side of the membrane to the other side which in turn results in voltage generation. The direction of H^+ transport in the new cell is not yet clearly understood and requires detailed study in the future.

We measured the output power to a capacitive load. In the first cell, an open circuit voltage of about 0.2 V across the membrane was measured repeatedly in separate experiments (Fig. 4), which is in agreement with the previously reported values (7).

Note that in the previous method the mixture that is brushed on the micropore did not contain POPC. Due to the amphiphilic characteristics of POPC lipid bioplymers, POPC self-assembles as bilayer membrane. Incorporation of MC and oxidized cholesterol in this membrane makes a significantly more stable structure than the mixture without POPC.

As discussed earlier, the configuration of the first cell is conventionally used to show the proton pumping capability of the MC dye. However, this cell has very large internal resistance likely because of the slow mobility of the protons in I_2/I^- electrolyte. Therefore, a new configuration was designed in which the I_2/I^- electrolyte was eliminated. In this new configuration, as the membrane is directly in contact with the electrode, protons immediately reach the cathode and the diffusional complications are circumvented. This results in a dramatic improvement in the cell open circuit voltage as well as the storage time. For comparison, Fig. 4 depicts the charging curves of a capacitor measured for the two cells. It can be seen that the charging time in the new cell is four orders of magnitude reduced from about 10^4 min in a conventional configuration to 10 min in a new one. In addition, the open circuit voltage of the new cell is more than two-fold higher than that obtained using the conventional configuration employing I_2/I^- solution. The maximum open circuit voltage for the cell with new configuration is about 580 mV whereas this value for the conventional configuration is about 200 mV. This observation unambiguously establishes the feasibility for exploiting the concept of pmf generated by the lipid/MC macromolecules.

CONCLUSION

In this study, a high-performance photoelectrochemical cell was designed based on the light induced proton pumping characteristic of hexadecyl MC dye which is embedded in lipid molecules. This newly designed cell differs from the conventional cell by eliminating the I_2/I^- solution as an electrolyte, which decreases the internal resistance caused by the slow mobility of protons in I_2/I^- electrolyte. Consequently, the current density and open circuit voltage were significantly improved in the new photoelectrochemical cell. These results demonstrate the enhanced energy harvesting capability of the stable macromolecular membrane of lipid/hexadecyl MC.

Acknowledgements—We thank Prof. Atul N. Parikh and Aref Shahini for their helpful discussions and insights. This report is supported by the Oklahoma Center for Advancement of Science and Technology (grant no. AR131-054 8161) and the AFOSR under grant no. FA9550-10-1-0010).

REFERENCES

1. Schulten, K. and P. Tavan (1978) Mechanism for light-driven proton pump of halobacterium-halobium. *Nature* **272**, 85–86.
2. Oesterhe, D. and W. Stoecken (1973) Functions of a new photoreceptor membrane. *Proc. Natl Acad. Sci. USA* **70**, 2853–2857.
3. Bhowmik, B. B., C. Senvarma and P. Nandy (1988) Photoinduced electron-transfer from lipid to dye in a liposome. *Phys. Lett. A* **130**, 55–57.
4. Bhowmik, B. B., R. Dutta and P. Nandy (1985) Photovoltage generation in dye-probed bilayer lipid-membranes. *Indian J. Chem., Sect A* **24**, 1046–1047.
5. Abdel-Kader, M. H. and U. Steiner (1983) A molecular reaction cycle with a solvatochromic merocyanine dye: An experiment in photochemistry, kinetics, and catalysis. *J. Chem. Educ.* **60**, 160–162.
6. Steiner, U., M. H. Abdel-Kader, P. Fischer and H. E. A. Kramer (1978) Photochemical cis/trans isomerization of a stilbazolium betaine. A protolytic/photochemical reaction cycle. *J. Am. Chem. Soc.* **100**, 3190–3197.
7. Basu, R., S. Das and P. Nandy (1993) Photoinduced proton transport mechanism in merocyanine-dye-probed planar lipid-membranes. *J. Photochem. Photobiol., B* **18**, 155–159.
8. Datta, R. and P. Nandy (1990) Role of merocyanine dye as a proton pump in photovoltage generation. *Photochem. Photobiol.* **52**, 907–909.
9. Das, S., R. Basu, A. Ghose, M. Minch and P. Nandy (1995) Heat-induced voltage generation in hexadecyl merocyanine dye-probed planar lipid-membranes. *Dyes Pigm.* **27**, 333–338.
10. Brown, G. H. (ed.) (1971) *Photochromism (Techniques of Chemistry)*, Vol. **3**. Wiley, New York.
11. Minch, M. J. and S. S. Shah (1979) Spectroscopic studies of hydrophobic association-merocyanine dyes in cationic and anionic micelles. *J. Org. Chem.* **44**, 3252–3255.
12. Morley, J. O., R. M. Morley, R. Docherty and M. H. Charlton (1997) Fundamental studies on Brooker's merocyanine. *J. Am. Chem. Soc.* **119**, 10192–10202.
13. Patnaik, L. and B. Sahu (1994) Solvent effects on the absorption spectra of merocyanine dyes. *J. Solution Chem.* **23**, 1317–1330.
14. Xiaochuan, L., K. Sung-Hoon and A. S. Young (2009) Optical properties of donor-π-(acceptor)$_n$ merocyanine dyes with dicyanovinylindane as acceptor group and triphenylamine as donor unit. *Dyes Pigm.* **82**, 293–298.

15. Svetlichnyi, V. A., A. A. Ishchenko, E. A. Vaitulevich, N. A. Derevyanko and A. V. Kulinich (2008) Nonlinear optical characteristics and lasing ability of merocyanine dyes having different solvatochromic behaviour. *Opt. Commun.* **281**, 6072–6079.
16. Beata, J., K. Janina and P. Jerzy (2007) Dichromophoric hemicyanine dyes. Synthesis and spectroscopic investigation. *Dyes Pigm.* **74**, 262–268.
17. Beata, J., K. Janina, P. Marek and P. C. Jerzy (2003) Hemicyanine dyes: Synthesis, structure and photophysical properties. *Dyes Pigm.* **58**, 47–58.
18. Zimmermann-Dimer, L. M. and V. G. Machado (2009) Chromogenic anionic chemosensors based on protonated merocyanine solvatochromic dyes: Influence of the medium on the quantitative and naked-eye selective detection of anionic species. *Dyes Pigm.* **82**, 187–195.
19. Mountz, J. M. and H. T. Tien (1978) Photoeffects of pigmented lipid-membranes in a microporous filter. *Photochem. Photobiol.* **28**, 395–400.
20. Brooker, L., A. C. Craig, D.W.S. Heseltine, P.W. Jonkins and L.L. Lincoln (1965) Color and constitution. XIII. Merocyanines as solvent property indicators. *J. Am. Chem. Sot.* **87**, 2443–2450.
21. Benson, H. G. and J. N. Murrell (1972) Some studies of benzenoid-quinonoid resonance. Part 2. The effect of solvent polarity on the structure and properties of merocyanine dyes. *J. Chem. Sot. Faraday Trans.* **68**, 137–143.
22. Calvin, M. and H. W. Alter (1951) Substituted stilbenes. I. Absorption Spectra. *J. Chem. Phys.* **19**, 765–768.