



Total protein measurement using a fiber-optic evanescent wave-based biosensor

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Abstract

A novel method and instrumental system to determine the total protein concentration in a liquid sample is described. It uses a fiber optic total protein sensor (FOPS) based on the principles of fiber optic evanescent wave spectroscopy. The FOPS applies a dye-immobilized porous glass coating on a multi-mode optical fiber. The evanescent waves at the fiber optic core-cladding interface are used to monitor the protein-induced changes in the sensor element. The FOPS offers a single-step method for quantifying protein concentrations without destroying the sample. The response time and reusability of the FOPS are evaluated. This unique sensing method presents a sensitive and accurate platform for the quantification of protein.

Introduction

Quantitative and qualitative analytical techniques are of considerable importance in various aspects of life sciences for the purpose of detection, identification and measurement of concentrations of biologically important molecules, such as proteins. Over the years, several methods based on standard colorimetric assay have been developed to detect and quantify protein concentration in biological samples (Lowry *et al.* 1951, Bradford *et al.* 1976). However, each of these methods presents certain limitations pertaining to its sensitivity, accuracy, and reproducibility (Sapan *et al.* 1999). The recent trend to improve biochemical analysis is to provide a platform, which is more sensitive, accurate, portable, rapid and economically viable.

Optical fibers are versatile due to their geometrical versatility, remote-sensing capability, small dimension, low weight and availability of inexpensive fibers. The fiber optics based biosensor can also facilitate the sensing of multiple parameters using a single optical fiber, without any cross talk in real-time (Cunningham 1998). The fiber optic total protein sensor (FOPS) de-

veloped in this study is based on the variation in the evanescent wave phenomenon at the core-cladding interface. The theoretical formalization of this concept can be found elsewhere (Ruddy *et al.* 1990). In the FOPS design, an optical fiber is used as the transduction element. Concomitantly, a chemical recognition unit is used to generate an analyte-dependent, spectroscopically detectable signal within the sensing region of the optical fiber. The chemical change that occurs because of the interaction between the analyte and immobilized indicator are measured by monitoring the electromagnetic radiation that returns from the sensing unit. In this work, the authors describe a fiber optic evanescent wave based protein sensor to determine the total protein concentration in a liquid sample. The response time and the reusability of the FOPS are also evaluated in this study.

Materials and methods

Coomassie Brilliant Blue G-250, bovine serum albumin (BSA), haemoglobin, cytochrome c, ovalbu-

min, multimode optic fiber, tetraethyl ortho-silicate (TEOS), ethanol, HF (50% v/v), phosphoric acid, phosphate buffered saline (PBS) pH 7.4 were used in this study. All chemicals were of analytical grade.

Fabrication of the fiber optic total protein sensor (FOPS)

Five plastic clad silica fibers of length 1 m each were used to fabricate the FOPS. They had a core diameter of 200 μm and a numerical aperture of 0.22. A length of 0.04 m of the outer protective sheath of the optical fiber was removed in the mid region of the selected optical fibers. The uncovered region of fiber was then soaked in a 50% (v/v) HF for 30 min. This facilitated the complete removal of the cladding as well as allowed uniform etching of the glass core of the optical fiber. The surface-prepared portion of the optical fiber was then washed and dried at room temperature. Once dried, the etched portions of the optical fibers were treated with 1 M HNO_3 for 10 min. This step activated the $-\text{OH}$ groups on the surface of the glass core and, consequently improved the adherence of sol-gel layer with it.

The sol-gel technique was utilized to form a porous glass, thin-film coating around the cladding denuded portion of the optical fiber. The preparation of the sol-gel was carried out at room temperature by the hydrolysis and condensation of tetraethyl ortho-silicate (TEOS) in an acidic environment to form siloxane polymer leading to gelation (Rabinovich 1994). The hydrolysis reaction proceeded by the replacement of the ethoxy groups in TEOS by the OH groups from water.

The starting solution was prepared by the partial hydrolysis of TEOS following a previously described procedure (Brinker *et al.* 1990). The ratio of TEOS, deionized water and ethanol was 12:3:1 by vol. One ml ethanol and 100 μl 1 M HCl were added to the mixture under constant stirring with a magnetic stirrer for 1 h and after that, the protein indicator solution, prepared from 500 mg of Coomassie Brilliant Blue G250, 50 ml ethanol and 150 ml H_3PO_4 in 1000 ml water, was added to the precursor liquid at a ratio of 3:1 (precursor liquid: indicator v/v). The entire mix was maintained under constant stirring for a period of 30 min at room temperature.

The polymer at this consistency was used to coat the prepared, uncladded portion of the optical fiber using dip coating equipment (KSV 3000 LB instruments, Seoul, Korea). The precursor solution containing the

indicator was placed in a 20 ml pipette. The surface prepared optical fiber, dried in a desiccator overnight was dipped into the pipette and drawn upwards using the dip coating equipment at a rate of 50 mm per min, at room temperature ($\sim 23^\circ\text{C}$). The coated fiber was then placed in a petri dish and allowed to dry for a period of 2 weeks. Prior to the experimentations with protein samples, the sensor portion was also examined to determine whether the Coomassie Brilliant Blue could be washed out of the porous glass matrix. The observed intensity of the Coomassie Brilliant Blue in water after repeated washing confirmed that this was not occurring.

The experiments in this study were conducted in two stages. In the first stage, experiments were carried out in order to characterize the FOPS, while in the second stage, experiments were conducted to evaluate the performance, response time and the reusability of the FOPS.

Characterization of the fiber optic protein sensor

In developing a quantitative method for determining an unknown concentration of a given species by absorption spectrometry, the first consideration is the choice of the absorption band at which absorbance measurements are made. An UV-visible absorption spectrum of the species to be determined can be obtained experimentally by means of a scanning double beam spectrophotometer. Absorptivity at any given wavelength is a constant and an inherent characteristic of the absorbing species. The numerical value of the absorptivity will determine the slope of the analytical curve and will demonstrate the concentration range over which the determination can be made. Towards this end, five glass slides were coated with sol-gel immobilized Coomassie Brilliant Blue and their absorption spectrums were determined using the UV-visible spectrophotometer. These experiments were conducted to characterize the dye immobilized sol-gel matrix and to evaluate the suitability of sol-gel as an inert matrix to immobilize protein indicator.

Figure 1 shows the schematic of the experimental system employed in this study. A xenon arc lamp (Hamamatsu C6979) was used as the light source. An objective lens with a numerical aperture similar to optical fiber was used to launch the light from the source into one end of the optical fiber. A broadband pass-filter was also introduced between the light source and objective lens. The other end of the optical fiber was connected to a photodetector (S390 Universal Op-

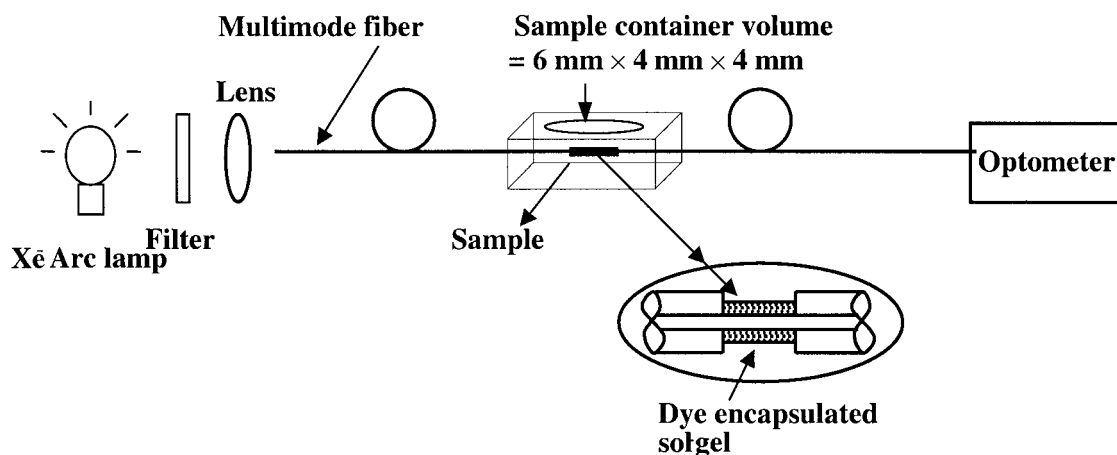


Fig. 1. Schematic diagram of the experimental set-up of the fiber optic total protein sensor.

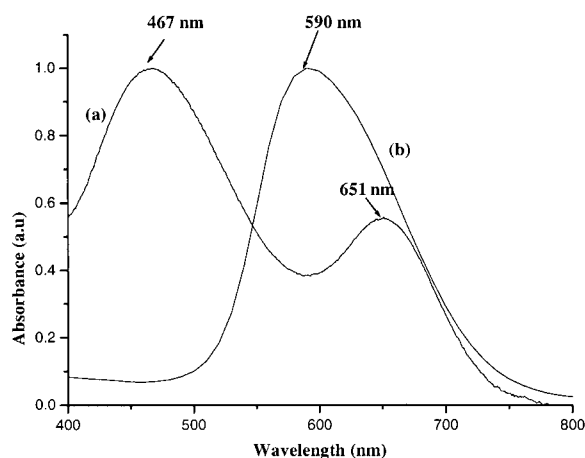


Fig. 2. Graph showing the UV-visible absorption spectrum of: (a) Coomassie Brilliant Blue (CBB) G-250, and (b) CBB when it binds with bovine serum albumin in a solution of ethanol, phosphoric acid and water.

tometer) linked to an optometer having a sensitivity of 10^{-12} W (spectral range of 310–700 nm). The photodetector along with the optometer aided in the monitoring photon variations at the end terminal of the optical fiber. A rectangular slot of 6 mm length, 4 mm width and 4 mm height was cut on a transparent perspex sheet of dimension 10 mm length, 6 mm width and 8 mm height was used as the container for the test sample. The dye-immobilized porous-glass coated portion of the optical fiber was secured on the base of the sample container using plastic wedges. This was done in such a way that a few drops (0.2 ml) of liquid sample would be sufficient to have the sensor portion immersed completely.

In order to evaluate the performance response time and reusability the experiments with the FOPS were carried out in two parts. In the first part of the experiment, the FOPS was tested with different concentrations of protein samples (stage 1). In the second part, experiments were conducted to determine the response time and the reusability of the FOPS (stage 2).

Stage 1: testing of protein samples with FOPS

Different protein samples such as BSA, haemoglobin, cytochrome c and ovalbumin were prepared in phosphate buffer saline at pH 7.4 to test the sensor. During experiments, each sample was separately added to the FOPS and the variation in the intensity of light at the output terminal was recorded. Each reading was taken after a stabilization period of 30 min from the onset, and each sample was tested with three different FOPS.

Stage 2: response time and reusability

The response time and reusability are considered crucial parameters in any biosensor. Therefore, experiments were conducted in this stage to determine the temporal response and possibility of reusability in the FOPS. BSA at $20 \mu\text{g ml}^{-1}$ was used as the protein sample. During testing, the sample was added to the FOPS portion, and the output intensity variation was monitored from the optometer. The optometer reading was recorded at every 5-min interval, for a period of 45 min.

The reusability of the FOPS was studied using 40% (v/v) methanol. [Methanol was used because of its ability to destain Coomassie Brilliant Blue (Ausubel 1991).] After each experiment, the sensing region of the FOPS was soaked in a 40% (v/v) methanol and the

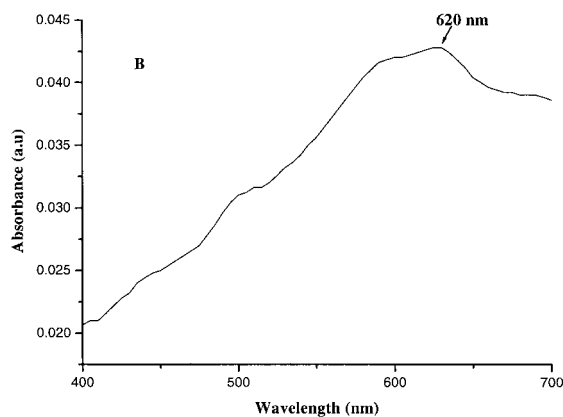
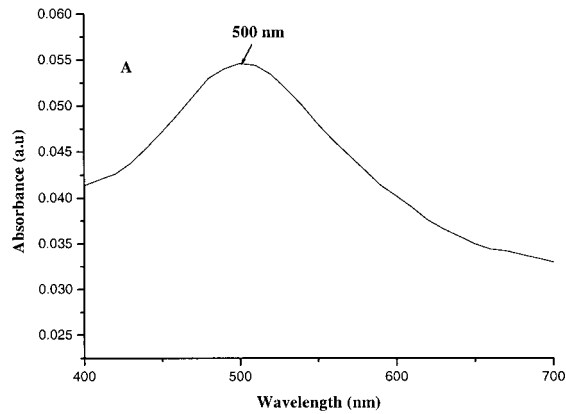


Fig. 3. Graph showing the UV-visible absorption spectrum of: (A) Coomassie Brilliant Blue (CBB) G-250 immobilized in sol-gel film, and (B) CBB in sol-gel film when it binds with bovine serum albumin.

output intensity variation displayed on the optometer was recorded at every 5-min interval, for a period of 1 h.

Results and discussion

Characterization of the fiber optic total protein sensor (FOPS)

Figure 2 shows the UV-visible absorption spectrum of Coomassie Brilliant Blue G-250 in a solution of ethanol and phosphoric acid. The Coomassie Brilliant Blue G-250 in the above composition exists in red and blue forms. Figure 2 displays the two absorption peaks for Coomassie Brilliant Blue at 467 nm and 651 nm

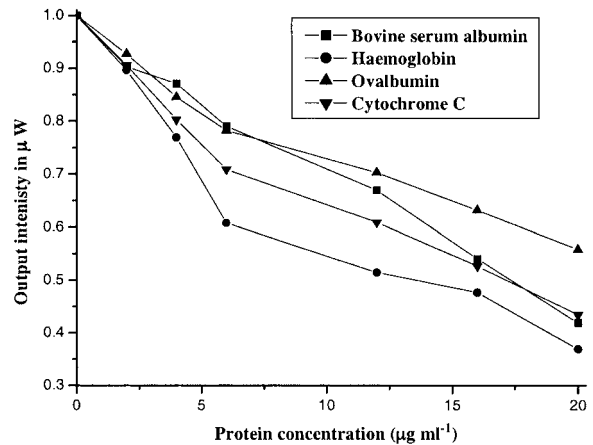


Fig. 4. Calibration curves of bovine serum albumin, haemoglobin, ovalbumin and cytochrome c.

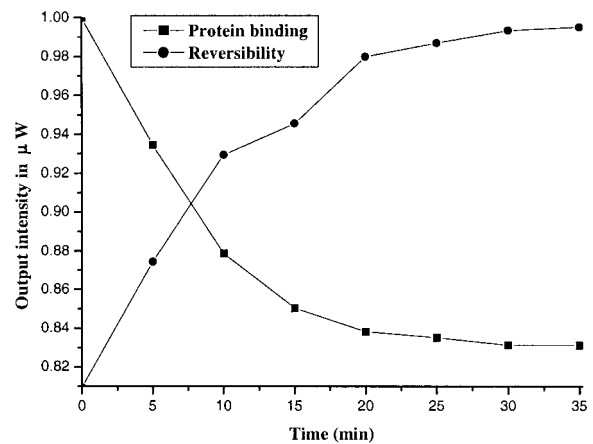


Fig. 5. Temporal response of the fiber optic total protein sensor when tested with bovine serum albumin at $20 \mu\text{g ml}^{-1}$.

wavelengths. Protein binding with the dye caused a shift in the above absorption peaks to 590 nm (Zor *et al.* 1996). This shift in the absorbance maximum was utilized in the FOPS to measure the protein concentration as explained in the following. Figure 3a represents the absorption spectra of Coomassie Brilliant Blue immobilized in a sol-gel film obtained from the UV-visible spectrophotometer. The maximum absorption peak was at 500 nm. Figure 3b shows the UV-visible absorption spectrum of the Coomassie Brilliant Blue immobilized in sol-gel after immersing in $100 \mu\text{g BSA ml}^{-1}$ for 5 min. There was a vivid shift in the absorption maximum towards 620 nm. Coomassie Brilliant Blue prepared at an acidic pH exists in a deprotonated form and when exposed to a protein, hydrophobic interactions form a dye-protein complex (Tal *et al.* 1985). This production causes the devel-

opment of a protonated form of Coomassie Brilliant Blue to display an absorption maximum at 590 nm (Sedmark *et al.* 1972).

The sol-gel immobilized dye differed from that in solution state. The difference in the maximum absorption peak value between the sol-gel immobilized dye and liquid dye can be attributed to the difference in the pK value of the dye in solution form and that in the sol-gel matrix (Gupta *et al.* 1997). The shift in absorption maximum of the Coomassie Brilliant Blue immobilized sol-gel formed the basis of the FOPS developed in this study.

Testing of the fiber optic total protein sensor (FOPS)

Stage 1: testing of protein samples with FOPS

Figure 4 illustrates the calibration curves of bovine serum albumin, haemoglobin, cytochrome c and ovalbumin obtained from the FOPS. There was a decrease in output intensity as the concentration of protein increased. This variation in output intensity was due to the dye-protein interaction at the sol-gel thin film, which caused a shift in the absorption peak maximum from 467 nm to 590 nm. Calibration of protein assay is crucial to ensure accuracy during testing with FOPS. Further, the protein concentrations should be within the dynamic range of the assay (Johnson *et al.* 1978).

Stage 2: response time and reusability

Figure 5 represents the temporal response of FOPS when tested with BSA at $20 \mu\text{g ml}^{-1}$. Output intensity decreased with time up to about 30 min. For the first 10 min, there was a significant reduction in output intensity due to the onset reaction of dye-protein complex. From 10 to 30 min, the gradient of output intensity reduction with time decreased as the dye-protein complex reached saturation. After 30 min, the output intensity reading began to stabilize upon the completion of the dye-protein reaction. Using this sensor method, the total protein concentration could be measured within 10 to 15 min. This is significantly faster than current techniques, such as the conventional colorimetric method, which takes about 1 h.

For the reusability tests for the FOPS, this was assessed by soaking the sensor in 40% (v/v) methanol. A total reversibility of the FOPS occurred after about 35 min soaking (Figure 5). For the first 10 min, there was a significant increase in output intensity. From 10 to 30 min, a more gradual increase in output intensity occurred. After 35 min, the output intensity stabilized

upon the completion of the reaction, suggesting that the dye-protein interaction had broken, thus resulting in the restoration of the spectral absorption maximum at 590 nm. The tests for reusability of used fibers were carried out twice to confirm repeatability. Both tests yielded the same results.

Conclusions

A new sensor technique, based on fiber optic spectroscopy for the rapid and sensitive detection of total protein, has been developed. The fiber optic total protein sensor enables single-step detection and quantification of microgram levels of total protein directly without destroying the sample. The highly sensitive Coomassie Brilliant Blue assay and fiber optic spectroscopy were utilized in this study to develop the novel biosensor. The evanescent waves from the optical fibers excited and sensed the presence of protein via color changes in the dye-immobilized porous-glass film along the cladding denuded section. The response time for the new sensor to determine concentration of protein in a sample was about 10 to 15 min. The sensor could also be reused by submerging it in 40% (v/v) methanol for about 35 min.

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