Organic Electronics 12 (2011) 751–755

Contents lists available at ScienceDirect

Organic Electronics

journal homepage: www.elsevier.com/locate/orgel

# Integrated semiconductor optoelectronic devices for real-time and indicator-free detection of aqueous nitric oxide

Yu-Chiang Chao [a], Shih-De Yeh [a], Hsiao-Wen Zan [b], Gao-Fong Chang [c,d], Hsin-Fei Meng [a,*], Chen-Hsiung Hung [e,*], Tzu-Ching Meng [f]

[a] Institute of Physics, National Chiao Tung University, Hsinchu 300, Taiwan
[b] Department of Photonics and Institute of Electro-Optical Engineering, National Chiao Tung University, Hsinchu 300, Taiwan
[c] Department of Chemistry, National Tsing Hua University, Hsinchu 300, Taiwan
[d] Molecular Science and Technology Program, Taiwan International Graduate Program, Academia Sinica, Taipei 115, Taiwan
[e] Institute of Chemistry, Academia Sinica, Taipei 105, Taiwan
[f] Institute of Biological Chemistry, Academia Sinica, Taipei 105, Taiwan

ARTICLE INFO

*Article history:*
Received 19 November 2010
Received in revised form 3 February 2011
Accepted 9 February 2011
Available online 20 February 2011

*Keywords:*
Nitric oxide
Signalling molecule
Biosensor
Real-time
Indicator-free

ABSTRACT

A real-time and indicator-free sensor specific to nitric oxide (NO) is realized for the first time by integrating a sensing hydrogel film, a top-emitting polymer light-emitting diode (PLED), and a silicon photodetector. The top-emitting PLED is utilized to excite the sensing film specific to NO, and the photoluminescence of the sensing film is transformed into electric signal by the photodetector. The influence of the excitation light on the out-put photocurrent of the photodetector is diminished by two delicately selected organic filters based on their absorption spectra. Lead phthalocyanine is deposited on a top-emitting PLED as a filter for generating an excitation light without overlapping the emission maximum of the sensing hydrogel film. Poly(3-hexylthiophene) is placed in front of the photodetector as another filter to remove the excitation light. This sensor demonstrated a fast photocurrent response to NO with response time within 5 min.



## 1. Introduction

Nitric oxide (NO), a short-lived free radical, is a signaling molecule transmitting signals in cardiovascular and nervous system [1,2]. NO plays an important role in a variety of physiological processes, such as smooth muscle relaxation and blood pressure regulation [3]. Immune system also generates NO as a defense against pathogens [4]. Unregulated NO production, however, is associated with pathological conditions, such as inflammation, septic shock, and cancer [5]. NO released after initial traumatic brain injury damages the brain and causes secondary brain injury [6]. Since protective and toxic influences of NO are frequently observed in parallel, generalization of the effects of NO on certain physiological function are quite difficult. Real-time detection of NO is important and helpful to unveil the correlation between NO concentration and physiological functions. Fluorescent microscopes and electron paramagnetic resonance spectrometers are conventionally utilized in fundamental research to inspect NO with the help of fluorescent indicators and spin-trapping compounds since NO does not fluoresce itself [7]. Dishes of cells and slices of brain have to be prepared in sequence to understand time-evolved NO distribution [6]. The specimen preparation time is too long for real-time detection of NO with lifetime around 5 s in physiological conditions. Besides, the influence of fluorescent indicator and spin-trapping compounds on cell functions cannot be completely avoided. Furthermore, fluorescent microscopes and electron paramagnetic resonance spectrometers are expensive, large-sized, and delicate systems. A small-sized semiconductor sensor for monitoring NO in a real-time and indicator-free manner is therefore essential not only for basic research but also for clinical diagnosis. Chemical

* Corresponding authors. Tel.: +886 3 5731955 (H.-F. Meng).
*E-mail addresses:* meng@mail.nctu.edu.tw (H.-F. Meng), chhung@chem.sinica.edu.tw (C.-H. Hung).

1566-1199/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.orgel.2011.02.003

and biological sensing has been demonstrated by an integrated semiconductor device consists of an excitation source, a photo detector (PD), and a sensing unit [8]. So far, the integrated semiconductor devices were used to detect glucose [9] and oxygen [8,10]. A sensor specific to NO has not been realized mainly because there is no sensing film for NO. In our recent work, a NO sensing hydrogel film was demonstrated possessing high selectivity, sensitivity, and stability in various pH values which could be used as the sensing unit for NO [11]. But so far no trial has been done to use the NO sensing film as the sensing unit. Besides, to miniaturize the integrated semiconductor device, a small-sized organic light-emitting diode (OLED) was used in glucose [9] and oxygen [8,10] sensor. However, the NO sensing film was characterized with a solid-state laser diode module as an excitation source, and no work has been done to show the NO sensing characteristics using OLED as an excitation source. Furthermore, unlike the laser with narrow emission spectrum, the emission spectrum of the OLED excitation light is broad, and hence the separation of reflected excitation light and the emission of the sensing unit is important in order to reduce the influence of the tail of excitation light on the emission of the sensing unit. However, optical filters based on glass substrate is conventionally used which is expensive and occupies volumes [8–10]. Light separation was never achieved in an easy way.

In this work, a NO sensor is realized for the first time by integrating a sensing hydrogel film specific to NO with a top-emitting polymer light-emitting diode (PLED), silicon (Si) PD, and two organic filters as shown in Fig. 1(a). The top-emitting PLED is used to excite the sensing hydrogel film, and the small-sized Si PD is used to detect the photoluminescence (PL) of the sensing hydrogel film. The influence of the tail of excitation light on the emission of the sensing unit is reduced by using two organic filters. Filter 1 is deposited above the transparent cathode of the top-emitting PLED to filter out the wavelength of the excitation light around the emission peak of the sensing hydrogel film. Filter 2 is placed in front of the Si PD to remove the light other than the sensing hydrogel film. The sensing film specific to NO developed in our group [11] is utilized as the sensing unit. The PL of the NO sensing hydrogel film changes after adsorbing NO, and such change in PL can be monitored by Si PD. Light signal is therefore transformed into electric signal for recording. Real-time and indicator-free detection of NO is demonstrated. All components except the NO sensing film can be sealed inside a water-proof container with electrical feedthroughs for driving PLED and monitoring photocurrent outputs from Si PD. The photocurrent response with response time within 5 min is demonstrated. In practice, after each usage, the sensing hydrogel film is the only component that needs to be replaced.

## 2. Experimental

Top-emitting PLED is fabricated on cleaned glass substrate. Schematic device structure and the image of the top-emitting PLED under forward bias are shown in Fig. 1(b) and (c), respectively. Aluminium (Al)/molybdenum oxide ($MoO_3$)/poly(3,4-ethylenedioxythiophene): poly(styrenesulfonate) (PEDOT:PSS) is used as an opaque anode. A striped-type shadow mask is used for preparing Al (100 nm) and MoO3 (3 nm). PEDOT:PSS (CLEVIOS™ P VP AI 4083) of 40 nm is then spin coated and annealed at 200 °C for 15 min. On top of the PEDOT:PSS surface, the PLED structure is cross-linkable TFB (20 nm)/Green B (65 nm)/Ca (10 nm)/Ag (15 nm)/PbPc (200 nm). The hole transport layer of cross-linkable poly[(9,9-dioctylfluorenyl-2,7-diyl)-co-(4,4′-(*N*-(p-butylphenyl))diphenylamine)] (CTFB) (in toluene) is formed by spin coating and then baked at 180 °C for 60 min. The emitting layer is also formed by spin coating polyfluorene derivative LUMATION Green B (GB) by Dow Chemical (in toluene) and then baking at 130 °C for 30 min. The transparent cathode Ca/Ag and lead phthalocyanine (PbPc) are deposited with striped-type shadow mask. Each active area is about 1 mm × 10 mm, and the width of gap between each active area is 1 mm. During the real-time detection, the luminescence of the top-emitting PLED is kept at 1600 cd/m$^2$. Fig. 1(d) shows 250 nm poly(3-hexylthiophene) (P3HT) Filter 2 prepared by drop casting P3HT dichlorobenzene solution on glass substrate. The sensing hydrogel film is a blend of host polymer poly(2-hydroxyethyl methacrylate) (pHEMA) and fluorescent probe 11,16-bisphenyl-6,6,21,21-tetramethyl-*m*-benzi-6,21-porphodimetheno-chloro-Zinc(II)

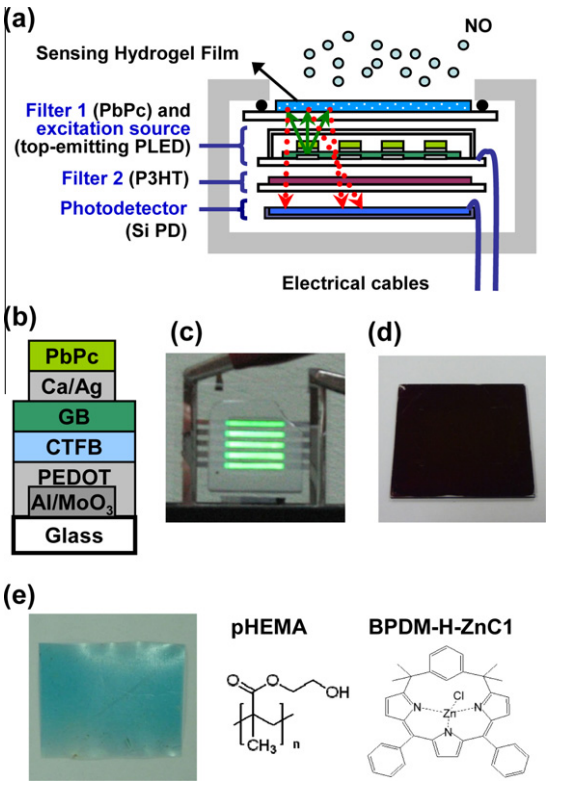


**Fig. 1.** (a) The schematic structure of the sensor integrated with a sensing hydrogel film, a top-emitting PLED, a Si PD, and two organic filters. (b) Device structure of the top-emitting PLED. PbPc is deposit on transparent cathode serving as filter. (c) Photograph of the top-emitting PLED. (d) Photograph of the P3HT filter. (e) Photograph of the sensing hydrogel film composed of pHEMA and BPDM–H–ZnC1. Molecule structures of pHEMA and BPDM–H–ZnC1 are also shown.

(BPDM–H–ZnCl). The image of the sensing hydrogel film and molecular structures of pHEMA and BPDM–H–ZnCl are shown in Fig. 1(e). BPDM–H–ZnCl (2 mg) is first dissolved in a mixture (10 ml) of 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), and azobisisobutyronitrile (AIBN), and then the blending solution is poured into a Teflon cell and annealed at 80 °C for 10 min to form a film of 180 micrometer. HEMA is used as a hydrophilic monomer, EGDMA is used as a hydrophobic cross-linker, and AIBN (1%) is used as thermal initiator. HEMA is blended with EGDMA in the weight ratio 12:1. BPDM–H–ZnCl is synthesized from *m*-benziporphodimethene based molecule.[12] The real-time detection of NO is carried out with a polydimethylsiloxane (PDMS) microfluidic system.[13] The dimension of the channel is $30 \times 15 \times 1$ mm (length × width × height). The PDMS with a sheet of sensing hydrogel film in the channel is sealed with a glass slide. Fluid inlet and outlet are located at two ends of the channel. NO bubbled water is prepared by bubbling 1000 ppm NO (in $N_2$ carrier gas) into DI water for 1 h. The electroluminescence (EL) spectra and current–luminance–voltage (*I–L–V*) characteristics of top-emitting PLED are measured by a Photo Research PR650 spectrophotometer integrated with Keithley 2400 multi-meter. Absorption spectra are recorded by Hewlett–Packard 845X UV–visible system. Emission spectrum of sensing hydrogel film is monitored by high resolution fiber optic spectrometer (EPP2000, StellarNet). Si PD (S2387, Hamamatsu Photonics), sensitive from visible light to infrared light, is biased at −10 V by Keithley 2400 multi-meter to monitor the photocurrent. In some experiment, 405 nm laser diode (Power Technology) is used to excite sensing hydrogel film.

## 3. Results and discussion

The selectivity of BPDM–H–ZnC1 is demonstrated in Fig. 2(a) and (b). Various gases are bubbled into $2 \times 10^{-6}$ M BPDM–H–ZnC1 acetonitrile solution for 2 min. No dramatic difference in PL spectrum can be observed after bubbling oxygen ($O_2$), carbon dioxide ($CO_2$), and carbon monoxide (CO). A decrease in PL is observed only after bubbling NO. BPDM–H–ZnC1 is specific to NO and it does not react with $O_2$ and $CO_2$ in the atmosphere and another important signalling molecule CO. In addition, since nitrate ($NO_3^-$) and nitrite ($NO_2^-$) are end products of NO metabolism, nitrate donor (PPN($NO_2$)) (PPN: bis(triphenylphosphine)iminium) and nitrite donor (PPN($NO_3$)) are added into BPDM–H–ZnCl solution to test the influence of nitrate and nitrite on the PL intensity. Hydrogen peroxide ($H_2O_2$), another free radical in human body, is also tested. *S*-nitroso-*N*-acetylpenicillamine (SNAP) is used as NO donor. The molecule structures of SNAP, PPN($NO_2$) and PPN($NO_3$) are shown in Fig. 2(c). Concentrations of donors are all $10^{-3}$ M. Except the SNAP, no one can induce continuing decrease in PL as shown in Fig. 2(b). This demonstrates the BPDM–H–ZnCl is highly selective which makes it a reliable reagent for future applications in biological systems.

As shown in Fig. 3(a), NO sensing characteristics of the hydrogel film are first demonstrated by using a microfluidic system and 405 nm laser as excitation source. The sensing hydrogel film is initially settled in deionized (DI) water in the microfluidic channel with stable PL at 675 nm. The PL spectrum of the sensing hydrogel film is similar to the one of BPDM–H–ZnC1 solution which means BPDM–H–ZnCl probe functions well even after being trapped in pHEMA. Dramatic decrease in PL of the sensing hydrogel film can be observed when the NO bubbled water is injected into the microfluidic channel at 16th and 51st minute. The changing in PL stops after refilling the channel with DI water at 36th and 66th minutes. These characteristics show that the sensing hydrogel film is suitable for real-time and indicator-free detection.

To separate excitation light and the emission light, the first step is to filter the EL of the top-emitting PLED to form

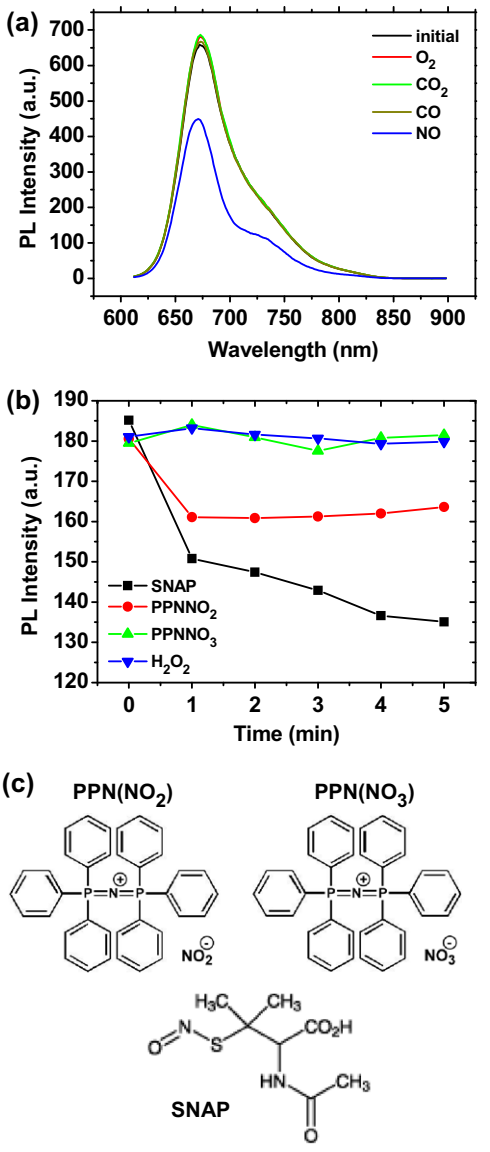


**Fig. 2.** (a) The PL spectra after bubbling different gases. (b) The PL intensities at 675 nm vary with time after adding different molecules. (c) The molecule structures of SNAP, PPN($NO_2$) and PPN($NO_3$).

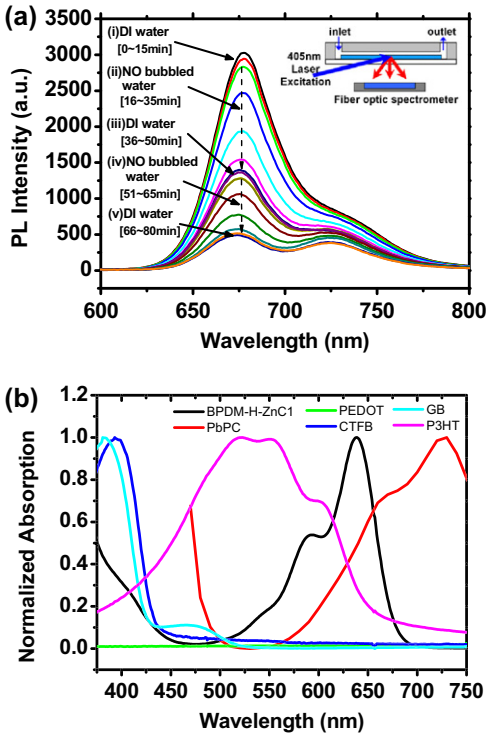


**Fig. 3.** (a) The PL spectra of the sensing hydrogel film placed in microfluidic channel. The inset shows the experimental setup. DI water or NO bubbled water is injected into microfluidic channel at different time. (b) Normalized absorption spectra of BPDM–H–ZnC1, PbPc, PEDOT, CTFB, GB, and P3HT.

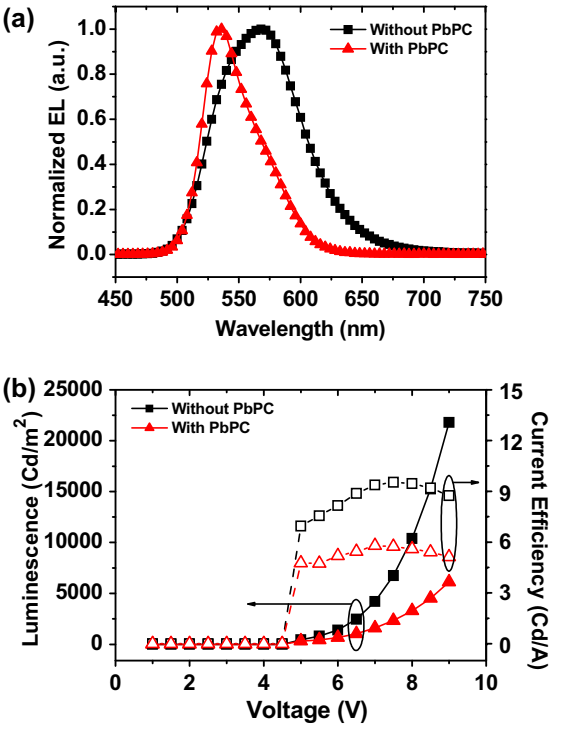


**Fig. 4.** (a) Normalized EL spectra and (b) luminescence and current efficiency of top-emitting PLED with or without PbPc on top of the transparent cathode.

an excitation light without the wavelength overlaps the emission of the sensing hydrogel film. Organic material PbPc is therefore deposited on top-emitting PLED as Filter 1 since it absorbs light above 600 nm with an absorption peak at 730 nm as shown in Fig. 3(b). Besides, PbPc is almost transparent for the light between 500 and 570 nm, and the emission of top-emitting PLED with PbPc on transparent cathode in that range can pass PbPc for excitation without large light intensity attenuation. Fig. 4(a) shows the EL spectra of top-emitting PLED with or without covering PbPc. The EL spectrum of the top-emitting PLED without PbPc ranges from 500 to 700 nm. Deposition of 200 nm PbPc on top-emitting PLED narrows its spectrum range and almost no emission around 675 nm can be observed. This ensures the 675 nm light that Si PD received comes mostly from the BPDM–H–ZnC1 fluorescent NO probe in the sensing hydrogel film. The luminescence and current efficiency of the top-emitting PLED with or without PbPc are shown in Fig. 4(b). Although both luminescence and current efficiency are decreased for the top-emitting PLED with PbPc filter because part of the emission is absorbed, stable current efficiency with maximum of 5.8 cd/A at 7 V can still be obtained. The luminescence at 7 V is 1600 cd/m$^2$ which is enough for this integrated semiconductor device.

The second step to separate excitation light and the emission light is to remove the rest of excitation light. As shown in Fig. 5(a), the spectrum shows two apparent peaks at 535 nm and 680 nm as P3HT Filter 2 is absent. Since each material used in top-emitting PLED does not have apparent absorption above 500 nm, the PL of the sensing film as well as the reflected excitation light can pass though the gaps between active areas of top-emitting PLED and received by Si PD without large light intensity attenuation. Except the PL of the sensing hydrogel film, the reflected excitation light can also be detected. In the presence of P3HT Filter 2 which absorbs most of the visible light below 650 nm, the reflected excitation light can be removed. However, the absorption of P3HT filter above 650 nm is not zero, and hence the emission from sensing hydrogel film also decreased a little but still detectable. The P3HT filter used in this work is deposited on a glass substrate and placed in front of Si PD to demonstrate its function. In practice, the P3HT filter can be deposited on Si PD or on the bottom of the PLED glass substrate for space saving.

After the separation of the excitation light and emission of the sensing hydrogel film is achieved, the change in photocurrent outputs from Si PD may represent mostly the change in PL of the sensing hydrogel film. Fig. 5(b) shows the real-time and indicator-free detection of NO by an integrated semiconductor device consisting of a sensing hydrogel film, a top-emitting PLED with PbPc filter, and a Si PD covered with P3HT filter. The photocurrent curve is smoothed by taking the average of 40 data points around each point as shown in Fig. 5(c). The photocurrent output from Si PD remains at almost the same value until the NO bubbled water is injected into the microfluidic channel. The photocurrent ceases to descend as DI water is refilled

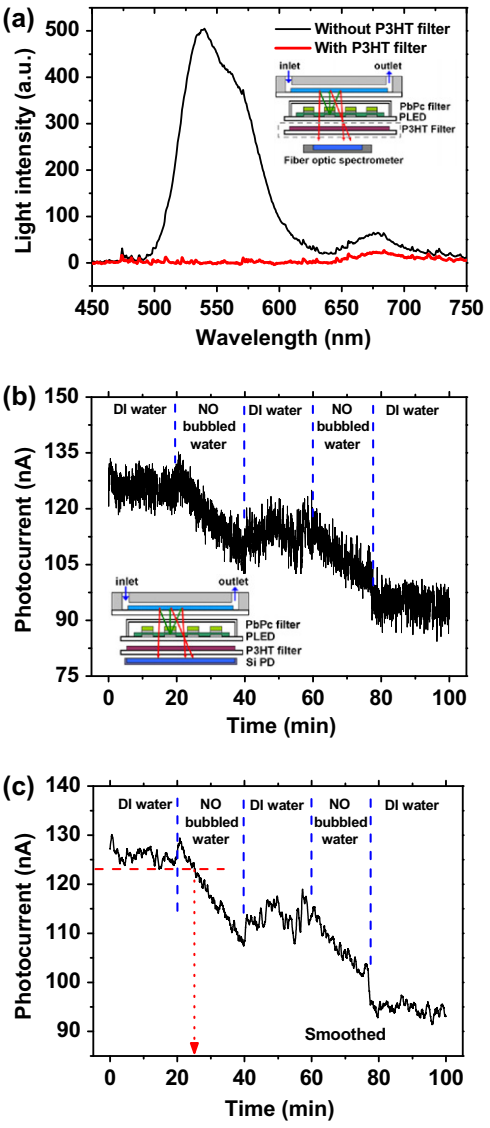


**Fig. 5.** (a) Spectra of the light received by Si PD with or without P3HT filter placed in front of it. (b) The photocurrent response to different environment. (c) The photocurrent response is smoothed for clarity.

into the microfluidic channel. The decrease in photocurrent corresponds to the decrease in PL intensity of the sensing hydrogel film as shown in Fig. 3(a). This is the first time that aqueous NO can be detected by an integrated solid-state optoelectronic sensor in a real-time and indicator-free manner. The NO concentration is estimated to be in the order of $10^{-3}$ M since it has been reported that NO solubility in oxygen-free water is 1.9 mM at 25 °C [7]. It has been demonstrated in our previous work that a higher NO concentration leads to a larger PL variation in the same time interval [11]. The slopes of the photocurrent obtained from the photocurrent vs. time plot are different for various NO concentrations. The slope of the photocurrent is therefore used to indicate the NO concentration, and the response time is the time needed for the slope of the photocurrent changes to another value and the photocurrent exceeds the noise level. Although immediate slope change is observed within few seconds after injecting NO bubbled water as shown in Fig. 4(c), the response time is still estimated to be 5 min which is determined by the time needed for the PD to output an photocurrent lower than the noise level. The response time can be improved by increasing the signal-to-noise ratio and enhancing the fluorescence of the NO probe. Increasing the surface area of the sensing film may also help. The realization of this integrated optoelectronic sensor makes the real-time and indicator-free detection of NO possible for the first time. Such a small-sized sensor is suitable for long-term and portable detection of NO, and it is a help to understand the relation between NO and physiological changes.

## 4. Conclusion

In conclusion, real-time and indicator-free detection of nitric oxide is achieved by an integrated semiconductor optoelectronic device consisting of a sensing film, an excitation source, and a photodetector. The influence of the excitation light on the output current of the photodetector is diminished by utilizing two organic filters. One filter is deposited above a top-emitting PLED to produce an excitation light without overlapping the emission maximum of the sensing hydrogel film. The other filter is placed in front of photodetector to prevent the excitation light get into the photodetector. The photocurrent changes as NO enters the environment and the response time is 5 min. Such integrated solid-state device is compact, small-size, and portable which can be used in fundamental research and clinical diagnosis.

## Acknowledgement

This work is supported by the National Science Council of Taiwan (NSC99-2628-M-009-001-), Academia Sinica (AS-100-TP-B03), and the Veterans General Hospitals and University System of Taiwan Joint Research Program (VGHUST100-G5-1-1). Authors are grateful to members in biomimetic system research center, National Chiao Tung University.

## References

[1] D.S. Bredt, S.H. Snyder, Annu. Rev. Biochem. 63 (1994) 175.
[2] S. Moncada, R.M. Palmer, E.A. Higgs, Pharmacol. Rev. 43 (1991) 109.
[3] R.M.J. Palmer, A.G. Ferrige, S. Moncada, Nature 327 (1987) 524.
[4] C. Bogdan, Nature Immunol 2 (2001) 907.
[5] L.L. Thomsen, D.W. Miles, L. Happerfield, L.G. Bobrow, R.G. Knowles, S. Moncada, Br. J. Cancer 72 (1995) 41.
[6] R.S. Clark, P.M. Kochanek, M.A. Schwarz, J.K. Schiding, D.S. Turner, M. Chen, T.M. Carlos, S.C. Watkins, Pediatr. Res. 39 (1996) 784.
[7] T. Nagano, Chem. Rev. 102 (2002) 1235.
[8] V. Savvate'ev, Z. Chen-Esterlit, J.W. Aylott, B. Choudhury, C.-H. Kim, L. Zou, J.H. Friedl, R. Shinar, J. Shinar, R. Kopelman, Appl. Phys. Lett. 81 (2002) 4652.
[9] B. Choudhury, R. Shinar, J. Shinar, J. Appl. Phys. 96 (2004) 2949.
[10] Z. Zhou, R. Shinar, A.J. Allison, J. Shinar, Adv. Funct. Mater. 17 (2007) 3530.
[11] Y.C. Chao, S.D. Yeh, H.W. Zan, G.F. Chang, H.F. Meng, C.H. Hung, T.C. Meng, C.S. Hsu, S.F. Horng, Appl. Phys. Lett. 96 (2010) 223702.
[12] C.H. Hung, G.F. Chang, A. Umar, G.F. Lin, L.Y. Luo, W.M. Ching, E.W.G. Diau, Chem. Comm. (2008) 978.
[13] F. Patolsky, G. Zheng, C.M. Lieber, Nat. Protoc. 1 (2006) 1711.