Sensors and Actuators B 207 (2015) 262–268

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Polyaniline nanoparticle based colorimetric sensor for monitoring bacterial growth

Bhawana Thakur[a], Chellachamy A. Amarnath[a], S.H. Mangoli[b], Shilpa N. Sawant[a,*]

[a] Chemistry Division, Bhabha Atomic Research Centre, Mumbai 400085, India
[b] Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai 400085, India

ARTICLE INFO

Article history:
Received 11 September 2014
Received in revised form 9 October 2014
Accepted 10 October 2014
Available online 23 October 2014

Keywords:
Polyaniline nanoparticles
Pathogen detection
Bacterial sensor
Colorimetric sensor
Generic sensor
pH sensor

ABSTRACT

Detection of pathogens in food and water is important for quality monitoring and disease prevention. Herein, we report a simple and novel colorimetric sensor for detection of bacterial growth relying on estimation of its metabolic products. As a proof of the concept, utility of the sensor for detection of *Escherichia coli* (*E. coli*) is demonstrated. All major groups of microorganisms including *E. coli* commonly use the glycolytic pathway for glucose degradation in the presence or absence of oxygen, eventually releasing mixed acids like succinate, acetate, malate, etc. as by-products. Polyaniline, a conducting polymer, is highly sensitive to the presence of protons in its microenvironment and its protonic doping results in a visible color change. Hence, polyaniline nanoparticles were synthesized and the sensor films were fabricated by incorporating these nanoparticles in agarose gel. The films when used for real time monitoring of bacterial growth exhibited a visible color change from blue to green. Since no specific antibodies or receptors are used, the sensor is generic in nature and has potential for adaptation to real life applications in the form of patch sensor on cartons to gauge integrity and freshness of food items and beverages in real time.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Pathogen detection and growth monitoring has gained utmost importance in recent years as a result of increasing incidences of disease outbreak due to contamination of drinking water and food. Infectious and toxigenic bacteria cause a great deal of human suffering and death [1]. The fact that they thrive in most common matrices (drinking water, food and air) make them unavoidable and thus more dangerous. The sensors for monitoring bacterial growth hold importance in many fields like food and beverage industry, bacteriology, public health, etc. There are numerous methodologies developed in past few years for rapid, specific and sensitive detection of pathogens [2]. Conventional methods like the culturing technique require almost 3–7 days for analysis whereas the polymerase chain reaction (PCR) based molecular detection techniques give rapid results but they are complex and require expensive instrumentation. Many other sensing techniques require use of labels, fluorescent probes [3] and special treatment of matrix before or during analysis.

There are several recent literature reports on specialized techniques and principles like surface plasmon resonance (SPR), piezoelectric and acoustic wave, electrochemistry, on chip polymerase chain reaction (PCR) and real time mass spectrometry [4–8]. Some of the above techniques are based on immunoassays and hence make use of antibody–antigen interaction, which provides high specificity in the sensing process. However, in such cases it becomes challenging to monitor viable bacteria in presence of dead bacteria as both participate in this type of interaction on sensor surface leading to erroneous results. Short self-life of such biosensors is again a limiting factor in terms of operational stability. Thus, there is an urgent requirement to develop new alternative methods and protocols that can aid simplicity, improve analysis time and detection limit of bacterial monitoring procedures. Colorimetric detection methods hold a great promise in this direction [9]. They can be used to detect bacterial contaminants in food that can help to monitor changes in packaging and storage conditions thus gauging the integrity and freshness of food items [10]. This is important from the point of view of quality control as well as food safety. However, opting for solution phase colorimetic methods using chromogenic or fluorescent probes [3] comes with constraints due to turbidity which is encountered during bacterial growth. Instead, it is advantageous to use sensor films which show a visual color change on decomposition of the food or on pathogen growth. Such films can

* Corresponding author. Tel.: +91 22 25590288.
E-mail address: stawde@barc.gov.in (S.N. Sawant).

http://dx.doi.org/10.1016/j.snb.2014.10.045
0925-4005/© 2014 Elsevier B.V. All rights reserved.

be used in the form of a 'patch' on food packages like a bar code to check if the content meets quality and safety standards. The colorimetric patches are extremely attractive because it is possible to directly observe the color change with the naked eye helping the consumer to decide about the freshness of the food items. The color change can be triggered either by binding of the pathogen to the receptor on the sensor film or due to binding of secreted toxins with the active material like nanoparticles present in the sensor [11]. Scindia et al., 2007 fabricated a glass supported polydiacetylene (PDA) based colorimetric sensor for detection of bacteria where the amphiphilic molecules secreted by proliferating bacteria induced the color change in the PDA matrix [12]. A colorimetric sensor based on chemoresponsive dyes was reported to identify eight strains of bacteria. The volatile metabolites released by the bacteria during their growth triggered the color change in the dyes. Although the amount of bacteria required to bring about the color change was less (1–10 CFU/ml), the time taken for the analysis was 24 h [13]. Luo et al., 2014 developed a colorimetric biosensor assay integrated with PCR technique for the detection of Salmonella using gold nanoparticles and species-specific invA gene. The method could detect Salmonella down to 3×10^3 CFU/ml in water, but involved complex fabrication steps including extended time for detection (~5 h) [14].

In the present work, color change of the sensor is triggered by interaction of the film with metabolic products released by the pathogens. Several bacteria like *E. coli* in addition to aerobic and anaerobic respiration undergo fermentation for energy production. During the fermentation process, they consume simple carbohydrate like glucose and release mixed acids like succinate, acetate, malate, etc. as by-products [15]. Conducting polymer polyaniline is highly sensitive to pH changes in its microenvironment [16] and its conductivity is known to increase by 8–10 orders of magnitude on doping with acids [17]. This is due to the transition of its emeraldine base (EB) form to emeraldine salt (ES) which is also accompanied by color change from blue to green. However, PAni is not easily soluble in aqueous or organic solvents, making fabrication of sensor film out of it difficult. This processibility issue can be solved to a great extent by preparing colloidal nanoparticles of polyaniline [18]. More recently, we have reported synthesis of polyaniline nanoparticles using biopolymer pectin as stabilizer [19] and demonstrated its application in glucose sensing [20] and as a supercapacitor electrode material for physiological fluids. The pectin coated polyaniline nanoparticles (PAni-Pec NPs) have shown very good biocompatibility in terms of cell viability and thus they were employed in the present study to develop colorimetric sensor film for detection of pathogenic bacteria. Moreover, pectin is a common additive used in the food as a gelling agent (in jams and jellies), stabilizer (in fruit juices and milk drinks) and as a source of dietary fiber. Hence, utilization of pectin for stabilization of PAni NPs helps to improve its biocompatibility for potential use as a colorimetric sensor for monitoring freshness of food commodities. In the present study, *E. coli* BW25113 was used as model system and the color changes in the sensor film were monitored by recording the optical absorption to provide a proof of concept. The current method involves simple sensor fabrication steps, without using any bio-recognition element. The sensor films demonstrated high sensitivity for detection of the bacteria and can be used for real time monitoring of bacterial growth.

2. Experimental

2.1. Chemicals and reagents

Aniline (Sigma–Aldrich) was distilled under vacuum before use. Agarose was purchased from Sigma–Aldrich. Pectin was purchased from Sisco Research Laboratory (SRL), India and d-(+)-glucose (99.5%) was obtained from Loba Chemie. Na_2HPO_4, KH_2PO_4, NaCl and KCl (for making phosphate buffer solution), and ammonium persulphate (APS) were procured from SRL, India. Tryptone and yeast extract used for bacterial media was from Difco. Aqueous solutions were prepared using deionized water from a Milli-Q purification system.

2.2. Instruments

Morphology of the PAni-PEC NPs sample was analyzed by field emission scanning electron microscopy (FE-SEM) Auriga, Zeiss, Germany. DLS measurements were performed using a Malvern 4800 Autosizer employing 7132 digital correlator. The light source was He–Ne laser operated at 633 nm with a maximum output power of 15 mW. The average decay rate was obtained by analyzing the electric field autocorrelation function, g1 (τ) vs. time data recorded at 90° using method of CONTIN [21]. FTIR characterization of the samples was carried out using Bomen Hartman and Braun, MB Series FTIR instrument. X-ray diffraction (XRD) patterns of the samples were recorded (using Philips Diffractometer PW 1710) from 2θ 10° to 90° at the step of 2°/min using monochromatic CuKα radiation at 40 kV excitation voltage and 30 mA tube current. For XPS measurement was done by drop casting of PAni-Pec on silicon substrate mounted on a specimen holder using silver paste. The conducting path was given from the bottom to the top of the surface of the sample by silver paste, to avoid the surface charging effect. The sample chamber was then evacuated to a vacuum better than 1×10^{-9} Torr. The sample was excited by Mg-K_α radiations ($h\nu$ = 1254.6 eV), photoelectron spectra were analyzed using a VG make CLAIM 2 analyzer system in the energy range of 0–1000 eV. UV–vis spectra was measured using JASCO V650 UV–vis spectrophotometer. pH of the LB was adjusted using digital pH meter (TOSHCON, India).

2.3. Bacterial cultivation

Wild Type *Escherichia coli* – K12 strain BW25113 were grown overnight in lysogeny broth (LB) [22] at 37 °C to get a stationary phase. The cell density of saturated culture was determined by measuring the optical density at 600 nm wavelength. These cells were diluted as required and grown in fresh LB supplemented with 2% glucose at room temperature.

2.4. Optical sensor fabrication

The procedure for fabrication of the optical sensor is schematically presented in Fig. 1.

2.5. Preparation of PAni-Pec NPs

25 ml of aqueous solution of ammonium persulfate (2.28 g) was added drop-wise to a 60 ml solution containing 1.862 g pectin (Pec), 9 ml HCl (11 N) and 0.9313 g of aniline (Aniline:Pec 1:2). The reaction was carried out at room temperature under constant stirring for 4 h to yield homogeneous PAni-Pec dispersion. The PAni-Pec was precipitated in 1:1 mixture of ethanol–water (500 ml) followed by filtration with Whatmann 41 filter paper and washing with 1:1 mixture of ethanol–water (500 ml) to get pure PAni-Pec precipitate. The precipitated PAni-Pec NPs were re-dispersed in nanopure water by ultrasonication where its final concentration was estimated to be 10 mg/ml.

2.6. Fabrication of sensor film

250 μL of PAni-Pec NPs is dispersed in 5 ml PBS having 2% agarose at 70 °C. A 1 cm × 2.5 cm polyethylene terephthalate (PET)

Download English Version:

https://daneshyari.com/en/article/10412714

Download Persian Version:

https://daneshyari.com/article/10412714

Daneshyari.com