



Development of simple and sensitive hydrogel based colorimetric sensor array for the real-time quantification of gaseous ammonia

Sanduru Thamarai Krishnan^a, Kuk Hui Son^b, Namhyoung Kim^c, Buddolla Viswanath^a, Sanghyo Kim^{a,*}, Jeong Ho An^{d,*}

^a Department of Bionanotechnology, Gachon University, San 65, Bokjeong-Dong, Sujeong-Gu, Seongnam-Si, Gyeonggi-Do 461-701, Republic of Korea

^b Department of Thoracic and Cardiovascular Surgery, Gachon University Gil Medical Center, Gachon University, 21 Namdong-daero 774beon-gi 1, Namdong-gu, Incheon 405-760, Republic of Korea

^c Department of Applied Statistics, Gachon University, San 65, Bokjeong-Dong, Sujeong-Gu, Seongnam-Si, Gyeonggi-Do 461-701, Republic of Korea

^d Department of Polymer Science & Engineering, Sungkyunkwan University, Suwon, Gyeonggi-Do 440-146, Republic of Korea

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ABSTRACT

A real-time colorimetric sensor array (CSA) offers the advantages of diversity and accuracy for the quantification of multiple analytes; however, traditional sensors require a complex fabrication process. Therefore, to take full advantage of this sensing platform, we have developed a simple CSA system composed of a polymer, a reducing agent, and different pH indicators. Distinctive color response patterns were classified by extracting the hidden information, (i.e., red, green, and blue (RGB) values) from the indicators. This triple-channel sensing platform is further applied for statistical analysis, to quantify different concentrations of ammonia and other analytes. The sensor array showed a limit of detection of 0.3 ppm, which is well below the diagnostic criteria for ammonia concentration in the breath of healthy individuals and of patients with end-stage renal disease. As this sensor would be able to quantify gaseous ammonia in the breath, it is relevant to the point-of-care diagnosis of patients with renal diseases.

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1. Introduction

Ammonia (NH₃), an essential inorganic nitrogen compound, is involved in various metabolic processes in the human body. It originates mainly from dietary protein that is broken down by bacteria in the intestine and converted to urea in the liver. The urea is then removed through urine [1–4]. However, an increase in the level of excess ammonia in the blood, may lead to serious health issues such as chronic kidney disease (CKD), hyperammonemia, and cirrhosis [5–7]. In patients with end-stage renal disease (ESRD), the kidneys are unable to excrete the urea completely [8,9] leading to an increase in blood urea nitrogen (BUN) levels [10]. Furthermore, the excess ammonia and ammonium ions enter into the blood-lung barrier and cause ammonia odor in the breath of ESRD patients [1]. In order to diagnose the stages of renal disease, monitoring of the ammonia level in the human body is essential. For this, different techniques such as urine and blood tests [11,12] are involved, but these are invasive, expensive and not well tolerated by patients. Therefore, there is a specific need to find a non-invasive diagnostic tool capable of monitoring diseases. Any approach must meet the aforementioned need for the detection of increased levels of specific

chemicals present in exhaled breath may indicate the presence of certain disease. Generally, exhaled breath contains numerous non-volatile and volatile compounds, including ammonia, acetone, isoprene, hexane and toluene in a certain amount [parts per billion (ppb) to parts per million (ppm)] [13,14]. Hence, for non-invasive and effective diagnosis, exhaled breath analysis could be a potential approach to monitor ammonia levels in the breath of renal patients [15]. The ammonia level in healthy individual ranges from 500 to 1500 ppb, whereas that in renal patients it may be approximately 1500 to 15,000 ppb [16–19]. These ammonia levels are variable according to the sex, age, and smoking and food habits of the individual [20].

Several analytical techniques are under investigation for the monitoring of chemical compounds present in exhaled breath, including selected ion flow tube mass spectrometry (SIFT-MS), laser photoacoustic spectroscopy, optical fiber sensor, metal-oxide semiconductors [21–24]. However, these instrumental techniques are complicated, highly expensive and required technical expertise. In addition, these techniques are prone to preanalytical errors due to sample transportation, storage, and handling [25]. For example, Turner et al. [16] studied the breath ammonia levels of 30 volunteers using the SIFT-MS technique and found this analytical instrument to be too complex to handle and expensive, and that sampling and storing the breath could affect the accuracy of the evaluations. Ogimoto et al. [26] were able to determine

* Corresponding authors.

E-mail addresses: samkim@gachon.ac.kr (S. Kim), jhahn1us@skku.edu (J.H. An).

ammonia concentrations using a porous film system made up of nanoparticles and two quartz crystal microbalances but this instrument failed to quantify concentrations of <3 ppm. In addition, these analytical techniques have poor sensitivity, sensor drifts, and they are expensive. Besides the summarized techniques, i.e., SIFT-MS, laser photoacoustic spectroscopy, optical fiber sensor and metal-oxide semiconductors, colorimetric methods have also been extensively reported in monitoring gaseous chemicals, including ammonia. In comparison to these traditional instruments, colorimetric sensors have a wide range of analytical responses, high selectivity, and high ease of use. Typically, traditional colorimetric sensors were developed using C2 reverse phase silica gel plates or polyethylene terephthalate (PET) as a substrate and chemically responsive sensitizers [27,28]. However, the visibility of color change of the sensitizers is less towards the gaseous analytes.

In order to take an advantage herein, we have developed a novel hydrogel film as a substrate (with the combination of polyvinyl alcohol (PVA), titanium oxide nanoparticles (TiO₂ NPs) and hydroquinone (HQ)) and sol-gel formulated pH indicator sensor array for the real-time quantification of ammonia which is not reported elsewhere. The substrate of the sensor was fabricated by incorporating the PVA with TiO₂ NPs and HQ, which is highly exhibits the

visibility of color change of the sensor array. For the preparation of pH indicators, we make use of sol-gel formulation to obtain better responsiveness to gaseous ammonia and then cast in an array on the hydrogel film. These formulations provide an ideal versatility of the matrix to minimize the effect of the external environment. Furthermore, we have successfully quantified various concentrations of gaseous ammonia with other breath-relevant analytes that are correlated to the exhaled breath of renal patients. In addition, we demonstrated the sensor with the real breath sample. The quantitative results of breath of two healthy controls show better correlation with gaseous ammonia (at 0.5 ppmv).

2. Experimental

2.1. Materials

All the reagents and solvents used throughout this work were of analytical grade, obtained from Sigma-Aldrich St. Louis, MO, USA, and used as without any further purification. This includes the TiO₂ NPs in the size range of 21 nm. Polyvinyl alcohol (PVA) was obtained from Junsei Chemical Co. Ltd., Japan.

Table 1
List of pH indicators used in this study to detect gaseous ammonia.

Sl. no.	Indicator	Structure	Amount of dye	Properties
1.	Bromocresol Purple (BCP)		148.08 μM	a) Molar mass: 540.22 g/mol. b) pH ranges from 5.2 (yellow) to 6.8 (purple)
2.	Bromophenol Red (BPR)		5.66 μM	a) Molar mass: 512.16 g/mol. b) pH ranges from 5.2 (orange-yellow) to pH 6.8 (red)
3.	Bromothymol Blue (BTB)		1.60 μM	a) Molar mass: 624.38 g/mol. b) pH ranges from 6.0 (yellow) to 7.6 (blue)
4.	Chlorophenol Red (CPR)		189.00 μM	a) Molar mass: 423.27 g/mol. b) pH ranges from 4.8 (yellow) to 6.7 (violet)
5.	Bromophenol Blue (BPB)		14.92 μM	a) Molar mass: 669.96 g/mol. b) pH ranges from 3.0 (yellow) to 4.6 (blue)
6.	Bromocresol Green (BCG)		96.09 μM	a) Molar mass: 624.38 g/mol. b) pH ranges from 6.0 (yellow) to 7.6 (blue)
7.	Methyl Red (MR)		3.71 μM	a) Molar mass: 269.30 g/mol. b) pH ranges from 4.4 (red) to 7.6 (yellow)
8.	Phenol Red (PR)		11.28 μM	a) Molar mass: 354.38 g/mol. b) pH ranges from 6.8 (yellow) to 8.2 (red)
9.	Cresol Red (CR)		2.47 μM	a) Molar mass: 404.41 g/mol. b) pH ranges from 7.2 (yellow) to 8.8 (red)

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