Malaysian Journal of Science **27** (1): 121 – 127 (2008)

Occurrence of the "Peaking Effect" Corresponding to the "Highest Range of Effective Intensities" exhibited by Bacteriorhodopsin (bR) – Carboxymethylcellulose (CMC) Biosensor upon Illumination

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Received 9th August 2007, accepted 13th December 2007.

ABSTRACT Photo-electrical characterization works were carried out to understand the basic functionality of the novel bacteriorhodopsin (bR) - Carboxymethylcellulose (CMC) photosensor proposed and fabricated earlier. A single-pixel sensor was fabricated and its photoresponse towards light intensities and thermal variations studied. The occurrence of the "peaking effect" corresponding to a certain "highest range of effective intensities" upon light illumination was also observed and discussed.

ABSTRAK Pencirian foto-elektrik telah dijalankan untuk memahami operasi am peranti perantis peka-cahaya bacteriorhodopsin (bR) – Carboxymethylcellulose (CMC) yang telah dicadangkan dan dibina sebelum ini. Suatu peranti peka-cahaya satu piksel telah dibina dan sifat tindakbalas foto peranti tersebut terhadap keamatan cahaya dan variasi haba dikaji. Kejadian "kesan memuncak" merujuk kepada suatu "julat keamatan berkesan paling tinggi" terhadap sinaran cahaya juga telah dicerap dan dibincangkan.

(Bacteriorhodopsin, bR-CMC photosensor, peaking effect, highest range of effective intensities)

INTRODUCTION

Much anticipation and interest is shown worldwide as to the manipulation of bio-molecular based technology into optical data processing systems. Since a high level of freedom in molecular reengineering is feasible, the native molecular material is easily scrutinized for independent parameter optimizations for various systems requirements. One such bio-material is bacteriorhodopsin (bR) [1 - 3] found in a certain type of bacteria living only in extremely salty water conditions. bR is fast becoming a popular research material for its high level of photosensitivity and photoreaction. As such, many bR-based sensors that mimic the natural functions of native bR by converting light energy into electrical energy have been investigated by various groups [4 - 7] for its photosensing capabilities.

In an earlier work [8], we had successfully acquired stable switching profiles from a novel bR-Carboxymethylcellulose (CMC) sensor fabricated according to a protocol developed to investigate the optical data processing capabilities of the biosensor. To further complement the earlier observations, investigations pertaining to understanding the photo-thermal relationship were carried-out using a single-pixel bR-CMC photosensor fabricated according to the earlier protocol.

MATERIALS AND METHODS

Materials Preparation

PM (molecular weight without retinal of 26784 Da) of variant type V1 containing lyophilized powder of bR molecules from Halobacterium salinarum were purchased from MIB, Munich Innovative Biomaterials, Germany. Provided product information stated absorption maximum of bR in deionized water after light adaptation at 566 nm, while absorbance ratio (referring to the ratio of maximum optical densities, ODs at 280 nm and 570 nm), OD280 / OD570 at 2/3. For the purpose of the study, bR was suspended in pure deionized water to form a bR suspension with a concentration of 0.2 mg/ml. The gel-like "artificial membrane" CMC-salt viscous gel electrolyte, bought in the form of powder from Sigma Chemicals (product number 419273-100G), was prepared by slowly dissolving 6% CMC in 1 M of KCl (pH 7.4) prepared earlier in purified deionized water. Homogeneity of the gel electrolyte and the bR suspension was achieved by means of using an agitator (Model 34524), supplied by Snijders Scientific, Holland. Gold coating (resistivity of 11.5 Ω/cm) formed on standard glass slides (2.50 cm x 7.50 cm) used in the work was achieved by using a BIO-RAD SEM sputtering system. The counter electrode used, the semi-transparent ITO conducting electrode (2.50 cm x 7.70 cm) had a surface resistivity of about 75.0 Ω/cm. Single pixel light-receptive area of 1.25 cm x 0.90 cm on the gold sputtered electrode was prepared by simply using a sharp metal point to define the dimensions of the light receiving area and thoroughly wiping of the rest of the sputtered gold on the slide with a filter paper. Electrical connections for the pixel from the edge to the underside of the pixel bearing glass slide were made using silver conductive paint (resistivity of 1.20 Ω/cm) obtained from RS Components, United Kingdom. Copper wires were then attached to the individual electrical connections using the silver conductive paint which acts both as glue and when fully dried the conductive connection material. To seal the sensor against humidity fluctuations, a silicon based adhesive sealant (Selley Silicone Sealant) was used.

Fabrication of the bR-CMC photosensor

A glass slide prepared and washed with methanol and deionized water is sputtered with gold and by using the sharp metal point as mentioned earlier, a single pixel configuration is prepared on the gold coated glass slide (working electrode), which acts as the gold electrode. Electrical connection is made by using silver conductive paint on the edge of the gold pixel (the side of the glass slide) to the underside of the gold electrode. A copper wire is then attached by using the silver conductive paint to the electrical connection made on the underside of the pixel bearing glass slide. Using a 1 ml syringe, a drop of the gel-like CMC solution (about 0.13 ml) was applied onto the gold pixel before a drop of bR suspension (about 0.1 ml) was deposited using a syringe directly onto the CMC droplet. To induce high molecular orientation of bR as it fuses into the CMC viscous droplet, the gold pixel bearing the bR-matrix was placed on an aluminium base and kept under a high electric field of 50 V/cm to utilise the bR's net negative charge and electric dipole moment. Silver paint connecting the edge of the gold pixel to the underside of the glass slide bearing the pixel enables electrical connection to the aluminium base, which is connected to the positive polarity of the dc supply as shown in Figure 1. The ability of the bR molecules in maintaining their molecular orientation even after the removal of the electric field and the drying of the electrode while retaining the natural intrinsic properties of bR has been shown by Puu *et al.* [9]. Photosensors prepared in such ways also enables a high efficiency in generating photoelectric current [10] since almost all proton pathways are orientated in the same direction.

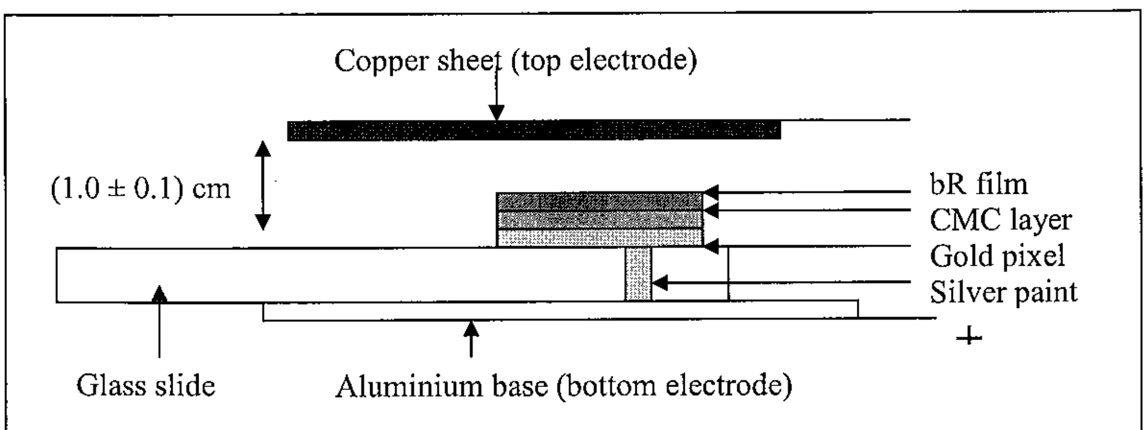

Figure 1. Diagram showing the top and bottom electrode to create electric field

To allow the pixel bearing electrode to dry naturally under the electric field and normal room temperature, the whole setup is kept in dark for about 48 hours. An ITO slide washed with methanol and deionized water is dried and used as a counter electrode by sandwiching it with the prepared bR-matrix on the gold pixel. To allow good adhesion, the working electrode is wetted using a drop of deionized water from a 1 ml syringe before sandwiching. If the weight of the ITO slide is placed immediately on top of the "gel-like" bR matrix before allowing complete drying, the viscous matrix will spread all over and flow out of the gold pixel. By keeping the fabricated bR-sensor overnight in dark conditions to dry naturally under the same electric field and room temperature, the electric dipole moment of bR will be oriented in the same direction. The edge of the sensor is then sealed using the silicon based adhesive sealant to reduce the effects of humidity fluctuations after complete drying. To give robust physical support, the entire sensor is placed permanently by using adhesive tapes onto a solid polystyrene base and kept in dry and dark conditions overnight [12] before any signal acquisition works are carried out.

Experimental setup for signal acquisition

In order to induce efficient photoreaction and therefore obtain good photoresponse, a high-powered 1000 W Metal Halide (MH) white light source was utilized. The light source recreates an almost "sunlight-like" continuous flow of high energy photons consisting of a broad spectrum of wavelengths. By simply exposing the bR-CMC sensor to the light source over a varying distance from the light source (155.0 to 70.0 cm), studies into the activation light intensity and heat effect profiles are carried out (detected at a vertical height of 14.0 cm). Measurements used to generate the various photo-electrical profiles were taken using the same sensor. Signal stability and reproducibility were ascertained by observing the peak values generated over a period of a few months. Initial investigations showed quite stable signal generation as was also demonstrated in the earlier work.

RESULTS AND DISCUSSIONS

The photoresponse-distance graph in Figure 2 shows an expected overall increasing profile up to about 85.0 cm away from the light source. This range of distances and therefore the intensities corresponds between the temperature ranges of 25.0 to 27.5°C generated a difference of 2.5°C.

Since the decreasing distances towards the light source represents rapidly increasing intensities (corresponding to the small temperature difference of about 2.5°C), a similar profile was also seen for the photoresponse-intensity (150.0 to 70.0 cm) profile shown in Figure 3. As the sensor is known to produce higher photoresponse towards medium strong sunlight with higher light intensities (exposure to sunlight registered values over 100.0 mV), the following sharp drop at 27.5°C (Figure 4) was attributed towards the increase in temperature. Distances at the points around the peak (85.0 cm), 90.0 and 80.0 cm registered the temperature values of 27.0°C and 27.5°C respectively. Therefore the peak photoresponse observed corresponds to a value of temperature between these two points.

However, small fluctuations in temperature as registered by the insensitive Hg-based thermometer between these points may not be very accurate. As such, the peak observed should be considered to occur at about 27.5°C. For efficient photoresponse for the proposed bR-CMC design, the activating range seems to be from about 150.0 to 85.0 cm distance (25.0 to 27.5°C) from the light source without jeopardising bR's intrinsic proton production and pumping mechanism. Beyond this point with further increase in surrounding heat, the bR-sensor will also work as expected in producing efficient photoresponse but with reduced outputs.

To further investigate this phenomenon, an experimental setup to increase light intensity while keeping the surrounding temperature at about 27.5°C to recreate the "peaking" phenomenon was prepared. This involved putting a square semi-transparent Perspex-curtain (15.0 cm x 15.0 cm), acting as an attenuator, in between the sensor and the light source. The inserted Perspex-curtain attenuates lower the intensity and heat received by the bR-sensor. Since light intensity shows a higher increase towards the light source as compared to temperature, higher values of intensities (and therefore the generated photovoltage) are achieved for each value of temperature measured (Figures 5 and 6). As such, higher intensities at lower temperatures can be controlled by simply moving the glass-curtain along the distance from the light source.

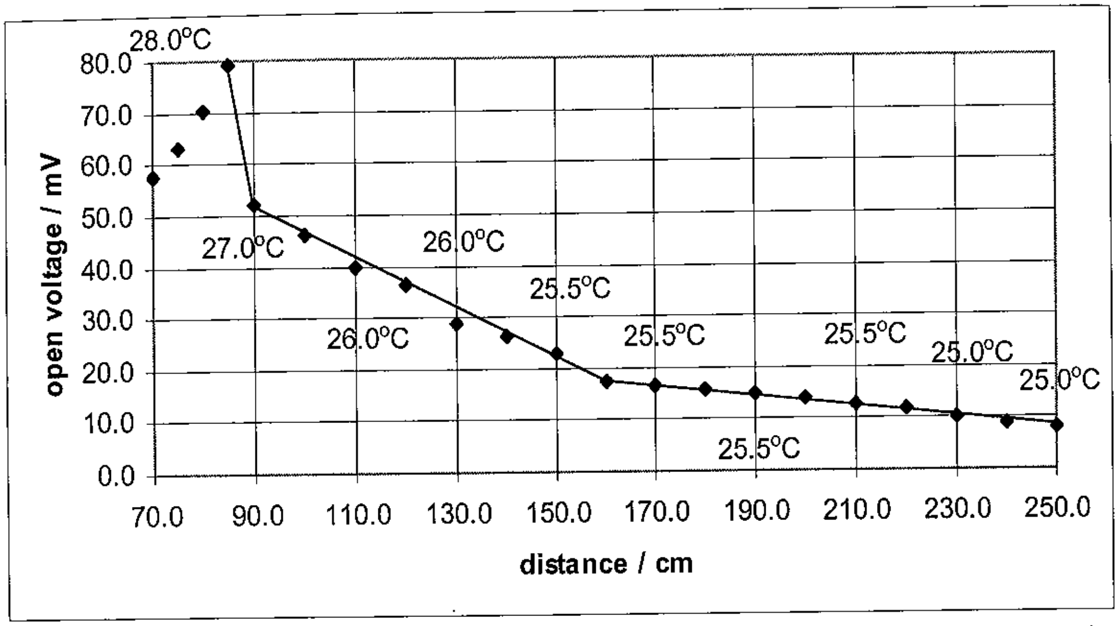

Figure 2. The open voltage-distance profile exhibited by the bR-CMC biosensor clearly shows the 3 gradients

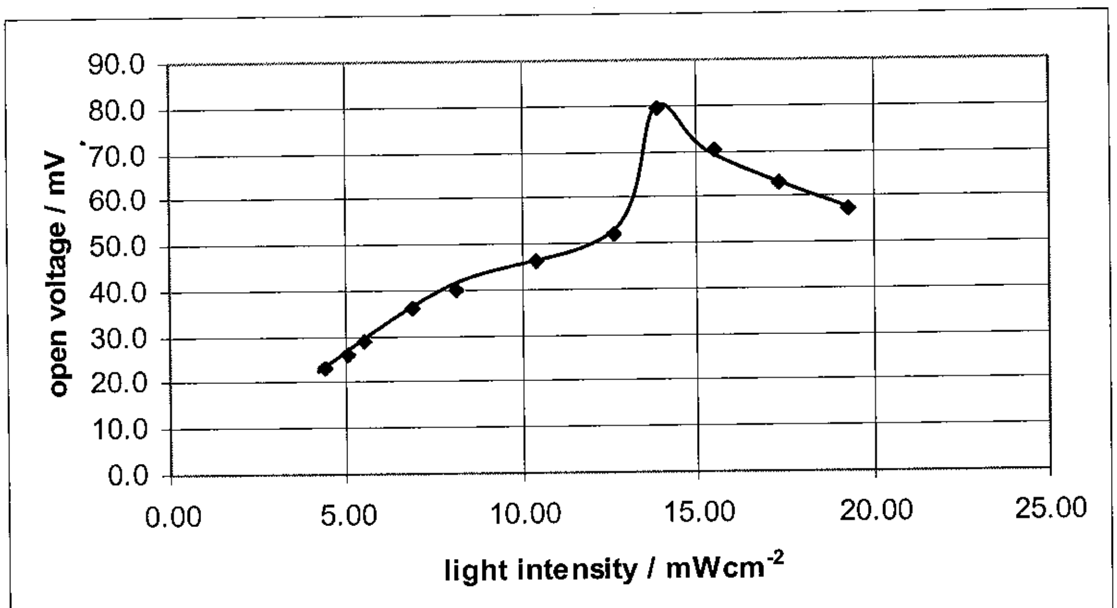

Figure 3. The 3rd gradient shown here actually represents a higher effective light intensity range for generating photoresponse. This range corresponds from about 12.62 to 13.87 $mWcm^{-2}$, which is the maximum photoresponse peak. The sharp peak seen occurs at about 85.0 cm away from the light source at an intensity of 13.87 $mWcm^{-2}$.

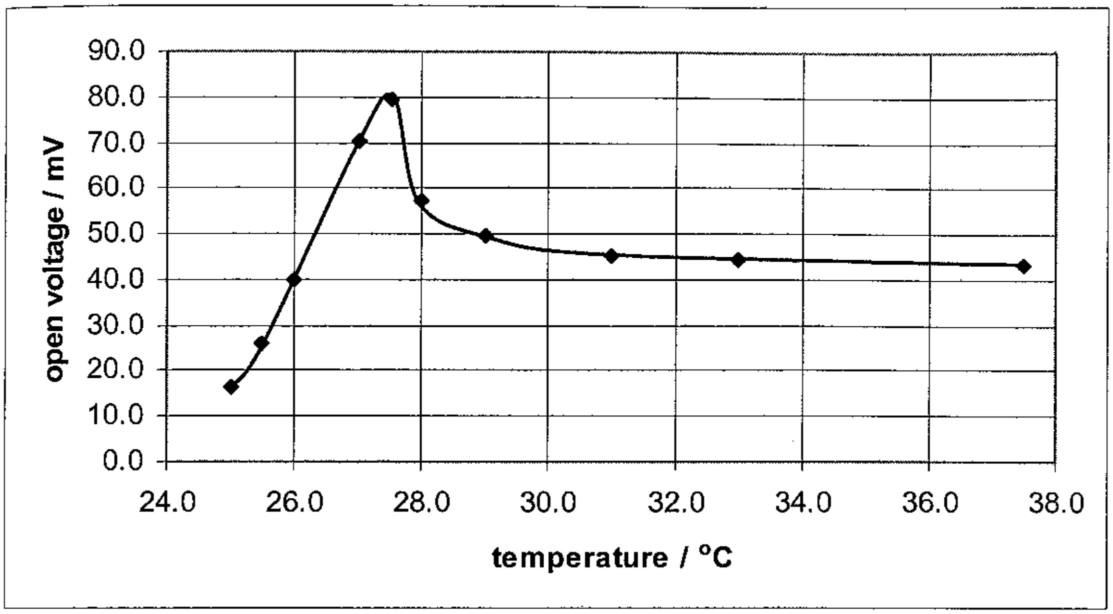

Figure 4. The photoresponse-temperature profile showing the "peaking effect" occurring at 27.5°C

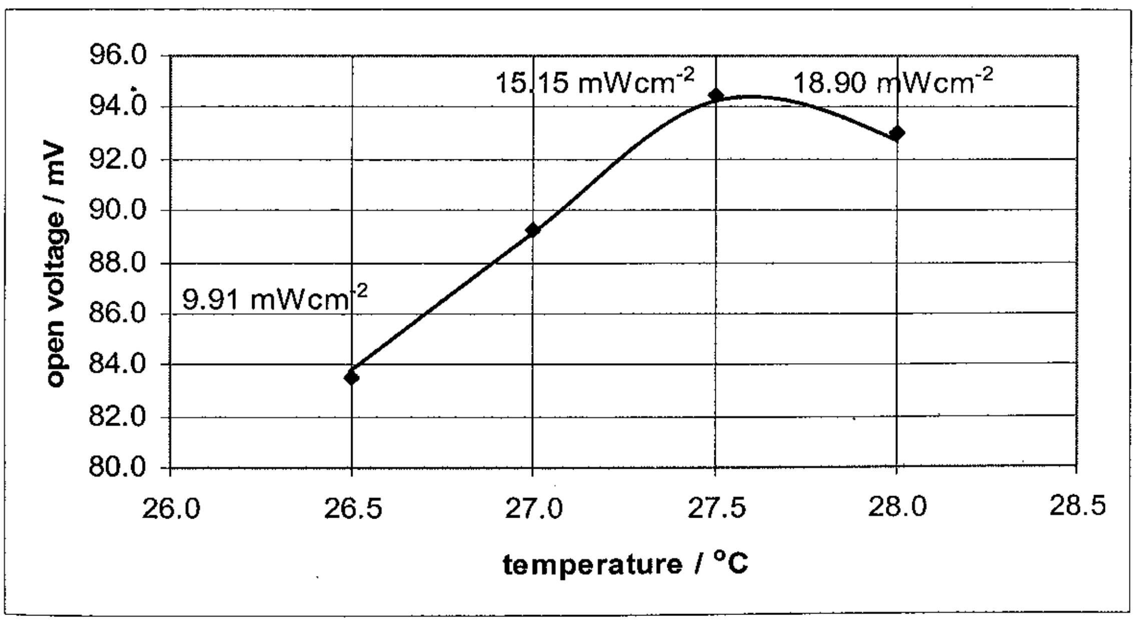

Figure 5. Photoresponse-temperature graph obtained clearly portrays the phenomenon with maximum photoresponse peaking at about 27.5°C.

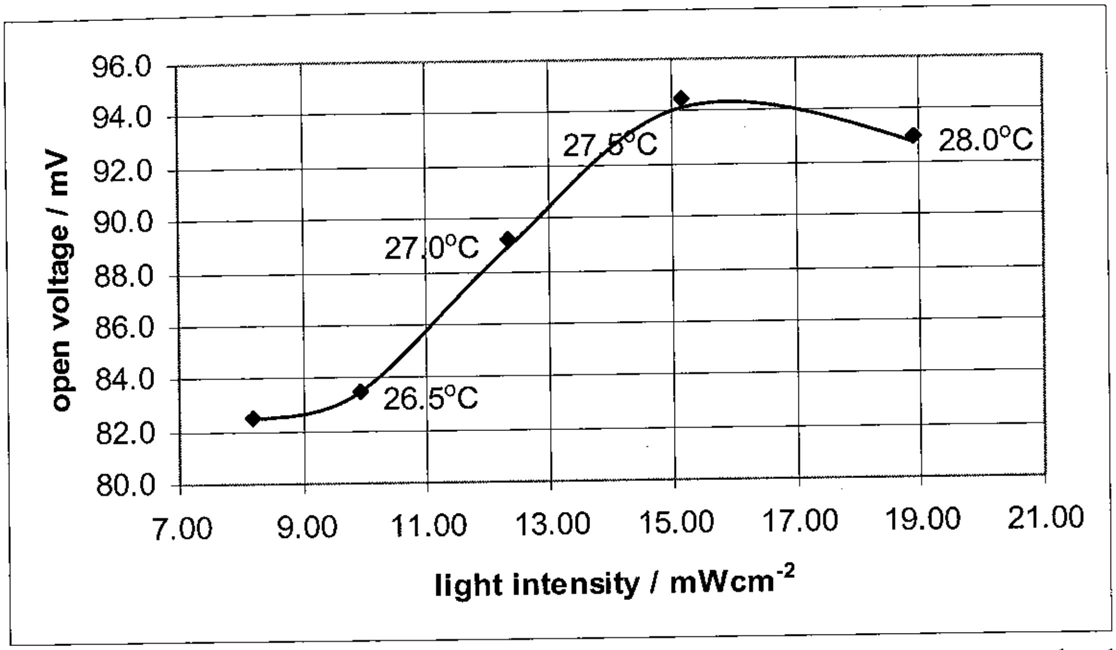

Figure 6. Photoresponse-intensity graph showing higher values of light intensities as compared to the surrounding temperature

By moving the attenuator forward or backward, higher intensities for a temperature range of 26.5 to 28.0°C were registered for decreasing distances from 100.0 to 60.0 cm. In an initial test with the photometer, intensity above 20.00 mWcm^{-2} was registered for a distance of 60.0 cm from the light source. With the attenuator inserted, the photometer showed a reading of 18.90 mWcm^{-2} for the same distance. Although the intensity was increased, the peak value remains at the same temperature as shown in Figure 5. The observations and results therefore prove that this "peaking" phenomenon is clearly heat related. At this point, it can be safely concluded that the effect of heat plays a much more important role on generating photoresponse outputs at very close distances compared to the effects of the continuously increasing intensities.

The photoresponse-light intensity graph shown in Figure 3 suggests the 3rd gradient as the "highest range of effective intensities" (12.62 to 13.87 mWcm^{-2} at about 27.0°C to below 27.5°C) to generate photoresponse. This explains the more effective nature of sunlight intensity to induce photoreaction. Higher sunlight intensities are transmitted at obviously lower temperatures since the vast surrounding environment absorbs and dissipates the generated heat to a much lower value. As thus, a higher effective range of intensities are generated at much lower temperatures. In the laboratory environment however, the light intensity from the MH light source was more forward oriented and as such generated much more heat into the surrounding areas. And since the overall heat generated by the MH light source is much higher at closer distances (so as to increase the light intensity), the most effective range of intensities to generate photoresponse was obviously shorter.

The "attenuator experiment" further proved this phenomenon. The graphs in Figure 5 and 6 show a higher value of intensity at a closer distance from the light source generating higher photoresponse value. It marks an increase in intensity from 13.87 to 15.15 mWcm^{-2}. This increase in intensity relates to a proportional increase in the photoresponse yield, which registered 94.50 mV as compared to the earlier value of 79.30 mV. Here the "peaking" still occurs at 27.5°C but when the attenuator was inserted between the sensor and the light source, the same temperature was obtained at a closer distance with a higher value of light intensity.

CONCLUSIONS

Initial characterization works show the existence of an optimum operating temperature. This "fixed" temperature value which corresponds to the "peaking effect" influenced by the "highest range of effective intensities" suggests a certain mechanism by which the bR-CMC biosensor operates. To fully understand the behavior of the biosensor under various operational parameters, an obvious level of clarity on the functioning mechanism is thus required. Therefore, the future characterization works would be directed towards better understanding the biosensor behavior by employing higher light intensities with controllable environment temperatures so as to emulate the "peaking effect" at higher intensities. As such, studies into the possibility of the occurrence of various "peaking effect" stages could be investigated and ascertained.

The bio-signals generated exhibited good reproducibility in terms of the "peaking effect" observed. Stable measurements acquired from a four months old sensor suggest good long-term stability. These crucial factors enable future realization of developing robust and practical bR-based photosensing devices.

ACKNOWLEDGEMENTS

This work was partially supported by VOTE-F grant IPP/UPDiT/Geran (PJP)/F0161/2003D. We would like to extend our gratitude to the staff at the Institute of Postgraduate Studies, University of Malaya for their assistance and all others for contributing positively towards the success of this project.

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