Development and Characterization of Carbonic Anhydrase-Based CO_2 Biosensor for Primary Diagnosis of Respiratory Health

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***Abstract*—It is a global need to realize noninvasive, simple, rapid, selective, inexpensive, and portable assessment methods for diagnosis of diseases. Enzyme-based bio-sensing system, compared with traditional analytical methods, has all such potential attributes. This paper proposes carbonic anhydrase enzyme (CA) (E.C. 4.2.1.1)-based cost-effective, highly selective, and reproducible CO_2 biosensing system that can measure CO_2 concentration (ppm level) in expired breath accurately to give valuable information for assessing the respiratory disorders of the subjects. CA is extracted from spinach leaves and immobilized on an electrode assembly. The assembly generates a sensible electrical signal (mV) when brought in contact with the aqueous CO_2. The sensor characterizes a linear response from 160–2677 ppm of CO_2 concentration dissolved in water, good sensitivity (~0.132mV/ppm) with excellent fast response time within 12 s. The features include repeatability, shelf life (~5 months), re-usability (~20 times), and selective responsiveness to the CO_2 molecules in the exhaled breath. The feasibility for the use of the biosensor in a suitable setup for home-based monitoring of CO_2 in exhaled breath has been proposed and justified. The device showed a good correlation between the results obtained from the sensor and established clinical test.**

***Index Terms*—Carbonic anhydrase enzyme, CO_2 biosensor, characterization of the sensor, exhaled breath.**

I. Introduction

EXHALED breath analysis is becoming an increasing area of interest for assessing the respiratory disorders.

It offers a valuable non-invasive diagnostic approach to draw clinical conclusions about the disorders of various human organs [1]. The exhaled breath due to the diffusion process of soluble gases in the blood at the alveolar-capillary junction is exploited as the noninvasive surrogates for a blood sample [2], [3]. Beyond the limit-concentrations of the trace gases in exhaled breath are associated with the pathogenesis of many diseases. Therefore, the quantitative analysis of gases in expired breath is important for effective clinical uses [3]. Normal human breath contains a few atmospheric molecules, in relatively high concentrations along with several volatile organic compounds at the parts per million or sub-ppm levels, and about four hundred major volatile organic compounds (VOCs) at the parts per billion or parts per trillion levels [4], [5].

Respiratory acidosis, also called respiratory failure or ventilatory failure, is a condition that occurs when the lungs cannot remove enough of the carbon dioxide (CO_2) produced by the body. Asthma, chronic obstructive pulmonary disease (COPD), pneumonia or sleep apnea etc. are responsible for such a situation. A measurement of CO_2 concentration is a primary approach to monitor the status of ventilation. It decides the degree of severity and effectiveness of the therapy provided. Moreover, it is of emergent need to monitor and control the physiological acid-base status and electrolyte balance of critically ill and surgical patients. Therefore, a sensory system that allows an accurate, rapid, selective, interference-free, cost-effective measurement of respiratory CO_2 concentration is likely to be useful for primary assessment of the respiratory health. The established literature survey indicates that poor selectivity, chemical interferences and response times are the major problems to develop CO_2 sensors to quantify respiratory CO_2 concentration [6]. Electrochemical [7], [8], Fiber-optic chemical [9], [10] and acoustic [11] sensors are capable to measure wide range of CO_2 concentration. However, they can partially meet the desired objective in clinical ambience. The infrared sensors used in conventional clinical capnography [12] are capable of sensing respiratory CO_2 concentration accurately. All the approaches satisfy a fraction of the basic demands in health care area. Development of enzymatic biosensor provides inexpensive, rapid, real-time, accurate and reliable information about the analyte of interest selectively present in a complex mixture of various constituents (exhaled breath). A number of enzymatic biosensors are available for monitoring clinically important parameters like blood glucose, urea, cholesterol, and uric acid etc. [13], [14]. Enzymes or enzyme-labeled antibodies are the most common bio-recognition components of biosensors whose presence in the bio-recognition layer provide electro-active substances for detection by physicochemical transducer to present measurable signal. Clark and Lyons (1962) [15] first reported the enzyme electrode to measure blood glucose level using glucose oxidase (an oxido-reductase enzyme). This approach was the basis of numerous variations of many other oxygen mediated glucose oxidase enzyme sensors [16], [17]. Rivas *et al.* [18] used carbon nanotube paste electrode (CNTPE) with glucose oxidase to obtain more sensitive glucose biosensor. Vöelkl *et al.* [19]

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