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Research Article

Advancement Towards Microfluidic Approach to Develop Economical Disposable Optical Biosensor for Lead Detection

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Abstract

An economical single use optical biosensor has been developed for lead detection using a microfluidic approach. The present study represents the initiative step for amalgamation of microfluidic and advanced biosensor technologies which offers rapid analysis with lab-on-a-chip policy. Urease producing *Bacillus sphaericus* was co-immobilized with phenol red (pH indicator) in the glass capillary which acted as a microchannel. For immobilization, a combination of sol-gel approach and calcium alginate method cited first time in literature was used which reduced the time of solidification to seconds as compared to hours with sol-gel alone. Bioassay principle was based on urease inhibition in the presence of lead. Fiber optic spectrophotometer was used as transducer which measured the intensity of color change. Linear relationship (10-1000 µg/L) was observed between logarithmic concentration of lead and absorbance. The study resulted in the development of cheap, miniaturized, sensitive and reliable lead biosensor with requirement of small sample volume (1 mL).

Keywords: *Bacillus sphaericus*; Microfluidic; Fiber optics; Lead; Sol-gel; Biosensor

Introduction

Increased environmental awareness and stringent environmental regulation led to the dire need of the techniques for the fast, easy and economic detection of various pollutants like heavy metals, pesticides and toxic gases. Heavy metals are among the most hazardous pollutants due to their ubiquitous presence in the biosphere, their bioavailability from both natural and anthropogenic sources as well as their high toxicity even at trace level [1,2]. Lead is among the toxic heavy metals which harm the body when present above the threshold concentration [3]. The higher concentration of lead (>18 µM) in the blood may cause coma and death [4]. It affects different parts of the body particularly brain and central nervous system. Accumulation of lead in the body produces damaging effects associated with hematology, neurology and nephrology which include paralysis, mental retardation and neural deafness. The substitution of calcium in the body with lead causes impairment in the development of bones and teeth [5]. Human exposure to lead occurs primarily through lead-based paints, industrial waste, water from lead-laden pipes, soil and dust generated from gasoline and food items like milk, dairy products and imported candies. The cattle grazing on the metal contaminated fields transmit lead contamination in the milk and dairy products [4,6,7]. A threshold lead limit of 10 µg/L was estimated in food and water by the International Agency for Research on Cancer (IARC) while 5 µg/L in drinking water by Environmental Protection Agency (EPA) [8,9]. It is mandatory, therefore, to detect the lead in food samples and drinking water to prevent the deleterious effects of lead. Conventional methods to detect heavy metals like differential pulse polarography, Atomic Absorption Spectrophotometry (AAS), Differential Pulse Cathodic Stripping Voltametry (DPCSV) are superseded by the use of biosensors [10,11].

Biosensors are analytical tools capable of providing either qualitative or quantitative results, consisting of an immobilized biological recognition element such as an enzyme, antibody or cell receptor immobilized to a physicochemical transducer to create a single unit. Now-a-days biosensor technology has emerged as the most promising tool for detection of heavy metals being cost-effective, fast, selective, sensitive, portable, easy to use and reliable [12]. A number of lead biosensors have been developed using various transducers like conductometer [13-15], electrochemical [16-22] and optical [3,23-27] based on whole cell, enzymes or DNAzyme.

The present study depicts the use of optical transducer along with microfluidic approach. Microfluidic system allows the controlled flow of operations like fluid transport, valving, mixing, separation, concentration and detection in Lab-on-a-Chip field [28] which helps to overcome the matrix effect, a major hindrance in the widespread use of biosensors for extra laboratory testing and drug arrays. Fluids are pumped into a particular channel known as microchannel at defined rate. Microfluidic is competent to analyze small sample volumes e.g. 10^{-9} – 10^{-18} L [29]. A few reports are available for use of microfluidic approach in biosensor technology. Chang and his workers [30] fabricated a microfluidic biosensor for lead detection using DNAzyme exhibiting linearity in the range of 0.1-100 µM (0.02-20.7 mg/L) concentration of lead with detection limit of 11 nM. The biosensor developed in this study demonstrated the linear range of 10-100 µg/L Pb^{2+} which is far lower than previously described biosensor making it suitable for the application in milk, water and

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clinical samples. Moreover, use of whole cell for the fabrication of biosensor rather than DNAzymes help to make it economical. The present approach helps to realize the miniaturization of biosensors which provides the advantages of portability, reduced cost, increased analysis speed, reduction in sample and reagent consumption.

Materials and Methods

All the chemicals and reagents used in the study were of analytical grade. Glass capillaries (Ø1mm) were used to prepare microchannels. Fiber Optic Spectrophotometer (Ocean Optics, Maya 2000 series) has been used as a transducer.

Biocomponent

Urease producing microbe *Bacillus sphaericus* MTCC 5100 (a novel soil isolate of Biosensor Technology Laboratory, Department of Biotechnology, Punjabi University, Patiala) was used as a biocomponent in the development of biosensor. *B. sphaericus* was grown on the media containing urea (2.5%), beef extract (1%), peptone (1%), sodium chloride (0.5%) at 37°C, pH 7.0 for 24 h under aerobic conditions (200 rpm). Microbial cells were harvested from 24 h grown culture by centrifugation at 5000 rpm for 10 min at 4°C and stored in 0.05M PBS buffer (pH 7.2). The urease activity of microbe was estimated by Nessler's method [31].

Immobilization strategy

The biocomponent was co-immobilized with phenol red indicator using a hydrosol gel method [14]. TMOS (Tetramethylorthosilicate) sol-gel stock mixture was prepared by mixing 20 µL whole cells of *B. sphaericus* (U 197.44), 600 µL ethanol, 100 µL TMOS, 30 µL of 5 mM NaOH, 80 µL Phenol Red (5mg /4 ml of 50% Ethanol) and 70 µL de-ionised water. 100 µL of 3% sodium alginate (dissolved in 0.05 M PBS buffer) was added in the stock mixture following incubation (1 hour) at 4°C. Immediately 60-60 µL of final mixture was dispensed into the glass capillaries in the centre and allowed to solidify.

Fabrication of biosensor

The microchannel with immobilized biocomponent was fitted in the hole of optic fiber dip probe (Figure 1A) which allowed the real time monitoring of the reaction taking place between the fluids passing through the microchannel and the immobilized biocomponent. The peristaltic pump was connected to adjust the flow rate. The whole assembly of the system is shown in the Figure 1B. The *Spectra Suite* software was used to measure the absorbance over the range of 400-700 nm to determine λ_{max} using fiber optic spectrophotometer. 2M urea solution was passed through the microchannel at a rate of 0.33 ml min^{-1} (20 ml h^{-1}) for 3 min to allow hydrolysis which resulted in color change of phenol red from yellowish to pink due to the formation of ammonium ions. The absorbance was measured at particular wavelength (555 nm). Later on, urea solutions (2 M) spiked with different concentrations of analyte i.e. lead (10-1000 µg/L) was allowed to pass through the microchannels at same flow rate and time and the absorbance was measured. To check the reusability of biosensor (microchannel with immobilized biocomponent), same microchannel was used to flow different solutions. The repeated use of microchannel required its regeneration which was performed with 0.1M EDTA solution.

Figure 1: (A) Fitting of microchannel within fiber optics dip probe (B) Assembly of the System.

Results and Discussion

An optical biosensor was fabricated to detect the presence of the lead. The microfluidic approach allowed very confined space for the immobilization of the biocomponent, flow of the analyte, reaction between analyte and biocomponent as well as real time monitoring of the reaction which ensured the miniaturization of the biosensor. A novel immobilization strategy has been employed combining two different approaches for the immobilization of the biocomponent within the microchannel. The hydrosol gel immobilization method was unified with calcium alginate method which has resulted in the drastic reduction in the solidification time from 90 -120 min to just few seconds. To the best of our knowledge, this is the first report of the blending two immobilization approaches which led to extreme decline in the solidification time. Besides this, a small volume of the sample (1 mL) was required as compared to conventional methods. The developed biosensor provided the quick and visible response with respect to analyte (lead).

The passage of standard urea (2 M) solution through the microchannel containing urease producing microbe *B. sphaericus* resulted in the color change of the phenol red from yellow to pink. The color change was less intense (Figure 2A) with lead spiked solution as compared to standard urea solution (Figure 2B & C) due to the inhibition of urease enzyme with lead.

λ_{max} was found to be 555 nm after scanning over 400 to 700 nm (Figure 3). Therefore, the absorbance was noted down at 555 nm for all samples (Table 1). Figure 3 shows the overlay of spectrum obtained after hydrolysis with urea and other synthetic solutions spiked with different concentrations of lead. The linear relation (R^2 = 0.9834) was observed between logarithmic concentration of lead (µg/L) and absorbance at 555 nm (Figure 4). The detection range of lead (10-1000 µg/L) with biosensor was found applicable for various water,

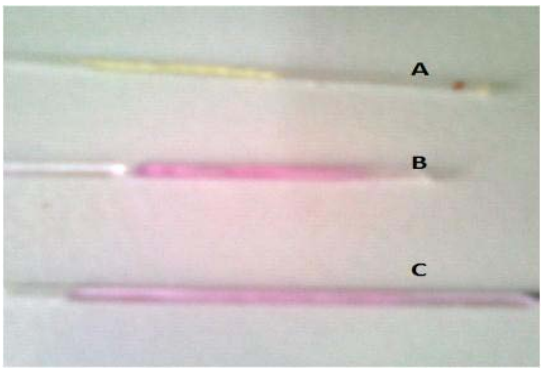

Figure 2: Color change in pH indicator, Phenol red (A) Fresh Microchannel (B) After Urea hydrolysis (C) Urea hydrolysis in the presence of lead.

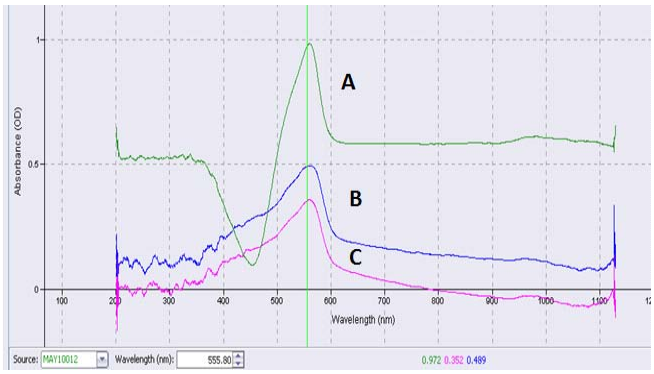

Figure 3: Scanning of maximum wavelength of color substance produced after urea hydrolysis (A) Standard Urea (B) Urea spiked with 100 µg/L of lead (C) Urea spiked with 1000 µg/L of lead.

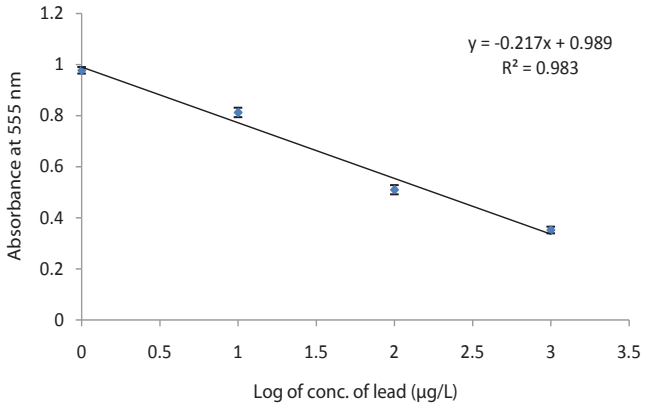

Figure 4: Performance of optical biosensor with different concentration of lead (10-1000 µg/L) in the synthetic samples.

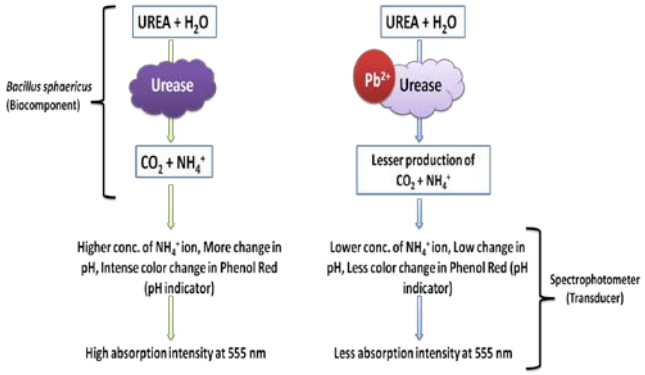

Figure 5: Bioassay Principle of the developed biosensor.

milk and clinical samples as the permissible limit of lead in milk and drinking water is 10 µg/L while Blood Lead Level (BLL) concentration is 100 µg/L [32].

The developed biosensor is based on the enzyme inhibition (Figure 5); urease catalyses the hydrolysis of urea leading to the production of NH_4^+ which results in the change of pH [10]. This change in the pH can be indicated by color change in the pH dependent dye, phenol red and absorbance can be measured with spectrophotometer. In the presence of lead (heavy metal as inhibitor), urease activity decreases

Table 1: Change in Absorbance (555 nm) with different concentration of lead (Single use of developed biosensor).

Samples	Absorbance (555nm)
Standard urea (2M)	0.977 ± 0.0128
Urea (2M) spiked with 10 µg/L of lead	0.812 ± 0.0184
Urea (2M) spiked with 100 µg/L of lead	0.509 ± 0.0180
Urea (2M) spiked with 1000 µg/L of lead	0.352 ± 0.0130

Table 2: Change in Absorbance (555 nm) with different concentrations of lead (Repeated use of developed biosensor).

Samples	Absorbance (555nm)
Standard urea (2M)	0.922
Urea (2M) spiked with 10 µg/L of lead	0.210
Urea (2M) spiked with 100 µg/L of lead	0.109

resulting in the production of less amount of NH_4^+, lesser change in pH and color and consequently less absorbance as compared to control. The greater the concentration of lead, the lesser will be absorbance of the sample.

The single use of the developed biosensor (microchannel with immobilized biocomponent) provided good linearity. To check the multiple use of developed biosensor, same microchannel was used for the analysis of different samples. As expected, absorbance decreases with increase in concentration of lead (Table 2) even when the same microchannel was used repeatedly. But the leaching of dye and wash-out of biocomponent from the microchannel was also observed which may lead to decrease in absorption rather than mere due to inhibition of urease. This process may give false indication about concentration of lead. Therefore, the single use of the developed biosensor was preferable as compared to repeated use. The cost effective nature of the developed biosensor surpasses the single use of the biosensor sinceone microchannel enclosed only 60 µl of immobilization mixture containing biocomponent and pH indicator. However, further improvement in immobilization method is recommended to prevent leaching of dye to make the biosensor reusable.

Microscale fluid regulators (e.g. valves, mixers and pumps) can be integrated on the lab-on-a-chip platform to increase the analytical performance of biosensors [29]. A DNAzyme based microfluidic biosensor was earlier developed for the lead detection using a microfluidic device with multifaceted microfluidic channels employing nanocapillaries to facilitate the efficient and expedient flow in the device [30]. The use of sophisticated device made it costlier. However, the microfluidic biosensor developed in the present study is cheap, miniaturized, sensitive and reliable.

Conclusion

An economical disposable optical biosensor was developed for lead detection in the synthetic media which can extend its application in different milk, water and clinical samples after minor optimizations. Urease producing *Bacillus sphaericus* was used in the fabrication of biosensor employing the technique of microfluidic. The blend of microfluidic and advanced biosensor technologies offers new promises including high-throughput analysis and portability. Enzyme inhibition was used as a bioassay principle and inhibition of the enzyme with lead was measured with change in absorbance of color

produced after the hydrolysis of substrate. A linear relationship was observed between logarithmic concentration of lead (10-1000 μg/L) and absorbance. A novel immobilization strategy of immobilizing biocomponent with pH indicator has been used which reduced the time of immobilization significantly from hours to few seconds. The study resulted in the development of cheap, miniaturized, sensitive and reliable lead biosensor.

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