Development and Evaluation of Portable Low Cost Testing System for Phthalates

Asif I. Zia, S.C. Mukhopadhyay, P.L. Yu and I. H. Al-Bahadly. School of Engineering and Advanced Technology, Massey University, Palmerston North. New Zealand.

S.C.Mukhopadhyay@massey.ac.nz

C. Gooneratne and Jürgen Kosel King Abdullah University of Science and Technology, Saudi Arabia.

Abstract— A portable low-cost microcontroller based testing system was designed and constructed to detect phthalates in aqueous media. Phthalates, especially di (2-ethylhexyl) phthalate is the most ubiquitous endocrine disrupting compound (EDC) posing highest reproductive toxicity threat to all living species on earth. Frequency response analyser (FRA) approach was used to develop the rapid response, non-invasive electrochemical impedimetric system for detection. A smart thin film gold interdigital electrodes capacitive sensor with enhanced penetration depth was used with the proposed system for detection of the said EDC. The performance of the developed system was evaluated by comparing the results to the commercially available electrochemical Impedimetric frequency response analyzer equipment. Complex nonlinear least square (CNLS) curve fitting algorithm was used to deduce the equivalent circuit for the developed system. The DEHP detection results by the developed system were validated using high performance liquid chromatography (HPLC) diode array detection confirming that the proposed system was able to detect the concentration of phthalates in aqueous medium.

Keywords-Interdigital; dielectric; sensors; sensing performance; Impedance spectroscopy; DEHP; Endocrine disrupting compounds; electrochemical; MEMS

I. INTRODUCTION

Endocrine Disruptor Compounds (EDCs), a term coined in 1990, are the exogenous chemicals or mixture of synthetic chemicals that interfere in the normal functioning of endocrine system and hormones creating hormonal imbalance in human beings and animal species. Among the known endocrine disruptors the most ubiquitous are the esters of 1-2-Benzenedicarboxylic acid commonly known as phthalates. The orthophthalate diesters are commonly produced by alcoholic esterification of phthalic anhydride and DEHP constitutes more than 80% of the total phthalate production all over the world. Its low synthesis cost and capability to induce mechanical strength and flexibility to Polyvinyl Chloride (PVC) products makes it a potential candidate for unlimited industrial applications. The major function of the endocrine system is to regulate nutrient supply to all cells, control growth and reproduction in a living organism. It had been concluded in research that DEHP pose highest toxicity and endocrine disruptive threat to human race, especially to the young children, infants, pregnant and nursing mothers [1]. A number of recent researches have suggested declining trend in

the reproductive hormonal levels, damage to sperm DNA and count in male adults [2], whereas, elevated risk of altered breast development and breast cancer, premature puberty and prostate changes in females[1, 3]. The abundance of phthalates in ecosystem has raised the public health concerns in the recent years, especially after endocrine disrupting properties of these estrogenic compounds were highlighted by research. [4] The penetration of these teratogenic and carcinogenic compounds into food chain, waste water and ecosystem occurs not only during their production phase but during their usage and even after their disposal via leaching, migration and volatilization. Since phthalate esters are not covalently bound to the poly vinyl chloride (PVC plastic) molecular structure they leach, migrate, and evaporate directly into foodstuffs and atmosphere exposing human beings through ingestion, inhalation and dermal routes.[5]. The extensive use of phthalates in medical products have paved their way to directly infuse into body fluids via medication and use of disposable medical plastic products [6]. Leaching of phthalates into food from packaging [7], from PET (PETE, polyethylene terephthalate) bottles into beverages and mineral water [5] and from corks of glass bottles [8] has been published. Guo et al. showed phthalates leaching into orange juice from tetra packaging increased 110 times above the safe intake limit of 6.0 µg/L set by US EPA (United States Environmental Protection Agency) during its shelf life and until close to its expiry date[9]. The guideline for safe drinking water given by World Health Organization (WHO) and European Union in their water policy is set to 8.0 µg/L DEHP as safe limit in fresh and drinking waters, published in a list of priority compounds posing endocrine disrupting hazards to human [10]. After ingestion, the phthalates are rapidly hydrolyzed to the corresponding monoester by the digestive system and excreted in urine and feces. In humans, short alkali chain phthalates are eliminated mostly within hours but long chain phthalates like DEHP require further metabolism via ωand ω -1- oxidation of the aliphatic side chain[11]. Blood and urine are the most common matrices used for biomonitoring of EDCs but the results of these biomonitors do not provide the complete picture of EDC intake, rather just provide the quantity of metabolites excreted losing information about the amounts of phthalate that have become the body burden. Therefore, it is necessary to have a rapid assay which may detect the quantity of phthalates present in food and beverages

as initial intake via the oral route which is deemed to be the biggest source of human phthalate exposure due to the extensive use of food plastics packaging in our everyday life style. The objective of this research is to develop a low cost real time testing system which is rapid, highly sensitive and involves minimal support electronics and should have a minimal level of complication in application. Presented design of portable low cost FRA device with interdigital capacitive sensor design, that was developed to selectively detect phthalates, possesses properties of speed, real time and in situ testing in aqueous medium.

II. DEVELOPMENT OF INTERDIGITAL SENSOR

Conventional ID capacitive sensors are structured as repeated patterns of single sensing and single excitation (working) finger-like electrode pairs fabricated on one side of a solid substrate to achieve single side access and non-invasive testing. Capacitive reactance, as a function of analyte properties, is produced as a consequence of applied alternating electric field perturbations exposed as frequency sweep to the sensor. The penetration depth of the fringing electric field is a function of the spatial wavelength (distance between sensing and working electrode) of the thin film electrodes [12]. In situ testing capability, single side access, field penetration depth control, signal strength control, simplified modeling, and low cost renders planar ID capacitive sensors the most suitable candidate for chemical and biological sensing applications. [13-15]. These type of sensors have already been used for hormone detection[16, 17], quality monitoring of dairy, meat and leather products[18-22] humidity sensors[23], chemical sensing[24-26], and position sensors[27].

A. Sensor Design for ID Capacitive Sensors

In order to maintain system linearity and reversibility, very low amplitude perturbation is applied to the ID sensors therefore; the spatial wavelength has to be kept in the range of few microns, consequently limiting the penetration depth of the fringing electric field. Penetration depth of the fringing electric field was achieved by employing a smart design with multiple sensing electrodes between working electrodes. The design optimization and performance evaluation was carried out by finite element modelling (FEM) software COMSOL® Multiphysics using its AC/DC module in 3D quasi-static mode. Simulated mathematical model depicted that new sensor design is better as compared to the conventional sensor design with respect to size, design, performance and sensitivity. Two designs with eleven and five sensing electrodes with pitch length (distance between two neighbouring electrodes) set at 50 µm showed better sensitivity. Figure 1 shows the sensor's geometric layout in comparison to the conventional sensors. New sensor was designed by keeping the substrate material and thickness, electrode material and thickness and effective sensing area constant. Design geometry was drawn in eleven sensing electrodes between two working electrodes with pitch length set at50µm. Sensing area was fixed to 2.5mm x 2.5mm, electrode area 25µm x 2.425mm and electrode thickness to 520 nm was taken as standard. COMSOL® Multiphysics

software estimated an average penetration depth of 437.6 microns for eleven electrode design therefore it was selected for the fabrication. Detailed analysis and comparison results for the new design have been discussed elsewhere.[28-30]

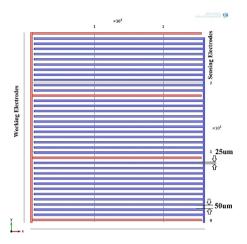


Fig.1 New smart design Si base ID sensor (1-11-50)

III. DEVELOPMENT OF PORTABLE FRA DEVICE

With an enormous research in the field of impedance measurements, frequency response analyser (FRA) has achieved a de facto standard for electrochemical impedance measurements. A dc potential overlaid by a small amplitude (5-15mV) ac sine wave applied to the excitation electrode and the consequent measurement of resulting ac current made is commonly termed as FRA.

A. Design

Direct digital synthesis (DDS) was used to produce the sine wave at a digital-to-analogue (DAC) interface. This is implemented by breaking a wave form into discrete points allowing for the waveform to be digitally replicated; the more points taken and the higher precision the DAC, the higher the resolution becomes compared to an analogue signal. 256 (8 bit) points were used to produce a sine wave that gave a compromise between resolution and frequency; as resolution increases, max frequency achievable decreases. The ability of Arduino microcontroller to set a whole port in one instruction allowed to be sent to the DAC attached to an 8 pin microcontroller port producing a reliable sine wave up to 1KHz., an R2R ladder DAC attached to an 8 pin port was used to produce the sine wave due to unavailability of built-in DAC in the Arduino microcontroller. This removed the need for a filter, and allowed for a controllable range of frequencies. However it gave issues when there were many interrupts present to monitor the sensor, which deformed the waveform. A second Arduino microcontroller was used for sine wave generation to overcome this allowing precise and reliable frequency control. The waveform required to be about the ground reference having positive and negative features to give the desired input. This was achieved by using a Non-inverting op-amp with a negative offset to both push the wave form

down, and to attenuate it to the required voltage. The op-amp needed to be attached to a negative voltage supply to output a negative voltage. This was achieved using a single power supply by using a 555 IC. Three operational amplifiers were used around the sensor to collect the signal and the current through the system was measured through a series resistor. Due to the sensor following resistors' small size the voltage drop over the resistor was significantly small, affecting the accuracy of the ADC. To reduce this issue an op-amp was used to give a positive gain of 7 times to amplify the signal. This op amp also isolated the sensor from any circuitry used in front of this op-amp. This was important not to affect the capacitance of the sensor as this is the main factor being measured. A DC offset needed to be added to the signal before Arduinos' ADC as it cannot handle a negative voltage. This offset was produced by a simple voltage divider, and a capacitor to decouple the offset from the output of the op-amp as shown below. Two diodes before the ADC were used to make sure the voltage stayed between 0 and 5V. If they went higher or lower than this, the voltage would be dumped into the supply lines protecting the ADC.

The phase shift was obtained by using two zero-crossing circuits and two rising edge external interrupts on the Arduino. The output of this circuit is then sent through a diode (D3 and D5) to limit the negative current to 0.7V and a resistor (R996 and R997) to limit the current to 0.5mV. A second diode pair (D4 and D6) are meant to provide extra protection against high negative voltage. The RC combination (R998, C999, R999, and C998) were inspired by Oscilloscope probes that use the same circuit. The affect produced is not losing the signal at high frequencies due to noise and spikes. The diagram for the designed circuit is shown in figure 2, whereas figure 3 shows the Assembled and fully operational testing system.

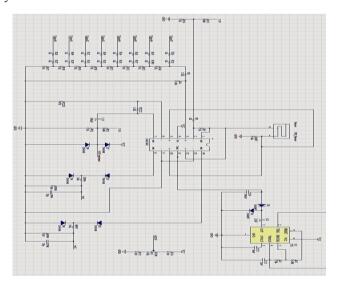


Fig.2 Circuit diagram for the designed Arduino microcontroller based portable FRA

A GUI interface was written in C++ that listens to the Serial port waiting for the starting frequency, and collects data until the starting frequency occurs again. The Microcontroller

formats the data being output in the following way "F#\n" "#\n" "for each reading. This allows the GUI to identify the starting point of the data with the F, and each measurement

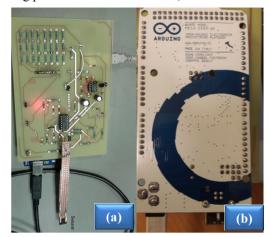


Fig.3 Circuit board (a) Component side with attached sensor (b) Arduino microcontroller board for portable FRA

ends with a line character i.e. print line (F + Frequency), print line (RMS), and print line (phase shift).

The information read by the GUI was converted for analytical use. The output of the device read is the Frequency, V_{rms} , and Phase shift (Θ). The absolute value of Impedance is calculated as follows

Impedance $|Z| = 1.18V_{rms}/70~000$

However this RMS value was amplified by 7 to make it readable by the ADC, therefore it is really 7 times smaller; hence 70 000, rather than 10000 (the resistor value). Figure 4

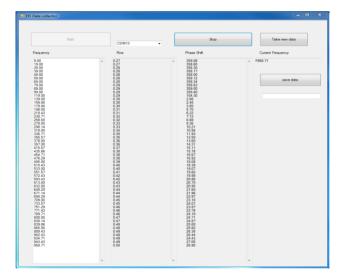


Fig.4 Graphical User Interface and Data acquisition for the designed Arduino microcontroller based portable FRA

displays the data acquisition graphical user interface.

IV. RESULTS AND DISCUSSIONS

To verify the functionality of the designed device, the phase and $V_{\rm rms}$ measurements were compared to an oscilloscope. It should be noted that only the $V_{\rm rms}$ response was expected to match as the Oscilloscope was measuring peak-peak, which is very susceptible to noise. Phase shift between the input and the output of the sensor is the most critical parameter in determining the impedance. The comparison results in Table 1 shows an error less than 2 percent for the designed device as compared to the phase shift measured by Hioki (Japan) high precision LCR meter.

TABLE1
PERFORMANCE COMPARISON MEASURED PARAMETERS

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F(Hz)	Phase (deg)		%erro	%erro V ₁		rms %error	
	Tester	LCR	r	Tester	OSC	/001101	
10	1.72	1.75	1.72	0.57	0.58	1.7	
100	1.07	1.05	1.90	0.57	0.58	1.72	
200	-4.95	-5.0	1	0.615	0.62	0.80	
300	-11.98	-12.0	0.16	0.63	0.64	1.56	
400	-16.1	-16.0	-0.62	0.68	0.67	-1.4	
500	-21.15	-21.1	-0.23	0.69	0.69	0	
600	-25.3	-25.1	-0.79	0.73	0.73	0	
700	-29.4	-29.2	-0.68	0.74	0.75	1.333	
800	-32.95	-32.8	-0.45	0.81	0.80	-1.25	
900	-35.32	-35.3	-0.05	0.82	0.81	-1.23	

A. Material and Method

Di (2-ethylhexyl) phthalate (DEHP) solution prepared gravimetrically at a concentration of $200\mu g/mL$ (200 ppm) in ethanol was received from ChemService® USA. Deionized water 18.2 $M\Omega\text{-cm}$ (MilliQ®) from Millipore USA was used to prepare the samples of lower concentration by serial dilution method. Pure deionized water with a 200ppm concentration of ethanol was used as control solution for the experiments. pH meter from IQ Scientific Instrument Inc. USA was used to measure pH levels of the test and control solutions. The pH meter was calibrated with buffer solutions provided by the manufacturer prior to the testing of pH of the test samples.

Three different concentrations of DEHP - 1, 10, and 50mgl⁻¹ (ppm) in deionized water were tested at controlled temperature (20.3°C) and humidity (44%) conditions. Control solution was 18MΩ-cm deionized MilliQ water with pH 6.71. The average pH value measured for all three samples was measured to be 6.52 at 20.3°C. The sensing system was profiled in air before its exposure to the different DEHP concentrations to create an experimental reference curve for the sensor performance. This curve plays an important role in resetting the sensor to its initial conditions after testing each concentration.

B. Electrochemical Impedence Spectroscopy Characterization

The outcome of an impedance measurement is represented as bode and Nyquist (Cole-Cole) plot which is further analyzed on basis of Randle's equivalent circuit model in order to deduce electrical equivalent of exchange and diffusion processes describing the system kinetics by complex nonlinear least square curve fitting

(CNLS) of the experimental data. Bode plot for the portable testing system is given in figure 5. The decreasing diameter of the Nyquist plot indicates the increasing concentration of the phthalate in the sample. It is observed that the sensor is sensitive to the adsorbed phthalate molecules at the electrode-solution interface at low

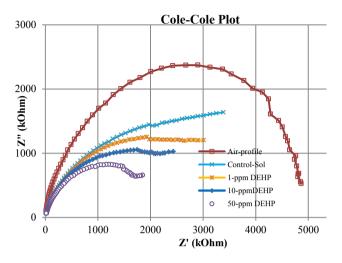


Fig.5 Nyquist plot for data measured by the designed Arduino microcontroller based portable FRA

frequencies. Real part of impedance curve dominates due to the presence of ionic concentration in the sample. The diffusion of ions in the electrode surface is a fast kinetic process which is taking place at low frequency range, on the other hand the capacitive behavior of the sensor is observed at relatively high frequency range. Due to the dielectric properties of DEHP the corresponding change in real and imaginary impedance values causes the diameter of the Nyquist plot to decrease with increasing concentration of DEHP in the sample solution. In order to investigate the dominance of ionic concentration in the test samples, real and

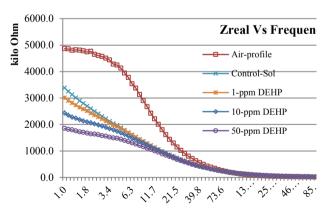


Fig.6 Z' vs F (Hz) plot for the data acquired by portable FRA

imaginary parts of impedance are plotted against frequency which provide a clear picture about the nature of the material under test. Figure 6 shows real part of the impedance plotted against frequency using new designed portable tester. It can be seen from the plot that the concentration change in analyte changes the real impedance but it is not as dominating as the imaginary part of the impedance. The change in imaginary impedance represents the dielectric nature of the material under test consequently increasing the capacitance value of the sensor. The plot for the imaginary part of impedance is shown in figure 7. The plot shows that with the increasing concentration of DEHP the imaginary impedance of the system is decreasing which describes the dielectric nature of the analyte molecule.

Figure 8 below shows the HPLC chromatograph for eluent after testing each sample. A DEHP peak is observed at 1.91 minutes with few initial noise peaks. The noise observed before the appearance of the DEHP peak is predominantly due

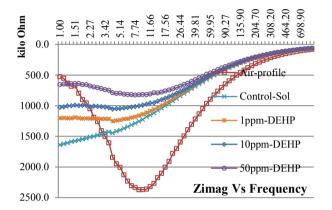


Fig 7. Z" vs F (Hz) plot for the data acquired by portable FRA

to the presence of traces of solvent in the eluent which appears even in the HPLC chromatograph obtained for the control

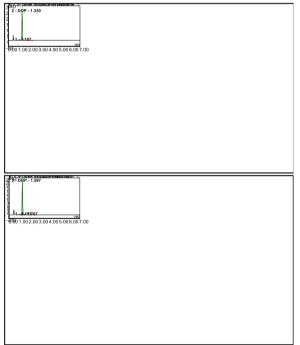


Fig. 8 HPLC-DAD for eluent from (a) 1ppm and (b) 10ppm DEHP in spiked DI water

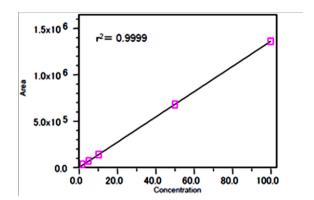


Fig 9. Calibration Plot shows good linearity for concentration from 0.1 to 100mg/L (0.1ppm to 100 ppm)

solution. The insolubility of DEHP in polar medium dictates the requirement for the use of solvent to dissolve it in aqueous medium.

CONCLUSIONS

A portable low-cost microcontroller based testing system has been designed and developed to detect phthalates in aqueous media. Frequency response analyser (FRA) approach has been used to develop the rapid response, non-invasive electrochemical impedimetric system for phthalate detection. A smart thin film gold interdigital electrodes capacitive sensor with enhanced penetration depth was used with the proposed system for detection of the said EDC. The performance of the developed system has been evaluated by comparing the results to the commercially available electrochemical Impedimetric frequency response analyzer equipment. The DEHP detection results by the developed system have been validated using high performance liquid chromatography (HPLC) diode array detection confirming that the proposed system was able to detect the concentration of phthalates in aqueous medium.

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