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Research Article

Fluorescence Detection 400–480 nm Using Microfluidic System Integrated GaP Photodiodes

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Ciprofloxacin is a commonly used antibiotic and the active ingredient in a veterinary antibiotic. Detecting its presence allows us to understand its absorption process in blood as well as tissue. A portable microfluidic system has been fabricated. It operates at low bias voltage and shows a linear relationship between concentration levels and system response. Detection of concentrations down to 1 ppb of ciprofloxacin in microliters of solution was achieved.

1. Introduction

As the medical industry has evolved, it has become necessary to have accurate testing available in the shortest possible time [1–5]. Miniaturizing UV and fluorescence detectors and extending them to fluid analysis is one way of bridging this gap [6–9].

Detecting ciprofloxacin has become increasingly important because it is the active ingredient in enrofloxacin an antibiotic used in veterinary medicine [10, 11]. Interest in monitoring ciprofloxacin levels in the tissues of animals raised for food and in the milk of cows has led to improvements in current technology and a search for new detection techniques. Ciprofloxacin absorbs strongly in the UV ($\leq$280 nm) and emits at $\sim$ 440 nm [10].

Currently, online detection is carried out by analytical methods such as high-performance liquid chromatography (HPLC), which involves a relatively costly investment in hardware [12, 13], or by capillary electrophoresis. Capillary electrophoresis (CE), while simpler than the aforementioned HPLC, still requires complex sample preparation methods utilizing pH adjustment to reach lower detection limits and requires very high bias voltage (kV) [12, 14]. These two methods usually employ photomultiplier tubes (PMTs) for photodetection.

PMTs have been used in applications such as low-level ultraviolet detection in laser-induced fluorescence biological-agent warning systems [15]. Other applications in this wavelength range are under-water detection at 400 nm, the wavelength at which water is transparent, and detection of 440 nm-wavelength light from scintillation crystals that are used to sense gamma rays from nuclear material.

While PMTs are among the most sensitive detectors currently available, semiconductor photodiodes offer the advantages of being less expensive and more robust. Si photodiodes have high responsivities at 440 nm; however, GaP exhibits a detection cutoff wavelength of 550 nm, which makes it an attractive alternative to Si, which requires expensive filters to reject extraneous wavelengths.

This paper expands on our previous success in integrating photodiodes with microfluidics for ciprofloxacin detection [16]. In that work, linear detection of 0.01 ppm ciprofloxacin was achieved. The best published results for the widely used techniques are 0.01 ppm HPLC and 0.015 ppm CE [12, 14]. We note however that these detection limits were obtained in physiological samples. It should also be pointed out that HPLC and CE provide richer spectral results than the technique reported here, which provides simple confirmation of the presence of ciprofloxacin.

2. Fabrication

GaP wafers were grown by metal organic chemical vapor deposition. The wafer structure was as follows: an n-type

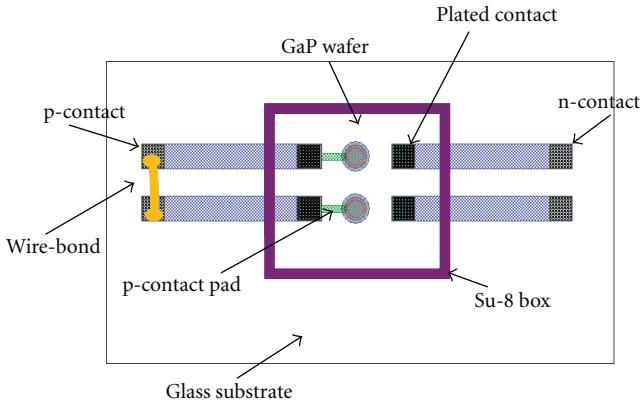

FIGURE 1: Detector system as viewed through the glass substrate.

substrate with a 650 nm-thick n^+-doped (1.4×10^{19} cm^{-3}) first layer, an 800 nm-thick unintentionally undoped (4×10^{16} cm^{-3}) second layer, and a 300 nm p^+-doped (1.1×10^{19} cm^{-3}) top layer.

Mesa structure devices were fabricated by standard clean-room processes. First, a mesa was defined by photolithography and etched to the bottom n^+ layer by inductively coupled plasma. This was followed by a 5-second etch in HNO_3 : HCl : DI water in equal parts to remove damage to the sidewall caused by the inductively coupled plasma etch. Plasma enhanced chemical-vapor deposition was used to deposit SiO_2 which served as both a passivation and an antireflection coating. After contacts were formed by metal evaporation of AuGe-Ni-Au (40 nm, 10 nm, 110 nm), the contacts were annealed for ten seconds at 430°C in a mixture of nitrogen and hydrogen [17]. A Ti-Au p-contact pad was evaporated and both the p-contact pad and n contact were Au plated to a final thickness of ~2 μm. The wafer was diced into 1 mm^2 chips consisting of a 2 × 1 array of GaP photodiodes. In order to flip the contacts a corresponding metal contact Ti-Au was evaporated on a thin glass slide. These contacts were Au plated at each end; the GaP photodiodes were bonded to the inner contacts and the outer contacts served as bias pads after flip-chip bonding. Using SU-8 photoresist, a box slightly larger than the chip and approximately 30 μm in height was defined by photolithography. Its function was to facilitate alignment of the contacts on the GaP chips with those on the glass. The chips were bonded to the contact pads at 220°C. The p-contact pads of two adjacent devices were wire bonded together placing the photodiodes in parallel with each other (see Figure 1).

3. Device Characteristics

The dark currents of the individual devices were 0.06 and 0.04 pA at 2 V reverse bias with breakdown voltage ~38 V. After wire bonding the devices in parallel, they had a combined dark current of ~0.15 pA at 2 V reverse bias. A peak quantum efficiency of 38% was achieved at 440 nm after bonding; this reflects a 10% loss in efficiency due to bonding. Figure 2 shows the device characteristics.

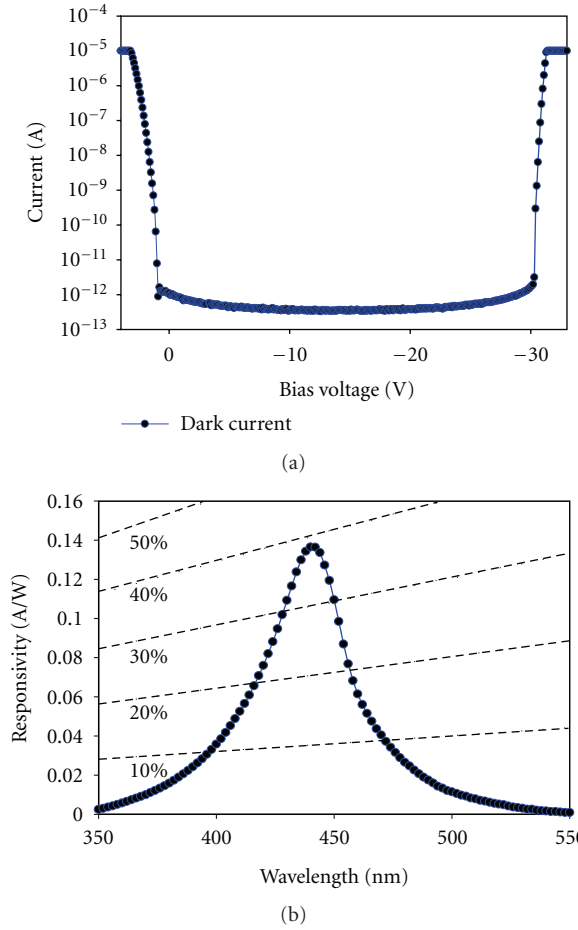

FIGURE 2: (a) Dark current versus voltage for two devices combined in parallel; (b) quantum efficiency versus wavelength for a single bonded device.

4. Measurement Setup

Fused silica capillaries with inner and outer diameters of 320 μm and 435 μm, respectively, were purchased from Polymicro Technologies. A window was opened in the capillary by removing a short strip of the polyimide coating. This window was aligned with the photodiodes, and the capillary was affixed to the detector system, thus integrating the two parts. Forced air was used to push solution from a vial in order to fill the capillary. The whole system was placed on a flat holder with openings for both the capillary and the excitation light. A 266 nm pulsed laser with 7.3 kHz repetition rate and a 400 ps pulse width was used as the excitation light source. The laser is a compact model and consists of controller box length 6.5 inches, width 5 inches, and height 4 inches as well as the actual laser which is 6 by 3 by 2 inches. The capillary was illuminated by focusing the laser signal with a microscope objective at 90 degrees to the photodiodes. Any light from the laser that is detected

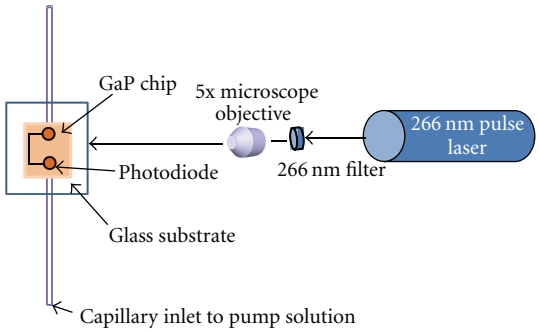

Figure 3: Schematic depiction of measurement setup.

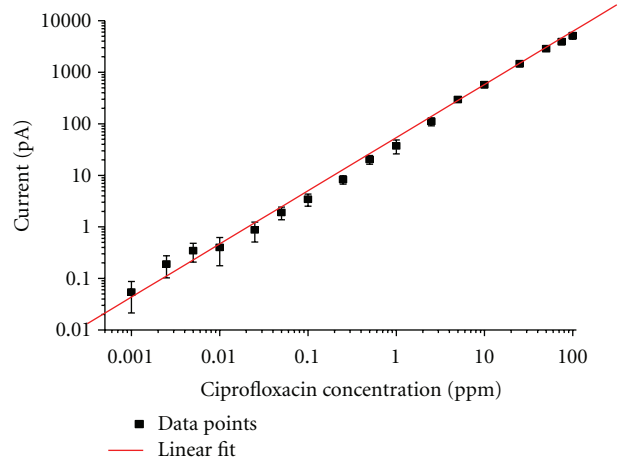

Figure 4: Linear detection photocurrent versus ciprofloxacin concentration.

by the photodiodes contributes to the background noise level; to reduce the scattered laser light, a narrow-band filter 266 ± 10 nm was placed before the microscope objective. A schematic of the measurement apparatus is depicted in Figure 3.

5. Linear Fluorescence Detection

Ciprofloxacin powder was purchased from Fluka, Sigma-Aldrich, and a stock solution of 100 ppm of ciprofloxacin with equal parts of methanol and deionized water was prepared. The stock solution was further diluted with equal parts of methanol and de-ionized to prepare varying concentrations.

Two GaP photodiodes connected in parallel were used for these measurements. Each device was 220 μm in diameter. For linear detection, the devices were biased at −2 V and the average current was measured with a Keithley 6430 SourceMeter. The laser was focused to a spot size of ~ 150 μm. For light incident perpendicular to the detector <1% of the scattered is detected. In operation, light is incident perpendicular to the detector and the scattered light accounts for <1% of the signal. When the laser is focused on the capillary directly adjacent to either detector, the scattered light signal in the other detector is below the detection limit. The lowest background current was therefore achieved when the laser was positioned on the capillary adjacent to one of the detectors. Since the fluorescence from the ciprofloxacin is anisotropic, the fluorescence was detected by both photodiodes even though the sample was illuminated only under the first detector. Placing the two detectors in parallel effectively increases the fluorescence detection area while the excitation was proximate to a single device, which significantly reduced the background level.

The photodiode current was measured as ciprofloxacin of different concentrations were pumped through the capillary. When the current reached a stable maximum, a ten data point average was recorded. Methanol was pumped through the capillary after each ciprofloxacin concentration. The methanol flushes any traces on ciprofloxacin from the capillary. This process was continued until the total current returned to the background level. Each concentration was measured three times, and the averaged data along with standard deviation bars is shown in Figure 4. Concentrations from 1 ppb to 100 ppm were measured. The system showed a linear response for concentrations in this range.

6. Conclusion

Integration of two parallel connected photodiodes with a microcapillary was investigated. Individual devices had a peak quantum efficiency of 38%, and the connected parallel devices had a combined dark current of 0.15 pA, both at reverse bias 2 V. This system is a simple and effective way of detecting fluorescence from solutions in the wavelength range 400–480 nm. It can potentially be used for initial measurements but, due to its inability to separate analytes, is not a replacement for HPLC or CE. Linear detection of ciprofloxacin concentrations from 100 ppm down to 1 ppb was achieved.

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