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Araştırma Makalesi / Research Article

A New Perspective towards Diagnosis of Sleep Apnea: Isoprene Detection Using PSSA-g-PANI based SAW Sensor

Cihat TAŞALTIN[1], Gülsen BAYTEMİR[2*], Nevin TAŞALTIN[2, 3*]

[1]TUBITAK Marmara Research Center, Materials Institute, 41470, Kocaeli, Turkey

[2]Maltepe University, Department of Electrical and Electronics Eng. 34857, Istanbul, Turkey

[3]Maltepe University, Department of Basic Sciences 34857, Istanbul, Turkey

**Corresponding author e-mail: gulsenbaytemir@maltepe.edu.tr ORCID ID: http://orcid.org/0000-0002-1143-0730*

**Corresponding author e-mail: nevintasaltin@maltepe.edu.tr ORCID ID: http://orcid.org/0000-0001-6788-1605*

e-mail: ctasaltin@gmail.com ORCID ID: http://orcid.org/0000-0002-8978-802X

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Keywords
Sleep apnea; Sensor; Exhaled breath; Isoprene

Abstract

Cellular metabolic changes triggered by Obstructive Sleep Apnea Syndrome (OSAS) are detected invasively by detecting isoprene as a biomarker of OSAS in the breath. When falling asleep, isoprene level begins to increase above 3 ppm. Therefore, monitoring isoprene levels in exhaled breath under normal conditions may provide a non-invasive method to detect, monitor, and control sleep disorders such as sleep apnea. In this study, PSSA-g-PANI based SAW gas sensors were prepared and tested against isoprene gas. Sensor measurements reveal that PSSA-g-PANI based SAW type sensor detected (1-150) ppm isoprene gas with high sensitivity and thus PSSA-g-PANI based SAW type sensor may enable monitor and control of sleep apnea.

Uyku Apnesi Teşhisine Yeni Bir Bakış Açısı: PSSA-g-PANI tabanlı SAW Sensörü Kullanarak İzopren Tespiti

Anahtar Kelimeler
Uyku apnesi; Sensor; Solunan nefes; Izopren

Öz

Obstrüktif Uyku Apne Sendromu (OSAS) tarafından tetiklenen hücresel metabolik değişiklikler, nefeste OSAS'ın biyobelirteci olarak izopren saptanarak invazif olarak tespit edilir. Uykuya dalındığında izopren seviyesi 3 ppm'nin üzerine çıkmaya başlar. Bu nedenle, normal koşullar altında solunan nefeste izopren seviyelerinin izlenmesi, uyku apnesi gibi uyku bozukluklarını tespit etmek, izlemek ve kontrol etmek için invazif olmayan bir yöntem sağlayabilir. Bu çalışmada, PSSA-g-PANI tabanlı SAW gaz sensörleri hazırlanmış ve izopren gaza karşı test edilmiştir. Sensör ölçümleri, PSSA-g-PANI tabanlı SAW tipi sensörün yüksek hassasiyetle (1-150) ppm izopren gazı algıladığını ve dolayısıyla PSSA-g-PANI tabanlı SAW tipi sensörün uyku apnesinin izlenmesini ve kontrolünü sağlayabileceğini ortaya koymaktadır.

1. Introduction

Obstructive Sleep Apnea Syndrome (OSAS) is characterized by cardiovascular diseases (Mehra *et al.* 2006, Gami *et al.* 2013), metabolic diseases (Peled *et al.* 2007), and neurological diseases characterized by an intermittent decrease in airflow (hypopnea) and/or interruption of airflow (apnea) due to problems in the upper airways (Stone *et al.* 2016, Redline 2010). OSAS is more common in children (Schlaud *et al.* 2004, Mitchell and Kelly 2006, Sabato *et al.* 2006, Holty *et al.* 2013). In exhaled breath, volatile organic compounds (VOCs), produced by cellular metabolism and acting as biomarkers, are abundant. The fact that cellular metabolic changes triggered by OSAS are detected invasively by detecting isoprene as a biomarker of OSAS in the breath. Under normal conditions, an increase in isoprene concentration is due to sleep and isoprene plays a role in sleep regulation (King *et al.* 2012, Cailleux and Allain 1989, Salerno-Kennedy and Cashman 2005). The normal concentration of isoprene at wakefulness is in the range of (509 ppb-1.43 ppm) for adults. When falling asleep, isoprene level begins to increase above 3 ppm (King *et al.* 2012). Therefore, monitoring isoprene levels in exhaled breath under normal conditions may provide a non-invasive method to detect, monitor, and control disorders of sleep. In the literature, there are very few studies on the development of sensors for the diagnosis of OSAS (Carpagnano 2011, Bikov *et al.* 2016, Finamore *et al.* 2019, Gouma *et al.* 2011, Gouma 2018). There has been increasing interest in the investigation of lungs by non-invasive means measuring the exhaled breath volatile mediators, such as nitric oxide (NO), carbon monoxide (CO), ethane and pentane and finally the non-volatile substances in the liquid phase of exhalate, termed breath condensate. Carpagnano *et al.* focused on exhaled breath analysis giving an update on its general aspects, its application in OSAS, and finally its actual clinical applicability and areas for future direction (Carpagnano 2011). Accelerated airway inflammation may play a crucial role in the pathophysiology of OSAS; however, this phenomenon has been investigated only in a limited number of studies. The analysis of exhaled breath represents a promising, non-invasive tool to evaluate airway inflammation in this context. The knowledge on exhaled biomarkers in OSAS has been growing with an emerging number of methodological studies which help to interpret exhaled breath data. Bikov *et al.* summarized the results of studies on exhaled breath condensate biomarkers, exhaled volatile compounds and exhaled monoxides in OSA, and critically reviewed methodological limitations (Bikov *et al.* 2016). Furthermore, Finamore *et al.* reported that exhaled breath analysis has demonstrated a clinical relevance in identifying individuals affected by the disease, in assessing the response to treatment and, potentially, to monitor patient's adherence to mechanical ventilation (Finamore *et al.* 2019). After these inspiring studies, Gouma *et al.* described a new concept of a microsystem with three-nanosensor array which potentially serving as a coarse diagnostic tool handheld breath analyzer to provide a first detection device. It was reported that by connecting the sensor array to an integrated circuit for electrical reading and temperature control provides a complete microsystem which is able to capture a single exhaled breath and analyze it according to the relative content of isoprene, ammonia and carbon dioxide gas. The sensor was able to detect and discriminate among various isoprene concentrations in the range of 300 ppb to 1 ppm and above-the range of interest for the targeted application (Gouma *et al.* 2011, Gouma 2018). In the light of the results obtained in these studies, it is necessary to produce economic sensors that can make the diagnosis of OSAS in a shorter time. Our research team focuses on the fabrication of novel non-invasive sensors for the detection of isoprene towards OSAS diagnosis.

SAW sensors are widely used in the detection of gaseous VOCs since they are highly effective for the discrimination of target chemical substances and, they are portable, cheap, and applicable for real-time analysis. Sensing material is expected to make an interaction with analyte molecules, resulting a shift in the resonance frequency (Tasaltin *et al.* 2012).

The gas sensing mechanism of a SAW sensor can be explained as follows: When the sensitive layer absorbs gas molecules from the ambient, the boundary conditions for the propagating surface are changed, and consequently the velocity and attenuation of the wave change. These changes can be detected with great accuracy by a SAW oscillator as a frequency shift. The velocity of propagation of the surface wave depends on various factors. Sensor responses are frequency shifts due to mass loading. Film conductivity is one of the parameters of wave attenuations.

As one of the conductive polymers, Poly (Styrene Sulfonic Acid) (PSSA) covalently grafted with Polyaniline (PSSA-g-PANI) is water-soluble, and it has attracted attention for advanced applications such as an electrode in supercapacitors, electromagnetic inherence shielding. Although PSSA-g-PANI has high chemical stability and charges transport properties, there is no report on PSSA-g-PANI based sensor. In this work, a novel PSSA-g-PANI based SAW sensor was prepared to give a new perspective to the diagnosis of sleep apnea for real-time monitoring of isoprene which is the sleep apnea biomarker in the breath.

2. Experimental

2.1. Materials

Di-tert-butyl dicarbonate (BoC_2O) (ReagentPlus®, ≥99%), amino styrene (97%), Dichloromethane (DCM), anhydrous sodium sulfate (Na_2SO_4), N-SuccinylAlaAlaAlapnitroanilide (SSNa), and 2,2′-Azobis(2-methylpropionitrile) (AIBN) were purchased from Sigma Aldrich Company (Germany). Dimethyl sulfoxide (DMSO) (99.9%) and hydrochloric acid (HCl) (37%) were purchased from Merck Company (Germany). The synthesis of PSSA-g-PANI consists of three experimental steps (synthesis of tert-butyl 4-vinyl phenyl carbamate (BOC-AMS, synthesis of P(SSNa-co-BOC-AMS), and synthesis of PSSA-g-PANI). 1.19 g of amino styrene solved in 10 ml of deionized water and stirred with 2.40 g of BoC_2O at 35 ^{0}C for 4 h. The amino styrene / BoC_2O solution was extracted with 25 ml of DCM. The solution was dried by Na2SO4, filtered by a membrane filter. 3 g SSNa, 0.2g tBOC-AMS, and 55 mg AIBN were mixed in DMSO and heated at 70 ^{0}C for 12 h under nitrogen. The sample was mixed with acetone and filtered. The sample dried at 50 ^{0}C for 24 h in a vacuum oven. 1.0 g P(SSNa-co-BOC-AMS) was added to 1 M HCl solution (30 ml) at 30 ^{0}C for 1 h and then the solution was cooled at 0 ^{0}C. The solution was mixed with 0.086 g of ANI for 0.5 h, stirred with ammonium persulfate / HCl solution for 6 h, and then filtered by dialysis membrane filters (molecular weight cutoff, 3500) (Bae *et al.* 2014) (**Figure 1**).

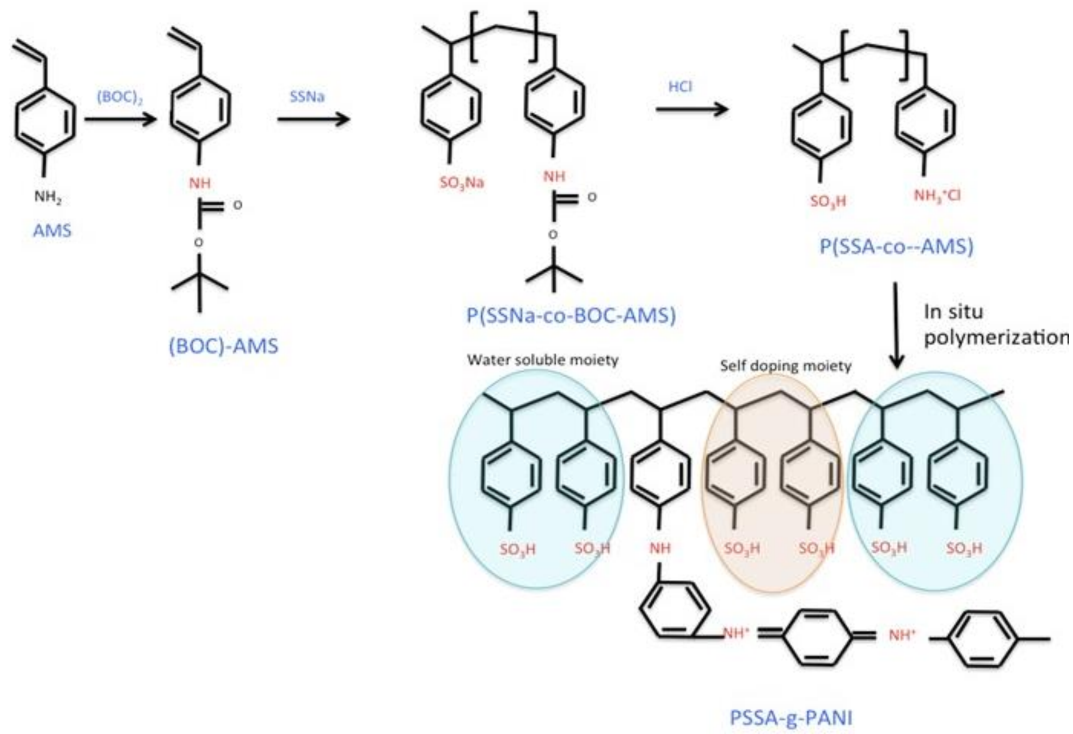

Figure 1. The synthesis of PSSA-g-PANI.

2.2. Sensor fabrication and measurement

Commercially SAW transducers with 433.3 MHz device (SAW Components GmbH, Dresden, Germany) fundamental resonance frequency were used. They were purchased from SAW Components GmbH, Dresden, Germany. At first, the synthesized PSSA-g-PANI as a sensing film was coated on the SAW transducer using an electro-spray coating system. The electrospray equipment is basically a two-chamber setup with a sample holder that rotates at 1.000 rpm and exposes the IDE to positive electrospray mist and a negative discharge cloud. The coating voltages were set as ~+3.5 kV for the needle and ~-1.5 kV for the tungsten tip (Tasaltin *et al.* 2010).

2.3. Sensor Measurement

Chemical detection against polar (water, propanol, etanol) and nonpolar (hexane, toluene, trichloroethylene, chloroform) VOCs was investigated. The gas stream consisting of VOC vapor was produced through cooled bubblers immersed in the thermally controlled bath. In order to adjust the gas concentration to the desired amount, analyte-saturated gas stream was diluted with pure synthetic air using computer-operated mass flow controllers at a constant flow rate of 300 ml/min. Typical experiments were performed as repeated exposure to analyte gas (10 min) followed by purging (10 min) to reset the baseline. The sensor chip temperature was kept at room temperature using a temperature controller (Lake Shore, USA). The concentrations in the range of of 100–5000 ppm were used for each VOC. To make the comparison more accurate, p_i/p_{0i} responses of relative concentrations of different vapor pressures were used (pi: actual analyte concentration, p_{0i}: saturation vapor pressure at the temperature during measurement). (Tasaltin *et al.* 2012).

Table 1. Properties of analytes: saturation vapor pressure at −10 ◦C, the tested concentration range; the environmental temperature of 22 ◦C as calculated using Antoine's equation.

Analyte	Dielectric constant (ε)	Dipole Moment (μ)	Concentration (ppm) Min.	Concentration (ppm) Max.	p^0(-10 ^{0}C) ppm	p^0(22 ^{0}C) ppm
Hexane (HEX)	1,89	0,08	860	5.160	34.600	177.130
Acetone (ACE)	20,7	2,88	1500	10.000	54.600	87.000
Isoprene	2,1	0,29	1500	11.600	87.000	22.300
Ethanol (ETH)	24,50	1,69	460	2.300	9.200	66.200
Water	80,10	1,85				

3. Results and Discussion

3.1. Materials Characterization

Synthesized PSSA-g-PANI was characterized via 1H NMR analysis (Figure 2) 1H NMR spectra (300 MHz, DMSO-d6) of PSSA-g-PANI shows multiple peaks at 7-7.3 ppm corresponding to H in PSSA-g-PANI. The peak at 1.5 ppm, which is related to $-CH_3$, disappeared because of removing BOC group in 1 M HCl solution.

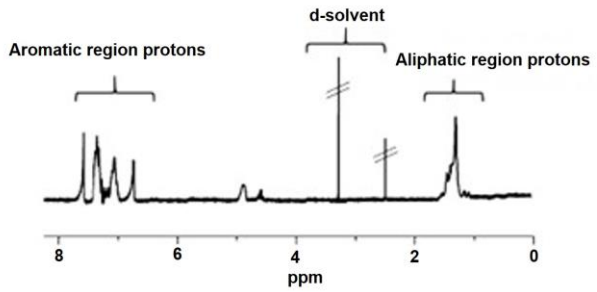

Figure 2. 1H NMR spectra of the synthesized PSSA-g-PANI.

3.2. Sensor Responses

The PSSA-g-PANI having two response regimes (linear and nonlinear) against isoprene and humidity. These interactions depend on the dipole moment and dielectric Constance of measured VOC's. In this study Isoprene having high responses than the other VOC's, Because, Isoprene has lowest dielectric and constancy of dipole moment comparing the others except hexane (Figure 3). Another parameter that should be to consider is the response and recovery times. This could be achieved by the analyzing of time-response data. As it is shown in the time-response plot in Figure 4, the recovery and response times for low concentrations are quite low, which is desirable. However, for high concentrations, the response time increases, and on the contrary, the recovery time is lower compared to the response time.

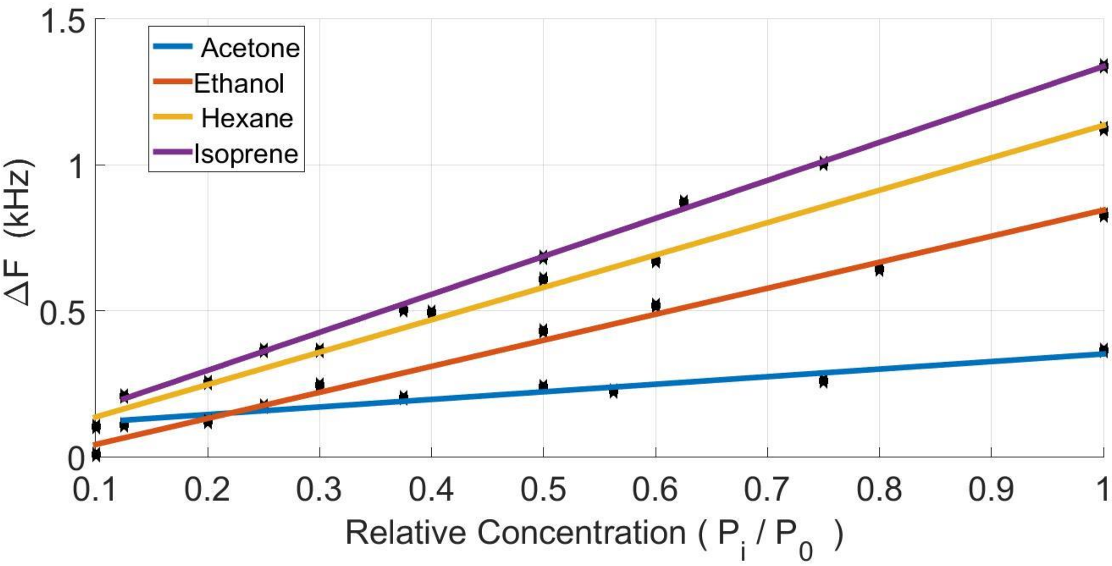

Figure 3. Frequency versus of relative concentration for PSSA-g-PANI.

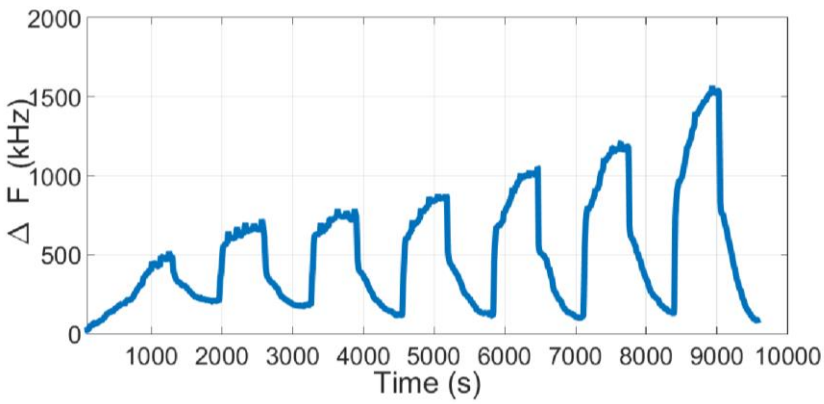

Figure 4. Isoprene response of PSSA-g-PANI coated SAW time versus frequency shift.

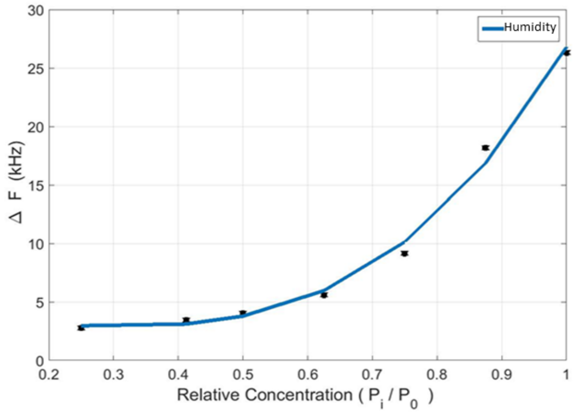

Figure 5. Response against different concentration of humidity.

The sensor response to humidity is quite different from VOC's. This could be account for the incomparably high dielectric coefficients of humidity. Water molecules with a high dielectric coefficient can be absorbed by PSSA-g-PANI. This situation increases the film conductivity and hence causes a shift in the SAW sensor frequency (Tasaltin *et al.* 2012).

To ascertain the stability results, the sensor experiments were carried out using the SAW transducers under similar conditions. The sensor responses for isoprene have not changed even after a week. It is revealed that PSSA-g-PANI based SAW sensor has good stability.

4. Conclusions

In this study, PSSA-g-PANI based SAW gas sensors were prepared and tested against isoprene gas. Sensor measurements reveal that PSSA-g-PANI based SAW type sensor detected (1-150) ppm isoprene gas in the 60s with high sensitivity. Sensor measurements reveal that a prepared PSSA-g-PANI based SAW type sensor may enable monitor and control of sleep apnea.

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