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Detection of Citral in some lemongrass essential oil with copolymer composites based QCM Sensor

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Abstract*— Citral, a significant aldehyde monoterpene found in various kinds of lemongrass (Cymbopogon sp.), is used as an aromatherapy tool to decrease tension, anxiety, and depression, as well as a fragrance in soaps and other personal care items. Moreover, recent research has revealed that this substance has numerous important health benefits, including anti-oxidant, anti-inflammatory, anti-hyperglycemic, and anti-diabetic qualities, to mention a few. Citral is the major component of the lemongrass essential oil (LGEO). The percentage of citral found in commercially marketed LGEOs are quite fluctuating, therefore it is important to quantify the citral molecules in the commercially available LGEOs. In this study, a pioneering detection method for citral is presented using copolymerization of Methacrylic Acid and Styrene on the surface of a Quartz Crystal Microbalance (QCM) sensor. The fabricated sensor has yielded a high sensitivity of 0.0833 Hz/ppm with a commendable correlation factor (R^2) of 0.9648. The applicability of this sensor is further enriched by its large linear operating range of (50-600) ppm. The potential of the sensor was further investigated by discriminating four four distinct commercially available LGEO depending on Citral c oncentration, which has produced a high separability index of 0.9562 when analyzed through Principal Component Analysis. This QCM-based measurement technique enhances citral detection possibilities with promising results thereby further widening the application areas for QCM based measurement.

Keywords*— Lemongrass Essential Oil, Citral, QCM, PCA

I. INTRODUCTION

Since ancient times, therapeutic plants, often known as medicinal herbs, have been found and employed in traditional medical procedures. Cymbopogon citratus (commonly known as lemongrass) is one such plant that found its widespread application in various traditional medicine and other industrial applications based on its essential oil. Lemongrass essential oil (LGEO) can be extracted from the leaves and stems of species of Cymbopogon like Cymbopogon citratus, Cymbopogon flexuosus, Cymbopogon winterianus, Cymbopogon martinii, Cymbopogon nardus, and Cymbopogon refractus [1] by different methods such as steam distillation, hydro distillation (HD), microwave-assisted hydrodistillation (MAHD), and supercritical fluid extraction (SFE) with CO_2 [2]. The main ingredients of essential oils are terpenes, which are naturally occurring volatile molecules with a strong aroma. Citral, [3,7-dimethyl-octa-2,6-dienal] is a monoterpene aldehyde which is the major component of LGEO comprised of two geometric isomers geranial (transcitral, citral A) and neral (cis-citral, citral B) [3-4]. The molecular structure of citral is shown in Fig1. It is found in many studies [1,2,4] that various LGEO contains geranial (20-40%), neral (15-35%), geraniol (8-20%), limonene (5-8%) and linalool (2-5%) as main components. Due to its distinctive lemon fragrance, citral has become a flavouring agent of significant importance, and a widely utilized raw material for the pharmaceutical, food, perfume, and cosmetics sectors [2, 22]. Apart from its several industrial uses citral has antimicrobial, antioxidant, anti-inflammatory, cardiovascular, antihyperglycemic, antidiabetic and antitumour properties [5-10]. The citral content of LGEO is typically used to assess the quality of the oil and must be at least 75% to be regarded as a high-quality product [24]. Admissible daily intake for citral is 0.5 mg/Kg [23].

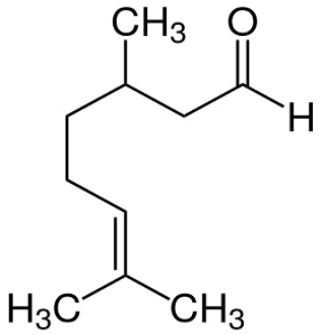

Fig.1. Molecular structure of citral.

To evaluate the quality of commercially available LGEO, the methods for its proper detection and quantification have become significant. Several chromatography and spectrometry techniques namely gas chromatography-ion mobility (GC-IMS), gas chromatography-mass spectroscopy (GC-MS), gas chromatography with flame ionization detector (GC-FID), liquid chromatography (LC) and Fourier Transform Infrared Spectroscopy (FTIR) have been used for this purpose [13-15]. However, these detection methods are reliable but they are frequently time-consuming and expensive, and when the kind of essential oil varies, they may require expensive modifications to the acquisition equipment. Additionally, these systems demand highly qualified

personnel for reliable operations. On the contrary, unconventional methods like QCM-based aromatic gas detection techniques have the advantages of being lightweight, affordable, and durable with specialized, extremely sensitive, and precise measuring capabilities [16]. The sensing component of QCM has undergone a number of advancements in recent years, which have greatly expanded the application fields it may be used. These areas now include drug identification in pharmaceutical applications and quality assessment of commercially available foods and drinks [17-19]. A QCM sensor functions by changing the resonance frequency (f_0) of an AT-cut quartz crystal as the quantity of gas sample adsorption varies. According to Sauerbrey's equation [20] which is shown in eq (1), the variation in frequency (Δf) and the quantity of mass deposited (Δm) on the surface are linearly related.

$$\Delta f = -\frac{2 f_0^2}{A\sqrt{P_q Q_q}} \Delta m \quad (1).$$

Where:

f_0 : Resonant frequency of the fundamental mode (Hz)

Δf : normalized frequency change (Hz)

Δm : Mass change (g)

A : Active crystal area (Area between electrodes, cm^2)

P_q : Density of quartz (2.648 g/cm^3)

Q_q : shear modulus of quartz (2.947×10^{11} $g \cdot cm^{-1} \cdot s^{-2}$ for AT-cut crystal).

The sensing procedure comprises passing a stable, inert environment over the coated sensor surface while a regulated flow of a gas mixture containing the target analyte is being passed through. The analyte is adsorbed on the surface of the coated sensor during this process and frequency variations are seen for a while before stabilizing. The adsorbed gas is then released in a brief purging session. After being calibrated, the developed sensor can be used again for measurements with great accuracy [21]. The system's effectiveness depends on the conception of suitable coating materials for the crystal surface that can discriminate the target analyte from a mixture of gases originating from a source.

The proposed sensor has been developed by a polymer-based coating using Methacrylic Acid and Styrene as monomers and Di vinyl benzene as co-polymer. The target analyte's propensity to attach to the adsorption sites on the polymer chain is increased by using tung oil during polymerization. The ideal ratio of monomers to co-polymer and the most suitable coating thickness for the sensor surface have both been investigated in order to optimize coating properly. Based on the concentration of citral, the developed sensor was used to differentiate between four different LGEO samples using PCA.

II. Experimental

A. Materials and reagents used

Methacrylic acid (MA), Styrene, Di-vinyl-benzene (DVB), Tung Oil, and Ethanol were acquired from Sigma Aldrich in India. Four distinct LGEO samples and quartz crystal with silver-plated AT-cut 10 MHz sensors were purchased from the neighborhood market. The highest analytical grade was employed for each and every component.

B. Sensor development

Pure ethanol was used to clean the surface of bare quartz crystals, which were then dried and placed in a desiccator to prepare the sensor. The coating material has been prepared using various mixtures of MA, Styrene, and DVB with Ethanol as a solvent. In TABLE I, the combinations and frequency deviation for each of them has been presented. The sensor layer on the crystal surface has been set by using an easy drop-coating method. The coated sensor was placed in a temperature-controlled oven at 85ºC for 25 minutes in order to polymerize it.

C. Measurement Setup

Static headspace sampling methodology was used in the present study of QCM-based measurement. The sensor was inserted into a 100 cc Teflon-coated airtight chamber. The analytes were injected into the sensor chamber through a glass syringe at different concentrations. The flow of analytes into the chamber was managed by regulators. The sensor was excited by a high frequency generator using a Schmitt trigger. A microcontroller chip called the Atmega-328p has been used to accumulate data. A computer was used to process and store the generated data. The temperature and humidity were both kept constant throughout the whole experiment. After each measurement, the adsorbed analytes were released by using an air purging technique, after which the sensor returns to its baseline frequency.

D. Characterization of the Sensing Film

Fourier-transform infrared spectroscopy and scanning electron microscopy were used to analyze the sensor layer that was placed on the surface of QCM. The polymer sample was created in a glass vial for FTIR analysis and upon polymerization, was crushed into a powder using a mortar and pestle. In a 1:1 ratio, potassium bromide (KBr) was thoroughly combined with the powdered sample before being finely ground once more. This fine powder mixture was dried at 105°–115°C for two hours, and then the sample was palletized using a pallet-forming die by exerting a force of around 4 tons under several mm Hg of vacuum. The pallet's formation required some time. Fourier transform infrared spectroscope (L160000F, Perkin Elmer Inc, USA) with two temperatures stabilized DTGS (deuterated triglycine sulfate) detectors and standard optical system with KBr windows for data collection over a spectral range of 8,300 – 350 cm^{-1} at the best resolution of 0.5 cm^{-1} were made. To account for infrared light scattering losses, the background measurement was performed using only a pallet of KBr (no sample), after which the prepared pallet was placed on the pallet holder. With a 15 kV acceleration voltage, a scanning electron microscope (ZEISS) was used to examine the surface morphology of the sensor film.

III. Results and Discussion

A. Ratio Optimization

Ten distinct sensors, designated CT1 to CT10, have been created by altering the amounts of the reagents MA, styrene, DVB, tung oil, benzoyl peroxide, and ethanol listed in TABLE I. It has been seen that the CT4 sensor has produced

a superior response from CT1 through CT10 responses at a particular 300 ppm gas concentration. As a result, further study has been performed using the CT4 sensor.

TABLE I. DIFFERENT COMPOSITION OF METHACRYLIC ACID – STYRENE - DIVINYL BENZENE-TUNG OIL COPOLYMERS

Sample No.	Monomers and Co-polymers used						Freq Dev (Hz)
	MA (µL)	Styrene (µL)	DVB (µL)	Tung oil (µL)	Benzoyl peroxide (g)	(Ethanol) (mL)	
CT1	50	20	10	20	0.05	10	8
CT2	50	20	20	20	0.05	10	16
CT3	50	20	30	20	0.05	10	12
CT4 [a]	**50**	**30**	**20**	**20**	**0.05**	**10**	**24**
CT5	50	30	10	20	0.05	10	20
CT6	50	40	10	20	0.05	10	12
CT7	60	40	20	20	0.05	10	20
CT8	60	40	30	20	0.05	10	16
CT9	60	40	20	20	0.05	10	12
CT10	70	40	20	20	0.05	10	16

[a] Denotes the optimum combinations of the monomers and co-polymers used.

B. *Optimization of coating thickness*

The responses of five distinct sensors to a constant concentration of citral have been demonstrated in Fig 2. These sensors were created with five different amounts of coating drop. This has led to the conclusion that a coating thickness of 2.4 µL is the amount at which the largest frequency variation occurs. With this, an optimal coating level has been achieved.

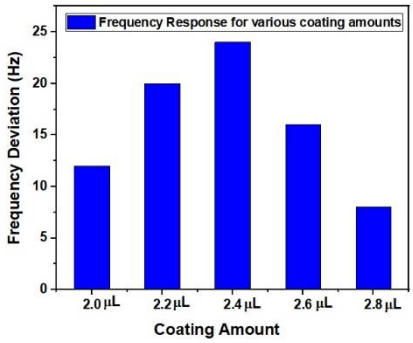

Fig.2 Coating amount optimization graph.

C. *Sensitivity Analysis*

Sensitivity dictates the response of the fabricated sensor in ppm. The optimized CT4 sensor has been exposed to varying concentrations of citral from (50-600) ppm using a calibrated glass syringe. The range of concentrations (in ppm) was calculated using eq (2), where V_1 and V_2 stand for the volume of the sensor chamber and the volume of the syringe respectively. S_1 and S_2 imply concentration (in ppm) of gas in the sensor chamber and the concentration (ppm) in the syringe respectively.

$$V_1 S_1 = V_2 S_2 \quad (2)$$

where,

V_1 = Volume of the sensor chamber

V_2 = Volume of syringe.

S_1 = Concentration (ppm) of gas in sensor chamber.

S_2 = Concentration (ppm) of gas in the syringe.

The sensitivity of CT4 was obtained by tracing its frequency response (in Hz) with respect to the range of concentrations (in ppm) of the target analyte starting from 50 to 600 ppm. Results are displayed in Fig.3. The sensitivity of CT4 sensor was calculated as 0.0833 Hz/ppm with a linear regression coefficient (R^2) of 0.9648. After each injection of the target analyte, the adsorbed gas on the surface of the sensor was removed through the standard air purging method, wherein the sensor was subjected to a continuous flow of fresh air, which removes the adhered target molecules thereby raising the working frequency of the crystal. The real time response profile for the fabricated CT4 sensor has been shown in Fig.4.

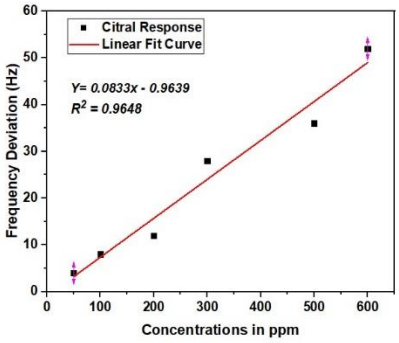

Fig.3. Sensitivity analysis curve

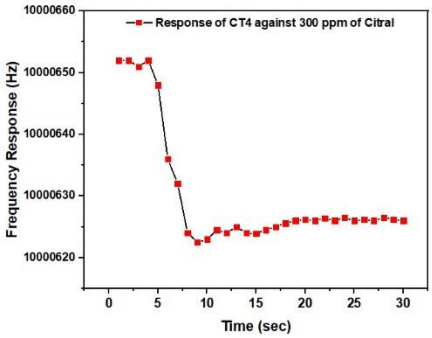

Fig.4. Real-time response profile of CT4 sensor for 300 ppm.

D. *Fourier Transform Infrared Spectroscopy*

The FTIR analysis of the synthesized methacrylic acid and styrene copolymer is illustrated in Fig. 5. As per the literature reference [25-26], the band observed at 1471 cm-1 corresponds to the symmetric stretching mode of the methyl group, while the distinctive features at 1895 cm-1 are indicative of carboxylic acid functionalities, representing simple carbonyl compounds featuring methyl (CH_3) bonds. Furthermore, the presence of a band at 2988 cm-1 can be attributed to the (vinyl) C-H stretch vibration originating from styrene.

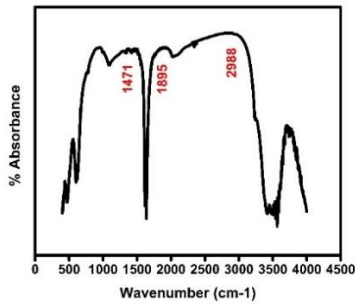

Fig.5. FTIR of synthesized methacrylic acid and styrene copolymer.

E. Scanning Electron Microscopy

The surface morphology of the bare crystal and crystal surface with sensing polymer has been shown in Fig 6(a) and 6(b). From the figures we can observe that the surface of the bare crystal is smooth, while the surface of the polymer is rough.

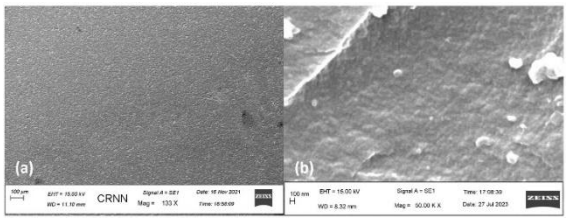

Fig.6. SEM images of (a) bare crystal and (b) methacrylic acid and styrene copolymer-based sensor.

F. Repeatability, Reproducibility and Reusability

Repeatability, reproducibility, and reusability are the three most significant criteria for a sensor. To evaluate the repeatability of the proposed sensor, different concentrations of citral were injected ten times at constant ambient conditions. Responses are presented in TABLE II. The maximum repeatability was attained at 600 ppm concentration (83.87%), while the average repeatability across all gas concentrations was 82.6%. Five distinct CT4 sensors were fabricated for the examination of reproducibility, and they were repeatedly exposed to the injected analyte. According to TABLE II, a maximum reproducibility of 80.92% was achieved at 300 ppm of injected citral vapor. Over the course of 80 days, static headspace sampling of the target gas was carried out under identical conditions at regular intervals. According to the response, which is shown in Fig. 7 the sensor is stable and reusable for a period above two months because the frequency variation is 12% on the 80th day from the response on the first day.

TABLE II. REPEATABILITY AND REPRODUCIBILITY RESULTS WITH 95% CONFIDENCE INTERVAL FOR CT4 SENSOR

Citral concentration (ppm)	**Repeatability**		**Reproducibility**	
	Rept (%)	***CI***	***Repd (%)***	***CI***
50	82.12	±3.99	80.23	±4.67
100	82.37	±4.65	79.98	±4.45
200	83.15	±5.43	80.26	±5.91
300	81.98	±4.97	80.92	±5.93
500	82.19	±5.32	79.95	±6.56
600	83.87	±5.86	80.73	±8.06
800	82.56	±8.96	80.58	±9.89

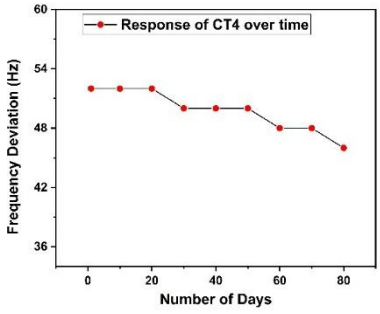

Fig.7. Reusability profile of CT4 sensor against citral.

G. Study of Limit of Detection

The limit of detection (LoD) of a sensor is another significant phenomenon. It is the smallest quantity of target gas that can be quantitatively measured and detected. It is based on the linear calibration curve's slope and the response's standard deviation (σ) as determined by a number of measurements (N). Mathematically it is expressed as,

$$\text{LoD} = 3.3\ \sigma / S \tag{3}$$

Based on eq (3), LoD was found at 7.15 ppm, which is the lowest limit of detection with the proposed measurement setup.

H. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a statistical technique that is essential for data exploration because it transforms multidimensional data into coordinates that optimize variance while decreasing correlation in the dataset [18]. Every data point X_n is projected onto a scalar value $U_1^T X_n$. The mean of the projected data is UX' where X' is the sample set mean given by,

$$X' = \frac{1}{N}\sum_{n=1}^{N} X_n \tag{4.1}$$

And the variance of the projected data is given by,

$$\frac{1}{N}\sum_{n=1}^{N}\{U_1^T X_n - UX'\}^2 = U_1^T S U_1 \tag{4.2}$$

Where S is the data covariance matrix defined by

$$S = \frac{1}{N}\sum_{n=1}^{N}(X_n - X')\,(X_n - X')^T \tag{4.3}$$

The biggest variance is seen in the first principal component, and this variance increases with each successive component. Two principal components, PC1 and PC2, or the first and second principal components, respectively, have been extracted from the dataset in this work, as shown in Fig. 8. In comparison to all other PCs, PC1 has been discovered to be 99.9993%, whereas PC2 is 0.0002% with a separability index of 0.9562.

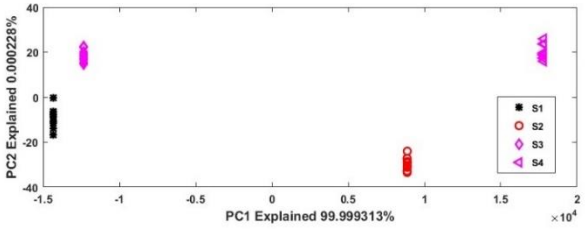

Fig.8. PCA Plot with 4 different concentrations of lemongrass essential oil.

IV. Conclusion

This study has introduced an innovative citral detection method, leveraging the copolymerization of Methacrylic Acid and Styrene on a Quartz Crystal Microbalance (QCM) sensor. The fabricated sensor has demonstrated exceptional sensitivity, registering at 0.0833 Hz/ppm, with a commendable correlation factor (R^2) of 0.9648. Furthermore, the sensor's versatility is underscored by its broad linear operating range of (50-600) ppm. The potential of this sensor has been further substantiated by its ability to distinguish between four different commercially available LGEO samples based on their citral content. Principal Component Analysis revealed a high separability index of 0.9562, showcasing the sensor's proficiency in discriminating citral levels effectively. This innovative QCM-based measurement technique opens up exciting possibilities for citral detection, offering promising results that extend the application areas for QCM-based measurements. It not only addresses the pressing need for citral quantification in LGEO but also has the potential to revolutionize the way we measure and utilize citral in various industries, further advancing its multifaceted applications.

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