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Preanodized Screen Printed Carbon Electrode for Detection of Linalool using Three Terminal Network

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Abstract*: Linalool is a very important flavouring compound found in plants which is used in food and beverages. Linalool has been traditionally detected by analytical instruments such as gas chromatography (GC) coupled with mass spectroscopy(MS) which are not suitable for routine tests. For fast and low cost detection of chemical compounds electrochemial sensors are most suitable. Screen printed carbon electrode (SPCE) is one of the most popular and low cost device used for detection of chemical compounds. In this article we present the detection of linalool using a low cost preannodized commercial screen printed carbon electrode (SPCE). Traditionally electrochemical sensors are used in two terminal mode, however three terminal analysis of electrochemical sensors are found to be more rationale and accurate. In this paper we have analyzed detection of linalool by an advanced three terminal analysis. First we have performed cyclic voltammetry(CV) of the SPCE which showed clear oxidation peaks at different concentration of linalool. The input-output data of the CV has been used for analysis of the impedance of the SPCE.The impedance model of the SPCE was estimated by autoregressive moving average with exogenous inputs(ARMAX) modelling technique using the CV data. The three terminal impedance fitting revealed the values of electrical parameters and the parasitic elements at different linalool concentration. The stability limits of the SPCE was also determined from the pole-zero and Nyquist plots of the estimated models. Impedance behaviour to frequency of the SPCE was further analyzed by impedance plot(Z vs $-Z'$) from which we are able to relate the CV scan rate to the impedance of the SPCE. Finally the sensitivity and repeatability of the SPCE was determined using a measurement circuit.

***Keywords*: Linalool detection, SPCE, three terminal analysis, ARMAX modeling, parasitic elements.**

I. INTRODUCTION

Linalool ($C_{10}H_{18}O$) is a hydrophobic and monoterpenoid compound which are extracted from plants for use as perfumes and flavouring food and beverages[1]. Detection and characterization of linallol are done by high performance liquid chromatography(HPLC),gas chromatography(GC) coupled to mass spectrometry or flame ionization detectors(FID) with different sample pretreatments[2-8]. These methods involve high cost method[6];instrumentation and they are not easily applicable in routine analysis. Literature available for detection of linalool are- GC−FID, GC−MS, and GC−olfactometry for linalool in the plant *Michelia alba*[2]; investigation of linalool using GC−FI, GC-MS, and GC−Olfactometry in white Austrian clover flowers[3]; quantitative analysis of linalool in wine[4]; determination of myristicin and linalool in plants exposed to microwave radiation byHPLC[5]; assay of linalool in coriander oil by proton NMR characterization and adulteration monitoring of linalool by Electrospray Ionization Mass Spectrometry[7].A new method based on fluidized-bed extraction combined with detection by GC-MS was developed to detect linalool [8].

Since the analytical methods as discussed above are not suitable for fast routine tests, electrochemical sensing has been established as a low cost and fast detection technique of odorant molecules such as a supported lipid bilayer membrane (s-BLMs) formed on a freshly cleaved metallic surface [9]. Although a variety of electrodes have been developed for electrochemical sensors using noble metals, carbon materials in Screen printed carbon electrodes (SPCE) deposited on rigid substrates to flexible materials like paper, fabric, wearable cloth etc have drawn attention. The SPCEs are becoming popular due to low manufacturing cost and their diverse design options using thick film technology. The SPCE features mixtures of two main components - carbon powder (graphite) acting as electroactive sensor and a liquid binder (lipophilic organic liquid). The organic liquid acts as a binder as well as influences the overall electrochemical behaviour of the paste [10]. It offers hydrophobic character of the carbon paste surface, which is the main reason for different behaviour of CPEs compared to carbon solid electrodes.

In the last decade SPCEs has become popular for detection of various compounds[11-16] such as – a preanodized SPCE for *propofol* at pH 7.0 of phosphate buffer solutions [11] ; a SPCE modified with the nanocomposite for *nilutamide*[12] ;graphite nanosheets and ZnO nanoparticles deposited SPCE for glucose oxidase[13]; Ag@GNRs film modified SPCE for pesticide *methyl parathion* [14]; a chitosan (CHT) crafted zinc ZnO nanoneedles modified SPCE for 4-nitrophenol in water[15]. Researchers have conducted experiment for comparing commercial SPCEs such as in a SPCE (C-110, Dropsens, Ltd. ,Spain) along with four other SPCE are tested to compare their performance[16].

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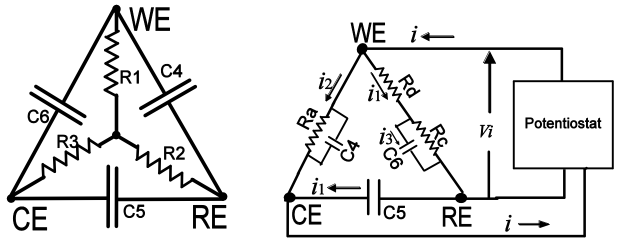
In their study the authors estimated the best performance in C-110 as-anoidic and cathoidic peak current ratio: 1.18, E_{pc}= 0.112V, Sensitivity =97 mV, 20 µA mM^{-1} .

Motivated by the advantages of SPCEs, in this paper we present a preannodized commercial SPCE for detection of linalool and analysis of its model electrical parameters using three terminal analysis similar to C-110[17].

Three terminal circuit analysis of SPCE

Fletcher [18] suggested that although conventionally the three terminal sensors are represented by a two terminal circuit is better represented by a three terminal circuit as shown in Fig.1(a) where R_1, R_2 and R_3 are node to node resistances while C_4, C_5 and C_6 are three inter-electrode stray capacitances. In our earlier work we have proposed an alternative delta equivalent three terminal circuit which fits best to a higher order (2nd and 3rd) model (Fig.1(b)) [17]. Further we consider that the basic Randle's circuit appears across WE and RE node with $\mathrm{R_d}$ representing the analyte resistance, R_c as the charge transfer resistance and C_6, the double layer capacitance. In this circuit we considered that the solution resistance between RE and CE is compensated by the potentiostat [19].



(a) (b)

Fig.1 Three terminal electrode networks (a) Original (b) proposed[14]

In the circuit of Fig.1(b) the network $R_d + (R_c.C_6)$ represents the WE impedance, while R_a, C_4 and C_5 represents interelectrode stray parasites.

In this paper we propose ARMAX model transfer functions $\mathrm{G(s)} = I(\mathrm{s})/V(\mathrm{s})$ of the three terminal network of the SPCE in linalool as the analyte, where $I(\mathrm{s})$ is the response current and $V(\mathrm{s})$ is the applied voltage. From the transfer function model we derive the impedances $\mathrm{Z(s)} = \mathrm{V(s)/I(s)}$ which are fitted to our three terminal impedance of Eq(1).

A. ARMAX Modeling

In our earlier work we have performed ARMAX modeling of five different metal electrodes of a custom made voltammetric e-tongue [20]. Motivated by the promising results on analysis of the performance of the five electrodes, in this paper we propose that ARMAX model can be estimated from the input and output cyclic voltammetry(CV) data of the SPCE.

We define a linear time-invariant (LTI) system with an input signal $u(n)$ and output $y(n)$ by eqn (1) as:

$$\sum_{k=1}^{na} a_k y(n-k) = \sum_{k=1}^{nb} b_k u(n-k) + e(n-k) \qquad (1)$$

where $e(n-k)$ is the moving average error, n_a is the number of poles; n_b is the number of zeroes and k is the moving average time delay. We generate the data of length n from measured input voltage (u) and output response current (i_0) of the SPCE from CV measurement given by :

$$u(k) = [u(k-1)u(k-2)\ldots..u(k-n)]$$
$$i_0(k) = [i_0(k-1)\; i_0(k-2)\ldots.i_0(k-n)] \qquad (2)$$

The prediction error minimization (PEM) system identification algorithm minimizes a cost function given by Eqn(3).

$$V_N(G,H) = \sum_{t=1}^{n} \in^2 (t) \qquad (3)$$

where ∈(t) is given by:

$$\in (t) = y(t) - \hat{y}(t) \qquad (4)$$

where $y(t)$ is the measured response current i_0(t) and $\hat{y}$(t) is the estimated response current $\widehat{i_0}$(t) of the electrodes.

II. MATERIALS AND METHODS

The experiments were conducted in steps as stated below-

i) Preparation of solutions
ii) Cyclic voltammetry of the SPCE at different linalool concentrations and storing of input voltage and output current
iii) ARMAX modeling using CV data for different concentrations of linalool
iv) Fitting of ARMAX model to three terminal network
v) Impedance analysis
vi) Amperometric measurement
vii) Repeatability test

B. Chemicals

All of the chemicals used in this work were of analytical grade and were used without further purification. Standard solutions of linalool ($C_{10}H_{18}O$,98%), sulfuric acid (H_2SO_4-95–97%) and potassium chloride (KCl) were purchased from Sigma Aldrich (USA).

C. Sensor and instrument

The screen-printed electrodes(SE-100) were purchased from Zensor(USA).The Zensor screen printed SPE has a planar carbon working electrode (central circle) printed on a PVC substrate, a carbon auxiliary electrode (outer annular crescent) and Ag/AgCl pellet reference electrode. In the SPCE relatively large tabs at the top of the SPE enable electrical contacts. Fig.2(a) shows the SPEC while Fig.2(b) shows the electrode dimensions. The surface of the SPCE was rinsed with ultrapure water between successive measurements. The SPCE was preannodized in a 0.05 mol H_2SO_4 solution by applying anodic potentials (ranging from -0.5 to +0.5 V) for five cycles with a scan rate of 10mV/s. A potentiostat (SP-300,BioLogic,France) was used for CV measurements of the SPCE.

D. Preparation of solution

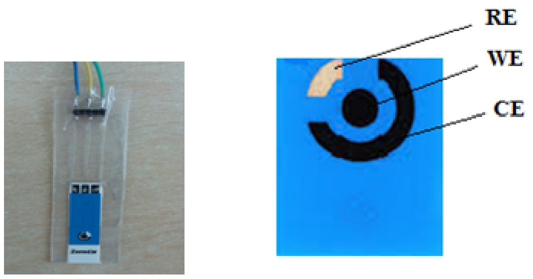
Potassium chloride solution (pH 7.0) was prepared from crystalline KCl as the supporting electrolyte with a concentration of 0.1M. Stock solution of 0.65 mM linalool was prepared by dissolving 14.35 µl linalool in 100 ml water containing 0.1 M KCl by constant stirring. Similarly 0.325 mM, 0.1625 mM , 0.0812mM solutions were prepared by half diluting the stock solution successively while keeping the concentration of KCl constant. The cell and the SPCE were cleaned and washed thoroughly by water. We have used deionized water purified from a Milli-Q purification system.

For ARMAX modeling of the SPCE we have used the time series input-output data of the CV for a total 2000 data points during one cycle(0-1-0 Volt) with a scan rate of 10mV/s. Therefore the input frequency of the CV is 20Hz.

III. RESULTS AND DISCUSSIONS

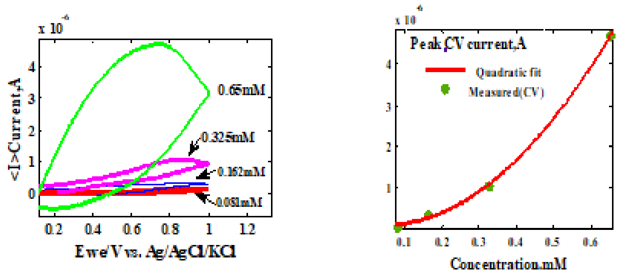
A. CV peak currents with linalool concentration

The sensitivity of the SPCE was evaluated from the CV current response with varying concentration of linalool. We have conducted CV with concentrations of 0.65mM, 0.325mM, 0.1625mM and 0.0812mM as shown in Fig.3(a),however below 0.0812mM the peaks are found to be noisy which are omitted. It is found that the peak oxidation current increases with concentration of linalool while the redox peak is almost constant. The oxidation peak currents for varying concentration at a voltage of 0.86V$_{rms}$ is shown in Fig.3(b). The quadratic regression equation for the concentration vs current relation is found as $i = 12.9c^2 - 1.2c + 0.02$ with r^2 = 0.9994(RMSE=9.19×10^{-8}),where i is the peak current (×10^{-6}A) and c is the concentration of linalool in mM. The highest ratio of peak anoidic and cathoidic current is found as 0.58 while the sensitivity is found as 0.25µAmM^{-1}.



(a) (b)

Fig.2 Zensor SPCE(SE-100) (a) after connecting with lead wires; package size:25. 4mm × 7mm × 0. 625mm (b) three electrodes of the SPCE ;central-WE, right-RE and circular-CE; exposed surface area of the WE- 0.0314 mm² and CE-0.1256 mm²



(a) (b)

Fig.3 Voltammograms of the SPCE (10 mVs^{-1} scan rate) solutions of linalool (a) CV for one cycle each (b) variatio peak current with concentration

B. Estimation of impedance

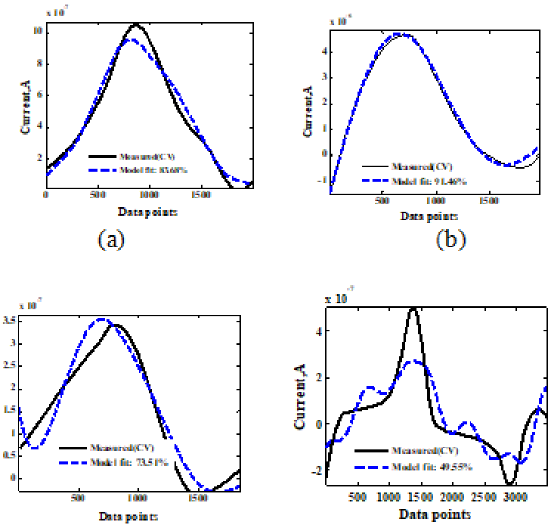
The estimated transfer function $G(s)$ of the SPCE (Table-I) that fits with the circuit of Fig.1 (c) is of the form:

$$G(s) = \frac{1 + a_1 s + a_2 s^2 + a_3 s^3}{1 + b_1 s + b_2 s^2} \tag{5}$$

from which we get the impedance equation as:

$$Z(s) = \frac{1 + b_1 s + b_2 s^2}{1 + a_1 s + a_2 s^2 + a_3 s^3} \tag{6}$$

The transfer functions estimated for the SPCE shown in Table I indicate that for different concentrations of linalool the model parameters are different. The impedance of the SPCE at four different concentrations as shown in Table-1 were fitted to the three terminal impedance Eqn(1) to estimate the electrical parameters. The three electrode parameters (R_{sol}, R_{ct} and C_{dl}) and the two parasitic parameters (R_p and C_p) estimated are shown in Fig.5 at various concentrations.



(a) (b)

(c) (d)

Fig.4 Model fits for the network (a) 0.0812mM (b) 0.1625mM (c) 0.325mM and (d) 0.65mM

TABLE I
IMPEDANCE FUNCTIONS Z(s)

C (mM)	Z(s)	Pole	Zero
0.0812	$\frac{134.2(s+1.16)(s^2+0.21s+22.74)}{(s-84.09)(s+0.22)}$	-0.0040 -0.0006 + j0.0094	-0.1274 -0.0013
0.1625	$\frac{276.2(s+0.36)(s^2+0.013s+0.019)}{(s+9.8)(s-0.14)}$	-0.3650 -0.0068 ± j0.1387	-9.8911 0.1482
0.325	$\frac{4167(s+0.37)(s^2-0.001s+0.057)}{(s+10.59)(s+0.18)}$	-0.0075 0.0000 ± j0.0048	-0.2117 -0.0036
0.65	$\frac{-4492.3(s^2-0.19s+0.12)}{(s+1.5)}$	0.0998 ± j0.3397	-1.57

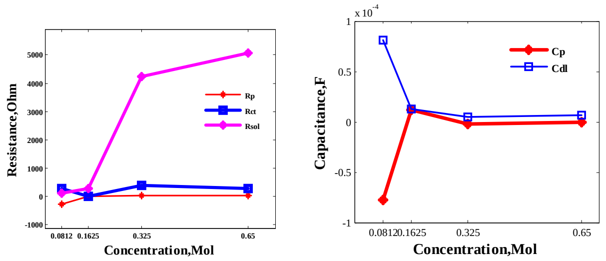


Fig.5 Variations of electrical parameters with concentration of linalool at 0.0812mM to 0.65mM (a)

It is observed that the analyte resistance R_{sol} increase to about 5KΩ when the concentration of linalool is increased from 0.081mM to 0.65mM. Similarly the parasitic resistance R_p also increases from -266.62Ω to 19.82Ω, while R_{ct} has its own charge transfer resistance values of 266.79Ω,15.66Ω,401.48Ω and 276.78Ω respectively .On the other hand the double layer capacitance (C_{dl}) decreases from 81.1μF to 6.7 μF, while the parasitic capacitance (C_p) monotonically decreases from 77.45 μF to 0.37 μF. From this it is evident that the parasitic resistance increases while parasitic capacitance decreases as linalool concentration increases in the SPCE.

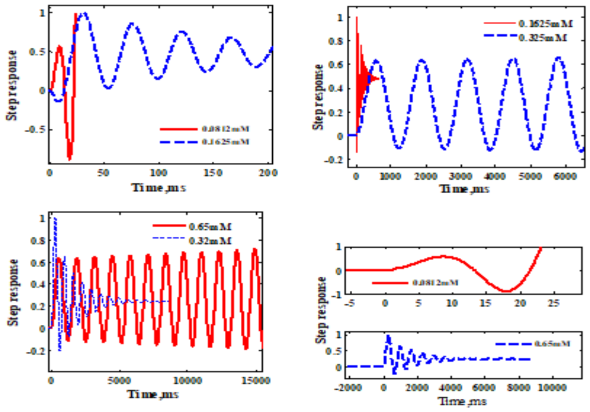


Fig.6 Comparison of step responses of the SPCE with higher concentration (a) 0.08125 mM with 0.162mM (b) 0.1625mM with 0.32mM (c) 0.32 mM with 0.65mM (d)).0812mM with 0.65mM.

C. Electrode stability

Fig.4 shows the fits of the models. It is observed that the fits for linalool concentrations of 0.0812mM, 0.1625mM, 0.325mM and 0.65mM are- 83.68%,91.46%,73.5% and 49.55% respectively. The reason for low fit in highest concentration (0.65mM) is inconsistent CV current of the SPCE may be due to degradation of the reference electrode during reaction of the Cl^- ion with linalool at higher concentration. The Cl^- ions are formed due to CV at 1V that reacts with linalool producing *10-chlorodecanoyl chloride:*

$C_{10}H_{18}O$ +Cl_2= C10H18Cl_2O. It may accumulate at the interface between electrode and electrolyte causing polarization and affects the Redox process. The unstability of the SPCE at higher concentration of linalool is also evident from the pole-zero positions (Table-1),the step response(Fig.6) and Nyquist plot(Fig.7). In Fig.6(a-c), the step responses are compared with the next higher concentration of linalool. It is observed from table 2 that as concentration increases the oscillatory response of the SPCE worsens. In Fig.6(b) the response at 0.162mM shows damped oscillations till 800ms while at 0.325mM till 6s and at 0.65mM till 1.5s(Fig.6(c)). Fig.6(d) shows the comparison of step response of the SPCE at lowest (0.0812mM) and highest(0.65mM) concentrations. Although from the pole positions (Table-1) the SPCE apparently shows stable, however from the Nyquist plots (Fig.7) the stability criterion can be analyzed. If there is a pole in right half plane(RHP) , for a system to be stable the following condition should be fulfilled:

$$Z = N + P \qquad (7)$$

Where Z is the numbers of poles in RHP,P is the poles in the RHP and N is the numbers of clockwise encirclements of the -1+j0 point. From this condition we find that-

0.08mM : $Z = 0, N = 0$ and $P = 0$ so the SPCE is stable
0.162mM : $Z = 1, N = 2$ and $P = 0$ so the SPCE is unstable
0.32mM : $Z = 0, N = 0$ and $P = 1$ so the SPCE is unstable
0.65mM : $Z = 0, N = 0$ and $P = 1$ so the SPCE is unstable

The gain margins of the SPCE models are also found as- 128.56dB,91.13dB, 94.00dB and infinity at concentrations of 0.0812mM, 0.1625mM, 0.325mM and 0.65mM. From this analysis it is evident that the SPCE shows an unstable model at higher concentration thereby producing inconsistent response.

D. Impedance spectra

The impedance behavior has been verified by impedance spectra as shown in Fig.8. Distinct impedance semicircles are observed at different quadrants (Q) specified as:

0.081mM: $Q, III(-R - jX)$

TABLE II
STABILITY AND IMPEDANCE PARAMETERS

Conc (mM)	Oscillation time(ms)	P	Z	N	Gain margin (dB)	Impedance
0.081	17	0	0	0	128.56	$-R + jX$
0.162	800	0	1	2	91.13	$+R + jX$
0.32	6000	1	0	0	94.00	$-R - jX$
0.65	1500	1	0	0	Inf	$-R - jX$

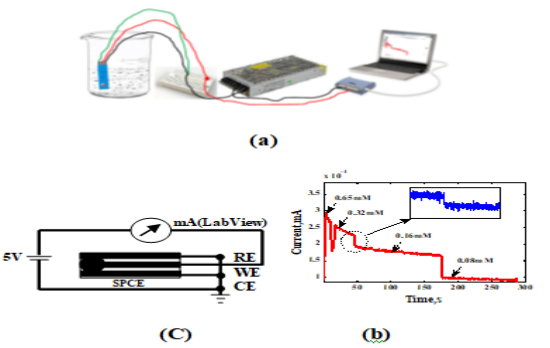
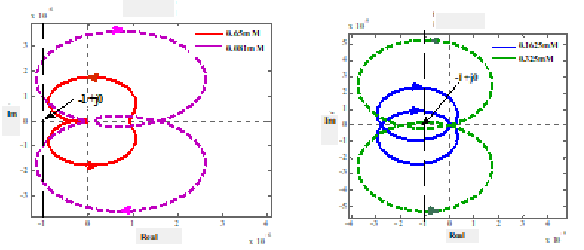


Fig.9 Amperometric measurement of the SPCE to linalool (a) schematic diagram of the setup. The WE is supplied with +5V and the RE-CE are grounded.The current through the WE is measured using a current channel of the DAQ card (b) measuring circuit (c) acquired current signal at successive dilution of the concentration. The inset figure shows the noise

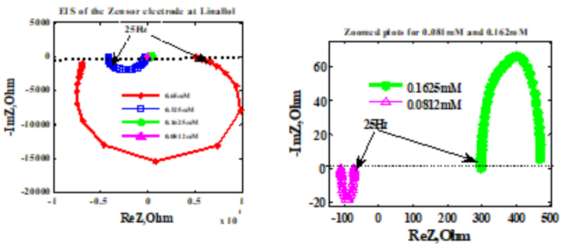
0.162mM: $Q, I(R + jX)$
0.32mM : $Q, III(-R - jX)$,
0.65mM : $Q, III(-R - jX)$ and $Q, IV(-R - jX)$
where R and X are the resistance and reactance of the SPCE respectively.

Prominent negative semicircles are seen in- third and fourth quadrants at 0.65mM, 0.325mM, 0.0812mM due to negative C_p and R_p while at 0.1625mM the semicircle is positive at first quadrant since all electrical parameters are positive. From the impedance spectra the real part of the impedances of the SPCE are found -109 Ω, 300Ω , –4113Ω and 5122Ω at 0.0812 mM, 0.1625 mM, 0.325mM and 0.65mM respectively. The CV scan rate used in our experiment is equivalent to 20Hz as discussed in section 2. However at nearest discrete frequency of 25Hz(shown by arrow) in impedance spectra indicates that negative electrical parameters cannot be avoided by changing the scan rate since neither of the spectra crosses zero-axis in both real and imaginary impedance.



(a) (b)

Fig.7 Nyquist plot of models of the SPCE. Due to better visibility the plots are shown for two pairs having equal scale values (a) at 0.065mM and 0.0812mM and (b) at 0.1625mM and 0.325mM



(a) (b)

Fig.8 Impedance spectra of SPCE (a) at 0.081-0.65mM (b) Zoomed for 0.0812 and 0.1625 mM; The impedance intercepts at 25Hz is shown to relate to CV scan rate of 20Hz

E. Amperometric analysis

From the CV and impedance spectra it is evident that the SPCE is able to detect linalool to a lowest level of 0.0812mM.We have used an amperometric circuit(Fig.9(a) and (c)) for sensing linalool using the three electrode SPCE based on a data acquisition(DAQ) card connected to a PC with LabView. The SPCE was used in two terminal mode by connecting the RE to the CE. The current flowing through the circuit on application of a voltage of 1 V was measured by the DAQ card using LabView. Stock solution of linalool was prepared in a borosil beaker of 500 ml containing 0.1 M KCl by diluting the stock solution successively as discussed in section II. The noise in the current signal was filtered by configuring a Butterworth filter in Labview. The acquired signal is shown in Fig.9(b). It is observed that the current monotonically decreases as 0.29mA, 0.26mA,0.16mA and 0.09mA when the concentration is decreased successively as- 0.65mM, 0.325mM,0.16mM and 0.0812mM.From this measurement the sensitivity of the SPCE to linalool is found as 0.3289mA/mM. This current can be converted to get a sensitivity of 328.9mV/mM across a load resistor of 1KΩ.

To test the repeatability of the sensor the sensor responses over ten repeated trials on 0.325mM linalool were observed as shown in Fig.10(a). The sensor was dipped and removed ten times over a period of 5min 30s period and the responses were recorded. The data during each response period was processed to determine the mean and the standard deviation. The errorbar plot shown in Fig.10(b) indicated a maximum deviation of 0.04μA in fifth trial which is almost 1.2% of the mean value. Therefore the SPCE is able to show a good repeatability in sensing linalool.

IV. CONCLUSION

In this work, a preannodized commercial SPCE in three terminal network analysis has been used for the first time to detect linalool concentration quantitatively. The study shows that the SPCE can detect linalool in two terminal mode also with a linear current sensitivity. The three terminal analysis shows that the parasitic resistance increases while the parasitic capacitance decreases with linalool concentration. The amperometric measurement method shows a good sensitivity and repeatability of the SPCE. The proposed method is useful for detection of linalool having great importance in food and chemical industries.

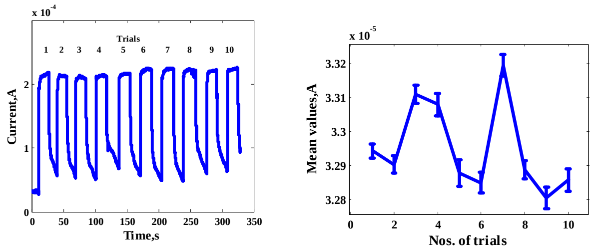


Fig.10 Repetition measurement (a) the SPCE response to ten trials with a concentration of 0.325mM of linalool (b) error bar plot over the mean values of the responses

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